N-Aryl 2-Cyanothioacetamide Intermediates in Heterocyclic Synthesis: Synthesis and AntimicrobialEvaluation of 3-Cyano-2(1H)-Pyridineth-Ione, Chromene-3-Carbothioamide and Chromeno[3,4-c]Pyridinethione Derivatives

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Accepted: May 18, 2011; Published: August 30, 2011

Abstract

A novel synthesis of 3-cyano-2(1H)-pyridinethiones, chromene-3-carbothioamide and chromeno[3,4-c]pyridines were obtained through interaction of *N*-aryl cyanothioacetamide derivatives with some electrophilic reagents. Most of the target compounds were then evaluated for their antimicrobial and antifungal activities.

Keywords: *N*-Aryl cyanothioacetamide derivatives; Michael addition; pyridine-2(1*H*)-thiones; chromenes-3-carbothioamide; biological activity.

1. Introduction

3-Cyano-2(1H)-pyridinethiones [1,2] and their related compounds were found to be very reactive substances for the synthesis of many different heterocyclic systems which exhibited biological activities such as antibacterial, pesticidal, antifungal, acaricidal and neurotropic activities [3-10]. Also, the 3-Cyano-pyridine-2(*1H*)-thiones compounds have gained considerable interest due to their importance as intermediates for the synthesis of the biologically active deazafolic acid and deaza amino protein ring system [11,12]. Thus, the synthesis of pyridines and their analogs has attracted much attention. Therefore, the development of a simple, one-pot, and directed synthetic route, which provides diverse of 3-cyano-pyridinethione compounds, is strongly desired for this important class of heterocycles. Also, chromene derivatives are widely used for production of highly effective fluorescent dyes for synthetic fiber and daylight fluorescent pigments [13-15]. They also play a vital role in electrophotographic and electroluminescent devices [16].

As a part of our program directed for the development of a new, simple and efficient procedure for the synthesis of biologically active heterocyclic nitrogenous compounds utilizing readily available intermediates [17-24], we have investigated the reaction of *N*-aryl cyanothioacetamide derivatives **1a,b** with some electrophilic reagents. The investigation has resulted in the development of novel procedure for the synthesis of 3-cyano-2(*1H*)-pyridinethiones and their fused derivatives. These compounds seem promising for further chemical transformation and biological evaluation studies.

2. Methods

All melting points are uncorrected. IR spectra (KBr) were measured on Shimadzu 440 spectrometer, ^{1}HNMR spectra were obtained in DMSO-d_6 on a Varian Gemini 200 MHz spectrometer using TMS as internal standard; chemical shifts are reported as (ppm). Mass spectra were obtained on GCMS\QP 1000 Ex mass spectrometer at 70 ev. Elemental analyses were carried out at the Microanalytical Center, Faculty of Science (Cairo University, Egypt). Microbiology screening was carried out in Botany Department, Faculty of Science, Al-Azhar University.

4,6-Diamino-1-(4-chlorophenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (3). A mixture of **1** (0.01 mole), malononitrile (0.01 mole) and a few drops of piperidine was refluxed for 2h in an oil bath at 160°C, then allowed to cool. The solid product was collected and recrystallized from dioxane as yellow crystals to give (**3).**

This compound was obtained in 65% yield, m.p. 256-258ºC. IR (KBr): ν = 3314, 3182 (NH_2), 2204 (C≡N), 1625 (C=N), 1301 cm^{-1}(C=S). ^{1}HNMR (DMSO-d_6): δ =5.40 (s, 1H, CH-pyridine), 5.80, 6.55 (2s, 4H, $2NH_2$), 6.10, 6.90 (2d, 4H, Ar-H). MS: *m/z* = 276 (M^+; 3.9), 170 (5.5), 153 (7.0), 127 (70.5), 65 (77), and 55 (100). Anal.Calcd.for$C_{12}H_9ClN_4S$ (276.5): C 52.08; H 3.28; N 20.24. Found: C 51.81; H 3.06; N 20.05.

1-p-Tolyl-4,6-dimethyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (5). Equimolar amounts of **1a** (0.01 mole) and acetylacetone (0.01 mole) with a few drops of piperidine in an oil bath were refluxed for 1h at 160°C, then allowed to cool. The solid product was collected and recrystallized from acetic acid as brown crystals to give **5**.

This compound was obtained in 60%yield, m.p. 288-290ºC. IR (KBr): ν =3031 (CH-arom.), 2929 (CH-aliph.), 2215 (C≡N), 1310 cm^{-1}(C=S). ^{1}HNMR (DMSO-d_6): δ =2.10, 2.25, 2.40 (3s, 9H, $3CH_3$), 6.30 (s, 1H, CH-pyridine), 6.15, 6.80 (2d, 4H, Ar-H). MS: *m/z* = 254 (M^+; 3.0), 239 (85), 237 (100), 91 (50) and 65 (37.5). Anal.Calcd.for$C_{15}H_{14}N_2S$ (254): C, 70.83; H, 5.55; N, 11.01. Found: C, 70.58; H, 5.34; N, 10.77.

1-p-Chlorophenyl-6-methyl-4-(2-methyl-4-oxopent-1-enyl)-2-thioxo-1,2-dihy-dropyridine-3-carbonitrile (7). A mixture of **1a** (0.01 mole) and acetyl acetone (0.02 mole) with a few drops of piperidine in an oil bath were refluxed for 2h at 160°C, then allowed to cool. The solid product was collected and recrystallized from acetic acid as brown crystals to give **7**.

