

Synthesis and antimicrobial activity of some nitrogen heterobicyclic systems: Part II

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The reaction of 3-chloro-5,6-diphenyl-1,2,4-triazine **1** with amino derivatives and with active methylene compounds have been studied in neutral and alkaline media to obtain 3-heteroaryl- 5,6-diphenyl-1,2,4-triazines. The structures of the new compounds have been elucidated with the help of elemental analysis and spectral data. Preliminary screening of some selected compounds for antimicrobial activity is reported.

As a part of a research program directed towards the preparation of some new 1,2,4-triazines as analogs of useful drugs¹⁻⁷ and keeping in view their biocidal effect, we report herein the reactions of 3-chloro-5,6-diphenyl-1,2,4-triazine **1** with amines and active methylene compounds followed by ring closure reactions with binitrogen compounds. An attempt is made to study the structure activity relationship.

3-Substituted amino-5,6-diphenyl-1,2,4-triazines **2a-h** were synthesized by treating triazine **1** with amino compounds such as, sulfanilamide, 4-aminoantipyrine, 4-aminoacetophenone, anthranilic acid, 5-chloroanthranilic acid, 5-bromoanthranilic acid, 5-iodoanthranilic acid and/or 4-substituted semicarbazide in DMF [Scheme I]. The structure of these compounds were based on elemental analysis and IR spectral data. The IR spectral of these compounds showed the presence of characteristic band due to bonded NH.

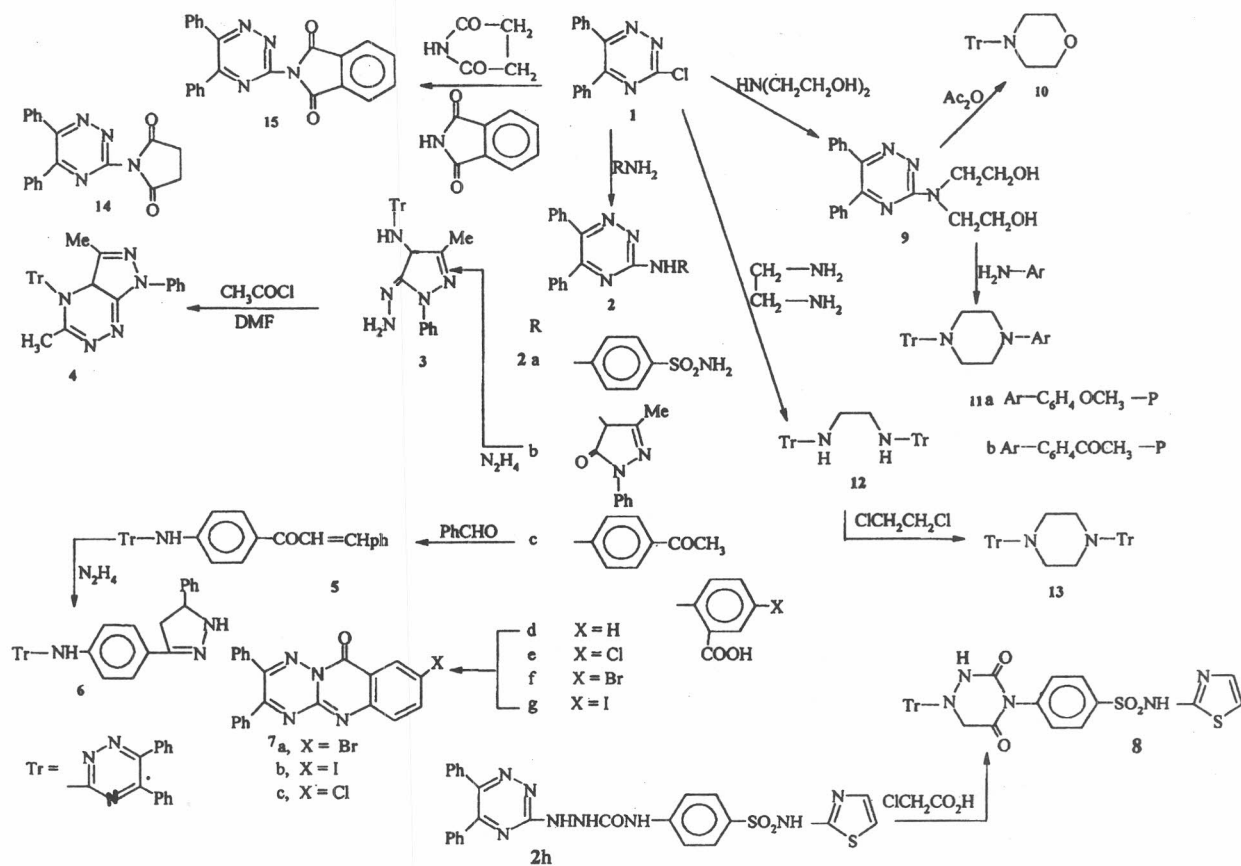
Hydrazinolysis of **2b** in dry ethanol gave the hydrazone **3** which on treatment with acetyl chloride in DMF afforded the pyrazolo [3,4-*e*] [1,2,4] triazine **4**. The structures of **3** and **4** were deduced from elemental analysis and IR spectral data. The IR spectrum of **3** showed the presence of NH₂, NH with exocyclic C=N groups. The absence of the absorption bands due to NH₂ and NH groups in the IR spectrum of **4** confirm the formation of fused bicyclic structure.

