Synthesis and Antimicrobial Activity of Novel Heterocycles Utilizing 3-(1,4-Dioxo-3,4-dihydrophthalazin-2(1*H*)-yl)-3-oxopropanenitrile as Precursors

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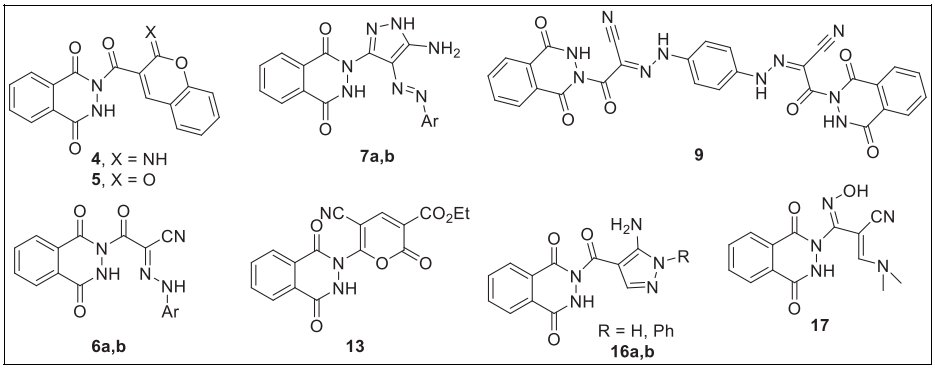
Received September 3, 2018
DOI 10.1002/jhet.3463
Published online 00 Month 2019 in Wiley Online Library (wileyonlinelibrary.com).



The reaction of 3-(1,4-dioxo-3,4-dihydrophthalazin-2(1*H*)-yl)-3-oxopropanenitrile **1** and salicyladehyde furnished coumarin derivatives **4** and **5**. Coupling reaction of **1** with aryl diazonium chlorides and benzene-1,4-bis (diazonium) chloride gave the corresponding hydrazones **6a**,**b** and bishydrazone **9**, respectively. Hydrazones **6** underwent intramolecular cyclization upon treating with hydrazine hydrate to give 3-aminopyrazoles **7**. Pyranyl phthalazine **13** was prepared from the reaction of **1** with ethyl 2-cyano-3-ethoxyacrylate **10**. Enaminonitrile **14** was reacted with hydrazine hydrate/phenylhydrazine and hydroxylamine to afford the corresponding pyrazoles **16** and oxime **17**. The antimicrobial evaluation revealed pyrazole derivatives **7a**,**b** and **16a**,**b** displayed a broad spectrum activity against most strains. 3-Aminopyrazole derivative **7b** showed potent antibacterial activity against all tested microorganisms.

INTRODUCTION

Phthalazines are important heterocycles that are known to possess diverse pharmacological properties like antimicrobial [1–3], antitumor [4], anticonvulsant [5], cardiotonic [6], and vasorelaxant activities [7]. Subramanian *et al*. [8] reported the synthesis of hydrazinyl phthalazine as anti-malaria parasite and *Plasmodium falciparum*. Moreover, 1,2,4-triazolo[3,4-*a*] phthalazines have been reported to have high-affinity ligands to the a2d-1 subunit of voltage-gated calcium channel [9]. 1,2,4-Triazolo[3,4-*a*]phthalazine-3-carboxamides were found to possess anti-inflammatory activities [10]. Chromeno pyrazolo[1,2-*b*]phthalazines have been reported to have antifungal activity [11]. *N*-substituted phthalazin-1-amine derivatives reported to have epidermal growth factor receptor inhibitors [12]. Furthermore, pyrazole containing heterocyclic are great interest because they show plenty pharmacological activities such as antidepressant [13], antioxidant [14], anti-inflammatory [15], anticancer [16], antimicrobial [17–19], antiviral [20,21], anticonvulsant [22], and insecticidal activities [23]. Recently, Sangani *et al*. [24] reported one-pot synthesis of pyrazolo[1,2-*b*]phthalazine-5,10-diones as antimicrobial, antituberculosis, and antioxidant agents.

In addition, coumarin derivatives occupied significant site either in natural products or in organic synthesis due to their exhibit plenty pharmacological activities [25–27]. Based on the earlier information and in continuing with our previous work [28,29], we have designed and synthesized coumarin, pyrane, pyrazole, and hydrazone derivatives bearing phthalazine moiety.

RESULTS AND DISCUSSION

Chemistry. The precursor 3-(1,4-dioxo-3,4-dihydrophthalazin-2(1*H*)-yl)-3-oxopropanenitrile (**1**) was prepared, in a very good yield, from the reaction of equimolar amount of phthalic anhydride with cyanoacetohydrazide in acetic acid at room temperature [30–32]. *N*-coumarinyl phthalazinyl ketones **4** and **5** were prepared from the reaction of **1** and salicylaldehyde either in EtOH containing piperidine at room temprature (RT) or in AcOH containing anhydrous sodium acetate at

reflux temperature, respectively. The IR spectrum of **4** and **5** showed the lack of nitrile absorption peak at 2258 cm^{-1} and the appearance of new peaks at 3350 and 1732 cm^{-1} attributed to C═NH (imino) and C═O, respectively. In addition ^1H NMR spectroscopy revealed increasing in the aromatic signals intensity because of coumarin moiety, and the disappearance of methylene protons at δ 3.35 ppm. Their ^{13}C NMR spectrum revealed, among others, signals resonate at δ 157.6 and 159.5 ppm owing to C═NH and C═O carbons, respectively. The mass spectrum of **4** and **5** gave the molecular ion peaks at *m/z* 333.9 and 334.1, respectively (Scheme 1).

