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Novel Fused and Spiro Heterocyclic Compounds Derived from 4-(4-Amino-5-Mercapto-4*H*-1,2,4-Triazol-3-yl)Phthalazin-1(2*H*)-One

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Abstract: Some new fused heterocyclic systems such as 1,2,4-triazolo[3,4-b]1,3,4-thiadiazoles and 1,3,4-thiadiazines were synthesized through the reaction of 4-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)phthalazin-1(2*H*)-one **4** with triethyl orthoformate, furoyl chloride, thiophene-2-aldehyde, phenylisothiocyanate, chloro acetonitrile and p-nitro-*w*-bromoacetophenone. Also spiro systems were achieved from reaction of compound **4** with isatine and fluorenone. The newly synthesized 1,3,4-thiadiazoles and 1,3,4-thiadiazines were obtained in good yields and their structures were elucidated by spectral data and elemental analysis.

Key words: Triazolothiadiazole · Triazolothiadiazine · Spiro thiadiazole derivatives · Phthalazin-1(2*H*)-one

INTRODUCTION

A survey of the literature revealed that compounds bearing 1,2,4-triazole ring are well known as antimicrobial, anticonvulsant, antidepressant, antihypertensive, antitumarial and analgesic agents [1-3]. Moreover, some 1,2,4-triazolo[3,4-b]1,3,4-thiadiazoles and triazolo-1,3,4-thiadiazines derived from 4-amino-3-mercapto-1,2,4-triazoles are associated with diverse pharmacological activities [4-6]. On the other hand, phthalazines and phthalazinones are *N*-heterocycles with a wide range of biological activities [7-13]. Continuing our efforts directed toward the synthesis of new heterocyclic compounds with anticipated biological activities [14-20]. Much attention has been directed in our laboratory for synthesis of heterocycles containing the phthalazine and triazolothiadiazole or triazolothiadiazine rings.

RESULTS AND DISCUSSIONS

We previously reported [19] that hydrazinolysis of the lactone **1** with hydrazine hydrate afforded the hydrazide **2** (unexpected product) which when treated with carbon disulphide in ethanol containing potassium hydroxide gave the corresponding potassium dithiocarbazate **3**, finally treatment of compound **3** with hydrazine hydrate in refluxing water afforded 4-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)phthalazin-1(2*H*)-one **4**. (Scheme 1).

The structure of **4** was deduced from the spectroscopic and analytical data. Thus, IR spectrum of compound **4** displayed $\nu_{NH2,\ NH}$ at 3245, 3162, 3108 cm^{-1}, $\nu_{C=O}$ at 1665 cm^{-1}. Moreover ^{1}H-NMR spectrum of compound 4 exhibited signals at δ (ppm) 14.2 (s, 1H, SH, exchangeable with D_2O), 13.27 (s, 1H, NH, exchangeable with D_2O), 8.33 (d, $1H_{arom.}$, J = 1.5 Hz), 7.95 (dd, $2H_{arom.}$, J = 10.8 Hz), 7.82 (d,$1H_{arom.}$, J = 3.6 Hz) and 5.74 (brs, 2H, NH_2, exchangeable with D_2O). Furthermore, the mass spectrum show the correct molecular ion peak at *m/z* = 260 (100%) which represent the base peak.

A plausible mechanism for the formation of compound 4 is shown in Chart 1.

The functionalities in 4-amino-5-mercapto-3-substituted-1,2,4-triazoles made them valuable key precursors for the formation of fused heterocyclic compounds containing 1,2,4-triazolo[3,4-b] 1,3,4-thiadiazoles and 1,3,4-thiadiazines [20, 21]. Treatment of **4** with thiophene-2-aldehyde in refluxing DMF yielded 4-[6-(thiophen-2-yl)-5,6-di-hydro[1,2,4]triazolo[3,4-b]1,3,4-thiadiazol-3-yl] phthalazin-1(2*H*)-one 5. (Scheme 2).

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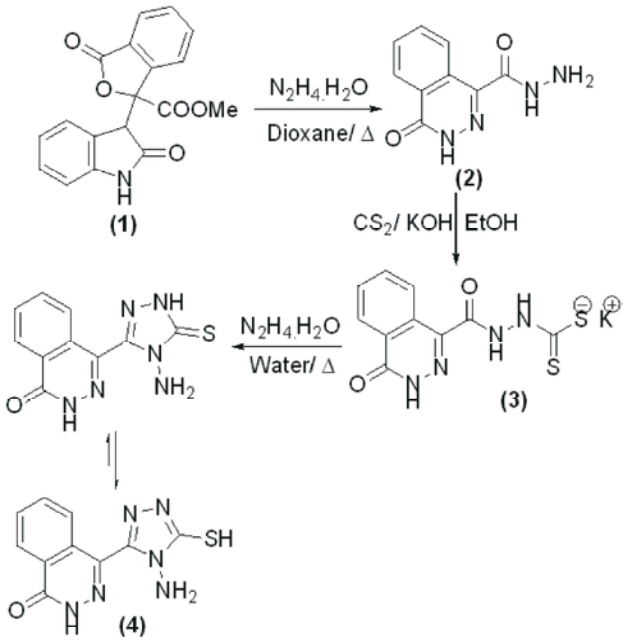


