Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Tetrazole Regioisomers in the Development of Nitro Group-Containing Antitubercular Agents

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CONTENT

Table S1. In vitro antibacterial activities of compounds **18b**, **23b**, **36b**, **40b** and **40d** expressed as MIC (μM).

Strains		Studied compounds – MIC (IC_{95}; μM)						
		18b	**23b**	**36b**	**40b**	**40d**	**VAN**	**GEN**
SA	24 h	>500	>500	>500	>500	>500	0.35	-
	48 h	>500	>500	>500	>500	>500	-	-
MRSA	24 h	>500	>500	>500	>500	>500	0.35	-
	48 h	>500	>500	>500	>500	>500	-	-
SE	24 h	>500	>500	>500	>500	>500	0.35	-
	48 h	>500	>500	>500	>500	>500	-	-
EF	24 h	>500	>500	>500	>500	>500	0.7	-
	48 h	>500	>500	>500	>500	>500	-	-
EC	24 h	>500	>500	>500	>500	>500	-	0.26
	48 h	>500	>500	>500	>500	>500	-	-
KP	24 h	>500	>500	>500	>500	>500	-	0.26
	48 h	>500	>500	>500	>500	>500	-	-
KP-E	24 h	>500	>500	>500	>500	>500	-	0.26
	48 h	>500	>500	>500	>500	>500	-	-
PA	24 h	>500	>500	>500	>500	>500	-	1
	48 h	>500	>500	>500	>500	>500	-	-

SA - *Staphylococcus aureus* ATCC 6538; **MRSA -** methicillin resistant *Staphylococcus aureus* H 5996/08; **SE -** *Staphylococcus epidermidis* H 6966/08; **EF -** *Enterococcus faecalis* J 14365/08; **EC -** *Escherichia coli* ATCC 8739; **KP -** *Klebsiella pneumoniae* D 11750/08; **KP-E** - ESBL positive *Klebsiella pneumoniae* J 14368/08; **PA -** *Pseudomonas aeruginosa* ATCC 9027; **VAN** – Vancomycin; **GEN** – Gentamicin

Table S2. In vitro antifungal activities of compounds 1**8b**, **23b**, **36b**, **40b** and **40d** expressed as MIC (μM).

Strains		Studied compounds - MIC* (IC_{80}/IC_{50}; μM)						
		18b	**23b**	**36b**	**40b**	**40d**	**FLU**	**AMB**
CA	24 h	>500	>500	>500	>500	>125	0.82	0.54
	48 h	>500	>500	>500	>500	>125	-	-
CT	24 h	>500	>500	>500	>500	>125	1.6	0.54
	48 h	>500	>500	>500	>500	>125	-	-
CK	24 h	>500	>500	>500	>500	>125	105	1
	48 h	>500	>500	>500	>500	>125	-	-
CG	24 h	>500	>500	>500	>500	>125	26	0.54
	48 h	>500	>500	>500	>500	>125	-	-
TA	24 h	>500	>500	>500	>500	>125	210	0.27
	48 h	>500	>500	>500	>500	>125	-	-
AF	24 h	>500	>500	>500	>500	>125	>500	0.54
	48 h	>500	>500	>500	>500	>125	-	-
AC	24 h	>500	>500	>500	>500	>125	>500	1
	48 h	>500	>500	>500	>500	>125	-	-
TM	72 h	>500	>500	>500	>500	>125	105	0.54
	120 h	>500	>500	>500	>500	>125	-	-

*IC_{50} for **AF**, **AC**, **TM**; IC_{80} for **CA**, **CT**, **CK**, **CG, TA**

CA - *Candida albicans* ATCC 44859; **CT** - *Candida tropicalis* 156; **CK** - *Candida krusei* E28; **CG** - *Candida glabrata* 20/I; **TA** - *Trichosporon asahii* 1188; **AF** - *Aspergillus fumigatus* 231; **AC** - *Absidia corymbifera* 272; **TM** - *Trichophyton mentagrophytes* 445; **FLU** – Fluconazole; **AMB** – Amphotericin B

Experimental section

Chemistry

The structures of the prepared compounds were identified using ^{1}H-NMR and ^{13}C-NMR spectroscopy. The purity of all compounds reported was determined by elemental analysis or HPLC-HRMS. All chemicals were obtained from Sigma-Aldrich (Schnelldorf, Germany) and were used without further purification. TLC was performed on Merck aluminum plates with silica gel 60 F_{254}. Merck Kieselgel 60 (0.040-0.063 mm) was used for column chromatography. Melting points were recorded with a Büchi B-545 apparatus (BUCHI Labortechnik AG, Flawil, Switzerland) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded by Varian Mercury Vx BB 300 or VNMR S500 NMR spectrometers (Varian, Palo Alto, CA, USA). Chemical shifts were reported as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal. The elemental analysis was carried out on an Automatic Microanalyser EA1110CE (Fisons Instruments S.p.A., Milano, Italy). HPLC-HRMS (ESI+) experiments were performed using the UltiMate3000 Rapid Separation Liquid Chromatography System with a Q-Exactive Plus Mass Spectrometer (Thermo Scientific, Bremen, Germany).

Although we did not observe any explosive or shock-sensitive properties of studied compounds, proper safety precautions should be applied while synthesizing and handling them!

Synthesis of alkyl selenocyanates (9-13):

A suspension of alkyl halide (10 mmol) and potassium selenocyanate (11 mmol) in 7 mL DMF or THF was stirred until completion under the conditions given below. The reaction mixture was then diluted with EtOAc (10 mL) and the organic layer was washed with 1%

aqueous NaOH (2 × 10 mL) and H_2O (1 × 10 mL). The organic solvent was dried over Na_2SO_4. If necessary, the crude product was purified by column chromatography (Mobile phase: Hexane/EtOAc).

*Benzyl selenocyanate (**9**)*: Benzyl bromide was used as a substrate. The reaction mixture was stirred in THF at room temperature (rt) for 1 h. Yield: 84% as a malodorous white solid; mp 68-69 °C (lit.[1] mp 68-70 °C). ^{1}H NMR (300 MHz, acetone) δ 7.53 – 7.24 (m, 5H), 4.43 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 138.62, 129.80, 129.57, 128.91, 103.04, 33.04.

*4-Nitrobenzyl selenocyanate (**10**)*:[2] 4-Nitrobenzyl chloride was used as a substrate. The reaction mixture was heated in DMF at 80 °C for 8 h. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 4:1). Yield: 66% as a yellow solid; mp 121-122 °C (lit.[3] mp 122 °C). ^{1}H NMR (300 MHz, acetone) δ 8.26 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 2H), 4.55 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 148.48, 146.63, 131.02, 124.64, 102.75, 31.48.

*2,4-Dinitrobenzyl selenocyanate (**11**)*: 2,4-Dinitrobenzyl chloride was used as a substrate. The reaction mixture was heated in THF at 60 °C for 10 h. Yield: 90% as a yellowish solid; mp 69-70 °C (lit.[4] mp 70-72 °C) . ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.06 (d, *J* = 2.4 Hz, 1H), 8.55 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 4.51 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.01, 146.60, 139.55, 133.32, 128.70, 121.70, 101.46, 29.29.

*3,5-Dinitrobenzyl selenocyanate (**12**)*: 3,5-Dinitrobenzyl chloride was used as a substrate. The reaction mixture was heated in DMF at 80 °C for 5 h. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 10:1). Yield: 82% as a yellow solid; mp 128-129 °C. ^{1}H NMR (300 MHz, acetone) δ 8.90 (t, *J* = 2.1 Hz, 1H), 8.77 (d, *J* = 2.1 Hz, 2H), 4.75 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 149.54, 143.87, 130.02, 118.85, 102.67, 30.54. Anal. Calcd for $C_8H_5N_3O_4Se$: C, 33.58; H, 1.76; N, 14.64. Found: C, 33.62; H, 1.97; N, 14.55.

*4-Methoxybenzyl selenocyanate (**13**)*: 4-Methoxybenzyl chloride was used as a substrate. The reaction mixture was stirred in THF at rt for 10 h. Yield: 97% as a brownish solid; mp 53-55 °C (lit.[5] mp 54-55 °C). ^{1}H NMR (300 MHz, acetone) δ 7.37 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 4.41 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, acetone) δ 160.59, 131.14, 130.22, 114.96, 103.20, 55.55, 33.19.

Synthesis of 5-[3,5-(dinitrobenzyl)selanyl]-1*H*-tetrazole (14):

3,5-Dinitrobenzyl selenocyanate **12** (0.286 g, 1 mmol) was added to a suspension of sodium azide (0.065 g, 1 mmol) and triethylammonium chloride (0.138 g, 1 mmol) in toluene (7 mL). The reaction mixture was stirred at 50 °C for 10 h. The reaction mixture was cooled to rt and extracted with 1% aqueous NaOH (15 mL). The aqueous layer then was washed with EtOAc (2 × 15 mL) and carefully acidified by HCl to pH 5.5-6, and the product was filtered and washed with water. Rapid acidification of the aqueous phase or acidification to a lower pH led to precipitation of selenium. Yield: 46% as white solid. ^{1}H NMR (500 MHz, acetone) δ 8.80 – 8.77 (m, 3H), 4.85 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 149.28, 144.96, 130.23, 118.14, 29.33. Anal. Calcd for $C_8H_6N_6O_4Se$: C, 29.19; H, 1.84; N, 25.53. Found: C, 29.55; H, 2.14; N, 25.49.

General procedure for the preparation of 1-alkyl-5-(alkylselanyl)-1*H*-tetrazoles (15-19) and 2-alkyl-5-(alkylselanyl)-2*H*-tetrazoles (20-24).

Alkyl selenocyanate **9**, **10**, **12** or **13** (1 mmol) was added to a suspension of sodium azide (1 mmol) and triethylammonium chloride (1 mmol) in toluene (7 mL). The reaction mixture was stirred at 80-90 °C for 2-6 h until completion, as determined by TLC. The reaction mixture was cooled to rt and extracted with 1% aqueous NaOH (15 mL). The aqueous layer then was

washed with EtOAc (2 × 15 mL). Alkyl halide (0.75 mmol) and TBAB (0.038 mmol) in CH_2Cl_2 (15 mL) were added to the aqueous phase. The reaction mixture was stirred at rt for 48 h. Subsequently, the organic layer was separated, washed with water (2 × 15 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the products, 1- and 2-isomers, were separated by silica gel column chromatography using the mobile phase hexane/EtOAc.

When 2,4-dinitrobenzyl selenocyanate **11** was used as a substrate, the reaction with sodium azide and triethylammonium chloride was stirred in THF at rt overnight and then THF was evaporated. Subsequently, the residue was dissolved in 1% aqueous NaOH (15 mL), and the procedure continued as described previously.

Dimethyl sulfate, benzyl bromide, 4-nitrobenzyl iodide, 3,5-dinitrobenzyl iodide and 2,4-dinitrobenzyl iodide were used as alkylating agents.

1-Alkyl-5-(alkylselanyl)-1*H*-tetrazoles (15-19):

*5-(Benzylselanyl)-1-(3,5-dinitrobenzyl)-1H-tetrazole (**15d**)*: Yield: 32% as a yellowish solid; mp 111-112 °C. ^{1}H NMR (500 MHz, acetone) δ 8.88 (t, *J* = 2.1 Hz, 1H), 8.54 (d, *J* = 2.1 Hz, 2H), 7.27 – 7.24 (m, 2H), 7.19 – 7.12 (m, 3H), 5.87 (s, 2H), 4.54 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 149.54, 147.66, 139.12, 138.80, 129.59, 129.57, 129.32, 128.35, 119.57, 50.40, 33.39. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 43.28; H, 2.92; N, 19.69.

*5-(Benzylselanyl)-1-(2,4-dinitrobenzyl)-1H-tetrazole (**15e**)*: Yield: 12% as a beige solid; mp 78-82 °C. ^{1}H NMR (500 MHz, acetone) δ 8.91 (d, *J* = 2.4 Hz, 1H), 8.44 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.34 – 7.32 (m, 2H), 7.30 – 7.23 (m, 3H), 7.11 (d, *J* = 8.6 Hz, 1H), 6.03 (s, 2H), 4.58 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 148.77, 148.75, 148.32, 138.87, 136.61, 132.10, 129.77, 129.48, 129.02, 128.49, 121.50, 48.98, 33.34. HRMS (ESI+) m/z calcd for $C_{15}H_{13}N_6O_4Se^+$:

421.01580 (100.0%), 419.01659 (47.9%), 417.01849 (18.9%), 423.01598 (17.6%); found 421.0141 (100%), 419.0155 (50%), 417.0173 (19%), 423.0139 (18%).

*5-[(4-Nitrobenzyl)selanyl]-1-(4-nitrobenzyl)-1H-tetrazole (**16c**)*: Yield: 16% as a yellowish solid; mp 132-133 °C (with decomposition). ^{1}H NMR (300 MHz, acetone) δ 8.18 (d, *J* = 8.8 Hz, 2H), 8.09 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 5.74 (s, 2H), 4.68 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 148.84, 148.06, 147.30, 147.16, 142.02, 130.96, 129.91, 124.70, 124.34, 51.12, 31.69. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 42.68; H, 3.05; N, 19.73.

