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# Synthesis, reactions and biological evaluation of benzyltriazolophthalazine derivatives

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ABSTRACT

A series of triazolophthalazine derivatives (**4**-**22**) were synthesized and characterized. The structures of the newly synthesized compounds were confirmed by spectral data. The newly synthesized compounds were also screened for their antimicrobial activity.

## 1. Introduction

Reports of the synthesis of phthalazine-1(*H*)-one derivative have been recently published [1-9]. Phthalazine derivatives are important heterocyclic and are known to possess variety of biological activities such as antimicrobial, anticonvulsant, cardiotonic, vasorelaxant, antifungal, anticancer, antitumor agent, antianxiety drug, and anti-inflammatory activities [10-19]. Multiple reports indicates that arylaminophthalazine derivative (Figure 1, A) act as inhibitor of vascular endothelial growth factor (VEGFR-2) has entered clinical testing against various cancers [20]. Also, a series of 1-(isoquinoline-5-yl)-4-arylaminophthalazine (Figure 1, B) were studied as a potent inhibitors of VEGFR and the later compound inhibit VEGFR-1, a related receptor tyrosine kinase [21,22]. γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain [23]. 3-Phenyl-6-(pyrid-2-ylmethloxy)-[1,2,4]triazolo[3,4-*a*] phthalazine (Figure 1, C) was identified as a class of GABA-A receptor ligands with large improvement in binding affinity due to hydrogen-bond-donating interaction from the receptor adjacent to that portion of the molecule [24] (Figure 1, C).

In view of the above aforementioned facts, the author, undertook synthesis of some newly benzo-fused ring of 4-benzylphthalazine, such derivatives could possess interesting and useful biological properties.

## 2. Experimental

### *2.1. Instrumentation*

Melting points were determined on a Stuart melting point apparatus and are uncorrected. IR spectra were recorded in KBr using a FT-IR 5300 spectrometer and Perkin Elmer spectrum RXIFT-IR system (ν, $cm^{-1}$). The $^1H$ NMR at 300 MHz and $^{13}C$ NMR spectra at 75 MHz were recorded in DMSO-$d_6$ on a Varian Mercury VX-300 NMR spectrometer. Chemical shifts (δ) are related to that of the solvent. Mass spectra were measured on a Shimadzu GC-MS-QP-1000 EX mass spectrometer at 70 eV. The elemental analyses were performed at the Microanalytical Center, Cairo University, Cairo, Egypt.

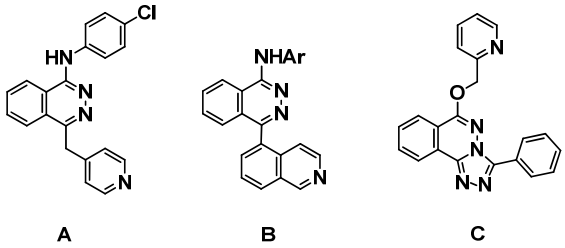


**Figure 1.** Structures of arylaminophthalazine (A and B) and triazolophthalazine derivatives (C).

### *2.2. General procedure for the synthesis of 4-benzyl-phthalazine derivatives* (2 and 3)

A mixture of 4-benzyl-1-chlorophthalazine (**1**) (0.25 g, 10 mmol) and thiosemicarbazide or thiocarbohydrazide (10 mmol) in absolute ethanol (30 mL) was refluxed for 3 hours. The solvent was evaporated in vacuum. The obtained solid was filtered off and washed with ethanol (Scheme 1).


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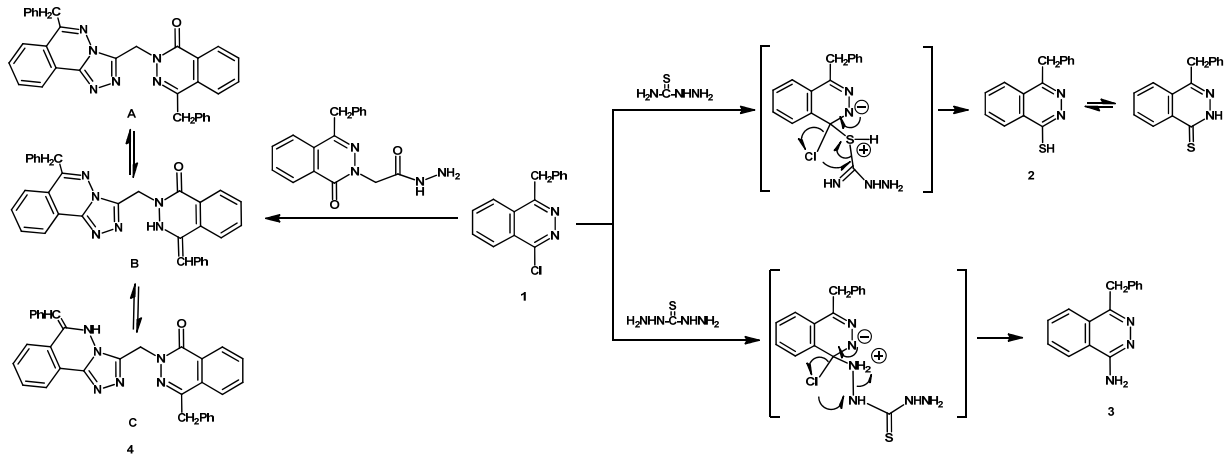


**Scheme 1**

*4-Benzylphthalazin-1-yl-thiol* (**2**): Yellow crystals. Crystallization from ethanol. Yield: 85%. M.p.: 160-162 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3150 (NH), 2908 (SH). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 14.32 (brs, 1H, SH, exchangeable by $D_2O$), 8.75 (d, 1H, Ar-H), 8.61 (brs, 1H, NH, cancelled by $D_2O$), 8.05-7.88 (m, 3H, Ar-H), 7.37-7.18 (m, 5H, Ar-H), 4.40 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 252 ($M^+$, 83.8), 251 (100), 220 (3.3), 219 (14.34), 218 (39.8), 191 (3.2), 165 (7.4), 89 (11.1), 76 (9.4). Anal. calcd. for $C_{15}H_{12}N_2S$: C, 71.40; H, 4.79; N, 11.10. Found: C, 71.33; H, 4.65; N, 11.04%.

*4-Benzylphthalazin-1-yl-amine* (**3**): Yellow crystals. Crystallization from ethanol. Yield: 65%. M.p.: 178-179 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3274, 3204 ($NH_2$), 1640 (C=N). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.65-7.28 (m, 9H, Ar-H), 5.25 (s, 2H, $CH_2Ph$), 4.48 (brs, 2H, $NH_2$, exchangeable by $D_2O$). MS (*m/z* (%)): 235 ($M^+$, 4.6), 107 (7.2), 106 (100), 105 (73.0), 76 (5.7). Anal. calcd. for $C_{15}H_{13}N_3$: C, 76.57; H, 5.57; N, 17.86. Found: C, 76.38; H, 5.36; N, 17.73%.

### *2.3. General procedure for the synthesis of triazolo-phthalazine derivatives* (4 and 5)

A mixture of 4-benzyl-1-chlorophthalazine (**1**) (0.25 g, 10 mmol) and (4-benzyl-1-oxo-1*H*-phthalazin-2yl)acetic acid hydrazide (0.30 g, 10 mmol) or cyanoacetohydrazide (0.09 g, 10 mmol) in absolute ethanol (30 mL) and 2 drops of TEA was refluxed for 3 hours. The solvent was evaporated in vacuum. The obtained solid was filtered off and washed with ethanol (Scheme 1 and 2).

*4-Benzyl-2-((6-benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl) methyl)phthalazin-1(2H)-one* (**4**): White crystals. Crystallization from dioxane. Yield: 85%. M.p.: 200-202 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3186 (NH), 3026 (C-H aromatic), 1650 (CO). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 11.35, 11.14 (2s, 1H, NH), 10.13, 9.93 (2s, 1H, NH), 7.90-7.15 (m, 18H, Ar-H), 5.31, 4.96 (2s, 1H, =CHPh), 4.33 (d, *J* = 4.5 Hz, 2H, $CH_2$), 4.16 (d, *J* = 11.7 Hz, 2H, $CH_2$). $^{13}C$ NMR (75 MHz, DMSO-$d_6$, δ, ppm): 167.77 (CO), 158.86, 144.87, 144.74, 143.83, 138.19, 136.77, 133.26, 131.27, 131.05, 128.79, 128.45, 128.39, 128.35, 128.29, 127.53, 127.46, 127.22, 126.42, 126.32, 125.68, 125.357, 125.136, 125.00, 123.58, 120.74, 120.69, 52.31 ($NCH_2C$), 37.66 ($CH_2Ph$), 37.56 ($CH_2Ph$). MS (*m/z* (%)): 508 ($M^+$, 95.6), 417 (100), (M-$CH_2Ph$), 361 (15.5), 259 (5.6), 249 (17.3), 92 (11.0), 91 (95.6). Anal. calcd. for $C_{32}H_{24}N_6O$: C, 75.57; H, 4.76; N, 16.52. Found: C, 75.34; H, 4.67; N, 16.42%.

