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Synthesis of Chromen-2-one, Pyrano[3,4-c]chromene and Pyridino[3,4-c]chromene Derivatives as Potent Antimicrobial Agents

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Abstract: In an attempt for development of new antimicrobial agents; new series of chromen-2-one, pyrano[3,4-c]chromene and pyridino[3,4-c]chromene derivatives bearing a diazo moiety were synthesized. Chromen-2-one derivatives were synthesized *via* treatment of 5-(aryldiazo) salicylaldehyde with different type of active methylene derivatives. Pyrano[3,4-c]chromene and pyridino[3,4-*c*]chromene derivatives were synthesized *via* treatment of chromen-2-one derivatives with another active methylene derivatives. The synthesized compounds were evaluated for their expected antimicrobial activity; where, the majority of these compounds showed potent antibacterial and antifungal activities against the tested strains of bacteria and fungi.

Keywords: Chromen-2-ones, Pyrano[3,4-c]chromenes, Pyridino[3,4-*c*]chromene; Antibacterial and antifungal activities.

INTRODUCTION

NATURAL and synthetic chromen-2-one derivatives have great interest from medicinal chemists, many of the chromene derivatives used as precursors for the synthesis of potentially biologically active compounds.[1–5] Chromen-2-one moiety is being the parent scaffold in many of biological derivatives. Some of chromene skeleton are well established as antimicrobial agents such as Novobiocin and Chlorobiocin.[6] Recently, chromene derivatives have great interesting due to their potential application such as anti-HIV,[7] antitubercular,[8] antioxidant,[9] anticancer,[10] cytotoxic agents,[11] antidyslipidemic agent,[12] antileishmanial,[13] anti-inflammatory agents.[14]

Diazo compounds showed many valuable medicinal and pharmaceutical activities such as inhibition of RNA, DNA and carcinogenesis, protein synthesis and nitrogen fixation.[15–18]

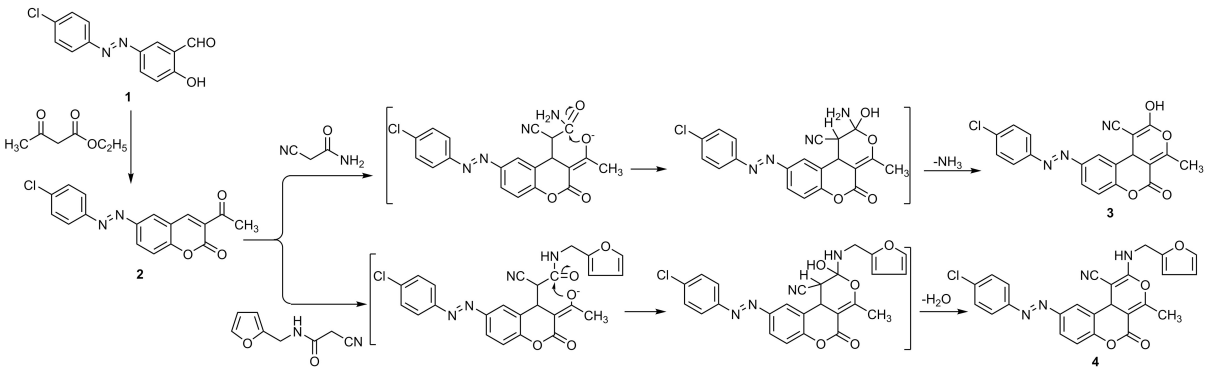
Bacterial and fungal resistance is one of the serious medical problems. Also, the levels rate of resistance is increasing to classical antibiotics. So, the development and discovery of effective antibacterial and antifungal drugs with novel mechanisms of action become urgent tasks for antimicrobial research programs.[19–22]

In light of these facts, the present study was designed to synthesize new chromene derivatives and evaluate their antimicrobial activity. As a trial to obtain novel class of antibacterial and antifungal agents, various groups were introduced into the target compounds

RESULTS AND DISCUSSION

Chemistry

Treatment salicylaldehyde derivative **1**[23] with ethyl acetoacetate in boiling EtOH/DMF led to the formation of chromen-2-one derivative **2** as yellow solid in high yield (Scheme 1). Its IR spectrum showed absorption bands at: 1740, 1650 cm^{-1} corresponding to 2C=O functional groups. ^1H NMR revealed signals at: δ = 2.86 and 8.87 ppm corresponding to CH_3 and chromene-H4, respectively. In

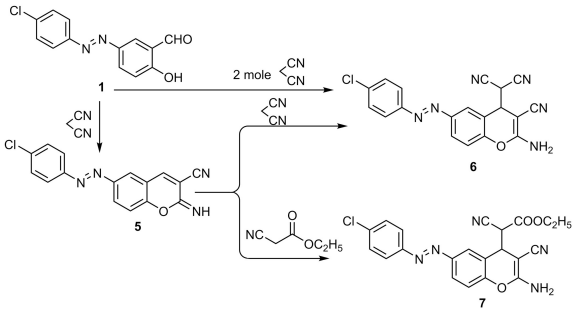


Scheme 1. Synthesis of pyrano[3,4-c]chromene derivatives **3** and **4**.

addition, the resulting chromene derivative was used for further chemical transformations as new routes for condensed chromene with possible biological activity. Acetylcoumarin derivative **2** was allowed to react with cyanoacetamide in presence of a catalytic amount of piperidine to give pyrano[3,4-c]chromene derivative **3**. IR spectrum of compound **3** revealed absorption bands at: δ = 3413 and 2209 cm^{-1} for the OH and cyano functional groups. Its ^{1}H NMR spectrum showed two characterized signals at: δ = 2.50 and δ = 9.04 ppm assigned for the CH_3 and OH protons.

Reaction of acetylcoumarin derivative **2** with 2-cyano-*N*-(furan-2-ylmethyl) acetamide[24] in the presence of few drops of piperidine under reflux yielded the corresponding pyrano[3,4-*c*]chromene derivative **4**. IR spectrum of **4** showed absorption bands at: 3253, 2209 and 1709 cm^{-1} corresponding to NH, C≡N and C=O functional groups, respectively. ^{1}H NMR spectrum showed three signals at: δ = 2.57, 3.9 and 5.07 ppm due to CH_3, CH_2, and CH protons, respectively. Formation of **4** may be proceed through the addition of methylene group to the activated double bond of chromene to give Michael adduct, which cyclized to the respective pyrano[3,4-*c*]chromene **4** as a final product *via* elimination of water molecule.

Interaction of **1** with malononitrile in the presence of piperidine, 3-cyano chromane derivative **5** was obtained in good yield (Scheme 2). Its IR spectrum showed two characterized absorption bands at: 3348 and 2210 cm^{-1} corresponding to NH and C≡N functional groups, respectively. Also, its ^{1}H NMR assigned multiple signals in the region 7.54–8.25 for Ar-H with H-pyrane. In addition, interaction of compound **5** with malononitrile or ethyl acetoacetate in presence of catalytic amount piperidine afforded chromenylmalononitrile derivative **6** and chromenyl ethyl cyanoacetate derivative **7,** respectively. Chromenylmalononitrile **6** can be prepared by treatment compound **1** with two moles of malononitrile. IR spectrum of **7** revealed the presence of cyano and carbonyl ester functional groups at: 2203 and 1737 cm^{-1}, respectively. Its ^{1}H NMR spectrum displayed in addition to the aromatic proton signals, two doubles signals at: δ = 3.93 and 4.16 ppm integrated for two protons due to the two CH protons. Moreover, its ^{13}C NMR confirmed the structure.

