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Original article

Synthesis of novel chromene derivatives of expected antitumor activity

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ABSTRACT

Inhibition of tubulin polymerization is one of the important tactics in cancer therapy. Since 4-aryl-4*H*-chromene derivatives are found to be microtubule-binding agents via interfering with tubulin polymerization so we decide to concentrate our exploration efforts on the combination of this nucleus with 5-, 6-, and/or 7-memebered heterocyclic moieties in a novel series of compounds to explore the effect that might result from this combination. Ten novel compounds were selected for anticancer screening assay against MCF-7 breast cancer cell line in comparison to colchicine as positive control and most of them showed excellent activity.

1. Introduction

Microtubule-binding agents (MBAs) are widely used in cancer chemotherapy [1]. There are two classes of MBAs; those that stabilize microtubules and promote polymerization, and those that destabilize the microtubules and promote depolymerization. Both types interfere with the mitotic spindle assembly during cell division, resulting in cell death. Colchicine (**I**), podophyllotoxin (**II**), and combretastatin A-4 (**III**) (Fig. 1) are representatives of compounds that inhibit microtubule assembly [2,3] and share a common structural feature; two aromatic rings directly bonded or separated by 1–4 carbon atoms, in such a way that they are close in space but not coplanar [4].

A major turning point in the development of MBAs as antitumor agents is the identification of the natural compound namely combretastatin A-4 (**III**) [4]. Compound **III** strongly inhibits the polymerization of tubulin by binding to the colchicine binding site [2,3]. Besides, it can inhibit angiogenesis, a process essential for tumor growth [5–7]. The synthesis of different 4-arylcoumarin analogs of combretastatin A-4 (**III**) lead to the discovery of promising series of potent apoptosis inducing agents possessing vascular targeting activity [8–14]. These compounds were found to be tubulin destabilizers, binding at or nearly at the binding site of colchicine. They were also active in drug-resistant cancer cell lines and highly active as single agents or in combination with other anticancer agents in several tumor models, so they could be developed into new therapeutic anticancer regimes [15–19]. On the other hand, numerous triazolo, triazino and triazepino pyrimidine derivatives have been reported as cell cycle arrest agents that act via interfering with the polymerization [20,21] or depolymerization [22] of microtubules assembly leading to cell death. Prompted by these findings, we intended to synthesize novel series of compounds containing both 4-aryl-4*H*-chromenes and triazolo, triazino and triazepino pyrimidine moieties to explore the synergistic effect that might result from this combination (Schemes 1–5).

2. Results and discussion

2.1. Chemistry

2-Amino-4-(4-chlorophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (**1**) was synthesized according to the reported method [23,24] and alkylated using methyl or ethyl iodide to afford compounds **2a** and **2b**. The IR spectra of **2a** and **2b** assured the presence of both amino functionality and cyano group at 3432 (**2a**), 3332 (**2b**) cm^{-1} and 2196 (**2a**), 2185 (**2b**) cm^{-1} respectively. Meanwhile, ^1H NMR spectra of compounds **2a** and **2b** revealed the presence of one exchangeable singlet signal at δ 6.98 and 6.54 ppm respectively corresponding to NH_2 protons. The ^{13}C NMR for

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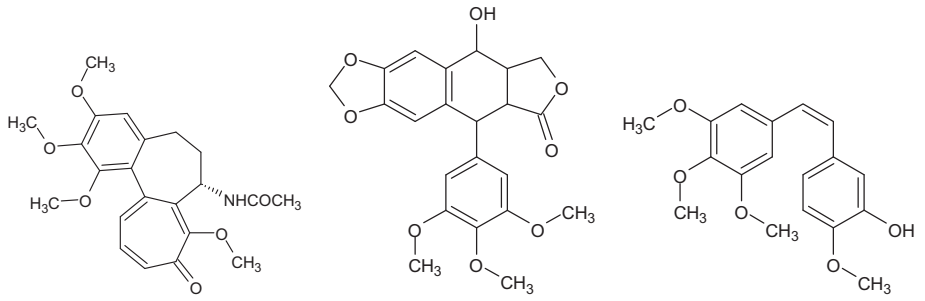


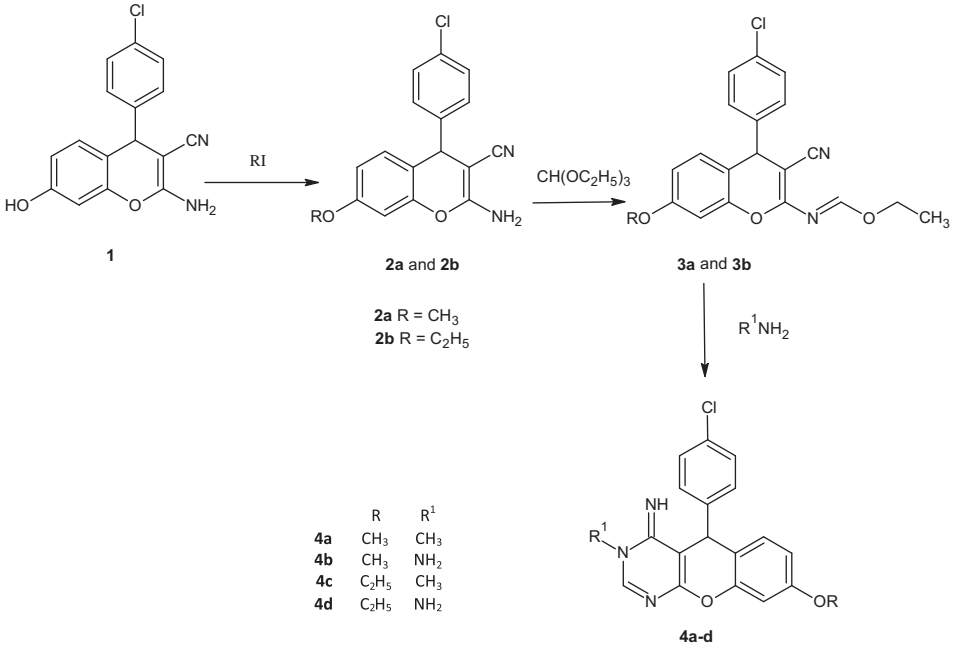
Fig. 1. Colchicine **I**, podophyllotoxin **II**, and combretastatin A-4 **III**.

compound **2a** disclosed three signals at δ 39.9 ppm, δ 55.5 ppm and δ 114.2 ppm corresponding to the stereogenic carbon of the chromene nucleus, CH_3 and CN, sequentially.

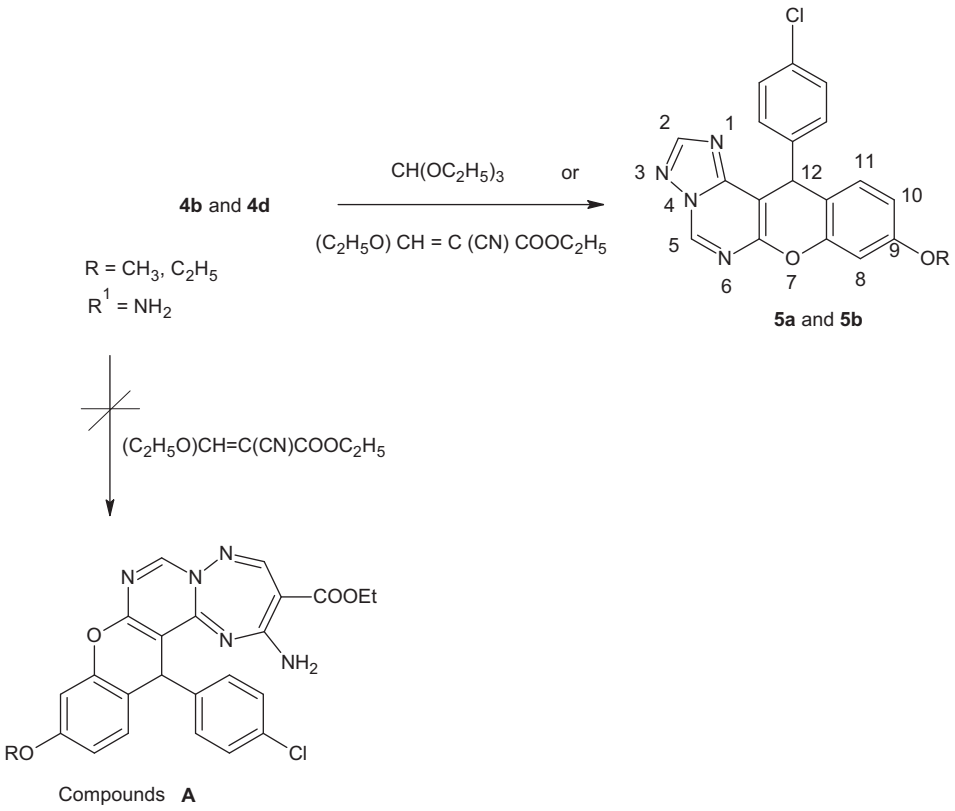
Compounds **2a** and **2b** were reacted with triethyl orthoformate to give **3a** and **3b**. IR spectra of compounds **3a** and **3b** revealed the disappearance of NH_2 group while, their ^{1}H NMR spectra showed a triplet at δ 1.39, 1.36 ppm (OCH_2CH_3), a quartet at δ 4.43, 3.49 ppm (OCH_2CH_3) and a singlet at δ 8.30, 8.38 ppm indicated the presence of imino group (N=C*H*). Interaction of **3a** and **3b** with equimolar amount of either methylamine or hydrazine hydrate in absolute ethanol at ambient temperature gave the key intermediates **4a**–**d** (Scheme 1).

