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Original article

Synthesis and antibacterial activity of some new heterocycles incorporating phthalazine

A.M. Khalil, M.A. Berghot, M.A. Gouda*

Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt

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ABSTRACT

3-(1,4-Dioxo-3,4,4e,5,10,10a-hexahydro-1*H*-5,10-benzeno-benzo[g]phthalazin-2-yl)-3-oxo-propiononitrile (**1**) was utilized as key intermediate for the synthesis of some new iminocoumarin **2**, chromenone **3**, aminothiazole **4**, triazepine **5a, b** and **6**, hydrazono-propiononitrile **7**, pyridopyrazotriazine **8**, monobromo **9**, dibromo **10** quinoxaline **11**, ketene *N,S*-acetal **13**, ketene *S,S*-diacetal **17** and **18a, b** and methyl dithioate **20** derivatives, respectively. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. Representative compounds of the synthesized product were tested and evaluated as antibacterial agent.

1. Introduction

In the past decades, the synthesis of heterocyclic compounds has been a subject of great interest due to their wide applicability. Heterocyclic compounds occur very widely in natural and are essential to life. Among a large variety of heterocyclic compounds, heterocycles containing phthalazine moiety are of interest because the show some pharmacological and biological activities (Fig. 1) [1–3]. Phthalazine derivatives were reported to possess anticonvulsant [4], cardiotonic [5], and vasorelaxant [6], activities.

In view of the above mentioned findings and as continuation of our effort [7–10], to identify new candidates that may be of value in designing new, potent, selective and less toxic antimicrobial agent, we report herein the synthesis of some new heterocycles incorporating phthalazine moiety starting from dibenzobarallene.

2. Results and discussion

2.1. Chemistry

The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1–3. Dibenzobarallene was prepared according to the previous reported method [7]. Cyanoacetic acid hydrazide was reacted with dibenzobarallene in dimethylformamide to afford 3-(1,4-dioxo-3,4,4e,5,10,10a-hexahydro-1*H*-5,10-benzeno-benzo[g]phthalazin-2-yl)-3-oxo-propiononitrile (**1**). The structure **1** was established on the basis of elemental and spectral data. The IR spectrum showed bands at 3200, 2250, 1727 and 1658 cm^{-1} due to NH, CN and 3CO groups, respectively. Its ^{1}H NMR spectrum displayed singlet signals at δ 3.7 and 9.8 ppm due to two protons at CH2 and one proton of NH, respectively. Moreover, the mass spectrum of **1** exhibited the molecular ion peak at *m*/*z* 357, which is in agreement with its molecular formula $C_{21}H_{15}N_3O_3$.

Thus, cyclocondensation of compound **1** with salicaldehyde in boiling dimethylformamide containing a catalytic amount of triethylamine afforded the iminocoumarin derivative **2**, which hydrolysis in a mixture of Conc. hydrochloric acid and ethanol to achieve 2-chromone derivative **3**.

The reaction of **1** with elemental sulfur and phenyl isothiocyanate in warming dimethylformamide containing a catalytic amount of triethylamine give the thiazole derivative **4**. Structure of the latter product based on analytical and spectral data. The IR spectrum showed bands at 3414, 3287, 3172 cm^{-1} (NH) and 1731, 1698 cm^{-1} (CO). Its ^{1}H NMR spectrum revealed the appearance of signals at δ 6.7 (s, 2H, NH_2), 7.1–7.5 (m, 13H, Ar–H) and 9.9 (br, 1H, NH). Moreover, the ^{13}C NMR spectrum characterized by the

* Corresponding author. Tel.: +2050 6432235; fax: +2050 2246781.
E-mail address: dr_mostafa_chem@yahoo.com (M.A. Gouda).

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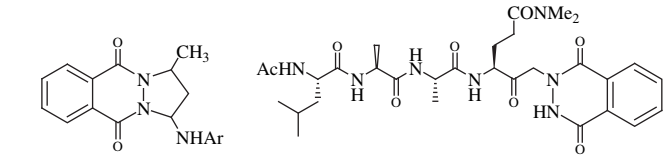


Fig. 1. (a) Antihypoxic and antipyretic agent. (b) HAV 3C inhibitor.

presence of three new signals at δ 186.1, 152.8 and 79.3 due to C_2, C_4 and C_5 of thiazole ring, respectively.

Cyclization of **4** by refluxing with either triethylorthoformate or acetic anhydride was studied in the aim of formation of 1,2,4-triazepine derivatives with potential biological activities [11,12]. Thus, it reacted with either triethylorthoformate or acetic anhydride to give 1,2,4-triazepine derivatives **5a, b**.

