One-step synthesis of chromene-3-carboxamide, bis-chromene, chromeno[3,4-*c*]pyridine and bischromeno[3,4-*c*]pyridine derivatives for antimicrobial evaluation

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Cyanoacetamide derivatives were reacted with salicylaldehyde under different conditions afforded chromenes and coumarin derivatives respectively. A number of chromeno[3,4-*c*]pyridine derivatives were prepared from the reaction of N-cyclohexyl-2-imino-2H-chromene-3-carboxamide with malononitrile and/or cynaothiocatemaide. Bischromeno[3,4-*c*]pyridine derivatives were prepared from the reaction of N,N′-(ethane-1,2-diyl)bis(2-imino-2H-chromene-3-carboxamide) with malononitrile and/or *N*-alkyl-2-cyanoacetamide derivative with 2-iminochroemen derivative, respectively. Some of these compounds were screened for their antimicrobial activities.

Keywords: chromenes, bischromene, chromeno[3,4-*c*]pyridines, bischromeno-[3,4-*c*]pyridines, antimicrobial activity

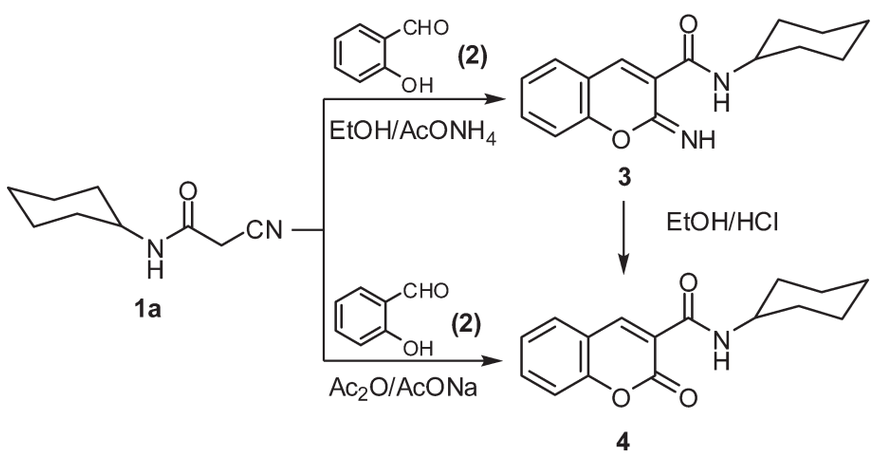
Antibacterial, coronary dilatory and hypothermal agents as well as potential laser dyes have been reported for many chromene derivatives.[1–4] Moreover, coumarin derivatives have anticoagulant and antibacterial activities.[5–9] Furthermore, benzocoumarins have analgesic and antihypertensive activities.[10] The present study is part of our program aimed at developing easy routes for the synthesis of fused heterocyclic compounds starting with cyanoacetanilides as simple organic compounds. We now report the synthesis of chromene carboxamide and bischromene derivatives and their use as building blocks in the synthesis of novel pyridochromene derivatives to evaluate their antimicrobial activity.

Results and discussion

N-Alkyl-2-cyanoacetamide derivatives **1a**,**b** were prepared in high yield from the reaction of aliphatic amines with ethyl cyanoacetate.[11] Cyclocondensation of cyanoacetamide derivative **1a** with salicylaldehdye **2** in refluxing ethanol containing a catalytic amount of ammonium acetate furnished **3**, Scheme 1. The structure of **3** was established on the basis of elemental analysis and spectral data. The reaction probably takes place through condensation of the aldehydic group with the active methylene function in compound **1a** followed by nucleophilic attack of the hydroxyl group on the neighbouring nitrile residue eventually giving the corresponding **3**. On the other hand, cyclocondensation of **1b** with salicylaldehdye **2** in refluxing acetic anhydride containing a catalytic amount of sodium acetate furnished **4**. Elemental analysis and spectral data were in complete accordance with compound **4**. Furthermore, the structure of **4** was showed the identity (m.p, and mixed m.p) of an authentic sample synthesised upon hydrolysis of **3** with EtOH/HCl.

