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Design, synthesis, anti-proliferative activity, and molecular docking studies of novel benzo[*f*]chromene, chromeno [2,3-d]pyrimidines and chromenotriazolo[1,5-c]pyrimidines

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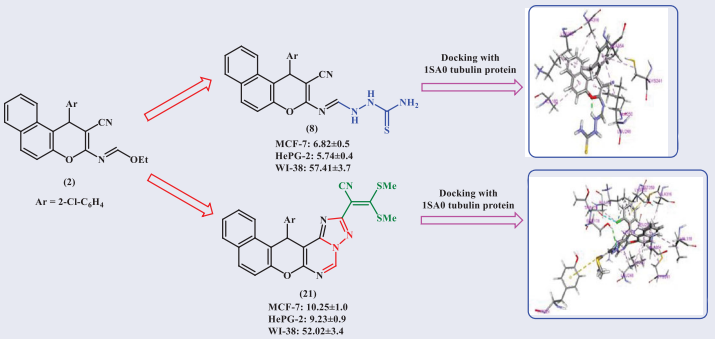
ABSTRACT

In seeking to establish new anticancer agents, a group of novel substituted chromeno[2,3-d]pyrimidine and chromenotriazolo[1,5-c]pyrimidine derivatives were designed and synthesized as potential anti-proliferative agents. Chromeno[2,3-d]pyrimidine derivatives were prepared via reaction of ethyl formimidate derivative **2** with different nitrogen nucleophiles and chromenotriazolo[1,5-c]pyrimidine derivatives were obtained from treatment of cyanomethyl derivative **14** with various electrophilic reagents. The structures of the synthesized compounds were substantiated on the basis of spectral data and elemental analysis. All the synthesized products were evaluated for their antiproliferative activity against two human tumor cell lines; breast adenocarcinoma (MCF-7) and hepatocellular carcinoma (HepG-2) in addition to normal fibroblasts (WI-38). Derivatives **8** and **21** had significant and selective anti-proliferative activity against liver and breast cell lines without harming the normal fibroblasts. The molecular docking studies of most active compounds **8**, **10**, and **21** were performed to examine their binding pattern with protein receptors (PDB: 1SA0).

GRAPHICAL ABSTRACT



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Introduction

Benzochromenes and triazolopyrimidines (TPs) serve as some important building blocks for biologically active molecules. Figure 1 has been disclosed by these two important biological active pharmacophore component systems.

The enormous medicinal and biological activities of fused 4*H*-chromene have stimulated much research in this scope.[1–3] Furthermore, functionally substituted chromenes have played growing part in synthetic approaches to promising compounds in the field of medicinal chemistry including anticancer,[4] antimicrobial,[5] aldose reductase inhibitors,[6] antiproliferative and apoptosis-inducing,[7] molluscicidal,[8] antileishmanicidal,[9] antitumor,[10] antioxidant,[11] anticonvulsant,[12] as well as treatment of Alzheimer's disease[13] and Schizophrenia disorder.[14] Fused chromene ring systems display blood platelet antiaggregating,[15] hypolipidemic,[16] analgesic,[17] vascular-disrupting activity,[18] DNA breaking activities and mutagenicity activities.[19]

The second pharmacophore component is pyrimidine or triazolopyrimidine that is an important category of heterocyclic compounds with a wide range of biological activities. Pyrimidines have acquired considerable attention because of their role in biological systems, especially in nucleic acids, which contain purines and pyrimidines as the major nucleobases.[20] It has been noticed that the incorporation of an additional ring to the pyrimidine core tends to exert a profound impact on conferring new biological activities in these molecules.[21] Thus, TPs, a subtype of purine analogs, have been the topic of biological and chemical studies due to their motivating pharmacology including cardiac stimulant, antihypertensive, antifungal, antimalarial, antimicrobial, anti-HBV, antipyretic, anticancer, antiinflammatory, analgesic, leishmanicidal and potential herbicidal activities.[22–28] In addition, the simple molecule of Trapidil, the most widely known triazolopyrimidine derivative acts as a platelet-derived growth factor antagonist and as a phosphodiesterase inhibitor.[22]

The various biological activities of these pharmacophores; benzochromenes and triazolopyrimidines, prompt the authors to continue their attempts to synthesize biologically active systems[29–35] and discover novel compounds containing the two pharmacophores in a single molecule to exhibit higher pharmacological activities. Therefore, we designed and synthesized new substituted chromenes bearing aryl group at the 4- position, and pyrimidine moieties at the 2,3- position as potential cytotoxic agents against a variety of human cancer cell lines. In addition, docking of three

