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Synthesis and Antimicrobial Evaluation of Some Annulated Chromeno[2,3-*b*]Pyridines using 6-Methylchromone-3-Carbonitrile

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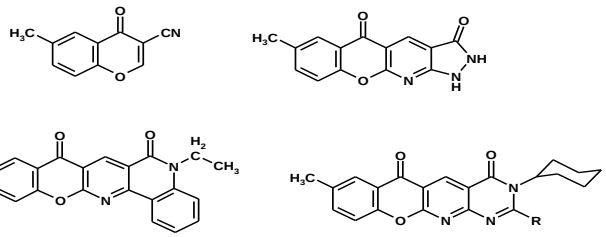
Abstract: A variety of heteroannulated chromeno[2,3-*b*]pyridines and their related compounds were efficiently synthesized from chemical transformations of 6-methylchromone-3-carbonitrile (**1**) with some cyclic and acyclic carbon nucleophiles. Also, the reactivity of carbonitrile **1** was studied towards 1-ethyl-4-hydroxy-3-nitroacetylquinolin-2(1*H*)-one (**8**) producing the unexpected 5-ethyl-10-methyl-8*H*-benzo[*h*]chromeno[2,3-*b*][1,6]naphthyridine-6(5*H*),8-dione (**9**). A diversity of 2-substituted-3-cyclohexyl-8-methyl-4*H*,6*H*-chromeno[3',2':5,6]pyrido[2,3-*d*]pyrimidine-4,6-diones were synthesized. Structures of the newly synthesized products have been deduced on the basis of elemental analysis and spectral data. The newly prepared compounds were screened for their antimicrobial activity.



Keywords: 6-Methylchromone-3-carbonitrile, basic rearrangement, RORC, chromeno[2,3-*b*]pyridines, chromeno[3',2':5,6]pyrido[2,3-*d*]pyrimidines.

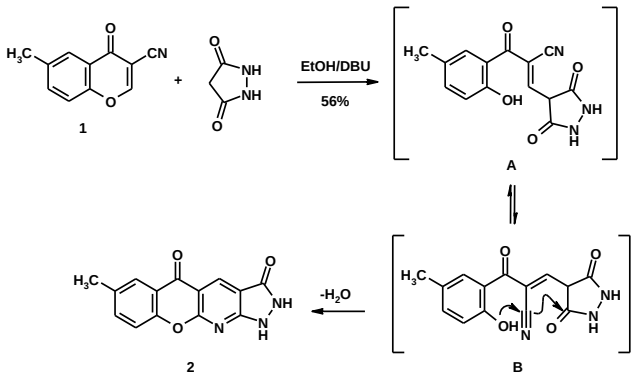
1 Introduction

Chromone derivatives represent the most common heterocycles in natural compounds of plant origin and serve as the basis of flavonoid structures [1-4]. Chromones exhibit wide range of biological activity including anticancer [5,6], antitumor [7], antioxidant [8], anti-inflammatory [9], antimalarial [10] and treatment of Alzheimer's disease [11]. The presence of the electron-withdrawing cyano function at the 3-position of the chromone nucleus changes the chemical reactivity of the γ-pyrone ring with respect to nucleophiles [12-14]. The diversity of properties of chromone-3-carbonitriles is attributed to the existence of highly reactive geminally activated push–pull alkenes (α,β-unsaturated ketones and nitriles simultaneously) with a good leaving group at the β-carbon atom, whose role is played by the phenolate anion [15-17]. Herein, we aimed to study the chemical transformations of 6-methylchromone-3-carbonitrile (**1**) [18] towards some carbon nucleophiles hoping to construct a variety of heteroannulated chromeno[2,3-*b*]pyridines and their related heterocyclic systems.

2 Results and Discussion

The present work aimed to study the chemical transformations of 6-methylchromone-3-carbonitrile (**1**) towards some carbon nucleophiles hoping to synthesize some novel heteroannulated chromone[2,3-*b*]pyridines. Thus, treating carbonitrile **1** with pyrazolidine-3,5-dione in boiling ethanol containing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a basic catalyst gave chromeno[2,3-*b*] pyrazolo[4,3-*e*]pyridine derivative **2** (Scheme 1). Formation of compound **2** may occur *via* nucleophilic attack at C-2 position with concomitant γ-pyrone ring opening leading to intermediate **A**, free rotation around the single bond produced intermediate **B**. Intramolecular cyclization through addition of hydroxyl group onto the nitrile function with concomitant cyclocondensation afforded the final product **2** (Scheme 1). The FT-IR spectrum of compound **2** showed specific absorption bands at 3445, 3293 (2NH), 1682 ($C=O_{cyclic\ amide}$), 1651 ($C=O_{\gamma\text{-pyrone}}$) and 1621 cm^{-1} (C=N). Its ^{1}HNMR spectrum showed distinctive singlet assignable to $H\text{-}4_{pyridine}$ at δ 8.65, in addition to two D_2O-exchangeable signals attributed to 2NH protons at δ 9.46 and 11.51, respectively. The mass spectrum of compound **2**

showed the molecular ion peak at m/z 267 which agrees well with the suggest formula weight (267.24) and confirms the identity of the proposed structure.

