

## Synthesis and Biological Evaluation of Some 1, 2, 4-Triazoles and [1, 2, 4]Triazolo[4, 3-*b*]-Pyridazine Derivatives

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**Abstract:** The synthesis and structure characterization of 1, 2, 4-triazoles and [1, 2, 4]triazolo[4, 3-*b*]pyridazine derivatives 2-10, 12-16 were prepared starting with 2-(5-phenyl-3*H*-1, 2, 4-triazol-3-ylsulfanyl)-acetohydrazide 1 and 11 as starting materials. Moreover, the prepared compounds were evaluated as antimicrobial agents and the results revealed that, compounds 4 and 6 revealed promising antimicrobial activities compared to the activity of the commonly used antimicrobial drug, Ciprofloxacin and Ketoconazole.

**Key words:** Triazoles • Triazolo[4, 3-*b*]pyridazine • Antimicrobial activity

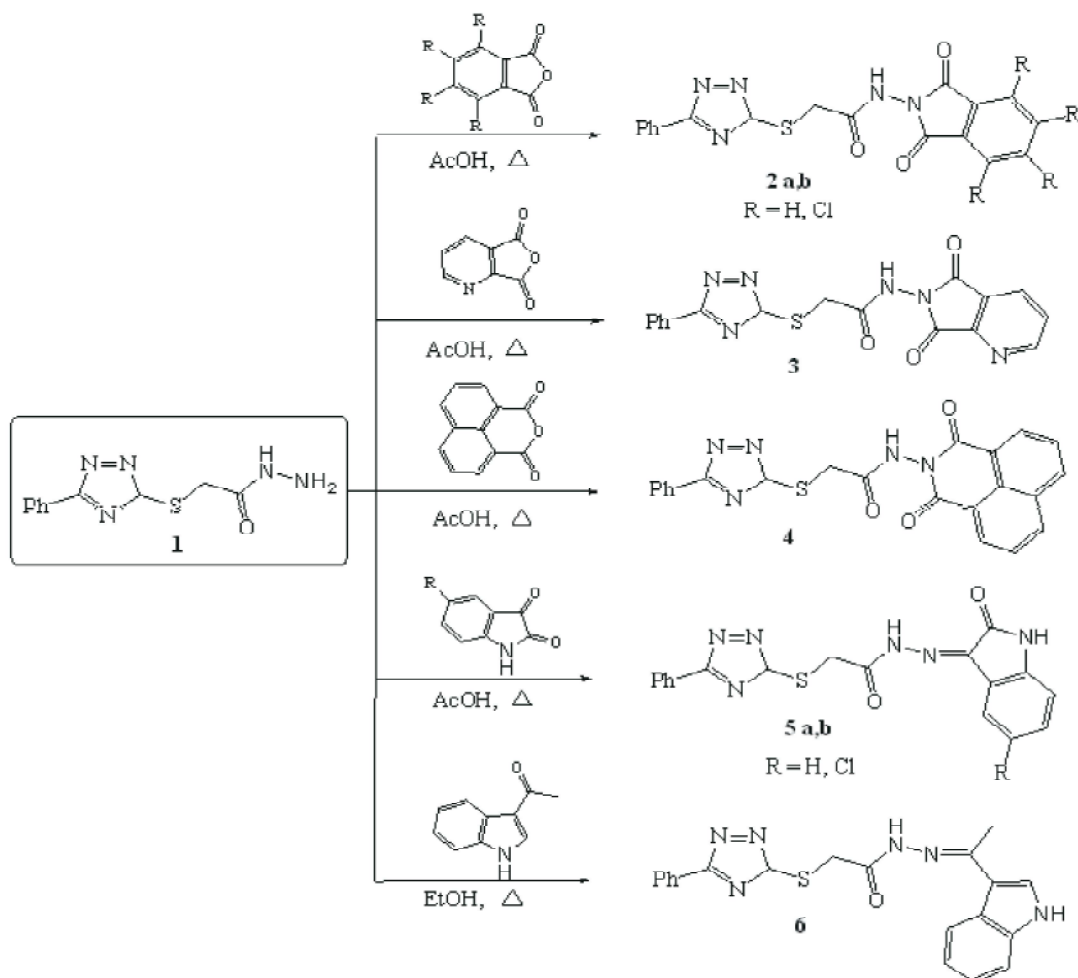
### INTRODUCTION

In the last few decades, the chemistry of 1, 2, 4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance [1-4]. For example, a large number of 1, 2, 4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants sedatives, antianxiety, antimicrobial agents [5] and anti-mycotic activity such as fluconazole, voriconazole [6]. Also, there are some known drugs containing the 1, 2, 4-triazole group [7]. Moreover, sulphur containing heterocycles represent an important group of sulphur compounds that are promising for use in practical applications [8]. Among these heterocycles, the mercapto- and thione-substituted 1, 2, 4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives, such as antibacterial [9], antifungal [10], antitubercular [11], antimycobacteria [12], anticancer [13], diuretic [14]. Based on the above mentioned research results, the goal of this study is to synthesize some novel 1, 2, 4-triazole thiol and their fused 1, 2, 4-triazole to obtain new compounds which are

expected to possess notable pharmacological applications.