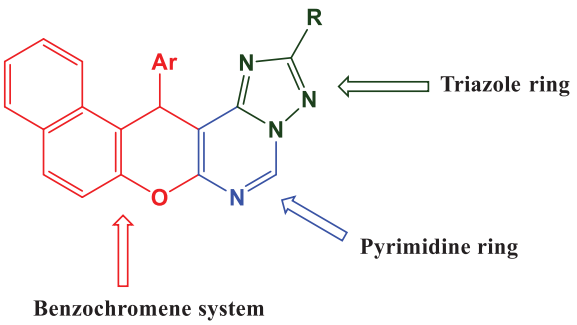
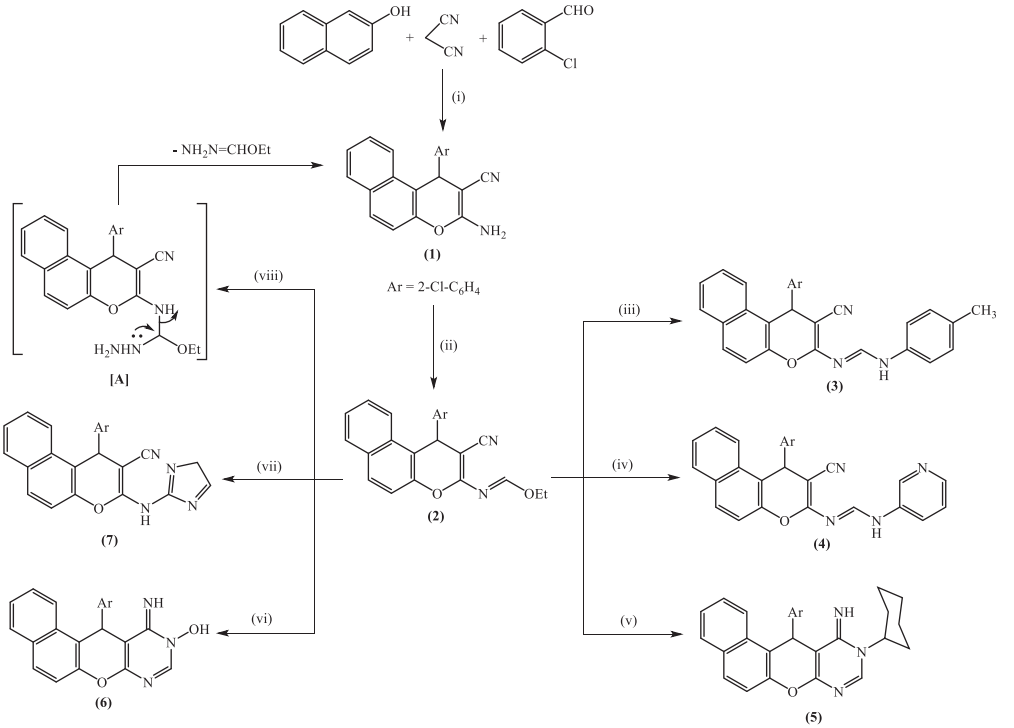


Figure 1. Benzochromene triazolopyrimidines scaffold.



(i) EtOH/pip.; (ii) $CH(OEt)_3/Ac_2O$; (iii) *p*-toluidine/dioxane/Δ; (iv) 3-aminopyridine/dioxane/Δ;(v) cyclohexylamine/pyridine/Δ; (vi) hydroxylamine hydrochloride/pyridine/Δ; (vii) ethylenediamine/dioxane/Δ; (viii) hydrazine hydrate/EtOH

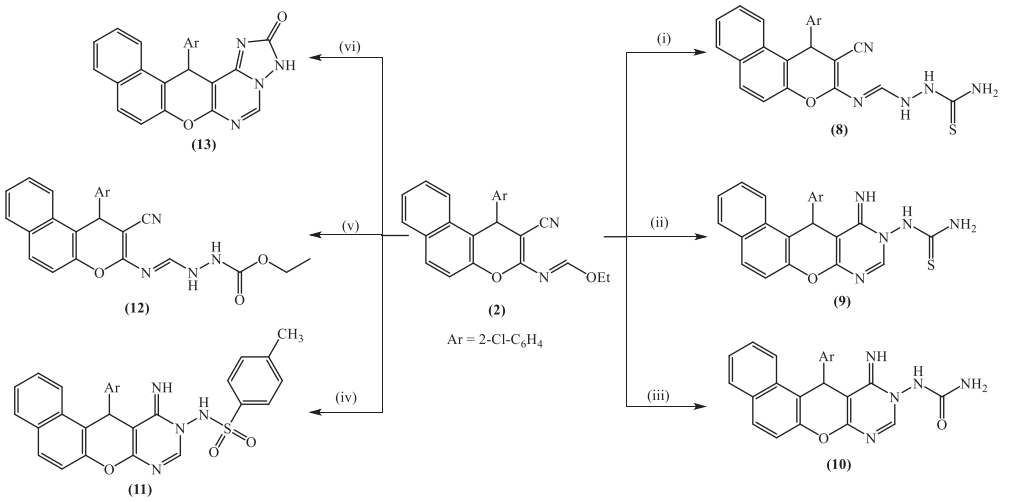
Scheme 1. Reaction of ethyl formimidate derivative **2** with primary amines.

synthesized compounds with 1SA0 tubulin protein was carried out in order to investigate their binding interactions and to explore their binding modes.

Results and discussion

3-Amino-1-(2-chlorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**1**) was prepared using our methodology[36] via one pot-three component cyclo condensation reaction of malononitrile, 2-chlorobenzaldehyde and β-naphthol in ethanol containing a catalytic amount of piperidine (Scheme 1). However, it was previously prepared via multi-component reaction of malononitrile, 2-chlorobenzaldehyde and β-naphthol using either mild basic ionic liquid 2-hydroxyethanaminium format[37] or bael fruit extract (BFE)[38] as a catalyst.

