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RESEARCH ARTICLE

Design, 'one-pot' synthesis, characterization, antibacterial and antifungal activities of novel 6-aryl-1,2,4,5-tetrazinan-3-thiones in dry media

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A novel 'one-pot' synthesis of 6-aryl-1,2,4,5-tetrazinan-3-thiones is carried out by the three-component coupling of thiourea, various structurally diverse aromatic aldehydes and ammonium acetate in the presence of reusable $NaHSO_4 \cdot SiO_2$ heterogeneous catalyst in dry media under microwave irradiation. FT-IR, ^{1}H NMR, D_2O Exchange, HOMOCOR, ^{13}C NMR, MS and elemental analysis characterize all the synthesized compounds. *In vitro* antibacterial/fungal activities are carried out for all the synthesized eight new compounds. All the compounds are more active against bacterial strains namely *Staphylococcus aureus, β-Heamolytic streptococcus, Vibreo cholerae, Salmonella typhii, Shigella felxneri, Klebsiella pneumonia* and *Pseudomonas* except compounds **1** and **6**, while compound **6** shows promising activity against *Salmonella typhii*. Moreover, of all the compounds tested, compounds **3** and **8** are more effectual against all the tested fungal strains.

Keywords: 6-Aryl-1,2,4,5-tetrazinan-3-thiones; Antibacterial activity; Antifungal activity; $NaHSO_4 \cdot SiO_2$ heterogeneous catalyst; 'One-pot' synthesis

1. Introduction

1,2,4,5-Tetrazines represent an important class of heterocyclic compounds that find many practical and synthetic applications [1]. 1,2,4,5-Tetrazines are now under investigation for their effectiveness against cancer by the National Cancer Institute, USA [2]. Numerous biological activities were reported for tetrazoloheterocycles, such as being useful as antiallergic, antiulcer [3], antiinflammatory, analgesic, bronchodilating, bactericidal [4], hypotensive [5] and pesticidal activities [6]. In addition, some tetrazoles derivatives have been introduced in the design of non-peptide ligands for GHS receptor [7], shows competitive inhibition potency for the Carbapenem and Cephamyci-resistant dinuclear zinc metallo-β-lactamase from *Bacteroides fragilis* [8], treatment of type 2 diabetes [9]. Such interesting results encouraged us to examine the following reaction. Since the formation of N–N bond is relatively difficult, 1,2,4,5-tetrazines were generally prepared form hydrazine derivatives or

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from nitrilimines. A number of mono hydrazones of simple aldehydes and ketone with thiocarbohydrazide and 6-Alkylhexahydro-1,2,4,5-tetrazine-3-thiones have been reported [10]. In this report, only aliphatic aldehydes gave 1,2,4,5-Tetrazines. But benzaldehyde gave only true monohydrazone with thiocarbohydrazide. In the light of these findings, the present work describes 'one-pot' construction of the novel tetrazoloheterocycles for potential pharmacological activities.

As there is a need for 'clean technology revolution' [11–14], there has been considerable interest in the microwave irradiation protocol for rapid synthesis of a variety of organic compounds due to the selective absorption of microwave energy by polar molecules. The use of heterogeneous catalyst, in particular $NaHSO_4 \cdot SiO_2$ [15–18] have made landmark in different areas of organic synthesis due to their environmental compatibility combined with good yield and selectivities that can be achieved.

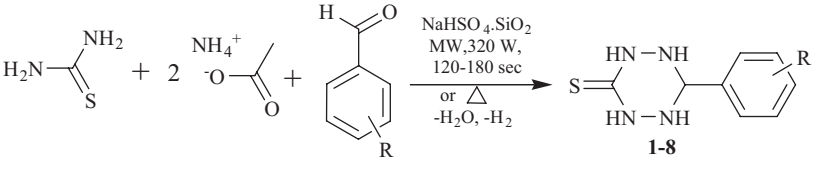
2. Results and discussion

2.1 *Chemistry*

6-Aryl-1,2,4,5-tetrazinan-3-thiones is synthesized by the multicomponent cyclocondensation reaction of one mole of thiourea, one mole of substituted benzaldehyde and two moles of ammonium acetate in the presence of $NaHSO_4 \cdot SiO_2$ heterogeneous catalyst in dry media under microwave irradiation and thermal conditions (scheme 1).