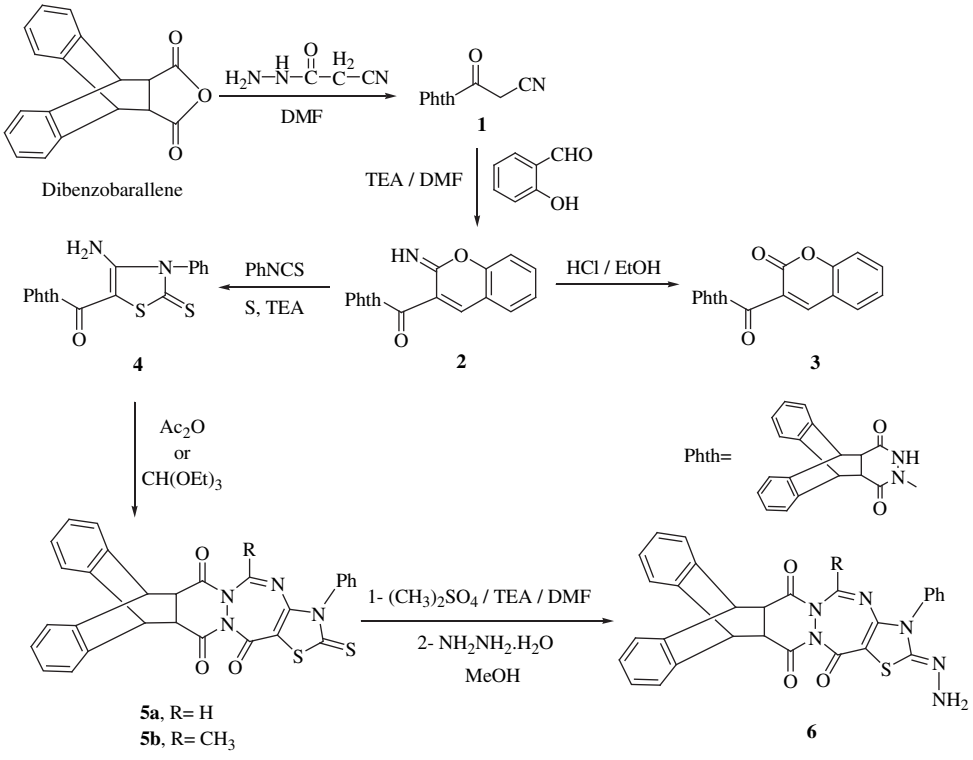
The analytical and spectral data are in agreement with the proposed structure. Thus, IR spectrum of **5a, b** showed absorption bands due to carbonyl groups at 1728–1718 cm^{-1} beside, the absence of (NH) and (NH_2) bands. The ^1H NMR of **5a** showed beside the expected signals, a characteristic signals at 7.1–7.8 (m, 14H, Ar–H, CH = N). Furthermore, the FAB mass spectrum of **5b** showed its quasi-molecular ion peak appeared at *m/z* 549 ($M^+ + 1$). When compound **5b** was heated with dimethylsulphate in dimethylformamide followed by stirring with hydrazine hydrate, the hydrazone derivative **6** was formed. Elemental analysis, IR, ^1H NMR, ^{13}C NMR and FAB mass are in agreement with the proposed structure.

We have found that diazotized aromatic amine is an excellent building block for the synthesis of the target compound. Thus, coupling of compound **1** with 4,6-dimethyl-1*H*-pyrazolo[3,4-b]pyridine-3-diazonium chloride [13], in pyridine at 0–5 °C afforded the corresponding hydrazono compound **7**. When compound **7** is refluxed in acetic acid, it can be cyclized to the corresponding triazine derivative **8**. The formation of **8** may be interpreted through the nucleophilic attack of ring nitrogen on cyano group. The IR spectrum of **8** showed three absorption bands at 3405, 3321 and 3271 due to the three (NH) groups besides three carbonyl absorption bands at 1731, 1692 and 1663.

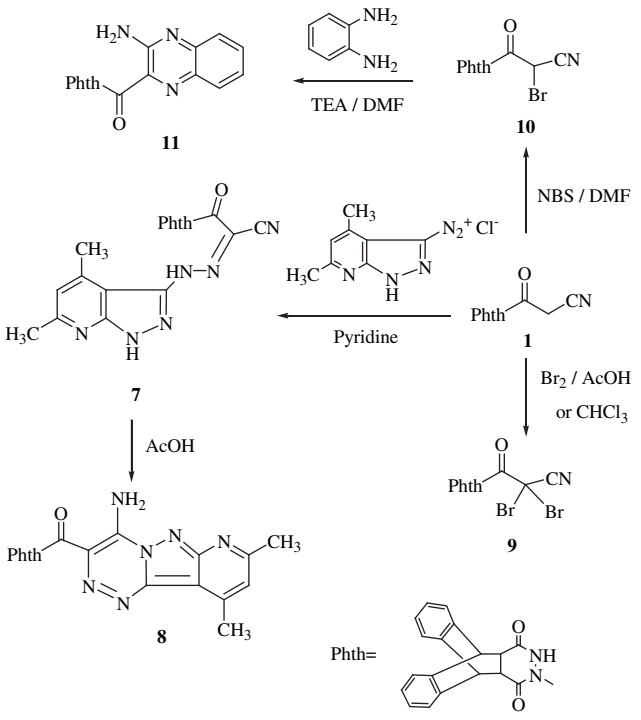
Also, its mass spectrum exhibited the molecular ion peak at 530 which is in agreement with the molecular formula $C_{29}H_{22}N_8O_3$, the molecular ion was undergo retro Diels–Alder reaction to revealing (M^+-anthracene) and anthracene peaks at *m/z* 352 and 178.

The remarkable importance of α-bromoacetonitrile derivatives [14,15], for the construction of variety of heterocyclic compounds promoted us to investigate the synthesis of new bromopropionitrile derivative. Thus, a suspension of **1** in acetic acid or chloroform reacted readily with bromine to yield dibromopropionitrile derivative **9**, while monobromopropionitrile derivative **10** was not obtained, but when the reaction take place with *N*-bromosuccinamide in dimethylformamide at room temperature compound **9** was obtained. The structures **9** and **10** were assigned on the basis of their spectral data. The mass spectrum of compound **10** exhibited the molecular ion peak at *m/z* 437 ($M^+ + 2$), which is in agreement with the molecular formula $C_{21}H_{14}BrN_3O_2$. Moreover, the ^1H NMR spectrum of compound **9** revealed beside the absence of CH_2 signal, signals of the remaining protons at the expected chemical shift. Condensation of compound **10** with *o*-phenylenediamine in dimethylformamide and in the presence of triethylamine afforded the aminoquinoxaline derivative **11**. The chemical structure of **11** was elucidated on the basis of elemental analysis and spectral data. The IR spectrum indicated the presence of absorption bands at 3433, 3385 (NH), 1730, 1685 (CO) and 1614 cm^{-1} (C=N).

