Heteroaromatization of Coumarin Part I: Design, Synthesis, Re-Actions, Antitumor Activities of Novel of Chromen-3-Yl-Pyridine and Chromen-3-Yl-Naphthyridine Derivatives

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Article

Heteroaromatization of Coumarin Part I: Design, Synthesis, Reactions, Antitumor Activities of Novel of Chromen-3-yl-pyridine and Chromen-3-yl-naphthyridine Derivatives

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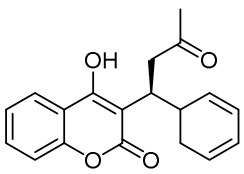
Abstract: Synthesis of a novel series of chromen-3-yl-pyridine moieties. IR, NMR, and MS spectroscopy were used to confirm the structure of these novel compounds and study of antitumor activity of these compounds. The structure activity relationship investigation demonstrated that 2,4-diamino-5-(3-methoxyphenyl)-7-(2-oxo-2H-chromen-3-yl)-1,8-naphthyridine-3-carbonitrile (**16**), was more effective and naphthyridine-3-carbonitrile derivatives **17, 18** and pyrido[2,3-d]pyrimidine derivative **12**, while compounds **5a,b, 9c, 11, 13 and 14** were moderate activity for antitumor activities.

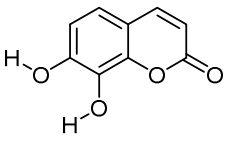
Keywords: 3-acetylcoumarin-2one; aromatic aldehydes; malononitrile; ethyl cyanoacetate; Acetic anhydride; carbon disulphide; and antitumor activities

Introduction

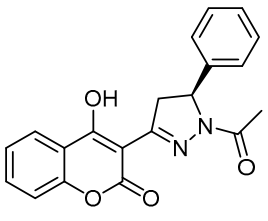
The attention medicinal chemists and organic has been attracted for many years to the coumarins and their derivatives synthesis as a large number of synthetic and natural products contain this heterocyclic nucleus. Coumarins have diverse biological and pharmacological activities as anti-inflammatory [3], antitumor [1], analgesic and ulcerogenic [2], anticoagulant [4], photo triggering [5] and fungicidal [6] properties, and can act as anticoagulants in the production of pesticides [7]. coumarin compounds have a antitumor activity which attract the attention of researchers because of their cytotoxic activity against numerous types of cancers like breast cancer cell progression, leukemia, cell carcinoma, malignant melanoma, renal and prostate. regardless the numerous attempts to search for more antitumor agents that is effective, coumarin still remains to be one of the most versatile class of compound against cancer cell lines [8-10], they are considered an important component among all the molecules in drug discovery. Warfarin (Figure 1) reduces metastases from intestinal carcinomas to a great extent [11] and is also used as an adjunct to the surgical treatment of malignant tumors [12]. In addition, daphnetin (Figure 1) inhibits tyrosine kinase, epidermal growth factor receptor, serine/threonine- specific protein kinase, and protein kinase C in vitro [13]. On the identified new class of MEK 1 kinase inhibitors is dihydropyrazole-substituted benzopyran-2-one [14] (Figure 1).



Warfarin



Daphnetin



Dihydropyrazole-substituted benzopyran-2-one

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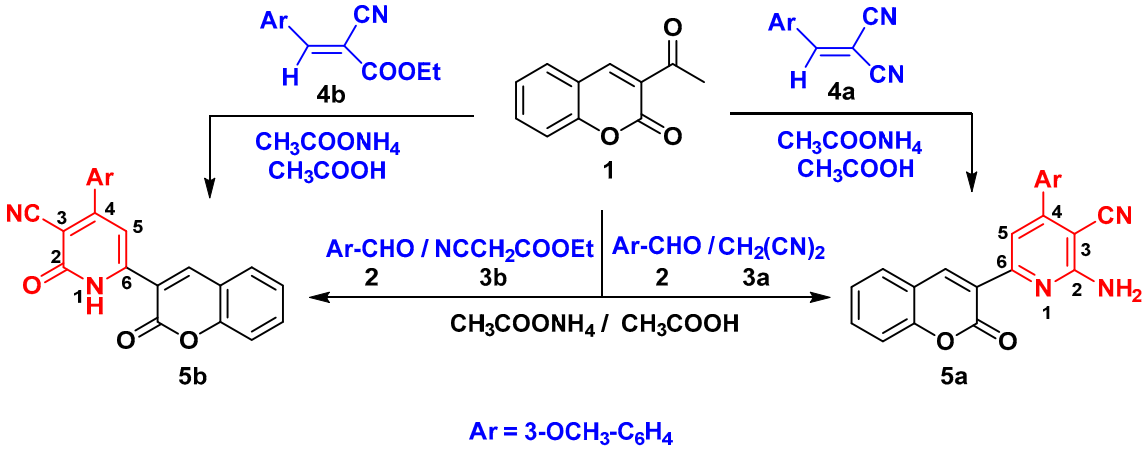
In continuation of our research program on the synthesis of novel heterocyclic compounds exhibiting antitumor activities [15–27], we attempted to design pyrimidine, tetrazole and triazolopyrimidine to position 3 of coumarin as a novel

3-heteroarylcoumarins, which have not been reported hitherto, to evaluate their *in vitro* antitumor activity against Human breast cancer cell line (MCF-7), human cervical cancer cell line (HeLa), and human prostate cancer (PC-3).

Results and Discussion

The condensation reaction of 3-acetyl-2H-chromen-2-one (1) with 3-methoxybenzaldehyde (2) malononitrile (**3a**) or ethyl cyanoacetate (**3b**), in the presence of ammonium acetate and acetic acid under reflux to give the corresponding 2-amino-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl) pyridine-3-carbonitrile (**5a**) or 4-(3-methoxyphenyl)-2-oxo-6-(2-oxo-2H-chromen-3-yl)-1,2-dihydropyridine-3-carbonitrile (**5b**). The other compound Support for structure **5a, b** was obtained its independent synthesis by the reaction of 3-acetylcoumarin-2one (1) with 2-(3-methoxy benzylidene) malononitrile (**4a**) or ethyl-3-(3-methoxyphenyl)-2-cyano acrylate (**4b**) in an ammonium acetate and acetic acid, solution under reflux (Scheme 1).



