# Cycloaddition of 4-Amino-3-mercapto-1,2,4-triazole to Heterocumulenes and Antifungal Activity of the Resulting 1,2,4-Triazolo[3,4-*c*]-1,2-dithia-4,5-diazines

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Phenacylation of 4-amino-5-aryl/[(aryloxy)methyl]-3-mercapto-1,2,4-triazole (Ia–c) furnished the requisite 4-amino-5-aryl/[(aryloxy)methyl]-3-(phenacylthio)-1,2,4-triazole (IIa–c). Compounds IIa–c underwent cycloaddition with $CS_2$ and aryl isothiocyanate, affording a novel class of compounds, 6-aryl/[(aryloxy)-methyl]-1,2,4-triazolo[3,4-*c*]-1,2-dithia-4,5-diazine-3-thione (IIIa–c) and 6-aryl/[(aryloxy)methyl]-3-(arylimino)-1,2,4-triazolo[3,4-*c*]-1,2-dithia-4,5-diazine (IVa–g), respectively. Compounds IIIa–c and IVa–g have been compared with Dithane M-45, a standard commercial fungicide, for their antifungal activity against *Aspergillus niger* and *Fusarium oxysporum*, and the screening results have been correlated with the structural features of the tested compounds.

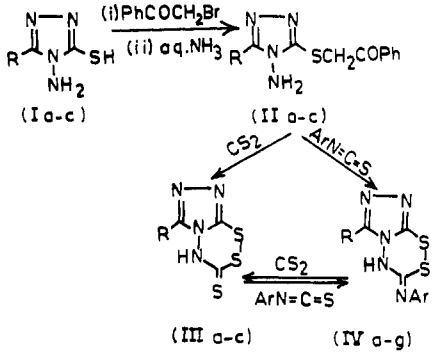
The appreciable antifungal activity displayed by some 1,2,4-triazolo[3,4-*c*]-1,2,4-dithiazoles and 1,3,4-oxadiazolo-[3,2-*d*]-1,3,4-dithia/thiadiazines reported in our earlier papers (Singh et al., 1983, 1985) prompted us to synthesize more compounds with certain structural modifications. During the course of our investigation into 1,2,4-triazolo-[3,4-*c*]-1,2-dithia-4,5-diazine as antifungal agent, we found that various thiadiazine derivatives possess antifungal activity (Singh et al., 1985; Lies et al., 1977). Likewise, a number of 1,2,4-triazole derivatives have also been found to be antifungal (Prasad et al., 1986; Greenfield et al., 1970; Buechel et al., 1975).

One would expect promising antifungal activity from a planar fused ring system with a dithiadiazine nucleus because the compact size and planarity of a molecule often enhance its pesticidal activity (Chatt et al., 1956; Fischer et al., 1976; Rothwell et al., 1963; Singh et al., 1981). The investigation appeared quite interesting as 6-aryl/[(aryloxy)methyl]-1,2,4-triazolo[3,4-*c*]-1,2-dithia-4,5-diazine-3-thiones (IIIa–c) and 6-aryl/[(aryloxy)methyl]-3-(arylimino)-1,2,4-triazolo[3,4-*c*]-1,2-dithia-4,5-diazines (IVa–g) reported herein have been synthesized for the first time.

The reaction sequence leading to the formation of IIIa–c and IVa–g is given in Scheme I. Ia–c were phenacylated (Shadbolt, 1971) to afford IIa–c, which as expected was a nucleophilic substrate for the addition of heterocumulenes, and reacted with $CS_2$ and aryl isothiocyanate to yield IIIa–c and IVa–g. The cycloaddition was accompanied by elimination of the phenacyl group, which was isolated as a 2,4-dinitrophenylhydrazone derivative. The bicyclic products IIIa–c/IVa–g were exclusively formed even when heterocumulenes ($CS_2$ or aryl isothiocyanates) and IIa–c were reacted in 1:2 molar ratio; this ruled out the possible addition for the second molecule.

