SYNTHESIS OF SOME NEW HETEROCYCLIC COMPOUNDS CONTAINING COUMARIN RING AND EVALUATION OF THEIRS PHARMACOLOGICAL EFFECT

Reda M. Fikry[a]*, Nabila A. Ismail[a], Mohammed El-Garby[a], Enaiat M. Kamel[a] and Ahmed D. H. Deeb[a,b]

Keywords: 3-Acetyl coumarin, Nicotino-nitrile, Pyridopyrimidine, Naphthyridine, Antitumor.

2-Amino-6-(2-oxo-2*H*-chromen-3-yl)-4-phenylnicotino-nitrile was reacted with cyclohexanone, formic acid, acetic anhydride, acetophenone, triethylorthoformate and hydrazine hydrate to give the corresponding pyridopyrimidine and pyridopyridine derivatives. On the other hand, 2-amino-4-(3-chloro-phenyl)-6-(2-oxo-2*H*-chromen-3-yl)nicotinonitrile was cyclized by reacting it with urea, thiourea, formamide, triethylorthoformate, hydrazine hydrate and cyclohexanone to give the corresponding cyclic pyridopyridine, pyridopyrimidine and pyridotriazine derivatives. The potential cytotoxicity activity of compounds 7, 8, 16 and 17 was tested against breast carcinoma cell line by SRB (Sulphorhodamine-B) assay.

* Corresponding Authors
E-Mail: redmof56@yahoo.com
[a] Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt
[b] Department of Chemistry, Faculty of Science, Jazan University, Jizan, 2097, Saudi Arabia

Introduction

Coumarin and coumarin-related compounds have been proved for many years to have significant therapeutic potential. Coumarins could be synthesized by various methods, such as Pechmann,[1] Perkin,[2] Knoevenagel,[3] Reformatsky,[4] Witting,[5] Claisen[6] and flash vacuum pyrolysis reaction.[7] It has shown numerous biological activities, such as antitumor,[8] anti-HIV (NNRTI),[9] antioxidation,[10] antimicrobial activity [11] and anticancer activity. [12]

Material and methods

All melting points are uncorrected and were determined on Gallenkamp electric melting point apparatus. IR spectra (KBr discs, cm^{-1}) were recorded on a FT/IR-400 spectrophotometer (Perkin Elmer). ^{1}HNMR spectra were recorded on a Varian-300 ($CDCl_3$, DMSO-d_6) solution. Chemical shifts are reported as δ (ppm) values relative to tetramethylsilane (TMS) as internal reference. The elemental analyses were carried out at Micro analytical center, Cairo University.

3-Acetyl-*2H*-chromen-2-one (3)

3-Acetylcoumarin was prepared according to the procedure reported.[13] A mixture of salicylaldehyde (50 mmol) and ethyl acetoacetate (50 mmol) was stirred with cooling. To this mixture, 1 g of piperidine was added with shaking. The mixture was maintained at freezing temperature for 2-3 h, resulting in a yellow colored solid mass, which was separated out. It was recrystallized from ethanol to get the target compound **3**.

2-Amino-6-(2-oxo-*2H*-chromen-3-yl)-4-phenylnicotinonitrile (4a)

A mixture of 3-acetylcoumarin (10 mmol), benzaldehyde (10 mmol), malononitrile (10 mmol) and ammonium acetate (20 mmol) in acetic acid 30 ml was refluxed for 3 hrs, cooled and filtered off to get the solid mass, dried and recrystallized from acetic acid. Yield 61 %, m.p. 262 °C. Anal. Calcd. For $C_{21}H_{13}N_3O_2$ (339.35): C, 74.33; H, 3.86; N, 12.38; O, 9.43. Found: C, 74.31; H, 3.81; N, 12.35;O, 9.41. IR (KBr): 3492, 3360, 2214 and 1726 cm^{-1} attributed to (NH_2), (CN) and (C=O), respectively.

2-Amino-4-(3-chlorophenyl)-6-(2-oxo-*2H*-chromen-3-yl)nicotinonitrile (4b)

A mixture of 3-acetyl coumarin (10 mmol), 3-chlorobenzaldehyde (10 mmol), malononitrile (10 mmol) and ammonium acetate (20 mmol) in acetic acid 30 ml was refluxed for 3 h, cooled and filtered off to get the solid mass, dried and recrystallized from acetic acid. Yield 58 %, m.p. 258-260°C. Anal. Calcd. For $C_{21}H_{12}ClN_3O_2$ (373.79): C, 67.48; H, 3.24; N, 11.24; O, 8.56. Found: C, 67.44; H, 3.21; N, 11.21; O, 8.51. IR (KBr): 3313, 3193, 2202 and 1732 cm^{-1} attributed to (NH_2), (CN) and (C=O), respectively, ^{1}H NMR of compound **4b** revealed signals at δ 7.1 (s, 2H, NH_2) and δ 7.4 - 8.4 (m, 10H, ArH's).

