HETEROCYCLES, Vol. 100, No. 7, 2020, pp. 1035 - 1047. © 2020 The Japan Institute of Heterocyclic Chemistry
Received, 13th April, 2020, Accepted, 25th May, 2020, Published online, 28th May, 2020
DOI: 10.3987/COM-20-14264

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SOME NOVEL CHROMENOPYRIDINE, PYRANOCHROMENE AND 3-HETARYLCOUMARIN DERIVATIVES

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Abstract- 3-Acetylcoumarin was used as a precursor for the synthesis of novel fused heterocyclic compounds as chromeno[3,4-*c*]pyridine, chromeno[4,3-*b*]pyridine and pyrano[3,4-*c*]chromene derivatives in addition to the synthesis of 3-hetarylchromene derivatives. The chemical structures of newly synthesized compounds were avowed by means of spectral and analysis data. The novel synthesized compounds were estimated as antibacterial agents against *Staphylococcus aureus* and *Escherichia coli* bacteria.

INTRODUCTION

Coumarins are naturally occurring compounds, exhibited various biological activities as anti-oxidant, anti-microbial, analgesic, antimalarial, anti-inflammatory, anti-HIV, anti-tuberculosis and anticancer.[1-11] Coumarin derivatives are main component in many drugs, for example, Warfarin (Figure 1) decreases metastases from intestinal carcinomas to a pronounced extent[12] and it is also used for treatment of malignant tumors.[13] Also, daphentin (7,8-dihydroxycoumarin) (Figure 1) inhibits tyrosine kinase.[14] In addition, 3-(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-4-hydroxy-2*H*-chromen-2-one (Figure 1) was identified as a class of MEK 1 kinase inhibitors.[15]

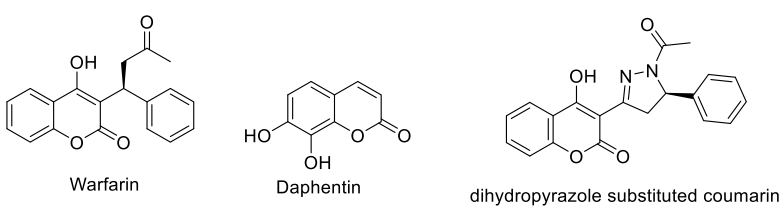
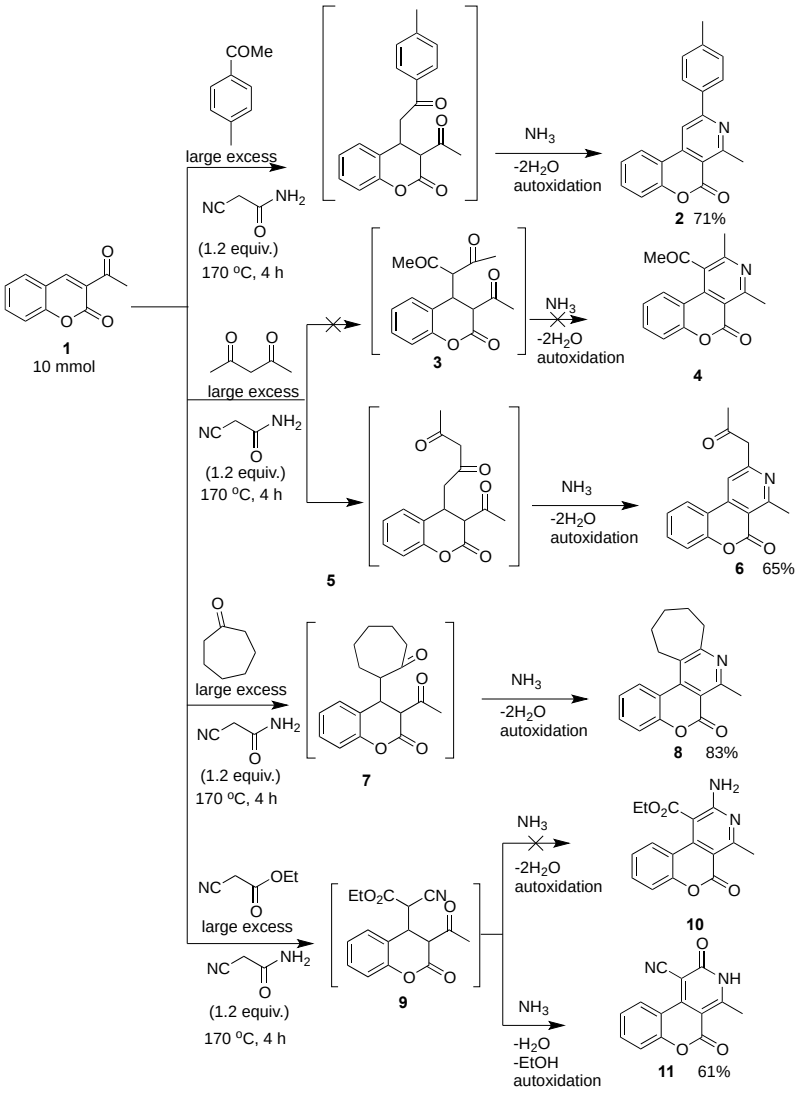


