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Synthesis and Antimicrobial Activity of Some Condensed [4-(2,4,6-Trimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl]acetic Acid Hydrazide

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Some new 1,2,4-triazolo-, 1,3,4-oxadiazolo-, 1,3,4-thiadiazol-, and pyrazolo-2,4,6-trimethylphenyl-1(2*H*)-oxo-phthalazine derivatives were synthesized and identified by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. The new compounds were synthesized with the objective of studying their antimicrobial activity.

Keywords 1(2*H*)-oxo-phthalazine, 1,2,4-triazole, 1,3,4-oxadiazole, 1,3,4-thiadiazole, antimicrobial activity

Introduction

Nitrogen containing heterocyclic compounds have received much attention as shown by the numerous studies published on their applicability in different areas, especially as drugs.[1,2] Phthalazines are examples of nitrogen heterocycles that possess exciting biological properties.[3-5] They form the structural profile for several biologically active compounds and hence they are considered as important key elements. Several reports in the literature have focused on the pharmacology of phthalazine derivatives. These reports have resulted in a great number of contributions in diverse area of interest.[6-11] Phthalazines have been reported to possess anticonvulsant,[12] cardiotonic[13] and vasorelaxant activities.[14,15] Additionally, phthalazines have recently been reported to potentially inhibit serotonin reuptake and considered as anti-depression agents.[16] Several approaches have been reported in the literature for synthesis of phthalazinone.[12,17] Generally, phthalazines are synthesized from either phthalic anhydride derivatves, 2-aryl-3-hydroxyindene-1-ones, or β-diketones via condensation with hydrazine hydrate by either heating[18] or applying microwave irradiation.[15] In view of the aforementioned facts, it seemed most interesting to synthesize some condensed [4-(2,4,6-trimethyl phenyl)-1(2*H*)-oxo-phthalazin-2-yl] acetic acid hydrazide with the aim to evaluate their antimicrobial activities.

Results and Discussion

Aroylation of an aromatic system by reaction with phthalic anhydride under Friedel Craft's conditions yields the *o*-aroylbenzoic acid.[19,20] Thus, reaction of mesitylene with phthalic anhydride in the presence of anhydrous aluminium chloride, was carried out to produce 2-(2,4,6-trimethyl benzoyl) benzoic acid (**2**). Merchant *et al.*[21] prepared phthalazin-l-ones via the condensation of the aroyl benzoic acid with hydrazine hydrate in boiling ethanol. Accordingly, adopting the Merchant *et al.* procedure, condensation of benzoic acid derivative **2** with hydrazine hydrate in boiling ethanol afforded the 4-(2,4,6-trimethyl phenyl)-2*H*-phthalazin-l-one (**3**) in 65% yield. The IR spectrum showed a characteristic absorption bands at ν 1654 cm^{-1} corresponding to CO group. The ^{1}H NMR spectrum of compound **3** showed NH at δ 11.17. Compound **3** was treated with ethyl bromoacetate to afford the corresponding phthalazine acetic acid ethyl ester **4** (Scheme 1). The structure of compound **4** was confirmed on the basis of their elemental analysis and spectral data. The IR spectrum showed a characteristic absorption band at ν=1731 cm^{-1} corresponding to CO of ester, CO of cyclic amide at ν=1659 cm^{-1} and devoid any band for NH. The ^{1}H NMR spectrum of compound **4** showed a triplet signal at δ 1.48 assigned for CH_3CH_2, a quartet signal at δ 4.18 assigned for CH_2CH_3, a singlet at δ 4.79 assigned for CH_2CO, beside 3 CH_3 and aromatic protons. The phthalazine acetic acid ethyl ester **4** was converted to the corresponding hydrazide **5** in high yield by the reaction with hydrazine hydrate (Scheme 1). The hydrazide derivative **5** revealed absorption bands at ν=1650, 3162 and 3301 cm^{-1} corresponding to CO and $NHNH_2$ groups. The ^{1}H NMR spectrum of compound **5** showed δ 4.40 (s, 2H, NH_2 exchangeable with D_2O), 4.71 (s, 2H,

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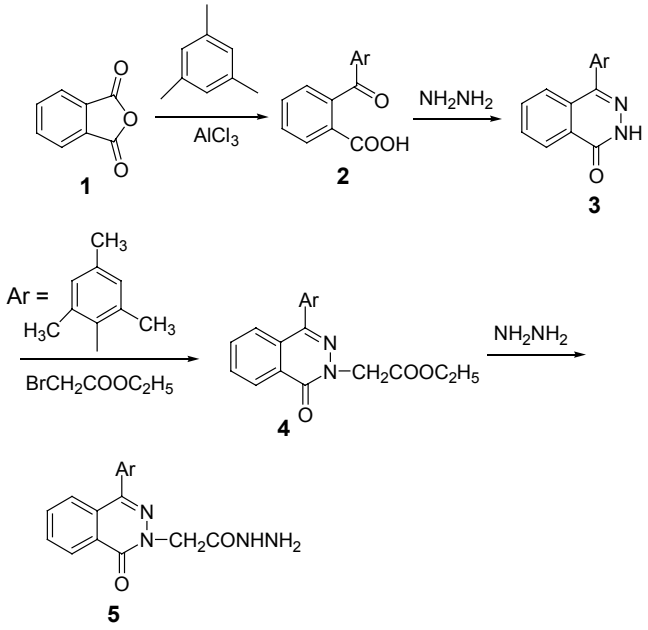
CH_2CO), 9.3 (s, 1H, NH exchangeable with D_2O) and aromatic protons. Whereas, the hydrazide derivatives constitute a class of compounds that have served as useful intermediates towards construction of different heterocyclic compounds.[22]

