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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Anticancer activities, molecular docking and structure–activity relationship of novel synthesized 4*H*-chromene, and 5*H*-chromeno [2,3-*d*]pyrimidine candidates

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Abstract In the present study, a series of 4*H*-chromene and 5*H*-chromeno[2,3-*d*]pyrimidine derivatives was synthesized and evaluated as potential cytotoxic agents. The cytotoxic activities of the target compounds were evaluated against four cancer cell lines MCF-7, HCT-116, HepG-2, and A549 in comparison with vinblastine and colchicine as reference drugs. We explored the structure–activity relationship of 4*H*-chromenes with modification at the 2-,4- or 7-position, and fused pyrimidine ring at 2,3-position. Most of the screened compounds showed marginal antitumor activity against the different cell lines in comparison to the standard drugs. The structure–activity relationship study revealed that the antitumor activity of the synthesized compounds was significantly affected by the lipophilicity of the substituent at the 2-,4- or 7-position for the 4*H*-chromenes, and 5,8-position or fused pyrimidine ring at 2,3-positions for 5*H*-chromeno[2,3-*d*]pyrimidines. Structure–activity relationship was elaborated with the help of molecular docking studies. The structures of the synthesized compounds were established on the basis of the spectral data, infrared, proton nuclear magnetic resonance, 13-Carbon nuclear magnetic resonance and mass spectroscopic data.

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Keywords 4*H*-Chromene · 5*H*-Chromeno[2,3-*d*] pyrimidine · Antitumor · Lipophilicity · SAR · Molecular docking

Introduction

Chromene (Benzopyran) is a heterocyclic ring system in which a benzene ring and a pyran ring are fused together. Moreover, the function of substituted chromenes plays a vital role in the synthetic approaches of promising compounds in the field of medicinal chemistry including antimicrobial (Vala et al. 2016; Bingi et al. 2015; Killander and Sterner 2014; Chetan et al. 2012; Kathrotiya and Patel 2012), anti-inflammatory (Thomas and Zachariah 2013), anti-proliferative (El-Agrody et al. 2016; Magedov et al. 2007), antioxidant (Singh et al. 2010; Vukovic et al. 2010), herbicidal, analgesic and anticonvulsant (Bhat et al. 2008), antitubercular (Nimesh et al. 2011), anticoagulant, estrogenic antispasmolytic, estrogenic (Nareshkumar et al. 2009), TNF-α inhibitor (Cheng et al. 2003) effects and activities, as well as inhibitor of diabetes-induced vascular dysfunction (Birch et al. 1996). Such diverse biological and pharmacological activities have made chromene derivatives important for further development in organic synthesis and medicinal studies (Thompson 2000; Nefzi et al. 1997).

Recently, 2-amino-4*H*-chromene derivatives are of great interest for their antitumor activities (Kheirollahi et al. 2014; Saffari et al. 2014; Zhang et al. 2014; Olczak et al. 2013; Patil et al. 2013; Akbarzadeh et al. 2012; Rafinejad et al. 2012; Sabry et al. 2011; Musa et al. 2010). In addition, other 4*H*-chromene derivatives showed some biological and pharmacological applications, such as, Crolibulin

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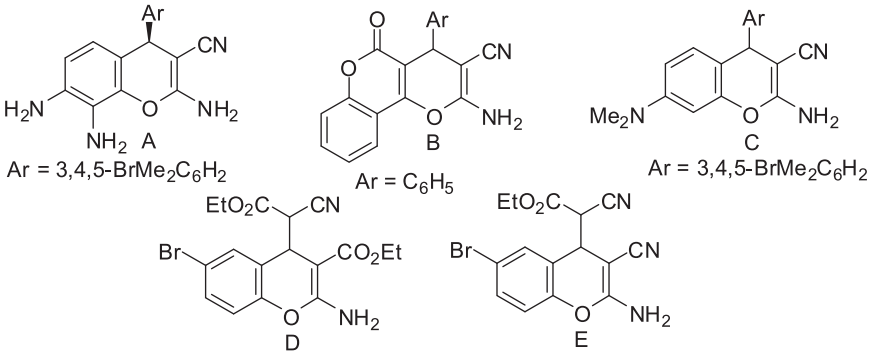
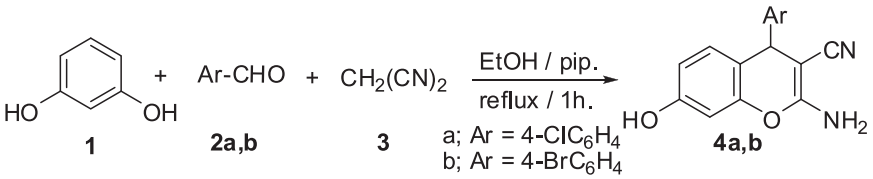
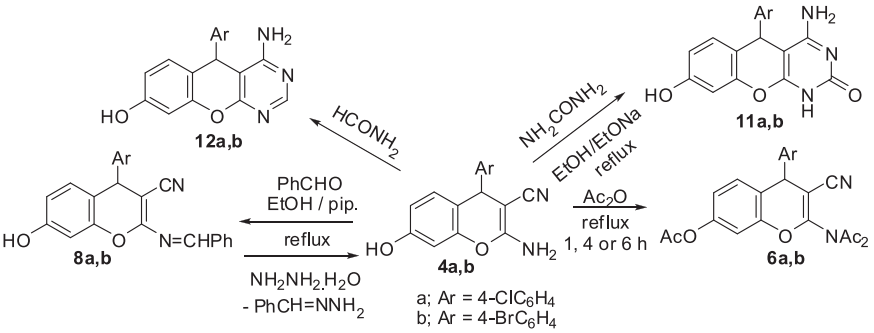


Fig. 1 Structures of some 2-amino-4*H*-chromenes with diverse biological and pharmacological activities



Scheme 1 Synthesis of halogenated 7-hydroxy-4*H*-chromene derivatives (**4a**, **b**)



Scheme 2 Synthetic protocol of compounds (**6**, **8**, **11**, **12**)

(EPC2407) (A) which is currently in Phase I/II clinical trials for the treatment of advanced solid tumors (Patil et al. 2013). Meanwhile, pyranopyranone (B) that served as precursor for the blood anticoagulant warfarin (Wiener et al. 1962), and benzopyrane (C) has been known for its anticancer therapeutic properties (Kemnitzer et al. 2005). Furthermore, ethyl 2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (D) and 2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carbonitrile (E) are well known as inhibitors of Bcl-2 protein and as apoptosis inducers (Doshi et al. 2006; Wang et al. 2000), as shown in Fig. 1.

Among the different types of chromenes, our interest has been focused on 2-amino-3-cyano-4*H*-chromene scaffolds, which is one of the most active species that proved to be a useful intermediate for the synthesis of 5*H*-chromeno[2,3-*d*] pyrimidine derivatives. In addition, 4*H*-chromene and 5*H*-chromeno[2,3-*d*]pyrimidine derivatives have been emerged as promising and attractive scaffold in the development of potent antitumor and antimicrobial agents (Fouda 2016; Parthiban et al. 2015; El-Agrody et al. 2014; Kandeel et al. 2013; Abd-El-Aziz et al. 2004).

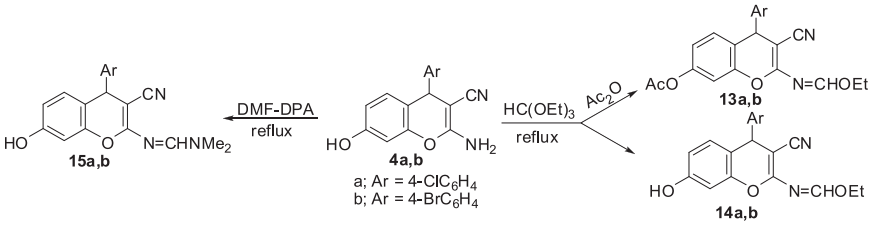
In view of the above-mentioned findings, we intend to synthesize novel series of 4*H*-chromenes and compounds containing both 4*H*-chromene and pyrimidine moieties (Schemes 1–4) to explore the synergistic effect that might result from this combination on the antitumor activities. The structure–activity relationship (SAR) of the 2-,4- or 7-position for 4*H*-chromene, 5,8-position or the fused pyrimidine ring at 2,3-positions for 5*H*-chromeno[2,3-*d*] pyrimidine was discussed and molecular docking was studied.

Results and discussion

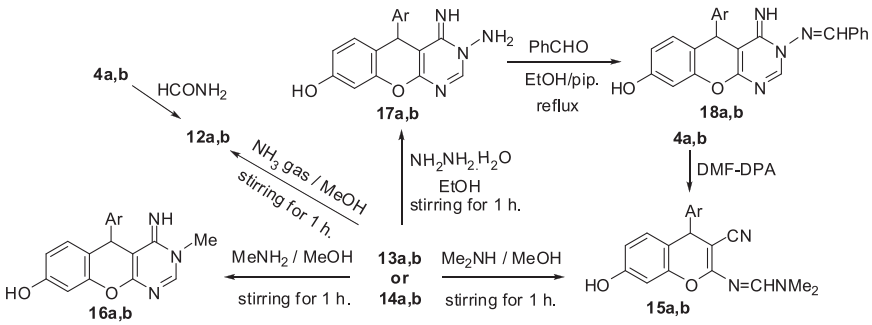
Chemistry

2-Amino-4-(4-chloro/bromophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (**4a**, **b**) were prepared via three-components condensation of resorcinol (**1**) with a mixture of 4-chlorobenzaldehyde (**2a**) or 4-bromobenzaldehyde (**2b**) and malononitrile (**3**) in ethanolic piperidine solution under reflux for 1 h as cited in Scheme 1.