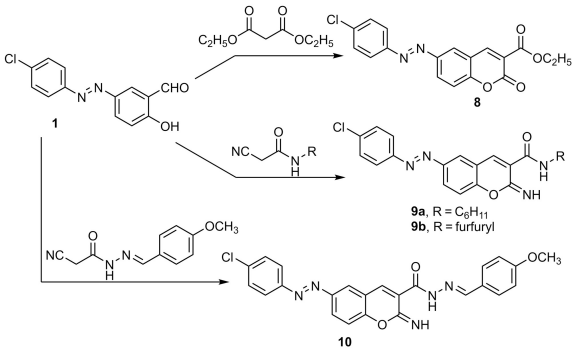


Scheme 2. Synthesis of chromene derivatives **5**–**7**.

Cyclocondensation of the salicylaldehyde derivative **1** with diethyl malonate in presence of piperidine as catalyst afforded chromene-3-carboxylate derivative **8** as colored solid in high yield (Scheme 3). IR spectrum of **8** revealed absorption band at: 1722 cm^{-1} attributed for C=O functional group. ^{1}H NMR spectrum was characterized by the presence of triplet and quartet signals at: δ = 1.20 and 4.29 ppm corresponding for ethyl protons. Iminochromene derivatives **9a** and **9b** were obtained through cyclocondensation of **1** with 2-cyano-*N*-(cyclohexyl)acetamide[25] and 2-cyano-*N*-(furan-3-ylmethyl)acetamide, respectively. IR spectrum of **9a** exhibited strong absorption bands at 3338 and 1672 cm^{-1} due to NH and C=O functional groups, respectively. Its ^{1}H NMR showed signals at: δ = 0.80–1.06, 2.65–2.71, 8.96 and 10.17 ppm corresponding to CH_2 cyclohexyl, CH–N cyclohexyl, NH and CONH protons, respectively.

On the same manner, interaction of **1** with 2-cyano-*N*'-(4-methoxybenzylidene)acetohydrazide afforded the corresponding iminochromene derivative **10**. IR spectrum of **10** showed bands for amino and carbonyl groups at: 3301 and 1660 cm^{-1}, respectively. ^{1}H NMR spectrum displayed

two characterized singlet signals at: δ = 3.80 and 8.75 ppm corresponding for OCH_3 and azomethine protons, respectively. In addition, ^{13}C NMR showed three signals at: 55.8, 151.0 and 160.9 corresponding to OCH_3, C=N and C=O carbons, respectively.



Scheme 3. Synthesis of chromene derivatives **8**–**10**.

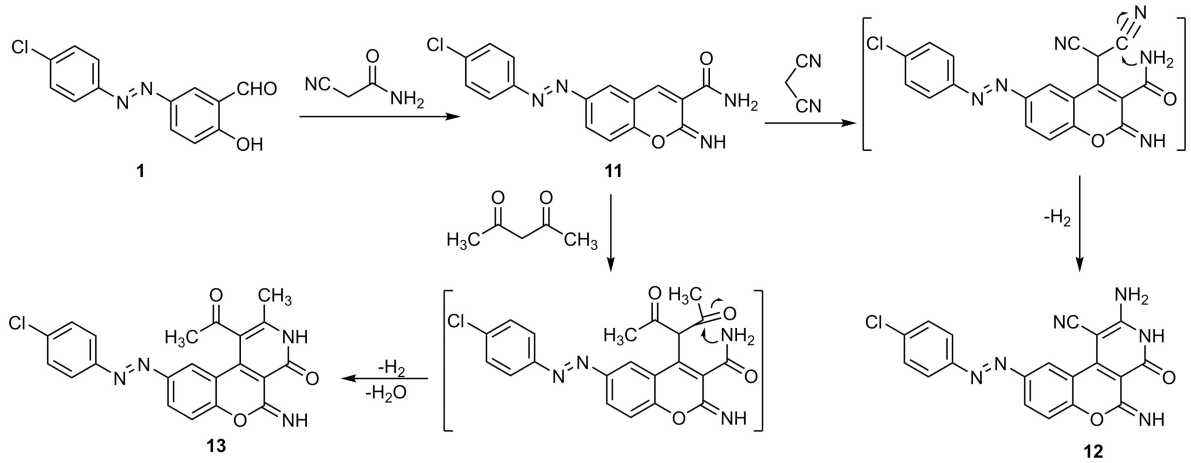
methylene group to the activated double bond in compound **11** to afford the acyclic Michael adduct that cyclized, then aromatized to yield the final products.

Finally, 2-iminochromene derivative **9b** was utilized as a starting material for further reaction. Hence, reaction of **9b** with malononitrile or ethyl cyanoacetate in presence of a catalytic amount of piperidine afforded pyridino[3,4-*c*]-chromene derivatives **14** and **15** (Scheme 5).

Antimicrobial activity

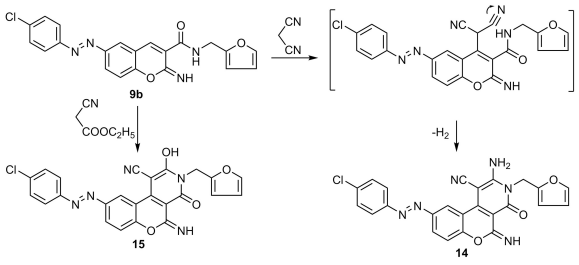
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

Fifteen test organisms representing three different microbial groups were used: Group 1: (Gram positive bacteria) *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024), *Streptococcus pyogenes* (RCMB 010015), and *Bacillis subtilis* (RCMB 010063); Group 2: (Gram negative bacteria) *Proteous vulgaris* (RCMB 010085), *Klebsiella pneumonia* (RCMB



Scheme 4. Synthesis of pyridino[3,4-*c*]chromene derivatives **12** and **13**.

In addition, chromene-3-carboxamide **11** was obtained *via* interaction of **1** with cyanoacetamide in EtOH/DMF containing catalytic amount of piperidine. ^1H NMR spectrum of **11** afforded two broad signals at: δ = 8.62, 9.04 ppm attributed to NH and NH_2 protons. Reaction of chromene derivative **11** with malononitrile or acetylacetone afforded products which formulated on the basis of elemental analysis and spectral data as chromenopyridine derivatives **12** and **13**, respectively (Scheme 4). ^1H NMR spectrum of **12** exhibited two broad signals at: δ = 4.31 and 9.54 ppm due to NH_2 and NH protons, respectively. ^1H NMR spectrum of **13** exhibited signals at: δ = 2.27, 2.47 and 8.70 ppm corresponding to CH_3, $COCH_3$ and NH protons, respectively. The formation of **12** and **13** is assumed through the Michael addition of the activated



Scheme 5. Synthesis of pyridino[3,4-*c*]chromene derivatives **14** and **15**.