3-(5-Amino-4-phenyl-6,7,8,9-tetrahydrobenzo[*b*][1,8]naphthyridin-2-yl)-*2H*-chromen-2-one (5)

To a solution of **4a** (10 mmol) in cyclohexanone (15 ml), anhydrous $ZnCl_2$ (10 mmol) was added and the reaction mixture was refluxed for 30 minutes. The complex with zinc

chloride was separated from the solution, the mixture dissolved in 40 % sodium hydroxide (20 ml), and extracted with benzene. The benzene layer was dried (Na_2SO_4) and evaporated to give **5**, which recrystallized from acetic acid. Yield 48 %, m.p. 312 ºC. Anal. Calcd. For $C_{27}H_{21}N_3O_2$ (419.47): C, 77.31; H, 5.05; N, 10.02; O, 7.63. Found: C, 77.27; H, 5.01; N, 10.05; O, 7.59. IR (KBr): 3384, 3210 and 1726 cm^{-1} attributed to (NH_2) and (C=O), respectively.

7-(2-Oxo-2H-chromen-3-yl)-5-phenylpyrido[2,3-d]pyrimidin-4(*3H*)-one (6)

A solution of **4a** (5 mmol) in formic acid (20 ml) was heated under reflux for 24 h then cooled, poured into crushed-ice, filtered off, dried and recrystallized from methanol to give **6**. Yield 52 %, m.p. 210-212 ºC. Anal. Calcd. For $C_{22}H_{13}N_3O_3$ (367.36): C, 71.93; H, 3.57; N, 11.44; O, 13.07. Found: C, 71.90; H, 3.52; N, 11.41; O, 13.02. IR (KBr): 3243, 1680 and 1738 cm^{-1} attributed to (NH), (C=O) and (C=O), respectively; ^{1}H NMR of compound **6** revealed signals at δ 6.6 – 8.2 (m, 12H, ArH's) and δ 12.00 (s, 1H, NH).

4-Amino-7-(2-oxo-*2H*-chromen-3-yl)-5-phenyl-1,8-naphthyridin-2(1*H*)-one (7)

A mixture of compound **4a** (5 mmol), acetic anhydride (8 ml) and phosphoric acid (8 ml) was heated under reflux for 10 h, cooled, poured into ice-water then neutralized with solid sodium carbonate to *pH* = 7, filtered off, dried and recrystallized from acetic acid. Yield 67 %, m.p. 300 ºC. Anal. Calcd. For $C_{23}H_{15}N_3O_3$ (381.38): C, 72.43; H, 3.96; N, 11.02; O, 12.59. Found: C, 72.45; H, 3.91; N, 11.00; O, 12.55. IR (KBr): 3434, 3313, 3193 and 1725 cm^{-1} attributed to (NH), (NH_2) and (C=O), respectively; ^{1}H NMR of compound **7** revealed signals at δ 2.88 (s, 2H, NH_2) and δ 7.36 - 8.66 (m, 12H, ArH's) and δ 11.8 (s, 1H, NH).

3-(5-Amino-4,7-diphenyl-1,8-naphthyridin-2-yl)-*2H*-chromen-2-one (8)

A mixture of **4a** (5 mmol) , acetophenone (5 mmol) and few drops of TEA in 1,4-dioxan (30 ml) was heated under reflux for 5 h then cooled and poured into ice-water to precipitate, filtered off, dried and recrystallized from 1,4-dioxan. Yield 63 %, m.p. 310-312 ºC. Anal. Calcd. For $C_{23}H_{19}N_3O_2$ (441.48): C, 78.90; H, 4.34; N, 9.52; O, 7.25. Found: C, 78.82; H, 4.31; N, 9.48; O, 7.21. IR (KBr): 3390, 3220 and 1726 cm^{-1} attributed to (NH_2) and (C=O), respectively; ^{1}H NMR of compound **8** revealed signals at δ 4.99 (s, 2H, NH_2) and δ 7.36 - 8.92 (m, 17H, ArH's).