*1-(3,5-Dinitrobenzyl)-5-[(4-nitrobenzyl)selanyl]-1H-tetrazole (**16d**)*: Yield: 30% as a light beige solid; mp 135-136 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.87 (t, *J* = 2.1 Hz, 1H), 8.56 (d, *J* = 2.1 Hz, 2H), 8.06 (d, *J* = 8.7 Hz, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 5.94 (s, 2H), 4.69 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 149.53, 148.00, 147.46, 147.19, 139.06, 130.88, 129.62, 124.31, 119.53, 50.47, 31.85. Anal. Calcd for $C_{15}H_{11}N_7O_6Se$: C, 38.81; H, 2.39; N, 21.12. Found: C, 38.84; H, 2.94; N, 20.59.

*1-(2,4-Dinitrobenzyl)-5-[(4-nitrobenzyl)selanyl]-1H-tetrazole (**16e**)*: Yield: 20% as an amorphous yellow solid. ^{1}H NMR (500 MHz, acetone) δ 8.91 (d, *J* = 2.4 Hz, 1H), 8.47 (dd, *J* = 8.6, 2.4 Hz, 1H), 8.13 (d, *J* = 8.7 Hz, 2H), 7.65 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.6 Hz, 1H), 6.09 (s, 2H), 4.71 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 148.83, 148.10, 148.05, 147.18, 136.42, 132.44, 131.02, 128.98, 124.41, 121.52, 49.13, 31.79. Anal. Calcd for $C_{15}H_{11}N_7O_6Se$: C, 38.81; H, 2.39; N, 21.12. Found: C, 38.87; H, 2.54; N, 21.43. HRMS (ESI+) m/z calcd for $C_{15}H_{12}N_7O_6Se^{+}$: 466.00088 (100.0%), 464.00167 (47.9%), 462.00357 (18.9%), 468.00106 (17.6%); found: 466.0009 (100%), 464.0021 (49%), 462.0037 (19%), 468.0010 (18%).

5-*[(2,4-Dinitrobenzyl)selanyl]-1-methyl-1H-tetrazole (**17a**)*: Yield: 16% as a yellow solid; mp 88-89 °C. ^{1}H NMR (500 MHz, acetone) δ 8.87 (d, *J* = 2.4 Hz, 1H), 8.53 (dd, *J* = 8.6, 2.4 Hz, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 4.91 (s, 2H), 3.98 (s, 3H); ^{13}C NMR (126 MHz, acetone) δ

148.37, 148.28, 147.14, 142.27, 134.82, 128.86, 121.57, 34.67, 28.89. Anal. Calcd for $C_9H_8N_6O_4Se$: C, 31.50; H, 2.35; N, 24.49. Found: C, 31.63; H, 2.69; N, 24.78.

*1-Benzyl-5-[(2,4-dinitrobenzyl)selanyl]-1H-tetrazole (**17b**)*: Yield: 13% as a light beige solid; mp 159-160 °C. ^{1}H NMR (500 MHz, acetone) δ 8.82 (d, *J* = 2.4 Hz, 1H), 8.46 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.35 – 7.30 (m, 3H), 7.25 – 7.23 (m, 2H), 5.57 (s, 2H), 4.89 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 148.23, 147.14, 142.15, 134.84, 134.69, 129.77, 129.46, 128.89, 128.83, 121.61, 52.13, 29.26. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 43.28; H, 3.18; N, 19.81.

*1-[(2,4-Dinitrobenzyl)selanyl]-1-(4-nitrobenzyl)-1H-tetrazole (**17c**):* Yield: 7% as an oil. ^{1}H NMR (300 MHz, acetone) δ 8.81 (d, *J* = 2.4 Hz, 1H), 8.48 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.20 (d, *J* = 8.7 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.51 (d, *J* = 8.7 Hz, 2H), 5.79 (s, 2H), 4.91 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 148.87, 148.23 (2C), 147.54, 142.01, 141.90, 134.76, 130.02, 128.85, 124.75, 121.55, 51.21, 29.44. HRMS (ESI+) m/z calcd for $C_{15}H_{12}N_7O_6Se^+$: 466.00088 (100.0%), 464.00167 (47.9%), 462.00357 (18.9%), 468.00106 (17.6%); found 466.0011 (100%), 464.0018 (47%), 462.0038 (19%), 468.0013 (18%).

*5-[(2,4-Dinitrobenzyl)selanyl]-1-(3,5-dinitrobenzyl)-1H-tetrazole (**17d**)*: Yield: 10% as an amorphous yellow solid. ^{1}H NMR (300 MHz, acetone) δ 8.89 (t, *J* = 2.1 Hz, 1H), 8.82 (d, *J* = 2.4 Hz, 1H), 8.60 (d, *J* = 2.1 Hz, 2H), 8.48 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 6.00 (s, 2H), 4.92 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 149.63, 148.30 (2C), 147.79, 142.05, 139.03, 134.82, 129.69, 128.91, 121.55, 119.63, 50.59, 29.58. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.66; H, 1.87; N, 22.04. HRMS (ESI+) m/z calcd for $C_{15}H_{11}N_8O_8Se^+$: 510.98596 (100.0%), 508.98674 (47.9%), 506.98865 (18.9%), 512.98613 (17.6%); found 510.9857 (100%), 508.9868 (49%), 506.9886 (19%), 512.9860 (19%).

*1-(2,4-Dinitrobenzyl)-5-[(2,4-dinitrobenyl)selanyl]-1H-tetrazole (**17e**):* Yield: 6% as an amorphous yellow solid. ^{1}H NMR (500 MHz, acetone) δ 8.92 (d, *J* = 2.4 Hz, 1H), 8.85 (d, *J* = 2.5 Hz, 1H), 8.55 – 8.51 (m, 2H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 6.14 (s, 2H), 4.95 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 148.92, 148.84, 148.36, 148.31, 142.03, 136.37, 134.88, 132.60, 129.07, 128.94, 121.60 (2c), 49.39, 29.54. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.55; H, 1.98; N, 21.72. HRMS (ESI+) m/z calcd for $C_{15}H_{11}N_8O_8Se^+$: 510.98596 (100.0%), 508.98674 (47.9%), 506.98865 (18.9%), 512.98613 (17.6%); found 510.9854 (100%), 508.9867 (48%), 506.9883 (18%), 512.9857 (18%).

*5-[(3,5-Dinitrobenzyl)selanyl]-1-methyl-1H-tetrazole (**18a**)*: Yield: 15% as a light grey solid; mp 118-119 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (t, *J* = 2.1 Hz, 1H), 8.70 (d, *J* = 2.1 Hz, 2H), 4.76 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.54, 145.62, 141.90, 129.39, 118.19, 34.12, 29.67. Anal. Calcd for $C_9H_8N_6O_4Se$: C, 31.50; H, 2.35; N, 24.49. Found: C, 31.42; H, 2.54; N, 24.74.

*1-Benzyl-5-[(3,5-dinitrobenzyl)selanyl]-1H-tetrazole (**18b**)*:[17] Yield: 12% as a light beige solid; mp 154-156 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.75 (t, *J* = 2.1 Hz, 1H), 8.68 (d, *J* = 2.1 Hz, 2H), 7.35 – 7.29 (m, 3H), 7.22 – 7.19 (m, 2H), 5.57 (s, 2H), 4.81 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 149.21, 146.86, 144.55, 134.90, 130.16, 129.72, 129.45, 128.81, 118.26, 52.09, 30.51. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 43.05; H, 2.80; N, 20.15.

*5-[(3,5-Dinitrobenzyl)selanyl]-1-(4-nitrobenzyl)-1H-tetrazole (**18c**):* Yield: 15% as a beige solid; mp 156-158 °C. ^{1}H NMR (500 MHz, acetone) δ 8.75 (t, *J* = 2.1 Hz, 1H), 8.69 (d, *J* = 2.1 Hz, 2H), 8.19 (d, *J* = 8.7 Hz, 2H), 7.49 (d, *J* = 8.7 Hz, 2H), 5.79 (s, 2H), 4.85 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 148.34, 148.01, 146.45, 143.66, 141.13, 129.34, 129.10, 123.87, 117.39, 50.34, 29.86. Anal. Calcd for $C_{15}H_{11}N_7O_6Se$: C, 38.81; H, 2.39; N, 21.12. Found: C,

39.1; H, 2.71; N, 20.8. HRMS (ESI+) m/z calcd for $C_{15}H_{12}N_7O_6Se^+$: 466.00088 (100.0%), 464.00167 (47.9%), 462.00357 (18.9%), 468.00106 (17.6%); found 466.0011 (100%), 464.0019 (46%), 462.0036 (18%), 468.0012 (18%).

*1-(3,5-Dinitrobenzyl)-5-[(3,5-dinitrobenzyl)selanyl]-1H-tetrazole (**18d**):* Yield: 12% as a beige solid; mp 140-143 °C. ^{1}H NMR (300 MHz, acetone) δ 8.89 (t, *J* = 2.1 Hz, 1H), 8.77 – 8.72 (m, 4H), 8.59 (d, *J* = 2.1 Hz, 1H), 5.99 (s, 2H), 4.88 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 149.59, 149.24, 147.54, 144.53, 139.00, 130.25, 129.64, 119.59, 118.26, 50.52, 30.77. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.74; H, 2.66; N, 21.51.

*1-(2,4-Dinitrobenzyl)-5-[(3,5-Dinitrobenzyl)selanyl]-1H-tetrazole (**18e**):* Yield: 12% as a beige solid; mp 137-138 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.91 (d, *J* = 2.4 Hz, 1H), 8.80 (t, *J* = 2.1 Hz, 1H), 8.75 (d, *J* = 2.1 Hz, 2H), 8.51 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 6.12 (s, 2H), 4.89 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 149.27, 148.93, 148.87, 148.00, 144.51, 136.30, 132.77, 130.31, 129.02, 121.55, 118.31, 49.29, 30.81. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.67; H, 2.26; N, 21.63.

*1-(3,5-dinitrobenzyl)-5-[(4-methoxybenzyl)selanyl]-1H-tetrazole (**19d**)*: Yield: 12% yield as a yellowish solid; mp 121-123 °C. ^{1}H NMR (500 MHz, acetone) δ 8.88 (t, *J* = 2.1 Hz, 1H), 8.54 (d, *J* = 2.1 Hz, 2H), 7.14 (d, *J* = 8.7 Hz, 2H), 6.69 (d, *J* = 8.7 Hz, 2H), 5.87 (s, 2H), 4.50 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (126 MHz, acetone) δ 160.04, 149.49, 147.74, 139.13, 130.79, 130.45, 129.57, 119.46, 114.60, 55.43, 50.38, 33.36. Anal. Calcd for $C_{16}H_{14}N_6O_5Se$: C, 42.77; H, 3.14; N, 18.71. Found: C, 42.72; H, 3.34; N, 18.48.

*1-(2,4-dinitrobenzyl)-5-[(4-methoxybenzyl)selanyl]-1H-tetrazole (**19e**)*: Yield: 11% as a yellow solid; 122-124 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.91 (d, *J* = 2.4 Hz, 1H), 8.42 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.22 (d, *J* = 8.6 Hz, 2H), 7.03 (d, *J* = 8.5 Hz, 1H),

6.79 (d, *J* = 8.6 Hz, 2H), 6.02 (s, 2H), 4.53 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (126 MHz, acetone) δ 160.22, 148.67, 148.38, 136.64, 131.97, 130.97, 130.53, 128.92, 121.46, 114.80, 55.51, 48.90, 33.32. Anal. Calcd for $C_{16}H_{14}N_6O_5Se$: C, 42.77; H, 3.14; N, 18.71. Found: C, 42.72; H, 3.43; N, 18.57.

2-Alkyl-5-(alkylselanyl)-2*H*-tetrazoles (20-24):

*5-(Benzylselanyl)-2-(3,5-dinitrobenzyl)-2H-tetrazole (**20d**)*: Yield: 34% as a yellow solid; mp 102-103 °C. ^{1}H NMR (500 MHz, acetone) δ 8.97 (t, *J* = 2.1 Hz, 1H), 8.78 (d, *J* = 2.1 Hz, 2H), 7.44 – 7.31 (m, 2H), 7.27 – 7.06 (m, 3H), 6.31 (s, 2H), 4.47 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 157.68, 149.68, 139.27, 138.74, 130.17, 129.76, 129.23, 128.04, 119.80, 55.55, 30.53. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 42.92; H, 3.17; N, 19.79.

*5-(Benzylselanyl)-2-(2,4-dinitrobenzyl)-2H-tetrazole (**20e**):* Yield: 15% as a beige solid; mp 75-77 °C. ^{1}H NMR (500 MHz, acetone) δ 8.97 (d, *J* = 2.4 Hz, 1H), 8.60 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.39 – 7.32 (m, 2H), 7.28 – 7.19 (m, 3H), 6.48 (s, 2H), 4.48 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 157.60, 149.11, 149.03, 139.26, 136.04, 133.59, 129.79, 129.29, 129.07, 128.08, 121.50, 54.19, 30.58. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 43.17; H, 3.39; N, 19.74.

