Received: 26 September 2019 | Revised: 11 December 2019 | Accepted: 23 December 2019

DOI: 10.1002/jhet.3892

WILEY

ARTICLE

Cyclization of *N*-benzyl cyanoacetamide: Novel synthesis and biological activity of pyrrole, pyrimidine, and pyran derivatives

Eman O. Hamed[1] | **Mohamed G. Assy**[1] | **Adel M. Shalaby**[2] | **Rawda E. Sayed**[1]

[1]Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt

[2]Central Laboratory for Revolution Research the Abbasid Fish, Zagazig, Egypt

Correspondence
Eman O. Hamed, Department of Chemistry, Faculty of Science, Zagazig University, Zagazig 44519, Egypt.
Email: dremanomar54@gmail.com

Abstract

Heteroannulation of *N*-Benzyl cyanoacetamide **1** to a new series of heterocycles has been developed. Thus, reaction of **1** with different polarized π systems afforded pyrrolo **4**, pyridone **6**, pyridine **8**, and diazapene **10** derivatives, respectively. *N*-Benzyl cyanoacetamide that undergo condensation reaction with salicylaldehyde yielded pyran derivative **11**. Nitrosation of **11** furnished condensed pyran **13**. Compound **11** reacted with benzaldehyde, carbon disulfide (cyclizing agent), and ammonium thiocyanate to provide pyrane **17**, thiazine **18**, and thiourea **20** derivatives, respectively. Cinnamoyl isothiocyanate was reacted with compound **11** to produce non-isolable thiourea derivative **21**. The newly synthesized compounds have been characterized by infrared (IR), proton nuclear magnetic resonance (^{1}H NMR), and carbon nuclear magnetic resonance (^{13}C NMR) spectral data. The compounds were then evaluated for antibacterial and anticancer activities.

1 | INTRODUCTION

Heterocycles constitute the largest part of organic chemistry. They have immense applications in biological and industrial fields. For example, numerous natural (eg, quinine, atropine, codeine, morphine, and theophylline) and synthetic (eg, diazepam, chlorpromazine, antipyrine, and isoniazid) drugs are heterocycles. Moreover, heterocycles are the main constitute of dyes (eg, mauveine), herbicides (eg, paraquat), luminophores (eg, acridine orange), and pesticides (eg, diazinon). Synthesis of a wide range of heterocycle including compounds has been a research interest for long time, and numerous approaches are described in the literature. Introduction of new, atom economic, and efficient routes for this chemistry branch is currently a popular research interest. Among the new synthetic routes are the uses of cyano acetamide as a reactive precursor for synthesis of multifunctional heterocyclic compounds. *N*-Benzyl cyanoacetamide (Figure 1) contains multilateral reactive centers as β-functional nitrile (C≡N), amide moiety (NH—C═O), and α-CH acid, which are favorable reactive centers to react with numerous nucleophiles and electrophiles providing heterocyclic systems with different ring sizes[1–8] that exhibit pharmaceuticals,[9,10] antibacterial,[11] anticoagulant,[12] antifungacidal,[13] antihistaminic agent,[14] antileishhumanial agent,[15] antimicrobial agent,[16] herbicides,[17] and dyes[18,19] properties. Recently, our research interest directed to design and synthesis of biologically active heterocyclic systems from readily available reagents.[20,21] Herein, we hope to use a facile and direct approach to synthesize a novel series of heterocyclic systems (namely, pyridine, pyrrole, furan, thiazine, benzopyrane, and pyrazole) utilizing *N*-benzyl cyanoacetamide as simple synthetic synthon via addition reactions involving polarized π systems.

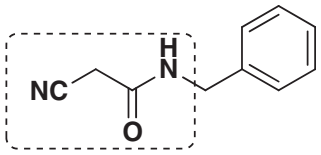


FIGURE 1 Favorable reactive centers of N-benzyl cyanoacetamide

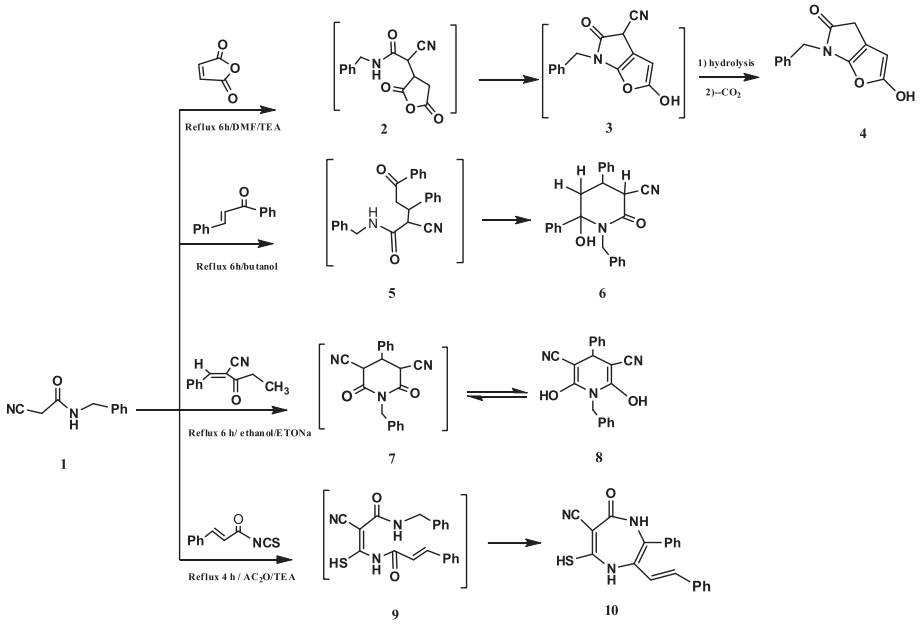
2 | RESULTS AND DISCUSSION

Compounds of types **4**, **6**, **8**, and **10** were prepared as a result of Michael-type addition of activated methylene of *N*-phenyl cyanoacetamide to different polarized π systems. Reaction of **1** with maleic anhydride afforded **4** via initial non-isolable Michael adduct **2** that undergo intramolecular cyclodehydration, cyanohydrolysis, and then evolution of CO_2 (Scheme 1).