Scheme 1: Synthesis of 4-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)phthalazin-1(2*H*)-one 4

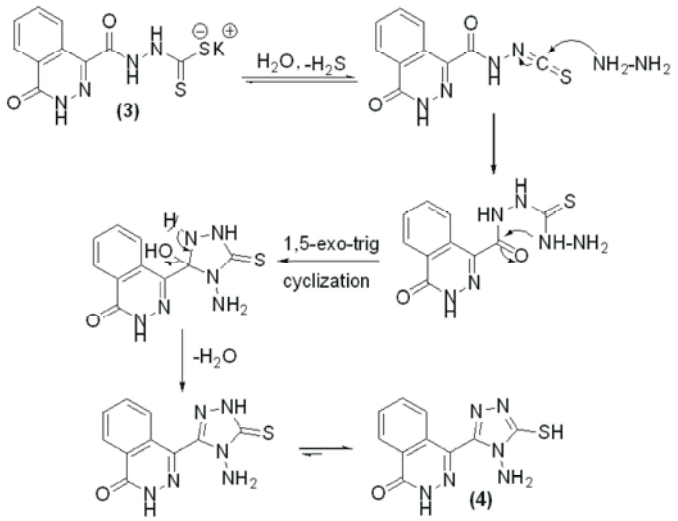
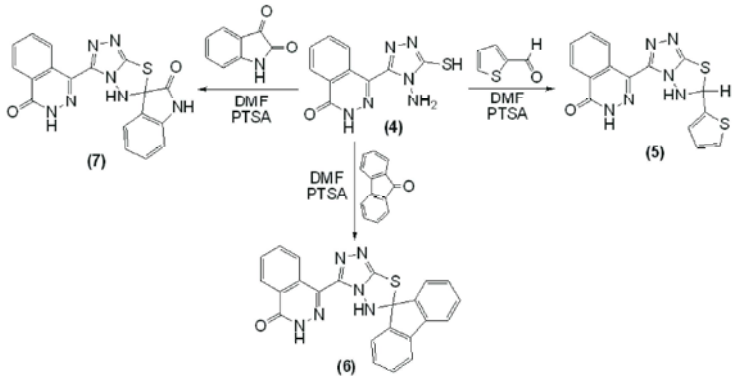


Chart 1: A plausible mechanism for conversion of 3 to 4



Scheme 2: Reactions of compound 4 with aldehyde and cyclic ketones

Treatment of compound **4** with cyclic ketones was found to give spiro thiadiazole derivatives. Thus, the reaction of 4-amino-5-mercapto-3-substituted-1,2,4-triazole **4** with fluorenone and/or isatin in refluxing DMF in the presence of the catalytic amount of *p*-toluene sulphonic acid [PTSA] resulted in the formation of 4-(5*H*-spiro-1,2,4-triazolo[3,4-b]1,3,4-thiadizole-6,9'-fluorene-3-yl] phthalazin-1(2*H*)-one **6** and 3-(4-oxo-3,4-dihydro-phthalazin-1-yl)-5*H*-spiro-1,2,4-triazolo[3,4-b]1,3,4-thiadizole-6,3'-indolin-2'-one 7, respectively (Scheme 2).

The conversion of **4** to compounds 5, 6 and 7 could be explained on the basis of nucleophilic addition by the nitrogen nucleophile of the amino group on the carbonyl group to give the corresponding Schiff's bases followed by cyclization process via the nucleophilic addition for the thiol group on the azomethine to give 5 and spiro compounds 6 and 7. (Chart 2).

The reaction of compound **4** with one carbon donors such as TEOF (triethyl orthoformate) and phenylisothiocyanate has been investigated. Thus, treatment of compound **4** with TEOF in freshly distilled acetic anhydride yielded the unexpected product which identified as 4-(6-ethoxy-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phthalazin-1(2*H*)-one **8** [22]. (Scheme 3).

The structure **8** was confirmed using the analytical and spectroscopic data (IR and ^{1}H-NMR). The IR spectrum of **8** displayed ν_{NH} at 3214, 3159 cm^{-1} and $\nu_{C=O}$ at 1667 cm^{-1}. The ^{1}H-NMR of this unexpected product confirmed the structure **8**, a result of possible formation of 8 indicated the presence of quartet and triplet of ethoxy protons and the absence of a signal corresponding to the thiol and formyl protons. The ^{1}H-NMR exhibited signals at δ (ppm) 13.3 (s, 1H, NH, exchangeable with D_2O), 8.96 (d, 1H$_{arom.}$, J = 7.8 Hz), 8.35 (d,1H$_{arom.}$, J = 6.9 Hz), 8.06 (dd, 1H$_{arom.}$), 7.9 (dd, 1H$_{arom.}$), 3.38 (q, 2H, CH_2, J = 7.5 Hz) and 1.4 (t, 3H, CH_3, J = 7.5 Hz).

