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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, cytotoxic evaluation, and molecular docking study of 4,5-diaryl-thiazole-2-thione analogs of combretastatin A-4 as microtubule-binding agents

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Abstract A series of combretastatin A-4 analogs in which *cis*-olefinic bond replaced by thiazole ring were prepared by reaction of α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones and dithiocarbamate derivatives. The cytotoxicity of these compounds was determined against three cancer cell lines (HT-29), (MCF-7), (AGS) as well as fibroblastic cell line (NIH-3T3) using MTT assay. Inhibition of tubulin polymerization for some potent compounds was evaluated. These biological studies proved that **6j** and **6o** were the most potent compounds in this series. Furthermore 2-(methylthio)-substituted compounds show moderate or no activity. Docking studies involving **6j** and **6o** demonstrated that this analogs could be successfully docked in the colchicine binding site of α,β-tubulin.

Keywords 4,5-Diaryl-thiazole · Tubulin · Cytotoxicity · Molecular docking

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Introduction

Microtubules are globular protein polymers compose of α and β-tubulin heterodimers. These fundamental components of all eukaryotic cells involved in critical cellular functions, such as maintenance of cellular shape, cell transport, mitosis, and cell signaling (Walczak 2000).

Microtubule-binding agents (MBAs) are widely used in cancer chemotherapy because the inhibition of tubulin polymerization leading to cell death by apoptosis. MBAs are tubulin binders that work by stabilization of microtubules and promote polymerization or by destabilization and prevent polymerization (Aryapour *et al.*, 2011). There are three different binding sites for compounds that interact with microtubules including; vinca, taxanes, and colchicines (Li *et al.*, 2011). Combretastatin A-4 (**7**) is *cis*-stilbene deivative that isolated from the African willow tree (*combretum caffrum*) (Fig. 1). This compound binds to colchicine site of tubulin and perturbs microtubule polymerization (Kumar *et al.*, 2012). Although **7** has strong cytotoxic effect against wide variety of human cancer cell lines, but does not show efficacy in vivo, because of its low aqueous solubility and the high tendency its *cis*-double bond to isomerizes into the more thermally stable and inactive *trans*-isomer (Romagnoli *et al.*, 2010).

Two aromatic rings that linked by a double bond in the *cis* configuration is necessary for a high antitumoral activity of combretastatin. Therefore many structural modifications of **7** have been reported that olefinic bond replaced by heterocyclic analogs which were more stable and soluble. Among the modifications, the replacement with five-membered rings is the promising target. Thiophene (Theeramunkong *et al.*, 2011), pyrazole (Ohsumi *et al.*, 1998), imidazole (Schobert *et al.*, 2010), isoxazole (Kaffy *et al.*, 2006), thiazole (Ohsumi *et al.*, 1998), triazole (Odlo *et al.*, 2008), and many other rings have been synthesized so far.

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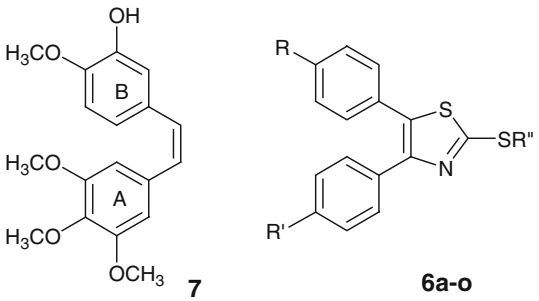


Fig. 1 Structure of combretastatin A-4 (**7**) and 4,5-diaryl-thiazol-2-thiones (compounds **6a–o**)

In this article, we designed and synthesized a new series of 4,5-diaryl-thiazol-2-thialkyl analogs of **7** (Fig. 1) and the cytotoxicity of all of synthesized compounds were determined against four cell lines. Additionally, some of the most potent compounds were selected for further evaluation of tubulin polymerization activity. Finally, molecular docking studies of two analogs were performed to investigate the binding mode of selected compounds with the active site of the protein.

Results and discussion

Chemistry

For synthesis of 1,2-(4-substituted)diaryl-1-ethanones **3a–h**, appropriate phenyl acetic acid **1a–d** were reacted with various substituted aromatic hydrocarbons **2a–c** in presence of TFAA and phosphoric acid (Veeramaneni *et al.*, 2003). Subsequently, **3a–h** were brominated using liquid bromine in glacial acetic acid to obtain related α-bromo-1,2-(*p*-substituted)diaryl-1-ethanones **4a–h** (Gadad *et al.*, 2008). In other reaction, ammonium dithiocarbamate was prepared by passing ammonia through a solution of carbon disulfide (Epple *et al.*, 2010). In the next step, ammonium dithiocarbamate reacted with alkyl and benzyl iodides to prepared dithiocarbamate derivatives **5a–c**. Finally, the title compounds 4,5-diaryl-2-(alkyl or benzyl)-thiazoles **6a–o** were prepared by the reaction related bromoketones **4a–h** with different dithiocarbamate derivatives under reflux condition (Scheme 1). Compound **7** was synthesized as a positive control according to the previous literature (Gaukroger *et al.*, 2001). All of synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, EI-Mass, and CHN analysis.