Figure 1. Some coumarin derivatives as anticancer and kinase inhibitors

In addition, coumarin derivatives are significant as photochemotherapeutic agents, which are used for treatment of skin diseases.[16] Chromenopyridine derivatives displayed a variety of important properties such as antimicrobial,[17] analgesic,[18] anti-tubercular[19] and antitumor activities.[20]

RESULTS AND DISCUSSION

Based on the previous findings, we report here the synthesis of novel chromeno[3,4-*c*]pyridine, chromeno[4,3-*b*]pyridine and 3-hetarylcoumarin derivatives starting from 3-acetylcoumarin (**1**). According to reported literature,[21] cyanoacetamide is best choice as ammonia source for reaction of 3-acetylcoumarin with ketonic compounds to form chromeno[3,4-*c*]pyridin-5-one derivatives, while use of alcoholic ammonia or ammonium acetate was not successful. Thus, heating of **1** with some ketonic compounds such as 4-methylaceophenone, acetylacetone, cycloheptanone and/or ethyl cyanoacetate as reagents and solvents in the presence of cycnoacetamide as ammonia source at 170 °C furnished chromeno[3,4-*c*]pyridine derivatives **2**, **6**, **8** and **11**, respectively[21] (Scheme 1).

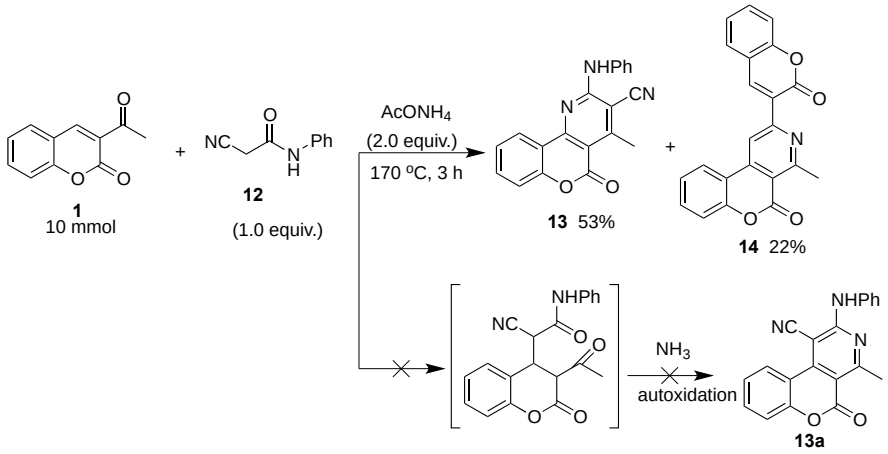


Scheme 1. Synthesis of chromeno[3,4-*c*]pyridin-5-one derivatives

The reaction of **1** with ketonic addendum was assumed that it proceeded *via* Michael addition to give 1,5-dicarbonyl compounds intermediates which cyclized in presence of ammonia to give finally chromeno[3,4-*c*]pyridine derivatives. Contrary to our expectation, the reaction of **1** with acetylacetone underwent Michael addition *via* methyl ketone and not activated methylene to give 4-methyl-2-(2-oxopropyl)-5*H*-chromeno[3,4-*c*]pyridin-5-one (**6**) (Scheme 1). The chemical structure of **6** was clearly confirmed by its ^{1}H NMR spectrum which exposed four singlet signals at δ 2.08, 2.90, 4.15 and 8.04 ppm due to two methyl groups, CH_2 and C_3-H pyridine.

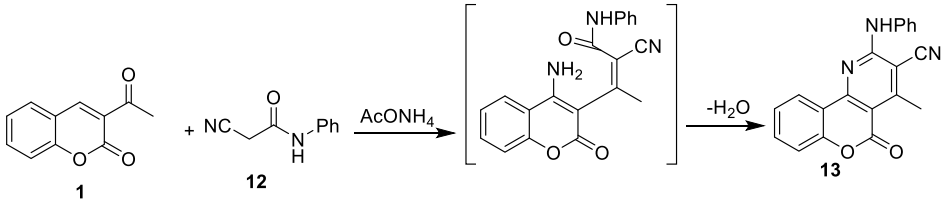
Additionally, treatment of **1** with ethyl cyanoacetate led to the Michael adduct intermediate **9** that has two possible routes to afford chromenopyridine derivative. The IR spectrum of reaction product **11** displayed an absorption peak at 2232 cm^{-1} due to nitrile group, which indicated that CN was not involved in the cyclization reaction and also IR spectrum of reaction product revealed two bands at 1645 and 1727 cm^{-1} assignable to amide and lactone, respectively that established the formation of compound **11**[22-24] rather than **10.** The structure of **11** was also recognized from its analytical and spectral data. According to reported literature,[21] treatment of **1** with cyanoacetamide afforded compound **14** instead of **11**. The mass spectra of compounds **2**, **6**, **8** and **11** gave molecular ion peaks accord with their proposed structures.