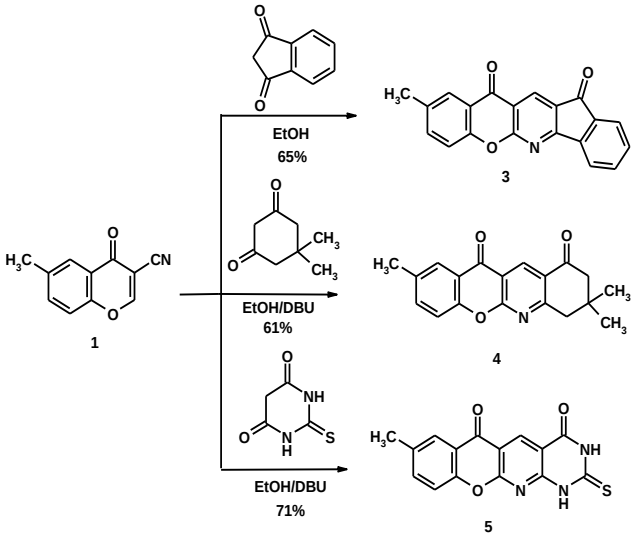


Scheme 1

Under the same reaction mechanism, the pentafused chromeno[2,3-*b*]indeno[2,1-*e*]pyridine derivative **3** was synthesized by reacting carbonitrile **1** with 1,3-indanedione in absolute ethanol without catalyst (Scheme 2). In this reaction, the use of basic catalyst such as DBU, triethylamine or piperidine should be avoided because addition of base in this reaction produce with 1,3-indanedione a purple color during the reaction and isolation of the product in a pure form was unsuccessful. The FT-IR spectrum of compound **3** showed distinctive absorption bands at 1718 ($C=O_{indanone}$), 1662 ($C=O_{\gamma\text{-pyrone}}$) and 1617 cm^{-1} (C=N). The ^1H NMR spectrum showed characteristic singlet at δ 8.51 assigned to H-6.

In the same manner, 5,5-dimethylcyclohexane-1,3-dione (dimedone) undergoes smooth and efficient ring opening and ring closure (RORC) reactions with carbonitrile **1**, yielding chromeno[2,3-*b*]quinoline-4,6-dione derivative **4**, in 61% yield (Scheme 2). The ^1H NMR spectrum showed singlet signal assigned to H-5 at δ 8.79. Structure of compound **4** was further deduced from its mass spectrum which revealed the molecular ion peak at m/z 307, and the base peak at m/z 251 corresponding to the molecular ion after loss of 2CO groups.

Likewise, 8-methyl-2-thioxo-chromeno[3`,2`:5,6] pyrido[2,3-*d*]pyrimidine-4,6(1*H*,3*H*)-dione (**5**) was prepared, in 71% yield, from basic rearrangement of carbonitrile **1** with thiobarbituric acid (Scheme 2). The FT-IR spectrum of compound **5** showed absorption bands at 1695 ($C=O_{pyrimidinone}$), 1648 ($C=O_{\gamma\text{-pyrone}}$) and 1609 cm^{-1} (C=N). Its ^1H NMR spectrum showed specific singlet at δ 8.87 corresponding to H-$4_{pyridine}$, in addition to an exchangeable signal at δ 11.42 attributed to 2NH protons. The mass spectrum of compound **5** showed the molecular ion peak, as the base peak, at m/z 311 and confirms the suggested structure.

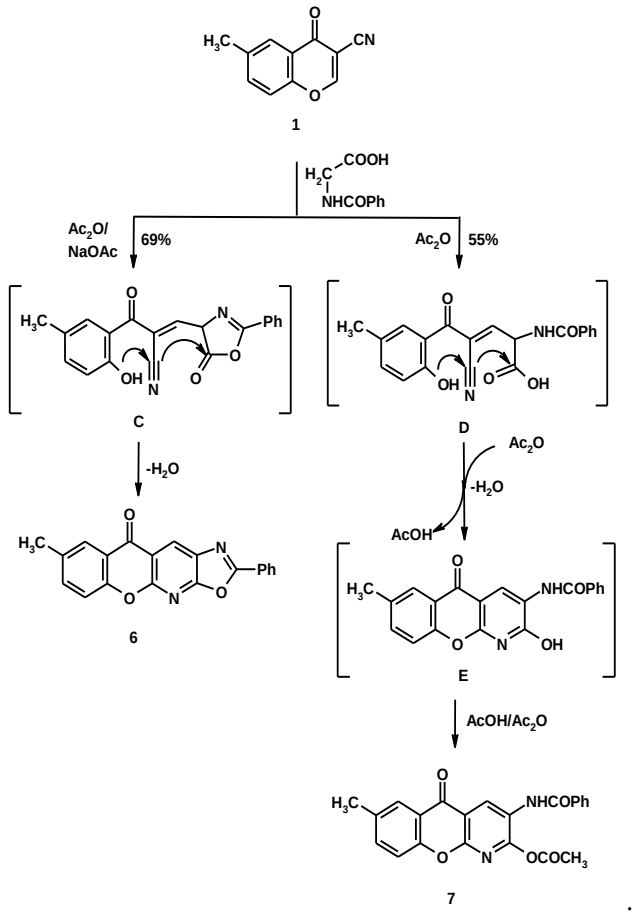


Scheme 2

On the other hand, condensation of 6-methylchromone-3-carbonitrile (**1**) with hippuric acid in boiling acetic anhydride containing freshly fused sodium acetate furnished 7-methyl-2-phenyl-5-oxo-5*H*-[1,3]oxazolo[5,4-*b*] chromeno[3,2-*e*]pyridine (**6**) as yellow crystals (Scheme 3). The reaction may proceed *via* the initial formation of 2-phenyloxazol-5-one (as a non isolable intermediate) which reacts with carbonitrile **1** producing intermediate **C** which underwent cycloaddition followed by cyclodehydration under the reaction condition giving the final product **6** (Scheme 3). Chromeno[2,3-*b*]pyridin-2-yl acetate **7** was obtained, as red crystals, from the reaction of carbonitrile **1** with hippuric acid in boiling acetic anhydride only, *via* the non isolable intermediates **D** and **E** as depicted in Scheme 3. The FT-IR spectrum of compound **6** showed absorption band at 1660 cm^{-1} assigned to $C=O_{\gamma\text{-pyrone}}$. Its ^1H NMR spectrum showed distinctive singlet attributable to H-$4_{pyridine}$ at δ 8.79. While the FT-IR spectrum of compound **7** showed three characteristic absorption bands at 1783, 1727 and 1666 cm^{-1} attributed to OC=O, $C=O_{amide}$ and $C=O_{\gamma\text{-pyrone}}$, respectively. The ^1H NMR spectrum showed singlet typical to H-$4_{pyridine}$ at δ 8.81, in addition to D_2O-exchangeable signal at δ 9.38 corresponding to NH proton.

