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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME NOVEL COUMARIN DERIVATIVES CONTAINING PYRIDINE MOIETY

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Abstract – The 2-acetyl-3*H*-benzo[*f*]chromen-3-one **(1)** was used as a key intermediate for the synthesis of 3-(1-amino-3-oxo-3*H*-benzo[*f*]chromen-2-yl)-but-2-enenitrile derivatives **3a-d** *via* condensation reactions with activated nitrile derivatives in the presence of ammonium acetate. Moreover, the **3a-d** underwent interamolecular cyclization to form 3-alkyl-2-amino-4-methyl-5-oxo-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridine **4a-d.** Compound **1** reacts with acetophenone and cyclohexanone in the presence of cyanoacetamide to afford the benzo[5,6]chromeno[3,4-*c*]pyridin-5-one derivatives **5** and **6**, respectively. Also, 4-aryl-6-[benzo[*f*]coumarin-3-yl]-3-cyano-2-pyridone derivatives **8a-d** were synthesized by an efficient and convenient method by the one-pot reaction of **1** with aromatic aldehydes **7a-d** and malononitrile in the presence of sodium hydroxide under a solvent free condition. This method has the advantages of mild reaction conditions, easy workup and inexpensive reagents. Moreover, 2-**(**4,6-diphenylpyridin-2-yl)-3*H*-benazo[*f*]chromen-3-one **(16)** was prepared *via* reaction of α-pyrdinium salt of methyl ketone of **1** with benzalacetophenone in the presence of ammonium acetate. The structures of the new synthesized compounds were confirmed by spectral data and elemental analyses. New compounds were tested for *in vitro* cytotoxicity against heptacelluar carcinoma (HepG2) and breast cancer (MCF-7) in addition to their antibacterial evaluation.

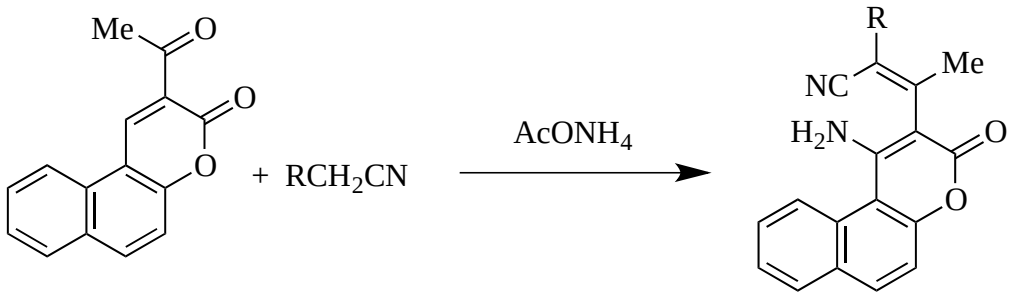
Coumarins or benzo-2-pyrone derivatives are one of the most significant families of natural product compounds and are important in synthetic organic chemistry. They have been widely used as starting materials or intermediates in the pharmaceutical, perfumery and agrochemical industries.

Coumarins are also used as fluorescent brighteners, efficient laser dyes and additives in food and cosmetics.[1] The coumarins represent a large group of compounds that have been reported to possess a wide range of biological activities,[2-4] including anticoagulant and antithrombotic properties.[5-7]

Recently, coumarins have attracted considerable attention for electronic and photonic applications[8,9] due to their inherent photochemical characteristics, reasonable stability and solubility in various organic solvents. Many coumarin derivatives have been commercialized as blue-green lasers for fluorescent labels, fluorescent probes[10-12] and enzymatic measurements.[13] They exhibit intense fluorescence upon substitution with various functional groups at different positions.[14, 15]

Fused heterocyclic scaffolds with nitrogen and oxygen atoms are fundamental to the medicinal chemistry for the development of several new drugs. To date, few reports are available in the literature regarding the synthesis of fused chromeno-pyridine or chromene ring bearing pyridine moiety. Owing to the necessity for an improved methodology for the synthesis of these fused scaffolds and prevalence of impressive biological properties of both pyridine and coumarins, we were interested in developing a mild synthetic protocol for the synthesis of the fused and isolated chromeno-pyridine.

In the present work, we have developed the synthesis of new 2-amino-4-methyl-5-oxo-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridine derivatives **4a-d** *via* interamolecular cyclization of 3-(1-amino-3-oxo-3*H*-benzo[*f*]chromen-2-yl)- but-2-enenitrile derivatives **3a-d**.



1 **2a-d** **3a-d**

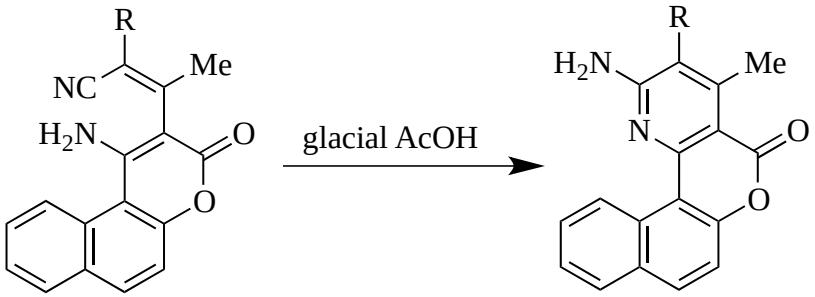
a, R = CN
b, R = SO_2Ph
c, R = 2-benzothiazolyl
d, R = 2-CN-C_6H_4-

Scheme 1

The expected crotononitrile derivatives **3a-d**, presumably formed by condensation reaction, Michael addition of ammonia to α, β-unsaturated lactone followed by autooxidation **(Scheme 1)**. The structures of the products **3a-d** were indicated by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analyses. The IR spectra of compounds **3a-d** showed a characteristic absorption band in the region 2000-2205 cm^{-1} corresponding to the stretching vibration of the cyano group. The high frequency region of the spectra showed two strong absorption bands at 3300-3400 cm^{-1} due to the stretching vibrations of the NH_2 group, in addition to a strong absorption band in the region 1710-1722 cm^{-1} corresponding to the stretching vibration of the α, β-unsaturated lactone in the coumarin ring.

