Egypt. J. Chem. **Vol. 66,** No. 1, pp. 113-120 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis and Biological Activity of Chromene Derivatives, chromeno[2,3-d][1,3]oxazine derivatives, and chromeno[2,3-d]pyrimidine derivatives

Mahmoud N. M. Yousif[1*], Reda M.Abdelhameed[2], Ameen A. Abu-Hashem[1], Nabil M. Yousif[1]

[1]*Photochemistry Department, Chemical Industries Research Institute, National Research Centre, Cairo, Egypt*

[2]*Department of Applied Organic Chemistry, Chemical Industries Research Institute, National Research Centre, Egypt*

Abstract

Chromene derivative 1 reacted with acetyl chloride and chloroacetyl chloride to give compounds **2a,b**. Acetyl derivatives **2a,b** were heated under reflux in dry xylene to give oxazine derivatives **3a,b** which reacted with hydrazine hydrate and hydroxylamine to afford compounds **4a,b** and **5a,b** respectively. Pyrimidine derivative **4a** reacted with xylose and glucose to give compounds **6a,b** which were converted to compounds **7a,b** by reaction with acetic anhydride. The structure of the prepared derivatives was elucidated through mass spectroscopy, 1H & 13C NMR, infrared spectroscopy and elemental analysis. Anticancer activity of some of prepared compounds was performed against three different cancer cell lines.

Keywords: chromene, chromeno[2,3-d][1,3]oxazin, oxazine, anticancer activity.

1. Introduction

Chromene derivatives have gained the attention of researchers due to their different applications in medicine. Chromene derivatives have different biological properities such as anticonvulsant, antimicrobial, anticancer, antituberculosis, anticholinesterase, antidiabetic, and inhibitor of monoamine oxidase activities [1-4]. Chromene derivatives promoted apoptosis through interaction with tubulin at sites of binding of colchicine [1-4]. They also inhibited tubulin polymerization and lead to caspase dependant apoptotic and G2/M cell cycle arrest [1-4]. 4H Chromene derivatives were selective inhibitor of formyl Ca+ peptide receptor-1 (FPR-1) leading to block of calcium ion flux and inhibited chemotaxis in human neutrophils. Chromene derivatives **Ia-c** were potent antitumor agents against cancer cell lines [5]. 3,4-Dihydronaphthalene-2-carboxylate derivative **II** had a good anticancer profile with an IC50 of 20 µM [6]. Chromene derivative **III** induced cell cycle detention at G2 and S cell cycle and induced cell death through apoptosis due to potent induction of caspase-mediated apoptosis in a p53-independent manner [7].

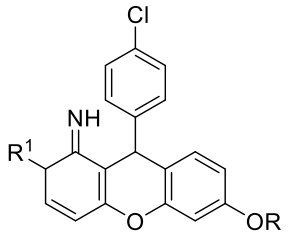
Chromeno[2,3-d]pyrimidine derivatives have also a wide range of biological activities such as anticancer activity, antimicrobial activity, antituberculosis activity, and antioxidant potential [8-10]. Chromeno[2,3-d]pyrimidine derivatives **IV,V** have potent activity against gram negative Staphylococcus Aureus as compared with standard drug cefotaxime [6].

All the aforementioned biological activities directed us to synthesize a series of novel chromene derivatives and we have evaluated the biological activity of some of the prepared compounds.

*Corresponding author e-mail: mahmoud_nabil18@yahoo.com,; (Mahmoud N. M. Yousif).

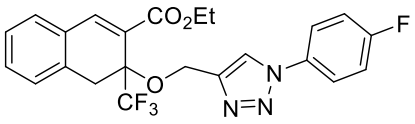
Receive Date: 05 January 2022, Revise Date: 14 March 2022, Accept Date: 13 April 2022.

DOI: 10.21608/EJCHEM.2022.114873.5214

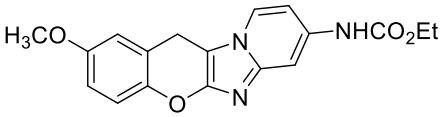


Ia-c

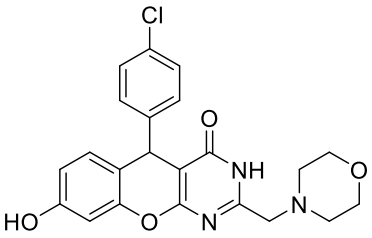
a, R=CH_3, R^1=CH_3
b, R=CH_3, R^1=NH_2
c, R=C_2H_5, R^1=CH_3
d, R=C_2H_5, R^1=NH_2



II



III



IV

Experimental

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). ^{1}H NMR was determined on a Jeol-Ex-400 NMR spectrometer (Jeol, Tokyo, Japan) and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal standard. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo electron corporation, USA). Microanalyses were operated using Mario El Mentar apparatus and satisfactory results were within the accepted range (±0.30 of the calculated values). Follow up the reactions and checking the purity of the compounds was made by TLC on silica gel-protected aluminium sheets (Type 60 F254, Merck). Mass spectra, and elemental analysis were done in Microanalytical Centre in Faculty of Science, Cairo University. ^{1}H &^{13}C NMR, IR spectra, and antimicrobial activity were done in National Research Centre, Cairo, Egypt. All used chemicals were of reagent grade and were used as supplied directly unless otherwise stated. Compound **1** was prepared according to literature [12].

