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Synthesis and cytotoxic activity of sulfur linked mono / bis heterocycles

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A variety of sulfur linked mono / bis heterocycles have been prepared and their cytotoxic activity on A549 lung adenocarcinoma cells studied. The imidazolyl and benzimidazolyl thiadiazoles (**7c**, **8c**) display appreciable cytotoxic activity against A549 cells.

Keywords: Cytotoxic activity, A549 lung adenocarcinoma cells, 1,2,4-thiadiazole

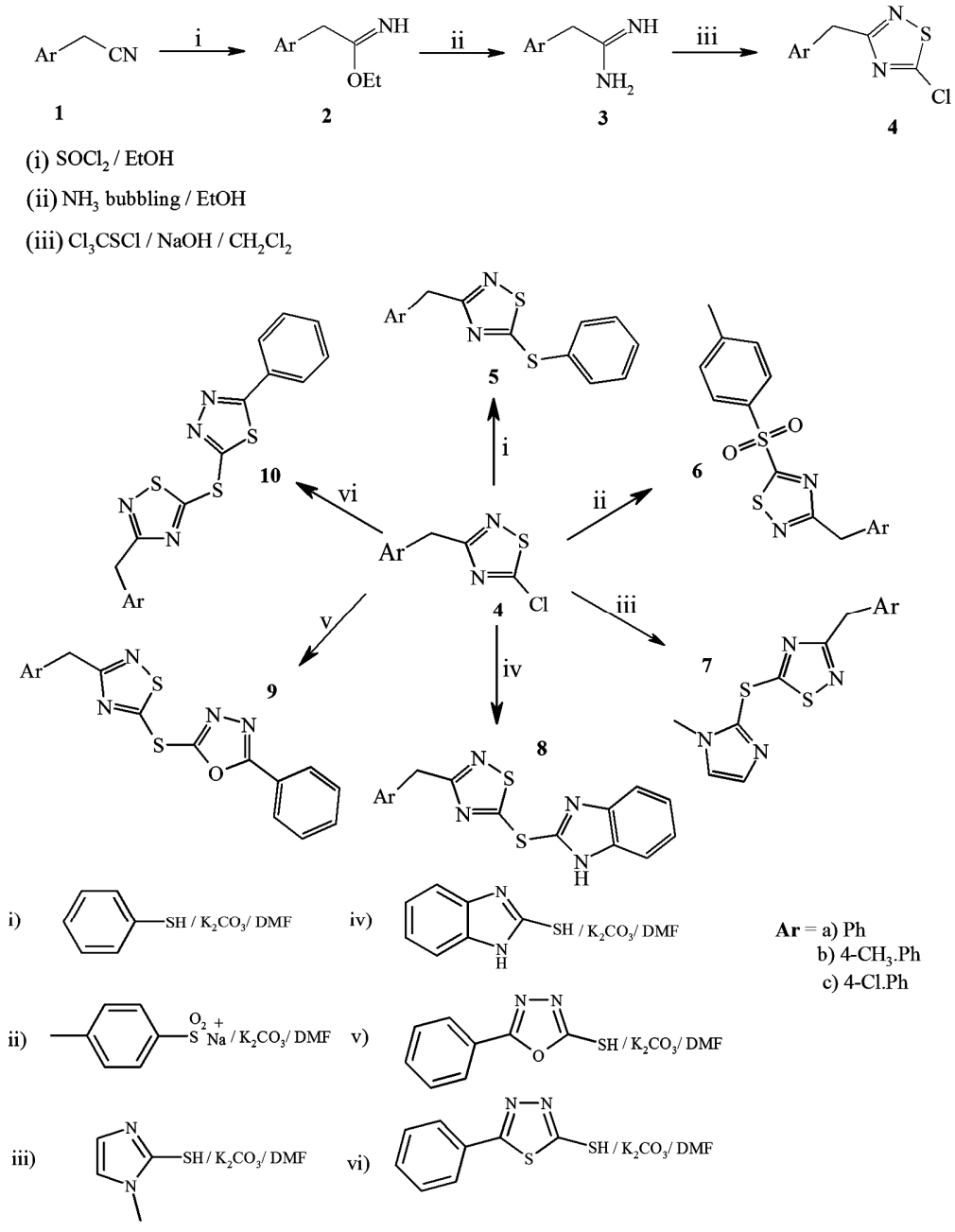
1,2,4-Thiadiazole is the core unit of many bioactive molecules of various therapeutic categories such as antimicrobial, antitubercular, antiviral, herbicidal and fungicidal[1–5]. Indolyl 1,2,4-thiadiazoles exhibit pronounced antitumor activity against a panel of cancer cell lines LnCaP, PC3 and PaCa2 (Ref 6,7). Although the only commercial 1,2,4-thiadiazole drug is the antibiotic cefozopram[8], there are a number of synthetic products related to this system with a broad range of biological activities towards the central nervous system (CNS)[9], G-protein coupled receptors[10], inflammation[11], cardiovascular system[12,13] and antibiotic activity[14,15]. Moreover, thiadiazoles are easily capable of crossing the cellular membranes due to their mesoionic nature and their better liposolubility is attributed to the presence of sulphur atom. The mesoionic compounds are capable of crossing the cellular membranes and interact with biological targets with distinct affinities. The good liposolubility of the sulphur atom in this heterocyclic moiety might also have a positive effect on the biological activity and pharmacokinetic properties of thiadiazole containing compounds. Fascinated by the diverse bioactivity of 1,2,4-thiadiazoles it was proposed to conjugate 5-aryl / heteroaryl moieties to 1,2,4-thiadiazole and to study their cytotoxic activity.