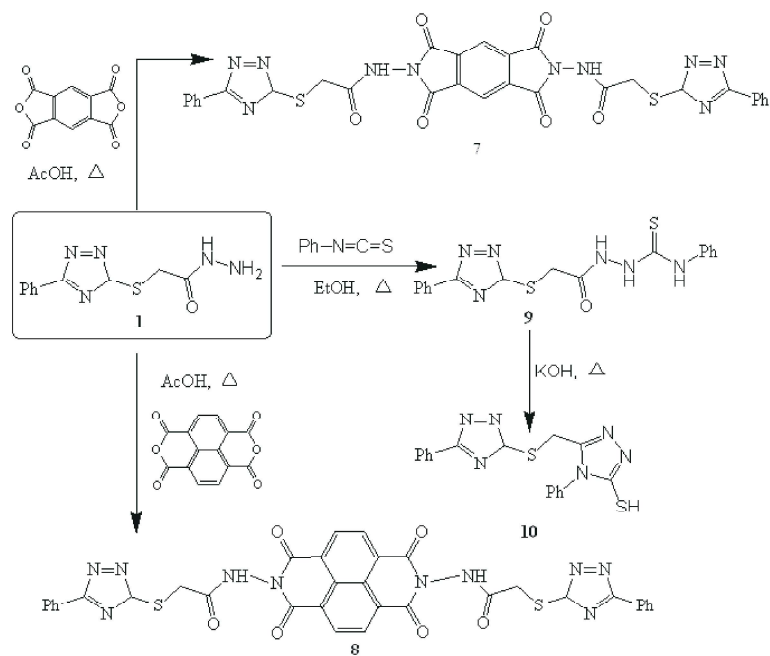
### RESULTS AND DISCUSSION

**Chemistry:** In this work the phenyl-1, 2, 4-triazole thiol has proved to be a rich source of various heterocyclic compounds. The starting material 2-(5-phenyl-3*H*-1, 2, 4-triazol-3-ylsulfanyl)acetohydrazide 1 was prepared in good yield according to the reported procedure [15] and treated with an equimolar amount of phthalic anhydride or 1, 2, 4, 5 tetrachlorophthalic anhydride, in glacial acetic acid, to give compound acetamide derivatives 2a, b, respectively. The structure of compounds 2a,b was supported by elemental analysis and data, especially the absence of the NH<sub>2</sub> group and the presence of C=O groups in IR spectra. Similarly, treatment of compound 1 with quinolinic anhydride or naphthalene-1, 8-dicarboxylic acid anhydride, in glacial acetic, afforded 3 or 4, respectively. Also, when the hydrazide 1 was reacted with indoline-2, 3-dione, 5-chloro-indoline-2, 3-dione reacted and 3-acetyl indol, it afforded compounds 5a, b and 6, respectively. The <sup>1</sup>HNMR spectra for these compounds revealed signal characteristic for NH aliphatic and NH aromatic (cf. Experimental).