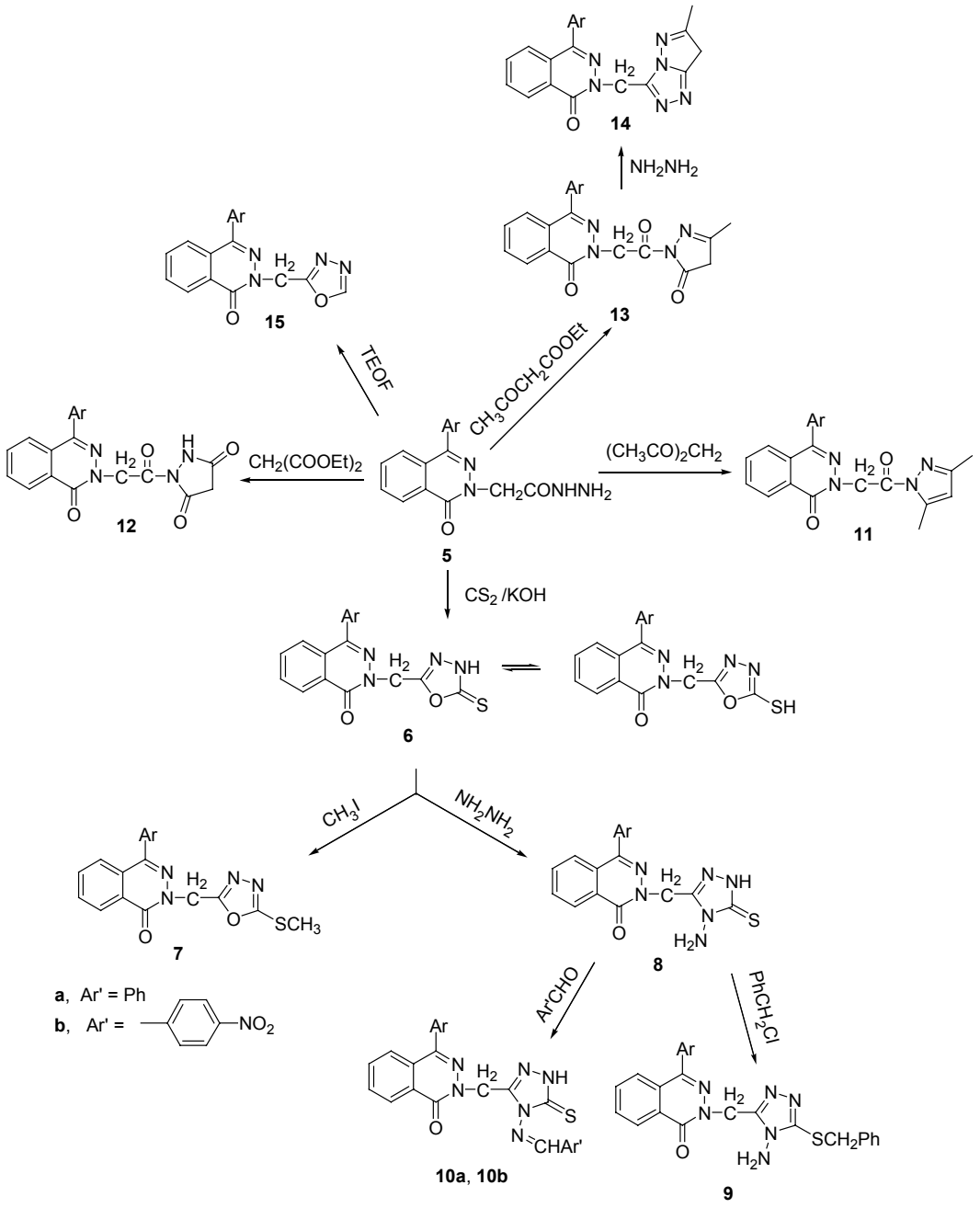
Scheme 1



Cyclization of **5** using carbon disulphide in alcoholic potassium hydroxide gave the corresponding oxadiazolo-2-thione derivative **6**. The IR spectrum of compound **6** revealed the presence of CO and NH groups at 1660 and 3196 cm^{-1} respectively. The ^1H NMR spectrum of compound **6** showed δ 5.01 for assigned CH_2 and 7.06 for assigned NH (exchangeable with D_2O) and aromatic protons. The ^{13}C NMR spectrum of **6** exhibited the expected number of signals for the aromatic carbons as well as three methyl signals and methylene signal at δ 20.1, 23.2 and 62.5. The structure of compound **6** was inferred chemically from (i) its reaction with methyl iodide afforded the methyl thiooxadiazolophthalazine **7**, its IR spectrum revealed no absorption for NH group, and the ^1H NMR of compound **7** showed the absence of NH and the signals at δ 2.58 assigned for S—CH_3; (ii) its reaction with hydrazine hydrate afforded the amino triazolo derivative **8**. The IR spectrum of compound **8** showed a characteristic absorption band at ν=3201 and 3301 cm^{-1} corresponding to NH_2 group, whereas its mass spectrum showed a peak corresponding to its molecular ion at *m/z* 392. Reaction of compound **8** with benzyl chloride in the presence of alcoholic potassium hydroxide yielded 2-[(4-amino-5-(benzylthio)-4*H*-1,2,4-triazolo-3-yl)methyl]-4-mesityl phthalazin-1(2*H*)-one (**9**). In the IR spectra of compound **9** no absorption at 1300 cm^{-1} was observed indicating the disappearance of the thione group. The ^1H NMR spectrum of compound **9** showed signals at δ 4.79 for assigned S—CH_2, 4.93 for assigned N—CH_2 and 5.6 for assigned NH_2 (exchangeable with D_2O). On the other hand condensation of compound **8** with aromatic aldehydes, namely, benzaldehyde and *p*-nitrobenzaldehyde in absolute ethanol afforded the corresponding Schiff's bases **10a**, **10b** respectively. The IR spectra of compounds **10a**, **10b** showed a characteristic absorption band at ν 1621 and 1630 cm^{-1} corresponding to C=N group. The ^1H NMR spectrum of compound **10a** showed the presence of NH and azomethin (CH=N) at δ 13.8 and 9.5 respectively. The mass spectrum of **10b** showed a peak corresponding to its molecular ion at *m/z* 525. Cyclization of hydrazide **5** with acetyl acetone, diethylmalonate and/or ethylacetoacetate afforded the corresponding pyrazole derivatives **11**—**13** respectively according to reported methods[23] (Scheme 2). The IR spectra of compounds **11**—**13** showed the presence of absorption band s for CO groups at 1661—1723 cm^{-1}. The ^1H NMR of compound **11** showed signals at δ 2.01 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.30 (s, 6H, $2CH_3$), 4.80 (s, 2H, CH_2) and 6.40 (s, 1H, CH pyrazole). The mass spectrum of **12** showed a peak corresponding to its molecular ion at *m/z* 404. The ^1H NMR of compound **13** showed signals at δ 1.95 assigned for CH_3, 4.97 assigned for CH_2, and δ 6.10 assigned for 4-CH_2. Cyclization of **13** using hydrazine hydrate in boiling ethanol gave the corresponding pyrazolotriazolophthalazine derivative **14**. The structure of **14** was verified by the spectral data where, the IR showed the absorption bands at ν 1660 cm^{-1} assigned for CO group, and the ^1H NMR showed signals at δ 1.97 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.4 (s, 6H, $2CH_3$), 3.05 (s, 2H, CH_2), 5.02 (s, 2H, CH_2CO) and 7.33—8.10 (m, 6H, aromatic protons). The hydrazide derivative **5** reacted with triethylorthoformate to afford the corresponding oxadiazolederivative **15** (Scheme 2). The IR spectrum of compound **15** showed the presence of absorption bands for CO group at 1666 cm^{-1} and devoid any bands for NH group. The ^{13}CNMR spectrum of **15** exhibited the expected number of signals for the aromatic carbons as well as three methyl signals and methylene signal at δ 20.5, 22.0 and 54.2. Its mass spectrum shows the molecular ion peak at *m/z* 346. The interaction of hydrazide **5** with acetic acid in presence of phosphorus oxychloride afforded oxadiazolophthalazine derivative **16** (Scheme 3), which displayed two bands at 1245 and 1080 cm^{-1} for the C—O—C asymmetric and symmetric stretching, respectively, in addition to the band at 1620 cm^{-1} for C=N stretching. The ^1H NMR spectrum of compound **16** showed signals at δ 2.10 for assigned CH_3 (oxadiazole moity), 4.52 for assigned N—CH_2.

Refluxing of compound **17** with phosphorus pentasulfide in dry xylene afforded thiadiazolo phthalazine derivative **18**, and its IR revealed no absorption for NH. The ^1H NMR spectrum of compound **18** showed δ 4.42 (s, 2H, CH_2), 6.60 (s, 1H, CH thiadiazole) and aromaticprotons. The hydrazide derivative **5** reacted with acrylonitrile to afford the corresponding cyanoethylhy-

Scheme 2



drazide derivative **19** in good yield. The structure of compound **19** was confirmed by the ^{1}H NMR, where the ethyl protons appear at δ 3.11 and 3.23. Treatment of **19** with carbon disulfide in pyridine afforded the corresponding oxadiazolethione derivative **20** (Scheme 3). Both IR and ^{1}H NMR spectra showed no signals corresponding to the NH groups thus confirming the structure of compound **20**. Also, the hydrazide derivative **5** reacted with phenyl isothiocyanate to give the corresponding thiocarbamate derivative **21**. Similarly, the hydrazide derivative **5** reacted with phenyl isocyanate to produce the corresponding carbamate derivative **22**. The ^{1}H NMR of compound **21** showed δ 10.11 (s, 1H, CONH, exchangeable with D_2O) and 12.86 (s, 1H, NHPh, exchangeable with D_2O). The ^{1}H NMR of compound **21** showed δ 9.53 assigned for NHPh (exchangeable with D_2O), and δ 10.33 assigned for CONH (exchangeable with D_2O). The thiocarbamate derivative **21** was cyclized to the corresponding triazole derivative **23** by treatment with sodium hydroxide, while it was cyclized to the corresponding thiadiazolo phthalazine derivative **24** by using conc. sulfuric acid (Scheme 3). The required structure was verified by spectral data. Fusion of hydrazide **5** with phthalic anhydride gave the corresponding N-amide derivative **25**. The ^{1}H NMR of compound **25** showed signals at δ 4.33 assigned for $COCH_2$ and δ 10.72 assigned for NH (exchangeable with D_2O). Its mass spectrum showed the molecular ion