This compound was obtained in 70% yield, m.p. 282-284ºC. IR (KBr): ν = 3062 (CH-arom.), 2942 (CH-aliph.), 2217 (C≡N), 1653 cm^{-1} (C=O).^{1}HNMR (DMSO-d_6): δ = 1.97, 2.05 (2s, 6H, $2CH_3$), 2.42 (s, 3H, $COCH_3$), 3.00 (s, 2H, CH_2), 6.45 (s, 1H, Olefinic-CH), 6.97 (s, 1H, CH-pyridine), 7.21-7.65 (m, 4H, Ar-H). ^{13}C-NMR (300 MHz) δ=20.1 ($\underline{C}H_3$); 22.3 ($\underline{C}H_3$); 36.1 ($\underline{C}H_3$); 50.4 ($\underline{C}H_2$), 98.4 ($\underline{C}H$); 103.5 ($\underline{C}H$); 108.4 (C); 114.6 ($\underline{C}N$); 126.3 (CH); 131.4 (C-Cl); 133.2 (CH); 135.2 (C); 137.3 (C); 140.1 (C); 168.4 (C); 177.4 (C=S); 206.4 (C=O). Anal.Calcd. for $C_{19}H_{17}ClN_2OS$ (356.5): C, 63.95; H, 4.80; N, 7.85. Found : C, 63.72; H, 4.66; N, 7.69.

6-Amino-1-aryl-2-thioxo-1,2-dihydropyridin-3,4,5-tricarbonitrile (10a,b). General procedure: A mixture of **1** (0.01 mole), tetracyanoethylene**2** (0.01 mole) and triethyl amine (0.5 ml) in dioxane (30 ml) was refluxed for 3h, then allowed to cool. The solid product was collected and recrystallized from the proper solvent to give **10a,b**.

Compound (10a). This compound was obtained in 60% yield as white crystals (ethanol), m.p. 240-242ºC. IR (KBr): ν = 3314, 3182 (NH_2), 2190, 2204 (C≡N), 1625 (C=N), 1312 cm^{-1}(C=S). ^{1}HNMR (DMSO-d_6): δ = 2.30 (s, 3H, CH_3), 6.51 (s, 2H, NH_2), 6.77-6.98 (m, 4H, Ar-H). MS: *m/z* = 291 (M^+; 3.50), 218 (4.0), 105 (6.0), 69 (15), 57 (73.4), and 54 (100). Anal.Calcd. for $C_{15}H_9N_5S$ (291): C, 61.85; H, 3.11; N, 24.04. Found: C, 61.66; H, 2.93; N, 23.81.

Compound (10b). This compound was obtained in 55% yield as brown crystals (ethanol), m.p. 251-253ºC. IR (KBr): ν = 3183, 3100 (NH_2), 2220, 2208 (C≡N), 1633 (C=N), 1300 cm^{-1} (C=S).^{1}HNMR (DMSO-d_6): δ = 6.34 (s, 2H, NH_2), 6.98-7.46 (m, 4H, Ar-H). MS: *m/z* = 311 (M^+; 5.26), 264 (4.0), 216 (3.0), 104 (21.5), 91 (46.50), 79 (75.0) and 57 (100). Anal.Calcd. for $C_{14}H_6ClN_5S$ (311.5): Calcd. C, 53.94; H, 1.94; N, 22.46. Found: C, 53.71; H, 1.74; N, 22.23.

6-Amino-4-(2-chlorophenyl)-2-thioxo-1-p-tolyl-1,2,3,4-tetrahydropyridine-3,5-dicarbonitrile (11); 6-amino-1,4-bis(4-chlorophenyl)-2-thioxo-1,2-dihydropyridine-3,5-dicarbonitrile (13). General procedure: A mixture of **1** (0.01 mole) and the appropriate arylidenemalononitrile (0.01 mole) in ethanol (30 ml) was treated with piperidine (0.5 ml) and the reaction mixture was refluxed for 3h. The solid product which produced on heating was filtered and recrystallized from the proper solvent to give **11** and **13** respectively.

Compound (11). This compound was obtained in 73% yield as white crystals (acetic acid), m.p. >300ºC. IR (KBr): ν = 3448, 3328 (NH_2), 3108 (CH-arom.), 2919 (CH-aliph.), 2189 cm^{-1} (C≡N). ^{1}HNMR (DMSO-d_6): δ =2.14 (s, 3H, CH_3),

4.12, 4.39 (2d, pyridine H-3, H-4; J = 12Hz)**,** 6.33-7.49 (m, 8H, Ar-H), 10.10 (s, 2H, NH_2; exchangeswithD_2O). Anal.Calcd. for $C_{20}H_{15}ClN_4S$ (378.5):C, 63.40; H, 3.99; N, 14.79. Found: C, 63.26; H, 3.75; N, 14.51.

Compound (13). This compound was obtained in 65% yield as gray crystals (acetic acid), m.p. >300ºC. IR (KBr): ν = 3314, 3212 (NH_2), 2214 (C≡N). ^{1}HNMR (DMSO-d_6): δ =7.38-7.71 (m, 8H, Ar-H), 8.25 (br, 2H, NH_2; exchangeswith D_2O). MS: *m/z* = 397 (M^+; 34.77), 396 (M-1; 40.50), 313 (38.5), 236 (37), 152 (43), 113 (75.0) and 75 (100). Anal.Calcd. fo r$C_{19}H_{10}Cl_2N_4S$ (397): C, 57.44; H, 2.54; N, 14.10. Found: C, 57.27; H, 2.32; N, 13.96.

