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Behavior of 3-hydrazino-6-aryl-1,2,4-triazin-5-one as a strong nucleophile towards active electrophilic compounds and their antibacterial evaluation

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Abstract: The behavior of 3-hydrazino-6-aryl-1,2,4-triazin-5-one towards the active electrophilic compounds in polar and/ or non-polar solvents and various times and temperatures, has been studied. *N*-[2-(3-(3/5-(4-Nitrophenyl)-5/3-thioxo-1*H*-1,2,4-triazol-1-yl)-5-oxo-1,2,4-triazin-6-yl)phenyl]pivalamides were obtained from the reaction of *N*-(2-(3-hydrazineyl-5-oxo-1,2,4-triazin-6-yl)phenyl)pivalamide with 4-nitrobenzoyl isothiocyanate in THF and/ or EtOH-piperidine respectively. Also, *N*-(2-(3-hydrazineyl-5-oxo-1,2,4-triazin-6-yl)phenyl)pivalamide was shown a strong nucleophilic behavior by reaction with *N*-phenylthiourea to produce *N*-[2-(5-oxo-3-(2-(phenylcarbamothioyl)hydrazineyl)-1,2,4-triazin-6-yl)phenyl]pivalamide, which upon cyclization with diethyl carbonate produced *N*-(2-(5-oxo-3-(5-oxo-4-phenyl-3-thioxo-1,2,4-triazolidin-1-yl)-1,2,4-triazin-6-yl)phenyl)pivalamide. Moreover, *N*-(2-(3-hydrazineyl-5-oxo-1,2,4-triazin-6-yl)phenyl)pivalamide studied its behavior by reaction with cyanoacetic acid, chloroacetonitrile, and/ or benzoyl carbonitrile to produce *N*-(2-(3-amino-4,8-dioxo-4*H*-[1,2,4]triazino[4,3-*b*][1,2,4]triazin-7-yl)phenyl)pivalamide,*N*-(2-(4-amino-8-oxo-2*H*-[1,2,4]triazino[4,3-*b*][1,2,4]triazin-7-yl)phenyl)pivalamide and *N*-(2-(4-imino-8-oxo-3-phenyl-4*H*-[1,2,4] triazino [4,3-*b*][1,2,4]triazin-7-yl)phenyl)pivalamide. Structure of the products was established upon their elemental analysis and FT-IR, ^{1}H/ ^{13}C NMR, and MS. The new compounds were evaluated as antibacterial agents some Gram-positive and negative bacteria. Some compounds were showed the highest inhibition activity towards *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, and *Sarcina lutea* bacteria and lowest inhibitory activity against *Escherichia coli* bacteria.

Keywords: Behavior of 3-hydrazino-triazinone; Antibacterial; Electrophiles; Inhibition activity; Gram-positive and negative; Strong nucleophile

1. Introduction

Polyfunctionalized 1,2,4-triazine has significant vital probes properties, as biological, pharmacological, and agriculture applications, as anti-HIV [1], anticancer [2], antimicrobial [3,4], photochemical probes for the inhibition of vitiligo,[5] antibiotic resistances [6]. and anti-inflammatory [6]. On the other hand, phosphorus-bearing 1,2,4-triazine mostly enhanced their physical, chemical, and biological properties.[7,8] Also, 3-hydrazino-6-aryl-1,2,4-triazin-5-one used to produce heterobicyclic as anti-HIV and/or molluscicidal activity against some snails.[7] As well as 3-thixio-1,2,4-traizin-5-one derivatives used as starting materials to obtain heteropolycyclic nitrogen systems as antioxidant and anti-inflammatory [9]. *o*-Diamines as hydrazino moiety are very active substrates for the building of various heterobicyclic nitrogen systems [9,10]. Abdel-Rahman *et al*. [11], have been investigated the behavior of hydrazino-groups towards various bi-electrophilic compounds. Based on these observations, and as a part of our continuing work in these areas [12,13], the present work describes other attempts for the behavior of 3-hydrazino-1,2,4-triazinone towards some activated electrophilic compounds in different medium and conditions in view of their bactericidal effects.

2. Results and Discussion

N-[2-(3-Hydrazino-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl)phenyl]pivalamide (**3**) as a starting material, was obtained from acylated 6-(2 aminophenyl)-3-thioxo-1,2,4-triazin-5(4*H*)one (**1**) [14] with *t*-butoyl chloride in warming DMF, followed by hydrazinolysis in EtOH Scheme 1.