*2-(4-Nitrobenzyl)-5-[(4-nitrobenzyl)selanyl]-2H-tetrazole (**21c**)*: Yield: 28% as a white solid; 132-133 °C. ^{1}H NMR (500 MHz, acetone) δ 8.28 (d, *J* = 8.7 Hz, 2H), 8.06 (d, *J* = 8.7 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.62 (d, *J* = 8.7 Hz, 2H), 6.10 (s, 2H), 4.58 (s, 2H). ^{13}C NMR (126 MHz, acetone) δ 156.75, 149.11, 147.85, 141.79, 130.98, 130.56, 124.77, 124.20, 56.41, 29.33. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 42.96; H, 3.07; N, 19.99. HRMS (ESI+) m/z calcd for $C_{15}H_{13}N_6O_4Se^+$: 421.01580 (100.0%), 419.01659 (47.9%), 417.01849 (18.9%), 423.01598 (17.6%); found 421.0156 (100%), 419.0164 (48%), 417.0184 (19%), 423.0155 (18%).

*2-(3,5-Dinitrobenzyl)-5-[(4-nitrobenzyl)selanyl]-2H-tetrazole (**21d**)*: Yield: 31% as a beige solid; mp 110-112 °C. ^{1}H NMR (500 MHz, acetone) δ 8.96 (t, *J* = 2.1 Hz, 1H), 8.77 (d, *J* = 2.1 Hz, 2H), 8.07 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 8.8 Hz, 2H), 6.32 (s, 2H), 4.59 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 157.08, 149.66, 147.85, 138.64, 130.98, 130.16, 124.20, 119.81, 55.62, 29.30. Anal. Calcd for $C_{15}H_{11}N_7O_6Se$: C, 38.81; H, 2.39; N, 21.12. Found: C, 38.81; H, 2.61; N, 21.25.

*2-(2,4-Dinitrobenzyl)-5-[(4-nitrobenzyl)selanyl]-2H-tetrazole (**21e**)*: Yield: 37% as an oil. ^{1}H NMR (500 MHz, acetone) δ 8.96 (d, *J* = 2.4 Hz, 1H), 8.61 (dd, *J* = 8.6, 2.4 Hz, 1H), 8.09 (d, *J* = 8.8 Hz, 2H), 7.64 – 7.61 (m, 3H), 6.47 (s, 2H), 4.58 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 156.91, 149.17, 149.13, 147.87, 147.83, 135.76, 133.86, 130.98, 129.07, 124.24, 121.51, 54.26, 29.37. HRMS (ESI+) m/z calcd for $C_{15}H_{12}N_7O_6Se^{+}$: 466.00088 (100.0%), 464.00167 (47.9%), 462.00357 (18.9%), 468.00106 (17.6%); found 466.0004 (100%), 464.0015 (50%), 462.0032 (19%), 468.0005 (18%).

*5-[(2,4-Dinitrobenzyl)selanyl]-2-methyl-2H-tetrazole (**22a**)*: Yield: 30% as a yellow solid; mp 91-93 °C. ^{1}H NMR (500 MHz, acetone) δ 8.84 (d, *J* = 2.5 Hz, 1H), 8.47 (dd, *J* = 8.5, 2.5 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 4.83 (s, 2H), 4.39 (s, 3H); ^{13}C NMR (126 MHz, acetone) δ 155.86, 148.58, 148.02, 142.97, 134.79, 128.55, 121.48, 40.09, 26.66. Anal. Calcd for $C_9H_8N_6O_4Se$: C, 31.50; H, 2.35; N, 24.49. Found: C, 31.58; H, 2.57; N, 24.3.

*2-Benzyl-5-[(2,4-dinitrobenzyl)selanyl]-2H-tetrazole (**22b**)*: Yield: 24% as an oil. ^{1}H NMR (500 MHz, acetone) δ 8.81 (d, *J* = 2.4 Hz, 1H), 8.33 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.44 – 7.39 (m, 5H), 5.90 (s, 2H), 4.80 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 156.24, 148.50, 147.95, 142.80, 134.78, 134.68, 129.74, 129.66, 129.45, 128.38, 121.51, 57.52, 26.81. HRMS (ESI+) m/z calcd for $C_{15}H_{13}N_6O_4Se^{+}$: 421.01580 (100.0%), 419.01659 (47.9%), 417.01849 (18.9%), 423.01598 (17.6%); found 421.0149 (100%), 419.0162 (49%), 417.0181 (19%), 423.0147 (18%).

*5-[(2,4-Dinitrobenzyl)selanyl]-2-(4-nitrobenzyl)-2H-tetrazole (**22c**):* Yield: 11% as an oil. ^{1}H NMR (300 MHz, acetone) δ 8.81 (d, *J* = 2.4 Hz, 1H), 8.39 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.28 (d, *J* = 8.7 Hz, 2H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.70 (d, *J* = 8.7 Hz, 2H), 6.12 (s, 2H), 4.83 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 156.73, 149.09, 148.58, 148.00, 142.76, 141.70, 134.77, 130.59, 128.45, 124.78, 121.50, 56.47, 26.84. HRMS (ESI+) m/z calcd for $C_{15}H_{12}N_7O_6Se^+$: 466.00088 (100.0%), 464.00167 (47.9%), 462.00357 (18.9%), 468.00106 (17.6%); found 466.0006 (100%), 464.0019 (52%), 462.0035 (19%), 468.0007 (19%).

*5-[(2,4-Dinitrobenzyl)selanyl]-2-(3,5-dinitrobenzyl)-2H-tetrazole (**22d**)*: Yield: 22% as a yellow solid; mp 142-144 °C. ^{1}H NMR (300 MHz, acetone) δ 8.97 (t, *J* = 2.1 Hz, 1H), 8.81 (d, *J* = 2.4 Hz, 1H), 8.79 (d, *J* = 2.1 Hz, 2H), 8.42 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 6.33 (s, 2H), 4.84 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 157.07, 149.69, 148.61, 148.00, 142.78, 138.56, 134.81, 130.21, 128.49, 121.47, 119.84, 55.70, 26.83. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.49; H, 2.14; N, 21.81.

*2-(2,4-Dinitrobenzyl)-5-[(2,4-dinitrobenyl)selanyl]-2H-tetrazole (**22e**):* Yield: 23% as a yellow solid; mp 94-97 °C. ^{1}H NMR (500 MHz, acetone) δ 8.97 (d, *J* = 2.4 Hz, 1H), 8.81 (d, *J* = 2.4 Hz, 1H), 8.62 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.42 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.64 (d, *J* = 8.5 Hz, 1H), 6.49 (s, 2H), 4.83 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 156.92, 149.19, 149.11, 148.55, 148.03, 142.70, 135.74, 134.77, 133.85, 129.11, 128.52, 121.53, 121.52, 54.36, 26.96. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.56; H, 2.16; N, 21.65.

*5-[(3,5-Dinitrobenzyl)selanyl]-2-methyl-2H-tetrazole (**23a**):* Yield: 23% as a beige solid; mp 102-103 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.89 (t, *J* = 2.1 Hz, 1H), 8.60 (d, *J* = 2.1 Hz, 2H), 4.54 (s, 2H), 4.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.74, 148.40, 143.24, 129.21, 117.67, 39.78, 27.70. Anal. Calcd for $C_9H_8N_6O_4Se$: C, 31.50; H, 2.35; N, 24.49. Found: C, 31.61; H, 2.51; N, 24.74.

*2-Benzyl-5-[(3,5-dinitrobenzyl)selanyl]-2H-tetrazole (**23b**)*: Yield: 20% as a beige solid; mp 96-97 °C. ^{1}H NMR (300 MHz, acetone) δ 8.77 – 8.69 (m, 3H), 7.36 (s, 5H), 5.87 (s, 2H), 4.74 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 156.18, 149.19, 145.24, 134.74, 130.19, 129.70, 129.28, 128.92, 117.96, 57.50, 28.39. Anal. Calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05. Found: C, 43.2; H, 3.0; N, 19.82.

*5-[(3,5-Dinitrobenzyl)selanyl]-2-(4-nitrobenzyl)-2H-tetrazole (**23c**):* Yield: 44% as a light grey solid; mp 101-103 °C. ^{1}H NMR (300 MHz, acetone) δ 8.74 (t, *J* = 2.1 Hz, 1H), 8.70 (d, *J* = 2.1 Hz, 2H), 8.24 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 8.6 Hz, 2H), 6.10 (s, 2H), 4.75 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 156.72, 149.22, 149.09, 145.24, 141.72, 130.46, 130.22, 124.79, 118.01, 56.50, 28.52. Anal. Calcd for $C_{15}H_{11}N_7O_6Se$: C, 38.81; H, 2.39; N, 21.12. Found: C, 39.16; H, 2.35; N, 21.25.

*2-(3,5-Dinitrobenzyl)-5-[(3,5-dinitrobenzyl)selanyl]-2H-tetrazole (**23d**):* Yield: 31% as a beige solid; mp 162-164 °C. ^{1}H NMR (300 MHz, acetone) δ 8.96 (t, *J* = 2.1 Hz, 1H), 8.77 – 8.69 (m, 5H), 6.32 (s, 2H), 4.76 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 155.68, 149.69, 149.21, 145.22, 138.55, 130.17, 130.10, 119.82, 117.96, 55.71, 28.48. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.68; H, 2.34; N, 22.18.

*2-(2,4-Dinitrobenzyl)-5-[(3,5-dinitrobenzyl)selanyl]-2H-tetrazole (**23e**)*: Yield: 35% as a white solid; mp 124-125 °C. ^{1}H NMR (500 MHz, acetone) δ 8.95 (d, *J* = 2.4 Hz, 1H), 8.75 (t, *J* = 2.1 Hz, 1H), 8.70 (d, *J* = 2.1 Hz, 2H), 8.57 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 6.47 (s, 2H), 4.75 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 156.82, 149.19, 149.16, 145.14, 135.69, 133.73, 130.19, 129.5, 121.5, 117.99, 54.34, 28.52. Anal. Calcd for $C_{15}H_{10}N_8O_8Se$: C, 35.38; H, 1.98; N; 22.0. Found: C, 35.53; H, 2.25; N, 21.84.

*2-(3,5-Dinitrobenzyl)-5-[(4-methoxybenzyl)selanyl]-2H-tetrazole (**24d**):* Yield: 18% as a yellow solid; mp 93-97 °C. ^{1}H NMR (300 MHz, acetone) δ 8.97 (t, *J* = 2.1 Hz, 1H), 8.78 (d, *J* = 2.1 Hz, 2H), 7.27 (d, *J* = 8.7 Hz, 2H), 6.75 (d, *J* = 8.7 Hz, 2H), 6.31 (s, 2H), 4.43 (s, 2H),

3.72 (s, 3H); ^{13}C NMR (75 MHz, acetone) δ 159.88, 157.84, 149.67, 138.77, 130.96, 130.86, 130.16, 119.79, 114.58, 55.52, 55.42, 30.28. Anal. Calcd for $C_{16}H_{14}N_6O_5Se$: C, 42.77; H, 3.14; N, 18.71. Found: C, 43.1; H, 3.46; N, 18.78.

*2-(2,4-dinitrobenzyl)-5-[(4-methoxybenzyl)selanyl]-2H-tetrazole (**24e**):* Yield: 5% as an oil. ^{1}H NMR (500 MHz, acetone) δ 8.97 (d, *J* = 2.4 Hz, 1H), 8.60 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.47 (s, 2H), 4.44 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (126 MHz, acetone) 159.90, 157.70, 149.09, 149.05, 136.11, 133.49, 131.01, 130.87, 129.06, 121.49, 114.64, 55.46, 54.15, 30.36. HRMS (ESI+) m/z calcd for $C_{16}H_{15}N_6O_5Se^+$: 451.02636 (100.0%), 449.02715 (47.9%), 447.02906 (18.9%), 453.02654 (17.6%); found 451.0265 (100%), 449.0273 (48%), 447.0293 (19%), 453.0266 (18%).

Synthesis of alkyl thiocyanates (25-28):

A suspension of alkyl halide (10 mmol) and potassium thiocyanate (11 mmol) in 7 mL DMF or THF was stirred until completion, as determined by TLC, under the conditions given below. The reaction mixture was then diluted with EtOAc (10 mL) and the organic layer was washed with 1% aqueous NaOH (2 × 10 mL) and H_2O (1 × 10 mL). The organic solvent was dried over Na_2SO_4. If necessary, the crude product was purified by column chromatography (Mobile phase: Hexane/EtOAc).