Ethyl-N-(3-cyano-6-(2-oxo-*2H*-chromen-3-yl)-4-phenylpyridin-2-yl)formimidate (9)

Compound **4a** (5 mmol) was refluxed in triethylorthoformate (30 ml) for 8 h, cooled, poured into petroleum ether, filtered off, dried and recrystallized from benzene to give **9**. Yield 65 %, m.p. 222-224 ºC. Anal. Calcd. For $C_{24}H_{17}N_3O_3$ (395.41): C, 72.90; H, 4.33; N, 10.63; O, 12.14. Found: C, 72.84; H, 4.35; N, 10.60; O, 12.12. IR (KBr): 2979, 2225, 1738 and 1640 cm^{-1} attributed to ($CH_{aliphatic}$), (CN), (C=O) and (C=N), respectively.

3-(4-Hydrazinyl-5-phenylpyrido[2,3-*d*]pyrimidin-7-yl)-*2H*-chromen-2-one (10)

A mixture of compound **9** (25 mmol), hydrazine hydrate (1.5 ml, 98 %) in dry benzene (20 ml) was stirred for 4 h. at room temperature, filtered off, dried and recrystallized from methanol to give compound **10**. Yield 42 %, m.p. 274-276 ºC. Anal. Calcd. For $C_{24}H_{21}N_5O_3$ (427.46): C, 67.44; H, 4.95; N, 16.38; O, 11.23. Found: C, 67.41; H, 4.91; N, 16.32; O, 11.23. IR (KBr): 3390, 3315, 3174 and 1723 cm^{-1} attributed to (NH_2), (NH) and (C=O), respectively; ^{1}H NMR of compound **10** revealed signals at δ 2.4 (s, 2H, NH_2) and δ 4.2 (s, 1H, NH) and δ 7.3-8.9 (m, 12H, ArH's).

2-(6-Amino-5-cyano-4-phenylpyridin-2-yl)-3-(2-hydroxyphenyl)acrylohydrazide (11)

A mixture of compound **4a** (25 mmol) and equivalent amount of hydrazine hydrate in ethanol (25 ml) was heated under reflux for 4 h, cooled, filtered off, dried and recrystallized from methanol to give **11**. Yield 57 %, m.p. 304-306 ºC. Anal. Calcd. For $C_{21}H_{17}N_5O_2$ (371.39): C, 67.91; H, 4.61; N, 18.86; O, 8.62. Found: C, 67.85; H, 4.58; N, 18.82; O, 8.61. IR (KBr): 3470, 3344, 3212, 2215 and 1691 cm^{-1} attributed to (NH, NH_2), (OH), (CN) and $(C=O)_{hydrazide}$, respectively; ^{1}H NMR of compound **11** revealed signals at δ 2.00 (s, 2H, NH_2 of $NHNH_2$) and δ 5.53 (s, 1H, OH) and δ 6.7 – 7.6 (m, 10H, ArH's and Pyridine proton) and δ 7.8 (s, 2H, NH_2) and δ 8.00 (s, 1H, NH) and δ 8.6 (s, 1H, cinnamoyl proton).

4-Amino-5-(3-chlorophenyl)-7-(2-oxo-*2H*-chromen-3-yl)pyrido[2,3-d]pyrimidin-2(*1H*)-one (12a)

A mixture of compound **4b** (25 mmol) and urea (30 mmol) was heated to be fused for 2 h, then dissolved in a solution of sodium hydroxide (10 %) with stirring for 2 h, then neutralized by HCl to obtain solid product, filtered off, washed with water, dried and recrystallized from acetic acid to give **12a**. Yield 45 %, m.p. 285 ºC. Anal. Calcd. For $C_{22}H_{13}ClN_4O_3$ (416.82): C, 63.39; H, 3.14; N, 13.44; O, 11.52. Found: C, 63.35; H, 3.11; N, 13.40; O, 11.53. IR (KBr):3475, 3410, 1728 and 1724 cm^{-1} attributed to (NH_2), (C=O) and (C=O), respectively; ^{1}H NMR of compound **12a** revealed signals at δ 2.8 (s, 2H, NH_2) and δ 7.36 - 8.9 (m, 10H, ArH's) and δ 9.2 (s, 1H, NH).