*General procedure for preparation of compounds **2a,b***

A mixture of compound **1** (0.01 mole) and acid chloride (0.01 mole) in 15 mL pyridine was refluxed for 1 hour. Then, the reaction mixture is poured onto ice / HCl. The formed solid was filtered and crystallized from ethanol to give compounds **2a,b**.

*N-(8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-3-cyano-5,6,7,8-tetrahydro-4H-chromen-2-yl)acetamide **2a***

Yield: 65%; m.p. 139-141 °C; IR (KBr) cm^{-1}, ν: 3340 (NH), 2212 (CN), 1655 (C=O); ^{1}H NMR (DMSO-d6) δ/ppm: 1.42 (m, 2H, CH_2), 1.87 (s, 3H, CH_3), 2.29 (t, 2H, *J*= 7.1 Hz, CH_2), 2.41 (t, 2H, *J*=7.1 Hz, CH_2), 2.91 (s, 1H, CH), 5.32 (s, 1H, CH=), 7.21-7.35 (m, 8 H, Ar), 8.12 (brs, 1H, NH). ^{13}C NMR (DMSO-d6) δ/ppm: 22.18, 23.17, 24.02, 25.30, 26.3 (3CH_2, CH, CH_3), 110.1, 110.8, 115.1, 115.8, 120.1, 121.8, 122.5, 123.7, 124.1, 125.9, 126.3, 127.1, 127.3, 128.1, 128.3, 129.1, 129.32, 130.7, 132.7 (18 C=, CN), 155 (C=O). MS (*m/z*): 451.3 (M^+, 31%). Anal. Calcd. for $C_{25}H_{20}Cl_2N_2O_2$: C, 66.53; H, 4.47; N, 6.21; Found: C, 66.61; H, 4.51; N, 6.30.

*2-Chloro-N-(8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-3-cyano-5,6,7,8-tetrahydro-4H-chromen-2-yl)acetamide **2b***

Yield: 65%; m.p. 200-202 °C; IR (KBr) cm^{-1}, ν: 3375 (NH), 2235 (CN), 1664 (C=O); ^{1}H NMR (DMSO-d6) δ/ppm: 1.41 (m, 2H, CH_2), 2.07 (t, 2H, *J* =7.1 Hz, CH_2), 2.40 (t, 2H, *J*=7.1 Hz, CH_2), 3.53 (s, 1H, CH), 4.30 (s, 2H, CH_2Cl), 5.91 (s, 1H, CH=), 7.20-7.80 (m, 8 H, Ar), 8.30 (brs, 1H, NH). ^{13}C NMR (DMSO-d6) δ/ppm: 21.2, 22.2, 23.1, 24.1, 25.5 ($3CH_2$, CH, CH_2), 111.2, 112.8, 114.2, 115.1, 120.3, 121.6, 122.1, 123.7, 124.2, 124.9, 125.3, 126.1, 126.8, 128.3, 128.6, 129.1, 129.5, 131.2, 132.6 (18 C=, CN), 157.1 (C=O). MS (*m/z*): 485.7 (M+, 41%). Anal. Calcd. for $C_{25}H_{19}Cl_3N_2O_2$: C, 61.81; H, 3.94; N, 5.77; Found: C, 61.91; H, 3.98; N, 5.81.

*General procedure for preparation of compounds **3a,b***

A mixture of compounds **2a,b** (0.01 mole) and 50 mL of dry xylene were refluxed for 3 hours. Dry xylene is prepared by heating sodium metal (3g.) with 50 mL xylene. The reaction mixture was concentrated to its third volume and the formed solid is filtered to give compounds **3a-c**.

*9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-6,7,8,9-tetrahydro-4H,5H-chromeno[2,3-d][1,3]oxazin-4-imine **3a***

Yield: 55%; m.p. 169-171 °C; IR (KBr) cm^{-1}, ν: 3355 (NH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.08 (s, 3H, CH_3), 1.47 (t, 2H, J =7.1 Hz, CH_2), 1.56 (m, 2H, CH_2), 1.63 (t, 2H, J=7.1 Hz, CH_2), 2.40 (s, 1H, CH), 5.40 (s, 1H, CH=), 7.31-7.56 (m, 8 H, Ar), 8.20 (brs, 1H, NH). ^{13}C NMR (DMSO-d6) δ/ppm: 21.2, 23.2, 24.1, 25.4, 26.1 ($3CH_2$, CH, CH_3), 110.2, 111.8, 114.2, 115.3, 120.2, 120.8, 121.5, 122.7, 123.2, 124.9, 126.1, 126.9, 127.1, 128.9, 129.0, 129.1, 129.8, 130.1, (18 C=), 160.1, 165.2 (2 C=N). MS (*m/z*): 451.3 (M^+, 25 %). Anal. Calcd. for $C_{25}H_{20}Cl_2N_2O_2$: C, 66.53; H, 4.47; N, 6.21; Found: C, 66.61; H, 4.51; N, 6.31.