On the other hand, heating a mixture of **1** and 2-cyano-*N*-phenylacetamide (**12**) in the presence of ammonium acetate at 170 °C yielded 4-methyl-5-oxo-2-(phenylamino)-5*H*-chromeno[4,3-*b*]pyridine-3-carbonitrile (**13**)[25,26] instead of its regioisomer **13a** and 4-methyl-2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[3,4-*c*]pyridin-5-one (**14**) that presumably formed due to reaction of two moles of **1** with ammonium acetate[21,27] (Scheme 2). Reported literature[25] confirmed that the reaction occur firstly by 1,4-aza Michael reaction that augment formation of **13** as shown in Scheme 3. The mixture of **13** and compound **14** was completely separated successfully by boiling in excess chloroform, which dissolved compound **14** only and compounds **13** remains as precipitate.



Scheme 2. Synthesis of 4-methyl-5-oxo-2-(phenylamino)-5*H*-chromeno[4,3-b]pyridine-3-carbonitrile (**13)**

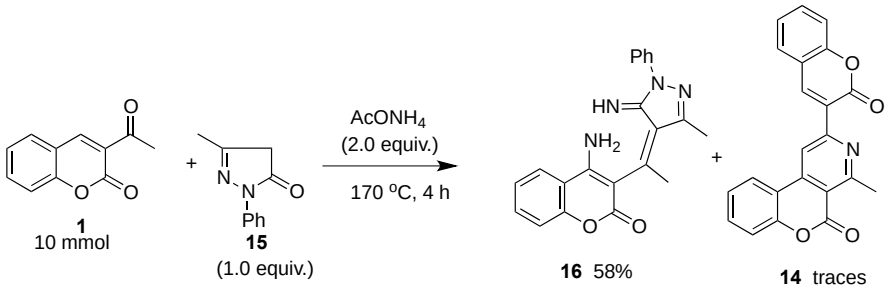
The structures of both **13** and **14** were authenticated from their analytical and spectral data. Thus, IR spectrum of **13** displayed strong absorption peak at 2195 cm^{-1} due to CN group, an absorption band at 3424 cm^{-1} equivalent to NH and strong absorption band at 1725 cm^{-1} attributable to coumarin carbonyl group. Mass spectrum of **13** delivered molecular ion peak at *m/z* 327 that accord with its supposed structure. ^{1}H NMR spectrum of compound **13** provided more evidence for its proposed structure by displaying two singlet signals at δ 2.04 and 10.69 ppm owing to Me and NH protons, respectively. The formation of compounds **13** could be explained by plausible mechanism was offered in scheme 3.



Scheme 3. Mechanism of formation of compound **13**

The structure of **14** was identified form its ^{1}H NMR and reported literature.[21] ^{1}H NMR spectrum of **14** raveled three singlet signals at δ 2.59, 9.23 and 9.51 ppm attributable to Me, C_3-H pyridine and C_4-H coumarin, respectively, in addition to aromatic protons signals.

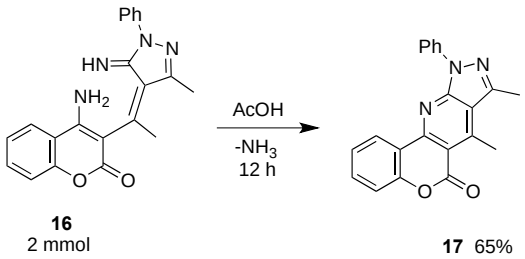
In a similar methodology, interaction of **1** with pyrazolone derivative **15** in the presence of ammonium acetate presented 4-amino-3-(1-(5-imino-3-methyl-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)ethyl)-2*H*-chromen-2-one (**16**) and traces of **14** (Scheme 4). The chemical structure of **16** was deduced based on its spectral data. Thus, its ^{1}H NMR spectrum, in addition to aromatic signals, exhibited two singlet signals for two methyl protons at δ 2.38 and 2.44 ppm and two singlet signals (D_2O exchangeable) at δ 5.04 and 9.77 ppm assignable to NH_2 and NH, respectively.



Scheme 4. Synthesis of 4-amino-3-(1-(5-imino-3-methyl-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)-ethyl)-2*H*-chromen-2-one (**16**)

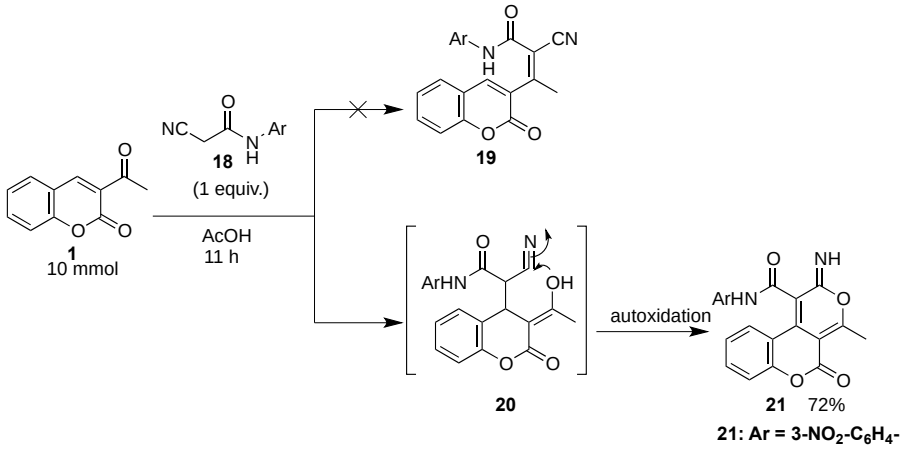
Subsequent heating a solution of **16** in glacial acetic acid under reflux presented the corresponding 7,8-dimethyl-10-phenylchromeno[4,3-*b*]pyrazolo[4,3-*e*]pyridin-6(10*H*)-one (**17**) (Scheme 5). The