The ^{1}H-NMR spectra of **3a-d** showed the presence of a singlet signal within the region δ 2.15-2.30 ppm due to the methyl protons and a multiplet signal within the region δ 7.28-8.20 ppm due to aromatic protons in addition to a broad singlet signal (D_2O exchangeable) in the region 6.00-6.20 ppm due to an amino group. In addition, the structure of compounds **3a-d** was confirmed by its mass spectroscopic measurement.

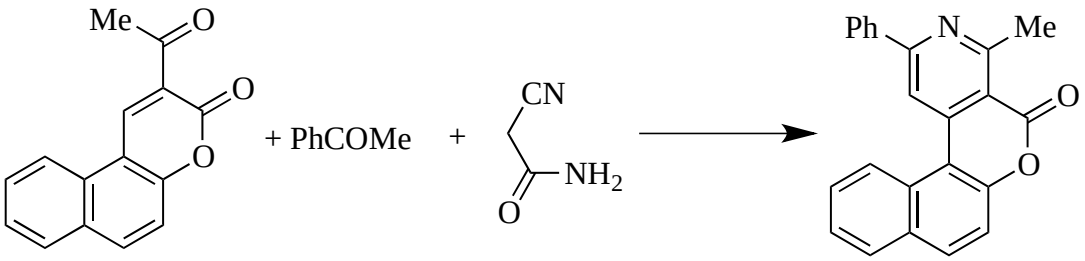
When compounds **3a-d** were refluxed in glacial acetic acid, they afforded 3-alkyl-2-amino-4-methyl-5-oxo-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridin **(4a-d) (Scheme 2)**.



3a-d **4a-d**

Scheme 2

The structure of compounds **4b** and **4c** was established on the basis of their IR spectra which showed the absence of any peak around 2100-2250 cm^{-1} due to a cyano group, which confirms that cyano group was involved in the reaction, while structure of compounds **4a** and **4d** was established on the basis of their IR spectra which showed the presence of one absorption band in the region 2100-2250 cm^{-1} due to a cyano group instead of two absorption bands in the region 2100-2250 cm^{-1} due to cyano group in **3a** and **3d.** Also, structure of compounds **4a-d** was established on the basis of their ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. When **1** was treated with acetophenone and cyanoacetamide for furnishing ammonia,[16] it afforded 2-phenyl-4-methyl-5*H*-benzo[5,6]chromeno[3,4-*c*]pyridin-5-one **(5) (Scheme 3)**.

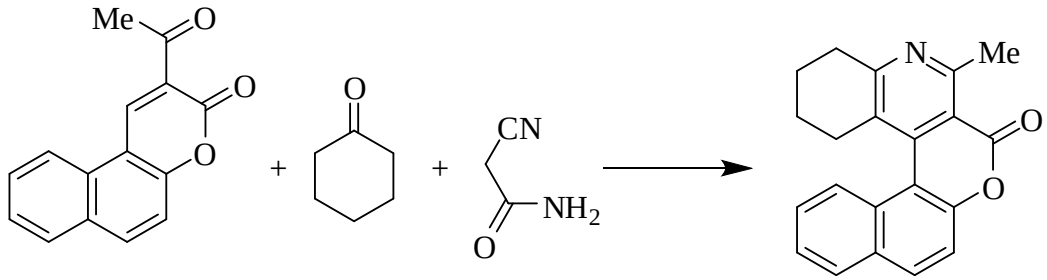


1 **5**

Scheme 3

The structure of the product **5** was inferred from its analytical and spectral data. Thus, its IR spectrum showed characteristic absorption band at 1718 cm^{-1} due to a carbonyl group. The ^{1}H-NMR spectra of **5** exhibited a singlet signal at δ 2.43 ppm due to methyl protons in addition to a multiplet signal in the region 7.10-8.40 ppm due to aromatic protons.

Typically, on treating **1** with cyclohexanone and cyanoacetamide as ammonia source, it gave 2-methyl-11,12,13,14-tetrahydro-3*H*-benzo[5,6]chromeno[3,4-*c*]quinolin-3-one **(6) (Scheme 4)**.



1 **6**

Scheme 4

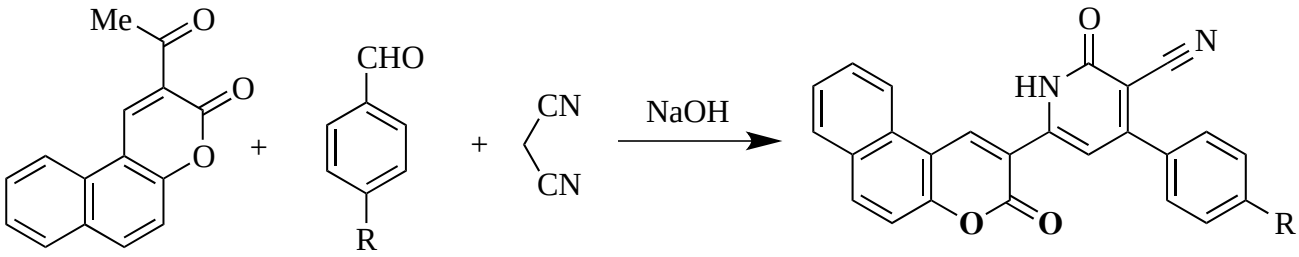
Newly synthesized compound **6** was characterized based on an elemental analysis, IR, ^{1}H-NMR and mass spectral data. The IR spectrum of compound **6** showed absorption bands in the range from 3090–2890 cm^{-1} due to aliphatic –CH_2- and CH aromatic. The strong band at 1725 cm^{-1} is attributed to the C=O stretching vibration. The ^{1}H-NMR spectrum of **6** showed a multiplet signal at δ 1.60-1.70 ppm due to CH_2-12 and CH_2-13, a singlet signal at δ 2.50 ppm due to a methyl group, a triplet signal at δ 2.60 ppm due to CH_2-11 and a triplet signal at δ 3.10 ppm due to CH_2-14, in addition to multiplet signals in region 7.20-8.40 ppm. The mass spectrum gave a molecular ion peak at *m/z* = 315 which confirm with the proposed structure. The combined spectral data gave strong support for the proposed structure.