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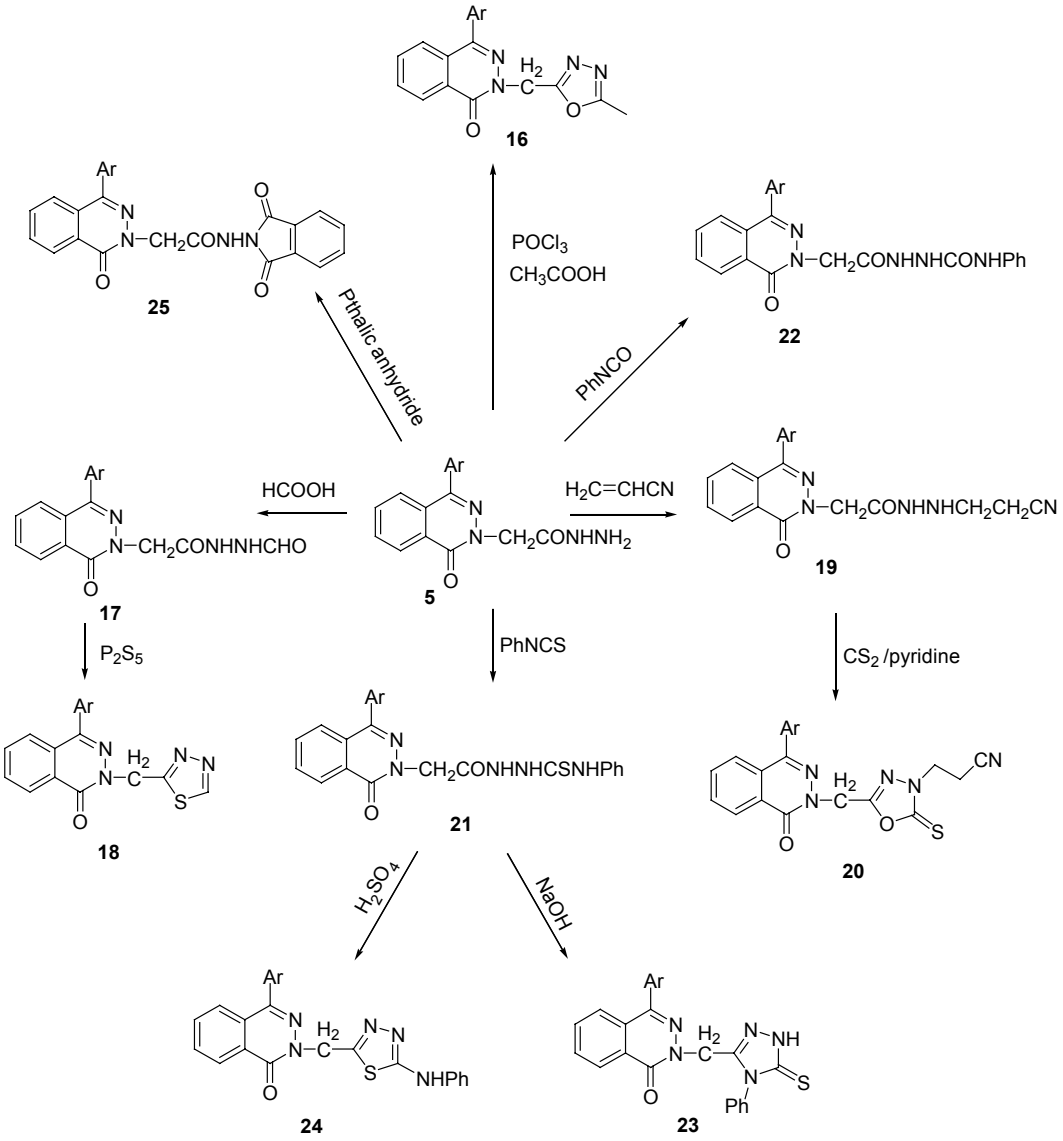
Scheme 3



peak at *m/z* 466.

To get a new series of expected biologically active Schiff's bases, it was interest to condense hydrazide **5** with different aromatic aldehydes namely benzaldehyde, *p*-chlorobenzaldehyde, thiophene-2-carboxaldehyde and furfural in ethanol to give the corresponding Schiff's bases **26a**—**26d** respectively. The IR spectrum of compounds **26a**—**26d** showed stretching bands at 1615—1629 cm^{-1} corresponding to C=N group. The ^1H NMR spectra of compounds **26a**—**26d** showed the methylidene protons at δ 8.55—8.98. On other hand *N*′-(4-chlorobenzylidene)-2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl) acetohydrazide **(26b)** reacted with thioglycollic acid to afford the corresponding thiazolidine derivative **27b** in modrate yield (Scheme 4). The ^1H NMR of compound **27b** showed δ 3.51 for assigned SCH_2, 5.62 for assigned CH, 8.84 for assigned NH, and the aromatic protons. Cyclization of benzylidene compound **26a** by its boiling with acetic anhydride afforded the oxadiazole derivative **28a**. The IR spectrum of compound **28a** showed the presence of absorption bands for CO groups at 1658 and 1720 cm^{-1} and devoid any bands for NH group, and its ^1H NMR showed CH of oxadiazole ring at δ 2.08. The reaction of hydrazide **5** with (ethoxymethylene)malononitrile gave the corresponding pyrazolophthalazine derivative **29**. The IR spectrum of compound **29** showed the absorption bands characteristic for NH_2 and CN groups at 3351 and 2230 cm^{-1}. The mass spectrum gave the molecular ion peak at *m/z*=412. Condensation of hydrazide **5** with *D*-glucose afforded the hydrazone derivative **30**. The compound **30** revealed absorption bands for OH and NH groups in IR spectra and its ^1H NMR spectrum showed the presence of the sugar protons, NH and azomethine (CH=N). Furthermore, when compound **5** was allowed to react with acetyl chloride it give *N*′-acetyl-1-{2-[4-(2,4,6-trimethyphenyl)-1(2*H*)-oxo-phthalazin-2-yl]-aceto}hydrazide (**31**). The ^1H NMR of compound **31**

showed $COCH_3$ at δ 2.05. The latter compound was subjected to a cyclization reaction by refluxing with an ethanolic sodium hydroxide solution to give the corresponding pyrazolophthalazine derivative **32**. The ^1H NMR of compound **32** showed the pyrazolo protons at δ 4.48 and 7.0. The hydrazide derivative **5** reacted with *D*-glucono-1,5-lactone to afford the C-nucleoside **33**. The IR spectrum of compound **33** revealed absorption bands at 1659 and 3440 cm^{-1} attributable to CO and OH groups. ^1H NMR spectrum showed the presence of the sugar protons.