Interestingly, new derivative of heteroannulated chromone identified as 5-ethyl-10-methyl-8*H*-benzo[*h*]chromeno[2,3-*b*][1,6]naphthyridine-6(5*H*),8-dione (**9**) was easily and efficiently synthesized from DBU catalyzed condensation reaction of 6-methylchromone-3-carbonitrile (**1**) with 1-ethyl-4-hydroxy-3-nitroacetylquinolin-2(1*H*)-one (**8**) [19] (Scheme 4). The reaction may proceed *via* nucleophilic attack of C-3 in 1-ethyl-4-hydroxy-3-nitroacetylquinolin-2(1*H*)-one (**8**) at C-2 position of chromone moiety with γ–pyrone ring opening followed by free rotation around single bond producing intermediate **F**. Cycloaddition of hydroxyl group onto the nitrile function generate intermediate **G**. Elimination of the side chain (intermediate **H**) followed by

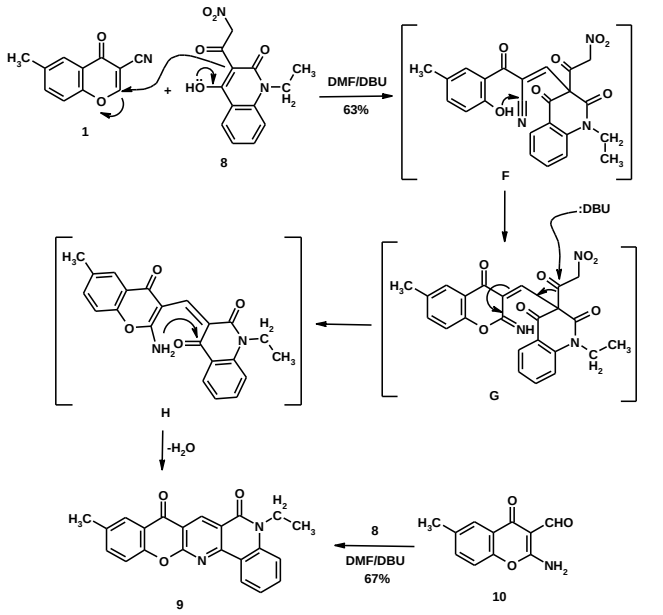
cyclodehydration yielded the final product **9**. Compound **9** was authentically prepared from the condensation reaction of 2-amino-6-methylchromone-3-carboxaldehyde (**10**) with 1-ethyl-4-hydroxy-3-nitroacetylquinolin-2(1*H*)-one (**8**) under the same reaction conditions (Scheme 4).



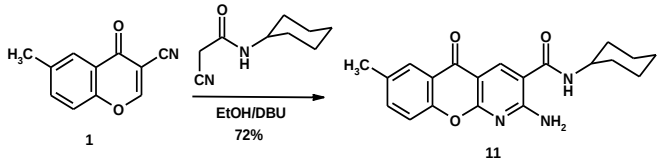
Scheme 3

The FT-IR spectrum of compound **9** showed characteristic absorption bands at 1670, 1652 1601 cm^{-1} assignable to $C=O_{quinolinone}$, $C=O_{\gamma\text{-pyrone}}$, and C=N, respectively. Its ^1H NMR spectrum revealed triplet and quartet signals at δ 1.29 and 4.34 attributable to ethyl protons, in addition to characteristic singlet at δ 9.20 attributable to H-7. Structure of compound **9** was further deduced from its mass spectrum which revealed the molecular ion peak at m/z 356 with high relative abundance (81%), and the base peak at m/z 328 attributable to M+ after loss of two CO group.

Next, RORC reaction of carbonitrile **1** with *N*-cyclohexylcyanoacetamide in boiling ethanol containing DBU afforded 2-amino-*N*-cyclohexyl-7-methyl-5-oxo-5*H*-chromeno[2,3-*b*]pyridine-3-carboxamide(**11**) (Scheme 5). The FT-IR spectrum of compound **11** showed characteristic absorption bands at 3440, 3310, 3145 (NH_2, NH), 1656 ($C=O_{\gamma\text{-pyrone}}$), 1633 ($C=O_{amide}$), 1595 cm^{-1} (C=N). The ^1H NMR spectrum of compound **11** showed singlet signals typical to H-6 and H-4 at δ 7.89 and 8.73, respectively. The spectrum also revealed D_2O-exchangeable signals attributable to NH_2 and NH at δ 8.25 and 8.64, respectively. Further, the mass spectrum of compound **11** showed the molecular ion peak at m/z 351 and confirms the suggested structure.