Scheme 3 Synthesis of 4*H*-chromene derivatives (**13–15**)



Scheme 4 Synthetic protocol of compounds (**12**, **15–18**)



Acylation of compounds **4a** and **4b** with refluxed acetic anhydride resulted in the isolation of the diacetyl derivatives (**6a**, **b**) with acylation of the hydroxyl group at 7-position instead of the monoacetyl (**5**) or pyrimidine derivatives (**7**), while condensation of compounds **4a**, **b** with benzaldehyde in refluxed ethanol under basic conditions afforded the Schiff base products (**8a**, **b**) as shown in Scheme 2. Hydrazinolysis of **8a**, **b** in ethanol at room temperature under stirring or at reflux returned to *β*-enaminonitrile **4** instead of the pyrimidine derivative (**10**) (Khafagy et al. 2002). The formation of the *β*-enaminonitrile **4** can be explained via elimination of benzaldehydehydrazone from the addition product intermediate **9**. Besides, reaction of compounds **4a**, **b** with urea in ethanol in the presence of sodium ethoxide under reflux gave the cycloaddition products 4-amino-5-(4-chloro/bromophenyl)-8-hydroxy-1*H*-chromeno[2,3-*d*]-pyrimidin-2(5 *H*)-one (**11a**, **b**), while reaction of compounds **4a**, **b** with formamide under reflux gave the cycloaddition products 4-amino-5-(4-chloro/bromophenyl)-5*H*-chromeno[2,3-*d*]pyrimi-din-8-ol (**12a**, **b**). These results are depicted in Scheme 2.

Treatment of compounds **4a** and **4b** with triethyl orthoformate in acetic anhydride under reflux afforded 7-acetoxy-2-ethoxymethyleneamino-4-(4-chloro/bromophenyl)-4*H*-chromene-3-carbonitrile (**13a**, **b**) with acylation of the hydroxyl group at 7-position, while reaction of compounds **4a**, **b** with the neat triethyl orthoformate afforded 2-ethoxymethyleneamino-4-(4-chloro/bromophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (**14a**, **b**). Furthermore, condensation of **4a**, **b** with *N,N*-dimethylformamide–dineopentylacetal (DMF–DPA) in benzene gave the 4-(4-chloro/bromophenyl)-2-dimethylaminomethyleneamino-7-hydroxy-4*H*-chromene-3-carbonitrile (**15a**, **b**) as shown in Scheme 3.

Reaction of the imidate **13a**, **b** and **14a**, **b** with dimethylamine in methanol at room temperature under stirring for 1 h yielded the imidine **15a**, **b** (m.p., mixed m.p., identical infrared (IR) and mass spectrum) with deacylation of the acetoxy group at 7-position into hydroxyl group in the case of imidate **13a**, **b** Scheme 4. This imidine **15a**, **b** can also be obtained as described before from the reaction of **4a**, **b** with DMF–DPA (m.p., mixed m.p., identical IR and MS spectrum) Scheme 3. In addition, treatment of the imidate **13a**, **b** and **14a**, **b** with methylamine in methanol at room temperature under stirring for 1 h gave the cycloaddition products 5-(4-chloro/bromophenyl)-4-imino-3-methyl-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (**16a**, **b**) (m.p., mixed m.p., identical IR and MS spectrum) with deacylation of the acetoxy group at 7-position into hydroxyl group in the case of imidate **13a**, **b** as shown in Scheme 4.

Hydrazinolysis of **13a**, **b** and **14a**, **b** afforded the cycloaddition products 3-amino-5-(4-chloro/bromophenyl)-4-imino-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (**17a**, **b**) (m.p., mixed m.p., identical IR and MS spectrum) with deacylation of the acetoxy group at 7-position into hydroxyl group in the case of imidate **13a**, **b**, while ammonolysis of **13a**, **b** and **14a**, **b** with NH_3 gas bubbled in methanol at room temperature under stirring for 1 h gave the cycloaddition products 4-amino-5-(4-chloro/bromophenyl)-5*H*-chromeno[2,3-*d*]pyrimidin-8-ol (**12a**, **b**) (m.p., mixed m.p., identical IR and MS spectrum) with deacylation of the acetoxy group at 7-position into hydroxyl group in the case of imidate **13a**, **b**. The aminopyrimidine derivatives (**12a**, **b**) can be obtained as described before from the reaction of **4a**, **b** with formamide (m.p., mixed m.p., identical IR and MS spectrum) (Scheme 2). Finally, interaction of the aminoiminopyrimidine derivative **17a**, **b** with benzaldehyde in ethanolic piperidine under reflux afforded the open chain

Table 1 SAR of the aryl group, 2-, 2, 3-positions and the inhibitory concentration (IC_{50}, in μg/ml) of target compounds against the four human cancer cell lines in comparison with vinblastine and colchicine as measured with the microculture tetrazolium (MTT) method

Compound	Log *P*	IC_{50} (μg/ml)[a]			
		MCF-7	HCT-116	HepG-2	A549
4a	3.08 ± 0.50	18.8 ± 0.3[b]	5.4 ± 0.11[b]	10.0 ± 0.1[b]	0.99 ± 0.2[c]
4b	3.26 ± 0.52	5.8 ± 0.16[b]	12.2 ± 0.3[b]	3.0 ± 0.11[b]	2.67 ± 0.18[c]
6a	2.60 ± 0.67	12.4 ± 0.05	38.1 ± 0.04	4.76 ± 0.03	6.13 ± 0.06
6b	2.77 ± 0.69	12.4 ± 0.02	20.9 ± 0.11	8.16 ± 0.06	10.2 ± 0.07
8a	5.78 ± 0.62	6.22 ± 0.4	12.4 ± 0.01	3.13 ± 0.2	2.24 ± 0.3
8b	5.95 ± 0.65	3.1 ± 0.23	18.2 ± 0.01	1.47 ± 0.03	2.06 ± 0.07
11a	8.14 ± 0.69	≥ 75	≥ 75	45.8 ± 0.02	64.2 ± 0.06
11b	8.31 ± 0.72	≥ 75	≥ 75	≥ 75	≥ 75
12a	3.20 ± 0.68	16 ± 0.13	13.8 ± 0.01	6.55 ± 0.11	8.97 ± 0.2
12b	3.37 ± 0.71	≥ 75	≥ 75	≥ 75	≥ 75
13a	4.90 ± 0.63	7.81 ± 0.18	10.0 ± 0.23	3.99 ± 0.2	2.43 ± 0.4
13b	5.08 ± 0.65	71.1 ± 0.2	≥ 75	12.1 ± 0.3	6.12 ± 0.11
14a	5.01 ± 0.63	3.39 ± 0.03	8.84 ± 0.3	1.77 ± 0.13	2.68 ± 0.2
14b	5.18 ± 0.65	12.4 ± 0.3	6.99 ± 0.4	3.26 ± 0.12	5.21 ± 0.13
15a	3.81 ± 0.64	38 ± 0.1	40.8 ± 0.2	31.6 ± 0.06	24.6 ± 0.2
15b	3.98 ± 0.66	6.49 ± 0.2	6.4 ± 0.14	4.31 ± 0.16	2.89 ± 0.3
16a	2.41 ± 0.75	2.95 ± 0.6	7.06 ± 0.13	1.5 ± 0.1	1.04 ± 0.06
16b	2.58 ± 0.79	5.85 ± 0.03	5.96 ± 0.2	1.81 ± 0.5	1.38 ± 0.03
17a	1.89 ± 0.75	6.15 ± 0.2	9.41 ± 0.4	2.98 ± 0.15	1.36 ± 0.6
17b	2.07 ± 0.79	4.85 ± 0.14	4.1 ± 0.3	1.1 ± 0.7	0.96 ± 0.5
18a	4.50 ± 0.78	2.54 ± 0.12	10.5 ± 0.5	1.55 ± 0.03	1.86 ± 0.4
18b	4.67 ± 0.82	3.8 ± 0.18	2.04 ± 0.4	1.59 ± 0.11	1.64 ± 0.3
Vinblastine	4.58 ± 0.66	6.1 ± 0.03	2.6 ± 0.08	4.6 ± 0.01	3.78 ± 0.01
Colchicine	0.92 ± 0.55	17.7 ± 0.03	42.8 ± 0.06	10.6 ± 0.02	21.3 ± 0.03

[a] IC_{50} values expressed in μg/ml as the mean values of triplicate wells from at least three experiments and are reported as the mean ± standard error

[b] El-Agrody et al. (2014) and

[c] Fouda (2016)

products, 3-(benzylideneamino)-5-(4-chloro/bromophenyl)-4-imino-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (**18a**, **b**). These results are depicted in Scheme 4.

The structure of compounds **6**, **8**, **11–18** was established on the basis of IR, proton nuclear magnetic resonance (^{1}H NMR), 13-Carbon nuclear magnetic resonance (^{13}C NMR) and MS data.