3-(4-Amino-5-(3-chlorophenyl)-2-thioxo-1,2-dihydropyrido[2,3-d]pyrimidin-7-yl)-*2H*-chromen-2-one (12b)

A mixture of compound **4b** (25 mmol) and thiourea (30 mmol) was heated to be fused for 2 h, then dissolved in a solution of sodium hydroxide (10 %) with stirring for 2 h, then neutralized by HCl to obtain solid product, filtered off, washed with water, dried and recrystallized from acetic acid to give **12b**. Yield 47 %, m.p. 235 ºC. Anal. Calcd. For $C_{22}H_{13}ClN_4O_2S$ (432.88): C, 61.04; H, 3.03; N, 12.94; O, 7.39. Found: C, 61.01; H, 3.05; N, 12.91; O, 7.36. IR (KBr):

3463, 3400, 1724 and 1270 cm^{-1} attributed to (NH_2), (C=O) and (C=S), respectively.

3-(4-Amino-5-(3-chlorophenyl)pyrido[2,3-*d*]pyrimidin-7-yl)-*2H*-chromen-2-one (13)

A mixture of compound **4b** (5 mmol) and formamide (15 ml) was refluxed for 90 minutes then cooled, poured into crushed-ice to obtain the solid product, filtered off, washed, dried and recrystallized from acetic acid to give **13**. Yield 53 %, m.p. 286-288 ºC. Anal. Calcd. For $C_{22}H_{13}ClN_4O_2$ (400.82): C, 65.92; H, 3.27; N, 13.98; O, 7.98. Found: C, 65.87; H, 3.22; N, 13.91; O, 7.95. IR (KBr): 3432, 3390, 1725 and 1604 cm^{-1} attributed to (NH_2), (C=O) and (C=N), respectively.

Ethyl-N-(4-(3-chlorophenyl)-3-cyano-6-(2-oxo-*2H*-chromen-3-yl)pyridin-2-yl)formimidate (14)

Compound **4b** (5 mmol) was heated under reflux in triethylorthoformate (30 ml) for 8 h, cooled, poured into petroleum ether to completely precipitation, filtered off, dried and recrystallized from benzene. Yield 50 %, m.p. 210 ºC. Anal. Calcd. For $C_{24}H_{16}ClN_3O_3$ (429.86): C, 67.06; H, 3.75; N, 9.78; O, 11.17. Found: C, 67.09; H, 3.71; N, 9.72; O, 11.18. IR (KBr): 3061, 2993, 2220, 1733 and 1651 cm^{-1} attributed to ($CH_{aromatic}$), ($CH_{aliphatic}$), (CN), (C=O) and (C=N), respectively.

3-(5-(3-Chlorophenyl)-4-hydrazinylpyrido[2,3-*d*]pyrimidin-7-yl)-*2H*-chromen-2-one (15)

A mixture of compound **15** (25 mmol) and hydrazine hydrate (1.5 ml) in dry benzene (25 ml) was stirred at room temperature for 4 hrs, filtered off, dried and recrystallized from benzene to give **15**. Yield 42 %, m.p. 298 ºC. Anal. Calcd. For $C_{22}H_{13}ClN_4O_2$ (415.83): C, 63.54; H, 3.39; N, 16.84; O, 7.70. Found: C, 63.55; H, 3.36; N, 16.80; O, 7.67. IR (KBr): 3390, 3340, 1725 and 1604 cm^{-1} attributed to (NH, NH_2), (C=O) and (C=N), respectively; ^{1}H NMR of compound **15** revealed signals at δ 2.5 (s, 2H, NH_2) and δ 3.1 (s, 1H, NH) and δ 7.4 - 9.08 (m, 11H, ArH's).

3-(5-(3-Chlorophenyl)-4-hydroxypyrido[2,3-*d*][1,2,3]triazin-7-yl)-2*H*-chromen-2-one (16)

A solution of sodium nitrite (10 mmol) in 10 ml of water was added to a cold solution of **4b** (5 mmol) in acetic acid (30 ml) and concentrated HCl (15 ml), after completion of addition, the ice bath was removed and stirring continued at room temperature for additional 2 h. The crude product obtained was recrystallized from acetic acid. Yield 61 %, m.p. 292-294 ºC. Anal. Calcd. For $C_{21}H_{11}ClN_4O_3$ (402.79): C, 62.62; H, 2.75; N, 13.91; O, 11.92. Found: C, 62.58; H, 2.80; N, 13.92; O, 11.88. IR (KBr): 3432, 1726 and 1607 cm^{-1} attributed to (OH), (C=O) and (C=N), respectively; ^{1}H NMR of compound **16** revealed signals at δ 7.4 - 9.00 (m, 10H, ArH's) and δ 9.06 (s, 1H, OH).