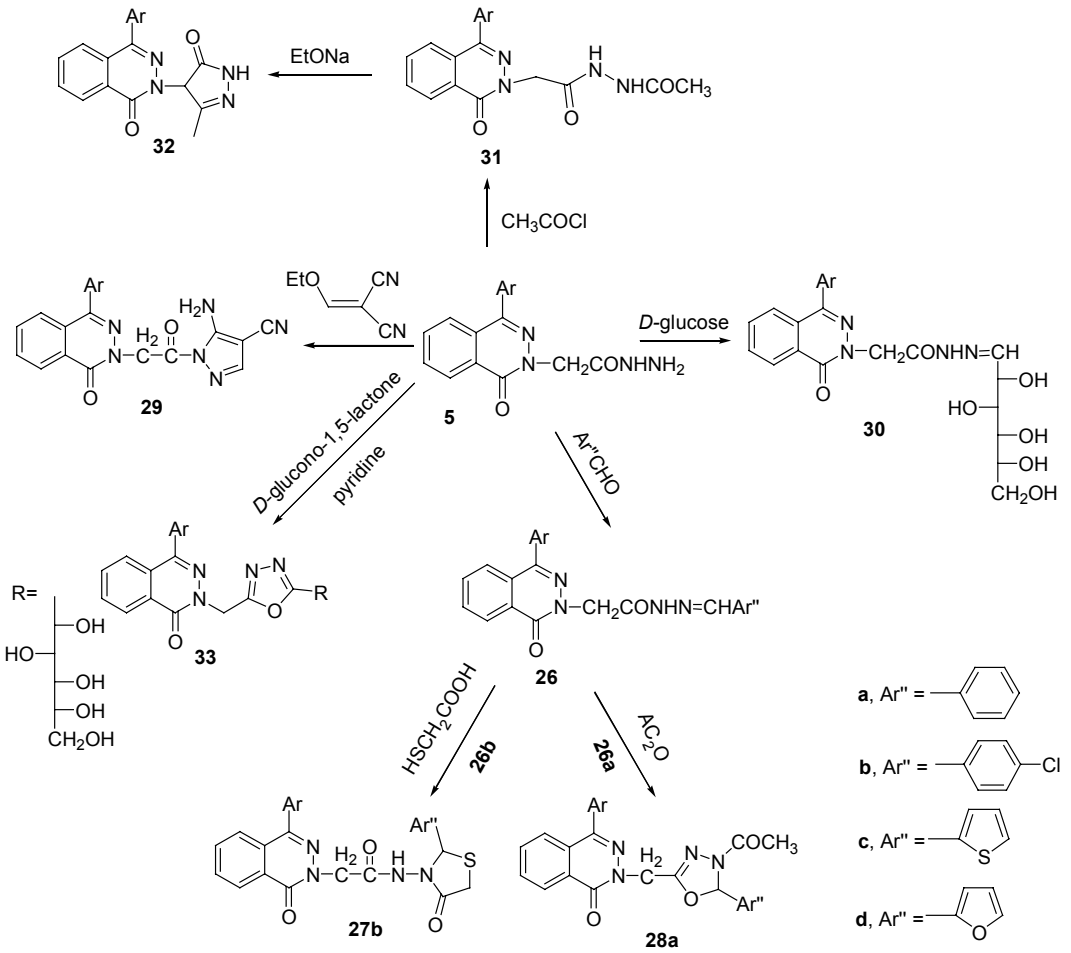
Experimental

Melting points were measured with a Gallenkamp apparatus and are uncorrected. The reactions were followed up and the purification of products was carried out on pre-coated TLC plates (Silica gel 60 F_{254}, Merck), visualizing the spots in ultraviolet light. IR spectra were recorded on a Shimadzu FT-IR 8101 PC infrared spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were determined in DMSO-d_6 at 300 MHz on a Varian Mercury VX 300 NMR spectrometer and their chemical shifts (δ) are reported with respect to TMS as internal standard. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University.

4-(2,4,6-Trimethylphenyl)phthalazin-1(2*H*)-one (3) Hydrazine hydrate (98%) 0.3 mL was added to a solution of 2.6 g **2** (0.01 mol) in 15 mL absolute ethanol. The reaction mixture was refluxed for 2 h, after cooling the obtained solid was filtered off and crystallized from ethanol to give 1.72 g **3** in 65% yield as colorless crystals: m.p. 235—236 ℃; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.39 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 7.27—8.50 (m, 6H, ArH), 11.17 (s, 1H, NH, exchangeable with D_2O); IR (KBr) ν: 3212, 1654 cm^{-1}; MS (70 eV) *m/z* (%): 264 (M^+, 18), 77 (100). Anal. calcd for $C_{17}H_{16}N_2O$: C 77.25, H 6.10, N 10.60; found C 77.20, H 6.13, N, 10.57.

[4-(2,4,6-Trimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl]acetic acid ethyl ester (4) A mixture of 2.6 g **3** (0.01 mol), 5 g ethyl bromoacetate (0.03 mol) and 4.1 g potassium carbonate (0.03 mol) in 30 mL dry acetone was heated under reflux for 30 h, cooled at room temperature and poured into water. The obtained solid was filtered off and crystallized from petroleum ether (40—60 ℃) to give 2.8 g **4** in yield 80% as colorless crystals: m.p. 124—125 ℃; ^1H NMR (DMSO-d_6, 300 MHz) δ: 1.48 (t, J=10 Hz, 3H, CH_3CH_2), 2.39 (s, 3H, CH_3), 2.51 (s, 6H, $2CH_3$), 4.18 (q, J=10 Hz, 2H, CH_2CH_3), 4.79 (s, 2H, CH_2CO), 7.10—8.0 (ArH); IR (KBr) ν: 1731, 1659 cm^{-1}; MS (70 eV) *m/z* (%): 350 (M^+, 15),

Scheme 4



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305 (100). Anal. calcd for $C_{21}H_{22}N_2O_3$: C 71.98, H 6.33, N 7.99; found C 71.93, H 6.31, N 8.03.

[4-(2,4,6-Trimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl]acetic acid hydrazide (5) A mixture of 3.5 g **4** (0.01 mol) and 2 mL hydrazine hydrate in 50 mL absolute ethanol was refluxed for 2 h and cooled at room temperature. The resultant solid was filtered and crystallized from ethanol to give 2.6 g **5** in yield 77% as colorless crystals: m.p. 227—228 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.30 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 4.40 (s, 2H, NH_2 exchangeable with D_2O), 4.71 (s, 2H, CH_2CO), 7.30—8.01 (ArH), 9.30 (s, 1H, NH exchangeable with D_2O); IR (KBr) v: 3301, 3298, 3162, 1650 cm^{-1}; MS (70 eV) *m/z* (%): 337 (M+1, 10), 77 (100). Anal. Calcd for $C_{19}H_{20}N_4O_2$: C 67.84, H 5.99, N 16.66; found C 67.79, H 6.02, N 16.67.

4-(2,4,6-Trimethylphenyl)-2-[(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)methyl]phthalazin-1(2*H*)-one (6) 0.6 g hydrazide **5** (0.002 mol) was added to 0.28 g potassium hydroxide solution in 40 mL absolute ethanol. Then, 6 mL carbon disulfide was added portion wise and the reaction mixture was refluxed till no odour of hydrogen sulfide evolved (18 h). The reaction mixture was poured onto ice water and rendered acidic with hydrochloric acid. The precipitated solid was filtered, dried and crystallized from ethanol to give 0.4 g **6** in yield 54% as yellow crystals: m.p. 180—181 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.35 (s, 3H, CH_3), 2.47 (s, 6H, $2CH_3$), 5.01 (s, 2H, CH_2CO), 7.06 (s, 1H, NH exchangeable with D_2O), 7.26—7.76 (ArH); ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 20.1, 23.2, 62.5, 123.8, 126.3, 127.7, 128.0, 129.8, 130.3, 131.4, 132.5, 134.2, 138.7, 141.0, 158.4, 158.6, 191; IR (KBr) v: 3196, 1660, 1274 cm^{-1}; MS (70 eV) *m/z* (%): 277 ($M^+-C_2N_2OS$, 23), 103 (41), 64 (100). Anal. calcd for $C_{20}H_{18}N_4O_2S$: C 63.47, H 4.79, N 14.80, S 8.47; found C 63.43, H 4.77, N 14.86, S 8.45.

1,2-Dihydro-4-(2,4,6-Trimethylphenyl)-2-[(5-methylthio)-1,3,4-oxadiazol-2-yl]phthalazine (7) To a mixture of 20 mL absolute ethanol containing KOH (0.05 g, 0.001 mol), compound **6** (0.4 g, 0.001 mol) and methyl iodide (0.001 mol) were added. The reaction mixture was stirred overnight and poured onto water. The precipitated solid was collected by filtration, dried and crystallized from ethanol to give 0.26 g **7** in yield 66%: m.p. 119—120 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.32 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 2.58 (s, 3H, SCH_3), 4.60 (s, 2H, CH_2), 7.10—7.92 (ArH); IR (KBr) v: 1667, 1602 cm^{-1}; MS (70 eV) *m/z* (%): 392 (M^+, 13), 263 (100). Anal. calcd for $C_{21}H_{20}N_4O_2S$: C 64.27, H 5.14, N 14.28, S 8.17; found C 64.29, H 5.16, N 14.24, S 8.15.

2-[(4-Amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]-4-mesitylphthalazin-1(2H)-one (8) A mixture of 3.7 g **6** (0.01 mol) and 2 mL hydrazine hydrate (0.03 mol) in 20 mL absolute ethanol was refluxed for 2 h. The solvent and the excess hydrazine hydrate were removed under reduced pressure, the residue was washed with ether, then recrystallized from ethanol to give 3 g **8** in yield 76% as colorless crystals: m.p. 260—261 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.36 (s, 3H, CH_3), 2.49 (s, 6H, $2CH_3$), 4.10 (s, 2H, CH_2), 5.22 (s, 2H, NH_2 exchangeable with D_2O), 7.16—8.12 (ArH), 13.3 (s, 1H, NH exchangeable with D_2O); IR (KBr) v: 3301, 3201, 3131, 2600, 1662, 1610, 1157 cm^{-1}; MS (70 eV) *m/z* (%): 392 (M^+, 10), 248 (100). Anal. calcd for $C_{20}H_{20}N_6OS$: C 61.20, H 5.14, N 21.41, S 8.17; found C 61.22, H 5.12, N 21.40, S 8.16.

