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Synthesis and Antimicrobial Activities of Some New Fully Substituted 1,2,4-Triazines

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A facile synthesis of fully substituted 1,2,4-triazines (***3a-3c***) involving the reaction of benzoinhydrazone **1** with isothiocyanates followed by alkylation, hydroxymethylation, aminolysis and hydrazinolysis has been described. The structure of the products has been deduced from their elemental analysis and spectral data (UV, IR, ^{1}H-NMR and mass spectra). Antimicrobial screening of some products revealed that only the compound **6** showed marked activity towards Streptomyces.

Key words: Benzoinhydrazide alkylation, 1,2,4-Triazines, Antimicrobial activity.

Introduction

The chemistry and chemotherapeutic properties of 1,2,4-triazines and related compounds were described recently. (Abdel Rehman *et al* 1990, 1993a; Abdel Rehman 1991, 1992; Abdel Halim *et al* 1995). The present work of the same authors reports synthesis and reactions of fully substituted 1,2,4-triazines. Such compounds are likely to show enhanced biological activities.

Experimental

Melting points are uncorrected. IR spectra were recorded in KBr on a FI-IR 1650 spectrophotometer (γ max in cm^{-1}), 90 MHz ^{1}H-NMR spectra in DMSO-d_6 on an JNM-PMX 60 spectrophotometer using TMS as internal standard (chemical shifts in δ ppm), UV spectra (λ_{max} in nm) in ethanol on Perkin Elmer Lampda 3B and mass spectra recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 ev. The purity of the compounds was checked by ascending TLC ($CHCl_3$-MeOH).

Preparation of benzoinhydrazone **(1).** A mixture of benzoin (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (50ml) was refluxed for 2 h; the reaction mixture was concentrated and cooled. The solid obtained was filtered off and recrystallized to give **1** (Table 1).

Preparation of 1,4-disubstituted thiosemicarbazide **(2a-c).** A mixture of compound **1** (0.01 mol) and the appropriate isothiocyanate (0.01 mol) in DMF (20 ml) was refluxed for 2 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **2a-c** (Table 1).

Preparation of 1,4-disubstituted semicarbazide **(2d).** A mixture of compound **1** (0.01 mol) and phenyl isocynate (0.01 mol) in dioxan (100 ml) was refluxed for 2 h. The reaction mixture was concentrated and cooled. The solid obtained was filtered off and recrystallized to give **2d** (Table 1).

Synthesis of 5H-5,6-diphenyl-4-substituted-1,2,4-triazin-3(2H)-thiones (3a-c) and 5H-5,6-diphenvl-4-substituted-1,2,4-triazine-3(2H)-one **(3d).** A suspension of **2a-d** (0.01 mol) in glacial acetic acid (50 ml)-fused sodium acetate (5 g)/ or DMF (20 ml) was heated under reflux for 4 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **3a-d** (Table 1).

Alkylation of compound 3a-c: Formation of S-alkyl derivatives **(4a-c).** A mixture of **3a-c** (0.01 mol) in monochloro-acetic acid (0.01 mol) was refluxed for 2 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **4a-c** (Table 1).

5H-5,6-Diphenyl-3-methylthio-4-methyl-1,2,4-triazine **(5).** A suspension of **4a** (0.01 mol) in aqueous K_2CO_3 (10%, 100 ml) was heated under reflux for 2 h. The solid obtained was filtered off and recrystallized to give 5 (Table 1).

5H-5,6-Diphenyl-4-methyl-3-(thiomethylbenzimidazol-2-yl)-1,2,4-triazine **(6).** A mixture of **4a** (0.01 mol) and o-phenylenediamine (0.01 mol) in dil HCl (4N, 10 ml) was refluxed for 2 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **6** (Table 1).

Preparation of 3-disubstituted mythyl 1,2,4-triazine **(7).** A mixture of **3a** (0.01 mol) and acetylacetanilide (0.01 mol) in ethanolic NaOH (10%, 100 ml) was refluxed for 2 h, cooled and acidified with dil HCl. The solid obtained was filtered

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off and recrystallized from the proper solvent to give **7** (Table 1).