3-(5-(3-Chlorophenyl)-4-hydroxypyrido[2,3-d]pyrimidin-7-yl)-*2H*-chromen-2-one (17)

A mixture of compound **4b** (5 mmol) and triethylorthoformate (10 ml) in dimethylformamide (20 ml) was heated under reflux for 45 minutes, cooled, filtered off, dried and recrystallized from acetic acid to give yellow crystals of **17**. Yield 70 %, m.p. 296-298 ºC. Anal. Calcd. For $C_{22}H_{12}ClN_3O_3$ (401.80): C, 65.76; H, 3.01; N, 10.46; O, 11.95. Found: C, 65.71; H, 3.04; N, 10.41; O, 11.91. IR (KBr): 3405 , 1724 and 1607 cm^{-1} attributed to (OH), (C=O) and (C=N), respectively; ^{1}H NMR of compound **17** revealed signals at δ 7.3 - 8.1 (m, 11H, ArH's) and δ 8.8 (s, 1H, OH).

2-(6-Amino-4-(3-chlorophenyl)-5-cyanopyridin-2-yl)-3-(2-hydroxyphenyl)acrylo-hydrazide (18)

A mixture of compound **4b** (25 mmol) and equivalent amount of hydrazine hydrate in ethanol (25 ml) was heated under reflux for 4 h, cooled, filtered off, dried and recrystallized from methanol to give **18.** Yield 69 %, m.p. 200 ºC. Anal. Calcd. For $C_{21}H_{16}ClN_5O_2$ (405.84): C, 62.15; H, 3.97; N, 17.26; O, 7.88. Found: C, 62.11; H, 3.92; N, 17.22; O, 7.86. IR (KBr): 3510, 3420, 3304, 3163, 2212 and 1652 cm^{-1} attributed to (OH), (NH), (NH, NH_2), (CN) and (C=O), respectively; ^{1}HNMR of compound **18** revealed signals at δ 2.00 (s, 2H, NH_2 of $NHNH_2$) and δ 5.53 (s, 1H, OH) and δ 6.7 – 7.6 (m, 9H, ArH's and Pyridine proton) and δ 7.8 (s, 2H, NH_2) and δ 8.00 (s, 1H, NH) and δ 8.6 (s, 1H, cinnamoyl proton).

3-(5-Amino-4-(3-chlorophenyl)-6,7,8,9-tetrahydrobenzo[*b*]-[1,8]naphthyridin-2-yl)-*2H*-chromen-2-one (19)

To a solution of **4b** (5 mmol) in cyclohexanone (8 ml) anhydrous $ZnCl_2$ (5 mmol) was added and the reaction mixture was refluxed for 50 minutes. The complex with zinc chloride was separated from the solution. Thereafter, the mixture was dissolved in 40 % sodium hydroxide (20 ml) and extracted with benzene and then benzene was evaporated to give **19** which was recrystallized from ethanol. Yield 56 %, m.p. >300ºC. Anal. Calcd. For $C_{27}H_{20}ClN_3O_2$ (453.92): C, 71.44; H, 4.44; N, 9.26; O, 7.05. Found: C, 71.38; H, 4.41; N, 9.21; O, 7.09. IR (KBr): 3480, 3395, 2932 and 1698 cm^{-1} attributed to (NH_2), ($CH_{aliphatic}$) and (C=O), respectively.

Antitumor assay

Reagents: Fetal bovine serum (FBS) and L-glutamine, were from GibcoIvitrogen Co. (Scotland, UK). RPMI-1640 medium was from cambrex (New Jersey, USA). Dimethylsulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures: Three human tumor cell lines, MCF-7 breast adenocarcinoma was obtained from the European Collection of cell cultures (ECACC, Salisbury, UK).

They grew as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5 % heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U mL^{-1}, streptomycin 100 µg mL^{-1}), at 37 ºC in a humidified atmosphere containing 5 % CO_2.

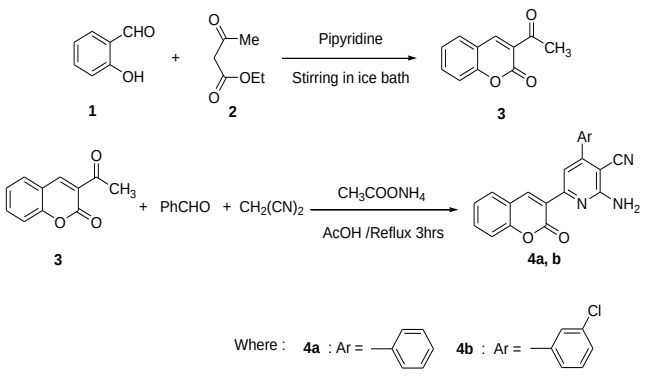
Exponentially growing cells were obtained by plating 1.5 x 105 cells mL^{-1} for MCF-7 followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5 %) of DMSO used in each assay.