010093), *Shigella flexneri* (RCMB 0100542) and *Pseudomonas aeruginosa* (RCMB 010043); Group 3: (Fungi) *Aspergillus fumigates* (RCMB 02564), *Aspergillus clavatus*

(RCMB02593), *Candida albicans* (RCMB 05035), and *Penicillium marneffei* (RCMB 01267). Agar-diffusion method[26] was used for the determination of the preliminary screening of antibacterial activity. The newly synthesized target compounds were evaluated against clinical isolates of standard strains of fungi by the broth dilution method according to NCCLs[27] Ampicillin, Gentamycin and Amphotericin B were used as reference drugs for Gram-positive bacteria, Gram-negative bacteria and fungi, respectively. The results were recorded for each tested compounds as the average diameter of inhibition zones of bacterial growth around the discs in mm. The inhibition zone diameters, attributed to the tested original concentration (5 mg/mL) as a preliminary test, are shown in Table 1.

Concerning the antimicrobial activity of chromene derivatives **2**, **5**, **8**, **9a**,**b**, **10**, **11**, the results displayed those derivatives showed moderate to good activities against most of all the screened organisms. Using the general structure provided in Figure 1, certain aspects of the structure activity relationships for these compounds can be more clearly highlighted.

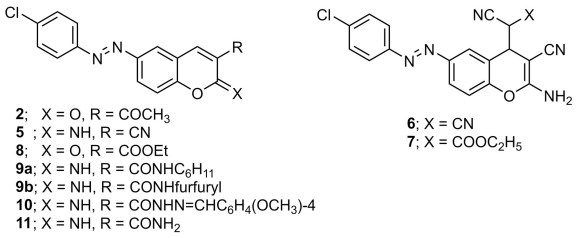
The results revealed that compound **2** showed antimicrobial activity nearly equipotent to the reference drug against all screened organisms. While compound **8** showed antimicrobial activity nearly equipotent to the reference drug only against gram –ve bacteria. Chromen-2-one **5**, **9a**,**b**, **10** and **11**; X was NH group and R were cyano or carboxamide derivatives. Regarding the effect of R group, compound **5** showed high antimicrobial activity against all screened organisms. Compound **9b** and **10** showed good antimicrobial activities only against gram +ve bacteria. Compound **11** showed high antimicrobial activity only against gram –ve bacteria. Compound **9a** showed high antimicrobial activity only against fungi. Using the general structure provided in Figure 1, compound **6** showed high antimicrobial activity against all screened bacteria while compound **7** showed good activity only against fungi.

Using the general structure provided in Figure 2, pyrano[3,4-c]chromene derivatives **3** and **4**; R were hydroxyl group (**3**) or $NHCH_2$furfuryl moiety (**4**). Regarding the effect of R group, pyrano[3,4-c]chromene **3** showed high antimicrobial activity against all screened bacteria. While, pyrano[3,4-c]chromene **4** showed moderate activity against most of tested organisms.

Concerning the antimicrobial activity of pyridino[3,4-*c*]-chromene derivatives **12**–**15**: The presence of pyridino[3,4-*c*]chromene moiety resulted in the highest antimicrobial activity among all the compounds investigated in this study. The presence of pyridino[3,4-*c*]-chromene moiety exhibited the highest antibacterial activity against most of the organisms; pyridino[3,4-*c*]-chromene moiety showed results greater than the reference drug against most of the

Table 1. Antimicrobial activities of the synthesized compounds against the pathological Gram +ve bacteria, Gram –ve bacteria and fungi expressed as inhibition diameter zones in millimeters

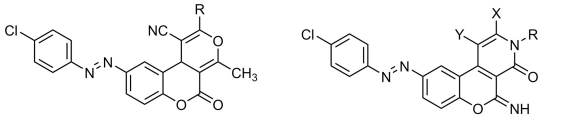
Compd. No.	Gram +ve bacteria				Gram –ve bacteria				Fungi			
	S. aureus	*S. epider.*	*S. pyoge.*	*B. subtili.*	*P. vulga.*	*K. pneu.*	*S. Flexn.*	*P. aerug.*	*A. fumig.*	*A. clavat.*	*C. albic.*	*P. neffei*
2	24.5±0.3	22.5±0.4	23.7±0.8	26.3±0.6	22.6±0.2	21.0±0.6	21.4±0.6	16.3±0.5	21.2±0.3	19.2±0.2	18.7±0.3	20.7±0.5
3	25.6±0.5	22.4±0.5	21.4±0.2	28.6±0.5	20.2±0.3	23.4±0.5	22.4±0.3	15.0±0.2	19.5±0.3	18.8±0.3	16.5±0.4	19.8±0.3
4	15.1±0.7	14.5±0.5	NA	13.1±0.3	12.3±0.2	13.5±0.5	NA	NA	14.5±0.6	11.7±0.5	10.3±0.3	13.5±0.8
5	24±0.5	20.9±0.6	18.9±0.3	24.8±0.2	21.2±0.7	22.8±0.2	21.6±0.5	16.8±0.6	20.4±0.6	18.9±0.3	21.9±0.6	20.2±0.3
6	15.3±0.4	14.7±0.4	NA	17.2±0.2	13.2±0.3	15.2±0.3	12.5±0.5	NA	21.1±0.3	19.1±0.2	23.8±0.4	18.9±0.3
7	27.8±0.2	23.2±0.5	23.2±0.3	27.9±0.4	21.2±0.7	23.8±0.3	22.9±0.3	16.6±0.4	14.2±0.5	12.6±0.2	NA	13.5±0.6
8	17.2±0.4	14.2±0.2	NA	18.4±0.1	22.5±0.3	24.3±0.1	22.4±0.3	16.9±0.3	13.5±0.5	11.8±0.4	10.9±0.3	12.2±0.6
9a	15.2±0.2	16.9±0.3	NA	17.1±0.2	12.5±0.5	13.9±0.4	11.9±0.3	NA	21.8±0.3	19.2±0.3	22.5±0.4	19.9±0.6
9b	26.7±0.6	22.0±0.2	21.7±0.3	28.8±0.3	14.8±0.4	15.5±0.7	14.7±0.4	NA	14.2±0.3	15.1±0.3	NA	13.4±0.1
10	24.3±0.5	23.3±0.5	24.2±0.3	28.3±0.5	14.2±0.6	12.6±0.4	11.5±0.6	NA	13.4±0.6	11.7±0.5	NA	16.8±0.2
11	17.7±0.3	14.4±0.2	NA	16.5±0.4	21.5±0.2	23.3±0.6	21.4±0.6	15.2±0.5	19.9±0.4	20.0±0.4	23.0±0.2	20.2±0.5
12	29.2±0.3	26.1±0.3	28.3±0.4	36.3±0.4	24.5±0.2	27.7±0.3	25.2±0.6	18.2±0.4	25.1±0.6	24.1±0.3	26.9±0.4	26.4±0.5
13	30.0±0.5	28.2±0.5	28.5±0.3	32.9±0.3	23.8±0.2	28.2±0.3	25.5±0.3	19.4±0.4	24.8±0.6	22.5±0.6	27.3±0.6	23.6±0.5
14	29.9±0.4	27.6±0.4	28.5±0.4	34.8±0.3	25.1±0.5	27.2±0.1	26.2±0.3	18.8±0.3	23.6±0.4	23.9±0.5	28.4±0.8	25.2±0.7
15	29.5±0.7	26.8±0.4	27.5±0.4	35.7±0.7	25.9±0.1	29.2±0.2	26.2±0.6	17.8±0.4	23.9±0.3	22.7±0.2	28.4±0.2	24.9±0.4
Ampicillin	28.9±1	25.4±0.1	26.4±0.3	34.6±0.3	-----	-----	-----	-----	-----	-----	-----	-----
Gentamycin	-----	-----	-----	-----	23.4±0.3	26.3±0.1	24.8±0.2	17.3±0.1	-----	-----	-----	-----
Amphotericin B	--	-----	-----	-----	-----	-----	-----	-----	23.7±0.1	21.9±0.1	26.4±0.2	22.6±0.3



2; X = O, R = $COCH_3$
5 ; X = NH, R = CN
8; X = O, R = COOEt
9a; X = NH, R = $CONHC_6H_{11}$
9b; X = NH, R = CONHfurfuryl
10; X = NH, R = $CONHN{=}CHC_6H_4(OCH_3)$-4
11; X = NH, R = $CONH_2$

6; X = CN
7; X = $COOC_2H_5$

Figure 1.