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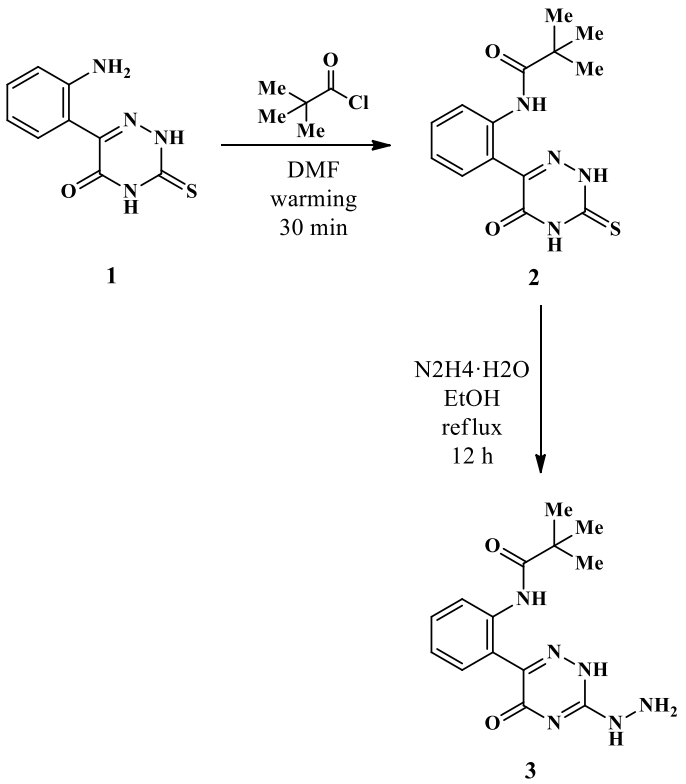
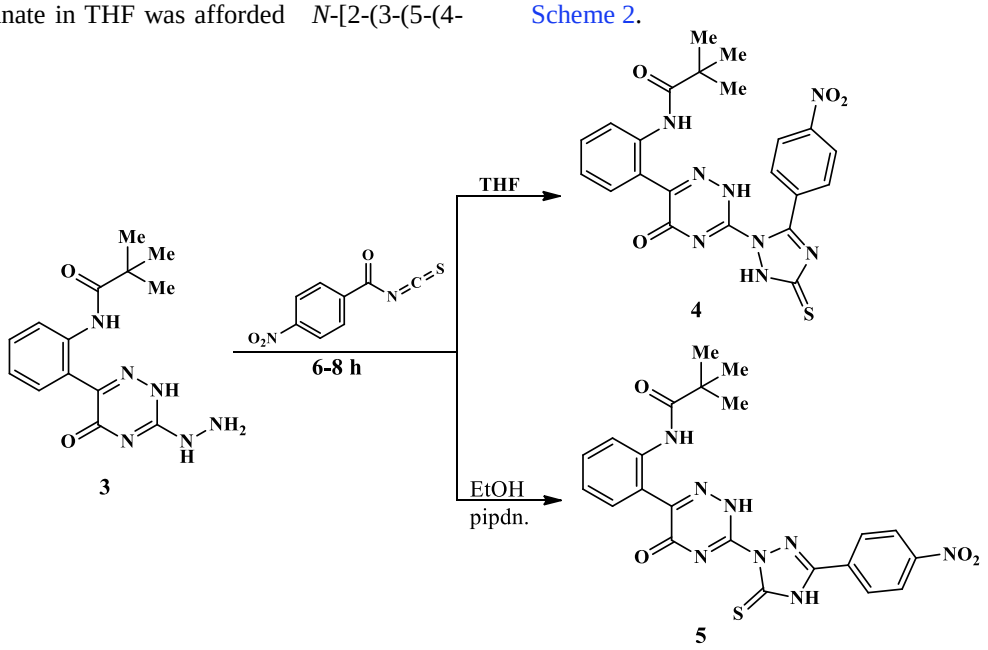


1 **2** **3**

Scheme 1. Synthesis of compounds **2** and **3**

The main aim of this work is a study of the behavior of strong bi-nucleophilic as hydrazine groups towards activated poly electrophilic centers in polar and nonpolar solvents. Thus, refluxing of *N*-[2-(3-hydrazino-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl) phenyl] pivalamide (**3**) with 4-nitrobenzoyl isothiocyanate in THF was afforded *N*-[2-(3-(5-(4-nitrophenyl)-3-thioxo-2,3-dihydro-1*H*-1,2,4-triazol-1-yl)-5-oxo-2,5 -dihydro-1,2,4- triazin-6-yl)phenyl] pivalamide (**4**), while that reaction in EtOH/ drops of piperidine, produced *N*-[2-(3-(3-(4-nitrophenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)-5-oxo-2,5 -dihydro-1,2,4-triazin-6-yl)phenyl]pivalamide (**5**) Scheme 2.



3 **4** **5**

Scheme 2. Synthesis of compounds **4** and **5**

Reaction of compound **3** took place *via* attacking a strong NH_2 of hydrazino group on the carbon of N=C=S group of 4-nitrobenzoyl isothiocyanate in nonpolar solvent (THF), followed by elimination one mol of H_2O to produce compound **4**, while in a polar solvent (EtOH-piperidine), the reaction took another way by attacking a strong NH_2 of hydrazino group on the carbon of O=C-N group of 4-nitrobenzoyl isothiocyanate followed by elimination one mol of H_2O (Fig. 1).