Antitumor assays

The synthesized compounds **6**, **8**, **11–18** were evaluated for their in vitro antitumor activity against four human cancer cell lines: breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116), hepatocellular carcinoma (HepG-2), and lung carcinoma (A549) at various concentrations ranging from 0 to 50 μg/ml and the cell viability was measured by the microculture tetrazolium (MTT) assay as described in the literature (Rahman et al. 2001; Mosmann 1983). In vitro cytotoxic evaluation using cell viability assay was performed at the Regional Center for Mycology & Biotechnology (RCMP), Al-Azhar University using vinblastine and colchicine as reference drugs. The results were expressed as growth inhibitory concentration (IC_{50}) values, which represent the compound concentrations required to produce a 50% inhibition of cell growth after 24 h of incubation compared to untreated controls as shown in Table 1 and Fig. 2.

The results from Table 1 explicated that some of the synthesized compounds displayed excellent to modest or fair growth inhibitory activity against the tested cell lines MCF-7, HCT-116, HepG-2 and A549. Investigations of the cytotoxic activity against MCF-7 indicated that compounds **18a**, **16a**, **8b**, **14a**, **18b**, **17b**, **4b**, and **16b** (IC_{50} = 2.54 ± 0.12, 2.95 ± 0.6, 3.1 ± 0.23, 3.39 ± 0.03, 3.8 ± 0.18, 4.85 ± 0.14, 5.8 ± 0.16 and 5.85 ± 0.03 μg/ml, respectively) were the most sensitive compared to the standard drugs

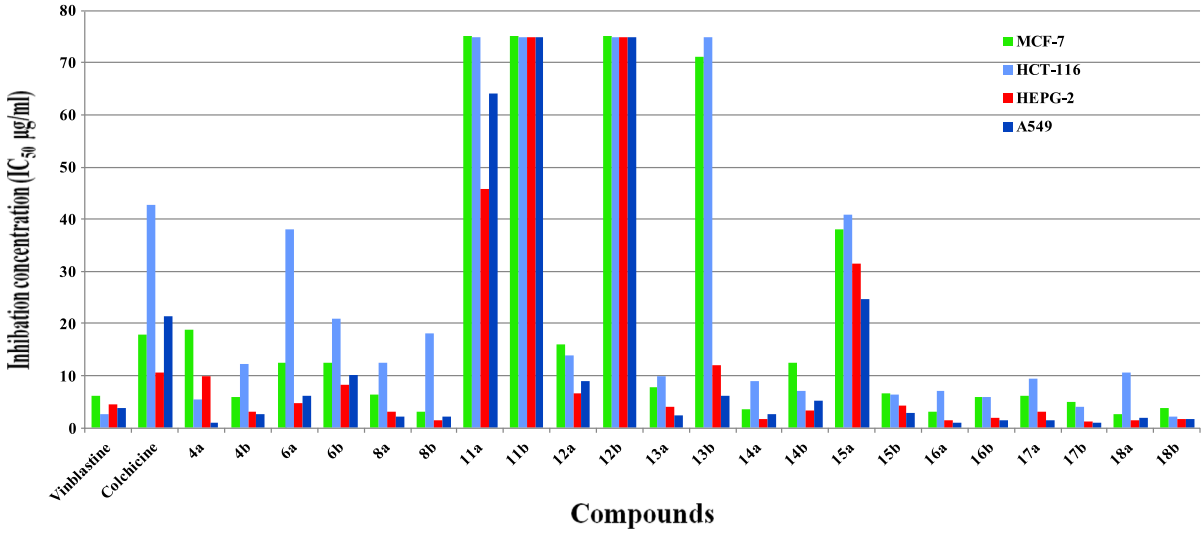


Fig. 2 IC_{50} values expressed in (µg/ml) of target compounds against the cell lines MCF-7, HCT-116, HepG-2 and A549

vinblastine ($IC_{50} = 6.1 \pm 0.03$ µg/ml), colchicine ($IC_{50} = 17.7 \pm 0.03$ µg/ml) and compounds **17a** and **8a** ($IC_{50} = 6.15 \pm 0.2$ and 6.22 ± 0.4 µg/ml) were almost equipotent as vinblastine, while compounds **17a**, **8a**, **13a**, **14b**, **6a**, **b** and **12a** ($IC_{50} = 6.15 \pm 0.2$, 6.22 ± 0.4, 7.81 ± 0.18, 12.4 ± 0.3, 12.4 ± 0.05, 12.4 ± 0.02 and 16 ± 0.13 µg/ml, respectively) were found to be the most potent derivative against MCF-7 compared to colchicine ($IC_{50} = 17.7 \pm 0.03$ µg/ml) and compound **4a** ($IC_{50} = 18.8 \pm 0.3$ µg/ml) was almost equipotent as colchicine. Besides, compound **18b** ($IC_{50} = 2.04 \pm 0.4$ µg/ml) possessed good cytotoxic activity against HCT-116 compared to vinblastine ($IC_{50} = 2.6 \pm 0.08$ µg/ml), while compounds **18b**, **17b**, **4a**, **16b**, **15b**, **14b**, **16a**, **14a**, **17a**, **13a**, **18a**, **4b**, **8a**, **12a**, **8b**, **6b**, **a** and **15a** ($IC_{50} = 2.04 \pm 0.4$, 4.1 ± 0.3, 5.4 ± 0.11, 5.96 ± 0.2, 6.4 ± 0.14, 6.99 ± 0.4, 7.06 ± 0.13, 8.84 ± 0.3, 9.41 ± 0.4, 10.0 ± 0.23, 10.5 ± 0.5, 12.2 ± 0.3, 12.4 ± 0.01, 13.8 ± 0.01, 18.2 ± 0.01, 20.9 ± 0.11, 38.1 ± 0.04, and 40.8 ± 0.2 µg/ml, respectively) were found to be the most potent derivative overall the tested compounds against HCT-116 compared to colchicine ($IC_{50} = 42.8 \pm 0.08$ µg/ml). On the other hand, cytotoxicity evaluation in HepG-2 cell line revealed that the compounds **17b**, **8b**, **16a**, **18a**, **b**, **14a**, **16b**, **17a**, **4b**, **8a**, **14b**, **13a** and **15b** ($IC_{50} = 1.1 \pm 0.7$, 1.47 ± 0.03, 1.5 ± 0.1, 1.55 ± 0.03, 1.59 ± 0.11, 1.77 ± 0.13, 1.81 ± 0.5, 2.98 ± 0.15, 3.0 ± 0.11, 3.13 ± 0.2, 3.26 ± 0.12, 3.99 ± 0.2 and 4.31 ± 0.16 µg/ml, respectively) were more potent and efficacious than vinblastine ($IC_{50} = 4.6 \pm 0.01$ µg/ml), colchicine ($IC_{50} = 10.6 \pm 0.01$ µg/ml) and compound **6a** ($IC_{50} = 4.76 \pm 0.03$ µg/ml) was almost equipotent as vinblastine, while compounds **6a**, **12a**, **6b** and **4a** (with $IC_{50} = 4.6 \pm 0.01$, 6.55 ± 0.11, 8.16 ± 0.06 and 10.0 ± 0.1 µg/ml, respectively) displayed good cytotoxicity compared to colchicine. Concerning activity against A549, compounds **17b**, **4a**, **16a**, **17a**, **16b**, **18b**, **a**, **8b**, **a**, **13a**, **4b**, **14a** and **15b** were the most active analogs through this study (with $IC_{50} = 0.96 \pm 0.5$, 0.99 ± 0.2, 1.04 ± 0.06, 1.36 ± 0.6, 1.38 ± 0.03, 1.64 ± 0.3, 1.86 ± 0.4, 2.06 ± 0.07, 2.24 ± 0.3, 2.43 ± 0.4, 2.67 ± 0.18, 2.68 ± 0.2 and 2.89 ± 0.3 µg/ml, respectively) compared to vinblastine ($IC_{50} = 3.78 \pm 0.01$ µg/ml) and colchicine ($IC_{50} = 21.3 \pm 0.03$ µg/ml), while compounds **14b**, **13b**, **6a**, **12a**, and **6b** were more potent and efficacious (with $IC_{50} = 5.21 \pm 0.13$, 6.12 ± 0.11, 6.13 ± 0.06, 8.97 ± 0.2 and 10.2 ± 0.07 µg/ml, respectively) than colchicine. In addition, the rest of compounds were moderately active or inactive for all the cell lines MCF-7, HCT-116, HepG-2 and A549.