Moreover, the resulting chromene derivatives have latent functional substituents which have the potential for their chemical transformations giving new routes for the preparation of condensed chromene with possible biological activity. Thus, the dihydrochromenopyridine derivative **7** was obtained up on treatment of chromene derivative **3** with malononitrile in presence of piperidine as a catalyst, Scheme 2. The structure of **7** was confirmed on the basis of elemental analysis and spectral data. Both elemental and spectral data supported the proposed structure **11** the other possible structures **9**, **10** were ruled out due to the odour of H_2S not observed during the reaction (lead acetate test). The formation of **7** may be assumed to proceed via the addition of an active methylene group to the double bond of chromene to give the Michael adduct **5** which underwent cyclisation to give dihydropyridine **7**.

From the literature, attention has increasingly been paid to the synthesis of bisheterocyclic compounds which exhibit various biological activities including antibacterial, fungicidal, tuberculostatic, and plant growth regulative properties.[12–15] Earlier work revealed that bisheterocyclic compounds displayed much better antibacterial activity than heterocyclic compounds.[16] Thus, bis 2-imino chromene derivative **12** was obtained via the reaction of cyanoacetamide **1b** with salicylaldehyde **2** (1:2 molar ratio) under reflux. Elemental analysis



Scheme 1

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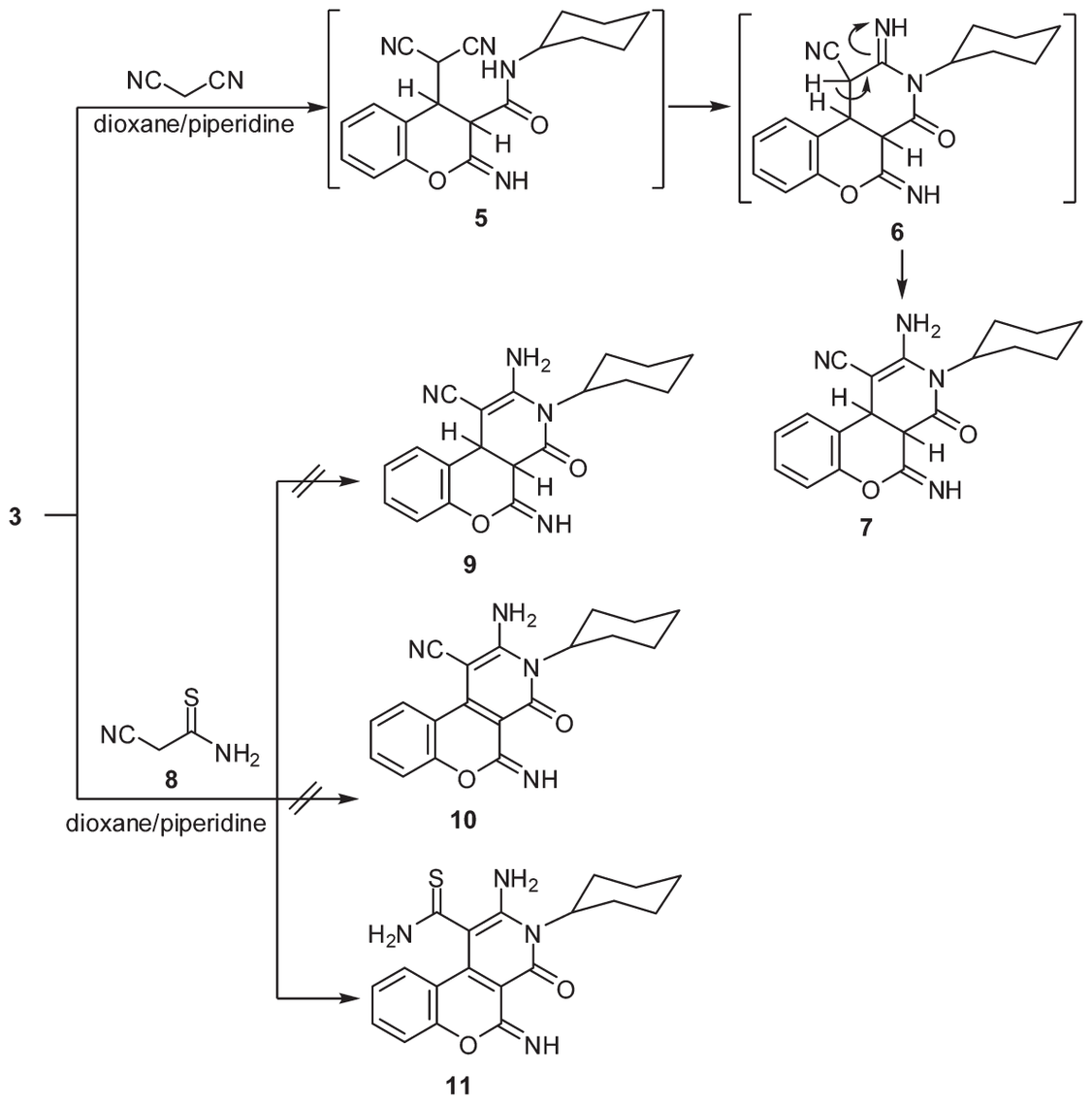