The structures of compounds **4a**–**d** were established on the basis of elemental analyses and spectral data. IR spectra of compounds **4a**–**d** showed no absorption band for CN group meanwhile revealed characteristic absorption bands at 3336–3156 cm^{-1} indicating the presence of NH, NH_2 functionality. Furthermore, ^{1}H NMR spectra of compounds **4a**–**d** revealed the appearance of singlet signals at δ 7.79–8.17 ppm corresponding to C2H of the pyrimidine ring. ^{1}H NMR spectrum of compound **4a** revealed two singlets at δ 3.40 and 3.77 ppm assigned to N–CH_3 and O–CH_3 protons, respectively, ^{1}H NMR spectrum of compound **4b** showed appearance of D_2O exchangeable broad signal at δ 4.74 ppm assigned to two protons of NH_2 group. It is worth to mention that, upon hydrazinolysis of **3a** and **3b** using excess hydrazine hydrate 99% instead of equimolar amounts, compounds **2a** and **2b** were recovered. This may be attributed to, after the reaction of 1 mol of hydrazine with the iminoether **3a** and **3b** a preferential elimination of ethyl formate hydrazone rather than cyclization to give the pyrimidine ring took place (Fig. 2).

Schemes 2–5 summarizes the synthesis of the triazolochromenopyrimidines with different substitution at C2 using different one-carbon donor cyclizing agents to react with the key intermediates **4b** and **4d**. Cyclization of **4b** and **4d** with either triethyl orthoformate, acetic anhydride or diethyl oxalate gave the corresponding 1,2, 4-triazolo[1,5-c]pyrimidines **5a** and **5b**; **5c** and **5d** and **5e** and **5f** respectively. Neither IR nor ^{1}H NMR spectra supported the presence of the NH or NH_2 protons that existed in the precursor **4b** and **4d**. IR spectra of compounds **5e** and **5f** showed a band at 1741, 1745 cm^{-1} confirming the presence of (C=O) of the ester moiety. Moreover, the ^{1}H NMR spectra of **5a**–**f** showed two singlet signals at δ 5.63, 5.81 ppm and δ 9.00, 9.75 ppm indicating C12H and C5H, respectively. The increased chemical shift for these signals-compared to their values in tricyclic system **4b** and **4d**



Scheme 1. Synthesis of compounds **2a** and **2b**, **3a** and **3b** and **4a**–**d**.



Scheme 2. Synthesis of compounds **5a** and **5b**.

δ 4.88–5.22 (C5H) and δ 8.09–8.11(C2H) ppm- can be attributed to the deshielding effect of the diamagnetic current of the aryl π-electrons.

The ^{1}H NMR spectra of compounds **5a** and **5b** revealed a singlet signal at δ 8.28 ppm which was assigned to the C2H. While, ^{1}H NMR spectra of **5c** and **5d**, the methyl protons (C2–CH_3) were found as a singlet signal at δ 2.55, 2.57 ppm. Also, the ^{1}H NMR spectra of **5e** and **5f** showed triplet and quartet signals corresponding to ester protons.

In the course of this study, attempts of synthesis of chromenopyrimidinotriazepines **A** via reacting compounds **4b** and **4d** with ethyl ethoxymethylene cyanoacetate were not successful. Instead, this reaction gave a product which was identical in all respects (m.p., mixed m.p., IR and ^{1}H NMR) with compounds **5a** and **5b** (Scheme 2).

The suggested mechanism for the reaction of **4b** and **4d** with ethyl ethoxymethylene cyanoacetate might be illustrated in Fig. 3.

Reacting compounds **4b** and **4d** with acetyl acetone for obtaining compounds **B** was unsuccessful and compounds **5c** and **5d** were resulted instead (Scheme 3).

The suggested mechanism for the reaction of **4b** and **4d** with acetyl acetone might be illustrated in Fig. 4.

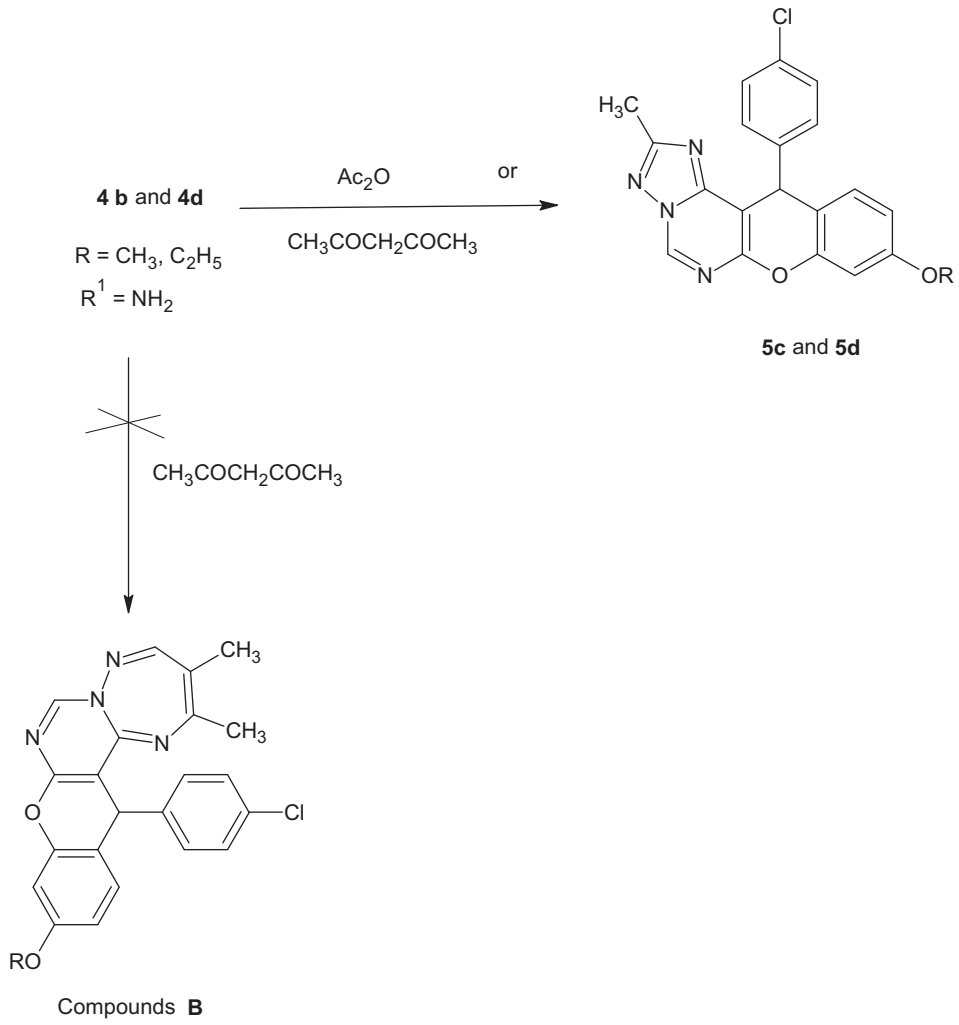
An attempt to prepare hydroxypyrimidotriazines **C** via heating compounds **4b** and **4d** with ethyl cyanoacetate in refluxing ethanol took place but unfortunately different products were obtained. These compounds were suggested to be the triazolopyrimidines **5g** and **5h** according to all available spectroscopic data and elemental analysis.

The IR spectra showed the presence of cyano group at 2251, 2259 cm^{-1}. Furthermore, ^{1}H NMR spectra revealed a singlet signal at δ 4.00 corresponding to two protons (CH_2CN) and no signals corresponding to OH either in IR or ^{1}H NMR spectra.

A suggested mechanism for the formation of **5g** and **5h** is shown (Fig. 5).

Preparation of compounds **D** was intended to be via the reaction of the key intermediates **4b** and **4d** with different chloro acid chlorides but interestingly, treating compounds **4b** and **4d** with equimolar amount of chloro acid chloride in dioxane containing catalytic amount of triethylamine did afford neither the starting materials nor compounds **D** but compounds **6a–d** were the products and this might be ascribed to the stability and aromaticity of the triazole ring system over the triazepine ring system (Scheme 5).

The structure of **6a–d** was suggested by different spectroscopic data. IR spectra displayed the absence of absorption bands corresponding to (C=O and NH) groups existing in the compounds **D**. ^{1}H NMR spectra of compounds **6a** and **6c** showed a singlet signal at δ 4.73 assigned to two protons of (CH_2Cl). Inspection of the ^{1}H NMR spectra of compounds **6b** and **6d** revealed the presence of pentet and two triplet signals at δ 2.22–2.38, 3.06–3.07 and 3.66 ppm due to (CH_2–CH_2–CH_2) protons respectively (Scheme 5). Compounds **7a–f** were prepared from the interaction of the key intermediates **4b** and **4d** with different arylidene malononitriles in the presence of piperidine under reflux in absolute ethanol. The structure of the given compounds was consistent with the IR, ^{1}H NMR and mass spectra. The absence of NH group as well as NH_2 group from the IR and ^{1}H NMR spectra of **7a–f** showed the increase in aromatic protons at δ 6.71–8.28 ppm confirmed their structures. Once more C12H and C5H of compounds **7a–f** showed increase in chemical shift at δ 5.72–5.76 (C12H) and δ 9.05–9.09 (C5H) ppm – compared to their values in tricyclic system **4b** and **4d** δ 4.88–5.22 (C5H) and δ 8.09–8.11 (C2H) ppm – and this can be attributed to the deshielding effect of the diamagnetic current of the aryl π-electrons.