3; R = OH
4; R = $NHCH_2$furfuryl

12; Y = CN, X = NH_2, R = H
13; Y = $COCH_3$, X = CH_3, R = H
14; Y = CN, X = NH_2, R = CH_2furfuryl
15; Y = CN, X = OH, R = CH_2furfuryl

Figure 2.

organisms. Using the general structure provided in Figure 2, a moderate difference in antimicrobial activity is noted between the tested pyridino[3,4-*c*]chromene derivatives, this indicate that the main effect related to the presence of the pyridino[3,4-*c*]chromene moiety. The comparison between the antimicrobial activity of pyridino[3,4-*c*]chromene and standard reference drugs against the used Gram positive, Gram negative bacteria and fungi is represented graphically in Figure 3.

MINIMUM INHIBITORY CONCENTRATIONS AGAINST GRAM POSITIVE BACTERIA, GRAM NEGATIVE BACTERIA AND FUNGI

The minimal inhibitory concentrations (MICs) for the promising compounds were determined using twofold serial dilution method[27] MICs results (μg/mL) of the most promising derivatives **2**, **3**, **5**, **6**, **9a**, and **11**–**15** are presented in Table 2. The majority of synthesized compounds showed varying degrees of inhibition against the test panel of species.

Pyridino[3,4-*c*]chromene **14** showed equipotent potency of standard drugs in inhibiting the growth of *S. pyogenes* (MIC 0.24 μg/mL), *P. vulgaris* (MIC 1.95 μg/mL), *S. flexneri* (MIC 0.48 μg/mL), *A. clavatus* (MIC 1.95 μg/mL) and *P. Marneffei* (MIC 1.95 μg/mL). Pyridino[3,4-*c*]-chromene **14** displayed 50 % less activity compared to standard drugs against *P. aeruginosa* (MIC 62.5 μg/mL).

Chromen-2-one **11** showed equipotent potency of standard drugs in inhibiting the growth of *S. flexneri* (MIC 0.48 μg/mL), *P. aeruginosa* (MIC 31.25 μg/mL) and *P. Marneffei* (MIC 1.95 μg/mL). Chromen-2-one **11** displayed 50 % less activity compared to standard drugs against *S. pyogenes* (MIC 0.48 μg/mL), *P. vulgaris* (MIC 3.9 μg/mL), *A. Fumigates* (MIC 1.95 μg/mL), *A. clavatus* (MIC 3.9 μg/mL) and *C. albicans* (MIC 0.48 μg/mL).

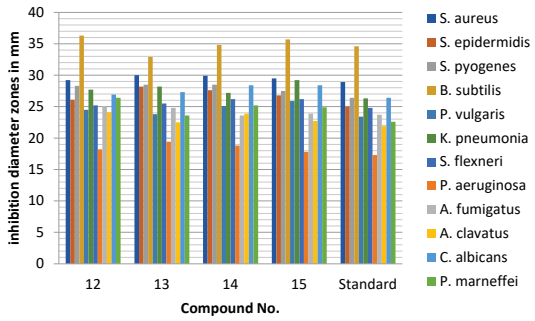


Figure 3. The comparison between the antimicrobial activities of pyridino[3,4-*c*]chromene and standard drug against the used Gram +ve, Gram –ve bacteria and fungi.

Pyridino[3,4-*c*]chromene **12** showed equipotent potency of standard drugs in inhibiting the growth of *S. pyogenes* (MIC 0.24 μg/mL) and *P. vulgaris* (MIC 1.95 μg/mL). Pyridino[3,4-*c*]chromene **12** displayed 50 % less activity compared to standard drugs against *S. epidermidis* (MIC 0.97 μg/mL), *K. pneumonia* (MIC 0.48 μg/mL), *P. aeruginosa* (MIC 62.5 μg/mL), *A. Fumigates* (MIC 1.95 μg/mL), *A. clavatus* (MIC 3.9 μg/mL) and *P. Marneffei* (MIC 3.9 μg/mL). Pyridino[3,4-*c*]chromene **13** showed equipotent potency of standard drugs in inhibiting the growth of *A. Fumigates* (MIC 0.97 μg/mL) and *P. Marneffei* (MIC 1.95 μg/mL). pyridino[3,4-*c*]chromene **13** displayed 50 % less activity compared to standard drugs against *C. albicans* (MIC 0.48 μg/mL).

Chromen-2-one **9a** showed equipotent potency of standard drugs in inhibiting the growth of *A. fumigates* (MIC 0.97 μg/mL). Chromen-2-one derivative **9a** displayed 50 % less activity compared to standard drugs against *S. pyogenes* (MIC 0.48 μg/mL), *P. vulgaris* (MIC 3.9 μg/mL), *P. aeruginosa* (MIC 62.5 μg/mL), *A. clavatus* (MIC 3.9 μg/mL), *C. albicans* (MIC 0.48 μg/mL) and *P. Marneffei* (MIC 3.9 μg/mL).

Chromene **5** displayed 50 % less activity compared to standard drugs against *S. epidermidis* (MIC 0.24 μg/mL), *P. vulgaris* (MIC 3.9 μg/mL), *K. pneumonia* (MIC 0.48 μg/mL), *A. clavatus* (MIC 3.9 μg/mL) and *P. Marneffei* (MIC 3.9 μg/mL).

Chromene **2** displayed 50 % less activity compared to standard drugs against *P. vulgaris* (MIC 3.9 μg/mL) and *P. Marneffei* (MIC 3.9 μg/mL).**T2**

CONCLUSION

Chromen-2-one, pyrano[3,4-c]chromene and pyridino[3,4-*c*]-chromene derivatives bearing a diazo moiety were synthesized in order to evaluate their antibacterial and antifungal activities. Regarding the effect of each derivative against bacterial and fungal strains, results of antimicrobial activity in this study revealed that: the majority of these

Table 2. Minimum inhibitory concentration (μg/mL) of the most potent synthesized compounds against the pathological organisms