Coumarins and 2-pyridones are classic heterocyclic scaffolds which constitute vital substructures of several natural products and received enormous admiration for their wide range of applications. Secondary metabolites and synthetic intermediates of 2- pyridone scaffolds demonstrate broad spectrum

of synthetic, material and biological applications.[17] Ricinine[18] with its remarkable CNS stimulant activity was the first isolated 2-pyridone natural product followed by the discovery of analogous antibiotic natural products such as elfamycin,[19] ilicolicin[20] and efratomycin,[21] with excellent vasodilating properties.[22]

Numerous methods[23] have been reported for the synthesis of 2-pyridone derivatives because of the biological importance associated with these compounds. However, these methods suffer from several drawbacks such as a long reaction time, an excess of a volatile organic solvent, lower product yields, and harsh refluxing conditions. Therefore, the development of a simple and efficient method for the preparation of 2- pyridone derivatives is an active area of research and there is scope for further improvement involving mild reaction conditions and higher product yields.

In recent years, solvent-free organic reactions[24] have caused great interests which have many advantages such as high efficiency and selectivity, easy separation and purification, mild reaction conditions and a benefit to industry as well as environment. Some solvent-free reactions can be carried out with just heating.[25] We herein describe a practical and simple method to prepare 4-aryl-6-[benzo[*h*]coumarin-3-yl]-3-cyano-2-pyridone **8a-d** with heating raw material under dry conditions. The synthesis of 4-aryl-6-[benzo[*f*]coumarin-3-yl]-3-cyano-2-pyridone **8a-d** is illustrated in the **Scheme 5.**



1 **7a-d** **8a-d**

8a, R = H
8b, R = OH
8c, R = OMe
8d, R = $N(Me)_2$

Scheme 5

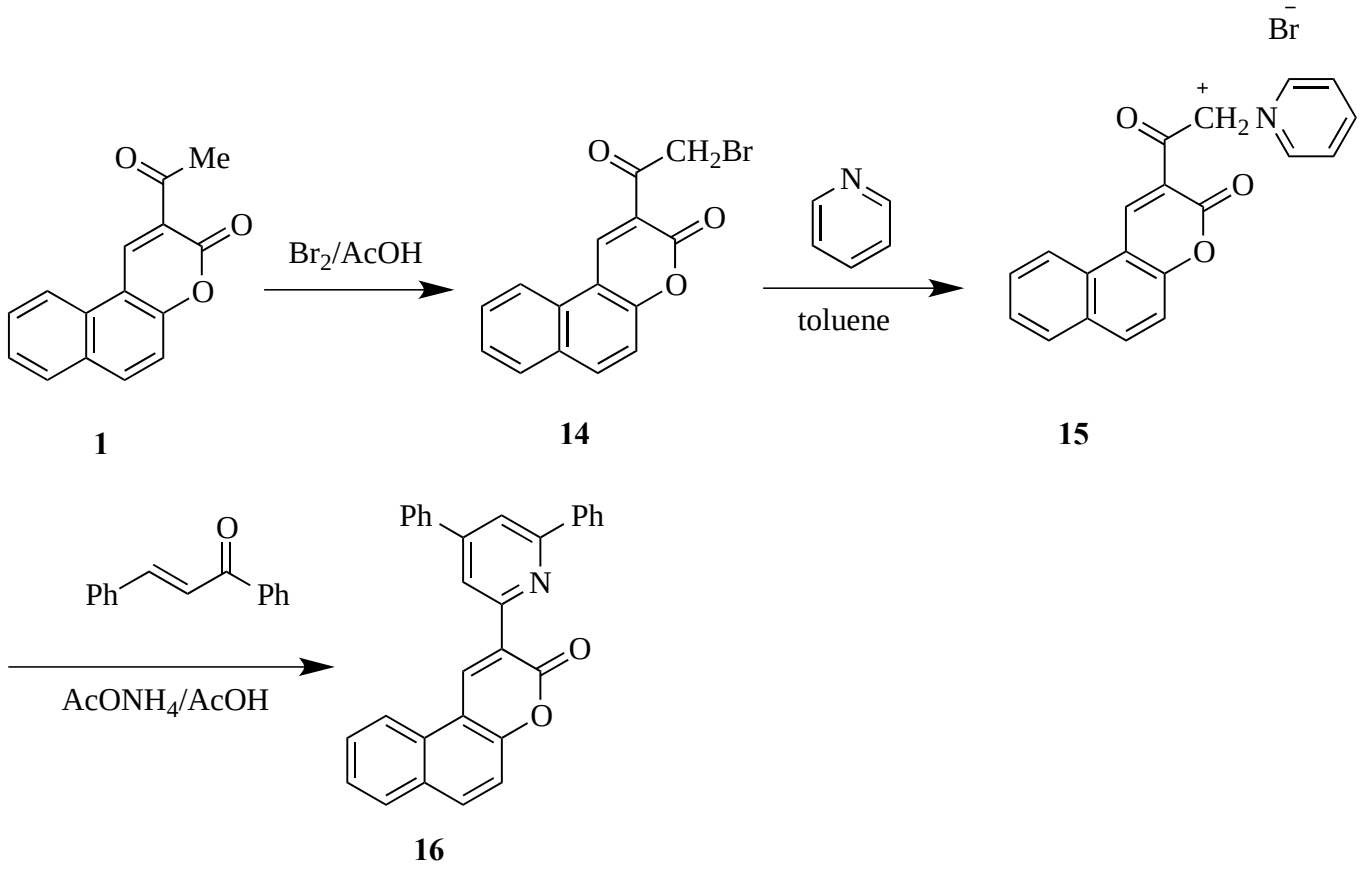
In the presence of sodium hydroxide, the reactions of various aromatic aldehydes **7a-d** and **1** with malononitrile were carried out respectively to afford the corresponding products **8a-d**. All reactions were completed within 30-45 min (TLC controlled) with high yields of products.