Scheme 1. Synthesis of chromen-3-yl-pyridine derivatives 5a, b.

plausible mechanism for the formation of chromen-3-yl pyridine derivatives catalyzed by ammonium acetate is shown in **Scheme 2**. The Knoevenagel reaction in the presence of ammonium acetate occurs via an initial formation of 2-(4-methoxy benzylidene)malononitrile or ethyl-3-(4-methoxy phenyl)-2-cyanoacrylate, after removal of one molecule of water from the condensation of p-methoxy benzaldehyde and malononitrile or ethyl cyanoacetate, while 3-acetyl-2H-chromen-2-one react ammonium acetate afforded 3-(1-iminoethyl)-2H-chromen-2-one with is converted to the 3-(1-aminovinyl)-2H-chromen-2-one form after tautomerisation and attacks the of 2-(4-chloro benzylidene)malononitrile or ethyl-3-(4-chlorophenyl)-2-cyano acrylate as Michael acceptor, to give intermediate and then furnished the intermediate product, which upon intramolecular cyclization and rearrangement gave rise to chromen-3-yl pyridine derivatives **4a,4b** (Scheme 2).

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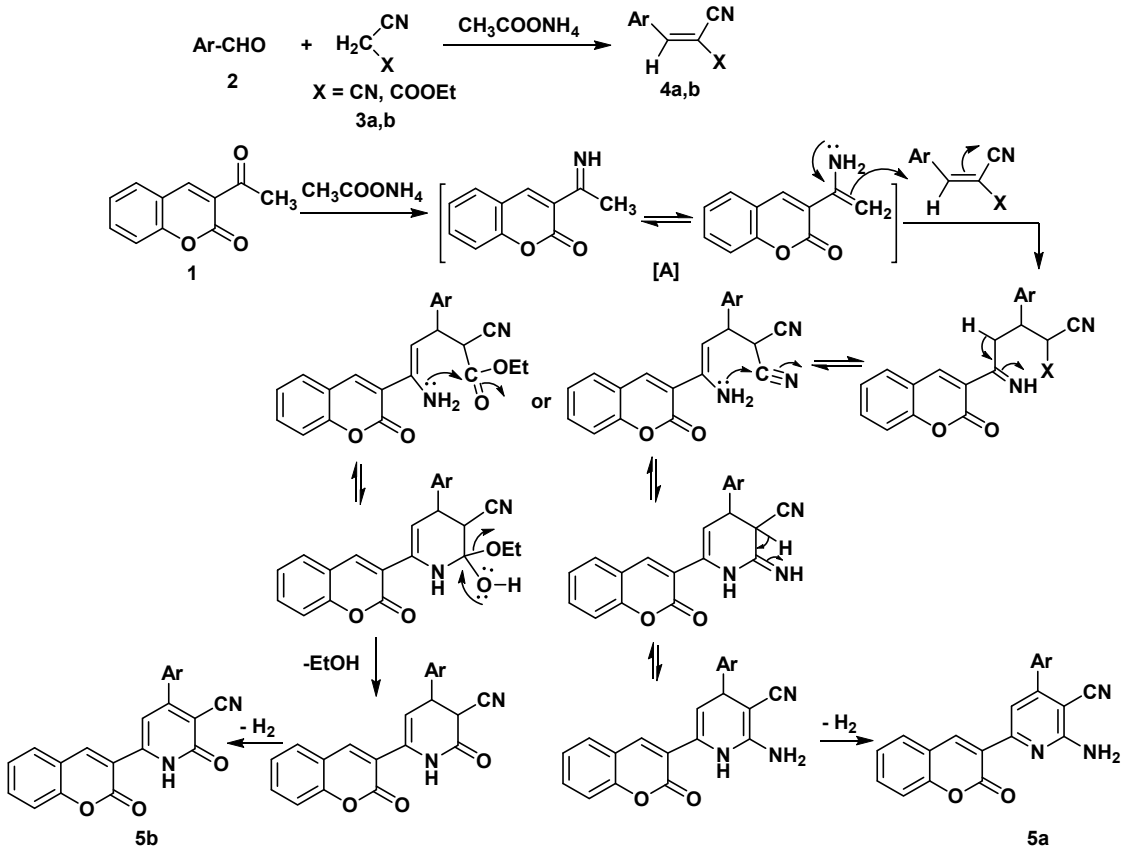

Scheme 2. Plausible mechanistic pathway of the formation of compounds **5a,b**.

Reaction of **5a** with triethyl orthoformate gave the corresponding formimidate derivative **6**. Hydrazinolysis of compound **6** in dry benzene at room temperature yielded the formimidohydrazide derivative **7**, and which upon cyclization of compound **7** by heating in absolute ethanol gave rise to aminoiminoderivatives **8**. Different aromatic aldehydes were condensed with compound **5a** in dioxane/Et_3N solution under reflux to give the corresponding arylidene amino derivatives **9a-c**. When arylidine amino derivatives **9a-c** were treated with hydrazine hydrate in dry benzene under stirring at room temperature, the addition product was formed from the intermediate [A], and aldehyde hydrazone was eliminated to give enaminonitrile **5a** instead of the pyrimidine derivatives **10a-c**. (Scheme 2).