Condensation of **2c** with benzaldehyde in sodium ethoxide⁸ yielded the chalcone **5**, which on hydrazinolysis led to the formation of reduced pyrazole **6**. The IR spectrum of **5** revealed the absorption bands that correspond to NH, C=O and exocyclic C=C functional groups. Presence of resonance signals at δ 4.5, 7-7.6 and 10.5 ppm due to CH=CH, aromatic and NH protons respectively confirms the structure of **5**.

The structural difference between compounds **5** and **6** was revealed from elemental analysis, IR and ¹H NMR spectral data. The IR spectrum of **6** showed the absence of characteristic bands due to C=O. ¹H NMR of **6** indicated signals due to CH₂, CH, aromatic and NH protons at δ 3.4, 4.6, 7.2-7.8, 8.0 and 10.5 ppm respectively.

Incorporation of quinazoline moiety in the heterocyclic systems as fused derivatives has led to compounds possessing significant biological activity. The present work also describes the synthesis of some new fused quinazolino-1,2,4-triazines **7a-c** starting from **2e-g** (Scheme I). IR spectra of **7a-c** revealed the absence of absorption due to OH, NH groups. The mass spectrum of **7b** (Chart I) showed M⁺ at m/z 476 (0.4%). Formation of ions at m/z 202, 178 or 68 due to diphenylene acetylene, iodobenzene or CN₄ moieties corroborate with the postulated structure for **7b** (Chart 1).

The point of interest in this investigation is that **2h** on reaction with monochloroacetic acid in aq.



Scheme I

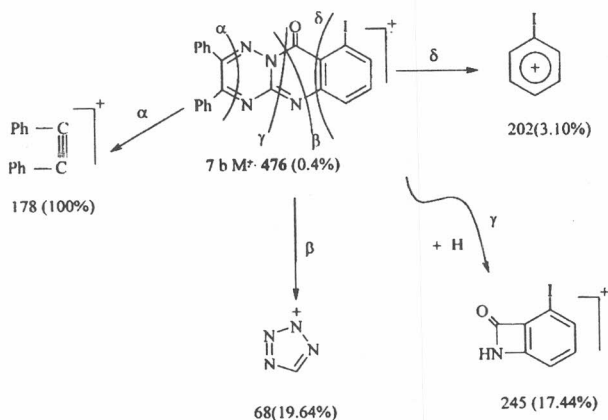


Chart 1

NaOH^9 yielded 1,4-di-substituted-6-dihydro-1,2,4-triazin-3,5-(2H, 6H)-dione **8**. IR spectrum of **8** showed a broad band for NH and bands due to SO_2NH_2 and cyclic $\text{C}=\text{O}$ groups. Its mass spectrum (Chart 2) revealed the molecular ion peak at m/z 584 (0.3%) and the base peak at 105 (100%), due to benzaldehyde imine moiety.

Interaction between **1** and diethanolamine in DMF afforded trisubstituted amine **9**, which on refluxing with acetic anhydride produced *N*-(5,6-diphenyl-1,2,4-triazin-3-yl) morpholine **10**. Compound **10** was also obtained directly from treatment of compound **1** with morpholine in DMF.