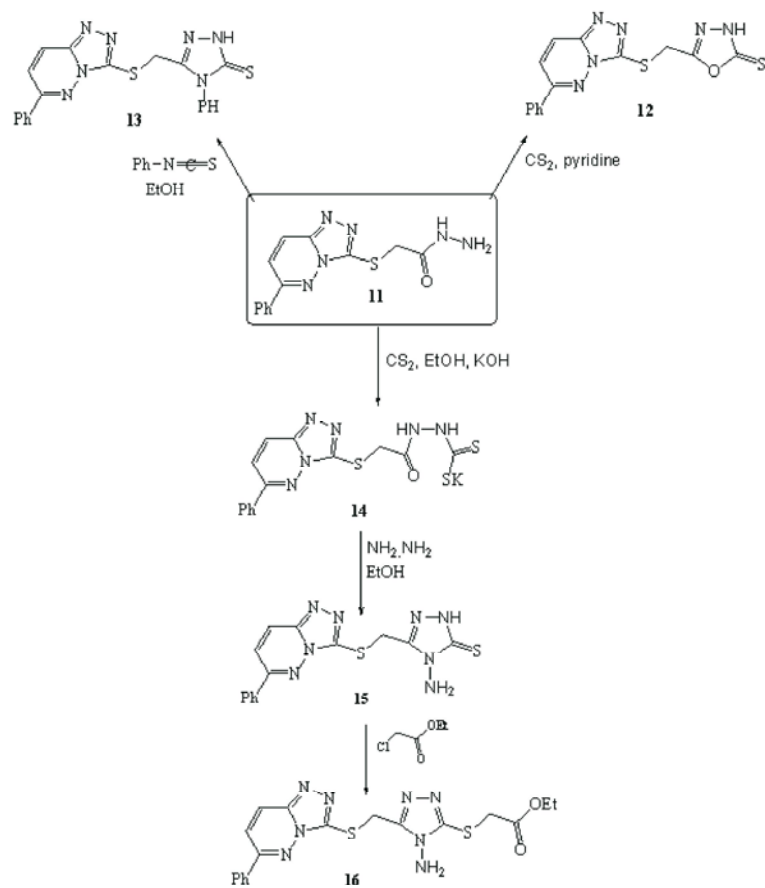


While, treatment of compound 1 with 1,2,4,5-benzenetetracarboxylic dianhydride and 1,4,5,8-naphthylenetetracarboxylic dianhydride, in glacial acetic acid, afforded bis-imide derivatives 7 and 8, respectively. The structure of compounds 7 and 8 was confirmed by elemental analysis and the spectral data. The mass spectrum of compound 8 showed molecular ion peak at  $m/z$  730 ( $M^+$ , 3.85%). Furthermore, the acid hydrazide 1 was reacted with phenyl isothiocyanate to afford semicarbazide derivatives 9. The  $^1\text{H}$  NMR spectrum for latter compound revealed signals characteristic for 3NH groups and  $^{13}\text{C}$  NMR spectrum showed signals at 182 ppm for (C=S). When the compound 9 was stirred in potassium hydroxide solution, it afforded 1,2,4-triazole-3-thiol derivative 10. The structure of compound 10 was confirmed by the absence of the NH groups and the presence of SH signal in  $^1\text{H}$  NMR spectrum and signal at 165.1 for (triazole SH) in  $^{13}\text{C}$  NMR spectrum (cf. Experimental). On the other side, a solution of

compound 11 [16] was treated with carbon disulfide in pyridine to afford oxadiazolethione 12. The IR spectrum of 12 showed the absence of the  $\text{NH}^2$ , and the presence of (C=S) and mass spectrum showed molecular ion peak at  $m/z$  342.31 ( $M^+$ , 1.38%) with a base peak at  $m/z$  55.11. Also, a solution of compound 11 was treated with phenyl isothiocyanate in pyridine to afford triazolethione 13. While, treatment of a solution of compound 11 with carbon disulfide in alcoholic potassium hydroxide afforded potassium salt 14. Treatment of this salt with hydrazine hydrate in absolute ethanol afforded the corresponding triazolethione 15. When compound 15 was treated with ethylchloroacetate in absolute ethanol, it afforded ethyl ester derivative 16. The IR spectrum of compound 16 showed the absence of C=S, and the presence of (C=O) and ( $\text{NH}_2$ , broad band). Also, the mass spectrum showed molecular ion peak at  $m/z$  443.28 ( $M^+$ , +1, 4.50%) with a base peak at  $m/z$  83.10 (cf. Experimental).



Scheme 2



Scheme 3

### Biological Activity

**Antimicrobial Screening:** The antibacterial activity of the synthesized compounds was tested against *Escherichia coli* NRRL B-210 (Gram -ve bacteria), *Bacillus subtilis* NRRL B-543 (Gram +ve bacteria), *Staphylococcus aureus* NRRL B-313 using nutrient agar medium. The antifungal activity of the compounds was tested against *Candida albicans* NRRL Y-477 using Sabouraud dextrose agar medium.

**Agar Diffusion Medium [17]:** A suspension of the organisms were added to a sterile nutrient agar media at 45°C and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer. An amount of 0.1ml of the synthesized compounds was poured inside the holes. A hole filled with DMSO was also used as control. The plates were left for 1 hour at room temperature as a period of pre-incubation diffusion to minimize the effects to variation in time between the applications of the different solutions. The plates were then incubated at 37°C for 24 hours and observed for antibacterial activity. The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin (50 µg/ml) and Ketoconazole (50 µg/ml) were used as standard for antibacterial and antifungal activity, respectively. The observed zone of inhibition is presented in Table 1.

**Minimum Inhibitory Concentration:** Minimum Inhibitory Concentration (MIC) of the tested compounds was determined by agar streak dilution method. 68 mg/ml stock solution of the synthesized compounds were made using DMSO as the solvent. From this stock solution, the following concentrations (0.17; 0.34; 0.68; 0.85 and 1.7 mg/ml) of the solutions of the tested compounds were mixed with the known quantities of molten sterile agar media aseptically. About 20 ml of the media containing the tested compound was dispensed into each sterile Petri dish. Then the media were allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking all the plates were incubated at 37°C for 24 h/48 h. for bacterial and fungus activity, respectively. Then the plates were observed for the growth of microorganisms. The lowest concentration of the synthesized compounds inhibiting the growth of the given bacteria/fungus was considered as minimum inhibitory concentration (MIC) of the test compounds against that bacteria or fungi on the plate. The MIC values of each compound against bacteria and fungus were tabulated in Table 2. The antimicrobial activity of some newly synthesized compounds 2a, 3, 4, 5b, 6 and 10 were tested and the results were shown in Table 1. Among the tested compounds, it was noticed that compound 4 showed more significant activity against Gram positive bacteria. While, compound 6 demonstrated inhibitory activity against Gram negative bacteria and fungi.

Table 1: Inhibition zone in mm as a criterion of antibacterial and antifungal activities of the newly synthesized compounds.