Scheme 3. Synthesis of compounds **5c** and **5d**.

2.2. Antitumor activity

In this study, ten of the newly synthesized compounds were subjected to cytotoxic evaluation against human breast tumor cell line (MCF-7) using Colchicine as the reference drug. The response parameter calculated was IC_{50} value (Table 1), which corresponds to the compound concentration causing 50% mortality in net cells.

Compounds **4a**, **4b**, **5f**, **7c** and **7f** turned to be particularly promising for the development of new pharmacological inhibitors for breast tumor cells. The most promising results were obtained with compounds **4a** and **5f** (IC_{50} = 0.007 μM) that showed almost double the activity of colchicine (IC_{50} = 0.013 μM) (Fig. 6).

Three of the tested compounds **4b**, **7c** and **7f** (IC_{50} = 0.008 μM) (Fig. 7) showed nearly one and half the activity of colchicine.

Compounds **5a** and **5h** (IC_{50} = 0.011 μM) (Fig. 8) showed almost equal inhibitory effect on breast tumor cells as than the positive control (IC_{50} = 0.013 μM).

Moreover, compound **6d** (IC_{50} = 0.029 μM) (Fig. 9) revealed nearly half the activity of the positive control. Finally compounds **5c** and **5g** (IC_{50} = 0.033 μM, 0.039 μM) (Fig. 10) are the least reactive ones.

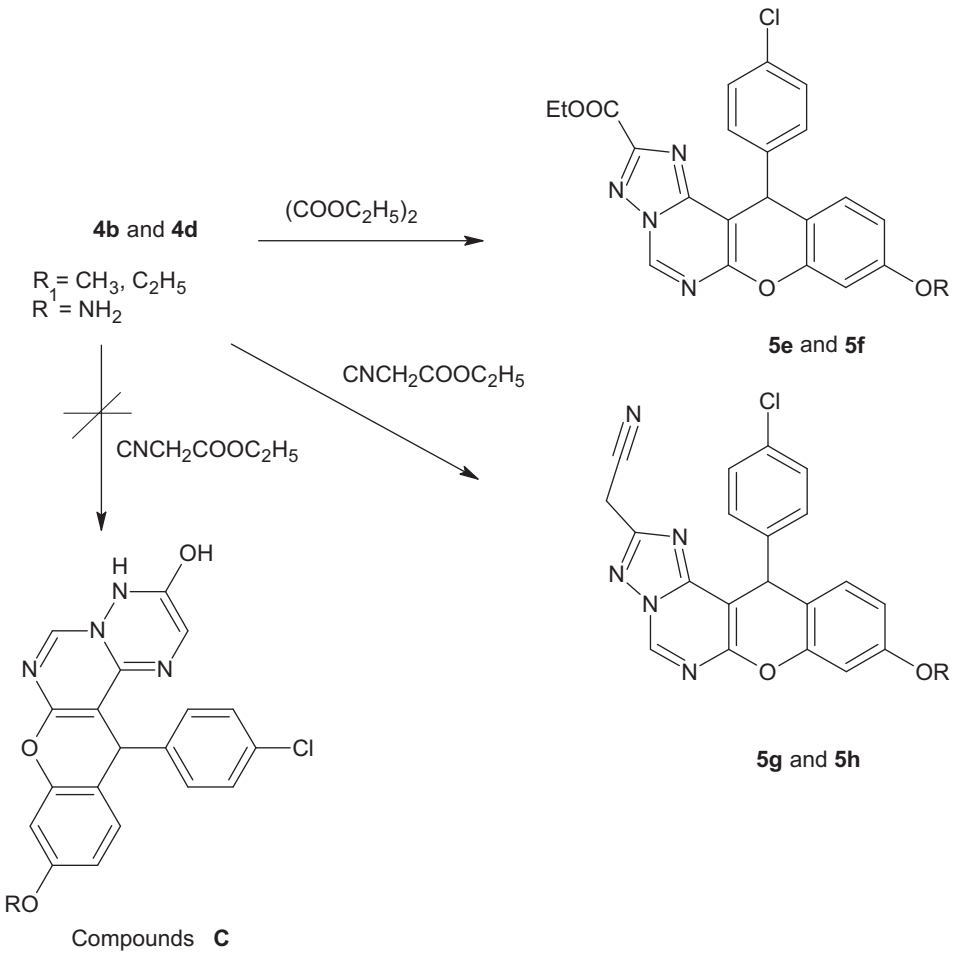
3. Conclusion

In summary, the objective of the present study was to design, synthesize a library of novel hybrid compounds comprising chromenes and different triazolopyrimidinyl moieties and investigate the anticancer activity of these new compounds. It is worth to mention, many of unexpected compounds were resulted during the reaction of the key intermediates **4b** and **4d** with different organic reagents and this may be attributed to the electronic nature of this aminocyanochromene ring system where the amino group is a very weak basic center and carbon number 2 is carrying a highly positive charge. The preliminary biological evaluations revealed that the newly synthesized chromene derivatives **4a**, **4b**, **5f**, **7c** and **7f** showed superior *in vitro* antitumor activity when compared to colchicine as a reference drug. We might conclude that, this superior *in vitro* anticancer activity and good potential of causing cancer cells death might be attributed to their structure similarity to colchicine (**I**) and podophyllotoxin (**II**) (Fig. 1).

4. Experimental protocols

4.1. Chemistry

Progress of the reactions was monitored using thin layer chromatography (TLC) sheets that precoated with UV fluorescent silica gel MERCK 60 F 254 that was visualized by UV lamp and I_2 vapor. Solvent system was chloroform: methanol (in different ratios). ^{1}H NMR and ^{13}C-NMR spectra were carried out on Varian Gemini 300 MHz Spectrometer, at the Microanalytical Center, Cairo University, Egypt. Using TMS as internal standard and chemical



Scheme 4. Synthesis of compounds **5e–h**.

shifts were recorded in ppm on δ scales. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm^{-1}. GC Mass spectra were run on Shimadzu QP-2010 spectrometer and Mass spectra were run on Hewlett Packard 5988 spectrometer at the Microanalytical Center, Cairo University, Egypt and National Research Center, Giza, Egypt. Melting points were determined on a Griffin instrument and are uncorrected. All products reported showed ^1H NMR spectra in agreement with the assigned structures. Elemental Analyses were performed by the Micro-analytical Laboratory, Cairo University, Egypt. Compound **1** was prepared according to reported method [23,24].

*4.1.1. General procedure for the preparation of compounds **2a** and **2b***

A mixture of compound **1** (2.98 g, 0.01 mol) and potassium hydroxide (0.56 g, 0.01 mol) in ethanol (20 mL) was stirred at room temperature for 1 h, then the appropriate methyl or ethyl iodide (0.015 mol) was added and the mixture was heated under reflux for 24 h. The reaction mixture was cooled, filtered and the formed precipitate was washed with ethanol and crystallized from absolute ethanol to yield **2a** and **2b**.

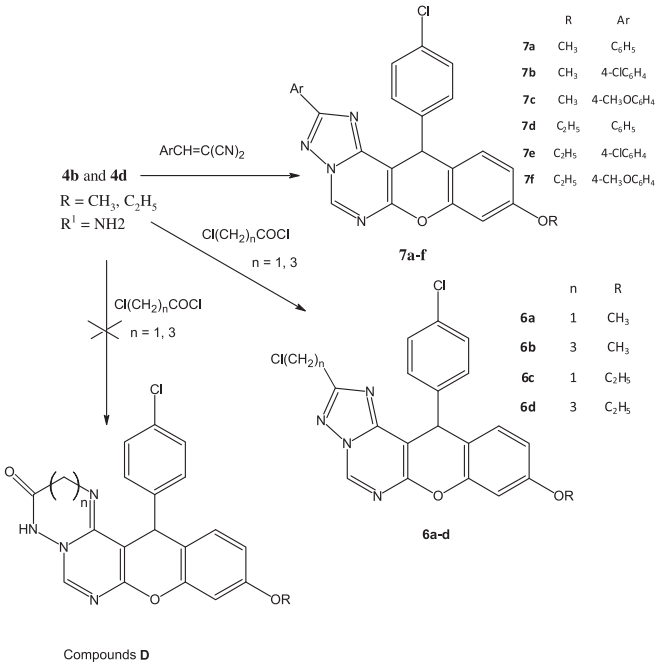
*4.1.1.1. (RS) 2-Amino-4-(4-chlorophenyl)-7-methoxy-4H-chromene-3-carbonitrile (**2a**).* Yield 84%. mp 167–168 °C. IR (KBr): 3432, 3347 (forked, NH_2), 3055 (CH arom.), 2960 (CH aliph.), 2185 (CN) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3); 4.72 (s, 1H, C4H); 6.55 (s, 1H, ArH); 6.66 (d, J = 8.7 Hz, 1H, ArH); 6.90 (d, J = 8.7 Hz, 1H, ArH); 6.98 (s, 2H, NH_2, D_2O exchangeable); 7.18 (d, J = 8.1 Hz, 2H, ArH); 7.35 (d, J = 8.1 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6): δ 39.9, 55.5, 76.5, 101.4, 111.7, 114.2, 119.5, 128.9, 129.2, 130.1, 133.0, 143.2, 149.1, 159.0, 159.5; EIMS: m/z (%) = 314 (M + 2$^{+\cdot}$, 2.8), 312 (M$^{+\cdot}$, 8.4), 201 (M–$C_6H_4Cl^{+\cdot}$, 100), 75 ($C_6H_3^{+\cdot}$, 24.3); Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$ (312.76): C 65.29, H 4.19, N 8.96; Found: C 65.50, H 4.31, N 9.16%.

