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DESIGN AND SYNTHESIS OF SUBSTITUTED CHROMENES AS POTENTIAL ANTICANCER AGENTS

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ABSTRACT
Inhibition of tubulin polymerization is among the important targets that are useful in cancer therapy. Since 4-aryl-4H-chromenes are found to be tubulin destabilizers, an idea of a great interest is to combine this 4-aryl-4H-chromene nucleus with other nuclei targeting tubulin to disclose the activity of the resulting compounds. The goal compounds were synthesized and evaluated for in vitro anticancer activity. Several compounds showed excellent-very good cytotoxic activity compared to the used reference drug. Docking of a group of these biologically active compounds was carried out at colchicine binding site of tubulin and very interesting results were obtained.

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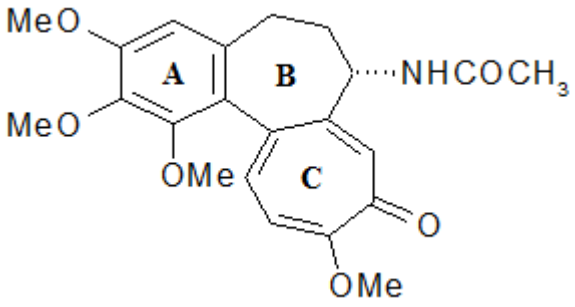
INTRODUCTION

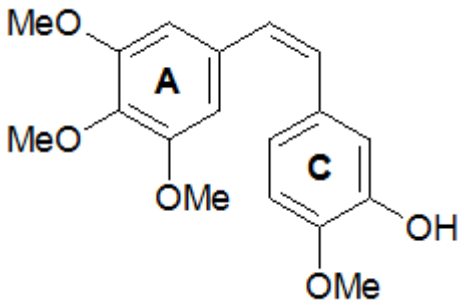
Microtubule interfering agents (MIAs) have a long history as cancer therapeutic agents by acting either as microtubule stabilizers or destabilizers[1,2]. They can be classified according to the site of action. Drugs binding to tubulin are either microtubule polymerization inhibitors or microtubule depolymerization inhibitors such as colchicine (figure 1) and its derivatives which are one of the most important classes of MIAs that interact with tubulin binding site at the interphase of α and β-subunits of the tubulin heterodimer[3].

Figure 1 colchicine



Drugs that bind to the colchicine binding site of tubulin and induce tubulin depolymerization hold promise as antitumor drugs through their ability to induce cell cycle arrest and to kill cancer cells selectively through apoptosis[1]. During the process of developing colchicine-like compounds for cancer treatment, a major turning point was the synthesis of a lead compound, combretastatin A (CA-4) (figure 2); not only for its highly cytotoxic activity against a variety of human cancer cell lines and its vascular disrupting activity [4], but also because it has been from the simplest compounds that can inhibit angiogenesis, a process essential for tumor growth [5]. CA-4 has a trimethoxyphenyl group as the A ring of colchicine. Instead of the B ring in colchicine, CA4 has an ethylene group. The double bond of this group was used to lock a different C ring in the *Trans* orientation which allows for the alignment of the C ring into the colchicine binding pocket of tubulin. Also, the C ring has a hydroxyl group that is critical for its biological function by formation of a hydrogen bond with an oxygen atom in the pocket of α-tubulin [1].

Figure 2 combretastatin A-CA-4



Moreover, structure activity relationship studies (SAR) of combretastatin A-4 led to the synthesis of different 4-arylcoumarin and 4-aryl-4*H*-chromene analogues, whereas the latter was discovered to have a promising series of potent apoptosis inducing agents possessing vascular targeting activity [6-9] .These compounds were found to be tubulin destabilizers, binding at or close to 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 agents [10-14]. It is worth to mention that, the presence of the 4-aryl moiety, the 3-cyano group, and the 2-amino functionality as well as the presence of a hydrophobic group at position 7 are essential for the anticancer activity of this nucleus[11-13]. All of The previously mentioned survey encouraged us to design and synthesis compounds bearing 4-aryl-4*H*-chromene hoping that they might have the potential to be developed into future anticancer drugs.

EXPERIMENTAL:

General

Melting points were determined on a Griffin apparatus and were uncorrected. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were carried out on Varian Gemini 300 MHZ Spectrometer, at the Microanalytical Center, Cairo University, Cairo, Egypt. Using TMS as internal standard and chemical shifts were recorded in ppm on δ scales. GC Mass was run on Shimadzu QP-2010 spectrometer at the Microanalytical Center, Cairo University, Cairo, Egypt and National Research Center, Giza, Egypt. Element analyses were carried out at the Microanalytical Center, Cairo University, Cairo, Egypt. 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 vapour. Solvent system was chloroform: methanol (in different ratios). The docking was performed using Mol soft ICM-pro software. Compound **1** was prepared according to a reported method [15].

Chemistry

(RS) Ethyl 2-(2-amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy) acetate *(2)*

To a well-stirred mixture of **1** (2.98 g, 0.01 mol) and anhydrous potassium carbonate (5.52 g, 0.04 mol) in dry dimethyl formamide (30 mL), ethyl chloroacetate (1.22 g, 0.01 mol) was added dropwise. After completion of the addition, the reaction mixture was heated under reflux for 2 h then cooled and poured into ice-cold water. The precipitated solid was filtered, washed with water, dried and crystallized from absolute ethanol to afford compound **2** in 3.58 g (93%) yield. mp 162-163 °C. IR (KBr): 3418, 3337 (forked, NH_2), 2977 (CH aliph.), 2189 (C≡N), 1750 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6,δ, ppm): *δ* 1.19 (t, 3H, CH_3); 4.15 (q, 2H, CH_2); 4.73 (s, 1H, C4H); 4.77 (s, 2H, OCH_2); 6.55 (s, 1H, ArH); 6.67 (d, 1H, ArH); 6.92 (d, 1H, ArH + 2H, NH_2, D_2O exchangeable); 7.20, 7.22 (d, J_{value} = 9 Hz, 2H, ArH); 7.36, 7.38 (d, J_{value} = 9Hz, 2H, ArH) ppm; EIMS: *m/z* (%) = 386 (M+2$^{\cdot+}$, 13.22), 384 (M$^{\cdot+}$, 17.31), 355 (M-C_2H_5 $^{\cdot+}$, 11.36), 297 (M-$C_4H_7O_2$ $^{\cdot+}$, 28.04), 273 (M-C_6H_4Cl $^{\cdot+}$, 100), 75 (C_6H_3 $^{\cdot+}$, 23.37); Anal.Calcd for $C_{20}H_{17}ClN_2O_4$ (384.81): C 62.42,H 4.45, N 7.28; Found: C 62.60, H 4.52, N 7.08%.