The reactions are performed at 320 W of MW power for 120–180 s (75 °C, conventional heating using oil bath, 30–45 min). After completion of the reaction as indicated by the TLC, the reaction mixture was extracted with ethyl acetate, concentrated *in vacuo* and purified by column chromatography using Petroleum ether (40:60): ethyl acetate [3:7] as eluent. The stoichiometry (1:2:1 thiourea: NH_4OAc: benzaldehyde) of the reaction is also supported by the formation of novel 6-aryl-1,2,4,5-tetrazinan-3-thiones (table 1, entries 1–8).

The structure of 6-aryl-1,2,4,5-tetrazinan-3-thiones have been elucidated on the basis of their melting points, elemental analysis, MS, IR, ^{1}H NMR, D_2O exchange, ^{13}C NMR and two-dimensional NMR spectral studies including Homonuclear Correlation (HOMOCOR) spectra. The plausible mechanistic pathway is given in scheme 2. In order to check the possibility of intervention of specific (non-purely thermal) microwave effects, the reaction has also been examined using oil bath. Significant lower yields are obtained. This observation demonstrates clearly that the effect of MW irradiation is not purely thermal. This behaviour is consistent with mechanistic considerations of the reaction [19–20]. As they are connected to polarity medium, specific MW effects are expected if transition state (TS) is more polar than the ground state (GS) of the reaction. Due to the dipole–dipole electrostatic interactions being more developed in the TS, the stabilization of the TS is superior to the GS, leading thus to a decrease in the activation energy. In the TS, the polarity of the system is enhanced as looser ion pairs are involved when compared to the GS.

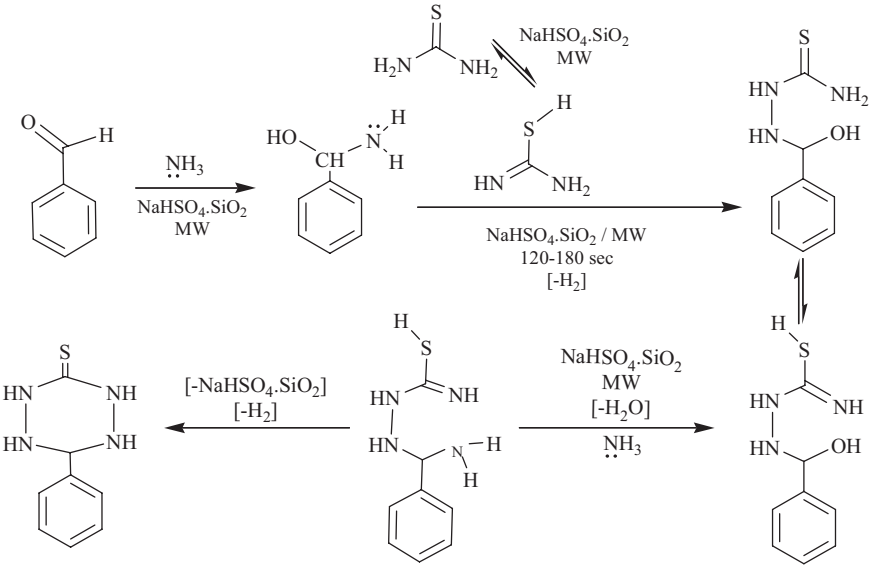


SCHEME 1. Novel 'one-pot' synthesis of some 6-aryl-1,2,4,5-tetrazinan-3-thiones catalyzed by $NaHSO_4 \cdot SiO_2$ heterogeneous catalyst in 'dry media'.

Table 1. Physical and analytical datas of 6-aryl-1,2,4,5-tetrazinan-3-thiones (**1–8**).