On the other hand, *N*¹,*N*⁴-disubstituted piperazine **11** was obtained¹⁰ by reaction of **9** with primary aromatic amine in the presence of conc. HCl.

Further, compound **1** on refluxing with 1,2-diaminoethane in DMF afforded the corresponding bis-compound **12** which on ring closure reaction with 1,2-dichloroethane led to 1,4-disubstituted-piperazine **13**. This was also obtained from **1** by reaction with piperazine. The synthesis of imide derivatives **14** or **15** have been made by treatment of **1** with succinimide and phthalimide respectively in DMF.

The replacement of chlorine atom in **1** with active methylene compounds and their reactivity towards nucleophilic nitrogen compounds¹¹

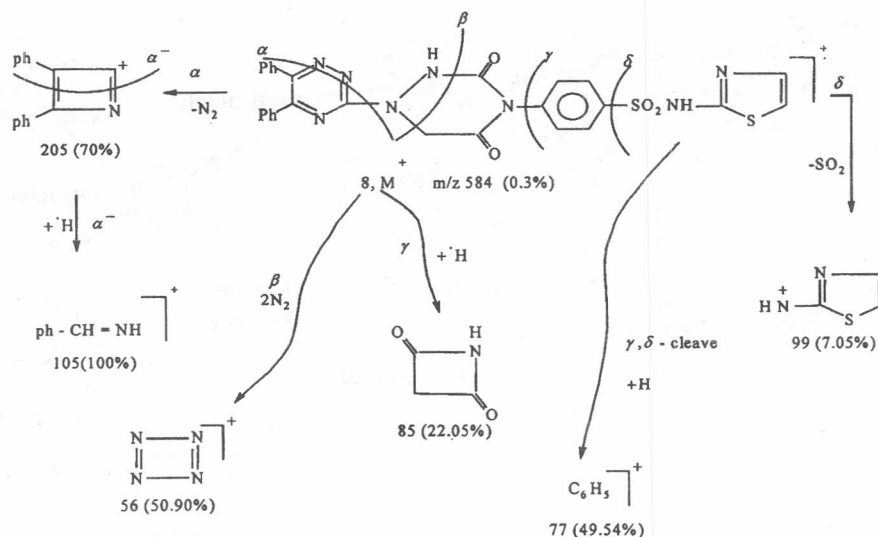
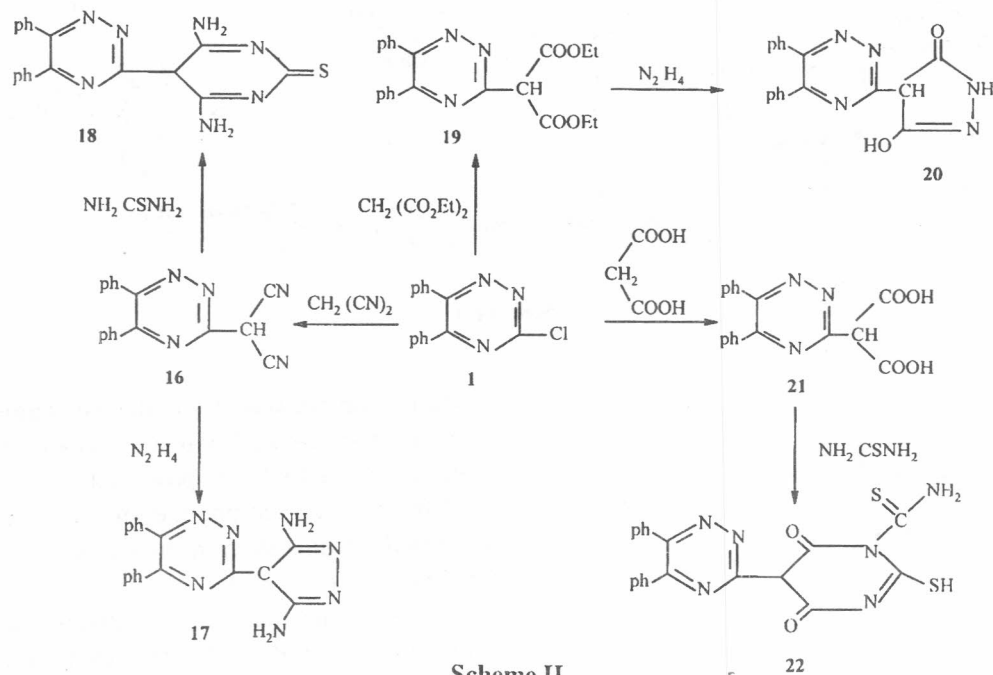