At first, the aldol condensation takes place from aromatic aldehydes **7a-d** and methyl ketone **1** to give intermediate **9**, or alternatively, the Knoevenagel condensation takes place by aldehydes **7a-d** and malononitrile to give intermediate **10**. Then, the intermediate **9** undergoes the Michael reaction with malononitrile or the intermediate **10** undergoes the Michael reaction with **1** to afford the intermediate **11.**

Partial hydrolysis of the intermediate **11** gives the intermediate **12,** which undergoes intramoleculer nucleophilic addition and dehydration. The formation product **8a-d** from **13** could be due to dehydrogenation by the atmospheric oxygen.

Because the reaction worked under a solvent-free condition, the handling procedure of reaction was very simple. The structure of compounds **8a-d** was established based on elemental and spectroscopic data. IR spectra of compounds **8a-d** showed a strong absorption peak in the region 3180-3300 cm^{-1} due to NH group, 2100-2250 cm^{-1} attributable to cyano group, 1625-1640 cm^{-1} due to amidic carbonyl. ^1H NMR showed a singlet signal 11.88-12.60 ppm due to NH, 6.70-6.90 ppm due to C_5-H pyridine, in addition to a multiplet in the region of 7.20-8.30 ppm due to aromatic protons and C_4-H in benzo[*f*]coumarin ring.

2-(4,6-Diphenylpyridin-2-yl)-3*H*-benzo[*f*]chromen-3-one **(16)** was synthesized by Kröhnke pyridine synthesis *via* the formation of α-pyridinium salt,[26] then reaction of the product **15** with chalcone in the presence of ammonium acetate as shown in **Scheme 6**.



1 **14** **15** **16**

Scheme 6

BIOLOGICAL ACTIVITY

ANTIBACTERIAL STUDIES

The newly synthesized compounds were checked for their *in vitro* against Grampositive bacteria such as *Bacillus sunbtills* and *Enterococcus faecalis E61* and Gram-negative bacteria such as Salmonella typhimurium and *Escherichia coli* in order to establish their bioactivities. In these tests, Ampicillin and Chloramphenicol were used as the standard drugs. Disk diffusion technique was used for the determination of the antibacterial. The results obtained against these microorganisms are given in **Table 1**

Table 1. Inhibition zone (mean diameter of inhibition in mm) as a criterion of antibacterial activities of the newly synthesized compounds

Compound no.	Inhibition zone (mm)			
	Gram positive bacteria		Gram negative bacteria	
	Bacillus sunbtills	Enterococcus faecalis E61	Salmonella typhimurium T 876	E. coli
3a	13	13	--	38
3b	12	7	--	--
3c	13	10	--	--
3d	11	12	--	10
4a	8	14	--	29
4b	11	14	--	34
4c	10	14	--	--
4d	8	13	--	--
5	7	12	--	--
6	--	10	--	--
8a	12	11	--	--
8b	13	9	8	--
8c	12	10	12	--
8d	10	10	11	--
16	9	8	--	--
Reference drugs				
Ampicillin	25	20	13	19
Chloramphenicol	24	23	18	21

The results obtained clearly show the efficiency of some of the new compounds. The results indicated that some of the synthesized compounds have higher activity than the standard such as **3a, 4a** and **4b** against *E. coli*.

We found that the activity of the synthesized compounds depends on their concentration and the strain of tested bacteria. Grampositive bacteria were more susceptible to the synthesized compounds than Gram negative ones.

This effect can be attributed in part to the great complexity of the double membrane containing cell envelope in Gram-negative bacteria, compared to the single membrane structure of positive ones.

The results depicted in **Table 1** revealed that most of tested compounds displayed variable moderately inhibitory effects on the growth of the tested Grampositive and low effect against Gramnegative bacterial strains. The compounds **3a, 3b, 3c, 8a, 8b and 8c** showed relative activity towards Gram-positive bacteria but less than the reference drugs. Regarding the structure-activity relationship, it was revealed that all compounds have higher activity than the others that contain either a polar group as NH_2 (**3a, 3b, 3c)** or NH in other compounds**.**

Compounds **3a, 4a** and **4b** revealed mean diameters of the clear inhibition zones 38, 29 and 34 mm against *E. Coli* Gram negative bacteria, respectively, i.e. greater clear inhibition zones than obtained by two reference drugs Ampicilin and Chloroamphincol 19 and 21 mm, respectively. The activity of **3a** may be due presence of ethylidenemalononitrile moiety**,** while **4b** may be attributed to presence of sulphonyl group in its structure.