chemical structure of **17** was established from its analytical and spectral data. Its ^{1}H NMR spectrum devoid any D_2O exchangeable singlet signals which indicate that NH_2 and NH were involved in the cyclization reaction, also its mass spectrum showed molecular ion peak at *m/z* 341 identical to its proposed structure.



Scheme 5. Synthesis of 7,8-dimethyl-10-phenylchromeno[4,3-*b*]pyrazolo[4,3-*e*]pyridin-6(10*H*)-one (**17**)

An attempt for the synthesis of 2-cyano-3-(2-oxo-2*H*-chromen-3-yl)-*N*-arylbut-2-enamide (**19**) to be used a synthon for synthesis of chromenopyridine was failed *via* reaction of **1** with 2-cyano-*N*-arylacetamide (**18**) in refluxing glacial acetic acid and instead, we obtained 2-imino-4-methyl-5-oxo-*N*-aryl-2*H*,5*H*-pyrano[3,4-*c*]chromene-1-carboxamide derivative **21** (Scheme 6).

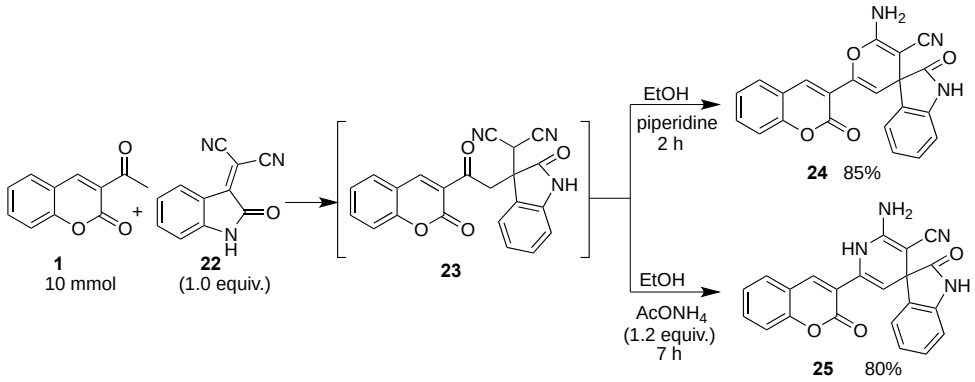


Scheme 6. Synthesis of 2-imino-4-methyl-5-oxo-*N*-(3-nitrophenyl)-2*H*,5*H*-pyrano[3,4-*c*]chromene-1-carboxamide (**21**)

The chemical structure of **21** was elucidated *via* its elemental and spectral data. IR spectrum of **21** devoid any absorption bands due to CN group, which confirm that nitrile group was involved in the cyclization reaction. The ^{1}H NMR spectra of **21** displayed two NH protons signals at δ 10.05 and 10.46 ppm in addition to singlet signal at 2.59 ppm attributable to Me. The mass spectrum molecular ion peak of structure **21** consent with their proposed structure.

3-Acetylcoumarin (**1**) had conventional significant attention as a synthon for the synthesis of pyran and

pyridine and other heterocyclic derivatives.[28-30] Therefore, in this work, we settle the above facts by synthesis of novel coumarin derivatives bearing pyran and pyridine moiety in position three by reaction of **1** with 2-(2-oxoindolin-3-ylidene)malononitrile (**22**) in refluxing EtOH catalyzed by piperidine to give pyran derivative **24**. On the other hand, when the same reaction occurred in presence of ammonium acetate instead of piperidine, it afforded 1,4-dihydropyridine derivative **25** (Scheme 7).



Scheme 7. Synthesis of 3-pyranylcoumarin derivative **24** and 3-pyridylcoumarin derivative **25**

The spectral and analytical data of **24** and **25** agreed with their anticipated structures. Thus, ^{1}H NMR spectrum of **24** (as an example) displayed four singlet signals at δ 6.18, 7.29, 8.47 and 10.60 ppm due to C_3-H pyran, NH_2, C_4-H coumarin and NH protons, respectively in addition to aromatic protons.