2-[(4-Amino-5-(benzylthio)-4*H*-1,2,4-triazol-3-yl)-methyl]-4-mesitylphthalazin-1(2*H*)-one (9) To a mixture of 3.9 g **8** (0.01 mol) and 0.67 g of KOH (0.012 mol) in 50 mL absolute ethanol was added 1.52 g of benzyl chloride (0.012 mol). The reaction mixture was heated under reflux for 2 h, cooled and dilute with 30 mL of water. The precipitated product was dried under vacuum and recrystallized from ethanol to give 3.2 g **9** in yield 66%: m.p. 184—185 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.28—2.46 (2s, 9H, $ArCH_3$), 4.79 (s, 2H, S-CH_2), 4.93 (s, 2H, N-CH_2), 5.6 (s, 2H, NH_2, exchangeable with D_2O) and 7.10—8.45 (m, 11H, aromatic protons); IR (KBr) v: 3320, 3300, 1660 cm^{-1}; MS (70 eV) *m/z* (%): 482 (M^+, 7), 91 (100). Anal. calcd for $C_{27}H_{26}N_6OS$: C 67.20, H 5.43, N 17.41, S 6.64; found C 67.15, H 5.39, N 17.44, S 6.65.

Reaction of 2-[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]-4-mesitylphthalazin-1(2*H*)-one (8) with some aromatic aldehydes

General procedure A mixture of 0.4 g **8** (0.001 mol) and an appropriate aromatic aldehyde, namely benzaldehyde or *p*-nitrobenzaldehyde (0.001 mol) was refluxed in 20 mL absolute ethanol for 4 h. After cooling the separated solid was collected by filtration, dried and crystallized from the proper solvent to give the title compounds **10a** and **10b**, respectively.

2-[(4-(benzylideneamino)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]-4-mesitylphthalazin-1(2*H*)-one (10a) 4 g **10a** in yield 83%, m.p. 200—201 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.39 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 4.43 (s, 2H, CH_2), 7.16—8.12 (ArH), 9.5 (s, 1H, CH=N), 13.8 (s, 1H, NH exchangeable with D_2O); IR (KBr) v: 3162, 1658, 1621 cm^{-1}; MS (70 eV) *m/z* (%): 480 (M^+, 5.2), 77 (100). Anal. calcd for $C_{27}H_{24}N_6OS$: C 67.48, H 5.03, N 17.49, S 6.67; found C 67.45, H 5.10, N 17.43, S 6.64.

4-Mesityl-2-[(4-(4-nitrobenzylideneamino)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]phthalazin-1(2*H*)-one (10b) 4.7 g **10b** in yield 90%, m.p. 191—192 ℃; IR (KBr) v: 3195, 1668, 1630, 1560, 1334 cm^{-1}; MS (70 eV) *m/z* (%): 525 (M^+, 4.1), 77 (100). Anal. calcd for $C_{27}H_{23}N_7O_3S$: C 61.70, H 4.41, N 18.65, S 6.10; found C 61.74, H 4.40, N 18.63, S 6.06.

2-(2-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-oxoethyl)-4-(2,4,6-trimethylphenyl)phthalazin-1(2*H*)-one (11) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 0.30 g acetyl acetone (0.003 mol) in 20 mL ethanol was refluxed for 10 h. After cooling the obtained solid was collected, dried and crystallized from

ethanol to give 0.66 g **11** in yield 82% as colorless crystals: m.p. 180—181 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.01 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.30 (s, 6H, $2CH_3$), 4.80 (s, 2H, CH_2), 6.40 (s, 1H, CH pyrazole), 7.50—8.31 (m, 6H, ArH); IR (KBr) v: 1720, 1677 cm^{-1}. Anal. calcd for $C_{24}H_{24}N_4O_2$: C 71.98, H 6.04, N 13.99; found C 71.95, H 6.08, N 14.02.

1-[2-(4-Mesityl-1-oxophthalazin-2(1*H*)-yl)acetyl]-pyrazolidine-3,5-dione (12) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 0.48 g diethylmalonate (0.003 mol) in 20 mL ethanol was refluxed for 10 h. After cooling the obtained solid was collected, dried and crystallized from ethanol to give 0.61 g **12** in yield 76% as colorless crystals: m.p. 290—291 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.35 (s, 3H, CH_3), 2.44 (s, 6H, $2CH_3$), 3.20 (s, 2H, CH_2), 4.50 (s, 2H, CH_2CO), 7.10—8.01 (m, 6H, ArH), 9.92 (s, 1H, NH exchangeable with D_2O); IR (KBr) v: 3179, 1690, 1661 cm^{-1}; MS (70 eV) *m/z* (%): 404 (M^+, 4.1), 305 (100). Anal. calcd for $C_{22}H_{20}N_4O_4$: C 65.34, H 4.98, N 13.85; found C 65.30, H 5.01, N 13.88.

5-Methyl-3-oxo-2-[1(2*H*)-oxo-4-(2,4,6-trimethylphenyl)phthalazin-2-ylmethylcarbonyl]-3,4-dihydropyrazol (13) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 0.39 g ethylacetoacetate (0.003 mol) in 20 mL ethanol was refluxed for 8 h. After cooling the obtained solid was collected, dried and crystallized from ethanol to give 0.58 g **13** in yield 75% as colorless crystals: m.p. 268—269 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.95 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 4.97 (s, 2H, CH_2CO), 6.10 (s, 2H, CH_2), 7.21—8.30 (m, 6H, ArH); IR (KBr) v: 1723, 1650, 1613 cm^{-1}; MS (70 eV) *m/z* (%): 402 (M^+, 6.0), 305 (100). Anal. calcd for $C_{23}H_{22}N_4O_3$: C 68.64, H 5.51, N 13.92; found C 68.60, H 5.53, N 13.93.

2-[(6-Methyl-7*H*-pyrazolo[5,1-*c*][1,2,4]triazol-3-yl)methyl]-4-(2,4,6-trimethylphenyl) phthalazin-1(2*H*)-one (14) Hydrazine hydrate (98%) 0.5 mL was added to a solution of 0.4 g **13** (0.001 mol) in 10 mL absolute ethanol. The reaction mixture was refluxed for 8 h, after cooling the obtained solid was filtered off and crystallized from ethanol to give 0.32 g **14** in yield 82% as colorless crystals: m.p. 239—240 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.97 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.40 (s, 6H, $2CH_3$), 3.05 (s, 2H, CH_2), 5.02 (s, 2H, CH_2CO), 7.09—7.85 (m, 6H, ArH); IR (KBr) v: 1660 cm^{-1}; MS (70 eV) *m/z* (%): 398 (M^+, 6.0), 263 (100). Anal. calcd for $C_{23}H_{22}N_6O$: C 69.33, H 5.57, N 21.09; found C 69.35, H 5.53, N 21.13.

2-[(1,3,4-Oxadiazol-2-yl)methyl]-4-mesitylphthalazin-1(2*H*)-one (15) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 5 mL triethylorthoformate was refluxed for 15 h on steam bath. The formed solid was collected, dried and crystallized from ethyl acetate to give 0.50 g **15** in yield 73%: m.p. 133—134 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.10 (s, H, CH oxadiazole), 2.37 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 5.11 (s, 2H, CH_2CO), 7.33—8.51 (m, 6H, ArH); ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 20.5, 22.0, 54.2, 124.1, 126.8, 128.2, 129.1, 129.8, 131.0, 131.9, 132.6, 134.9, 139.1, 141.5, 155.2, 159.0, 164.3; IR (KBr) v: 1666 cm^{-1}; MS (70 eV) *m/z* (%): 346 (M^+, 13.0), 263 (100). Anal. calcd for $C_{20}H_{18}N_4O_2$: C 69.35, H 5.24, N 16.17; found C 69.30, H 5.28, N 16.19.

4-Mesityl-2-[(5-methyl-1,3,4-oxadiazol-2-yl)methyl]phthalazin-1(2*H*)-one (16) A solution of 3.3 g **5** (0.01 mol), and 0.6 g acetic acid (0.01 mol) in phosphorous oxychloride (1 mL), was refluxed for 6—8 h. After cooling, the excess phosphorous oxychloride was evaporated under reduced pressure. The residue obtained was diluted with ice water (50 mL), neutralized with saturated sodium bicarbonate, and extracted with ethyl acetate (50 mL×3). The combined organic layer was dried over anhydrous sodium sulphate. After filtration, the solvent was evaporated to get crude product, which was purified by using CombiFlash® Companion® flash chromatography system using ethyl acetate/hexane as mobile phase to get 2.5 g **16** in yield 69% as colorless crystals: m.p. 188—189 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.10 (s, 3H, CH_3), 2.26 (s, 6H, $2CH_3$), 2.74 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 7.09—7.91 (m, 6H, ArH); IR (KBr) v: 1653, 1620 cm^{-1}; MS (70 eV) *m/z* (%): 360 (M^+, 6.0), 263 (100). Anal. calcd for $C_{21}H_{20}N_4O_2$: C 69.98, H 5.59, N 15.55; found C 70.01, H 5.60, N 15.50.