6'-Amino-2'-thioxo-1'-(4-chloro-phenyl)-1',2',3',4'-tetrahydrospiro[cyclohexane-1,4'-pyridine]-3',5'-dicarbonitrile (14). A mixture of **1b** (0.01 mole), cyclohexanone (0.01 mole) and malononitrile (0.01 mole) in ethanol (30 mL) containing piperidine (0.5 mL) was refluxed for 3h. The solid product produced on heating was filtered then recrystallized from dioxane as beige crystals to give **14**.

This compound was obtained in 60% yield, m.p. 290-292ºC. IR (KBr): ν = 3304, 3166 (NH_2), 2206 cm^{-1} (C≡N).^{1}HNMR (DMSO-d_6): δ =2.20-2.59 (m, 10H, cyclohexyl), 6.04 (s, 1H, pyridine H-3), 6.91 (s, 4H, Ar-H), 12.50 (br, 2H, NH_2; exchanges with D_2O). Anal.Calcd. for $C_{18}H_{17}ClN_4S$ (356.5): C, 60.58; H, 4.80; N, 15.70. Found: C, 60.42; H, 4.61; N, 15.46.

2-Imino-3-N-(4-chlorophenyl)-thioxocarboxamido-2H-chromene (15), N-(4-chloro-phenyl)-3-imino-3H-benzo[f]chromene-2-carbothioamide (17). General procedure: A mixture of compound **1b** (0.01 mole), appropriate aldehyde (salicylaldehyde or 2-hydroxynaphthaldehyde; 0.01 mole) and ammonium acetate (0.01 mole) in ethanol (30 ml) was refluxed for 1h. The solid product which produced on heating was collected and recrystallized to furnish **15** and **17**.

Compound (15). This compound was obtained in 75% yield as white crystals (dioxane), m.p. 185-187ºC. IR (KBr): ν = 3306 (NH), 1620 cm^{-1} (C=N).^{1}HNMR (DMSO-d_6): δ =7.22-8.01 (m, 8H, Ar-H), 8.56 (s, 1H, CH-chromene), 9.65, 14.90 (2s, 2H, 2NH; exchanges with D_2O). MS: *m/z* =314 (52.50), 313 (100), 281 (30.0), 171 (30.0), 118 (27.5), and 75 (40.0). Anal.Calcd. for $C_{16}H_{11}ClN_2OS$ (314.5): C, 61.05; H, 3.52; N, 8.90. Found: C, 60.79; H, 3.28; N, 8.71.

Compound (17). This compound was obtained in 60% yield as beige crystals (dioxane), m.p. 280-282ºC. IR (KBr): ν = 3242 (NH), 3046 (CH-arom.), 1630 (C=N) and 1314 cm^{-1} (C=S). ^{1}HNMR (DMSO-d_6): δ =7.36-8.34 (m, 10H, Ar-H), 9.00 (s, 1H, CH-chromene), 9.20, 12.5 (2s, 2H, 2NH; exchanges with D_2O). Anal.Calcd. for $C_{20}H_{13}ClN_2OS$ (364.5): C, 65.84; H, 3.59; N, 7.68. Found: C, 65.59; H, 3.43; N, 7.45.

***N*-(4-Substituted-phenyl)-2-oxo-2H-chromene-3-carbothioamide (16a,b), N-(4-chlorophenyl)-3-oxo-3H-benzo[f]chromene-2-carbothioamide (18). General procedure:**

Method A: A mixture of compound **1** (0.01 mole), appropriate o-hydroxyaldehyde (salicylaldehyde or 2-hydroxynaphthaldehyde; 0.01 mole) and sodium acetate (0.01 mole) was refluxed in acetic anhydride (30 ml) for 1h. The resulting solid was filtered off and recrystallized from the suitable solvent.

Method B: A suspension of **15** or **17** (0.01 mole) in ethanol (30 ml), hydrochloric acid (5 ml) was refluxed for 1h, then cooled and the solid product was filtered off and recrystallized from the proper solvent.

Compound (16a). This compound was obtained in 50% yield as white crystals (dioxane), m.p. 230-232ºC. IR (KBr): ν =3275 (NH), 3031 (CH-arom.), 2921 (CH-aliph.), 1712 cm^{-1} (C=O).^{1}HNMR (DMSO-d_6): δ = 2.28 (s, 3H, CH_3), 7.16-8.02 (m, 8H, Ar-H), 8.90 (s, 1H, CH-chromene), 10.58 (s, 1H, NH; exchanges with D_2O). Anal.Calcd. for $C_{17}H_{13}NO_2S$ (295): C, 69.13; H, 4.44; N, 4.74. Found: C, 68.92; H, 4.26; N, 4.53.

Compound (16b). This compound was obtained in 70% yield as white crystals (dioxane), m.p.190-192ºC. IR (KBr): ν =3192 (NH), 3052 (CH-arom.), 1698 cm^{-1} (C=O). ^{1}HNMR (DMSO-d_6): δ =7.48-8.00 (m, 8H, Ar-H), 8.90 (s, 1H, CH-chromene), 10.70 (s, 1H, NH; exchanges with D_2O). Anal.Calcd. for $C_{16}H_{10}ClNO_2S$ (315.5): C, 60.86; H, 3.19; N, 4.44. Found: C, 60.62; H, 3.04; N, 4.18.

Compound (18). This compound was obtained in 65% yield as brown crystals (DMF), m.p. >300ºC. IR (KBr): ν =3180 (NH), 3054 (CH-arom.) and 1688 cm^{-1} (C=O). ^{1}HNMR (DMSO-d_6): δ =7.46-8.62 (m, 10H, Ar-H), 9.36 (s, 1H, CH-chromene), 12.30 (s, 1H, NH; exchanges with D_2O). Anal.Calcd. for $C_{20}H_{12}ClNO_2S$ (365.5): C, 65.66; H, 3.31; N, 3.83. Found: C, 65.34; H, 3.19; N, 3.68.