ANTIMICROBIAL ASSAY

The antibacterial activity of novel synthesized heterocycles under examination was assessed in *vitro* against Gram-positive bacteria (*Staphylococcus aurous)* and Gram-negative bacteria (*Escherichia coli*) using filter paper disc diffusion method.[31] Ampicillin was used as a control reference for *in vitro* antibacterial activity. Antibacterial activity was expressed in diameter inhibition zones in millimeters (mm). The percentage activity index for the complex was calculated by the formula as under:

$$\%\ Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ compound\ (diametre)}{Zone\ of\ inhibition\ by\ standard\ (diametre)}\ \text{X}100$$

Table 1. Inhibition diameter zone (mm) of the novel synthesized heterocycles.

Compounds	**Gram (+ve) bacteria**		**Gram (-ve) bacteria**	
	S. aureus **(mg/mL)**		***E. coli*** **(mg/mL)**	
	Diameter of inhibition zone (mm)	**% Activity index**	**Diameter of inhibition zone (mm)**	**% Activity index**

2	3	13.0	NA	----
6	20	86.9	19	79.2
8	13	56.5	10	41.7
11	9	39.1	7	29.2
13	17	73.9	16	66.7
14	5	21.7	NA	----
16	17	73.9	18	75.0
17	NA	----	NA	----
21	11	47.8	13	54.2
24	NA	----	NA	----
25	10	43.5	9	37.5
Ampicillin	23	100	24	100

"NA": No Activity

According to data in Table 1, the tested compounds showed more strong results against Gram-positive bacteria (*Staphylococcus aureus*) than Gram-negative bacteria (*Escherichia coli*).

Compounds **6**, **13** and **16** exhibited the best results (86.9, 73.9 and 73.9% respectively) against Gram-positive bacteria compared with standard drug. Compounds **6** and **16** displayed good results against Gram-negative bacteria (79.2, 66.7 and 75.0% respectively)**,** while compound **13** provided moderate result.

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

Structure activity relationship for chromeno[3,4-*c*]pyridine derivatives **2**, **6**, **8** and **11** displayed obviously that presence aliphatic substituent incorporating electron withdrawing group as 2-oxopropyl in compound **6** compared with other chromeno[3,4-*c*]pyridine derivatives. On the other hand, presence of fused cycloheptane ring in **8** exhibited moderate antimicrobial activities. Cyclocondensation of compound **16** to construct chromeno[4,3-*b*]pyrazolo[4,3-*e*]pyridine derivative **17** terminate the antimicrobial activity, which demonstrate that presence of isolated pyrazole ring moiety showed remarkably activity than fused chromenopyrazole. Comparing the antimicrobial activity of pyranochromene derivative **21** with those of chromenopyridine derivatives **6**, **8** and **13** showed that presence of pyridine ring exhibit more reactivity than pyran ring. Additionally, 3-pyridylchromene derivative **25** has antimicrobial activity while 3-pyranylchromene derivative **24** was biologically inactive.

CONCLUSION

We report here simple and efficient synthetic routes to prepare chromeno[3,4-*c*]pyridine, chromeno[4,3-*b*]pyridine and pyrano[3,4-*c*]chromene derivatives utilizing 3-acetylcouumarin. The antimicrobial activities of the novel synthesized compounds were evaluated and compared with ampicillin as standard drug. Compound **6** exposed the best results against Gram-positive and Gram-negative bacteria.

EXPERIMENTAL

All melting points were determined on Gallenkamp electric melting point instrument and are uncorrected. Precoated Merck silica gel 60F-254 plates were used for TLC. The IR (KBr) spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer (Thermoelectron Co. Egelsbach, Germany). The ^{1}H NMR spectra (400 and 500 MHz) and ^{13}C NMR (100 and 125 MHz) were recorded on Brucker. DMSO-d_6 and $CDCl_3$ were used as solvents, chemical shifts, tetramethylsilane was used as an internal standard ($\delta = 0$). Mass spectra (EI) were recorded on a gas chromatography/mass spectrometry QP-100 EX Shimadzu (Japan). Elemental analyses were carried out at the Micro-Analytical Center at Cairo University, Cairo, Egypt.

Synthesis of 3-acetylcoumain (1)[32]

A mixture of salicylaldehyde (12.2 g, 0.1 mol) and ethyl acetoacetate (13 g, 0.1 mol) was stirred under cooling conditions. To this mixture, few drops of piperidine were added and stirring continued for 15 min. The mixture was kept at freezing temperature for 2 h. The resulting yellow mass solid was separated out and recrystallized from EtOH to give **1** in 85% yield; mp 122 ºC.

General procedure for synthesis of 4-methyl--5*H*-chromeno[3,4-*c*]pyridin-5-one derivatives 2, 6, 8 and 11.

A mixture of 3-acetylcoumarin (**1**) (1.88 g, 10 mmol), cyanoacetamide (1.0 g, 12 mmol) and ketonic compounds namely: 4-methylacetopheneone, acetylacetone, cycloheptanone and/or ethyl cyanoacetate (15 mL) was heated in a sealed tube at 170 ºC for 4 h. The reaction mixture was left to cool at room temperature overnight. The solids were precipitated were washed with cold EtOH for several times, filtered off and dried. The obtainable solid products were recrystallized from EtOH to give the corresponding compounds (**2**, **6**, **8** and **11**).