Compound	*R*	Reaction time MW[a] (sec)/ Δ[b] (min)	Yield[c] (%) MW/Δ	m.p°C	Elemental analysis (%) C Found (calculated)	H Found (calculated)	N Found (calculated)	m/z (M^+) Molecular formula
1	H	120/30	78/35	187–188	49.52 (49.48)	5.13 (5.15)	28.88 (28.86)	(195) $C_8H_{10}N_4S$
2	*p*-Cl	160/35	76/30	170–172	42.03 (42.01)	3.95 (3.97)	24.51 (24.50)	(229) $C_8H_9N_4ClS$
3	*o*-Cl	180/38	74/33	158–160	42.07 (42.01)	3.93 (3.97)	24.55 (24.50)	(229) $C_8H_9N_4ClS$
4	*p*-F	140/33	80/40	170–172	45.29 (45.27)	4.25 (4.27)	26.41 (26.40)	(213) $C_8H_9N_4FS$
5	*p*-CH_3	150/40	75/35	146–148	51.92 (51.90)	5.79 (5.81)	26.92 (26.90)	(209) $C_9H_{12}N_4S$
6	*p*-OCH_3	155/41	78/32	170–174	48.19 (48.20)	5.37 (5.39)	24.96 (24.98)	(225) $C_9H_{12}N_4OS$
7	*o*-CH_3	160/38	74/35	160–164	51.94 (51.90)	5.83 (5.81)	26.94 (26.90)	(209) $C_9H_{12}N_4S$
8	*m*-OC_6H_5	180/45	72/30	148–150	58.70 (28.72)	4.92 (4.93)	19.55 (19.57)	(287) $C_{14}H_{14}N_4OS$

[a] MW = Microwave irradiation.
[b] Δ = Conventional heating.
[c] A power level of 320 W is applied for all the reactions performed under microwave irradiation and a temperature of 75°C is applied for thermal reactions.



SCHEME 2 Possible mechanistic pathway for the formation of 6-phenyl-1,2,4,5-tetrazinan-3-thione.

Washing easily regenerated the $NaHSO_4{\cdot}SiO_2$ catalyst with ethyl acetate, followed by drying at 110 °C for 1 h. It was shown that $NaHSO_4{\cdot}SiO_2$ remained catalytic active for 6 runs and the yields are 80, 75, 70, 60, 50 and 40%, respectively for 6-phenyl-1,2,4,5-tetrazinan-3-thione (table 1, entry-1). However, there was noteworthy decrease in the activity after 4 uses was observed since recycling led to loss of efficiency of the catalyst owing to absorption of organics. Organics on the solid surface result in the reduction of the number of active centers. Also $NaHSO_4$ on the SiO_2 was lost during washing. Organic species could be removed at higher temperature reactivating the catalyst.

2.1.1 Spectral analysis of 6-phenyl-1,2,4,5-tetrazinan-3-thione (table 1, entry 1). The IR spectrum of 6-phenyl-1,2,4,5-tetrazinan-3-thione exhibits characteristic NH bands at 3398 cm^{-1}, in addition to thioamide (C=S) stretching at 1178 cm^{-1}. The band of carbonyl group of aldehyde disappeared in the IR spectra of all compounds and the presence of weak bands at 1450 cm^{-1} (N-N) are more evident for the formation of 6-phenyl-1,2,4,5-tetrazinan-3-thione. The ^1H NMR spectrum of 6-phenyl-1,2,4,5-tetrazinan-3-thione shows the presence of two isomers, one major and one minor. For the major isomer a doublet at 4.96 ppm is due to the benzylic proton. A triplet like signal observed at 3.90 ppm is due to NH protons adjacent to benzylic carbon. A singlet at 8.67 ppm is due to thioimide protons. Aromatic protons are appeared in the range of 7.30–7.44 ppm. The minor isomer signals are observed at 5.42, 3.22 and 8.46 ppm. The pattern of the spectrum is almost similar to the major isomer. Hence the signals are assigned to benzylic, amino and thioimide protons. Moreover, to confirm the NH proton signals, ^1H NMR spectrum is recorded after adding D_2O. The signals observed at 3.20, 3.90, 8.46 and 8.67 ppm are exchanged with D_2O.

In order to confirm the assignment of signals, HOMOCOR is also recorded for 6-phenyl-1,2,4,5-tetrazinan-3-thione. The signal at 4.96 ppm shows cross peaks with signals at 3.90 and 8.67 ppm. The cross peak signal at 3.90 ppm is having two protons.