Biological study

Cell growth inhibitory assay

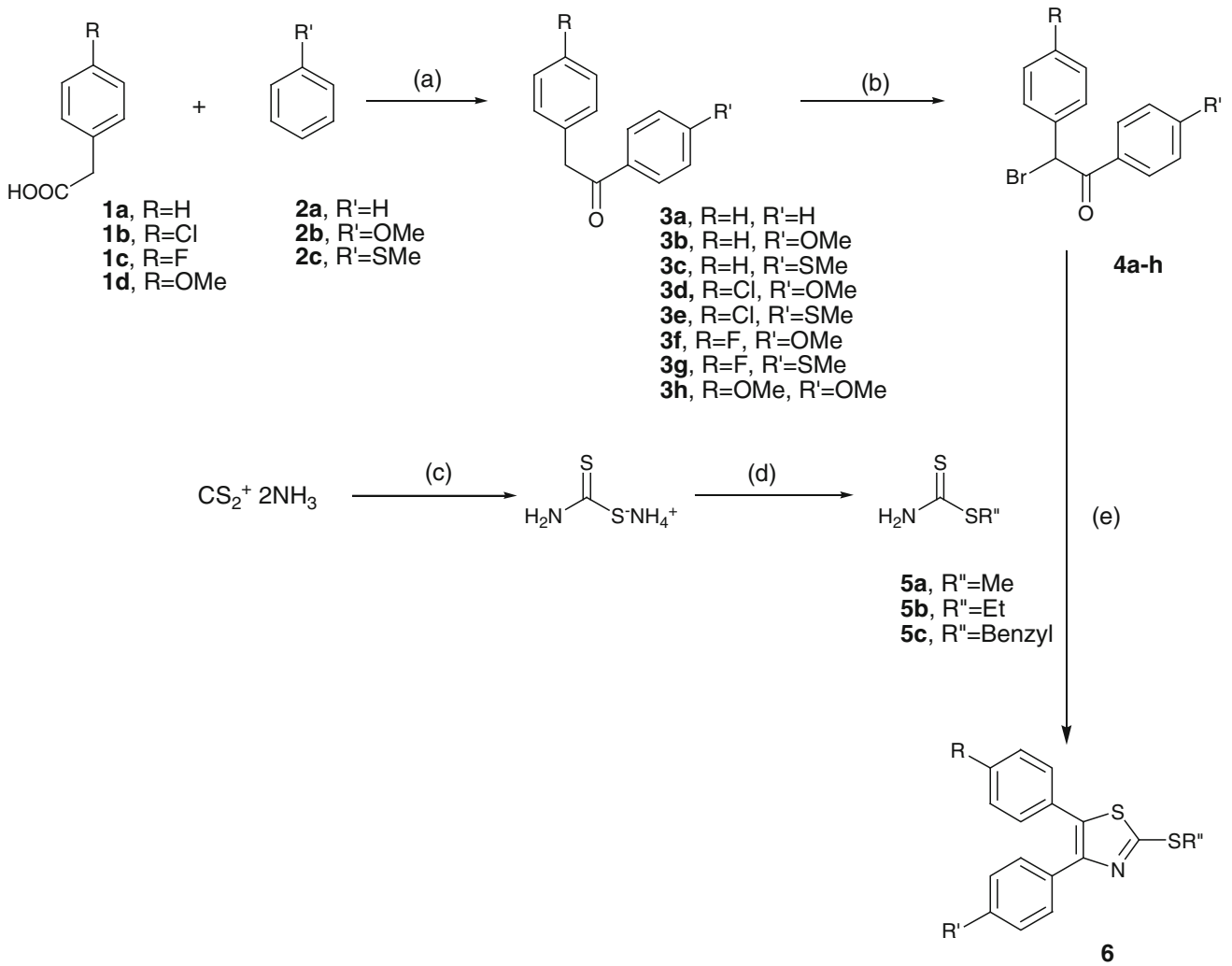
MTT colorimetric assay (Scudiero *et al.*, 1988; Aliabadi *et al.*, 2010) was employed to evaluate the ability of synthetic compounds **6a–o** to inhibit the growth of three cancer cell lines including the human colon adenocarcinoma (HT-29), human breast adenocarcinoma (MCF-7), and human stomach adenocarcinoma (AGS) as well as mouse embryonic fibroblast cell line (NIH-3T3). The culture cells were treated with several concentrations of test compounds for 48 h. The ability of these analogs to inhibit the growth of cells is summarized in Table 1. These results revealed that proliferation of HT-29 cell line was not affected by any of the synthesized compounds; expect the **6j** which showed a weak cytotoxicity. There is an interesting point that **6j** showed very low toxcicity against NIH-3T3 cell line compared to **7**. The compounds **6j** with 4-chloro and 4-thiomethyl substituted on phenyl ring had greater cytotoxic activity (IC_{50} = 7.1 μM) against MCF-7 cell line than other compounds and **6o** with methoxy group on two phenyl rings and 2-(benzylthio) group exhibited moderate antiproliferative effect (IC_{50} = 23.8 μM, IC_{50} = 23.9 μM) against MCF-7 and AGS cell lines. In contrast, **6n** with two para methoxy groups on phenyl and 2-(methyl thio) substitution on thiazole ring had the lowest cytotoxic activity.

In this study we synthesized 15 new compounds and tested them against three cancer cell lines, human colon carcinoma cell line HT-29, human breast adenocarcinoma cell line MCF-7, and human caucasian gastric adenocarcinoma cell line AGS as well as fibroblast cell line NIH-3T3 by MTT test.

Structure activity relationship (SAR) studies of **7** and some related structures have shown that trimethoxy phenyl (A-ring, Fig. 1) and *cis*-configuration are important for cytotoxic and antitubulin activities. These studies demonstrated that reducing the number of methoxy groups or replacement by other groups in A-ring reduced the cytotoxic activity. The SAR studies has also been underlined that the changes of the substituted group B-ring are acceptable, so that 3-hydroxy group is not necessary for antiproliferative activity and 4-methoxy group of B-ring could be replaced with different groups (Assadieskandar *et al.*, 2013; Pandit *et al.*, 2006).

Impacts of the number of OCH_3 in some analogous of **7** in which 1*H*-indole-2-one was used as central heterocyclic ring, showed that tri-OCH_3 in A-ring is optimum for the activity against PC-3 and MDA-MB-231 cell lines. Reducing the number of methoxy groups from 3 to 1 decreased the activity of the compounds by 2–3 order of magnitude. However, there is no data for the cytotoxicity of 1*H*-indole-2-one derivatives in normal cell lines (Pandit *et al.*, 2006).

The thiazole ring has been reported as a suitable mimic for the *cis*-olefin present in **7** (Romagnoli *et al.*, 2012). The same results were reported about the role of the number of OCH_3 groups in A-ring.