SAR studies

The partition coefficient Log *P* (factor of the lipophilicity), which is well known as an index of lipophilicity, is an important physicochemical parameter that was measured by ACD/Labs Log *P* calculated, ver.14.02 and is cited in Table 1. The preliminary SAR study has focused on the effect of the substituent at 2-, 4- or 7-position of the 4*H*-chromene moiety and the substituent at 5-, 8-position or the fused pyrimidine ring at 2,3-positions of the 5*H*-chromeno [2,3-*d*]pyrimidine moiety, on the antitumor activities of the synthesized compounds. Comparison of the cytotoxic activities of the target compounds **4a**, **b** and their analogs **6**, **8**, **11–18** against MCF-7, HCT-116, HepG-2, and A549, we found that incorporating a pyrimidine nucleus at 2,3-positions with hydrophobic groups, 4-chlorophenyl or 4-bromophenyl at 5-position, and more hydrophobic groups (=NH-4, –N=CHPh-3; =NH-4, –NMe-3 and =NH-4, $–NH_2$-3) for compounds **18a**, **16a**, **18b**, **17b** and **16b** ($IC_{50} = 2.54 \pm 0.12$, 2.95 ± 0.6, 3.8 ± 0.18, 4.85 ± 0.14 and 5.85 ± 0.03 µg/ml, respectively), and a hydrophilic group

hydroxy group at 8-position has caused a remarkable enhancement in the antitumor activity against MCF-7 compared to vinblastine and colchicine ($IC_{50} = 6.1 \pm 0.03$ and 17.7 ± 0.03 μg/ml) with decreasing of the partition coefficient Log *P* as shown in Table 1, while introduction of hydrophobic groups (–N=CHPh and –N=CHOEt) at 2-position with hydroxy group at 7-positions and 4-bromophenyl or 4-chlorophenyl at 4-position for compounds **8b** and **14a** ($IC_{50} = 3.1 \pm 0.23$ and 3.39 ± 0.03 μg/ml) showed more activity than the standard drugs and compound **17a** ($IC_{50} = 6.15 \pm 0.2$ μg/ml), with a pyrimidine nucleus at 2,3-positions, hydrophobic groups (=NH-4, $–NH_2$-3) and 4-chlorophenyl at 5-position was almost equipotent as vinblastine and active than colchicines, beside introduction of hydrophobic groups (–N=CHPh, -N=CHOEt and $-NAc_2$) at 2-position with hydroxy or acetoxy groups at 7-positions and 4-chlorophenyl or 4-bromophenyl at 4-position for compounds **8a**, **13a**, **14b** and **6a**, **b** ($IC_{50} = 6.22 \pm 0.4$, 7.81 ± 0.18, 12.4 ± 0.3, 12.4 ± 0.05, and 12.4 ± 0.02 μg/ml) or incorporating a pyrimidine nucleus at 2,3-positions with hydrophobic group ($–NH_2$-4) and 4-chlorophenyl at 5-position with a hydroxy group at 8-positions for compound **12a** ($IC_{50} = 16 \pm 0.13$ μg/ml) represented the most active derivatives against MCF-7 compared to colchicine, suggesting that the pyrimidine nucleus at 2,3-positions or 4*H*-chromene nucleus with certain hydrophobic or hydrophilic groups respectively, was indispensable for the activities against breast adenocarcinoma and 4-chloro-phenyl is favorable than 4-bromophenyl at 5- or 4-position with decreasing of the partition coefficient Log *P* and the 5*H*-chromeno[2,3-*d*]pyrimidine derivatives showed superior in vitro antitumor activity than the 4*H*-chromene derivatives

Further investigation of the impact of the substitution at different positions, on the (SAR), the result from Table 1, revealed that compound **18b** ($IC_{50} = 2.04 \pm 0.4$ μg/ml) showed good activity against human colon carcinoma (HCT-116) compared to vinblastine and colchicine ($IC_{50} = 2.6 \pm 0.08$ and 42.8 ± 0.06 μg/ml), while compounds **17b**, **4a**, **16b**, **15b**, **14b**, **16a**, **14a**, **17a**, **13a**, **18a**, **4b**, **8a**, **12a**, **8b**, **6b**, **a** and **15a** with ($IC_{50} =$, 4.1 ± 0.3, 5.4 ± 0.11, 5.96 ± 0.2, 6.4 ± 0.14, 6.99 ± 0.4, 7.06 ± 0.13, 8.84 ± 0.3, 9.41 ± 0.4, 10.0 ± 0.23, 10.5 ± 0.5, 12.2 ± 0.3, 12.4 ± 0.01, 13.8 ± 0.01, 18.2 ± 0.01, 20.9 ± 0.11, 38.1 ± 0.04 and 40.8 ± 0.2 μg/ml, respectively) were found to be the most potent derivatives against HCT-116 compared to colchicines with decreasing partition coefficient Log *P* as shown in Table 1, suggesting that the antitumor activity is significantly affected by the presence of the pyrimidine nucleus at 2,3-positions with certain hydrophobic or hydrophilic groups; some of the hydrophobic groups at 2-positions of the 4*H*-chromene moiety and 4-bromophenyl are favorable than 4-chlorophenyl at 5- or 4-position with decreasing partition coefficient Log *P*, and the 5*H*-chromeno[2,3-*d*]pyrimidine derivatives showed superior in vitro antitumor activity than the 4*H*-chromene derivatives.

Concerning activity against hepatocellular carcinoma (HepG-2), compounds **17b**, **8b**, **16a**, **18a**, **b**, **14a**, **16b**, **17a**, **4b**, **8a**, **14b**, **13a** and **15b** with ($IC_{50} = 1.1 \pm 0.7$, 1.47 ± 0.03, 1.5 ± 0.1, 1.55 ± 0.03, 1.59 ± 0.11, 1.77 ± 0.13, 1.81 ± 0.5, 2.98 ± 0.15, 3.0 ± 0.11, 3.13 ± 0.2, 3.26 ± 0.12, 3.99 ± 0.2, and 4.31 ± 0.16 μg/ml, respectively) were more potent and efficacious than vinblastine ($IC_{50} = 4.6 \pm 0.01$ μg/ml), colchicine ($IC_{50} = 10.6 \pm 0.02$ μg/ml) and compound **6a** ($IC_{50} = 4.76 \pm 0.03$ μg/ml) was almost equipotent as vinblastine with decreasing partition coefficient Log *P* as shown in Table 1, whiles compounds **6a**, **12a**, **6b** and **4a** with ($IC_{50} = 4.6 \pm 0.01$, 6.55 ± 0.11, 8.16 ± 0.06 and 10.0 ± 0.1 μg/ml, respectively) displayed good cytotoxicity compared to colchicines, implying that incorporating the pyrimidine nucleus at 2,3-positions with hydrophobic groups (=NH-4, $-NH_2$-3; =NH-4, –NMe-3 and =NH-4, –N=CHPh-3) and a hydroxy group at 8-position showed superior in vitro antitumor activity than the 4*H*-chromene derivatives with hydrophobic groups (–N=CHPh-2, –N=CHOEt-2, NH_2-2 and $–N=CHNMe_2$-2) and a hydroxy or acetoxy groups at 7-positions against hepatocellular carcinoma, and 4-bromophenyl is favorable than 4-chlorophenyl at 5- or 4-position with decreasing of the partition coefficient Log *P*.

Further investigation of the impact of substitution pattern at the 2-,4-,7-position and 2-,3- 5-,8-positions on the prepared chromenes and chromenopyrimidines activities against lung carcinoma (A549) was then conducted. Incorporating the pyrimidine nucleus at 2,3-positions with hydrophobic groups (=NH-4, $–NH_2$-3; =NH-4, –NMe-3 and =NH-4, –N=CHPh-3) and a hydroxy group at 8-position for compounds **17b**, **16a**, **17a**, **16b**, **18b**, **a** with ($IC_{50} = 0.96 \pm 0.5$, 1.04 ± 0.06, 1.36 ± 0.6, 1.38 ± 0.03, 1.64 ± 0.3 and 1.86 ± 0.4 μg/ml, respectively) or introduction of the hydrophobic group (NH_2-2, –N=CHPh-2, –N=CHOEt-2 and $–N=CHNMe_2$-2) and hydroxy or acetoxy groups at 7-position for compounds **4a**, **8b**, **a**, **13a**, **4b**, **14a** and **15b** with ($IC_{50} = 0.99 \pm 0.2$, 2.06 ± 0.07, 2.24 ± 0.3, 2.43 ± 0.4, 2.67 ± 0.18, 2.68 ± 0.2 and 2.89 ± 0.3 μg/ml, respectively) showed increased in activity against A549 compared to vinblastine and colchicine ($IC_{50} = 3.78 \pm 0.01$and 21.3 ± 0.03 μg/ml) with decreasing partition coefficient Log *P* as shown in Table 1, suggesting that the antitumor activity is significantly affected by the presence of 5*H*-chromeno[2,3-*d*]pyrimidine with a hydroxy group at 8-position more than the 4*H*-chromene with hydrophobic group at 2-position, a hydroxy or acetoxy groups at 7-position and 4-bromophenyl is favorable than 4-chlorophenyl at 5- or 4-position with decreasing partition coefficient Log *P*.

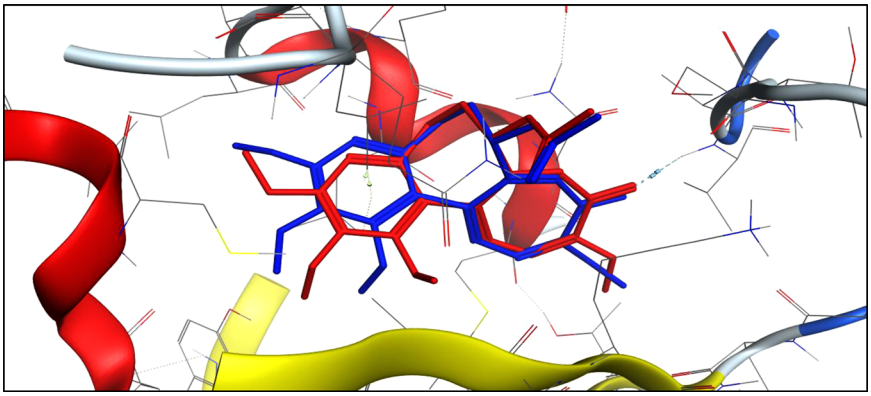


Fig. 3 Superimposition of the co-crystallized (*red*) and the docking pose (*blue*) of colchicine in the tubulin active site with RMSD of 0.942 Å (color figure online)

Finally, we discovered that the substitution pattern at 2-,4- or 7-position of the 4*H*-chromene moiety and the substituent at 5-,8-position or the fused pyrimidine ring at 2,3-positionpositions of the 5*H*-chromeno[2,3-*d*]pyrimidine moiety are crucial elements for the antitumor activity. The incorporation of pyrimidine nucleus at 2,3-positions with hydrophobic groups as (=NH-4, –N=CHPh-3; =NH-4, $–NH_2$-3 and =NH-4, –NMe-3) and a hydroxy group at 8-position or (–N=CHPh2, –N=CHOEt-2, NH_2-2 and $–N=CHNMe_2$-2) and a hydroxy or acetoxy groups at 7-position of the chromene nucleus are favorable and greatly enhanced the antitumor activity than the other hydrophobic groups and 4-bromophenyl is favorable than 4-chlorophenyl at 5- or 4-position with decreasing of the partition coefficient Log *P*.