*6-Benzyl([1,2,4]triazolo[3,4-a]phthalazin-3-yl)acetonitrile* (**5**): Yellow crystals. Crystallization from ethanol. Yield: 85%. M.p.: 160-162 °C. FT-IR (KBr, *v*, $cm^{-1}$): 2900 (CH aliphatic), 2250 (CN). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.48, 8.22 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 1H, Ar-H), 7.99-7.19 (m, 4H, Ar-H), 7.62, 7.28 (2d, *J* = 7.2 Hz, 2H, Ar-H), 4.72 (s, 2H, $CH_2Ph$), 4.57 (s, 2H, $CH_2CN$). MS (*m/z* (%)): 299 ($M^+$, 59.5), 298 (100), 271 (16.8), 205 (7.0), 128 (15.2), 102 (18.1), 91 (74.7), 66 (5.1), 53 (3.5). Anal. calcd. for $C_{18}H_{13}N_5$: C, 72.23; H, 4.38; N, 23.40. Found: C, 72.07; H, 4.20; N, 23.29%.

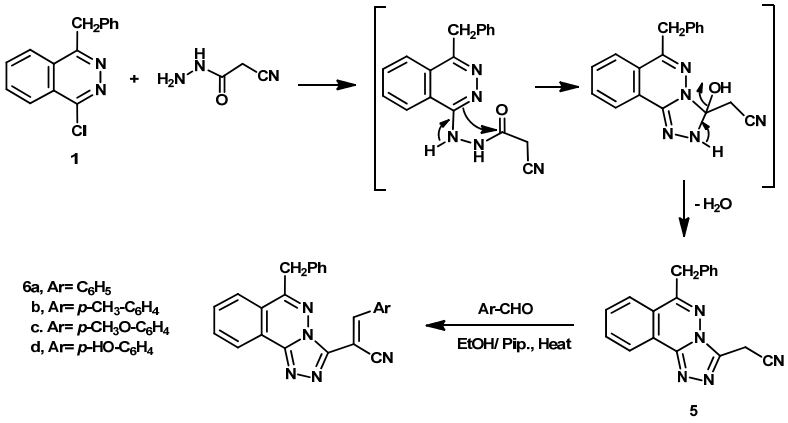
### *2.4. General procedure for the synthesis of triazolo-phthalazineacrylonitrile derivatives* (6a-d)

A mixture of (6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)aceto-nitrile (**5**) (0.29 g, 10 mmol) and aromatic aldehydes (10 mmol) in absolute ethanol (30 mL) and 2 drops of piperidine was refluxed for 2 hours. The solvent was evaporated in vacuum. The obtained solid was filtered off and washed with ethanol (Scheme 2).

*2-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-phenyl-acrylonitrile* (**6a**): Yellow crystals. Crystallization from dioxane. Yield: 80%. M.p.: 230-232 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3038 (CH-aromatic), 2917 (CH aliphatic), 2223 (CN). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.48, 8.22 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 1H, Ar-H), 8.00-7.22 (m, 9H, Ar-H), 7.64, 7.30 (2d, *J* = 7.2 Hz, 2H, Ar-H), 7.05 (s, 1H, =CH), 4.75 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 387 ($M^+$, 5.3), 386 (12.3), 91 (100). Anal. calcd. for $C_{25}H_{17}N_5$: C, 77.50; H, 4.42; N, 18.08. Found: C, 77.41; H, 4.35; N, 17.97%.

*2-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-p-tolyl-acrylonitrile* (**6b**): Yellow crystals. Crystallization from dioxane. Yield: 90%. M.p.: 258-260 °C. FT-IR (KBr, *v*, $cm^{-1}$): 2910 (CH aliphatic), 2216 (CN). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.49, 8.27 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.85 (d, *J* = 7.2, 1H, Ar-H), 8.02-7.21 (m, 8H, Ar-H), 7.63, 7.29 (2d, *J* = 7.2 Hz, 2H, Ar-H), 7.08 (s, 1H, =CH), 4.74 (s, 2H, $CH_2Ph$), 2.36 (s, 3H, $CH_3$). MS (*m/z* (%)): 401 ($M^+$, 40.6), 400 (100), 91 (70.7). Anal. calcd. for $C_{26}H_{19}N_5$: C, 77.79; H, 4.77; N, 17.44. Found: C, 77.65; H, 4.64; N, 17.37%.

*2-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-(4-meth-oxyphenyl)acrylonitrile* (**6c**): Yellow crystals. Crystallization from dioxane. Yield: 87%. M.p.: 264-266 °C. FT-IR (KBr, *v*, $cm^{-1}$): 2924 (CH aliphatic), 2216 (CN). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.45, 8.19 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.88 (d, *J* = 7.2, 1H, Ar-H), 8.11-7.24 (m, 8H, Ar-H), 7.61, 7.27 (2d, *J* = 7.2 Hz, 2H, Ar-H), 7.14 (s, 1H, =CH), 4.72 (s, 2H, $CH_2Ph$), 3.82 (s, 3H, $OCH_3$). MS (*m/z* (%)): 417 ($M^+$, 6.6), 416 (49.5), 91 (100). Anal. calcd. for $C_{26}H_{19}N_5O$: C, 74.80; H, 4.59; N, 16.78. Found: C, 74.63; H, 4.7; N, 16.66%.




**Scheme 2**

*2-(6-Benzyl[1,2,4]tri-azolo[3,4-a]phthalazin-3-yl)-3-(4-hydroxyphenyl)acrylonitrile* (**6d**): Yellow crystals. Crystallization from dioxane. Yield: 85%. M.p.: 298-300 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3432 (OH), 2920 (CH aliphatic), 2220 (CN). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 9. 80 (s, 1H, OH), 8.65, 8.41 (2d, *J* = 8.1 Hz, 2H, Ar-H), 8.25 (s. 1H, =CH), 7.79 (d, *J* = 7.2, 1H, Ar-H), 8.00-7.54 (m, 8H, Ar-H), 7.61, 7.32 (2d, *J* = 7.2 Hz, 2H, Ar-H), 4.69 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 403 ($M^+$, 13.8), 402 (48), 401 (51.4), 91 (100), 90 (81). Anal. calcd. for $C_{25}H_{17}N_5O$: C, 74.43; H, 4.25; N, 17.36. Found: C, 74.22; H, 4.09; N, 17.25%.

### *2.5. General procedure for the synthesis of triazolo-phthalazinechromene derivatives* (7-10)

A mixture of (6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)acetonitrile (**5**) (0.29 g, 10 mmol) and phenolic aldehydes (salicylaldehyde, 2-hydroxy-1-naphthaldehyde and 2,7-dihydroxy-1-naphthaldehyde) (10 mmol) in absolute ethanol (30 mL) and 2 drops of piperidine was refluxed for 2 hours. The obtained solid was filtered off and washed with ethanol (Scheme 3).

*3-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-2H-chromen-2-imine* (**7**): Yellow crystals. Crystallization from ethanol. Yield: 95%. M.p.: 266-268 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3228 (NH), 3056 (CH aromatic), 1642 (C=N), 2924 (CH aliphatic). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 10.20 (s, 1H, NH, exchangeable by $D_2O$), 9.20 (s, 1H, 4H, chromene), 8.48 (s, 1H, Ar-H), 8.63-7.23 (m, 12H, Ar-H), 4.71 (s, 2H, $CH_2Ph$). Anal. calcd. for $C_{25}H_{17}N_5O$: C, 74.43; H, 4.25; N, 17.36. Found: C, 74.16; H, 4.07; N, 17.23%.

*3-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-chromen-2-one* (**8**): Yellow crystals. Crystallization from DMF. Yield: 90%. M.p.: > 340 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3046 (CH aromatic), 2925 (CH aliphatic), 1732 (CO). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 9.20 (s, 1H, 4H, chromene), 8.48 (s, 1H, Ar-H), 8.63-7.23 (m, 12H, Ar-H), 4.71 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 404 ($M^+$, 100), 403 (99.9), 204 (11.3), 91 (60.4). Anal. calcd. for $C_{25}H_{16}N_4O_4$: C, 74.25; H, 3.99; N, 13.85. Found: C, 74.02; H, 3.84; N, 13.76%.