*9-(2-Chlorobenzylidene)-2-(chloromethyl)-5-(2-chlorophenyl)-6,7,8,9-tetrahydro-4H,5H-chromeno[2,3-d][1,3]oxazin-4-imine **3b***

Yield: 45%; m.p. 278-280 °C; IR (KBr) cm^{-1}, ν: 3385 (NH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.08 (t, 2H, *J* =7.1 Hz, CH_2), 1.47 (m, 2H, CH_2), 1.29 (t, 2H, *J*= 7.1Hz, CH2), 2.43 (s, 1H, CH), 3.17 (s, 2H, CH_2Cl), 5.41 (s, 1H, CH=), 7.31-7.53 (m, 8 H, Ar), 8.41 (brs, 1H, NH). ^{13}C NMR (DMSO-d6) δ/ppm: 20.1, 22.1, 23.5, 24.6, 25.3 ($4CH_2$, CH), 110.1, 112.7, 113.2, 114.1, 118.3, 120.6, 121.1, 122.4, 123.2, 124.5, 125.5, 126.2, 126.9, 127.3, 127.6, 128.2, 129.3, 131.1, 132.3 (18 C=), 160.1, 163.5(2 C=N). MS (*m/z*): 485.7 (M^+, 45 %). Anal. Calcd. for $C_{25}H_{19}Cl_3N_2O_2$: C, 61.81; H, 3.94; N, 5.77; Found: C, 61.90; H, 3.98; N, 5.81.

General procedure for preparation of compounds **4a,b**

A mixture of compounds **3a,b** (0.0l mole) and 1 mL hydrazine hydrate in 50 mL ethanol was refluxed for 1 hour. The reaction mixture was cooled to room temperature and the formed solid is filtered to afford compounds **4a,b**.

*9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-4-imino-2-methyl-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-amine **4a***

Yield: 71%; m.p. 218-220 °C; IR (KBr) cm^{-1}, ν: 3364 (NH_2), 3342 (NH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.30 (t, 2H, *J* =7.1 Hz, CH_2), 1.70 (m, 2H, CH_2), 2.00 (t, 2H, *J*=7.1 Hz, CH_2), 2.10 (s, 3H, CH_3), 2.40 (s, 1H, CH), 4.81 (s, 1H, CH=), 7.30-7.60 (m, 8 H, Ar), 8.34 (brs, 3H, NH, NH_2). ^{13}C NMR (DMSO-d6) δ/ppm: 20.1, 21.4, 22.3, 22.8, 27.1 ($3CH_2$, CH, CH_3), 110.3, 110.7, 113.1, 114.3, 121.2, 121.7, 121.9, 122.2, 123.7, 123.9, 124.2, 124.9, 126.1, 128.1, 129.3, 129.6, 129.8, 130.1, (18 C=), 160.5, 165.4 (2 C=N). MS (*m/z*): 465.3 (M^+, 36%). Anal. Calcd. for $C_{25}H_{22}Cl_2N_4O$: C, 64.52; H, 4.77; N, 12.04; Found: C, 64.61; H, 4.83; N, 12.10.

*9-(2-Chlorobenzylidene)-2-(chloromethyl)-5-(2-chlorophenyl)-4-imino-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-amine **4b***

Yield: 73%; m.p. 248-250 °C; IR (KBr) cm^{-1}, ν: 3324 (NH_2), 3345 (NH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.31 (m, 2H, CH_2), 1.50 (t, 2H, *J* =7.1 Hz, CH_2), 1.79 (t, 2H, *J* =7.1 Hz, CH_2), 2.00 (s, 1H, CH), 3.20 (s, 2H, CH_2Cl), 4.80 (s, 1H, CH=), 7.35-7.61 (m, 8 H, Ar), 8.34 (brs, 3H, NH, NH_2). ^{13}C NMR (DMSO-d6) δ/ppm: 21.2, 22.3, 23.4, 24.0, 24.7 ($4CH_2$, CH), 111.5, 112.5, 113.8, 114.0, 115.1, 121.6, 121.8, 122.4, 123.1, 123.5, 125.4, 125.9, 126.3, 127.1, 127.7, 128.1, 129.2, 131.2 (18 C=), 158.1, 163.4 (2 C=N). MS (*m/z*): 499.8 (M^+, 51%).

Anal. Calcd. for $C_{25}H_{21}Cl_3N_4O$: C, 60.08; H, 4.24; N, 11.21; Found: C, 60.13; H, 4.29; N, 11.29.