2-Amino-3-(4-chlorophenyl)-5-imino-4-thioxo-4,5-dihydro-3H-chromeno[3,4-c]-pyridine-1-carbonitrile (20), 3-(4-chlorophneyl)-5-imino-2-oxo-4-thioxo-2,3, 4a,5, 10b-hexahydro-1H-chromeno[3,4-c]pyridine-1-carbonitrile (22) and 5-imino-2-(4-chlorophenylamino)-3-(4-chlorophenyl)-4-thioxo-4,6-dihydro-3H-chromeno[3,4-c]-pyridine-1-carbonitrile (24). General procedure: A mixture of **15** (0.01 mole), active methylene compounds (namely, malononitrile, ethyl cyanoacetate, 2-cyano-*N*-tolylthio-acetamide **1a**; 0.01 mole) and a few drops of piperidine in ethanol (30 ml) was heated under reflux for 3h. The solid product which produced on heating was collected by filtration and recrystallized from the proper solvent.

Compound (20). This compound was obtained in 60% yield as brown crystals (DMF), m.p. >300ºC. IR (KBr): ν =3434, 3338, 3188 (NH_2/NH), 2202 (C≡N), 1610 cm^{-1} (C=N).^{1}HNMR (DMSO-d_6): δ =6.52 (s, 2H, NH_2; exchanges with D_2O), 7.32-7.88 (m, 8H, Ar-H), 9.98 (s, 1H, NH; cancelled with D_2O). MS: *m/z* =378 (M^+; 2.50), 379 (M+1; 3.0), 380 (M+2; 3.0), 349 (4.0), 300 (100), 194 (17.5), 127 (20) and 55 (25). Anal.Calcd. for $C_{19}H_{11}ClN_4OS$ (378.5): C, 60.24; H, 2.93; N, 14.79. Found: C, 60.02; H, 2.56; N, 14.47.

Compound (22). This compound was obtained in 50% yield as yellow crystals (dioxane), m.p. >300ºC. IR (KBr): ν =3202 (NH), 2204 (C≡N), 1698 cm^{-1} (C=O). ^{1}HNMR (DMSO-d_6): δ =7.45-7.82 (m, 8H, Ar-H), 8.02, 9.14 (2d, chromene H-3, H-4), 8.94 (s, 1H, pyridine H-3), 10.00 (s, 1H, NH; exchanges with D_2O). Anal.Calcd. for $C_{19}H_{12}ClN_3O_2S$ (381.5): C, 59.76; H, 3.17; N, 11.00. Found: C, 59.54; H, 3.06; N, 10.77.

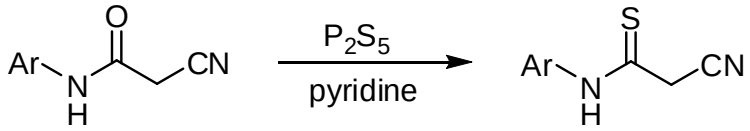
Compound (24). This compound was obtained in 50% yield as yellow crystals (dioxane), m.p. >300ºC. IR (KBr): ν =3432 (NH), 2926 (CH-aliph.), 2198 (C≡N), 1624 cm^{-1} (C=N). ^{1}HNMR (DMSO-d_6): δ =2.31 (s, 3H, CH_3), 6.72-7.93 (m, 12H, Ar-H), 8.15, 9.33 (2s, 2H, 2NH; exchanges with D_2O). MS: *m/z* =469 (M^+; 11.64), 367 (10), 267 (20.5), 190 (50), 173 (100), 111 (60) and 75 (80). Anal.Calcd. for $C_{26}H_{17}ClN_4OS$ (468.5): C, 66.59; H, 3.65; N, 11.95. Found: C, 66.36; H, 3.41; N, 11.78.

2-(4-Chlorophenylamino)-5-imino-4-oxo-4,5-dihydro-3H-chromeno[3,4-c]pyridine-1-carbonitrile (27). A mixture of **1b** (0.01 mole), 2-imino-chromene-3-carboxamide **25** (0.01 mole) and a few drops of piperidine in dioxane (30 ml) was refluxed for 3h. The solid product produced on heating was collected and recrystallized from DMF as brown crystals to give **27**.

This compound was obtained in 65% yield, m.p. >300ºC. IR (KBr): ν = 3365, 3165 (NH), 2997 (CH-arom.), 2201 (C≡N), 1676 cm^{-1} (C=O). ^{1}HNMR (DMSO-d_6): δ =7.45-7.78 (m, 8H, Ar-H), 8.99 (s, 1H, NH), 10.34 (br, 2H, 2NH; exchanges with D_2O). MS: *m/z* =362 (M^+; 0.3), 363 (M+1; 0.3), 253 (base peak), 225 (10.5), 170 (15.5) and 127 (30). Anal.Calcd. for $C_{19}H_{11}ClN_4O_2$ (362.5): C, 62.91; H, 3.06; N, 15.44. Found: C, 62.77; H, 2.84; N, 15.21.

3. Results and Discussion

The key intermediate N-aryl cyanothioacetamide derivatives **1a,b** were prepared in high yield from the thionation of N-aryl 2-cyanoacetamide derivatives with P_2S_5 in pyridine at reflux temperature[24].