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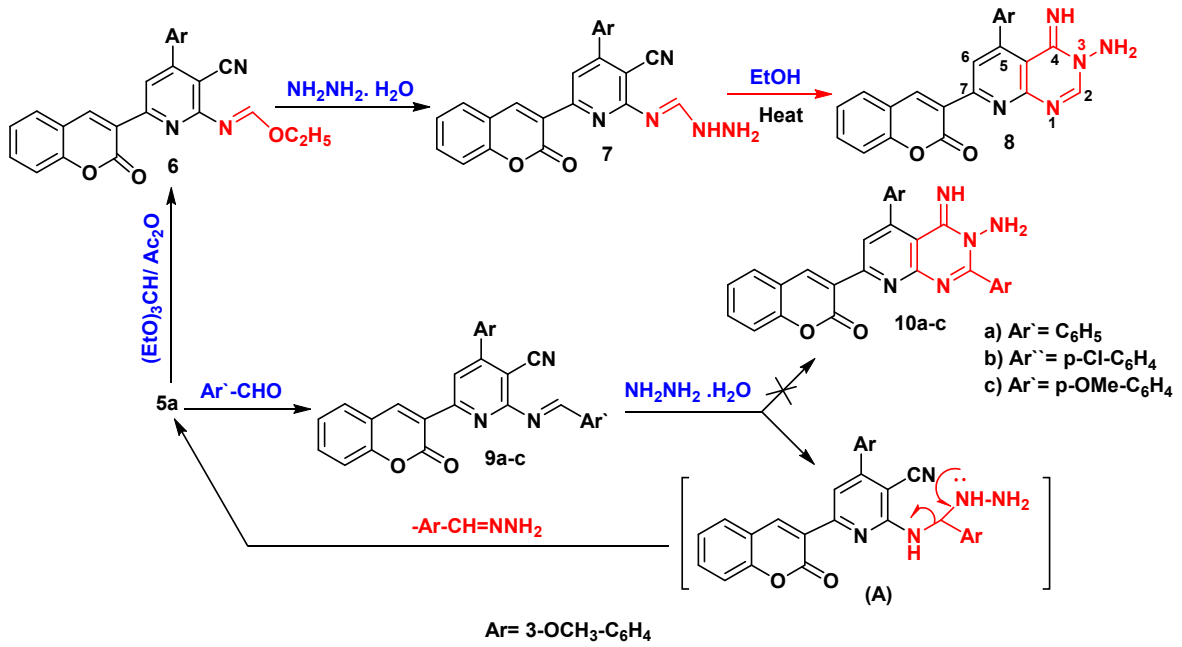
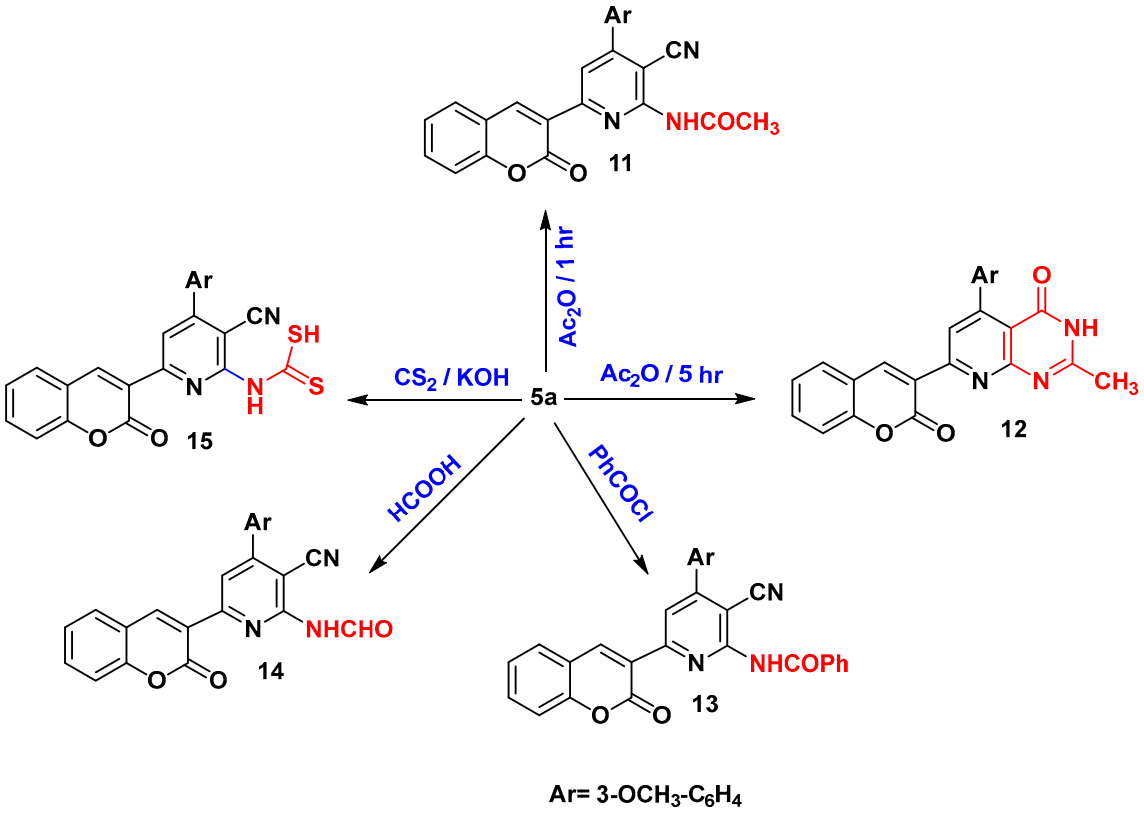


Scheme 3. Reaction of the compound **5a** with $(EtO)_3CH$ and NH_2-NH_2. H_2O.

Treatment of **5a** with Ac_2O gave two products, depending on the reaction time; the first was identified as N-(3-cyano-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)acetamide (**11**, 1/2 hour), while the last was identified as 5-(3-methoxyphenyl)-2-methyl-7-(2-oxo-2H-chromen-3-yl)pyrido[2,3-d]pyrimidin-4(3H)-one (**12**, 5 hours), while react of compound **5a** with benzoyl chloride in DMF at reflux 6 hr afforded benzamide derivative **13**, and formylation of compound **5a** with formic acid reflux 5 hours to give formamide derivative **14**. Reaction of **5a** with carbon disulfide in ethanol/alc.KOH afforded carbamodithioic acid **15** scheme 4.



Scheme 4. Reaction of compound **5a** with Ac2O, PhCOCl, Frmic acid and CS_2.