*2-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3H-benzo[f]chromen-3-imine* (**9a**): Yellow crystals. Crystallization from ethanol. Yield: 92%. M.p.: 276-278 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3248 (NH), 2928 (CH aliphatic), 1638 (C=N).$^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 10.03 (s, 1H, NH; exchangeable by $D_2O$), 9.46 (s, 1H, benzochromene-4-H), 8.6 (d, *J* = 8 Hz, 1H, Ar-H), 8.38 (d, *J* = 7.4 Hz, 1H, Ar-H), 8.16 (d, *J* = 9.3 Hz, 1H, Ar-H), 7.93 (d, *J* = 7.2 Hz, 1H, Ar-H) 8.07-7.15 (m, 11H, Ar-H), 4.73 (s, 2H, $CH_2Ph$). Anal. calcd. for $C_{29}H_{19}N_5O$: C, 76.81.; H, 4.22; N, 15.44. Found: C, 76.55; H, 4.13; N, 15.30%.

*2-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-imino-3H-benzo[f]chromen-9-ol* (**9b**): White crystals. Crystallization from DMF. Yield: 85%. M.p.: > 340 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3280 (NH), 3476 (OH), 1638 (C=N). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 10.09 (brs, 1H, NH, exchangeable by $D_2O$), 9.42 (brs, 1H, OH, exchangeable by $D_2O$), 9.28 (s, 1H, benzochromene - 4H), 8.61, 8.34 (2d, *J* = 7.8 Hz, 2H, Ar-H), 8.29-7.10 (m, 12H, Ar-H), 4.75 (s, 2H, $CH_2Ph$). Anal. calcd. for $C_{29}H_{19}N_5O_2$: C, 74.19; H, 4.08; N, 14.92. Found: C, 74.03; H, 3.95; N, 14.85%.

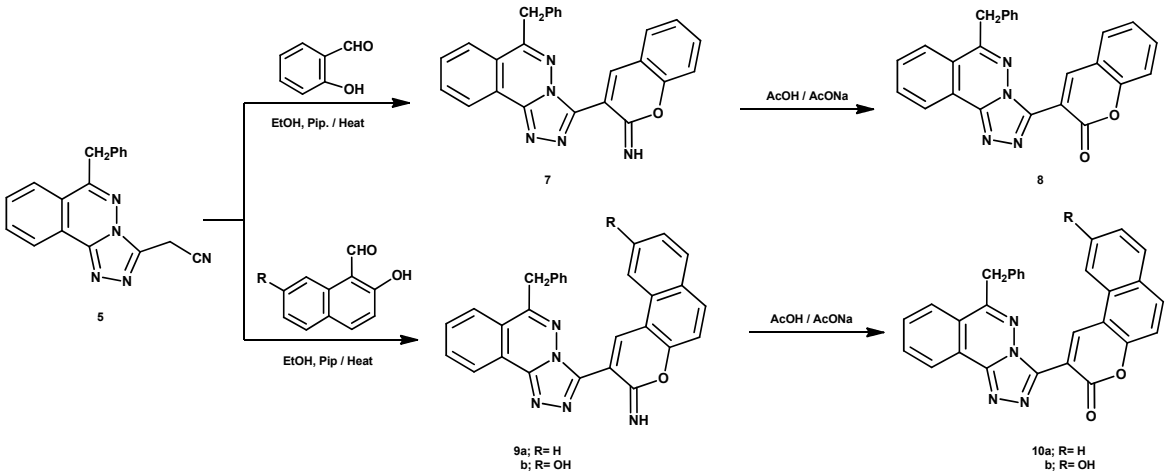
*2-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-benzo[f]chromen-3-one* (**10a**): Yellow crystals. Crystallization from dioxane. Yield: 90%. M.p.: 298-300 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3043, (CH aromatic), 1726 (CO). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 9.48 (s, 1H, benzochromene-4-H), 8.62 (d, *J* = 8 Hz, 1H, Ar-H), 8.37 (d, *J* = 7.4 Hz, 1H, Ar-H), 8.18 (d, *J* = 9.3 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.2 Hz, 1H, Ar-H) 8.08-7.16 (m, 11H, Ar-H), 4.74 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 454 ($M^+$, 100), 426 (25.9), 410 (3.9), 382 (3.3), 35 (3.3), 268 (3.5), 232 (3.6), 205 (2.2), 189 (5), 164 (5.1), 91 (27.2). Anal. calcd. for $C_{29}H_{18}N_4O_2$: C, 76.64; H, 3.99; N, 12.33. Found: C, 76.50; H, 3.87; N, 12.18 %.

*2-(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-9-hydroxy benzo[f]chromen-3-one* (**10b**): White crystals. Crystallization from DMF. Yield: 85%. M.p.: > 340 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3452 (OH), 2928 (CH aliphatic), 1720 (CO). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 9.50 (brs, 1H, OH, exchangeable by $D_2O$), 9.30 (s, 1H, benzochromene - 4H), 8.60, 8.35 (2d, *J* = 7.8 Hz, 2H, Ar-H), 8.29-7.12 (m, 12H, Ar-H), 4.76 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 470 ($M^+$, 3.09), 445 (35.26), 400 (13.61), 385 (19.59), 368 (15.05), 341 (27.01), 311 (11.55), 296 (34.43), 284 (29.07), 224 (8.87), 193 (49.9), 149 (39.59), 104 (100), 76 (30.93). Anal. calcd. for $C_{29}H_{18}N_4O_3$: C, 74.03; H, 3.86; N, 11.91. Found: C, 73.90; H, 3.73; N, 11.76%.

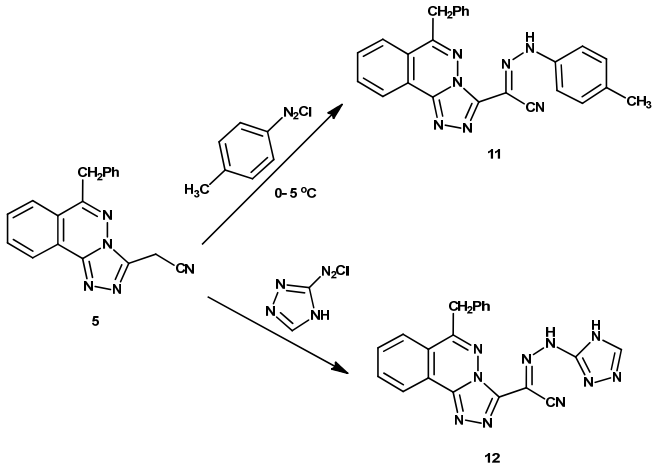
### *2.6. General procedure for the synthesis of phthalazine-hydrazo acetonitrile derivatives* (11 and 12)

To a stirred solution of (6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)acetonitrile (**5**) (0.29 g, 10 mmol) in ethanol (50 mL) containing, sodium acetate (3 g) *p*-tolyldiazonium salt or triazolediazonium salt (prepared by adding sodium nitrite (10 mmol) to *p*-toluidine (0.12 g, 10 mmol) or 1,2,4-triazol-3-amine (0.13 g, 10 mmol) in conc. HCl (6 mL) at 0~5 °C under stirring was added dropwise The reaction mixture was then left at room temperature for 2 hours. and the solid product formed was collected by filtration (Scheme 4).

*6-Benzyl-N'-(p-tolyl)-[1,2,4]triazolo[3,4-a]phthalazine-3-carbohydrazonoyl cyanide* (**11**): Yellow crystals. Crystallization from ethanol. Yield: 80%. M.p.: 172-174 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3082 (NH), 2220 (CN), 1590 (C=N).




**Scheme 3**




**Scheme 4**

$^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 14.01, 13.60 (2s, 2H, NH, exchangeable by $D_2O$), 8.63, 8.26(2d, $J$ = 7.2 Hz, 2H, Ar-H), 8.51, 8.33 (2d, $J$ = 8.1 Hz, 2H, Ar-H), 8.04- 7.18 (m, 9H, Ar-H), 4.72 (s, 2H, $CH_2Ph$), 1.32 (s, 3H, $CH_3$). MS (*m/z* (%)): 417 ($M^+$, 13.1), 416 (13.3), 388 (5.3), 203 (2.1), 128 (2.3), 103 (2.1), 91 (100), 90 (96.0), 77 (8.3), 76 (7.6), 65 (29.1). Anal. calcd. for $C_{25}H_{19}N_7$: C, 71.93; H, 4.59; N, 23.49. Found: C, 71.35; H, 4.38; N, 23.33%.