Chart 2



Scheme II

prompted us to investigate their reaction further. Thus, treatment of 1 with malononitrile in DMF resulted in the formation of trisubstituted methane 16 (Scheme II). Hydrazinolysis of 16 in absolute ethanol produced 3,5-diamino-4-(5,6-diphenyl-1,2,4-triazin-3-yl) pyrazoline 17 while its reaction with thiourea in methanol gave 4,6-diamino-5-(5,6-diphenyl-1,2,4-triazin-3-yl) pyrimidin-2-thione 18 (Scheme II). The structures of 16-18 were established by analytical and IR spectral data.

Based on the reactivity of diethyl malonate and

malonic acid for the synthesis of a large number of heterocyclic systems, 3-hydroxy-4-(5,6-diphenyl-1,2,4-triazin-3-yl) pyrazolin-5-one 20 was obtained from reaction of 1 with diethyl malonate to give 19 followed by hydrazinolysis. The compounds 19 and 20 exhibited IR absorption bands in the range $1700\text{--}1600\text{ cm}^{-1}$ (C=O) and at 3100 cm^{-1} (NH). The structure of 20 was supported by ^1H NMR which showed signals at δ 3.4, 7.4 - 8.0 and 10, due to $-\text{CH}$, aromatic, OH and NH protons, respectively.

El-Gendy *et al.* have reported the preparation

of heterocyclic system having 1,2,4-triazine moiety^{12,13}. In view of this we reacted compound **1** with malonic acid in DMF to give **21** which on treatment with thiourea in methanol afforded¹¹ 2-mercapto-3-thiocarbamido-5-(5, 6-diphenyl-1, 2, 4-triazin-3-yl) pyrimidin-4,6 (1*H*, 3*H*) dione **22**. IR spectrum of **22** indicated the presence of NH₂, NH, C=O, CS functional groups. Its mass spectrum exhibited molecular ion at *m/z* 437 (0.2%) corresponding to (M+1) due to presence of sulfur, and base peak at *m/z* 178 (100%) corresponding to diphenyl-acetylene (Chart 3). The probable mechanism for this type of reaction may involve nucleophilic attack of nitrogen on thiocarbamide center followed by elimination of one mole of NH₃ (Chart 4).

Antimicrobial activities

The antimicrobial activities of some of these compounds were tested against Gram positive bacterium *Bacillus subtilis* and fungus *Aspergillus niger* by the disc method¹⁴, using DMF as a solvent at three different concentrations 25, 50 and 100 µg/mL. Growth inhibition was calculated with reference to control¹⁵.

The results have shown that compounds in which 4,6-dihydroxy- pyrimidine having mercapto group in the position 3 of 1,2,4-triazine nucleus have exhibited significant antibacterial activity but less activity against the tested fungus (Table I).