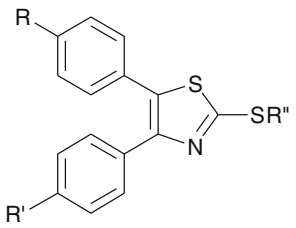
Scheme 1 Reagents and conditions: *a* H_3PO_4, $(CF_3CO)_2O$, 25 °C, 1 min; *b* Br_2, CH_3COOH, 25 °C, 2 h; *c* THF, 25 °C; *d* Methanol, alkyl, or benzyl iodide, 25 °C; *e* Methanol, reflux, 24 h

Our results are in agreement with previous studies. However, the cytotoxic effects of the synthesized compounds **6a–o** are less than reference **7**, in all tested cell lines, there are some interesting results related to selectivity of their cytotoxic properties. A comparison of the biologic activities of the tested compounds and reference compound clearly shows that **7** is very toxic for normal cell, NIH-3T3 as well as three tested cell lines. Compounds **6d**, **6i**, **6j**, and **6l** showed a partial selectivity effects for one or more of the tested cell lines. Attention to the chemical structure of above compounds illustrates that all of them were substituted with benzyl group in 2-position of thiazole ring. The replacement of benzyl group with methyl group was not useful for increasing the activity (**6n** and **6o**, **6k** and **6l**, **6e** and **6f**, **6c** and **6d**, **6a** and **6b**). The most active compound was **6j** that showed the partial selectivity on all tested cell lines. Although the activity of compound **6j** is not remarkable to comparison of the reference compounds, it seems that the substitution of SCH_3 and Cl in 4-position of phenyl groups could be conducted to the selectivity. The structure of compound **6j** could be considered as a probe for further development studies because design and synthesis of new diaryl-heterocyclic analogs would still merit consideration in order to increase the richness of structure repertoire and optimizing the lead structure.

Inhibition of tubulin polymerization

To investigate whether these compounds bind to tubulin and inhibit its polymerization, the inhibitory effect of three potent compounds **6h**, **6j**, **6o** on the polymerization of purified tubulin was evaluated. Compound **7** was also evaluated by this assay as a positive control. Figure 2 shows that **6h** was ineffective in the tubulin assay but in the presence of two other compounds **6j** and **6o**, polymerization activity of microtubule was significantly lower than

Table 1 Structure and cytotoxicity of analogs of combretastatin A-4



Compounds	R	R′	R″	(IC_{50}, μM)[a]			
				HT-29	MCF-7	AGS	NIH-3T3
6a	H	H	CH_3	55.4 ± 2.5	88.3 ± 2.2	53.2 ± 1.4	75.6 ± 2.4
6b	H	H	CH_2ph	>100	N.D	>100	86.3 ± 1.8
6c	H	OCH_3	CH_3	83.0 ± 1.9	>100	38.0 ± 1.1	72.4 ± 1.2
6d	H	OCH_3	CH_2ph	82.3 ± 0.9	>100	51.7 ± 1.2	>100
6e	H	SCH_3	CH_3	45.9 ± 1.2	N.D	>100	>100
6f	H	SCH_3	CH_2ph	>100	N.D	>100	>100
6g	Cl	OCH_3	CH_3	50.1 ± 1.0	48.8 ± 1.7	27.1 ± 1.3	29.2 ± 0.8
6h	Cl	OCH_3	CH_2CH_3	49.5 ± 1.5	41.2 ± 1.3	37.3 ± 1.9	53.7 ± 1.1
6i	Cl	OCH_3	CH_2ph	>100	>100	79.1 ± 2.4	>100
6j	Cl	SCH_3	CH_2ph	31.9 ± 0.8	7.1 ± 0.6	35.4 ± 1.8	>100
6k	F	OCH_3	CH_3	69.5 ± 1.7	45.4 ± 1.0	82.7 ± 1.4	29.2 ± 1.3
6l	F	OCH_3	CH_2ph	>100	53.2 ± 1.5	94.2 ± 2.0	>100
6m	F	SCH_3	CH_2ph	>100	N.D	>100	>100
6n	OCH_3	OCH_3	CH_3	>100	>100	>100	>100
6o	OCH_3	OCH_3	CH_2ph	87.5 ± 1.8	23.8 ± 0.9	23.9 ± 1.3	38.3 ± 0.9
7				1.58 ± 0.5	0.67 ± 0.2	0.064 ± 0.04	0.097 ± 0.08

N.D not determined

[a] Values are the mean ± SD. All experiments were performed at least three times

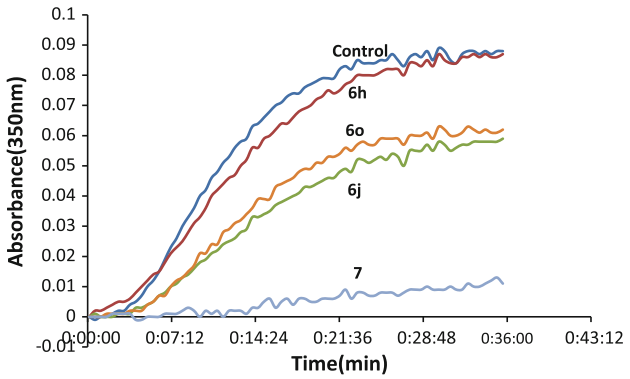


Fig. 2 Effect of 10 μM each of compounds (*6h*), (*6j*), (*6o*) on the polymerization of tubulin, compound *7* represents as a positive control

control experiment. However, the inhibition activity level of these compounds was less than that of **7** in final concentration of 10 μM. Comparing the tubulin inhibition with corresponding cytotoxic activities revealed that the two data were correlated with together and it seems that tubulin is a fair target for 4,5-diaryl-thiazole-2-thione analogs of reference **7**.

Molecular modeling

Molecular docking of the most potent inhibitors **6j** and **6o** in the colchicine binding site of tubulin was performed on the binding model based on the tubulin structure (PDB code: 1SA0) (Ravelli *et al.*, 2004). The binding model observed for this series of compounds is very similar to the one observed for the cocrystallized DAMA-colchicine (Fig. 3). The phenyl group on 4-position of thiazole ring mimics the three methoxy phenyl moiety of the colchicine and was buried in the α,β-tubulin structure near the cys249, while the aromatic ring in the 5-position of the thiazole ring establishes a π–π interaction with Asn258 and a series of hydrophobic interactions with Met259 and Lys352. These results postulated that the cytotoxic activity of this

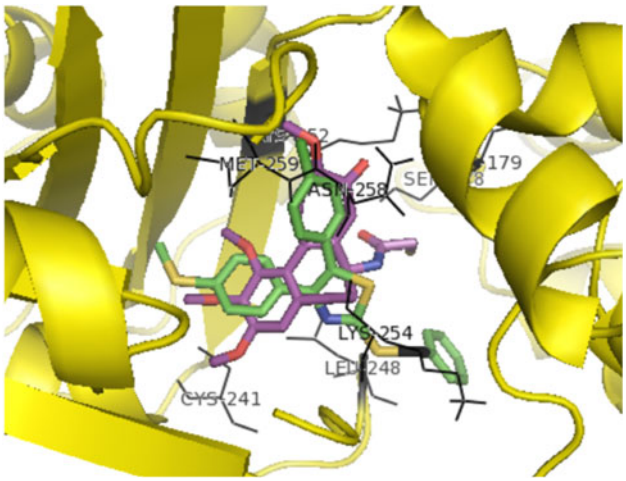


Fig. 3 Docked pose of **6j** (*green*) overlapped with DAMA-colchicine (*magneta*) in the tubulin-binding site

series may be attributed to interaction with colchicines binding site between α and β subunit of tubulin protein.