The structure assigned for product **4** is confirmed by analytical and spectral data. The infrared (IR) spectrum of **4** provided C═O broad absorption peaks at 1643 cm^{-1} and also displayed downfield signal at 8.71 for OH group, aromatic multiplet in region δ = 7.26 to 7.35 ppm, and doublet of CH_2 in addition to methylene proton that was observed at 2.23 ppm. Carbon nuclear magnetic resonance (^{13}C NMR) indicated the presence of sp^2 C═O and SP C≡N at δ = 162.64 and 139.02 ppm, respectively. Conjugated addition of **1** to benzal acetophenone: the reaction started with formation of non-isolable acyclic Michael adduct **5** followed by intramolecular nucleophilic addition of imino gp to C═O, affording *N*-benzylpyridine derivatives **6** (Scheme 1); absorption frequencies of C≡N and C═O were observed at 2410 and 1621 cm^{-1}, respectively. Proton nuclear magnetic resonance (^1H NMR) spectrum of the compound **6** showed the sp^3 aliphatic protons system in addition to OH. Parent peak for heterocyclic **6** was observed at 140.13 ppm, in addition to carbon signals that was detected at 165 ppm for sp^2 of C═O and sp of CN at 140 ppm. Benzylidene ethyl cyanoacetate underwent heterocyclization, affording *N*-benzylpyridone **7** followed by enolization providing **8** (Scheme 1). Thus, ^1H NMR leads to 4 H pyridine at 6.62 and also to no C═O absorption, where CN peak was located at 2210 cm^{-1}. Spectral analysis was in agreement with the proved structure. Cyclization of **1** to diazepne derivative **10** was achieved via base-mediated addition of carbanion of **1** to acyl heteroallene electrophilic carbon followed by addition of nucleophilic benzylic carbanion to oxo-electrophilic carbon and subsequent dehydration (Scheme 1). The structure was achieved by the presence of C≡N, C═O, and SH functions in IR spectrum, in addition to the absence of benzyl CH_2 protons and in addition to carbon signals that were detected at 165 ppm for sp^2 C═O and sp of C≡N at 182 ppm in IR spectra at 1645 and 2257 cm^{-1}, respectively.

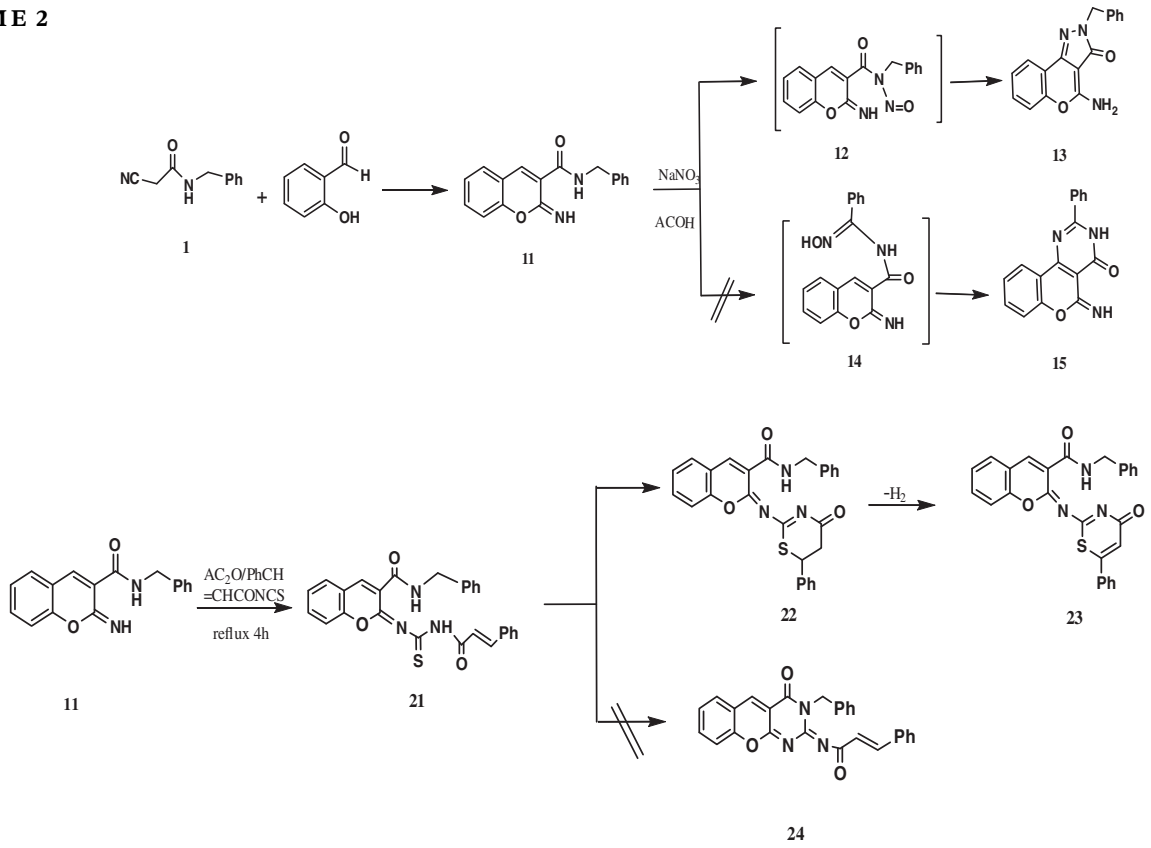
The target pyran derivative **11** was obtained from the condensation reaction of *N*-benzylcyanoacetamide **1** with salicylaldehyde followed by intramolecular addition of nucleophilic OH to CN function (Scheme 2). Nitrosation of **11** provided condensed pyran **13**, the reaction started by formation of non-isolable *N*-nitroso derivative followed by losing of H_2O providing **13**; non-oxazine derivative **15** was observed (Scheme 2).