5H-5,6-Diphenyl-4-methyl-3-(2-mercapto-4-substituted amino-6-methylpyrimidin-5-yl)-1,2,4-triazine **(8)**. Equimolar mixture of **7** and thiorea in ethanolic NaOH (10%, 100 ml) was refluxed for 2h, cooled and acidified with dil HC1. The solid obtained was filtered off and recrystallized to give **8** (Table 1).

Preparation of sulphanilamide derivatives **(9a,b)**. A mixture of **3b,c** (0.01 mol) and sulphanilamide (0.01 mol) in ethanolic NaOH (10%, 100 ml) was refluxed for 4 h, cooled and poured onto crushed ice-HC1. The solid obtained was filtered off and recrystallized to give **9a-b** (Table 1).

5H-5,6-Diphenyl-4-substituted-3-hydrazino-1,2,4-triazine **(10a-c)**. A mixture of **3a-c** (0.01 mol) and hydrazine hydrate (0.01 mol) in isopropyl alcohol (50 ml) was refluxed for 4 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **10a-c** (Table 1).

6,7-Diphenyl-3-mercapto-7H-s-methyl triazolo [4,3-b]-[1,2,4] triazine **(11)**. Compound **10a** (0.01 mol) and carbon disulphide (10 ml) in ethanolic KOH (10%, 100 ml) was refluxed for 4h, cooled and poured onto crushed ice-HC1. The solid obtained was filtered off and recrystallized to give **11** (Table 1).

5H-3-chloro-4,5,6-triphenyl-1-1,2,4-triazine **(12)**. A suspension of 3d (0.003 mol) in $POC1_3$ (2 ml) was heated under reflux for 1 h, cooled and poured dropwise onto crushed ice with stirring for 1 h. The solid obtained was filtered off and recrystallized to give **12** (Table 1).

Preparation of 5H-4,5,6-triphenyl-2-hydroxymethyl-1,2,4-triazine-3-one **(13a)**. A mixture of **3d** (0.003 mol) and formaldehyde (1 ml) in methanol (10 ml) was refluxed for 5 h and cooled. The solid obtained was filtered and recrystallized to give **13a** (Table 1).

5H-2-Ethyl-4,5,6-triphenyl--1,2,4-triazine-3-one **(13b)**. A mixture of 3d (0.003 mol) and ethyl iodide (1 ml) in ethanol (10 ml) was refluxed for 3 h and cooled. The solid obtained was filtered and recrystallized to give **13b** (Table 1).

5H-2-Benzyl-4,5,6-triphenyl-1,2,4-triazine-3-one **(13c)**. A mixture of 3d (0.01 mol) and benzyl chloride (0.01 mol) in DMF (10 ml) was refluxed for 5 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **13c** (Table 1).

Preparation of N1 Cyanoethyl benzoin hydrazone **(14d)**. Compound **1** (0.01 mol), acrylonitrile (0.01 mol) in pyridine (40 ml) and H_2O (10 ml) were refluxed for 4 h, cooled and poured onto crushed ice-HC1. The solid obtained was filtered off and recrystallized to give **14d** (Table 1).

Preparation of N^1-Benzenesulfonyl benzoin hydrazone **(14e)**. Compound **1** (0.01 mol) and benzenesulphonyl chloride (0.01 mol) in pyridine (40 ml), were refluxed for 10 min, cooled and poured onto crushed ice-HC1. The solid obtained was filtered off and recrystallized to give **14e** (Table 1).

Preparation of N^4-phenyl-semicarbazone **(15d,e)**. A mixture of N^1-substituted hydrazone **14d** & **14e** (0.01 mol) and phenylisocyanate (0.01 mol) in DMF (20 ml) was refluxed for 2 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **15d, e** (Table 1).

5H-2-Substituted 4,5,6-triphenyl-1,2,4-triazine-3-one **(13d,e)**. A mixture of N^4-phenyl semicarbazone **15d** and **15e** (0.01 mol), glacial acetic acid (100 ml) and sodium acetate (10 g) was refluxed for 2 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **13d** and **13e** (Table 1).