The existence of two isomeric structures of thiones is due to different conformations in solution. Unlike carbocyclic six membered systems, the tetrazines, which have four nitrogen

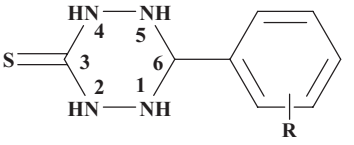


Figure 1.

atoms including four lone pairs, expected to exists in non-chair conformations due to lone pair–lone pair interactions. Among these conformations, the phenyl group occupies axial like or equatorial like orientation. Due to nitrogen quadrapole effect, it is difficult to calculate the coupling constant between the protons attached to nitrogen atoms. The coupling constant of proton attached to nitrogens and H-6 is only measurable, we expected triplet or double-doublet for H-6 protons. But only two separate doublets at 4.96 and 5.42 ppm are observed for with coupling constants 8.6 and 10.7 Hz. This clearly indicates that N-1 proton or N-5 proton is coupled with H-6 proton. The phenyl group, which occupies equatorial like orientation, has N-5 proton couple with H-6 proton, but N-1 proton does not couple with H-6 proton (the dihedral angle is nearly 90°). The phenyl group, which occupies axial like orientation, have N-5 proton does not couple but N-1 proton couple with H-6 proton. The above spectral analysis reveals that existence of two isomers. Between the two isomers, one isomer with phenyl ring in equatorial like is major and phenyl ring in axial like orientation is minor.

The ^{13}C NMR spectrum of 6-phenyl-1,2,4,5-tetrazinan-3-thione shows the presence of two isomers, one major and one minor. For the major isomer the signal at 64.9 ppm is due to the benzylic carbon. The signal appeared at 176.0 ppm is assigned to thioamide carbonyl carbon. The *ipso* carbon appeared at 140.1 ppm. The aromatic carbons appeared in the region of 126.9–128.5 ppm. The minor isomer signals are observed at 68.8 and 177.6 ppm. The pattern of the spectrum is almost similar to the major isomer. Hence the signals are assigned to benzylic and thioamide carbonyl carbon.

To grasp structure activity relationship well, numberings of the target compound are done (figure 1).

2.2 *Structure activity relationship results*

2.2.1 Antibacterial activity. All the synthesized novel target molecule 6-aryl-1,2,4,5-tetrazinan-3-thiones (**1–8**) were tested for their antibacterial activity (tables 2 and 3) *in vitro* against *Staphylococcus aureus, β-Heamolytic streptococcus, Vibreo cholerae, Salmonella typhii, Shigella felxneri, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas*. Ciprofloxacin was used as standard drug; whose zone of inhibition (mm) values for *S. aureus, β-H. streptococcus, V. cholerae, S. typhii, S. felxneri, E. coli, K. pneumonia* and *Pseudomonas are* 25, 28, 23, 22, 23, 24, 26 and 23, *respectively*. In general all the synthesized novel 6-aryl-1,2,4,5-tetrazinan-3-thiones (**1–8**) exerted a wide range of modest antibacterial activity *in vitro* against the tested organisms. But their activity decreased upon dilution. All the compounds are less active against *E. coli* and more active against *S. aureus, β-H. streptococcus, V. cholerae, S. typhii, S. felxneri, K. pneumonia* and *Pseudomonas* except compounds **1** and **6**, while compound **6** shows promising activity against *S. typhii*.

Table 2. *In vitro* profile of compounds 1–4 against test bacteria and fungi.

	Compound 1			Compound 2			Compound 3			Compound 4		
Microorganisms	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm
Staphylococcus aureus	−	+	+++	−	−	++++	+	+++	++++	−	++	++++
β-Heamolytic streptococcus	+	++	+++	−	+	++++	+	+++	++++	+	+++	++++
Vibreo cholerae	−	++	+++	+	+	++++	+	+++	++++	+	+++	++++
Salmonella typhii	−	+	+++	+	+	++++	−	+++	++++	+	+++	++++
Shigella felxneri	−	+	+++	+	+	++++	++	++	++++	−	++	++++
Escherichia coli	−	++	+++	+	−	++++	+	++	+++	+	++	+++
Klebsiella pneumonia	+	+	+++	+	−	++++	+	+++	++++	+	++++	++++
Pseudomonas	+	++	+++	+	+	++++	+	++	++++	+	++	++++
Aspergillus flavus	+	++	+++	+	+	++++	−	++	++++	+	++	++++
Mucor	−	+	+++	+	+	++++	+	++	++++	+	++	++++
Rhizopus	−	+	+++	+	−	++++	−	++	++++	+	++	++++
Microsporum gypsuem	−	+	+++	+	−	++++	+	++	++++	+	++	++++

(−) = inactive, (+) = weakly active (12–16 mm), (+)(+) = moderately active (17–21 mm), (+)(+)(+) = strong active (22–29), (+)(+)(+)(+) = highly active (30–33).

