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# Synthesis of Novel Chromenes as Cytotoxic Agents

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**ABSTRACT**

*Novel substituted chromenes, chromenopyrimidine derivatives and chromenotriazolo-pyrimidines were synthesized. Several compounds were evaluated for their antitumor activity, most of them revealed promising cytotoxic activity against breast cancer cell line MCF-7 in comparison to colchicine as positive control.*

**Key Words:** Heterocycles, Substituted chromenes, Chromenopyrimidine, Chromenotriazolo-pyrimidines, Cytotoxic activity.

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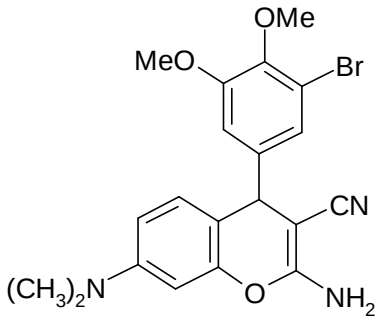
## INTRODUCTION

The cell protein tubulin is one of the most important molecular targets of antitumor agents. Two subunits of this protein, α- and β-tubulins can undergo polymerization to give microtubules. The control over cell division processes is among their important and various functions in cells. Inhibition of these processes by intervening into the tubulin system of tumor cells provides the basis of one of types of antitumor therapy. Antitumor agents can bind to different sites of tubulin and cause either its uncontrolled polymerization or inhibit tubulin polymerization. Tubulin polymerization is inhibited by colchicine and its analogs. The action of colchicine is based on binding in a particular site of tubulin (the colchicine site), resulting in deformation of the α, β- dimer structure, which hinders the tubulin assembly into microtubules [1,2]. In recent years, several new structural classes of ligands of the colchicine binding site of tubulin have been found. For example, this activity would be expected for compound **I** (Figure 1) belonging to the 4-aryl-4*H*-chromene series [3].




Compound **I**

**Figure 1**

4-aryl-4*H*-chromenes have been discovered as a promising lead of potent apoptosis inducing agents possessing vascular targeting activity [4-7]. 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 [8-12].

Keeping this in mind, it was aimed in this work to synthesize a new series of heterocyclic compounds containing 4-aryl-4*H*-chromene moiety and this was achieved via Schemes 1 and 2.

## MATERIALS AND METHODS

### 1.1.Chemistry

Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel sheets that precoated with UV fluorescent silica (MERCK 60 F 254) and spots were developed using $I_2$ vapour / UV light as visualizing agents. Solvent system was chloroform: methanol (in different ratio). $^1$H NMR spectra were determined in $CDCl_3$, or DMSO-$d_6$ solvent with Varian Gemini 300 MHZ Spectrometer. Peak positions were given in parts per million ($\delta$) downfield the tetramethylsilane as internal standard. $^{13}$C NMR spectra were carried out on Gemini 300 MHZ Spectrometer. 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. X-ray crystallography was performed by the X-ray laboratory of National Research Center, Cairo, Egypt. Melting points were determined on a Griffin instrument and are uncorrected. All reported products showed $^1$H NMR spectra in agreement with the assigned structures. Elemental analyses were performed at the Micro-analytical Center, Cairo University, Egypt. Compound **1a&b** was prepared adopting a reported procedure [13, 14].

#### 2.1.1 General procedure for the preparation of compounds (*RS)* 8-alkoxy-5-(4-chlorophenyl)-5*H*-chromeno[2,3-*d*]pyrimidin-4-amines (2a&b)

A solution of **1a&b** (0.01 mol) in formamide (20 mL) was heated under reflux for 3 h then cooled and poured into ice-cold water (20 mL). The precipitated solid was filtered, washed with water and crystallized from the appropriate solvent to afford **2a&b**.

#### 2.1.1.1 *(RS)* 5-(4-Chlorophenyl)-8-methoxy-5*H*-chromeno[2,3-*d*]pyrimidin-4-amine (2a)

Compound **2a** was crystallized from benzene: acetone (1: 1) mixture, the following spectral data were recorded for compound **3a**: IR (KBr): 3206 ($NH_2$), 1626 (C=N) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$): $\delta$ 3.80 (s, 3H, $OCH_3$); 5.30 (s, 1H, C5H); 6.72-7.42 (m, 7H, ArH + 2H, $NH_2$, $D_2O$ exchangeable); 8.18 (s, 1H, C2H) ppm.

#### 2.1.1.2 *(RS)* 5-(4-Chlorophenyl)-8-ethoxy-5*H*-chromeno[2,3-*d*]pyrimidin-4-amine (2b)

Compound **2b** was crystallized from benzene, the following spectral data were recorded for compound **2b**: IR (KBr): 3385, 3335 ($NH_2$), 3165 (CH arom.), 2979 (CH aliph.), 1653 (C=N)cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38 (t, $J_{value}$ = 7.2 Hz, 3H, $CH_3$); 3.98 (q, $J_{value}$ = 7.2 Hz, 2H, $CH_2$); 4.85 (s, 2H, $NH_2$, $D_2O$ exchangeable); 4.89 (s, 1H, C5H); 6.57-7.27 (m, 7H, ArH), 8.09 (s, 1H, C2H) ppm; MS: *m/z* 355 (M+2 $^{+\cdot}$, 7.93), 353 (M $^{+\cdot}$, 24.88), 242 (M-$C_6H_4Cl$ $^{+\cdot}$,100).