Scheme 2

and spectral data were in complete agreement with compound **12** (see Figs 1 and 2).

Treatment of 2-iminochromene derivative **13**[17] with cyanoacetamide derivative **1b** afforded bischromeno[3,4-*c*]pyridine derivative **14** and the other possible structure **15** was excluded on the basis of elemental and spectral data. Also, bischromenopyridine derivative **16** was obtained via the reaction of **12** with malononitrile in ethanol in the presence of a catalytic amount of piperidine. Infrared spectrum of compound **16** displayed absorption bands at 3436, 3344, 3186 (NH and NH_2), 2206 (C≡N) and 1660 (C=O). The ^{1}H NMR spectrum revealed three singlets at δ 4.01, 6.57, and 9.21 for CH_2, NH_2, and NH, respectively, Scheme 3.

Antimicrobial activity

Most synthesised compounds were screened for their antimicrobial activity. The diameter of inhibition zone was measured as an indicator for the activity of the compounds; ampicillin is used as reference drug.

The results for antibacterial activities depicted in Table 1 revealed that compounds **3**, **4**, **7**, **11**, **12**, **14** and **16** exhibited good activities against the reference chemotherapeutics. On the other hand, most of the prepared compounds exhibited good antifungal activities against the reference drugs.

We report here a simple and convenient route for the synthesis of some heterocyclic bases on chromene, bischromene, chromeno[3,4-*c*]-pyridine, and bischromeno[3,4-*c*]pyridines derivatives for antimicrobial evaluation.

The tested compounds were evaluated by the agar diffusion technique using a 1 mg mL^{-1} solution in DMSO.[18] The test organisms were four bacterial strains: *Bacillius theringiensis, Serratia marcescens, Klebsiella pneumoniae,* and *Proteus mirabilis* and two fungi: *Fusarium oxysporum*, and *Aspergillus ochraceus.* A control using DMSO without the test compound was included for each organism. Ampicillin was purchased from the Egyptian market and used in a concentration 2 mg mL^{-1} as reference drugs. The bacterial and fungi were tested on nutrient agar and potato dextrose agar media, respectively. Three plates were used for each compound as replicates. The plates were incubated for 24 h, and seven days for bacteria and fungi, respectively. After the incubation period, the diameter of inhibition zone was measured as an indicator of the activity of the compounds.

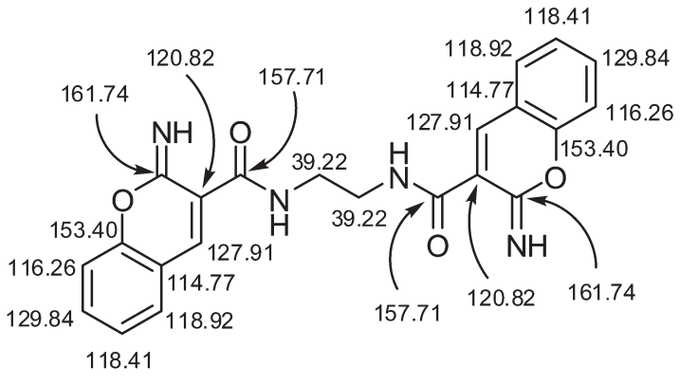


Fig. 1 ^{13}C NMR spectrum of compound **12**.