Refluxing compound 4 with phenylisothiocyanate in the presence of sodium hydroxide in DMF afforded 4-(6-(phenylamino)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl) phthalazin-1(2*H*)-one 9. (Scheme 3).

Stirring compound **4** with furoyl chloride in dry dimethylformamide in the presence of triethyl amine at room temperature followed by acidification yielded the triazolothiadiazole derivative **10**. (Scheme 3).

The formation of compound **10** could be formulated as shown in Chart 3.

Carboethoxymethylation of **4** with ethyl chloroacetate in boiling dioxane in the presence of fused sodium acetate for 2 hrs resulted in the *S*-alkylation and afforded the uncyclized product **11** rather than the cyclized product **12** or **13**. (Scheme 4).

The uncyclized nature of **11** was confirmed from its elemental analysis which was consistent with the molecular formula $C_{14}H_{14}N_6O_3S$ and the existence of $\nu_{NH2, NH}$ at 3315, 3217, 3160, 3106 cm^{-1}, $\nu_{C=O(ester)}$ at 1734 cm^{-1} in the IR spectrum. Moreover, the presence of one singlet in ^{1}H-NMR spectrum at δ 6.108 ppm integrated for two protons disappeared in D_2O corresponding to the NH_2 protons which ruled out the existence of the cyclized compound 12 and 13. Furthermore, the recorded peaks in the mass spectrum at *m/z* = 346 (20.4%) and *m/z* = 273 (100%) represent the molecular ion peak and the base peak [M-C_2H_4, -CO_2] which completely in accord with the assigned structure.

Cyanomethylation of 4 with chloroacetonitrile in boiling dioxane in the presence of fused sodium acetate for 10 hrs yielded the cyclized product 4-[6-amino-6,7-dihydro-5*H*-1,2,4-triazolo[3,4-b]1,3,4-thiadiazin-3-yl]phthalazin-1(2*H*)-one 14. (Scheme 4).

The absence of the stretching absorption band for the nitrile group in the IR spectrum ruled out the uncyclized product.

4-[6-(4-Nitrophenyl)-7*H*-1,2,4-triazolo[3,4-b]1,3,4-thiadiazin-3-yl]phthalazin-1(2*H*)-one **15** was prepared in quantitative yield upon treatment of compound **4** with *p*-nitro-*w*-bromoacetophenone in refluxing dioxane. (Scheme 4).

The absence of the stretching absorption bands for NH_2 in the IR and ^{1}H-NMR confirm the cyclized structure **15**. The IR spectrum of **15** displayed one absorption band for NH group at 3301 cm^{-1} and one absorption band for carbonyl group (phthalazinone) at 1674 cm^{-1}. The formation of compound **14** and **15** may proceed via nucleophilic substitution reaction (S_N2) followed by 1,6-exo-dig and exo-trig cyclization, respectively (Chart 4).

Antimicrobial Evaluation: The antibiotic resistance is a growing problem; this is due the overuse of antibiotics in human and the use of antibiotics as growth promoters in food of animals, so there is a growing demand for new antibiotics. Some selected examples were evaluated for their in vitro antimicrobial activity against two strains of bacteria and two fungus strains; tetracycline was used as standard drug for bacteria and amphotericin was used as standard drug for fungi. Preliminary screening of the synthetic derivatives and standard drugs were performed at fixed concentration 20 mg/ ml, inhibition was recorded