*4.1.1.2. (RS)-2-Amino-4-(4-chlorophenyl)-7-ethoxy-4H-chromene-3-carbonitrile (**2b**).* Yield 85%. mp 181–182 °C. IR (KBr): 3424, 3332 (forked, NH_2), 3070 (CH arom.), 2976 (CH aliph.), 2196(CN) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.29 (t, 3H, CH_3); 3.98 (q, 2H, CH_2); 4.72 (s, 1H, C4H); 6.54 (s, 1H, ArH); 6.65 (d, 1H, ArH + 2H, NH_2, D_2O exchangeable); 6.90 (d, 1H, ArH); 7.18 (d, J = 8.4 Hz, 2H, ArH); 7.36 (d, J = 8.4 Hz, 2H, ArH) ppm; Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$ (326.79): C 66.16, H 4.63, N 8.57; Found: C 66.42, H 4.42, N 8.43%.

*4.1.2. General procedure for the preparation of compounds (**3a** and **3b**)*

A mixture of compound **2a** and **2b** (0.01 mol) and triethyl orthoformate (20 mL) was heated under reflux for 4 h. The excess of orthoformate was removed under reduced pressure, and then the residue was washed with ethanol and crystallized from the appropriate solvent to give **3a** and **3b**.

*4.1.2.1. Ethyl (RS) (ZE) N-4-(4-chlorophenyl)-3-cyano-7-methoxy-4H-chromen-2-ylformimidate (**3a**).* Crystallized from absolute



Scheme 5. Synthesis of compounds **6a**–**d** and **7a**–**f**.

ethanol. Yield 88%. mp 131–132 °C. IR (KBr): 3068 (CH arom.), 2965 (CH aliph.), 2200 (CN), 1644 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.39 (t, 3H, CH_2CH_3); 3.80 (s, 3H, OCH_3); 4.43 (q, 2H, CH_2); 4.80 (s, 1H, C4H); 6.60 (s, 1H, ArH); 6.65 (d, J = 8.7 Hz, 1H, ArH); 6.84 (d, J = 8.7 Hz, 1H, ArH); 7.14 (d, J = 8.7 Hz, 2H, ArH); 7.29 (d, J = 8.7 Hz, 2H, ArH); 8.38 (s, 1H, N=CH) ppm; Anal. Calcd. for $C_{20}H_{17}ClN_2O_3$ (368.82): C 65.13, H 4.65, N 7.60; Found: C 65.28, H 4.66, N 7.80%.

*4.1.2.2. (ZE) (RS)-Ethyl N-4-(4-chlorophenyl)-3-cyano-7-ethoxy-4H-chromen-2-ylformimidate (**3b**).* Crystallized from methanol. Yield 61%. mp 135–136 °C. IR (KBr): 3067 (CH arom.), 2981 (CH aliph.), 2208 (CN), 1619 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.28–1.36 (m, 6H, $2CH_2CH_3$); 3.94 (q, 2H, OCH_2CH_3); 4.35 (q, 2H, N=CHOCH_2CH_3); 4.71 (s, 1H, C4H); 6.49 (s, 1H, ArH); 6.54 (d, J = 8.4 Hz, 1H, ArH); 6.74 (d, J = 8.4 Hz, 1H, ArH); 7.06 (d, J = 8.4 Hz, 2H, ArH); 7.20 (d, J = 8.4 Hz, 2H, ArH); 8.30 (s, 1H, N=CH) ppm; ^{13}C NMR ($CDCl_3$): δ 14.3, 14.4, 32.2, 62.9, 65.2, 62.8, 95.0, 103.3, 108.9, 123.3, 129.4, 129.7, 129.8, 131.5, 141.3, 156.5, 157.0, 157.3, 163.6; EIMS: m/z (%) = 384 (M + 2$^{+\cdot}$, 7.31), 382 (M$^{+\cdot}$, 20.40), 271 (M–$C_6H_4Cl^{+\cdot}$, 100); Anal. Calcd. for $C_{21}H_{19}ClN_2O_3$ (382.85): C 65.88, H 5.00, N 7.32; Found: C 66.09, H 4.80, N 7.12%.

*4.1.3. General procedure for the preparation of compounds **4a**–**d***

A solution of **3a** and **3b** (0.01 mol) and either methylamine or hydrazine hydrate (99%, 0.01 mol) in absolute ethanol (30 mL) was stirred at room temperature for 1 h. The solid formed was filtered and crystallized from benzene to give **4a**–**d**.

*4.1.3.1. (ZE) (RS)-5-(4-Chlorophenyl) 8-methoxy-3-methyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-imine (**4a**).* Yield 95%. mp 128–129 °C. IR (KBr): 3221 (NH), 2966 (CH aliph.), 1643 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.40 (s, 3H, N–CH_3); 3.77 (s, 3H, OCH_3); 4.86 (s, 1H, C5H); 6.59–7.28 (m, 7H, ArH + 1H, NH, D_2O exchangeable); 7.79 (s, 1H, C2H) ppm; EIMS: m/z (%) = 355 (M + 2$^{+\cdot}$, 10.41), 353 (M$^{+\cdot}$, 28.27), 338 (M–$CH_3^{+\cdot}$, 29.34), 105 (M–$C_{12}H_{11}ClN_3O^{+\cdot}$, 100); Anal. Calcd. for $C_{19}H_{16}ClN_3O_2$ (353.81): C 64.50, H 4.56, N 11.88; Found: C 64.63, H 4.49, N 11.58%.

*4.1.3.2. (ZE) (RS)-5-(4-Chlorophenyl)-4,5-dihydro-4-imino -8-methoxy-3H-chromeno[2,3-d]pyrimidin-3-amine (**4b**).* Yield 93%. mp 187–189 °C. IR (KBr): 3336, 3156 (NH, NH_2), 2928 (CH aliph.), 1639 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.78 (s, 3H, OCH_3); 4.74 (br s, 2H, NH_2, D_2O exchangeable); 4.88 (s, 1H, C5H); 6.60–7.36 (m, 7H, ArH + 1H, NH, D_2O exchangeable); 8.09 (s, 1H, C2H) ppm Anal. Calcd. for $C_{18}H_{15}ClN_4O_2$ (354.80): C 60.94, H 4.26, N 15.79; Found: C 60.76, H 4.20, N 15.49%.

*4.1.3.3. (RS) (ZE) -5-(4-Chlorophenyl)-8-ethoxy-3-methyl-3H-chromeno[2,3-d]pyrimidin-4(5H)-imine (**4c**).* Yield 84%. mp 169–170 °C. IR (KBr): 3324 (NH), 3028 (CH arom.), 2976 (CH aliph.), 1640 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.40 (t, J = 7.2 Hz, 3H, CH_2CH_3); 3.59 (s, 3H, N–CH_3); 3.99 (q, J = 7.2 Hz, 2H, CH_2CH_3); 5.64 (s, 1H, C5H); 6.32–7.37 (m, 7H, ArH); 7.87 (s, 1H, NH, D_2O exchangeable); 8.17 (s, 1H, C2H) ppm; Anal. Calcd. for $C_{20}H_{18}ClN_3O_2$ (367.84): C 65.31, H 4.93, N 11.42; Found: C 65.54, H 4.81, N 11.12%.

*4.1.3.4. (RS) (ZE) -5-(4-Chlorophenyl)-4-imino-4,5-dihydro-8-ethoxy-3H-chromeno[2,3-d]pyrimidin-3-amine (**4d**).* Yield 89%. mp 168–169 °C. IR (KBr): 3333, 3180 (NH, NH_2), 3087 (CH arom.), 2981 (CH aliph.), 1629 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.29 (t, J = 7.2 Hz, 3H, CH_2CH_3); 4.00 (q, J = 7.2 Hz, 2H, CH_2CH_3); 5.22 (s, 1H, C5H); 6.65–7.36 (m, 7H, ArH + 3H, NH, NH_2, D_2O exchangeable); 8.11 (s, 1H, C2H) ppm;. ^{13}C NMR ($CDCl_3$): δ 14.3, 25.7, 65.1, 95.0, 103.2, 108.9, 123.1, 129.4, 129.7, 129.8, 131.8, 141.3, 157.0, 157.2, 163.0, 164.0; EIMS: m/z (%) = 370 (M + 2$^{+\cdot}$, 11.63), 368 (M$^{+\cdot}$, 33.67), 352 (M–$NH_2^{+\cdot}$, 100); Anal. Calcd. for $C_{19}H_{17}ClN_4O_2$ (368.83): C 61.88, H 4.65, N 15.19; Found: C 61.90, H 4.70, N 14.91%.