(RS) 2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)acetohydrazide (3)

A mixture of compound **2** (3.84 g, 0.01 mol) and hydrazine hydrate (0.055 g, 0.011 mol) in absolute ethanol (30 mL) was heated under reflux for 4 h. The solid formed while hot was filtered and crystallized from absolute ethanol to give compound **3** in 3.33 g (90%) yield. mp: 127-128 °C. IR (KBr): 3327 (2NH_2, NH), 2922 (CH aliph.), 2188 (C≡N), 1652 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6,δ, ppm): *δ* 4.30 (s, 2H, $NHNH_2$, D_2O exchangeable); 4.47 (s, 2H, OCH_2); 4.72 (s, 1H, C4H); 6.59 (s, 1H, ArH); 6.69 (d, 1H, ArH); 6.92 (d, 1H, ArH + 2H, NH_2, D_2O exchangeable); 7.18, 7.21 (d, J_{value} = 7.8 Hz, 2H, ArH); 7.35, 7.38 (d, J_{value} = 7.8 Hz, 2H, ArH); 9.30 (s, 1H, NH, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 372 (M+2$^{\cdot+}$, 3.78), 370 (M$^{\cdot+}$, 11.30), 273 (M-$C_3H_5N_4$ $^{\cdot+}$, 100); Anal.Calcd for $C_{18}H_{15}ClN_4O_3$ (370.79):C 58.31, H 4.08, N 15.11; Found: C 58.40, H 4.10, N 15.43%.

General method for the preparation of (RS)-2-Amino-7-(2-(3-amino-4-(ZE)-arylidene-5-imino-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)-4-(4-chlorophenyl)-4H-chromene-3-carbonitriles *(4a-d)*

To a mixture of **3** (1.48 g, 0.004 mol) and the respective arylidene malononitrile (0.004 mol) in absolute ethanol (25 mL), a few drops of piperidine were added. The reaction mixture was heated under reflux for 2 h. The solid formed on hot was collected by filtration and crystallized from the appropriate solvent to afford **4a-d**.

(RS)-2-Amino-7-(2-(3-amino-4-(ZE)-benzylidene-5-imino-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)-4-(4-chlorophenyl)-4H-chromene-3-carbonitriles *(4a)*

Crystallized from benzene: acetone (1:1), Yield 93%. mp 244-245 °C. IR (KBr): 3406, 3322, 3235 (2NH_2, NH), 3062 (CH arom.), 2932 (CH aliph.), 2184 (C≡N), 1684 (C=O), 1642 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): *δ* 4.69 (d, 2H, OCH_2); 5.13 (s, 1H, C4H); 6.52-7.67 (m, 12H, ArH + 3H, NH_2, NH, D_2O exchangeable); 7.99 (s, ½ H, benzylidene CH); 8.30 (s, ½ H, arylidene CH); 11.52 (s, 2H, NH_2, D_2O exchangeable) ppm; ^{13}C NMR ($CDCl_3$, δ, ppm): *δ* 39.47 (C-4), 55.82 (C-3), 65.01 (OCH_2), 115.13 (C≡N), 101.65, 111.89, 112.04, 120.29, 126.87, 127.07, 128.55, 128.70, 129.24, 129.80, 129.91, 130.13, 131.33, 133.84, 143.95, 144.94, 148.01, 148.69, 157.90, 160.18, 163.95 (C-2, arylidene C, pyrazolyl-C & Ar-C), 168.70 (C=O); EIMS*: m/z*(%) = 523 (M-H$^{\cdot+}$, 2.48), 57 (M-$C_{26}H_{16}ClN_4O_3$ $^{\cdot+}$, 100); Anal.Calcd for $C_{28}H_{21}ClN_6O_3$ (524.96): C 64.06, H4.03, N 16.01; Found: C 64.35, H 4.29, N 15.78%.

(RS)-2-Amino-7-(2-(3-amino-4-(ZE)-(4-chlorobenzylidene)-5-imino-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)-4-(4-chlorophenyl)-4H-chromene-3-carbonitriles *(4b)*

Crystallized from chloroform, Yield 84%. mp 267-268°C. IR (KBr): 3406, 3314, 3206 (2NH_2, NH), 3055 (CH arom.), 2183 (C≡N), 1682 (C=O), 1639 (C=N)cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): *δ* 4.70 (s, 2H, OCH_2); 5.12 (s, 1H, C4H); 6.52-7.68 (m, 11H, ArH + 3H, NH_2, NH, D_2O exchangeable); 7.97 (s, ½ H, arylidene

CH); 8.28 (s, ½ H, arylidene CH); 11.55 (br s, 2H, NH_2, D_2O exchangeable) ppm; Anal.Calcd for $C_{28}H_{20}Cl_2N_6O_3$ (559.40): C60.12, H 3.60, N 15.02; Found: C 60.41, H 3.79, N 15.23%.

(RS)-2-Amino-7-(2-(3-amino-4-(ZE)-(4-methoxybenzylidene)-5-imino-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)-4-(4-chlorophenyl)-4H-chromene-3-carbonitriles ***(4c)***

Crystallized from chloroform, Yield 88%. mp 255-256°C. IR (KBr): 3422, 3314, 3206 ($2NH_2$, NH), 3048 (CH arom.), 2955 (CH aliph.), 2187 (C≡N), 1674 (C=O), 1643 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): *δ* 3.79 (s, 3H, OCH_3); 4.68 (d, 2H, OCH_2); 5.11 (s, 1H, C4H); 6.52-7.22 (m, 7H, ArH + 3H, NH_2, NH, D_2O exchangeable); 7.35, 7.38 (d, J_{value} = 8.1 Hz, 2H, ArH); 7.61, 7.64 (d, J_{value} = 8.1 Hz, 2H, ArH); 7.94 (s, ½ H, arylidene CH); 8.24 (s, ½ H, arylidene CH); 11.42 (d, 2H, NH_2, D_2O exchangeable) ppm; Anal.Calcd for $C_{29}H_{23}ClN_6O_4$ (554.98): C 62.76, H 4.18, N 15.14; Found: C 63.00, H 4.19, N 14.82%.