Treatment of compound **1** with phenyl isothiocyanate in dimethylformamide and in the presence of potassium hydroxide, at room temperature give the non-isolated sodium salt which methylated by treatment with methyl iodide to afford the novel ketene *N,S*-acetal **13**. The structure of **13** was established on the basis of its elemental analysis and spectral data. Its IR spectrum showed absorption bands at 3286, 3152, 2205 and 1728, 1684 cm^{-1} due to two (NH) groups, nitrile and carbonyl functions. The ^1H NMR



Scheme 1.

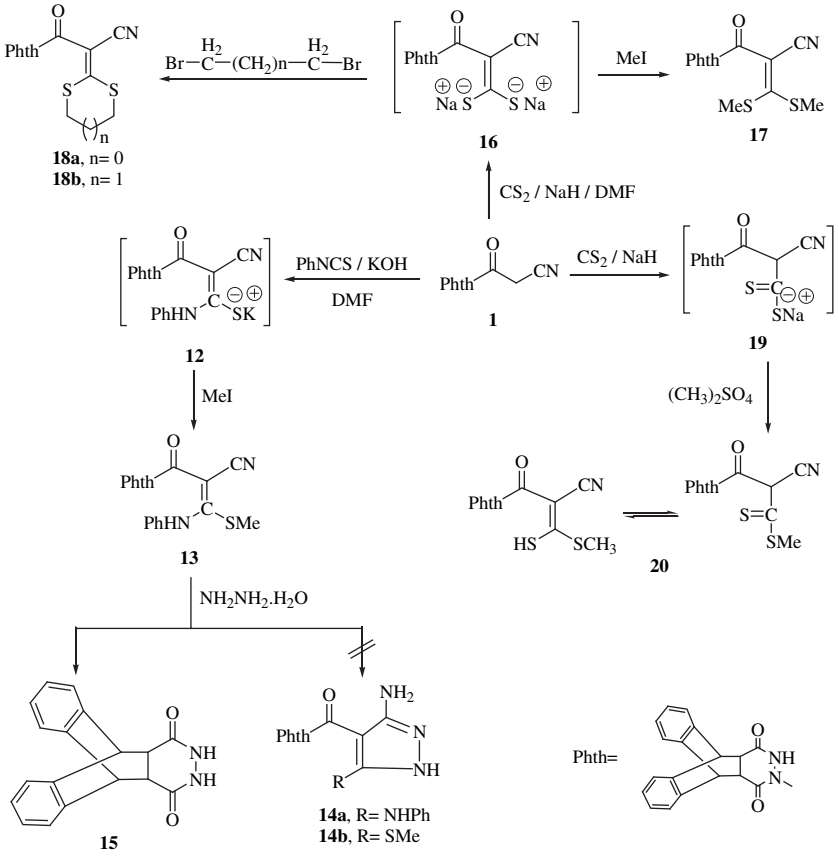


Scheme 2.

spectrum displayed beside the expected signals, a characteristic singlet signal at δ 2.1 for three methyl protons. Reaction of **13** with hydrazine in dimethylformamide not afforded 3-aminopyrazole **14a** as expected, but the known phthalazinedione derivative **15** [16], was achieved. Compound **15** was confirmed by its mass spectrum and comparison of its melting point and mixed melting point with authentic sample.

Oxoketene dithioacetals, especially dimethyl thioacetal have considerable attention due to their synthetic importance for the construction of variety of alicyclic, aromatic and heterocyclic compounds [17,18]. Thus, propiononitrile derivative **1** treated with carbon disulphide in the presence of two equivalent sodium hydride to give the non-isolated sodium salt **16** which was allowed to react with halogenated compounds namely, methyl iodide, 1,2-dibromomethane or 1,3-dibromopropane to give the corresponding ketene dithioacetal **17** and **18a, b**, respectively. IR, ^{1}H NMR and mass spectra have characterized the structure of these compounds. The IR spectra exhibited bands at 3440–3300 (NH), 2204–2202 (CN) and 1746–1642 cm^{-1} (CO). The ^{1}H NMR spectrum of **17** displayed characteristic two singlet signals at δ 2.47 and 2.51 corresponding to six protons of two methyl groups. Also, ^{1}H NMR spectrum of **18a** showed signals at δ 3.6 (br, 4H, CH_2CH_2) and 10.4 (s, 1H, NH). Furthermore, the (+)-ESI mass spectrum of **18b** showed four quasi-molecular ion peaks at 474.1 $[M+H]^+$, 496.1 $[M+Na]^+$, 946.8 $[2M+H]^+$ and 968.9 $[2M+Na]^+$, and (−)-ESI mass spectrum showed two quasi-molecular ion peaks at 472.2 $[M-H]^-$ and 944.9 $[2\,M-H]^-$ pointing 473 as the molecular mass of the compound.