The coupling reaction of **1** with 4-chlorobenzenediazonium chloride and 4-methylbenzenediazonium chloride was performed in EtOH/AcONa at 0–5°C to afford hydrazones **6a** and **6b**, respectively. ^1H NMR spectrum showed new D_2O exchangeable singlet signals at δ 10.85 and 10.91 ppm owing to HNN═ of **6a** and **6b**, respectively, and the disappearances CH_2 singlet signal. The mass spectra of **6a**,**b** gave molecular ion peaks at *m/z* 367.1 and 347.1, respectively. The geometry of **6a** was studied using the quantum mechanical calculations using DFT, B3LYP, and 6-31G** as basis set. The latter calculation showed *E*-configuration is more stable than *Z*-configuration (Fig. 1). Hydrazones **6a**,**b** underwent intramolecular cyclization upon treating with hydrazine hydrate in ethanol to give 3-aminopyrazoles **7a**,**b**. The IR spectrum of **7b** displayed the lack of nitrile and carbonyl absorption peaks and the appearance three peaks in the region 3320–3127 cm^{-1} because of NH_2 and NH. Also, ^1H NMR exhibited D_2O exchangeable broad singlet signal at δ 5.62 ppm owing to four exchangeable hydrogens (NH_2 and 2NH), as reported previously [33–35]. Alternatively, compounds **7a**,**b** were prepared from coupling reaction of 5-aminopyrazolyl phthalazine **8**, which was synthesized from the reaction of **1** with hydrazine hydrate in EtOH at reflux temperature, with diazotized aromatic amine. Similarly, compound **1** was treated with benzene-1,4-bis(diazonium) chloride to give the corresponding bishydrazone **9** (Scheme 2). The quantum mechanical calculations of bishydrazone **9**

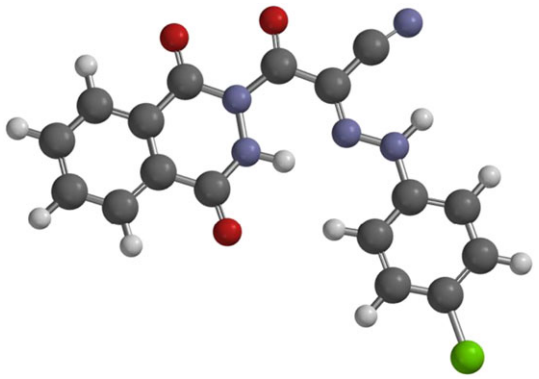


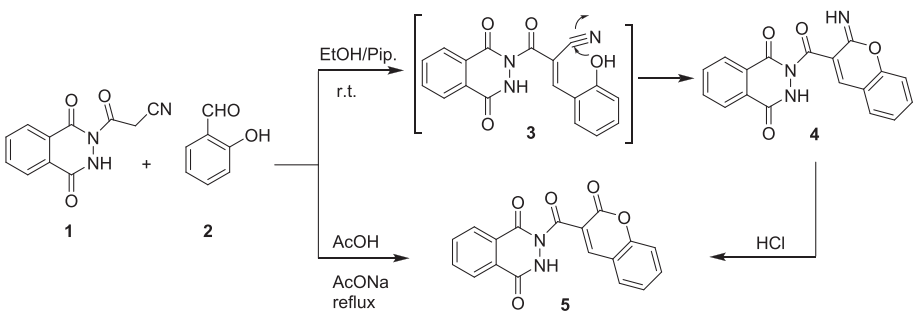
Figure 1. Energy of *E*-configuration of compound **6a**. [Color figure can be viewed at wileyonlinelibrary.com]

showed that *E*,*Z*-configuration is the most stable isomer (Fig. 2).

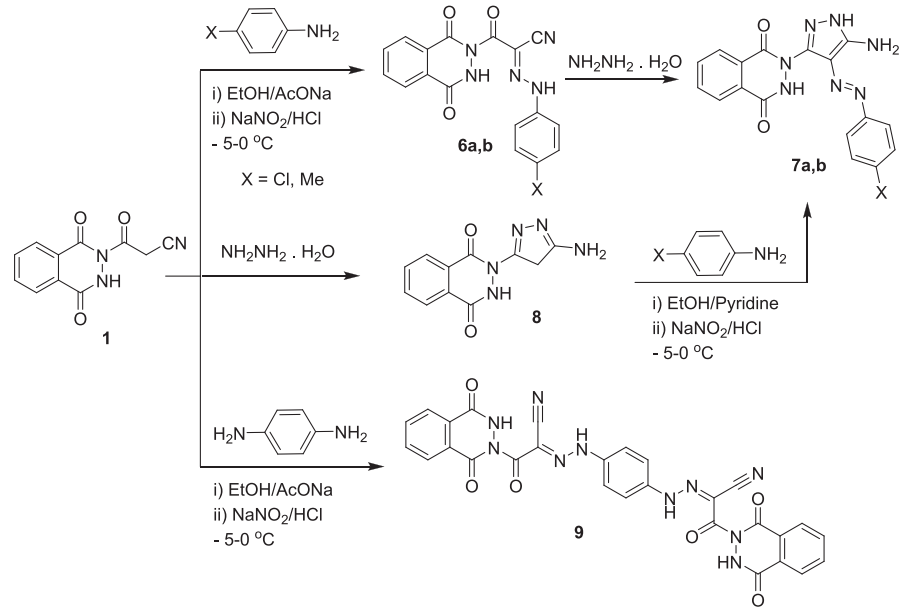
On the other hand, compound **1** was treated with ethyl 2-cyano-3-ethoxyacrylate **10** in ethanol containing EtONa at reflux temperature to furnish ethyl 2-iminopyran-3-carboxylate **13**, in a good yield (Scheme 3). The mechanism of formation compound **13** involves Michael addition reaction of **1** to **10** to afford intermediate **11** then intramolecular cyclization of enol form **12** to give product **13**. The structure of **13** was elucidated using spectral and analytical data. The IR spectrum exhibited peaks at 3286, 2224, 1713, 1700, and 1681 because of NH, CN, (C═O, ester), (C═O, pyranone), and (C═O, amide) functions, respectively. Also, ^1H NMR spectrum exhibited new triplet, quartet, and singlet signals at δ 1.30, 4.22, and 8.13 ppm because of ethoxy and pyranone protons, respectively, besides multiplet signals at 7.20–7.34 ppm owing to phthalazine protons. ^{13}C NMR spectrum showed peaks at 14.4, 60, 157.6, 162.8, 163.6, and 169.2 owing to CH_3, CH_2O, C═O of ester, C═O of lactone, and 2C═O of phthalazine, respectively. Its mass spectrum revealed *m/z* 339 $[M - Me]^+$ (20%), 296.77 $[M - (Me+CN)]^+$, (45%), 172 (100%).