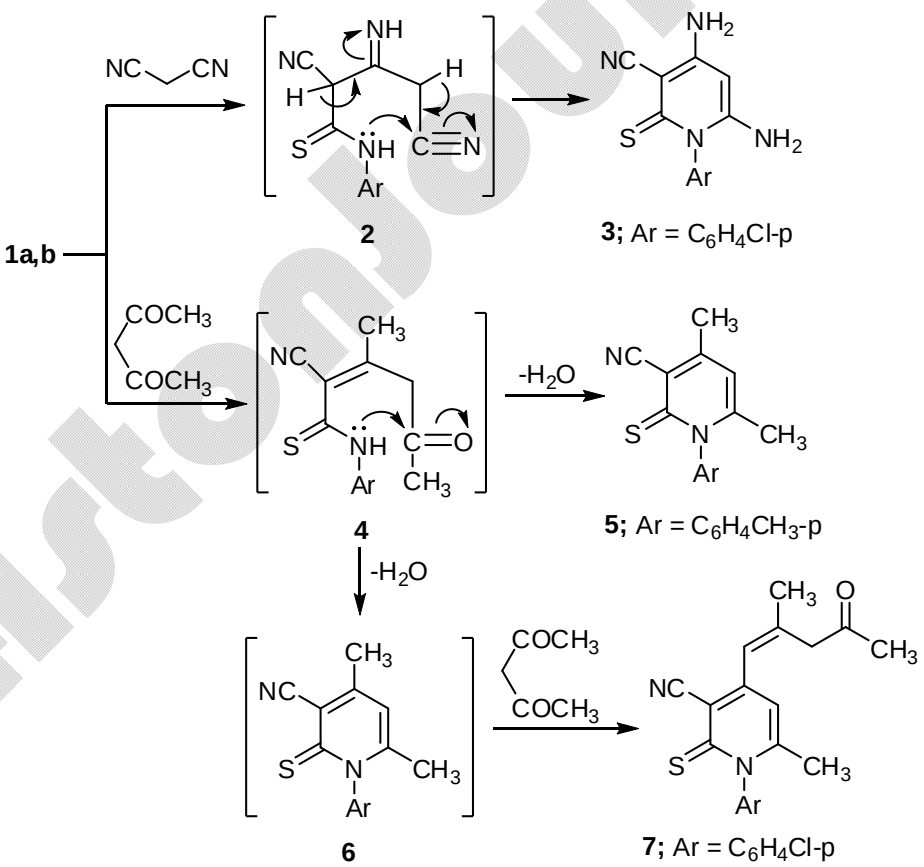


(1a,b)

a; Ar = $C_6H_4CH_3$-p
b; Ar = C_6H_4Cl-p

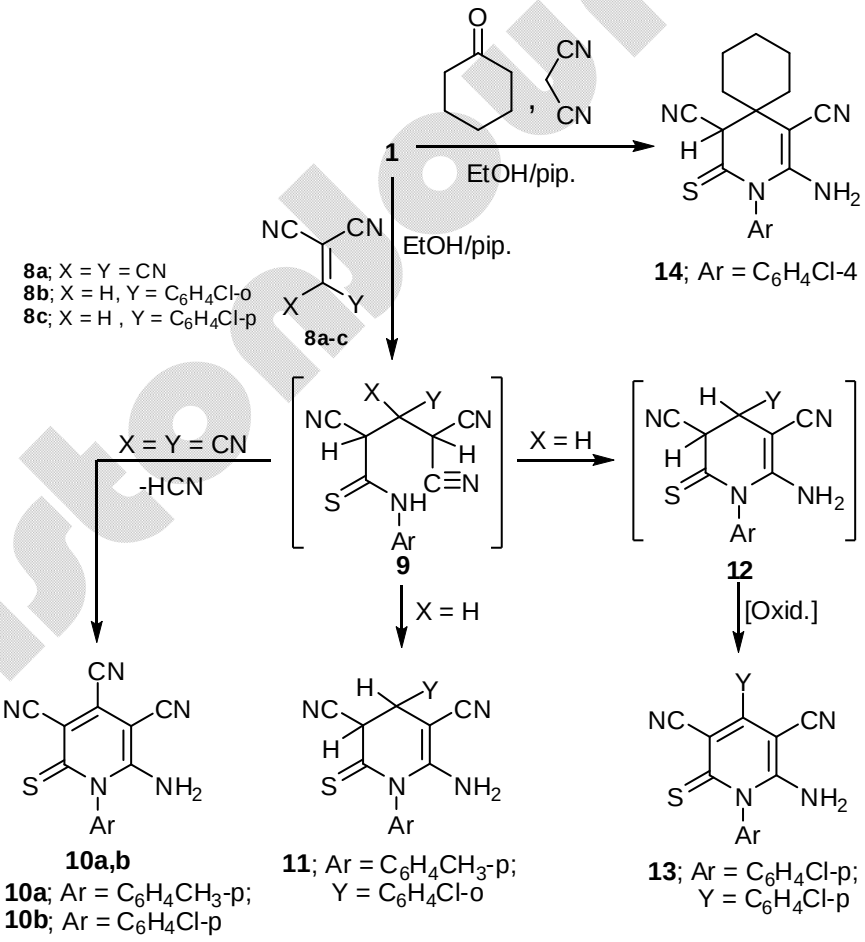
The reactivity of **1a,b** toward some active methylene reagents was also investigated. Thus, cyanothioacetamide derivative**1b** was reacted with malononitrile (1: 1 molar ratio) in an oil bath at 160°C to yield 4, 6-diamino-2-pyridinethione derivative **3**. The infrared spectrum of compound **3** revealed absorption bands at 3314, 3182 (NH_2), 2204 cm^{-1} (C≡N). ^{1}HNMR spectrum of **3** showed singlet at 5.40 ppm due to pyridine-H5 with two singlets at 5.80, 6.55 ppm for $2NH_2$. Also, its mass spectrum showed a molecular ion peak at *m/z* 276 (3.90%).