CYTOTOXIC SCREENING

The *in vitro* cytotoxicity **IC_{50} (µmol/L)** of the new synthesized compounds was studied using the 5-fluorouracil as reference drug, including MCF-7 (breast) and HePG2 (liver). The results are listed in **Table 2**

Table 2. Cytotoxic activity of the newly synthesized compounds

Compounds	*In vitro* Cytotoxicity IC_{50} (µmol/L)	
	HePG2	MCF-7
5-FU	9.30	13.1
3a	8.5	100
3b	69.2	73.4
3c	13.4	29.5
3d	22.0	22.4
4a	48.7	47.1
4b	67.5	94.7
4c	67.4	70.3

4d	69.3	70.4
8a	77.6	78.3
8b	58.1	59.9
8c	53.6	56.3

IC_{50} (µmol/L): (1-10) very strong, 11-25 (strong), 26-50 (moderate), 51-100 (very weak), 200 (non-cytotoxicity), 5-Fu= 5 florocurcil.

All compounds showed cytotoxicity against MCF-7 (breast) and HePG2 (liver). Compound **3a** showed a very strong cytotoxicity against HePG2 (liver) even more strong than 5-FU, while compound **3a** showed very weak cytotoxicity against MCF-7 (breast). Compounds **3c** and **3d** showed strong cytotoxicity against HePG2 (liver)**.** Also compound **3d** showed strong cytotoxicity against MCF-7 (breast). Compound **3c** showed moderate cytotoxicity against MCF-7 and **4a** showed moderate cytotoxicity against MCF-7 (breast) and HePG2 (liver). The other compounds showed a weak cytotoxicity against MCF-7 (breast) and HePG2 (liver). From above data, compounds, which contain crotonitrile moiety, have the strong cytotoxicity effect.

In conclusion, we have developed a simple and novel method for the synthesis of 4,6-diaryl-2-pyridone under a solvent-free condition by one-pot reactions of **1**, aromatic aldehydes **7a-d**, and malononitrile. Because of avoiding the use of a toxic organic solvent, this protocol has advantages of cheap starting materials, an excellent yield, mild reaction conditions, a simple experimental procedure and a friendly environment. We believe that the present methodology addresses the current devise toward green chemistry.

EXPERIMENTAL

Melting points were recorded on Gallenkamp electric melting point apparatus (Electronic Melting Point Apparatus, Great Britain, London) and are uncorrected. Precoated Merck silica gel 60F-254 plates were used for thin-layer chromatography (TLC) and the spots were detected under UV light (254 nm). The infrared spectra were obtained from potassium bromide triturate containing 0.5% of the product on Pye Unicam SP 1000 IR spectrophotometer (Thermoelectron Co. Egelsbach, Germany. The ^{1}H-NMR spectra were determined on Varian Gemini 400 MHz (Varian Co., Cairo university, Egypt), ^{13}C-NMR = 100 MHz. Deuterated DMSO-d_6 and $CDCl_3$ was used as a solvents, tetramethylsilane (TMS) was used as an internal standard and chemical shifts were measured in δ ppm. Mass spectra were determined on a GC-MS.QP-100 EX Shimadzu (Japan). Elemental analyses were recorded on Perkin-Elmer 2400 Elemental analyzer at the Micro-analytical Center at Cairo University, Cairo, Egypt.

Synthesis of 3-(1-amino-3-oxo-3*H*-benzo[*f*]chromen-2-yl)-but-2-enenitrile derivatives 3a-d. A

mixture of each of **1** (2.38 g, 0.01 mol) and appropriate nitriles **2a-d** (0.01 mol) in the presence of ammonium acetate (1.54 g, 0.02 mol) was heated in an oil-bath at 150 ºC for 45 min. The reaction mixture was poured onto ice/HCl. The solid that separated out was filtered, dried and recrystallized from EtOH-DMF to give compounds **3a-d.**

2-(1-(1-Amino-3-oxo-3*H*-benzo[*f*]chromen-2-yl)ethylidene)malononitrile (3a). Dark brown solid; yield (85%); mp 200 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3358, 3312,(NH_2), 2203, 2189 (two CN), 1720 (C=O); ^{1}H-NMR (400 MHz , DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3), 6.10 (s, 2H, NH_2), 7.28-8.20 (m, 6H, Ar-H); ^{13}C NMR (100 MHz , DMSO-d_6) δ (ppm): 11.9, 84.6, 104.4, 113.9, 115.0, 117.8, 121.5, 123.1, 124.4, 127.5, 128.9, 130.2, 132.1, 153.6, 154.00, 159.2, 161.00, 175.1; MS (EI, 70 eV) *m/z* = 301 (M^+). Anal. Calcd for $C_{18}H_{11}N_3O_2$ (301.31): C, 71.75; H, 3.68; N, 13.95. Found: C, 71.72; H, 3.70; N, 13.97.

3-(1-Amino-3-oxo-3*H*-benzo[*f*]chromen-2-yl)-2-(phenylsulfonyl)but-2-enenitrile (3b). Dark brown solid; yield (88%); mp 104 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3368, 3315 (NH_2), 2187 (CN),1718 (C=O); ^{1}H-NMR (400 MHz , DMSO-d_6) δ (ppm): δ 2.23 (s, 3H, CH_3), 6.14 (s, 2H, NH_2), 7.28-8.20 (m, 11H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm): 12.2 , 104.5, 114.9, 115.3, 117.1, 122.0, 122.6, 123.9, 127.2, 128.5 (2C), 128.9, 129.1, 129.9 (2C), 130.7, 132.1, 135.2, 144.1, 150.5, 158.7, 159.5, 161.5; MS (EI, 70 eV) *m/z* = 390 (M^+- CN). Anal. Calcd for $C_{23}H_{16}N_2O_4S$ (416.45): C, 66.34; H, 3.87; N, 6.73; S, 7.70. Found: C, 66.35; H, 3.84; N, 6.70; S, 7.70.