Refluxing the enaminonitrile **1** with triethylorthoformate in the presence of freshly distilled acetic anhydride gave ethyl formimidate derivative **2**, which then exploited for the syntheses of novel chromeno[2,3-d]pyrimidines and chromenotriazolo[1,5-c]pyrimidines. The ^{1}H NMR spectrum of **2** revealed the presence of triplet and quartet signals referring to the presence of ethoxy group protons. Treatment of the key intermediate ethyl formimidate derivative **2** with either *p*-toluidine or 3-aminopyridine in refluxing dioxane afforded formamidine derivatives **3** and **4**, respectively. The existence of $\nu_{C\equiv N}$ at 2207, 2188 cm^{-1} in IR spectra of formamidines **3** and **4** in addition to the appearance of singlet signals of NH exchangeable with D_2O in ^{1}H NMR spectra reinforce the proposed structures. Furthermore,



(i) thiosemicarbazide/dioxane/30 min; (ii) thiosemicarbazide/dioxane/6 h; (iii) semicarbazide hydrochloride/pyridine/Δ; (iv) *p*-toluene sulphonohydrazide/pyridine/Δ; (v) ethyl carbazate/dioxane/Δ; (vi) ethyl carbazate/pyridine/Δ

Scheme 2. Reaction of ethyl formimidate derivative **2** with hydrazides.

aminolysis of formimidate derivative **2** with cyclohexyl amine in refluxing pyridine gave the corresponding iminopyrimidine derivative **5**. Also, the reaction of formimidate derivative **2** with hydroxylamine hydrochloride in refluxing pyridine afforded 12-(2-chlorophenyl)-10-hydroxy-benzo[5,6]chromeno[2,3-d]pyrimidine-11(12*H*)-imine (**6**) (Scheme 1). Good evidence to formation of the imine structure is obtained from the IR spectra of both iminopyrimidine derivatives **5** and **6** which are devoid of stretching frequency of C≡N group.

On the other hand, heating the formimidate derivative **2** with diamine such as ethylenediamine in boiling dioxane afforded the imidazole derivative **7**. The existence of $\nu_{C\equiv N}$ at 2188 cm^{-1} in the IR spectrum of compound **7** supported the suggested structure. In addition to the ^1H NMR ($CDCl_3$) spectrum which showed the disappearance of the triplet and quartet signals attributed to the ethyl protons of its precursor **2**. Hydrazinolysis of compound **2** with hydrazine hydrate in ethanol at room temperature or under reflux yielded the enaminonitrile **1** (much more thermodynamically stable) instead of expected pyrimidine derivative. According to our speculation, the formation of compound **1** is believed to proceed via addition of hydrazine to azomethine functionality to give nonisolable addition product [**A**] followed by elimination of nonisolable ethyl formohydrazonate moiety [NH_2N=CHOEt].

A number of novel chromeno[2,3-d]pyrimidines were also synthesized using the readily obtainable ethyl formimidate derivative **2** upon treatment with different hydrazine derivatives. Thus, boiling ethyl formimidate **2** with thiosemicarbazide in dioxane yielded a white solid which was formed after thirty minutes and identified as thiosemicarbazide derivative **8**. However, on continuing heating, the formed acyclic thiosemicarbazide derivative **8** dissolved and the reaction mixture was heated for further 6 h and the product obtained was identified as iminopyrimidine derivative **9** (Scheme 2). Moreover, when compound **2** was allowed to react with semicarbazide hydrochloride in boiling pyridine, iminopyrimidine derivative **10** was obtained. Likewise, benzo[5,6]chromeno[2,3-d]pyrimidine derivative **11** was obtained from treatment of formimidate