Table 3. *In vitro* profile of compounds 5–8 against test bacteria and fungi.

	Compound 5			Compound 6			Compound 7			Compound 8		
Microorganisms	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm	100 ppm	200 ppm	500 ppm
Staphylococcus aureus	−	+	+ + ++	−	+	+ + +	−	+	+ + +	+	++	+ + ++
β-Heamolytic streptococcus	−	+	+ + ++	−	+	+ + +	−	+	+ + +	+	+ + +	+ + ++
Vibreo cholerae	−	+	+ + ++	+	++	+ + +	−	+	+ + +	+	++	+ + ++
Salmonella typhii	−	+	+ + +	+	++	+ + ++	−	+	+ + +	+	+ + +	+ + ++
Shigella felxneri	−	+	+ + +	−	++	+ + +	+	++	+ + +	+	++	+ + ++
Escherichia coli	−	+	+ + +	−	++	+ + +	−	++	+ + +	+	+ + +	+ + +
Klebsiella pneumonia	−	+	+ + +	−	+	+ + +	−	++	+ + +	+	+ + +	+ + ++
Pseudomonas	+	+	+ + +	−	+	+ + +	−	+	+ + +	+	+ + +	+ + ++
Aspergillus flavus	+	+	+ + ++	+	++	+ + ++	+	++	+ + +	+	++	+ + ++
Mucor	−	+	+ + +	−	++	+ + +	−	++	+ + +	+	++	+ + ++
Rhizopus	−	+	+ + +	−	+	+ + +	−	+	+ + +	+	++	+ + ++
Microsporum gypsuem	+	+	+ + +	−	++	+ + ++	+	+	+ + +	+	++	+ + ++

2.2.2 Antifungal activity. The *in vitro* antifungal activity (tables 2 and 3) of the synthesized novel heterocyclic compounds **1–8** was studied against the fungal strains viz., *Aspergillus flavus, Mucor, Rhizopus* and *Microsporum gypsuem*. Fluconazole was used as a standard drug whose zone of inhibition (mm) values for *A. flavus, Mucor, Rhizopus* and *M. gypsuem* are 20 ± 0.5 zone of inhibition (mm) against all the tested fungi. Generally all the synthesized compounds exerted a wide range of modest *in vitro* antifungal activity against all the tested organisms. But their activity decreased upon dilution. Besides compounds **1**, **6** and **7**, all other compounds are having more biological activity. Moreover, of all the compounds tested, compounds **3** and **8** are more effectual against all the tested fungal strains.

3. Conclusion

Results of this study show that the nature of substituent on the phenyl ring viz., chloro, fluoro, methyl, methoxy and phenoxy moiety in 6-aryl-1,2,4,5-tetrazinan-3-thiones is determinant for the nature and extent of the activity of the synthesized compounds, which might have influences on their inhibiting mechanism of actions. The method of action of these compounds is unknown. These observations may promote a further development of our research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection.

4. Experiment

4.1 *Microbiology*

4.1.1 Materials. All the bacterial strains namely *S. aureus, β-H. streptococcus, V. cholerae, S. typhii, S. felxneri, E. coli, K. pneumonia, Pseudomonas* and fungal strains namely *A. flavus, Mucor, Rhizopus* and *M. gypsuem* are get hold of from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