*(6-Benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-[(4H-[1,2,4] triazolo-3-yl)hydrazono]acetonitrile* (**12**): Orange crystals. Crystallization from ethanol. Yield: 80%. M.p.: 180-182 °C. FT-IR (KBr, $v$, $cm^{-1}$): 3076 (NH), 2224 (CN), 1658 (C=N). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 14.00, 13.60 (2s, 2H, NH, exchangeable by $D_2O$), 12.60 (s, 1H, triazolo-H), 8.64, 8.25 (2d, $J$ = 7.2 Hz, 2H, Ar-H), 8.52, 8.35 (2d, $J$ = 8.1 Hz, 2H, Ar-H), 8.04-7.18 (m, 5H, Ar-H), 4.72 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 394 (61.9), 393 (51.2), 286 (7.7), 231 (6.6), 129 (7.7), 128 (4.8), 103 (7.1), 91 (100). Anal. calcd. for $C_{20}H_{14}N_{10}$: C, 60.91; H, 3.58; N, 35.51. Found: C, 60.78; H, 3.45; N, 35.42%.

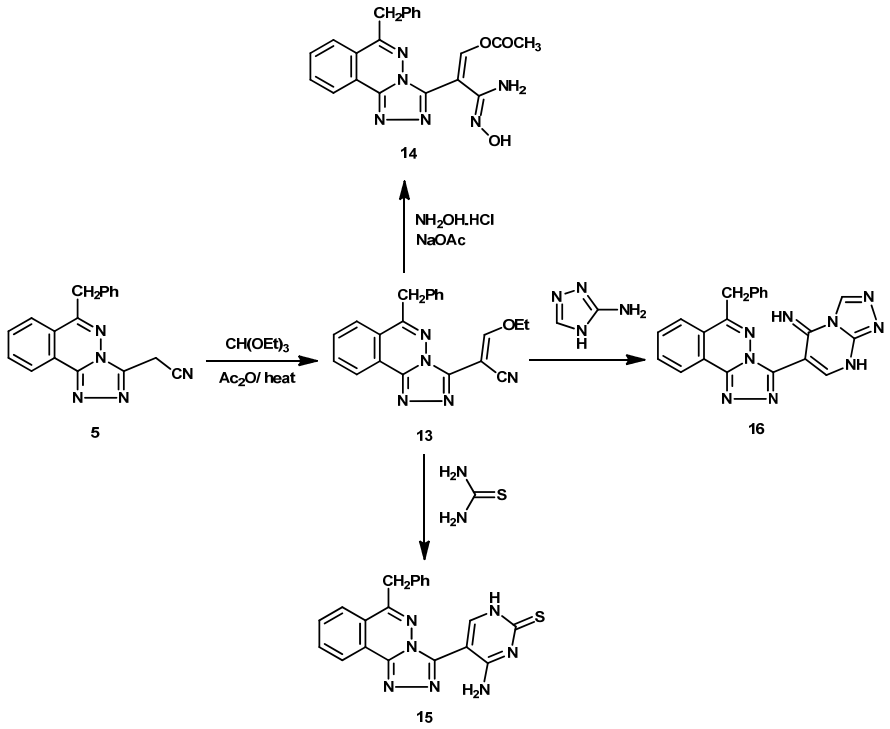
### *2.7. Synthesis of 2-(6-benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-ethoxyacrylonitrile* (13)

A mixture of (6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)acetonitrile (**5**) (10 mmol) and triethyl orthoformate in acetic anhydride (15 mL) was refluxed for 5 hours. The solvent was evaporated in vacuum. The solid product was collected by filtration and washed with ethanol. Yellow crystals. Crystallization from benzene (Scheme 5). Yield: 95%. M.p.: 170-171 °C. FT-IR (KBr, $v$, $cm^{-1}$): 3070 (CH-aromatic), 2936 (CH-aliphatic), 2256 (CN). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.25 (s, 1H, =CH), 8.53-7.19 (m, 9H, Ar-H), 4.72 (s, 2H, $CH_2Ph$), 4.28 (q, 2H, $CH_2CH_3$), 1.30 (t, 3H, $CH_2CH_3$). Anal. calcd. for $C_{21}H_{17}N_5O$: C, 70.97; H, 4.82; N, 19.71. Found: C, 70.82; H, 4.76; N, 19.58%.

### *2.8. General procedure for the synthesis of triazolo-phthalzine derivatives* (14 and 15)

A mixture of 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-3-ethoxyacrylonitrile (**13**) (10 mmol) and $NH_2OH{\cdot}HCl$ (15 m mol) or $NH_2CSNH_2$ (10 mmol) in ethanol (30 mL) fused sodium acetate (25 mmol) was added. The resulting mixture was refluxed for 3 hrs and then allowed at cool to room temperature and diluted with water (20 mL). The obtained solid was filtered off and washed with ethanol (Scheme 5).

*3-Amino-2-(6-benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-(hydroxyimino)prop-1-enyl acetate* (**14**): White crystals. Crystallization from ethanol. Yield: 80 %. M.p.: 220-222 °C. FT-IR (KBr, $v$, $cm^{-1}$): 3382 (OH), 3284, 3192 ($NH_2$), 1644 (CO), 1612 (C=N).




**Scheme 5**

$^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 10.15 (s, 1H, OH), 8.80 (s, 1H,=CH), 8.48, 8.22 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 1H, Ar-H), 8.14-7.23 (m, 4H, Ar-H), 7.62, 7.28 (2d, *J* = 7.2 Hz, 2H, Ar-H), 6.50 (br, 2H, $NH_2$), 4.74 (s, 2H, $CH_2Ph$), 3.37 (s, 3H, $COCH_3$). MS (*m/z* (%)): 402 ($M^+$, 100), 370 (80.9), 345 (57.4), 344 (53.2), 328 (40.4), 274 (85.1), 236 (59.6), 192 (21.3), 118 (21.8), 55 (36.2). Anal. calcd. for $C_{21}H_{18}N_6O_3$: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.56; H, 4.39; N, 20.74%.

*4-Amino-5-(6-benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-1H-pyrimidine-2-thione* (**15**): Yellow crystals. Crystallization from dioxane. Yield: 85%. M.p.: 275- 277 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3244 ($NH_2$), 3056 (NH), 1640 (C=S). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 9.32 (s,1H, NH), 8.67 (s, 1H,CH), 8.50, 8.24 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 1H, Ar-H), 7.99-7.19 (m, 4H, Ar-H), 7.62, 7.28 (2d, *J* = 7.2 Hz, 2H, Ar-H), 6.40 (br, 2H, $NH_2$), 4.72 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 385 ($M^+$, 19.4), 327 (100), 326 (14.7), 163 (15.3), 91 (17.9). Anal. calcd. for $C_{20}H_{15}N_7S$: C, 62.32; H, 3.92; N, 25.44. Found: C, 62.11; H, 3.76; N, 25.35%.

### *2.9. Synthesis of 6-(6-benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-8H-[1,2,4]triazolo[4,3-a]pyrimidin-5-ylideneamine* (16)

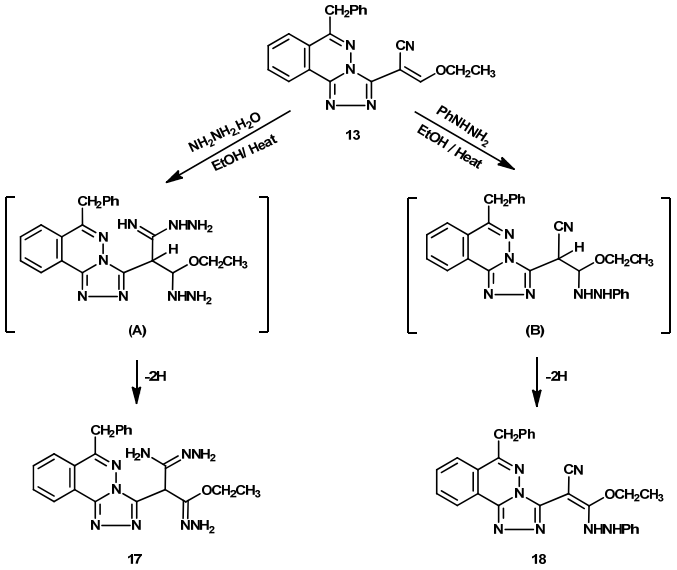
A mixture of 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-3-ethoxyacrylonitrile (**2**) (10 mmol) and 3-amino-1,2,4-triazolo (10 mmol) in DMF (30 mL) was refluxed for 3 hrs. The solvent was evaporated in vacuum. The solid product was collected by filtration and washed with ethanol. Brown crystals. Crystallization from dioxane (Scheme 5). Yield: 85%. M.p.: 280-282 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3184 (NH), 1632, 1602 (C=N). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 11.34 (s, 1H, CH=N-triazol), 10.02 (s,1H, NH), 8.88 (s, 1H, CH-pyrimidine), 8.48, 8.22 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 1H, Ar-H), 7.99-7.19 (m, 4H, Ar-H), 7.63, 7.29 (2d, *J* = 7.2 Hz, 2H, Ar-H), 5.70 (s, 1H, NH), 4.72 (s, 2H, $CH_2Ph$). MS (*m/z* (%)): 393 ($M^+$, 51.4), 90 (100). Anal. calcd. for $C_{21}H_{15}N_9$: C, 64.11; H, 3.84; N, 32.04. Found: C, 64.03; H, 3.76; N, 31.87%.