(RS)-2-Amino-7-(2-(3-amino-4-(ZE)-(2,3-dimethoxybenzylidene)-5-imino-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)-4-(4-chlorophenyl)-4H-chromene-3-carbonitriles ***(4d)***

Crystallized from absolute ethanol, Yield 79%. mp 224-225°C. IR (KBr): 3429, 3318, 3206 ($2NH_2$, NH), 3067 (CH arom.), 2970 (CH aliph.), 2187 (C≡N), 1686 (C=O), 1643 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): *δ* 3.75 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 4.68 (d, 2H, OCH_2); 5.11 (s, 1H, C4H); 6.51-7.41 (m, 10H, ArH + 3H, NH_2, NH, D_2O exchangeable); 8.27 (s, ½ H, arylidene CH); 8.57 (s, ½ H, arylidene CH); 11.46 (s, 2H, NH_2, D_2O exchangeable) ppm; Anal.Calcd for $C_{30}H_{25}ClN_6O_5$ (585.01): 61.59, H 4.31, N 14.37; Found: C 61.59, H 4.53, N 14.18%.

General method for the preparation of (RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-arylideneacetohydrazides (**5a-f**)

A mixture of compound **3** (1.85 g, 0.005 mol) and the appropriate aromatic aldehyde (0.005 mol) in absolute ethanol (25 mL) was treated with triethyl amine (2-3 drops) and heated under reflux for 2-4 h. The solid formed on hot was filtered, dried and crystallized from the absolute ethanol to give compounds **5a-f**.

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-[2(2-phenyl)vinyl]acetohydrazides (**5a**)

Yield 88%. mp 240-241°C. IR (KBr): 3402, 3237, 3206 (NH, NH_2), 3055 (CH arom.), 2940 (CH aliph.), 2183 (C≡N), 1686 (C=O), 1643 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): *δ* 4.70 (d, 2H, OCH_2); 5.14 (s, 1H, C4H); 6.53-7.70 (m, 12H, ArH + 2H, NH_2, D_2O exchangeable); 8.00 (s, ½ H, N=CH); 8.32 (s, ½ H, N=CH); 11.58 (d, 1H, NH, D_2O exchangeable) ppm; Anal.Calcd for $C_{25}H_{19}ClN_4O_3$ (458.90): C 65.43, H 4.17, N 12.21; Found: C 65.37
, H 4.66, N 11.92%.

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-[2(4-chlorophenyl)vinyl] acetohydrazides ***(5b)***

Yield 90%. mp 264-265°C. IR (KBr): 3406, 3314, 3206 (NH, NH_2), 3059 (CH arom.), 2920 (CH aliph.), 2183 (C≡N), 1686 (C=O), 1643 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): *δ* 4.70 (d, 2H, OCH_2); 5.15 (s, 1H, C4H); 6.53-7.74 (m, 11H, ArH + 2H, NH_2, D_2O exchangeable); 7.99 (s, ½ H, N=CH); 8.30 (s, ½ H, N=CH); 11.64 (d, 1H, NH, D_2O exchangeable) ppm; Anal.Calcd for $C_{25}H_{18}Cl_2N_4O_3$ (493.34): C 60.86, H 3.68, N 11.36; Found: C 60.90, H 3.80, N 11.17%.

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-[2(3-hydroxyphenyl)vinyl] acetohydrazides ***(5c)***

Yield 85%. mp 279-280°C. IR (KBr): 3429, 3325, 3231 (OH, NH, NH_2), 3068 (CH arom.), 2863 (CH aliph.), 2183 (C≡N), 1648 (C=O), 1582 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): *δ* 4.69 (d, 2H, OCH_2); 5.12 (s, 1H, C4H); 6.52-7.14 (m, 7H, ArH + 2H, NH_2, D_2O exchangeable); 7.19, 7.22 (d, J_{value} = 8.4 Hz, 2H, ArH); 7.35, 7.38 (d, J_{value} = 8.4 Hz, 2H, ArH), 7.91 (s, ½ H, N=CH); 8.21 (s, ½ H, N=CH); 9.58 (s, 1H, OH, D_2O exchangeable); 11.47 (d, 1H, NH, D_2O exchangeable) ppm; Anal.Calcd for $C_{25}H_{19}ClN_4O_4$ (474.90): C 63.23, H 4.03, N 11.80; Found: C 63.10, H 4.10, N 12.13%.

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-[2(4-hydroxyphenyl)vinyl] acetohydrazides ***(5d)***

Yield 77%. mp 226-227°C. IR (KBr): 3429, 3318, 3202 (OH, NH, NH_2), 2978 (CH aliph.), 2187 (C≡N), 1686 (C=O), 1647 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ,

ppm): δ 4.67 (d, 2H, OCH_2); 5.09 (s, 1H, C4H); 6.51-7.52 (m, 11H, ArH + 2H, NH_2, D_2O exchangeable); 7.89 (s, ½ H, N=CH); 8.19 (s, ½ H, N=CH); 9.95 (br. s, 1H, OH, D_2O exchangeable); 11.32 (d, 1H, NH, D_2O exchangeable) ppm; Anal.Calcd for $C_{25}H_{19}ClN_4O_4$ (474.90): C 63.23, H 4.03, N 11.80; Found: C 63.01, H 4.49, N 11.72%.

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-[2(4-methoxyphenyl)vinyl] acetohydrazides ***(5e)***

Yield 96%. mp 251-252°C. IR (KBr): 3422, 3314, 3206 (NH, NH_2), 2187 (C≡N), 1674 (C=O), 1643 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): δ 3.79 (s, 3H, OCH_3); 4.68 (d, 2H, OCH_2); 5.11 (s, 1H, C4H); 6.52-7.64 (m, 11H, ArH + 2H, NH_2, D_2O exchangeable); 7.94 (s, ½ H, N=CH); 8.24 (s, ½ H, N=CH); 11.41 (d, 1H, NH, D_2O exchangeable) ppm; EIMS*: m/z*(%) = 490 (M+2†, 14.17), 488 (M†, 40.91), 377 (M-$C_6H_4Cl^{\dagger}$, 100); Anal.Calcd for $C_{26}H_{21}ClN_4O_4$ (488.92): C 63.87, H 4.33, N 11.46; Found: C 63.90, H 4.40, N 11.23%.