*4.1.4. General procedure for the preparation of compounds **5a** and **5b***

A solution of **4b** and **4d** (0.001 mol) and triethyl orthoformate (10 mL) was heated under reflux for 6 h. The precipitated solid formed after cooling were filtered, washed with ethanol, dried and recrystallized from absolute ethanol to afford compounds **5a** and **5b**.

*4.1.4.1. (RS)-12-(4-Chlorophenyl)-9-methoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**5a**).* Yield 86%. mp 215–216 °C. IR (KBr): 3073 (CH arom.), 2932 (CH aliph.), 1626 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.84 (s, 3H, OCH_3); 5.67 (s, 1H, C12H); 6.73 (d, J = 8.4 Hz, 1H, ArH); 6.85 (s, 1H, ArH); 7.06 (d, J = 8.4 Hz, 1H, ArH); 7.23 (d, J = 8.7 Hz, 2H, ArH); 7.27 (d, J = 8.7 Hz, 2H, ArH); 8.28 (s, 1H, C2H); 9.14 (s, 1H, C5H) ppm; Anal. Calcd. for $C_{19}H_{13}ClN_4O_2$ (364.79): C 62.56, H 3.59, N 15.36; Found: C 62.70, H 3.40, N 15.56%.

*4.1.4.2. (RS)-12-(4-Chlorophenyl)-9-ethoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**5b**).* Yield 81%. mp 177–178 °C. IR (KBr): 3077 (CH arom.), 2979 (CH aliph.), 1626 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.44 (t, J = 7.2 Hz, 3H, CH_2CH_3); 4.06 (q, J = 7.2 Hz, 2H, CH_2CH_3); 5.67 (s, 1H, C12H); 6.72 (d, J = 8.4 Hz, 1H, ArH); 6.84 (s, 1H, ArH); 7.05 (d, J = 8.4 Hz, 1H, ArH); 7.24 (d, J = 8.1 Hz, 2H, ArH); 7.29 (d, J = 8.1 Hz, 2H, ArH); 8.28 (s, 1H, C2H); 9.14 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$): δ 29.8, 56.0, 107.2, 111.6, 118.5, 127.6, 129.1, 129.6, 129.9, 130.8, 132.0, 144.1, 147.2, 147.9, 155.5, 160.8; EIMS: m/z (%) = 380 (M + 2$^{+\cdot}$, 8.27), 378 (M$^{+\cdot}$, 22.04), 267 (M–$C_6H_4Cl^{+\cdot}$, 100); Anal. Calcd. for $C_{20}H_{15}ClN_4O_2$ (378.82): C 63.41, H 3.99, N 14.79; Found: C 63.30, H 3.80, N 14.49%.

*4.1.5. General procedure for the preparation of compounds **5c** and **5d***

A mixture of **4b** and **4d** (0.001 mol) and acetic anhydride (10 mL) was heated under reflux for 6 h. The precipitated solid formed after cooling were filtered, washed with ethanol, dried and recrystallized to afford compounds **5c** and **5d**.

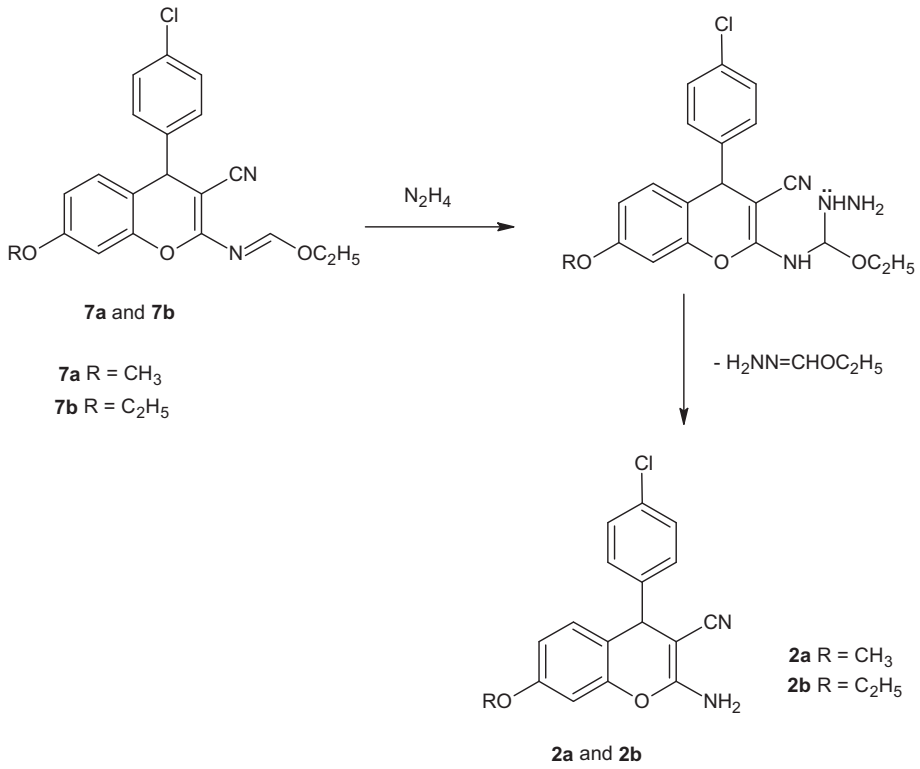


Fig. 2. The suggested mechanism for the reaction of compounds **3a** and **3b** with excess hydrazine hydrate.

*4.1.5.1. (RS)-12-(4-Chlorophenyl)-2-methyl-9-methoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**5c**).* Crystallized from methanol. Yield 85%. mp 231–232 °C. IR (KBr): 3061 (CH arom.), 2960 (CH aliph.), 1628 (C═N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.57 (s, 3H, C2CH_3); 3.84 (s, 3H, OCH_3); 5.65 (s, 1H, C12H); 6.73 (d, J = 8.7 Hz, 1H, ArH); 6.85 (s, 1H, ArH); 7.07 (d, J = 8.7 Hz, 1H, ArH); 7.24 (d, J = 8.4 Hz, 2H, ArH); 7.31 (d, J = 8.4 Hz, 2H, ArH); 9.01 (s, 1H, C5H) ppm; EIMS: m/z (%) = 380 (M + 2$^{+\cdot}$, 7.06), 378 (M$^{+\cdot}$, 19.72), 267 (M–$C_6H_4Cl^{+}$, 100); Anal. Calcd. for $C_{20}H_{15}ClN_4O_2$ (378.82): C 63.41, H 3.99, N 14.79; Found: C 63.50, H 4.10, N 14.70%.

*4.1.5.2. (RS)-12-(4-Chlorophenyl)-9-ethoxy-2-methyl-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**5d**).* Crystallized from methanol: chloroform (1:1). Yield 89%. mp 214–215 °C. IR (KBr): 3060 (CH arom.), 2981 (CH aliph.), 1626 (C═N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.43 (t, J = 7.2 Hz, 3H, CH_2CH_3); 2.55 (s, 3H, C2CH_3); 4.05 (q,

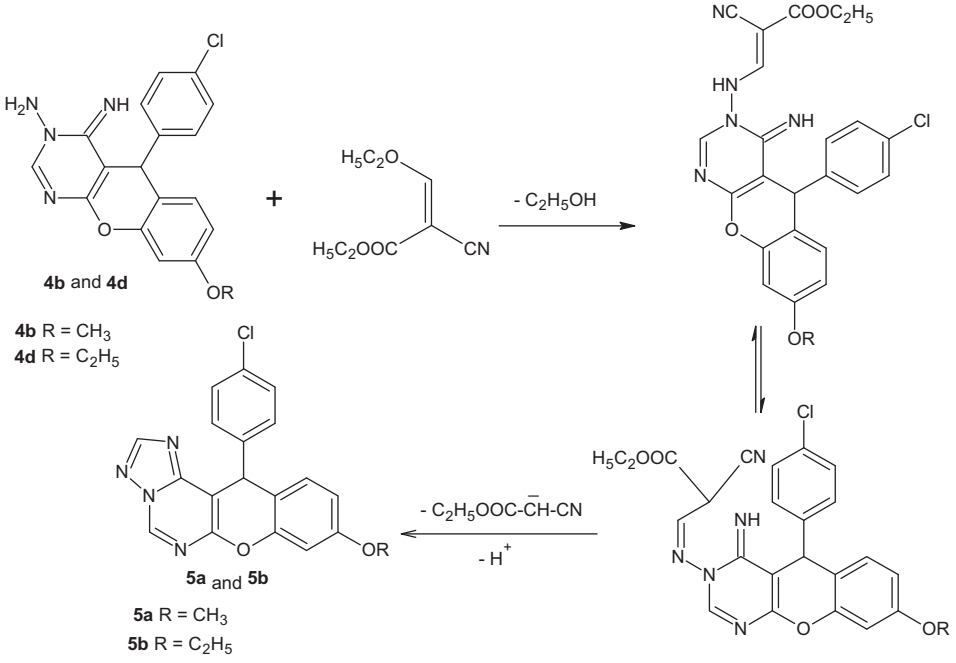


Fig. 3. The suggested mechanism for the reaction of **4b** and **4d** with ethyl ethoxymethylene cyanoacetate.