Compd. No.	*S. aureus*	*S. epidermidis*	*S. pyogenes*	*B. subtilis*	*P. vulgaris*	*K. pneumonia*	*S. flexneri*	*P. aeruginosa*	*A. fumigatus*	*A. clavatus*	*C. albicans*	*P. marneffei*
2	0.97	15.63	3.9	1.95	3.9	1.95	3.9	125	3.9	15.63	31.25	3.9
3	3.9	3.9	31.25	1.95	7.81	3.9	7.81	250	3.9	31.25	62.5	7.81
5	0.24	0.24	3.9	0.06	3.9	0.48	3.9	500	15.63	3.9	31.25	3.9
6	3.9	1.95	62.5	0.97	7.81	1.95	7.81	250	7.81	15.63	31.25	15.63
9a	0.24	1.95	0.48	0.06	3.9	0.84	1.95	62.5	0.97	3.9	0.48	3.9
11	0.97	1.95	0.48	0.06	3.9	1.95	0.48	31.25	1.95	3.9	0.48	1.95
12	0.24	0.97	0.24	0.06	1.95	0.48	3.9	62.5	1.95	3.9	0.97	3.9
13	3.9	62.5	125	62.5	7.81	1.95	3.9	250	0.97	7.81	0.48	1.95
14	0.24	3.9	0.24	0.06	1.95	0.97	0.48	62.5	0.24	1.95	0.97	1.95
15	3.9	62.5	31.25	125	7.81	3.9	15.63	250	62.5	7.81	62.5	125
Ampicillin	0.06	0.48	0.24	0.007	-----	-----	-----	-----	-----	-----	-----	-----
Gentamycin	-----	-----	-----	-----	1.95	0.24	0.48	31.25	-----	-----	-----	-----
Amphotericin B		-----	-----	-----	-----	-----	-----	-----	0.97	1.95	0.24	1.95

compounds showed potent antibacterial and antifungal activities against the tested strains of bacteria and fungi. Pyridino[3,4-*c*]chromene moiety resulted in the highest antimicrobial activity among all the compounds investigated in this study. Pyridino[3,4-*c*]chromene moiety showed results greater than the reference drug against most of the organisms.

Experimental Section

All melting points are recorded on digital Gallen Kamp MFB-595 instrument and may be uncorrected. The IR spectra (KBr) (cm^{-1}) were measured on a JASCO spectrophotometer. ^{1}H NMR spectra were recorded on Bruker spectrometers (at 500 MHz) and are reported relative to deuterated solvent signals in deuterated dimethylsulfoxide (DMSO-d_6). ^{13}C NMR spectra were recorded on Bruker Spectrometers (at 125 MHz) in deuterated dimethylsulfoxide (DMSO-d_6). The purity of the synthesized compounds was monitored by TLC. Elemental analyses were carried out by the Microanalytical Research Center, Faculty of Science, Cairo University. Analytical results for C, H and N were within –/+ 0.4 of the calculated values. The antimicrobial screening and minimal inhibitory concentrations of the tested compounds were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

SYNTHESIS OF 3-ACETYL-6-((4-CHLOROPHENYL)-DIAZENYL)-2*H*-CHROMEN-2-ONE (2)

Equimolar amounts of compound **1** (0.01 mol) and ethyl acetoacetate (0.01 mol) in a mixture of 15 mL EtOH and 5 mL DMF was treated with few drops of piperidine. The reaction mixture was heated under reflux for 4 h, and then allowed to cool. The precipitate that formed was filtered, washed with ethanol, dried and crystallized from ethanol to give compound **2** as yellow crystals. Yield 85 %; m.p. 235–237 °C; IR: ν/cm^{-1} = 1740, 1650 (2C=O); ^{1}H NMR: δ/ppm = 2.86 (s, 3H, CH_3), 7.58-8.25 (m, 7H, Ar-H), 8.87 (s, 1H, CH–pyrane); ^{13}C NMR: 29.3 (CH_3), 118.0, 119.5, 122.2, 123.6, 124.4 (2C), 129.3 (2C), 131.0, 136.1, 138.4, 150.2, 150.8, 155.7, 159.4, 188.7 (C=O); MS: m/z = 326 (M^+; 3.9), 170 (5.5), 153 (7.0), 127 (70.5), 65 (77), 55 (100); *Anal.* Calcd. For $C_{17}H_{11}ClN_2O_3$ (326.74): C, 62.49; H, 3.39; N, 8.57; found: C, 62.65; H, 3.24; N, 8.87

SYNTHESIS OF 9-((4-CHLOROPHENYL)DIAZENYL)-2-HYDROXY-4-METHYL-5-OXO-5,10b-DIHYDRO-PYRANO[3,4-c]CHROMENE-1-CARBONITRILE (3)

Cyanoacetamide (0.01 mol) was added to a solution of compound **2** (0.01 mol) in mixture of 15 mL EtOH, 5 mL DMF and 3 drops of piperidine. The reaction mixture was heated under reflux for 4 hours and concentrated to its half-volume. The solid product was filtered off and recrystallized from ethanol to give compound **3** as pale yellow crystals. Yield 70 %; m.p. 265–267 °C; IR: ν/cm^{-1} = 3413 (broad, OH), 2925 (CH-aliphatic), 2209 (C≡N), 1671 (C=O); ^{1}H NMR: δ/ppm = 2.5 (s, 3H, CH_3), 5.06 (s, 1H, H-pyrane), 7.59–8.22 (m, 7H, Ar-H), 9.04 (br, 1H, OH); ^{13}C NMR: 18.0 (CH_3), 52.1, 106.7, 117.4, 121.4, 121.8, 124.4, 124.6, 125.0, 128.6, 129.1, 130.2 (2C), 136.5, 149.4, 151.2, 152.8, 159.0, 162.3, 185.2; *Anal.* Calc.: $C_{20}H_{12}ClN_3O_4$: C, 61.00; H, 3.07; N, 10.67; Found: C, 60.89; H, 3.34; N, 10.97.

SYNTHESIS OF 9-((4-CHLOROPHENYL)DIAZENYL)-2-(FURAN-2-YLMETHYLAMINO)-4-METHYL-5-OXO-5,10b-DIHYDROPYRANO[3,4-c]CHROMENE-1-CARBONITRILE (4)

A mixture of **2** (0.01 mol) and 2-cyano-*N*-(furan-2-ylmethyl) acetamide (0.01 mol) in a mixture of 15 mL EtOH and 5 mL DMF containing a catalytic amount of piperidine was heated under reflux for 4 h, then left to cool. The solid product formed was filtrated off, washed with EtOH, dried,

and finally crystallized from EtOH/DMF to give compound **4** as pale yellow crystals. Yield 60 %; m.p. 290–292 °C; IR: ν/cm^{-1} = 3253 (NH), 2925 (aliphatic CH), 2209 (C≡N), 1709 (C=O); ^{1}H NMR: δ/ppm = 2.57 (s, 3H, CH_3), 3.9 (s, 2H, CH_2), 5.07 (s, 1H, H-4-pyrane), 5.15 (d, 1H, CH-furfuryl), 6.40 (d, 1H, CH-furfuryl), 7.50-8.56 (m, 8H, 7Ar-H + CHO-furfuryl), 9.6 (br, 1H, NH cancelled with D_2O); ^{13}C NMR: 17.3 (CH_3), 37.7 (CH_2), 45.2 (CH), 106.0, 110.6, 111.4, 119.1, 120.4, 121.8, 124.4 (2C), 124.9, 128.6 (2C), 129.1 (2C), 136.8, 142.3, 145.8, 150.2, 151.2, 152.5, 159.3, 161.9, 176.6; *Anal.* Calc: $C_{25}H_{17}ClN_4O_4$ (472.89): C, 63.50; H, 3.62; N, 11.85; Found: C, 63.36; H, 3.66; N, 11.22.