4.1.2 *In vitro* antibacterial and antifungal activity. The *in vitro* activities of the compounds were tested in Sabourauds dextrose broth (SDB) (Hi-media, Mumbai) for fungi and nutrient broth (NB) (Hi-media, Mumbai) for bacteria by the Disc Diffusion method [21]. The respective hydrochlorides of the test compounds **1–8** were dissolved in water to obtain 1 mg mL^{-1} stock solution and the different concentrations (100, 200 and 500 ppm) are prepared from the stock solution. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1 °C while fungal spores from 1 to 7 days old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in SDB. Sterile paper disc of 5 mm diameter is saturated with the three different concentrations and such discs are placed in each seeded agar plates. The petri plates are incubated in BOD incubator at 37 °C for bacteria and at 28 °C for fungi. The zone of inhibition is recorded by visual observations after 24 h of inhibition for bacteria and after 72–96 h of inhibition for fungi. Moreover, the zone of inhibition is measured by excluding the diameter of the paper disc. Ciprofloxacin was used as standards for bacteria and fluconazole as standard for fungi under analogous conditions.

4.2 *Chemistry*

Performing TLC assessed the reactions and the purity of the products. All the reported melting points were taken in open capillaries and were uncorrected. IR spectra were recorded in KBr (pellet forms) on a Nicolet-Avatar–360 FT-IR spectrophotometer and note worthy absorption values (cm^{-1}) alone are listed. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively on Bruker AMX 400 NMR spectrometer using DMSO as solvent. HOMOCOR Spectrum was recorded on Bruker DRX 500 NMR spectrometer using standard parameters. The ESI +ve MS spectra were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalysis was obtained on Carlo Erba 1106 CHN analyzer. A conventional (unmodified) domestic microwave oven equipped with a turntable (LG, MG-395 WA, 230 V∼50 Hz and 760 W) was used for the irradiation.

4.2.1 General procedure for the synthesis of 6-aryl-1,2,4,5-tetrazinan-3-thiones catalyzed by $NaHSO_4{\cdot}SiO_2$ under microwave irradiation (entries 1–8). A mixture containing thiourea (10 mmol), substituted benzaldehyde (10 mmol), ammonium acetate (20 mmol) and $NaHSO_4{\cdot}SiO_2$ (100 mg) was added in an alumina bath and mixed properly with the aid of glass rod (10 s) and then irritated in a microwave oven for the appropriate period of time as mentioned in table 1 at 320 W (monitored by TLC). After completion of the reaction, the reaction mixture was extracted with ethyl acetate (3 × 5 mL). The catalyst was removed by filtration and reused. The combined organic layer was washed with water three times and then dried over anhydrous $MgSO_4$. The organic layer was concentrated *in vacuo* to furnish the products, which were purified by column chromatography using Petroleum ether (40:60): ethyl acetate [3:7] as eluent.

4.2.2 6-Phenyl-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3398, 3211, 3162, 3033, 2896, 1521, 1450, 1178, 700; ^1H NMR (δ ppm): 3.90 (t, J = 8.7 Hz, 2H, $H_{1\&5}$), [3.22, J = 10.9 Hz], 8.60 (s, 2H, $H_{2\&4}$), [8.46], 4.96 (d, J = 8.6 Hz, 1H, H_6), [5.42, J = 10.7 Hz], 7.30–7.44 (m, 5H, H_{arom}). ^{13}C NMR (δ ppm): 64.9 [68.8] -C_6, 176.0 [177.6] -C=S, 140.1 -*ipso* C, 126.9–128.5 -C_{arom}. (The values in parentheses [], represent the minor isomer).

The compounds 2–8 were synthesized similarly.

4.2.3 6-(4-Chlorophenyl)-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3388, 3228, 3180, 3054, 2967, 1593, 1489, 1166, 821; ^1H NMR (δ ppm): 4.04 (t, J = 8.6 Hz, 2H, $H_{1\&5}$), [3.65, J = 9.9 Hz], 8.8 (s, 2H, $H_{2\&4}$), [8.64], 4.95 (d, J = 8.4 Hz, 1H, H_6), [5.40, J = 9.8 Hz], 7.32–7.47 (m, 4H, H_{arom}). ^{13}C NMR (δ ppm): 64.0 [67.3] -C_6, 176.1 [177.1] -C=S, 132.6, 138.2 -*ipso* C, 128.8–129.3 -C_{arom}.