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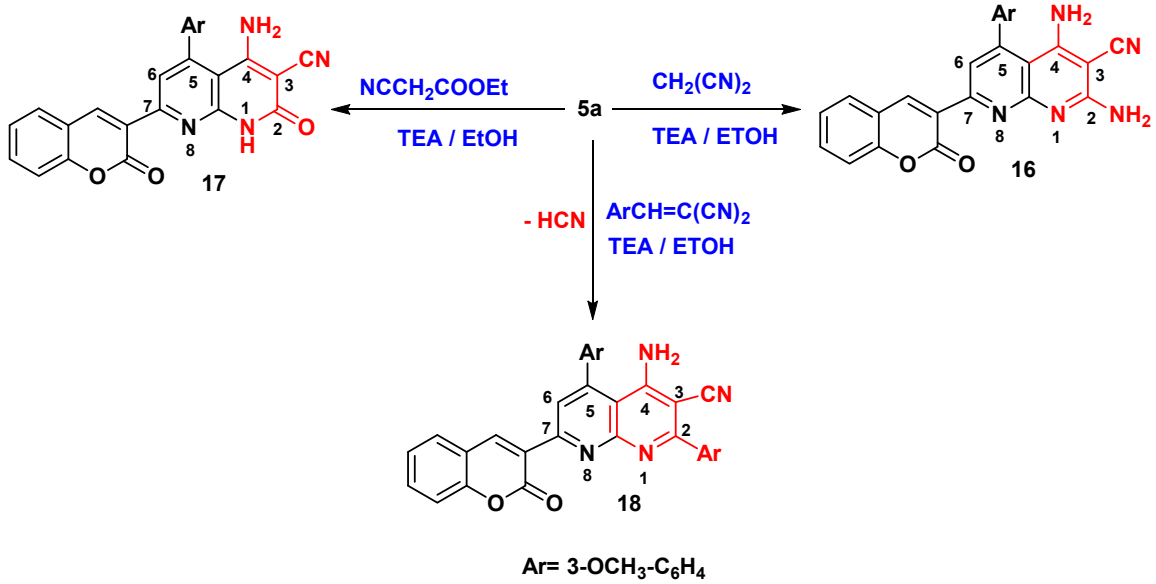
Reaction of compound **5a** with malononitrile or ethyl cyanoacetate in an absolute EtOH / triethyl amine reflux 5 hours afforded 2,4-diamino-5-(3-methoxyphenyl)-7-(2-oxo-2H-chromen-3-yl)-1,8-naphthyridine-3-carbonitrile (**16**) or 4-amino-5-(3-methoxyphenyl)-2-oxo-7-(2-oxo-2H-chromen-3-yl)-1,2-dihydro-1,8-naphthyridine-3-carbonitrile (**17**), while reaction of compound 5a with 2-(3-methoxybenzylidene)malononitrile in an absolute EtOH/ Et_3N afforded 4-amino-2,5-bis(3-methoxyphenyl)-7-(2-oxo-2H-chromen-3-yl)-1,8-naphthyridine-3-carbonitrile (**18**) and elimination of hydrogen cyanide (Scheme 5).



Scheme 5. Synthesis of 1,8-naphthyridine-3-carbonitrile derivatives.

Experimental Section

3.1. Materials and Equipment

A Stuart Scientific Co., Ltd. device was used to determine melting points. On a Jasco FT/IR 460 additional spectrophotometer, IR spectra of KBr pellets were evaluated. A Bruker AV 400 MHz spectrometer was used to record ^1H and ^{13}C-NMR spectra. A Shimadzu GC/MSQP5050A spectrometer was used to measure mass spectra. Sigma Aldrich or S.D. fine chemicals limited provided all of the reagents and solvents, which were used without additional purification. Al-Azhar University, Cairo, Egypt, Regional Centre for Mycology (RCM).

Synthesis

General Procedure: Synthesis of chromen-3-yl pyridine Derivatives 5a,b

Method A: To the mixture of 3-methoxy benzaldehyde(0.01mole), 3-acetyl coumarin-2one (1.88 g, 0.01 mole), malononitrile (0.66 g, 0.01 mole), or ethyl cyanoacetate (0.01mole) and ammonium acetate (0.015 mole), in acetic acid glacial (30 ml) was heated until precipitation was completed.

Method B: To the mixture of, 2-(3-methoxy benzylidene)malononitrile or ethyl 3-(3-methoxy phenyl)-2-cyanoacrylate) (1.84 g, , 0.01 mole or 2.31 g, 0.01mmol, respectively), 3-acetyl coumarin (1,88 g, 10mmole), and ammonium acetate (0.015 mole), in acetic acid glacial (30 ml) was heated until precipitation was completed. The precipitated solid was removed, washed with methanol, and recrystallized from dioxane

2-amino-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl) pyridine-3-carbonitrile (5a)

Yellow powder (dioxane); yield 90%, mp =260–263 °C; IR: 3,360, 3,331 (NH_2), 3,160 (CH-aromatic), 3,092 (CH-aliphatic), 2,201 (C≡N), 1,660 (C=C). ^1H-NMR δ: 3.83 (s, 3H, OCH_3), 7.01 (br, 2H,

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NH_2, exchangeable by D_2O), 8.82 (s, 1H, coumarin 4H) 7.11–9,06 (m, 9H, Ar-H+ pyridine 5H). ^{13}C-NMR δ: 55.77 (OCH_3), 88.85 (C-3), 113.28 (CN), 114.19, 115.65,116.45, 117.06, 119.23, 120.76, 124.42, 125.39, 128.78, 130.35, 133.36, 133.60, 138.64, 144.62, 152.48, 153.98, (Ar-C), 158.97 (C-4),159.42(C-6), 159.77 (C-2), 160.66 (CO). MS m/z (%): 369 (M^+, 100). Anal. Calcd. for $C_{22}H_{15}N_3O_3$ (369.38): C, 71.54; H, 4.04; N, 11.38%. Found: C, 71.05; H, 3.65; N, 11.01

4-(3-methoxyphenyl)-2-oxo-6-(2-oxo-2H-chromen-3-yl)-1,2-dihydropyridine-3-carbonitrile (5b)

Yellow powder (dioxane); yield 85%, mp =260–263 °C; IR: 3,350, (NH), 3,154 (CH-aromatic), 2,987 (CH-aliphatic), 2,204 (C≡N), 1728 (CO), 1685 (CO), 1,656 (C=C). ^{1}H-NMR δ: 3.85 (s, 3H, OCH_3), 7.37 (s, 1H, pyridine 5H), 7.45–9.09 (m, 10H, Ar-H + Coumarin 4H + NH). ^{13}C-NMR δ: 55.45 (OCH_3), 114.48 (CN), 115.99 (C-3), 118.53, 118.96, 125.83, 128.55, 128.88, 129.29, 129.95, 130.37, 130.80, 131.07, 132.50, (Ar-C), 146.00 (C-4), 147.45 (C-6),160.70 (CO), 168.38 (CO). MS m/z (%): 370 (M^+, 100). Anal. Calcd. for $C_{22}H_{14}N_2O_4$ (370.36): C, 71.35; H, 3.81; N, 7.56%. Found: C, 70.43; H, 3.07; N, 7.20

Ethyl (E)-N-(3-cyano-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)formimidate (6)

A mixture of **5a** (3.69 g, 0.01mole) and triethyl orthoformate (2 mL) in acetic anhydride (10 mL) was refluxed for 3 hours. After cooling, the precipitated product was filtered off and washed several times with cold EtOH. The solid obtained was filtered off and recrystallized from benzene. Colourless crystals, yield 70%, mp 210 °C; IR: 2,980 (CH-aromatic), 2,835 (CH-aliphatic), 2,220 (CN), 1,725 (CO), 1,631 (C=N), 1,597 (C=C). 1H-NMR δ: 1.39 (t, 3H, CH_3, J = 7.1 Hz), 3.33 (q, 2H, CH2, J = 7.1 Hz), 3.84 (s, 3H, OCH_3), 7.05-8.29 (m, 8H, Ar-H), 8.83 (s, 1H, pyridine 5H), 9.12 (s, 1H, coumarin 4H), 9.47 (s, 1H, N = CH).

