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Synthesis of new 4[4-(4-nitrophenoxy)phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones and their evaluation as anthelmintics

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Abstract A library of novel 4-[4-(4-nitrophenoxy)phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones containing different substituents at the 5-position is synthesized. The mechanochemical treatment is followed to synthesize target compounds **8 (a–p),** and the yields are compared by both the grinding method and the conventional method. The structure of the intermediate, isothiocyanato-4-(4-nitrophenoxy)benzene **6**, is analyzed by single crystal X-ray studies. The title compounds are characterized by spectral and elemental analyses and further evaluated for their efficacy as anthelmintics in vitro. Compounds **8c**, **8j**, **8k**, **8l,** and **8m** exhibit significant anthelmintic activity.

Keywords Anthelmintic · Grinding · Nitroscanate · Triazole

Introduction

Nitroscanate, chemically called isothiocyanato-4-(4-nitrophenoxy)benzene is an anthelmintic drug active against nematodes and cestodes in cats and dogs. Close intimacy of the immunocompromised human population with cats and dogs has

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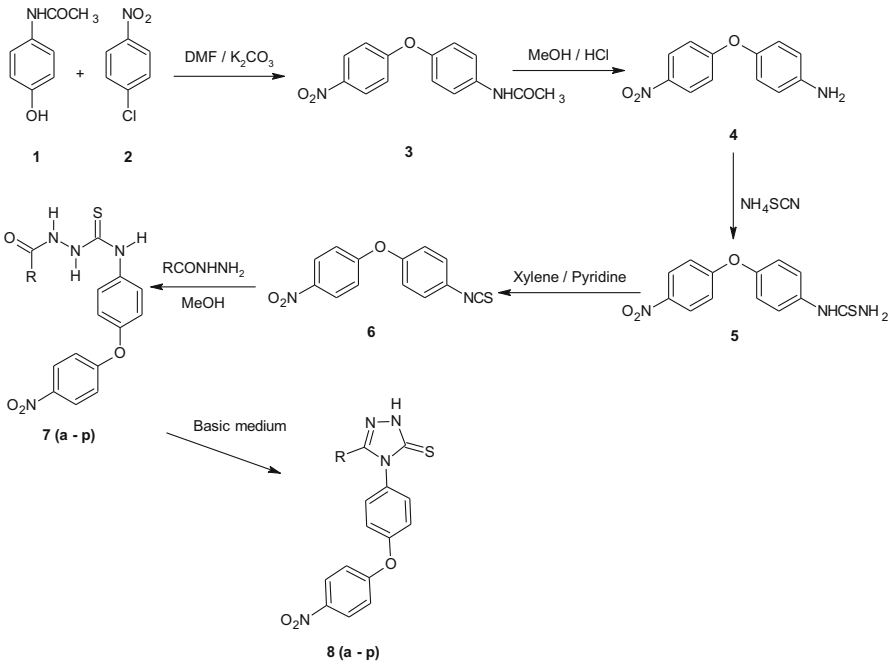
considerably increased the risk of transmission of parasitic infections [1, 2]. Nitroscanate is structurally related to an herbicide named nitrofen, which is described to be a carcinogen [3] and a teratogen [4]. It belongs to the nitro phenyls class of anthelmintic drugs, a class that comprises a number of anthelmintics like amoscanate, amocarzine, and nitrodan [5]. Pharmacological profiles of the nitroaryls and nitroheterocycles include the antibacterials and anthelmintics [6]. These activities are ascribed to their ability to undergo cytochrome P-450 dependent reduction to form reactive species like Ar–NHOH and Ar–NH_2, which interact with DNA and perturb its normal functions in different pathogens. This property of nitro compounds is certainly related to their ability to kill a variety of microbes and parasites; concurrently it may impart to them mutagenicity [7] and carcinogenicity [8]. This is the reason why some of the nitro phenyl drugs are withdrawn from the market. There are findings suggesting that while designing a drug with a biphenyl ether moiety, the simultaneous presence of –NO_2 and –NCS functions should be avoided. The presence of the latter group may enhance the genetic toxicity of the former while any modification of –NCS function leads to a greater reduction of mutagenicity [9]. Hence, we have tried to modify –NCS function for mercapto triazoles, considering the high biological significance of the triazole moiety [10–15].

In congruence with microwave-assisted synthesis [16], grinding-accelerated synthesis is gaining recognition. It is the chemical synthesis [17, 18] using mechanical force [19]. Shorter reaction times, higher yields, environmental friendliness, and operational simplicity form the salient features of the grinding synthesis. In continuance of our work on the synthesis of interesting five-membered heterocycles [20–23], we report the study describing the grinding synthesis of novel 4[4-(4-nitrophenoxy)phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones, their characterization, and progression of their in vitro anthelmintic activities.