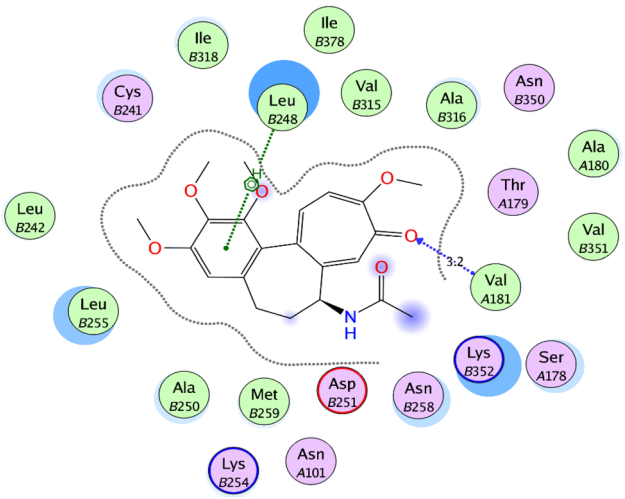


Fig. 4 2D Interaction diagram showing colchicine docking pose interactions in the tubulin active site (distances in Å)

Molecular docking studies

Several crystal structures are available in the protein data bank for tubulin, for this work we choose (PDB ID: 5EYP) (Ahmad et al. 2016) which have tubulin co-crystallized with colchicine as inhibitor.

First, the molecular docking setup was validated by carrying out re-docking of colchicine near the tubulin active site. The re-docking validation step reproduced the experimental binding mode of the co-crystallized ligand quite efficiently indicating the suitability of the used setup for the intended docking study and this is indicated by the small RMSD of 0.942 Å (<2 Å) between the docked pose and the co-crystallized ligand (energy score (S) = −12.85 kcal/mol) and by the ability of the docking poses to reproduce the key interactions accomplished by the co-crystallized ligand with the hot spots in the active site, Val181A Figs. 3 and 4.

As seen in Figs. 3 and 4, colchicine binds to *β*-tubulin at its interface with α-tubulin, resulting in inhibition of tubulin polymerization (Lu et al. 2012). The trimethoxyphenyl group of colchicine (Ring A) is located in the *β*-tubulin structure near the amino acid residue Cys241B. Colchicine binds by its carbonyl moiety in ring A through hydrogen bonding with the backbone NH of Val181A. The hydrophobic tricyclic structure of colchicine interacts through hydrophobic interaction with the hydrophobic amino acids in chain B lining the binding site (Leu242, Leu248, Ala250, Leu255, Met259, Val315, ALa316, Ilu318 and Ilu378).

The ability of the synthesized compounds to interact with the key amino acids in the active site rationalizes their good activity as indicated by their docking pattern and docking score compared to that of colchicine as shown in Table 2.

Table 2 shows that most of the newly synthesized compounds are showing better docking score than that of colchicine indicating that they have better tubulin inhibition and so better antitumor activity as proven experimentally in the cell line test.

The docking study showed that the aryl rings in the newly synthesized compounds overlap with ring A and ring C of colchicine, and so share colchicine in their ability to interact with the hydrophobic amino acids lining the

Table 2 Docking energy scores (*S*) in kcal/mol for the newly synthesized compounds in tubulin Colchicine binding site

Compound	Energy score (*S*) (kcal/mol)
4a	−12.01
4b	−12.26
6a	−13.77
6b	−13.77
8a	−13.87
8b	−13.99
11a	−12.63
11b	−13.04
12a	−12.58
12b	−12.93
13a	−12.70
13b	−13.20
14a	−12.45
14b	−13.11
15a	−12.71
15b	−13.04
16a	−13.10
16b	−13.23
17a	−13.13
17b	−13.26
18a	−14.29
18b	−14.49
Colchicine	−12.85

binding site (Leu242, Leu248, Ala250, Leu255, Met259, Val315, ALa316, Ilu318, and Ilu378) as shown in Figs. 5–7.

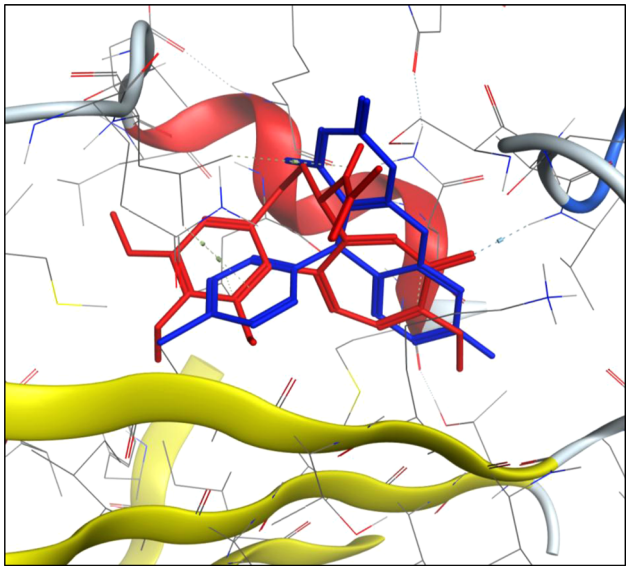


Fig. 5 Superimposition of the co-crystallized colchicine ligand (*red*) and the docking pose of compound **11a** (*blue*) in the tubulin active site showing the overlap of the aryl rings in the newly synthesized compound with ring A and ring C of colchicine (color figure online)

Conclusion

New series of 4*H*-chromenes **6**, **8**, **13–15** and 5*H*-chromeno [2,3-*d*]pyrimidines **11**, **12**, **16–18** were synthesized and evaluated for their cytotoxic activity against four cancer cell lines, namely breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116), hepatocellular carcinoma (HepG-2) and lung carcinoma (A549). The results revealed that compounds **18a**, **16a**, **8b**, **14a**, **18b**, **17b**, **4b**, and **16b** displayed high growth inhibitory activity against MCF-7 cells, while compound **18b** showed relatively potent antitumor activity against HCT-116 as compared to vinblastine and colchicine. Whereas, compounds **17b**, **8b**, **16a**, **18a**, **b**, **14a**, **16b**, **17a**, **4b**, **8a**, **14b**, **13a** and **15b** were more potent and efficacious against HepG-2 than vinblastine and colchicines, besides compounds **17b**, **4a**, **16a**, **17a**, **16b**, **18b**, **a**, **13a**, **4b**, **14a**, and **15b** exhibited good antitumor profile against A549 than the standard drugs vinblastine and colchicines. From the SARs, we discovered that the incorporation of pyrimidine nucleus at 2,3-positions with hydrophobic or hydrophilic group and some of hydrophobic group at 2-position or hydrophilic group at 7-position of the chromene nucleus is favorable, and greatly enhanced the activity than the other hydrophobic groups and 4-bromophenyl is favorable than 4-chlorophenyl at 5- or 4-position with decreasing of the partition coefficient Log *P*. Also, the compounds containing both 4*H*-chromenes and pyrimidine moieties showed superior in vitro antitumor activity than the 4*H*-chromene derivatives.

Experimental

All chemicals were purchased from Sigma-Aldrich Chemical Co. Melting points were determined with a Stuart Scientific Co. Ltd apparatus and are uncorrected. IR spectra were determined as KBr pellets on a Jasco FT/IR 460 plus spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker High Performance Digital FT-NMR Spectrometer Avance III 400 MHz spectrometer and a Bruker AV 500 MHz spectrometer or Varian Gemini-300BB at 300 MHz. The MS were measured on a Shimadzu GC/MS-QP5 spectrometer. Elemental analyses were carried out at the Regional Centre for Mycology & Biotechnology (RCMP), Al-Azhar University, Cairo, Egypt and the results were within ± 0.3%.