*General procedure for the preparation of compounds **5a,b***

A mixture of compounds **4a,b** (0.01 mole) and hydroxylamine hydrochloride (0.01 mole) in 40 mL pyridine were refluxed for 3 hours. The reaction mixture was cooled to room temperature and poured to ice. The formed solid was filtered and crystallized from ethanol to afford compounds **5a,b**.

*9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-4-imino-2-methyl-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-ol **5a***

Yield: 61%; m.p. 279-281 °C; IR (KBr) cm^{-1}, ν: 3326 (NH), 3225 (OH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.28 (t, 2H, *J* =7.1 Hz, CH_2), 1.67 (t, 2H, *J* =7.1 Hz, CH_2), 2.10 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 3.17 (s, 1H, CH), 4.81 (s, 1H, CH=), 7.10-7.50 (m, 8 H, Ar), 8.50 (brs, 2H, NH, OH). ^{13}C NMR (DMSO-d6) δ/ppm: 19.2, 20.4, 21.3, 23.8, 25.1 ($3CH_2$, CH, CH_3), 111.3, 111.7, 112.1, 113.3, 120.2, 121.6, 121.8, 122.1, 122.8, 123.1, 124.5, 125.9, 126.3, 127.1, 128.3, 128.6, 129.8, 130.4 (18 C=), 161.5, 164.4 (2 C=N). MS (*m/z*): 466.3 (M^+, 41 %). Anal. Calcd. for $C_{25}H_{21}Cl_2N_3O_2$: C, 64.39; H, 4.54; N, 9.01; Found: C, 64.43; H, 4.61; N, 9.10.

*9-(2-Chlorobenzylidene)-2-(chloromethyl)-5-(2-chlorophenyl)-4-imino-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-ol **5b***

Yield: 62%; m.p. 248-250 °C; IR (KBr) cm^{-1}, ν: 3410 (NH), 3237 (OH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.28 (t, 2H, *J* =7.1 Hz, CH_2), 1.52 (t, 2H, *J* =7.1 Hz, CH_2), 1.60 (m, 2H, CH_2), 2.10 (s, 1H, CH), 3.27 (s, 2H, CH_2Cl), 4.81 (s, 1H, CH=), 7.20-7.50 (m, 8 H, Ar), 7.80 (brs, 2H, NH, OH). ^{13}C NMR (DMSO-d6) δ/ppm: 20.2, 21.3, 22.4, 23.0, 25.7 ($4CH_2$, CH), 110.1, 111.2, 113.4, 113.8, 114.1, 120.6, 121.9, 122.6, 123.4, 123.9, 124.1, 125.3, 126.4, 127.2, 127.9, 128.2, 129.1, 135.2 (18 C=), 159.2, 160.4 (2 C=N). MS (*m/z*): 500.8 (M^+, 51 %). Anal. Calcd. for $C_{25}H_{20}Cl_3N_3O_2$: C, 59.96; H, 4.03; N, 8.39; Found: C, 60.05; H, 4.10; N, 8.45.

*General procedure for the preparation of compounds **6a,b***

A mixture of compound **4a** (0.01 mole), and 0.01 mole of sugar in 40 mL acetic acid were refluxed for 6 hours. The reaction mixture was cooled to room temperature and the formed solid was filtered and crystallized from ethanol to afford compounds **6a,b**.

*5-((9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-4-imino-2-methyl-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-yl)imino)pentane-1,2,3,4-tetraol **6a***

Yield: 51%; m.p. 135-137 °C; IR (KBr) cm^{-1}, ν: 3410 (NH), 3280 (OH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.18 (t, 2H, *J* =7.1 Hz, CH_2), 1.51 (t, 2H, *J* =7.1 Hz, CH_2), 2.10 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 2.57 (s, 1H, CH), 3.11 (t, 1H, *J*=7 Hz, CHOH), 3.20 (t, 1H, *J*= 7 Hz, CHOH), 3.40 (m, 1H, CHOH), 3.70 (d, 2H, *J*=7 Hz, CH_2OH), 4.89 (s, 1H, CH=), 7.10-7.470 (m, 8 H, Ar), 8.20 (d, 1H, J=6.2 Hz, CH=N), 8.57 (brs, 5H, NH, 4OH). ^{13}C NMR (DMSO-d6) δ/ppm: 20.1, 20.9, 22.4, 24.8, 26.1 ($3CH_2$, CH, CH_3), 60.2, 60.9, 65.3, 70.5 (4COH), 110.2, 111.6, 112.3, 113.6, 120.1, 122.4, 122.8, 123.1, 123.8, 124.2, 124.7, 125.8, 126.1, 126.8, 128.1, 129.6, 129.8, 152.4 (18 C=), 160.4, 160.9, 164.6 (3 C=N). Anal. Calcd. for $C_{30}H_{30}Cl_2N_4O_5$: C, 60.31; H, 5.06; N, 9.38; Found: C, 60.39; H, 5.12; N, 9.43.