Results and discussion

Chemistry

The target compounds are synthesized as per Scheme 1. *P*-Hydroxy acetanilide **1** is condensed with *p*-chloronitrobenzene **2** in DMF at 135–140 °C in presence of anhydrous K_2CO_3. The excess amount of unreacted *p*-hydroxy acetanilide **1** is removed by washing with hot water. The second step involves hydrolysis of *N*-[4-(4-nitrophenoxy)phenyl]acetamide **3** with hydrochloric acid in methanol. The 4-(4-Nitrophenoxy)aniline **4** is converted to 4-(4-Nitrophenoxy)phenyl thiourea **5** in an environmentally benign method by heating **4** with ammonium thiocyanate in water at 100 °C. The 4-(4-nitrophenoxy)phenyl thiourea **5** is refluxed in a nonpolar solvent, xylene, in the presence of pyridine to afford isothiocyanato-4-(4-nitrophenoxy)benzene **6**. Thus, the use of hazardous chemicals like thiophosgene, CS_2, and NaOCl for the conversion of 4-(4-nitrophenoxy)aniline **4** to isothiocyanato-4-(4-nitrophenoxy)benzene **6** can be avoided. Isothiocyanato-4-(4-nitrophenoxy)benzene **6** is then treated with different synthesized aryl carbohydrazides to yield *N*-[4-(4-nitrophenoxy)phenyl]-2-(phenylcarbonyl)hydrazinecarbothioamide **7 (a–p)**.



Scheme 1 Where $R = a$ phenyl, b 4-methylphenyl, c 4-methoxyphenyl, d 4-nitrophenyl, e 4-bromophenyl, f 4-chlorophenyl, g 2-furyl, h 2-thiophenyl, i 3-methoxyphenyl, j 2,4-dimethoxyphenyl, k 3,4-dimethoxyphenyl, l 4-fluorophenyl, m 2-benzofuryl, n 3-chlorophenyl, o 3-bromophenyl, p 2,4-dichlorophenyl

Cyclodehydration of **7 (a–p)** in basic medium affords 4[4-(4-nitrophenoxy)phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones **8 (a–p)**. This reaction is carried out by both the grinding method and the conventional method. The grinding method is a solid-phase synthesis which requires a shorter time (12 min) and occurs at room temperature resulting in comparable yields. On the other hand, conventional synthesis needs heating in a solvent at 80 °C for about an hour. A comparison of the yields obtained by grinding and conventional methods is given in Table 1. In the IR spectra of compounds **8 (a–p)**, there is NH stretching in the characteristic region of 3400 cm^{-1}. Their ^{1}H NMR signals for NH emerging downfield around δ 14.0 authenticates the existence of the ring in thione form. In the ^{13}C NMR spectra, C-3 carbons have shown up around δ 168 and C-5 carbons around δ 162 denoting the formation of target compounds. Other signals are observed in the respective regions.

Crystal structure analysis

In order to confirm the orientation of biphenyl groups, crystal structure analysis of compound 6 is undertaken. Figure 1 displays the ORTEP diagram of **6** at 50 % probability with atom numbering while Fig. 2 depicts its packing diagram viewed along the b-axis. X-ray data of compound **6** was obtained to characterize the

Table 1 Physical data of synthesized compounds **8 (a–p)**

Compound	Melting point (°C)	Yield (%)	
		Conventional	Grinding
8a	192–194	80	82
8b	226–228	89	93
8c	248–250	87	89
8d	268–270	74	75
8e	230–232	81	84
8f	254–256	79	83
8g	202–204	82	85
8h	212–214	84	89
8i	236–238	85	87
8j	250–252	82	85
8k	260–262	82	84
8l	218–220	85	88
8m	206–208	84	88
8n	244–246	76	76
8o	240–242	82	83
8p	218–220	74	74

compound and to know the orientation of biphenyl groups. The C6–O1–C5 bond angle is 119°. This shows that the two biphenyl groups are out of the plane.

Biology

The results of the anthelmintic activity exhibited by compounds **8(a–p)** and standard drug on *Pheretima posthuma* are shown in Table 2. The anthelmintic activity screening data reveal that compound **8c** with a *p*-OMe phenyl group attached to the 5th position of 3-mercapto-1,2,4-triazole ring exhibits significant anthelmintic activity compared to nitroscanate, the standard drug. This suggests that besides the nitro group, perhaps the *p*-OMe phenyl group is also influential in exhibiting anthelmintic activity. Duplication of the methoxy groups as in **8j** and **8k** has caused similar results as in the monosubstituted compound. Similarly, there is no much variation in the result of the compound **8i** monomethoxylated at the *meta* position. Compound **8l** with *p*-fluoro phenyl group has induced a pronounced anthelmintic activity with respect to the standard. This effect can be attributed to the presence of a *p*-fluoro group as in the anthelmintic drug flubendazole. Compounds **8a** and **8d** have shown moderate activities. Meanwhile, compounds **8b**, **8e**, and **8f** have demonstrated better activities owing to the presence of *p*-Me, *p*-Br, and *p*-Cl phenyl groups, respectively. Similar results are observed with the *meta* substitutions of chloro **8n** and bromo **8o** groups as well as with the duplication of chloro groups **8p**. The replacement of phenyl group with the heteroaryl moiety has created varied effects. Compounds **8g** and **8h** have shown average activities but the presence of the benzofuran unit as in **8m** demonstrated potent anthelmintic activity.