4.2.4 6-(2-Chlorophenyl)-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3368,3240, 3186,3058, 2968,1588, 1480,818; ^1H NMR (δ ppm): 4.10 (t, J = 8.8 Hz, 2H, $H_{1\&5}$), [3.61, J = 10.1 Hz] 8.82 (s, 2H, $H_{2\&4}$), [8.67], 4.94 (d, J = 8.4 Hz, 1H, H_6), [5.47, J = 9.8 Hz] 7.32–7.47 (m, 4H, H_{arom}). ^{13}C NMR (δ ppm): 61.9 [65.6] -C_6, 176.9 [177.6] -C=S, 135.5, 136.6 -*ipso* C, 125.9–130.5 -C_{arom}.

4.2.5 6-(4-Fluorophenyl)-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3380,3245, 3199,3067, 2920,1541, 1450,1178, 779; ^1H NMR (δ ppm): 4.02 (t, J = 8.7 Hz, 2H, $H_{1\&5}$),

8.71(s, 2H, $H_{2\&4}$), 4.92 (d, J = 8.2 Hz, 1H, H_6), 7.22–7.41 (m, 4H, H_{arom}). ^{13}C NMR (δ ppm): 64.0 [67.5] -C_6, 175.9 [173.3] -C=S, 135.8, 162.7 -*ipso* C, 128.3–129.1 -C_{arom}.

4.2.6 6-(4-Methylphenyl)-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3368, 3198, 3166, 3065, 2920, 1538, 1460, 1175, 768; ^{1}H NMR (δ ppm): 2.31 (s, 3H, CH_3), 3.85 (t, J = 8.3 Hz, 2H, $H_{1\&5}$), 8.64 (s, 2H, $H_{2\&4}$), 4.91 (d, J = 8.2 Hz, 1H, H_6), 7.13–7.26 (m, 4H, H_{arom}). ^{13}C NMR (δ ppm): 21.0 $-CH_3$, 64.5 -C_6, 175.9 -C=S, 137.1, 137.3 -*ipso* C, 126.9–128.7 -C_{arom}.

4.2.7 6-(4-Methoxyphenyl)-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3316, 3211, 3168, 3071, 2933, 1510, 1463, 1174, 835; ^{1}H NMR (δ ppm): 3.81 (s, 3H, OCH_3), 3.83 (t, J = 8.9 Hz, 2H, $H_{1\&5}$), [3.10, J = 10.3 Hz], 8.61 (s, 2H, $H_{2\&4}$), [8.36], 4.88 (d, J = 8.2 Hz, 1H, H_6), [5.34, J = 10.8 Hz], 7.11–7.37 (m, 4H, H_{arom}). ^{13}C NMR (δ ppm): 55.05 $-OCH_3$, 63.9 [66.8] -C_6, 175.9 [173.3] -C=S, 130.8, 157.9 -*ipso* C, 114.0–128.5 -C_{arom}.

4.2.8 6-(2-Methylphenyl)-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3320, 3216, 3153, 3065, 2920, 1538, 1463, 1175, 760; ^{1}H NMR (δ ppm): 2.09 (s, 3H, CH_3), 3.61 (t, J = 8.8 Hz, 2H, $H_{1\&5}$), 8.58 (s, 2H, $H_{2\&4}$), 5.08 (d, J = 8.5 Hz, 1H, H_6), 7.15–7.33 (m, 4H, H_{arom}). ^{13}C NMR (δ ppm): 17.71 $-OCH_3$, 62.2 -C_6, 176.9 -C=S, 135.6, 137.6 -*ipso* C, 125.4–130.3 -C_{arom}.

4.2.9 6-(3-Phenoxyphenyl)-1,2,4,5-tetrazinan-3-thione. IR (KBr) (cm^{-1}): 3407, 3171, 3039, 2902, 1543, 1450, 1248, 783; ^{1}H NMR (δ ppm): 3.68 (t, J = 8.4 Hz, 2H, $H_{1\&5}$), [3.98, J = 9.9 Hz], 8.65 (s, 2H, $H_{2\&4}$), [8.80], 5.35 (d, J = 8.3 Hz, 1H, H_6), [4.92, J = 9.7 Hz], 6.92–7.45 (m, 9H, H_{arom}). ^{13}C NMR (δ ppm): 67.4 [64.2] -C_6, 177.0 [176.0] -C=S, 160.0, 157.4 -*ipso* C, 122.9–137.8 -C_{arom}.

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