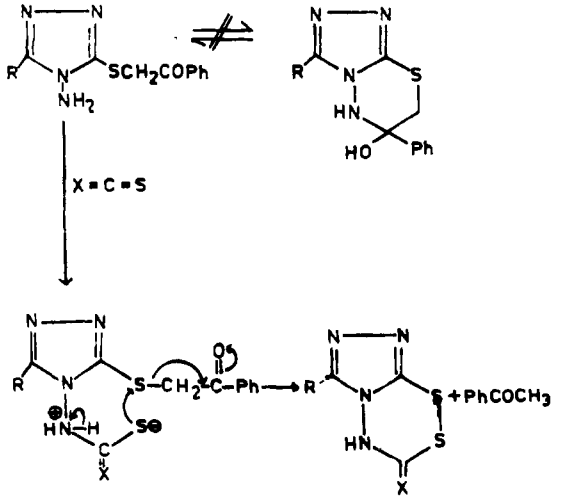
A plausible mechanism for the cycloaddition of IIa–c to heterocumulenes is depicted in Scheme II. $^1H$ NMR

**Scheme I**




I-IV (a-g): R = $C_6H_5$, 4-$CH_3C_6H_4OCH_2$, 2-$ClC_6H_4$; Ar = $C_6H_5$, 2-$CH_3OC_6H_4$, 4-$CH_3OC_6H_4$

**Scheme II**




spectra of IIa–c showed that these exist entirely as the keto form, unlike many other *S*-phenacylthiourea which have

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Table I. Yields, Melting Points, Molecular Formulas, and Elemental Analyses of Compounds IIIa–c and IVa–g

| compd | R | yield, % | mp, °C | mol. formula | found (calcd), % C | H | N |
|---|---|---|---|---|---|---|---|
| IIIa | $C_6H_5$ | 78 | 183 | $C_9H_6N_4S_3$ | 40.56 (40.60) | 2.21 2.26 | 21.00 21.05) |
| IIIb | 4-$CH_3C_6H_4OCH_2$ | 69 | 142 | $C_{11}H_{10}N_4OS_3$ | 42.51 (42.58 | 3.30 3.23 | 18.10 18.06) |
| IIIc | 2-$ClC_6H_4$ | 68 | 100 | $C_9H_5N_4ClS_3$ | 35.86 (35.94 | 1.70 1.66 | 18.56 18.64) |
| | | | Ar = $C_6H_5$ | | | | |
| IVa | $C_6H_5$ | 81 | 185 | $C_{15}H_{11}N_5S_2$ | 55.40 (55.38 | 3.36 3.38 | 21.60 21.54) |
| IVb | 4-$CH_3C_6H_4OCH_2$ | 72 | 138–139 | $C_{17}H_{15}N_5OS_2$ | 55.22 (55.28 | 4.02 4.07 | 19.00 18.97) |
| IVc | 2-$ClC_6H_4$ | 70 | 108 | $C_{15}H_{10}N_5ClS_2$ | 50.04 (50.00 | 2.75 2.78 | 19.40 19.44) |
| | | | Ar = 2-$CH_3OC_6H_4$ | | | | |
| IVd | $C_6H_5$ | 82 | 195 | $C_{16}H_{13}N_5OS_2$ | 54.00 (54.08 | 3.68 3.66 | 19.78 19.72) |
| IVe | 4-$CH_3C_6H_4OCH_2$ | 76 | 135 | $C_{18}H_{17}N_5O_2S_2$ | 54.20 (54.14 | 4.18 4.26 | 17.48 17.54) |
| IVf | 2-$ClC_6H_4$ | 71 | 105 | $C_{16}H_{12}N_5ClOS_2$ | 49.28 (49.29 | 3.00 3.08 | 18.00 17.97) |
| | | | Ar = 4-$CH_3OC_6H_4$ | | | | |
| IVg | $C_6H_5$ | 80 | 192 | $C_{16}H_{13}N_5OS_2$ | 54.10 (54.08 | 3.68 3.66 | 19.78 19.72) |

been reported to be a mixture of the keto and cyclized aminocarbinol form (Shadbolt, 1971; Fefer et al., 1961).