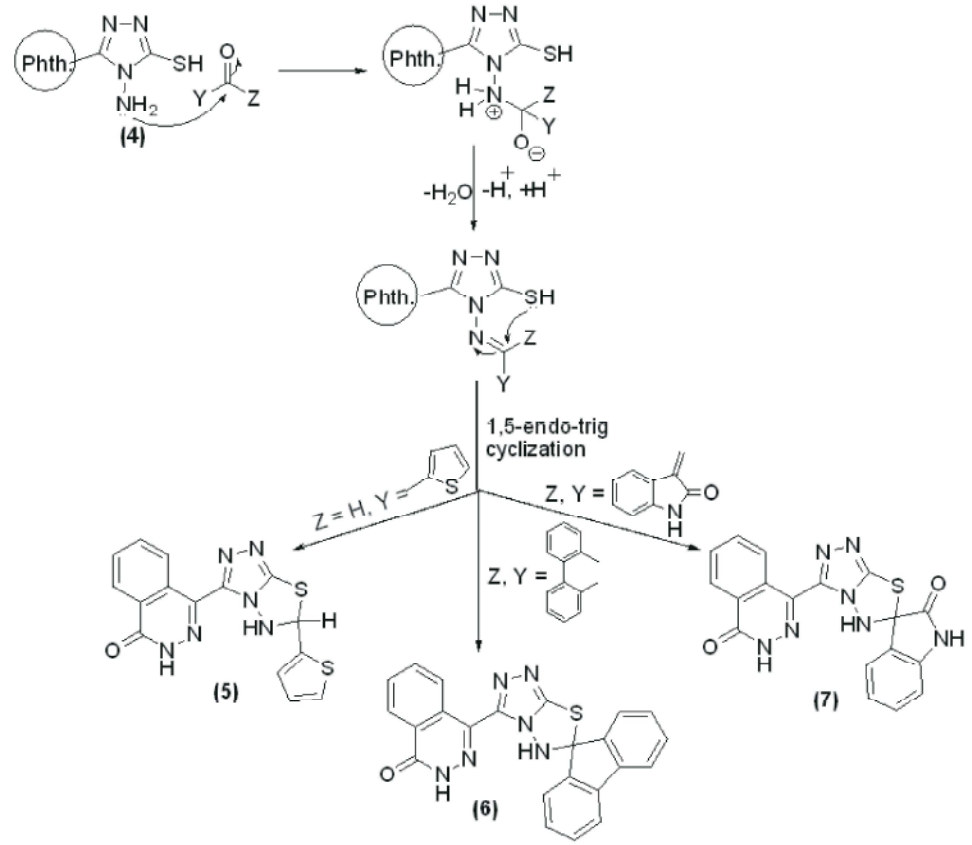
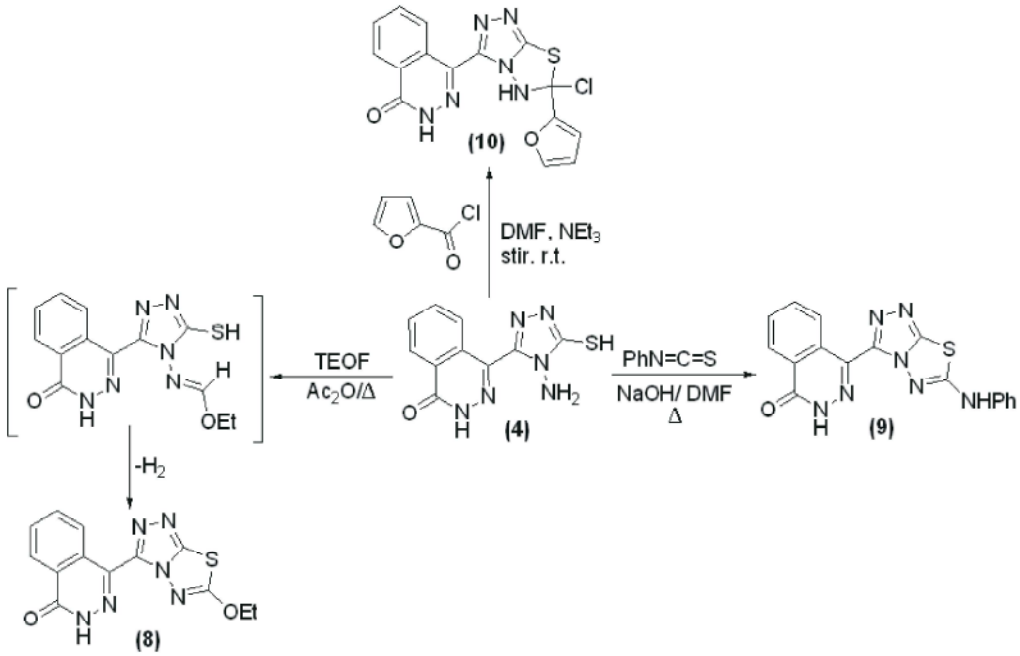


Chart 2: The expected mechanism for the formation of 5 and the spiro compounds 6 and 7
In chart 2 Z,Y= complete in middile ring ….. C=O



Scheme 3: Synthesis of 1,2,4-triazolo[3,4-b]1,3,4-thiadiazol-3-yl)phthalazin-1(2*H*)-ones (8-10)
In Scheme 3 Net_3……???