*Benzyl thiocyanate (**25**)*:[6] Benzyl bromide was used as the starting material. The reaction mixture was stirred in DMF at 100 °C for 1 h. Yield: 95% as a white solid; mp 40-41 °C (lit.[7] mp 39-40 °C). ^{1}H NMR (300 MHz, DMSO) δ 7.48 – 7.28 (m, 5H), 4.35 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 136.33, 129.21, 128.93, 128.46, 113.15, 37.18.

*4-Nitrobenzyl thiocyanate (**26**)*:[8] 4-Nitrobenzyl chloride was used as a substrate. The reaction mixture was heated in DMF at 100 °C for 6 h. Yield: 96% as a yellowish solid; mp 79-81 °C (lit.[9] mp 85-86 °C). ^{1}H NMR (300 MHz, DMSO) δ 8.26 (d, *J* = 8.7 Hz, 2H), 7.69 (d, *J* = 8.7

Hz, 2H), 4.48 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 147.44, 144.24, 130.56, 124.11, 112.82, 35.94.

*3,5-Dinitrobenzyl thiocyanate (**27**)*: 3,5-Dinitrobenzyl chloride was used as a substrate. The reaction mixture was heated in DMF at 100 °C for 3 h. The product was purified by column chromatography (Mobile phase: Hexane/EtOAc, 7:1). Yield: 82% as a white solid; mp 120-122 °C (lit.[10] mp 120-121 °C). ^{1}H NMR (500 MHz, acetone) δ 8.95 (t, *J* = 2.1 Hz, 1H), 8.82 (d, *J* = 2.1 Hz, 2H), 4.74 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 149.66, 141.82, 130.29, 119.37, 111.87, 36.31. Anal. Calcd for $C_8H_5N_3O_4S$: C, 40.17; H, 2.11; N, 17.57; S, 13.40. Found: C, 40.15; H, 2.06; N, 17.42; S, 13.53.

*2,4-Dinitrobenzyl thiocyanate (**28**)*: 2,4-Dinitrobenzyl chloride was used as the starting material. The reaction mixture was refluxed in THF for 10 h. Yield: 75% as a yellowish solid; mp 84-86 °C (lit.[8] mp 86-87 °C). ^{1}H NMR (300 MHz, DMSO) δ 8.82 (d, *J* = 2.4 Hz, 1H), 8.66 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 4.74 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 147.71, 147.62, 137.98, 134.32, 128.64, 121.11, 112.39, 33.83.

General procedure for the synthesis of 5-(alkylsulfanyl)-1*H*-tetrazoles (29-31):

Acetic acid (0.39 g, 6.5 mmol) was added to a suspension of alkyl thiocyanate **25-27** (5 mmol) and sodium azide (0.42 g, 6.5 mmol) in *n*-butanol (15 mL). The reaction mixture was heated at 100 °C for 4-8 h. Subsequently, the solvent was evaporated under reduced pressure. The obtained solid was dissolved in 3% NaOH (10 mL) and washed with EtOAc (2 × 15 mL). The aqueous layer was acidified to pH 2-3, and the product was filtered and washed with water.

*5-(Benzylsulfanyl)-1H-tetrazole (**29**)*:[11] Yield: 50% as a white solid; mp 138-139 °C (lit.[12] mp 134-136 °C). ^{1}H NMR (300 MHz, DMSO) δ 7.44 – 7.20 (m, 5H), 4.50 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 153.88, 136.85, 129.09, 128.73, 127.82, 36.17.

*5-[(4-Nitrobenzyl)sulfanyl]-1H-tetrazole (**30**)*: Yield: 51% as a yellow solid; mp 147-148 °C. ^{1}H NMR (300 MHz, DMSO) δ 8.16 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 8.7 Hz, 2H), 4.62 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 153.74, 146.97, 145.35, 130.37, 123.80, 35.21. Anal. Calcd for $C_8H_7N_5O_2S$: C, 40.5; H, 2.97; N, 29.52; S, 13.52. Found: C, 40.66; H, 3.23; N, 29.81; S, 13.85.

*5-[(3,5-Dinitrobenzyl)sulfanyl]-1H-tetrazole (**31**)*: Yield: 68% as a white solid; mp 151-152 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.75 (t, *J* = 2.1 Hz, 1H), 8.71 (d, *J* = 2.1 Hz, 2H), 4.75 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 148.09, 142.46, 129.7, 126.96, 117.92, 34.39. Anal. Calcd for $C_8H_6N_6O_4S$: C, 34.04; H, 2.14; N, 29.78; S, 11.36. Found: C, 34.06; H, 2.1; N, 29.46; S, 11.34.

*Synthesis of 5-[(2,4-dinitrobenzyl)sulfanyl]-1H-tetrazole (**32**)*

2,4-Dinitrobenzyl thiocyanate **28** (0.72 g, 3 mmol) was added to a suspension of sodium azide (0.23 g, 3.6 mmol) and triethylammonium chloride (0.5 g, 3.6 mmol) in THF. The reaction mixture was stirred at rt overnight. Subsequently, the solvent was evaporated under reduced pressure. The crude product was dissolved in 1% NaOH (10 mL), washed with EtOAc (2 × 15 mL) and the aqueous layer was acidified to pH 1-2. The product was filtered and washed with water. Yield: 80% as a yellowish solid; mp 167-168 °C. ^{1}H NMR (300 MHz, DMSO) δ 8.75 (d, *J* = 2.4 Hz, 1H), 8.52 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 4.87 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 153.80, 148.10, 147.15, 139.47, 134.11, 128.15, 120.72, 33.19. Anal. Calcd for $C_8H_6N_6O_4S$: C, 34.04; H, 2.14; N, 29.78; S, 11.36. Found: C, 34.28; H, 2.31; N, 29.66; S, 11.68.

General procedure for the preparation of 1-alkyl-5-(alkylsulfanyl)-1*H*-tetrazoles (33-36) and 2-alkyl-5-(alkylsulfanyl)-2*H*-tetrazoles (37-40).

An alkylating agent (1 mmol) was added to a solution of 5-(alkylsulfanyl)-1*H*-tetrazole **29-32** (1.1 mmol) and KOH (1.2 mmol) in 7 mL of THF (for 2,4-dinitrobenzyl derivatives) or DMF (for other derivatives). The reaction mixture was heated at 75 °C for 2-10 h until completion, as determined by TLC. The solvent was evaporated under reduced pressure and the resulting mass was dissolved in EtOAc (20 mL), washed with 1% NaOH (2 × 20 mL) and water (1 × 20 mL) and dried over sodium sulfate Na_2SO_4. The solvent was evaporated and the products, 1- and 2-isomers, were separated using silica gel column chromatography (Mobile phase: Hexane/EtOAc, 10:1 - 5:1).

Dimethyl sulfate, benzyl bromide, 4-nitrobenzyl iodide, 3,5-dinitrobenzyl iodide, 4-chlorobenzyl chloride and 3,4-dichlorobenzyl chloride were used as alkylating agents.

1-Alkyl-5-(alkylsulfanyl)-1*H*-tetrazoles (33-36):

*5-(Benzylsulfanyl)-1-(3,5-dinitrobenzyl)-1H-tetrazole (**33d**):* The reaction mixture was heated for 2 h. Yield: 25% as a white solid; mp 112-113 °C.^{1}H NMR (300 MHz, acetone) δ 8.90 (t, *J* = 2.1 Hz, 1H), 8.56 (d, *J* = 2.1 Hz, 2H), 7.37 – 7.32 (m, 2H), 7.27 – 7.19 (m, 3H), 5.89 (s, 2H), 4.56 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 154.65, 149.60, 138.99, 137.32, 129.78, 129.54, 129.40, 128.66, 119.60, 49.80, 38.31. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.63; H, 3.38; N, 22.93; S, 8.75.

*1-(4-Nitrobenzyl)-5-[(4-nitrobenzyl)sulfanyl]-1H-tetrazole (**34c**):* The reaction mixture was heated for 4 h. Yield: 28% as a white solid; mp 126-127 °C. ^{1}H NMR (300 MHz, acetone) δ 8.21 (d, *J* = 8.7 Hz, 2H), 8.13 (d, *J* = 8.7 Hz, 2H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.49 (d, *J* = 8.7 Hz, 2H), 5.74 (s, 2H), 4.71 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 154.10, 148.87, 148.31, 145.41, 141.84, 131.10, 129.92, 124.74, 124.36, 50.54, 36.94. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.59; H, 3.59; N, 22.76; S, 8.69.

*5-[(4-Nitrobenzyl)sulfanyl]-1-(3,5-dinitrobenzyl)-1H-tetrazole (**34d**):* The reaction mixture was heated for 5 h. Yield: 27% as a white solid; mp 160-161 °C. ^{1}H NMR (300 MHz, acetone) δ 8.91 (t, *J* = 2.1 Hz, 1H), 8.59 (d, *J* = 2.1 Hz, 2H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 5.95 (s, 2H), 4.74 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 154.31, 149.63, 148.33, 145.45, 138.92, 131.10, 129.59, 124.36, 119.61, 49.92, 37.04. Anal. Calcd for $C_{15}H_{11}N_7O_6S$: C, 43.17; H, 2.66; N, 23.49; S, 7.68. Found: C, 42.9; H, 2.74; N, 23.14; S, 7.83.

*5-[(2,4-Dinitrobenzyl)sulfanyl]-1-methyl-1H-tetrazole (**35a**)*: The reaction mixture was heated for 4 h. Yield: 15% as a white solid; mp 111-112 °C .^{1}H NMR (300 MHz, acetone) δ 8.87 (d, *J* = 2.4 Hz, 1H), 8.55 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 5.00 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (75 MHz, acetone) δ 153.77, 149.00, 148.51, 140.28, 135.27, 128.63, 121.47, 34.77, 33.96. Anal. Calcd for $C_9H_8N_6O_4S$: C, 36.49; H, 2.72; N, 28.37; S, 10.82. Found: C, 36.72; H, 3.01; N, 27.99; S, 10.51.

*1-Benzyl-5-[(2,4-dinitrobenzyl)sulfanyl]-1H-tetrazole (**35b**):* The reaction mixture was heated for 7 h. Yield: 19% as a yellowish solid; mp 145-146 °C .^{1}H NMR (300 MHz, acetone) δ 8.82 (d, *J* = 2.4 Hz, 1H), 8.48 (dd, *J* = 8.6, 2.4 Hz, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.37 – 7.29 (m, 3H), 7.26 – 7.21 (m, 2H), 5.53 (s, 2H), 4.98 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 153.72, 148.85, 148.48, 140.14, 135.12, 134.71, 129.75, 129.42, 128.82, 128.60, 121.51, 51.51, 35.08. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.16; H, 3.34; N, 22.22; S, 8.48.

*5-[(2,4-Dinitrobenzyl)sulfanyl]-1-(4-nitrobenzyl)-1H-tetrazole (**35c**):* The reaction mixture was heated for 8 h. Yield: 10% as a brownish solid. ^{1}H NMR (300 MHz, acetone) δ 8.81 (d, *J* = 2.4 Hz, 1H), 8.50 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.20 (d, *J* = 8.9 Hz, 2H), 8.07 (d, *J* = 8.5 Hz, 1H), 7.50 (d, *J* = 8.9 Hz, 2H), 5.75 (s, 2H), 5.00 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 154.15, 148.89, 148.81, 148.48, 141.75, 140.01, 135.22, 129.95, 128.65, 124.76, 121.46, 50.61, 35.12. Anal. Calcd for $C_{15}H_{11}N_7O_6S$: C, 43.17; H, 2.66; N, 23.49; S, 7.68. Found: C,

43.6; H, 2.82; N, 23.73; S, 7.48. HRMS (ESI+) m/z calcd for $C_{15}H_{12}N_7O_6S^+$: 418.05643 (100.0%), 419.05978 (16.2%); found 418.0557 (100%), 419.0580 (17%).

*5-[(2,4-Dinitrobenzyl)sulfanyl]-1-(3,5-dinitrobenzyl)-1H-tetrazole (**35d**):* The reaction mixture was heated for 10 h. The product was purified by preparative TLC. Yield: 16% as a brownish solid. ^{1}H NMR (300 MHz, acetone) δ 8.91 (t, *J* = 2.0 Hz, 1H), 8.81 (d, *J* = 2.4 Hz, 1H), 8.58 (d, *J* = 2.0 Hz, 2H), 8.51 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 5.96 (s, 2H), 5.02 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 154.38, 149.64, 148.91, 148.53, 139.96, 138.82, 135.27, 129.58, 128.64, 121.42, 119.61, 49.94, 35.14. Anal. Calcd for $C_{15}H_{10}N_8O_8S$: C, 38.97; H, 2.18; N, 24.24; S, 6.94. Found: C, 39.17; H, 2.55; N, 24.29; S, 6.7. HRMS (ESI+) m/z calcd for $C_{15}H_{11}N_8O_8S^+$: 463.04151 (100.0%), 464.04486 (16.2%); found 463.0408 (100%), 464.0440 (18%).