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(ZE)-[2(2,3-dimethoxyphenyl)vinyl] acetohydrazides ***(5f)***

Yield 89%. mp 231-232°C. IR (KBr): 3460, 3329, 3244 (NH, NH_2), 2986 (CH aliph.), 2195 (C≡N), 1682 (C=O), 1612 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): δ 3.75 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 4.68 (d, 2H, OCH_2); 5.11 (s, 1H, C4H); 6.51-7.38 (m, 10H, ArH + 2H, NH_2, D_2O exchangeable); 8.28 (s, ½ H, N=CH); 8.58 (s, ½ H, N=CH); 11.53 (d, 1H, NH, D_2O exchangeable) ppm; Anal.Calcd for $C_{27}H_{23}ClN_4O_5$ (518.95): C 62.49, H 4.47, N 10.80; Found: C 62.21, H 4.58, N 10.76%.

General method for the preparation of (RS)-2-(2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)acetyl) substituted hydrazinecarbothioamide ***(6a&b)***

To a solution of the acid hydrazide **3** (1.48 g, 0.004 mol) in absolute ethanol (20 mL), was added the appropriate isothiocyanate (0.004 mol). The reaction mixture was heated under reflux for 4 h; the solid formed while hot was collected by filtration, washed with ethanol and crystallized to afford **6a-c**.

(RS)-2-(2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)acetyl) N-ethylhydrazinecarbothioamide ***(6a)***

Crystallized from methanol, Yield 88%. mp 144-145°C. IR (KBr): 3438 (3NH, NH_2,), 2975 (CH aliph.), 2190 (C≡N), 1654 (C=O), 1285, 1232 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) δ 1.04 (t, 3H, CH_3); 3.44 (q, 2H, CH_2CH_3); 4.57 (s, 2H, OCH_2); 4.73 (s, 1H, C4H); 6.64 (s, 1H, ArH); 6.73 (d, 1H, ArH); 6.93 (d, 1H, ArH + 2H, NH_2, D_2O exchangeable); 7.19, 7.21 (d, J_{value} = 8.1 Hz, 2H, ArH); 7.36, 7.38 (d, J_{value} = 8.1 Hz, 2H, ArH); 7.89 (s, 1H, NH, D_2O exchangeable); 9.16 (s, 1H, NH, D_2O exchangeable); 9.97 (s, 1H, NH, D_2O exchangeable) ppm; Anal.Calcd for $C_{21}H_{20}ClN_5O_3S$(457.93): C 55.08, H 4.40, N 15.29; Found: C 54.86, H 4.45, N 15.00%.

(RS)-2-(2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)acetyl) N'-phenylhydrazine-carbothioamide ***(6b)***

Crystallized from methanol: acetone (1: 1). Yield 73%. mp 142-143°C. IR (KBr): 3449, 3325, 3248, 3206 (3NH, NH_2), 3090, 3067 (CH arom.), 2191 (C≡N), 1655 (C=O), 1292, 1242 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) δ 4.62 (s, 2H, OCH_2); 4.74 (s, 1H, C4H); 6.66-7.39 (m, 12H, ArH + 2H, NH_2, D_2O exchangeable); 9.62 (s, 2H, 2NH, D_2O exchangeable); 10.23 (s, 1H, NH, D_2O exchangeable) ppm; EIMS*: m/z*(%) = 506 (M+1†, 1.57), 134 (M-$C_{18}H_{16}ClN_4O_3^{\dagger}$, 100); Anal.Calcd for $C_{25}H_{20}ClN_5O_3S$(505.98): C 59.34, H 3.98, N 13.84; Found: C 59.33, H 3.98, N 13.62%.

General method for the preparation of (RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(4-oxo-3-substitutedthiazolidin-(ZE)-2-ylidene)acetohydrazides ***(7a&b)***

A mixture of appropriate thiosemicarbazide **6b&c** (0.01mol) and ethyl chloroacetate (1.34 g, 0.011 mol) was heated under reflux in dimethyl formamide (30 mL) in the presence of anhydrous sodium acetate (1.64 g, 0.02 mol) for 4 h. The reaction mixture was cooled and poured into ice-cold water. The solid precipitated was filtered, washed with water, dried and crystallized from the suitable solvent to furnish **7a&b**

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(4-oxo-3-ethylthiazolidin-(ZE)-2-ylidene)acetohydrazides *(7a)*

Crystallized from chloroform: acetone (1: 1). Yield 93%. mp 191-192°C. IR (KBr): 3400, 3333 (NH, NH_2), 2191 (C≡N), 1728 (thiazolidinone C=O), 1659 (amidic C=O), 1582 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 1.12 (t, 3H, CH_3); 3.66 (q, 2H, CH_2CH_3); 4.02 (s, 2H, CH_2 thiazolidinone); 4.62 (s, 2H, OCH_2); 4.73 (s, 1H, C4H); 6.60 (s, 1H, ArH); 6.72 (d, 1H, ArH); 6.93 (d, 1H, ArH + 2H, NH_2, D_2O exchangeable); 7.19, 7.21 (d, J_{value} = 8.4 Hz, 2H, ArH); 7.36, 7.38 (d, J_{value} = 8.4 Hz, 2H, ArH); 10.47 (s, 1H, NH, D_2O exchangeable) ppm; EIMS*: m/z*(%) = 499 ($M+2^{\cdot+}$, 8.20), 497 ($M^{\cdot+}$, 24.70), 386 ($M-C_6H_4Cl^{\cdot+}$,100); Anal.Calcd for $C_{23}H_{20}ClN_5O_4S$(497.95): C 55.48, H 4.05, N 14.06; Found: C 55.42, H 3.60, N 13.70%.

(RS)-2-(2-Amino-4-(4-chlorophenyl)-3-cyano-4H-chromen-7-yloxy)-N'-(4-oxo-3-phenylthiazolidin-(ZE)-2-ylidene)acetohydrazides *(7b)*

Crystallized from chloroform. Yield 76%. mp 181-182°C. IR (KBr): 3410, 3332 (NH, NH_2), 2977 (CH aliph.), 2188 (C≡N), 1712 (thiazolidinone C=O), 1648 (amidic C=O) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 4.18 (s, 2H, CH_2 thiazolidinone); 4.73 (s, 2H, OCH_2); 4.79 (s, 1H, C4H); 6.68-7.35 (m, 12H, ArH + 2H, NH_2, D_2O exchangeable); 11.11 (s, 1H, NH, D_2O exchangeable) ppm; Anal.Calcd for $C_{27}H_{20}ClN_5O_4S$(546.00): C 59.39, H 3.69, N 12.83; Found: C 59.56, H 3.89, N 12.61%.

General method for the preparation of (RS)-2-Amino-4-(4-chlorophenyl)-7-((4-substituted-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(8a&b)*

To a mixture of **3** (1.48 g, 0.004 mol) and the respective isothiocyanate (0.004 mol) in absolute ethanol (25 mL), a few drops of triethyl amine were added. The reaction mixture was heated under reflux for 4 h then cooled; the solid formed was collected by filtration, washed with ethanol and crystallized from absolute ethanol to afford **8a&b**.