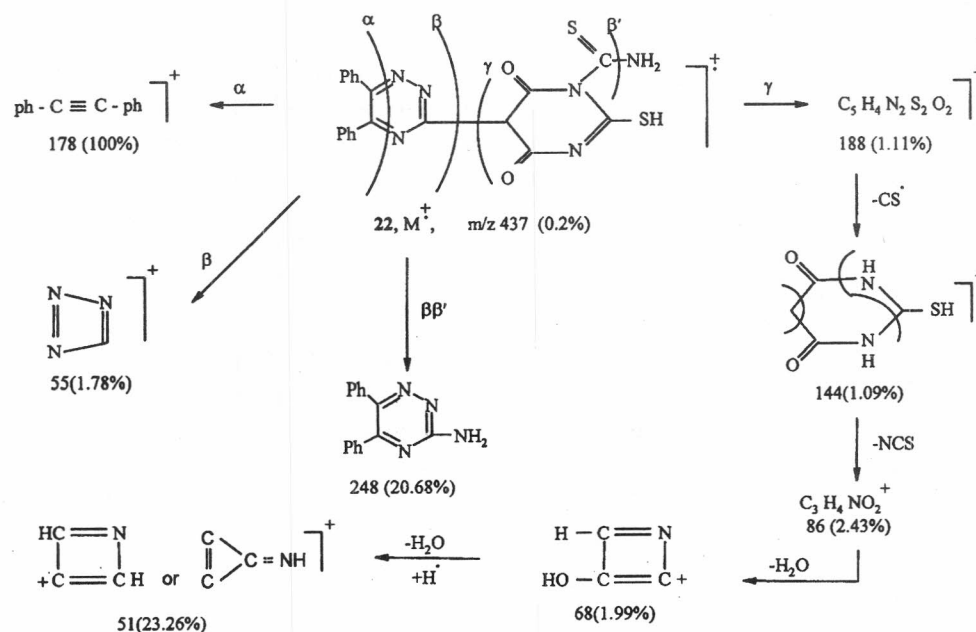
The replacement of hydroxy or mercapto group by amino or nitrile group resulted in the enhancement of antibacterial activity. Moderate antibacterial activity was observed for the substituted amino compounds, while no activity was observed between tested compounds and evaluated fungi at any concentration.

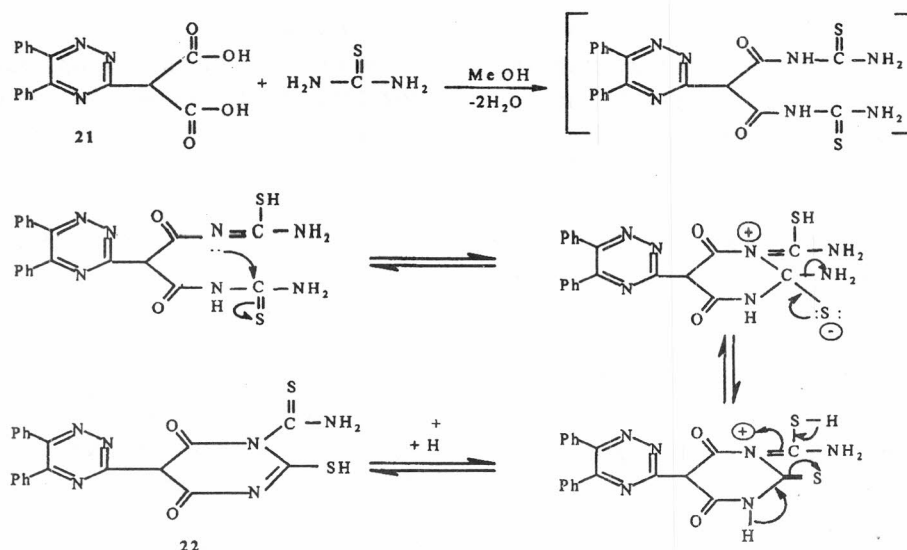
Experimental Section

Melting points are uncorrected. Microanalysis were carried out at microanalytical data unit at Cairo University on Heroeus instrument. All the new compounds gave satisfactory C, H, N, S and halogens values within ± 0.5% difference from the calculated values. IR spectra were recorded on a Pye Unicam SP 1000 spectrophotometer in KBr,

Table I—The effect of some synthesized compounds on the growth of *Bacillus subtilis* and *Aspergillus niger*

Compd	Diameter of inhibition zone (mm)					
	<i>Bacillus subtilis</i>			<i>Aspergillus niger</i>		
	25 µg/mL	50 µg/mL	100 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
2h	2.0	3.0	3.0	0.0	0.0	0.0
8	3.0	6.0	7.0	0.0	0.0	0.0
15	1.0	2.0	2.0	0.0	1.0	1.0
16	0.0	1.0	1.0	0.0	0.0	1.0
17	2.0	2.0	2.0	0.0	0.0	0.0
18	0.0	1.0	1.0	0.0	0.0	0.0
20	1.0	3.0	4.0	0.0	0.0	0.0
22	0.0	0.0	1.0	0.0	0.0	0.0





^1H NMR spectra were measured on a Varian A-66 and JEOL-90 MHz spectrometers using TMS as internal reference. Chemical shifts are expressed in δ ppm and the mass spectra on a FINNI-GAN 3300 mass spectrometer by direct inlet (Source temp: 90-300°C beam energy 70 eV). 3-Chloro-5,6-diphenyl-1,2,4-triazine **1** was pre-pared as described in literature¹.