Results and Discussion

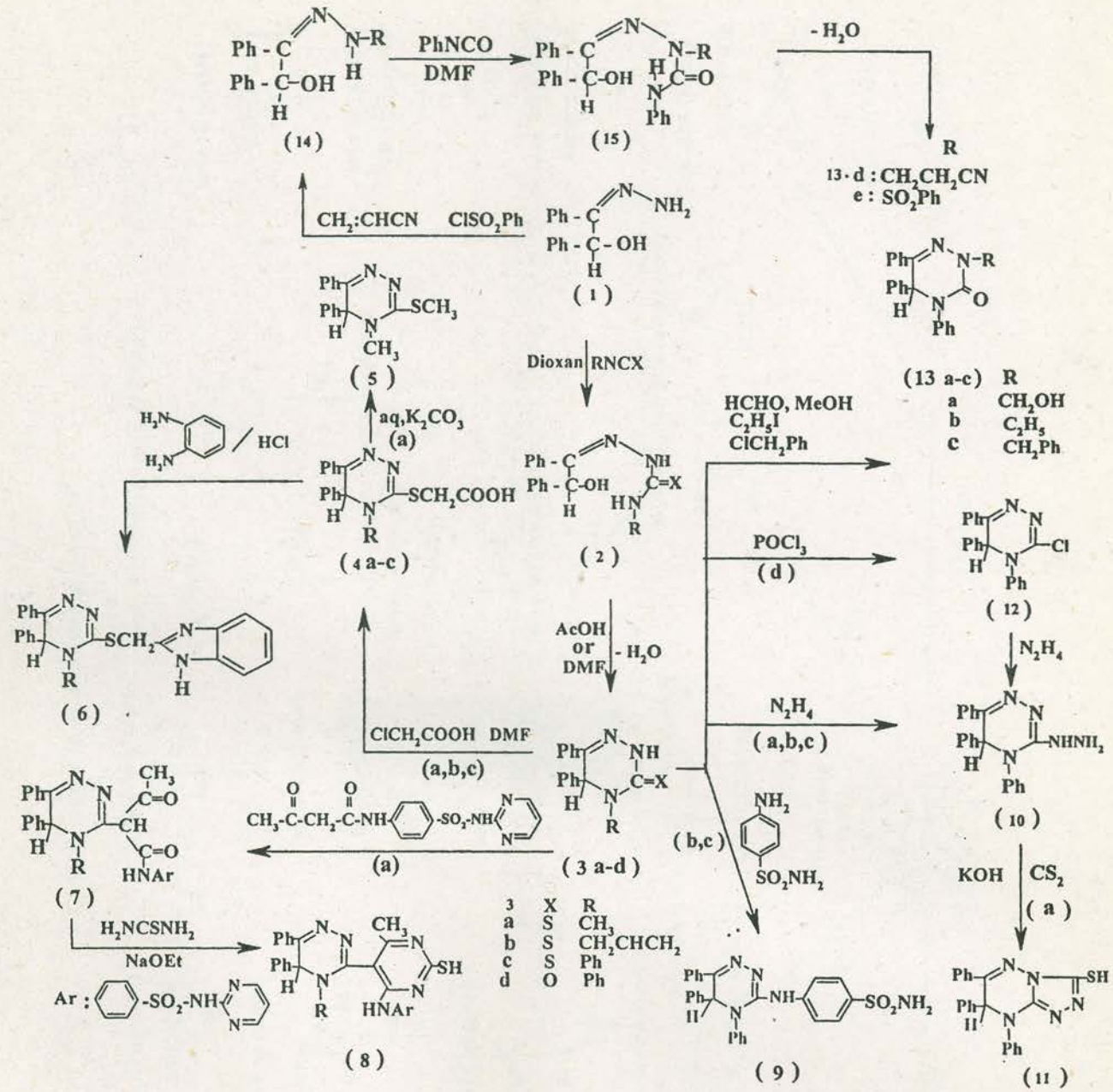
The addition of isothiocyanate or isocyanate derivatives namely, methyl isothiocyanate, allyl isothiocyanate, phenyl isothiocyanate and phenyl isocyanate to benzoinhydrazone (1) in dioxan furnished N^4-substituted thiosemicarbazone and substituted semicarbazone derivatives **2a-d** which underwent cyclization by refluxing with glacial acetic acid-fused sodium acetate or DMF to produce 5H-5,6-diphenyl-4-substituted-1,2,4-triazine-3 (2H) thiones **(3a-c)** and 5H-5,6-diphenyl-4-substituted-1,2,4-triazine-3 (2H) one **(3d)** [Scheme 1].