(RS)-2-Amino-4-(4-chlorophenyl)-7-((4-ethyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(8a)*

Yield 75%. mp 187-188°C. IR (KBr): 3372, 3314, 3186 (NH, NH_2), 2191 (C≡N), 1651 (C=N), 1362, 1269 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 1.22 (t, 3H, CH_3); 3.99 (q, 2H, CH_2CH_3); 4.74 (s, 1H, C4H); 5.22 (s, 2H, OCH_2); 6.75 (s, 1H, ArH); 6.78 (d, 1H, ArH); 6.93 (d, 1H, ArH + 2H, NH_2, D_2O exchangeable); 7.19, 7.22 (d, J_{value} = 8.4 Hz, 2H, ArH); 7.35, 7.38 (d, J_{value} = 8.4 Hz, 2H, ArH); 13.90 (br. s, 1H, NH, D_2O exchangeable) ppm; EIMS*: m/z*(%) = 441 ($M+2^{\cdot+}$, 0.43), 439 ($M^{\cdot+}$, 1.06), 297 ($M-C_5H_8N_3S^{\cdot+}$, 100); Anal.Calcd for $C_{21}H_{18}ClN_5O_2$ S (439.92): C 57.33, H 4.12, N 15.92; Found: C 57.09, H 4.29, N 15.78%.

(RS)-2-Amino-4-(4-chlorophenyl)-7-((4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(8b)*

Yield 69%. mp 156-157°C. IR (KBr): 3314, 3171 (NH, NH_2), 3051 (CH arom.), 2932 (CH aliph.), 2191 (C≡N), 1647 (C=N), 1327, 1242 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 4.69 (s, 1H, C4H); 4.97 (s, 2H, OCH_2); 6.52-7.50 (m, 12H, ArH + 2H, NH_2, D_2O exchangeable); 14.00 (br s, 1H, NH, D_2O exchangeable) ppm; EIMS*: m/z*(%) = 486 ($M-1^{\cdot+}$, 0.3), 187 ($M-C_{15}H_{11}ClN_3S^{\cdot+}$, 100); Anal.Calcd for $C_{25}H_{18}ClN_5O_2S$ (487.96): C 61.54, H 3.72, N 14.35; Found: C 61.49, H 3.84, N 14.11%.

General method for the preparation of (RS)-2-Amino-4-(4-chlorophenyl)-7-((4-substituted-5-substitutedthio-4H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(9a-d)*

The respective alkyl halide (0.001mol) was added to a mixture of **8a&b** (0.001mol) and anhydrous sodium acetate (0.41 g, 0.005mol) in absolute ethanol (15 mL) and the reaction mixture was heated under reflux for 4 h. After cooling, the solid product formed was filtered, washed with water and crystallized from absolute ethanol to afford **9a-d**.

(RS)-2-Amino-4-(4-chlorophenyl)-7-((4-ethyl-5-ethylthio-4H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(9a)*

Yield 72%. mp 176-177°C. IR (KBr): 3318 (NH_2), 3166 (CH arom.), 2975 (CH aliph.), 2188 (C≡N), 1652 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 1.24 (t, 3H, SCH_2CH_3); 1.31 (t, 3H, NCH_2CH_3); 3.17

(q, 2H, SCH_2CH_3); 3.96 (q, 2H, NCH_2CH_3); 4.74 (s, 1H, C4H); 5.29 (s, 2H, OCH_2); 6.76-6.97 (m, 3H, ArH + 2H, NH_2, D_2O exchangeable); 7.20, 7.22 (d, J_{value} = 8.7 Hz, 2H, ArH); 7.36, 7.39 (d, J_{value} = 8.7 Hz, 2H, ArH) ppm; EIMS*: m/z*(%) = 469 ($M+2^{\ddagger}$, 3.46), 467 ($M^{\ddagger}$, 10.06), 170 ($M\text{-}C_{16}H_{10}ClN_2O_2^{\ddagger}$, 100); Anal.Calcd for $C_{23}H_{22}ClN_5O_2S$ (467.97): C 59.03, H 4.74, N 14.97; Found: C 58.80, H 4.50, N 14.80%.

(RS)-2-Amino-4-(4-chlorophenyl)-7-((4-ethyl-5-acetamidothio-4H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(9b)*

Yield 81%. mp 145-146°C. IR (KBr): 3324 ($2NH_2$), 3180 (CH arom.), 2985 (CH aliph.), 2186 (C≡N), 1652 (C=O), 1582 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 1.25 (t, 3H, CH_3); 3.91 (s, 2H, SCH_2); 4.02 (q, 2H, NCH_2); 4.73 (s, 1H, C4H); 5.28 (s, 2H, OCH_2); 6.79 (s, 1H, ArH); 6.80 (d, 1H, ArH); 6.96 (d, 1H, ArH + 2H, NH_2, D_2O exchangeable); 7.19, 7.22 (d, J_{value} = 8.4 Hz, 2H, ArH); 7.36, 7.39 (d, J_{value} = 8.4 Hz, 2H, ArH); 7.60 (s, 2H, $CONH_2$, D_2O exchangeable) ppm; Anal.Calcd for $C_{23}H_{21}ClN_6O_3S$ (496.97): C 55.59, H 4.26, N 16.91; Found: C 55.67, H 4.31, N 16.60%.

(RS)-2-Amino-4-(4-chlorophenyl)-7-((4-phenyl-5-ethylthio-4H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(9c)*

Yield 83%. mp 222-223°C. IR (KBr): 3310 (NH_2), 3144 (CH arom.), 2973 (CH aliph.), 2191 (C≡N), 1651 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 1.28 (t, 3H, CH_3); 3.11 (q, 2H, SCH_2); 4.69 (s, 1H, C4H); 5.08 (s, 2H, OCH_2); 6.60-7.52 (m, 12H, ArH + 2H, NH_2, D_2O exchangeable) ppm; Anal.Calcd for $C_{27}H_{22}ClN_5O_2S$ (516.01): C 62.84, H 4.30, N 13.57; Found: C 62.99, H 4.65, N 13.62%.