Compound **11** displayed deshielded signal at 10.69 and 8.90 for NH protons. Also, C═O frequency appeared at 1673 cm^{-1} in addition to carbon signal of C═O that was observed at 163.73 ppm. Pyrazolo pyran **13** contained signal for NH_2 at 9.11 ppm, and carbonyl frequency was



SCHEME 1

SCHEME 2



SCHEME 3

located at 1655 cm^{-1}; carbonyl carbon signal was detected at 161.74 ppm.

The target **17** showed signals at 9.13 and 9.11 for NH_2 protons; carbonyl absorption was located at 1699 cm^{-1}, while ^{13}C leads to carbonyl carbon at 161. ppm. Using carbon disulfide as cyclizing agent provided thiazine derivative **19** presumably via the non-isolable thiazine **18** that suffers air oxidation (Scheme 3). IR spectrum of compound **19** provided NH, C═O and C═S absorption peaks at 3300, 1695 cm^{-1}, and 1284 cm^{-1}. Also, compound **19** shows δ = 9.13 ppm for NH proton, and double doublet at 4.54 and 4.50 ppm for benzylic CH_2. ^{13}C NMR showed sp^2 of C═O at 161 ppm. Compound **11** was added to ammonium thiocyanate to provide thiourea derivative **20** (Scheme 3). Compound **20** revealed downfield signal for NH and NH_2 in addition to C═S and C═O sp^2 carbon signals at 161.75 and 154.36 ppm, respectively.

Cinnamoyl isothiocyanate was reacted with exocyclic nucleophilic nitrogen pyrimidine derivative of **11** to produce non-isolable thiourea derivative **21** that cyclized to produce thiazine derivative **23**, and non-**24** was isolated (Scheme 4). IR of the target **23** led to the presence of NH and CO in addition to C═N. ^1H NMR showed signals at δ = 10.43 ppm for NH and δ = 7.36 to 7.20 ppm for ArH'S, and double doublet at δ = 4.53 to 4.38 ppm for benzylic protons. ^{13}C NMR indicate C═O sp^2 carbon at 163 and 153 ppm.

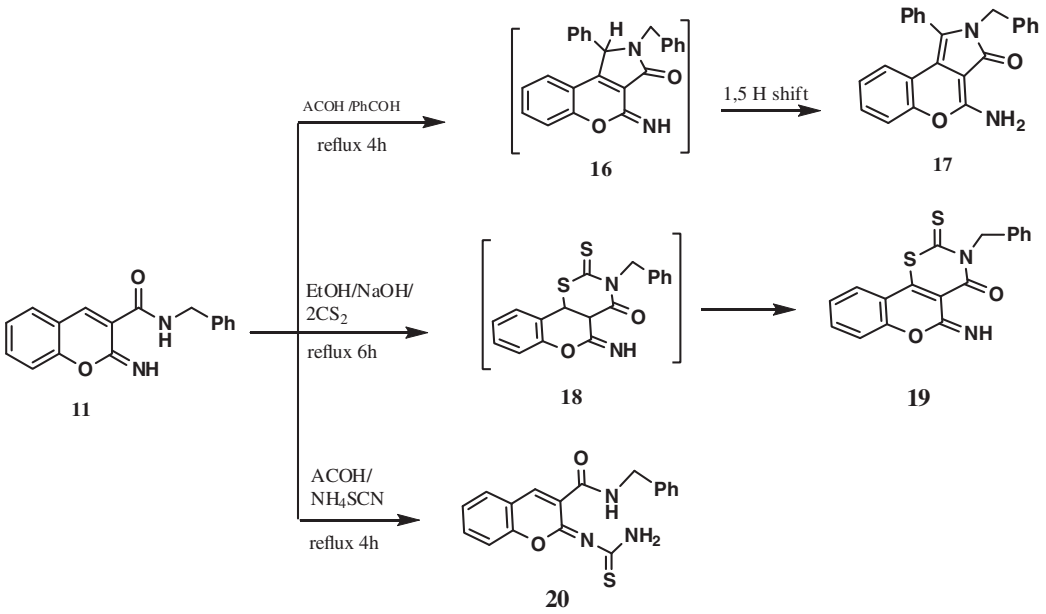
3 | EXPERIMENTAL

3.1 | Chemistry

Melting points were measured using an Electro thermal IA 9100 apparatus with open capillary tubes and are uncorrected. All experiments were carried out using drying solvents. Products were purified by crystallization. The IR spectra (KBr disk) were recorded on a Pye Unicom Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The $^1H/(^{13}C)$ NMR spectra were recorded at Varian Mercury VX-300 NMR 300 (75.4) MHz spectrometer using dimethyl sulfoxide (DMSO)-d_6 as a solvent. All chemical shifts were expressed on the δ (ppm) scale using tetramethylsilane (TMS) as an internal standard reference. The coupling constant (*J*) values are given in Hz. Mass spectrometer and analytical data were obtained from the Microanalysis Center at Cairo University, Giza, Egypt.

3.1.1 | 6-Benzyl-2-hydroxy-4, 6-dihydro-5H-furo[2,3-b] pyrrole-5-one (4)

A solution of maleic anhydride (0.01 mol), compound **1** (0.01 mol), and tetraethylammonium (TEA) (three



SCHEME 4

drops) in dimethylformamide (DMF) 20 mL was refluxed for 6 hours and then poured onto diluted glacial acetic acid. The formed solid was filtered off, washed with water, dried, and crystallized from glacial acetic acid to give brown powder of **4** in 79% yield. mp 128-132°C; IR (KBr) ν_{max} 3299 (OH) broad, 1643 (C═O), and 1542 (C═C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 8.0-7.3 (S, 1H, OH), 7.26-7.35 (m, 6H, ArH's), 3.33 (S, 1H, CH), 2.5 (S, 2H, CH_2Ph), 2.2 (d, 4H, $2CH_2$); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 162.64, 139.02, 128.84, 127.85, 116.67, 43.14, 39.98, 25.77; Anal. Calcd. for $C_{13}H_{11}NO_3$: C, 68.12; H, 4.84; N, 6.11; O, 20.92. Found: C, 68.00; H, 4.85; N, 6.00.