Experimental

Chemistry

The melting points were ascertained on Thomas Hoover apparatus and remain uncorrected. IR (KBr) spectra were recorded on a Shimadzu 8300 Fourier transform

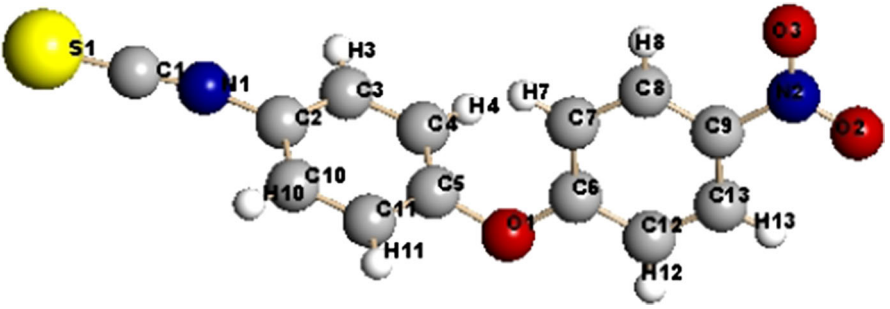


Fig. 1 ORTEP diagram of **6** at 50 % probability with atom numbering

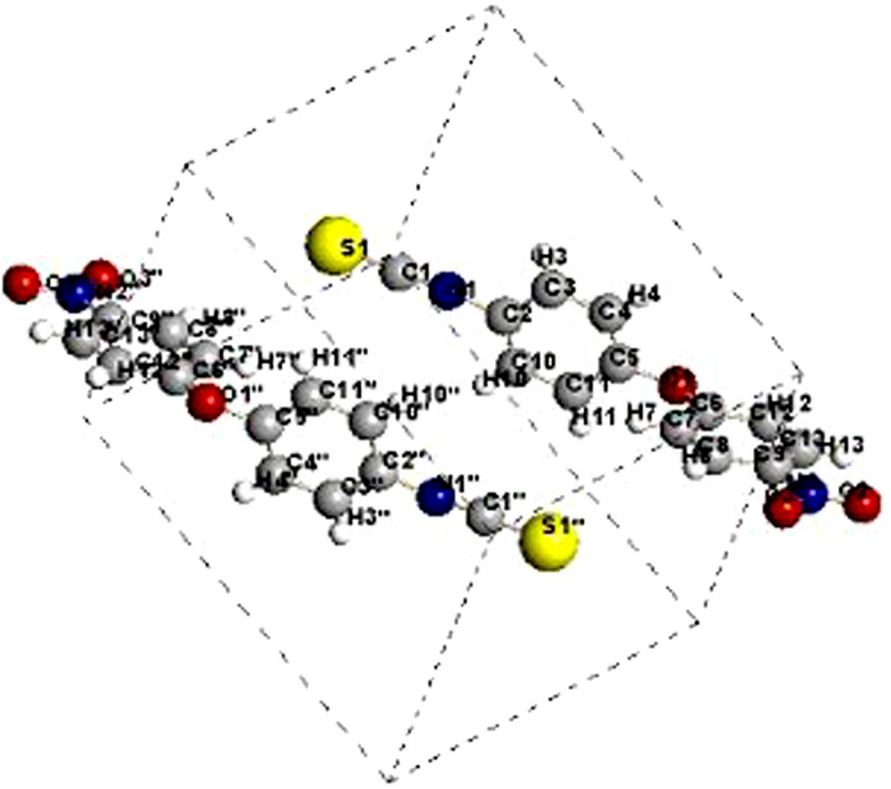


Fig. 2 Packing diagram of **6** viewed along b-axis

Table 2 Effects of compounds **8 (a–p)** and standard drug on *Pheretima posthuma*

Compound	Paralysis time (min)		Death time (min)	
	25 mg/50 mL	50 mg/50 mL	25 mg/50 mL	50 mg/50 mL
Control	–	–	–	–
8a	20.04 ± 0.003	17.38 ± 0.015	25.23 ± 0.037	22.23 ± 0.008
8b	10.44 ± 0.006	12.06 ± 0.005	17.17 ± 0.008	19.17 ± 0.042
8c	9.52 ± 0.012	6.12 ± 0.014	15.05 ± 0.006	12.17 ± 0.037
8d	21.03 ± 0.008	18.32 ± 0.033	27.12 ± 0.014	23.16 ± 0.006
8e	11.35 ± 0.006	8.49 ± 0.035	16.09 ± 0.003	12.53 ± 0.018
8f	11.50 ± 0.028	9.09 ± 0.015	18.18 ± 0.053	14.29 ± 0.037
8g	15.42 ± 0.014	12.04 ± 0.028	21.35 ± 0.006	17.19 ± 0.008
8h	17.18 ± 0.020	15.10 ± 0.008	24.08 ± 0.035	20.07 ± 0.014
8i	10.05 ± 0.012	7.12 ± 0.023	15.045 ± 0.010	12.50 ± 0.024
8j	9.57 ± 0.006	6.16 ± 0.024	15.03 ± 0.010	12.16 ± 0.009
8k	9.52 ± 0.010	6.15 ± 0.005	15.15 ± 0.015	12.25 ± 0.020
8l	9.43 ± 0.014	6.01 ± 0.020	14.16 ± 0.013	11.34 ± 0.017
8m	9.50 ± 0.026	6.17 ± 0.015	15.00 ± 0.010	11.55 ± 0.029
8n	12.13 ± 0.015	9.24 ± 0.008	18.36 ± 0.025	14.50 ± 0.010
8o	11.50 ± 0.025	9.00 ± 0.010	16.20 ± 0.036	13.03 ± 0.005
8p	11.55 ± 0.011	9.15 ± 0.025	18.10 ± 0.036	14.25 ± 0.005
Nitroscanate	9.45 ± 0.024	6.05 ± 0.017	14.17 ± 0.014	11.36 ± 0.023

infrared spectrometer. ^{1}H NMR spectra were recorded on a Bruker AM 400 MHz spectrometer and ^{13}C NMR spectra on a Bruker AM 100 MHz spectrometer using DMSO-d_6 as solvent and TMS as an internal standard (Chemical shift in ppm). ESI mass spectra were recorded on an Agilent 6520 ESIQTOF instrument at ionization potential of 110 V and acetonitrile as solvent. Elemental analyses were obtained on a Vario-EL instrument. Thin layer chromatography (TLC) was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck). Visualization is made with ultraviolet light (UV. R-340). The solvents were evaporated with a Buchi rotary evaporator.