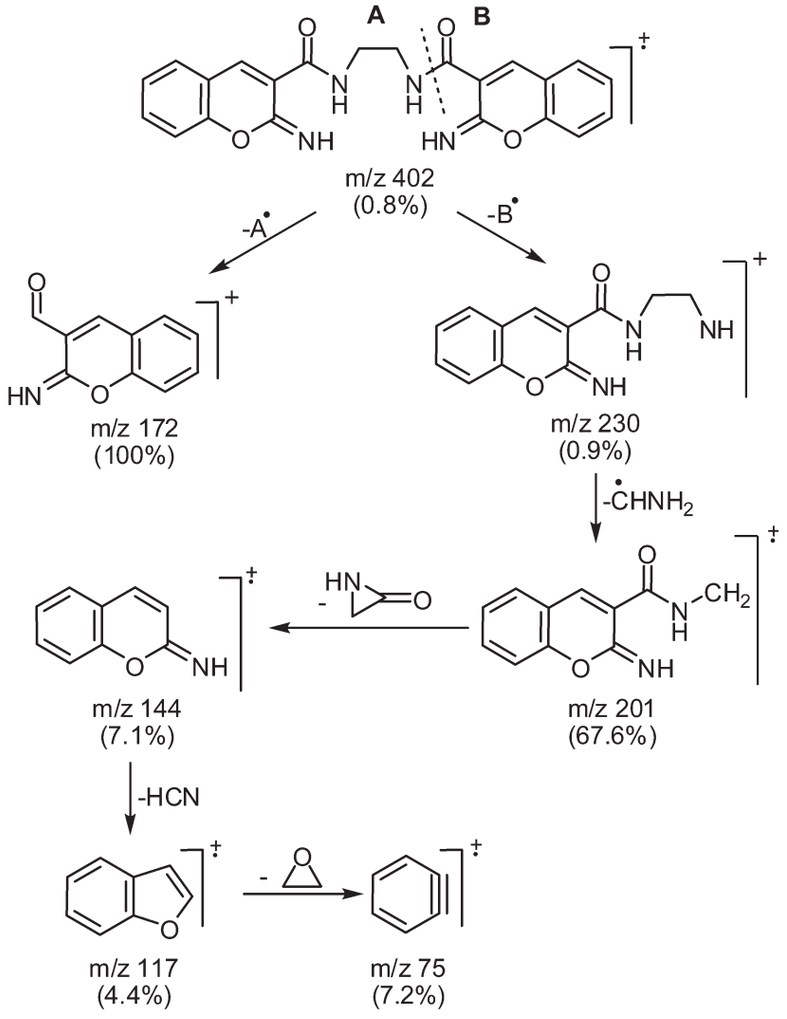
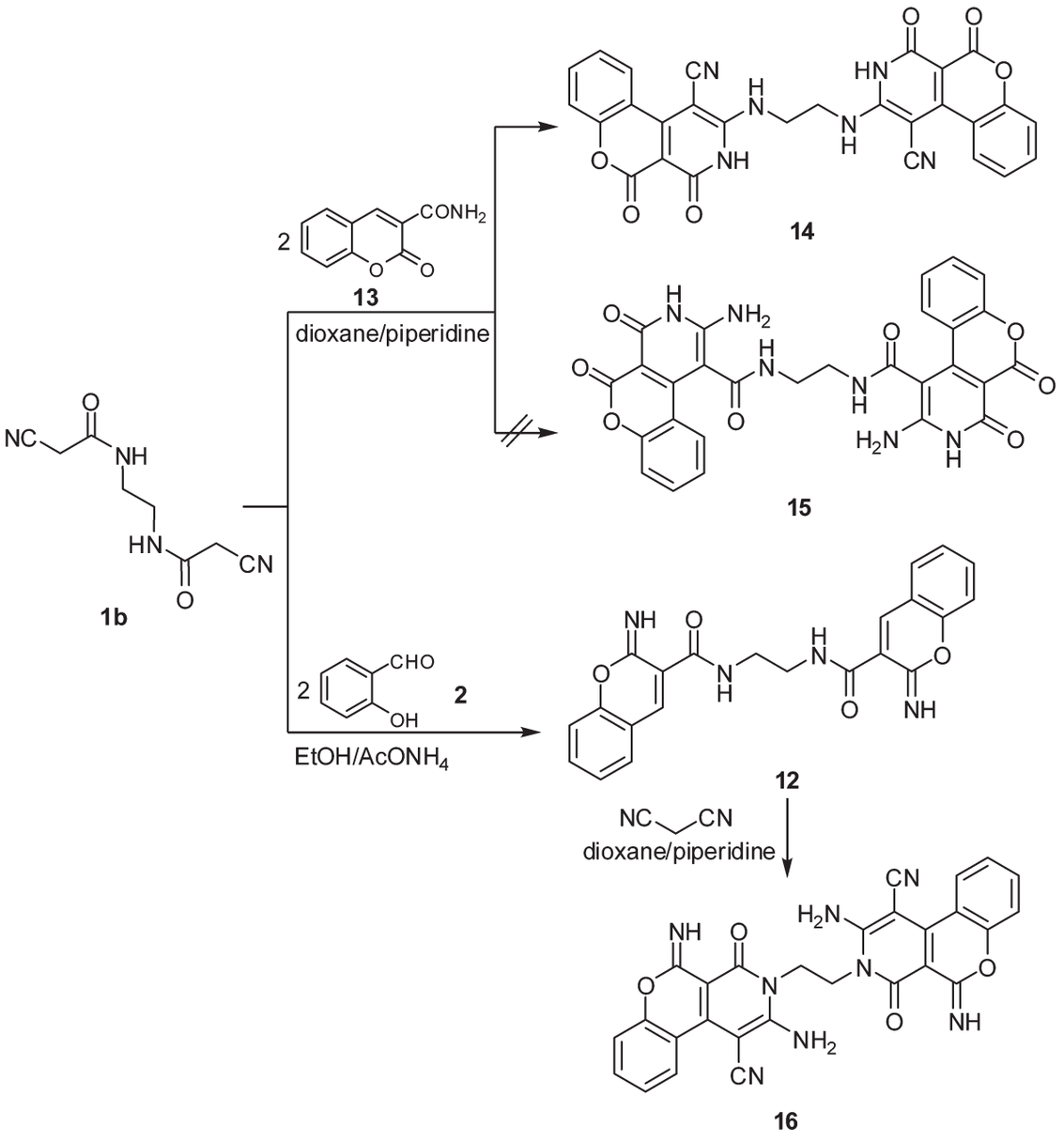