The formation of **3** is assumed to proceed via the nucleophilic addition of **1** to the cyano function of malononitrile to form the acyclic adduct **2** followed by intramolecular cyclization. Also, cyclocondensation of **1a** with acetylacetone under reflux conditions furnished 4,6-dimethyl-2-pyridinethione derivatives **5** via intramolecular cyclization of non-isolable intermediate **4** followed by dehydration, scheme 1. ^{1}HNMR spectrum of **5** showed three signals at 2.10, 2.25, and 2.40 ppm for three CH_3 with singlet at 6.30 for pyridine-H5. The mass spectrum of **5** was compatible with the molecular formula $C_{15}H_{14}N_2S$ (M = 254). While cyclocondensation of **1b** with acetylacetone under the same condition, two moles of acetylacetone were consumed and 2-pyridine-thione derivative **7** was obtained. The infrared spectrum of **7** revealed absorption bands at 2217, 1653 and 1305 cm^{-1} for nitrile, carbonyl and thiocarbonyl function groups, respectively. ^{1}HNMR spectrum of **7** showed signals at δ = 1.97, 2.05 and 2.42 ppm for three methyl groups with singlet at 3.00 ppm for methylene group and two singlets at 6.45, and 6.97 ppm due to olefinic-H and pyridine-H, in addition to aromatic protons. Furthermore, the structure of compound **7** was supported by ^{13}C-NMR spectrum (DMSO-d_6) which showed signals at δ = 20.1, 22.3, 36.1 ppm corresponding to three carbons of the three methyl groups in addition to the presence of a signal at 206.4ppm corresponding to (C=O) which aided the consumption of another mole of acetylacetone.



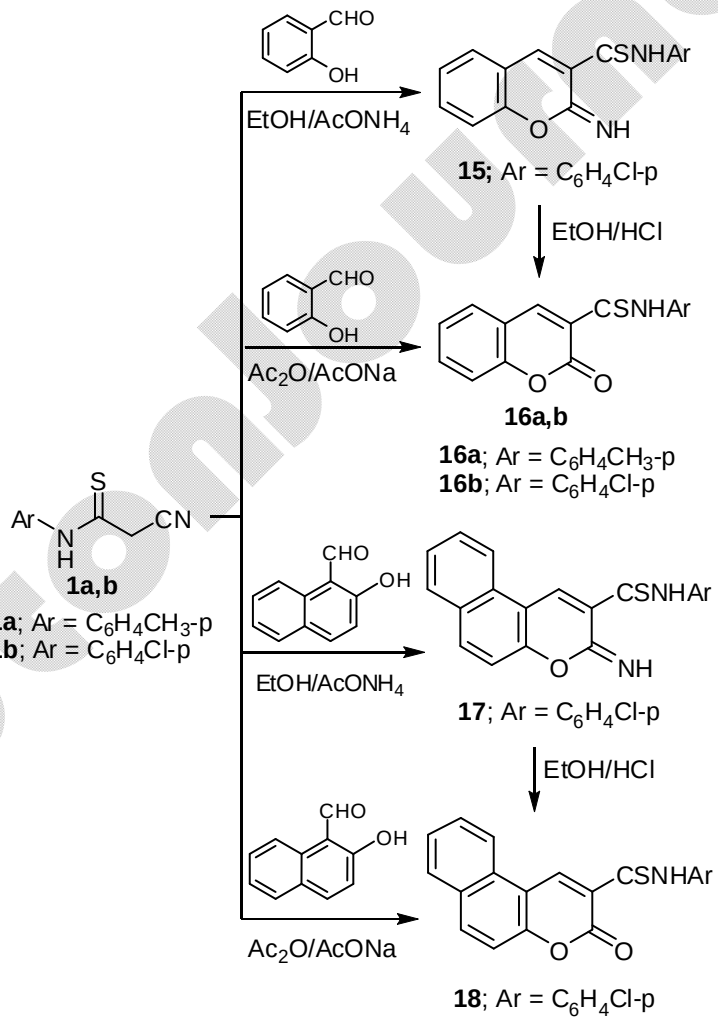
Scheme 1

As a part of this research, the reaction of N-aryl cyanothioacetamide derivatives with unsaturated nitriles was also investigated. Thus, when equimolecular amounts of N-aryl cyanothioacetamides **1a,b** and tetracyanoethylene**8a** were reacted in the presence of a catalytic amount of triethylamine, pyridinethione of type **10** was obtained. The structures of **10a,b** were confirmed on the basis of elemental analysis and spectral data. The infrared spectrum of **10a** showed absorption bands at 3314, 3182 (NH_2), 2190, 2204 cm^{-1} (C≡N). ^{1}HNMR spectrum (DMSO-d_6) of **10a** revealed singlet at δ 2.30 corresponding CH_3 in addition to the presence of aromatic and NH_2 protons. Furthermore, the mass spectrum supported the structure of **10a** and exhibited a molecular ion peak at *m/z* 291 (3.50%). The formation of **10** was assumed to proceed via Michael addition of the active methylene of **1** to the activated double bond of tetracyanoethylene **8a** to form the non-isolable intermediate **9** followed by intramolecular cyclization and loss of hydrogen cyanide**,** Scheme 2. In addition, it has been found that compound **1a** was reacted with *o*-chlorocinnamonitrile **8b** to yield 1 : 1 Michael adduct **9** as intermediate which underwent cyclization to afford the dihydropyridinethione derivative **11**. The infrared spectrum showed bands at 3448, 3328 (NH_2), and 2189 cm^{-1} (C≡N). Also, ^{1}HNMR spectrum exhibited signals at δ: 2.14 (s, 3H, CH_3), 4.12, 4.39 (2d, 2H, pyridine H*3*, H4; J=12Hz). On the other hand, reaction of cyanothioacetamide derivative **1b** with *p*-chloro-cinnamonitrile **8c** afforded pyridinethione derivative **13** through intermediate **12**. The mass spectrum of compound **13** showed a molecular ion peak at *m/z* 397 (34.77%) which is in agreement with its molecular formula $C_{19}H_{10}Cl_2N_3S$. It seems that **13** was formed via the formation of Michael adduct **9** which underwent cyclization and oxidation through intermediate **12**. Furthermore, ternary condensation of compound **1b**, cyclohexanone and malononitrile (1: 1: 1 molar ratio) afforded the pyridinethione of type **14,** Scheme 2.