N-[4-(4-Nitrophenoxy)phenyl]acetamide (**3**) *p*-Hydroxy acetanilide **1** (6.00 g, 0.039 mol) was dissolved in DMF (20 mL) under room temperature (r.t.) stirring. The *p*-Chloronitrobenzene (6.26 g, 0.039 mol) **2** and anhydrous K_2CO_3 (2.75 g, 0.0198 mol) were then added to the reaction mixture. This brown reaction mass was stirred at r.t. for 30 min. The temperature of the reaction mass was slowly raised to 135–140 °C under stirring. This temperature was maintained at 135–140 °C for 1 h as monitored by TLC (hexane: EtOAc; 1:1). The reaction mass was cooled and the yellow precipitate that formed was washed with 25 % NaOH solution. The solid was suck dried upon a hot water wash and crystallized from methanol to afford *N*-[4-(4-nitrophenoxy)phenyl]acetamide **3** as yellow solid (10.17 g, 94 %); mp 146–148 °C; R_f 0.81 (hexane: EtOAc; 1:1); IR (KBr): ν_{max} 3291, 1656, 1342,

1235 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.32$ (2H, d, ArH, $J = 8$ Hz), 8.24 (2H, d, ArH, $J = 8$ Hz), 7.76 (1H, s, NH), 7.64 (2H, d, ArH, $J = 8$ Hz), 7.36 (2H, d, ArH, $J = 8$ Hz), 2.20 (3H, s, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz,) : $\delta = 186.7$, 162.4, 153.6, 143.5, 128.2, 126.9, 126.1, 121.3, 117.5; Anal. Calcd. for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.72; H, 4.49; N, 10.27.

4-(4-Nitrophenoxy)aniline (**4**) Refluxed *N*-[4-(4-Nitrophenoxy)phenyl]acetamide **3** (8.00 g, 0.029 mol) and Conc. HCl (18 mL) in methanol (16 mL) for 20 min. Upon completion of reaction as marked by TLC (hexane: EtOAc; 1:1), reaction mass was cooled down and the yellow solid formed was washed with chilled water. This solid was crystallized from methanol to yield 4-(4-nitrophenoxy)aniline **4** (6.94 g, 89 %); mp 228–230 °C; R_f 0.76 (hexane: EtOAc; 1:1); IR (KBr): ν_{max} 3120, 1451, 1335, 1244 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,) : $\delta = 8.26$ (2H, d, ArH, $J = 8$ Hz), 7.58(2H, d, ArH, $J = 8$ Hz), 7.25 (2H, d, ArH, $J = 8$ Hz), 7.14 (2H, d, ArH, $J = 8$ Hz), 3.71 (2H, bs, NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz,) : $\delta = 162.2$, 154.6, 143.7, 128.4, 127.3, 126.5, 121.1, 118.1; Anal. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17. Found: C, 62.55; H, 4.33; N, 12.13.

4-(4-Nitrophenoxy)phenyl thiourea (**5**) Ammonium thiocyanate (4.27 g, 0.056 mol) was stirred in water (20 mL) for 15 min at r.t. to get a clear solution. Added 4-(4-nitrophenoxy)aniline **4** (5.00 g, 0.018 mol) and refluxed at 100 °C for 2 h. As indicated by TLC (hexane: EtOAc; 1:1), the yellow solid formed was cooled, filtered off, and washed with hot water. Recrystallized from methanol to offer 4-(4-nitrophenoxy)phenyl thiourea **5** (4.60 g, 85 %); mp 190–192 °C; R_f 0.4; IR (KBr): ν_{max} 3352, 3280, 1428, 1351, 1328, 1212 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.02$ (2H, s, NH), 8.99 (1H, bs, NH), 8.26 (2H, d, ArH, $J = 8$ Hz), 7.44 (2H, d, ArH, $J = 8$ Hz), 7.26 (2H, d, ArH, $J = 8$ Hz), 7.13 (2H, d, ArH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz,) $\delta = 172.5$, 166.4, 153.7, 143.5, 127.7, 127.3, 126.1, 121.9, 117.3; Anal. Calcd. for $C_{13}H_{11}N_3O_3S$:C, 53.97; H, 3.83; N, 14.52. Found: C, 53.99; H, 3.80; N, 14.56.

Isothiocyanato-4-(4-nitrophenoxy)benzene (**6**) Dissolved 4-(4-nitrophenoxy)phenyl thiourea **5** (4.0 g, 0.013 mol) in xylene (20 mL) and added catalytic amount of pyridine (0.1 mL), refluxed at 140 °C for 45 min as indicated by TLC (hexane: EtOAc; 1:1). Gradually cooled the reaction mass and stirred at r.t. for 30 min. Filtered off the insoluble solid and removed xylene *in vacuo*. The yellow solid thus obtained was crystallized from methanol to provide isothiocyanato-4-(4-nitrophenoxy)benzene **6** (3.08 g, 82 %); mp 120–122 °C; R_f 0.73 (hexane: EtOAc; 1:1); IR (KBr): ν_{max} 2115, 1489, 1358, 1238, 842 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,):$\delta = 8.26$ (2H, d, ArH, $J = 8$ Hz), 7.51(2H, d, ArH, $J = 8$ Hz), 7.25 (2H, d, ArH, $J = 8$ Hz), 7.14 (2H, d, ArH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 162.4$, 153.9, 143.1, 134.1, 128.4, 127.2, 126.3, 121.1, 117.7; Anal. Calcd. for $C_{13}H_8N_2O_3S$:C, 57.35; H, 2.96; N, 10.29. Found: C, 57.30; H, 2.99; N, 10.22.