SYNTHESIS OF 6-((4-CHLOROPHENYL)DIAZENYL)-2-IMINO-2H-CHROMENE-3-CARBONITRILE (5)

A mixture of compound **1** (0.01 mol), malononitrile (0.01 mol) and drops of piperidine in a mixture of 15 mL EtOH and 5 mL DMF was heated under reflux for 2 hours. The solid product formed on hot collected by filtration and recrystallized from ethanol/DMF to give compound **5** as brown crystals. Yield 80 %; m.p. > 300 °C; IR: ν/cm^{-1} = 3348 (NH), 2210 (C≡N); ^{1}H NMR: δ/ppm = 7.54–7.78 (m, 3H, Ar-H), 7.87-8.25 (m, 5H, Ar-H & H-pyrane), 9.53 (br, 1H, NH exchangeable with D_2O); ^{13}C NMR: 110.3, 112.7, 115.4, 116.8, 117.5, 121.5, 123.6, 123.7, 124.4, 129.1, 136.5, 145.2, 150.8, 156.5, 164.4; *Anal.* Calc. $C_{16}H_9ClN_4O$ (308.73): C, 62.25; H, 2.94; N, 18.15; Found: C, 62.43; H, 2.65; N, 18.22.

SYNTHESIS OF 2-(6-((4-CHLOROPHENYL)DIAZENYL)-3-CYANO-2-IMINO-2H-CHROMEN-4-YL)MALONONITRILE (6)

Method A: A mixture of compound **1** (0.01 mol), malononitrile (0.02 mol) in a mixture of 15 mL EtOH, 5 mL DMF and 3 drops of piperidine was heated under refluxed for 3 h. The solid product formed on hot, collected by filtration and recrystallized from ethanol as orange crystals. Yield 82 %. Method B: A mixture of compound **5** (0.01 mol), malononitrile (0.01 mol) in a mixture of 15 mL EtOH, 5 mL DMF and 3 drops of piperidine was heated under refluxed for 2 h. The solid product formed on hot, collected by filtration and recrystallized from ethanol as orange crystals. Yield 79 %; m.p. > 300 °C; IR: ν/cm^{-1} = 3335 (NH_2), 2209 (C≡N); ^{1}H NMR: δ/ppm = 3.76 (d, 1H, J = 3.7 Hz, CH), 4.01 (d, 1H, J = 3.7 Hz, pyrane-H-4), 6.57–7.88 (m, 7H, Ar-H), 9.53 (br, 2H, NH_2); ^{13}C NMR: 50.1, 63.8, 113.7, 117.1, 118.4, 121.1, 124.2, 124.4, 129.1, 130.3, 136.5, 146.1, 150.8, 151.2, 176.3; *Anal.* Calc. $C_{19}H_{11}ClN_6O$ (374.79): C, 60.89; H, 2.96; N, 22.42; Found: C, 60.46; H, 2.54; N, 22.42.

SYNTHESIS OF ETHYL 2-(2-AMINO-6-((4-CHLORO–PHENYL)DIAZENYL)-3-CYANO-4H-CHROMEN-4-YL)-2-CYANOACETATE (7)

A solution of compound **5** (0.01 mol) and ethyl cyanoacetate (0.01 mol) in a mixture of 15 mL EtOH and 5 mL DMF and 0.5 mL of piperidine was heated under reflux for 4 h. The solid that formed was filtered and recrystallized from ethanol as brown crystals**.** Yield 50 %; m.p. 250–251°C; IR: ν/cm^{-1} = 3364, 3260 (NH_2), 2990 (aliphatic CH), 2203 (C≡N), 1737 (C=O ester); ^{1}H NMR: δ/ppm = 1.07 (t, 3H, J = 7.5 Hz, CH_3), 3.93 (d, 1H, J = 3.8 Hz, CH), 4.16 (d, 1H, J = 3.8 Hz, CH-pyrane), 4.21 (q, 2H, J = 7.5 Hz, CH_2 ester), 7.6–8.22 (m, 7H, Ar-H), 9.22 (br, 2H, NH_2); ^{13}C NMR: 14.8 (CH_3), 39.4 (CH), 71.9 (CH), 60.5 (CH_2), 118.4 (C≡N), 118.8 (C≡N), 123.9, 124.8, 127.4, 130.2, 137.0, 144.7, 148.9, 166.9 (C=O); *Anal.* Calc. $C_{21}H_{16}ClN_5O_3$ (421.84): C, 59.79; H, 3.82; N, 16.60; Found: C, 59.45; H, 3.64; N, 16.02.

SYNTHESIS OF ETHYL 6-((4-CHLOROPHENYL)DIAZENYL)-2-OXO-2H-CHROMENE-3-CARBOXYLATE (8)

A mixture of compound **1** (0.01 mol), diethyl malonate (0.01 mol) and catalytic amount of piperidine in EtOH/DMF (15/5 mL) was heated under reflux for 3 h. The solid product was collected by filtration and recrystallized from ethanol as brown crystals. Yield 75 %; m.p. 265–267 °C; IR: ν/cm^{-1} = 2923 (CH-aliphatic), 1722 (C=O); ^{1}H NMR: δ/ppm = 1.20 (t, 3H; J = 7.5 Hz, CH_3), 4.29 (q, 2H, J = 7.5 Hz, CH_2), 7.59–8.20 (m, 8H, 7Ar-H & H-pyrane); ^{13}C NMR: 15.4, 62.4, 113.5, 118.3, 119.5, 117.8, 122.5, 123.0, 124.4, 129.1, 136.5, 149.4, 150.8, 155.2, 156.2, 167.1; *Anal.* Calc. $C_{18}H_{13}ClN_2O_4$: C, 60.60; H, 3.67; N, 7.85. Found: C, 60.84; H, 3.53; N, 7.54.

SYNTHESIS OF 2-IMINO-2*H*-CHROMENE-3-CARBOXAMIDES 9a,b

To a solution of **1** (0.01 mol), 2-cyano-*N*-(cyclohexyl)acetamide or 2-cyano-*N*-(furan-3-ylmethyl)acetamide (0.01 mol) in EtOH/DMF (15/5 mL) and catalytic amount of piperidine was heated under reflux for 4 h, then cooled and poured into ice water. The resulting solid was filtered off, washed with water, dried and crystalized from ethanol.

6-((4-Chlorophenyl)diazenyl)-*N*-cyclohexyl-2-imino-2*H*-chromene-3-carboxamide (9a): Pale yellow crystals. Yield 65 %; m.p.130–132 °C; IR: ν/cm^{-1} = 3338 (NH), 2922, 2852 (CH-aliph), 1672 (C=O); ^{1}H NMR: δ/ppm = 0.8–1.06 (m, 10H, CH_2 cyclohexyl), 2.65–2.71 (m, 1H, CH-N cyclohexyl), 7.57–8.13 (m, 8H, Ar-H & H-pyrane), 8.96, 10.17 (2br, 2H, 2NH; cancelled with D_2O); ^{13}C NMR: 23.2, 24.7, 33.1, 49.4, 113.1, 118.3, 120.5, 120.9, 124.1, 124.4, 128.7, 129.1, 137.5, 146.2, 151.2, 157.0, 161.3, 164.4; *Anal.* Calc. $C_{22}H_{21}ClN_4O_2$ (408.89): C, 64.62; H, 5.18; N, 13.70. Found: C, 64.85; H, 5.33; N, 13.55.