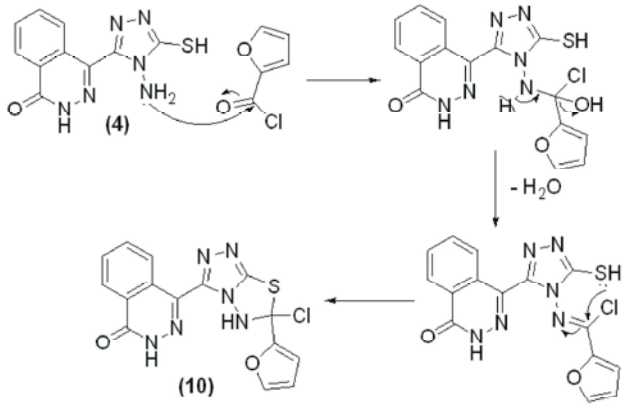
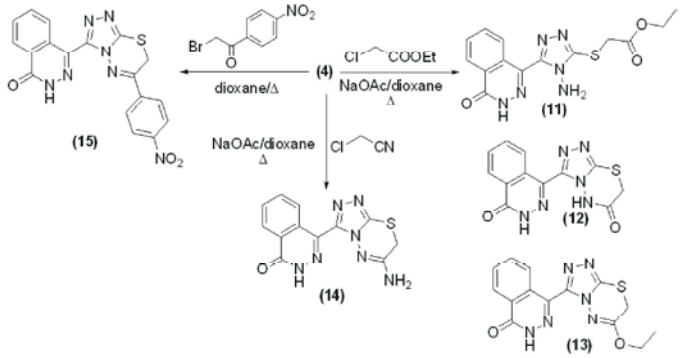


Chart 3: A plausible mechanism for the synthesis of 10



Scheme 4: *S*-Alkylation of compound 4

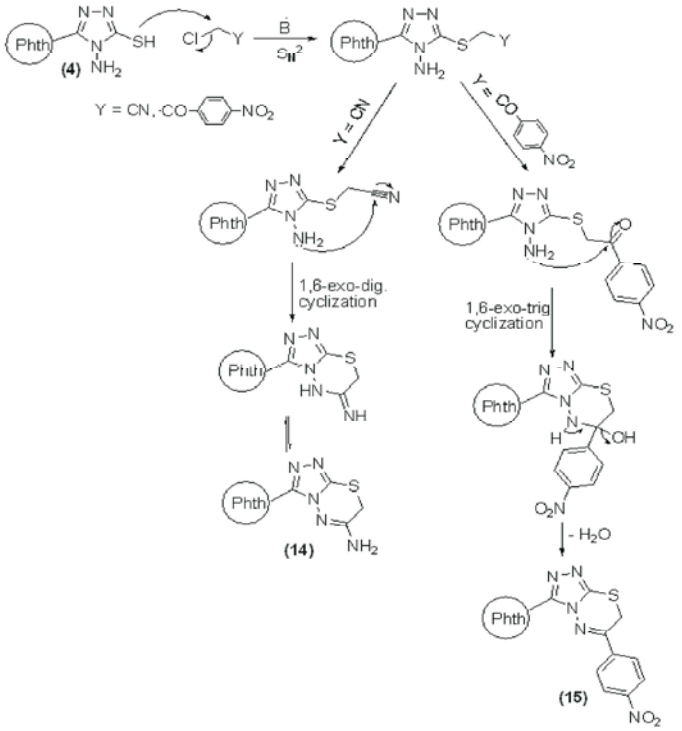


Chart 4: A plausible mechanism for the formation of 1,2,4-triazolo[3,4-b]1,3,4-thiadiazine derivatives 14, 15
In chart 4 x=Cl, Br

Table 1: Antimicrobial screening results of the tested compounds

	Inhibition zone diameter (mm / mg sample)			
Sample	*Escherichia coli* (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus* (Fungus)	*Candida albicans* (Fungus)
Control: DMSO	0.0	0.0	0.0	0.0
4	9	9	0.0	9
5	10	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0
9	10	12	0.0	9
10	12	0.0	0.0	0.0
11	11	12	33	12
14	0.0	0.0	0.0	0.0
Tetracycline	32	30	0.0	0.0
Amphotericin B	0.0	0.0	18	20

-G: Gram reaction.

- Solvent: DMSO

• 0.0: no activity (inhibition zone less than 7mm)

• 7-10 weak activity

• 11-15 moderate activity

• More than 15 strong activity

by measuring the diameter of the inhibition zoon at the end of 18hrs for bacteria. Based on the results of the inhibition zoon, data in table 1 revealed that compounds 4, 5, 9, 10 and 11 exhibited moderate antibacterial and antifungal activities compared with the standard drugs. Compounds 6, 7, 8 and 14 exhibited no antimicrobial activity.

CONCLUSION

From the foregoing survey, it seems that functionalities in 4-amino-5-mercapto-3-substituted-1,2,4-triazoles provide a useful and convenient strategy for synthesis of numerous fused heterocyclic compounds containing 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole and 1,3,4-thiadiazine derivatives. The subject of such reactions is still ongoing and undoubtedly will provide new fused functionalized 1,3,4-thiadiazoles of both industrial and biological interests.