Representative procedure for *N*-[4-(4-nitrophenoxy)phenyl]-2-(phenylcarbonyl)hydrazinecarbothioamide **7 (a–p)**: Refluxed an equimolar proportion of compound **6** and

aryl carbohydrazide in methanol (10 vol.) for 1 h. Upon cooling, the resulting solid was filtered off and crystallized from methanol to afford pure *N*-[4-(4-nitrophenoxy)phenyl]-2-(phenylcarbonyl)hydrazinecarbothioamide **7 (a–p)**.

Characterization data of *N*-[4-(4-nitrophenoxy)phenyl]-2-[(4-methoxyphenyl)-carbonyl]hydrazinecarbothioamide (**7c**): R_f 0.21 (toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3021, 1499, 1311, 1240 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 10.41$ (1H, s, NH), 9.84(1H, s, NH), 9.73 (1H, s, NH), 8.26 (2H, d, ArH, $J = 8$ Hz), 7.93 (2H, d, ArH, $J = 8.8$ Hz), 7.56 (2H, d, ArH, $J = 6.4$ Hz), 7.11–7.17 (4H, m, ArH), 7.04 (2H, d, ArH, $J = 8$ Hz), 3.82 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 166.0$, 163.5, 162.5, 154.8, 151.5, 142.6, 137.1, 130.2, 128.2, 126.6, 125.1, 120.4, 117.6, 113.9, 55.9; Anal. Calcd. for $C_{21}H_{18}N_4O_5S$:C, 56.46; H, 4.03; N, 13.17. Found: C, 56.41; H, 4.08; N, 13.14.

4-[4-(4-nitrophenoxy)phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones 8 (a–p): typical procedure

Conventional method

Refluxed hydrazinecarbothioamide **7 (a–p)** (0.3 mmol) with aqueous 5 % NaOH solution (10 mL) for 1 h; checked the clear solution with TLC (toluene: EtOAc; 2:1). Cooled and acidified with 6 N HCl and crystallized from ethanol.

Grinding method

Ground hydrazinecarbothioamide **7 (a–p)** (0.3 mmol) and NaOH pellets (0.6 mmol) in a porcelain mortar with a pestle for 12 min at r.t. Monitored the pasty reaction mixture by TLC (toluene: EtOAc; 2:1), poured into ice cold water, and acidified with 6 N HCl. Filtered off the crude solid and crystallized from ethanol.

4-[4-(4-Nitrophenoxy)phenyl]-5-phenyl-2H-1,2,4-triazole-3-thione (**8a**) R_f 0.57 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3474, 3142, 1554, 1542, 1358, 1224 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,):$\delta = 14.07$ (1H, s, NH), 8.36 (2H, d, ArH, $J = 8$ Hz), 8.12 (2H, d, ArH, $J = 7.5$), 7.68 (2H, d, ArH, $J = 8$ Hz), 7.64 (2H, d, ArH, $J = 8$ Hz), 7.61 (1H, t, ArH, $J = 7.2$), 7.56 (2H, t, ArH, $J = 7.6$), 7.40 (2H, d, ArH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 167.4$, 160.2, 156.7, 149.3, 143.1, 131.6, 130.6, 129.1, 128.6, 128.5, 128.3, 126.9, 122.6, 118.7; Anal. Calcd. for $C_{20}H_{14}N_4O_3S$:C, 61.53; H, 3.61; N, 14.35. Found: C, 61.57; H, 3.63; N, 14.38.

4-[4-(4-Nitrophenoxy)phenyl]-5-(4-methylphenyl)-2H-1,2,4-triazole-3-thione (**8b**) R_f 0.68 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3452, 3052, 1526, 1502, 1326, 1240 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.12$ (1H, s, NH), 8.32 (2H, d, ArH, $J = 8$ Hz), 7.61 (2H, d, ArH, $J = 8$ Hz), 7.32 (2H, d, ArH, $J = 8$ Hz), 7.25 (2H, d, ArH, $J = 8$ Hz), 7.21 (2H, d, ArH, $J = 8$ Hz), 6.98 (2H, d, ArH, $J = 8$ Hz), 2.68 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 168.5$, 161.4, 160.7, 155.2, 143.4, 138.3, 132.7, 130.2, 129.6, 128.7, 126.1, 122.6, 118.2, 116.8, 21.5;

Anal. Calcd. for $C_{21}H_{16}N_4O_3S$:C, 62.36; H, 3.99; N, 13.85. Found: C, 62.35; H, 3.91; N, 13.80.