### *2.10. General procedure for the synthesis of hydrazonate and acrylonitrile derivatives* (17 and 18)

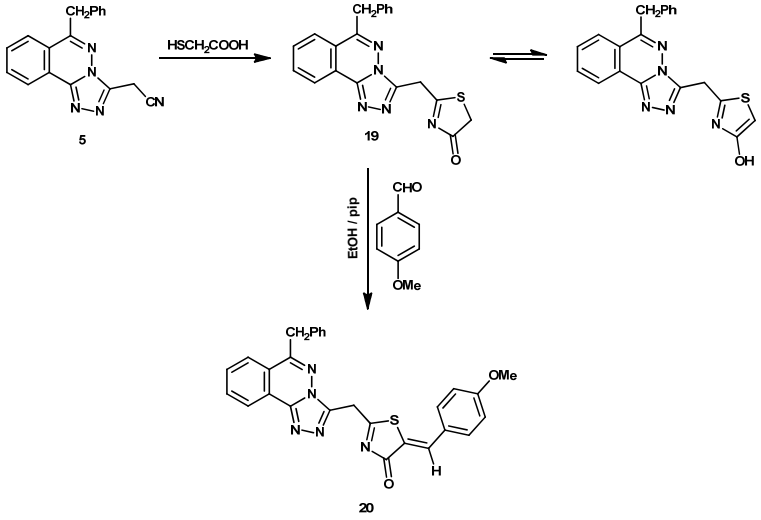
A mixture of 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-3-ethoxyacrylonitrile (**13**) (10 mmol) and hydrazine hydrate (20 mmol) or phenylhydrazine (10 mmol) in ethanol (30 mL). Solvent was removed by rotary evaporation and the obtained solid was filtered off and washed with dil. ethanol (Scheme 6).

*Ethyl 3-amino-2-(6-benzyl[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-hydrazonopropane hydrazonate* (**17**): While crystals. Crystallization from benzene. Yield: 75%. M.p.: 270- 272 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3198 (NH), 1598 (C=N). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.48 (s, 1H,CH), 8.50, 8.24 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 1H, Ar-H), 8.27-7.25 (m, 8H, Ar-H and $2NH_2$), 7.62, 7.28 (2d, *J* = 7.2 Hz, 2H, Ar-H), 6.48 (br, 2H, $NH_2$), 4.72 (s, 2H, $CH_2Ph$), 3.34 (q, *J* = 7.1 Hz, 2H, $CH_2$), 15.1 (t, *J* = 7.1 Hz, 3H,$CH_3$). MS (*m/z* (%)): 417 ($M^+$, 32.6), 371 ($M^+$-$C_2H_5OH$, 17.4), 339 ($M^+$- $C_2H_5OH$, $N_2H_4$, 63.6), 298 ($M^+$- $C_2H_5OH$, $N_2H_4$, $N_2CH$, 22.5), 272 (15.3), 234 (7.6), 128 (4.2), 91 ($PhCH_2^+$, 100). Anal. calcd. for $C_{21}H_{23}N_9O$: C, 60.42; H, 5.55; N, 30.20. Found: C, 60.20; H, 5.35; N, 30.05%.

*2-(6-Benzyl-[1,2,4]triazolo[3,4-a]phthalazin-3-yl)-3-ethoxy-3-(2-phenylhydrazinyl)acrylonitrile* (**18**): While crystals. Crystallization from dioxane. Yield: 70%. M.p.: 278- 280 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3212 (NH), 2198 (CN), 1526 (C=C), 1643 (C=N). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.56, 8.32 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.76 (d, *J* = 7.2, 1H, Ar-H), 8.30-7.41 (m, 10H, Ar-H and NH), 7.57, 7.21 (2d, *J* = 7.2 Hz, 2H, Ar-H), 6.78 (br, 1H, NH), 4.62 (s, 2H, $CH_2Ph$), 3.31 (q, *J* = 7.1 Hz, 2H, $CH_2$), 1.40 (t, *J* = 7.1 Hz, 3H, $CH_3$). MS (*m/z* (%)): 461 ($M^+$, 100), 371 ($M^+$-$C_7H_6$, 96.9), 298 ($M^+$-$C_7H_6$, -$CH_3OC$=N.$NH_2$, 40.9), 206 (13.4), 120 (18.1). Anal. calcd. for $C_{27}H_{23}N_7O$: C, 70.27; H, 5.02; N, 21.24. Found: C, 70.04; H, 4.89; N, 21.13%.



Scheme 6



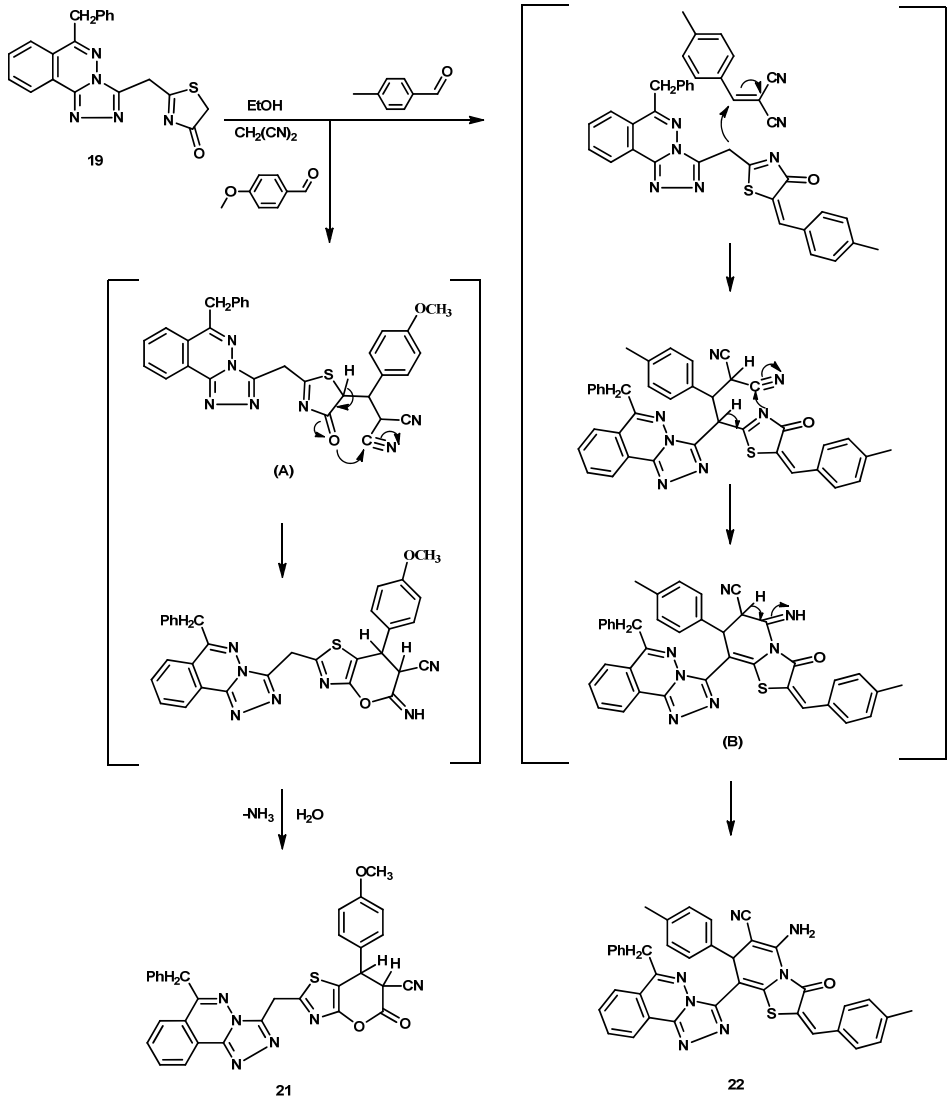
Scheme 7

### *2.11. Synthesis of 2-(6-benzyl[1,2,4]triazolo[3,4-a] phthalazin-3-ylmethyl)thiazol-4-one* (19)

A mixture of (6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)acetonitrile (**13**) (10 mmol) and thioglycolic acid (10 mmol) in pyridine (10 mL) was refluxed for 3 hours. The solvent was removed on rotary evaporation the solid obtained was filtered off, washed with ethanol. Gray crystals. Crystallization from dioxane (Scheme 7). Yield: 85%. M.p.: 260-262 °C. FT-IR (KBr, *v*, $cm^{-1}$): 2924 (CH-aliphatic), 1726 (CO). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 11.43 (s, 1H, OH, exchangeable by $D_2O$), 8.44, 7.92 (2d, 2H, Ar-H), 8.15 (d, 1H, Ar-H), 7.84-7.18 (m, 6H, Ar-H), 6.45 (s, 1H, CH-thiazolidinone), 4.55 (s, 2H, $CH_2Ph$), 4.08 (s, 2H, $CH_2$), 3.92 (s, 2H, $CH_2$). MS (*m/z* (%)): 373 ($M^+$, 13.1), 374 (M+1, 4.1), 375 (M+2, 2.6), 372 (1.9), 340 (2.4), 300 (2.1), 299 (3.4), 271 (0.6), 128 (2.1), 102 (4.5), 91 (100), 66 (7.5), 53 (2.8). Anal. calcd. for $C_{20}H_{15}N_5OS$: C, 64.33; H, 4.05; N, 18.75. Found: C, 64.20; H, 3.96; N, 18.62%.