Materials and methods

Chemistry

All starting compounds were purchased from Merck. Melting points determined by using a Thomas Hoover melting point apparatus. The ^{1}H NMR spectra were record on Bruker FT-500 MHz spectrometer. The instrument was set as 125 MHz for acquiring ^{13}C NMR spectra. Chemical shifts are given in parts per million (δ, ppm) with respect to TMS as internal standard. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus. Elemental analyses were within ±0.4 % of theoretical values for C, H, and N. Infrared spectra were acquired on a Nicolet Magna 550-FT spectrometer. EI-Mass was recorded on a Agilent 5975C with triple-axis detector.

General method for preparation of 4,5-diaryl-2-(alkyl or arylthio)-thiazole

To a solution of ammonium dithiocarbamate (10 mmol) in methanol, benzyl, or alkyl iodide (15 mmol) was added and the mixture was stirred at room temperature (RT). After 1 h, methanol was removed in vacuo and the remnant was extracted with dichloromethane. After distillation of solvent under reduced pressure, prepared oil compound (12 mmol) was added to a solution of α-bromo-1,2-(*p*-substituted)diaryl-1-ethanone (10 mmol) in methanol. The mixture was heated to reflux overnight. Then the reaction was cooled to RT and the precipitated product was filtered and recrystallized from ethanol to obtain 4,5-diaryl-2-(alkyl or aryl thio)-thiazole compounds.

4,5-Bis(phenyl)-2-(methylthio)-thiazole ***(6a)*** Yield: 65 %. Mp: 78–81 °C, IR (KBr, cm^{-1}) *v* 3061, 2967, 2919, 1595, 1473, 1436, 1408, 1319, 1221, 1306, 964, 754, 705. ^{1}H NMR ($CDCl_3$) δ: 2.77 (s, 3H, SCH_3), 7.28–7.31 (m, 6H, Ar), 7.55–7.59 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 16.68 (CH_3), 27.89 (C_4-Ar_5), 128.15 (C_4-Ar_4), 128.29 ($C_{3,5}$-Ar_4), 128.79 ($C_{3,5}$-Ar_5), 129.02 ($C_{3,5}$-Ar_4), 129.56 ($C_{2,6}$-Ar_5), 131.81 (C_1-Ar_5), 132.2 (C_5-thiazole), 134.52 (C_1-Ar_4), 149.7 (C_4-thiazole), 164.07 (C_2-thiazole). EI-Mass, *m/z* (%); 283 (100), 250 (55), 210 (40), 179 (15), 165 (36), 77 (10). Anal. Calcd. For $C_{16}H_{13}NS_2$: C, 67.81; H, 4.62; N, 4.94. Found: C, 67.98; H, 4.83; N, 5.15.

4,5-Bis(phenyl)-2-(benzylthio)-thiazole ***(6b)*** Yield: 55 %. Mp: 101–102 °C, IR (KBr, cm^{-1}) *v* 3027, 2934, 2837, 1606, 1501, 1474, 1402, 1294, 1255, 1171, 1092, 1031, 960, 818, 713. ^{1}H NMR ($CDCl_3$) δ: 4.51 (s, 2H, CH_2), 7.27–7.42 (m, 11H, Ar), 7.49 (d, $J = 7.5$ Hz, 2H, $H_{2,6}$–Ar_4), 7.57 (d, $J = 7.5$ Hz, 2H, $H_{2,6}$–Ar_5). ^{13}C NMR ($CDCl_3$) δ: 38.61 (CH_2), 127.70 (C_4-Benzyl), 127.92 (C_4-Ar_5), 128.22 (C_4-Ar_4), 128.31 ($C_{3,5}$-Ar_4), 128.69 ($C_{2,6}$-Benzyl), 128.80 ($C_{3,5}$-Ar_5), 129.02 ($C_{2,6}$-Ar_4), 129.21 ($C_{3,5}$-Benzyl), 129.57 ($C_{2,6}$-Ar_5), 131.71 (C_1-Ar_5), 132.93 (C_5-thiazole), 134.47 (C_1-Ar_4), 136.61 (C_1-Benzyl), 149.63 (C_4-thiazole), 161.86 (C_2-thiazole). EI-Mass, *m/z* (%); 359 (92), 326 (85), 210 (100), 165 (40), 91 (78), 65 (29). Anal. Calcd. For $C_{22}H_{17}NS_2$: C, 73.50; H, 4.77; N, 3.90. Found: C, 73.65; H, 4.96; N, 5.32.

4-(4-Methoxyphenyl)-5-(phenyl)-2-(methylthio)-thiazole ***(6c)*** Yield: 64 %. Mp: 68–69 °C, IR (KBr, cm^{-1}) *v* 3057, 2965, 2924, 2842, 1607, 1505, 1476, 1415, 1327, 1296, 1248, 1169, 1028, 829, 757. ^{1}H NMR ($CDCl_3$) δ: 2.75 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 6.83 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_4), 7.30–7.36 (m, 5H, Ar_5), 7.48 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_4). ^{13}C NMR ($CDCl_3$) δ: 16.8 (SCH_3), 55.32 (OCH_3), 113.55 ($C_{3,5}$-Ar_4), 127.04 (C_1-Ar_4), 128.01 (C_4-Ar_5), 128.81 ($C_{3,5}$-Ar_5), 129.53 ($C_{2,6}$-Ar_5), 130.28 ($C_{2,6}$-Ar_4), 130.81 (C_1-Ar_5),132.0 (C_5-thiazole), 149.51 (C_4-thiazole), 159.29 (C_4-Ar_4), 163.98 (C_2-thiazole). EI-Mass *m/z* (%); 313 (100), 280 (25), 240 (28), 225 (20). Anal. Calcd. For $C_{17}H_{15}NOS_2$: C, 65.14; H, 4.82; N, 4.47. Found: C, 65.32; H, 4.93; N, 4.55.