Tumour cell growth assay

The effects of compounds of **7, 8, 16** and **17** on the in vitro growth of human tumor cell lines were evaluated according to the procedure[14-17] adopted by the National Cancer Institute (NCI, USA) in the "In vitro Anticancer Drug Discovery Screen" that uses the protein-binding dye Sulforhodamine B to assess cell growth. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 µM. Following this exposure period adherent cells were fixed, washed and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Wincoski, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50 % (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

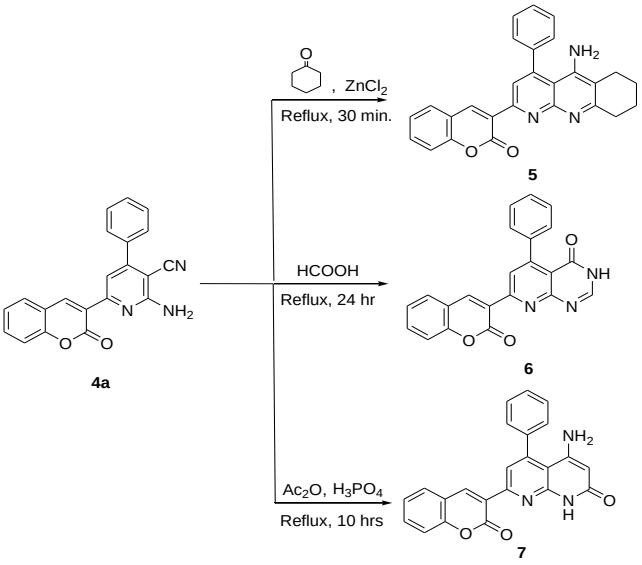
Results and discussion

3-Acetylcoumarin-2-one **3** was prepared and was allowed to react with malononitrile, benzaldehyde and/or 3-chlorobenzaldehyde in the presence of ammonium acetate and acetic acid under reflux to give the corresponding 2-amino-6-(2-oxo-2*H*-chromen-3-yl)-4-phenylnicotino-nitrile **4a**[18] or 2-amino-4-(3-chloro-phenyl)-6-(2-oxo-2*H*-chromen-3-yl)nicotinonitrile **4b** (Scheme 1).

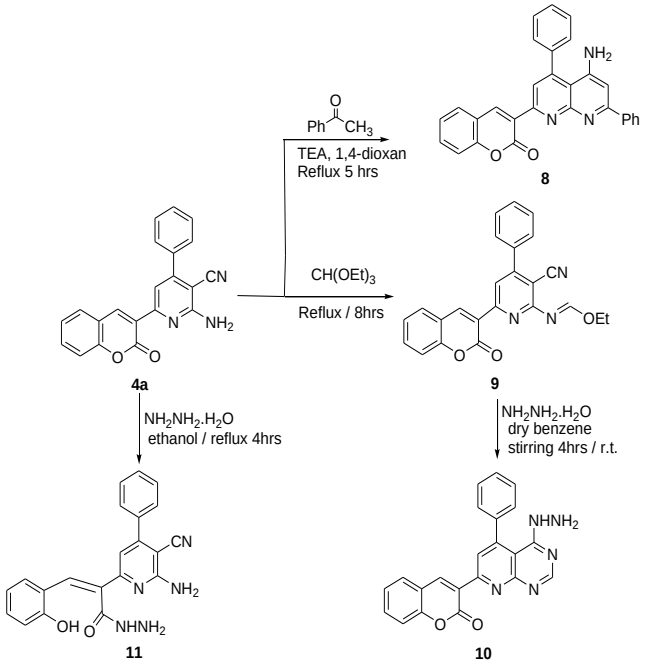


Scheme 1

Continually, compound **4a** was condensed with cyclohexanone in the presence of anhydrous zinc chloride to give the corresponding 3-(5-amino-4-phenyl-6,7,8,9-tetrahydrobenzo[*b*][1,8]naphthyridin-2-yl)-2*H*-chromen-2-one **5**. The synthesis of 7-(2-oxo-2*H*-chromen-3-yl)-5-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one **6** was achieved by the reaction of compound **4a** with formic acid. Treatment of compound **4a** with acetic anhydride in the presence of phosphoric acid underwent cyclization affording 4-amino-7-(2-oxo-*2H*-chromen-3-yl)-5-phenyl-1,8-naphthy-ridin-2(*1H*)-one **7** (Scheme 2).