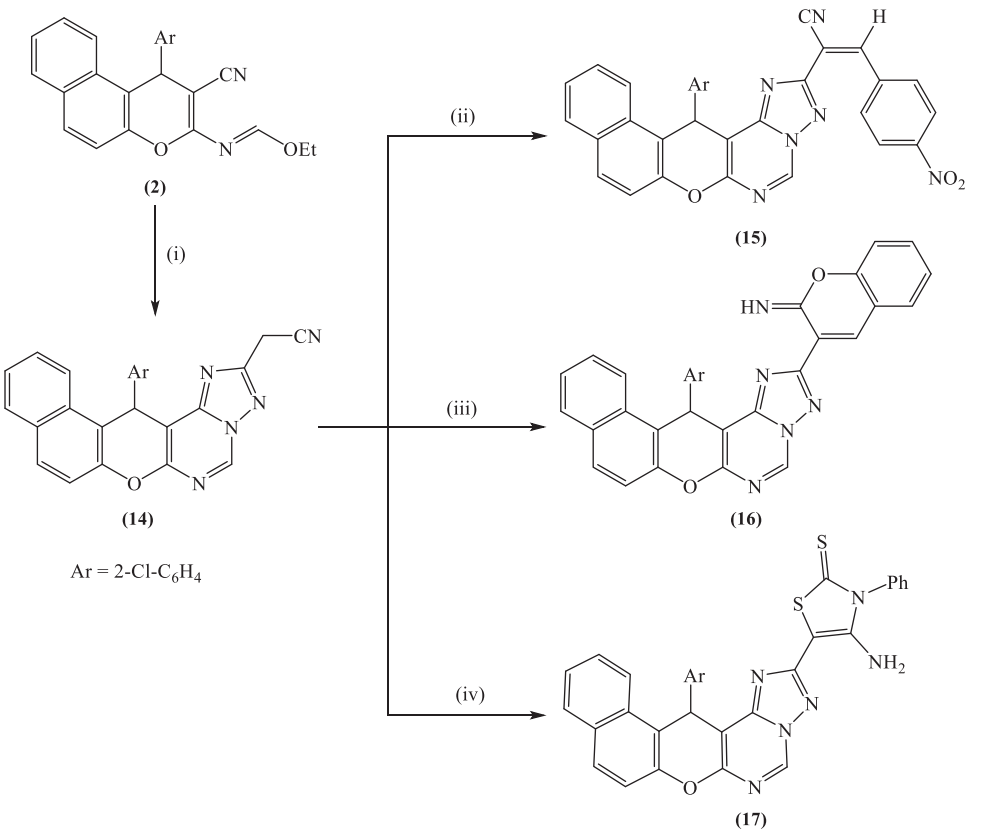
derivative **2** with *p*-toluene sulphonohydrazide in refluxing pyridine. On the other hand, treatment of ethyl formimidate **2** with ethyl carbazate in refluxing dioxane afforded ethyl 2-{[(1-(2-chlorophenyl)-2-cyano-1*H*-benzo[*f*]chromen-3-yl)imino]methyl} hydrazine-1-carboxylate (**12**) as a sole product. However, when the reaction was conducted in pyridine, chromenotriazolo[1,5-*c*]pyrimidine derivative **13** was yielded as a sole product. This behavior can be explained based on the basic nature of pyridine which is needed to promote the 1,6-exo-dig cyclization, which involve proton transfer from –NH to CN group, followed by 1,5-exo-trig cyclization to give **13**.

The reaction of ethyl formimidate derivative with acid hydrazides was examined and found to afford two ring annulations leading to the formation of fused triazolopyrimidine systems.[39–41] Thus, 2-(14-(2-chlorophenyl)-14*H*-benzo[5,6]chromeno[3,2-e][1,2,4]triazolo[1,5-c] pyrimidin-2-yl)acetonitrile (**14**) was isolated via interaction of ethyl formimidate **2** with 2-cyanoacetohydrazide in refluxing dioxane (Scheme 3). The structure **14** was substantiated from studying analytical and spectroscopic data. Thus, the IR spectrum displayed $\nu_{C\equiv N}$ at 2257 cm^{-1} (saturated nitrile) and $\nu_{C=N}$ at 1634 cm^{-1}. Strong support of structure **14** was forthcoming from ^{1}H NMR ($CDCl_3$) spectrum which revealed the disappearance of the triplet and quartet signals attributed to the ethyl protons of its precursor **2** and exhibited the appearance of signals at (δ, ppm): 4.08 (s, 2H, CH_2), 6.74 (s, 1H, HC_4-pyran), 7.07–8.20 (m, 10H, Ar–H), 9.11 (s, 1H, pyrimidine moiety).

The presence of cyanomethyl functionality in **14** was utilized to insert and construct new heterocyclic systems via reaction with different electrophilic reagents, namely, *p*-nitrobenzaldehyde, salicylaldehyde, phenyl isothiocyanate, and carbon disulfide. It has been reported that cyanomethyl derivatives underwent base-catalyzed condensation with aromatic aldehydes to give Knoevenagel condensation product and reacted with salicylaldehyde under basic condition to afford coumarin derivatives.[42] Similarly, cyanomethyl derivative **14** was subjected to react with *p*-nitrobenzaldehyde and/or salicylaldehyde in dioxane containing a catalytic amount of piperidine to afford arylidene derivative **15** and iminochromene derivative **16**, respectively. The IR spectrum of compound **16** showed sharp stretching absorption bands at 3301 and 1648 cm^{-1} characteristic for NH and C=N group frequencies and absence of absorption band of cyano-group. Moreover, ^{1}H-NMR spectrum of the product is devoid of methylene protons of its precursor **14** and showed signals attributed to NH proton at δ 10.18 ppm as singlet exchanged with D_2O, singlet at δ 9.66 ppm for HC_2-pyrimidine, singlet for HC_4-chromene proton at δ 8.99 ppm, multiplet for aromatic protons (14 H) at δ 7.79–8.21 ppm and singlet at δ 6.57 ppm for HC_4-pyran.

Meanwhile, when compound **14** was allowed to react with phenyl isothiocyanate and elemental sulfur in the presence of a catalytic amount of triethylamine, thiazole-2-thione derivative **17** was obtained. IR spectrum exhibited group frequencies at 3446, 3298, 1629 and 1240 cm^{-1} representing the NH_2, C=N together with C=S groups, respectively. ^{1}H NMR spectrum displayed two singlet signals integrated for one proton at δ 9.51 and 6.49 ppm for HC_2-pyrimidine moiety and HC_4-pyran, respectively, in addition to singlet signal at δ 6.82 ppm integrated for two protons exchangeable with D_2O for NH_2-group and multiplet for 15H at δ 7.11–8.03 ppm.