#### 2.1.2 General procedure for the preparation of compounds *(RS) N*-acetyl-*N*-[7-alkoxy-4-(4-chlorophenyl)-3-cyano-4*H*-chromen-2-yl]acetamides (3a&b)

A mixture of **1a&b** (0.01 mol) and acetic anhydride (20 mL) was heated under reflux for 5 h. The precipitated crystals formed after cooling were filtered and recrystallized from ethanol to give compounds **3a&b**.

#### 2.1.2.1 *(RS) N*-Acetyl-*N*-[4-(4-chlorophenyl)-3-cyano-7-methoxy-4*H*-chromen-2-yl]acetamide (3a)

**3a**: IR (KBr): 3069 (CH arom.), 2949 (CH aliph.), 2220 (C≡N), 1744 (2 acetyl C=O) cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 2.46 (s, 6H, $2COCH_3$); 3.79 (s, 3H, $OCH_3$); 4.90 (s, 1H, C4H); 6.60 (s, 1H, ArH); 6.70 (d, $J_{value}$ = 8.7 Hz, 1H, ArH); 6.87 (d, $J_{value}$ = 8.7 Hz, 1H, ArH); 7.22 (d, $J_{value}$ = 8.4 Hz, 2H, ArH); 7.35 (d, $J_{value}$ = 8.4 Hz, 2H, ArH).

#### 2.1.2.2 *(RS) N*-Acetyl-*N*-[4-(4-chlorophenyl)-3-cyano-7-ethoxy-4*H*-chromen-2-yl]acetamide (3b)

**3b**: IR (KBr): 3106 (CH arom.), 2982 (CH aliph.), 2219 (C≡N), 1744 (2 acetyl C=O) cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 1.40 (t, $J_{value}$ = 7.2 Hz, 3H, $CH_3$); 2.46 (s, 6H, $2COCH_3$); 4.01 (q, $J_{value}$ = 7.2 Hz 2H, $CH_2$); 4.90 (s, 1H, C4H); 6.58 (s, 1H, ArH); 6.69 (d, $J_{value}$ = 8.7 Hz, 1H, ArH); 6.86 (d, $J_{value}$ = 8.7 Hz, 1H, ArH); 7.25 (d, $J_{value}$ = 8.1 Hz, 2H, ArH); 7.34 (d, $J_{value}$ = 8.1 Hz, 2H, ArH); GCMS: *m/z* 412 (M+2 $^{+\cdot}$, 1.54), 410 (M $^{+\cdot}$, 4.71), 215 (M-$C_{10}H_8ClO_2$ $^{+\cdot}$, 100).

**2.1.3 General procedure for the preparation of compounds 7-alkoxy-4-(4-chlorophenyl)-2-oxo-2*H*-chromene-3-carbonitriles (4a&b)**

A mixture of **1a&b** (0.01mol), the appropriate acid chloride (0.01 mol) and anhydrous potassium carbonate (2.07 g, 0.015 mol) in tetrahydrofuran (30 mL) was heated under reflux for 2 h. The solid that separated after cooling was collected by filtration, washed with water, dried and crystallized from ethanol to afford compounds **4a&b**

**2.1.3.1 4-(4-Chlorophenyl)-7-methoxy-2-oxo-2*H*-chromene-3-carbonitrile (4a)**

**4a**: IR (KBr): 3096 (CH arom.), 2990 (CH aliph.), 2219 (C≡N), 1727 (C=O) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 3.94 (s, 3H, $OCH_3$); 6.85 (s, 1H, ArH); 6.90 (d, $J_{value}$ = 9 Hz,1H, ArH); 7.24 (d, $J_{value}$ = 9 Hz, 1H, ArH); 7.43 (d, $J_{value}$ = 8.4 Hz, 2H, ArH); 7.60 (d, $J_{value}$ = 8.4 Hz, 2H, ArH); $^{13}$C NMR ($CDCl_3$): $\delta$ 56.24, 97.92, 101.27, 111.49, 113.79, 114.11, 129.53, 129.86, 130.44, 137.41, 156.43, 157.29, 162.58, 165.72; GCMS: *m/z* 313 (M+2 $^{\cdot+}$, 35.63), 311 (M $^{\cdot+}$, 100), 283 (M-CO $^{\cdot+}$, 46.37).

**2.1.3.2 4-(4-Chlorophenyl)-7-ethoxy-2-oxo-2*H*-chromene-3-carbonitrile (4b)**

**4b**: IR (KBr): 3095 (CH arom.), 2944 (CH aliph.), 2224 (C≡N), 1730 (C=O) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 1.48 (t, $J_{value}$ = 7.2 Hz, 3H, $CH_3$); 4.15 (q, $J_{value}$ = 7.2 Hz, 2H, $CH_2$); 6.83 (s, 1H, ArH); 6.88 (d, $J_{value}$ = 9 Hz, 1H, ArH); 7.24 (d, $J_{value}$ = 9 Hz, 1H, ArH); 7.41 (d, $J_{value}$ = 8.7 Hz, 2H, ArH); 7.59 (d, $J_{value}$ = 8.7 Hz, 2H, ArH).