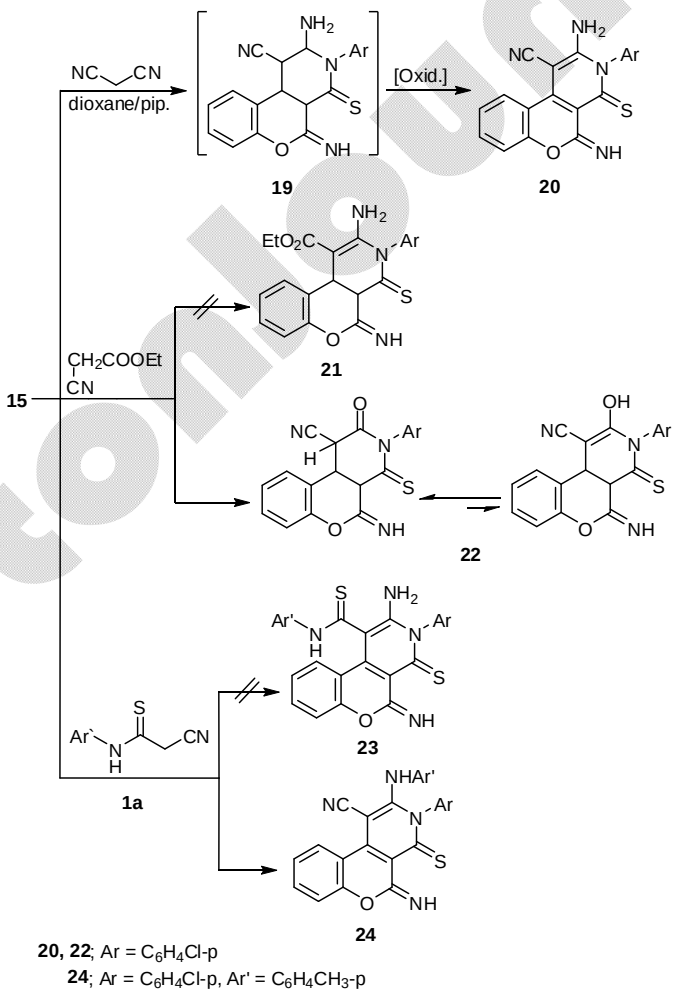
Scheme 2

This part of research was aimed at the synthesis of condensed pyridinethione derivatives containing chromene moiety, thus, cyclocondensation of cyanothioacetamide derivative **1** with salicylaldehyde and 2-hydroxynaphthaldehyde in refluxing ethanolic ammonium acetate afforded the corresponding 2-iminochromone **15** and 2-imino-benzo[f]chromene **17**, respectively. The absence of the nitrile group in IR spectra of compounds **15** and **17** confirms the cyclization process. ^{1}HNMRof compound **15** revealed singlets at δ 8.56 ppm for chromene-H. Also, the mass spectrum of **15** exhibited a molecular ion peak at *m/z* 314 (52.5%) together with a base peak at *m/z* 313 (M-1; 100%). On the other hand, cyclocondensation of cyanothioacetamide derivatives **1a,b** with salicylaldehyde and 2-hydroxynapththaldehyde in refluxing acetic anhydride containing a catalytic amount of sodium acetate afforded chromene-2-one derivatives **16a,b** and benzo[f]chromene-2-one derivative **18**. Infrared spectrum of **16a,b** showed the absence of C≡N absorption band and showed the presence of a characteristic absorption band at 1702, 1698 cm^{-1} for (C=O; lactone), corresponded to **a** and **b** respectively. ^{1}HNMR spectrum of **18** revealed two singlet signals at δ 9.36, 12.30 ppm for chromene-H and NH. Compounds **16b** and **18** could also be obtained in a good yield via the hydrolysis of compounds **15** and **17** in refluxing ethanolic-HCl, Scheme 3. The isolation of the chromenes **15-18** from the reaction supports the intermediacy of the E-geometrical isomer during the reaction course. The nitrile group must be in a *trans*position to the olefinic proton in the intermediate ylidene structure, as only this geometrical isomer can easily undergo cyclization to the corresponding chromenes **15-18**.