Stirring cyanomethyl derivative **14** with carbon disulfide in ethanolic potassium hydroxide and *N*,*N*-dimethylformamide afforded the dipotassium disulfide salt **18** which

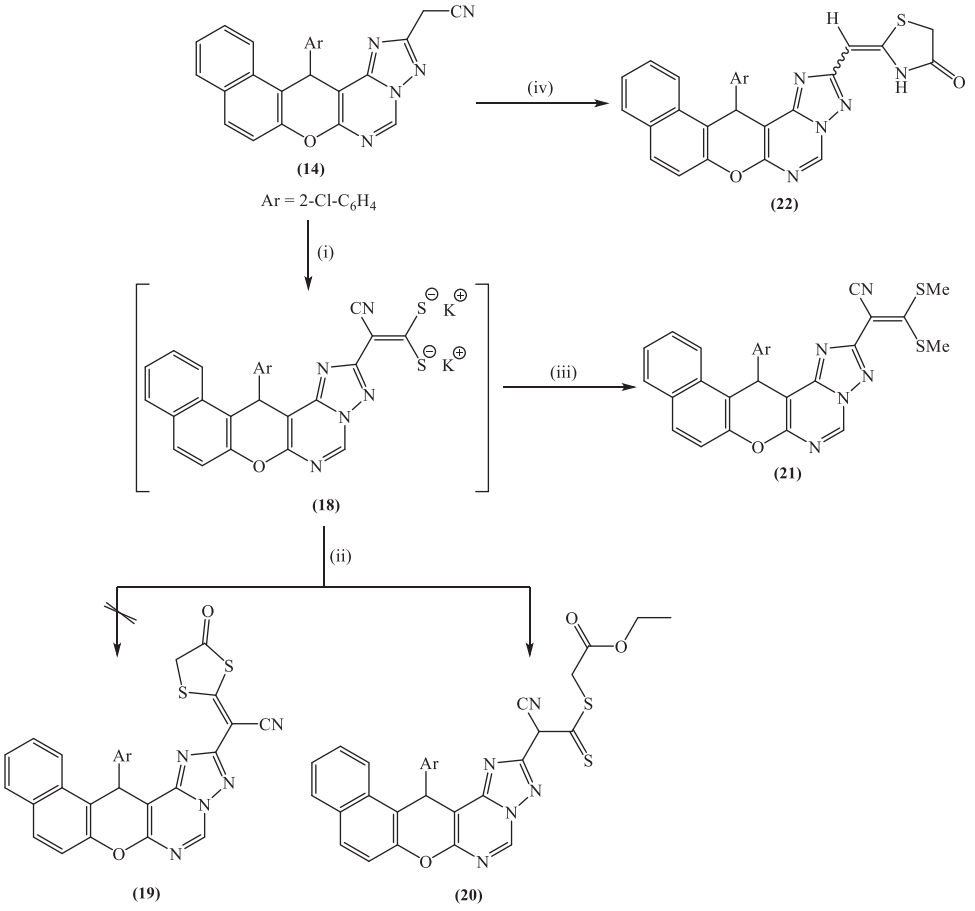


(i) 2-cyanoacetohydrazide/dioxane/Δ; (ii) *p*-nitrobenzaldehyde/dioxane/pip.; (iii) salicylaldehyde/dioxane/pip.; (iv) PhNCS/ S/ Et_3N

Scheme 3. Synthesis of some chromenotriazolo[1,5-c]pyrimidines.

reacted *in situ* with ethyl chloroacetate to give uncyclized product **20** instead of 1,3-dithiolan-4-one derivative **19** (Scheme 4). Moreover, under the same conditions treatment of dipotassium disulfide salt **18** with dimethyl sulfate yielded 2-{14-(2-chlorophenyl)-14*H*-benzo[5,6]chromeno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl}-3,3-bis-(methylthio)acrylonitrile (**21**).

On the other hand, refluxing compound **14** with thioglycollic acid in pyridine afforded thiazolidin-4-one derivative **22**. IR spectrum displayed bands attributed to NH (br, due to H-bonding), CO (five membered-aza lactone) and C=N groups at 3427, 3242, 1717, 1631 cm^{-1}, respectively. Inspection of ^1H NMR (DMSO-d_6) revealed the existence of signals correlated with protons of CH_2, CH, CH=, N=CH, and NH groups as well as multiplet for the aromatic protons together with extra signals for methylene, methane, olefinic and iminomethine protons. This is in accord with its existence as a mixture of *E*- and *Z*-isomers in the ratio of 67:33. The higher percentage of the *E*-isomer as compared with *Z*-isomer may be attributed to the high stability of the former as a result of the extension of conjugation and the existence of intramolecular H-bonding.[43] Attempts to separate the isomers from the mixture by fractional crystallization or column chromatography failed probably due to the small difference in the R_f of the two isomers (Fig. 2).