4-(4-Methoxyphenyl)-5-(phenyl)-2-(benzylthio)-thiazole ***(6d)*** Yield: 67 %. Mp: 75–77 °C, IR (KBr, cm^{-1}) *v* 3030, 2929, 2832, 1604, 1507, 1474, 1326, 1245, 1175, 1030, 830, 750, 689. ^{1}H NMR ($CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 4.52 (s, 2H, CH_2), 6.85 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_4),

7.27–7.44 (m, 8H, Ar), 7.45–7.56 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 38.61 (CH_2), 55.26 (OCH_3), 113.69 ($C_{3,5}$-Ar_4), 127.10 (C_1-Ar_4), 127.68 (C_4-Benzyl), 128.07 (C_4-Ar_5), 128.68 ($C_{2,6}$-Benzyl), 128.78 ($C_{3,5}$-Ar_5), 129.20 ($C_{3,5}$-Benzyl), 129.55 ($C_{2,6}$-Ar_5), 130.27 ($C_{2,6}$-Ar_4), 131.55 (C_1-Ar_5), 131.96 (C_5-thiazole), 136.66 (C_1-Benzyl), 149.49 (C_4-thiazole), 159.29 (C_4-Ar_4), 161.57 (C_2-thiazole). EI-Mass *m/z* (%); 389 (100), 356 (70), 240 (91), 226 (70), 197(20), 165 (16), 121 (12), 91 (40). Anal. Calcd. For $C_{23}H_{19}NOS_2$: C, 70.92; H, 4.92; N, 3.60. Found: C, 71.05; H, 4.91; N, 3.52.

4-(4-Methylthiophenyl)-5-(phenyl)-2-(methylthio)-thiazole ***(6e)*** Yield: 46 %. Mp: 89–90 °C, IR (KBr, cm^{-1}) *v* 3073, 2917, 1595, 1491, 1432, 1327, 1031, 853, 816, 750. ^{1}H NMR ($CDCl_3$) δ: 2.48 (s, 3H, SCH_3), 2.75 (s, 3H, SCH_3), 7.16 (d, $J = 8.9$ Hz, 2H, $H_{2,6}$-Ar_4), 7.32-7.36 (m, 5H, Ar_5), 7.47 (d, $J = 8.9$ Hz, 2H, $H_{3,5}$-Ar_4). ^{13}C NMR ($CDCl_3$) δ: 15.53 (SCH_3), 16.68 (SCH_3), 125.96 ($C_{3,5}$-Ar_4), 128.19 (C_4-Ar_5), 128.85 ($C_{3,5}$-Ar_5), 129.30 ($C_{2,6}$-Ar_4), 129.53 ($C_{2,6}$-Ar_5), 131.13 (C_1-Ar_4), 131.78 (C_1-Ar_5), 131.78 (C_5-thiazole), 138.29 (C_4-Ar_4), 149.07 (C_4-thiazole), 164.16 (C_2-thiazole). EI-Mass *m/z* (%); 329 (100), 296 (25), 267 (22), 241 (23), 208 (24), 165(17), 121 (12), 77 (10). Anal. Calcd. For $C_{17}H_{15}NS_3$: C, 61.97; H, 4.59; N, 4.25. Found: C, 62.15; H, 4.51; N, 4.18.

4-(4-Methylthiophenyl)-5-(phenyl)-2-(benzylthio)-thiazole ***(6f)*** Yield: 67 %. Mp: 92–93 °C, IR (KBr, cm^{-1}) *v* 3028, 2914, 1593, 1492, 1473, 1399, 1321, 1244, 1116, 1085, 1024, 958, 821, 751. ^{1}H NMR ($CDCl_3$) δ: 2.49 (s, 3H, SCH_3), 4.52 (s, 2H, CH_2), 7.16 (d, $J = 7.0$ Hz, 2H, $H_{2,6}$-Ar_4), 7.28–7.39 (m, 8H, Ar), 7.44–7.51 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 15.53 (SCH_3), 38.59 (CH_2), 125.95 ($C_{3,5}$-Ar_4), 127.70 (C_4-Benzyl), 128.27 (C_4-Ar_5), 128.69 ($C_{2,6}$-Benzyl), 128.84 ($C_{3,5}$-Ar_5), 129.17 ($C_{3,5}$-Benzyl), 129.29 ($C_{2,6}$-Ar_4), 129.55 ($C_{2,6}$-Ar_5), 131.02 (C_1-Ar_4), 131.66 (C_1-Ar_5), 132.50 (C_5-thiazole), 136.54 (C_1-Benzyl), 138.36 (C_4-Ar_4), 148.95 (C_4-thiazole), 162.02 (C_2-thiazole). EI-Mass *m/z* (%); 357 (18), 341 (40), 267 (80), 234 (26), 207 (100), 165 (15), 135 (44), 91 (65), 77 (42). Anal. Calcd. For $C_{23}H_{19}NS_3$: C, 68.11; H, 4.72; N, 3.45. Found: C, 68.25; H, 4.81; N, 3.38.

4-(4-Methoxyphenyl)-5-(4-chlorophenyl)-2-(methylthio)-thiazole ***(6g)*** Yield: 78 %. Mp: 95–96 °C, IR (KBr, cm^{-1}) *v* 3007, 2967, 2831, 1605, 1531, 1472, 1425, 1410, 1173, 1091, 1030, 960, 866, 821. ^{1}H NMR ($CDCl_3$) δ: 2.74 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 6.84 (d, $J = 8.5$ Hz, 2H, $H_{3,5}$-Ar_4), 7.22–7.34 (m, 4H, Ar), 7.45 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_5). ^{13}C NMR ($CDCl_3$) δ: 15.48 (SCH_3), 54.20 (OCH_3), 114.1 ($C_{3,5}$-Ar_4), 125.97 (C_1-Ar_4), 129.13 ($C_{3,5}$-Ar_5), 130.12 ($C_{2,6}$-Ar_4), 130.48 (C_1-Ar_5), 130.75 ($C_{2,6}$-Ar_5), 132.0 (C_5-thiazole), 134.30 (C_4-Ar_5), 149.30 (C_4-thiazole), 157.20 (C_4-Ar_4), 162.77 (C_2-thiazole). EI-Mass *m/z* (%); 349 (33), 347 (100), 316 (20), 314 (60), 276 (22), 274 (66), 261 (60), 231 (18), 195 (26), 152 (28). Anal. Calcd. For $C_{17}H_{14}ClNOS_2$: C, 58.69; H, 4.06; N, 4.03. Found: C, 58.89; H, 3.97; N, 3.88.