4-[4-(4-Nitrophenoxy)phenyl]-5-(4-methoxyphenyl)-2H-1,2,4-triazole-3-thione (**8c**) R_f 0.62 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3448, 3247, 1596, 1504,1334, 1249 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.07$ (1H, s, NH), 8.33 (2H, d, ArH, $J = 8$ Hz), 7.47 (2H, d, ArH, $J = 8$ Hz), 7.32 (2H, d, ArH, $J = 8$ Hz), 7.27 (2H, d, ArH, $J = 8$ Hz), 7.21 (2H, d, ArH, $J = 9.2$ Hz), 6.96 (2H, d, ArH, $J = 8$ Hz), 3.71(3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 168.8$, 162.4, 161.1, 155.3, 150.9, 143.2, 131.9, 131.5, 130.2, 126.8, 121.2, 118.5, 118.3, 114.5, 55.7; Mass spectrum, *m/z* (*I*rel, %): 421 [M + H] + (19), 374 (100), 344 (24), 283 (3.9), 210 (3.7), 149 (5.1), 77 (2.4). Anal. Calcd. for $C_{21}H_{16}N_4O_4S$: C, 59.99; H, 3.84; N, 13.33. Found: C, 59.91; H, 3.87; N, 13.39.

4-[4-(4-Nitrophenoxy)phenyl]-5-(4-nitrophenyl)-2H-1,2,4-triazole-3-thione (**8d**) R_f 0.61 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3409, 3070, 1596, 1504, 1334, 1242 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.21$ (1H, s, NH), 8.34 (2H, d, ArH, $J = 8$ Hz), 8.16 (2H, d, ArH, $J = 8$ Hz), 7.54 (2H, d, ArH, $J = 8$ Hz), 7.42 (2H, d, ArH, $J = 8$ Hz), 7.26–6.99 (4H, m, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 170.1$, 164.8, 155.5, 150.2, 148.6, 145.3, 135.2, 130.7, 130.1, 129.9, 126.4, 123.7, 122.8, 119.9; Anal. Calcd. for $C_{20}H_{13}N_5O_5S$: C, 55.17; H, 3.01; N, 16.08. Found: C, 55.12; H, 3.08; N, 16.03.

4-[4-(4-Nitrophenoxy)phenyl]-5-(4-bromophenyl)-2H-1,2,4-triazole-3-thione (**8e**) R_f 0.71 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3389, 1589, 1514, 1366, 1241 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.14$ (1H, s, NH), 8.30 (2H, d, ArH, $J = 8$ Hz), 7.82 (2H, d, $J = 7.5$, ArH); 7.70 (2H, d, $J = 7.5$, ArH), 7.60 (2H, d, ArH, $J = 8$ Hz), 7.40 (2H, d, ArH, $J = 8$ Hz), 7.21 (2H, d, ArH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 167.2$, 163.6, 161.9, 154.6, 148.3, 143.6, 134.5, 133.6, 132.0, 128.1, 123.5, 122.1, 118.6, 114.2; Anal. Calcd. for $C_{20}H_{13}BrN_4O_3S$: C,51.18; H, 2.79; N, 11.94. Found: C, 51.14; H, 2.75; N, 11.89.

4-[4-(4-Nitrophenoxy)phenyl]-5-(4-chlorophenyl)-2H-1,2,4-triazole-3-thione (**8f**) R_f 0.73 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3415, 3072, 1591, 1507, 1340, 1251, 745 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.21$ (1H, s, NH), 8.34 (2H, d, ArH, $J = 8$ Hz), 7.81 (2H, d, ArH, $J = 8$ Hz), 7.68 (2H, d, ArH, $J = 8$ Hz), 7.48 - 7.28 (6H, m, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 169.6$, 162.1, 154.8, 149.7, 143.4, 135.4, 132.6, 131.8, 130.2, 129.9, 127.4, 126.4, 121.5, 116.7; Anal. Calcd. for $C_{20}H_{13}ClN_4O_3S$: C, 56.54; H, 3.08; N, 13.19. Found: C, 56.60; H, 3.06; N, 13.10.

4-[4-(4-Nitrophenoxy)phenyl]-5-(furan-2-yl)-2H-1,2,4-triazole-3-thione (**8g**) R_f 0.63 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3438, 3042, 1591, 1504, 1334, 1244 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.24$ (1H, s, NH), 8.26 (2H, d, ArH, $J = 8$ Hz), 8.02 (2H, d, H Furan, $J = 4.3$ Hz), 7.68 (2H, d, ArH, $J = 8$ Hz), 7.62 (2H, d, ArH, $J = 8$ Hz), 7.25 7(1H, t, H Furan, $J = 4.0$ Hz), 7.36 (2H, d, ArH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 168.2$, 162.5, 158.8, 151.6,

149.5, 143.7, 140.9, 132.3, 130.6, 127.9, 122.8, 117.5, 110.3, 106.1; Anal. Calcd. for $C_{18}H_{12}N_4O_4S$:C, 56.84; H, 3.18; N, 14.73. Found: C, 56.80; H, 3.11; N, 14.75.

4-[4-(4-Nitrophenoxy)phenyl]-5-(thiophen-2-yl)-2H-1,2,4-triazole-3-thione (**8h**) R_f 0.63 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3432, 3241, 1573, 1512, 1339, 1245 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.18$ (1H, s, NH), 8.14 (2H, d, ArH, $J = 8$ Hz), 7.88 (2H, d, H Thiophene, $J = 4.8$ Hz), 7.76 (2H, d, ArH, $J = 8$ Hz), 7.68 (2H, d, ArH, $J = 8$ Hz), 7.46 (2H, d, ArH, $J = 8$ Hz), 7.25 (1H, t, H Thiophene, $J = 4.0$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 169.1$, 162.6, 158.2, 149.9, 144.7, 133.3, 130.4, 129.3, 126.6, 126.0, 124.3, 122.8, 118.3, 117.6; Anal. Calcd. for $C_{18}H_{12}N_4O_3S_2$: C, 54.53; H, 3.05; N, 14.13. Found: C, 54.59; H, 3.01; N, 14.16.