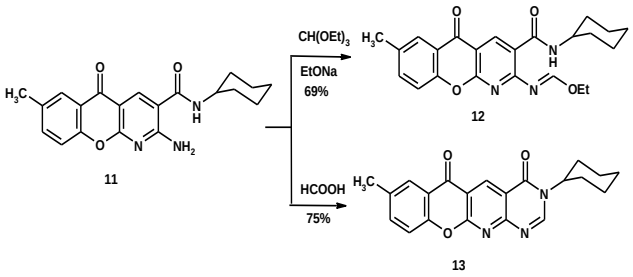


Scheme 4



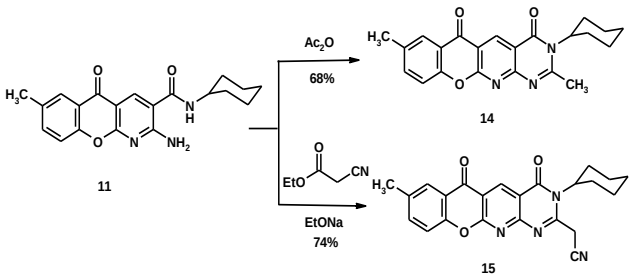
Scheme 5

Chromeno[2,3-*b*]pyridine derivative **11** used a good precursor to synthesize some new chromeno[3',2':5,6] pyrido[2,3-*d*]pyrimidine derivatives *via* the reaction with mono electrophilic reagents. Therefore, reaction of compound **11** with triethylorthoformate in sodium ethoxide afforded ethyl [7-methyl-5-oxo-3-(cyclohexylcarbamoyl)-5*H*-chromeno[2,3-*b*]pyridin-2-yl]imidoformate (**12**). While, fusion of compound **11** with formic acid afforded the target product; 3-cyclohexyl-8-methyl-4*H*,6*H*-chromeno[3',2':5,6] pyrido[2,3-*d*]pyrimidine-4,6-dione (**13**) (Scheme 6). The FT-IR spectrum of compound **12** showed absorption bands at 3383, 1688, 1659 and 1617 cm^{-1} assignable to NH, $C=O_{amide}$, $C=O_{\gamma\text{-pyrone}}$ and C=N, respectively. Its ^1H NMR spectrum showed triplet and quartet signals at δ 1.18 and 4.56 typical to ethoxy protons, in addition to doublet exchangeable signal attributed to NH proton at δ 10.29. The FT-IR spectrum of compound **13** showed characteristic absorption bands at 1689 ($C=O_{pyrimidine}$), 1663 ($C=O_{\gamma\text{-pyrone}}$) and 1619 cm^{-1} (C=N). The ^1H NMR spectrum of compound **13** showed distinctive singlet signals at corresponding to H-7, H-2 and H-5 at δ 7.91, 8.63 and 8.79, respectively.



Scheme 6

Also, boiling chromeno[2,3-*b*]pyridine **11** with acetic anhydride gave 3-cyclohexyl-2,8-dimethyl-4*H*,6*H*-chromeno[3',2':5,6]pyrido[2,3-*d*]pyrimidine-4,6-dione (**14**) (Scheme 7). The FT-IR spectrum of compound **14** showed characteristic absorption bands at 1692 ($C=O_{pyrimidine}$), 1670 ($C=O_{\gamma\text{-pyrone}}$), 1617 cm^{-1} (C=N). Its ^1H NMR spectrum showed distinctive singlet signals at δ 2.36 (CH_3), 2.47 (CH_3), 7.95 (H-7) and 8.91 (H-5).



Scheme 7

Finally, treatment of chromeno[2,3-*b*]pyridine **11** with ethyl cyanoacetate in sodium ethoxide solution produced 2-cyanomethyl-3-cyclohexyl-8-methyl-4*H*,6*H*-chromeno[3',2':5,6]pyrido[2,3-*d*]pyrimidine-4,6-dione (**15**) (Scheme 7). The ^1H NMR spectrum of compound **15** showed four singlet signals at δ 2.38 (CH_3), 3.97 (CH_2CN), 7.87 (H-7) and 8.78 (H-5). The FT-IR spectrum showed particular absorption bands at 2236 (C≡N), 1655 ($C=O_{pyrimidine}$), 1626 ($C=O_{\gamma\text{-pyrone}}$) and 1601 cm^{-1} (C=N).

3 Antimicrobial Evaluation

The newly synthesized compounds were evaluated for their in *vitro* antibacterial activity against *Staphylococcus aureus* (ATCC25923) and *Bacillus subtilis* (ATCC6635) as examples of Gram-positive bacteria and *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) as examples of Gram-negative bacteria. They were also evaluated against *Candida albicans* (ATCC 10231) as the fungal strain. The test was performed on medium potato dextrose agar (PDA) which contained infusion of 200 g potatoes, 6 g dextrose and 15 g agar [20]. Uniform size filter paper disks (3 disks per compound) were impregnated by equal volume (10 µl) from the concentrations of 500 and 1000 µg/mL dissolved compounds in dimethylformamide (DMF) and carefully placed on inoculated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and for 48 h at 24°C in the case of fungi. Cephalothin, Chloramphenicol and Cycloheximide were used as reference drugs for Gram-positive bacteria, Gram-negative bacteria and the fungus, respectively. The obtained results were recorded for each tested compound as average diameter of inhibition zones of the bacteria and fungus around the disks in mm at the concentrations 500 and 1000 µg/mL. The antimicrobial activities were determined by measuring the inhibition zones (Table 1).

Table 1: In *vitro* antimicrobial activities of the synthesized compounds 2-15 at 500 and 1000 µg/mL by disc diffusion assay.

	Mean* of zone diameter, nearest whole mm.									
	Gram - positive bacteria				**Gram - negative bacteria**				**Fungi**	
Organisms	*Staphylococcus aureus* (ATCC 25923)		*Bacillus subtilis* (ATCC 6635)		*Salmonella typhimurium* (ATCC 14028)		*Escherichia coli* (ATCC 25922)		*Candida albicans* (ATCC 10231)	
	1000 µg/ml	500 µg/ml	1000 µg/ml	500 µg/ml	1000 µg/ml	500 µg/ml	1000µg /ml	500 µg/ml	1000 µg/ml	500 µg/ml
2	11 L	8 L	9 L	6 L	9 L	7 L	15 I	11 I	12 I	9 L
3	13 I	8 L	8 L	4 L	11 L	6 L	10 L	7 L	9 L	7 L
4	14 I	10 I	12 I	7 L	6 L	3 L	14 I	12 I	13 I	10 I
5	8 L	6 L	10 L	6 L	7 L	5 L	6 L	2 L	10 L	7 L
6	14 I	11 I	12 I	10 I	8 L	4 L	7 L	3 L	14 I	11 I
7	13 I	10 I	13 I	9 I	14 I	9 I	10 L	6 L	15 I	9 L
9	14 I	11 I	16 I	11 I	8 L	3 L	8 L	3 L	11 L	6 L
11	13 I	10 I	14 I	9 I	8 L	4 L	7 L	3 L	12 I	7 L
12	17 I	13 I	11 L	7 L	13 I	8 L	10 L	5 L	14 I	10 I
13	9 L	6 L	12 I	7 L	9 L	4 L	13 I	9 I	17 I	11 I
14	15 I	11 I	16 I	12 L	5 L	2 L	12 I	9 I	14 I	10 I
15	17 I	12 I	15 I	11 I	17 I	15 I	16 I	10 I	18 I	14 I
(S)	35	26	35	25	36	28	38	27	35	28

* Calculated from 3 values.