Treatment enaminonitrile **14**, which was prepared according to the literature procedures [32], with hydrazine hydrate or phenylhydrazine in EtOH at reflux temperature to afford 5-aminopyrazoles **16a** and **16b**,

Scheme 1. Synthesis of compounds **4** and **5**.



Scheme 2. Synthesis of compounds **6**, **7**, **8**, and **9**.



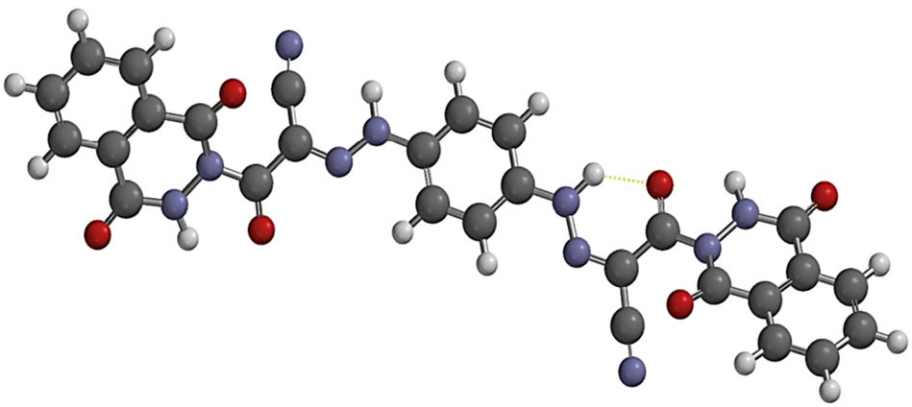


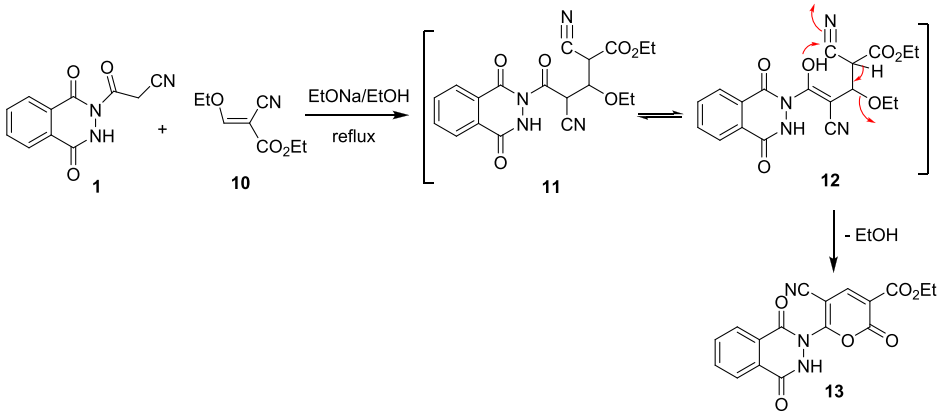
Figure 2. The *E*,*Z*-configuration of bishydrazone **9**. [Color figure can be viewed at wileyonlinelibrary.com]

respectively, in over 85% yields. The IR spectrum of **16b** showed the disappearance of the nitrile peak. The ^{1}H NMR spectrum revealed the lack of olefinic singlet and two methyl protons of enamine moiety, in addition, NH_2 and NH protons appeared at δ 5.54 and 11.49 ppm, respectively. Moreover, the mass spectrum of **16b** displayed [M^+] at *m/z* 374. When **14** was treated with hydroxylamine HCl in ethanol containing potassium carbonate at reflux temperature, gave the corresponding oxime **17** and the expected isoxazole derivative **18** not obtained (Scheme 4). It is compatible with the previously reported by Al-Zaydi *et al*. [36]. In addition, Shawali *et al*. [37] and Farag *et al*. [38] reported that the reaction of enaminone and hydroxylamine firstly yield the corresponding oxime followed by intramolecular cyclocondensation to afford the corresponding isoxazole.