4-[4-(4-Nitrophenoxy)phenyl]-5-(3-methoxyphenyl)-2H-1,2,4-triazole-3-thione (**8i**) R_f 0.61 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3436, 3240, 1588, 1503, 1368, 1226 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.03$ (1H, s, NH), 8.36 (2H, d, ArH, $J = 8$ Hz), 7.44 (2H, d, ArH, $J = 8$ Hz), 7.33 (2H, d, ArH, $J = 8$ Hz), 7.26 (2H, d, ArH, $J = 8$ Hz), 7.11-6.83 (4H, m, ArH), 3.75(3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 167.9$, 162.1, 160.9, 155.3, 150.6, 145.7, 131.8, 131.5, 130.5, 126.9, 122.4, 119.1, 118.2, 114.5, 53.2; Anal. Calcd. for $C_{21}H_{16}N_4O_4S$: C, 59.99; H, 3.84; N, 13.33. Found: C, 59.90; H, 3.86; N, 13.37.

4-[4-(4-Nitrophenoxy)phenyl]-5-(2,4-dimethoxyphenyl)-2H-1,2,4-triazole-3-thione (**8j**) R_f 0.62 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3413, 3245, 1587, 1513, 1345, 1268 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.01$ (1H, s, NH), 8.15 (2H, d, ArH, $J = 8$ Hz), 7.25 (2H, d, ArH, $J = 8$ Hz), 7.16 (2H, d, ArH, $J = 8$ Hz), 7.08 (2H, d, ArH, $J = 8$ Hz), 6.88–6.80 (3H, m, ArH), 3.02 (6H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 164.8$, 161.4, 160.9, 159.0, 154.2, 150.6, 147.9, 130.7, 127.6, 126.8, 124.0, 123.7, 119.3, 116.1, 115.1, 114.8, 54.7; Anal. Calcd. for $C_{22}H_{18}N_4O_5S$: C, 58.66; H, 4.03; N, 12.44. Found: C, 58.60; H, 4.05; N, 12.49.

4-[4-(4-Nitrophenoxy)phenyl]-5-(3,4-dimethoxyphenyl)-2H-1,2,4-triazole-3-thione (**8k**) R_f 0.62 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3455, 3211, 1579, 1498, 1328, 1214 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.01$ (1H, s, NH), 8.10 (2H, d, ArH, $J = 8$ Hz), 7.61 (2H, d, ArH, $J = 8$ Hz), 7.18 (2H, d, ArH, $J = 8$ Hz), 6.88 (2H, d, ArH, $J = 8$ Hz), 6.73–6.68 (3H, m, ArH), 3.11 (6H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 165.2$, 162.4, 157.9, 153.3, 150.2, 146.2, 145.5, 144.5, 133.7, 127.6, 125.5, 121.8, 119.1, 114.8, 113.6, 113.2, 51.1; Anal. Calcd. for $C_{22}H_{18}N_4O_5S$: C, 58.66; H, 4.03; N, 12.44. Found: C, 58.62; H, 4.09; N, 12.41.

4-[4-(4-Nitrophenoxy)phenyl]-5-(4-fluorophenyl)-2H-1,2,4-triazole-3-thione (**8l**) R_f 0.69 (toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3448, 3078, 1596, 1504, 1342, 1242 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.23$ (1H, s, NH), 8.33 (2H, d, ArH, $J = 8$ Hz), 7.65 (2H, d, ArH, $J = 8$ Hz), 7.49 (2H, d, ArH, $J = 8$ Hz), 7.33–7.20 (6H, m, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 169.4$, 162.6, 154.8, 149.9, 143.1, 132.9, 132.0, 131.3, 130.1, 126.9, 126.6, 123.4, 121.1, 118.4;

Anal. Calcd. for $C_{20}H_{13}FN_4O_3S$: C, 58.82; H, 3.21; N, 13.72. Found: C, 58.86; H, 3.21; N, 13.79.

4-[4-(4-Nitrophenoxy)phenyl]-5-(benzofuran-2-yl)-2H-1,2,4-triazole-3-thione (**8m**) R_f 0.66 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3481, 3042, 1565, 1492, 1313, 1269 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.20$ (1H, s, NH), 8.24(2H, d, ArH, $J = 8$ Hz), 7.81 (1H, d, H Benzofuran, $J = 7.6$ Hz); 7.79 (1H, s, H Benzofuran), 7.75 (1H, d, H Benzofuran, $J = 8.0$ Hz), 7.66 (2H, d, ArH, $J = 8$ Hz), 7.60 (2H, d, ArH, $J = 8$ Hz), 7.55–7.51 (1H, t, H Benzofuran, $J = 7.6$ Hz), 7.41–7.37 (1H, t, H Benzofuran, $J = 7.6$ Hz), 7.32 (2H, *d*, ArH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 167.9$, 161.5, 156.0, 155.7, 149.1, 143.2, 134.6, 130.9, 127.9, 127.8, 127.3, 126.9, 125.4, 124.7, 123.2, 117.3, 113.5, 111.7; Anal. Calcd. for $C_{22}H_{14}N_4O_4S$: C, 61.39; H, 3.28; N, 13.02. Found: C, 61.38; H, 3.24; N, 13.05.