The structure of **2** and **3** was established from their elemental analysis and spectral study. IR spectra of **2** and **3** revealed the NH bands in addition to the absorption bands characterized by NCS functions, while that of **3d** revealed the presence of NH, C=O and aromatic groups. UV spectrum of **3b** showed nmax at 255nm attributable to $\pi\,\pi$–π^* and n-π^* electronic transition. ^{1}H-NMR spectrum of **3b** recorded signal due to CH_2, allyl, aromatic, or H-5 and NH protons of 1,2,4-triazine. Mass spectrum of **3b** showed the molecular ion peak at m/e 307 which underwent further fragmentation process to give the mercapto triazole at m/e 128, in addition to the diphenyl acetylene at m/e 176 (Palmer *et al* 1971) and the iminobenzyl ion as a base peak at m/e 105 (Chart I) whose subsequent fragmentation was found to be consistent with the fragmentation pattern previously reported for such type of compounds (Abdel Rehman *et al* 1993b). Moreover, mass spectrum of **3d** also recorded the molecular ion peak at m/e 327 and the base peak at m/e 77 (Chart II).

Table 1
Physical and analytical data of various compounds 3-15

Compound	m.p [°C]	Yield(%) solvent of crystallization	Mol. formula*	Mol. weight	Analysis(%) Found/(Calcd)			X-ray analysis S % (found / calcd.)
					C	H	N	
3a	207	65a	$C_{16}H_{15}N_3S$	281	68.00	5.15	14.55	10.18
					68.32	5.33	14.94	11.38
3b	182	70b	$C_{18}H_{17}N_3S$	307	69.85	5.25	13.35	10.49
					70.35	5.53	13.68	10.42
3c	239	75b	$C_{21}H_{17}N_3S$	343	73.08	4.85	12.01	8.66
					73.46	4.95	12.24	9.32
3d	212	60b	$C_{21}H_{17}N_3O$	327	76.91	4.88	12.55	——
					77.06	5.19	12.84	——
4a	above 280	70c	$C_{18}H_{17}N_3SO_2$	339	63.51	4.59	12.12	8.55
					63.71	5.01	12.38	9.44
4b	193	70b	$C_{20}H_{19}N_3SO_2$	365	65.29	4.95	11.35	7.95
					65.75	5.20	11.50	8.76
4c	125	65d	$C_{23}H_{19}N_3SO_2$	401	68.70	4.52	10.15	6.99
					68.82	4.73	10.47	7.98
5	above 185	80c	$C_{17}H_{17}N_3S$	295	68.85	5.66	13.95	10.00
					69.15	5.76	14.23	10.84
6	129-130	74e	$C_{24}H_{21}N_5S$	411	69.79	4.89	16.75	6.86
					70.07	5.20	17.03	7.78
7	90	67f	$C_{30}H_{27}N_7SO_4$	581	61.59	4.40	16.59	5.00
					61.96	4.64	16.86	5.51
8	107	60g	$C_{31}H_{27}N_9SO_2$	621	59.55	4.18	20.01	10.60
					59.90	4.34	20.28	10.30
9b	125	65h	$C_{24}H_{23}N_5SO_2$	445	64.50	4.88	15.34	6.39
					64.71	5.16	15.73	7.19
9c	27	70g	$C_{27}H_{23}N_5SO_2$	481	67.15	4.70	14.09	5.95
					67.35	4.78	14.55	6.65
10a	242-243	78e	$C_{16}H_{17}N_5$	279	68.39	5.88	24.75	——
					68.81	6.09	25.08	——
10b	243	68b	$C_{18}H_{19}N_5$	305	70.80	5.95	22.79	——
					70.81	6.22	22.95	——
10c	240	65b	$C_{21}H_{19}N_5$	341	73.45	5.29	20.12	——
					73.90	5.57	20.52	——
11	170	65b	$C_{17}H_{15}N_5S$	321	63.31	4.37	21.51	8.77
					63.55	4.67	21.80	9.67
12	178	70i	$C_{21}H_{16}N_3Cl^+$	345	72.88	4.40	11.91	——
					73.04	4.63	12.17	——
13a	90	72a	$C_{22}H_{19}N_3O_2$	357	73.59	5.01	11.29	——
					73.94	5.32	11.76	——
13b	272	60a	$C_{23}H_{21}N_3O$	355	77.45	5.55	11.71	——
					77.74	5.91	11.83	——
13c	192	67a	$C_{28}H_{23}N_3O$	417	80.23	5.19	10.00	——
					80.57	5.51	10.67	——
13d	242	65e	$C_{24}H_{20}N_4O$	380	75.00	4.85	14.44	——
					75.18	5.26	14.73	——
13e	125	70a	$C_{27}H_{21}N_3SO_3$	467	69.01	4.15	8.81	6.05
					69.37	4.49	8.99	6.85
14d	above 280	78e	$C_{17}H_{17}N_3O$	279	72.92	5.45	14.43	——
					73.11	6.09	15.05	——
14e	235	75a	$C_{20}H_{18}N_2SO_2$	350	68.17	4.8	7.78	7.99
					68.57	5.14	8.00	8.74
15d	190	80e	$C_{24}H_{22}N_4O_2$	398	72.02	5.41	13.90	——
					72.36	5.52	14.07	——
15e	179	75a	$C_{27}H_{23}N_3SO_4$	485	66.39	4.50	8.39	5.80
					66.80	4.74	8.65	6.56