Fig. 2 Fragmentation pattern of compound **12**.

Experimental

All melting points are uncorrected. IR spectra (KBr) were measured on a Shimadzu 440 spectrometer, ^{1}H NMR spectra were obtained in DMSO on a Varian Gemini 200 MHz spectrometer using TMS as internal standard; chemical shifts are reported as (ppm). Mass spectra were obtained on a GCMS\QP 1000 Ex mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Centre, Faculty of Science (Cairo University, Egypt). Microbiology screening carried out in Botany Department, Faculty of Science, Al-Azhar University.

N-Cyclohexyl-2-imino-2H-chromene-3-carboxamide (**3**): A mixture of cyanoacetamide derivative (**1a**) (0.01 mol), salicylaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in ethanol (30 mL) for 2 h. The solid product which produced on heating was collected and recrystallised from dioxane as white yellow crystals; 70% yield, m.p. 172–174 °C. IR (KBr): ν = 3180 (NH), 2924 (stretching CH) and 1654 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6): δ = 1.32–1.36 (m, 6H, cyclohexyl protons), 1.66–1.86 (m, 4H, cyclohexyl protons), 3.80 (hump, 1H, cyclohexyl proton), 7.40–8.01 (m, 4H, ArH), 8.26 (s, 1H, chromene-H), 8.85, 10.49 (2s, 2H, 2NH; exchangeable with D_2O).