3.1.2 | 1-Benzyl-6-hydroxy-2-oxo-4,6-diphenylpiperidine-3-carbonitrile (6)

A mixture of benzal acetophenone (0.01 mol), compound **1** (0.01 mol), and three drops (TEA) was refluxed in butanol (20 mL) for 6 hours and then concentrated and acidified by hydrochloric acid (HCl). The formed precipitate was filtered off, washed in water, dried, and crystallized from butanol to give off white crystals of **6** in 80% yield. mp 134-138°C, IR (KBr) v_{max} 3320 (OH), 2241(CN), and 1621(CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 8.94 (S, 1H, OH), 8.91-6.85 (m, 15H, ArH's), 4.3-3.84 (m, 6H, sp^3H); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 197.41, 164.27, 140.13, 138.84, 136.73, 133.88, 129.23, 128.86, 128.62, 128.40, 128.30, 127.98, 127.82, 127.55, 127.41; Anal. Calcd. for $C_{25}H_{22}N_2O_2$: C, 78.50; H, 5.80; N, 7.33; O, 8.37. Found: C, 78.00; H, 5.70; N, 7.20.

3.1.3 | 1-Benzyl-2,6-dihydroxy-4-phenyl-1,4-dihydropyridine-3,5 dicarbonitrile (8)

A solution of (Z)-2-benzylidene-3-oxopentanenitrile (0.01 mol), compound **1** (0.01 mol), and ethoxide (0.01 mol) in absolute ethanol (20 mL) was refluxed for 6 hours, then poured into diluted glacial acetic acid hydrochloric acid, stirring till the formation of precipitate was filtered off, and then crystallized from ethanol to give pale yellow crystal of **8** in 97% yield; mp 158-162°C, IR (KBr) v_{max} 3429 (OH), 2210 (CN), and1621 (C═O) cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 11.87 (S, 1H, OH), 7.46-6.63 (m, 10H, ArH's), 4.7 (d, 1H, CH, J = 0.6 HZ), 4.60 (d, 1H, CH, J = 29.6 HZ), 4.10 (dd, 2H, CH_2, J = 5.6 HZ); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 182.36, 168.03, 166.15, 163.18, 149.10, 144.45, 134.3, 131.13, 129.47, 128.64, 120.58, 117.35; Anal. Calcd. for $C_{20}H_{15}N_3O_2$: C, 72.93; H, 4.59; N, 12.76; O, 9.72. Found: C, 73.00; H, 4.50; N, 12.00.

3.1.4 | 5-oxo-3-phenyl-2-[(E)-2-phenylethenyl]-7-sulfonyl-4,5-dihydro-1H-1,4-diazepine-6-carbonitrile (10)

A mixture of cinnamoyl isothiocyanate (0.01 mol) and compound **1** (0.01 mole) was refluxed for 4 hours in dry

acetone in presence of three drops (TEA) and then poured into diluted glacial acetic acid, and the formed precipitate was crystallized from glacial acetic acid to give yellow crystal of **10** in 95% yield. mp 208-212°C, IR (KBr) v_{max} 3303 (NH), 2257 (C═N), 1645 (C═O), 1548 (C═C), and 1333 cm^{-1}(SH), ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 11.19 (S, 1H, SH), 9.81 (S, 1H, NH), 9.47(S, 1H, NH), 7.94-6.82 (m, 12H, ArH's, ^{+2}H styrly), ^{13}C NMR (300 MHz, DMSO-*d*6) δ (ppm) 182.36, 166.15, 149.1, 144.39, 134.05, 131.13, 129.36, 128, 120.58, 117.35, 40.40, 39.57); Anal. Calcd. for $C_{20}H_{15}N_3OS$: C, 69.54; H, 4.38; N, 12.17; O, 4.63; S, 9.28. Found: C, 69.50; H, 4.27; N, 12.17; O, 4.63; S, 9.20.

3.1.5 | *N*-Benzyl-2-imino-2H-chromene-3-carboxamide (11)

A solution of compound **1** (0.01 mol), salicylaldehyde (0.01 mol), and sodium ethoxide (0.01 mole) in absolute ethanol (20 mL) was refluxed for 8 hours, and the reddish solution was poured into diluted HCl. The formed reddish-brown precipitate was filtered off, washed with water, dried, and crystallized from absolute ethanol to give reddish-brown powder of **11** in 96% yield. mp 148-152°C, IR (KBr) v_{max} 3307 (NH), 1673 (C═O), 1641 (C═N), and 1608 (C═C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.699 (S, 1H, C═NH), 8.992 (S, 1H, CONH), 8.50-6.90 (m, 10H, ArH's), 4.54-4.53 (dd, 1H, CH_2, J = 5.2 HZ), 4.39-4.38 (dd, 1H,CH_2, J = 5.2 HZ); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 140.18, 135, 133.44, 128.86, 128.35, 127.81, 127.14, 124.54, 120.70, 119.83, 116.61, 115.36, 112.64, 43.44, 40.19, 39.36; Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.36; H, 5.07; N, 10.068; O, 11.5. Found: C, 73.00; H, 5.00; N, 10.1.

3.1.6 | 4-Amino-2-benzylchromeno[4,3-c] pyrazol-3(2H)-one (13)

A solution of compound **11** (0.01 mol) and sodium nitrate (0.01 mole) was stirred in the presence of glacial acetic acid (20 mL) for 6 hours and then poured stepwise into ice water; the formed precipitate was filtered off, washed in water, dried, and crystallized from glacial acetic acid to give pale yellow powder of **13** in 85% yield. mp 163-167°C, IR (KBr) ν_{max} 3250 (NH_2), 1655 (C═O), and 1610 (C═C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.12 (S, 2H, NH_2), 7.99-7.26 (m, 9H, ArH's), 4.55-4.54 (d, 2H, CH_2, J = 5.6 HZ), ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 161.74, 160.81, 154.36, 148, 139.38, 134.56, 13.72, 128.86, 127.86, 125.60, 119.58, 118.95, 116.61, 43.22, 40.41, 39.58. Anal. Calcd. for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 3.80; N, 14.43; O, 11.00. Found: C, 70.00; H, 4.50, N, 14.50.