### *2.12. Synthesis of 2-((6-benzyl-[1,2,4]triazolo[3,4-a] phthalazin-3-yl)methyl)-5-(4-methoxybenzylidene)thiazol-4-one* (20)

A mixture of 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-ylmeth-yl)thiazolidin-4-one (**19**) (10 mmol) and *p*-anisaldehyde (10 mmol) in ethanol (20 mL) and few drops of piperidine was refluxed for 3 hours. The mixture then cooled and the separated solid was filtered off washed with ethanol. Yellow crystals. Crystallization from dioxane (Scheme 7). Yield: 90%. M.p.: 280-282 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3058 (CH-aromatic), 2936 (CH-aliphatic), 1682 (CO), 1626 (C=N). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 9. 01 (s, 1H, =CH), 8.48, 8.22 (2d, *J*= 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 1H, Ar-H), 7.99-7.94, 7.86-7.83, 7.27-7.19 (m, 8H, Ar-H), 7.62, 7.28 (2d, *J* = 7.2 Hz, 2H, Ar-H), 4.72 (s, 2H, $CH_2Ph$), 3.74 (s, 3H, $OCH_3$), 3.90 (s, 2H, $CH_2$). MS (*m/z* (%)): 491 ($M^+$, 95.1), 459 (2.0), 300 (23.2), 299 (95.1), 193 (32.0), 165 (20.1), 164 (20), 149 (20.7), 91 (100). Anal. calcd. for $C_{28}H_{21}N_5O_2S$: C, 68.41; H, 4.31; N, 14.25. Found: C, 68.30; H, 4.22; N, 14.18%.



**Scheme 8**

### 2.13. *Synthesis of* 2-((6-benzyl-[1,2,4]triazolo[3,4-a] phthalazin-3-yl)methyl)-7-(4-methoxyphenyl)-5-oxo-6,7-dihydro-5H-pyrano[2,3-d]thiazole-6-carbonitrile (21)

A mixture of 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-ylmeth-yl)thiazolidin-4-one (**19**) (10 mmol) and *p*-anisaldehyde (10 mmol) and malononitrile (10 mmol) in absolute ethanol (30 mL) and few drops of piperidine was refluxed for 3 hours. The mixture then cooled and the separated solid was filtered off washed with ethanol (Scheme 8). Yellow crystals. Crystallization from dioxane. Yield: 80%. M.p.: 302-304 °C. FT-IR (KBr, *v*, $cm^{-1}$): 2924 (CH-aliphatic), 2190 (CN), 1690 (CO). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.42 (d, 1H, Ar-H), 8.25 (d, 1H, Ar-H), 7.97-7.66 (m, 11H, Ar-H), 5.38 (s, 2H, $CH_2Ph$), 4.72, 4.63 (2d, 2H, H-3 & H-4-pyran), 3.78 (s, 2H, -$CH_2$-), 3.58 (s, 3H, $OCH_3$). Anal. calcd. for $C_{31}H_{22}N_6O_3S$: C, 66.65; H, 3.97; N, 15.04. Found: C, 66.48; H, 3.81; N, 14.88%.

### 2.14. *Synthesis of* 5-amino-8-(6-benzyl-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-2-(4-methylbenzylidene)-3-oxo-7-(p-tolyl)-3,7-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-6-carbonitrile (22)

A mixture of 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl-methyl)thiazolidin-4-one (**19**) (10 mmol) and *p*-tolualdehyde (20 mmol) and malononitrile (10 mmol) in absolute ethanol / dioxane (30 mL) and few drops of piperidine was refluxed for 4 hours. The mixture then cooled and the separated solid was filtered off washed with ethanol (Scheme 8). Yellow crystals. Crystallization from DMF. Yield: 70%. M.p.: > 320 °C. FT-IR (KBr, *v*, $cm^{-1}$): 3428, 3338 ($NH_2$), 3026 (CH-aromatic), 2918 (CH-aliphatic), 2188 (CN), 1702 (CO). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 9.01 (s, 1H, =CH), 8.48, 8.22 (2d, *J* = 8.1 Hz, 2H, Ar-H), 7.81 (d, *J* = 7.2, 2H, Ar-H), 7.99-7.19 (m, 13H, Ar-H), 4.72 (s, 2H, $CH_2Ph$), 5.60 (br, 2H, $NH_2$, exchangeable by $D_2O$), 4.50 (s, 1H, pyridine), 3.72 (s, 3H, $CH_3$), 3.70 (s, 3H, $CH_3$). MS (*m/z* (%)): 643 (M+, 0.9), 615 (1.1), 579 (4.1), 476 (10.0), 408 (11.5), 299 (13.6), 148 (11.9), 91 (100). Anal. calcd. for $C_{39}H_{29}N_7OS$: C, 72.76; H, 4.54; N, 15.23. Found: C, 72.56; H, 4.32; N, 15.05%.

**Table 1.** Antimicrobial activity of the new compounds.

| Compound | Minimum inhibitory concentration (MIC) in µg/mL | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gram-negative bacteria | | Gram-positive bacteria | | | | Fungi | |
| | *P. aeruginosa* (MTCC 741) | *E. coli* (NCTC-10410) | *B. cereus* (ATGG 14579) | *B. subtilis* (MTCC 441) | *B. sphaericus* (MTCC 11) | *Staphylococcus* (MTCC 96) | *A.O. Wilhelm* (AUCC-230) | *P.C. Thom* (AUCC-530) |
| 2 | 500 | 500 | 500 | 200 | 500 | 200 | - | - |
| 3 | 500 | 250 | 500 | 500 | 250 | 500 | - | - |
| 4 | 25 | 25 | 50 | 25 | 50 | 25 | 125 | 100 |
| 5 | 200 | 500 | 250 | 500 | 250 | 250 | - | 500 |
| 6a | 250 | 200 | 200 | 500 | 250 | 200 | - | 500 |
| 6b | 500 | 250 | 500 | 500 | 500 | 250 | - | - |
| 6c | 500 | 500 | 200 | 250 | 500 | 250 | - | - |
| 6d | 200 | 200 | 250 | 200 | 250 | 200 | 500 | 250 |
| 7 | 250 | 200 | 500 | 500 | 250 | 500 | - | - |
| 8 | 25 | 50 | 50 | 25 | 25 | 50 | - | - |
| 9a | 500 | 500 | 200 | 250 | 500 | 500 | - | 500 |
| 9b | 500 | 500 | 250 | 250 | 200 | 500 | - | - |
| 10a | 50 | 25 | 25 | 100 | 100 | 25 | - | 250 |
| 10b | 25 | 100 | 50 | 50 | 25 | 50 | 100 | 100 |
| 11 | 250 | 250 | 250 | 200 | 500 | 500 | - | - |
| 12 | 50 | 50 | 100 | 100 | 25 | 25 | - | - |
| 13 | 250 | 200 | 200 | 500 | 250 | 500 | - | - |
| 14 | 500 | 250 | 500 | 500 | 200 | 500 | - | - |
| 15 | 500 | 500 | 500 | 250 | 500 | 500 | - | - |
| 16 | 25 | 25 | 50 | 25 | 50 | 50 | 125 | 100 |
| 17 | 500 | 500 | 250 | 200 | 500 | 250 | - | - |
| 18 | 250 | 250 | 500 | 500 | 500 | 200 | - | - |
| 19 | 25 | 25 | 50 | 25 | 50 | 50 | 125 | 100 |
| 20 | 50 | 50 | 25 | 50 | 50 | 25 | 100 | 100 |
| 21 | 25 | 25 | 25 | 25 | 100 | 100 | - | 500 |
| 22 | 100 | 25 | 50 | 100 | 50 | 50 | 100 | 100 |
| Ampicillin | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | - | - |
| Mycostatin | - | - | - | - | - | - | 31.25 | 31.25 |

### *2.15. Antimicrobial assay*

The antimicrobial activity of the newly synthesized compounds **2**-**22** were evaluated against two species of Gram-negative bacteria *Pseudomonas aeruginosa* (MTCC 741) and *Escherichia coli* (NCTC-10410); four Gram-positive bacteria, *Bacillus cereus* (ATGG 14579), *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 11) and *Staphylococcus* (MTCC 96); and two fungus, *Aspergillus ochraceus Wilhelm* (AUCC-230) and *Penicillium chrysogenum Thom* (AUCC-530) strains by disk diffusion method. Ampicillin and Mycostatin were used as standard drugs for the bacteria and fungi, respectively [27,28]. Preliminary screening of phthalazine derivatives and standard drugs was 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 **2**-**22** against all bacterial and fungal strains was determined by liquid dilution method. Stock solutions of tested compounds with 500, 250, 200, 100, 50, 25, 12.5, and 6.25 µg/mL concentrations were prepared with DMSO solvent. The solutions of standard drugs, Ampicillin and Mycostatin are used 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 and 0.2 mL of the inoculums was added. Further 3.8 mL of sterile water was added to each of the test tubes. These test tubes were incubated for 24 h at 37 °C and observed for the presence of turbidity. This method was repeated by changing phthalazine compounds with standard drugs Ampicillin and Mycostatin for comparison. The minimum inhibitory concentration at which no growth was observed was taken as the MIC value (Table 1).