3-Substituted amino-5,6-diphenyl-1,2,4-triazines 2a-g. A mixture of **1** (0.01 mole) and the appropriate amino compound (0.01 mole) in DMF (20 mL) was heated under reflux for 15 min, cooled and poured on ice. The mixture was filtered and the solid obtained was crystallized from a proper solvent to give **2a-g**. Melting point crystallization solvent and % yield for these compounds were found to be: **2a**: 218-20°C, (benzene) 94%; **2b**: 95-96°C (benzene), 78%; **2c**: 208-10°C (ethyl acetate), 62%, **2e**: 98-100°C, (acetic acid), 82%; **2f**: 179-81°C (acetic acid), 91%; **2g**: 101-103°C (acetic acid), 90%, IR (**2a**): 3390, 3280, 3210 (NH), 3080 (CH aromatic), 1600 (C=N) and 1300, 1225 (SO_2) cm^{-1} ; IR (**2b**): 3250 (NH), 3080 (CH aromatic), 2900 (CH aliphatic), 1670 (C=O) and 1600 (C=N) cm^{-1} ; IR (**2c**): 3250 (NH), 3090 (CH aromatic), 2960 (CH aliphatic), 1660 (C=O) and 1610 (C=N); IR (**2e**): 3475 (OH), 3330 (NH), 3090 (CH aromatic), 1770 (C=O) and 1610 (C=N) cm^{-1} ; IR (**2f**): 3480 (OH), 3320 (NH),

3080 (CH aromatic), 1700 (C=O) and 1620 (C=N) cm^{-1} .

***N*¹-(5,6-Diphenyl-1,2,4-triazin-3-yl)-*N*⁴-(4-phenyl-sulfonamidothiazol-2-yl) semicarbazide 2h.** A mixture of **1** (0.01 mole) and appropriate *N*¹,*N*⁴-disubstituted semicarbazide (0.01 mole) in DMF (20 mL) was refluxed for 10 min, cooled and poured on ice. The solid obtained was filtered and crystallized from ethanol to give **2h**: m.p. 99-100°C, yield 84%; IR: 3400 (NH), 3090 (CH aromatic), 2930 (CH aliphatic), 1780 (C=O) and 1320, 1245 (SO_2) cm^{-1} .

3-(1-Phenyl-2-dimethyl-4*H*-5-hydrazonopyrazolin-4-yl) amino-5,6-diphenyl-1,2,4-triazine 3. A mixture of **2b** (0.01 mole) and hydrazine hydrate (0.01 mole) in absolute ethanol (50 mL) was refluxed for 2 hr. The solid obtained was collected by filtration and crystallized from ethanol to give **3**, m.p. 205-206°C, 69%; IR: 3350-3280 (NH), 3010 (CH aromatic) and 1610 (C=N) cm^{-1} .

2,3,5-Trimethyl-1-phenyl-4-(5,6-diphenyl-1,2,4-triazin-3-yl)-4*H*-pyrazolo [4,3-*e*]-1,2,4-triazine 4. A mixture of **3** (0.01 mole) and acetyl chloride (0.01 mole) in DMF was heated under reflux for 4 hr, cooled and poured on ice. The solid obtained was recrystallized from toluene to give **4**, m.p. 128-30°C, yield 65%; IR: 3040 (CH aromatic), 2900 (CH aliphatic) and 1590 (C=N) cm^{-1} .

3-(Cinnamoylphenyl) amino-5,6-diphenyl-1,

2,4-triazine 5. A mixture of **4** (0.01 mole) and benzaldehyde (0.01 mole) in ethanolic NaOH (5%, 50 mL) was stirred at room temperature for 2 hr and acidified with dil. HCl. The precipitated solid was collected by filtration and crystallized from benzene to give **5**, m.p. 250-52°C, yield 72%; IR: 3280 (NH), 3050 (CH aromatic), 2960 (CH aliphatic), 1660 (C=O) and 1600 (C=N) cm^{-1} .