*5-[(3,5-Dinitrobenzyl)sulfanyl]-1-methyl-1H-tetrazole (**36a**)*: The solution of dimethyl sulfate (0.126g, 1 mmol) and TBAB (0.02 g, 0.05 mmol) in CH_2Cl_2 (5 mL) was added to the solution of 5-[(3,5-dinitrobenzyl)sulfanyl]-1*H*-tetrazole **31** (0.31 g, 1.1. mmol) and NaOH (0.048 g, 1.2 mmol) in water (5 mL). The reaction mixture was stirred 48 h at rt. The organic layer was separated, washed with 1M NaOH (1 × 5 mL) and water (1 × 10 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the product was isolated by column chromatography (hexane/EtOAc, 3:1). Yield: 30% as a white solid; mp 106-108 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.96 (t, *J* = 2.1 Hz, 1H), 8.72 (d, *J* = 2.1 Hz, 2H), 4.73 (s, 2H), 3.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.33, 148.56, 140.71, 129.35, 118.45, 35.37, 33.51. Anal. Calcd for $C_9H_8N_6O_4S$: C, 36.49; H, 2.72; N, 28.37; S, 10.82. Found: C, 36.70; H, 2.88; N, 28.48; S, 11.09.

*1-Benzyl-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**36b**)*:[17] Compound was prepared analogously to **36a**. Yield: 21% as a beige solid; mp 154-156 °C (with decomposition). ^{1}H NMR (500 MHz, DMSO) δ 8.72 – 8.64 (m, 3H), 7.33 – 7.27 (m, 3H), 7.17 – 7.11 (m, 2H),

5.54 (s, 2H), 4.77 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 153.06, 147.97, 141.97, 133.92, 129.70, 128.99, 128.62, 127.95, 117.99, 50.51, 35.00. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.26; H, 3.21; N, 22.49; S, 8.58.

*5-[(3,5-Dinitrobenzyl)sulfanyl]-1-(4-nitrobenzyl)-1H-tetrazole (**36c**):* The reaction mixture was heated for 10 h. Yield: 35% as a yellowish solid; mp 140-143 °C. ^{1}H NMR (500 MHz, acetone) δ 8.80 (t, *J* = 2.1 Hz, 1H), 8.76 (d, *J* = 2.1 Hz, 2H), 8.21 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 5.77 (s, 2H), 4.89 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 153.91, 149.25, 148.89, 142.87, 141.77, 130.32, 129.90, 124.75, 118.63, 50.61, 36.13. Anal. Calcd for $C_{15}H_{11}N_7O_6S$: C, 43.17; H, 2.66; N, 23.49; S, 7.68. Found: C, 43.46; H, 2.86; N, 23.22; S, 7.95.

*1-(3,5-Dinitrobenzyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**36d**):* The reaction mixture was heated for 7 h. Yield: 22% as a yellowish solid; mp 165-168 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.91 (t, *J* = 2.1 Hz, 1H), 8.81 (t, *J* = 2.1 Hz, 1H), 8.79 (d, *J* = 2.1 Hz, 2H), 8.60 (d, *J* = 2.1 Hz, 2H), 5.97 (s, 2H), 4.92 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 153.30, 148.77, 148.42, 142.02, 137.93, 129.53, 128.79, 118.78, 117.80, 49.11, 35.28. Anal. Calcd for $C_{15}H_{10}N_8O_8S$: C, 38.97; H, 2.18; N, 24.24; S, 6.94. Found: C, 38.87; H, 2.52; N, 23.94; S, 7.01.

*1-(4-chlorobenzyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**36f**):* The reaction mixture was heated for 5 h. Yield: 14% as a white solid; mp 130-131 °C. ^{1}H NMR (500 MHz, acetone) δ 8.81 (t, *J* = 2.1 Hz, 1H), 8.76 (d, *J* = 2.1 Hz, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 2H), 5.57 (s, 2H), 4.87 (s, 2H). ^{13}C NMR (126 MHz, acetone) δ 153.58, 149.28, 142.95, 134.97, 133.60, 130.63, 130.32, 129.81, 118.61, 50.78, 36.12. Anal.Calcd for $C_{15}H_{11}ClN_6O_4S$: C, 44.29; H, 2.73; N, 20.66; S, 7.88. Found: C, 44.6; H, 2.88; N, 20.67; S, 7.63.

*1-(3,4-dichlorobenzyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazole (**36g**):* The reaction mixture was heated for 5 h. Yield: 15% as a white solid; mp 121-124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (t, *J* = 2.0 Hz, 1H), 8.65 (d, *J* = 2.0 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.33 (d, *J* = 2.0 Hz, 1H), 7.08 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.35 (s, 2H), 4.69 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.15, 148.56, 140.41, 133.89, 133.55, 132.16, 131.29, 130.03, 129.30, 127.30, 118.52, 50.00, 35.66. Anal.Calcd for $C_{15}H_{10}Cl_2N_6O_4S$: C, 40.83; H, 2.28; N, 19.05; S, 7.27. Found: C, 41.19; H, 2.68; N, 18.78; S, 7.26.

2-Alkyl-5-(alkylsulfanyl)-2*H*-tetrazoles (37-40).

*5-(Benzylsulfanyl)-2-(3,5-dinitrobenzyl)-2H-tetrazole (**37d**):* Yield: 55% as a white solid; mp 116-117 °C. ^{1}H NMR (300 MHz, acetone) δ 8.97 (t, *J* = 2.1 Hz, 1H), 8.76 (d, *J* = 2.1 Hz, 2H), 7.56 – 7.33 (m, 2H), 7.29 – 7.10 (m, 3H), 6.27 (s, 2H), 4.44 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 165.02, 149.66, 138.66, 138.00, 130.16, 129.78, 129.24, 128.28, 119.79, 55.61, 36.50. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.53; H, 3.39; N, 22.71; S, 8.57.

*2-(4-Nitrobenzyl)-5-(4-nitrobenzyl)sulfanyl-2H-tetrazole (**38c**):* Yield: 52% as a white solid; mp 131-132 °C. ^{1}H NMR (300 MHz, acetone) δ 8.26 (d, *J* = 8.7 Hz, 2H), 8.11 (d, *J* = 8.7 Hz, 2H), 7.72 – 7.62 (m, 4H), 6.06 (s, 2H), 4.57 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 164.06, 149.07, 148.09, 146.30, 141.70, 131.03, 130.53, 124.74, 124.21, 56.45, 35.64. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.45; H, 3.32; N, 22.77; S, 8.84.

*5-[(4-Nitrobenzyl)sulfanyl]-2-(3,5-dinitrobenzyl)-2H-tetrazole (**38d**):* Yield: 53% as a light beige solid; mp 111-112 °C. ^{1}H NMR (300 MHz, acetone) δ 8.95 (t, *J* = 2.0 Hz, 1H), 8.75 (d, *J* = 2.0 Hz, 2H), 8.11 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.7 Hz, 2H), 6.27 (s, 2H), 4.58 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 164.37, 149.66, 148.11, 146.29, 138.56, 131.01, 130.16,

124.21, 119.81, 55.69, 35.57. Anal. Calcd for $C_{15}H_{11}N_7O_6S$: C, 43.17; H, 2.66; N, 23.49; S, 7.68. Found: C, 43.42; H, 2.88; N, 23.55; S, 7.82.

*5-[(2,4-Dinitrobenzyl)sulfanyl]-2-methyl-2H-tetrazole (**39a**):* Yield: 32% as a yellowish solid; 87-88 °C. ^{1}H NMR (300 MHz, acetone) δ 8.82 (d, *J* = 2.4 Hz, 1H), 8.50 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 4.89 (s, 2H), 4.33 (s, 3H); ^{13}C NMR (75 MHz, acetone) δ 162.96, 149.39, 148.23, 141.00, 135.01, 128.31, 121.31, 40.14, 33.31. Anal. Calcd for $C_9H_8N_6O_4S$: C, 36.49; H, 2.72; N, 28.37; S, 10.82. Found: C, 36.71; H, 2.89; N, 28.02; S, 10.91.

*2-Benzyl-5-[(2,4-dinitrobenzyl)sulfanyl]-2H-tetrazole (**39b**):* Yield: 44% as a yellowish solid; 62-64 °C. ^{1}H NMR (300 MHz, acetone) δ 8.78 (d, *J* = 2.4 Hz, 1H), 8.37 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.38 (s, 5H), 5.83 (s, 2H), 4.86 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 163.35, 149.30, 148.15, 140.86, 134.92, 134.67, 129.70, 129.42, 128.16, 121.33, 57.57, 33.43. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.69; H, 3.37; N, 22.42; S, 8.61.

*5-[(2,4-Dinitrobenzyl)sulfanyl]-2-(4-nitrobenzyl)-2H-tetrazole (**39c**):* Yield: 44% as an oil. ^{1}H NMR (500 MHz, acetone) δ 8.78 (d, *J* = 2.4 Hz, 1H), 8.42 (dd, *J* = 8.6, 2.4 Hz, 1H), 8.26 (d, *J* = 8.7 Hz, 2H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 6.05 (s, 2H), 4.88 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 163.83, 149.36, 149.10, 148.19, 141.58, 140.79, 134.98, 130.57, 128.21, 124.74, 121.30, 56.53, 33.41. HRMS (ESI+) m/z calcd for $C_{15}H_{12}N_7O_6S^+$: 418.05643 (100.0%), 419.05978 (16.2%); found 418.0562 (100%), 419.0594 (17%).

*5-[(2,4-Dinitrobenzyl)sulfanyl]-2-(3,5-dinitrobenzyl)-2H-tetrazole (**39d**):* Yield: 42% as a white solid; mp 127-130 °C. ^{1}H NMR (500 MHz, acetone) δ 8.96 (t, *J* = 2.1 Hz, 1H), 8.78 (d, *J* = 2.3 Hz, 1H), 8.75 (d, *J* = 2.1 Hz, 2H), 8.44 (dd, *J* = 8.6, 2.3 Hz, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 6.26 (s, 2H), 4.89 (s, 2H).; ^{13}C NMR (126 MHz, acetone) δ 163.28, 148.81, 148.55,

147.35, 139.93, 137.61, 134.15, 129.32, 127.38, 120.39, 118.97, 54.90, 32.47. Anal. Calcd for $C_{15}H_{10}N_8O_8S$: C, 38.97; H, 2.18; N, 24.24; S, 6.94. Found: C, 38.7; H, 2.44; N, 23.91; S, 7.01.

*5-[(3,5-Dinitrobenzyl)sulfanyl]-2-methyl-2H-tetrazole (**40a**):* Yield: 58% as a light beige solid; mp 94-96 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 (t, *J* = 2.1 Hz, 1H), 8.66 (d, *J* = 2.1 Hz, 2H), 4.55 (s, 2H), 4.30 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.02, 148.45, 142.07, 129.21, 117.99, 39.82, 34.62. Anal. Calcd for $C_9H_8N_6O_4S$: C, 36.49; H, 2.72; N, 28.37; S, 10.82. Found: C, 36.54; H, 2.82; N, 28.46; S, 10.52.

*2-Benzyl-5-[(3,5-dinitrobenzyl)sulfanyl]-2H-tetrazole (**40b**):* Yield: 47% as a beige solid; mp 109-111 °C. ^{1}H NMR (300 MHz, acetone) δ 8.80 – 8.75 (m, 3H), 7.34 (s, 5H), 5.83 (s, 2H), 4.74 (s, 2H); ^{13}C NMR (75 MHz, acetone) δ 163.34, 149.20, 143.78, 134.64, 130.24, 129.66, 129.60, 129.24, 118.31, 57.54, 34.94. Anal. Calcd for $C_{15}H_{12}N_6O_4S$: C, 48.38; H, 3.25; N, 22.57; S, 8.61. Found: C, 48.6; H, 3.39; N, 22.43; S, 8.7.

*5-[(3,5-dinitrobenzyl)sulfanyl]-2-(4-nitrobenzyl)-2H-tetrazole (**40c**):* Yield: 58% as a light beige solid; mp 130-132 °C. ^{1}H NMR (500 MHz, acetone) δ 8.77 (t, *J* = 2.1 Hz, 1H), 8.74 (d, *J* = 2.1 Hz, 2H), 8.22 (d, *J* = 8.7 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 2H), 6.05 (s, 2H), 4.75 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 163.83, 149.20, 149.03, 143.72, 141.60, 130.39, 130.24, 124.71, 118.32, 56.51, 34.98. Anal. Calcd for $C_{15}H_{11}N_7O_6S$: C, 43.17; H, 2.66; N, 23.49; S, 7.68. Found: C, 43.58; H, 2.89; N, 23.14; S, 7.68.