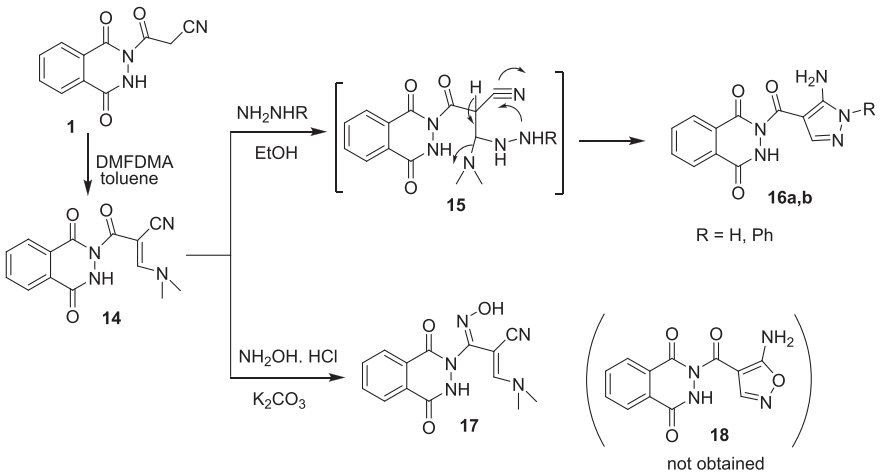
The structure of oxime **17** was elucidated using quantum mechanical calculations and spectral data. The quantum calculation of **17** revealed it has four stereoisomers (*E*,*E*; *E*,*Z*; *Z*,*E*; and *Z*,*Z*). *Z*,*Z*-isomer is the most stable one because of intramolecular hydrogen bonding (Fig. 3). The IR spectrum of **17** showed two bands at 2229 and 2191 cm^{-1} owing to nitrile. In addition, the ^{1}H NMR spectrum exhibited two D_2O exchangeable signals at δ 9.95 and 11.57 ppm assigned to OH and NH protons, respectively. Moreover, the mass spectrum of **17** gave molecular ion peaks at *m/z* 301.3 $[M + 2]^+$ (2%) and 283.9 $[M - 15]^+$ (100%).

Antimicrobial activity. *In vitro* antimicrobial screening of the newly synthesized compounds was carried out using cultures of two Gram-positive bacteria and two Gram-negative bacteria, as well as, two fungal strains using the

Scheme 3. Synthesis of compound **13**. [Color figure can be viewed at wileyonlinelibrary.com]



Scheme 4. Synthesis of compounds **16a**, **16b**, and **17**.



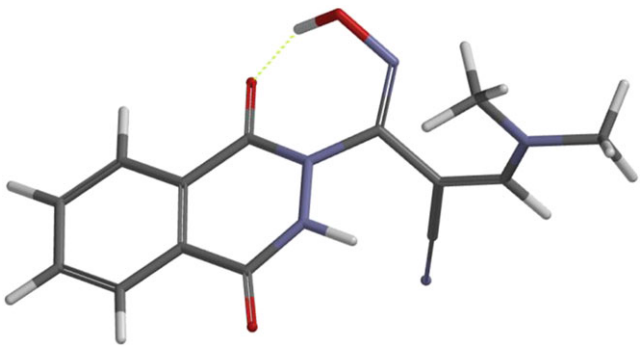


Figure 3. Energy of *Z*,*Z*-isomers of compound **17**. [Color figure can be viewed at wileyonlinelibrary.com]

agar well diffusion method [39]. Ampicillin and Colitrimazole are antibacterial and antifungal agents, respectively, were used as controls to evaluate the potency of the compounds being studied under the same conditions as shown in Table 1.

The results revealed that some compounds displayed high antibacterial activity toward Gram-positive bacteria than Gram-negative bacteria. However, compounds **5**, **14**, and **17** were inactive with most strains. Pyrazole derivatives **7a**, **7b**, **16a**, and **16b** displayed a broad

Table 1

In vitro antimicrobial activity of the synthesized compounds[a,b].

	Gram-negative bacteria				Gram-positive bacteria				Fungi			
	Escherichi coli		*Pseudomonas aeuroginosa*		*Staphylococcus aureus*		*Bacillus subtilis*		*Candida albicans*		*Aspergillus flavus*	
Entry	I. Z.	Activity index (%)	I.Z.	Activity index (%)	I.Z.	Activity index (%)	I.Z.	Activity index (%)	I.Z.	Activity index (%)	I.Z.	Activity index (%)
1	6	24.0	9	39.1	7	29.2	10	43.5	4	14.8	5	20.0
4	2	8.0	6	26.1	7	29.2	11	47.8	10	37.0	13	52.0
5	NA[c]	—	NA	—	NA	—	2	8.7	NA	—	NA	—
6b	6	24.0	10	43.5	12	50.0	14	60.9	15	55.5	17	68.0
7a	12	48.0	15	65.2	20	83.3	18	78.3	17	63.0	19	76.0
7b	13	52.0	17	73.9	21	87.5	19	82.6	20	74.1	22	88.0
8	4	16.0	7	30.4	9	37.5	11	47.8	7	25.9	10	40.0
9	9	36.0	12	52.2	10	41.7	12	52.2	13	48.1	15	60.0
14	NA	—	3	13.0	6	25.0	7	30.4	6	22.2	8	32.0
16a	7	28.0	11	47.8	16	66.7	17	73.9	24	88.9	23	92.0
16b	10	40.0	12	52.2	14	58.3	15	65.2	16	59.2	18	72.0
17	NA	—	NA	—	2	8.3	3	13.0	NA	—	2	8.0
Ampicillin	25	100	23	100	24	100	23	100	NA	—	NA	—
Colitrimazole	NA	—	NA	—	NA	—	NA	—	27	100	25	100

[a]Antimicrobial activity expressed as inhibition diameter zones (I.Z.) in millimeters (mm).
[b]The experiment was carried out in triplicate and the average zone of inhibition was calculated.
[c]NA, no activity.

Table 2

Minimum inhibitory concentration in (μg/mL) for compounds **7a**, **7b**, **16a**, and **16b**.

	Minimum inhibitory concentration					
Entry	*Escherichi coli*	*Pseudomonas aeuroginosa*	*Staphylococcus aureus*	*Bacillus subtilis*	*Candida albicans*	*Aspergillus flavus*
7a	187.5	125	250	93.7	15.6	11.7
7b	93.7	62.5	125	62.5	7.8	3.9
16a	250	187.5	375	187.5	23.4	15.6
16b	750	500	500	375	46.9	31.2
Ampicillin	125	125	187.5	125	—	—
Colitrimazole	—	—	—	—	5.8	3.9

spectrum activity against most strains. Pyrazoles **7a** and **7b** showed higher activity toward *Pseudomonas aeuroginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* than **16a** and **16b**. Compound **7b** exhibited greater activity than **7a**. The fungal strain *Candida albicans and Aspergillus flavus* showed a potent sensitivity toward compounds **7a**, **7b**, **16a**, and **16b** with activity index (63, 74.1, 88.9, 59.2), and (76, 88, 92, 72), respectively. Also, it was observed that compound **16a** displayed higher activity than **7b**.