3-[4-(2,3,4-trihydro-3-phenylpyrazolo-5-yl)-phenyl]amino-5,6-diphenyl-1,2,4-triazine 6. To a solution of **5** (0.01 mole) in ethanol (20 mL), hydrazine hydrate (0.01 mole) was added and the reaction mixture refluxed for 5 hr. The solid that separated on cooling was collected and recrystallized from ethanol to give **6**, m.p. 180-82°C, yield 70%; IR: 3250, 3150 (NH), 3030 (CH aromatic), 2980 (CH aliphatic) and 1605 (C=N) cm^{-1} .

9,10-Diphenyl-5-halo-7-oxoquinazolino [3,2-*b*]-1,2,4-triazines 7a-c. Compound **2e,f** and **2g** (0.5 g) in glacial acetic acid (25 mL) was refluxed for 4 hr, cooled and poured on ice. The solid obtained was recrystallized to give **7a-c** respectively. Melting point, crystallization solvent and % yield for these compounds were found to be: **7a**: 88-90°C (benzene), 82%; **7b**: 115-16°C (benzene), 86%; **7c**: 132-35°C (benzene), 84%; IR (**7a**): 3090 (CH aromatic), 1680 (C=O) and 1610 (C=N) cm^{-1} .

1-(5,6-Diphenyl-1,2,4-triazin-3-yl)-4-(4-phenyl-sulfonamidothiazol-2-yl)-6-dihydro-1,2,4-triazin-3,5-(2*H*) dione 8. A mixture of **2h** (0.01 mole) and monochloroacetic acid (0.01 mole) in aq. NaOH (5%, 20 mL) was refluxed for 4 hr, cooled, diluted with cold water and neutralized with dil. HCl. The precipitated solid was crystallized from ethanol to give **8**, m.p. 88-90°C, yield 69%; IR: 3500-3100 (OH,NH), 1715 (C=O) and 1290, 1230 (SO_2) cm^{-1} .

3-(Diethanol) amino-5,6-diphenyl-1,2,4-triazine 9. A mixture of **1** (0.01 mole) and diethanolamine (0.01 mole) in DMF (20 mL) was refluxed for 1h, cooled and poured on ice. The solid obtained was recrystallized from ethanol to give **9**, m.p. 141-42°C, yield 72%; IR: 3430 (OH), 2960-2900 (CH aliphatic), 1620 (C=N) cm^{-1} .

***N*-(5,6-Diphenyl-1,2,4-triazin-3-yl) morpholine 10.** Compound **9** (0.5 g) in acetic anhydride (20 mL) was refluxed for 4 hr, cooled and poured on ice with stirring. The solid obtained was recrystallized from benzene to give **10**, m.p. 138-

40°C, yield 84%; IR: 3050 (CH aromatic), 2850-2800 (CH aliphatic) and 1610 (C=N) cm^{-1} .

***N*¹-(5,6-Diphenyl-1,2,4-triazin-3-yl)-*N*⁴-aryl-piperazine 11a,b.** A mixture of **9** (0.01 mole) and the appropriate primary aromatic amine (0.01 mole) in ethanol (50 mL) and HCl (4*N*, 10 mL) was refluxed for 4 hr, cooled and poured on ice. The solid that precipitated crystallized from benzene to give **11a,b**: **11a**: m.p. 125-126°C, yield 96%; **11b**: m.p. 140-41°C, yield 90%; IR (**11b**): 1700 (C=O), 1600 (C=N), 1480 (def. CH_2 , CH_3) cm^{-1} .

Reaction of 1 with 1,2-diaminoethane: Formation of bis-compound 12. A mixture of **1** (0.02 mole) and 1,2-diaminoethane (0.01 mole) in DMF (20 mL) was heated under reflux for 1 hr, cooled and poured on ice and filtered. The solid obtained was crystallized from ethanol to give **12**, m.p. 90-91°C, yield 94%; IR: 3100 (NH), 2900 (CH_2), 1620 (C=N), 1440 (def. CH_2) cm^{-1} .

***N*¹,*N*⁴-Di (5,6-diphenyl-1,2,4-triazin-3-yl) piperazine 13.** A mixture of **12** (0.01 mole) and 1,2-dichloroethane (0.01 mole) in DMF (20 mL) was refluxed for 2 hr, cooled and poured on ice and the resultant solid was crystallized from DMF to give **13**, m.p. 214-15°C, yield 86%; IR: 2900-2850 ($\text{CH}_2\text{-CH}_2$), 1610-1590 (C=N), 1470 (def. $\text{CH}_2\text{-CH}_2$) cm^{-1} .