3.1.7 | 4-Amino-2-benzyl-1-phenylchromeno[3,4-c] pyrrol-3(2H)-one (17)

A mixture of compound **11** (0.01 mol) and benzaldehyde (0.01 mol) was refluxed in glacial acetic acid (20 mL) for 6 hours and then poured stepwise into ice water, and the precipitate obtained was filtered off, washed in water, dried, and crystallized from glacial acetic acid to give pale yellow powder of **17** in 75% yield, mp 158-162°C; IR (KBr) v_{max} 3308 (NH_2), 1699 (C═O), and 1654 (C═C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.13-9.11 (S, 1H, NH_2), 7.99-7.25 (m, 14H, ArH's), 4.55-4.54 (d, 2H, J = 6.0 HZ); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 161.73, 154.36, 154.36, 148, 139.37, 134.56, 130.72, 128.86, 127.87, 125.60, 119.57, 116.61, 43.22, 40.21, 39.58; Anal. Calcd. for $C_{24}H_{18}N_2O_2$: C, 78.66; H, 4.95; N, 7.65; O, 8.73. Found: C, 78.70; H, 5.00, N, 7.6

3.1.8 | 3-Benzyl-5-imino-2-thioxo-2,3-dihydro-4H,5H-chromeno[3,4-e][1,3] thiazin-4-one (19)

A solution of compound **11** (0.01 mol) and carbon disulfide (0.02 mol) was refluxed in presence TEA (0.01 mol) in ethanol (20 mL) for 6 hours and then poured into diluted glacial acetic acid; the formed precipitate was filtered off, washed in water, dried, and crystallized from ethanol to give pale brown powder of **19** in 96% yield; mp 298-302°C; IR (KBr) v_{max} 3300 (NH), 1695(C═O), and 1607 (C═N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.13 (S, 1H, NH), 7.99-7.24 (m, 9H, ArH's), 4.55-4.54 (d, 2H, CH_2, J = 6.0 HZ); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 161.74, 160.81, 154.36, 148, 139.37, 134.56, 130.72, 128.86, 127.86, 125.60, 119.58, 116.61, 43.22, 40.21, 39.58; Anal. Calcd. for $C_{18}H_{12}N_2O_2S_2$: C, 61.34; H, 3.43; N, 7.95; O, 9.08; S, 18.20. Found: C, 61.30; H, 3.50; N, 8.00; S, 18.00.

3.1.9 | *N*-Benzyl-2-(carbamothioylimino)-2H-chromene-3-carboxamide (20)

A solution of compound **11** (0.01 mol) and ammonium thiocyanate (0.01 mol) in glacial acetic acid (20 mL) was

refluxed for 8 hours, then poured stepwise into ice water, and the formed was stirred. It was filtered off, washed in water, dried solid, and was crystallized from glacial acetic acid to give dark brown of **20** in 78% yield; mp 168-172°C; IR (KBr) ν_{max} 3410 (NH), 3264 (NH_2), 1678 (C═O), 1607 (C═C), and 1354 (C═S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.99 (S, 1H, NH), 8.88-8.86 (S, 2H, NH_2, J = 5.2 HZ), 7.35-7.20 (m, 10H, ArH's), 5.11-5.06 (d, 2H, CH_2Ph, J = 20HZ); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 161.75, 154.36, 147.99, 139.37, 138.30, 130.72, 128.86, 128.04, 127.28, 12.60, 119.59, 117.56, 116.61, 43.22, 39.97, 23.79; Anal. Calcd. for $C_{18}H_{15}N_3O_2S$: C, 64.07; H, 4.48; N, 12.46; O, 9.48; S, 9.51. Found: C, 64.00; H, 4.40; N, 12.40; S, 9.40.

3.1.10 | *N*-Benzyl-2-imino-*N*-(4-oxo-6-phenyl-5,6-dihydro-4H-1,3-thiazin-2-yl)-2H-chromene-3-carboxamide (23)

The solution of compound **11** (0.01 mol) and cinnamoyl isothiocyanate (0.01 mol) in dioxane (20 mL) was refluxed for 8 hours and then poured into ice water; the obtainable solid was filtered off, washed with water, dried, and crystallized to form beige powder of 23 in 95% yield; mp 178-182°C; IR (KBr) ν_{max} 3367 (NH), 1726 (C═O) and 1337 (C═S) cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*6) δ (ppm) 10.43 (S, 1H, NH), 7.36-7.20 (m, 16H, ArH's), 5.75 (S, 1H, CH), 4.84 (S, 1H, NH), 4.53-4.38 (d, 2H, CH_2, J = 5.2 HZ); ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 163.25, 153.06, 151.30, 138.68, 138.37, 129.01, 128.62, 127.71, 127.25, 73.77, 45.36, 40.20, 39.37; Anal. Calcd. for $C_{27}H_{19}N_3O_3S$: C, 70.42; H, 4.01; N, 8.801; O, 10.051; S, 6.72. Found: C, 70.40; H, 4.00; N, 8.90; S, 6.72.