Minimum inhibitory concentration. Compounds that showed greater antibacterial and antifungal activities are further assayed for minimum inhibitory concentration (MIC), and the values are listed in Table 2. MIC value of compound **7b** against *Escherichi coli*, *P. aeuroginosa*, *B. subtilis*, and *S. aureus* is lower than standard antibacterial drug Ampicillin while displaying MIC value against *A. flavus* equal to standard antifungal drug Colitrimazole. Moreover, compound **7a** displayed low MIC value on *B. subtilis* than standard drug Ampicillin and showed MIC value on *P. aeuroginosa* equal to Ampicillin.

Structure–activity relationship. The structure–activity relationship revealed that compounds with pyrazole unit **7a**,**b** and **16a**,**b** showed higher activity than other compounds. Furthermore, the presence of aryldiazene substituents increases the activity as in **7a** and **7b**. The presence of electron donating substituents on the aromatic ring increased its antimicrobial activity as **7b**.

CONCLUSION

In this study, we synthesized a novel coumarins **4**, and **5**; hydrazone **6**; bishyrazone **9**; pyrazole **7**, **8**, and **16**;

pyran-2-one **13** and oxime **17** derivatives bearing phthalazine scaffold, in good yields, utilizing phthalazinyl-3-oxopropanenitrile **1**. The new compounds are characterized by spectral and CHN analysis. The quantum mechanical calculations of hydarzone **6**, bishydrazone **9**, and oxime **17** exhibited the most stable configuration is *E*-configuration; *E*,*Z*-configuration; and *Z*,*Z*-configuration, respectively. The antimicrobial evaluation showed compounds **7a**, **7b**, **16a**, and **16b** have prominent antibacterial activity against against most strains.

EXPERIMENTAL

Chemistry. ***General.*** All melting points were determined on digital Gallen-Kamp MFB-595 instrument using open capillary tubes and are uncorrected. IR spectra were recorded on Schimadzu FTIR 440 spectrometer using KBr pellets. Mass spectra were performed at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer provided with a data system. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker model (500 MHz) Ultra Shield NMR spectrometer in $CDCl_3$ or DMSO-d_6 using tetramethylsilane as an internal standard; chemical shifts are reported as δ ppm units. Solvents were dried by standard techniques. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds was carried out and was run using thin layer chromatography (TLC) aluminum sheets silica gel 60 F_{254} (Merck). Compounds **1** [31,32] and **14** [32] were synthesized based on the reported procedures.

2-(2-Imino-2H-chromene-3-carbonyl)-2,3-dihydrophthalazine-1,4-dione (4). A mixture of *N*-cyanoacetophthalazine **1** (0.458 g, 2 mmol) and salicylaldehyde **2** (0.244 g, 2 mmol) in EtOH (25 mL) containing a catalytic amount of piperidine (0.5 mL) was stirred at room temperature for 4 h (TLC). The solid formed was filtered off, washed with EtOH, dried, and crystallized from EtOH. Colorless powder, yield 85%, mp 243–244°C (EtOH); IR (ν_{max}, cm^{-1}): 3350w, 3161w (NH), 1699s, 1681s, 1668s (C═O), 1605s (C═N), 1541-1496w (C═C); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 4.35 (s, D_2O exchangeable, 1H, ═NH), 6.77 (t, 1H, *J* 7 Hz, coumarin-H_6), 7.15 (t, 1H, *J* 7.5 Hz, coumarin-H_8), 7.24 (m, 1H, coumarin-H_7), 7.40–7.43 (t, 1H, *J* 7.5 Hz, coumarin-H_5), 7.54 (dd, 3H, *J* 7, 4.5 Hz, phthalazine-H), 7.75 (dd, 1H, *J* 8, 1.5 Hz, phthalazine-H), 8.15 (s, 1H, coumarin-H_4), 9.90 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 115.7, 118.8, 124.2, 126.7, 128.1, 129.6, 129.9, 131.3, 133.3, 137.6, 153.2, 155.8 (C═O), 157.6 (C═NH), 165.3 (C═O), 168 (C═O); MS *m/z* (%): 333.9 [M$^+$] (10%), 291 (30), 266.1 (100); *Anal*. Calcd for $C_{18}H_{11}N_3O_4$ (333.30): C, 64.87; H, 3.33; N, 12.61, Found: C, 64.55; H, 3.11; N, 12.49%.

2-(2-Oxo-2H-chromene-3-carbonyl)-2,3-dihydrophthalazine-1,4-dione (5). *Method A.* To a solution of **1** (0.458 g, 2 mmol) in acetic acid (30 mL) containing 0.5 g of fused sodium acetate, salicylaldehyde **2** (0.244 g, 2 mmol) was added. The mixture was heated under reflux for 3 h (TLC). After cooling, the formed product was filtered off, washed with EtOH, dried, and crystallized from mixture EtOH. Yellow crystals, yield 86%, mp 300°C (EtOH); IR (ν_{max}, cm^{-1}): 3275w (NH), 1732s, 1716s, 1690s (C═O), 1608–1510s (C═C); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 7.46 (t, 1H, *J* 7.5 Hz, coumarin-H_6), 7.55 (d, 1H, *J* 9 Hz, coumarin-H_8), 7.80 (t, 1H, *J* 7.5, coumarin-H_7), 7.99 (m, 5H, phthalazine-H and coumarin-H_5), 8.99 (s, 1H, coumarin-H_4), 10.78 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 116.3, 117, 118.2, 123.9, 125.3, 129.5, 130.7, 135, 135.4, 147.5, 149.9, 154.3 (C═O), 159.5 (C═O), 161.1 (C═O), 164.7 (C═O); MS *m/z* (%): 334.1 [M$^+$] (100%), 335 (26%), 173.1 (45); *Anal*. Calcd for $C_{18}H_{10}N_2O_5$ (334.29): C, 64.67; H, 3.02; N, 8.38, Found: C, 64.35; H, 2.92; N, 8.10%.