***N*-(5,6-Diphenyl-1,2,4-triazin-3-yl) imides 14, 15.** A mixture of **1** (0.01 mole) and succinimide and/or phthalimide (0.01 mole) in DMF (20 mL) was refluxed for 1 hr, cooled and poured on ice. The resultant solid was crystallized from ethyl acetate to give **14** and/or **15**; **14**: m.p. 220-222°C, yield 75%; **15**: m.p. 188-90°C, yield 80%; IR (**14**): 1750 (C=O), 1480 (def. CH_2) cm^{-1} .

Reaction of 1 with malononitrile: Preparation of 16. A mixture of **1** (0.01 mole) and malononitril (0.01 mole) in DMF (20 mL) was refluxed for 15 min, cooled and poured on ice. The solid thus obtained crystallized from benzene to give **16**, m.p. 180-82°C, yield 87%; IR: 3020 (CH aromatic), 2300-2280 (C $\equiv$ N) and 1580 (C=N) cm^{-1} .

3,5-Diamino-4*H*-4-(5,6-diphenyl-1,2,4-triazin-3-yl) pyrazole 17. A mixture of **16** (0.01 mole) and hydrazine hydrate (0.01 mole) in absolute ethanol (50 mL) was refluxed for 4 hr, cooled and poured on ice. The solid thus obtained was crystallized from ethanol to give **17**, m.p. 233-35°C, yield 86%;

IR: 3300-3200 (NH), 3100 (CH aromatic) and 1620 (C=N) cm^{-1} .

4,6-Diamino-5H-5-(5,6-diphenyl-1,2,4-triazin-3-yl) pyrimidin-2-thione 18. A mixture of **16** (0.01 mole) and thiourea (0.01 mole) in absolute ethanol (50 mL) was heated under reflux for 4 hr, cooled and poured on ice. The solid obtained was recrystallized from ethanol to give **18**, m.p. 175-76°C, yield 92%; IR: 3300-3200 (NH), 3100 (CH aromatic), 1620 (C=N) and 1200 (C-S) cm^{-1} .

Preparation of substituted diethyl malonate 19. A mixture of **1** (0.01 mole) and diethyl malonate (0.01 mole) in DMF (20 mL) was refluxed for 2 hr, and the excess solvent was removed. The oily residue was solidified with pet. ether (100-20°C). The solid thus obtained was crystallized from ethyl acetate to give **19**, m.p. 215-17°C, yield 95%; IR: 3080 (CH aromatic), 2910 (CH aliphatic), 1700-1660 (C=O), and 1610 (C=N) cm^{-1} .

4H-4-(5,6-Diphenyl-1,2,4-triazin-3-yl)-5-hydroxypyrazolin-3 (2H)one 20. A mixture of **19** (0.01 mole) and hydrazine hydrate (0.01 mole) in dry benzene (100 mL) was heated under reflux for 8 hr. The excess solvent was removed. The solid obtained upon dilution was collected by filtration and then crystallized from ethanol to give **20**, m.p. 194-95°C, yield 78%; IR: 3100 (NH), 1700 (C=O) and 1600 (C=N) cm^{-1} .

Preparation of substituted malonic acid 21. A mixture of **1** (0.01 mole), malonic acid (0.01 mole) in DMF (20 mL) was refluxed for 2 hr, cooled and poured on ice. The resultant solid was crystallized from acetic acid to give **21**, m.p. 204-205°C, yield 88%; IR: 3430, 3310 (OH), 1670, 1650 (C=O) and 1600 (C=N) cm^{-1} .

5H-5-(Diphenyl-1,2,4-triazin-3-yl)-2-mercapto-3-thiocarbamido pyrimidin-4,6-dione 22. A

mixture of **21** (0.01 mole) and thiourea (0.02 mole) in methanol (100 mL) was heated under reflux for 4 hr, cooled and poured on ice. The solid obtained was recrystallized from ethanol to give **22**, m.p. 225-27°C, yield 81%; IR: 3320-3100 (NH₂,NH), 3010 (CH aromatic), 2500 (SH), 1700, 1670 (C=O) and 1300 (C=S) cm^{-1} .

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