Scheme 2



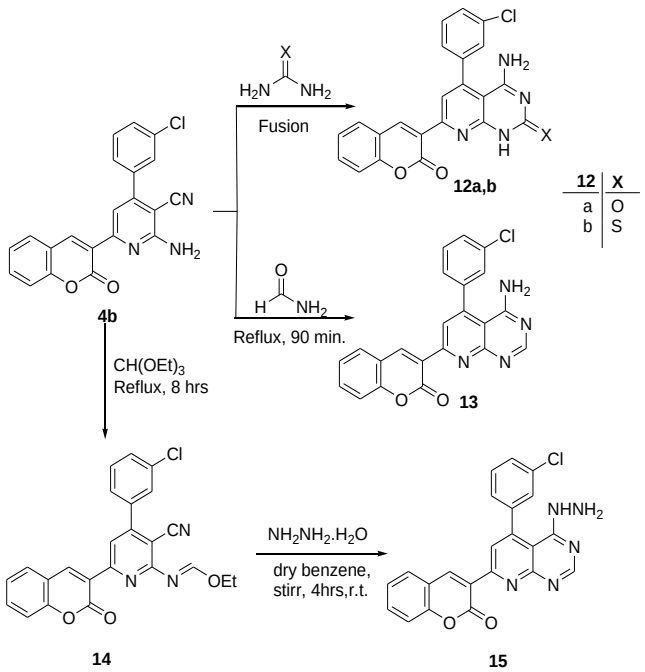
Scheme 3.

Table 1. The antitumor activity of compounds **7, 8, 16** and **17** against breast carcinoma cell line (MCF-7)

Compound	Viability rate (%)			IC_{50} (mg mL^{-1})
	0.1 µg mL^{-1}	1 µg mL^{-1}	10 µg mL^{-1}	
7	54.22 ± 6.42	50.06 ± 4.87	46.49 ± 4.20	56.34 ± 4.82
8	77.58 ± 4.36	72.43 ± 4.36	68.30 ± 8.63	14.32 ± 2.58
16	80.27 ± 10.63	83.46 ± 8.69	70.19 ± 6.48	28.50 ± 2.46
17	62.34 ± 4.38	58.28 ± 7.56	52.39 ± 8.69	2.32 ± 3.87

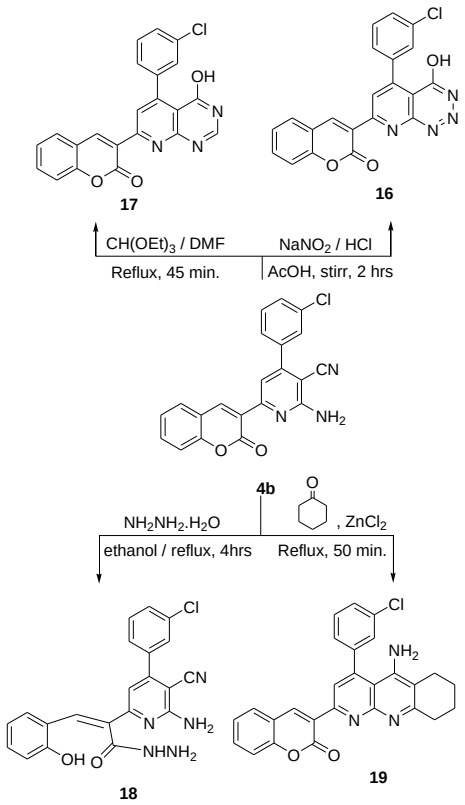
Also cyclization of compound **4a** with acetophenone in the presence of triethylamine yielded 3-(5-amino-4,7-diphenyl-1,8-naphthyridin-2-yl)-*2H*-chromen-2-one **8**. The condensation of compound **4a** with triethylorthoformate gave ethyl-N-3-cyano-6-(2-oxo-*2H*-chromen-3-yl)-4-phenyl pyridine-2-yl formmidiate **9** which reacted with hydrazine hydrate under reflux giving compound **10**. On the other hand, when compound **4a** reacted with hydrazine hydrate, the coumarin ring opened to obtain the corresponding compound 2-(6-amino-5-cyano-4-phenylpyridin-2-yl)-3-(2-hydroxyphenyl)acrylohydrazide **11** (Scheme 3).