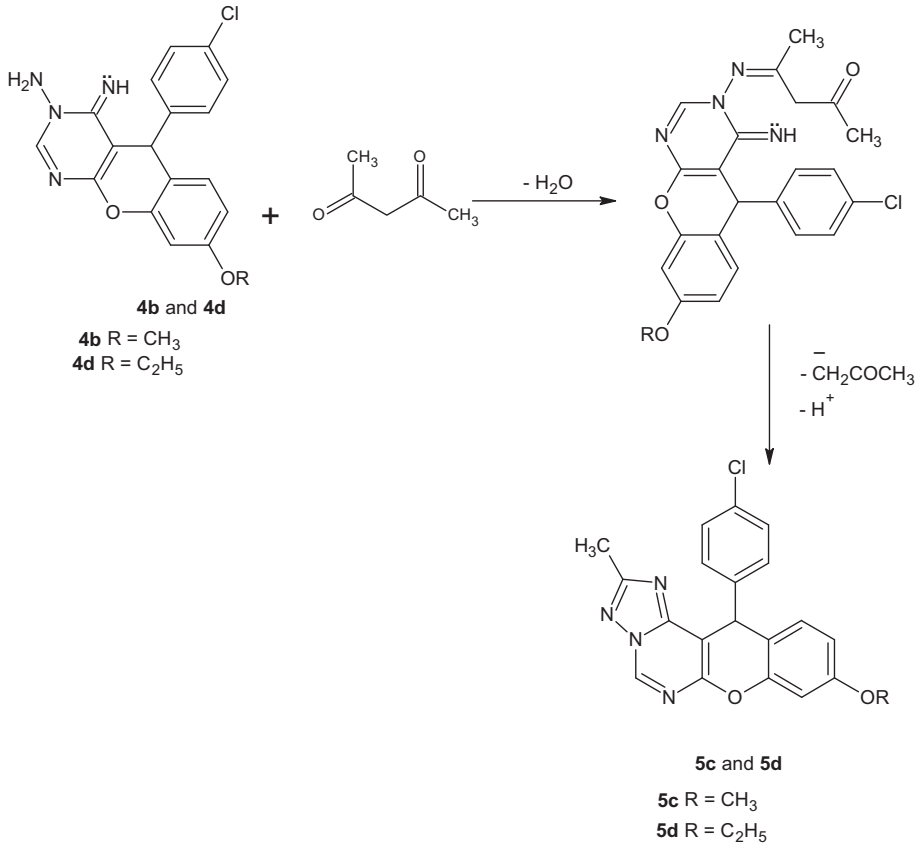


Fig. 4. The suggested mechanism for the reaction of **4b** and **4d** with acetyl acetone.

$J = 7.2$ Hz, 2H, CH_2–CH_3); 5.63 (s, 1H, C12H); 6.71 (d, $J = 8.4$ Hz, 1H, ArH); 6.83 (s, 1H, ArH); 7.05 (d, $J = 8.4$ Hz, 1H, ArH); 7.21 (d, $J = 8.4$ Hz, 2H, ArH); 7.29 (d, $J = 8.4$ Hz, 2H, ArH); 9.00 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$): δ 14.2, 29.8, 65.3, 107.3, 110.6, 118.2, 127.2, 129.4, 129.8, 130.0, 131.3, 142.1, 147.8, 153.5, 154.8, 157.3, 160.4, 171.6; EIMS: m/z (%) = 394 (M + 2$^{+\cdot}$, 8.34), 392 (M$^{+\cdot}$, 22.62), 281 (M–$C_6H_4Cl^{+}$, 100); Anal. Calcd. for $C_{21}H_{17}ClN_4O_2$ (392.85): C 64.21, H 4.36, N 14.26; Found: C 64.10, H 4.50, N 13.96%.

*4.1.6. General procedure for the preparation of compounds **5e–h***

A mixture of **4b** and **4d** (0.001 mol) and either diethyl oxalate or ethyl cyanoacetate (0.001 mol) in absolute ethanol (15 mL) was

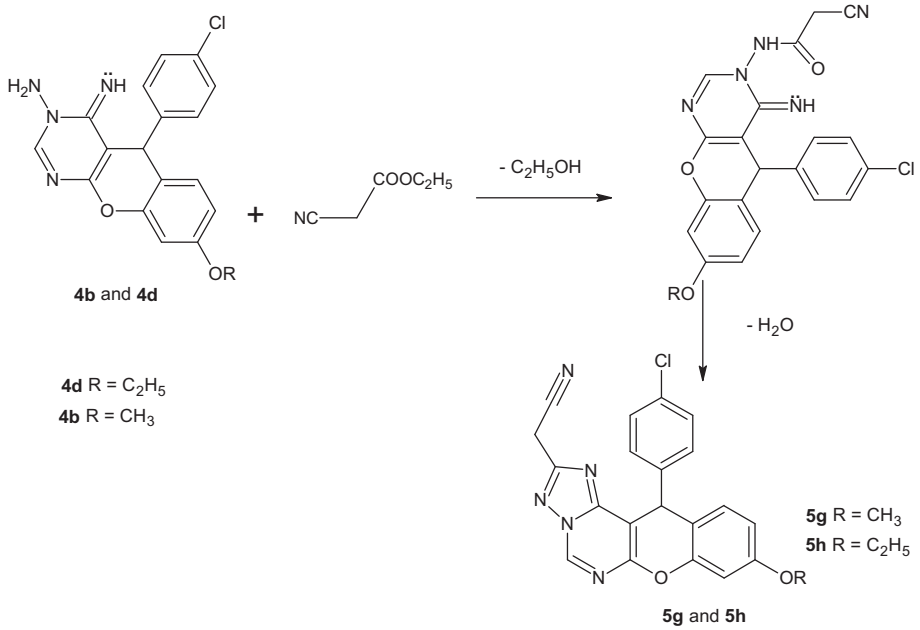


Fig. 5. The suggested mechanism for the reaction of **4b** and **4d** with ethyl cyanoacetate.

Table 1
Results of *in vitro* cytotoxic activity of reference drug and the tested compounds on human breast tumor cell line (MCF-7).

Cpd no	IC_{50} μM
Colchicine	0.013
4a	0.007
4b	0.008
5a	0.011
5c	0.033
5f	0.007
5g	0.039
5h	0.011
6d	0.029
7c	0.008
7f	0.008

IC_{50}: concentration of a drug that is required for 50% inhibition.

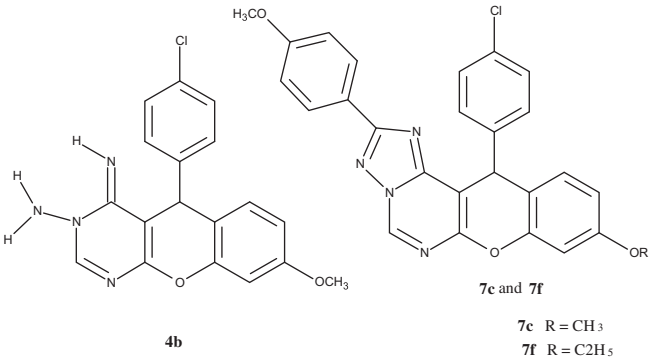


Fig. 7. Structures of compounds **4b**, **7c** and **7f**.

heated under reflux for 4 h. After cooling, the solid formed was collected and crystallized from the appropriate solvent to afford compounds **5e**–**h**.

*4.1.6.1. (RS)-Ethyl 12-(4-chlorophenyl)-9-methoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine-2-carboxylate (**5e**).* Crystallized from a mixture of methanol: benzene (3:1). Yield 75%. mp 229–230 °C. IR (KBr): 3067 (CH arom.), 2956 (CH aliph.), 1741 (C=O), 1626 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.32 (t, J = 7.2 Hz, 3H, CH_2CH_3); 3.78 (s, 3H, OCH_3); 4.38 (q, J = 7.2 Hz, 2H, CH_2CH_3); 5.81 (s, 1H, C12H); 6.77–7.34 (m, 7H, ArH); 9.75 (s, 1H, C5H) ppm; EIMS: m/z (%) = 438 (M + 2┐$^+$, 11.99), 436 (M┐$^+$, 31.30), 325 (M–C_6H_4Cl┐$^+$, 100); Anal. Calcd. for $C_{22}H_{17}ClN_4O_4$ (436.85): C 60.49, H 3.92, N 12.83; Found: C 60.69, H 4.10, N 12.86%.

*4.1.6.2. (RS)-Ethyl 12-(4-chlorophenyl)-9-ethoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine-2-carboxylate (**5f**).*
Crystallized from a mixture of methanol: benzene (3:1). Yield 60%. mp 279–280 °C. IR (KBr): 3063 (CH arom.), 2983 (CH aliph.), 1745 (C=O), 1627 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.42–1.50 (m, 6H, 2 CH_3); 4.06 (q, J = 7.2 Hz, 2H, OCH_2–CH_3); 4.55 (q, J = 7.2 Hz, 2H, $COOCH_2CH_3$); 5.78 (s, 1H, C12H); 6.73–7.36 (m, 7H, ArH); 9.18 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$): δ 13.6, 14.3, 29.8, 59.1, 56.1, 107.3, 110.6, 118.7, 127.2, 129.4, 129.8, 130.0, 131.3, 141.1, 150.5, 151.9, 153.5, 154.8, 157.3, 167.0, 171.6; Anal. Calcd. for $C_{23}H_{19}ClN_4O_4$ (450.87): C 61.27, H 4.25, N 12.43; Found: C 61.13, H 4.28, N 12.61%.