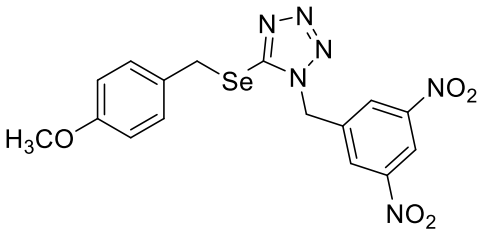
*2-(3,5-dinitrobenzyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-2H-tetrazole (**40d**):* Yield: 51% as a white solid; mp 156-159 °C (with decomposition). ^{1}H NMR (500 MHz, acetone) δ 8.95 (t, *J* = 2.1 Hz, 1H), 8.77 (t, *J* = 2.1 Hz, 1H), 8.74 (d, *J* = 2.1 Hz, 2H), 8.72 (d, *J* = 2.1 Hz, 2H), 6.27 (s, 2H), 4.77 (s, 2H); ^{13}C NMR (126 MHz, acetone) δ 163.25, 148.78, 148.37, 142.86, 137.61, 129.37, 129.22, 118.94, 117.46, 54.92, 34.09. Anal. Calcd for $C_{15}H_{10}N_8O_8S$: C, 38.97; H, 2.18; N, 24.24; S, 6.94. Found: C, 39.04; H, 2.43; N, 24.05; S, 7.02.

*2-(4-chlorobenzyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-2H-tetrazole (**40f**):* Yield: 52% as a white solid; mp 113-114 °C. ^{1}H NMR (500 MHz, acetone) δ 8.79 (t, *J* = 2.1 Hz, 1H), 8.75 (d, *J* = 2.1 Hz, 2H), 7.38 (s, 4H), 5.85 (s, 2H), 4.75 (s, 2H). ^{13}C NMR (126 MHz, acetone) δ 163.52, 149.24, 143.78, 135.16, 133.51, 131.13, 130.24, 129.77, 118.32, 56.74, 34.99. Anal.Calcd for $C_{15}H_{11}ClN_6O_4S$: C, 44.29; H, 2.73; N, 20.66; S, 7.88. Found: C, 44.40; H, 2.85; N, 20.57; s, 8.27.

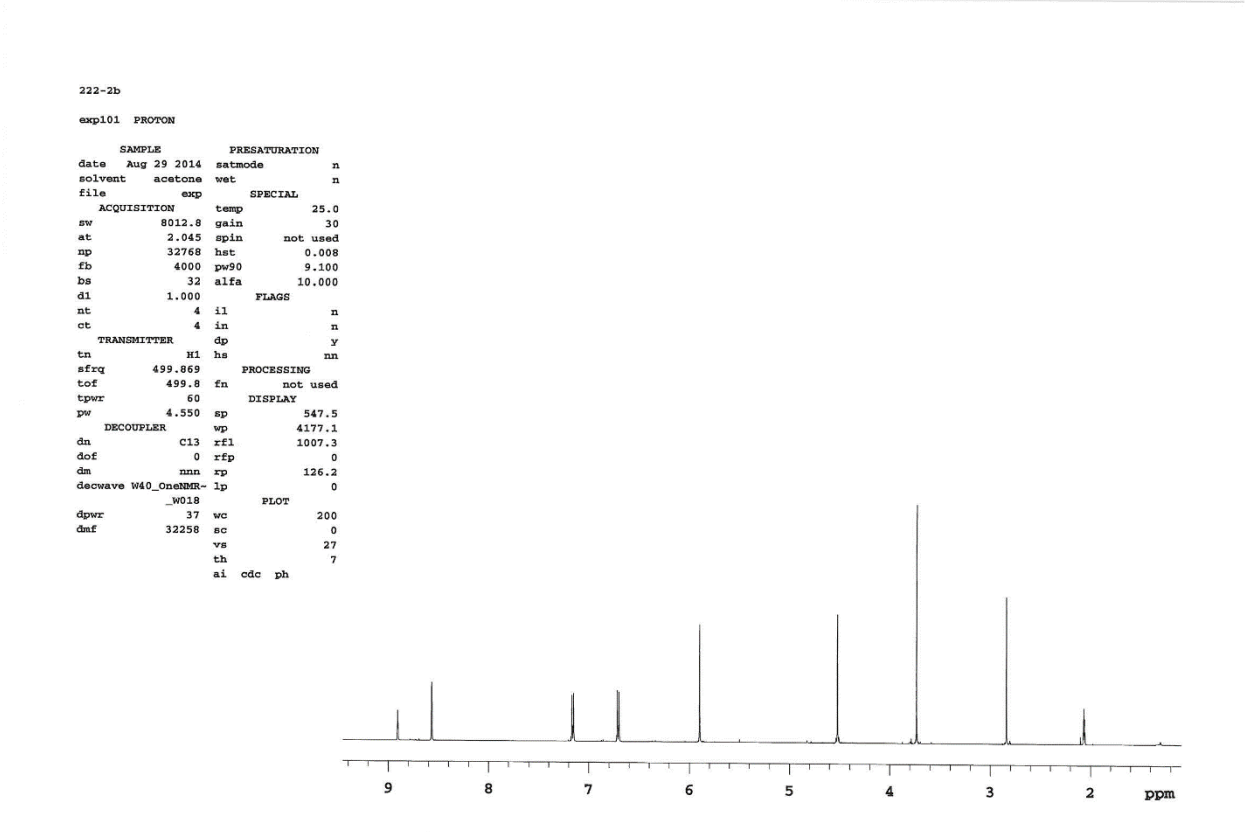
*2-(3,4-dichlorobenzyl)-5-[(3,5-dinitrobenzyl)sulfanyl]-2H-tetrazole (**40g**):* Yield: 40% as a white solid; mp 112-113 °C. ^{1}H NMR (500 MHz, acetone) δ 8.79 (t, *J* = 2.1 Hz, 1H), 8.76 (d, *J* = 2.1 Hz, 2H), 7.60 (d, *J* = 2.1 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.34 (dd, *J* = 8.3, 2.1 Hz, 1H), 5.89 (s, 2H), 4.76 (s, 2H). ^{13}C NMR (126 MHz, acetone) δ 163.70, 149.24, 143.75, 135.34, 133.28, 133.04, 131.87, 131.49, 130.24, 129.43, 118.32, 56.13, 34.97. Anal.Calcd for $C_{15}H_{10}Cl_2N_6O_4S$: C, 40.83; H, 2.28; N, 19.05; S, 7.27. Found: C, 40.76; H, 2.45; N, 18.87; S, 7.49.

NOESY experiments

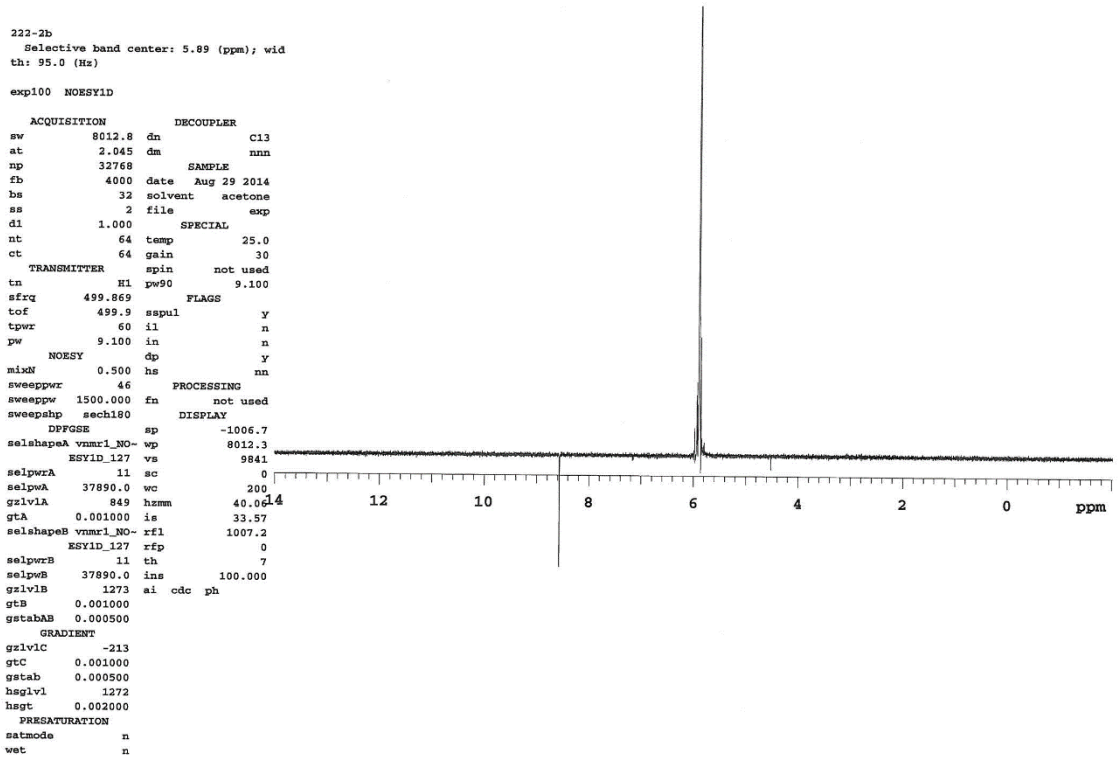
*1-(3,5-dinitrobenzyl)-5-[(4-methoxybenzyl)selanyl]-1H-tetrazole (**19d**)*



^{1}H NMR (**19d**)

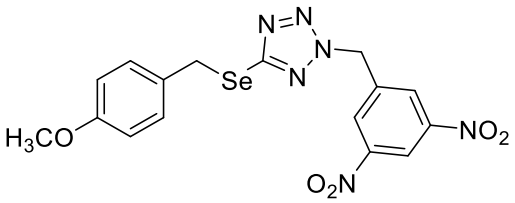


1D NOESY (**19d**)

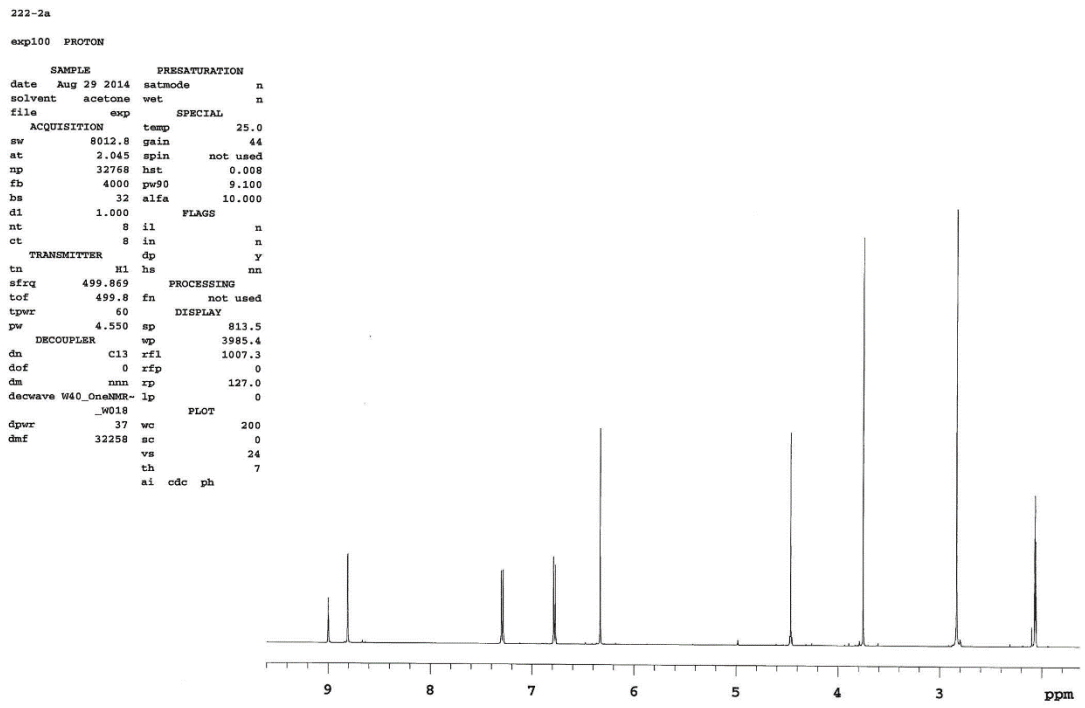


```
222-2b
  Selective band center: 5.89 (ppm); wid
th: 95.0 (Hz)

exp100  NOESY1D

   ACQUISITION
sw          8012.8
at           2.045
np           32768
fb            4000
bs              32
ss               2
d1           1.000
nt              64
ct              64
   TRANSMITTER
tn              H1
sfrq       499.869
tof          499.9
tpwr            60
pw           9.100
      NOESY
mixN         0.500
sweeppwr        46
sweeppw   1500.000
sweepshp   sech180
      DPFGSE
selshapeA vnmr1_NO~
        ESY1D_127
selpwrA         11
selpwA     37890.0
gzlvlA          849
gtA       0.001000
selshapeB vnmr1_NO~
        ESY1D_127
selpwrB         11
selpwB     37890.0
gzlvlB          1273
gtB       0.001000
gstabAB   0.000500
    GRADIENT
gzlvlC          -213
gtC       0.001000
gstab     0.000500
hsglvl          1272
hsgt      0.002000
  PRESATURATION
satmode          n
wet              n

      DECOUPLER
dn              C13
dm              nnn
       SAMPLE
date   Aug 29 2014
solvent    acetone
file            exp
      SPECIAL
temp          25.0
gain            30
spin      not used
pw90         9.100
      FLAGS
sspul            y
il               n
in               n
dp               y
hs              nn
    PROCESSING
fn        not used
     DISPLAY
sp         -1006.7
wp          8012.3
vs            9841
sc               0
wc             200
hzmm         40.06
is           33.57
rfl          1007.2
rfp              0
th               7
ins        100.000
ai  cdc  ph
```