^{13}C-NMR δ: 14.40 (CH_3), 55.82 (OCH_3), 64.10 (CH_2),114.48 (CN), 115.98 (C-3), 120.09 (C-5), 121.00, 123.17, 125.40, 128.79, 130.37, 130.55, 133.93, 134.11, 137.67, 137.82, 145.41, 145.79 (Ar-C), 155.05 (C-4), 159.80 (C-6), 161.10 (CO), 162.57 (N=CH), 170.63 (C-2). MS m/z (%): 425 (M+, 100), Anal. Calcd. for $C_{25}H_{19}N_3O_4$ (425.44): C, 70.58; H, 4.50; N, 9.88 %. Found C, 70.03; H, 4.12; N, 9.27

(E)-N''-(3-cyano-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)formimidohydrazide (7)

A mixture of **6** (4.25 g, 0.01 mole), hydrazine hydrate (5 mL, 99%) or in dry benzene (50 mL) was stirred for 3 hrs at room temperature. The solid obtained was filtered off and recrystallized from dioxane.

Pale yellow powder, yield 84%, mp 280 °C; IR: 3,307, 3,276 (NH_2), 3,200(NH), 2,980 (CH-aromatic), 1,728 (CO), 1,647 (C=N).

^{1}H-NMR δ: 3.82 (s, 3H, OCH_3), 4.28 (br, 1H, NH, exchangeable by D_2O), 6.70- 8.89 (m, 10H, Ar-H and NH_2, exchangeable by D_2O), 7.87 (s, 1H, pyrimidine 5H), 8.89 (s, 1H, Coumrain 4H). ^{13}C-NMR δ: 55.84 (OCH3), 114.76 (CN), 116.03 (C-3), 120.44 (C-5), 121.23, 123.65, 125.98, 128.34, 130.40, 130.87, 133.63, 136.01, 137.64, 138.04, 144.60, 145.45 (Ar-C), 156.32 (C-4), 158.11 (C-6), 159.20 (CO), 163.04 (N=CH), 168.05 (C-2). MS m/z (%): 411 (M+, 100), Anal. Calcd. for $C_{23}H_{17}N_5O_3$ (411.42): C, 67.15; H, 4.17; N, 17.02 %. Found C, 66.64; H, 3.59; N, 16.87

3-(3-amino-4-imino-5-(3-methoxyphenyl)-3,4-dihydropyrido[2,3-d]pyrimidin-7-yl)-2H-chromen-2-one (8)

Heating of 7 (2.05 g, 0.005 mol), in absolute ethanol (50 mL) was reflux for 2 hrs. The solid obtained was filtered off and recrystallized from dioxane.

Pale yellow powder, yield 84%, mp 270 °C; IR: 3,316, 3,300 (NH_2), 3,178(NH), 2,983 (CH-aromatic), 1,726 (CO), 1,659 (C=N).

^{1}H-NMR δ: 3.83 (s, 3H, OCH_3), 5.72 (s, 2H, NH, exchangeable by D_2O), 7.25- 8.19 (m, 10H, Ar-H + NH and pyridine CH), 8.83 (s, 1H, coumarin 4H), 8.89 (s, 1H, pyrimidine CH). ^{13}C-NMR δ: 55.94 (OCH_3), 117.15 (C-6), 120.14, 121.26, 123.70, 125.88, 128.12, 130.43, 130.97, 133.43, 136.31, 137.64, 138.04, 144.60 (Ar-C), 146.77 (C-2), 156.32 (C-4), 158.11 (C-6), 158.94 (C-7), 163.32 (CO). MS m/z (%): 411 (M+,

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100), Anal. Calcd. for $C_{23}H_{17}N_5O_3$ (411.42): C, 67.15; H, 4.17; N, 17.02 %. Found C, 66.80; H, 3.70; N, 16.70.

3.2.8. Synthesis of Benzylideneamino Derivatives (9a-c).

A mixture of compound **5a** (3.69 g, 0.01 mole), benzaldehyde, 4-chlorobenzaldehyde, or 4-methoxybenzaldehyde (1.06 g , 1.40 g , 1.36 g , 0.01 mol, respectively), ethanol (20 mL), and triethylamine (0.5 mL) were refluxed for 4 hr then cooled to room temperature. The solid product was collected by filtration and recrystallized from dioxan to give compounds **9a-c.**

(E)-2-(benzylideneamino)-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl) pyridine-3-carbonitrile (9a)

Pale yellow powder (dioxane); yield 83%, mp 252°C; IR: 3,184 (CH-aromatic), 2,201 (C≡N), 1,720 (CO), 1,657 (C= C).^{1}H-NMR δ: 3.84 (s, 3H, OCH_3), 7.07–8.24 (m, 14H, Ar-H + pyridine 5H), 8.82 (s, 1H, coumarin 4H),10.02 (s, 1H, N=CH).

^{13}C-NMR δ: 55.85 (OCH_3), 114.90 (CN), 116.05 (C-3), 121.10 (C-5), 122.01, 123.54, 125.76, 128.53, 130.05, 131.32, 133.80, 134.60, 137.05, 137.60, 145.56, 146.10 (Ar-C), 155.71 (C-4), 158.98 (C-6), 161.78 (CO), 163.04 (N=CH), 169.23. (C-2). MS m/z (%): 457 (M^+, 100), Anal. Calcd. for $C_{29}H_{19}N_3O_3$ (457.14): C, 76.14; H, 4.19; N, 9.19%. Found: C, 75.76; H, 3.63; N, 8.82 %.

(E)-2-((4-chlorobenzylidene)amino)-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl) pyridine-3-carbonitrile (9b)

Pale yellow powder (dioxane); yield 75%, mp 282°C; IR: 3,190 (CH-aromatic), 2,203 (C≡N), 1,726 (CO), 1,658 (C=C).^{1}H-NMR δ: 3.86 (s, 3H, OCH_3), 7.09–8.26 (m, 13H, Ar-H + pyridine 5H), 8.90 (s, 1H, coumarin 4H),10.45 (s, 1H, N=CH).