Method B. The iminochromene derivative **6** (2 mmol) was dissolved in EtOH (30 mL) and treated with HCl (5 mL). The reaction mixture was heated under reflux for 3 h, left to cool. The obtained product was filtered off, washed with cold water, dried, and crystallized from mixture EtOH.

General procedure for synthesis of hydrazones 6a, 6b, and 9. To a stirred solution of *N*-cyanoacetophthalazine **1** (0.458 g, 2 mmol) in ethanol (30 mL), sodium acetate trihydrate (0.26 g, 2 mmol) was added. After stirring for 15 min, the mixture was chilled at 0°C and treated with cold solution of 4-chloroaniline (0.254 g, 2 mmol), 4-methylaniline (0.214 g, 2 mmol), or 1,4-phenylene diamine (0.108 g, 1 mmol) in 6 M hydrochloric acid (1.5 mL) with sodium nitrite solution (0.14 g, 2 mmol) in water (3 mL). The addition of the diazonium salt was stirred for an additional 2 h at 0–5°C and then left for 8 h in a refrigerator (4°C). The resulting solid was collected by filtration, washed thoroughly with water, and dried. The crude product was crystallized from ethanol to give hydrazones **6a**, **6b**, and **9**, respectively.

(E)-N-(4-chlorophenyl)-2-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-2-oxoacetohydrazonoyl cyanide (6a). Yellow crystals, yield 83%, mp 259–260°C (EtOH); IR (ν_{max}, cm^{-1}): 3235w (NH), 2222w (CN), 1737s, 1681s (C═O), 1640–1600w (C═N), 1552–1485 m (C═C); ^{1}H NMR (500 MHz, CDMSO-d_6) δ_H (ppm): 7.31 (d, 2H, *J* 7.5 Hz, Ar—H), 7.43 (d, 2H, *J* 7 Hz, Ar—H), 7.73 (dd, 2H, *J* 8, 2.5 Hz, phthalazine-H), 7.93 (dd, 2H, *J* 8 Hz, phthalazine-H), 10.85 (s, D_2O exchangeable, 1H, NH),

12.11 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 103.9, 110.8, 117.5, 123.9, 128, 128.6, 129.6, 132.3, 141, 158.6 (C═O), 161.7 (C═O), 167.3 (C═O); MS *m/z* (%): 367.1 [M^+] (97), 368 [M + H]$^+$ (32); *Anal*. Calcd for $C_{17}H_{10}ClN_5O_3$ (367.75): C, 55.52; H, 2.74; N, 19.04, Found: C, 55.17; H, 2.48; N, 18.83%.

(E)-2-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-2-oxo-N-(p-tolyl) acetohydrazonoyl cyanide (6b). Yellow crystals, yield 82%, mp 251–252°C (EtOH); IR (ν_{max}, cm^{-1}): 3232w (NH), 2220w (CN), 1735s, 1678s (C═O), 1640–1600w (C═N), 1552–1485 m (C═C); ^1H NMR (500 MHz, CDMSO-d_6) δ_H (ppm): 2.29 (s, 3H, CH_3), 7.21 (d, 2H, *J* 8.5 Hz, Ar—H), 7.64 (d, 2H, *J* 8.5, Ar—H), 7.96 (dd, 2H, *J* 8.5, 3 Hz, phthalazine-H), 8 (dd, 2H, *J* 5.5, 3 Hz, phthalazine-H), 10.91 (s, 1H, D_2O exchangeable, 1H, NH), 12.25 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 20.5 (CH_3), 103.9, 110.9, 116.4, 124, 129.3, 129.7, 134.3, 135.4, 139.6, 160.7(C═O), 163.3 (C═O), 165.3 (C═O); MS *m/z* (%): 347.1 [M^+] (97%), 348 [M + H]$^+$ (40), 347.1 [M]$^+$ (97), 346.5 (100); *Anal*. Calcd for $C_{18}H_{13}N_5O_3$ (347.33): C, 62.24; H, 3.77; N, 20.16, Found: C, 62.06; H, 3.51; N, 19.96%.

General procedure for synthesis 4-arylazopyrazoles 7.

Method A. To a solution of the appropriate hydrazone **6a** or **6b** (2 mmol) in EtOH (20 mL) was added hydrazine hydrate (2 mmol). The reaction mixture was refluxed for 6 h and then left to cool. The solid product was collected, washed with EtOH, dried, and finally recrystallized from EtOH to afford the corresponding 4-arylazopyrazole derivatives **7a** and **7b**, respectively.

Method B. To a stirred cold solution of the pyrazole derivative **8** (0.486 g, 2 mmol) in pyridine (10 mL) was added the appropriate arenediazonium chloride (2 mmol) portion wise over a period of 30 min at 0–5°C. After complete addition, the reaction mixture was stirred for further 3 h at 0–5°C. The solid product was collected, washed with water, dried, and finally recrystallized from EtOH afforded the corresponding 4-arylazopyrazole derivatives **7a** and **7b**.