Comp. No.	Inhibition zone in mm			
	Gram positive bacteria		Gram negative bacteria	Fungi
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
2a	22	-ve	14	-ve
3	20	21	20	-ve
4	24	23	23	-ve
5a	10	13	-ve	-ve
5b	22	20	20	17
6	21	-ve	25	22
7	12	11	16	-ve
8	13	21	23	-ve
10	22	21	24	22
Ciprofloxacin (50 µg/ml)	23	23	25	-ve
Ketaconazole (50µg/ml)	-ve	-ve	-ve	23

Highly active (inhibition zone > 20 mm), Moderately active (inhibition zone 16-19.2 mm), Slightly active (inhibition zone 11-15 mm), (-) no inhibition zone

Table 2: MIC in mg/ml of the newly synthesized compounds

Compound no.	Minimum inhibitory concentration in mg/ml			
	Gram positive bacteria		Gram negative bacteria	Fungi
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
2a	0.25	0.15	0.05	0.11
3	0.15	0.16	0.09	0.07
4	0.13	0.16	0.15	0.06
5b	0.11	0.13	0.05	0.05
6	0.19	0.15	0.02	0.06
10	0.21	0.21	0.04	0.05
Reference drugs				
Ciprofloxacin (µg/ml)	0.12	0.15	0.01	-
Ketoconazole (µg/ml)	-	-	-	0.03

## Experimental Section

**Chemistry:** All melting points are uncorrected and measured using Electro-Thermal IA 9100 apparatus (Shimadzu, Japan). Infrared spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were determined on a Jeol-Ex-400 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part per million; (δ values, ppm) against TMS as internal reference. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo Electron Corporation, USA). Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalysis Unit and the results were within the accepted range (± 0.20) of the calculated values. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoated aluminum sheets (Type 60 F<sub>254</sub>; Merck, Darmstadt, Germany). The above spectral data were made at central labs of faculty of science, Saudi Arabia and the Regional Centre for Mycology and Biotechnology, Egypt. Compounds 1 and 11 were prepared according to reported methods, compound 1 (m.p 148-151 °C and *lit.* m.p 152-155°C [15]) and compound 11 (m.p 297-300 °C and *lit.* m.p 299-300°C [16])

**Synthesis of 2-5: General Procedure:** A mixture of compound 1 (1 mmol) and phthalic anhydride, tetrachlorophthalic anhydride, quinolinic anhydride, naphthalene-1, 8-dicarboxylic acid anhydride, indoline-2, 3-dione, or 5-chloroindoline-2, 3-dione (1 mmol) in glacial acetic acid (50 ml) was refluxed for 2-4 h. The formed precipitates were filtered on hot, dried and recrystallized from appropriate solvents to give compounds 2-5, respectively.

**2-(5-Phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)-N-(1, 3-dioxoisindolin-2-ylsulfanyl)acetamide (2a):** White crystals; (Yield 58 %); m.p. 191-193°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1680 (C=N triazole), 1740 (C=O), 1796 (C=O), 3201 (NH); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ ppm: 3.34 (s, 2H, SCH<sub>2</sub>), 4.47 (s, 1H, CH triazole), 7.69-7.95 (m, 7H, Ar-H), 11.17 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ ppm: 38.2 (aliphatic SCH<sub>2</sub>), 89.3 (triazole SCH), 122.1-138.2 (Ar-C), 154.4 (triazole), 164.5 (anhydride C=O), 174.6 (amide C=O); Anal. calcd. for C<sub>18</sub>H<sub>13</sub>N<sub>5</sub>O<sub>3</sub>S (379.07) (%): C, 56.98; H, 3.45; N, 18.46; S, 8.45. Found (%): C, 56.76; H, 3.68; N, 18.68; S, 8.29.

**2-(5-Phenyl-3H-1, 2, 4-triazole-3-sulfanyl)-N-(4, 5, 6, 7-tetrachloro-1, 3-dioxoisindolin-2-ylsulfanyl)acetamide (2b):** White crystals; (Yield 73 %); m.p. 185-187°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1638 (C=N triazole), 1748 (C=O), 1717 (C=O), 3315 (NH); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ ppm: 3.36 (s, 2H, SCH<sub>2</sub>), 4.43 (s, 1H, CH triazole), 7.69 (m, 7H, Ar-H), 11.3 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ ppm: 37.3 (aliphatic SCH<sub>2</sub>), 91.4 (triazole SCH), 122.2-140.2 (Ar-C), 160.2 (triazole), 165.8 (anhydride), 172.8 (C=O amide); Anal. calcd. for C<sub>18</sub>H<sub>9</sub>Cl<sub>4</sub>N<sub>5</sub>O<sub>3</sub>S (517.17) (%): C, 41.80; H, 1.75; N, 13.54; S, 6.20. Found (%): C, 42.05; H, 1.54; N, 13.76; S, 6.39.