**2.1.4 General procedure for the preparation of compounds *(ZE) (RS)*-ethyl *N*-7-alkoxy-4-(4-chlorophenyl)-3-cyano-4*H*-chromen-2-ylformimidates (5a&b)**

A mixture of compound **2a&b** (0.01 mol) and triethyl orthoformate (20 mL) was heated under reflux for 4 h. The reaction mixture was evaporated under reduced pressure, and then the residue was washed with ethanol and crystallized from the appropriate solvent to give **5a&b**.

**2.1.4.1 *(ZE) (RS)*-Ethyl *N*-4-(4-chlorophenyl)-3-cyano-7-methoxy-4*H*-chromen-2-ylformimidate (5a)**

Compound **5a** was crystallized from absolute ethanol, the following spectral data were recorded for compound **5a**: IR (KBr): 3068 (CH arom.), 2965 (CH aliph.), 2200 (C≡N), 1644 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 1.39 (t, $J_{value}$ = 7.6 Hz, 3H, $CH_2C\underline{H}_3$); 3.80 (s, 3H, $OCH_3$); 4.43 (q, $J_{value}$ = 7.6 Hz, 2H, $CH_2$); 4.80 (s, 1H, C4H); 6.60 (s, 1H, ArH); 6.65 (d, $J_{value}$ = 8.7 Hz, 1H, ArH); 6.84 (d, $J_{value}$ = 8.7 Hz, 1H, ArH); 7.15 (d, $J_{value}$ = 8.7 Hz, 2H, ArH); 7.30 (d, $J_{value}$ = 8.7 Hz, 2H, ArH); 8.38 (s, 1H, N=CH).

**2.1.4.2 *(ZE) (RS)*-Ethyl *N*-4-(4-chlorophenyl)-3-cyano-7-ethoxy-4*H*-chromen-2-ylformimidate (5b)**

Compound **5b** was crystallized from methanol, the following spectral data were recorded for compound **5b**: IR (KBr): 3067 (CH arom.), 2981 (CH aliph.), 2208 (C≡N), 1619 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 1.28-1.36 (m, 6H, $2CH_2C\underline{H}_3$); 3.94 (q, $J_{value}$ = 7.2 Hz, 2H, $OC\underline{H}_2CH_3$); 4.35 (q, $J_{value}$ = 7.6 Hz, 2H, $N{=}CHOC\underline{H}_2CH_3$); 4.71 (s, 1H, C4H); 6.49 (s, 1H, ArH); 6.54 (d, $J_{value}$ = 8.4 Hz, 1H, ArH); 6.74 (d, $J_{value}$ = 8.4 Hz, 1H, ArH); 7.07 (d, $J_{value}$ = 8.4 Hz, 2H, ArH); 7.21 (d, $J_{value}$ = 8.4 Hz, 2H, ArH); 8.30 (s, 1H, N=CH); MS: *m/z* 384 (M+2 $^{\cdot+}$, 7.31), 382 (M $^{\cdot+}$, 20.40), 271 (M-$C_6H_4Cl$ $^{\cdot+}$, 100).

**2.1.5 General procedure for the preparation of compounds *(ZE) (RS)-N'*-(7-alkoxy-4-(4-chlorophenyl)-3-cyano-4*H*-chromen-2-yl)-*N*-carbamothioylformimidamides (6a&b)**

To a mixture of the iminoether **5a&b** (0.01 mol) and thiourea (0.76 g, 0.01 mol) in absolute ethanol (30 mL), sodium ethoxide (0.01 mol) [sodium metal (0.23 g, 0.01 mol) and absolute ethanol (5 mL)] was added. 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 benzene to afford **6a&b**.

**2.1.5.1 *(ZE) (RS)-N'*-(4-(4-Chlorophenyl)-3-cyano-7-methoxy-4*H*-chromen-2-yl)-*N*-carbamothioylformimid amide (6a)**

**6a**: IR (KBr): 3481, 3376, 3340 (NH, $NH_2$), 3186 (CH arom.), 2939 (CH aliph.), 2193 (C≡N), 1629 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 2.34 ( br. s, 1H, NH, $D_2O$ exchangeable); 3.78 (s, 3H, $OCH_3$); 4.95 (s, 1H, C4H); 5.02 (s, 2H, $NH_2$, $D_2O$ exchangeable); 6.59-7.36 (m, 7H, ArH); 8.31 (s, 1H, N=CH).