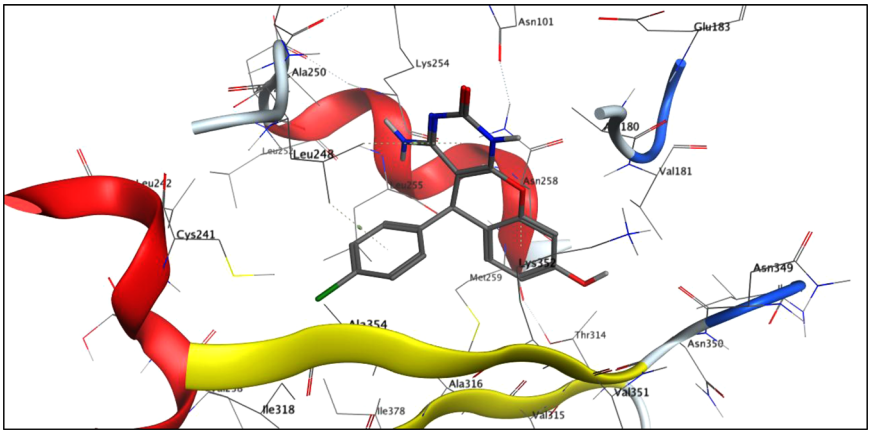


Fig. 6 3D diagram of compound **11a** showing its interaction with the tubulin active site

2-Amino-4-(4-chlorophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (4a)

Compound **4a** was synthesized according to the literature procedure (Makarem et al. 2008).

2-Amino-4-(4-bromophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (4b)

Compound **4b** was synthesized according to the literature procedure (Makarem et al. 2008).

*Preparation of (**6a**, **b**)*

A solution of 2-amino-4-(4-chloro/bromophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (**4a**, **b**) (0.01 mol) in acetic anhydride (20 ml) was refluxed for ½, 3 or 6 h. The solvent was removed under reduced pressure, and the solid obtained was collected and washed with cooled methanol, filtered, dried, and recrystallized from ethanol/benzene to afforded **6a**, **b**. The physical and spectral data of compounds **6a**, **b** are as follows:

7-Acetoxy-4-(4-chlorophenyl)-2-diacetylamino-4*H*-chromene-3-carbonitrile (6a)

Colorless crystals; m.p. 200–201 °C; yield 79%; IR (KBr) υ (cm^{-1}): 3061, 3018, 2946 (CH stretching), 2221 (CN), 1732, 1721 (CO); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta =$ 7.51–6.98 (7 H, m, aromatic), 5.36 (1 H, s, H-4), 2.44 (6 H, s, $2COCH_3$), 2.25 (3 H, s, $OCOCH_3$); MS *m/z* (%): 426 ($M^+ + 2$, 0.66), 424 (M^+, 2.00) with a base peak at 43 (100); anal. calcd for $C_{22}H_{17}ClN_2O_5$: C, 62.20; H, 8.35; N, 6.59. Found: C, 62.48; H, 8.61; N, 6.81%.

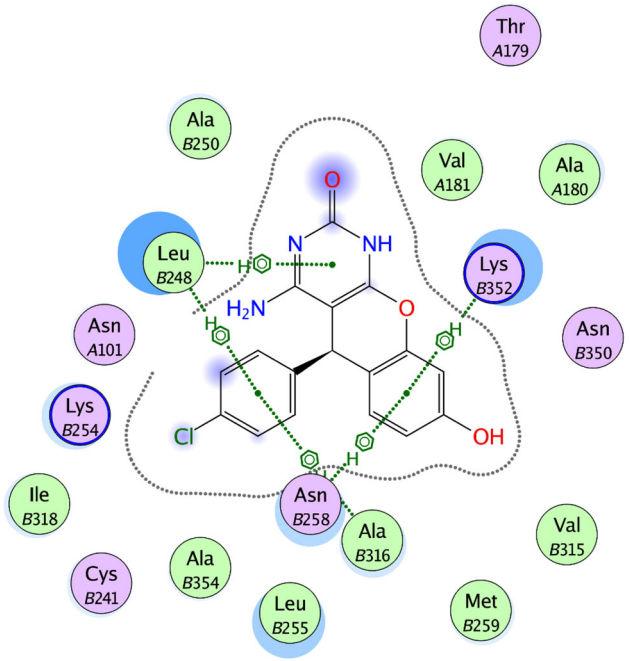


Fig. 7 2D representation of compound **11a** showing its interaction with the tubulin active site

7-Acetoxy-4-(4-bromophenyl)-2-diacetylamino-4*H*-chromene-3-carbonitrile (6b)

Colorless needles; m.p. 206–207 °C; yield 77%; IR (KBr) υ (cm^{-1}): 3068, 3011, 2938 (CH stretching), 2223 (CN), 1745, 1725 (CO); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta =$ 7.65–6.55 (7 H, m, aromatic), 5.36 (1 H, s, H-4), 2.45 (6 H, s, $2COCH_3$), 2.26 (3 H, s, $OCOCH_3$); MS *m/z* (%): 470 ($M^+ + 2$, 7.85), 468 (M^+, 8.05) with a base peak at 187 (100); anal. calcd for $C_{22}H_{17}BrN_2O_5$: C, 56.31; H, 3.65; N, 5.97. Found: C, 56.06; H, 3.42; N, 5.73%.

*Synthesis of the Schiff base (**8a, b**)*

Compounds **4a** and **4b** (0.01 mol), benzaldehyde (0.01 mol), piperidine (0.5 ml) in absolute ethanol (20 ml) was refluxed for 2 h. The solid product which formed was filtered, washed with cooled methanol, dried and recrystallized from ethanol to give **8a**, **b**. The physical and spectral data of compounds **8a**, **b** are as follows:

2-Benzylideneamino-4-(4-chlorophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (8a)

Yellow needles; m.p. 217–218 °C; yield 83%; IR (KBr) υ (cm^{-1}): 3332, (OH), 3070, 3017, 2966 (CH stretching), 2183 (CN); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.76$ (1 H, bs, OH), 9.11 (1 H, s, N=CH), 7.38–6.40 (12 H, m, aromatic), 4.66 (1 H, s, H-4); MS *m*/*z* (%): 388 ($M^+ + 2$, 0.61), 386 (M^+, 1.80) with a base peak at 187 (100); anal. calcd for $C_{23}H_{15}ClN_2O_2$: C, 71.41; H, 3.91; N, 7.24. Found: C, 71.19; H, 3.68; N, 7.02%.

2-Benzylideneamino-4-(4-bromophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (8b)

Yellow needles; m.p. 269–270 °C; yield 80%; IR (KBr) υ (cm^{-1}): 3382, (OH), 3055, 3007, 2796 (CH stretching), 2202 (CN); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.92$ (1 H, bs, OH), 9.12 (1 H, s, N=CH), 8.06–6.58 (12 H, m, aromatic), 5.10 (1 H, s, H-4); MS *m*/*z* (%): 432 ($M^+ + 2$, 16.13), 430 (M^+, 16.92) with a base peak at 275 (100); anal. calcd for $C_{23}H_{15}BrN_2O_2$: C, 64.05; H, 3.51; N, 6.50. Found: C, 63.85; H, 3.25; N, 6.29%.

Synthesis of 1*H*-chromeno[2,3-*d*]pyrimidin-2(5*H*)-one derivatives (11a, b)

A mixture of compounds **4a** and **4b** (0.01 mol) and urea (0.01 mol) with catalytic amount of sodium ethoxide in ethanol (15 ml) was refluxed on water bath for 6–7 h (monitored by thin-layer chromatography (TLC)). After completion of reaction, the reaction mixture was poured in crushed ice (50 g) and neutralized by the diluted hydrochloric acid (1:1). The separated product was collected by the filtration and washed with water. The crude product was purified by crystallization from absolute ethanol to afforded **11a,b**. The physical and spectral data of compounds **11a**, **b** are as follows:

4-Amino-5-(4-chlorophenyl)-8-hydroxy-1*H*-chromeno [2,3-*d*]pyrimidin-2(5 H)-one (11a)

Brown powder; m.p. > 300 °C; yield 80%; IR (KBr) υ (cm^{-1}): 3448, 3367, 3339, 3218 (OH, NH and NH_2), 3071, 2898, 2838, 2798 (CH stretching), 1747 (CO), 1627 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 10.50$ (1 H, bs, NH), 9.65 (1 H, bs, OH), 7.32–6.52 (7 H, m, aromatic), 6.50 (2 H, s, NH_2), 5.04 (1 H, s, H-5); MS *m*/*z* (%): 343 ($M^+ + 2$, 1.33), 341(M^+, 4.02) with a base peak at 230 (100); anal. calcd for $C_{17}H_{12}ClN_3O_3$: C, 59.75; H, 3.54; N, 12.30. Found: C, 60.00; H, 3.80; N, 12.56%.

4-Amino-5-(4-bromophenyl)-8-hydroxy-1*H*-chromeno [2,3-*d*]pyrimidin-2(5 H)-one (11b)

Brown powder; m.p. > 300 °C; yield 79%; IR (KBr) υ (cm^{-1}): 3452, 3382, 3331, 3211 (OH, NH & NH_2), 3030, 2888, 2835, 2781 (CH stretching), 1752 (CO), 1622 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 11.13$ (1 H, bs, NH), 9.87 (1 H, bs, OH), 7.46–6.80 (7 H, m, aromatic), 6.50 (2 H, s, NH_2), 5.02 (1 H, s, H-5); 387 ($M^+ + 2$, 14.65), 385 (M^+, 14.77) with a base peak at 275 (100); anal. calcd for $C_{17}H_{12}BrN_3O_3$: C, 52.87; H, 3.13; N, 10.88. Found: C, 53.04; H, 3.34; N, 11.01%.