4-[4-(4-Nitrophenoxy)phenyl]-5-(3-bromophenyl)-2H-1,2,4-triazole-3-thione (**8n**) R_f 0.71 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3476, 1586, 1504, 1342, 1242, 667 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.26$ (1H, s, NH), 8.64 (2H, d, ArH, $J = 8$ Hz), 7.91(2H, d, ArH, $J = 8$ Hz), 7.68 (2H, d, ArH, $J = 8$ Hz), 7.48 (2H, d, ArH, $J = 8$ Hz), 7.18-7.10 (4H, m, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 168.1$, 164.8, 154.8, 148.3, 146.1, 140.2, 136.7, 132.9, 130.2, 127.6, 126.6, 123.9, 120.9, 114.8, 114.1, 112.2; Anal. Calcd. for $C_{20}H_{13}BrN_4O_3S$: C, 51.18; H, 2.79; N, 11.94. Found: C, 51.19; H, 2.70; N, 11.92.

4-[4-(4-Nitrophenoxy)phenyl]-5-(3-chlorophenyl)-2H-1,2,4-triazole-3-thione (**8o**) R_f 0.73 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3428, 3065, 1587, 1413, 1244, 781 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.23$ (1H, s, NH), 8.34(2H, d, ArH, $J = 8$ Hz), 7.64 (2H, d, ArH, $J = 8$ Hz), 7.50 (2H, *d*, ArH, $J = 8$ Hz), 7.31(2H, d, ArH, $J = 8$ Hz), 7.29-7.20 (4H, m, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 169.4$, 161.8, 159.7, 150.3, 143.4, 140.9, 139.0, 135.6, 130.2, 128.7, 128.5,126.9, 126.7, 124.6, 121.5, 117.0; Anal. Calcd. for $C_{20}H_{13}ClN_4O_3S$: C, 56.54; H, 3.08; N, 13.19. Found: C, 56.56; H, 3.10; N, 13.13.

4-[4-(4-Nitrophenoxy)phenyl]-5-(2,4-dichlorophenyl)-2H-1,2,4-triazole-3-thione (**8p**) R_f 0.73 (Toluene: EtOAc; 2:1); IR (KBr): ν_{max} 3430, 3012, 1515, 1489, 1246, 730 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 14.19$ (1H, s, NH), 8.31 (2H, d, ArH, $J = 8$ Hz), 7.72(2H, d, ArH, $J = 8$ Hz), 7.58 (2H, d, ArH, $J = 8$ Hz), 7.39 (2H, d, ArH, $J = 8$ Hz), 7.22-7.14 (3H, m, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 167.4$, 161.4, 154.9, 146.1, 143.2, 136.3, 135.9, 135.0, 134.0, 129.7, 129.5, 128.8, 128.6, 126.9, 123.5, 116.6; Anal. Calcd. for $C_{20}H_{12}Cl_2N_4O_3S$: C, 52.30; H, 2.63; N, 12.20. Found: C, 52.28; H, 2.60; N, 12.26.

X-ray data collection

Suitable crystals are obtained by dissolving the title compound in ether followed by slow evaporation. A specimen of $C_{13}H_8N_2O_3S$ **6** is used for the X-ray crystallographic analysis. The X-ray intensity data are measured. The total exposure time is

3.96 h. The frames are integrated with the Bruker SAINT software package using a narrow-frame algorithm. The structure is solved and refined using the Bruker SHELXTL Software Package, using the space group P -1, with $Z = 2$ for the formula unit, $C_{13}H_8N_2O_3S$. This structure is deposited with the Cambridge Structural Data Base having CCDC NO. 1006102 as a supplementary publication.

Biology

Anthelmintic activity

We have adopted the anthelmintic assay as per the method already illustrated [24, 25] with the necessary modifications. Indian earthworm *Pheretima posthuma* is used for this study due to its anatomical and physiological resemblance with the roundworms. The earthworms of 5–6 cm length and 0.3–0.4 cm width are used for all experimental protocols. The worms are divided into ten sets with one earthworm per Petri dish at r.t. Nitroscanate solution is used as a standard drug and saline water as control. The test compounds **8(a–p)** and Nitroscanate are dissolved in a minimum quantity of dimethyl sulfoxide (DMSO) in a 50 mL standard flask and made up to the mark with saline water. All the test solutions and standard solution are prepared freshly before beginning an experiment. Then 50 mL of test solution is poured into each Petri dish. Each experiment is repeated thrice and the results are expressed as mean ± S.E.M [26]. We have observed the time taken for paralysis and death of individual worms (Table 2). The stage of paralysis is the time when no movement can be observed while the worms are shaken vigorously. Death is concluded to have occurred when the worms are observed to have lost their motility even when poked with a needle.

Conclusion

We report the interesting compounds, 4-[4-(4-nitrophenoxy)phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones. The crystal structure of the key intermediate, isothiocyanato-4-(4-nitrophenoxy)benzene **6** has been presented. All the target compounds are characterized by analytical techniques. Target compounds **8(a–p)** are synthesized by solid-phase grinding synthesis as well as by the conventional method. Grinding synthesis has offered comparable yields in a shorter reaction time at room temperature. Preliminary structure–activity relationship studies have helped to highlight the effectiveness of 4-[4-(4-nitrophenoxy)phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones as anthelmintics in vitro compared to nitroscanate.

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