L: Low activity = Mean of zone diameter ≤ 1/3 of mean zone diameter of control.

I: Intermediate activity = Mean of zone diameter ≤ 2/3 of mean zone diameter ofcontrol.

H: High activity = Mean of zone diameter > 2/3 of mean zone diameter of control.

S: Standard drug such as Chloramphencol in the case of Gram-positive bacteria, Cephalothinin the case of Gram-negative bacteria and cycloheximide in the case of fungi.

[1] The results depicted in Table 1 revealed that, most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against antifungal strain.

[2] In general, most of the tested compounds revealed moderate to low activities against the microorganism strains.

[3] The tested compounds recorded moderate activities against Gram-positive bacteria, except **2**, **5** and **13** for *Staphylococcus aureus*, and **2**, **3**, **5** and **12** for *Bacillus subtilis* which recorded low activities.

[4] The tested compounds recorded low activities against Gram-negative bacteria except **7**, **12** and **15** for *Salmonella typhimurium*, and **2**, **4**, **13, 14** and **12** for *Escherichia coli* which recorded moderate activities.

[5] The tested compounds recorded moderate activities against *Candida albicans* as the fungal strain except compounds **3**, **5** and **9** which recorded low activities.

[6] In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some new chromeno[2,3-*b*]pyridines and 2-substituted-3-cyclohexylchromeno[3',2':5,6]pyrido[2,3-*d*]pyrimidines with the hope of discovering new structure leads serving as antimicrobial agents. Generally, the prepared compounds showed moderate to low activities. However, none of the new synthesized compounds showed high or superior activity than the utilize reference drug.

4 Conclusion

In conclusion, A new series of heteroannulated chromeno[2,3-*b*]pyridines were efficiently synthesized from basic rearrangement of 6-methylchromone-3-carbonitrile (**1**) and some cyclic and acyclic active methylene nucleophiles. Also, a variety of 2-substituted-3-cyclohexyl-8-methyl-4*H*,6*H*-chromeno[3',2':5,6]pyrido[2,3-*d*]pyrimidine-4,6-diones were synthesized from heterocyclization of 2-amino-*N*-cyclohexyl-7-methyl-5-oxo-5*H*-chromeno[2,3-*b*]pyridine-3-carboxamide (**11**) with some mono-electrophilic reagents.

5 Experimental Section

General: Melting points were determined on a digital Stuart SMP3 apparatus. Infrared spectra were measured on FT-IR Nicolet IS10 spectrophotometer (cm^{-1}), using KBr disks. ^1H NMR spectra were measured on Mercury-300BB (300MHz), using DMSO-d_6 as a solvent and TMS (δ) as the internal standard. Mass spectra were obtained using GC-2010 Shimadzu Gas chromatography instrument mass spectrometer (70 eV). Elemental microanalyses were performed on a Perkin–Elmer CHN-2400 analyzer.

7-Methyl-1,2-dihydrochromeno[2,3-*b*]pyrazolo[4,3-*e*] pyridine-3,5-dione (2)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) and pyrazolidine-3,5-dione (0.33 g, 3 mmol) in absolute ethanol (20 mL) containing DBU (0.1 mL) was heated under reflux for 1h. The pale yellow crystals obtained during heating were filtered and recrystallized from DMF to give compound **2** as white crystals, mp > 300 ºC, yield (0.45 g, 56%). FT-IR (KBr, cm^{-1}): 3445, 3293 (2NH), 1682 ($C=O_{cyclic\ amide}$), 1651 ($C=O_{\gamma\text{-pyrone}}$), 1621 (C=N), 1576 (C=C). ^1H NMR (DMSO, δ): 7.32 (d, 1H, *J* = 8.1, H-9), 7.49 (d, 1H, *J* = 8.1, H-8), 7.78 (s, 1H, H-6), 8.65 (s, 1H, H-4), 9.46 (s, 1H, NH exchangeable with D_2O), 11.51 (s, 1H, NH exchangeable with D_2O). Mass spectrum (*m/z*, *I* %): 267 (8), 266 (18), 249 (38), 221 (9), 194 (9), 185 (6), 137 (10), 104 (6), 84 (100), 77 (15), 64 (67). Anal. Calcd for $C_{14}H_9N_3O_3$ (267.24): C, 62.92; H, 3.39; N, 15.72%. Found: C, 62.65; H, 3.16; N, 15.55%.

3-Methylchromeno[2,3-*b*]indeno[2,1-*e*]pyridine-5,7(5*H*,7*H*)-dione (3)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) and 1,3-indanedione (0.44 g, 3 mmol) in absolute ethanol (20 mL) was heated under reflux for 30 min. The white crystals obtained during heating were filtered and recrystallized from DMF/MeOH to give compound **3** yellow crystals, mp > 300 ºC, yield (0.61 g, 65%).IR (KBr, cm^{-1}): 2920, 2860 ($CH_{aliph.}$), 1718 ($C=O_{indanone}$), 1662 ($C=O_{\gamma\text{-pyrone}}$), 1617 (C=N), 1580 (C=C). ^1H NMR (DMSO, δ): 2.44 (s, 3H, CH_3), 7.29 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.50-7.78 (m, 4H, Ar-H), 7.93 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.63 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.04 (s, 1H, H-4), 8.51 (s, 1H, H-6). Anal. Calcd for $C_{20}H_{11}NO_3$ (313.31): C, 76.67; H, 3.54; N, 4.47%. Found: C, 76.40; H, 3.38; N, 4.25%.