3.2 | Biological activity

3.2.1 | Antibacterial activity

The synthesized compound was tested for its in vitro antibacterial activity against the Gram-positive and the Gram-negative bacteria, *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Aspergillus flavus* (fungus), and *Candida albicans* (fungus), using the liquid serial dilutions method for determination of minimal inhibitory concentration (MIC). The latter is defined according to the Antibiogram Committee of a modified Kirby-Bauer disk diffusion method.[22] As being the lowest concentration that results in the inhibition of visible bacterial growth, the determination of the minimum inhibitory concentration (MIC) was realized by the preparation of a series of dilutions of 1/2 of the synthetic product to test on liquid medium (microdilution). The minimum bactericidal concentration (MBC) was regarded as being the lowest concentration, in product tested, having shown an absence of growth. According to our study, compounds **17** and **19** have an inhibitory activity on the *S. aureus*, *E. coli*, *S. faecalis*, *B. subtilis*, *N. gonorrhoeae*, *P. aeruginosa*, *A. flavus*, and *C. albicans* strains.

3.3 | Biological activity (sensitivity tests) by Kirby-Bauer method

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disk diffusion method.[22] Briefly, 100 μL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately107 cells per milliliter for bacteria or 105 cells per milliliter for fungi.[23] A 100 μL of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disk diffusion method.[24,25] Of the many media available, National Committee for Clinical Laboratory Standards (NCCLS) recommends Mueller-Hinton agar because it results in good batch-to-batch reproducibility. Disc diffusion method for filamentous fungi was tested by using approved standard method (M38-A) developed by the study of David, Vishnu, Espinel, and Daniel[26] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the study of Julia, George, Max, and Wendy.[27] Plates were inoculated with filamentous fungi such as *A. flavus* at 25°C for 48 hours. Gram-positive bacteria such as *S. aureus* and *B. subtilis* and Gram-negative bacteria such as *E. coli* and *P. aeuroginosa* were incubated at 35 to 37°C for 24 to 48 hours, and yeast such as *C. albicans* was incubated at 30°C for 24 to 48 hours, and then the diameters of the inhibition zones were measured in millimeters.[22] Standard disks of ampicillin (antibacterial agent) and amphotericin B (antifungal agent) served as positive controls for antimicrobial activity, but filter disks impregnated with 10 μL of solvent (distilled water, chloroform, and DMSO) were used as a negative control. The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further, the depth of the agar in the plate is a factor to be considered in the disk diffusion method. This method is well documented, and standard zones of inhibition have been determined for susceptible and resistant values. Blank

paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10 μ of tested concentration of the stock solutions. When a filter paper disk impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disk into the agar. This diffusion will place the chemical in the agar only around the disk. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disk. If an organism is placed on the agar, it will not grow in the area around the disk if it is susceptible to the chemical. This area of no growth around the disk is known as a "zone of inhibition" or "clear zone." For the disk diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards.[27] Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods.[28,29]

Crystal violet stain (1%). It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH_2O and filtered through a Whatman No.1 filter paper.

Cell line propagation. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer, and 50-μg/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cytotoxicity evaluation using viability assay. For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 μL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 hours of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates[30] using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 hours. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 hours, and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated, and the crystal violet solution (1%) was

Sample		Inhibition Zone Diameter (mm/mg Sample)							
		Bacterial Species						Fungi	
		G^+			G^-				
		Bacillus subtilis	*Staphylococcus aureus*	*Streptococcus faecalis*	*Escherichia coli*	*Neisseria gonorrhoeae*	*Pseudomonas aeruginosa*	*Aspergillus flavus* (Fungus)	*Candida albicans* (Fungus)
Standard	Ampicillin (Antibacterial Agent)	26	21	27	25	28	26	—	—
	Amphotericin B (Antifungal Agent)	—	—	—	—	—	—	17	21
Control: DMSO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17		13	14	14	13	12	13	0.0	15
19		13	9	0.0	9	0.0	9	0.0	0.0

3.3.1 | Anticancer activity

Evaluation of cytotoxic effects of certain chemical compound

Mammalian cell lines. HepG-2 cells (human hepatocellular cancer cell line) and HCT-116 cells (human colon cancer cell line) were obtained from VACSERA Tissue Culture Unit.

Chemicals used. DMSO, crystal violet, and trypan blue dye were purchased from Sigma.[30] Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-ethylene diamine tetraacetic acid (EDTA) were purchased from Lonza.

added to each well for at least 30 minutes. The stain was removed, and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after it was gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader[31] to determine the number of viable cells, and the percentage of viability was calculated as [(ODt/ODc)]×100%, where ODt is the mean optical density of wells treated with the tested sample, and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC50), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA. USA).

Antitumor activity tests against HepG-2 cells

The effect of the newly synthesized compounds **11**, **13**, **17**, and **19** was evaluated on the in vitro growth of HepG-2 cells (human hepatocellular cancer cell line) after a continuous exposure for 48 hours. The results were introduced in Table 1 and Figure 2.

TABLE 1 Effect of the obtained compounds on the growth of HepG-2 cell line

Compound	GI50, μmol L^{-1} HepG-2
Doxorubicin	0.36
11	71.4
13	60.6
17	27.2
19	18.8

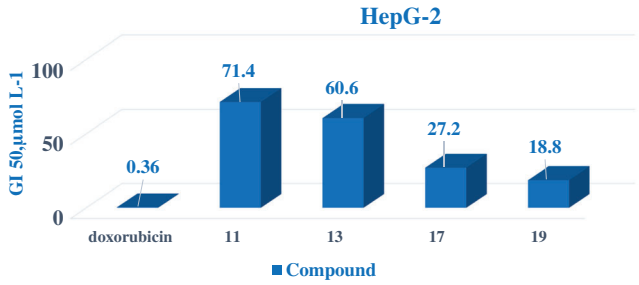


FIGURE 2 The inhibitory activities against HepG-2 cell lin

TABLE 3 Effect of the obtained compounds on the growth of HCT-116 cell line

Compound	GI50, μmol L^{-1} HCT-116
Doxorubicin	0.49
11	55.5
13	49.5
17	29.1
19	28.4