*2-(3,5-Dinitrobenzyl)-5-[(4-methoxybenzyl)selanyl]-2H-tetrazole (**24d**)*

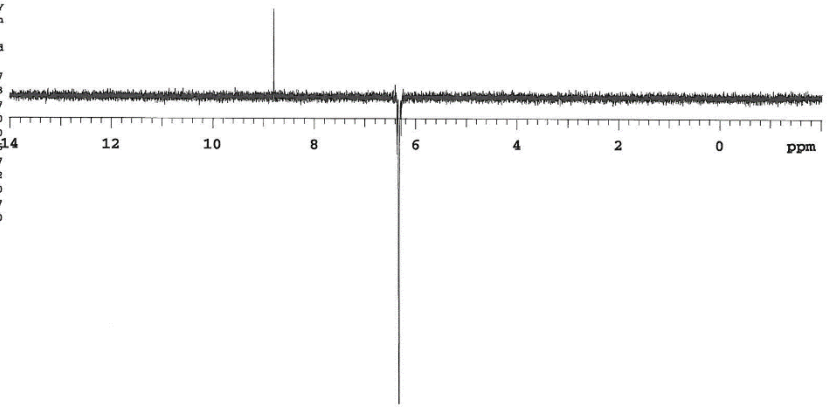


^{1}H NMR (**24d**)

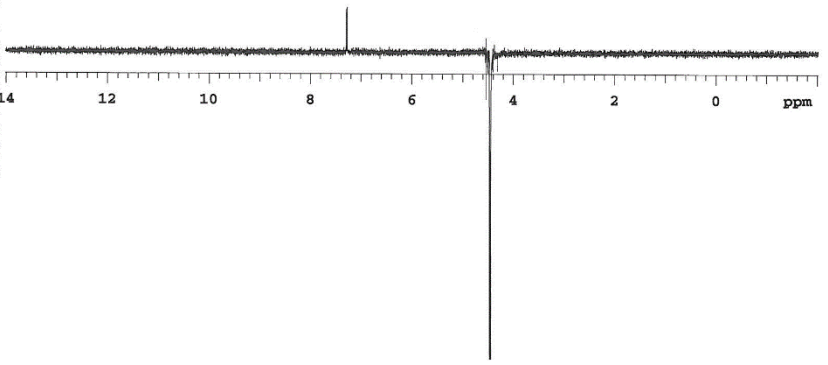


1D NOESY (**24d**)

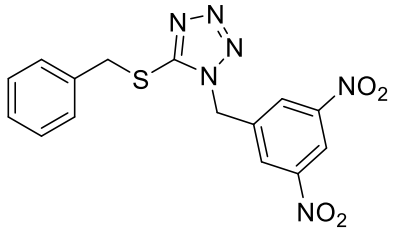
```
222-2a
  Selective band center: 6.33 (ppm); wid
th: 89.0 (Hz)

exp101  NOESY1D

   ACQUISITION          DECOUPLER
sw          8012.8  dn              C13
at           2.045  dm              nnn
np           32768        SAMPLE
fb            4000  date   Sep  2 2014
bs              32  solvent    acetone
ss               2  file            exp
d1           1.000       SPECIAL
nt              64  temp           25.0
ct              64  gain             30
   TRANSMITTER      spin       not used
tn              H1  pw90          9.100
sfrq       499.869        FLAGS
tof           499.9  sspul            y
tpwr             60  il               n
pw           9.100  in               n
     NOESY          dp               y
mixN         0.500  hs              nn
sweeppwr        46      PROCESSING
sweeppw    1500.000  fn         not used
sweepshp    sech180       DISPLAY
     DPFGSE          sp          -1006.7
selshapeA vnmr1_NO~ wp           8012.3
        ESY1D_129    vs            23667
selpwrA          10  sc                0
selpwA      40450.0  wc              200
gzlvlA           849  hzmm         40.06
gtA         0.001000  is            33.57
selshapeB vnmr1_NO~ rf1          1007.2
        ESY1D_129    rfp               0
selpwrB          10  th                7
selpwB      40450.0  ins         100.000
gzlvlB          1273  ai  cdc  ph
gtB         0.001000
gstabAB     0.000500
    GRADIENT
gzlvlC          -213
gtC         0.001000
gstab       0.000500
hsglvl          1272
hsgt        0.002000
  PRESATURATION
satmode           n
wet               n
```




```
222-2a
  Selective band center: 4.45 (ppm); wid
th: 119.0 (Hz)

exp101  NOESY1D

   ACQUISITION          DECOUPLER
sw          8012.8  dn              C13
at           2.045  dm              nnn
np           32768        SAMPLE
fb            4000  date   Sep  2 2014
bs              32  solvent    acetone
ss               2  file            exp
d1           1.000       SPECIAL
nt              64  temp           25.0
ct              64  gain             30
   TRANSMITTER      spin       not used
tn              H1  pw90          9.100
sfrq       499.869        FLAGS
tof           499.9  sspul            y
tpwr             60  il               n
pw           9.100  in               n
     NOESY          dp               y
mixN         0.500  hs              nn
sweeppwr        46      PROCESSING
sweeppw    1500.000  fn         not used
sweepshp    sech180       DISPLAY
     DPFGSE          sp          -1006.7
selshapeA vnmr1_NO~ wp           8012.3
        ESY1D_128    vs            15534
selpwrA          12  sc                0
selpwA      30251.0  wc              200
gzlvlA           849  hzmm         40.06
gtA         0.001000  is            33.57
selshapeB vnmr1_NO~ rf1          1007.2
        ESY1D_128    rfp               0
selpwrB          12  th                7
selpwB      30251.0  ins         100.000
gzlvlB          1273  ai  cdc  ph
gtB         0.001000
gstabAB     0.000500
    GRADIENT
gzlvlC          -213
gtC         0.001000
gstab       0.000500
hsglvl          1272
hsgt        0.002000
  PRESATURATION
satmode           n
wet               n
```



*5-(Benzylsulfanyl)-1-(3,5-dinitrobenzyl)-1H-tetrazole (**33d**)*

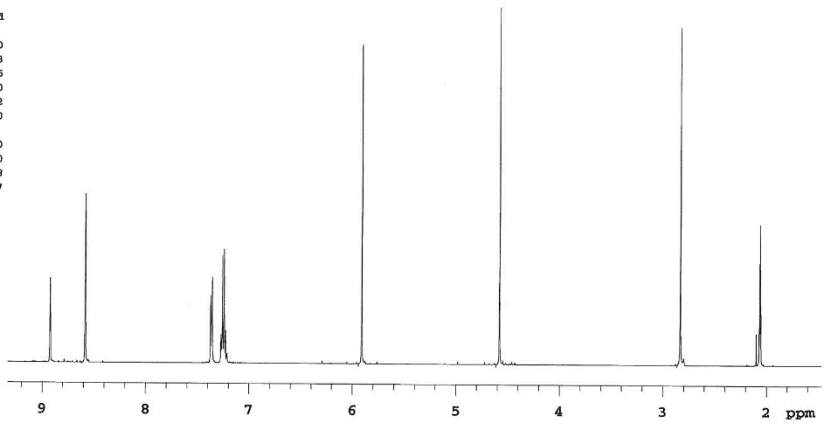


^{1}H NMR (**33d**)

GK237-1b

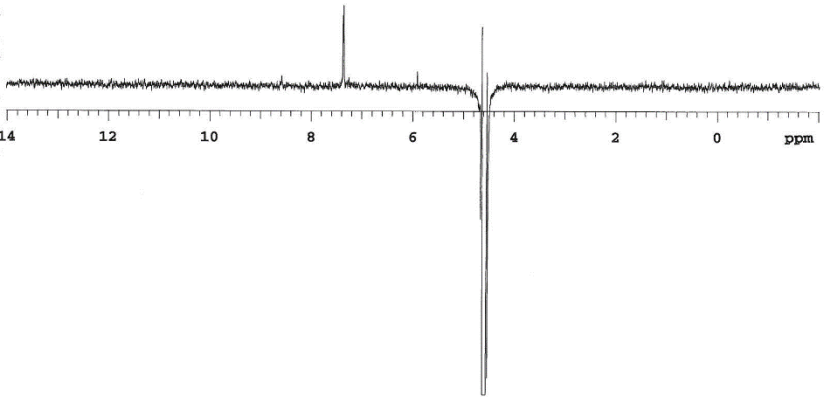
exp70 PROTON

SAMPLE		PRESATURATION	
date	Aug 26 2014	satmode	n
solvent	acetone	wet	n
file	exp	SPECIAL	
ACQUISITION		temp	25.0
sw	3930.8	gain	30
at	2.084	spin	20
np	16384	hst	0.008
fb	4000	pw90	9.100
bs	32	alfa	10.000
d1	1.000	FLAGS	
nt	4	il	n
ct	4	in	n
TRANSMITTER		dp	y
tn	H1	hs	nn
sfrq	499.869	PROCESSING	
tof	200.6	fn	not used
tpwr	60	DISPLAY	
pw	4.550	sp	735.0
DECOUPLER		wp	3930.3
dn	C13	rfl	-734.5
dof	0	rfp	0
dm	nnn	rp	98.2
decwave	W40_OneNMR-_W018	lp	0
		PLOT	
dpwr	37	wc	200
dmf	32258	sc	0
		vs	188
		th	7
		ai cdc ph	

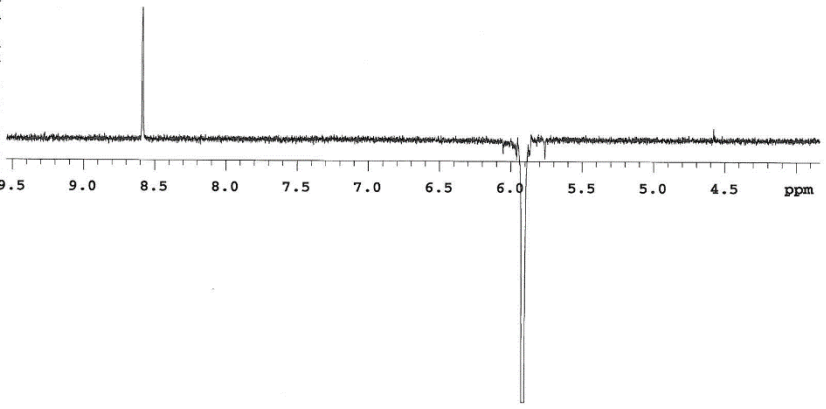


1D NOESY (**33d**)

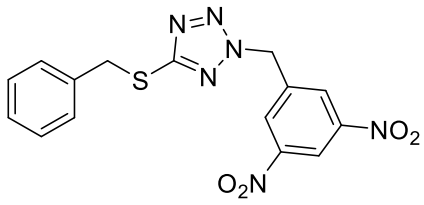
```
237-1b
  Selective band center: 4.59 (ppm); wid
th: 77.0 (Hz)

exp101  NOESY1D

   ACQUISITION          DECOUPLER
sw          8012.8  dn             C13
at           2.045  dm             nnn
np           32768       SAMPLE
fb            4000  date   Sep  2 2014
bs              32  solvent    acetone
ss               2  file           exp
d1           1.000       SPECIAL
nt              64  temp          25.0
ct              64  gain            30
   TRANSMITTER      spin       not used
tn              H1  pw90         9.100
sfrq       499.869       FLAGS
tof           499.9  sspul            y
tpwr             60  il               n
pw           9.100  in               n
      NOESY         dp               y
mixN         0.500  hs              nn
sweeppwr        46     PROCESSING
sweeppw   1500.000  lb            2.00
sweepshp   sech180  fn        not used
      DPFGSE             DISPLAY
selshapeA vnmr1_NO~  sp         -1006.7
        ESY1D_135  wp          8012.3
selpwrA          9  vs           83516
selpwA       46759.4  sc               0
gzlvlA           849  wc             200
gtA        0.001000  hzmm         40.06
selshapeB vnmr1_NO~  is           33.57
        ESY1D_135  rf1          1007.2
selpwrB          9  rfp              0
selpwB       46759.4  th               7
gzlvlB          1273  ins         100.000
gtB        0.001000  ai  cdc  ph
gstabAB    0.000500
     GRADIENT
gzlvlC          -213
gtC        0.001000
gstab      0.000500
hsglv1          1272
hsgt       0.002000
  PRESATURATION
satmode           n
wet               n
```




```
GX237-1b
  Selective band center: 5.90 (ppm); wid
th: 130.0 (Hz)

exp75  NOESY1D

   ACQUISITION          DECOUPLER
sw          8012.8  dn             C13
at           2.045  dm             nnn
np           32768       SAMPLE
fb            4000  date   Aug 27 2014
bs              32  solvent    acetone
ss               2  file           exp
d1           1.000       SPECIAL
nt              64  temp          25.0
ct              64  gain            30
   TRANSMITTER      spin             0
tn              H1  pw90         9.100
sfrq       499.869       FLAGS
tof           499.9  sspul            y
tpwr             60  il               n
pw           9.100  in               n
      NOESY         dp               y
mixN         0.700  hs              nn
sweeppwr        46     PROCESSING
sweeppw   1500.000  lb        not used
sweepshp   sech180  fn        not used
      DPFGSE             DISPLAY
selshapeA vnmr1_NO~  sp          1916.9
        ESY1D_123  wp          2852.2
selpwrA         13  vs           16517
selpwA       27690.0  sc               0
gzlvlA           849  wc             200
gtA        0.001000  hzmm         14.26
selshapeB vnmr1_NO~  is           33.57
        ESY1D_123  rf1          1007.2
selpwrB         13  rfp              0
selpwB       27690.0  th               7
gzlvlB          1273  ins         100.000
gtB        0.001000  ai  cdc  ph
gstabAB    0.000500
     GRADIENT
gzlvlC          -213
gtC        0.001000
gstab      0.000500
hsglv1          1272
hsgt       0.002000
  PRESATURATION
satmode           n
wet               n
```