The structural assignments of the synthesized compounds were based on elemental analyses, IR, $^1H$ NMR, and mass spectral data (Tables I and II).

## EXPERIMENTAL PROCEDURES

Melting points were determined in open glass capillaries and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 157 infrared spectrophotometer. $^1H$ NMR spectra were recorded on an EM-360 L (60 MHz) NMR spectrophotometer in $CDCl_3$ and DMSO-$d_6$ using TMS as internal reference; chemical shifts are expressed in $\delta$ (ppm). Mass spectra were recorded on a JEOL JMS-D 300 instrument.

**4-Amino-5-aryl/[(aryloxy)methyl]-3-mercapto-1,2,4-triazole (Ia–c).** These were prepared by a mixture of an appropriate acid hydrazide, potassium hydroxide, and carbon disulfide in ethanol. It was stirred for 2–3 h at 35–40 °C and then at room temperature for 15–18 h (Hoggarth, 1952). The potassium dithiacarbazinate thus obtained was treated with methyl iodide to furnish methyl dithiocarbazinate which was refluxed with $NH_2NH_2{\cdot}H_2O$ for 4 h in ethanol followed by acidification with hydrochloric acid (Hoggarth, 1952). Triazoles thus obtained were recrystallized from ethanol. The data for Ia agreed well with the analytical data already reported in the literature (Hoggarth, 1952); Ib and Ic are new, Ib: yield 73%; mp 190–191 °C (Found: C, 50.82; H, 5.03; N, 23.52. Calcd for $C_{10}H_{12}N_4OS$: C, 50.85; H, 5.08; N, 23.73). Ic: yield 65%; mp 152 °C (Found: C, 42.36; H, 3.10; N, 24.69. Calcd for $C_8H_7ClN_4S$: C, 42.38; H, 3.09; N, 24.72); IR 1620 (C=N), 2560 $cm^{-1}$ (SH).

**5-Aryl/[(aryloxy)methyl]-4-amino-3-(phenacylthio)-1,2,4-triazole (IIa–c).** A mixture of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole (Ia) (3.84 g, 0.02 M) and phenacyl bromide (3.98, 0.02 M) was stirred in acetone (250 mL) for 30 min to give IIa (Shadbolt, 1971). The free base was liberated by action of ammonium hydroxide and recrystallized from ethanol: yield 5.3 g (85.48%); mp 95 °C (Found: C, 61.98; H, 4.50; N, 18.00. Calcd for $C_{16}H_{14}N_4OS$: C, 61.94; H, 4.52; N, 18.06); IR 1615 (C=N), 1700 $cm^{-1}$ (C=O); $^1H$ NMR 4.84 (s, 2 H, $SCH_2$), 7.50–8.20 (m, 10 H, aromatic *H*), 8.60 (br, 2 H, $NH_2$).

Similarly, compounds IIb and IIc were prepared and recrystallized from ethanol. IIb: yield 78%; mp 80 °C (Found: C, 60.86; H, 5.00; N, 15.86. Calcd for $C_{18}H_{18}N_4O_2S$: C, 61.02; H, 5.08; N, 15.82); IR 1620 (C=N), 1705 $cm^{-1}$ (C=O); $^1H$ NMR 2.38 (s, 3 H, $CH_3$), 4.82 (s, 2 H, $SCH_2$), 5.20 (s, 2 H, $OCH_2$), 7.02–8.20 (m, 9 H, aromatic *H*), 8.62 (br, 2 H, $NH_2$). IIc: yield 73%; mp 110–112 °C (Found: C, 55.71; H, 3.72; N, 16.22. Calcd for $C_{16}H_{13}N_4ClOS$: C, 55.73; H, 3.77; N, 16.26); IR 1625 (C=N), 1702 $cm^{-1}$ (C=O); $^1H$ NMR 4.84 (s, 2 H, $SCH_2$), 7.42–8.22 (m, 9 H, aromatic *H*), 8.60 (br, 2 H, $NH_2$).