3-(1-Amino-3-oxo-3*H*-benzo[*f*]chromen-2-yl)-2-(benzo[d]thiazol-2-yl)but-2-enenitrile (3c) Yellow solid; yield (70 %); mp 233 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3397, 3323 (NH_2), 2200 (CN), 1721 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6,) δ (ppm): 2.30 (s, 3H, CH_3), 6.20 (s, 2H, NH_2), 7.28-8.20 (m, 10H, Ar-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 12.5, 103.1, 106.6, 115.0, 117.2, 118.4, 121.7, 122.6, 123.4, 124.5, 124.7, 125.8, 126.9, 128.2, 128.6, 130.7, 133.2, 137.8, 151.5, 153.8, 156.1, 159.2, 159.7, 162.7; MS (EI, 70 eV) *m/z* = 393 (M^+- NH_2). Anal. Calcd for $C_{24}H_{15}N_3O_2S$ (409.46): C, 70.40; H, 3.69; N, 10.26; S, 7.83. Found: C, 70.42; H, 3.70; N, 10.26; S, 7.84.

2-(2-(1-Amino-3-oxo-3*H*-benzo[*f*]chromen-2-yl)-1-cyanoprop-1-en-1-yl)benzonitrile (3d). Brown solid; yield (81 %); mp 118 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3377, 3342 (NH_2), 2203, 2199 (two CN), 1720 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6,) δ (ppm): 2.30 (s, 3H, CH_3), 6.20 (s, 2H, NH_2), 7.28-8.20 (m, 10H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm): 12.8, 100.9, 104.1, 108.9, 114.5, 115.9, 117.2, 118.8, 122.4, 125.0, 126.2, 127.7, 128.3, 128.6, 128.9, 130.4, 131.5, 132.4, 132.9, 139.9, 147.2, 152.8, 159.4, 161.6; MS (EI, 70 eV) *m/z* = 377 (M^+). Anal. Calcd for $C_{24}H_{15}N_3O_2$ (377.40): C, 76.38; H, 4.01; N, 11.13. Found: C, 76.30; H, 3.99; N, 11.10.

Synthesis of 3-alkyl-2-amino-4-methyl-5-oxo-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridine 4a-d. A solution of **3a-d** (0.01 mol) in glacial acetic acid (30 mL) was refluxed for 3 h. The solids that separated on concentration the solvent and cooling the solution were filtered off and recrystallized from EtOH-DMF as

compounds **4a-d**.

2-Amino-4-methyl-5-oxo-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridine-3-carbonitrile (4a). Dark brown solid; yield (30%); mp > 300 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3398, 3315 (NH_2), 2202 (CN),1722 (C=O); ^{1}H-NMR (400 MHz , DMSO-d_6,) δ (ppm): 2.50 (s, 3H, CH_3), 7.10 (s, 2H, NH_2), 7.28-8.20 (m, 6H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm): 16.8, 84. 6, 104.4, 113.9, 115.0, 117.8, 121.5, 123.1, 124.4, 127. 5, 128.9, 130.2, 132.1, 153.6, 154.00, 159.2, 175.1, 161.00; MS (EI, 70 eV) *m/z* = 301 (M^+). Anal. Calcd for $C_{18}H_{11}N_3O_2$ (301.31): C, 71.75; H, 3.68; N, 13.95. Found: C, 71.74; H, 3.67; N, 13.97.

2-Amino-4-methyl-3-(phenylsulfonyl)-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridin-5-one (4b). Dark brown solid; yield (55%); mp >300 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3364, 3349 (NH_2), 1725 (C=O); ^{1}H-NMR (400 MHz , DMSO-d_6,) δ (ppm): 2.50 (s, 3H, CH_3), 7.04 (s, 2H, NH_2), 7.28-8.20 (m, 11H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm): 17.1, 116.6, 116.9, 118.8, 123.9, 124.8, 126.0, 127.2, 128.6 (2C), 128.9, 129.4, 129.9 (2C), 133.7, 135.1, 137.3, 141.4, 148.8, 153.4, 155.8, 158.9, 163.1; MS (EI, 70 eV) *m/z* = 416 (M^+). Anal. Calcd for $C_{23}H_{16}N_2O_4S$ (416.45): C, 66.34; H, 3.87; N, 6.73; S, 7.70. Found: C, 66.35; H, 3.84; N, 6.70; S, 7.70.

2-Amino-3-(benzo[d]thiazol-2-yl)-4-methyl-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridin-5-one (4c). Red solid; yield (32%); mp 280 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3412, 3356 (NH_2), 1725 (C=O); ^{1}H-NMR (400 MHz , DMSO-d_6) δ (ppm): 2.51 (s, 3H, CH_3), 6.98 (s, 2H, NH_2), 7.28-8.20 (m, 10H, Ar-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm):18.9, 115.4, 116.3, 116.9, 119.2, 121.0, 121.8, 123.7, 124.1, 124.9, 125.5, 127.4, 128.2, 128.8, 129.6, 133.1, 133.8, 142.5, 148.7, 151.6, 153.3, 153.9, 158.5, 162.7; MS (EI, 70 eV) *m/z* = 409 (M^+). Anal. Calcd for $C_{24}H_{15}N_3O_2S$ (409.46): C, 70.40; H, 3.69; N, 10.26; S, 7.83. Found: C, 70.42; H, 3.70; N, 10.26; S, 7.84.