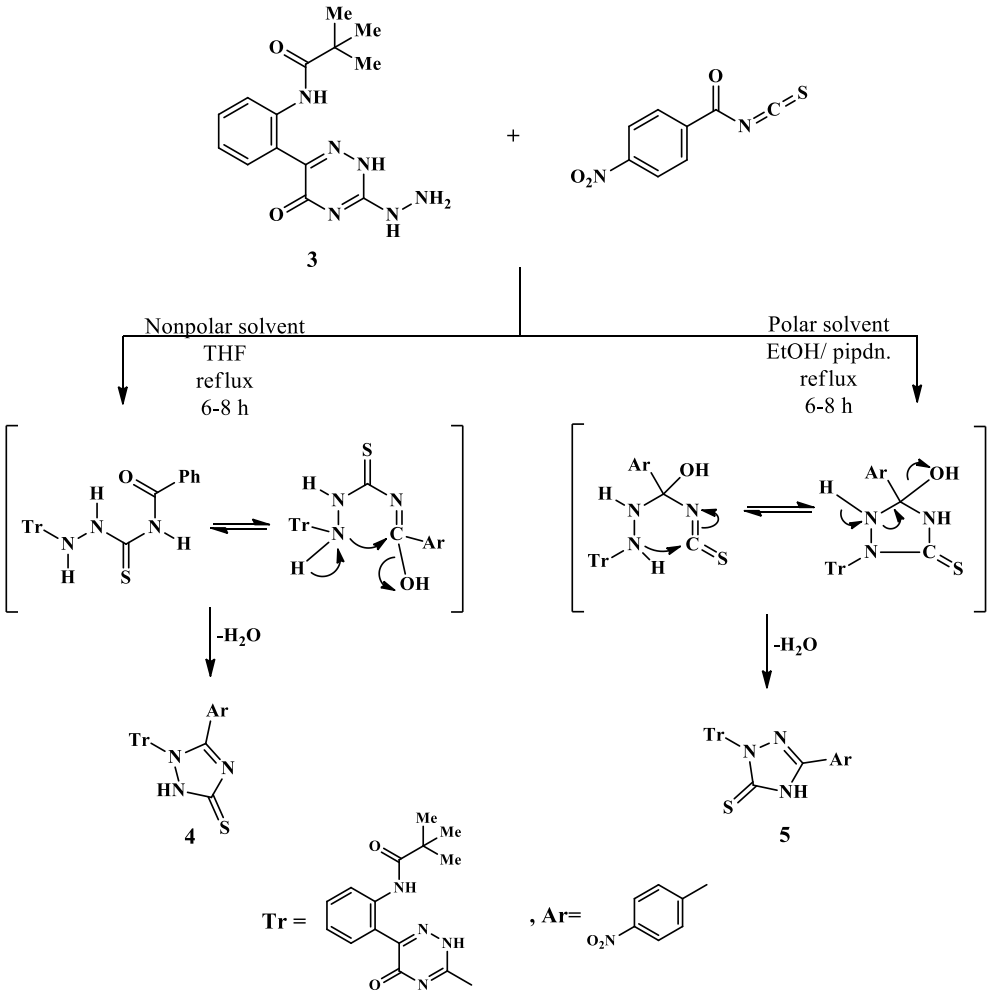


Figure 1. Formation of compounds **4** and **5** from **3**

The isomeric structures **4** & **5** have a different only in their melting points and/or mass fragmentation pattern (Fig. 2 & 3).

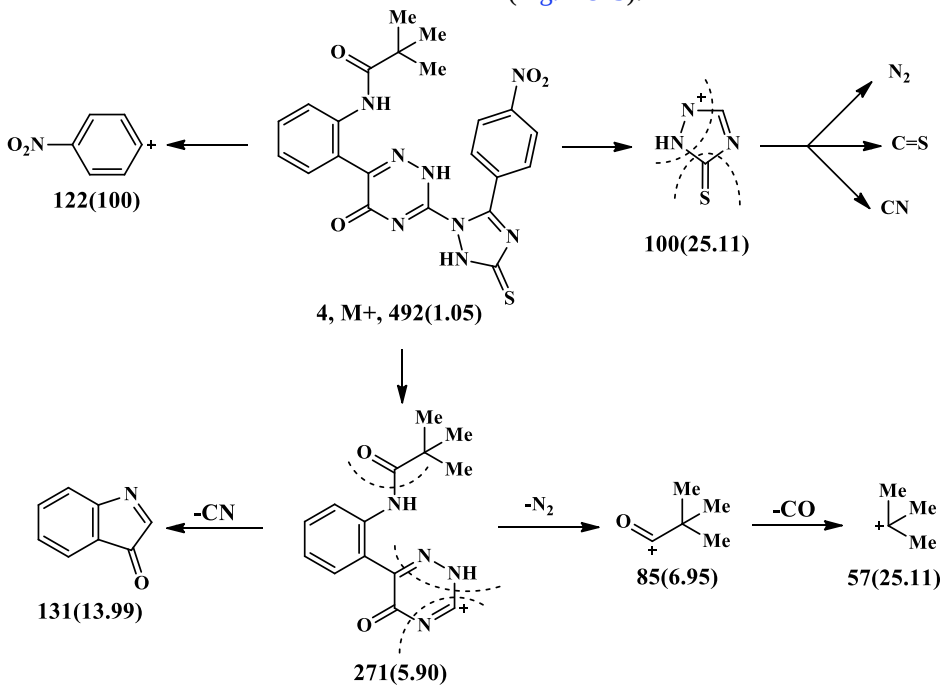


Figure 2. Mass fragmentation of compound **4**

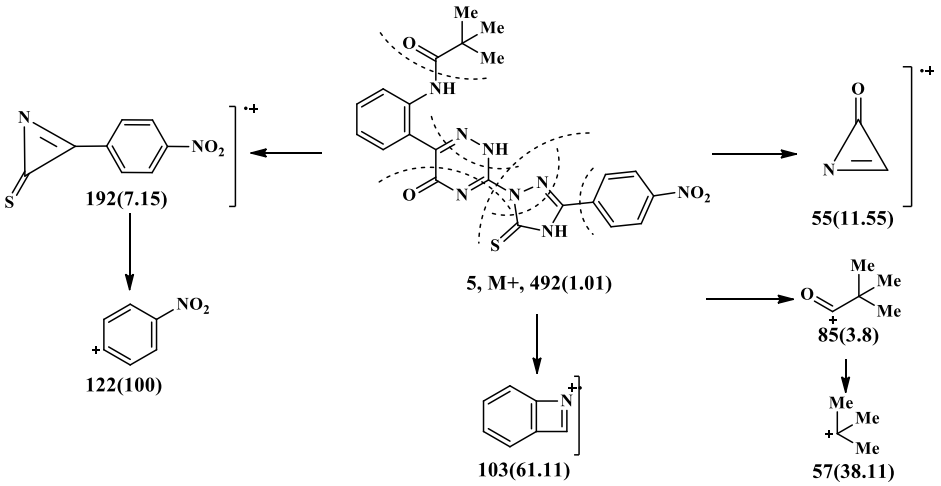


Figure 3. Mass fragmentation of compound **5**

Formation of compound **7** may take place *via* a nucleophilic attack of NH_2 of hydrazino moiety to a more electrophilic position (C=S) with the elimination of two mol of EtOH (Fig. 4).