Experimental: All melting points were taken on Griffin and Geory melting point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer using KBr Wafer technique. ^{1}H-NMR spectra were determined on a Varian Gemini 300 MHz using TMS as internal standard (chemical shifts in δ-scale). EI-MS were measured on a Schimadzu-GC-MS operating at 70 eV. Elemental analyses were carried out at the Microanalytical unit, Faculty of Science, Ain Shams University by using Perkin-Elmer 2400 CHN elemental analyzer and satisfactory analytical data (± 0.4) were obtained for all compounds. Biological activities were carried out at microanalytical center, Faculty of Science, Cairo University. The homogeneity of the synthesized compounds was controlled by TLC [Using TLC aluminum sheets silica gel F_{254} (Merck)].

***Synthesis of 4-(4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)phthalazin-1(2H)-one 4*:** To a stirred solution of 4-oxo-3,4-dihydrophthalazine-1-carbohydrazide **2** (2 g, 10 mmol) and potassium hydroxide (0.84 g, 15 mmol) in absolute ethanol (30 ml), carbon disulfide (0.88 mL, 15 mmol) was added dropwise and the mixture was stirred at room temperature for 6 h. The precipitated potassium dithiocarbazate salt **3** was collected by filtration, washed with dry ether and dried under vacuum. This salt was obtained in quantitative yield and was used in the next step without further purification. To a suspension of the potassium salt **3** (1 g, 2.8 mmol) in water (10 ml), hydrazine hydrate (0.28 mL, 5.7 mmol) was added and the whole mixture then heated under reflux until all hydrogen sulfide evolved. The reaction mixture was cooled then acidified with cold dilute hydrochloric acid and the precipitated product was filtered off, dried and then recrystallized from EtOH/dioxane to give **4** as beige crystals; mp: 240-2°C, yield: 65%. ^{1}H-NMR (DMSO-d_6) δ (ppm): 14.2 (s, 1H, SH, exchangeable with D_2O), 13.27 (s, 1H, NH, exchangeable with D_2O), 8.33 (d, 1$H_{arom.}$, J = 1.5 Hz), 7.95 (dd, 2$H_{arom.}$, J = 10.8 Hz), 7.82 (d,1$H_{arom.}$, J = 3.6 Hz), 5.74 (brs, 2H, NH_2, exchangeable with D_2O). IR (Kbr) υ_{max}: 3245, 3162, 3108

(NH_2, NH), 1665 (C=O) cm^{-1}. MS m/z (%): 260 (M^+; 100), 172 (38.7), 145 (4.5), 115 (18), 102 (15.2), 89 (11.4). Anal. Calcd. for $C_{10}H_8N_6OS$ (260.28): C, 46.15; H, 3.10; N, 32.29; S, 12.32. Found: C, 46.03; H, 2.98; N, 32.11; S, 12.21.

***Synthesis of 4-[6-(Thiophen-2-yl)-5,6-dihydro[1,2,4]triazolo[3,4-b]1,3,4-thiadiazol-3-yl] phthalazin-1(2H)-one 5*:** A mixture of **4** (1 g, 3 mmol), thiophene-2-aldehyde (0.4 mL, 3 mmol) and catalytic amount of *p*-toluene sulphonic acid (0.3 g) in DMF (20 ml) was heated under reflux for 16 hrs. The reaction mixture was concentrated and then poured onto cold water and the deposited solid was collected by filtration and recrystallized from EtOH/dioxane to give 5 as pale yellow crystals; mp: 334-336°C, yield: 72%. ^{1}H-NMR (DMSO-d_6) δ (ppm): 14.2 (s, 1H, NH, exchangeable with D_2O), 13.2 (s, 1H, NH, exchangeable with D_2O), 9.9 (brs,1H),8.3 (d, 1H, C_4-$H_{\text{thiophene ring}}$, J = 1.2 Hz), 8.0-7.75 (m, 4Harom.), 7.7(d, 1H, C_2-$H_{\text{thiophene ring}}$, J = 1.2 Hz), 7.2 (dd, 1H, C_3-$H_{\text{thiophene ring}}$, J = 3.6 Hz). IR (KBr) υ_{max}: 3142, 3101 (NH), 1649 (C=O) cm^{-1}. MS m/z (%): 354 (M^+; 7.3), 245 (100), 109 (93.1), 101 (16.3). Anal. Calcd. for $C_{15}H_{10}N_6OS_2$ (354.41): C, 50.83; H, 2.84; N, 23.71; S, 18.09. Found: C, 50.77; H, 2.63; N, 23.55; S, 18.12.

***Synthesis of 4-(5H-Spiro-1,2,4-triazolo[3,4-b]1,3,4-thiadizole-6,9'-fluorene-3-yl]phthalazin-1(2H)-one 6*:** A mixture of **4** (1 g, 3 mmol), fluorenone (0.69 g, 3 mmol) and catalytic amount of *p*-toluene sulphonic acid (0.3 g) in DMF (20 ml) was heated under reflux for 18 hrs. The reaction mixture was concentrated and then poured onto cold water and the precipitated product was filtered off, dried and recrystallized from dioxane/DMF to give 6 as yellow crystals; mp: >300°C, yield: 78%. IR (KBr) υ_{max}: 3372(NH), 1700 (C=O), 1666 (C=N) cm^{-1}. MS m/z (%): 422 (M^+; 0.8), 421 (72.9), 366 (67), 173 (100), 145 (58.7). Anal. Calcd. for $C_{23}H_{14}N_6OS$(422.46): C, 65.39; H, 3.34; N, 19.89; S, 7.59. Found: C, 65.45; H, 3.23; N, 19.76; S, 7.49.