2-(2-Amino-4-methyl-5-oxo-5*H*-benzo[5,6]chromeno[4,3-*b*]pyridin-3-yl)benzonitrile (4d). Brown solid; yield (35%); mp >300 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3403, 3353 (NH_2), 2208 (CN), 1724 (C=O); ^{1}H-NMR (400 MHz , DMSO-d_6) δ (ppm): 2.51 (s, 3H, CH_3),7.10 (s, 2H, NH_2), 7.28-8.20 (m, 10H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm): 18.9, 105.3, 107.4, 114.8, 116.0, 116.9, 117.5, 118.8, 124.2, 126.9, 127.8, 128.3, 158.7, 128.9, 129.2, 130.5, 132.9, 133.3, 134.9, 141.6, 142.8, 149.4, 153.5, 162.7; MS (EI, 70 eV) *m/z* = 377 (M^+). Anal. Calcd for $C_{24}H_{15}N_3O_2$ (377.40): C, 76.38; H, 4.01; N, 11.13. Found: C, 76.36; H, 4.04; N, 11.14.

Synthesis of 2-phenyl-4-methyl-5*H*-benzo[5,6]chromeno[3,4-*c*]pyridin-5-one (5). A solution of compound **1** (1.19 g, 5 mmol) and 2-cyanoacetamide (0.42 g, 5 mmol) was heated to reflux in 20 mL acetophenone for 1 h on an oil bath in 170 °C (monitored by TLC). The solid product was filtered off and recrystallized from EtOH-DMF to give compound **5**; dark yellow solid; yield (34%); mp 257 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 1718 (C=O); ^{1}H-NMR (400 MHz , DMSO-d_6) δ (ppm): 2.43 (s, 3H, CH_3), 7.10-8.40 (m, 12H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm): 23.7, 119.1, 121.9, 123.3,

124.8, 126.0, 126.9, 127.1, 127.4 (2C), 129.8 (2C), 130.5, 132.6, 136.7, 141.7, 152.2, 154.0, 157.9, 159.2, 164.2; MS (EI, 70 eV) *m/z* = 337 (M^+)Anal. Calcd for $C_{23}H_{15}NO_2$ (337.38): C, 81.88; H, 4.48; N, 4.15. Found: C, 81.79; H, 4.45; N, 4.13.

Synthesis of 2-methyl-11,12,13,14-tetrahydro-3*H*-benzo[5,6]chromeno[3,4-*c*]quinolin-3-one (6). A solution of compound **1** (1.19 g, 5 mmol) and 2-cyanoacetamide (0.42 g, 5 mmol) was heated to reflux in 20 mL cyclohexanone for 8 h on an oil bath in 170 °C (monitored by TLC). The solid product was filtered off and recrystallized from EtOH-DMF to give compound **6**; Dark yellow solid; yield (41%); mp 216 °C (EtOH-DMF); IR (KBr): *v*/cm^{-1} = 2890-3000 (CH_2 aliphatic), 3000-3090 (CH aromatic), 1725 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.60-1.70 (m, 4H, CH_2 -12,CH_2-13), 2.50 (s, 3H, CH_3), 2.60 (t, *J* = 7.80 Hz, 2H, CH_2-11), 3.10 (t, *J* = 7.50 Hz, 2H, CH_2-14) , 7.20-8.40 (m, 12H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm): 22.4, 22.9, 23.8, 25.1, 30.4, 119.6, 122.4, 124.3, 125.8, 126.2, 127.1, 128.9, 129.3, 131.2, 132.8, 135.3, 151.9, 154.7, 158.2, 159.7, 163.4; MS (EI, 70 eV) *m/z* = 315 (M^+). Anal. Calcd for $C_{21}H_{17}NO_2$ (315.37): C, 79.98; H, 5.43; N, 4.44. Found: C, 79.96; H, 5.46; N, 4.43.

Synthesis of 4-aryl-6-[benzo[*f*]coumarin-3-yl]-3-cyano-2-pyridone derivatives 8a-d. A mixture of aromatic aldehydes **7a-d** (1 mmol), **1** (1 mmol), malononitrile **3** (1.5 mmol) and NaOH (1.5 mmol) was put in a reaction flask and heated to a temperature of 75 °C for about 45 min. After completing the reaction, the reaction mixture was poured into water, and then washed with water thoroughly. The product was collected by filtration, dried and recrystallized from 95% EtOH.

2-Oxo-6-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)-4-phenyl-1,2-dihydropyridine-3-carbonitrile (8a). Dark brown solid; yield (87%); mp 80 °C (EtOH); IR (KBr): *v*/cm^{-1} =3288 (NH), 2200 (CN), 1724, 1633 (two C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 6.80 (s, 1H, C_5-H pyridine), 7.20-8.30 (m, 12H, Ar-H), 12.55 (s, 1H, NH) ; ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 101.1, 114.7, 115.9, 118.6, 120.7, 121.6, 122.1, 123.9, 125.0, 126.8, 127.4, 128.2, 128.8 (2C), 129.1, 129.8(2C), 132.3, 134.9, 136.6, 139.4, 155.3, 159.6, 161.4, 169.5; MS (EI, 70 eV) *m/z* = 390 (M^+). Anal. Calcd for $C_{25}H_{14}N_2O_3$ (390.40): C, 76.92; H, 3.61; N, 7.18. Found: C, 76.95; H, 3.58; N, 7.15.

4-(4-Hydroxyphenyl)-2-oxo-6-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)-1,2-dihydropyridine-3-carbonitrile (8b). Brown solid; yield (85%), mp 143 °C (EtOH); IR (KBr): *v*/cm^{-1} = br 3405 (OH), 3219 (NH), 2196 (CN), 1725, 1636 (two C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 6.80 (s, 1H, C_5-H pyridine), 7.20-8.30 (m, 11H, Ar-H), 10.20 (s, 1H, OH), 12.24 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 101.3, 114.8, 115.9, 117.3 (2C), 118.6, 120.7, 121.6, 122.2, 123.9, 125.4, 126.8, 127.4, 128.5, 129.6, 131.1(2C), 132.6, 134.9, 136.6, 139.9, 157.8, 159.6, 161.4, 169.6; MS (EI, 70 eV) *m/z* = 406 (M^+). Anal. Calcd for $C_{25}H_{14}N_2O_4$ (406.40): C, 73.89; H, 3.47; N, 6.89. Found: C, 73.90; H, 3.44; N, 6.86.