6-((4-Chlorophenyl)diazenyl)-*N*-(furan-2-ylmethyl)-2-imino-2*H*-chromene-3-carboxamide (9b): Yellow crystals. Yield 78 %; m.p. 230–232 °C; IR: ν/cm^{-1} = 3310, 3202 (2NH), 2928 (CH-aliph), 1670 (C=O); ^{1}H NMR: δ/ppm = 4.30 (s, 2H, CH_2), 6.39 (d, 1H, CH-furfuryl), 6.59 (d, 1H, CH-furfuryl), 7.14–8.03 (m, H, 7Ar-H & CH-O furfuryl & H-pyrane), 8.52,

10.17 (2br, 2H, 2NH cancelled with D_2O); ^{13}C NMR: 37.4, 111.2, 111.9, 112.4, 116.2, 120.8, 121.4, 124.5, 125.2, 128.2, 130.2, 136.5, 142.4, 145.8, 148.2, 151.8, 156.8, 160.2, 164.3; *Anal.* Calc. $C_{21}H_{15}ClN_4O_3$ (406.83): C, 62.00; H, 3.72; N, 13.77. Found: C, 62.33; H, 3.85; N, 13.54.

SYNTHESIS OF 6-((4-CHLOROPHENYL)DIAZENYL)-2-IMINO-*N*'-(4-METHOXYBENZYLIDENE)-2*H*-CHROMENE-3-CARBOHYDRAZIDE (10)

To a solution of compound **1** (0.01 mol) and 2-cyano-*N*'-(4-methoxybenzylidene)acetohydrazide (0.01 mol) in 20 mL of EtOH/DMF (3/1), a catalytic amount of piperidine was added. The reaction mixture was heated under reflux for 3 h. The solid product formed on hot, collected by filtration and recrystallized from ethanol/DMF as orange crystals. Yield 82 %; m.p. > 300 °C; IR: ν/cm^{-1} = 3301 (NH), 2931 (CH-aliph), 1660 (C=O); ^1H NMR: δ/ppm = 3.80 (s, 3H, OCH_3), 6.82-8.60 (m, 12H, Ar-H & pyrane), 8.75 (s, 1H, N=CH), 8.89, 9.10 (2br, 2H, 2NH; cancelled with D_2O); ^{13}C NMR: 55.8 (CH_3), 114.5, 114.8, 114.9, 115.6, 124.2, 124.3, 124.3, 124.6, 124.7, 128.9, 129.7, 129.7, 129.8, 129.9, 130.0, 130.1, 130.4, 136.1, 151.0 (C=N), 160.9 (C=O); *Anal.* Calc. $C_{24}H_{18}ClN_5O_3$ (459.89): C, 62.68; H, 3.95; N, 15.23; Found: C, 62.56; H, 3.57; N, 15.44.

SYNTHESIS OF 6-((4-CHLOROPHENYL)DIAZENYL)-2-IMINO-2*H*-CHROMENE-3-CARBOXAMIDE (11)

A solution of compound **1** (0.01 mol) and cyanoacetamide (0.01 mol) in 20 mL EtOH/DMF (3/1) containing 0.5 mL piperidine was heated under reflux for 4 h. The solid was filtered off and recrystallized from ethanol as pale yellow crystals. Yield 82 %; m.p. 270–272 °C; IR: ν/cm^{-1} = 3400 (NH_2+NH), 1651 (C=O); ^1H NMR: δ/ppm = 7.11–8.04 (m, 8H, Ar-H & H-pyrane), 8.62 (br, 1H, NH cancelled with D_2O), 9.04 (br, 2H, $CONH_2$ cancelled with D_2O); ^{13}C NMR: 113.2, 117.3, 120.8, 121.0, 123.0, 124.8, 128.1, 129.8, 137.5, 146.2, 151.2, 157.1, 165.5, 172.7; *Anal.* Calc. $C_{16}H_{11}ClN_4O_2$ (326.74): C, 58.82; H, 3.39; N, 17.15. Found: C, 58.95; H, 3.65; N, 17.43.

SYNTHESIS OF 2-AMINO-9-((4-CHLOROPHENYL)-DIAZENYL)-5-IMINO-4-OXO-4,5-DIHYDRO-3*H*-CHROMENO[3,4-C]PYRIDINE-1-CARBONITRILE (12)

A mixture of **11** (0.01 mol) and malononitrile (0.01 mol) in 20 mL EtOH/DMF (3/1) was heated under reflux for 4 h, then cooled and poured into ice water. The resulting solid was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compound **12** as red crystals. Yield 50 %; m.p. >300 °C; IR: ν/cm^{-1} = 3438, 3349, 3242, 3190 (NH_2 & 2NH), 2211 (C≡N), 1642 (C=O); ^1H NMR: δ/ppm = 4.31 (br, 2H, NH_2), 7.61–8.13 (m, 7H, Ar-H), 9.53 (br, 2H, 2NH cancelled with D_2O); ^{13}C NMR: 77.1, 112.5, 113.4, 116.8, 121.5, 121.8, 123.6, 124.4, 129.1, 136.5, 145.7, 151.2, 155, 156.5, 161.1, 162.3, 164.4; Anal. Calc: $C_{19}H_{11}ClN_6O_2$ (390.79): C, 58.40; H, 2.84; N, 21.51; Found: C, 58.65; H, 2.54; N, 21.75.

SYNTHESIS OF 1-ACETYL-9-((4-CHLOROPHENYL)-DIAZENYL)-5-IMINO-2-METHYL-3H-CHROMENO[3,4-C]PYRIDIN-4(5H)-ONE (13)

A mixture of **11** (0.01 mol) and acetyl acetone (0.01 mol) in 20 mL EtOH/DMF (3/1) was heated under reflux for 4 h, then cooled and poured into ice water. The resulting solid was filtered off, washed with water, dried and purified by recrystallization from ethanol to give **13** as pall brown crystals. Yield 66 %; m.p. 140–142 °C; IR: ν/cm^{-1} = 3338 (NH), 2929 (CH-aliph), 1742, 1664 (2C=O); ^1H NMR: δ/ppm = 2.27 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 7.20–8.13 (m, 7H, Ar-H), 8.7 (br, 2H, 2NH cancelled with D_2O); ^{13}C NMR: 21.7, 32.6, 112.7, 113.7, 118.7, 121.5, 121.9, 123.6, 124.4, 129.1, 136.5, 145.2, 149.1, 151.5, 155.4, 157.1, 162.5, 164.5, 175.7; *Anal.* Calc: $C_{21}H_{15}ClN_4O_3$ (406.83): C, 62.0; H, 3.72; N, 13.77; Found: C, 62.83; H, 3.43; N, 13.54

SYNTHESIS OF 2-AMINO-9-((4-CHLOROPHENYL)-DIAZENYL)-3-(FURAN-2-YLMETHYL)-5-IMINO-4-OXO-4,5-DIHYDRO-3H-CHROMENO[3,4-C]PYRIDINE-1-CARBONITRILE (14)