***Synthesis of 3-(4-Oxo-3,4-dihydrophthalazin-1-yl)-5H-spiro-1,2,4-triazolo[3,4-b]1,3,4-thiadizole-6,3'-indolin-2'-one 7*:** A mixture of **4** (1 g, 3 mmol), isatin (0.56 g, 3 mmol) and catalytic amount of *p*-toluene sulphonic acid (0.3 g) in DMF (20 ml) was heated under reflux for 3 hrs. The deposited solid on hot was filtered off, dried and recrystallized from DMF to give 7as white crystals; mp: >300°C, yield: 55%. IR (KBr) υ_{max}: 3157, 3113 (NH), 1671 (C=O) cm^{-1}. MS m/z (%): 387 (M-2; 75), 245 (50), 172 (87.5), 129 (87.5), 102 (75), 57 (100). Anal. Calcd. for $C_{18}H_{11}N_7O_2S$(389.39): C, 55.52; H, 2.85; N, 25.18; S, 8.23. Found: C, 55.46; H, 2.72; N, 25.03; S, 8.15.

***Synthesis of 4-(6-Ethoxy-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phthalazin-1(2H)-one 8*:** A mixture of **4** (1 g, 3 mmol), triethylorthoformate (10 mL) and freshly distilled acetic anhydride (10 mL) was heated under reflux for 10 hrs. The reaction mixture was concentrated and the deposited product was filtered off, washed with petroleum ether (b.p. 60-80°C), dried and recrystallized from dioxane to give **8** as beige crystals; mp:>300°C, yield: 48%. ^{1}H-NMR (DMSO-d_6) δ (ppm): 13.3 (s, 1H, NH, exchangeable with D_2O), 8.96 (d, $1H_{arom.}$, J = 7.8 Hz), 8.35 (d, $1H_{arom.}$, J = 6.9 Hz), 8.06 (d, $1H_{arom.}$, J = 3.7 Hz),7.9 (d, $1H_{arom.}$, J = 2.9 Hz),3.38 (q, 2H, CH_2, J = 7.5 Hz), 1.4 (t, 3H, CH_3, J = 7.5 Hz). IR (KBr) υ_{max}: 3214, 3159(NH), 1667 (C=O) cm^{-1}. MS m/z (%): 314 (M^+; 23.6), 286 (17.7), 270 (38.8), 171 (91.3), 145 (100), 119 (12.6). Anal. Calcd. for $C_{13}H_{10}N_6O_2S$(314.32): C, 49.67; H, 3.21; N, 26.74; S, 10.20. Found: C, 49.55; H, 3.11; N, 26.60; S, 10.03.

***Synthesis of 4-(6-(Phenylamino)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phthalazin-1(2H)-one 9*:** A mixture of **4** (1 g, 3 mmol), phenylisothiocyanate (0.4 mL, 3 mmol) and sodium hydroxide (0.8 g) in DMF (25 mL) was heated under reflux for 4 hrs. The reaction mixture was acidified with cold dilute hydrochloric acid and the precipitated solid was filtered off, dried and recrystallized from dioxane to give **9** as beige crystals; mp: >300°C, yield: 51%. IR (KBr) υ_{max}: 3160(NH), 1687 (C=O) cm^{-1}. MS m/z (%): 361 (M^+; 13.5), 171 (35.1), 128 (70.3), 102 (37.8), 92 (21.6), 91 (45.9), 64 (100). Anal. Calcd. for $C_{17}H_{11}N_7OS$(361.83): C, 56.50; H, 3.07; N, 27.13; S, 8.87. Found: C, 56.39; H, 2.97; N, 27.02; S, 8.72.

***Synthesis of 4-(6-Chloro-6-(furan-2-yl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)phthalazin-1(2H)-one 10*:** To a solution of **4** (1 g, 3 mmol) and triethyl amine (0.5 mL) in DMF (10 mL), furoyl chloride (0.3 mL, 3 mmol) was added dropwise in ice bath and the mixture was stirred at room temperature for 2 hrs. The reaction mixture was acidified with cold dilute hydrochloric acid and the precipitated product was filtered off, dried and then recrystallized from EtOH/dioxane to give **10** as beige crystals; mp: 290-292°C,yield: 43%. IR (KBr) υ_{max}: 3160, 3108 (NH), 1688 (C=O) cm^{-1}. MS m/z (%):370 (M-2; 2),246 (100), 186 (58.2), 145 (15.2), 102 (52.0). Anal. Calcd. for $C_{15}H_9N_6O_2SCl$ (372.79): C, 48.33; H, 2.43; N, 22.54; S, 8.60; Cl, 9.51. Found: C, 48.23; H, 2.29; N, 22.48; S, 8.50; Cl, 9.44.