4-Methyl-2-(*p*-tolyl)-5*H*-chromeno[3,4-*c*]pyridin-5-one (2).

Buff solid; yield 71%; mp 215-216 °C; IR (KBr, ν_{max}, cm^{-1}): 1719 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.44 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 7.35-7.39 (m, 4H, Ar-H), 7.60-7.63 (m, 1H, Ar-H), 8.07-8.17 (m, 3H, Ar-H), 8.21 (s, 1H, C_3-H pyridine); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 21.49, 26.02, 117.44, 118.05, 121.28, 123.53, 125.66, 128.14, 129.08, 130.72, 131.09, 133.53, 136.72, 151.88, 152.29,

157.48, 159.79, 168.29; MS *m/z* 301 (M^+). Anal. Calcd for $C_{20}H_{15}NO_2$ (301.35): C, 79.72; H, 5.02; N, 4.65%. Found: C, 79.78; H, 5.05; N, 4.69%.

4-Methyl-2-(2-oxopropyl)-5*H*-chromeno[3,4-*c*]pyridin-5-one (6).

Red solid; yield 65%; mp 172 °C; IR (KBr, ν_{max}, cm^{-1}): 1708, 1712 (2 C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.08 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 7.08 (d, 1H, *J* = 9 Hz, Ar-H), 7.15 (t, 1H, *J* = 7.5 Hz, Ar-H), 7.46 (t, 1H, *J* = 8 Hz, Ar-H), 7.88 (d, 1H, *J* = 7.5 Hz, Ar-H), 8.04 (s, 1H, C_3-H pyridine); ^{13}C NMR (125 MHz, DMSO-*d6*) δ (ppm): 25.92, 29.86, 53.46, 107.38, 117.21, 122.18, 125.30, 129.14, 129.62, 135.92, 151.17, 152.02, 158.19, 159.77, 168.24, 198.55; MS *m/z* 267 (M^+). Anal. Calcd for $C_{16}H_{13}NO_3$ (267.28): C, 71.90; H, 4.90; N, 5.24%. Found: C, 71.87; H, 4.92; N, 5.21%.

7-Methyl-10,11,12,13-tetrahydrochromeno[4,3-*d*]cyclohepta[*b*]pyridin-6(9*H*)-one (8).

White solid; yield 83%; mp 170 °C; IR (KBr, ν_{max}, cm^{-1}): 1730 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.84-1.86 (m, 2H, CH_2), 1.91-1.96 (m, 2H, CH_2), 2.00-2.04 (m, 2H, CH_2), 3.01 (s, 3H, CH_3), 3.22-3.24 (m, 4H, $2CH_2$ aliphatic), 7.29-7.30 (m, 1H, Ar-H), 7.36 (d, 1H, *J* = 8 Hz, Ar-H), 7.50-7.53 (m, 1H, Ar-H), 7.91-7.92 (m, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 26.01, 26.35, 30.45, 31.62, 39.08, 114.04, 117.43, 123.49, 127.85, 130.75, 131.20, 151.97, 159.81, 168.26; MS *m/z* 281 (M^++2), 280 (M^++1), 279 (M^+, base peak). Anal. Calcd for $C_{18}H_{17}NO_2$ (279.34): C, 77.40; H, 6.13; N, 5.01%. Found: C, 77.41; H, 6.08; N, 5.04%.

4-Methyl-2,5-dioxo-3,5-dihydro-2*H*-chromeno[3,4-*c*]pyridine-1-carbonitrile (11).

Brown solid; yield 61%; mp 285 °C; IR (KBr, ν_{max}, cm^{-1}): 1645, 1727 (2 C=O), 2232 (CN), 3448 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.83 (s, 3H, CH_3), 7.45-7.53 (m, 2H, Ar-H), 7.77-7.81 (m, 2H, Ar-H), 11.28 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 17.85, 105.59, 112.07, 115.86, 118.49, 123.48, 127.37, 128.48, 129.61, 151.27, 153.89, 160.08, 163.42, 168.92; MS *m/z* 252 (M^+). Anal. Calcd for $C_{14}H_8N_2O_3$ (252.23): C, 66.67; H, 3.20; N, 11.11%. Found: C, 66.60; H, 3.25; N, 11.14%.

Synthesis of 4-methyl-5-oxo-2-(phenylamino)-5*H*-chromeno[4,3-*b*]pyridine-3-carbonitrile (13).

A mixture of **1** (1.88 g, 10 mmol), 2-cyano-*N*-phenylacetamide **12** (10 mmol) and ammonium acetate (1.54 g, 20 mmol) was placed in a sealed tube and heated in an oil bath at 170 ºC for 3 h (the reaction progress was monitored by TLC). Upon competition, the reaction mixture was poured onto cold dilute HCl. The solid that separated was washed with water, filtered off and dried. The dried solid was heated in excess $CHCl_3$, then filter the precipitate, which identified as **13** and recrystallized from glacial acetic acid. When the $CHCl_3$ filtrate was left to cool, other solid product **14** was precipitated in purity enough that did not require further recrystallization.