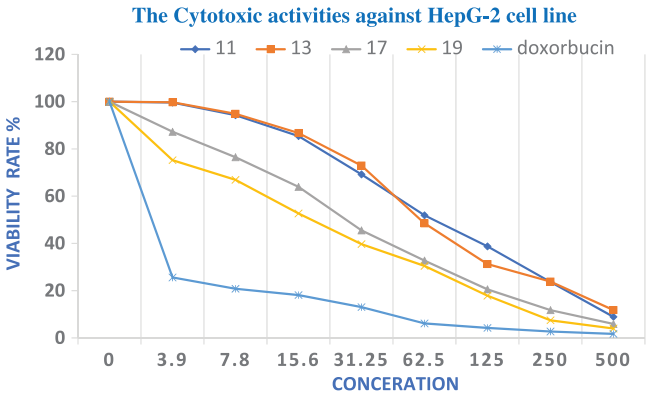


FIGURE 3 The cytotoxic activities against HepG-2 cell line

TABLE 2 Evaluation of cytotoxicity of the obtained compounds against HepG-2 cell line

Compound	Viability Rate (%) 0 (μg/mL)	3.9 (μg/mL)	7.8 (μg/mL)	15.6 (μg/mL)	31.25 (μg/mL)	62.5 (μg/mL)	125 (μg/mL)	250 (μg/mL)	500 (μg/mL)	IC50 (mg/mL)
Doxorubicin	100	25.59	20.81	18.13	13.05	6.13	4.22	2.70	1.72	0.36
11	100	99.64	94.27	85.41	69.23	51.88	38.75	23.67	8.92	71.4
13	100	99.72	94.86	86.73	72.94	48.51	31.27	23.82	11.79	60.6
17	100	87.26	76.49	63.91	45.51	32.73	20.56	11.78	5.92	27.2
19	100	75.23	66.91	52.67	39.72	30.46	17.89	7.43	3.97	18.8

TABLE 4 Evaluation of cytotoxicity of the obtained compounds against HCT-116 cell line

Compound	Viability Rate (%) 0 (μg/mL)	3.9 (μg/mL)	7.8 (μg/mL)	15.6 (μg/mL)	31.25 (μg/mL)	62.5 (μg/mL)	125 (μg/mL)	250 (μg/mL)	500 (μg/mL)	IC50 (mg/mL)
Doxorubicin	100	28.86	24.82	19.38	11.04	6.51	4.86	3.36	2.08	0.49
11	100	98.06	92.42	78.15	62.59	46.37	34.62	19.21	7.34	55.5
13	100	98.06	91.42	79.23	60.18	42.75	29.83	17.56	6.94	49.5
17	100	85.19	74.93	62.37	48.06	34.59	23.65	12.14	5.38	29.1
19	100	92.37	81.46	68.92	45.87	36.45	20.38	8.67	4.59	28.4

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI50) after a continuous exposure of 48 hours and to show means ± SEM of three-independent experiments performed in duplicate. All the examined compounds were able to inhibit the growth of the tested human tumor cell line in a dose-dependent manner. The results indicated through Table 1 and Figure 2 revealed that compound **19** showed high inhibitory effects against HepG-2 cell line, which is less than the corresponding reference doxorubicin. Compound **11** showed the lowest inhibitory effect, while the compounds **13** and **17** exhibited a moderate growth inhibitory effect. The cytotoxic and antitumor activities of prepared compounds **11**, **13**, **17**, and **19** were tested against HepG-2 cells line. The inhibitory activities were detected by using different concentrations of the tested compounds (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, and 500 μg), and the viability cells (%) were determined by colorimetric method. Also, the (IC50) was calculated as shown in Table 2 and Figure 3.

Results presented in Table 2 and Figure 3 revealed that compounds **19** and **17** have strong cytotoxic antitumor activity, and compound **13** and **11** have moderate cytotoxic antitumor activity against HepG-2 cell line.

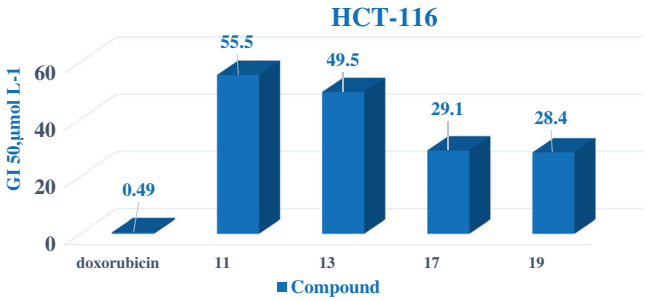


FIGURE 4 The inhibitory activities against HCT-116 cell line

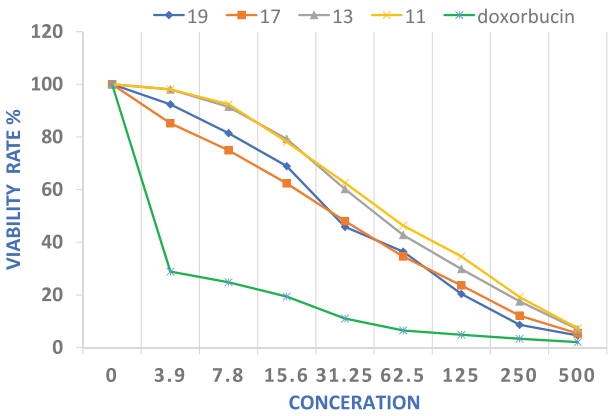


FIGURE 5 The cytotoxic activities against HCT-116 cell line

Antitumor activity tests against HCT-116 cells

The effect of the newly synthesized compounds **11**, **13**, **17**, and **19** was evaluated on the in vitro growth of HCT-116 cells (colon carcinoma cancer cell line) after a continuous exposure for 48 hours. The results were introduced in Table 3 and Figure 4.