**2-(5-Phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)-N-(5, 7-dioxo-5H-pyrrolo[3, 4-b]pyridin-6(7H)-yl-sulfanyl)acetamide (3):** White crystals; (Yield 51%); m.p. 182-184°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1604 (C=N triazole), 1640 (C=O), 1752 (C=O), 3198 (NH); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ ppm: 3.59 (s, 2H, SCH<sub>2</sub>), 4.48 (s, 1H, CH triazole), 7.80-7.94 (m, 7H, Ar-H), 11.00 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ ppm: 39.2 (aliphatic SCH<sub>2</sub>), 84.0 (triazol SCH), 123.0-138.3 (Ar-C), 150.3 (triazole C=N), 158.2 (anhydride C=O), 170.0 (amide C=O); Anal. calcd. for C<sub>17</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>S (380.38) (%): C, 53.68; H, 3.18; N, 22.09; S, 8.43. Found (%): C, 53.95; H, 3.31; N, 21.81; S, 8.62.

**(1, 3-Dioxo-1H-benzoisoquinolin-2(3H)-yl)-2-[(5-phenyl-3H-1, 2, 4-triazol-3-yl)sulfanyl]acetamide (4):** Pale yellow crystals; (Yield 56 %); m.p. 196-198°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1640 (C=N triazole), 1735 (C=O), 1774 (C=O), 3277 (NH); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 3.32 (s, 2H, SCH<sub>2</sub>), 4.51 (s, 1H, CH triazole), 7.68-7.94 (m, 5H, Ar-H), 8.54-8.57 (m, 9H, naphthalene), 11.20 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 38.5 (aliphatic SCH<sub>2</sub>), 80.1 (triazole SCH), 120.1-139.2 (Ar-C), 154.3 (triazole C=N), 162.0 (anhydride C=O), 174.0 (amide C=O); Anal. calcd. for C<sub>22</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>S (429.45) (%): C, 61.53; H, 3.52; N, 16.31; S, 7.47. Found (%): C, 61.76; H, 3.78; N, 16.68; S, 7.69.

**2-(5-Phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)-N-(2-oxoindolin-3-yliden)acetohydrazide (5a):** Bright yellow crystals; (Yield 85 %); m.p. 177-179°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1621 (C=N triazole), 1691 (C=O), 1710 (C=O), 3148 (NH aromatic), 3448 (NH aliphatic); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 3.20 ppm (s, 2H, SCH<sub>2</sub>), 4.10 (s, 1H, CH triazole), 6.96-7.71 (m, 9H, Ar-H), 11.31 (s, 1H, NH aliphatic), 13.40 (s, 1H, NH aromatic); Anal. calcd. for C<sub>18</sub>H<sub>14</sub>N<sub>6</sub>O<sub>2</sub>S (378.41) (%): C, 57.13; H, 3.73; N, 22.21; S, 8.47. Found (%): C, 57.25; H, 3.56; N, 22.46; S, 8.26.

**2-(5-Phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)-N-(2-oxoindolin-3-yliden)acetohydrazide (5b):** Yellow crystals; (Yield 88 %); m.p. 181-182°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1623 (C=N triazole), 1654 (C=O), 1695 (C=O aromatic), 3220 (NH aromatic), 3448 (NH aliphatic); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 3.36 (s, 2H, SCH<sub>2</sub>), 4.8 (s, 1H, CH triazole), 7.01-7.67 (m, 9H, Ar-H), 11.45 (s, 1H, NH aliphatic), 11.66 (s, 1H, NH aromatic); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 38.2 (aliphatic SCH<sub>2</sub>), 85.2 (triazole SCH), 113.9-145.1 (Ar-C), 132.2 (aromatic C=N), 165.3 (triazole C=N), 176.6 (amide C=O); Anal. calcd. for C<sub>18</sub>H<sub>13</sub>N<sub>6</sub>O<sub>2</sub>S (412.85) (%): C, 52.37; H, 3.17; N, 20.36; S, 7.77. Found (%): C, 52.05; H, 2.97; N, 20.56; S, 7.98.

**1-(1H-Indol-3-yl)ethylidene)-2-(5-phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)acetohydrazide (6):** A solution of compound 1 (1 mmol) in absolute ethanol (50 mL) and 3-acetyl indole (1 mmol) in the presence a catalytic amount of acetic acid was heated at 80°C for 1 h. On cooling, a precipitate formed which was filtered off, dried and recrystallized from methanol to give compound 6 as a pale yellow crystals, 65% yield, m.p. 201- 204°C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 1630 (C=N triazole), 1685 (C=O), 3294 (NH aromatic), 3345 (NH aromatic); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 2.51 (CH<sub>3</sub>, aliphatic), 3.35 (s, 2H, SCH<sub>2</sub>), 4.10 (s, 1H, CH triazole), 4.33 (s, 1H, CH indole), 7.70-7.90 (m, 8H, Ar-H),

8.8 (s, H, NH aliphatic), 9.41 (s, 1H, NH aromatic); Anal. calcd. for C<sub>20</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub>S (406.46) (%): C, 59.10; H, 4.46; N, 20.68; S, 7.89. Found (%): C, 59.28; H, 4.25; N, 20.85; S, 8.21.