4-Methyl-5-oxo-2-(phenylamino)-5*H*-chromeno[4,3-*b*]pyridine-3-carbonitrile (13).

Brown solid; yield 53%; mp 248 °C; IR (KBr, ν_{max}, cm^{-1}): 1725 (C=O), 2195 (CN), 3424 (NH); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.04 (s, 3H, CH_3), 7.09-7.15 (m, 2H, Ar-H), 7.22 (t, 2H, *J* = 9.5 Hz,

Ar-H), 7.31-7.34 (m, 3H, Ar-H), 7.72 (d, 1H, *J* = 5.5 Hz, Ar-H), 7.80 (d, 1H, *J* = 7.5 Hz, Ar-H), 10.69 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO) δ (ppm): 18.27, 80.19, 115.22, 117.14, 119.72, 121.13, 122.53, 125.84, 126.35, 127.39, 131.75, 135.88, 128.31, 152.46, 153.98, 157.06, 157.64, 162.54; MS *m/z* 327 (M^+). Anal. Calcd for $C_{20}H_{13}N_3O_2$ (327.34): C, 73.38; H, 4.00; N, 12.84%. Found: C, 73.34; H, 4.05; N, 12.80%.

4-Methyl-2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[3,4-*c*]pyridin-5-one (14).

Yellow solid; yield 22%; mp 297-298 °C; IR (KBr, ν_{max}, cm^{-1}): 1727, 1731 (2 C=O); ^{1}H NMR (500 MHz, CF_3CO_2D): 2.59 (s, 3H, CH_3), 7.62 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.69-7.73 (m, 3H , Ar-H), 7.99-8.06 (m, 3H, Ar-H), 8.52 (d, 1H, *J* = 8.5 Hz, Ar-H), 9.23 (s, 1H, C_3-H pyridine), 9.51 (s, 1H, C_4-H coumarin); MS *m/z* 355 (M^+). Anal. Calcd for $C_{22}H_{13}NO_4$ (355.35): C, 74.36; H, 3.69; N, 3.94%. Found: C, 74.36; H, 3.69; N, 3.94%.

Synthesis of 4-amino-3-(1-(5-imino-3-methyl-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)ethyl)-2*H*-chromen-2-one (16).

A mixture of **1** (1.88 g, 10 mmol), 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **15** (1.74 g, 10 mmol) and ammonium acetate (1.54 g, 20 mmol) was heated in an oil bath at 170 ^{o}C for 4 h (the reaction progress was monitored by TLC). Upon competition, the reaction mixture was poured onto cold dilute HCl. The solid that separated was washed with water, filtered off and dried. The dried solid was heated in excess $CHCl_3$, then filter the precipitate, which identified as **16** and recrystallized from glacial acetic acid. When the $CHCl_3$ filtrate is left to cool, other solid product **14** was precipitated in purity enough that did not require further recrystallization.

4-Amino-3-(1-(5-imino-3-methyl-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)ethyl)-2*H*-chromen-2-one (16).

Red solid; 58% yield; mp 172-173 °C; IR (KBr, ν_{max}, cm^{-1}): 1729 (C=O), 3323 (NH), 3423, 3447 (NH_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.38 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 5.04 (s, 2H, NH_2), 7.09-7.87 (m, 9H, Ar-H), 9.77 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.06, 28.64, 101.01, 112.36, 117.22, 119.31, 121.18, 124.98, 127.67, 128.32, 128.57, 129.33, 129.84, 130.00, 144.73, 148.61, 151.07, 155.00, 169.03; MS *m/z* 358 (M^+). Anal. Calcd for $C_{21}H_{18}N_4O_2$ (358.40): C, 70.38; H, 5.06; N, 15.63%. Found: C, 70.36; H, 5.09; N, 15.66%.

Synthesis of 7,8-dimethyl-10-phenylchromeno[4,3-*b*]pyrazolo[4,3-*e*]pyridin-6(10*H*)-one (17).

A solution of **16** (0.72, 2 mmol) in glacial acetic acid (10 mL) was refluxed for 12 h, (the reaction progress was monitored by TLC) then left to cool at room temperature. The precipitated solid product was filtered off, dried and recrystallized from DMF-EtOH to give the corresponding compounds (**17**) as yellow powder in 65% yield; mp 288 °C; IR (KBr, ν_{max}, cm^{-1}): 1727 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): 2.08 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 7.09-7.34 (m, 7H, Ar-H), 7.72 (d, 1H, *J* = 7.5 Hz, Ar-H),

7.80 (d, 1H, *J* = 7.5 Hz, Ar-H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$): 15.49, 19.93, 108.68, 120.53, 124.83, 125.72, 127.01, 128.14, 128.59, 129.29, 129.77, 130.78, 140.13, 144.29, 149.88, 151.80, 155.04, 158.27, 167.16; MS *m/z* 341 (M^+). Anal. Calcd for $C_{21}H_{15}N_3O_2$ (341.37): C, 73.89; H, 4.43; N, 12.31%. Found: C, 73.85; H, 4.48; N, 12.34%.