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI50) after a continuous exposure of 48 hours and to show means ± SEM of three-independent experiments performed in duplicate. All the examined compounds were able to inhibit the growth of the tested human tumor cell line in a dose-dependent manner. The results indicated through Table 3 and Figure 4 revealed that compound **19** showed high inhibitory effects against HCT-116 cell line, which is less than the corresponding reference doxorubicin. Compound **11** showed the lowest inhibitory effect, while the compounds **13** and **17** exhibited a moderate growth inhibitory effect. The cytotoxic and antitumor activities of prepared compounds **11**, **13**, **17**, and **19** were tested against HepG-2 cells line. The inhibitory activities were detected by using different concentrations of the tested compounds (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, and 500 μg), and the viability cells (%) were determined by colorimetric method. Also, the (IC50) was calculated as shown in Table 4 and Figure 5.

Results presented in Table 4 and Figure 5 revealed that compounds **19** and **17** have strong cytotoxic antitumor activity, and compound **13** and **11** have moderate cytotoxic antitumor activity against HCT-116 cell line.

ORCID

Eman O. Hamed https://orcid.org/0000-0002-9424-0405

REFERENCES

[1] B. Samir, T. Abd El-Gaber, A. F. Ahmed, *J. Arkivoc*, **2006**, *2006* (ix), 113.
[2] M. Poulomi, P. Anita, P. Manabendra, K. B. Rajani, K. B. Ajaya, *J. Chem. Rev.* **2014**, *114*, 2942.
[3] Z. S. Hoda, M. M. Rafat, H. H. Maher, E. M. Amira, *Phosphorus Sulfur Silicon* **2007**, *182*, 237.
[4] M. E. Sanaa, A. M. Asmaa, M. B. Wahid, *Arch. Pharmacal Res.* **1993**, *16*, 318.
[5] P.-Y. Baskar, K. K. Dakas, *Org. Lett.* **1988**–1991, *2011*, 13.
[6] J. He, J. Zheng, J. Liu, X. She, X. Pan, *Org. Lett.* **2006**, *20*, 4637.
[7] Hohmann, K. Schneider,; Bruntner, C,; Brown, R,; Jones, A. L, *J. Antibiot.*, **2009**, *62*, 75–79.
[8] A. C. Jain, P. K. Gupta, Tetrahedron. **1975**, *31*, 1695.
[9] M. H. Elnagdi, A. W. Erian, *Arch. Pharmacal Res.* **1991**, *324*, 853.
[10] A. G. Gilman, L. S. Goodman, *Occup. Environ. Med.* **1985**, *12*, 1109.
[11] R. M. De Marinis, J. R. E. Hoover, G. L. Dunn, P. Actor, J. V. Uri, J. A. Weisbach, *J. Antibiot.* **1975**, *28*, 463.
[12] T. I. Verhoef, W. K. Redekop, A. K. Daly, A. De Boer, *Br. J. Clin. Pharmacol.* **2014**, *77*, 626.
[13] Z. Lv, C. Cheng, Y. Zhang, T. Wang, J. Feng, H. Zhong, M. Zhang, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7106.
[14] N. A. Farag, S. R. Mohamed, G. A. H. Soliman, *Bioorg. Med. Chem.* **2008**, *16*, 9009.
[15] X. Fan, D. Feng, Y. Qu, X. Zhang, J. Wang, G. Andrei, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 809.
[16] R. Sakhuja, S. S. Panda, L. Khanna, S. Khurana, S. C. Jain, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5465.
[17] Costales, M. J.; Kleschick, W. A.; Ehr, R. J.; Weimer, M. R. U.S. Patent, 1998 5,763,359,; Chem. Abstr, 1998, 129, 67792k.
[18] S. M. Fahmy, A. H. Badran, M. H. Elnagdi, *J. Chem. Technol. Biotechnol.* **1980**, *30*, 390.
[19] S. M. Fahmy, M. El-Hosami, S. Elgamal, M. H. Elnagdi, *J. Chem. Technol. Biotechnol.* **1982**, *32*, 104.
[20] H. A. El-Sayed, A. M. Abdel Hamid, G. A. Mohammed., T. S. Faraj, *Synth. Commun.* **2018**, *48*, 786.
[21] A. E. Hassan, A. M. Hesham, *J. Iran. Chem. Soc.* **2018**, *12* (16), 4.
[22] A. W. Bauer, W. M. Kirby, C. Sherris, M. Turck, *Am. J. Clin. Pathol.* **1966**, *45*, 493.
[23] M. A. Pfaller, L. Burmeister, M. A. Bartlett, M. G. Rinaldi, *J. Clin. Microbiol.* **1988**, *26*, 1437.
[24] C. Jen-Chyi, H. Po-Ren, W. Jiunn-Jong, H. Shen-Wu, *Antimicrob. Agents Chemother.* **1997**, *12*, 1301.
[25] P. W. Melvin, L. Brandi, B. P. Jean, B. Carey-Ann, G. J. Stephen, *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, Clinical and Laboratory Standard Institute: Wayne, PA, **2018**, p. MO7.
[26] W. W. David, C. Vishnu, I. A. Espinel, J. S. Daniel, *Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi*, Vol. 22, Clinical and Laboratory Standard Institute: Wayne, PA, **2002**, p. M38.
[27] A. K. Julia, E. H. George, S. Max, A. Wendy, M. Catherine, *J. Clin. Microbiol.* **2000**, *38*, 3341.
[28] D. R. Vianna, L. Hamerski, F. Figueiró, A. Bernardi, L. C. Visentin, *Eur. J. Med. Chem.* **2012**, *57*, 268.
[29] L. D. Liebowitz, H. R. Ashbee, E. G. V. Evans, Y. Chong, N. Mallatova, Global Antifungal Surveillance Group, *Diagn. Microbiol. Infect. Dis.* **2001**, *4*, 27.
[30] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[31] S. M. Gomha, S. M. Riyadh, E. A. Mahmoud, M. M. Elaasser, *Heterocycles* **2015**, *91*, 1227.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Hamed EO, Assy MG, Shalaby AM, Sayed RE. Cyclization of *N*-benzyl cyanoacetamide: Novel synthesis and biological activity of pyrrole, pyrimidine, and pyran derivatives. *J Heterocyclic Chem*. 2020;1–10. https://doi.org/10.1002/jhet.3892