*6-((9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-4-imino-2-methyl-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-yl)imino)hexane-1,2,3,4,5-pentaol **6b***

Yield: 56%; m.p. 195-197 °C; IR (KBr) cm^{-1}, ν: 3380 (NH), 3270 (OH); ^{1}H NMR (DMSO-d6) δ/ppm: 1.21 (t, 2H, *J* =7.1 Hz, CH_2), 1.60 (t, 2H, *J* =7.1 Hz, CH_2), 2.20 (m, 2H, CH_2), 2.57 (s, 1H, CH), 3.10 (t, 1H, *J*=7 Hz, CHOH), 3.25 (t, 1H, *J*= 7 Hz, CHOH), 3.44 (t, 1H, J=7 Hz, CHOH), 3.50 (m, 1H, CHOH), 3.81 (d, 2H, *J*=7 Hz, CH_2OH), 3.81 (s, 2H, CH_2Cl), 4.89 (s, 1H, CH=), 7.20-7.37 (m, 8 H, Ar), 8.30 (d, 1H, *J*=6.2 Hz, CH=N), 8.61 (brs, 6H, NH, 5OH). ^{13}C NMR (DMSO-d6) δ/ppm: 19.1, 20.1, 22.3, 24.9, 25.1 ($3CH_2$, CH, CH3), 61.1, 63.1, 64.2, 70.5, 73.6 (5COH), 111.2, 111.8, 112.1, 113.2, 121.1, 122.5, 122.9, 123.2, 124.7, 125.1, 125.7, 126.8, 126.9, 127.8, 128.2, 129.3, 129.8,

152.3 (18 C=), 161.2, 160.3, 165.2 (3 C=N). Anal. Calcd. for $C_{31}H_{32}Cl_2N_4O_6$: C, 59.34; H, 5.14; N, 8.93; Found: C, 59.39; H, 5.19; N, 8.98.

*General procedure for the preparation of compounds **7a,b***

A mixture of compounds **6a,b** (0.01 mole) and 15 mL acetic anhydride were refluxed for 20 hours. The reaction mixture was cooled and poured onto ice. The formed solid was filtered and crystallized from ethanol to afford compounds **7a,b**.

*5-((9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-4-imino-2-methyl-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-yl)imino)pentane-1,2,3,4-tetrayl tetraacetate **7a***

Yield: 75%; m.p. 100-101 °C; IR (KBr) cm^{-1}, ν: 3374 (NH), 1748 (C=O); ^{1}H NMR (DMSO-d6) δ/ppm: 1.20 (t, 2H, *J* =7.1 Hz, CH_2), 1.41 (t, 2H, *J* =7.1 Hz, CH_2), 1.80 (s, 12 H, CH_3), 2.10 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 2.41 (s, 1H, CH), 3.21 (t, 1H, *J*=7 Hz, CHOH), 3.30 (t, 1H, *J*= 7 Hz, CHOH), 3.50 (m, 1H, CHOH), 3.82 (d, 2H, J=7 Hz, CH_2OH), 4.94 (s, 1H, CH=), 7.20-7.46 (m, 8 H, Ar), 8.10 (d, 1H, *J*=6.2 Hz, CH=N), 8.57 (brs, 1H, NH). ^{13}C NMR (DMSO-d6) δ/ppm: 21.1, 21.9, 22.3, 24.9, 26.2, 27.3, 28.6, 29.2, 30.1 (3CH_2, CH, 5 CH_3), 61.2, 62.9, 63.3, 71.5 (4COAc), 111.2, 111.7, 112.4, 113.9, 121.1, 122.6, 122.9, 123.2, 123.7, 124.1, 124.6, 125.7, 126.3, 126.9, 128.3, 129.1, 129.9, 153.4 (18 C=), 161.4, 162.9, 165.6 (3 C=N), 170.2 (4 C=O). Anal. Calcd. for $C_{38}H_{38}Cl_2N_4O_9$: C, 59.61; H, 5.00; N, 7.32; Found: C, 59.68; H, 5.10; N, 7.39.

*6-((9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-4-imino-2-methyl-6,7,8,9-tetrahydro-4H-chromeno[2,3-d]pyrimidin-3(5H)-yl)imino)hexane-1,2,3,4,5-pentayl pentaacetate **7b***