**6-Aryl/[(aryloxy)methyl]-1,2,4-triazolo[3,4-*c*]-1,2-dithia-4,5-diazine-3-thiones (IIIa–c).** The compounds IIa–c (0.01 M) and carbon disulfide (100 mL) were refluxed for 8–10 h. The brown residue obtained after evaporation of $CS_2$ was recrystallized from ethanol to furnish light yellow needles of the desired products. The compounds thus prepared are recorded in Table I. The spectral data are given in Table II.

**6-Aryl/[(aryloxy)methyl]-3-(arylimino)-1,2,4-triazolo-[3,4-*c*]-1,2-dithia-4,5-diazine (IVa–g).** A mixture of IIa–c (0.01 mol) and aryl isothiocyanate (0.01 M) was heated in an oil bath at 80–110 °C for 3 h. The reaction mixture was cooled and extracted with chloroform, and the extract was evaporated. The dark brown residue thus obtained was recrystallized from EtOH as yellow needles of IVa–g, which are listed in Table I. The spectral data are given in Table II.

**Antifungal Screening.** The pure cultures of test fungi (*Aspergillus niger*, *Fusarium oxysporum*), the pathogenicity of which was already verified, were obtained from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, Delhi. Agar (bacteriological grade) supplied by Sarabhai M. Chemicals was used as such. Compounds IIIa–c and IVa–g were screened by the agar plate technique (Horsfall, 1945) using Czapek's agar medium.

Suspensions of different concentrations of each compound, viz. 10 000, 1000, and 100 ppm, were prepared in an acetone–water (20:80 v/v) mixture. One milliliter of each concentration of the test compound was added separately to presterilized Petri plates containing 9 mL of the sterilized Czapek's agar medium to maintain the final concentration of 1000, 100, and 10 ppm. The compound was thoroughly mixed with the medium by rotating the plates of table top, thus swirling the contents. A fungal disc of 5-mm diameter, cut out with the help of a sterilized cork borer from the periphery of 1-week-old culture of the test fungus already planted on the Czapek's medium, was inoculated in the center of each plate in inverted position to bring the mycelia in direct contact with the medium. Petri plates containing 9 mL of Czapek's medium and 1 mL of acetone–water (20:80 v/v) mixture served as controls. The number of replicate assays in each case was three, whereas six replications of the controls were provided. The plates were incubated at 28 °C (±1 °C) for 96 h. No remarkable morphological change was observed in the developing fungi. After 96 h, four diameters of the

**Table II. Spectral Data of Compounds IIIa–c and IVa–g**

| compd | IR (KBr), $cm^{-1}$ C=N | C=S* and C=N (exocyclic) | NH | $^1H$ NMR, $\delta$ | MS/M$^+$ $m/z$ |
|---|---|---|---|---|---|
| IIIa | 1610, 1635 | 1205* | 3290 | 7.42–7.92 (m, 5 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 266 |
| IIIb | 1605, 1630 | 1195* | 3285 | 2.34 (s, 3 H, $CH_3$), 5.20 (s, 2 H, $OCH_2$), 7.02–8.20 (m, 4 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 310 |
| IIIc | 1610, 1635 | 1200* | 3290 | 7.00–8.22 (m, 4 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 301–302 |
| IVa | 1610, 1635 | 1668 | 3290 | 7.00–8.24 (m, 10 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 325 |
| IVb | 1605, 1630 | 1670 | 3290 | 2.40 (s, 3 H, $CH_3$), 5.20 (s, 2 H, $OCH_2$), 7.00–8.20 (m, 9 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 369 |
| IVc | 1610, 1635 | 1675 | 3285 | 7.00–8.22 (m, 9 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 360–361 |
| IVd | 1615, 1630 | 1670 | 3290 | 3.76 (s, 3 H, $OCH_3$), 7.20–8.22 (m, 9 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 355 |
| IVe | 1610, 1635 | 1672 | 3285 | 2.34 (s, 3 H, $CH_3$), 3.76 (s, 3 H, $OCH_3$), 5.20 (s, 2 H, $OCH_2$), 7.00–8.20 (m, 8 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 399 |
| IVf | 1610, 1635 | 1675 | 3285 | 3.76 (s, 3 H, $OCH_3$), 7.20–8.24 (m, 8 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 389–391 |
| IVg | 1605, 1635 | 1675 | 3290 | 3.76 (s, 3 H, $OCH_3$), 7.00–8.20 (m, 9 H, aromatic *H*), 13.8 (br, 1 H, N*H*) | 355 |