*4.1.6.3. (RS)-2-(12-(4-Chlorophenyl)-9-methoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl)acetonitrile (**5g**).* Crystallized from benzene. Yield 80%. mp 207–208 °C. IR (KBr): 3072 (CH arom.), 2966 (CH aliph.), 2259 (CN), 1630 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.85 (s, 3H, OCH_3); 4.00 (s, 2H, CH_2CN); 5.64 (s, 1H, C12H); 6.75 (d, J = 8.7 Hz, 1H, ArH); 6.86 (s, 1H, ArH); 7.06 (d, J = 8.7 Hz, 1H, ArH); 7.21 (d, J = 8.4 Hz, 2H, ArH); 7.26 (d, J = 8.4 Hz, 2H, ArH); 9.08 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$): δ 18.4, 29.5, 56.0, 107.4, 110.5, 114.9, 118.2, 127.9, 129.0, 129.4, 129.8, 130.4, 131.3, 141.1, 147.8, 153.5, 155.2, 160.5, 171.6; EIMS: m/z (%) = 405 (M + 2┐$^+$, 9.51), 403 (M┐$^+$, 25.87), 292 (M–C_6H_4Cl┐$^+$, 100); Anal. Calcd. for $C_{21}H_{14}ClN_5O_2$ (403.82): C 62.46, H 3.49, N 17.34; Found: C 62.51, H 3.59, N 17.06%.

*4.1.6.4. (RS)-2-(12-(4-Chlorophenyl)-9-ethoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl)acetonitrile (**5h**).*
Crystallized from benzene Yield 78%. mp 218–219 °C. IR (KBr): 3060 (CH arom.), 2982 (CH aliph.), 2251 (CN), 1628 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.44 (t, J = 7.2 Hz, 3H, CH_2CH_3); 4.00 (s, 2H, CH_2CN); 4.08 (q, J = 7.2 Hz, 2H, CH_2–CH_3); 5.63 (s, 1H, C12H); 6.73 (d, J = 8.4 Hz, 1H, ArH); 6.84 (s, 1H, ArH); 7.05 (d, J = 8.4 Hz, 1H, ArH); 7.21 (d, J = 8.7 Hz, 2H, ArH); 7.29 (d, J = 8.7 Hz, 2H, ArH); 9.08 (s, 1H, C5H) ppm; Anal. Calcd. for $C_{22}H_{16}ClN_5O_2$ (417.85): C 63.24, H 3.86, N 16.76; Found: C 63.23, H 4.09, N 16.53%.

*4.1.7. General procedure for the preparation of compounds **6a**–**d***

To a solution of **4b** and **4d** (0.004 mol) in dioxane (15 mL) was added the appropriate acid chloride (0.004 mol), followed by the addition of triethylamine (0.40 g, 0.004 mol). The reaction mixture was stirred at room temperature for 24 h then poured into ice-cold water. The solid product that formed was filtered, washed with water, dried and crystallized from a mixture of ethanol: chloroform (3:1) to give **6a**–**d**.

*4.1.7.1. (RS)-2-(Chloromethyl)-12-(4-chlorophenyl)-9-methoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**6a**).* Yield 80%. mp 200–201 °C. IR (KBr): 3056 (CH arom.), 2959 (CH aliph.), 1628 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.85 (s, 3H, OCH_3); 4.73 (s, 2H, CH_2); 5.68 (s, 1H, C12H); 6.75 (d, J = 8.7 Hz, 1H, ArH); 6.86 (s, 1H, ArH); 7.07 (d, J = 8.7 Hz, 1H, ArH); 7.23 (d, J = 8.4 Hz, 2H, ArH); 7.27 (d,

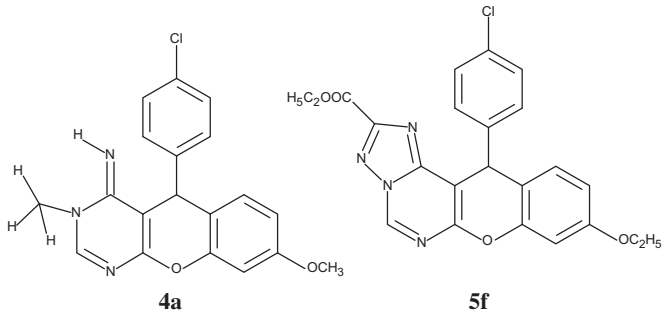


Fig. 6. Structures of compounds **4a** and **5f**.

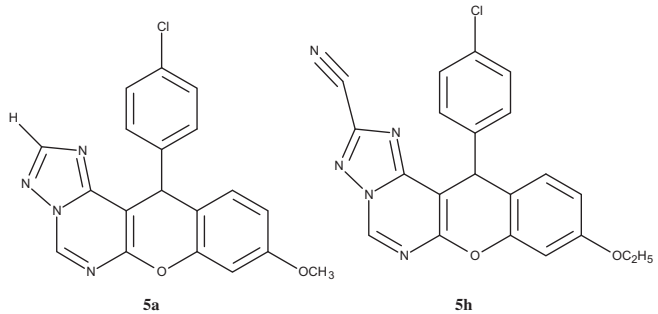
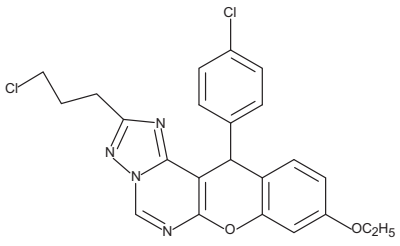


Fig. 8. Structure of compound **5a** and **5h**.



6d

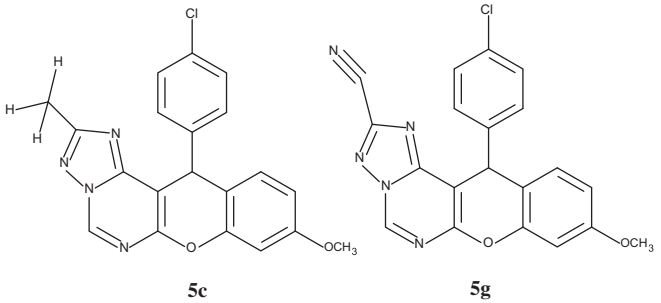
Fig. 9. Structure of compound **6d**.

J = 8.4 Hz, 2H, ArH); 9.07 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$): δ 29.8, 45.1, 56.2, 107.2, 110.6, 118.5, 127.7, 129.4, 129.6, 129.8, 130.9, 132.0, 141.1, 147.8, 153.5, 155.4, 160.4, 171.6; Anal. Calcd. for $C_{20}H_{14}Cl_2N_4O_2$ (413.26): C 58.13, H 3.41, N 13.56; Found: C 58.05, H 3.71, N 13.91%.

*4.1.7.2. (RS)-12-(4-Chlorophenyl)-2-(chloropropyl)-9-methoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**6b**).* Yield 81%. mp 175–176 °C. IR (KBr): 3057 (CH arom.), 2951 (CH aliph.), 1628 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.22 (pentet, J = 7.5 Hz, 2H, CH_2); 3.07 (t, J = 7.5 Hz, 2H, CH_2); 3.66 (t, J = 7.5 Hz, 2H, CH_2); 3.84 (s, 3H, OCH_3); 5.67 (s, 1H, C12H); 6.75 (d, J = 8.4 Hz, 1H, ArH); 6.85 (s, 1H, ArH); 7.07 (d, J = 8.4 Hz, 1H, ArH); 7.19 (d, J = 8.7 Hz, 2H, ArH); 7.29 (d, J = 8.7 Hz, 2H, ArH); 9.01 (s, 1H, C5H) ppm; Anal. Calcd. for $C_{22}H_{18}Cl_2N_4O_2$ (441.31): C 59.88, H 4.11, N 12.70; Found: C 60.09, H 4.25, N 12.50%.

*4.1.7.3. (RS)-2-(Chloromethyl)-12-(4-chlorophenyl)-9-ethoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**6c**).* Yield 87%. mp 197–198 °C. IR (KBr): 3059 (CH arom.), 2980 (CH aliph.), 1628 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.44 (t, J = 7.2 Hz, 3H, CH_2CH_3); 4.06 (q, J = 7.2 Hz, 2H, CH_2–CH_3); 4.73 (s, 2H, CH_2Cl); 5.67 (s, 1H, C12H); 6.73 (d, J = 8.4 Hz, 1H, ArH); 6.84 (s, 1H, ArH); 7.05 (d, J = 8.4 Hz, 1H, ArH); 7.20 (d, J = 8.7 Hz, 2H, ArH); 7.29 (d, J = 8.7 Hz, 2H, ArH); 9.06 (s, 1H, C5H) ppm; EIMS: m/z (%) = 431 (M + 4$^{+\cdot}$, 9.96), 429 (M + 2$^{+\cdot}$, 25.76), 427 (M$^{+\cdot}$, 39.64), 315 (M–$C_6H_4Cl^{+}$, 100); Anal. Calcd. for $C_{21}H_{16}Cl_2N_4O_2$ (427.28): C 59.03, H 3.77, N 13.11; Found: C 59.09, H 3.89, N 13.02%.