**Synthesis of 7 and 8. General Procedure:** A mixture of compound 1 (1 mmol) and 1, 2, 4, 5-benzenetetracarboxylic dianhydride or 1, 4, 5, 8-naphthylenetetracarboxylic di-anhydride (1 mmol) in glacial acetic acid (50 ml) was refluxed for 1-3 h. The formed precipitates were filtered on hot, dried and recrystallized from appropriate solvents to give compounds 7 and 8, respectively.

**Di-(2-(5-phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)acetamide)-1, 2, 4, 5-benzenetetracarboxylic di-anhydride (bis-imide derivative) (7):** Pale yellow crystals; (Yield 64 %); m.p. 231- 233°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1654, 1686 (2C=N triazole), 1718 (C=O), 1742 (C=O), 3422 (NH, broad band); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 3.40 (s, 2H, SCH<sub>2</sub>), 4.22 (s, 1H, CH triazole), 7.68-7.93 (m, 12H, Ar-H), 11.40 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 38.2 ppm (aliphatic SCH<sub>2</sub>), 84.86 (triazole SCH), 123.1-137.0 (Ar-C), 153.0 (triazole), 168.2 (anhydride), 172.1 (C=O amide); Anal. calcd. for C<sub>30</sub>H<sub>20</sub>N<sub>10</sub>O<sub>6</sub>S<sub>2</sub> (680.67) (%): C, 52.94; H, 2.96; N, 20.58; S, 9.42. Found (%): C, 52.62; H, 3.14; N, 20.34; S, 9.21.

**Di-(2-(5-phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)acetamide)-1, 4, 5, 8-naphthylenetetracarboxylic di-anhydride (bis-imide derivative) (8):** White crystals; (Yield 60%); m.p. 270-272°C; IR (KBr)  $\nu$  cm<sup>-1</sup>: 1625 (2C=N triazole), 1747 (C=O), 1830 (C=O), 2299 (NH); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 3.36 (s, 2H, SCH<sub>2</sub>), 4.43 (s, 1H, CH triazole), 7.69 (m, 7H, Ar-H), 11.3 (s, 1H, NH); MS m/z (%): 730.53 (M<sup>+</sup>, 3.85%); Anal. calcd. for C<sub>34</sub>H<sub>22</sub>N<sub>10</sub>O<sub>6</sub>S<sub>2</sub> (730.35) (%): C, 41.80; H, 1.75; N, 13.54; S, 6.20. Found (%): C, 42.05; H, 1.54; N, 13.76; S, 6.39.

**1-(2-(5-Phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)acetyl)-4-phenylthiosemicarbazide (9):** A mixture of compound 1 (1 mmol) and phenylisocyanate (2 mmol) in absolute ethanol (50 ml) was refluxed for 10 hours on steam bath. The excess solvent was evaporated and the reaction mixture was crystallized from ethanol to give compound 9 as pale yellow crystals, yield 76.2%, m.p. 197-199°C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 1654 (C=N triazole), 1715 (C=O), 3264 (NH, broad band); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  ppm: 2.19 (s, NHC=S, aliphatic), 3.34 (s, 2H, SCH<sub>2</sub>), 4.28 (s, NHph, aliphatic),

4.42 (s, 1H, CH triazole), 7.19-7.70 (m, 9H, Ar-H), 10.49 (s, H, NH aliphatic);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 35.2 (aliphatic  $\text{SCH}_2$ ), 84.1 (triazole SCH), 125.1-139.4 (Ar-C), 163.1 (triazol), 172.4 (C=O amide), 182.54 (C=S); Anal. calcd. for  $\text{C}_{17}\text{H}_{16}\text{N}_6\text{OS}_2$  (384.48) (%): C, 53.11; H, 4.19; N, 21.86; S, 16.68. Found (%): C, 52.82; H, 4.39; N, 21.65; S, 16.41.

**5-(5-Phenyl-3H-1, 2, 4-triazol-3-ylsulfanyl)methyl)-4-phenyl-4H-1, 2, 4-triazole-3-thiol (10):** The thiosemicarbazide 9 (3 mmol) was refluxed in potassium hydroxide solution (5%, 25 ml) for 3 h. The reaction mixture was cooled and acidified with hydrochloric acid to pH = 5. The precipitate formed was filtered off, washed with water, dried and recrystallized from ethanol to give the compound 10 as white crystals, yield 70.4%, m.p. 190-192°C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 1630.85 (C=N triazole), 1708 (C=O), 2925 (CH aliphatic), 3082 (CH aromatic), 3412 (SH);  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 3.31 (s, SH, aliphatic), 3.56 (s, 2H,  $\text{SCH}_2$ ), 3.76 (s, 1H, CH triazole), 7.35-7.87 (m, 10H, Ar-H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 39.0 ppm (aliphatic  $\text{SCH}_2$ ), 78.1 (triazole SCH), 120.0-129.2 (Ar-C), 165.1 (triazole SH), 168.0 (triazole), 172.5 (CHSH triazole), 188.76 (C=O amide); Anal. calcd. for  $\text{C}_{17}\text{H}_{12}\text{N}_6\text{OS}_2$  (380.45) (%): C, 53.67; H, 3.18; N, 22.09; S, 16.86. Found (%): C, 53.83; H, 3.35; N, 22.25; S, 17.07.