Refluxing of **13** with hydrazine hydrate not afforded the aminopyrazole derivative **14** as expected [19], but the phthalazine derivative **15**. While the reaction of compound **1** with carbon



Scheme 3.

disulphide and one equivalent sodium hydride in dimethylformamide gave non-isolated sodium dithioacetal derivative **19**, which was alkylated using dimethylsulphate to give methyl dithioate derivative **20**. Compound **20** was confirmed by elemental analysis, IR, and ^{1}H NMR spectra. The IR spectrum revealed bands at 3136 (NH), 2975 (CH aliphatic), 2202 (CN) and 1730, 1665 (CO). Also, its ^{1}H NMR spectrum displayed characteristic signals at δ 3.9 (s, 3H, CH_3), 7.1–7.9 (m, 8H, Ar–H), 10.8 (s, 1H, SH) and 11.12 (s, 1H, NH) beside the expected signals for other protons.

3. Pharmacology

Thirteen compounds were screened *in vitro* for their antimicrobial activities against *Bacillus thuringiensis* and *Escherichia coli* by the agar diffusion technique [20]. The bacteria were maintained on nutrient agar. DMSO showed no inhibition zones. The agar media were incubated with different microorganism culture tested. After 24 h of incubation at 30 °C, the diameter of inhibition zone (mm) was measured (Table 1). Ampicillin and chloramphenicol were purchased from Egyptian market and used in a concentration 2 mg ml^{-1} as references.

The results depicted in Table 1 revealed that compounds **2**, **6**, **7**, **9**, **10**, **13** and **20** exhibited interesting high activities against the reference chemotherapeutics. It is worth mentioning that incorporation of phthalazine moiety to coumarin, bromo, thiomethyl and hydrazo group caused significant activity against both *B. thuringiensis* and *E. coli*.

In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocyclic based on phthalazine for antibacterial evaluation.

4. Experimental

4.1. General

All melting points are in degree centigrade and are uncorrected. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets Infrared spectra were recorded on FTIR 5300 spectrometer and Perkin Elmer spectrum RXIFT-IR system (λ, cm^{-1}) using potassium bromide wafer technique, faculty of science El Azhar university. ^{1}H NMR spectra were determined on a Varian XL 200 MHz, Faculty of Science, Cairo University, a Brucker WP 300 Georg-August University Goettingen, Germany and a Brucker AC 300 Eberhard-Karls University, Tuebingen, Germany, in $CDCl_3$ or DMSO solvent using TMS as internal standard. ^{13}C NMR spectra were determined on Brucker AC 300 Eberhard-Karls University, Tuebingen, Germany, in $CDCl_3$ or DMSO solvent using TMS as internal standard. Mass spectra were recorded on GC–MS QP-1000 EX. Schimadzye (Japan), Faculty of Science, Cairo University, ESI MS with Quattro Triple Quadruple Mass Finingan MAT–Incos 50 ESILCQ (Finingan), Georg-August University Goettingen, Germany and FAB + Q_3MS LMR UP LR, Eberhard-Karls University, Tuebingen, Germany, Elemental analyses (C, H, and N) were carried out at the microanalytical center of Cairo Univ., Giza, Egypt, the results were found to be in good agreement (±0.12%) with the calculated values.

Table 1
Inhibition zone (mean diameter of inhibition in mm) as a criterion of antibacterial activities of the newly synthesized compounds.

Compound no.	Inhibition zone in mm	
	Gram-positive bacteria "*Bacillus thuringiensis*"	Gram-negative bacteria "*Escherichia coli*"
2	12	20
3	15	16
4	11	16
5a	12	13
5b	11	16
6	13	19
7	30	20
9	29	27
10	20	16
13	30	26
18a	–	13
18b	16	15
20	22	22
Reference drugs		
Ampicillin	18	19
Chloramphenicol	23	20

4.1.1. Synthesis of 3-(1,4-dioxo-3,4,4e,5,10,10a-hexahydro-1H-5,10-benzeno-benzo[g]phthalazin-2-yl)-3-oxo-propiononitrile (**1**)

A solution of dibenzobarallene (8.28 g, 0.03 mole) and cyanoacetic acid hydrazide (3.17 g, 0.032 mole) in dimethylformamide was refluxed for 4–5 h. The separated product was recrystallized from benzene-dimethylformamide to give **1**.

Yield 65%; 7 g; mp 310 °C; IR (KBr): ν/cm^{-1} = 3200 (NH), 2250 (CN), 1727, (2CO), and 1658 (CO); ^{1}H NMR (DMSO): δ_{ppm} = 3.1 (s, 2H, C_{11}–H, C_{12}–H), 3.7 (s, 2H, CH_2–CN), 4.6 (s, 2H, (C_9–H, C_{10}–H)), 7.20–7.61 (m, 8H, Ar–H) and 9.8 (s, 1H, NH); EIMS (*m*/*z*) (%) = 357 (M^+, 2.6), 318 (2.9), 290 (4.0), 259 (8.6), 231 (4.0), 202 (8.0), 178 (100), 152 (9.3), 112 (4.0), 82 (5.3) and 55 (3.3). Anal. for $C_{21}H_{15}N_3O_3$ (357.36): calcd.: C, 70.58; H, 4.23; N, 11.76%; found: C 70.64, H 4.27, N 11.83%.

4.1.2. Synthesis of 2-[(2-imino-2H-chromen-3-yl) carbonyl]-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazine-1,4-dione (**2**)

To a mixture of **1** (1.78; 0.005 mole) and salicyaldehyde (0.61 g; 0.005 mole) in DMF (15 ml) was added TEA (0.3 ml). The reaction was heated on water bath for 4 h, and poured into ice water. The obtained product was filtered, washed with water and crystallized from ethanol-benzene to give **2**.