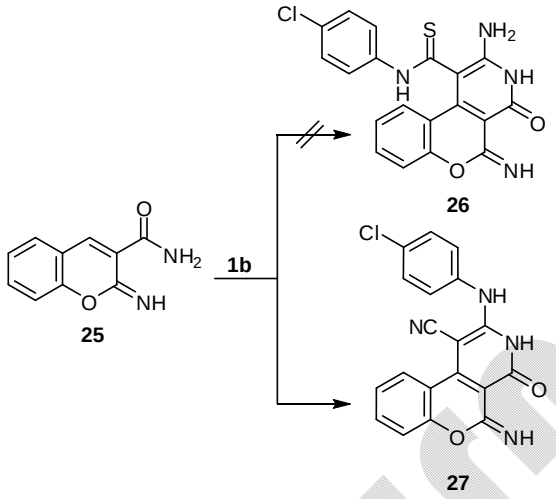
Scheme 3

Moreover, the resulting chromene derivative **15** have latent functional substituents which have the potential for further chemical transformations giving new routes for the preparation of condensed chromenopyridinethione derivatives. Thus, 2-iminochromene derivatives **15** was reacted with malononitrile in refluxing ethanol containing a catalytic amount of ammonium acetate to afford the chromeno[3,4-c]pyridine derivative **20**. Infrared spectrum of **20** showed (NH/NH_2) at 3434, 3338, 3188 and (C≡N) at 2202 cm^{-1}. Mass spectrum of **20** showed a molecular ion peak at *m/z* 378 (2.50%) with a base peak at *m/z* 300. The formation of **20** may be assumed via the formation of a cyclic Michael adduct **19** which underwent cyclization and aromatization. On the other hand, reaction of **15** with ethyl cyanoacetate afforded dihydrochromeno[3,4-c]pyridine derivative **22**, the other possible structure **21** was excluded on the basis of analytical and spectral data. The infrared spectrum exhibited bands at 3202 cm^{-1} (NH), 2204 (C≡N) and 1698 cm^{-1} (C=O) which confirm the keto form. ^{1}HNMR spectrum exhibited singlet signals at 8.02, 9.14 (chromene H-3, H-4), 8.94 (pyridine H-3) in addition to presence of a singlet at 10.00 ppm (NH), and its mass was compatible with the molecular formula $C_{19}H_{12}ClN_3O_2S$ (M^+; 381). Also, chromeno[3,4-c]pyridinethione derivative of type **24** was obtained via reaction of 2-iminochromene derivative **15** with cyanothioacetamide derivative **1a** in boiling ethanol containing piperidine as a catalyst. Both elemental and spectral data supported the proposed structure **24** and ruled out the other possible structure **23**. IR spectrum showed bands at 3432 (NH) and 2198 cm^{-1} (C≡N). Also, mass spectrum of **24** showed a molecular ion peak at *m/z* 469 (11.68%). formation of **24** was assumed to proceed via Michael addition of active methylene of **1a** to α,β-unsaturated double bond of 2-iminochromene derivative **15** followed by intramolecular cyclization with elimination of H_2S molecule and oxidation, Scheme 4.



Scheme 4

Finally, treatment of cyanothioacetamide derivative **1b** with 2-iminochromene-3-carboxamide **25** [25] in the presence of piperidine as catalyst afforded the chromeno[3,4-c]pyridine derivative **27**, and the other possible structure **26** was excluded on the basis of analytical and spectral data, scheme 5. Infrared spectrum of **27** showed absorption bands at 3365, 3165 (NH), 2201 (C≡N) and 1676 cm^{-1} (C=O). ^{1}HNMR spectrum of **27** revealed signals at δ: 8.99 (s, 1H, NH) and 10.34 ppm (broad s, 2H, 2NH) and the mass spectrum were compatible with the molecular formula $C_{19}H_{11}ClN_4O_2$ (M+1; 363).



Scheme 5

All the synthesized compounds were evaluated in vitro for their antibacterial activity against *Escherichia coli* NCTC-10418 and *Bacillus subtilis* NCTC-10400. Also, the antifungal activity against *Candida albicans* CBS-652, and *Aspergillus flavus* LTV.131 was evaluated using the agar-diffusion technique [26]. A 1 mg mL^{-1} solution in dimethyl-formamide (DMF) was used. The bacteria and fungi were grown on nutrient agar and Czapek's–Dox agar media, respectively. DMF as a negative control did not show inhibition zones. The agar media were inoculated with different micro-organism cultures tested. After 25 h of incubation at 30ºC for bacteria and 48 h for fungi, the diameter of inhibition zone (mm) was measured. Ampicillin (25 µg mL^{-1}) and Mycostatine (30 µg mL^{-1}) were used as reference drugs for anti-bacterial and antifungal activities, respectively. Most of the synthesized compounds were found to possess various antimicrobial activities towards all the microorganisms used (Table 1). Compounds **3, 7** and **11** were found to possess highest antibacterial activity towards *E. coli* and *Candida albicans* with minimal inhibitory concentration (MIC) values <50 mg mL^{-1}. In addition, compounds **5, 13, 15, 18, 20** and **22** revealed high activity against *E. coli*, *Bacillus subtilis* and *Candida albicans*, MIC values <50 mg mL^{-1}. However, none of the test compounds show superior activity than the reference drugs.

Table 1: Antimicrobial activity of the synthesized compounds (diameter zones in mm).

Compd. No.	Bacteria		Fungi	
	Gram negative bacteria	Gram positive bacteria	Unicellular	Multicellular
	E. coli NCTC-10418	*Bacillus subtilis* NCTC-10400	*Candida albicans* CBS-652	*Aspergillus flavus* LTV.131
3	++	+	+	++
5	-	+	+	+
7	+	+	-	++
13	+	+	+	+
11	++	+	++	+
15	+	+	+	-
18	+	-	-	+
20	+	+	+	+
22	+	+	+	+
Ampicillin	++++	++++	++++	++++

Competing Interests
Authors declare that they have no competing interests.

Authors' Contributions
All authors contributed equally to this work.

Acknowledgement
Plant and Microbiology Department, Faculty of Science, Al-Azhar University, carried out antimicrobial screening.

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