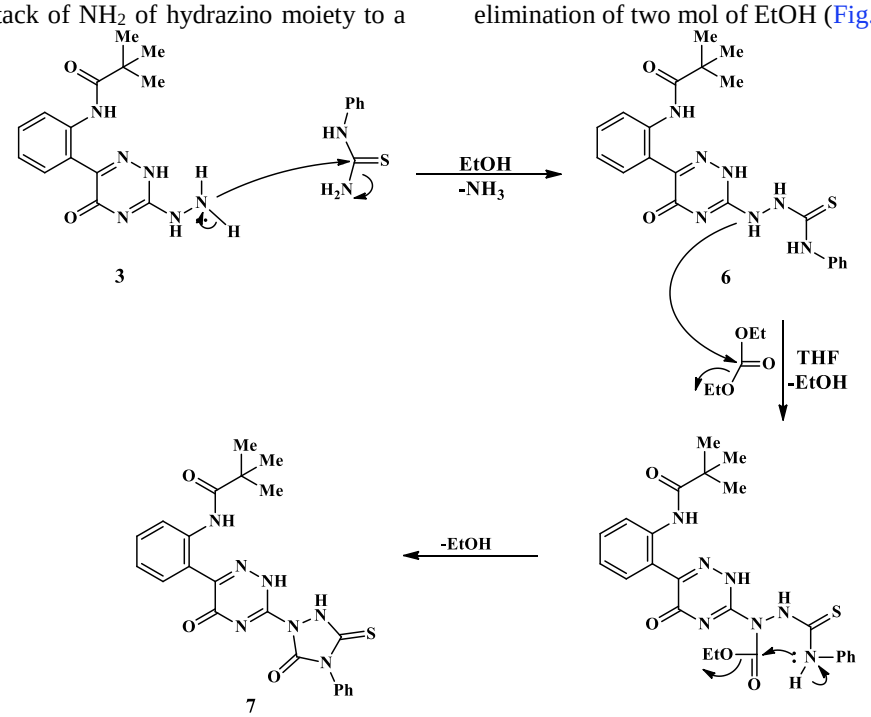
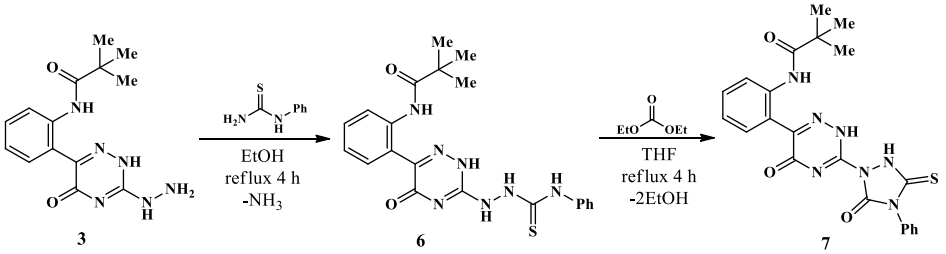


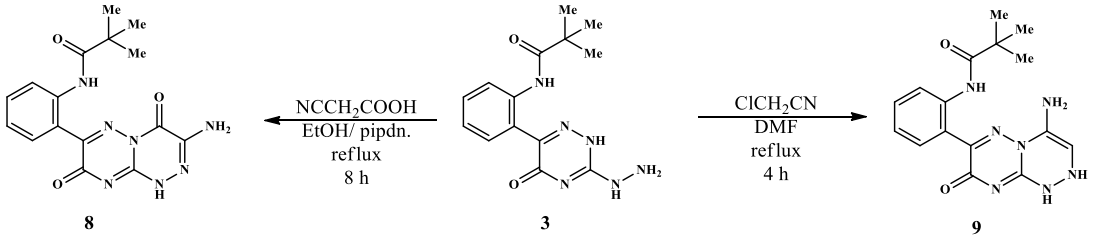
Figure 4. Formation of compound **7** from **3**

It is known that thiourea derivatives are as amides, so the amino group is easily removable. Thus, thiourea is considered weak bi-electrophilic agents. Based upon this fact, refluxing of *N*-[2-(3-hydrazino-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl) phenyl]pivalamide (**3**) with *N*-phenylthiourea in abs. EtOH was yielded *N*-[2-(5-oxo-3-(2-(phenyl carbamothioyl)hydrazino)-2,5-dihydro-1,2,4-triazin -6-yl)phenyl]pivalamide (**6**), which upon full heterocyclization by refluxing with diethyl carbonate in THF, led to the direct formation of *N*-[2-(5-oxo-3-(5-oxo-4-phenyl-3-thioxo-1,2,4-triazolidin-1-yl)-2,5-dihydro-1,2,4-triazin-6-yl) phenyl]pivalamide (**7**) Scheme 3.



Scheme 3. Synthesis of compounds **6** and **7**

Moreover, the interaction between compound **3** as a strong nucleophile and cyanoacetic acid as bi-electrophile in ethanol with few drops of piperidine as a catalyst, yielded[11] *N*-(2-(3-amino-4,8-dioxo-1,8-dihydro-4*H*-[1,2,4]triazino[4,3-*b*][1,2,4]triazin-7-yl)phenyl)pivalamide (**8**), while treatment with 2-chloroacetonitrile in DMF, afforded [12] *N*-(2-(4-amino-8-oxo-1,8-dihydro-2*H*-[1,2,4]triazino[4,3-*b*][1,2,4]triazin-7-yl)phenyl)pivalamide(**9**) respectively Scheme 4.



Scheme 4. Synthesis of compounds **8** and **9**

Formation of both compounds **8** and **9** may be shown in (Fig. 5 & 6).