**2.1.5.2 *(ZE) (RS)-N'*-(4-(4-Chlorophenyl)-3-cyano-7-ethoxy-4*H*-chromen-2-yl)-*N*-carbamothioylformimid amide (6b)**

**6b**: IR (KBr): 3381, 3336 (NH, $NH_2$), 3180 (CH arom.), 2979 (CH aliph.), 2194 (C≡N), 1621 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 1.38 (t, $J_{value}$ = 7.2 Hz, 3H, $CH_3$); 2.60 (br. s, 1H, NH, $D_2O$ exchangeable); 4.00 (q, $J_{value}$ = 7.2 Hz, 2H, $CH_2$); 4.96 (s, 1H, C4H); 5.26 (br.s, 2H, $NH_2$, $D_2O$ exchangeable); 6.59-7.34 (m, 7H, ArH); 8.32 (s, 1H, N=CH); MS: *m/z* 414 (M+2 $^{\cdot+}$, 30.86), 412 (M $^{\cdot+}$, 37.88), 411 (M-1 $^{\cdot+}$, 42.01), 55 ( M-$C_{18}H_{14}ClN_2O_2S$ $^{\cdot+}$, 100).

**2.1.6 General procedure for the preparation of compounds *(ZE) (RS)*-7-alkoxy-4-(4-chlorophenyl)-2-(morpholinomethyleneamino)-4*H*-chromene-3-carbonitriles (6c&d)**
A solution of compound **5a&b** (0.01mol) and morpholine (0.87 g, 0.01 mol) in ethanol (30 mL) was stirred at room temperature for 1 h. The solid formed was filtered, dried and crystallized from ethanol to give **6c&d**.

**2.1.6.1 *(ZE) (RS)* 4-(4-Chlorophenyl)-7-methoxy-2-(morpholinomethyleneamino)-4*H*-chromene-3-carbonitrile (6c)**
**6c**: IR (KBr): 3100 (CH arom.), 2907(CH aliph.), 2186 (C≡N), 1595 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 3.51 (t, $J_{value}$ = 6.4 Hz, 2H, $CH_2$ morpholine); 3.72-3.83 (m, 9H, $3CH_2$ morpholine and $OCH_3$); 4.76 (s, 1H, C4H); 6.58-7.28 (m, 7H, ArH); 8.23 (s, 1H, N=CH).

**2.1.6.2 *(ZE) (RS)* 4-(4-Chlorophenyl)-7-ethoxy-2-(morpholinomethyleneamino)-4*H*-chromene-3-carbonitrile (6d)**
**6d**: IR (KBr): 3100 (CH arom.), 2923 (CH aliph.), 2197 (C≡N), 1603 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 1.40 (t, $J_{value}$ = 7.2 Hz, 3H, $CH_3$); 3.50 (t, $J_{value}$ = 6.4 Hz 2H, $CH_2$ morpholine); 3.72-3.85 (m, 6H, $3CH_2$ morpholine); 4.00 (q, $J_{value}$ = 7.2 Hz, 2H, $C\underline{H}_2$-$CH_3$ ); 4.75 (s, 1H, C4H); 6.56 (s, 1H, ArH); 6.59 (d, $J_{value}$ = 8.4 Hz, 1H, ArH); 6.83 (d, $J_{value}$ = 8.4 Hz, 1H, ArH); 7.14 (d, $J_{value}$ = 9 Hz, 2H, ArH); 7.26 (d, $J_{value}$ = 9 Hz, 2H, ArH); 8.22 (s, 1H, N=CH); MS: *m/z* 425 (M+2 $^{\cdot+}$, 14.17), 423 (M $^{\cdot+}$, 37.64), 312 (M-$C_6H_4Cl$ $^{\cdot+}$, 100).

**2.1.7 General procedure for the preparation of compounds *(RS)*-9-alkoxy-12-(4-chlorophenyl)-2-(pyridin-4-yl)-12*H*-chromeno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (7a&b)**
To a mixture of **5a&b** (0.01mol) and the isonicotinic acid hydrazide (1.37 g, 0.01 mol) in dioxane (30 mL), a few drops of triethylamine were added. The reaction mixture was heated under reflux for 12 h then cooled and poured into ice-cold water. The precipitated solid was filtered, washed with water, dried and crystallized from a mixture of methanol: chloroform (3:1) to afford **7a&b**.

**2.1.7.1*(RS)*12-(4-Chlorophenyl)-9-methoxy-2-(pyridin-4-yl)-12*H*-chromeno[3,2-*e*][1,2,4]triazolo [1,5-*c*]pyrimi -dine (7a)**
**7a**: IR (KBr): 3047 (CH arom.), 2964 (CH aliph.), 1627 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ $\delta$ 3.81 (s, 3H, $OCH_3$); 5.73 (s, 1H, C12H); 6.74-7.36 (m, 7H, ArH); 8.17 (d, $J_{value}$ = 6Hz, 2H, pyridinyl 3,5-H); 8.79 (d, $J_{value}$ = 6Hz , 2H, pyridinyl 2,6-H); 9.14 (s, 1H, C5H)**.**