*Cl: Found, 9.85; Calcd, 10.52%. Solvents: a, Methanol; b, Isopropanol; c, Dimethylformamide; d, dil Isopropanol; e, dil Dimethylformamide; f, Dioxane; g, dil Methanol; h, dil ethanol; i, Toluene.



Scheme I.

According to Mansour *et al* (1994), the reaction of 1,2,4-triazinthione proceeds via nucleophilic attack either at SH or NH leading to 3-substituted mercapto-1,2,4-triazines. Thus, alkylation of **3a-c** using monochloroacetic acid in presence of DMF afforded S-alkyl derivatives **4a-c. 4a** on boiling with aqueous K_2CO_3 via decarboxylation gave 5H-5,6-diphenyl-3-methylthio-4-methyl-1,2,4-triazine (**5**) [Scheme I].

The IR spectra of **4a-c** showed the presence of frequencies at γ3297, 1735-1675 and 1490 cm^{-1} attributable to OH of COOH group, while that of **5** showed the absence of a-carboxylic group.

Treatment of **4a** with o-phenylenediamine in the presence of HC1 led to formation 5H-5,6-diphenyl-4-methyl-3-thiomethylbenzimidazol-2-yl-1,2,4-triazine (**6**). Structure of **6** was confirmed from analytical and spectral data. IR spectrum of **6** showed bands at 3100 and 1170 cm^{-1} due to the frequencies of NH, C-S groups.

Pyrimidine derivatives are known to possess physiological activities (Allimony *et al* 1995; Hou *et al* 1995) while on the other hand 1,2,4-triazine derivatives also have potential biological activities. Based on these observations **3a** reacted with acetylacetanilide derivative in sodium ethoxide to give 3-substituted methyl-1,2,4-triazine **7** which on treatment with