Yield 80%; 1.84 g; mp 285 °C; IR (KBr): ν/cm^{-1} = 3353, 3294 (2NH), 1723, 1702, (CO), 1630 (C=N) and 1610 cm^{-1} (C=C); ^{1}H NMR (DMSO): δ_{ppm} = 3.5 (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, (C_9–H, C_{10}–H)), 7.0–8.5 (m, 13H, Ar–H), 9.0 (br, 1H, NH (imino)) and 11.8 (s, 1H, NH); EIMS (*m*/*z*) (%) = 461 (M^+, 4.68), 295 (0.78), 203 (2.42), 202 (3.37), 178 (100), 172 (3.77), 102 (2.92) and 77 (3.51). Anal. for $C_{28}H_{19}N_3O_4$ (461.47): calcd.: C 72.88, H 4.15, N 9.11%; found: C 72.86, H 4.13, N 9.12%.

4.1.3. Synthesis of 2-[(2-chromenon-3-yl)carbonyl]-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazine-1,4-dione (**3**)

Compound **2** (0.92 g; 0.002 mole) was heated in a mixture of conc. HCl and ethanol (10 ml, 1:1 by vol.) for 15 min. on water bath. The reaction mixture was left to stand at room temperature overnight, and the solid product was filtered and crystallized from ethanol-benzene to give **3**.

Yield 83%; 0.76 g; mp > 320 °C; IR (KBr): ν/cm^{-1} = 3256 (NH), 1800, 1726 (CO) and 1610 cm^{-1} (C=C); ^{1}H NMR (DMSO): δ_{ppm} = 3.3 (br, 2H, C_{11}–H, C_{12}-H), 4.8 (br, 2H, (C_9–H, C_{10}–H)), 7.0–8.6 (m, 13H, Ar–H), and 10.0 (s, 1H, NH). Anal. for $C_{28}H_{18}N_2O_5$ (462.45): calcd.: C 72.72, H 3.92, N 6.06%; found: C 72.73, H 3.90, N 6.01%.

4.1.4. Synthesis of 2-[(4-amino-3-phenyl-2-thio-(3H)-5-thiazol-5-yl)carbonyl]-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazine-1,4-dione (**4**)

To a stirred solution of propiononitrile derivative **1** (3.57 g; 0.01 mole), finally divided sulfur (0.35 g; 0.011 mole) and TEA (1.2 ml) in a mixture of DMF and EtOH (20 ml, 1:1 by vol.), the phenyl isothiocyanate (1.35 g; 0.01 mole) was added. The reaction mixture

was heated in water bath at 60 °C for 3 h, and then cooled. The obtained product was filtered, washed with ethanol, dried and recrystallized from ethyl acetate to give **4**.

Yield 75%, 3.93 g; mp 286 °C; IR (KBr): $\nu/cm^{-1} = 3414, 3287, 3172$ (NH_2, NH) and 1731, 1698, 1652 (CO) cm^{-1}; ^1H NMR (DMSO): $\delta_{ppm} = 3.3$ (s, 2H, C_{11}–H, C_{12}–H), 4.7 (s, 2H, C_9–H, C_{10}–H), 6.7 (s, 2H, NH_2), 7.1–7.5 (m, 13H, Ar–H), and 9.9 (s, 1H, NH). ^{13}C NMR (DMSO): $\delta_{ppm} = 186.1, 173.3, 169.9, 152.8, 141.7, 138.8, 134.4, 129.8, 126.5, 126.0$, 124.5, 123.9, 79.3, 44.1 and 44.2. Anal. for $C_{28}H_{20}N_4O_3S_2$ (524.61): calcd.: C 64.10, H 3.84, N 10.68%; found: C 64.13, H 3.79, N 10.62%.

4.1.5. Synthesis of 6-substituted -4-phenyl-3-thio-1, 8,15-trioxo-8,8$_a$,9,14,14$_a$,15-hexahydro-3H [1′,3′] thiazolo [4′,5′-e]-1H-[1,2,4] triazepino [1,2-b]-9,14-benzeno-benzo-[g] phthalazine (**5a, b**)

A solution of compound **4** (1 g; 0.002 mole) in a mixture of triethylorthoformate and acetic anhydride (15 ml, 3:1 by vol.) or acetic anhydride was heated under reflux for 3hrs, and then cooled. The obtained product was filtered, dried and crystallized from ethanol-benzene to give **5a, b**.

5a; Yield 82%, 0.87 g; mp > 320 °C; IR (KBr): $\nu/cm^{-1} = 2953$ (C–H alphatic) and 1718 (br, CO) cm^{-1}; ^1H NMR (DMSO): $\delta_{ppm} = 3.7$ (s, 2H, C_{11}–H, C_{12}–H), 4.9 (s, 2H, C_9–H, C_{10}–H) and 7.1–7.5 (m, 14H, Ar–H, N–CH═N). Anal. for $C_{29}H_{18}N_4O_3S_2$ (534.61): calcd.: C 65.15, H 3.39, N 10.48%; found: C 65.17, H 3.4, N 10.35%.

5b; Yield 93%, 1.0 g; mp > 320 °C; IR (KBr): $\nu/cm^{-1} = 2950, 2900$ (C–H aliphatic) and 1728 (br, CO) cm^{-1}; FABMS (m/z) (%) = 549, ($M^+ + 1$, 9.60), 460 (4.80), 307 (100), 289 (73), 215 (24.3), 204 (12.1), 202 (16.5), 178 (38.7) and 165 (97.8). Anal. for $C_{30}H_{20}N_4O_3S_2$ (548.63): calcd.: C 65.68, H 3.67, N 10.21%; found: C 65.69, H 3.63, N 10.20%.