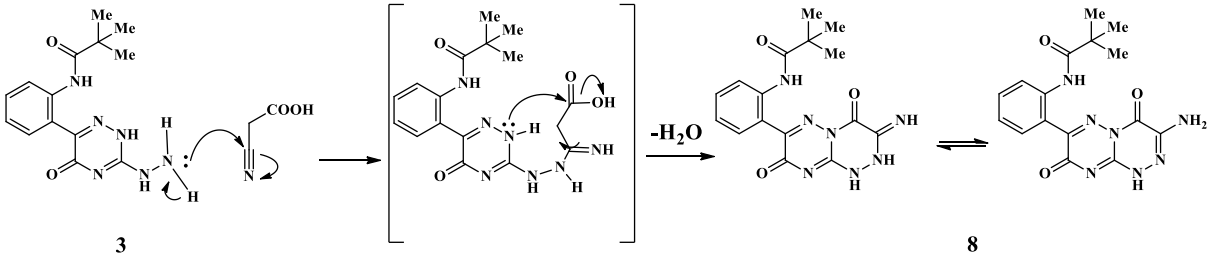


Figure 5. Formation of compound **8** from **3**

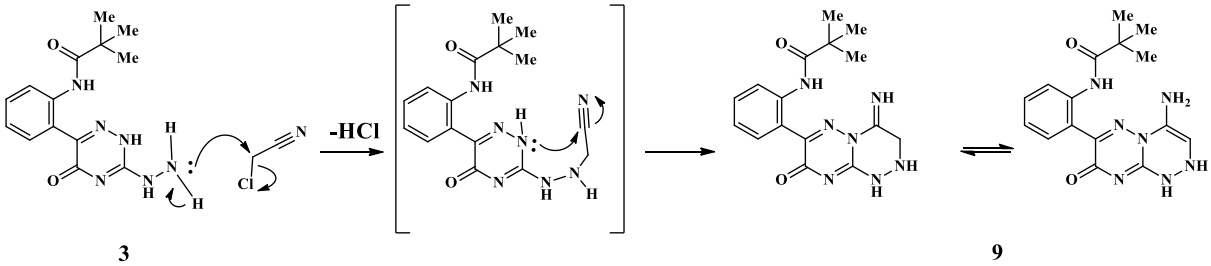
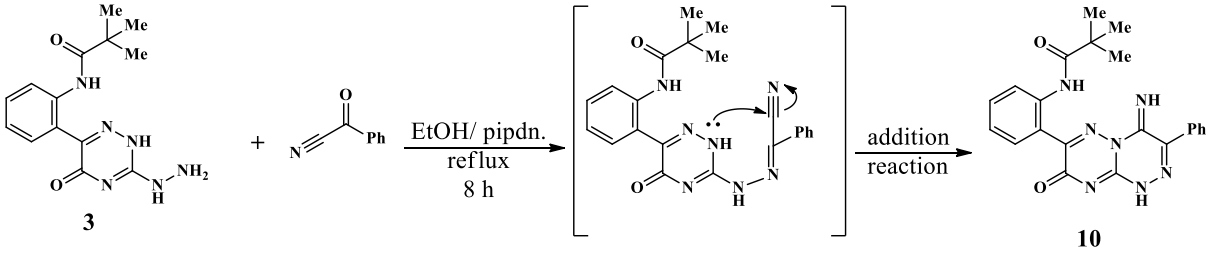


Figure 6. Formation of compound **9** from **3**

Abdel-Rahman *et al*.[11] studied the reactivity of strong nucleophile reagents towards active carbonitriles as electrophile reagents. Similarly, treatment of 3-hydrazino-1,2,4-triazinone **3** with benzoyl carbonitrile in ethanol with drops of piperidine, yielded *N*-(2-(4-imino-8-oxo-3-phenyl-1,8-dihydro-4*H*-[1,2,4]triazino[4,3-*b*][1,2,4]triazin-7-yl)phenyl)pivalamide (**10**) Scheme 5.



Scheme 5. Synthesis of compound **10** from **3**

Former structures of new compounds obtained were deduced from their elemental analysis and spectral measurements. FT-IR absorption spectra of all compounds were showed ῡ at 3300, 3250, 3150 cm^{-1} for NH functional groups, with ῡ at 1670, 1660, &1620, 1600~1570, &1200~1188 cm^{-1} attribute to C=O, C=N, and C=S respectively. Only, the compound **4** and **5** were recorded ῡ at 1530 & 1350 cm^{-1} for asymmetric and symmetric NO_2. ^{1}H NMR spectra of all new compounds were recorded resonated signals at δ 12.11, 10.55, and 8.55 ppm for exo NHCO, endo NHCO, and NHCS protons with *t*-butyl protons at δ 1.2, 1.11, and 0.99 ppm, besides the aromatic protons at δ 7.90-6.99 ppm.

Moreover, ^{13}C NMR spectra of the targets were showed mainly δ at 188-180, 168-152, and 142 ppm attribute to C=S, C=O, & C=N carbons, with aromatic carbons at δ at 132-126 ppm. The two isomers **4** and **5** have differences in melting points and mass spectra. So, mass fragmentation pattern gives us a good indication about these structures, compounds **4** and **5** were showed the molecular ion peak at *m*/*z* 492 with the base peak at *m*/*z* 122 (Fig. 2 & 3).

The former structures of compounds **6** and **7** have been confirmed from that FT-IR spectra were showed ῡ at 1200-1180 cm^{-1} for C=S functional group, while that of compounds **8** and **9** recorded ῡ at 3200-3100 and 1640-1630 cm^{-1} for NH_2 group. All compounds **8**-**10** showed a lacks CN group, which confirms the addition reactions have happened. ^1H NMR spectra of compounds **8** and **9** recorded at 3.5 ppm for NH_2 protons while that of compound **10** showed at 5.5 ppm for =NH proton.

Finally, the synthesized compounds were exhibited antibacterial activity, thus, from the obtained results in Table 1, we can be concluded that: the compounds **4**, **5**, & **7** are observed high inhibition activity towards *P. aeruginosa*, *B. subtilis*, *B. cereus*, and *S. lutea* bacteria and lowest inhibitory activity against *E. coli* bacteria. The MIC study of compounds **4**, **5**, and **7** shown in Table 2.

QSAR study showed that the higher activities of compounds **4**, **5**, & **7** might be due to the presence of thioxo-1,2,4-triazole moiety as well as 4-nitrophenol groups which as bactericidal agents. Moreover, the presence of 6-aryl-1,2,4-triazin-5-one nucleus enhanced that activity. Also, compound **4** had a bioconjugated system between the two heterocyclic 1,2,4-triazole and 1,2,4-triazine nucleus.