*5-(Benzylsulfanyl)-2-(3,5-dinitrobenzyl)-2H-tetrazole (**37d**)*

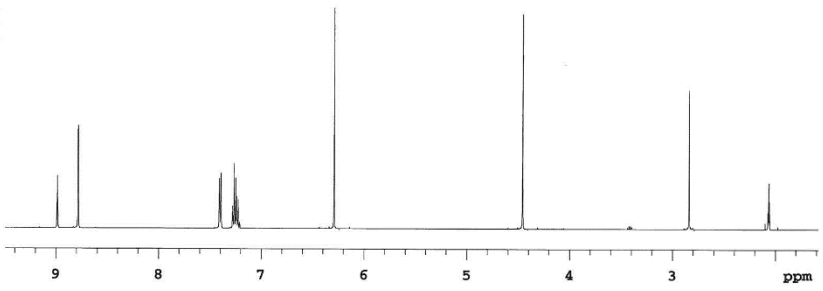


^{1}H NMR (**37d**)

GK237-1a

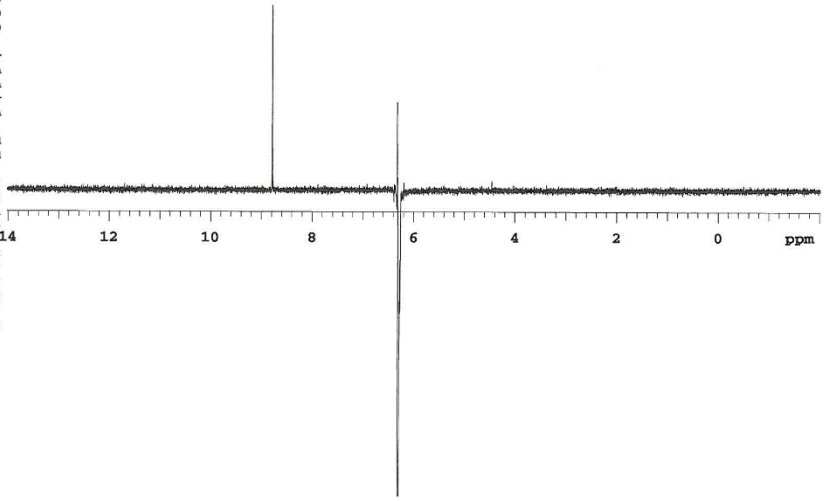
exp90 PROTON

SAMPLE		PRESATURATION	
date	Aug 27 2014	satmode	n
solvent	acetone	wet	n
file	exp	SPECIAL	
ACQUISITION		temp	25.0
sw	3955.7	gain	30
at	2.071	spin	20
np	16384	hst	0.008
fb	4000	pw90	9.100
bs	32	alfa	10.000
d1	1.000	FLAGS	
nt	8	il	n
ct	8	in	n
TRANSMITTER		dp	y
tn	H1	hs	nn
sfrq	499.869	PROCESSING	
tof	268.8	fn	not used
tpwr	60	DISPLAY	
pw	4.550	sp	790.8
DECOUPLER		wp	3955.2
dn	C13	rfl	-790.3
dof	0	rfp	0
dm	nnn	rp	99.8
decwave	W40_OneNMR~_W018	lp	0
		PLOT	
dpwr	37	wc	200
dmf	32258	sc	0
		vs	65
		th	7
		ai cdc ph	



1D NOESY (**37d**)

```
GK237-1a
  Selective band center: 6.28 (ppm); wid
th: 91.0 (Hz)

exp94  NOESY1D

   ACQUISITION
sw          8012.8
at           2.045
np           32768
fb            4000
bs              32
ss               2
d1           1.000
nt              64
ct              64
   TRANSMITTER
tn              H1
sfrq       499.869
tof          499.9
tpwr            60
pw           9.100
      NOESY
mixN         0.500
sweeppwr        46
sweeppw   1500.000
sweepshp   sech180
      DPFGSE
selshapeA vnmr1_NO~
         ESY1D_124
selpwrA         10
selpwA     39560.0
gzlvlA          849
gtA       0.001000
selshapeB vnmr1_NO~
         ESY1D_124
selpwrB         10
selpwB     39560.0
gzlvlB         1273
gtB       0.001000
gstabAB   0.000500
     GRADIENT
gzlvlC          -213
gtC       0.001000
gstab     0.000500
hsglvl          1272
hsgt      0.002000
  PRESATURATION
satmode          n
wet              n

     DECOUPLER
dn              C13
dm              nnn
       SAMPLE
date   Aug 28 2014
solvent     acetone
file            exp
      SPECIAL
temp          25.0
gain            30
spin             0
pw90         9.100
       FLAGS
sspul            y
il               n
in               n
dp               y
hs              nn
     PROCESSING
lb        not used
fn        not used
      DISPLAY
sp         -1006.7
wp          8012.3
vs           16151
sc               0
wc             200
hzmm         40.06
is           33.57
rf1         1007.2
rfp              0
th               7
ins         100.000
ai  cdc  ph
```



In vitro antimycobacterial assay.

Mycobacterial strains *M.tb* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80 and *M. avium* CNCTC My 330/88 from the Czech National Collection of Type Cultures (CNCTC) and the clinical isolate *M. kansasii* 6509/96 were used to evaluate the antimycobacterial activity of the prepared compounds. Basic suspensions of the mycobacterial strains were prepared according to the 1.0 McFarland standard. From the basic suspension, subsequent dilutions of each strain were made: *M.tb* 10^{-3}, *M. avium* 10^{-5}, and *M. kansasii* 10^{-4}. The appropriate dilutions of the strains (0.1 mL) were added to each well of the microtiter plates containing the studied compounds.

The compounds were dissolved in dimethyl sulfoxide (DMSO) and added to the medium at concentrations of 1000, 500, 250, 125, 62, 32, 16, 8, 4, 2 and 1 μmol/L. The activities were determined via the micromethod for the determination of the minimum inhibitory concentration in Sula's semisynthetic medium (SEVAC, Prague). MICs, i.e., the lowest concentration of a substance at which mycobacterial growth inhibition occurred (the concentration that inhibited >99% of the mycobacterial population), were determined after incubation at 37 °C for 7, 14, and 21 days for the *M. kansasii* strains and after 14 and 21 days for *M.tb* and *M. avium*. Isoniazid (INH) was used as a prototype drug.

In vitro antibacterial and antifungal assays.

The broth microdilution method was used for the evaluation of in vitro antibacterial and antifungal activity. The bacteria strains included 4 Gram positive cocci (*Staphylococcus aureus* ATCC 6538 (SA), Methicillin resistant *Staphylococcus aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08 (SE) and *Enterococcus faecalis* J 14365/08 (EF)) and 4 Gram negative rods (*Escherichia coli* ATCC 8739 (EC), *Klebsiella pneumoniae* D 11750/08 (KP), *Klebsiella pneumoniae* (a producer of extended-spectrum beta-lactamases) (ESBL) J 14368/08 (KP-E) and *Pseudomonas aeruginosa* ATCC 9027 (PA)). The compounds were dissolved in DMSO, and the final concentrations of the substances ranged from 0.488 to 500 μM. Mueller Hinton broth was used as the culture medium. The MIC was defined as a 95% or greater reduction of growth compared with the control. The MIC values were determined after 24 and 48 h of static incubation at 35 °C. Vancomycin was used as a prototype drug for Gram positive cocci, and gentamicin was used for Gram negative rods.

The fungi strains included 5 yeasts and yeast-like organisms (*Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156(CT), *Candida krusei* E28 (CK), *Candida glabrata* 20/I (CG), *Trichosporon asahii* 1188 (TA)) and 3 molds (*Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC), and *Trichophyton mentagrophytes* 445 (TM)). The procedure was performed in RPMI 1640 medium buffered to pH 7.0 with 0.165 mol of 3-morpholinopropane-1-sulfonic acid. The concentration range was the same as that used for the aforementioned bacteria. The MIC for yeasts and yeasts-like organisms was defined as an 80% or greater (IC_{80}), and for molds 50% or greater (IC_{50}), reduction of the fungal growth compared with the control. The MIC values were determined after 24 and 48 h of incubation at 35 °C. For *T. mentagrophytes*, the final MICs were determined after 72 and 120 h of incubation. Amphotericin B and fluconazole were used as prototype drugs.

In vitro cell proliferation/viability assessment.

The standard MTT assay (Sigma Aldrich) was used according to the manufacturer´s protocol to evaluate the effects of the studied compounds on the viability of CHO-K1 cells (Chinese hamster ovary, ECACC, Salisbury, UK). The cells were cultured according to the conditions recommended by ECACC (Ham's F12 with 2 mM glutamine and 10% (v/v) fetal bovine serum) and seeded at a density of 8,000 cells per well, under standard conditions, i.e., at 37 °C under 5% CO_2. The tested compounds were dissolved in DMSO and added to the growth medium with a final concentration of DMSO below 0.5% (v/v). The cells were incubated with the compounds or vehicle alone for 24 h. Then, the medium was replaced by 10 μM MTT and the cells were allowed to convert MTT into the colored formazan product for approximately 3 h. Thereafter, the MTT-containing medium was removed and the crystals of formazan were dissolved in DMSO (100 μL). The cell viability was measured as the amount of formazan produced by mitochondria and was assessed spectrophotometrically at 570 nm with 650 nm reference wavelength on Synergy HT (BioTek, USA). Cells treated with 0.1% Triton X-100 solution for at least 15 minutes were used as a toxic control. The half maximal inhibitory concentration (IC_{50}) was then calculated using non-linear regression of at least four concentration points (in triplicate, values of the toxic controls were subtracted from each sample´s absorbance) using GraphPad Prism 5 software. The final IC_{50} value is reported as the mean and SEM of 2-4 independent measurements.

Ames Fluctuation test

The mutagenic activity of the selected substances was detected using the commercial available Muta-ChromoPlate™ Bacterial strain Kit (ebpi, Mississauga, Ontario, Canada), which is the 96-well micro-plate version of the *Salmonella typhimurium* Ames Test. The test was performed and evaluated according to the manufacturer´s instructions. The final concentration of tested substances was 50 µM (the highest concentration that enables full solubility and that showed limited cytotoxicity in mammalian cell lines). *Salmonella typhimurium* tester strains TA 98 (detection of frame shift mutagens) and TA 100 (detection of base-exchange mutations) were used. The substances were dissolved in DMSO and the final amounts of DMSO in the whole reaction mixture was 0,1%. The following standard direct-acting mutagens were used as positive controls: sodium azide for use with the strain TA100 (final amount 0.5 µg, 25ng/mL) and 2-nitrofluorene for use with the strain TA98 (final amount 30 µg; 1.5 ng/mL). A blank plate as a sterility control and plates without any tested compounds for each strain were used as a spontaneous mutation control (background control).

Results

Table S3: Evaluation of mutagenicity in Ames fluctuation assay performed with selected substances on *Salmonella typhimurium* TA100 and TA98 strains

Substance tested	**Ames fluctuation assay**	
	TA100	**TA98**
Sodium azide	+	n.d.
2-nitrofluorene	n.d.	+
18b	-	+
23b	-	+
36b	-	-
40b	-	+
36c	-	+
40c	-	+
36f	-	+
40f	-	+

- negative mutagenicity
+ positive mutagenicity
n.d. not determined

Statistical significance of mutagenicity for all the positive substances was at the level of 0.001.

Using the Ames fluctuation assay, except the **36b**, all the tested chemicals displayed statistically significant mutagenic effects on the strain TA98 as indicated by the number of reverse-mutated colonies. Thus, the selected chemical agents seem to act as frame-shift mutagens although we observed significant difference in potency (a number of reverse-mutated colonies) to induce reverse mutation by tested substances. On the other hand, none of the selected substances revealed genotoxic effect in strain S. typhimurium TA100.

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