Scheme 3

Table 1 Zone (mean diameter of inhibition in mm) as a criterion of antibacetrial and antifungal activities of the newly synthesised compounds

Compound	Bacteria				Fungi	
	Bacillius theringiensis	*Serratia marcescens*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Fusarium oxysporum*	*Aspergillus ochraceus*
3	12	30	18	34	12	8
4	11	32	17	35	11	7
7	15	29	19	30	10	8
11	14.5	31	16	29	11	6
12	13	32	17	30	11	6
14	14	33	16	34	12	6.5
16	15	34	15	32	14	7
Ampicillin	17	40	20	40	15	10

MS: *m/z* = 269 (M-1, 25%), 76 (100%). Anal. Calcd for $C_{16}H_{18}N_2O_2$ (270.33): C, 71.09; H, 6.71; N, 10.36. Found: C, 71.00; H, 6.60; N, 10.30%.

N-Cyclohexyl-2-oxo-2H-chromene-3-carboxamide (**4**)
Method A: A mixture of cyanoacetamide derivative (**1a**) (0.01 mol), salicyl-aldehyde (0.01 mol) and sodium acetate (0.01 mol) was refluxed in acetic anhydride (30 mL) for 1hr. The solid product which produced on heating was collected and recrystallised from dioxane as white yellow crystals; 65% yield, m.p. 194–196 °C. IR (KBr): ν = 3260 (NH), 3057 (CH-arom.), 2970, 2924 (stretching CH) and 1695, 1640 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): δ = 1.21–1.39 (m, 6H, cyclohexyl protons), 1.54–1.87 (m, 4H, cyclohexyl protons), 3.80–3.82 (m, 1H, cyclohexyl proton), 7.41–8.00 (m, 4H, ArH), 8.84 (s, 1H, chromene-H), 8.60 (d, 1H, NH; exchangeable with D_2O). Anal. Calcd for $C_{16}H_{17}NO_3$ (271.31): C, 70.83; H, 6.32; N, 5.16. Found: C, 70.80; H, 6.20; N, 5.10%.

Method B: A solution of the corresponding iminochromene derivatives (**3**) (0.01 mol) in ethanol (30 mL), hydrochloric acid (5 mL) was refluxed for 1h. The solid product which produced on heating was collected and worked up as above.

2-Amino-3-cyclohexyl-5-imino-4-oxo-4,4a,5,10b-tetrahydro-3H-chromeno[3,4-c]pyridine-1-carbonitrile (**7**): A mixture of compound (**4**) (0.01 mol), malononitrile (0.01 mol) and piperidine (0.01 mol) was refluxed in dioxane (30 mL) for 3h. The solid product which produced on heating was collected and recrystallised from acetic acid as white yellow crystals; 70% yield, m.p. 211–212 °C. IR (KBr): ν = 3440, 3348, 3164 (NH&NH_2), 2984 (stretching CH), 2208 (C≡N), and 1650 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): δ = 1.21–1.39 (m, 6H, cyclohexyl protons), 1.56–1.87 (m, 4H, cyclohexyl protons), 3.68 (d, 1H, chromene H-3), 3.73 (hump, 1H, cyclohexyl proton), 4.67 (d, 1H, chromene H-4), 6.95 (s, 2H, NH_2; exchangeable with D_2O), 7.08–8.50 (m, 4H, ArH), 10.80 (s, 1H, NH; exchangeable with D_2O). MS: *m/z* = 320 (M-NH_2, 20%), 60 (100%). Anal. Calcd for $C_{19}H_{20}N_4O_2$ (336.39): C, 67.84; H, 5.99; N, 16.66. Found: C, 67.80; H, 5.85; N, 16.70%.