Yield: 81%; m.p. 118-120 °C; IR (KBr) cm^{-1}, ν: 3395 (NH), 1741 (C=O); ^{1}H NMR (DMSO-d6) δ/ppm: 1.10 (t, 2H, *J* =7.1 Hz, CH_2), 1.40 (t, 2H, *J* =7.1 Hz, CH_2), 1.80 (s, 15 H, 5 CH_3), 2.10 (m, 2H, CH_2), 2.47 (s, 1H, CH), 3.04 (t, 1H, *J*=7 Hz, CHOH), 3.21 (t, 1H, *J*= 7 Hz, CHOH), 3.51 (t, 1H, *J*=7 Hz, CHOH), 3.70 (m, 1H, CHOH), 3.90 (d, 2H, *J*=7 Hz, CH_2OH), 4.11 (s, 2H, CH_2Cl), 4.92 (s, 1H, CH=), 7.15-7.37 (m, 8 H, Ar), 8.40 (d, 1H, *J*=6.2 Hz, CH=N), 8.61 (brs, 1H, NH). ^{13}C NMR (DMSO-d6) δ/ppm: 20.1, 21.5, 22.1, 23.9, 25.2, 26.3, 27.6, 28.2, 30.1, 31.8 (3CH_2, CH, 6 CH_3), 61.3, 62.4, 63.4, 72.5, 75.2 (5COAc), 110.2, 111.4, 112.3, 113.5, 120.1, 121.6, 121.9, 122.2, 123.5, 124.3, 124.9, 125.5, 126.1, 126.2, 129.1, 129.5, 129.9, 151.4 (18 C=), 160.4, 161.9, 163.6 (3 C=N), 169.2 (4 C=O). Anal. Calcd. for $C_{41}H_{42}Cl_2N_4O_{11}$: C, 58.79; H, 5.05; N, 6.69; Found: C, 58.83; H, 5.12; N, 6.74.