Synthesis of 2-imino-4-methyl-*N*-(3-nitrophenyl)-5-oxo-2*H*,5*H*-pyrano[3,4-*c*]chromene-1-carboxamide (21).

A mixture of **1** (1.88 g, 10 mmol) and 2-cyano-*N*-(3-nitrophenyl)acetamide (**18**) (2.00 g, 10 mmol) in 15 mL of glacial acetic acid was heated under reflux for 11 h (the reaction progress was monitored by TLC), then left to cool at room temperature. The precipitated solid product was filtered off, washed with EtOH and dried to obtain buff solid in yield 72%; mp 135-137 °C; IR (KBr, ν_{max}, cm^{-1}): 1677, 1740 (2 C=O), 3264, 3303 (2 NH); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.59 (s, 3H, CH_3), 7.31-7.46 (m, 4H, Ar-H), 7.58 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.72 (t, 1H, *J* = 8 Hz, Ar-H), 7.93 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.65 (s, 1H, Ar-H), 10.05 (s, 1H, NH), 10.46 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ (ppm): 19.49, 97.88, 108.23, 112.46, 116.08, 120.38, 124.92, 127.03, 127.55, 128.69, 129.47, 130.53, 137.72, 148.16, 151.08, 159.48, 160.05, 161.22, 168.85, 197.03; MS *m/z* 391 (M^+). Anal. Calcd for $C_{20}H_{13}N_3O_6$ (391.34): C, 61.38; H, 3.35; N, 10.74%. Found: C, 61.35; H, 3.30; N, 10.78%.

Synthesis of 2'-amino-2-oxo-6'-(2-oxo-2*H*-chromen-3-yl)spiro[indoline-3,4'-pyran]-3'-carbonitrile (24). A mixture of **1** (1.88 g, 10 mmol) and 2-(2-oxo-1,2-dihydroindol-3-ylidene)malononitrile (1.95 g, 10 mmol) was dissolved in absolute EtOH (20 mL) in the presence of 3 drops of piperidine. The reaction mixture was heated under reflux condition for 2 h to confirm completion of the reaction under TLC controlled. The solid precipitate formed on cooling the reaction mixture to room temperature was filtered off, dried and recrystallized from EtOH as pale green solid in 85% yield; mp > 300 °C; IR (KBr, ν_{max}, cm^{-1}): 1664, 1718 (2 C=O), 2187 (CN), 3282 (NH), 3323, 3420 (NH_2); ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 6.18 (s, 1H, C_3-H pyran), 6.88 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.01 (t, 1H, *J* = 7.5 Hz, Ar-H), 7.16 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.24-7.27 (m, 1H, Ar-H), 7.29 (s, 2H, NH_2), 7.42-7.46 (m, 2H, Ar-H), 7.66-7.71 (m, 2H, Ar-H), 8.47 (s, 1H, C_4-H coumarin), 10.60 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ (ppm): 49.29, 53.30, 105.23, 109.96, 116.13, 117.19, 118.40, 122.50, 124.97, 125.17, 128.96, 129.22, 132.96, 133.72, 139.19, 141.11, 142.20, 152.65, 157.36, 160.98, 178.03; MS *m/z* 383 (M^+). Anal. Calcd for $C_{22}H_{13}N_3O_4$ (383.36): C, 68.93; H, 3.42; N, 10.96%. Found: C, 68.99; H, 3.45; N, 10.93%.

Synthesis of 2'-amino-2-oxo-6'-(2-oxo-2*H*-chromen-3-yl)-1'*H*-spiro[indoline-3,4'-pyridine]-3'-carbonitrile (25).

A mixture of **1** (1.88 g, 10 mmol), 2-(2-oxo-1,2-dihydroindol-3-ylidene)malononitrile (1.95 g, 10 mmol) and ammonium acetate (0.92 g, 12 mmol) was refluxed in EtOH (20 mL) for 7 h (the reaction progress was monitored by TLC). The reaction mixture was left to cool in room temperature and then poured into

ice HCl. The formed precipitate was filtered off, washed with water, dried and recrystalized from EtOH to give compound **25** in 80% yield as pale green solid; mp 223 °C; IR (KBr, ν_{max}, cm^{-1}): 1666, 1707 (2 C=O), 2184 (CN), 3140, 3201 (2 NH), 3277, 3363 (NH_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.20 (s, 1H, C_3-H pyridine), 6.90-7.21 (m, 3H, Ar-H), 7.28 (s, 2H, NH_2), 7.45-7.73 (m, 6H, Ar-H and NH), 8.50 (s, 1H, C_4-H coumarin), 10.53 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 49.44, 53.82, 105.26, 110.04, 116.11, 117.19, 118.45, 122.51, 124.99, 125.22, 128.96, 129.24, 132.92, 133.77, 139.19, 141.14, 142.28, 152.65, 157.38, 161.04, 178.07; MS *m/z* 382 (M^+). Anal. Calcd for $C_{22}H_{14}N_4O_3$ (382.38): C, 69.10; H, 3.69; N, 14.65%. Found: C, 69.15; H, 3.64; N, 14.64%.

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