**2.1.7.2 *(RS)* 12-(4-Chlorophenyl)-9-ethoxy-2-(pyridin-4-yl)-12*H*-chromeno[3,2*e*][1,2,4] triazolo[1,5-*c*]pyrimi -dine (7b)**
**7b**: IR (KBr): 3052 (CH arom.), 2981 (CH aliph.), 1625 (C=N) $cm^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 1.44 (t, $J_{value}$ = 7.2 Hz, 3H, $CH_3$); 4.07 (q, $J_{value}$ = 7.2 Hz, 2H, $CH_2$); 5.72 (s, 1H, C12H); 6.74 (d, $J_{value}$ = 8.1 Hz, 1H, ArH); 6.85 (s, 1H, ArH); 7.07 (d, $J_{value}$ = 8.1 Hz, 1H, ArH); 7.23 (d, $J_{value}$ = 8.4 Hz, 2H, ArH); 7.35 (d, $J_{value}$ = 8.4 Hz, 2H, ArH); 8.16 (d, $J_{value}$ = 6Hz, 2H, pyridinyl 3,5-H); 8.80 (d, $J_{value}$ = 6Hz, 2H, pyridinyl 2,6-H); 9.13 (s, 1H, C5H); MS: *m/z* 457 (M+2 $^{\cdot+}$, 15.87), 455 (M $^{\cdot+}$, 38.86), 344 (M-$C_6H_4Cl$ $^{\cdot+}$, 100), 316 (M- $C_8H_8Cl$ $^{\cdot+}$, 51.71).

## 1.2.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 1mg/mL Serial dilutions were made reaching final concentration of the compounds to 0, 5, 12.5, 25 and 50µg/mL Previous experiments have shown that DMSO at this concentration does not modify the cellular activities that we are analyzing. All chemicals used in this study are of high analytical grade. They either obtained from (Sigma-Alderich or Biorad)

**2.2.1. Measurement of potential cytotoxic activity**
The cytotoxic activity was measured *in vitro* on human breast tumour cell line (MCF-7) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan, *et al* [15].

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. Different 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 was fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color 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.

## RESULTS AND DISCUSSION

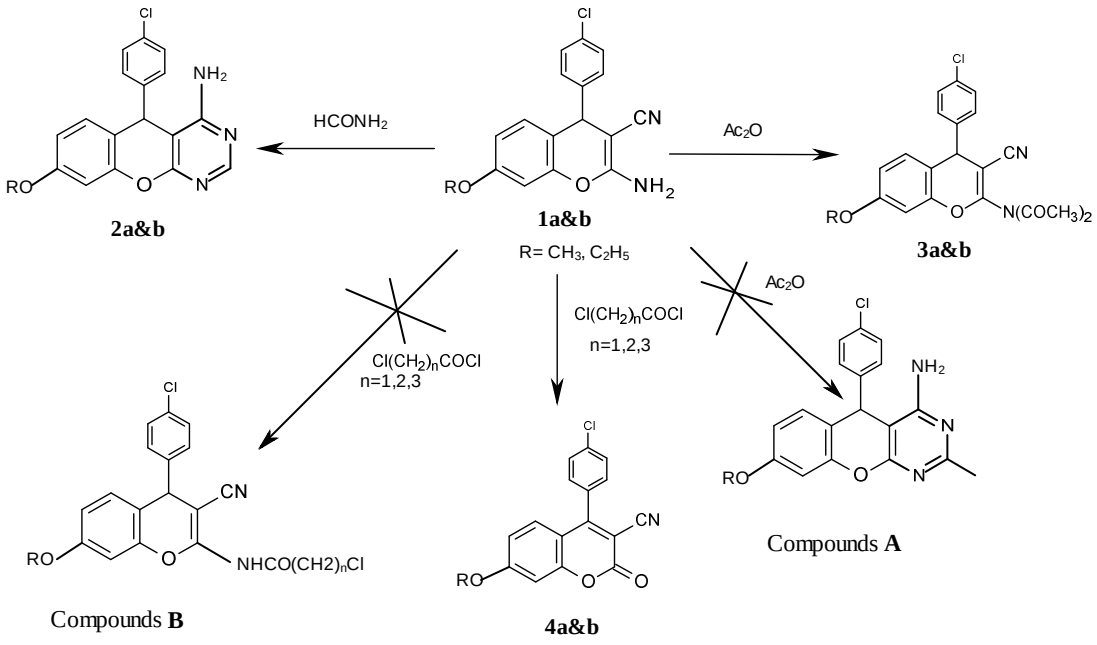
### 1.3.Chemistry

2-amino-4-(4-chlorophenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile alongside with its alkylated derivatives **1a&b** adopting the reported methods[13,14]. Reacting **1a&b** with formamide smoothly yielded 4-aminochromeno[2,3-*d*]pyrimidines **2a&b** (Scheme 1) that were confirmed using microanalyses (Table 1) and spectral data. The IR spectra showed the disappearance of the cyano group as well as the presence of an absorption band at 3385-3206 $cm^{-1}$ due to $NH_2$ group. While, the $^1H$ NMR spectra showed the presence of C2H proton signal at $\delta$ 8.09-8.18 ppm and C5H proton signal at $\delta$ 4.89-5.30 ppm. The increased chemical shift of C5H signal, compared to compounds **1a&b** can be attributed to the deshielding effect of the diamagnetic current of the aryl π-electrons.