In comparison between the activity of compounds, we can be concluded that 3-thioxo-1,2,4-triazole moiety gave a high activity than 5-thioxo-1,2,4-triazole moiety, which may be the presence of cyclic NCSN part within the bio-conjugated systems.

3. Conclusion

The behavior of 3-hydrazino-1,2,4-triazin-5-one as a strong nucleophile towards the active electrophilic compounds, in different media, has been investigated. Where the primary amino group firstly attacked the more electropositive atoms. *N*-(2-(3-Hydrazineyl-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl)phenyl)pival-amide was given tow reaction ways in polar and nonpolar solvents. All heterobicyclic systems obtained, were exhibited as *in vitro* antibacterial activity, especially Gram-positive bacteria, in comparing with Tetracycline as an antibiotic standard. Some compounds were showed a good result.

4. Experimental

All chemicals were purchased from Merck and BDH and used without any further purifications. The melting points were recorded on Stuart scientific SMP30 (Bibby, UK) melting point apparatus and reported as uncorrected. A Perkin Elmer model RXI-FT-IR 55,529 cm^{-1} was used for recording the FT-IR spectra. A Brucker advance DPX 400 MHz using TMS as an internal standard was used for recording the ^1H and ^{13}C NMR spectra in deuterated DMSO (δ in ppm) as a solvent. AGC-MS-QP 1000 ex-model was used for recording the mass spectra. Elemental microanalysis was performed on a Perkin-Elmer CHN-2400 analyzer. All reactions were monitored by TLC, using silica gel coated Al plates with fluorescent indicator F254. 4-Nitrobenzoyl isothiocyanate was obtained from refluxing 4-nitrobenzoyl chloride with ammonium thiocyanate in dry acetone [11], and 6-(2-aminophenyl)-3-thioxo-1,2,4-triazin-5(4*H*)one (**1**) also obtained from refluxing isatin with thiosemicarbazide in *aq*. NaOH [14], according to the reported methods.

Table 1. The *in vitro* antibacterial activities of compounds **4**-**8**.

Compound.	**Bacteria branch*/ Inhibition Zone (Hz)****							
	(+ve)-Bacteria				**(-ve)-Bacteria**			
	B. s.	***B. c.***	***S. l.***	***M. l.***	***P. a.***	***E. c.***	***A. j.***	***X. o.***
4	25	20	12	18	17	12	16	11
5	18	17	11	14	15	10	11	10
6	15	15	10	16	14	9	10	8
7	20	17	11	15	16	11	16	11
8	18	17	10	17	14	9	12	9
Tetracycline (Control)	15	15	10	20	15	10	15	10

*: *B. s.*: *Bacillus subtilis*, *B. c.*: *Bacillus cereus*, *S. l.*: *Sarcina lutea*, *M. l.*: *Micrococcus luteus*, *P. a.*: *Pseudomonas aeruginosa*, *E. c.*: *Escherichia coli*, *A. j.*: *Acinetobacter johnsonii*, *X. o.*: *Xanthomonas oryzae*. **: 50%, used as a selective concentration.

Table 2. The MCI study of activated compounds towards the selected microorganisms.

Organism*	Compound								
	4			**5**			**7**		
	50%	30%	10%	50%	30%	10%	50%	30%	10%
P. a.	17	12	11	15	11	8	16	12	8
B. s.	25	18	10	18	16	12	20	13	10
B. c.	20	14	9	17	12	9	17	15	12

*: *P. a.*: *Pseudomonas aeruginosa*, *B. s.*: *Bacillus subtilis*, *B. c.*: *Bacillus cereus*.

N-[2-(5-Oxo-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl) phenyl] pivalamide (2)

Compound **1** (17.5 g, 79.54 mmol) and *t*-butoyl chloride (9.54 g, 79.54 mmol) in DMF (150 ml) were warmed for 30 min. The reaction mixture was cooled to the room temperature then poured onto ice. The solid obtained was filtered off and crystallized from EtOH to give **2** as

yellowish crystals. Yield 18.37 g, 76 %, m.p: 344-345°C.

FT-IR (ATR, ῡ, cm^{-1}): 3300, 3180(2NH), 3050(ArH), 2960, 2880(aliphatic CH), 1670(C=O), 1650(CONH), 1580(C=N), 1330(cyclic NCSN), 1188(C=S), 880, 810(aromatic ring).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.55(s, 1H, NH), 11.02(s, 1H, NH), 10.85(s, 1H, NHCO), 7.70-7.42(m, 4H, aromatic), 1.90, 1.88, 1.21(each s, 9H, $3CH_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 188(C=S), 162, 154(C=O), 141(C=N), 132-128(aromatic carbons), 119, 116(C=C of 1,2,4-trizinone), 19.10, 18.88, and 18.79 (aliphatic carbons).

Calculated, $C_{14}H_{16}N_4O_2S$ (M^+ 305), %: C, 55.25; H, 5.30; N, 18.41; S, 10.53. Found, %: C, 55.11; H, 5.19; N, 18.32; S, 10.44.

N-[2-(3-Hydrazino-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl)phenyl]pivalamide (3)

A mixture of compound **2** (17.00 g, 55.92 mmol) and hydrazine hydrate (25 ml) in ethanol (150 ml) was heated under reflux for 12 h. The reaction mixture was cooled to the room temperature then poured onto ice. The yielded solid, was filtered off and crystallized from EtOH to give **3** as ball

yellow crystals. Yield 11.99 g, 71%, m.p: 359-360°C.

FT-IR (ATR, ῡ, cm^{-1}): 3300, 3180, 3090(NH, NH_2), 3060(ArH), 2980, 2870(aliphatic CH), 1660(C=O), 1640(CONH), 1620(deform. NH_2), 1580(C=N), 1480(deform. CH_3), 860, 810(aromatic ring).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.10(s, 1H, NH of 1,2,4-triazinone), 10.80(s, 1H, NHCO), 8.7(1H, NH), 7.55-7.41(m, 4H, aromatic), 3.49(s, 2H, NH_2), 1.11, 1.00, 0.95(each s, 3H, $3CH_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160, 152 (2C=O), 142(C=N), 139(C-N), 130-128(aromatic carbons), 19.10, 18.80, 18.77(aliphatic carbons).