*Synthesis of the 4-aminopyrimidine derivatives (**12a, b**)*

Method (a) A mixture of compounds **4a**, **b** (0.01 mol) and formamide (0.02 mol) was stirred at reflux for 6 h. The solvent was removed under vacuum. The solid obtained was recrystallized from benzene to give **12a**, **b**. The physical and spectral data of compounds **12a**, **b** are as follows:

4-Amino-5-(4-chlorophenyl)-5H-chromeno[2,3-d]pyrimidin-8-ol (**12a**) Colorless crystals; m.p. 281–282 °C; yield 71%; IR (KBr) υ (cm^{-1}): 3464, 3340, 3301 (OH & NH_2), 3097, 2819, 2762 (CH stretching), 1643 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.72$ (1 H, s, OH), 8.10 (1 H, s, H-2), 7.33–6.52 (7 H, m, aromatic), 6.80 (2 H, bs, NH_2), 5.18 (1 H, s, H-5); MS *m*/*z* (%): 327 ($M^+ + 2$, 8.61), 325 (M^+, 25.24) with a base peak at 276 (100); anal. calcd for $C_{17}H_{12}ClN_3O_2$: C, 62.68; H, 3.71; N, 12.90. Found: C, 62.91; H, 3.97; N, 13.16%.

4-Amino-5-(4-bromophenyl)-5H-chromeno[2,3-d]pyrimidin-8-ol (**12b**) Colorless crystals; m.p. 292–293 °C; yield 70%; IR (KBr) υ (cm^{-1}): 3464, 3352, 3307 (OH & NH_2), 3093, 2931, 2817 (CH stretching), 1648 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.72$ (1 H, s, OH), 8.10 (1 H, s, H-2), 7.46–6.52 (7 H, m, aromatic), 6.80 (2 H, bs, NH_2), 5.17 (1 H, s, H-5); MS *m*/*z* (%): 371 ($M^+ + 2$, 93.01), 369 (M^+, 93.15) with a base peak at 104 (100); anal. calcd for $C_{17}H_{12}BrN_3O_2$: C, 55.15; H, 3.27; N, 11.35. Found: C, 54.89; H, 3.04; N, 11.16%.

Method (b) A mixture of the imadate **13a**, **b** or **14a**, **b** (0.01 mol) and NH_3 gas in methanol was stirred for 2 h, then

the mixture was left overnight. The solid product was collected and crystallized from benzene to give **12a**, **b** (m.p., mixed m.p., identical IR and MS spectrum).

*Synthesis of the imidate **13a, b** and **14a, b***

A mixture of compounds **4a** and **4b** (0.01 mol) with triethyl orthoformate (0.01 mol) and acetic anhydride (30 ml) or without acetic anhydride was refluxed for 2 h. The solvent was removed under reduced pressure and the resulting solid was washed with methanol and recrystallized from benzene to give 7-acetoxy-2-ethoxymethyleneamino-4-(4-chlorophenyl)-4*H*-chromene-3-carbonitrile (**13a**) (Abd-El-Aziz et al. 2004), **13b** and **14a**, **b**, respectively. The physical and spectral data of compounds **13b** and **14a**, **b** are as follows:

7-Acetoxy-2-ethoxymethyleneamino-4-(4-bromophenyl)-4*H*-chromene-3-carbonitrile (13b)

Colorless needles; m.p. 173–174 °C; yield 86%; IR (KBr) υ (cm^{-1}): 3070, 2985, 2866, 2800 (CH stretching), 2210 (CN), 1767 (CO); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 8.67$ (1 H, s, N=CH), 7.59–6.90 (7 H, m, aromatic), 5.10 (1 H, s, H-4), 4.35 (2 H, q, $J = 7.1$ Hz, O–CH_2CH_3), 2.26 (3 H, s, $OCOCH_3$), 1.33 (3 H, t, $J = 7.1$ Hz, O–CH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 169.36 (C, COMe), 162.47 (C, C-2), 158.20 (C, C-7), 150.50 (CH, N=CH), 148.91 (C, C-8a), 130.58 (CH, C-5), 119.52 (C, CN), 119.42 (C, C-4a), 118.19 (CH, C-6), 110.94 (CH, C-8), 79.53 (C, C-3), 64.50 (CH_2, O-CH_2CH_3), 41.00 (CH, C-4), 21.23 (CH_3, COMe), 14.31 (CH_3, O–CH_2CH_3), 143.75 (C, C-1′), 132.33 (CH, C-3′, C-5′), 130.74 (CH, C-2′, C-6′), 121.21 (C, C-4′); MS *m*/*z* (%): 442 ($M^+ + 2$, 24.27), 440 (M^+, 24.27) with a base peak at 243 (100); anal. calcd for $C_{21}H_{17}BrN_2O_4$: C, 57.16; H, 3.88; N, 6.35. Found: C, 57.41; H, 4.11; N, 6.62%.

2-Ethoxymethyleneamino-4-(4-chlorophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (14a)

Pale yellow needles; m.p. 203–204 °C; yield 78%; IR (KBr) υ (cm^{-1}): 3396, (OH), 3076, 2985, 2869, 2804 (CH stretching), 2211 (CN); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.79$ (1 H, bs, OH), 8.66 (1 H, s, N=CH), 7.43–6.42 (7 H, m, aromatic), 5.18 (1 H, s, H-4), 4.33 (2 H, q, $J = 7.1$ Hz, O-CH_2CH_3), 1.32 (3 H, t, $J = 7.1$ Hz, O-CH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 162.31 (C, C-2), 158.30 (C, C-7), 157.98 (CH, N=CH), 149.36 (C, C-8a), 130.48 (CH, C-5), 118.48 (C, CN), 113.69 (C, C-4a), 112.23 (CH, C-6), 103.29 (CH, C-8), 79.74 (C, C-3), 64.35 (CH_2, O–CH_2CH_3), 40.80 (CH, C-4), 14.34 (CH_3, O–CH_2CH_3), 144.03 (C, C-1′), 132.30 (C, C-4′), 130.26 (CH, C-2′, C-6′), 129.25 (CH, C-3′, C-5′); MS *m*/*z* (%): 356 ($M^+ + 2$, 1.43), 354 (M^+, 4.38) with a base peak at 43 (100); anal. calcd for $C_{19}H_{15}ClN_2O_3$: C, 64.32; H, 4.26; N, 7.90. Found: C, 64.22; H, 4.16; N, 7.80%.

2-Ethoxymethyleneamino-4-(4-bromophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (14b)

Pale yellow needles; m.p. 189–190 °C; yield 77%; IR (KBr) υ (cm^{-1}): IR (KBr) υ (cm^{-1}): 3371, (OH), 3071, 2974, 2925, 2858 (CH stretching), 2211 (CN); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.83$ (1 H, bs, OH), 8.66 (1 H, s, N=CH), 7.88–6.53 (7 H, m, aromatic), 4.93 (1 H, s, H-4), 4.33 (2 H, q, $J = 7.1$ Hz, O-CH_2CH_3), 1.31 (3 H, t, $J = 7.1$ Hz, O-CH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 162.27 (C, C-2), 158.28 (C, C-7), 157.99 (CH, N=CH), 149.35 (C, C-8a), 130.47 (CH, C-5), 118.48 (C, CN), 113.69 (C, C-4a), 112.14 (CH, C-6), 103.30 (CH, C-8), 79.69 (C, C-3), 64.36 (CH_2, O-CH_2CH_3), 40.91 (CH, C-4), 14.33 (CH_3, O-CH_2CH_3), 144.42 (C, C-1′), 132.17 (CH, C-3′, C-5′), 130.62 (CH, C-2′, C-6′), 120.86 (C, C-4′); MS *m*/*z* (%): 400 ($M^+ + 2$, 10.01), 398 (M^+, 10.55) with a base peak at 187 (100); anal. calcd for $C_{19}H_{15}BrN_2O_3$: C, 57.16; H, 3.79; N, 7.02. Found: C, 57.01; H, 3.65; N, 6.89%.

*Synthesis of the imidine **15a, b***

Method (a) A mixture of compounds **4a**, **b** (0.01 mol) and DMF–DPA (0.01 mol) in benzene (30 ml) was refluxed for 3 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from benzene to give the imidine **15a**, **b**. The physical and spectral data of compounds **15a**, **b** are as follows:

4-(4-Chlorophenyl)-2-dimethylaminomethyleneamino-7-hydroxy-4*H*-chromene-3-carbonitrile (**15a**) Colorless crystals; m.p. 216–217 °C; yield 72%; IR (KBr) υ (cm^{-1}): 3464 (OH), 2931, 2896 (CH stretching), 2191 (CN); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.72$ (1 H, bs, OH), 8.41 (1 H, s, N=CH), 7.38–6.42 (7 H, m, aromatic), 4.67 (1 H, s, H-4), 3.15, 3.00 (6 H, s, $2CH_3$); MS *m*/*z* (%): 355 ($M^+ + 2$, 4.71), 353 (M^+, 14.88) with a base peak at 326 (100); anal. calcd for $C_{19}H_{16}ClN_3O_2$: C, 64.50; H, 4.56; N, 11.88. Found: 64.27; H, 4.30; N, 11.61%.