Compounds **A** were intended to be prepared via reacting compounds **1a&b** with acetic anhydride. Several attempts were carried out to prepare these target compounds **A** but these attempts were unsuccessful since it was found out during monitoring the reaction using (TLC) that both the mono and diacetyl derivatives were formed at the exact same time. However, in the reaction of compounds **1a&b** with acetic anhydride either in a boiling water bath or under reflux temperature for five hours, the formation of diacetyl derivatives **3a&b** was considered (Scheme 1). The absence of NH and $NH_2$ absorption bands and the presence of an absorption band corresponding to C≡N group at 2220, 2219 $cm^{-1}$ and also the appearance of a broad absorption band at 1744 $cm^{-1}$ corresponding to (2 C=O) in IR spectra confirming the formed product was neither compounds **A** nor the starting materials. $^1H$ NMR spectra of compounds **3a&b** indicated a singlet signal at $\delta$ 2.46 characteristic for six protons of diacetyl moiety (2 $COCH_3$) and the disappearance of $D_2O$ exchangeable signals of either $NH_2$ or NH group. In addition, the mass spectrum of compound **3b** revealed ion peaks at *m/z* 410 and at *m/z* 412 corresponding to $(M)^{+\cdot}$ and $(M+2)^{+\cdot}$, respectively in ratio of 3:1 (Cl pattern).

For the preparation of chloroacylaminochromenes **B** (Scheme 1)**;** reacting the aminocyano-chromenes **1a&b** with the appropriate chloro acid chloride was carried out but unexpectedly this reaction did not afford the expected acyl derivatives **B** but gave compounds that identified as 2-oxo-2*H*-chromene derivatives **4a**&**b** (Scheme 2).

Scheme 1

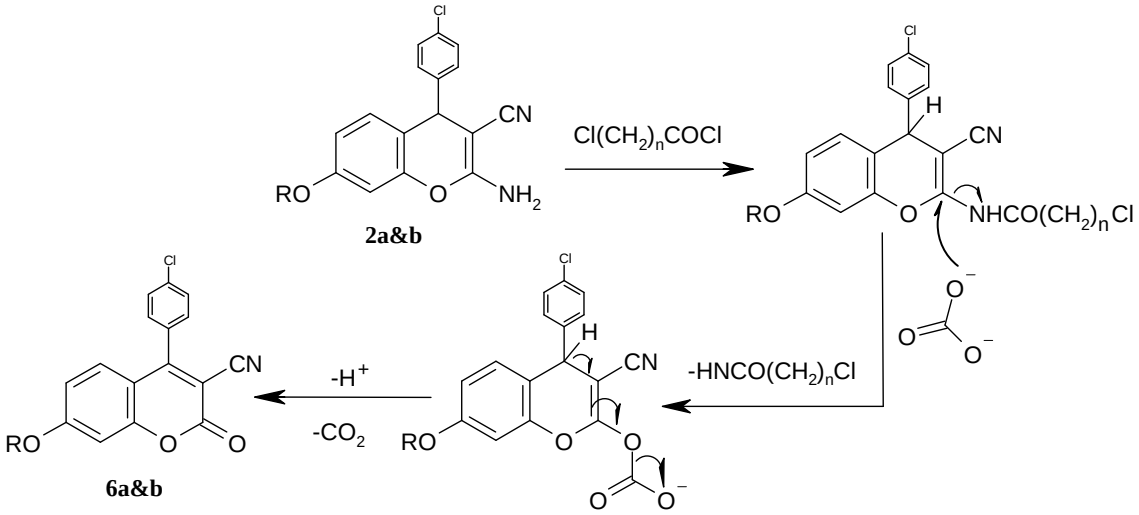



The formation of the target acyl derivatives **B** was eliminated on the basis of spectral data and element analysis (Table 1). IR spectra revealed the absence of NH group signal and the appearance of signal at 1727, 1730 $cm^{-1}$

attributed to C=O of **4a&b**, respectively. $^1$H NMR spectra showed the absence of the NH group as well as the absence of C4H and the CH acyl protons that existed in the target compounds **B**. Additional support for the structures of compounds **4a&b** was provided by $^{13}$C NMR spectrum of compound **4a**, hence the disappearance of peak corresponding to C-4 in chromene ring and the appearance of C=C instead confirming the structure of these compounds.

A suggested mechanism for the formation of compounds **4a&b** is thought to be, after the formation of the chloroacylaminochromene the bond become very weak due to the inductive effect and carbon number 2 become highly partially positive center and hence the attack with the conjugated carbonate base was easier with simultaneous break of the bond of the good leaving amide group (Figure 2).

**Figure 2**




Formimidic acid ethyl esters **5a&b** were prepared by heating the corresponding *o*-aminonitrile derivatives **1a&b** with triethyl orthoformate under reflux temperature. The IR spectra of compounds **5a&b** showed the absence of absorption bands due to $NH_2$ group. Moreover, the $^1$H NMR spectra of **5a&b** revealed the appearance of a triplet at $\delta$ 1.28-1.39 and a quartet at $\delta$ 4.35-4.43 for $CH_2CH_3$ protons and a singlet at $\delta$ 8.30-8.38 for N=CH proton. The mass spectrum of compound **5b** showed that the molecular ion peaks at *m/z* 382 $(M)^{+\cdot}$ and 384 $(M+2^{+\cdot})$ in ratio of 3:1 (Cl pattern).