## 3. Results and discussion

### *3.1. Synthesis*

Treatment of 4-benzyl-1-chlorophthalazine (**1**) [25] with thiosemicarbazide furnished 4-benzylphthalazin-1-ylthiol (**2**). While interaction of compound **1** with thiocarbohydrazide, 4-benzylphthalazin-1-ylamine (**3**) was the only isolable product. The formation of these products **2** and **3** pointed out that the less negative atom (S in case of thiosemicarbazide and terminal N in case of thiocarohydrazide) attacks the electrophilic carbon attached to Cl and = N groups (Scheme 1).

Interaction of (4-benzyl-1-oxo-1*H*-phthalazin-2-yl)acetic acid hydrazide [26] with compound **1** afforded the corresponding 4-benzyl-2-((6-benzyl[1,2,4]-triazolo-[3,4-*a*]phthalazin-3-yl)methyl)phthalazin-1(2*H*)-one (**4**) (Scheme 1).

The structure of compounds **2**-**4** were confirmed by IR, $^1$H NMR, $^{13}$C NMR and MS. The IR spectra of compound **2** showed ν at 2908 cm$^{-1}$ (SH), for compound **3** showed ν at 3274, 3204 cm$^{-1}$ ($NH_2$), 1640 cm$^{-1}$ (C=N), respectively. $^1$H NMR spectra of compound **2** showed *δ* at 8.61 (brs, 1H, NH), 14.32 (s, 1H, SH), for compound **3** showed *δ* at 4.48 (brs, 2H, $NH_2$), for compound **4** indicates that its structure is a mixture of three tautomeric forms (**A**) , (**B**) and (**C**) in different contributions showed *δ* at 11.14, 11.35 (2s, 1H, NH), 10.13, 9.93 (2s, 1H, NH), respectively.

$^{13}$C NMR spectra of compound **4** showed *δ* at 167.77 (CO), 52.31 ($NCH_2C$), 37.66, 37.56 ($CH_2Ph$). The mass spectra of compounds **2**-**4** showed the corresponding molecular ion peaks at *m/z* **=** 252 ($M^+$, 83.8), *m/z* = 235 ($M^+$, 4.6) and *m/z* = 508 ($M^+$, 95.6), respectively.

Reaction of compound **1** with cyanoacetohydrazide to give the corresponding (6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)acetonitrile (**5**). Treatment of compound **5** with aromatic aldehydes in boiling ethanol in the presence of piperidine as a catalyst afforded the corresponding triazolophthalazin-3-ylacrylonitrile derivatives, **6a**-**d** (Scheme 2). The structure of compounds **5** and **6** were confirmed by IR, $^1$H NMR and MS.

Condensation of compound **5** with phenolic aldehydes (salicylaldehyde, 2-hydroxy-1-naphthaldehyde and 2,7-dihydroxy-1-naphthaldehyde) under Knoevenagel reaction conditions afforded 3-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-2*H*-chromen-2-imine (**7**), 2-(6-benzyl[1,2,4]triazolo [3,4-*a*]phthalazin-3-yl)-3*H*-benzo[*f*]chromen-3-imine (**9a**) and 2-(6-benzyl[1,2,4]triazolo-[3,4-*a*]phthalazin-3-yl)-3-imino-3*H*-benzo[*f*]chromen-9-ol (**9b**), respectively (Scheme 3). Refluxing compound **7** and **9a**,**b** in glacial acetic acid/sodium acetate gave

3-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-chromen-2-one (**8**), 2-(6-benzyl[1,2,4]triazolo-[3,4-*a*]phthalazin-3-yl)-3-imino-3*H*-benzo[*f*]chromen-9-ol (**9b**) and 2-(6-benzyl[1,2,4] triazolo[3,4-*a*]phthala-zin-3-yl)-9-hydroxybenzo[*f*]chromen-3-one (**10b**), respectively (Scheme 3).

The structure of compounds **7**-**10** were confirmed by IR, $^1$H NMR and MS. The IR spectra of compound **7** showed ν at 3228 cm$^{-1}$ (NH), for compound **8** showed ν at 1732 cm$^{-1}$ (CO), for compound **9b** showed ν at 3280 cm$^{-1}$ (NH), 3476 cm$^{-1}$ (OH), for compound **10a** showed ν at 1726 cm$^{-1}$ (CO), compound **10b** showed ν at 3452 cm$^{-1}$ (OH), 1720 cm$^{-1}$(CO). $^1$H NMR spectra of compound **7** showed *δ* at 10.20 (s, 1H, NH), for compound **9a** showed *δ* at 10.03 (s, 1H, NH), for compound **9b** showed *δ* at 10.09 (brs, 1H, NH), 9.42 (brs, 1H, OH), respectively. The mass spectra of compounds **8** and **10a**,**b** and showed the corresponding molecular ion peaks at *m/z* = 404 (M$^+$, 100), *m/z* = 454 (M$^+$, 100), *m/z* = 470 (M$^+$, 3.09), respectively.

Since, compound **5** contains very reactive methylene group, so, this derivative underwent coupling with equimolar amount of *p*-tolyldiazonium chloride in ethanol and sodium acetate at (0-5 °C), to afford a colored product which was identified as (6-benzyl-*N*'-(*p*-tolyl)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-carbo-hydrazonoyl cyanide (**11**). In the same manner, triazolo-phthalazin-3-ylacetonitrile (**5**) couples with a buffered solution of 1*H*-[1,2,4]triazolo-3-yldiazonium chloride to afford the corresponding (6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-[(4*H*-[1,2,4]triazolo-3-yl)hydrazono]acetonitrile (**12**) (Scheme 4).

The structure of compounds **11** and **12** were confirmed by IR, $^1$H NMR and MS. The IR spectra of compound **11** showed ν at 3082 cm$^{-1}$ (NH), 2220 cm$^{-1}$ (CN), for compound **12** showed ν at 3076 cm$^{-1}$ (NH, br), 2224 cm$^{-1}$ (CN), respectively. $^1$H-NMR spectra of compound **12** showed *δ* at 14.00, 13.60 (s, 2H, NH), 12.60 (s, 1H, triazolo-H). The mass spectra of compounds **11** and **12** and showed the corresponding molecular ion peaks at *m/z* = 417 (M$^+$, 13.1), *m/z* = 394 (M$^+$, 61.9), respectively.

Reaction of compound **5** with triethyl-orthoformate in boiling acetic anhydride to give 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-3-ethoxyacrylonitrile (**13**) and treatment of compound **13** with hydroxylamine hydrochloride in boiling ethanol-fused sodium acetate gave 3-amino-2-(6-benzyl[1,2,4] triazolo[3,4-*a*]phthalazin-3-yl)-3-(hydroxyimino)prop-1-enyl acetate (**14**). The formation of compound **14** can be attributed to the nucleophilic addition of $NH_2OH$ to the CN group and nucleophilic exchange of the ethoxy group by the acetoxy nucleophile. Compound **13** undergoes cycloaddition with thiourea to furnished the corresponding 4-amino-5-(6-benz-yl [1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-1*H*-pyrimidine-2-thione (**15**) (Scheme 5). Similarly, compound **5** reacted with 3-amino-1,2,4-triazole to yield the corresponding 6-(6-benzyl[1,2,4] triazolo[3,4-*a*]phthalazin-3-yl)-8*H*-[1,2,4]triazolo[4,3-*a*]pyrimi-din-5-ylideneamine (**16**) (Scheme 5).