**Table III. Fungicidal Screening Results of Compounds IIIa–c and IVa–g**

| compd | av % inhibition against *A. niger* at 1000 ppm | 100 ppm | 10 ppm | *F. oxysporum* at 1000 ppm | 100 ppm | 10 ppm |
|---|---|---|---|---|---|---|
| IIIa | 87 | 59 | 45 | 85 | 58 | 43 |
| IIIb | 94 | 66 | 46 | 92 | 64 | 45 |
| IIIc | 100 | 74 | 51 | 100 | 72 | 50 |
| IVa | 69 | 45 | 20 | 66 | 42 | 18 |
| IVb | 73 | 49 | 33 | 71 | 46 | 30 |
| IVc | 81 | 67 | 47 | 79 | 64 | 45 |
| IVd | 75 | 54 | 35 | 73 | 40 | 32 |
| IVe | 80 | 50 | 31 | 78 | 46 | 30 |
| IVf | 100 | 70 | 50 | 99 | 68 | 48 |
| IVg | 79 | 46 | 36 | 76 | 45 | 33 |
| dithane M-45 (Maneb) | 100 | 86 | 70 | 100 | 85 | 68 |

fungal colony, intersecting one another at about 45°, were measured with a millimeter scale and percent inhibition of mycelial growth was calculated by

$$\% \text{ inhibition} = [(C - T) \times 100]/C$$

where $C$ is the average diameter of the fungal colony (mm) in control plates and $T$ is the average diameter of the fungal colony (mm) in treated plates.

Dithane M-45, a standard commercial fungicide (commonly known as Maneb), was also tested under similar conditions for comparing the results. The antifungal activity displayed by the tested compounds IIIa–c and IVa–g is summarized in Table III.

## RESULTS AND DISCUSSION

Compounds IIIa–c and IVa–g were evaluated for antifungal activity in vitro against *A. niger* and *F. oxysporum*, and the antifungal assays are summarized in Table III. All the tested compounds inhibited more than 66% growth of both the test fungi at 1000 ppm, but their activity decreased markedly at lower concentrations (100 and 10 ppm). Of these, the most active compounds, IIIb, IIIc, and IVf, exhibited antifungal activity almost equivalent to that of Dithane M-45 (Maneb) at 1000 ppm and inhibited 45–51% growth of both fungal species even at 10 ppm.

Although some of the screened compounds, IIIb, IIIc, and IVf, were highly toxic to *A. niger* and *F. oxysporum* at higher concentrations (1000 ppm), the overall results are not so encouraging as one would expect from the combined performance of the two biolabile nuclei, i.e., 1,2,4-triazole and 1,2-dithia-4,5-diazine. This might be attributed to the partial saturation in the triazolo-1,2-dithia-4,5-diazine ring system resulting in the loss of planarity.

It is, however, noteworthy that the introduction of chloro, methoxy, and methyl groups in the aryl moiety of these compounds tends to augment the antifungal activity. The antifungal activity imparted by these groups is chloro > methoxy > methyl.

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**Registry No.** Ia, 22706-11-2; Ib, 113766-05-5; Ic, 13229-02-2; IIa, 127399-28-4; IIb, 127399-29-5; IIc, 127399-30-8; IIIa, 127399-31-9; IIIb, 127399-32-0; IIIc, 127399-33-1; IVa, 127399-34-2; IVb, 127399-35-3; IVc, 127399-36-4; IVd, 127399-37-5; IVe, 127399-38-6; IVf, 127399-39-7; IVg, 127399-40-0.