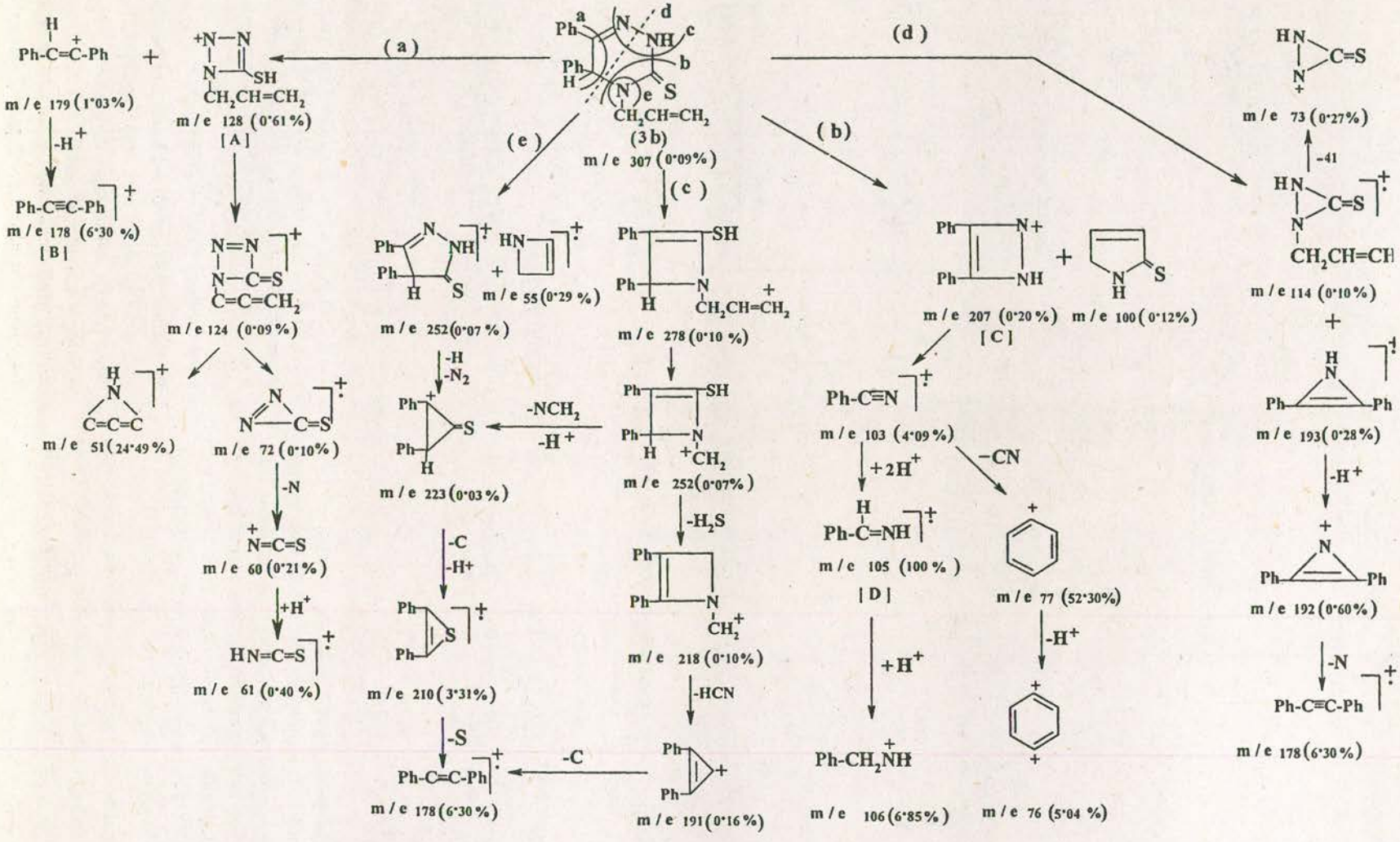


Chart 1 - Mass fragmentation pattern of 3b

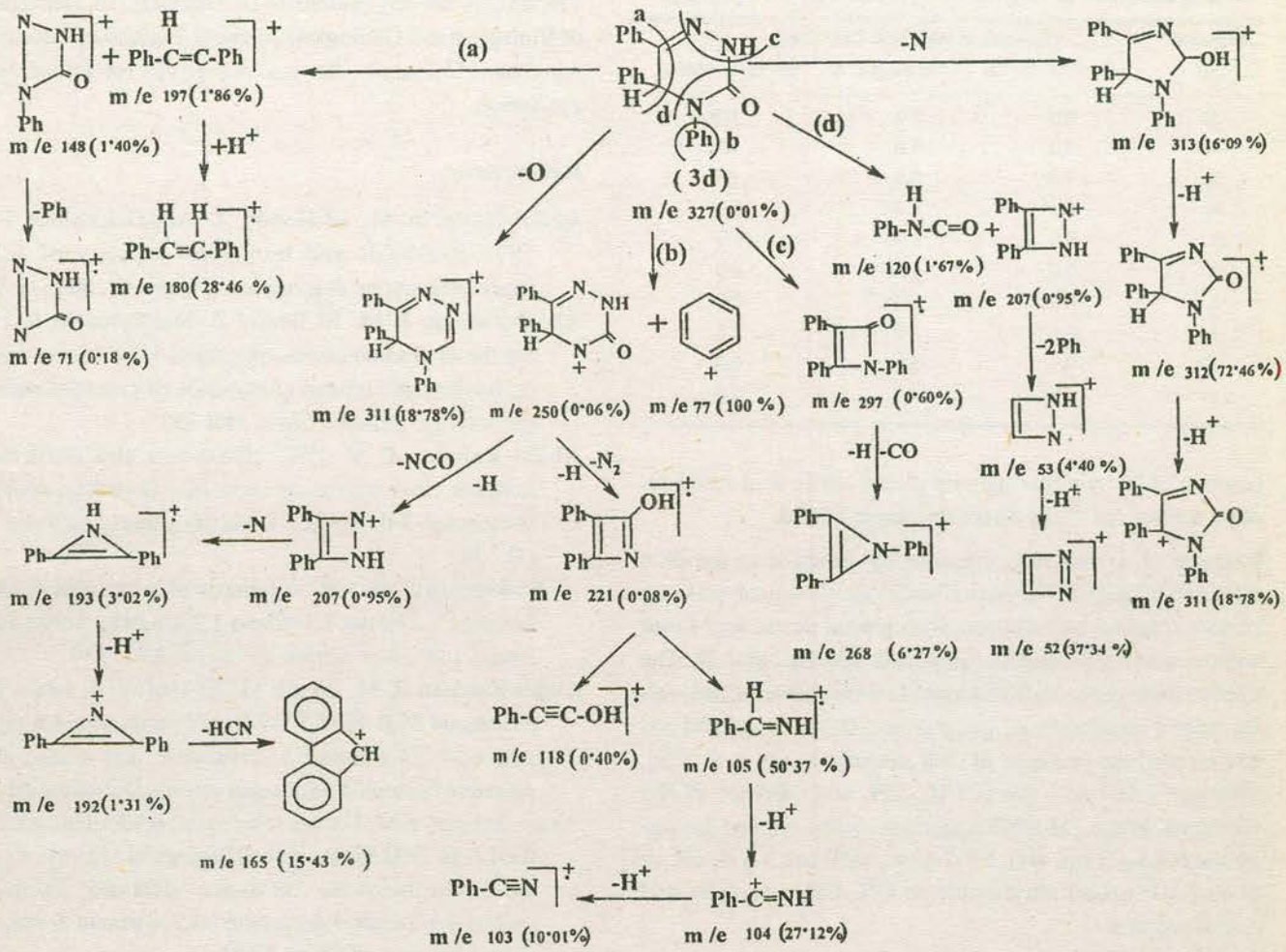


Chart II. The mass spectral fragmentation pattern of 3d

thiourea in sodium ethoxide afforded 5H-5,6-diphenyl-4-methyl-3-(2-mercapto-4-substituted amino-6-methyl-pyrimidin-5-yl)-1,2,4-triazine (**8**).

Structures of **7** and **8** were deduced from elemental analysis and spectral data. IR spectrum of **7** showed the absorption bands at γ 3050, 1680, 1350 and 1180 cm^{-1} to NH, C=O, SO_2 and C-S groups while that of **8** revealed the laking characteristic bands of CH_2, CO groups. UV absorption spectrum of 7 recorded λ_{max} at 261.2nm while that of **8** showed λ_{max} at 268nm. Also ^{1}H-NMR spectrum of **8** showed a resonated signals at δ 1.2,1.5,5.5,6.8-7.8 and 12 ppm attributable to CH_3, SH, aromatic, 5H of 1,2,4-triazine and NH protons.