4.1.6. Synthesis of 3-hydazino-6-methyl-4-phenyl-1,8,15-trioxo-8,8$_a$,9,14,14$_a$,15-hexahydro-3H-[1′,3′] thiazolo [4′,5′-e]-1H-[1,2,4] triazepino [1,2-b]-9,14-benzeno-benzo-[g] phthalazine (**6**)

To a solution of compound **5b** (5.5 g; 0.01 mole) in DMF (20 ml) dimethylsulphate (1.89 g; 0.015 mol) was added the reaction mixture was heated on water bath at 90 °C for 3 h. It was then poured portion wise to a stirred solution of hydrazine hydrate (2 ml) in methanol (10 ml). The stirring was continued for 2 h. The obtained product after adding ice cold-water was filtered washed with EtOH, dried and crystallized form DMF–methanol to give **6**.

Yield 78%, 4.25 g; mp 295 °C; IR (KBr): $\nu/cm^{-1} = 3400, 3336$, 3261 (NH, NH_2), 1772, 1704, 1682 (CO) and 1642 cm^{-1} (C═N); ^1H NMR (DMSO): $\delta_{ppm} = 2.45$ (s, 3H, CH_3), 3.2 (s, 2H, NH_2), 3.4 (d, 2H, C_{11}–H, C_{12}–H), 4.8 (d, 2H, C_9–H, C_{10}-H) and 7.1–7.6 (m, 13H, Ar–H); ^{13}C NMR (DMSO): $\delta_{ppm} = 173.8, 160.0, 154.1, 153.6, 141.8, 139.0$, 135.4, 129.5, 129.3, 128.7, 126.4, 126.1, 124.5, 124.1, 44.4, 44.2, 40.5, 40.1 and 21.7; FABMS (m/z) (%) = 547 ($M^+ + 1$, 1), 460 (3), 444 (3.5), 307 (33.9), 291 (85.6), 289 (31.3), 215 (7.8), 204 (11.3), 202 (15.6) and 178 (100). Anal. for $C_{30}H_{22}N_6O_3S$ (546.60): calcd.: C 65.92, H 4.06, N 15.38%; found: C 65.89, H 4.02, N 15.39%.

4.1.7. Synthesis of 3-(1,4-dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-3-oxo-2-[3-(4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine)hydrazono]propiononitrile **7**

A well stirred solution of 3-amino-(4,6-dimethyl-1*H*-pyrazolo[3,4-b]pyridine) (0.362 g; 0.002 mole) in (0.6 ml) concentrated HCl and H_2O (2 ml) was cold in ice-bath and diazotized with the solution of $NaNO_2$ (0.15 g; 0.002 mole) in H_2O (2 ml). The cold diazonium solution was added slowly to a well stirred solution of **1** (0.70 g; 0.002 mole) in pyridine (10 ml). The reaction mixture was stirred for another 2 hrs. The crude product was filtered off, dried well and recrystallized from ethanol-benzene **7**.

Yellow crystals; yield 76%; mp > 320; IR (KBr): $\nu/cm^{-1} = 3382$, 3202 (NH), 2235 (CN), 1732, 1686 (CO) and 1508 (N═N); ^1H NMR (DMSO): $\delta_{ppm} = 2.6$ (s, 3H, CH_3), 2.9(s, 3H, CH_3–C═N), 3.4 (d, 2H, C_{11}–H, C_{12}–H), 4.8 (d, 2H, C_9–H, C_{10}–H), 6.9–7.4 (m, 10H, Ar–H), 8.8 (br., 1H, NH–N═C) and 12.2 (s, 1H. NH). Anal. for $C_{29}H_{22}N_8O_3$ (530.54): calcd.: C 65.65, H 4.18, N 21.12%; found: C 65.62, H 4.14, N 21.1%.

4.1.8. Synthesis of pyridopyrazolotriazine derivative **8**

7 (1.06 g; 0.002 mole) was refluxed in glacial acetic acid (15 ml) for 4 h. The reaction mixture was left to stand at room temperature overnight, and the solid product was filtered and crystallized from ethanol-benzene to give **8**.

Yield 83%, 0.88 g; mp > 320 °C; IR (KBr): $\nu/cm^{-1} = 3405, 3221$, 3271 (NH_2, NH), 1731, 1692, 1663 (CO) and 1628 cm^{-1} (C═N); EIMS (m/z) (%) = 530 (M^+, 26.9), 352 (14.8), 241 (12.7), 178 (100), 174 (12.3), 146 (17.5) and 78 (11.8). Anal. for $C_{29}H_{22}N_8O_3$ (530.54): calcd.: C 65.65, H 4.18, N 21.12%; found: C 65.66, H 4.17, N 21.1%.

4.1.9. Synthesis of 2,2-dibromo-3-(1,4-dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-3-oxo-propiononitrile (**9**)

A mixture of bromine (1.6 g; 0.01 mole) in glacial acetic acid or chloroform (15 ml) was added drop wise over a period of 20 min. to a hot solution of **1** (3.57 g; 0.01 mole) in acetic acid (75 ml). The reaction was heated on water bath for 3 hrs; the separated product after cooling was filtered, washed with water and crystallized from DMF/MeOH to give **9**.

Yield 79%, 4.06 g; mp 221 °C; IR (KBr): $\nu/cm^{-1} = 3250$ (NH), 2260 (CN) and 1722, 1662 cm^{-1}(CO). ^1H NMR (DMSO): $\delta_{ppm} = 3.4$ (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, (C_9–H, C_{10}–H)), 7.1–7.5 (m, 8H, Ar–H), and 11.6–11.8 (br, 1H, NH). Anal. for $C_{21}H_{13}Br_2N_3O_3$ (515.15): calcd.: C 48.96, H 2.54, N 8.16%; found: C 48.86, H 2.50, N 8.20%.