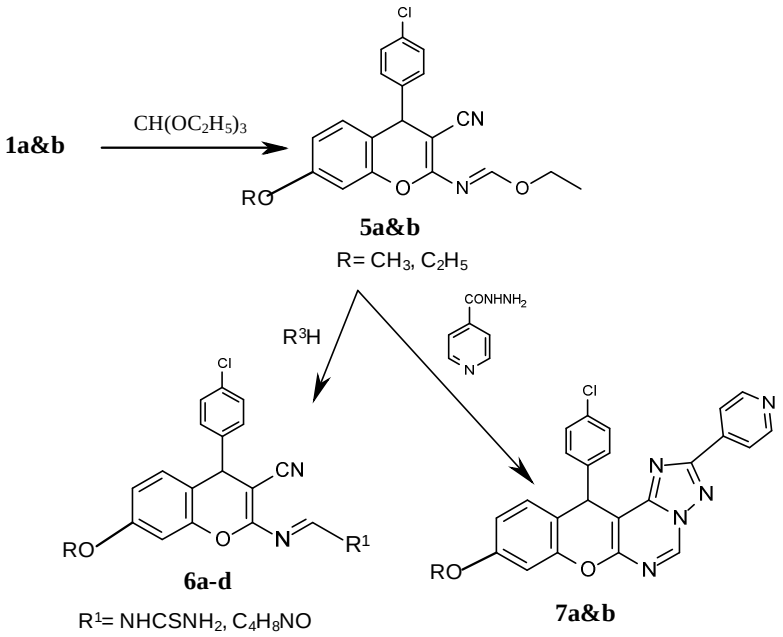
the appearance of a singlet signal at $\delta$ 7.79-8.17 attributed to (C2H) and the downfield chemical shift for the signal of C5H proton at $\delta$ 4.86 -5.64 compared to the expected value at $\delta$ 4.71-4.80 in the $^1$H NMR spectra confirmed the formation of tricyclic compounds.

**Table 1: Physical and microanalytical data of the compounds 2a&b-5a&b.**

| Compounds | R | Yield % | m.p (°C) | Mol. Formula ( M. Wt.) | Analysis % Calculated (found) C | H | N |
|---|---|---|---|---|---|---|---|
| **2a** | $CH_3$ | 96 | 226-227 | $C_{18}H_{14}ClN_3O_2$ (339.78) | 63.63 (63.92) | 4.15 (4.29) | 12.37 (12.08) |
| **2b** | $C_2H_5$ | 92 | 209-210 | $C_{19}H_{16}ClN_3O_2$ (353.81) | 64.50 (64.80) | 4.56 (4.69) | 11.88 (11.98) |
| **3a** | $CH_3$ | 78 | 134-135 | $C_{21}H_{17}ClN_2O_4$ (396.83) | 63.56 (63.81) | 4.32 (4.24) | 7.06 (7.05) |
| **3b** | $C_2H_5$ | 74 | 164-165 | $C_{22}H_{19}ClN_2O_4$ (410.86) | 64.32 (64.20) | 4.66 (4.80) | 6.82 (6.80) |
| **4a** | $CH_3$ | 83 | 207-208 | $C_{17}H_{10}ClNO_3$ (311.73) | 65.50 (65.30) | 3.23 (3.52) | 4.49 (4.39) |
| **4b** | $C_2H_5$ | 78 | 171-172 | $C_{18}H_{12}ClNO_3$ (325.75) | 66.37 (66.14) | 3.71 (3.84) | 4.30 (3.99) |
| **5a** | $CH_3$ | 88 | 130-132 | $C_{20}H_{17}ClN_2O_3$ (368.82) | 65.13 (65.28) | 4.65 (4.66) | 7.60 (7.80) |
| **5b** | $C_2H_5$ | 61 | 134-136 | $C_{21}H_{19}ClN_2O_3$ (382.85) | 65.88 (66.09) | 5.00 (4.80) | 7.32 (7.12) |

Condensation of the key intermediate iminoethers **5a&b** with different nitrogenous reagents such as thiourea and morpholine was adopted. Interaction of **5a&b** and these reagents was carried out either in ethanolic solution of iminoethers **5a&b** and thiourea then sodium ethoxide was added to help in reaction completion under reflux temperature to afford **6a&b** or just stirring the iminoethers **5a&b** and morpholine in absolute ethanol for 1 hour at room temperature to give **6c&d** (Scheme 2). The contradiction of the reaction conditions between the two reactions may be attributed to the difference in reactivity of thiourea and morpholine, since the latter is much more basic than the former reagent. The structure of compounds **6a-d** was established on the basis of spectroscopic data and element analysis (Table 2). The IR spectra of **6a&b** showed characteristic absorption bands at range of 3481-3336 $cm^{-1}$ indicating the presence of amino functionality. $^1$H NMR spectra of **6a-d** revealed the disappearance of the triplet and quartet signals attributed to the ethyl protons of the intermediates. In addition, $^1$H NMR spectra of **6a&b** showed $D_2O$ exchangeable signals at $\delta$ 2.34-2.60 and 5.02-5.26 for NH and $NH_2$, respectively while The $^1$H NMR spectra of **6c&d** demonstrated a triplet peak at $\delta$ 3.51 and 3.50 attributed to two protons of $CH_2$ of morpholine moiety and a multiplet peak at $\delta$ 3.72-3.83 attributed to nine protons ($3CH_2$ of morpholine moiety and $OCH_3$) and at $\delta$ 3.72-3.85 corresponding to six protons of $3CH_2$ of morpholine moiety, sequentially for **6c&d**.