***N′*-Formyl-2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (17)** A solution of 3.3 g **5** (0.01 mol) in formic acid (20 mL) was refluxed for 30 min. The solvent was evaporated and the residue was crystallized from methanol to give 3.0 g **17** in yield 83%: m.p. 249—250 ℃; IR (KBr) v: 3211, 1720, 1660 cm^{-1}; MS (70 eV) *m/z* (%): 364 (M^+, 11), 305 (100). Anal. calcd for $C_{20}H_{20}N_4O_3$: C 65.92, H 5.53, N 15.38; found C 65.88, H 5.55, N 15.40.

2-[(1,3,4-Thiadiazol-2-yl)methyl]-4-mesitylphthalazin-1(2*H*)-one (18) To a solution of 3.6 g **17** (0.01 mol) in xylene (150 mL), phosphorous pentasulphide (0.01 mol) was added. The mixture was refluxed for 1 h. The solvent was evaporated, water (10 mL) was added and the mixture was extracted with chloroform. The solvent was evaporated and the residue was recrystallized from benzene to give 2.9 g **18** in yield 80%: m.p. 130—131 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.25 (s, 3H, CH_3), 2.40 (s, 6H, $2CH_3$), 4.42 (s, 2H, CH_2), 6.6 (s, 1H, thiadiazole), 7.11—8.06 (m, 6H, ArH); IR (KBr) v: 1665, 1610 cm^{-1}; MS (70 eV) *m/z* (%): 362 (M^+, 13.0), 263 (100). Anal. calcd for $C_{20}H_{18}N_4OS$: C 66.28, H 5.01, N 15.46, S 8.85; found C 66.30, H 5.00, N 15.44, S 8.87.

***N′*-(2-Cyanoethyl)-2-(4-(2,4,6-trimethylphenyl)-1-oxophthalazin-2(1*H*)-yl) acetohydrazide (19)** A mixture of 0.3 g the acetic acid hydrazide **5** (0.001 mol) and 0.1 g acrylonitrile (0.001 mol) in 20 mL pyridine was refluxed for 13 h. After cooling, the reaction mixture was poured onto ice water and HCl. The precipitated solid was collected by filtration, dried and crystallized from methanol to give 0.28 g **19** in yield 76% as pale yellow crystals: m.p. 165—165 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.0 (s, 1H, $NHCH_2$, ex-

changeable with D_2O), 2.21 (s, 3H, CH_3), 2.35 (s, 6H, $2CH_3$), 3.11 (t, J=10.0 Hz, 2H, $NHCH_2$), 3.23 (t, J=10.0 Hz, 2H, CH_2CN), 5.00 (s, 2H, CH_2CO), 7.20—8.36 (m, 6H, ArH), 8.0 (s, 1H, CONH, exchangeable with D_2O); IR (KBr) ν: 3209, 2200, 1642 cm^{-1}. Anal. calcd for $C_{22}H_{23}N_5O_2$: C 67.85, H 5.59, N 17.98; found C 67.81, H 5.61, N 18.00.

3-[5-((4-Mesityl-1-oxophthalazin-2(1*H*)-yl)-methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl]propanenitrile (20) A suspension of **19** (0.38 g, 0.001 mol) and carbon disulfide (2 mL) in pyridine (5 mL) was heated under reflux on water bath for 24 h. The solvent was eliminated under reduced pressure and the residue was triturated with ice-cold water and neutralized with diluted HCl. The solid product obtained was filtered off and recrystallized from petroleum ether/ethyl acetate (2∶1) to to give 0.306 g **20** in yield 69% as buff crystals: m.p. 110—111 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.25 (s, 3H, CH_3), 2.40 (s, 6H, $2CH_3$), 3.01 (t, J=10.0 Hz, 2H, $NHCH_2$), 3.40 (t, J=10.0 Hz, 2H, CH_2CN), 4.10 (s, 2H, CH_2CO), 7.02—8.05 (m, 6H, ArH); IR (KBr) ν: 2220, 1667, 1630, 1160 cm^{-1}. Anal. calcd for $C_{23}H_{21}N_5O_2S$: C 64.02, H 4.91, N 16.23, S 7.43; found C 64.05, H 4.89, N 16.25, S 7.40.

1-{2-[4-(2,4,6-Trimethylphenyl)-1-oxophthalazin-2(1*H*)-yl]acetyl}-4-phenyl thiosemicarbazide (21) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and phenyl isothiocyanate (0.002 mol) in 20 mL dry *N*,*N*-dimethylformamide containing few drops of triethylamine was refluxed for 24 h. After cooling, the reaction mixture was poured onto ice water and HCl. The precipitated solid was collected by filtration, dried and crystallized from toluene to give 0.68 g **21** in yield 72% as colorless crystals: m.p.＞300 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.0 (s, 1H, NHCS, exchangeable with D_2O), 2.22 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 5.10 (s, 2H, $COCH_2$), 6.81—8.19 (m, 11H, ArH), 10.11 (s, 1H, CONH, exchangeable with D_2O), 12.86 (s, 1H, NHPh, exchangeable with D_2O); IR (KBr) ν: 3310, 3294, 3147, 1655 cm^{-1}; MS (70 eV) *m/z* (%): 471 (M^+, 22), 77 (100). Anal. calcd for $C_{26}H_{25}N_5O_2S$: C 66.22, H 5.34, N 14.85, S 6.80; found C 66.25, H 5.33, N 14.83, S 6.83.

1-{2-[4-(2,4,6-Trimethylphenyl)-1-oxophthalazin-2(1*H*)-yl] acetyl}-4-phenyl semicarbazide (22) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and phenyl isocyanate (0.004 mol) in 20 mL dry *N*,*N*-dimethylformamide was refluxed for 20 h. After cooling, the reaction mixture was poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from toluene to give 0.686 g **22** in yield 73% as colorless crystals: m.p. 200—201 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.25 (s, 3H, CH_3), 2.42 (s, 6H, $2CH_3$), 4.96 (s, 2H, $COCH_2$), 6.10 (s, 1H, NHCO, exchangeable with D_2O), 7.10—8.40 (m, 11H, ArH), 9.53 (s, 1H, NHPh, exchangeable with D_2O), 10.33 (s, 1H, CONH, exchangeable with D_2O); IR (KBr) ν: 3210, 1668 cm^{-1}; MS (70 eV) *m/z* (%): 455 (M^+, 18), 77 (100). Anal. calcd for $C_{26}H_{25}N_5O_3$: C 68.56, H 5.53, N 15.37; found C 68.52, H 5.56, N 15.40.

2-[(5-Mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)-methyl]-4-(2,4,6-trimethylphenyl)phthalazin-1(2*H*)-one (23) 0.001 mol of thiocarbamate derivative **21** was dissolved in sodium hydroxide solution (2 mol/L, 20 mL). The clear solution was heated on a water bath for 3 h, filtered after cooling and neutralized with dilute hydrochloric acid. The precipitated solid was collected by filtration, dried and crystallized from ethanol to give 0.32 g **23** in yield 71% as colorless crystals: m.p. 297—298 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.26 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 4.55 (s, 2H, $COCH_2$), 7.00—8.10 (m, 11H, ArH), 13.8 (s, 1H, NH, exchangeable with D_2O); IR (KBr) ν: 3234, 1669 cm^{-1}; MS (70 eV) *m/z* (%): 453 (M^+, 9.0), 77 (100). Anal. calcd for $C_{26}H_{23}N_5OS$: C 68.85, H 5.11, N 15.44, S 7.07; found C 68.80, H 5.14, N 15.46, S 7.10.

4-(2,4,6-Trimethylphenyl)-2-[(5-phenylamino-1,3,4-thiadiazol-2-yl)methyl]phthalazin-1-(2*H*)-one (24) 0.001 mol of of thiocarbamate derivatives **21** was mixed with conc. H_2SO_4 (5 mL), left overnight. The reaction mixture was poured onto ice water, the precipitated solid was collected by filtration, dried and crystallized from ethanol to give 0.30 g **24** in yield 66% as colorless crystals: m.p. 148—149 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.29 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 5.11 (s, 2H, $COCH_2$), 6.84—8.19 (m, 11H, ArH), 10.1 (s, 1H, NH, exchangeable with D_2O); IR (KBr) ν: 3196, 1660 cm^{-1}; MS (70 eV) *m/z* (%): 453 (M^+, 9.0), 77 (100). Anal. calcd for $C_{26}H_{23}N_5OS$: C 68.85, H 5.11, N 15.44, S 7.07; found C 68.81, H 5.13, N 15.47, S 7.09.