4-(4-Methoxyphenyl)-5-(4-chlorophenyl)-2-(ethylthio)-thiazole ***(6h)*** Yield: 42 %. Mp: 74–75 °C, IR (KBr, cm^{-1}) *v* 3011, 2970, 2841, 1603, 1528, 1470, 1449, 1416, 1170, 1085, 1038, 970, 865, 829. ^{1}H NMR ($CDCl_3$) δ: 1.50 (t, $J = 7.50$ Hz, 3H, CH_3), 3.27 (q, $J = 7.50$ Hz, 2H, CH_2), 3.82 (s, 3H, OCH_3), 6.84 (d, $J = 8.5$ Hz, 2H, $H_{3,5}$-Ar_4), 7.25 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_5), 7.29 (d, $J = 8.5$ Hz, 2H, $H_{2,6}$-Ar_4), 7.45 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_5). ^{13}C NMR ($CDCl_3$) δ: 14.67 (CH_3), 28.74 (CH_2), 55.26 (OCH_3), 113.77 ($C_{3,5}$-Ar_4), 126.73 (C_1-Ar_4), 129.01 ($C_{3,5}$-Ar_5), 129.55 (C_5-thiazole), 130.26 ($C_{2,6}$-Ar_4), 130.54 (C_1-Ar_5), 130.73 ($C_{2,6}$-Ar_5), 133.91 (C_4-Ar_5), 149.20 (C_4-thiazole), 159.41 (C_4-Ar_4), 162.96 (C_2-thiazole). EI-Mass *m/z* (%); 363 (33), 361 (100), 330 (60), 328 (20), 261 (13), 259 (41), 231 (10), 195 (12), 152 (16). Anal. Calcd. For $C_{18}H_{16}ClNOS_2$: C, 59.74; H, 4.46; N, 3.87. Found: C, 59.89; H, 4.52; N, 3.78.

4-(4-Methoxyphenyl)-5-(4-chlorophenyl)-2-(benzylthio)-thiazole ***(6i)*** Yield: 72 %. Mp: 94–95 °C, IR (KBr, cm^{-1}) *v* 2850, 2837, 1603, 1531, 1495, 1326, 1244, 1173, 1086, 953, 830. ^{1}H NMR ($CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 4.52 (s, 2H, CH_2), 6.86 (d, $J = 8.5$ Hz, 2H, $H_{3,5}$-Ar_4), 7.21–7.39 (m, 7H, Ar), 7.43–7.50 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 38.54 (CH_2), 55.28 (OCH_3), 113.82 ($C_{3,5}$-Ar_4), 126.70 (C_1-Ar_4), 127.71 (C_4-Benzyl), 128.68 ($C_{2,6}$-Benzyl), 129.03 ($C_{3,5}$-Ar_5), 129.17 ($C_{3,5}$-Benzyl), 130.27 ($C_{2,6}$-Ar_4), 130.46 (C_1-Ar_5), 130.76 ($C_{2,6}$-Ar_5), 131.54 (C_5-thiazole), 134.00 (C_4-Ar_5), 136.52 (C_1-Benzyl), 149.83 (C_4-thiazole), 159.47 (C_4-Ar_4), 162.10 (C_2-thiazole). EI-Mass *m/z* (%); 423 (100), 390 (86), 341(26), 274 (95), 207 (71), 135(35), 91 (95), 77 (41), 65 (32). Anal. Calcd. For $C_{23}H_{18}ClNOS_2$: C, 65.16; H, 4.28; N, 3.30. Found: C, 65.35; H, 4.18; N, 3.12.

4-(4-Methylthiophenyl)-5-(4-chlorophenyl)-2-(benzylthio)-thiazole ***(6j)*** Yield: 92 %. Mp: 112–113 °C, IR (KBr, cm^{-1}) *v* 3080, 3031, 2952, 1592, 1496, 1469, 1423, 1399, 1187, 1120, 1087, 863, 700. ^{1}H NMR ($CDCl_3$) δ: 2.50 (s, 3H, SCH_3), 4.51 (s, 2H, CH_2), 7.18 (d, $J = 8.5$ Hz, 2H, $H_{2,6}$-Ar_4), 7.22–7.38 (m, 7H, Ar), 7.42–7.48 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 15.48 (SCH_3), 38.63 (CH_2), 125.97 ($C_{3,5}$-Ar_4), 127.76 (C_4-Benzyl), 128.70 ($C_{2,6}$-Benzyl), 129.13 ($C_{3,5}$-Ar_5), 129.17 ($C_{3,5}$-Benzyl), 129.29 ($C_{2,6}$-Ar_4), 130.12 (C_1-Ar_4), 130.48 (C_1-Ar_5), 130.75 ($C_{2,6}$-Ar_5), 131.90 (C_5-thiazole), 134.30 (C_4-Ar_5), 136.39 (C_1-Benzyl),

138.84 (C_4-Ar_4), 149.30 (C_4-thiazole), 162.77 (C_2-thiazole). EI-Mass *m/z* (%); 441 (71), 439 (48), 281 (50), 253 (91), 207 (100), 164 (10), 135 (35), 91 (47), 73 (52). Anal. Calcd. For $C_{23}H_{18}ClNS_3$: C, 62.78; H, 4.12; N, 3.18. Found: C, 62.91; H, 4.05; N, 2.99.