(RS)-2-Amino-4-(4-chlorophenyl)-7-((4-phenyl-5-acetamidothio-4H-1,2,4-triazol-3-yl)methoxy)-4H-chromene-3-carbonitriles *(9d)*

Yield 77%. mp 151-152°C. IR (KBr): 3320 ($2NH_2$), 3177 (CH arom.), 2185 (C≡N), 1651 (C=O), 1582 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm) *δ* 3.92 (s, 2H, SCH_2); 4.70 (s, 1H, C4H); 5.09 (s, 2H, OCH_2); 6.58-7.56 (m, 12H, ArH + 2H, NH_2, D_2O exchangeable); 7.60 (s, 2H, $CONH_2$, D_2O exchangeable) ppm; Anal.Calcd for $C_{27}H_{21}ClN_6O_3S$ (545.01): C 59.50, H 3.88, N 15.42; Found: C 59.37, H 3.99, N 15.10%.

Cytotoxic Screening

The cytotoxic activity was measured *in vitro* on human breast tumour cell line (MCF-7) using colchicine as a positive control and the title compounds were dissolved in 20% DMSO in concentration 1mg/mL then Serial dilutions were made reaching final concentration of the compounds to 5, 12.5, 25, 50µg/mL . The cytotoxic activity was measured using Sulforhodamine-B stain (SRB) assay applying the method of Skehan *et al*. [16] Cells were plated in 96 multiwell plates (104 cell/ well) for 24 hour before treatment with the compounds to allow attachment of the cells to the wall of the plate. The five concentrations of the compound under test (0, 5, 12.5, 25, and 50µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37°C and in atmosphere of 5% CO_2. After 48 hours cell were fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted and IC_{50} [the concentration required for 50% inhibition of cell viability] was calculated for each compound. The IC_{50} values were calculated using sigmodial dose response curve fitting models (GraphPad, Prizm software incorporated).

Docking studies:

The X-ray structure of *α, β*-tubulin/*N*-deacetyl-*N*-(2-mercaptoacetyl)-colchicine (DAMA-colchicine) complex [17] was used in this study. To validate the docking reliability, the known X-ray structure of tubulin in complex with the DAMA-colchicine was used. The ligand DAMA-colchicine was flexibly docked to the binding site of tubulin, and the docking conformation corresponding to the lowest energy score value was selected as the most probable binding conformation with root mean standard deviation (rmsd) of 0.67 Aº and as a result, the docked DAMA-colchicine and crystal DAMA-colchicine are almost at the same position in the active site of tubulin suggesting a high docking reliability of MOE. Therefore, the MOE

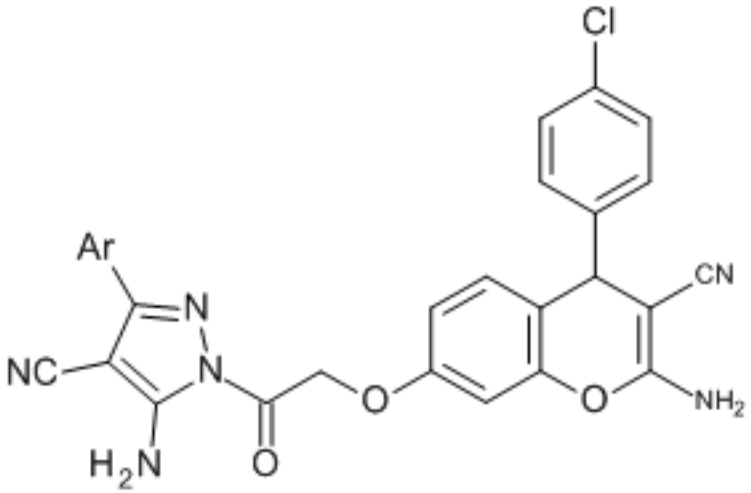
docking protocol and the used parameters could be extended to search the tubulin binding conformations of other synthesized compounds. Several steps took place before studying the interaction of the ligand with the amino acids of the active site, first acting on only one chain of amino acids containing one molecule of ligand, then deleting all water of crystallization away from the active site followed by isolation of the active site and recognition of the amino acids and finally studying the interaction of the ligand with the amino acids of the active site.Eight of the most active compounds were evaluated by docking on the colchicine binding site. Preparation of the synthesized compounds for docking was achieved *via* their 3D structure built by MOE. The same docking protocol used with the ligand was applied to the selected least energetic conformers that were subjected to conformational analysis using system search.

RESULT AND DISCUSSION

Chemistry

Preparation of compound **1** was via a three component one-pot condensation of malononitrile with 4-chlorobenzaldehyde and resorcinol in ethanol with few drops of piperidine [15, 18]. Reaction of *o*-aminocyanochromene **1** with ethyl chloroacetate was carried out in dry dimethyl formamide in the presence of anhydrous potassium carbonate as a catalyst to give **2** in an excellent yield. There is no conflict of interest between both the hydroxyl group and the amino group during the alkylation process since; the amino group is very weak to an extent that could not undergo a simple condensation reaction with an aldehyde or benzene sulphonyl chloride[19]. During this work, preparation of the key intermediate **3** was achieved by refluxing both the substrate **2** and hydrazine hydrate in ethanol for 4 hours. The IR spectrum revealed band at 3327 cm^{-1} for (NH_2, NH) groups and at 1652 cm^{-1} for (amidic C=O) group. ^1H NMR spectrum of compound **3**, showed singlet signals derived from hydrazide structure appeared at δ 4.30 (NH_2) and δ 9.30 (NH) with the integration for two protons and one proton, respectively. *o*-Aminocyano pyrazole **A** (figure 3) was expected to be the product of the reaction between the acid hydrazide **3** and arylidene malononitrile but surprisingly, upon applying this method herein, unexpected product was resulted and identified as compounds **4a-d** rather than the *o*-aminocyano pyrazole derivatives **A** (Figure 1) and this was attested by the spectroscopic data of these compounds.