***N′*-(1,3-Dioxoisoindolin-2-yl)-2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl)acetamide (25)** A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and phthalic anhydride (0.002 mol) in 15 mL acetic acid was refluxed for 10 h. After cooling the obtained solid was filtered off and crystallized from acetic acid to give 0.75 g **25** in yield 81% as colorless crystals: m.p.＞300 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.12 (s, 3H, CH_3), 2.41 (s, 6H, $2CH_3$), 4.33 (s, 2H, $COCH_2$), 6.94—8.29 (m, 11H, ArH), 10.72 (s, 1H, CONH, exchangeable with D_2O); IR (KBr) ν: 3187, 1793, 1744, 1684 cm^{-1}; MS (70 eV) *m/z* (%): 466 (M^+, 12), 305 (100). Anal. calcd for $C_{27}H_{22}N_4O_4$: C 69.52, H 4.75, N 12.01; found C 69.55, H 4.72, N 12.05.

Reaction of phthalazine hydrazide derivative 5 with some aromatic aldehydes

A mixture of 0.3 g the acetic acid hydrazide **5** (0.001 mol) and an appropriate aromatic aldehyde, namely benzaldehyde, *p*-chlorobenzaldehyde, thiophene-2-carboxaldehyde or furfural (0.001 mol) was refluxed in 20 mL absolute ethanol for 4 h. After cooling the separated solid was collected by filtration, dried and crystallized from the proper solvent to give aryl methylidene hydrazide derivatives **26a—26d**.

***N′*-Benzylidene-2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (26a)** 0.32 g **26a** in yield

76% as colorless crystals from ethanol: m.p. 195—196 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.19 (s, 3H, CH_3), 2.38 (s, 6H, $2CH_3$), 5.27 (s, 1H, NH, exchangeable with D_2O), 5.50 (s, 2H, CH_2), 6.80—7.85 (m, 10H, ArH), 8.66 (s, 1H, CH); IR (KBr) v: 3188, 1660, 1615 cm^{-1}; Anal. calcd for $C_{26}H_{24}N_4O_2$: C 73.56, H 5.70, N 13.20; found C 73.50, H 5.73, N 13.24.

***N′*-(4-Chlorobenzylidene)-2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (26b)** 0.37 g **26b** in yield 82% as colorless crystals from ethanol: m.p. 152—153 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.19 (s, 3H, CH_3), 2.38 (s, 6H, $2CH_3$), 5.01 (s, 2H, CH_2), 6.09 (s, 1H, NH, exchangeable with D_2O), 6.75—8.00 (m, 10H, ArH), 8.98 (s, 1H, CH); IR (KBr) v: 3282, 1649, 1618 cm^{-1}; MS (70 eV) *m/z* (%): 460 (M^+Cl^{37}, 1), 458 (M^+Cl^{35}, 4), 263 (100). Anal. calcd for $C_{26}H_{23}ClN_4O_2$: C 68.04, H 5.05, N 12.21; found C 68.08, H 5.01, N 12.23.

2-(4-Mesityl-1-oxophthalazin-2(1*H*)-yl)-*N′*-(thiophen-2-ylmethylene)acetohydrazide (26c) 0.31 g **26c** in yield 72% as faint brown crystals from toluene: m.p. 144—145 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.18 (s, 3H, CH_3), 2.33 (s, 6H, $2CH_3$), 5.05 (s, 2H, CH_2), 5.70 (s, 1H, NH, exchangeable with D_2O), 6.69—8.07 (m, 9H, ArH), 8.55 (s, 1H, CH); IR (KBr) v: 3188, 1651, 1619 cm^{-1}. Anal. calcd for $C_{24}H_{22}N_4O_2S$: C 66.96, H 5.15, N 13.01, S 7.45; found C 67.00, H 5.10, N 13.05, S 7.47.

***N′*-(Furan-2-ylmethylene)-2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (26d)** 0.33 g **26d** in yield 80% as faint brown crystals from toluene: m.p. 174—175 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.26 (s, 3H, CH_3), 2.43 (s, 6H, $2CH_3$), 5.45 (s, 2H, CH_2), 5.70 (s, 1H, NH, exchangeable with D_2O), 6.39—7.97 (m, 9H, ArH), 8.97 (s, 1H, CH); IR (KBr) v: 3271,1659, 1629 cm^{-1}. Anal. calcd for $C_{24}H_{22}N_4O_3$: C 69.55, H 5.35, N 13.52; found C 69.58, H 5.37, N 13.48.

[4-(2,4,6-Trimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl]-*N*-[4-oxo-2-(4-chlorophenyl) thiazolidin-3-yl]-acetamide (27b) A mixture of 0.6 g chloromethylidene hydrazide derivative **26b** (0.0013 mol), 0.1 mL thioglycolic acid (0.0013 mol), 0.5 g anhydrous zinc chloride and few drops of piperidine was refluxed in 20 mL dry *N*,*N*-dimethylformamide for 24 h. The reaction mixture was then poured onto water and the precipitated solid was filtered off, washed several times with water, dried to give 0.36 g **27b** in yield 69% as yellowish brown crystals from toluene: m.p. 299—300 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.28 (s, 3H, CH_3), 2.47 (s, 6H, $2CH_3$), 3.51 (s, 2H, SCH_2), 5.00 (s, 2H, CH_2), 5.62 (s, 1H, CH), 6.67—8.50 (m, 10H, ArH), 8.84 (s, 1H, NH, exchangeable with D_2O); IR (KBr) v: 3172, 1719, 1662 cm^{-1}. Anal. calcd for $C_{28}H_{25}ClN_4O_3S$: C 63.09, H 4.73, N 10.51, S 6.02; found C 63.12, H 4.70, N 10.53, S 6.05.

2-[(4-Acetyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-4-mesitylphthalazin-1(2*H*)-one (28a) Compound **26a** (0.6 g, 0.002 mol) and redistilled acetic anhydride (2 mL) were heated under reflux for 5 h. The reaction mixture was cooled, poured onto water and allowed to stand at room temperature for 3 h. The solid product formed was collected and recrystallized from petroleum ether/ethyl acetate mixture to give 0.68 g **28a** in yield 73% as colorless crystals: m.p. 101—102 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.08 (s, 1H, CH oxadiazole), 2.18 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.35 (s, 6H, $2CH_3$), 5.06 (s, 2H, CH_2), 7.15—8.12 (m, 11H, ArH); IR (KBr) v: 1720, 1658, 1612 cm^{-1}; MS (70 eV) *m/z* (%): 466 (M^+, 5), 43 (100). Anal. calcd for $C_{28}H_{26}N_4O_3$: C 72.09, H 5.62, N 12.01; found C 72.05, H 5.60, N 12.05.

5-Amino-1-(2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl)acetyl)-1*H*-pyrazole-4-carbonitrile (29) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and ethoxymethylene malononitrile (0.24 g, 0.002 mol) in 20 mL absolute ethanol was refluxed for 8 h. After cooling, the solvent was removed *in vacuo* and the solid residue was recrystallized from ethanol to give 0.68 g **29** in yield 82% as colorless crystals: m.p. 280—281 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.21 (s, 3H, CH_3), 2.40 (s, 6H, $2CH_3$), 4.66 (s, 2H, CH_2), 7.01—7.90 (m, 6H, ArH and NH_2, exchangeable with D_2O), 8.13 (s, 1H, CH pyrazole); IR (KBr) v: 3351, 2230, 1657 cm^{-1}; MS (70 eV) *m/z* (%): 412 (M^+, 19), 305 (100). Anal. calcd for $C_{23}H_{20}N_6O_2$: C 66.98, H 4.89, N 20.38; found C 67.01, H 4.85, N 20.40.