The compound **9** was obtained from treatment of **3a-c** with sulphanilamide in the presence of sodium ethoxide. Hydrazinolysis of **3a-c** by refluxing with hydrazine hydrate in isopropyl alcohol afforded 5H-5,6-diphenyl-3-hydrazino-1,2,4-triazine (**10**) which on refluxing with CS_2 in ethanolic KOH produced 3-mercapto-6,7-diphenyl-7H-8-methyl-triazolo [4,3-b][1,2,4] triazine (**11**) [Scheme I]. IR spectra of **10** and **11** showed absorption bands due to NH at 3150 cm^{-1} and a band at γ 1171 cm^{-1} due to C=S group (Sharvile 1961).

Through chlorination of **3d** using $POCl_3$, 5H-3-chloro-4,5,6-triphenyl-1,2,4-triazine (**12**) was obtained. Hydrazino-triazine **10** can also be obtained from interaction of 3-chloro derivative **12** and hydrazine hydrate.

Some displacement reactions of compound **3d** were also investigated. Thus, treatment of 3d with formaldehyde-methanol gave N^2 hydroxymethyl **13a**. N-alkyl derivatives **13b** and **13c** were obtained from treatment of **3d** with ethyl iodide and/or benzyl chloride.

On the other hand refluxing of benzoinhydrazine (**1**) with acrylonitrile in pyridine-water yielded N^1-disubstituted hydrazone **14** which upon addition of phenylisocyanate in DMF produced N^4-phenyl semicarbazone **15** Ring closure

Table 2
Antimicrobial activities of synthesized compounds

Compound No.	Diameter of Inhibition Zone (mm)		
	Bacillus subtilis	*Streptomyces sp.*	*Aspergillus niger*
3a	0.0	2.0	0.0
3b	0.0	6.0	0.0
3c	0.0	0.0	0.0
4a	0.0	9.0	1.0
5	0.0	2.0	0.0
6	3.0	12.0	6.0
8	0.0	0.0	0.0
9	0.0	2.0	2.0
10	0.0	3.0	0.0
11	0.0	4.0	2.0

reaction of **15** by refluxing with glacial acetic acid-fused sodium acetate led to the direct formation of **13d.**

Reaction of **1** with benzenesulphonyl chloride in pyridine followed by addition of phenylisocyanate in dioxan and ring closure reaction by refluxing with glacial acetic acid-fused sodium acetate furnished compound **13e** [Scheme I]. The synthesized compounds **13d** and **13e** were characterized with the help of elemental and spectral data. IR spectra of **13d** and **13e** showed the presence of characteristic bands at γ, 2220, 2900 and 1440 cm^{-1} due to CH_2, CN, with absence of NH functional group. ^{1}H-NMR spectrum of **13d** showed the signals at δ 3.5-4.2 (m, 4H), 6.9-7.4 (m, 15H) and 8.5 (s, 1H, of 5H-1,2,4-triazine) attributable to CH_2-CH_2, aromatic and hetero protons.

Antimicrobial activity. Some new prepared compounds **3-11** were evaluated for their antimicrobial activity against *Bacillus subtilis, Streptomyces spp.* and *Aspergillus niger* employing cup-diffusion technique (Abdel Rehman *et al* 1990) using DMF as solvent. The minimal inhibition concentration (MIC) and growth inhibition were determined with reference to the control (Sharvile 1961) of their tested compounds.

Quantitative structure activity relationship showed that compounds having sulfur showed potent antimicrobial activity, and compound **6** which has also benzimidazol moiety bearing 1,2,4-triazin-thione exhibited maximum activity, towards the tested organisms. Moreover, aryl group when attached to sulfur atom led to increase the activity as compound to methyl group (Schmeling 1966) (Table 2).

On the other hand with the increase of sulfur percentage in compound **6**, inhibition, decreased; while a mild sulfur percentage has a moderate inhibition by compound **4**.

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