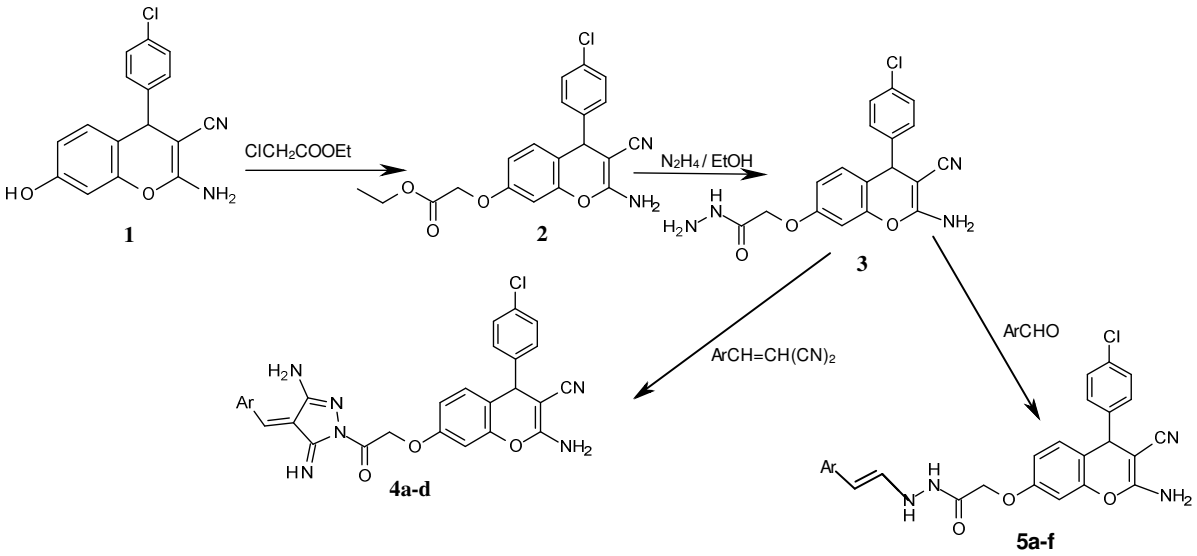
Figure 3 Compound **A**



This is may be due to; after the formation of the intermediate that resulted from the reaction between the hydrazide **3** and one of the cyano groups of the arylidene malononitriles, two sites of attack were available; either the olifenic carbon or the other cyano group, a preferential attack of the

lone pair of the nitrogen atom of the hydrazide **3** on the cyano group rather than the olifenic carbon took place since the carbon atom of the cyano group is more electropositive than that of the olifenic carbon to give compounds **4a-d** rather than compound **A**. The ^{1}H NMR spectra of compounds **4a-d** displayed two adjacent single peaks at δ 7.94-8.27 and 8.24-8.57 belonging to (arylidene CH). Compounds **5a-f** were synthesized by heating equimolar amounts of the hydrazide **3** with the corresponding aromatic aldehyde in absolute ethanol containing catalytic amount of triethyl amine. The structure of the synthesized compounds **5a-f** was consistent with the element analysis and spectral data. ^{1}H NMR spectra of compounds **5a-f** indicated the presence of the two isomers (*ZE*) in equimolar amounts, this is obviously showed by presence of two CH_2 groups as the peak is forked at its apex and by the appearance of two adjacent single peaks at δ 7.89-8.28 and 8.19-8.58 ppm attributed to azomethine proton (N=CH) and a doublet signal which was D_2O exchangeable indicating NH proton (Scheme 1).

Scheme (1) Synthesis of compounds **2, 3, 4a-d** and **5a-f**

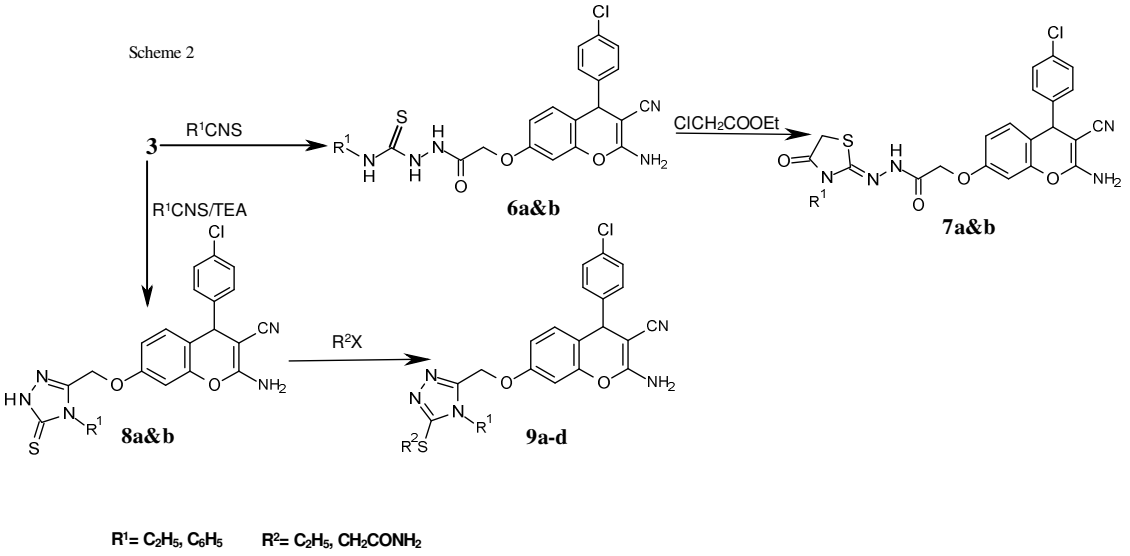


Ar= C_6H_5, 4-ClC_6H_4, 3-OHC_6H_4, 4-OHC_6H_4, 4-$OCH_3C_6H_4$, 2,3-$(OCH_3)_2C_6H_3$

Compounds **6a&b** was prepared via reacting isothiocyanates with the acid hydrazide **3** in refluxing ethanol. The ^{1}H NMR spectra of **6a&b** displayed D_2O exchangeable singlet signals due to three different –NH protons at δ 7.89-10.23. In addition, ^{1}H NMR spectrum of **6a** showed the appearance of a triplet and a quartet signal at δ 1.04 and δ 3.44 due to ethyl group. In the present research, preparation of **7a&b** from the corresponding thiosemicarbazide **6a&b** and ethyl chloroacetate in dry dimethyl formamide. Microanalytical and spectral data were in accordance with the expected structures, hence the IR spectra showed absorption bands at 1728-1712 cm^{-1} and at 1659- 1648 cm^{-1} attributed to thiazolidinone (C=O) and amidic (C=O) groups, respectively. ^{1}H NMR spectra showed the appearance of singlet signals at δ 4.02- 4.18 corresponding to two CH_2 protons of thiazolidinone ring of compounds**7a&b**. Synthesis of compounds **8a&b** took place by refluxing the acid hydrazide **3** and the appropriate isothiocyanate in ethanol and catalytic amount of triethyl amine where thiosemicarbazide was first formed and underwent intramolecular cyclization due to the presence of a base to triazolothione. The formation of thione tautomer demonstrated by the presence of two absorption bands at 1327–1362 and 1242–1269

cm^{-1} attributed to the (C=S) group in the IR spectra of compounds **8a&b**. In addition, ^{1}H NMR spectra showed D_2O exchangeable singlet signals at δ 13.90-14.00 due to the NH protons of 1,2,4-triazole ring. S-Alkylation of compounds **8a&b** with the respective alkyl halide in absolute ethanol in the presence of sodium acetate afforded the target compounds **9a-d**. The ^{1}H NMR spectra of **9a&c** revealed the existence of triplet and quartet signal at δ 1.24-1.28 and δ 3.11-3.17 for the protons of (SCH_2CH_3) group. Also, ^{1}H NMR spectra of **9b&d** showed a singlet signal at δ 3.91-3.92 corresponding to (SCH_2) and D_2O exchangeable signal at δ 7.60 indicating ($CONH_2$) group (Scheme 2).