***N′*-*D*-Aldehydoglucosyl-2-(4-mesityl-1-oxophthalazin-2(1*H*)-yl)acetohydrazide (30)** A mixture of 3.0 g the acetic acid hydrazide **5** (0.01 mol), *D*-glucose (1.8 g, 0.01 mol), absolute ethanol (40 mL) and a catalytic amount of glacial acetic acid was heated under refluxed for 2 h. After cooling the separated solid was collected by filtration, dried and crystallized from ethanol to 3.5 g **30** in yield 71% as colorless crystals: m.p. 300—301 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.29 (s, 3H, CH_3), 2.35 (s, 6H, $2CH_3$), 3.20—3.60 (protons of the alditol congregated with the solvent absorption), 3.70—3.80 (m, 2H, CH_2OH), 4.40—5.05 (m, 5H, 5OH, exchangeable with D_2O), 5.31 (s, 2H, CH_2CO), 7.26—7.90 (m, 7H, ArH and NH, exchangeable with D_2O), 8.35 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 20.1, 22.2, 58.2, 61.2, 70.7, 72.7, 77.1, 92.6, 124.0, 127.1, 128.0, 128.7, 129.8, 131.3, 131.9, 133.3, 135.0, 139.8, 142.1, 160.1, 162.3, 173.6; IR (KBr) v: 3353, 3220, 1661, 1613 cm^{-1}. Anal. calcd for $C_{25}H_{30}N_4O_7$: C 60.23, H 6.07, N 11.24; found C 60.27, H 6.11, N 11.20.

***N′*-Acetyl-1-{2-[4-(2,4,6-trimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl]aceto}hydrazide (31)** A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and acetyl chloride (0.004 mol) in 20 mL dry *N*,*N*-dimethylformamide containing few drops of triethylamine was refluxed for 14 h. After cooling, the reaction mixture was poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from toluene to give 0.48 g **31** in yield 64% as colorless crystals:

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m.p. 300—301 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.05 (s, 3H, $COCH_3$), 2.29 (s, 3H, CH_3), 2.35 (s, 6H, $2CH_3$), 5.31 (s, 2H, CH_2), 7.26—7.74 (m, 6H, ArH), 10.10 (s, 2H, 2 NH, exchangeable with D_2O); IR (KBr) v: 3298, 3165, 1661 cm^{-1}. Anal. calcd for $C_{21}H_{22}N_4O_3$: C 66.65, H 5.86, N 14.81; found C 66.60, H 5.89, N 14.83.

2-(4,5-Dihydro-3-methyl-5-oxo-1*H*-pyrazol-4-yl)-4-(2,4,6-trimethylphenyl)phthalazin-1(2*H*)-one (32) To a solution of sodium ethoxide (0.23 g Na in 20 mL abs. ethanol), compound **31** (0.001 mol) was added. The reaction mixture was refluxed for 5 h. After cooling, the reaction mixture was poured onto ice/HCl. The precipitated solid was collected by filtration, dried and crystallized from petroleum ether (40—60 ℃) to give 0.28 g **32** in yield 77% as colorless crystals: m.p. 190—191 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.94 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.40 (s, 6H, $2CH_3$), 4.48 (s, 1H, CH), 7.0 (s, 1H, NH, exchangeable with D_2O), 7.20—8.00 (m, 6H, ArH); IR (KBr) v: 3219, 1701, 1632 cm^{-1}. Anal. calcd for $C_{21}H_{20}N_4O_2$: C 69.98, H 5.59, N 15.55; found C 70.01, H 6.02, N 15.50.

4-Mesityl-2-((5-(1,2,3,4,5-pentahydroxypentyl)-1,3,4-oxadiazol-2-yl)methyl)phthalazin-1(2*H*)-one (33) A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and *D*-glucono-1,5-lactone (0.002 mol) in 20 mL pyridine was refluxed for 6 h. After cooling, the reaction mixture was poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from ethanol to give 0.30 g **33** in yield 61% as colorless crystals: m.p. 298—299 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.23 (s, 3H, CH_3), 2.41 (s, 6H, $2CH_3$), 3.11—3.62 (protons of the alditol congregated with the solvent absorption), 3.75—3.91 (m, 2H, CH_2OH), 4.55—5.05 (m, 5H, 5OH, exchangeable with D_2O), 5.20 (s, 2H, CH_2CO), 7.33—8.06 (m, 6H, ArH); IR (KBr) v: 3440, 3165, 1659 cm^{-1}. Anal. calcd for $C_{25}H_{28}N_4O_7$: C 60.48, H 5.68, N 11.28; found C 60.50, H 5.71, N 11.26.

Antimicrobial assay

The antimicrobial activity of the newly synthesized compounds **5**—**16**, **18**, **20**, **23**, **24**, **26b**, **26c**, **27b**, **28a**, **29**, **30**, **32** and **33** were evaluated against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Salmonella typhi* (*S. typhi*), bacterial stains and *Aspergillums niger* (*A. niger*), *Candida albicans* (*C. albicans*), fungal strains by disc diffusion method. Amoxicillin and Ketoconazole were used as standard drugs for bacteria and fungi respectively. Preliminary screening of phthalazine-derivatives and standard drugs were performed at fixed concentrations of 500 μg/mL. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and 72 h for fungi. Each experiment was repeated twice. Based on the results of zone of inhibition, the minimum inhibitory concentration (MIC) of compounds **5**—**16**, **18**, **20**, **23**, **24**, **26b**, **26c**, **27b**, **28a**, **29**, **30**, **32** and **33** against all bacterial and fungal strains was determined by liquid dilution method. Stock solutions of tested compounds with 200, 100, 50, 25, 12.5 and 6.25 μg·mL^{-1} concentrations were prepared with DMSO solvent. The solutions of standard drugs, Amoxicillin and Ketoconazole were prepared in the same concentrations. Inoculums of the bacterial and fungal culture were also prepared. To a series of tubes containing 1 mL each of phthalazine compound solution with different concentrations, 0.2 mL of the inoculums was added. Further 3.8 mL of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 h at 37 ℃ and observed for the presence of turbidity. This method was repeated by changing phthalazine compounds with standard drugs Amoxicillin and Ketoconazole for comparison. The minimum inhibitory concentration at which no growth was observed was taken as the MIC values (Table 1). The comparison of the MICs (in μg/mL) of potent compounds and standard drugs against tested strains is presented in Table 1.

Table 1 Antimicrobial activity of compounds **5**—**16**, **18**, **20**, **23**, **24**, **26b**, **26c**, **27b**, **28a**, **29**, **30**, **32** and **33**

Compound	Minimum inhibitory concentration (MIC) in μg/mL					
	Bacterial strain				Fungal strain	
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*A. niger*	*C. albican*
5	200	250	250	500	250	500
6	100	100	50	50	250	250
7	50	50	50	100	125	125
8	50	50	25	250	250	125
9	50	100	25	25	62.5	125
10a	25	25	50	50	62.5	62.5
10b	25	25	25	250	250	125
11	50	100	100	250	62.5	125
12	200	200	250	200	250	250
13	200	200	100	200	125	125
14	100	250	200	100	250	250
15	100	100	100	50	250	250
16	50	50	100	100	250	250
18	50	50	50	100	250	125
20	50	50	50	100	100	250
23	25	50	25	250	250	125
24	50	50	25	250	250	250
26a	50	50	25	50	250	125
26b	50	25	25	100	125	100
26c	25	25	50	50	62.5	62.5
26d	25	100	25	50	125	125
27b	50	50	50	50	100	250
28a	100	100	50	50	250	125
29	100	100	100	200	62.5	125
30	25	25	25	100	62.5	62.5
32	100	200	200	250	250	125
33	50	50	50	50	62.5	62.5
Amoxicillin	6.25	6.25	6.25	6.25	—	—
Ketaconazole	—	—	—	—	31.25	31.25

Investigation on antibacterial screening data (Table 1)

showed some of the compounds were active against four pathogenic bacteria. Triazolo derivatives **10a**, **10b**, **23**, and Schiff's bases **26c**, **26d**, **30** exhibited good activity against *S. aureus*. Similarly Triazolo derivatives **10a**, **10b**, and Schiff's bases **26b**, **26c**, **30** exhibited good activity against *B. subtilis*. The Triazolo derivatives **8**, **9**, **10b**, **23** and thiadiazolo derivatives **24** and Schiff's bases **26a**, **26b**, **26d**, **30** exhibited good activity against *S. typhi*. Also Triazolo derivative **9** exhibited good activity against *E. coli*. From these results it could be generalized that triazolo derivatives and Shiff's bases of phthalazinone **3** show higher activity compared to oxadiazolo, thiadiazolo and pyrazolo derivatives.

The antifungal results (Table 1) revealed that the synthesized compounds showed variable degree of inhibition against the tested fungi. Compounds **9**, **10a**, **11**, **26c**, **29**, **30** and **33** possessed good antifungal activity against *A. niger and C. albican*. From the results it was conclude that the triazolophthalazinone and Schiff's bases of phthalazinone derivative showed better activity.

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