2-(5-Amino-4-((4-chlorophenyl)diazenyl)-1H-pyrazol-3-yl)-2,3-dihydrophthalazine-1,4-dione (7a). Brown crystals, yield 80%, 265–267°C (EtOH); IR (ν_{max}, cm^{-1}): 3325–3165 m (NH_2 and NH), 1664 (C═O), 1618–1560s (C═N), 1500–1458 (C═C); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 5.61 (s, broad D_2O exchangeable, 4H, NH_2 and 2NH, appear together) [33–35], 7.13 (d, 2H, *J* 7.5 Hz, Ar—H), 7.45 (d, 2H, *J* 7 Hz, Ar—H), 7.70 (dd, 2H, *J* 7, 3 Hz, phthalazine-H), 8.10 (dd, 2H, *J* 7.5, 3 Hz, phthalazine-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 117.9, 124.6, 125.8, 128.5, 128.7, 131.9, 133.5, 138.2, 151.5, 154.5, 158.5 (C═O), 159 (C═O); MS *m/z* (%): 381 [M]$^+$(12%), 217 (100%); *Anal*. Calcd for $C_{17}H_{12}ClN_7O_2$ (381.78): C, 53.48; H, 3.17; N, 25.68, Found: C, 53.29; H, 3.03; N, 25.49%.

2-(5-Amino-4-(p-tolyldiazenyl)-1H-pyrazol-3-yl)-2,3-dihydrophthalazine-1,4-dione (7b). Brown crystals, yield 79%, mp 255–257°C (EtOH); IR (ν_{max}, cm^{-1}): 3320–3172 m (NH_2 and NH), 1660–1643 (C═O), 1595–1556s (C═N), 1495 (C═C); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 2.26 (s, 3H, CH_3), 5.62 (s, broad, D_2O exchangeable, 4H, NH_2, and 2NH, appear together) [33–35], 7.17 (d, 2H, *J* 8 Hz, Ar—H), 7.39 (d, 2H, *J* 7.5 Hz, Ar—H), 7.82 (dd, 2H, *J* 9.5, 3.5 Hz, phthalazine-H), 8.06 (dd, 2H, *J* 9.5, 3.5 Hz, phthalazine-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 20.5 (CH_3), 115.1, 125.3, 128, 129.3, 129.8, 132.1, 133.5, 139.6, 149.9, 155.7, 158.8 (C═O), 159 (C═O); MS *m/z* (%): 346 [M-15]$^+$ (2%), 217 (100%); *Anal*. Calcd for $C_{18}H_{15}N_7O_2$ (361.37): C, 59.83; H, 4.18; N, 27.13, Found: C, 59.71; H, 4.05; N, 26.98%.

2-(5-Amino-4H-pyrazol-3-yl)-2,3-dihydrophthalazine-1,4-dione (8). To a solution of *N*-cyanoacetophthalazine **1** (1.145 g, 5 mmol) in EtOH (30 mL), hydrazine hydrate (80%, 0.5 ml) was added. The mixture was heated at reflux 5 h and then allowed to cool. The precipitated product was filtered, washed (EtOH), dried, and recrystallized from EtOH. Colorless powder, (89%), mp 300°C (EtOH); IR (ν_{max}, cm^{-1}): 3320–3167 (NH_2 and NH) 1662 (C═O), 1600–1556s (C═N), 1492–1458 (C═C); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 1.78 (s, 2H, CH_2), 6.30 (s, broad D_2O exchangeable, 3H, NH_2, and NH, appear together) [33–35], 7.79 (m, 2H, phthalazine-H), 8.05 (m, 2H, phthalazine-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 23.8 (CH_2), 125.4, 125.6, 128.6, 131.9, 132.18, 156.6, 175 (C═O); MS *m/z* (%): 216.9 [M-26]$^+$ (100%), 161.6 (27%); *Anal*. Calcd for $C_{11}H_9N_5O_2$ (243.23): C, 54.32; H, 3.73; N, 28.79, Found: C, 54.06; H, 3.47; N, 28.68%.

(E)-N-(4-(2-((Z)-1-cyano-2-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-2-oxoethylidene)hydrazineyl) phenyl)-2-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-2-oxoacetohydrazonoyl cyanide (9). Colorless powder, yield 81%, mp 195–196°C (EtOH); IR (ν_{max}, cm^{-1}): 3275, 3254, (NH), 2216 (CN), 1737s, 1681s (C═O), 1604 (C═N), 1546–1469s (C═C); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 7.53 (s, 4H, Ar—H), 7.79 (dd, 4H, *J* 8.5, 3.5 Hz, phthalazine-H), 8.11 (dd, 4H, *J* 8.5, 3.5 Hz, phthalazine-H), 10.91 (s, D_2O exchangeable, 2H, NH), 11.20 (s, D_2O exchangeable, 2H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 104.5, 110.9, 118.6, 123.8, 129.6, 130.5, 133.3, 159.4 (C═O), 162.5 (C═O), 166.3 (C═O); MS *m/z* (%): 588 [M]$^+$ (20%), 229 (100%); *Anal*. Calcd for $C_{28}H_{16}N_{10}O_6$ (588.50): C, 57.15; H, 2.74; N, 23.80, Found: C, 56.96; H, 2.52; N, 23.56%.