2,2,8-Trimethyl-1,3-dihydro-chromeno[2,3-*b*]quinoline-4,6(4*H*,6*H*)-dione (4)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) and 5,5-dimethylcyclohexane-1,3-dione (0.42 g, 3 mmol) in absolute ethanol (20 mL) containing DBU (0.1 mL) was heated under reflux for 15 min. The white crystals obtained during heating were filtered and recrystallized from DMF/MeOH to give compound **4** as white crystals, mp 285-286 ºC, yield (0.56 g, 61%).IR (KBr, cm^{-1}): 3073 ($CH_{arom.}$), 2952, 2872 ($CH_{aliph.}$), 1685 ($C=O_{quinolinone}$), 1665 ($C=O_{\gamma\text{-pyrone}}$), 1602 (C=N), 1587 (C=C). ^1H NMR (DMSO, δ): 1.07 (s, 6H, $2CH_3$), 2.42 (s, 3H, CH_3), 2.63 (s, 2H, CH_2), 3.08 (s, 2H, CH_2), 7.56 (d, 1H, *J* = 8.1 Hz, H-10), 7.70 (d, 1H, *J* = 8.7 Hz, H-9), 7.88 (s, 1H, H-7), 8.79 (s, 1H, H-5). Mass spectrum (*m/z*, *I* %): 307 (72), 292 (14), 279 (14), 251 (100), 223 (16), 166 (10), 118 (7), 105 (4), 90 (8), 77 (10). Anal. Calcd for $C_{19}H_{17}NO_3$ (307.34): C, 74.25; H, 5.58; N, 4.56%. Found: C, 74.22; H, 5.47; N, 4.38%.

8-Methyl-2-thioxo-chromeno[3',2':5,6]pyrido[2,3-*d*] pyrimidine-4,6(1*H*,3*H*)-dione (5)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) and thiobarbituric acid (0.43 g, 3 mmol) in absolute ethanol (20

mL) containing DBU (0.1 mL) was heated under reflux for 30 min. The yellow crystals obtained during heating were filtered and recrystallized from DMF/H_2O to give compound **5** as yellow crystals, mp > 300 ºC, yield (0.66 g, 71%). IR (KBr, cm^{-1}): 3447 (2NH), 3069 ($CH_{arom.}$), 2952, 1850 ($CH_{aliph.}$), 1695 ($C=O_{pyrimidinone}$), 1648 ($C=O_{\gamma\text{-}pyrone}$), 1609 (C=N), 1583 (C=C), 1270 (C=S). ^{1}H NMR (DMSO, δ): 2.41 (s, 3H, CH_3), 7.42 (d, 1H, *J* = 9.0 Hz, H-10), 7.55 (d, 1H, H-9), 7.74 (s, 1H, H-6), 8.87 (s, 1H, H-4), 11.42 (bs, 2H, 2NH exchangeable with D_2O). Mass spectrum (*m/z*, *I* %): 311 (100), 283 (5), 278 (12), 253 (47), 224 (24), 195 (12), 134 (12), 105 (13), 80 (68), 64 (59). Anal. Calcd for $C_{15}H_9N_3O_3S$ (311.32): C, 57.87; H, 2.91; N, 13.50; S, 10.30%. Found: C, 57.52; H, 2.65; N, 13.29; S, 10.05%.

7-Methyl-2-phenyl-5-oxo-5*H*-[1,3]oxazolo[5,4-*b*] chromeno[3,2-*e*]pyridine (6)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) and hippuric acid (0.54 g, 3 mmol) in acetic anhydride (15 mL) and freshly fused sodium acetate (0.3 g) was heated under reflux for 2 h. The yellow crystals obtained after cooling were filtered, washed several times with water and recrystallized from acetic acid to give compound **6** as yellow crystals, mp 275-276 ºC, yield (0.68 g, 69%). IR (KBr, cm^{-1}): 3063 ($CH_{arom.}$), 2929, 2870 ($CH_{aliph.}$), 1660 ($C=O_{\gamma\text{-}pyrone}$), 1633 (C=N), 1590 (C=C). ^{1}H NMR (DMSO, δ): 2.47 (s, 3H, CH_3), 7.32 (t, 1H, Ar-H), 7.66-7.77 (m, 6H, Ar-H), 8.00 (s, 1H, H-6), 8.79 (s, 1H, H-4). Mass spectrum (*m/z*, *I* %): 328 (1), 266 (100), 251 (5), 237 (10), 197 (10), 169 (12), 140 (15), 134 (4), 119 (10), 105 (10), 90 (6), 77 (14). Anal. Calcd for $C_{20}H_{12}N_2O_3$ (328.33): C, 73.17; H, 3.68; N, 8.53%. Found: C, 72.86; H, 3.42; N, 8.36%.