***Synthesis of Ethyl 2-(4-amino-5-(4-oxo-3,4-dihydrophthalazin-1-yl)-4H-1,2,4-triazol-3-ylthio)acetate 11*:** A mixture of **4** (1 g, 3 mmol), ethyl chloroacetate (0.37 mL, 3mmol)in dioxane (20 mL) in the presence of fused sodium acetate (0.5 g) was heated under reflux for 2 hrs. The reaction mixture was concentrated and then poured onto cold water and the precipitated product was filtered off, dried and recrystallized from EtOH/dioxane to give **11** as beige crystals; mp: 194-196°C,yield: 80%. ^{1}H-NMR (DMSO-d_6) δ (ppm): 13.38 (s, 1H, NH, exchangeable with D_2O), 8.93 (d, 1$H_{arom.}$, J = 7.8 Hz), 8.3 (d, 1$H_{arom.}$, J = 7.8 Hz), 8.07-7.9 (dd, 2$H_{arom.}$), 6.1 (s, 2H, NH_2, exchangeable with D_2O), 4.3 (s, 2H, SCH_2),4.21-4.14 (q, 2H, CH_2,J = 7.2 Hz, J= 3.6 Hz), 1.23 (t, 3H, CH_3, J = 7.2 Hz). IR (KBr) υ_{max}: 3315, 3217, 3160, 3106 (NH_2, NH), 1734 (C=O_{ester}) cm^{-1}. MS m/z (%): 346 (M^+; 20.4), 300 (21.7), 273 (100), 172 (55.4), 145 (16.5), 130 (21.8), 115 (31.1). Anal. Calcd. for $C_{14}H_{14}N_6O_3S$ (346.36): C, 48.55; H, 4.07; N, 24.26; S, 9.26. Found: C, 48.31; H, 3.97; N, 24.13; S, 9.11.

***Synthesis of 4-[6-Amino-6,7-dihydro-5H-1,2,4-triazolo[3,4-b][1,3,4-thiadiazin-3-yl]phthalazin-1(2H)-one 14*:** A mixture of **4** (1 g, 3 mmol), chloroacetonitrile (0.22 mL, 3 mmol) in dioxane (20 mL) in the presence of fused sodium acetate (0.5 g) was refluxed for 10 hrs. The reaction mixture was concentrated and then poured onto cold water and the precipitated product was filtered off, dried and recrystallized from dioxane/DMF to give **14** as white crystals; mp: 254-256°C, yield: 59%. ^{1}H-NMR (DMSO-d_6) δ (ppm): 13.9 (s, 1H, NH, exchangeable with D_2O), 8.38-7.93 (m,4$H_{arom.}$),6.17 (s, 2H, NH_2, exchangeable with D_2O), 4.39 (s, 2H, CH_2). IR (KBr) υ_{max}: 3347, 3287, 3161(NH_2, NH), 1662 (C=O) cm^{-1}. MS m/z (%): 299 (M^+; 70.8), 283 (63.2), 258 (11), 228 (14.7), 198 (24.6), 172 (100), 130 (34.2), 114 (70), 88 (62.3), 50 (33.6). Anal. Calcd. for $C_{12}H_9N_7OS$ (299.31): C, 48.15; H, 3.03; N, 32.76; S, 10.71. Found: C, 48.03; H, 2.91; N, 32.58; S, 10.64.

***Synthesis of 4-[6-(4-Nitrophenyl)-7H-1,2,4-triazolo[3,4-b][1,3,4-thiadiazin-3-yl]phthalazin-1(2H)-one 15*:** A mixture of **4** (1 g, 3 mmol) and*p*-nitro-*w*-bromoacetophenone (0.73 g, 3 mmol) in dioxane (20 mL) was heated under reflux for 6 hrs. The reaction mixture was cooled and the deposited solid was filtered off, dried and recrystallized from dioxane/DMF to give **15** as beige crystals; mp: 246-248°C,yield: 71%. ^{1}H-NMR (DMSO-d_6) δ (ppm): 13.36 (s, 1H, NH, exchangeable with D_2O), 8.9-8.3 (dd, 4$H_{arom.}$),8.3-7.9 (m,4$H_{arom.}$),5.3 (s, 2H, CH_2). IR (KBr) υ_{max}: 3301(NH), 1674 (C=O) cm^{-1}. Anal. Calcd. for $C_{18}H_{11}N_7O_3S$ (405.39): C, 53.33; H, 2.73; N, 24.19; S, 7.91. Found: C, 53.24; H, 2.62; N, 24.03; S, 7.80.

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