Calculated, $C_{14}H_{18}N_6O_2$ (M^+302), %: C, 55.62; H, 6.00; N, 27.80. Found, %: C, 55.51; H, 5.89; N, 27.76.

N-[2-(3-(5-(4-Nitrophenyl)-3-thioxo-2,3-dihydro-1H-1,2,4-triazol-1-yl)-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl)phenyl]pivalamide (4)

A mixture of compound **3** (1.50 g, 4.96 mmol) and 4-nitrobenzoyl isothiocyanate (1.028 g, 4.96 mmol) in THF (20 ml) was heated under reflux for 6-8 h. The reaction mixture was cooled to the room temperature. The solid produced was filtered off and crystallized from dioxane to give **4** as

orange crystals. Yield 1.539 g, 63%, m.p: 328-330°C.

FT-IR (ATR, ῡ, cm^{-1}): 3200, 3180(NH, NH), 3060(ArH), 2980, 2890 (aliphatic CH), 1665(C=O), 1610(CONH), 1580(C=N), 1560, 1380(asym, & sym. NO_2), 1550(NCSN), 1190(C=S), 910, 870, 825(aromatic ring).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.10, 11.20, 8.90(each s, 3H, 3NH), 7.90, 7.78(d,d, 2H, aromatic), 7.71-7.66(m, 2H, aromatic), 7.28-7.42(m, 4H, aromatic), 1.20, 1.01, 0.99(each s, 9H, $3CH_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 182(C=S), 168(C=O), 150(C=O), 142(C=N), 140(C=N), 139 (C-N), 131-127(aromatic carbons), 19.10, 18.88, 18.86($3CH_3$).

Calculated, $C_{22}H_{20}N_8O_4S$ (M^+ 492), %: C, 53.65; H, 4.09; N, 22.75; S, 6.51. Found, %: C, 53.59; H, 3.99; N, 22.59; S, 6.41. M/S (*Int.* %): 492(M+, 1.05), 271(5.90), 131(13.99), 122(100), 100(25.11), 85(6.95), 57(25.11).

N-[2-(3-(3-(4-Nitrophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)-5-oxo-2,5-dihydro-1,2,4-triazin-6-yl)phenyl]pivalamide (5)

A mixture of compound **3** (1.50 g, 4.96 mmol) and 4-nitrobenzoyl isothiocyanate (1.028 g, 4.96 mmol) in EtOH (20 ml) with a few drops of piperidine was heated under reflux for 6-8 h. The reaction mixture was cooled to the room temperature. The solid produced was filtered off and crystallized from dioxane to give **5** as

deep-yellowish crystals. Yield 1.661 g, 68%, m.p: >360°C.

FT-IR (ATR, ῡ, cm^{-1}): 3300, 3250, 3180(3NH), 3080(ArH), 2980, 2870(aliphatic CH), 1670(C=O), 1650(CONH), 1580, 1540(C=N), 1550, 1350(asym, & sym. NO_2), 1188(C=S), 920, 880, 835(aromatic ring).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.33, 11.20, 8.88(each s, 3H, 3NH), 7.88, 7.82(d,d, 2H, aromatic), 7.68-7.59(m, 2H, aromatic), 7.55-7.40(m, 4H, aromatic), 1.11, 1.01, 0.98(each s, 9H, $3CH_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 180(C=S), 162(C=O), 150(C=O), 142(C=N), 140(C=N), 132-127(aromatic carbons), 18.88, 18.20, 18.00($3CH_3$).

Calculated, $C_{22}H_{20}N_8O_4S$ (M^+ 492), %: C, 53.65; H, 4.09; N, 22.75; S, 6.51. Found, %: C, 53.55; H, 3.98; N, 22.55; S, 6.11. M/S (*Int.* %): 492(M+, 1.01),

192(7.15), 122(100), 103(81.11), 85(3.80), 57(38.11), 55(11.55).

N-[2-(5-Oxo-3-(2-(phenylcarbamothioyl)hydrazino) -2,5-dihydro-1,2,4-triazin-6-yl)phenyl]pivalamide (6)

A mixture of compound **3** (2.50 g, 8.27 mmol) and *N*-phenylthiourea (1.258 g, 8.27 mmol) in EtOH (30 ml) was heated under reflux for 4h. The reaction mixture was cooled to the room temperature. The solid produced was filtered off and crystallized from dioxane to give **6** as

yellow crystals. Yield 2.025 g, 56%, m.p: 366-368°C.

FT-IR (ATR, ῡ, cm^{-1}): 3300, 3210, 3150, 3100(NH), 3060(ArH), 2970, 2880(aliphatic CH), 1680(C=O), 1660(CONH), 1600, 1580(C=N), 1188(C=S), 920, 880, 840, 810(aromatic ring).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.85(s, 1H, NH), 10.55(s, 1H, CONH), 8.80(s, 1H, NH), 7.83-7.77(m, 4H, aromatic), 7.74-742(m, 5H, Ph), 1.12, 1.08, 0.99(each s, $3CH_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 180(C=S), 162(C=O), 154(C=O), 142(C=N), 139(C-N), 132-126(aromatic carbons), 19.11, 18.88, 18.51($3CH_3$).

Calculated, $C_{21}H_{23}N_7O_2S$ (M^+ 437), %: C, 57.65; H, 5.30; N, 22.41; S, 7.33. Found, %: C, 57.60; H, 5.11; N, 22.12; S, 7.25.