4.1.10. Synthesis of 2-bromo-3(1,4-dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-3-oxo-propiononitrile (**10**)

A mixture of **1** (3.57 g; 0.01 mole) and *N*-bromosuccinamide (1.78 g; 0.01 mole) in DMF (30 ml) was stirred for 48 h, at room temperature. The obtained product after dilution with water was filtered, washed with water and crystallized from ethanol-benzene to give **10**.

Yield 75%, 3.27 g, mp 306 °C; IR (KBr): $\nu/cm^{-1} = 3186, 3186$ (NH), 2255 (CN) and 1796, 1728 cm^{-1}(CO); EIMS (m/z) (%) = 437 ($M^+ + 2$, 0.19), 435 (M^+, 0.2), 355 (0.11), 313 (0.72), 204 (1.09), 203 (4.39), 202 (6.62) and 178 (100). Anal. for $C_{21}H_{14}BrN_3O_3$ (436.26): calcd.: C 57.82, H 3.23, N, 9.63%; found: C 57.73, H 3.33, N 9.68%.

4.1.11. Synthesis of 2-[(3-amino-benzopyridazin-2-yl)carbonyl]-1,4-dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]phthalazine (**11**)

A mixture of **9** (0.87 g; 0.002 mole), *o*-phenylenediamine (0.21 g, 0.02 mole) and triethylamine (3 drops) in DMF (15 ml) was refluxed for 24 h. The obtained product after dilution with water was filtered, washed with water and crystallized from ethanol-benzene to give **11**.

Yield 76%, 0.7 g; mp 273 °C; IR (KBr): $\nu/cm^{-1} = 3433, 3335$ (NH, NH_2), 1730, 1685, (CO) and 1614 cm^{-1}(C = N); EIMS (m/z) (%) = 461 (M^+, 18.3), 290 (1.7), 283 (6.9), 255 (0.7), 204 (1), 203 (4.7), 202 (5.4) 178 (100), 144 (1.7) and 97 (2.5). Anal. for $C_{27}H_{19}N_5O_3$ (461.47): calcd.: C 70.27, H 4.15, N 15.18%; found: C 70.3, H 4.18, N 15.22%.

4.1.12. Synthesis of 2-methylthio-2-phenylaminomethylene-3-(1,4-dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-3-oxo-propiononitrile (**13**)

To a cold suspension of finally divided KOH (0.28 g; 0.005 mole) in dry dimethylformamide (25 ml) were added the nitrile derivative **1** (1.78 g; 0.005 mole) followed by phenyl isothiocyante. The mixture was stirred at room temperature for 12 h, then cooled

again to 0 °C, treated with the methyl iodide (0.7 g; 0.005 mole) and left to stand at room temperature for 8 h. The mixture was poured into ice cold-water. The resulting precipitate was filtered off, dried and crystallized from DMF-MeOH to give **13**.

Yield 74%, 1.87 g; mp 278 °C; IR (KBr): $\nu/cm^{-1} = 3286$, 3152 (2NH), 2205(CN) and 1728, 1684, cm^{-1} (CO); ^1H NMR (DMSO): $\delta_{ppm} = 2.1$ (s, 3H, CH_3), 3.4 (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H), 7.1–7.5 (m, 13H, Ar–H),10.4 (s, 1H, NHPh) and 11.7 (s, 1H, NH); EIMS (*m/z*) (%) = 508 ($M^+ + 2$, 0.19), 506 (M^+, 1.55), 432 (0.47), 290 (1.14), 275 (0.29), 231 (0.49), 204 (1.10), 202 (4.44), 178 (100), 97 (0.42) and 77 (57.8). Anal. for $C_{29}H_{22}N_4O_3S$ (506.57): calcd.: C 68.76, H 4.38, N 11.06%; found: C 68.63, H 4.30, N 11.00%.

*4.1.13. Synthesis of 2,3,4,4a,5,10,10$_a$-heptahydro-1H-5,10-benzeno-benzo[g]-phthalazine-1,4-dione (**15**)*

To suspension of **13** (1.01 g; 0.002 mole) or **17** (2.90 g; 0.002 mole) in DMF/EtOH (10 ml; 1.3 by vol.), hydrazine hydrate (0.1 ml; 0.002 mole) was added. The reaction mixture was heated under reflux for 2 h. The obtained product after cooling was filtered, dried and crystallized from the EtOH-benzene to give **15**.

Yield 81%; mp 290 °C; IR (KBr): $\nu/cm^{-1} = 3336$, 3260 (2NH), and 1774, 1696 cm^{-1} (CO); EIMS (*m/z*) (%) = 290 (M^+, 2.70), 231 (0.2), 204 (0.6), 202 (5.60), 178 (100), 112 (2.40) and 77 (1.70). Anal. for $C_{18}H_{14}N_2O_2$ (290.32): calcd.: C 74.47, H 4.86, N 9.65%; found: C 74.51, H 4.82, N 9.60%.