A solution of compound **9b** (0.01 mol) and malononitrile (0.01 mol) in EtOH/DMF (15/5 mL) containing catalytic amount of piperidine was heated under reflux for 4 h. The solid was filtered off and recrystallized from ethanol/DMF as yellow crystals. Yield 76 %; IR: ν/cm^{-1} = 3437, 3343, 3228 (NH_2 & NH), 2931(CH-aliph), 2207(C≡N), 1656 (C=O); ^1H NMR: δ/ppm = 4.15 (s, 2H, CH_2), 5.54 (br, 2H, NH_2), 7.00–8.25 (m, 10H, Ar-H), 9.53 (br, 1H, NH cancelled with D_2O); ^{13}C NMR: 43.1, 77.3, 110.4, 111.7, 112.4, 116.8, 118.8, 120.5, 121.2, 123.9, 124.1, 129.5, 136.4, 142.0, 142.9, 145.1, 148.2, 153.8, 155.4, 156.5, 156.9, 164.7; *Anal.* Calc. $C_{24}H_{15}ClN_6O_3$ (470.87): C, 61.22; H, 3.21; N, 17.85. Found: C, 61.35; H, 3.53; N, 17.43

SYNTHESIS OF 9-((4-CHLOROPHENYL)DIAZENYL)-3-(FURAN-2-YLMETHYL)-2-HYDROXY-5-IMINO-4-OXO-4,5-DIHYDRO-3H-CHROMENO[3,4-C]PYRIDINE-1-CARBONITRILE (15)

A solution of compound **9b** (0.01 mol) and ethyl cyanoacetate (0.01 mol) in 20 mL (EtOH/DMF; 15/5 mL) containing catalytic amount of piperidine was heated under reflux for 5 h. The solid was filtered off and recrystallized from ethanol as brown crystals. Yield 72 %; m.p. 250–252 °C; IR: ν/cm^{-1} = 3364 (broad, OH), 3228 (NH), 2945 (CH-aliph), 2201 (C≡N), 1646 (C=O); ^1H NMR: δ/ppm = 4.21 (s, 2H, CH_2), 7.51–8.60 (m, 10H, Ar-H), 9.2, 9.6 (2br, 2H, NH, OH cancelled with D_2O); ^{13}C NMR: 56.7, 110.8, 111.2, 112.8, 113.4, 115.8, 120.4, 121.8, 123.4, 124.4, 130.2, 137.5, 143.1, 146.2, 148.7, 151.8, 154.5, 156.0, 156.9, 165.4, 172; *Anal.* Calc. $C_{24}H_{14}ClN_5O_4$ (471.86): C, 61.09; H, 2.99; N, 14.84. Found: C, 61.31; H, 2.57; N, 14.77

REFERENCES

[1] S. A. Hessein, M. A. M. El-Sharief, S. Y. Abbas, H. Kh. Thabet, Y. A. Ammar, *Croat. Chem. Acta* **2016**, *89*, 91.

[2] B. S. Kuarm, Y. T. Reddy, J. V. Madhav, P. A. Crooks, B. Rajitha, *Bioorg. Med. Chem. Lett.* **2011**, *21,* 524.

[3] R. Pratap, V. J. Ram, *Tetrahedron* **2017**, *73*, 2529.

[4] A. Ustalar, M. Yilmaz, A. Osman, S. A. Kecel, *Turk. J. Chem.* **2017**, *41*, 80.

[5] A. Latifa, K. Mohamed, C. Geffroy-Rodier, G. Jérôme, Z. Sami, *Pharmacognosy Communications*. **2015**, *5*, 237.

[6] Y. K. Al-Majedy, A. H. Kadhum, A. A. Al-Amiery, A. Mohamad, *Sys, Rev Pharm*. **2017**, *8*, 62.

[7] D. Bhavsar, J. Trivedi, S. Parekh, M. Savant, S. Thakrar, A. Bavishi, A. Radadiya, H. Vala, J. Lunagariya, M. Parmar, L. Paresh, R. Loddo, A. Shah, *Bioorg. Med. Chem. Lett.* **2011**, *21,* 3443.

[8] N. R. Kamdar, D. D. Haveliwala, P. T. Mistry, S. K. Patel, *Med. Chem. Res*. **2012**, *20*, 854.

[9] O. M. Singh, N. S. Devi, D. S. Thokchom, G. J. Sharma, *Eur. J. Med. Chem*. **2010**, *45*, 2250.

[10] B. C. Raju, R. N. Rao, P. Suman, P. Yogeeswari, D. Sriram, T. B. Shaik, S. V. Kalivendi, *Bioorg. Med. Chem. Lett*. **2011**, *21,* 2855.

[11] T. Raj, R. K. Bhatia, A. Kapur, M. Sharma, A. K. Saxena, M. P. S. Ishar, *Eur. J. Med*. *Chem*. **2010**, *45,* 790.

[12] K. V. Sashidhara, J. N. Rosaiah, G. Bhatia, J. K. Saxena, *Eur. J. Med. Chem*. **2008**, *43*, 2592.

[13] Z. Nazarian, S. Emami, S. Heydari, S. K. Ardestani, M. Nakhjiri, F. Poorrajab, A. Shafiee, A. Foroumadi, *Eur. J. Med. Chem*. **2010**, *45,* 1424.

[14] P. Gebhardt, K. Dornberger, F. A. Gollmick, U. Grafe, A. Hartl, H. Gorls, B. Schlegela, C. Hertwecka, *Bioorg. Med. Chem. Lett*. **2007**, *17,* 2558.

[15] C. Park, J. Lim, Y. Lee, B. Lee, S. Kim, J. Lee, S. Kim, *Enzy. Microb. Tech*. **2007**, *40*, 1758.

[16] G. Chandravadivelu, P. Senniappan, *Int. J. Res. Pharm. Chem*. **2011**, *1*, 1082.

[17] S. Concilio, L. Sessa, A. M. Petrone, A. Porta, R. Diana, P. Iannelli, S. Piotto, *Molecules* **2017**, *22*, 875.

[18] A. Thoraya, Z.A Abdallah, *Arkivoc* **2008**, *17*, 295.

[19] Y. A. Ammar, M. A. M. Sh. El-Sharief, M. M. Ghorab, Y. A. Mohamed, A. Ragab, S. Y Abbas, *Cur. Org. Syn*. **2016**, *13,* 466.

[20] M. F. El Shehry, M. M. Ghorab, S. Y. Abbas, E. A. Fayed, S. A. Shedid, Y. A. Ammar, *Eur. J. Med. Chem*., **2018**, *143*, 1463.

[21] M. A. M. Sh. El-Sharief, S. Y. Abbas, M. A. Zahran, Y. A. Mohamed, A. Ragab, Y. A. Ammar, Z. *Naturforschung* **2016**, *71*, 875.

[22] Z. Moussa, M. A. M. S. El-Sharief, S. Y. Abbas, *Eur. J. Med. Chem*. **2016**, *122*, 419.

[23] B. S. Furniss, A. J. Hannaferd, and V. Rogers, Vogel's Textbook of Practical Organic Chemistry, Longman, Inc., New York, NY, USA, 4th edition, **1981**.

[24] A. A. Farag*, Der Pharma Chemica* **2015**, *7(11*), 130.

[25] S. A. Fouad, *Int. J. Adv. Res.* **2014**, 2, 442.

[26] R. E. Cooper, Analytical Microbiology, Ed. Kavangeh, F.W. Vol. 1&2, Academic press, New York and London, **1972**.

[27] Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards NCCLS); Method M27-A2, 2nd Edition; Wayne, Ed.; NCCLS: Pennsylvania, 22 (**2002**) 1–29.