^{13}C-NMR δ: 55.87 (OCH_3), 114.95 (CN), 116.12 (C-3), 121.45 (C-5), 122.23, 123.60, 125.83, 128.89, 130.67, 131.70, 133.53, 134.04, 137.23, 137.21, 145.34, 146.53 (Ar-C), 155.84 (C-4), 158.71 (C-6), 161.63 (CO), 163.23 (N=CH), 169.34 (C-2). MS m/z (%): 491 (M^+, 100), Anal. Calcd. for $C_{29}H_{18}ClN_3O_3$ (491.93): C, 70.81; H, 3.69; N, 8.54%. Found: C, 70.10; H, 3.04; N, 8.09 %.

(E)-2-((4-methoxybenzylidene)amino)-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl) pyridine-3-carbonitrile (9c)

Pale yellow powder (dioxane); yield 81%, mp 270°C; IR: 3,187 (CH-aromatic), 2,208 (C≡N), 1,708 (CO), 1,661 (C=C).^{1}H-NMR δ: 3.72 (s, 3H, OCH3). 3.82 (s, 3H, OCH_3), 7.39–8.18 (m, 13H, Ar-H + pyridine 5H), 8.95 (s, 1H, coumarin 4H),9.01 (s, 1H, N=CH).

^{13}C-NMR δ: 55.80 (OCH3), 55.88 (OCH_3), 114.90 (CN), 117.01 (C-3), 121.22 (C-5), 122.62, 123.62, 125.77, 128.61, 130.53, 131.32, 133.04, 134.63, 137.42, 137.66, 145.54, 146.78 (Ar-C), 155.63 (C-4), 158.60 (C-6), 162.05 (CO), 163.68 (N=CH), 169.34 (C-2). MS m/z (%): 487 (M^+, 100), Anal. Calcd. for $C_{30}H_{21}N_3O_3$ (487.52): C, 73.91; H, 4.34; N, 8.62%. Found: C, 73.32; H, 3.67; N, 8.10 %.

N-(3-cyano-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)acetamide (11)

A solution of **5a** (3.69 g, 0.01 mol) in Ac_2O (20 mL) was heated under reflux for 1h. The solid product formed was filtered off and washed with cold EtOH, the solid obtained was filtered off and recrystallized from dioxane.

Pale yellow powder, yield 68%, mp 220 °C; IR: 3,345 (NH), 3,122 (CH-aromatic), 2,940 (CH-aliphatic), 2,207 (C≡N), 1,710 (C=O), 1,690 (C=O). ^{1}H-NMR δ: 2.98 (s, 3H, CH3), 3.84 (s, 3H, OCH3), 7.05-9.12(m, 13H, Ar-H + pyridine 5H), 9.47 (s, 1H, coumarin 4H),11.02 (s, 1H, NH). ^{13}C-NMR δ: 27.03 (CH_3), 55.77 (OCH_3), 88.65 (C-3),112.85 (CN), 118.1, 118.9, 121.00, 123.17, 125.40, 128.79, 130.37, 130.55, 133.93, 134.11, 137.67, 137.82, 145.41, 145.79 (Ar-C), 159.42 (C-4), 159.77 (C-6), 160.66 (C=O), 162.78 (NHCO). MS m/z (%): 411 (M+, 100). Anal. Calcd. for $C_{24}H_{17}N_3O_3$ (411.42): C, 70.07; H, 4.17; N, 10.21%. Found: C, 69.54; H, 3.38; N, 10.02%.

5-(3-methoxyphenyl)-2-methyl-7-(2-oxo-2H-chromen-3-yl)pyrido[2,3-d]pyrimidin-4(3H)-one (12)

A solution of **5a** (3.69 g, 0.01 mol) in Ac_2O (20 mL) was heated under reflux for 3h. The solid product formed was filtered off and washed with cold EtOH, the solid obtained was filtered off and recrystallized from dioxane.

Pale yellow powder, yield 68%, mp 292 °C; IR: 3,360 (NH), 3,155 (CH-aromatic), 2,950(CH-aliphatic), 1,721(C=O), 1,695 (C=O).

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^{1}H-NMR δ: 2.90 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.36-8.92(m, 13H, Ar-H + pyridine 5H), 8.06 (s, 1H, coumarin 4H),10.80 (s, 1H, NH). ^{13}C-NMR δ: 27.03 (CH_3), 55.77 (OCH_3), 118.1, 118.9, 121.00, 123.17, 125.40, 128.79, 130.37, 130.55, 133.93, 134.11, 137.67, 137.82, 145.41, 145.79, 154.91158.97, (Ar-C), 159.42 (C-4), 159.57 (C-6), 160.66 (C=O), 167.35 (C=O). MS m/z (%): 411 (M+, 100). Anal. Calcd. for $C_{24}H_{17}N_3O_3$ (411.42): C, 70.07; H, 4.17; N, 10.21%. Found: C, 69.30; H, 3.54; N, 9.56%.

N-(3-cyano-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)benzamide (13)

A mixture of **5a** (3.69 g, 0.01 mol), benzoyl chloride (0.005 mol) in dry benzene (20 mL), was refluxed for 5 hours. The solid obtained was filtered off and recrystallized from dioxane.

Pale yellow powder, yield 60%, mp 195 °C; IR: 3,339 (NH), 3,129 (CH-aromatic), 2,957 (CH-aliphatic), 2,200 (C≡N), 1,720 (C=O), 1,698 (C=O). ^{1}H-NMR δ: 3.84 (s, 3H, OCH3), 7.99-8.93(m, 18H, Ar-H + pyridine 5H + coumarin 4H), 9.83 (s, 1H, NH). ^{13}C-NMR δ: 55.77 (OCH_3), 88.40 (C-3),113.79 (CN), 118.17, 118.60, 121.45, 123.32, 125.09, 128.32,129.43, 130.06, 130.67, 131.34, 133.07, 134.54, 135.27,137.43, 137.60, 145.23, 145.60 (Ar-C), 159.74 (C-4), 160.01 (C-6), 161.32(C=O), 164.04 (NHCO). MS m/z (%): 473 (M+, 100). Anal. Calcd. for C29H19N3O4 (473.49): C, 73.56; H, 4.04; N, 8.87%. Found: C, 73.03; H, 3.78; N, 8.32%.

N-(3-cyano-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)formamide (14)

A solution of compound **5a** (3.69 g, 0.01 mol), in formic acid (30 mL), was heated under reflux for 6h. The solid product formed was filtered off and washed with cold EtOH, the solid obtained was filtered off and recrystallized from dioxane.