2-Imimo-3-cyclohexyl-5-imino-4-oxo-4,5-dihydo-3H-chromeno [3,4-c]pyridine-1-carbothioamide (**11**): A mixture of compound (**4**) (0.01 mol), cyanothioacetamide (0.01 mol) and piperidine (0.01 mol) was refluxed in dioxane (30 mL) for 3h. The solid product which produced on heating was collected and recrystallised from acetic acid as white yellow crystals; 70% yield, m.p. 246–247 °C. IR (KBr): ν = 3432, 3360, 3170 (NH&NH_2), and 1656 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): δ = 1.21–1.37 (m, 6H, cyclohexyl protons), 1.53–1.88 (m, 4H, cyclohexyl protons), 3.75 (hump, 1H, cyclohexyl proton), 6.85 (s, 2H, NH_2), 7.11–8.70 (m, 6H, ArH + NH_2), 10.77 (s, 1H, NH). MS: *m/z* = 334 (M-H_2S, 12%), 283 (68%), 266 (35%), 253 (40%), 227 (76%0, 190 (40%), 173 (75%), 146 (125%), 127(25%), 77 (58%), 63 (100%). Anal. Calcd for $C_{19}H_{20}N_4O_2S$ (368.45): C, 61.94; H, 5.47; N, 15.21. Found: C, 61.90; H, 5.50; N, 15.10%.

N,N′-(Ethane-1,2-diyl)bis(2-imino-2H-chromene-3-carboxamide) (**12**): A mixture of compound (**1b**) (0.01 mol), salicylaldehyde (0.02 mol) and piperidine (0.01 mol) was refluxed in ethanol (30 mL) for 3h. The solid product which produced on heating was collected and recrystallised from dioxane as white yellow crystals; 61% yield, m.p. 261–263 °C. IR (KBr): ν = 3319 (NH), 3058 (CH-arom.), and 1647 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): δ = 4.21 (s, 4H, $2CH_2$), 6.24–8.42 (m, 10H, ArH + chromene H-4), 8.92, 10.44 (2s, 4H, 4NH; exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): δ = 39.22, 114.77, 116.26, 118.41, 120.82, 127.91, 129, 84, 153.40, and 161.74. MS: *m/z* = 402 (M^+, 0.8%) 172 (100%). Anal. Calcd for $C_{22}H_{18}N_4O_4$ (402.40): C, 65.66; H, 4.51; N, 13.92. Found: C, 65.60; H, 4.40; N, 13.80%.

2,2′-(Ethane-1,2-diylbis(azanediyl))bis(4,5-dioxo-4,5-dihydro-3H-chromeno-[3,4-c]pyridine-1-carbonitrile) (**14**): A mixture of compound (**1b**) (0.01 mol), 2-imino-chromene derivative (**13**) (0.02 mol) and piperidine (0.01 mol) was refluxed in dioxane (30 mL) for 3 h. The solid product which produced on heating was collected and recrystallised from acetic acid as white yellow crystals; 62% yield as brown crystals, m.p. 296–298 °C. IR (KBr): ν = 3312 (NH), 2196 (C≡N), and 1664 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): δ = 4.00 (s, 4H, $2CH_2$), 6.56–8.22 (m, 8H, ArH), 8.83, 10.32 (2s, 4H, 4NH). MS: *m/z* = 530 (M^+-2, 7%), 497 (20%), 304 (18%), 218 (15%), 173 (75%), 89 (100%). Anal. Calcd for $C_{28}H_{16}N_6O_6$ (532.46): C, 63.16; H, 3.03; N, 15.78. Found: C, 62.98; H, 2.82; N, 15.54%.

3,3′-(Ethane-1,2-diyl)bis(2-amino-5-imino-4-oxo-4,5-dihydro-3H-chromeno-[3,4-c]pyridine-1-carbonitrile) (**16**): A mixture of compound (**12**) (0.01 mol), malononitrile (0.02 mol) and piperidine (0.01 mol) in dioxane (30 mL) was heated under reflux for 3h. The solid product which produced on heating was collected by filtration and recrystallised from acetic acid as brown crystals; 60% yield, m.p. >300 °C. IR (KBr): ν = 3436, 3344, 3184 (NH and NH_2), 2206 (C≡N), and 1660 cm^{-1} (C=O). ^1H NMR (DMSO-d_6): δ = 4.01 (s, 4H, $2CH_2$), 6.57 (s, 4H, $2NH_2$; exchangeable with D_2O), 7.11–7.63 (m, 8H, ArH), 9.21 (s, 2H, 2NH; exchangeable with D_2O). Anal. Calcd for $C_{28}H_{18}N_8O_4$ (530.49): C, 63.39; H, 3.42; N, 21.12. Found: C, 63.30; H, 3.45; N, 21.16%.

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