Scheme 4. Reactivity of cyanomethyl derivative **14** towards carbon disulphide and thioglycollic acid.

Antitumor assays

Anti-proliferative activity of the newly synthesized compounds **1–12**, **14–17**, and **20–22** were screened in two human cancer cell lines, namely, breast adenocarcinoma (MCF-7), and hepatocellular carcinoma (HepG-2) in addition to the normal fibroblast (WI-38) using MTT colorimetric assay. *In-vitro* cytotoxicity evaluation using viability assay was performed using doxorubicin as a reference cytotoxic compound. 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 as shown in Table 1. The data showed that doxorubicin had an IC_{50} at ~4–7 μM against all cells investigated with no differentiation between cancer and normal cells. The results from Table 1 explicated that some of the prepared compounds displayed an excellent to modest or fair growth inhibitory activity against the tested cancer cell lines. Compounds **8** and **21** were the most potent derivatives as active as doxorubicin against MCF-7 and HepG2 cell lines with IC_{50} (6.82 ± 0.5, 5.74 ± 0.4) and

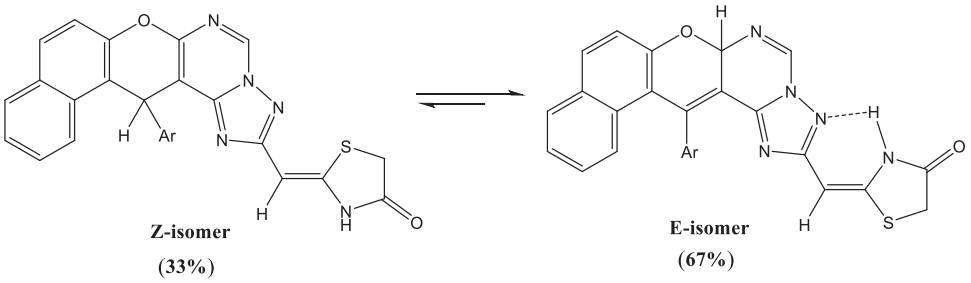


Figure 2. Z/E isomers of compound **22**.

Table 1. Cytotoxicity (IC_{50}) of the prepared compounds on different cell lines.

	IC_{50} (μM)[a]		
Compd. no.	MCF-7	HePG2	WI-38
1	92.43 ± 5.6	83.50 ± 4.8	55.13 ± 1.6
2	>100	>100	68.28 ± 4.0
3	51.55 ± 3.8	49.34 ± 3.1	13.84 ± 1.2
4	32.71 ± 2.4	29.33 ± 2.2	>100
5	39.96 ± 3.1	34.72 ± 2.4	73.62 ± 4.3
6	78.59 ± 4.9	72.64 ± 4.0	29.78 ± 2.1
7	30.85 ± 2.3	23.50 ± 1.9	85.02 ± 4.6
8	6.82 ± 0.5	5.74 ± 0.4	57.41 ± 3.7
9	24.21 ± 2.0	18.14 ± 1.5	49.13 ± 3.2
10	12.56 ± 1.3	11.08 ± 1.1	45.23 ± 2.9
11	28.14 ± 2.2	31.59 ± 2.3	91.31 ± 5.1
12	27.07 ± 2.1	16.18 ± 1.4	83.16 ± 2.7
14	>100	>100	62.30 ± 3.8
15	60.38 ± 4.2	54.76 ± 3.3	34.65 ± 2.3
16	52.14 ± 3.9	58.23 ± 3.6	24.29 ± 1.8
17	48.36 ± 3.6	35.01 ± 2.5	66.45 ± 3.9
20	33.79 ± 2.5	43.28 ± 2.7	19.82 ± 1.5
21	10.25 ± 1.0	9.23 ± 0.9	52.02 ± 3.4
22	38.98 ± 2.9	89.34 ± 4.8	38.17 ± 2.6
DOX	4.17 ± 0.2	4.50 ± 0.3	6.72 ± 0.5

[a]IC_{50} (μM): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), above 100 (non-cytotoxic). DOX: Doxorubicin.

(10.25 ± 1.0, 9.23 ± 0.9) μM, respectively. Compounds **8** and **21** were safe to the normal fibroblasts with IC_{50} at ∼52–57 μM. On the other hand, compounds **10** exhibited strong cytotoxic activity against MCF-7 and HepG2 cell lines with IC_{50} (12.56 ± 1.3, 11.08 ± 1.1) μM, respectively. Also compounds **9** and **12** showed strong cytotoxic activity against HepG2 cell line with IC_{50} (18.14 ± 1.5, 16.18 ± 1.4) μM, respectively, while compounds **3**-**5**, **7**, **11**, **17**, and **20** showed moderate activities toward the two cell lines. Weak activity toward MCF-7 and HePG-2 cell lines was observed with compounds **1**, **6**, **15**, and **16**.