The structure of compounds **13**-**16** were confirmed by IR, $^1$H NMR and MS. The IR spectrum of compound **13** showed absorptions at 2256 cm$^{-1}$ (CN), while for compound **14** the characteristic bands were at 3382 cm$^{-1}$ (OH), 3284, 3192 cm$^{-1}$ ($NH_2$), compound **16** at 1602, 1632 cm$^{-1}$ (C=N), 3184 cm$^{-1}$ (NH), and compound **15** at 1640 cm$^{-1}$ (C=S), 3244 cm$^{-1}$ ($NH_2$), 3056 cm$^{-1}$ (NH), respectively. The $^1$H-NMR of compound **13** showed characteristic signals at *δ* 1.310 (t, 3H, $CH_2CH_3$), 4.28 (q, 2H, $CH_2CH_3$), $CH_2$Ph (4.72, singlet signal), (7.19-8.53) (sets of multiplets, 9H, Ar-H), 8.25 (s, 1H, =CH), The mass spectra of compounds **14**-**16** showed the corresponding molecular ion peaks *m/z* = 402 (M$^+$, 100), *m/z* = 385 (19.4) and *m/z* = 393 (M$^+$, 51.4), respectively.

Treatment of 3-ethoxyacrylonitrile derivative (**13**) with hydrazine hydrate in ethanol at room temperature was unsuccessful, while when under reflux, the reaction was successful and the reaction product was identified as ethyl 3-amino-2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-3-hydrazonopropane hydrazonate (**17**).

The formation of such compound indicated that the reaction proceeds through nucleophilic addition of two hydrazine molecules to the vinyl and the nitrile groups leading to the formation of non-isolable intermediate (**A**), which in turn, undergoes spontaneous dehydrogenation rather than elimination of ethanol molecule to give compound **17** as the only isolable product (Scheme 6).

Also, treatment of compound **13** with ethanolic phenylhydrazine under the conditions of reflux gave the isolable product which was identified as 2-(6-benzyl-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-3-ethoxy-3-(2-phenyl hydrazinyl)acrylonitrile (**18**). The formation of this product could be explained through nucleophilic addition of one phenylhydrazine molecule to vinyl group followed by oxidation of the intermediate (**B**) to the final product (Scheme 6).

The structure of compounds **17** and **18** were confirmed by IR and MS. The IR spectrum of compound **17** showed absorptions at 3198 cm$^{-1}$ (NH), 1598 cm$^{-1}$ (C=N), while for compound **18** showed absorptions at 3212 (NH), 2198 cm$^{-1}$ (CN). The mass spectra of compounds **17** and **18** showed the corresponding molecular ion peaks *m/z* (%): 417 (M$^+$, 32.6) and 461 (M$^+$, 100), respectively.

Treatment of compound **5** with thioglycolic acid, the given reaction in Scheme 7 would occur, where 2-(6-benzyl[1,2,4] triazolo[3,4-*a*]phthalazin-3-ylmeth-yl)thiazol-4-one (**19**) is formed. Condensation of **19** with anisaldehyde in boiling ethanol/piperidine gave the corresponding 2-((6-benzyl-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)methyl)-5-(4-methoxybenzylidene)thiazol-4-one (**20**) (Scheme 7).

The structure of compounds **19** and **20** were confirmed by IR, $^1$H NMR and MS. The IR spectrum of compound **19** showed absorptions at 1726 cm$^{-1}$ (CO), while for compound **20** showed absorptions at 1682 cm$^{-1}$ (CO). $^1$H NMR spectra of compound **19** showed δ at 11.43 (s, 1H, OH, exchangeable by $D_2O$), 6.45 (s, 1H, CH-thiazolidinone), for compound **20** showed δ at 9. 01 (s, 1H, =CH), 3.74 (s, 3H, $OCH_3$). The mass spectra of compounds **19** and **20** showed the corresponding molecular ion peaks *m/z* = 373 (M$^+$, 13.1) and 491 (M$^+$, 95.1), respectively.

Treatment of compound **19** with a mixture of malononitrile and *p*-anisalodehyde in equimolar ratio in boiling ethanol-piperidine was successful and the corresponding 2-(6-benzyl[1,2,4]triazolo[3,4-*a*]phthalazin-3-ylmethyl)-5-oxo-6,7-dihydro-7-(4-methoxyphenyl)-5*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (**21**). The formation of compound **21** can be rationalized by addition of active methylene group of 4-thiazolinone **19** at the activated ethylenic double bond of benzylidene malononitrile forming an adduct (**A**) which undergoes intramolecular cyclization and spontaneous hydrolysis of the imino function into the carbonyl group under the experimental reaction conditions employed (Scheme 8). Compound **19** reacted with malononitrile and *p*-tolaldehyde in boiling ethanol-piperidine in molar ratio 1:1:2 to give 5-amino-8-(6-benzyl-[1,2,4]triazolo[3,4-*a*]phthalazin-3-yl)-2-(4-methyl benzylidene)-3-oxo-7-(*p*-tolyl)-3,7-dihydro-2*H*-thiazolo[3,2-*a*] pyridine-6-carbonitrile (**22**). The formation of compound **22** is rationalized by the condensation of two molecules of aromatic aldehyde with 4-thiazolinone and malononitrile, respectively followed by the nucleophilic addition of methylene bridge of the 1$^{st}$ derivative to the activated ethylenic double bond of the second one and the formed adduct (**B**) undergoes intramolecular cyclization into the final product **22** (Scheme 8).

The structure of compounds **21** and **22** were confirmed by IR, $^1$H NMR and MS. The IR spectrum of compound **21** showed absorptions at 2190 (CN), 1690 cm$^{-1}$ (CO), while for compound **22** showed absorptions at 2188 (CN) and 1702 cm$^{-1}$ (CO), respectively. The $^1$H NMR of compound **21** showed $\delta_H$ 8.42 (d, 1H, Ar-H), 8.25 (d, 1H, Ar-H), 7.97- 7.66 (m, 11H, Ar-H), 5.38 (s, 2H, $CH_2$Ph), 4.72, 4.63 (2d, 3H, 4H-pyran), 3.78 (s, 2H, -$CH_2$-), 3.58 (s, 3H, $OCH_3$). The mass spectra of compound **22** showed the corresponding molecular ion peak *m/z* = 643 (M$^+$, 0.9).

### *3.2. Antimicrobial activity*

The antimicrobial activity of the newly synthesized compounds **2**-**22** were evaluated against two species of Gram-negative bacteria *Pseudomonas aeruginosa* (MTCC 741); *Escherichia coli* (NCTC-10410); and four Gram-positive bacteria, *Bacillus cereus* (ATGG 14579); *Bacillus subtilis* (MTCC 441); *Bacillus sphaericus* (MTCC 11); *Staphylococcus* (MTCC 96); and two fungus, *Aspergillus ochraceus Wilhelm* (AUCC-230) and *Penicillium chrysogenum Thom* (AUCC-530) strains by disk diffusion method. Ampicillin and Mycostatin were used as standard drugs for the bacteria and fungi, respectively.

The comparison of the MICs (in $\mu$g/mL) of potent compounds and standard drugs against tested strains are presented in the (Table 1). Investigation of the antibacterial screening data (Table 1) showed that some of the compounds were active against some pathogenic bacteria. Compounds **4**, **8**, **10b**, **16, 19** and **21** exhibited good activity against *Pseudomonas aeruginosa,* while compounds **4**, **10a**, **16**, **19**, **21** and **22** exhibited good activity against *Escherichia coli* and compounds **10a**, **20** and **21** exhibited good activity against *Bacillus cereus*. In addition, compounds **4**, **8**, **16**, **19** and **21** exhibited good activity against *Bacillus subtilis* and compounds **8**, **10b** and **12** exhibited good activity against *Bacillus sphaericus*, while compounds **4**, **10a**, **12** and **20** exhibited good activity against *Staphylococcus*.

The antifungal results (Table 1) revealed that the synthesized compounds showed variable degrees of inhibition against the tested fungi. Compounds **4**, **10b**, **16**, **19**, **20** and **22** possessed moderate antifungal activity against *Aspergillus ochraceus Wilhelm* and *Penicillium chrysogenum Thom.*

## 4. Conclusion

Our interest in synthesis of heterocyclic compounds is to focus on their antimicrobial activity as a part of our program which aimed at the development of new and more potent antimicrobial agents. Thus, in this paper, we revealed the synthesis of some triazolophthalazine derivatives and antimicrobial evaluation of all the novel compounds. The structures of the novel compounds were elucidated on the basis of IR, $^{1}$H NMR, $^{13}$C NMR and MS data. A series of novel triazolophthalazine derivatives were prepared. The antimicrobial activity of these compounds was evaluated against various Gram-positive, Gram-negative bacteria and fungi. Triazolophthalazine and triazolophthalazinethiazole derivatives are strong activities against any of the test microorganisms.

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