Scheme 2 Synthesis of compounds **6a&b, 7a&b, 8a&b** and **9a-d**



3.2. Preliminary anticancer screening

Screening nine of the target compounds against human breast tumor cell line (MCF-7) was carried out using colchicine as a reference drug with IC_{50} (2.97µg/mL). Compounds **8a, 5f, 4c, 9b** and **5d** are more potent than the reference drug with IC_{50} (1.33, 2.23, 2.28, 2.50, and 2.53µg/mL). Additionally compound **9a** and **5e** are nearly as active as the control positive drug with IC_{50} (2.82, 2.88 µg/mL). On the other hand compound **4d** is less active than colchicine with IC_{50} (3.48µg/mL) (table 1).

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.	Percentage of the surviving MCF7 cells at each concentration(in µg/mL)				IC_{50} (µg/mL)
	5	12.5	25	50	
Colchicine	0.194273	0.171715	0.185526	0.201330	2.97
4c	0.345000	0.320000	0.205146	0.217526	2.28
4d	0.434000	0.307000	0.128380	0.142180	3.48
5d	0.271000	0.314000	0.205146	0.217526	2.53
5e	0.376000	0.427000	0.193355	0.307277	2.88
5f	0.377000	0.514000	0.473000	0.214993	2.23
7a	0.395000	0.434000	0.298730	0.277220	4.98
8a	0.244000	0.293000	0.214993	0.126791	1.33
9a	0.116524	0.096052	0.263305	0.450771	2.82
9b	0.090000	0.164035	0.381757	0.384086	2.50

Docking studies

Literature survey [2] revealed that the binding models of 15 ligand were used to derive a seven-point pharmacophore connecting the different structural classes of colchicine site inhibitors (CSI). The common pharmacophores consisted of three hydrogen bond acceptors with the backbone NH of the amino acid residues, one hydrogen bond donor, two hydrophobic centers which were located within the hydrophobic pocket and establishing hydrophobic contacts with side chains of the amino acid residues. Finally, one planar group embedded into a second hydrophobic pocket [10-14]. In the present work, a molecular modelling study has been performed to investigate the possible binding conformation for the newly prepared 4-aryl-*4H*-chromenes to the colchicine binding site of tubulin, which may give a clue about their proposed mechanism of action as antitubulin agents. The automated docking program of MOE 2008.10 was used to dock the active compounds on the colchicine-binding site. The most stable docking model was selected according to the best scored conformation predicted by the MOE scoring function. The complexes were energy-minimized with a MMFF94 force field till the gradient convergence 0.05 kcal/mol was reached. The structure of *N*-deacetyl-*N*-(2-mercaptoacetyl)-colchicine (DAMA-colchicine) was used as a reference ligand along the docking studies. All docked compounds showed good-excellent binding score and fitting to the active site pocket with good interactions with the amino acid residues of the active site (table 2) suggesting that their anticancer activity may be due to their proposed mechanism of action as antitubulin agents.

Table 2 Docking study data and cytotoxic activity (IC_{50}) for DAMA-colchicine and novel compounds

Cpd no.	Number of H-bonds	Amino acid residues forming H-bonds (H-bond Length in Aº)	Binding Energy Score*	IC_{50} (µg/mL)
ligand	-	----------	-8.86	-
4c	1	Ser^{178} (2.98 Aº)	-21.48	2.28
4d	2	Ser^{178} (2.65 Aº), Lys^{254} (3.00 Aº)	-19.46	3.48
5d	4	Val^{238} (2.48 Aº), Lys^{254} (2.81 Aº), Asn^{249} (3.08 Aº), Asn^{249} (2.81 Aº)	-14.89	2.53
5e	3	Thr^{179} (2.82 Aº), Asn^{258} (3.16 Aº), Lys^{352} (3.01 Aº)	-14.10	2.88
5f	2	Ser^{140} (3.39 Aº), Asn^{258} (2.97 Aº)	-18.15	2.23
8a	3	Ser^{178} (3.00 Aº), Asn^{101} (2.93 Aº), Gly^{144} (3.01 Aº)	-25.95	1.33
9a	1	Ser^{178} (2.80 Aº)	-19.94	2.82
9b	3	Val^{238} (2.70 Aº), Gln^{11} (2.78 Aº), Gln^{247} (2.69 Aº)	-19.18	2.50

* Binding Energy score (kcal/mol): energy of interaction of the ligand in the active site

* The higher the score in negative terms, the better the binding affinity.

Compound **8a** (IC_{50} = 1.33 µg/mL) produces a deep moving into the hydrophobic pocket of colchicine binding site with which the triazolothione moiety is able to reach the other hydrophobic pocket and is

involved in three hydrogen bonding with Ser^{178} (3.00 Aº), Asn^{101} (2.93 Aº), Gly^{144} (3.01 Aº) accompanied with an excellent binding energy score – 25.95 kcal/mol. All the other docked compounds showed moderate binding energy scores (-14.10 kcal/mol - -21.48 kcal/mol) and bond to 1-4 amino acid residues. In spite of binding to 4 amino acid residues, compound **5d** showed a moderate cytotoxic activity since its superimposition to the pocket is not complete.

CONCLUSION

Since no precedent publication described the synthesis and the cytotoxic activity of the 7-O-substituted 4-aryl-4*H*-aminocyanochromenes, the objective of this work was to synthesize and investigate the cytotoxic activity of a novel group of these compounds. The cytotoxic studies revealed that **8a** is the most active compound while compounds **5f, 4c, 9b** and **5d** are more potent than the reference drug and compound **9a** and **5e** are as active as the control positive drug. Docking the active compounds in the colchicine binding site of tubulin, may give an idea about the mechanism of how those active compounds might exert their cytotoxic activity.

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