Synthesis of ethyl 5-cyano-6-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-2-oxo-2H-pyran-3-carboxylate (13). Sodium (0.023 g, 1 mmol) was dissolved in

absolute EtOH (30 mL), to this solution, *N*-cyanoacetophthalazine **1** (0.229 g, 1 mmol) was added and stirred for 10 min at room temperature. Ethyl 2-cyano-3-ethoxyacrylate **10** (0.169 g, 1 mmol) was added to this mixture and refluxed for 6 h. The solid formed was filtered off, washed with EtOH, dried, and crystallized from EtOH. Yellow, yield 75%, mp 300°C (EtOH); IR (ν_{max}, cm^{-1}): 3286w (NH), 2224 m (CN), 1713s (C═O, ester), 1700s (C═O, pyrone), 1681s (C═O, amide), 1454–1402 m (C═C); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 1.30 (t, 3H, CH_3), 4.22 (q, 2H, CH_2), 7.27 (m, 4H, phthalazine-H), 8.13 (s, 1H, pyran-H), 11.33 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm):14.4 (CH_3), 60 (CH_2), 80.2, 117, 125.7, 129.1, 140.5, 144.3, 152.5, 154.2, 157.6 (CO), 162.8 (CO), 163.7 (CO), 169.2 (CO); MS *m/z* (%): 339 $[M - Me]^+$ (20%), 296.8 $[M - (Me + CN)]^+$, (45%), 172 (100%); *Anal*. Calcd for $C_{17}H_{11}N_3O_6$ (353.29): C, 57.80; H, 3.14; N, 11.89, Found: C, 57.53; H, 3.05; N, 11.69%.

General procedure for synthesis of 5-aminopyrazoles 16. To a solution of enaminonitrile **14** (0.568 g, 2 mmol) in EtOH (20 mL), hydrazine hydrate (80%, 0.2 mL) or phenylhydrazine (0.2 mL, 2 mmol) was added. The mixture was heated at reflux for 5 h (TLC) and then allowed to cool. The precipitated product was filtered, washed (EtOH), dried, and recrystallized from EtOH to give **16a** and **16b**, respectively.

2-(5-Amino-1H-pyrazole-4-carbonyl)-2,3-dihydrophthalazine-1,4-dione (16a). Yellow powder, yield 85%, mp 300°C (EtOH); IR (ν_{max}, cm^{-1}): 3273–3165 m (NH_2 and NH), 1700–1650 (C═O), 1600–1500 m (C═N); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 5.64 (s, D_2O exchangeable, 3H, NH_2 and NH, appear together), 7.96 (dd, 2H, *J* 8.5, 3.5 Hz, phthalazine-H), 8.21 (dd, 2H, *J* 8.5, 3 Hz, phthalazine-H), 8.23 (s, 1H, pyrazole-H), 11.62 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm):115.6, 123.2, 128.2, 130.5, 132.4, 136.4, 158.6 (CO), 166.7 (CO), 169.4 (CO); MS *m/z* (%): 347 $[M]^+$(3%), 161.6 ($C_8H_5N_2O_2$, phthalazine, 100%); *Anal*. Calcd for $C_{12}H_9N_5O_3$ (271.24): C, 53.14; H, 3.34; N, 25.82, Found: C, 52.96; H, 3.17; N, 25.67%.

2-(5-Amino-1-phenyl-1H-pyrazole-4-carbonyl)-2,3-dihydrophthalazine-1,4-dione (16b). Yellow powder, yield 87%, mp 300°C (EtOH); IR (ν_{max}, cm^{-1}): 3263–3165 m (NH_2 and NH), 1680–1650 (C═O), 1600–1556 m (C═N), 1492s (C═C); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 5.54 (s, D_2O exchangeable, 2H, NH_2), 7.87 (m, 5H, Ph-H), 8.05 (m, 4H, phthalazine-H), 8.19 (s, 1H, pyrazole-H), 11.49 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 117.9, 125.2, 127.2, 128.2, 132.4, 132.6, 142.6, 154.7, 154.9, 156.4, 159.4 (CO), 165.7 (CO); MS *m/z* (%): 347 $[M]^+$ (3%), 161.6 ($C_8H_5N_2O_2$, phthalazine, 100%); *Anal*. Calcd for $C_{18}H_{13}N_5O_3$ (347.33): C, 62.24; H, 3.77; N, 20.16, Found: C, 62.02; H, 3.52; N, 19.96%.

3-(Dimethylamino)-2-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)(hydroxyimino)methyl) acrylonitrile (17). To a solution of enaminonitrile **14** (0.568 g, 2 mmol) in absolute ethanol (30 mL) was added hydroxylamine hydrochloride (0.139 g, 2 mmol) in the presence of anhydrous potassium carbonate (0.276 g, 2 mmol). The reaction mixture was then refluxed for 5 h. The solid formed was collected by filtration and crystallized from ethanol. Colorless crystals, yield 88%, mp 259–260°C (EtOH); IR (ν_{max}, cm^{-1}): 3367w (OH), 3275w (NH), 2229w, 2191w (CN), 1728s, 1662s (C═O), 1616s (C═N), 1504 (C═C); ^1H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 3.21 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 7.79 (m, 5H, pthalazine-H and C═CH), 9.95 (s, D_2O exchangeable, 1H, OH), 11.57 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 38.3, 47.2, 67.1, 118.5, 123.9, 129.5, 135.3, 157.6, 164.5, 165.5, 167.3; MS *m/z* (%): 301.3 $[M + 2]^+$ (3%), 283.9 $[M-15]^+$ (100%), 161.6 (87); *Anal*. Calcd for $C_{14}H_{13}N_5O_3$ (299.29): C, 56.18; H, 4.38; N, 23.40, Found: C, 55.97; H, 4.19; N, 23.17%.

Antimicrobial activity. The antibacterial activity of the synthesized compounds was tested against a panel of two Gram-positive bacteria (*S. aureus*, *B. subtilis*), two Gram-negative bacteria (*E. coli*, *P. aeuroginosa*), and two fungi (*C. albicans*, *Aspergillus flavus*) was determined using agar well diffusion method as described in the literature [39] and the result was cited in Tables 1 and 2.

Acknowledgment. The authors are thankful to Department of Pharmacology, Faculty of Pharmacy, Mansoura University, Egypt for performing the antimicrobial evaluation.

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