4-(4-Methoxyphenyl)-2-oxo-6-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)-1,2-dihydropyridine-3-carbonitrile (8c). Dark brown solid; yield (79%); mp 110 °C (EtOH); IR (KBr): *v*/cm^{-1} = 3198 (NH), 2204 (CN), 1725,

1635 (two C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.90 (s, 3H, OCH_3), 6.80 (s, 1H, C_5-H pyridine), 7.20-8.30 (m, 11H, Ar-H), 11.98 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 59.2, 101.3, 114.8, 115.9, 116.4 (2C), 118.5, 120.5, 121.8, 122.2, 123.9, 125.6, 126.8, 127.4, 128.5, 129.6, 130.4 (2C), 132.6, 134.9, 136.8, 140.2, 157.8, 159.6, 161.4, 168.8; MS (EI, 70 eV) *m/z* = 420 (M^+). Anal. Calcd for $C_{26}H_{16}N_2O_4$ (420.42) : C, 74.28; H, 3.84; N, 6.66. Found: C, 74.26; H, 3.85; N, 6.64.

4-(4-(Dimethylamino)phenyl)-2-oxo-6-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)-1,2-dihydropyridine-3-carbonitrile (8d). Red solid; yield (71%); mp 136 °C (EtOH); IR (KBr): *ν*/cm^{-1} = 3188 (NH), 2194 (CN), 1725, 1640 (two C=O); ^{1}H-NMR (400 MHz , $CDCl_3$) δ (ppm): 3.10 (s, 6H, $2CH_3$), 6.80 (s, 1H, C_5-H pyridine), 7.20-8.30 (m, 12H, Ar-H), 11.90 (s, 1H, NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 42.6 (2C), 101.3, 113.5 (2C), 114.4, 116.0, 118.5, 120.7, 121.8, 122.2, 124.0, 125.6, 126.9, 127.4, 128.7, 129.8, 130.1 (2C), 132.9, 134.4, 136.8, 140.5, 157.9, 159.8, 161.7 , 168.9; MS (EI, 70 eV) *m/z* = 433 (M^+). Anal. Calcd for $C_{27}H_{19}N_3O_3$ (433.47): C, 74.81; H, 4.42; N, 9.69. Found: C, 74.80; H, 4.44; N, 9.70.

Synthesis of 2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (14)

The compound **14** was prepared according to the reported work[26]

Synthesis of 1-(2-oxo-2-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)ethyl)pyridin-1-ium bromide (15)

The compound **15** was prepared according to the reported work[26]

Synthesis of 2-(4,6-diphenylpyridin-2-yl)-3*H*-benzo[*f*]chromen-3-one (16). A solution of **15** (0.395g, 1 mmol), benzalacetophenone (0.208 g, 1 mmol) and ammonium acetate (0.77 g, 1 mmol) in 10 mL glacial acetic acid was refluxed for about 6 h. The solid product was isolated by filtration. The solid product was washed with EtOH. The crude product was dried and crystallized from DMF-EtOH to furnish pure solid product; Dark brown solid; yield (42%); mp 255 °C (DMF-EtOH); IR (KBr): *ν*/cm^{-1} =3000-3090 (CH aromatic), 1725 (C=O), 1623 (C=N); ^{1}H-NMR (400 MHz , DMSO-d_6) δ (ppm): 7.20-8.40 (m, 19H, Ar-H); ^{13}C-NMR (100 MHz , DMSO-d_6) δ (ppm):112.3, 115.9, 118.3, 119.7, 121.9, 123.8, 127.1 (2C), 127.3, 127.8 (2C), 128.7, 128.9, 129.2 (2C), 129.3, 129.4, 129.8 (2C), 130.7, 131.4, 133.3, 136.8, 144.1, 145.4, 150.9, 152.6, 154.9, 157.2 162.5; MS (EI, 70 eV) *m/z* = 425 (M^+). Anal. Calcd for $C_{30}H_{19}NO_2$ (425.49): C, 84.69; H, 4.50; N, 3.29. Found: C, 84.68; H, 4.52; N, 3.31.

ANTIMICROBIAL SCREENING

ANTIBACTERIAL ASSAY

The newly synthesized compounds obtained were preliminary evaluated for their *in vitro* antibacterial activity against a narrow spectrum of bacterial species procured from the Laboratory of Microbial Biochemistry (*Faculty of Pharmacy, Mansoura Univ*.). The paper disc assay described by Cooper[27] using nutrient agar medium was applied. Suspensions of each microorganism were prepared from their

24h-cultures to obtain approximately 106 colony forming units (cfu) per mL for plating. Paper discs (Whatman No.1) of 8 mm diameter were loaded individually with a constant amount (100 µg/disc) of the compounds to be tested. Discs were aseptically transferred and applied onto the dry surface of the inoculated plates and then incubated at 37 °C for overnight (~18-20 h). This assay was performed in duplicates and the mean diameters of the clear inhibition zones (mm) were recorded disregard a single colony or a faint haze caused by the inoculums.

CYTOTOXICITY ACTIVITY

The synthesized compounds were evaluated for their *in-vitro* anticancer effect *via* the standard MTT method,[28] against a panel of two human tumor cell lines namely; heptacelluar carcinoma (liver) HepG and, mammary gland (breast) MCF-7.

The cell lines were obtained from ATCC *via* the Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-Fluorouracil (5-Fu) was used as a standard anticancer drug for comparison.

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