Scheme 2




Synthesis of chromenotriazolopyrimidines **7a&b** was achieved *via* the interaction of the backbone intermediates **5a&b** with isonicotinic acid hydrazide in dioxane containing few drops of triethyl amine (Scheme 3). Spectral data of the obtained compounds confirmed their structures as **7a&b**. $^1$H NMR spectra showed the disappearance of the triplet and quartet signals attributed to the ethyl protons of its precursor **5a&b** and the presence of two doublet peaks in the aromatic region at $\delta$ 8.16-8.17 and $\delta$ 8.79-8.80 corresponding to four protons of pyridinyl moiety and two singlet signals at $\delta$ 5.72-5.73 and $\delta$ 9.13-9.14 indicating C12H and C5H, respectively. Once more, the increased chemical shift of C5H and C12H signals can be attributed to the deshielding effect of the diamagnetic current of the aryl π-electrons. Moreover, the mass spectrum of **7b** showed its ($M^{\ddagger}$) peak at *m/z* 455.

## 1.4. Antitumor activity

Colchicine was the reference drug that used in this study. The response parameter calculated was $IC_{50}$ value (Table 3), which corresponds to the compound concentration causing 50% mortality in human breast tumor cell line (MCF-7). Six of the newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity on human breast tumor cell line (MCF-7). Compounds **3a** ($IC_{50}$ = 0.007 µM) and **6a** ($IC_{50}$ = 0.008 µM) were double the reactivity of colchicine ($IC_{50}$ = 0.013 µM) while compounds **4a** ($IC_{50}$ = 0.014 µM) and **6c** ($IC_{50}$ = 0.011 µM) were nearly as active as colchicine. Moreover, Compounds **2b** ($IC_{50}$ = 0.026 µM) and **7b** ($IC_{50}$ = 0.026 µM) had half the reactivity of the positive control drug.

**Table 2: Physical and microanalytical data of the compounds 6a-d and 7a&b.**

| Compound | R | $R^1$ | Yield % | m.p (°C) | Mol. Formula ( M. Wt.) | Analysis % Calculated (found) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | N |
| **6a** | $CH_3$ | $NHCSNH_2$ | 95 | 230-229 | $C_{19}H_{15}ClN_4O_2S$ (398.87) | 57.21 (57.30) | 3.79 (4.10) | 14.05 (13.83) |
| **6b** | $C_2H_5$ | $NHCSNH_2$ | 84 | 236-235 | $C_{20}H_{17}ClN_4O_2S$ (412.89) | 58.18 (58.00) | 4.15 (4.00) | 13.57 (13.57) |
| **6c** | $CH_3$ |  | 66 | 192-191 | $C_{22}H_{20}ClN_3O_3$ (409.87) | 64.47 (64.69) | 4.92 (4.95) | 10.25 (10.05) |
| **6d** | $C_2H_5$ |  | 84 | 208-207 | $C_{23}H_{22}ClN_3O_3$ (423.89) | 65.17 (65.46) | 5.23 (5.10) | 9.91 (9.74) |
| **7a** | $CH_3$ | - | 66 | 261-262 | $C_{24}H_{16}ClN_5O_2$ (441.87) | 65.24 (65.50) | 3.65 (3.79) | 15.85 (15.71) |
| **7b** | $C_2H_5$ | - | 94 | 231-233 | $C_{25}H_{18}ClN_5O_2$ (455.90) | 65.86 (65.56) | 3.98 (3.99) | 15.36 (15.49) |

**Table 3: Results of *in vitro* cytotoxic activity of test compounds**

| **Compound no.** | **$IC_{50}$ µM** |
|---|---|
| Colchicine | 0.013 |
| **2b** | 0.026 |
| **3a** | 0.007 |
| **4a** | 0.014 |
| **6a** | 0.008 |
| **6c** | 0.011 |
| **7b** | 0.026 |

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

## CONCLUSION

In conclusion, the objective of the present study was to synthesize and investigate the anticancer activity of novel substituted chromenes, chromenopyrimidine derivatives and chromenotriazolopyrimidines. The results revealed that the corresponding chromene derivatives **3a** and **6a** showed the highest *in vitro* cytotoxic activity when compared to other tested compounds and colchicine as a reference drug. Additionally, compounds **4a** and **6c** showed moderate activity while **2b** and **7b** showed the least antitumor activity against the reference drug. It is worth to mention that compounds **3a, 6a, 4a** and **6c** have nearly the same structure as compound **I** (Figure 1) showed promising cytotoxic activity while in case of the tricyclic compounds the activity is reduced nearly by half.

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