4-(4-Bromophenyl)-2-dimethylaminomethyleneamino-7-hydroxy-4*H*-chromene-3-carbonitrile (**15b**) Colorless crystals; m.p. 250–250 °C; yield 71%; IR (KBr) υ (cm^{-1}): 3482, (OH), 3062, 2972, 2932, 2899 (CH stretching), 2191 (CN); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.76$ (1 H, bs, OH), 8.40 (1 H, s, N=CH), 7.52–6.42 (7 H, m, aromatic), 4.78 (1 H, s, H-4), 3.15, 3.00 (6 H, s, $2CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.32 (C, C-2), 160.74 (C, C-7), 157.78 (CH, N=CH), 149.28 (C, C-8a), 130.30 (CH, C-5),

120.25 (C, CN), 113.20 (C, C-4a), 103.71 (CH, C-6), 102.78 (CH, C-8), 72.25 (C, C-3), 37.91(CH, C-4), 34.99 [CH_3,N(CH_3)$_2$], 145.61 (C, C-1′), 131.99 (CH, C-3′, C-5′), 130.35 (CH, C-2′, C-6′), 120.78 (C, C-4′); MS *m/z* (%): 399 ($M^+ + 2$, 20.21), 397 (M^+, 20.40) with a base peak at 187 (100); anal. calcd for $C_{19}H_{16}BrN_3O_2$: C, 57.30; H, 4.05; N, 10.55. Found: C, 57.58; H, 4.31; N, 10.81%.

Method (b) A mixture of imadate **13a**, **b** or **14a**, **b** (0.01 mol) and dimethylamine in methanol (30 ml), was stirred for 1 h and the mixture was left overnight. The solid product was collected by filtration, washed with methanol and recrystallized from ethanol/benzene to afford **15a**, **b** (m.p., mixed m.p., identical IR and MS spectrum).

*Synthesis of the 3-methylpyrimidine derivatives (**16a, b**)*

Compound **16a**, **b** were prepared from imadate **13a**, **b** or **14a**, **b** (0.01 mol) and methylamine in methanol (30 ml) according to the procedure described for **15** (*Method b*) and recrystallized from ethanol/benzene to afford **16a**, **b**. The physical and spectral data of compounds **16a**, **b** are as follows:

5-(4-Chlorophenyl)-4-imino-3-methyl-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (16a)

Pale yellow crystals; m.p. 285–286 °C; yield 87%; IR (KBr) υ (cm^{-1}): 3466, 3340 (OH & NH), 2939, 2898 (CH stretching), 1635 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.70$ (1 H, bs, OH), 8.11 (1 H, s, H-2), 7.33–6.47 (8 H, m, aromatic, NH), 5.10 (1 H, s, H-5), 3.27 (3 H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 157.63 (C,C-4), 157.24 (C, C-8), 151.83 (C, C-10a), 149.68 (C, C-9a), 144.67 (C-2), 129.66 (C, C-6), 114.51 (C, C-5a), 113.04 (C-7), 103.18 (C-9), 98.14 (C-4a), 38.02 (CH, C-5), 35.81 (CH_3), 131.75 (C, C-1′), 130.47 (C, C-4′), 129.10 (CH, C-2′, C-6′), 127.47 (CH, C-3′, C-5′); MS *m/z* (%): 341 ($M^+ + 2$, 28.01), 339 (M^+, 84.10) with a base peak at 187 (100); anal. calcd for $C_{18}H_{14}ClN_3O_2$: C, 63.63; H, 4.15; N, 12.37. Found: C, 63.41; H, 3.95; N, 12.09%.

5-(4-Bromophenyl)-4-imino-3-methyl-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (16b)

Pale yellow crystals; m.p. 271–273 °C; yield 85%; IR (KBr) υ (cm^{-1}): 3469, 3348 (OH and NH), 3001, 2931, 2800 (CH stretching), 1639 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.72$ (1 H, bs, OH), 8.11 (1 H,s, H-2), 7.47–6.50 (8 H, m, aromatic, NH), 5.08 (1 H, s, H-5), 3.18 (3 H, s, CH_3); MS *m/z* (%): 385 ($M^+ + 2$, 98.55), 383 (M^+, 100); anal. calcd for $C_{18}H_{14}BrN_3O_2$: C, 56.27; H, 3.67; N, 10.94. Found: C, 56.50; H, 3.91; N, 11.18%.

*Synthesis of the 3-amino-4-iminopyrimidine derivatives (**17a, b**)*

Compound **17a**, **b** were prepared from imadate **13a**, **b** or **14a**, **b** (0.01 mol) and methylamine (0.01 mol) according to the procedure described for **15** (Method b) and recrystallized from ethanol/benzene to afford 3-amino-5-(4-chlorophenyl)-4-imino-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (**17a**) (Abd-El-Aziz et al. 2004) and **17b**. The physical and spectral data of compound **17b** was as follows:

3-amino-5-(4-bromophenyl)-4-imino-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (17b)

Colorless crystals; m.p. 234–235 °C; yield 81%; IR (KBr) υ (cm^{-1}): IR (KBr) υ (cm^{-1}): 3633, 3454, 3201 (OH, NH & NH_2), 3001, 2931, 2800 (CH stretching), 1639 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 9.71$ (1 H, bs, OH), 8.07 (1 H, s, H-2), 7.45–6.50 (7 H, m, aromatic), 6.63 (1 H, s, NH), 6.50 (2 H, s, NH_2), 5.10 (1 H, s, H-5); MS *m/z* (%): 386 ($M^+ + 2$, 25.39), 384 (M^+, 26.04) with a base peak at 368 (100); anal. calcd for $C_{17}H_{13}BrN_4O_2$: C, 53.00; H, 3.40; N, 14.54. Found: C, 52.81; H, 3.20; N, 14.32%.

*Synthesis of the Schiff base (**18a, b**)*

A mixture of imino compounds **17a**, **b** (0.01 mol) and benzaldehyde (0.01 mol) in ethanol (30 ml) and piperidine (0.5 ml) was refluxed for 2 h. (TLC monitoring). The solvent was removed under reduced pressure and the resulting solid was recrystallized from ethanol/benzene to give the open chain product **18a**, **b**. The physical data of the compounds **18a**, **b** are as follows:

3-(Benzylideneamino)-5-(4-chlorophenyl)-4-imino-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (18a)

Yellow crystals; m.p. 296–297 °C; yield 87%; IR (KBr) υ (cm^{-1}): IR (KBr) υ (cm^{-1}): 3459, 3201 (OH & NH), 3011, 2937 (CH stretching), 1647 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 10.93$ (1 H, s, NH), 9.74 (1 H, s, OH), 8.37 (1 H, s, N=CH), 8.16 (1 H, s, H-2), 7.71–6.61 (12 H, m, aromatic), 5.99 (1 H, s, H-5); MS *m/z* (%): 430 ($M^+ + 2$, 5.93), 428 (M^+, 13.40) with a base peak at 324 (100); anal. calcd for $C_{24}H_{17}ClN_4O_2$: C, 67.21; H, 4.00; N, 13.06. Found: C, 67.45; H, 4.23; N, 13.24%.

3-(Benzylideneamino)-5-(4-bromophenyl)-4-imino-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-8-ol (18b)

Yellow crystals; m.p. 311–312 °C; yield 84%; IR (KBr) υ (cm^{-1}): 3413, 3197 (OH & NH), 3058, 2980, 2825 (CH stretching), 1638 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) $\delta = 10.96$ (1 H, s, NH), 9.78 (1 H, s, OH), 8.37 (1 H, s, N=CH), 8.17 (1 H, s, H-2), 7.71–6.60 (12 H, m, aromatic), 5.98 (1 H, s, H-5); MS *m/z* (%): 474 ($M^+ + 2$, 5.76), 472 (M^+, 5.85) with a base peak at 368 (100); anal. calcd for $C_{24}H_{17}BrN_4O_2$: C, 60.90; H, 3.62; N, 11.84. Found: C, 61.14; H, 3.85; N, 12.06%.

Antitumor screening

Cell culture

The tumor cell lines breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116), hepatocellular carcinoma (HepG-2) and lung carcinoma (A549) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subculture two to three times a week.

Cytotoxicity evaluation using viability assay

The tumor cell lines were suspended in medium at concentration 5×104 cell/well in Corning® 96-well tissue culture plates and then incubated for 24 h. The tested compounds with concentrations ranging from 0 to 50 µg/ml were then added into 96-well plates (six replicates) to achieve different conc. for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96-well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96-well plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 ml of PBS) to each well including the untreated controls. The 96-well plates were then incubated at 37 °C and 5% CO_2 for 4 h. An 85-µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1 - (ODt/ODc)] × 100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph pad Prism software (San Diego, CA., USA) (Mosmann 1983).

Molecular docking study

All the molecular modeling studies were carried out using Molecular Operating Environment (MOE, 10.2008) software. All minimizations were performed with MOE until an RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$ with MMFF94x force field and the partial charges were automatically calculated. The X-ray crystallographic structures of tubulin co-crystallized with colchicine as inhibitor (PDB ID: 5EYP) (Ahmed et al. 2016) were downloaded from the protein data bank (http://www.rcsb.org/). The protein was prepared for docking study by removal of chain F and keeping chains A and B (α and β tubulin chains) as colchicine binding site is at the interface between these two chains. Water molecules and ligands that are not involved in the binding were also removed. Then the protein was prepared using *Protonate 3D* protocol in MOE with default options. The co-crystallized ligand was used to define the active site for docking. Triangle Matcher placement method and London dG scoring function were used for docking. Docking setup was first validated by re-docking of the co-crystallized ligand (Colchicine) in the vicinity of the active site of the protein with energy score $(S) = -12.85$ kcal/mol and RMSD of 0.942 Å. The validated setup was then used in predicting the binding mode and the binding interactions of the newly synthesized ligands at the active site of tubulin.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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