*4.1.7.4. (RS)-12-(4-Chlorophenyl)-2-(chloropropyl)-9-ethoxy-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**6d**).* Yield 88%. mp 171–172 °C. IR (KBr): 3071 (CH arom.), 2974 (CH aliph.), 1628 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.44 (t, J = 7.2 Hz, 3H, CH_2CH_3); 2.25 (pentet, J = 7.2 Hz, 2H, CH_2); 3.06 (t, J = 7.2 Hz, 2H, CH_2); 3.66 (t, J = 7.2 Hz, 2H, CH_2); 4.06 (q, J = 7.2 Hz, 2H, CH_2–CH_3); 5.65 (s, 1H, C12H); 6.72 (d, J = 8.4 Hz, 1H, ArH); 6.84 (s, 1H, ArH); 7.05 (d, J = 8.4 Hz, 1H, ArH); 7.22 (d, J = 8.7 Hz, 2H, ArH); 7.30 (d, J = 8.7 Hz, 2H, ArH); 9.01 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$): δ 14.3, 29.6, 56.1, 107.1, 111.6, 118.2, 127.0, 127.5, 128.4, 129.0, 129.4129.8, 130.0, 131.3, 136.9, 141.1, 148.3, 153.9, 154.8, 157.7, 171.6; Anal. Calcd. for $C_{23}H_{20}Cl_2N_4O_2$(455.34): C 60.67, H 4.43, N 12.30; Found: C 60.51, H 4.51, N 12.51%.



5c **5g**

Fig. 10. Structure of compounds **5c** and **5g**.

*4.1.8. General procedure for the preparation of compounds **7a–f***

To a mixture of **4b** and **4d** (0.004 mol) and the respective arylidene malononitrile (0.004 mol) in absolute ethanol (25 mL), piperidine (0.2 mL) was added. The reaction mixture was heated under reflux for 2 h. The precipitate that formed was collected by filtration while hot and crystallized from a mixture of methanol: chloroform (3:1) to afford **7a–f**.

*4.1.8.1. (RS)-12-(4-Chlorophenyl)-9-methoxy-2-phenyl-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**7a**).* Yield 75%. mp 264–265 °C. IR (KBr): 3058 (CH arom.), 2964 (CH aliph.), 1627 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H, OCH_3); 5.75 (s, 1H, C12H); 6.73–8.26 (m, 12H, ArH); 9.09 (s, 1H, C5H) ppm; Anal. Calcd. for $C_{25}H_{17}ClN_4O_2$ (440.88): C 68.11, H 3.89, N 12.71; Found: C 68.07, H 3.88, N 13.04%.

*4.1.8.2. (RS)-2,12-bis-(4-Chlorophenyl)-9-methoxy-12H-chromeno [3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**7b**).* Yield 82%. mp 280–281 °C. IR (KBr): 3078 (CH arom.), 2970 (CH aliph.), 1625 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.85 (s, 3H, OCH_3); 5.72 (s, 1H, C12H); 6.74 (d, J = 8.7 Hz, 1H, ArH); 6.86 (s, 1H, ArH); 7.08 (d, J = 8.7 Hz, 1H, ArH); 7.21 (d, J = 8.4 Hz, 2H, ArH); 7.36 (d, J = 8.4 Hz, 2H, ArH); 7.48 (d, J = 8.4 Hz, 2H, ArH); 8.20 (d, J = 8.4 Hz, 2H, ArH); 9.08 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$) δ: 29.8, 56.0, 107.3, 110.5, 118.2, 127.9, 128.4, 129.4, 129.8, 130.4, 131.2, 133.8, 134.7, 141.1, 148.0, 153.5, 155.2, 160.5, 171.6; Anal. Calcd. for $C_{25}H_{16}Cl_2N_4O_2$ (475.33): C 63.17, H 3.39, N 11.79; Found: C 63.44, H 3.59, N 11.94%.

*4.1.8.3. (RS)-12-(4-Chlorophenyl)-9-methoxy-2-(4-methoxyphenyl)-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**7c**).* Yield 69%. mp 256–257 °C. IR (KBr): 3060 (CH arom.), 2933 (CH aliph.), 1619 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3); 5.77 (s, 1H, C12H); 6.76 (d, J = 9 Hz, 1H, ArH); 6.87 (s, 1H, ArH); 7.02 (d, J = 9 Hz, 1H, ArH); 7.07 (d, J = 8.4 Hz, 2H, ArH); 7.21 (d, J = 8.4 Hz, 2H, ArH); 7.37 (d, J = 8.7 Hz, 2H, ArH); 8.21 (d, J = 8.7 Hz, 2H, ArH); 9.06 (s, 1H, C5H) ppm; Anal. Calcd. for $C_{26}H_{19}ClN_4O_3$ (470.91): C 66.31, H 4.07, N 11.90; Found: C 66.20, H 4.20, N 12.20%.

*4.1.8.4. (RS)-12-(4-Chlorophenyl)-9-ethoxy-2-phenyl-12H-chromeno [3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**7d**).* Yield 87%. mp 219–220 °C. IR (KBr): 3058 (CH arom.), 2982 (CH aliph.), 1625 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.45 (t, J = 7.2 Hz, 3H, CH_2CH_3); 4.06 (q, J = 7.2 Hz, 2H, CH_2–CH_3); 5.74 (s, 1H, C12H); 6.71–8.28 (m, 12H, ArH); 9.08 (s, 1H, C5H) ppm; ^{13}C NMR ($CDCl_3$) δ: 14.3, 29.8, 56.0, 107.3, 110.8, 118.2, 127.0, 127.9, 128.4, 129.4, 129.8, 130.4, 131.2, 136.7, 141.1, 153.5, 154.8, 155.2, 157.3, 160.6, 171.6; Anal. Calcd. for $C_{26}H_{19}ClN_4O_2$ (454.91): C 68.65, H 4.21, N 12.32; Found: C 68.61, H 4.30, N 12.60%.

*4.1.8.5. (RS)-2,12-bis-(4-Chlorophenyl)-9-ethoxy-12H-chromeno [3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**7e**).* Yield 88%. mp 264–265 °C. IR (KBr): 3067 (CH arom.), 2983 (CH aliph.), 1627 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.45 (t, J = 7.2 Hz, 3H, CH_2CH_3); 4.06 (q, J = 7.2 Hz, 2H, CH_2–CH_3); 5.76 (s, 1H, C12H); 6.74 (d, J = 8.4 Hz, 1H, ArH); 6.85 (s, 1H, ArH); 7.08 (d, J = 8.4 Hz, 1H, ArH); 7.22 (d, J = 8.4 Hz, 2H, ArH); 7.35 (d, J = 8.4 Hz, 2H, ArH); 7.48 (d, J = 8.4 Hz, 2H, ArH); 8.21 (d, J = 8.4 Hz, 2H, ArH); 9.09 (s, 1H, C5H) ppm; EIMS: m/z (%) = 493 (M + 4$^{+\cdot}$, 1.05), 491(M + 2$^{+\cdot}$, 6.86), 489 (M$^{+\cdot}$, 10.56), 488 (M − 1$^{+\cdot}$, 17.68), 459 (M–$C_2H_5^{+\cdot}$, 100); Anal. Calcd. for $C_{26}H_{18}Cl_2N_4O_2$ (489.35): C 63.81, H 3.71, N 11.45; Found: C 64.02, H 3.92, N 11.66%.

*4.1.8.6. (RS)-12-(4-Chlorophenyl)-9-ethoxy-2-(4-methoxyphenyl)-12H-chromeno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**7f**).* Yield 73%. mp 174–175 °C. IR (KBr): 2969 (CH aliph.), 1608 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.45 (t, $J = 7.2$ Hz, 3H, CH_2CH_3); 3.89 (s, 3H, OCH_3); 4.07 (q, $J = 7.2$ Hz, 2H, CH_2–CH_3); 5.72 (s, 1H, C12H); 6.74–7.27 (m, 7H, ArH); 7.36 (d, $J = 8.7$ Hz, 2H, ArH); 8.20 (d, $J = 8.7$ Hz, 2H, ArH); 9.05 (s, 1H, C5H) ppm; Anal. Calcd. for $C_{27}H_{21}ClN_4O_3$ (484.93): C 66.87, H 4.36, N 11.55; Found: C 66.92, H 4.72, N 11.19%.

4.2. Antitumor activity

The breast tumor cell line was obtained frozen in liquid nitrogen (−180 °C) from the American Type Culture Collection (ATCC) and was maintained at the National Cancer Institute, Cairo, Egypt, by serial sub culturing. Colchicine was used in this experiment as a positive control. The tested compounds were dissolved in 20% DMSO in concentration 1 mg/mL.

Serial dilutions were made reaching final concentration of the compounds to 0, 5, 12.5, 25, 50 μg/mL. All chemicals used in this study are of high analytical grade. They either obtained from (Sigma–Alderich or Biorad).

4.2.1. Measurement of potential cytotoxic activity

The cytotoxic activity was measured *in vitro* on human breast tumor cell line (MCF-7) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan et al. [25]. The results of *in vitro* cytotoxic activity experiments are presented in (Table 1).

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