While compound **4b** underwent cyclization upon treatment with urea and/or thiourea giving 4-amino-5-(3-chlorophenyl)-7-(2-oxo-*2H*-chromen-3-yl)pyrido-[2,3-*d*]pyrimidin-2(*1H*)-one **12a** and 3-(4-amino-5-(3-chlorophenyl)-2-thioxo-1,2-dihydropyrido[2,3-*d*]pyrimidin-7-yl)-*2H*-chromen-2-one **12b**. Cyclization of compound **4b** also can be achieved by allowing to react with formamide to afford 3-(4-amino-5-(3-chlorophenyl)pyrido[2,3-*d*]pyrimi-din-7-yl)-*2H*-chromen-2-one **13**. By the same way, compound **4b** reacted with triethylorthoformate to yield compound **14** which underwent further cyclization upon treatment with hydrazine hydrate at room temperature affording 3-(5-(3-chlorophenyl)-4-hydrazinylpyrido-[2,3-*d*]pyrimidin-7-yl)-*2H*-chromen-2-one **15** (Scheme 4).



Scheme 4

Diazotization of compound **4b** with sodium nitrite and conc. Hydrochloric acid in the presence of acetic acid led to formation of 3-(5-(3-chlorophenyl)-4-hydroxypyrido[2,3-*d*][1,2,3]triazin-7-yl)-*2H*-chromen-2-one **16**. The reaction of compound **4b** with triethylorthoformate in the presence of dimethylformamide underwent cyclization yielding compound **17** and the structure was established by infrared spectrum revealed no absorption in the CN region, furthermore, it displayed absorption bands at (3448-3400cm^{-1}) as a broad band (NH_2, OH). By the same way compound **4b** reacted with hydrazine hydrate and cyclohexanone to give compounds **18**, **19**, respectively (Scheme 5).



Scheme 5

Antitumor activity

The potential cytotoxicity activity of compounds **7, 8, 16** and **17** was tested against breast carcinoma cell line (MCF-7) by SRB (Sulphorhodamine-B) assay. Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

References

[1]Pechmann, V. H., Duisberg, C., *Chem. Ber.*, **1884**, *17*, 929.

[2]Johnson, J. R., *Org. React.*, **1942**, *1*, 210.

[3]Brufola, G., Fringuelli, F., Piermatti, O., Pizzo, F., *Heterocycles*, **1996**, *43*, 1257.

[4]Shirner, R. L., *Org. React.*, **1942**, *1*, 1.

[5]Yavari, I., Hekmat, S. R., Zonouzi, A., *Tetrahedron Lett.*, **1998**, *39*, 2391.

[6]Cairns, N., Harwood, L. M., Astles, D. P., *J. Chem. Soc., Perkin Trans*., **1994**, *1*, 3101.Cartwright, G. A., McNab, W., *J. Chem. Res.*, **1997**, *S*, 296.

[7]Weber, U. S., Steffen, B., Siegers, C. P., *Res. Commun. Mol. Pathol. Pharmacol*, **1998**, *99*, 193.

[8]Patil, A. D., Freyer, A. J., Drake, S. E., Haltiwanger, R. C., Bean, M. F., Taylor, P. B., Caranfa, M. J., Breen, A. L., Bartus, H. R., Johnson, R. K., Hertzberg, R. P., Westley, J. W., *J. Med. Chem.*, **1993**, *36*, 4131.

[9]Yun, B. S., Lee, I. K., Ryoo, I. J., Yoo, I. D., *J. Nat. Prod.*, **2001**, *64*, 1238.

[10]Zaha, A. A., Hazem, A., *Microbiologica*., **2002**, *25*, 213.

[11]Maly, D. J., Leonetti, F., Backes, B. J., Dauber, D. S., Harris, J. L., Craik, C. S., Ellman, J. A., *J. Org. Chem.*, **2002,** *67*, 910.

[13]Heravi, M. M., Sadjadi, S., Oskooie, H. A., Shoar, R. H., Bamoharram, F. F., *Catal. Commun.*, **2008**, *9*, 470–474.

[14]Campaigne, E., *Compr.. Heterocycl. Chem.*, **1984**, *4*, 863.

[15]Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenny, S., Boyd, M. R., *J. Natl. Cancer Inst.*, **1990**, *82*, 1107.

[16]Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paul, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, M., *J. Natl. Cancer Inst.*, **1991**, *83*, 757-776.

[17]Campbell, M., Mayo, H., Boyd, J., *J. Natl. Cancer Inst.*, **1991**, *83*,757.

[18]Zhou, J. F., Gong G. X., Zhu, F. X., Zhi, S. J., *Chin. Chem. Lett.*, **2009**, *20*, 37-39.

Received: 20.04.2015.
Accepted: 14.06.2015.