4-(4-Methoxyphenyl)-5-(4-fluorophenyl)-2-(methylthio)-thiazole ***(6k)*** Yield: 42 %. Mp: 72–74 °C, IR (KBr, cm^{-1}) *v* 3030, 2965, 2849, 1610, 1570, 1510, 1460, 1333, 1269, 1222, 1172, 1031, 991, 831, 784. ^{1}H NMR ($CDCl_3$) δ: 2.74 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 6.95 (d, $J = 8.5$ Hz, 2H, $H_{3,5}$-Ar_4), 7.03 (t, $J = 8.5$ Hz, 2H, $H_{3,5}$-Ar_5), 7.23 (dd, $J = 8.5$ Hz, $J = 5.5$ Hz, 2H, $H_{2,6}$-Ar_5), 8.00 (d, $J = 8.5$ Hz, 2H, $H_{2,6}$-Ar_4). ^{13}C NMR ($CDCl_3$) δ: 16.66 (SCH_3), 55.49 (OCH_3), 113.86 ($C_{3,5}$-Ar_4), 115.41 (d, $J_{CF} = 21.25$ Hz,$C_{3,5}$-Ar_5), 126.48 (C_1-Ar_4), 127.61 (C_1-Ar_5), 130.51 (d, $J_{CF} = 7.52$ Hz,$C_{2,6}$-Ar_5), 130.86 ($C_{2,6}$-Ar_4), 131.39 (C_5-thiazole), 149.41 (C_4-thiazole), 159.25 (C_4-Ar_4), 161.97 (C_2-thiazole), 163.45(d, $J_{CF} = 250.34$ Hz,C_4-Ar_5). EI-Mass *m/z* (%); 331 (100), 281 (35), 258 (38), 207 (45), 207 (45), 135(30), 73 (41). Anal. Calcd. For $C_{17}H_{14}FNOS_2$: C, 61.61; H, 4.26; N, 4.23. Found: C, 61.82; H, 4.15; N, 4.13.

4-(4-Methoxyphenyl)-5-(4-fluorophenyl)-2-(benzylthio)-thiazole ***(6l)*** Yield: 55 %. Mp: 84–85 °C, IR (KBr, cm^{-1}) *v* 2834, 2822, 1604, 1510, 1484, 1450, 1417, 1296, 1247, 1223, 1178, 1026, 829, 708. ^{1}H NMR ($CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 4.51 (s, 2H, CH_2), 6.85 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_4), 7.03 (t, $J = 8.5$ Hz, 2H, $H_{3,5}$-Ar_5), 7.27–7.39 (m, 5H, Ar), 7.43–7.49 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 38.54 (CH_2), 55.26 (OCH_3), 113.74 ($C_{3,5}$-Ar_4), 115.90 (d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-Ar_5), 126.82 (C_1-Ar_4), 127.68 (C_4-Benzyl), 127.96 (C_1-Ar_5), 128.67 ($C_{2,6}$-Benzyl), 129.18 ($C_{3,5}$-Benzyl), 130.22 ($C_{2,6}$-Ar_4), 131.35 (d, $J_{CF} = 10.0$ Hz, $C_{2,6}$-Ar_5), 131.67 (C_5-thiazole), 136.57 (C_1-Benzyl), 149.59 (C_4-thiazole), 159.38 (C_4-Ar_4), 161.69 (C_2-thiazole), 162.48 (d, $J_{CF} = 247.0$ Hz, C_4-Ar_5). EI-Mass *m/z* (%); 407 (100), 374 (70), 258 (95), 243 (70), 215 (70), 183 (12), 139 (14), 91 (70), 65 (11). Anal. Calcd. For $C_{23}H_{18}FNOS_2$: C, 67.79; H, 4.45; N, 3.44. Found: C, 67.85; H, 4.37; N, 3.30.

4-(4-Methylthiophenyl)-5-(4-fluorophenyl)-2-(benzylthio)-thiazole ***(6m)*** Yield: 71 %. Mp: 92–94 °C, IR (KBr, cm^{-1}) *v* 3050, 2919, 1594, 1501, 1471, 1425, 1223, 1155, 1028, 821, 702. ^{1}H NMR ($CDCl_3$) δ: 2.49 (s, 3H, SCH_3), 4.51 (s, 2H, CH_2), 7.03 (t, $J = 8.5$ Hz, 2H, $H_{3,5}$-Ar_5), 7.17 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_4), 7.27–7.37 (m, 5H, Ar), 7.44–7.47 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 15.45 (SCH_3), 38.53 (CH_2), 115.97 (d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-Ar_5), 125.94 ($C_{3,5}$-Ar_4), 127.72 (C_4-Benzyl), 128.69 ($C_{2,6}$-Benzyl), 129.19 ($C_{3,5}$-Benzyl), 129.24 ($C_{2,6}$-Ar_4), 130.83 (C_5-thiazole), 131.16 (C_1-Ar_4), 131.39 (d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-Ar_5), 131.72 (C_1-Ar_5), 136.52 (C_1-Benzyl), 138.53 (C_4-Ar_4), 149.19 (C_4-thiazole), 162.08 (C_2-thiazole), 162.60 (d, $J_{CF} = 251.25$ Hz, C_4-Ar_5). EI-Mass *m/z* (%); 423 (100), 390 (66), 341(10), 285 (95), 253 (35), 226 (20), 207 (35), 135 (10), 91 (80), 77 (20), 65 (17). Anal. Calcd. For $C_{23}H_{18}FNS_3$: C, 65.22; H, 4.28; N, 3.31. Found: C, 65.43; H, 4.37; N, 3.18.

4,5-Bis(4-methoxyphenyl)-2-(methylthio)-thiazole ***(6n)*** Yield: 42 %. Mp: 96–99 °C, IR (KBr, cm^{-1}) *v* 3035, 2914, 2838, 1604, 1511, 1480, 1460, 1418, 1292, 1250, 1175, 1024, 834. ^{1}H NMR ($CDCl_3$) δ: 2.73 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.83 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_5), 6.84 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_4), 7.26 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_5), 7.48 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_4). ^{13}C NMR ($CDCl_3$) δ: 16.70 (SCH_3), 55.23 (OCH_3), 55.30 (OCH_3), 113.64 ($C_{3,5}$-Ar_4), 114.21 ($C_{3,5}$-Ar_5), 124.17 (C_1-Ar_5), 127.27 (C_1-Ar_4), 130.15 ($C_{2,6}$-Ar_4), 130.78 ($C_{2,6}$-Ar_5), 130.31 (C_5-thiazole), 148.98 (C_4-thiazole), 159.13 [C_4-Ar_5 or (C_4-Ar_4)], 159.41 [C_4-Ar_4 or (C_4-Ar_5)], 163.09 (C_2-thiazole). EI-Mass *m/z* (%); 343 (100), 310 (34), 255 (31), 195 (20), 135 (18), 113 (22). Anal. Calcd. For $C_{18}H_{17}NO_2S_2$: C, 62.94; H, 4.99; N, 4.08. Found: C, 63.14; H, 4.87; N, 3.98.