Pale yellow powder, yield 70%, mp 190 °C; IR: 3,340 (NH), 3,160 (CH-aromatic), 2,960 (CH-aliphatic), 2,200 (C≡N), 1,727 (C=O), 1,670 (C=O).

1H-NMR δ: 3.84 (s, 3H, OCH_3), 7.07- 9.07(m, 11H, Ar-H + pyridine 5H+ coumarin 4H + CHO), 11.94 (s, 1H, NH). ^{13}C-NMR δ: 55.80 (OCH_3), 88.70 (C-3),113.94 (CN), 118.30, 119.22, 121.11, 123.04, 125.42, 128.70,129.90, 130.45, 130.98, 131.20, 133.11, 135.11,137.40, , 145.23, (Ar-C), 158.68 (C-4), 159.09(C-6), 160.40(C=O), 162.07 (NHCO). MS m/z (%): 397 (M+, 100). Anal. Calcd. for $C_{23}H_{15}N_3O_4$ (397.39): C, 69.52; H, 3.80; N, 10.56%. Found: C, 69.32; H, 3.04; N, 10.05%.

(3-cyano-4-(3-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)carbamodithioic acid (15)

A mixture of **5a** (3.69 g, 0.01 mol), ethanol (30 mL), KOH (0.3 g) and carbon disulfide (3 mL) was refluxed for 8 h. After removal of the ethanol, water was added and the alkaline solution was acidified with acetic acid, recrystallized from dioxane.

yellow powder, yield 65%, mp 250 °C; IR: 3,340 (NH), 3,160 (CH-aromatic), 2,960 (CH-aliphatic), 2,200 (C≡N), 1,727 (C=O), 1,670 (C=O), 1,050 (C=S).

^{1}H-NMR δ: 1.17(s, 1H, SH), 3.81 (s, 3H, OCH_3), 7.07- 7.89(m, 10H, Ar-H + pyridine 5H+ coumarin 4H), 11.94 (s, 1H, NH). ^{13}C-NMR δ: 55.80 (OCH_3), 88.70 (C-3),113.90 (CN), 118.38 119.30, 121.41, 123.23, 125.49, 128.81,129.95, 130.83, 131.43, 133.86, 135.80,137.39, 145.79, 146.20 (Ar-C), 159.74 (C-4), 160.12(C-6), 162.38(C=O), 170.01 (C=S). MS m/z (%): 445 (M+, 100). Anal. Calcd. for $C_{23}H_{15}N_3O_3S_2$ (445.51): C, 62.01; H, 3.39; N, 9.43%. Found: C, 61.70; H, 3.02; N, 9.08%.

Synthesis of 1,8-Naphthyridine-3-carbonitrile Derivatives (16-18)

To the mixture of 5a (3.69 g, 0.01mol), malononitrile (0.66 g, 0.01 mole), ethyl cyanoacetate (0.01 mol) or2-(4-methoxybenzylidene)malononitrile (1.84 g, 0.01 mole), absolute ethanol (30 ml) in present triethyl amine, was heated until precipitation was completed.

2,4-diamino-5-(3-methoxyphenyl)-7-(2-oxo-2H-chromen-3-yl)-1,8-naphthyridine-3-carbonitrile (16)

Pale yellow powder, yield 73%, mp 255 °C; IR: 3,440, 3,360, 3,340 (NH_2), 3,160 (CH-aromatic), 2,960 (CH-aliphatic), 2,205 (C≡N), 1,728 (C=O). 1H-NMR δ: 3.83 (s, 3H, OCH_3), 7.03- 8.92 (m, 14H, Ar-H + naphthyridine 6H+ coumarin 4H), 11.94 (s, 1H, NH). ^{13}C-NMR δ: 55.82 (OCH3), 88.70 (C-3),116.01 (CN), 118.60 119.05, 120.34, 121.40, 123.73, 125.60, 128.71,129.91, 130.80, 131.40, 133.48, 135.80,137.39, 145.79, 146.20, 146.75 (Ar-C), (C-4), 160.54(C-6), 163.54(C=O). MS m/z (%): 445 (M+, 100). Anal. Calcd. for C25H17N5O3 (435.44): C, 68.96; H, 3.94; N, 16.08%. Found: C, 68.30; H, 3.45; N, 15.41%.

4-amino-5-(3-methoxyphenyl)-2-oxo-7-(2-oxo-2H-chromen-3-yl)-1,2-dihydro-1,8-naphthyridine-3-carbonitrile (17)

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Pale yellow powder, yield 73%, mp 230 °C; IR: 3,410, 3,370 (NH_2), 3,153 (CH-aromatic), 2,957 (CH-aliphatic), 2,100 (C≡N), 1,728 (C=O), 1,680 (C=O). ^{1}H-NMR δ: 3.84 (s, 3H, OCH_3), 7.07- 7.89(m, 10H, Ar-H + naphthyridine 6H+ coumarin 4H), 8.82 (s, 2H, NH_2), 10.03 (s, 1H, NH). ^{13}C-NMR δ: 55.80 (OCH_3), 116.28 (CN), 118.61 119.25, 120.38, 121.42, 123.70, 125.61, 128.70,129.91, 130.70, 131.65, 133.41, 135.81,137.34, 145.72, 146.26, 146.73 (Ar-C), 160.54(C-6), 163.54(C=O), 164.42 (C=O). MS m/z (%): 436 (M^+, 100). Anal. Calcd. for C25H16N4O4 (436.43): C, 68.80; H, 3.70; N, 12.84%. Found: C, 68.30; H, 3.04; N, 12.04%.

4-amino-2,5-bis(3-methoxyphenyl)-7-(2-oxo-2H-chromen-3-yl)-1,8-naphthyridine-3-carbonitrile (18)

Pale yellow powder, yield 73%, mp 230 °C; IR: 3,400, 3,380 (NH2), 3,152 (CH-aromatic), 2,961 (CH-aliphatic), 2,103 (C≡N), 1,728 (C=O). 1H-NMR δ: 3.84 (s, 3H, OCH3), 3.57 (s, 3H, OCH3),7.07-9.07(m, 12H, Ar-H + naphthyridine 6H+ coumarin 4H + NH_2). ^{13}C-NMR δ: 55.80 (OCH_3), 55. 73 (OCH_3), 116.28 (CN), 118.61 119.25, 120.38, 121.43, 123.78, 125.69, 128.70,129.91, 130.65, 131.34, 133.70, 135.81,137.34, 144.70, 146.26, 147.02 (Ar-C), 160.51(C-6), 162.01(C=O). MS m/z (%): 526 (M^+, 100). Anal. Calcd. for $C_{32}H_{22}N_4O_4$ (526.55): C, 72.99; H, 4.21; N, 10.64%. Found: C, 72.04; H, 3.83; N, 10.10 %.