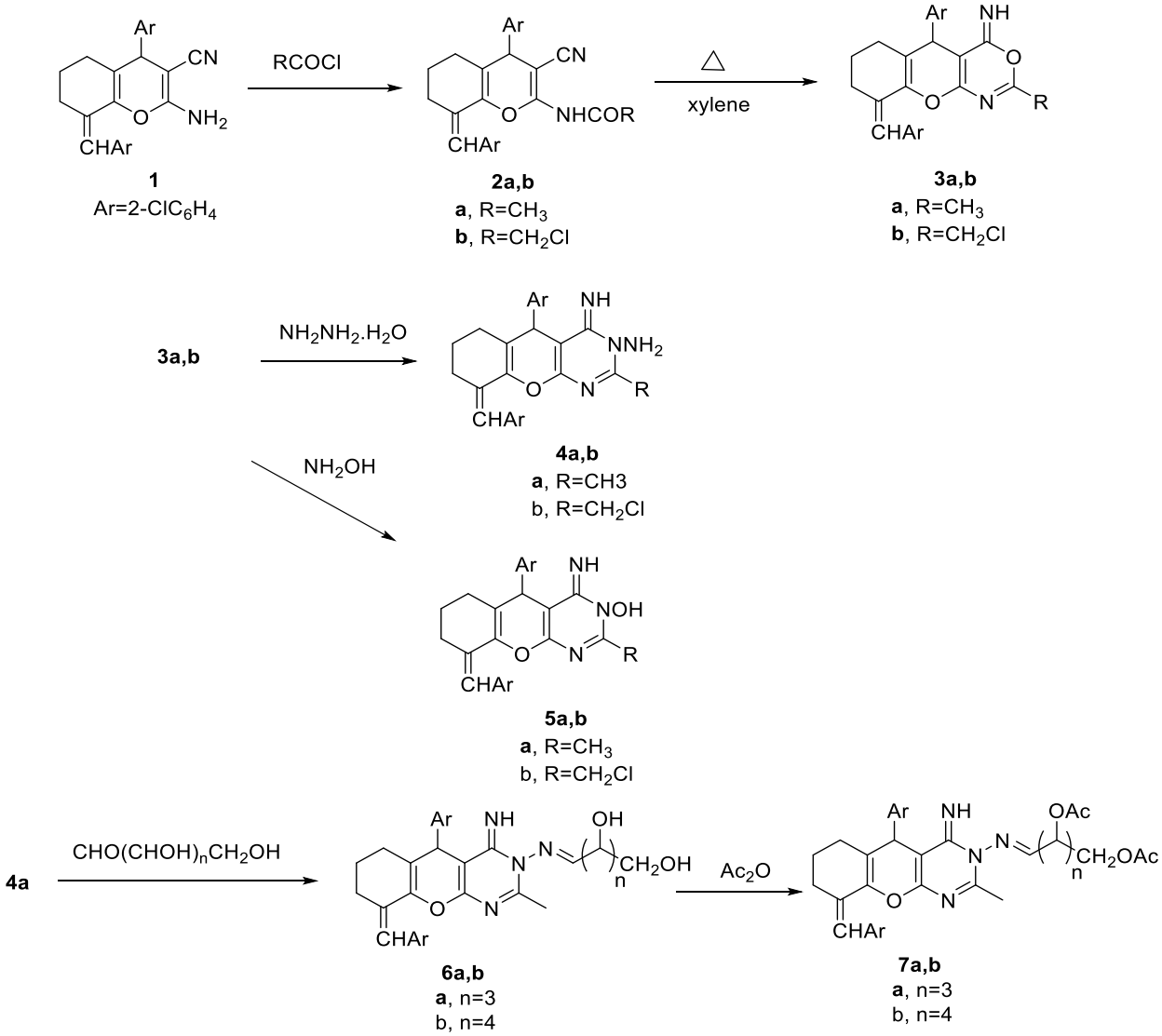
Results and Discussion

Aminocyano derivative **1** reacted with acetyl chloride and chloroacetyl chloride to give compounds **2a,b.** Acetyl derivatives **2a,b** were cyclized into oxazine derivatives **3a,b**. Spectroscopic data (^{1}H and ^{13}C NMR, infrared spectroscopy, and elemental analysis) were in agreement with the proposed structures (*cf*. experimental). The infrared spectrum of compound **2a** showed appearance of carbonyl group absorption at 1655 cm^{-1}. Compound **2a** showed characteristic chemical shift at 1.87 ppm as singlet corresponding to CH_3 in the ^{1}H NMR. Compound **2a** exhibited characteristic chemical shift at 155 ppm corresponding to the carbonyl group in the ^{13}C NMR. The absorption band of the cyano group has disappeared in the infrared spectrum of compound **3a**. Derivative **3a** exhibited disappearance of chemical shift for the carbonyl group in the ^{13}C NMR.

Oxazine derivatives **3a,b** reacted with hydrazine hydrate and hydroxylamine to afford compounds **4a,b** and **5a,b** respectively. The structures of compounds **4a-f** and **5a,b** were confirmed from ^{1}H NMR, IR, and mass spectral data. The infrared spectrum of compound **4a** exhibits the absorption band for the amino group at v 3364 cm^{-1}. The mass spectroscopy of compound **4a** shows M^+ at *m/z* 465.3. Compound **5a** showed a characteristic band for the hydroxyl group at v 3225 cm^{-1}**.** The mass spectroscopy of compound **5a** showed molecular ion peak at *m/z* 466.3.

Pyrimidine derivative **4a** reacted with xylose and glucose to give compounds **6a,b** which were converted to compounds **7a,b** by reaction with acetic anhydride. The spectroscopic results of acetylated compounds **6a-b** and **7a,b** were in compatibility with the suggested structure. The infrared spectrum of compound **6a** exhibited disappearance of the absorption band for the amino group and the appearance of hydroxyl group

characteristic band at v 3280 cm^{-1}. Derivative **6a** showed different chemical shifts corresponding to the sugar moiety in the ^{1}H NMR. The elemental analysis of compound **6a** confirmed the molecular formula of the compound. The infrared spectrum of derivative **7a** exhibited hydroxyl group absorption band disappearance and carbonyl group absorption band appearance at 1748 cm^{-1}. Derivative **7a** has a characteristic chemical shift for the carbonyl group at 170.2 ppm in the ^{13}C NMR. Derivative **7a** has a chemical shift at 1.80 ppm as a singlet signal corresponding to methyl moiety of the acetyl group in the ^{1}H NMR.



Anticancer activity

The anticancer activity of some of the prepared compounds was performed on three tumor cell lines namely human epithelial colorectal adenocarcinoma cells CaCo-2, adenocarcinomic human alveolar basal epithelial cells A-549, and human colorectal adenocarcinoma cell line HT-29. The anticancer activity is presented in table 1 at 100 µM.

Table 1. Cytotoxicity percentile of prepared derivatives on tumor cell lines at 100 μM.

Compounds No.	A-549	CaCo-2	HT-29
2a	20±1.2	71±0.6	31±0.9
2b	24±0.7	63±0.8	39±1.2
3a	31±0.5	61±1.2	38±0.9
3b	35±0.9	63±1.6	46±1.2
4a	41±0.4	59±1.2	56±1.6
4b	47±1.5	52±1.7	23±1.4
5a	49±1.8	89±1.3	90±1.5
5b	51±2.1	54±1.2	84±1.3
6a	81±0.6	38±1.8	86±1.7
6b	75±0.9	90.1±1.4	71±0.9
Doxorubicin	90	90	90

*Results are presented as average percentile cytotoxicity ± SD, n=3

Derivatives **6a**, and **6b** have anticancer activity against A-549 cell lines near to reference drug doxorubicin. Compounds **2a,b**, **3a,b**, **4a,b**, and **5a,b** have weak anticancer activity against A-549 cell lines towards reference drug doxorubicin. Compound **6b** has nearly the same cytotoxic activity towards CaCo-2 cell lines as compared to doxorubicin. Compound **5a** has an anticancer activity towards CaCo-2 cell lines similar to that of doxorubicin. Compounds **2a,b**, **3a,b**, **4a,b**, **5b**, and **6a** have weak anticancer activity towards CaCo-2 cell lines as compared to doxorubicin. Compound **5a** has anticancer activity towards HT-29 cell lines the same as doxorubicin. Compounds **5b**, **6a** have anticancer activities towards HT-29 cell lines close to that of doxorubicin. Compounds **2a,b**, **3a,b**, **4a,b**, and **6b** displayed weak anticancer activities towards HT-29 cell lines as compared with doxorubicin.