Molecular docking studies

In this work, docking of three synthesized compounds with 1SA0 tubulin protein was carried out in order to predict the proposed binding mode, affinity and the preferred orientation of each docking pose. Molecular docking study of the compound **8**; (Z)-2-

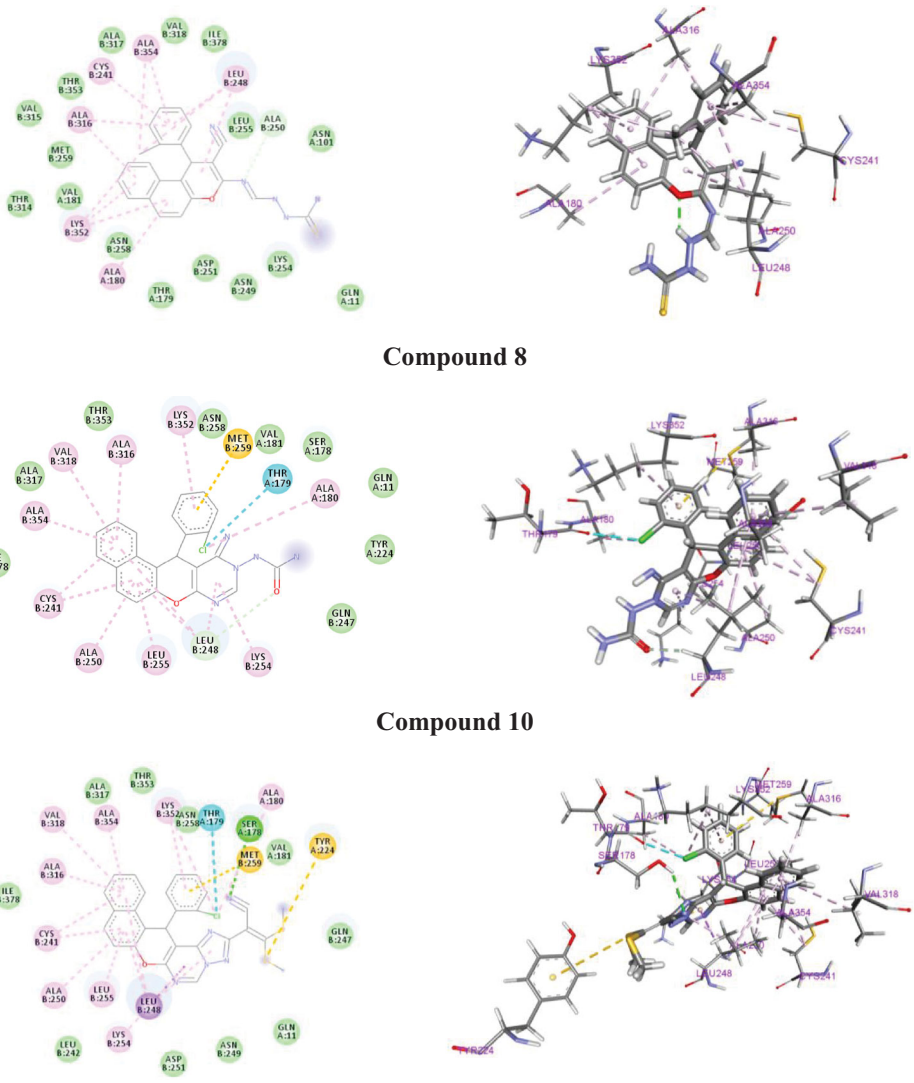


Figure 3. 2D and 3D interaction diagrams of compounds **8**, **10**, and **21** with tubulin (PDB: 1SA0) active sites.

(((4-(2-chlorophenyl)-3-cyano-4*H*-benzo[*f*]chromen-2-yl)imino)methyl)hydrazinecarbothioamide revealed that it has hydrophobic (pi-alkyl-Alkyl) interaction with the potential CYS-B-241, Ala-B-354, Leu-B-248, Ala-B-316, Lys-B-352 and Ala-B-180. It also shows interaction of the carbon-hydrogen weak bonding with the Ala-B-250 with the imine nitrogen at position 2 of the chromene moiety. It shows van der Waals interaction with many amino acids (Fig. 3).

Furthermore, molecular docking studies of the compound **10**; 1-(12-(2-chlorophenyl)-11-imino-11,12-dihydro-10*H*-benzo[5,6]chromeno[2,3-d]pyrimidin-10-yl)urea showed that it has hydrophobic (pi-alkyl-Alkyl) interaction with the potential CYS-B-241, Ala-B-354, Lys-B-254, Leu-B-255, Ala-B-250, Val-B-318, Ala-B-316, Lyy-B-352, and

Ala-B-180. It shows the interaction of the halogen bonding of chlorine with the Thr-A-179 with the oxygen moiety. It also shows van der Waals interaction with many amino acids (Fig. 3) Finally, molecular docking studies of the compound **21**; 2-(14-(2-chlorophenyl)-14*H*-benzo[5,6]chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-3,3-bis(methylthio)acrylonitrile detected that it has hydrophobic (pi-alkyl-Alkyl) interaction with the potential CYS-B-241, Lys-B-254, Leu-B-255, Ala-B-250, Ala-B-316, Val-B-318, Ala-B-354, Lys-B-352 and Ala-B-180. It shows the interaction of the pi-sulfur bonding with the Met-B-259 between benzene moiety and pi-sulfur interaction of Tyr-A-224 with one of the methyl sulfur group's sulfur. Leu-B-248 shows pi-sigma interactions and Thr-A-179 interacted with Cl. Ser-B-178 shows hydrogen bonding interaction. It also shows van der Waals interaction with many amino acids (Fig. 3).