7-Methyl-5-oxo-3-[(phenylcarbonyl)amino]-5*H*-chromeno[2,3-*b*]pyridin-2-yl acetate (7)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) and hippuric acid (0.54 g, 3 mmol) in acetic anhydride (15 mL) was heated under reflux for 2 h. The red crystals obtained during heating were filtered, washed several times with water and recrystallized from AcOH to give compound **7** as red crystals, mp 260-261 ºC, yield (0.64 g, 55%). IR (KBr, cm^{-1}): 3439 (NH), 3108 ($CH_{arom.}$), 2919, 2840 ($CH_{aliph.}$), 1783 (OC=O), 1727 ($C=O_{amide}$), 1666 ($C=O_{\gamma\text{-}pyrone}$), 1632 (C=N), 1574 (C=C). ^{1}H NMR (DMSO, δ): 1.91 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.43-7.71 (m, 5H, Ar-H), 7.89 (s, 1H, H-6), 7.95 (d, 1H, *J* = 6.6 Hz, H-6), 8.55 (d, 1H, Ar-H), 8.81 (s, 1H, H-4), 9.38 (s, 1H, NH exchangeable with D_2O). Mass spectrum (*m/z*, *I*%): 388 (3), 373 (2), 346 (6), 135 (4), 105 (100), 77 (38). Anal. Calcd for $C_{22}H_{16}N_2O_5$ (388.37): C, 68.04; H, 4.15; N, 7.21%. Found: C, 67.85; H, 4.12; N, 7.09%.

5-Ethyl-10-methyl-8*H*-benzo[*h*]chromeno[2,3-*b*][1,6] naphthyridine-6(5*H*),8-dione (9)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) or 6-methyl-2-aminochromone-3-carboxaldehyde (**10**) (0.61 g, 3 mmol) and 1-ethyl-4-hydroxy-3-nitroacetylquinolin-2(1*H*)-one (**8**) (0.83 g, 3 mmol), in DMF (15 mL) containing DBU (0.1 mL), was heated under reflux for 2 h. The yellow crystals obtained after cooling were filtered and crystallized from DMF/EtOH to give compound **9** asyellow crystals, yield (63-67%), mp 293-294 °C. IR (KBr, cm^{-1}): 3070 ($CH_{arom.}$), 2975, 2950 ($CH_{aliph.}$), 1670 ($C=O_{quinolinone}$), 1652 ($C=O_{\gamma\text{-}pyrone}$), 1601 (C=N) and 1558 (C=C). ^{1}H NMR (DMSO-d_6, δ): 1.29 (t, 3H, *J* = 7.2 Hz, $CH_2\mathit{CH_3}$), 2.42 (s, 3H, CH_3), 4.34 (q, 3H, *J* = 7.2 Hz, $\mathit{CH_2}CH_3$), 7.36 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.59-7.63 (m, 2H, Ar-H), 7.69-7.75 (m, 2H, Ar-H), 7.89 (s, 1H, H-9), 8.62 (d, 1H, Ar-H), 9.20 (s, 1H, H-7). Mass spectrum (m/z, *I* %): 356 (81), 328 (100), 311 (44), 300 (20), 271 (5), 255 (5), 228 (3), 163 (14), 155 (22), 89 (10), 91 (5), 77 (10), 63 (4). Anal. Calcd for $C_{22}H_{16}N_2O_3$ (356.38): C, 74.15; H, 4.53; N, 7.86%. Found: C, 72.90; H, 4.41; N, 7.56%.

2-Amino-*N*-cyclohexyl-7-methyl-5-oxo-5*H*-chromeno[2,3-*b*]pyridine-3-carboxamide (11)

A mixture of carbonitrile **1** (0.55 g, 3 mmol) and 2-cyano-*N*-cyclohexylacetamide (0.50 g, 3 mmol) in absolute ethanol (20 ml) containing DBU (0.1 ml) was heated under reflux for 30 min. The yellow crystals obtained during heating were filtered and crystallized from DMF/H_2O to give compound **11** as pale yellow crystals, yield 0.76 g (72%), mp 296-297 °C. IR (KBr, cm^{-1}): 3440, 3310, 3145 (NH_2, NH), 3050 ($CH_{arom.}$), 2933, 2860 ($CH_{aliph.}$), 1656 ($C=O_{\gamma\text{-}pyrone}$), 1633 ($C=O_{amide}$), 1595 (C=N), 1557 (C=C). ^{1}H NMR (DMSO, δ): 1.10-1.35 (m, 5H, $H_{axial\ cyclohexyl}$), 1.58-1.74 (m, 5H, $H_{equatorial\ cyclohexyl}$), 2.41 (s, 3H, CH_3), 3.76-3.75 (m, 1H, $H_{cyclohexyl}$), 7.46 (d, 1H, *J* = 8.4 Hz, H-9), 7.60 (d, 1H, *J* = 8.8 Hz, H-8), 7.89 (s, 1H, H-6), 8.25 (bs, 2H, NH_2 exchangeable with D_2O), 8.64 (d, 1H, NH exchangeable with D_2O), 8.73 (s, 1H, H-4). Mass spectrum (m/z, *I* %): 351 (20), 350 (24), 295 (43), 269 (30), 253 (100), 226 (26), 198 (45), 170 (7), 156 (74), 135 (20), 98 (79), 77 (12). Anal. Calcd for $C_{20}H_{21}N_3O_3$ (351.39): C, 68.36; H, 6.02; N, 11.96%. Found: C, 68.10; H, 5.85; N, 11.72%.

Ethyl [7-methyl-5-oxo-3-(cyclohexylcarbamoyl)-5*H*-chromeno[2,3-*b*]pyridin-2-yl]imidoformate (12)

A mixture of compound **11** (0.70 g, 2 mmol) and triethyl orthoformate (0.45 g, 3 mmol) in sodium ethoxide (0.5 g sodium in 30 mL absolute ethanol) was heated under reflux for 3 h. after cooling the reaction mixture was poured onto crushed ice and neutralized with cocn. HCl. The solid so formed was filtered and crystallized from DMF/EtOH to give compound **12** as yellow crystals, yield 0.56 g (69%), mp 306-307 °C. IR (KBr, cm^{-1}): 3383 (NH), 3027 ($CH_{arom.}$), 2927 ($CH_{aliph.}$), 1688 ($C=O_{amide}$), 1659 ($C=O_{\gamma\text{-}pyrone}$), 1617 (C=N), 1577 (C=C). ^{1}H NMR (DMSO, δ): 1.18 (t, 1H, $CH_2\mathit{CH_3}$), 1.22-1.43 (m, 5H, $H_{axial\ cyclohexyl}$), 1.69-1.96 (m, 5H, $H_{equatorial\ cyclohexyl}$), 2.43 (s, 3H, CH_3), 3.73-3.75 (m, 1H,