N-[2-(5-Oxo-3-(5-oxo-4-phenyl-3-thioxo-1,2,4-triazolidin-1-yl)-2,5-dihydro-1,2,4-triazin-6-yl) phenyl]pivalamide (7)

A mixture of **6** (1.50 g, 3.43 mmol) and diethyl carbonate (0.405 g, 3.43 mmol) in THF (20 ml) was heated under reflux for 4h. The reaction mixture was cooled to the room temperature. The solid produced was filtered off and crystallized from dioxane to give **7** as

deep-yellow crystals. Yield 0.794 g, 50%, m.p: 349-350°C.

FT-IR (ATR, ῡ, cm^{-1}): 3300, 3180, 3150(NH), 3080(ArH), 2980, 2870(aliphatic CH), 1670(C=O), 1660(C=O), 1620(CONH), 1590(C=N), 1330(cyclic NCSN), 1195(C=S), 880, 840, 810, 790(aromatic ring).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.50, 11.30(each s, 2H, 2NH), 7.21-6.99(m, benzo), 1.01, 0.99, 0.89(each s, $3CH_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 188(C=S), 168, 162, 154(3C=O), 142(C=N), 131-128(aromatic carbons), 119, 116(C5, C6 of 1,2,4-trizine), 20.10, 19.01, 18.95, 18.88(aliphatic carbons).

Calculated, $C_{22}H_{21}N_7O_3S$ (M^+ 463), %: C, 57.01; H, 4.57; N, 21.15; S, 6.92. Found, %: C, 56.98; H, 4.42; N, 21.09; S, 6.80.

N-[2-(3-Amino-4,8-dioxo-1,8-dihydro-4H-[1,2,4] triazino[4,3-b][1,2,4]triazin-7-yl) phenyl]pivalamide (8)

A mixture of compound **3** (1.50 g, 4.96 mmol) and cyanoacetic acid (0.421 g, 4.96 mmol) in absolute EtOH (25 ml) with drops of piperidine was heated under reflux for 8 h. The reaction mixture was cooled to the room temperature. The solid produced was filtered off and crystallized from dioxane to give **8**.

Yield 1.304 g, 74 %, m.p: 278-280°C.

FT-IR (ATR, ῡ, cm^{-1}): 3180(NH), 3080(NH_2), 1688, 1670(2C=O), 1590(C=N), 1470, 1441(deformation Me), 860(substituted Ph).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.2, 8.99(NH), 7.88-7.55(m, 4H, aromatic protons), 3.45(s, 2H NH_2), 1.01, 0.98, 0.90(each s, $3CH_3$).

Calculated, $C_{16}H_{17}N_7O_3$ (M^+ 355), %: C, 54.08; H, 4.82; N, 27.59. Found, %: C, 53.91; H, 4.66; N, 27.40.

N-[2-(4-Amino-8-oxo-1,8-dihydro-2H-[1,2,4] triazino[4,3-b][1,2,4]triazin-7-yl) phenyl]pivalamide (9)

A mixture of compound **3** (1.50 g, 4.96 mmol) and 2-chloroacetonitrile (0.372 g, 4.96 mmol) in DMF (25 ml) was heated under reflux for 4 h. The reaction mixture was cooled to the room temperature, then poured onto ice. The solid produced was filtered off and crystallized from EtOH to give **9**.

Yield 1.168 g, 69 %, m.p: 288-290°C.

FT-IR (ATR, ῡ, cm^{-1}): 3300, 3250, 3150(NH_2, NH), 1680, (C=O), 1650(CONH), 1630(NH_2), 1480, 1440(deformation Me), 860(substituted Ph).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.20, 11.11, 9.5(each s, 3NH), 7.88-7.51(m, 4H, aromatic protons), 3.51(s, 2H NH_2), 1.01, 0.98, 0.95(each s, $3CH_3$).

Calculated, $C_{16}H_{19}N_7O_2$ (M^+ 341), %: C, 56.29; H, 5.61; N, 28.72. Found, %: C, 55.95; H, 5.47; N, 28.67.

N-[2-(4-imino-8-oxo-3-phenyl-1,8-dihydro-4H-[1,2,4]triazino[4,3-b][1,2,4]triazin-7-yl)phenyl] pivalamide (10)

A mixture of compound **3** (1.50 g, 4.96 mmol) and (0.650 g, 4.96 mmol) in EtOH (25 ml) with drops of piperidine was heated under reflux for 8 h. The reaction mixture was cooled to the room temperature, then added dil. HCl. The solid produced was filtered off and crystallized from EtOH to give **9**.

Yield 1.319 g, 64 %, M.p: 170-272°C.

FT-IR (ATR, ῡ, cm^{-1}): 3400(OH), 3150(NH), 3080(NHCO), 1680, (C=O), 1660(C=O), 1580(C=N), 1480, 1440 (deformation Me), 880(substituted Ph).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.11, 10.50(each s, NH, NHCO), 8.90(OH), 7.80-7.66 (m, 4H, aromatic protons), 1.01, 0.90, 0.88(each s, $3CH_3$).

Calculated, $C_{22}H_{21}N_7O_2$ (M^+ 415), %: C, 63.60; H, 5.10; N, 23.60. Found, %: C, 63.49; H, 5.02; N, 23.37.

5. The *in vitro* antibacterial evaluation

The new synthesized systems were investigated as *in vitro* antibacterial agents such as Gram-positive bacteria involved *Bacillus subtilis*, *Bacillus cereus*, *Sarcina lutea*, and *Micrococcus luteus*; and Gram-negative bacteria involved *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter johnsonii*, and *Xanthomonas oryzae*, by using the conventional well/disc.[3] Tetracycline (30 µg/dis., 6 mm in diameter) used as a control.

The new compounds were dissolved in 5% DMSO to obtain a 0.5% stock solution. Grown cultures were used sterile nutrient agar medium in each Petri plate. The applied concentration in each 10, 30, and 50 mL of stock solution added. All the plates were incubated at 28°C for 24 h and the size of the resulted zone of inhibition determined.

6. Conflict of interest

The authors declare no conflicts of interest.

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