The interactions of standard drug doxorubicin with tubulin (PDB: 1SA0) active site were reported in previous studies.[32] From the previous, it is found that the compounds **8**, **10**, and **21** exhibit clear interactions with many amino acids of the tubulin active site; this visualization depicts the binding mode and probable cause of activity.

Experimental

All melting points were taken on a Griffin and George melting-point apparatus (Griffin & George Ltd., Wembley, Middlesex, UK) and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer (Pye Unicam Ltd., Cambridge, UK) by using the KBr wafer technique. ^{1}H-NMR spectra were determined on a Varian Gemini 300 MHz (Santa Clara, CA) by using tetramethylsilane as internal standard (chemical shifts in δ scale). EI-MS was measured on a Shimadzu GC-MS (Columbia, MD) operating at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer (Waltham, MA), and satisfactory analytical data (±0.4) were obtained for all compounds. The homogeneity of the synthesized compounds was controlled by thin-layer chromatography (TLC), using aluminum sheet silica gel F254 (Merck, Darmstadt, Germany). The antitumor activities were performed at Microanalytical Center of Mansoura University, Egypt.

3-Amino-1-(2-chlorophenyl)-1H-benzo[f]chromene-2-carbonitrile 1

A solution of malononitrile (1.98 g, 30 mmol), *o*-chlorobenzaldehyde (4.23 g, 30 mmol), and β-naphthol (4.32 g, 30 mmol) in absolute ethanol (30 mL) containing piperedine (0.5 mL) was heated under reflux for 2 h. Most of the solvent was distilled off and the reaction solution was left to cool at room temperature. The yielded solid was filtered off, dried and then crystallized from ethanol to give **1** as white crystals, mp 272–273 °C [Lit.[44] mp: 265 °C], yield: 92%.

Ethyl N-[1-(2-chlorophenyl)-2-cyano-1H-benzo[f]chromen-3-yl]formimidate 2

A solution of enaminonitrile **1** (1.75 g, 5 mmol) and triethylorthoformate (1.5 mL, 10 mmol) in acetic anhydride (5 mL) was heated at reflux for 2 h. The formed solid

while reflux was filtered off, washed with petroleum ether (60-80 °C), dried, and then crystallized from ethanol to afford **2** as beige crystals, mp 235–236 °C [Lit.[44] mp: 228 °C], yield: 89%. IR (KBr, ν, cm^{-1}): 2210 (CN), 1652 (HC=N). ^1H NMR (300 MHz, $CDCl_3$-d_6) δ (ppm): 1.39–1.43 (t, 3H, CH_3), 4.44–4.49 (q, 2H, CH_3), 5.97 (s, 1H, CH-pyran), 6.91–8.17(m, 10H, Ar–H), 8.46 (s, 1H, N=CH). MS (*m/z*, %): 389 (4), 388 (4), 368(100). Anal. Calcd. for $C_{23}H_{17}ClN_2O_2$ (388.851): C, 71.04; H, 4.41; Cl, 9.12; N, 7.20. Found: C, 71.25; H, 4.47; Cl, 9.30; N, 7.29.

Pharmacological activity

Cytotoxicity assay

The cytotoxic activity of thirteen compounds was tested against two human tumor cell lines namely: mammary gland (breast) MCF-7 and hepatocellular carcinoma (liver) HePG-2 in addition to normal fibroblasts (WI-38). The cell lines were obtained from the ATCC via the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Doxorubicin was used as a standard anticancer drug for comparison. The reagents used were RPMI-1640 medium, MTT, DMSO and Doxorubicin (Sigma Co., St. Louis, MO), and Fetal Bovine Serum (GIBCO, Paisley, UK). The different cell lines[45,46] mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 µg/mL streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded[47] in a 96-well plate at a density of 1.0×10^4 cells/well at 37 °C for 48 h under 5% CO_2 incubator. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µL of MTT solution at 5 mg/mL was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in a volume of 100 µL was added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at an absorbance of 570 nm using a plate reader (EXL 800, BioTech, Winoosky, VT).

Molecular docking

Computational molecular docking studies were performed using MOE 2014 software[48] on an octa-core machine using windows 10 OS (Microsoft, Redmond, WA). The software was used to find the putative binding modes of potential drug candidates. The PDB structure of the 1SA0 tubulin protein was downloaded from RCSB Data Bank (DOI: 10.2210/pdb1SA0/pdb). The resolution was 3.58 Å. The tetramer was resolved to dimmer of α1 and β1 and the docking site was located in the β1 subunit of the dimmer. The cavity was marked in MOE and the ligands (tested compounds) were minimized and saved to the database. Visualization was performed in Discovery Studio 4.0[49] which is free for this purpose.

Full experimental details and spectroscopic data (IR spectra, ^{1}H-NMR and MS) for compounds **3–17**, **20–22** can be found via the Supplementary Content section of this article's Web page.

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