$H_{cyclohexyl}$), 4.56 (q, 2H, *J* = 6.0 Hz, CH_2CH_3), 7.47 (d, 1H, *J* = 8.8 Hz, H-9), 7.72 (d, 1H, *J*= 8.4 Hz, H-8), 7.93 (s, 1H, H-6), 8.81 (s, 1H, H-4), 10.29 (d, 1H, *J*= 6.4 Hz, NH exchangeable with D_2O). Anal. Calcd for $C_{23}H_{25}N_3O_4$ (407.46): C, 67.80; H, 6.18; N, 10.31%. Found: C, 67.55; H, 5.90; N, 10.18%.

3-Cyclohexyl-8-methyl-4*H*,6*H*-chromeno[3',2':5,6] pyrido[2,3-*d*]pyrimidine-4,6-dione (13)

A mixture of compound **11** (0.70 g, 2 mmol) and formic acid (15 ml) was heated under reflux for 4 h. The yellow crystals obtained during heating were filtered and crystallized from DMF/EtOH to give compound **13** as yellow crystals, yield 0.54 g (75%), yellow crystals, mp > 310 °C. IR (KBr, cm^{-1}): 3063 ($CH_{arom.}$), 2928, 2851 ($CH_{aliph.}$), 1689 (C=O $_{pyrimidine}$), 1663 ($C=O_{\gamma\text{-pyrone}}$), 1619 (C=N), 1581 (C=C). ^{1}H NMR (DMSO, δ): 1.24-1.31 (m, 5H, H $_{axial\ cyclohexyl}$), 1.58-1.87 (m, 5H, H $_{equatorial\ cyclohexyl}$),2.40 (s, 3H, CH_3), 3.77 (br, 1H, $H_{cyclohexyl}$), 7.56 (d, 1H, *J* = 8.8 Hz, H-10), 7.69 (d, 1H, J= 8.8 Hz, H-9), 7.91 (s, 1H, H-7), 8.63 (s, 1H, H-2), 8.79 (s, 1H, H-5). Anal. Calcd for $C_{21}H_{19}N_3O_3$ (361.39): C, 69.79; H, 5.30; N, 11.63%. Found: C, 69.45; H, 5.09; N, 11.40%.

3-Cyclohexyl-2,8-dimethyl-4*H*,6*H*-chromeno[3',2':5,6] pyrido[2,3-*d*]pyrimidine-4,6-dione (14)

A mixture of compound **11** (0.70 g, 2 mmol) and acetic anhydride (10 ml) was heated under reflux for 4 h. The yellow crystals obtained during heating were filtered and crystallized from DMF/H_2O to give compound **14** as yellow crystals, yield 0.51 g (68%), yellow crystals, mp > 310 °C. IR (KBr, cm^{-1}): 3057 ($CH_{arom.}$), 2937, 2855 ($CH_{aliph.}$), 1692 (C=O $_{pyrimidine}$), 1670 ($C=O_{\gamma\text{-pyrone}}$), 1617 (C=N), 1551 (C=C). ^{1}H NMR (DMSO, δ): 1.27-1.36 (m, 5H, H $_{axial\ cyclohexyl}$), 1.59-1.89 (m, 5H, H $_{equatorial\ cyclohexyl}$), 2.36 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.76-3.78 (m, 1H, $H_{cyclohexyl}$), 7.62 (d, 1H, *J* = 8.4 Hz, H-10), 7.70 (d, 1H, *J* = 8.8 Hz, H-9), 7.95 (s, 1H, H-7), 8.91 (s, 1H, H-5). Anal. Calcd for $C_{22}H_{21}N_3O_3$ (375.42): C, 70.38; H, 5.64; N, 11.19%. Found: C, 70.06; H, 5.33; N, 11.10%.

2-Cyanomethyl-3-cyclohexyl-8-methyl-4*H*,6*H*-chromeno [3',2':5,6]pyrido[2,3-*d*]pyrimidine-4,6-dione (15)

A mixture of compound **11** (0.70 g, 2 mmol) and ethyl cyanoacetate (0.23 g, 3 mmol) in sodium ethoxide (0.5 g in 30 mL absolute ethanol) was heated under reflux for 3 h. after cooling the reaction mixture was poured onto crushed ice and neutralized with cocn. HCl. The solid so formed was filtered and crystallized from DMF to give compound **15** as yellow crystals, yield 0.59 g (74%), mp > 310 °C. IR (KBr, cm^{-1}): 3078 ($CH_{arom.}$), 2958, 2856 ($CH_{aliph.}$), 2236 (C≡N), 1655 ($C=O_{pyrimidine}$), 1626 ($C=O_{\gamma\text{-pyrone}}$), 1601 (C=N), 1548 (C=C). ^{1}H NMR (DMSO, δ): 1.18-1.35 (m, 5H, H $_{axial\ cyclohexyl}$), 1.62-1.81 (m, 5H, H $_{equarorial\ cyclohexyl}$), 2.38 (s, 3H, CH_3), 3.72-3.79 (m, 1H, H $_{cyclohexyl}$), 3.97 (s, 2H, CH_2CN), 7.47 (d, 1H, J= 8.4 Hz, H-10), 7.67 (d, 1H, *J*= 8.0 Hz, H-9), 7.87 (s, 1H, H-7), 8.78 (s, 1H, H-5). Anal. Calcd for $C_{23}H_{20}N_4O_3$ (400.43): C, 68.99; H, 5.03; N, 13.99%. Found: C, 68.75; H, 4.80; N, 13.72%.

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