**5-(6-Phenyl-[1, 2, 4]triazolo[4, 3-b]pyridazin-3-ylsulfanyl)methyl)-1, 3, 4-oxadiazole-2(3H)thione (12):** A mixture of compound 11 (1 mmol) and carbon disulfide (10 ml) in dry pyridine (30 ml) was refluxed for 10 h. The reaction mixture was cooled and poured into ice water (100 mL) and acidified with hydrochloric acid (3 ml, 10%). The precipitated material was filtered off, washed with water, dried and recrystallized from ethanol to give compound 12. Yield 75.4%, m.p. 235-237°C and *lit.* m.p. 236-238°C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 1543 (C=S), 1699 (C=N triazole), 3394 (NH); MS  $m/z$  (%): 342 [ $\text{M}^+$ ] (10.15). Anal. calcd. for  $\text{C}_{14}\text{H}_{10}\text{N}_8\text{OS}_2$  (342.4) (%): C 49.11, H 2.94, N 24.54, S 18.73. Found: C 49.34, H 2.73, N 24.31, S 18.52.

**5-(6-Phenyl-[1, 2, 4]triazolo[4, 3-b]pyridazin-3-ylsulfanyl)methyl)-4-phenyl-2H-1, 2, 4-triazole-3(4H)-thione (13):** A solution of compound 11 (1 mmol) was treated with phenyl isothiocyanate (1 mmol), in pyridine (20 ml), the reaction mixture was refluxed for 3 h, poured onto crushed ice, acidified with HCl, filtered, dried, recrystallized from ethanol to give compound 13 as white

crystals, 54% yield, m.p. 180-182°C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 1535 (C=S), 1697 (C=N triazole), 3055 (NH); MS  $m/z$  (%): 317.18 [ $\text{M}^+$ ] (1.26). Anal. calcd. for  $\text{C}_{20}\text{H}_{15}\text{N}_7\text{S}_2$  (417.51) (%): C, 57.53; H, 3.62; N, 23.48; S, 15.36. Found (%): C, 57.25; H, 3.81; N, 23.23; S, 15.52.

**(6-Phenyl-[1, 2, 4]triazolo[4, 3-b]pyridazine-3-ylsulfanyl)acetic acid hydrazino-potassium methanedithioate (14):** A solution of compound 11 (1 mmol) was treated with carbon disulfide (1 mmol) and potassium hydroxide (1 mmol) in absolute ethanol (40 mL), the reaction mixture was heated on water bath for 5 h, cooled, filtered, dried, to give potassium salt 14 as dark brown powder, 70 % yield. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 1539.20 (C=S), 1627 (C=N triazole), 1689 (C=O), 3051 (NH, broad band); MS  $m/z$  (%): 414.57 [ $\text{M}^+$ ] (0.71) with a base peak at  $m/z$  227.05. Anal. calcd. for  $\text{C}_{14}\text{H}_{11}\text{N}_6\text{OS}_3$  (414.57) (%): C, 40.56; H, 2.67; N, 20.77; S, 23.20. Found (%): C, 40.64; H, 2.46; N, 20.30; S, 23.51.

**3-(6-Phenyl-[1, 2, 4]triazolo[4, 3-b]pyridazin-3-ylsulfanyl)methyl)-4-amino-1H-triazole-5(4H)-thione (15):** A solution of compound 14 (1 mmol) was treated with hydrazine hydrate (1 mmol), in absolute ethanol (40 ml), the reaction mixture was refluxed for 3 h, poured onto crushed ice then acidified with HCl filtered, dried, recrystallized from ethanol to give compound 15 as black crystals, 50% yield, m.p. 280-282°C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 1543 (C=S), 1631 (C=N triazole), 3228 (NH, broad band), 3390 ( $\text{NH}_2$ , broad band); MS  $m/z$  (%): 356.08 [ $\text{M}^++1$ ] (0.73) with a base peak at  $m/z$  89.07. Anal. calcd. for  $\text{C}_{14}\text{H}_{12}\text{N}_8\text{S}_2$  (356.43) (%): C, 47.18; H, 3.39; N, 31.44; S, 17.99. Found (%): C, 47.54; H, 3.64; N, 31.29; S, 17.78.

**Ethyl-2-(3-(6-phenyl-[1, 2, 4]triazolo[4, 3-b]pyridazin-3-ylsulfanyl)methyl)-4-amino-4, 5-dihydro-1H-triazole-5-ylsulfanyl)acetate (16):** A solution of compound 15 (1 mmol) was treated with ethylchloroacetate (1 mmol) and sodium hydroxide (1 mmol), in absolute ethanol (40 ml), the reaction mixture was refluxed for 2 h, cooled, filtered, dried, recrystallized from ethanol to give compound 16 as brownish red crystals, 47% yield, m.p. 290-292°C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 1604 (C=N triazole), 1720 (C=O), 3429 (NH, broad band), 3444 ( $\text{NH}_2$ , broad band); MS  $m/z$  (%): 443.28 [ $\text{M}^++1$ ] (4.50) with a base peak at  $m/z$  83.10. Anal. calcd. for  $\text{C}_{18}\text{H}_{20}\text{N}_8\text{O}_2\text{S}_2$  (444.53) (%): C, 48.63; H, 4.53; N, 25.21; S, 14.43. Found (%): C, 48.94; H, 4.86; N, 25.48; S, 14.25.