Conclusion

A series of novel heterocyclic compounds **2a,b, 3a,b, 4a,b, 5a,b, 6a,b,** and **7a,b** have been prepared and characterized using mass spectroscopy, infrared spectroscopy, ^{1}H NMR, ^{13}C NMR and elemental analysis. The anticancer activity of some of the prepared compounds was performed on three tumor cell lines namely human epithelial colorectal adenocarcinoma cells CaCo-2, adenocarcinomic human alveolar basal epithelial cells A-549, and human colorectal adenocarcinoma cell line HT-29.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

The authors acknowledge National Research Centre.

Funding

The manuscript didn't receive any funding.

References

1- Raj V., Lee J., 2H/4H-Chromenes-A Versatile Biologically Attractive Scaffold. *Frontiers in Chemistry*, **8**: 623-646 (2020). doi: 10.3389/fchem.2020.00623

2-Abd-El-Aziz A., El-Ghezlani E., Elaasser M., Afifi T., and Okasha R., First example of cationic cyclopentadienyliron based chromene complexes and polymers: synthesis, characterization, and biological applications. *J. Inorg. Organomet. Polym. Mater.*, **30**: 131–146 (2020). doi: 10.1007/s10904-019-01295-w

3- Afifi T. H., Riyadh S. M., Deawaly A. A., and Naqvi A., Novel chromenes and benzochromenes bearing arylazo moiety: molecular docking, in-silico admet, in-vitro antimicrobial and anticancer screening. *Med. Chem. Res.*, **28**: 1471–1487 (2019). doi: 10.1007/s00044-019-02387-5

4- Ahmed H. E., El-Nassag M. A., Hassan A. H., Mohamed H. M., Halawa A. H., Okasha R. M., Ihmaid S., Abd El-Gilil, S. M., Khattab, E. S. A. E. H., Fouda A. M., El-Agrody A. M., Aljuhanie A., Afifie T. H., Developing lipophilic aromatic halogenated fused systems with specific ring orientations, leading to potent anticancer analogs and targeting the c-Src kinase enzyme. *J. Mol. Struct.* **1186**, 212–223 (2019). doi: 10.1016/j.molstruc.2019.03.012

5- Kandeel M. M., Kamal A. M., Abdelall E. K., Elshemy H.A., Synthesis of novel chromene derivatives of expected antitumor activity. *Eur. J. Med. Chem.*, **59**: 183–193 (2013). doi: 10.1016/j.ejmech.2012.11.011

6- Reddy K. R., Rao P.S., Dev G.J., Poornachandra Y., Kumar C. G., Rao P.S., Narsaiah B., Synthesis of novel 1, 2, 3-triazole/isoxazole functionalized 2h-chromene derivatives and their cytotoxic

activity. *Bioorg.Med. Chem. Lett.*, **24**: 1661–1663 (2014). doi: 10.1016/j.bmcl.2014.02.069

7- Lima C.F., Costa M., Proença M., Pereira-Wilson C. C., Novel structurally similar chromene derivatives with opposing effects on p53 and apoptosis mechanisms in colorectal HCT116 cancer cells. *Eur. J. Pharm. Sci.*, **72**: 34–45 (2015). doi: 10.1016/j.ejps.2015.02.019

8- Karimi N., Davoodnia A., and Pordel M., Synthesis of new 3H-chromeno[2,3-d]pyrimidine-4,6(5H,7H)-diones via the tandem intramolecular Pinner/Dimroth rearrangement. *Heterocycl. Commun.*, **24** (1): 31–35 (2018). doi: 10.1515/hc-2017-0228

9- Nemr M. T. M. and AboulMagd A. M., New fused pyrimidine derivatives with anticancer activity:synthesis, topoisomerase II inhibition, apoptotic inducing activity and molecular modeling study. *Bioorganic Chemistry*, **103**, 104134-104142 (2020). doi:10.1016/j.bioorg.2020.104134

10- Nemr M. T. M., Sonousi A., and Marzouk A. A., Design, synthesis and antiproliferative evaluation of newtricyclic fused thiazolopyrimidines targeting topoisomerase II: Molecular docking and apoptosis inducing activity. *Bioorganic Chemistry*, **105**, 104446-104455 (2020). doi: 10.1016/j.bioorg.2020.104446

11- Moustafa A. H., Mohammed S. M., Abd El-Salam E. A., and El-Sayed H. A., Synthesis and Antimicrobial Activity of New 3H-Chromeno[2,3-d]pyrimidine Derivatives. *Russian Journal of General Chemistry*, **90** (8): 1566-1572 (2020). doi:10.1134/S1070363220080277

12- Yousif M. N. M., El-Gazzar A. B. A., Fayed A. A., El-Manawaty M. A., Yousif N. M., Synthesis and Cytotoxic Evaluation of Novel Chromenes, and Chromene(2,3-d)pyrimidines. *Journal of Applied Pharmaceutical science*, **10** (12): 35-43 (2020).