Cytotoxicity Activities

From the newly synthesized compounds ten compounds were selected to be screened for their in vitro antitumor activity namely. Human breast cancer cell line (MCF-7), human cervical cancer cell line (HeLa), and human prostate cancer (PC-3). The cytotoxicity of the compounds was determined by calculation their IC_{50} values, which is the concentrations that caused a 50% reduction in cell growth compared with untreated control cells. ***5-Fluororacil*** was used as positive control for the comparison of cytotoxicity of synthesized compounds. Assays was performed in triplicate in three independent experiments, and their mean values are presented in (table 1). It is worth noting that most of the tested compounds possess significant cytotoxic activity toward the tested three tumor cell lines. The preliminary results reveled that compound **15** is more potent and effective with IC_{50} to be nearly as potent as the positive control ***5-Fluorouracil*** with IC50= 5.02, 8.30 and 6.60 μg/mL as the most active compound among the screened series.

Also, the data represented in (Table 1) showed that the highest activities were observed for compounds **16-18** with IC_{50} less than 10.0 μg/ mL, while compounds **5a,b** and **12** are moderate actives with IC_{50} less than 20.0 μg/ mL, toward the tested three tumor cell lines. Further, interpretation of the results declared that compounds **9c, 11, 13 and 14** showed the less potency against the three tumor cell lines with IC_{50}> 20.0 μg/mL. However, the obtained results were compared with ***5-Fluorouracil*** that has cytotoxic activity at concentrations 3.70, 6.48 and 4.92 ug/mL against **MCF-7, HaLe** and **PC-3**, respectively.

Structure activity relationship.

Subsequently, structure activity relationship (SAR) studies were performed to determine the effect of substituents on cytotoxic activities of the synthesized compounds. The obtained results of the antitumor evaluation declared implicitly some definite and interesting facts about the SAR of synthesized compounds. Generally, activity profile dependence on structural amendments of the molecule is dramatic and clear. The remarkable highlights of SAR are summarized here.

The presence of a basic skeleton of chromen-3-yl-pyridine is essential for the broad spectrum of cytotoxic activity toward the investigated three cell lines.

Table 1. Cytotoxicity (IC50 values of tested compounds against different tumor cell lines.

	In vitro cytotoxicity IC50 (μg/mL)		
Comp.	**MCF-7**	**HeLa**	**PC-3**
5a	18.32 ± 0. 41	20.61 ± 0. 23	19.64 ± 0.08
5b	16.05 ± 0. 82	19.01 ± 0.03	15.90 ± 0.42
9c	21.17 ± 0.50	26.01 ± 0.02	24.06 ± 0.03

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11	20.07 ± 0.82	24.61 ± 0.01	23.63 ± 0.03
12	13.04 ± 0. 08	16.09 ± 0.21	12.05 ± 0.85
13	27.05 ± 0.22	28.01 ± 0.87	26.08 ± 0.65
14	25.61 ± 0.13	26.77 ± 0.01	28.60 ± 0.42
16	5.02 ± 0.07	8.30± 0.61	5.60 ± 0.01
17	8.42 ± 0.30	10.31 ± 0. 10	7.96 ± 0.01
18	13.04 ± 0. 08	16.09 ± 0.21	12.05 ± 0.85
5-Fluorourcail	3.70 ± 0.50	6.48 ±0. 10	4.92 ± 0.40

1-10 (very strong), 11-25 (strong), 26-50 (moderate), 51-100 (very weak), and IC50 concentration of drug that decrease the viability of the cells by 50% compared with non-treated control cells, each values represents mean of three readings ±SD. Human breast cancer cell line (MCF-7), human cervical cancer cell line (HeLa), and human prostate cancer (PC-3) and 5-Fluorourcail; positive control compound.

2.3. Methodology. Cell culture.

Three mammalian cell lines **HeLa** (human cervical cancer cell line); **MCF-7** (human breast cancer cell line); and **PC-3** (human prostate cancer) were grown in RPMI-1640 medium, supplemented with 10% heat inactivated FBS. 50 units/mL of penicillin, 50 g/mL of streptomycin and maintained at 37 °C in a humidified atmosphere containing 50% CO_2. The cells were maintained as " monolayer culture" by serial subculturing

Sulforhodamine-B cytotoxicity assay

Preliminary cytotoxicity was performed using **Sulforhodamine-B** method as previously described [28,29]. Exponentially growing cells were collected using 0.25% Trysin-EDTA and seeded in 96-well plates at 1000-2000 cells/ well in RPMI-1640 supplemented medium. After 24 hr, cells were incubated for 72 hr with various concentrations of the tested compounds (1000, 500, 200, 100, 50, 20, and 10 µg/mL). following 72 hr treatment, the cells will be fixed with 10% trichloroacetic acid for 1 hr at 4 °C. Wells were stained for 10 min at room temperature with 0.4% with Sulforhodamine-B stain dissolved in 1% acetic acid. The plates were air dried for 24 hr, and the dye was solubilized with tris-HCl for 5 min on a shaker at 1600 rpm. The color intensity of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader ChroMate-4300, FL, USA). The relation between surviving fraction and during concentration is platted to get the survived curve of each tumor cell line. The dose response curve of compound was analyzed using E max model. IC50 was defined as the drug concentration required to reduce fluorescence to 50% of that of the control.

CONCLUSION

The synthesis of new compounds with potential applications as drug replacements is an area of high interest in the literature to overcome the drug resistance issue. For that reason, novel 2,4-diamino-5-(3-methoxyphenyl)-7-(2-oxo-2H-chromen-3-yl)-1,8-naphthyridine-3-carbonitrile (**16**), was more effective and naphthyridine-3-carbonitrile derivatives **17, 18** and pyrido[2,3-d]pyrimidine derivative **12**, starting from chromen-3-yl-pyridine derivatives (5a). Show the data of antitumor, 2,4-diamino-5-(3-methoxyphenyl)-7-(2-oxo-2H-chromen-3-yl)-1,8-naphthyridine-3-carbonitrile (**16**), exhibited potent anti-tumor activity, while naphthyridine-3-carbonitrile derivatives **17, 18** and pyrido[2,3-d]pyrimidine derivative **12** were the most active antitumor

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