4,5-Bis(4-methoxyphenyl)-2-(benzylthio)-thiazole ***(6o)*** Yield: 38 %. Mp: 106–107 °C. IR (KBr, cm^{-1}) *v* 3025, 2929, 2830, 1606, 1512, 1486, 1455, 1413, 1292, 1247, 1177, 1030, 827. ^{1}H NMR ($CDCl_3$) δ: 3.55 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 6.84 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_5), 6.86 (d, $J = 8.0$ Hz, 2H, $H_{3,5}$-Ar_4), 7.26 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_5), 7.29-7.39 (m, 3H, Benzyl), 7.42–7.48 (m, 2H, Benzyl), 7.50 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_4). ^{13}C NMR ($CDCl_3$) δ: 39.09 (CH_2), 55.45 (OCH_3-$Ar_{4,5}$), 113.5 ($C_{3,5}$-Ar_4), 114.20 ($C_{3,5}$-Ar_5), 123.88 (C_1-Ar_5), 126.65 (C_1-Ar_4), 127.66 (C_4-Benzyl), 128.67 ($C_{2,6}$-Benzyl), 129.28 ($C_{3,5}$-Benzyl), 130.30 ($C_{2,6}$-Ar_4), 130.84 ($C_{2,6}$-Ar_5), 131.65 (C_5-thiazole), 136.45 (C_1-Benzyl), 148.54 (C_4-thiazole), 159.36 (C_4-Ar_5), 159.69 (C_4-Ar_4), 161.45 (C_2-thiazole). EI-Mass *m/z* (%); 419 (83), 386 (60), 270 (100), 258 (68), 207 (30), 91 (42). Anal. Calcd. For $C_{24}H_{21}NO_2S_2$: C, 68.70; H, 5.04; N, 3.34. Found: C, 68.85; H, 4.89; N, 3.19.

Docking studies

Molecular docking of three derivatives into the three-dimensional X-ray structure of *Escherichia coli* FabH (PDB code: 1SA0) was carried out using the AUTODOCK software package (version 4.0) using a Lamarkian genetic algorithm (Huey *et al.*, 2007). For macromolecule pdbqt file generated and saved. The 3D structure of ligand molecules were built optimized and saved in Mol2 format with

the hyperchem8.0 software. The partial charges of Mol2 files were further modified by using Autodock. The box center was set to the center of cocrystalized ligand, DAMA colchicines ($x = 117.2187$, $y = 90.1800$, $z = 6.2898$) and the box size in all directions was set to 60 Å. To validate the method for docking ligand into the active site, DAMA colchicine was built using the hyperchem, energy minimized, and docked to the active site using the above parameter and the best scored pose of DAMA colchcine compared with the crystal structure. The derivatives were docked as flexible-ligand on the rigid α,β-tubulin conformation.

Biological study

Cell culture

Four cell lines namely AGS (stomach carcinoma cell), HT-29 (colon carcinoma cell), MCF-7 (breast carcinoma cells), and NIH-3T3 (mouse fibroblast cells) obtained from Pasteur Institute (Tehran, Iran). The cells were cultured in RPMI-1640 medium (Sigma) supplemented with 10 % heat-inactivated fetal bovine serum (FBS; Gibco, USA), penicillin (100 U/ml), and (100 µg/ml) streptomycin (Roche, Germany) at 37 °C in a humidified incubator with 5 % CO_2.

Growth inhibition assay

All compounds were tested for cytotoxic activity at 0.001–100 µM concentrations. Two controls were performed within each micro titer plate: a solvent (DMSO) control without drug and **7** as a positive control. Cells from different cell lines were seeded in 96-well plates (Nunc, Roskilde, Denmark) at the density of 8,000–10,000 viable cells per well and incubated for 48 h to allow cell attachment. The stock solutions of compounds in DMSO were diluted with media and added into each well of the plate. Cells were then incubated for another 24 or 48 h (depends to cell cycle of each cell line). The response of cells to compounds were evaluated by determining cell survival using 3-(4,5-dimethylthiazoyl-2-yl) 2,5-diphenyl tetrazolium bromide (MTT, Carl Roth, Karlsruhe, Germany). For this purpose, cells were washed in PBS, and 20 µl of MTT reagent (5 mg/ml) in phosphate buffered serum (PBS) was added to each well. After 4 h incubation at 37 °C, the medium was discarded and dimethyl sulfoxide (100 µl) was added to each well, and the solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each well was measured by plate reader (Anthous 2020; Austria) at a test wavelength of 550 nm against a standard reference solution at 690 nm. Assays were performed in triplicate in three independent experiments and the concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated by plotting the percentage cytotoxicity versus concentration on a logarithmic graph.

Tubulin preparation

Ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA), guanosine-5′-triphosphate type II-S (GTP), adenosine-5′-triphosphate (ATP), phenylmethylsulphonyl fluoride (PMSF), glycerol, $MgSO_4$ were purchased from Sigma (Deisenhofen, Germany). Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). $MgSO_4$, 1 M, was added to both GTP and ATP 100 mM stock solutions, as a ratio of 1:10 (v/v). Deionized and nanopure water was used in all buffers.

Microtubule protein (MTP) was isolated from fresh sheep brain (Aryapour *et al.*, 2011) after homogenization in the PIPES buffer (100 mM PIPES, pH 6.9, 1 mM EGTA, 1 mM MgSO4, 1 mM PMSF, and 1 mM MgATP), followed by two cycles of temperature dependent assembly and disassembly induced by 1 mM MgGTP (Williams and Lee, 1982) PEMG (100 mM PIPES, pH 6.9, 2 mM MgSO4, 1 mM EGTA, and 3.4 M glycerol) was used as assembly buffer. To obtain purified and MAP-free tubulin, the crude tubulin sample was applied to a phosphocellulose column (Weingarten *et al.*, 1975). Eluted fractions were aliquot and stored in the liquid nitrogen when not in use and stored at −70 °C for further experiments within two weeks. Concentration was assessed by the Bradford reagent (Bio-Rad Laboratories, Hercules, CA), using serum albumin as standard.

In vitro tubulin polymerization assay

For investigation of tubulin polymerization, tubulin (12 µM) in PMG buffer (80 mM Na-pipes, pH 6.9, 1 mM EGTA, 2 mM $MgSO_4$, and 8 mM glycerol) was first added to each well of a pre warmed 96-well plate. Then, some of potent derivatives and **7** (as a positive control) in final concentration of 10 µM were added. Then 1 µm of GTP was added to each sample in 0 °C and polymerization process was monitored by observing the variations in absorbance at 350 nm every 1 min for 50 min with a microplate reader (Williams and Lee, 1982).

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