4.1.14. Synthesis of ketene S,S-*acetal (**17, 18a, 18b**)*

4.1.14.1. General procedure. To a cold suspension of NaH (0.24 g; 0.01 mole) in dry dimethylformamide (25 ml) were added the nitrile derivative **1** (1.78 g; 0.005 mole) followed by carbon disulphide (1.14 g; 0.15 ml) slowly drop wise under stirring over a period of 15 min. while the temperature of the mixture was maintained at 5–10 °C. The mixture was stirred at room temperature for 12 h. Then cooled again to 0 °C, methyl iodide (1.42 g; 0.01 mole) or dihalocompound (0.005 mole) namely dibromoethane or dibromopropane was added drop wise over a period of 10 min and left to stand at room temperature for 24 h. The mixture was poured into ice cold-water. The resulting precipitate was filtered off, dried and crystallized from ethanol-benzene.

*4.1.14.1.1. 2-[Bis(methylthio)-methylene]-3-(1,4-dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-3-oxo-propiononitrile (**17**).* Yield 75%; 1.72 g, mp 270 °C; IR (KBr): $\nu/cm^{-1} = 3440$ (NH), 2202(CN), 1746, 1694 (CO) and 1638 cm^{-1} (C = C); ^1H NMR (DMSO): $\delta_{ppm} = 2.47$ (s, 3H, CH_3), 2.51(s, 3H, CH_3), 3.4 (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H), 7.1–7.5 (m, 8H, Ar–H) and 10.9 (s, 1H, NH); EIMS (*m/z*) (%) = 461 (M^+, 1.69), 453 (3.16), 378 (4.24), 313 (8.71), 264 (6.40), 178 (100), 152 (1.23) and 57 (38.44). Anal. for $C_{24}H_{19}N_3O_3S_2$ (461.56): calcd.: C 62.45, H 4.15, N 9.10%; found: C 62.59, H 3.96, N 9.1%.

*4.1.14.1.2. 3-(1,4-Dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-2-(1,3-dithiolan-2-ylidene)-3-oxo-propiononitrile (**18a**).* Yield 91%, 2.08; mp 320 °C; IR (KBr): $\nu/cm^{-1} = 3350$ (NH), 2204(CN), 1706 (CO) and 1632 cm^{-1}(C=C). ^1H NMR (DMSO): $\delta_{ppm} = 3.4$ (d, 2H, C_{11}–H, C_{12}–H), 3.6 (br, 4H, CH_2–CH_2), 4.8 (d, 2H, C_9–H, C_{10}–H), 7.1–7.4 (m, 8H, Ar–H) and 10.4 (s, 1H, NH). Anal. for $C_{24}H_{17}N_3O_3S_2$ (459.54): calcd.: C 62.73, H 3.73, N 9.14%; found: C 62.69, H 3.64, N 9.12%.

*4.1.14.1.3. 3-(1,4-Dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-2-(1,3-dithian-2-ylidene)-3-oxo-propiononitrile (**18b**).* Yield 85%, 2.01; mp > 320 °C; IR (KBr): $\nu/cm^{-1} = 3320$ (NH), 2200(CN) and 1734, 1664 cm^{-1} (CO). (+)-ESI mass spectrum showed four quasi-molecular ion peaks at 474.1 ($[M+H]^+$), 496.1 ($[M+Na]^+$), 946.8 ($[2M+H]^+$) and 968.9 ($[2M+Na]^+$), the (−)-ESI mass spectrum showed two quasi-molecular ion peaks at 472.2 ($[M–H]^-$) and 944.9 ($[2M–H]^-$) pointing 473 as the molecular mass of the compound. Anal. for $C_{25}H_{19}N_3O_3S_2$ (473.57): calcd.: C 63.41, H 4.04, N 8.87%; found: C 63.52, H 4.00, N 8.82%.

*4.1.15. Synthesis of methyl-2-cyano-3-(1,4-dioxo-3,4,4$_a$,5,10,10$_a$-hexahydro-1H-5,10-benzeno-benzo[g]-phthalazin-2-yl)-3-oxo-propane dithioate (**20**)*

To a cold suspension of NaH (0.12 g; 0.005 mole) in dry dimethylformamide (25 ml) were added the nitrile derivative **1** (1.78 g; 0.005 mole) followed by carbon disulphide (1.14 g; 0.15 ml) slowly drop wise under stirring over a period of 15 min while the temperature of the mixture was maintained at 5–10 °C. The mixture was stirred at room temperature for 12 h, then cooled again to 0 °C, dimethylsulphate (1.5 g; 0.01 mole) was added drop wise over a period of 10 min. The reaction mixture was stirred for 4 hrs, at room temperature and then poured into ice cold-water. The resulting precipitate was filtered off, dried and crystallized from DMF-MeOH to give **20**.

Yield 85%, 1.9 g; mp 276 °C; IR (KBr): $\nu/cm^{-1} = 3136$ (NH), 2975(C–H aliphatic), 2202 (CN), and 1730, 1665 cm^{-1} (CO); ^1H NMR (DMSO): $\delta_{ppm} = 3.5$ (d, 2H, C_{11}–H, C_{12}–H), 3.9 (s, 3H, CH_3), 4.8 (d, 2H, C_9–H, C_{10}–H), 7.1–7.9 (m, 8H, Ar–H), 10.8 (s, 1H, SH) and 11.2 (s, 1H, NH). Anal. for $C_{23}H_{17}N_3O_3S_2$ (447.53): calcd.: C 61.73, H 3.83, N 9.39%; found: C 61.83, H 3.92, N 9.42%.

4.2. In vitro antimicrobial activity

The tested compounds were evaluated by the agar diffusion technique [20] using a 2 mg ml^{-1} solution in DMSO. The test organisms were *B. thuringiensis* (Gram-positive) and *E. coli* (Gram-negative). A control using DMSO without the test compound was included for each organism. Ampicillin and chloramphenicol in DMSO were used as reference drugs.

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