## REFERENCES

1. Rashad, A.E., A.E. Mahmoud and M.M. Ali, 2011. Synthesis and anticancer effects of some novel pyrazolo[3, 4-d]pyrimidine derivatives by generating reactive oxygen species in human breast adenocarcinoma cells. *Eur. J. Med. Chem.*, 46(4): 1019-1026.
2. Shamroukh, A.H., M.E. Zaki, E.M. Morsy, F.M.E. Abdel-Motti and F.M. Abdel-Megeid, 2007. Synthesis of pyrazolo[4', 3':5, 6]pyrano[2, 3-d]pyrimidine derivatives for antiviral evaluation. *Arch. Pharm. Chem. Life Sci.*, 340(5): 236-243.
3. Singh, B., A. Maheshwari, G. Dak, K. Sharma and G. Talesara, 2010. Studies of antimicrobial activities of some 4-thiazolidinone fused pyrimidines, [1, 5]-benzodiazepines and their oxygen substituted hydroxylamine derivatives. *Indian J. Pharm. Sci.*, 72(5): 607-612.
4. Zhang, Y., P. Martens and K. Denkin, 2006. New drug candidates and therapeutic targets for tuberculosis therapy. *Drug Discov. Today*, 38(7-8): 759-767.
5. Holla, B.S., B. Veerendra, M.K. Shivananda and B. Poojary, 2003. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1, 2, 4-triazoles. *Eur. J. Med. Chem.*, 38(7-8): 759-767.
6. Duran, A., H.N. Dogan and S. Rollas, 2002. Synthesis and preliminary anticancer activity of new 1, 4-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-5H-1, 2, 4-triazoline-5-thiones. *Farmaco*, 57(7): 559-564.
7. Shah, M.H., M.Y. Mhasalkar, M.V. Palki, C.V. Deliwala and U.K. Sheth, 1969. New 1, 2, 4(H)-triazole derivatives as diuretic agent. *J. Pharm. Sci.*, 58(11): 1398-1401.
8. Mhasalkar, M.Y., M.H. Shah, S.T. Nikam, K.G. Anantanarayanan and C.V. Deliwala, 1970. 4-Alkyl-5-aryl-4H-1, 2, 4-triazole-3-thiols as hypoglycemic agents, *Eur. J. Med. Chem.*, 13(4): 672-674.
9. Lee, J., S.J. Kim, H. Choi, Y.H. Kim, I.T. Lim, H. Yang, C.S. Lee, H.R. Kang, S.K. Ahn, S.K. Moon, D.H. Kim and S. Lee, 2010. Identification of CKD-516: a potent tubulin polymerization inhibitor with marked antitumor activity against murine and human solid tumors. *Eur. J. Med. Chem.*, 53(17): 6337-6354.
10. Amir, M. and S. Kumar, 2007. Synthesis and evaluation of anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation properties of ibuprofen derivatives. *Acta Pharm.*, 57(1): 31-45.
11. Meyer, D. and T. Strassner, 2011. 1, 2, 4-Triazole-based tunable aryl/alkyl ionic liquids. *J. Org. Chem.*, 76(1): 305-308.
12. Schneider, S., T. Hawkins, M. Rosander, J. Mills, A. Brand, L. Hudgens, G. Warmoth and A. Vij, 2008. Liquid azide salts, *Inorg. Chem.*, 47(9): 3617-3624.
13. Emami, S., A. Foroumadi, M. Falahati, E. Lotfali, S. Rajabalian, S.A. Ebrahimi, S. Farahyar and A. Shafiee, 2008. 2-Hydroxyphenacyl azoles and related azolium derivatives as antifungal agents. *Bio. Org. Med. Chem. Lett.*, 18(1): 141-146.
14. Li, H., G.X. Cai and Z.J. Shi, 2010. LiCl-Promoted Pd(II)-catalyzed ortho carbonylation of N, N-dimethylbenzylamines. *Dalton Trans.*, 39(43): 10442-10446.
15. Guda, D.R., 2012. Trimethylsilyl isothiocyanate (TMSNCS): an efficient reagent for the one-pot synthesis of mercapto-1, 2, 4-triazoles. *Tetrahedron Letters*, 53: 5238-5242.
16. Shamroukh, A.H. and M.A. Ali, 2008. Anti-HAV activity of some newly synthesized triazolo[4, 3-b]pyridazines. *Arch. Pharm.*, 341(4): 223-230.
17. Venturini, T.P., L. Rossato, T.B. Spader, G.R. Tronco-Alves, M.I. Azevedo, C.B. Weiler, J.M. Santurio and S.H. Alves, 2011. *In vitro* synergisms obtained by amphotericin B and voriconazole associated with non-antifungal agents against *Fusarium* spp. *Diagn. Microbiol. Infect. Dis.*, 71(2): 126-130.