Results and Discussion

The synthetic intermediate 3-benzyl-5-chloro-1,2,4-thiadiazole **4** was prepared by the reaction of 2-phenylacetamidine **3** with perchloromethylmercaptane in the presence of NaOH (Scheme I). The amidine derivative was prepared by bubbling ammonia into ethyl 2-arylimidoacetate **2** in ethanol. The latter compound was obtained by the reaction of 2-arylacetonitrile **1** with thionyl chloride[16]. The aryl and heteroaryl 1,2,4-thiadiazoles **5-10** were prepared by the reaction of compound **4** with respective thiols. Thus, 3-benzyl-5-(phenylthio)-1,2,4-thiadiazole **5** and 3-benzyl-5-tosyl-1,2,4-thiadiazole **6** were prepared by the reaction of **4** with benzenethiol and sodium 4-methylbenzenesulfinate in the presence of K_2CO_3 in DMF. Similarly 5-(1-methyl-1*H*-imidazol-2-ylthio)-3-benzyl-1,2,4-thiadiazole (**7**) and 2-(3-benzyl-1,2,4-thiadiazol-5-ylthio)-1*H*-benzimidazole **8** were prepared by the treatment of **4** with 1-methyl-1*H*-imidazole-2-thiol and 1*H*-benzimidazole-2-thiol. Likewise, 2-(3-benzyl-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-oxadiazole **9** and 2-(3-benzyl-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-thiadiazole **10** were obtained by the reaction of **4** with 5-phenyl-1,3,4-oxadiazole-2-thiol and 5-phenyl-1,3,4-thiadiazole-2-thiol. The structures of all the compounds were confirmed by IR, ^{1}H and ^{13}C NMR, mass spectroscopy and elemental analyses.

Cytotoxic activity

The compounds **5-10** were subjected to MTT assay to determine growth inhibitory/cytotoxic capability (Table I). The compounds **7c** and **8c** showed appreciable cytotoxic activity on A549 cells with IC_{50} values 3.9 μM and 4.9 μM, respectively. However,



Scheme I — Synthesis of sulfur linked mono and bis heterocycles

the remaining compounds do not display any cytotoxicity when used up to 50 µM concentration. Figure 1 and Figure 2 shows the results of cytotoxicity of **7c** and **8c** using MTT assay on A549 lung adenocarcinoma cells. The cytotoxic activity observed with compounds **7c** and **8c** is concentration dependent. The compounds **7c** and **8c** at concentrations 5-50 µM showed lowest viability, while viability more than 50% is observed when this compound is used at a concentration below 5 µM on A549 cells. This suggests that compounds **7c** and **8c** as noticeable lead molecules for cytotoxic activity against tumor cells. A549 cells have a mutated K-ras oncogene. Further modifications to these molecules may provide a more potent molecule that could be developed as a therapeutic drug.

Table I — Cytotoxic activity statistical data

Compd	IC_{50} (μM)	SD
5c	3.7	±0.025
6c	3.8	±0.154
7c	3.9	±0.051
8c	4.9	±0.034
9c	3.7	±0.1
10c	3.6	±0.158

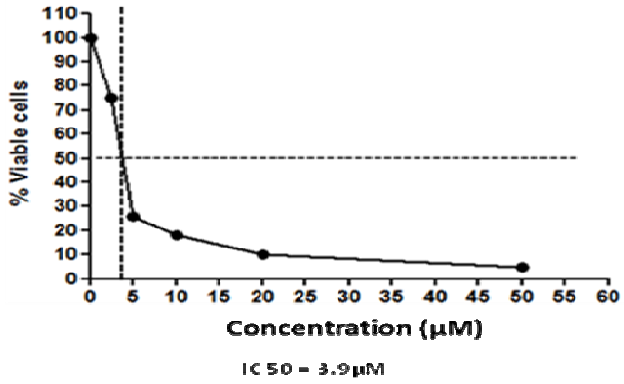


Figure 1 — The dose-response curve of compound **7c** measured by MTT assay on A549 lung carcinoma cells. X-axis shows the concentration of the compound, and Y-axis, the cell viability

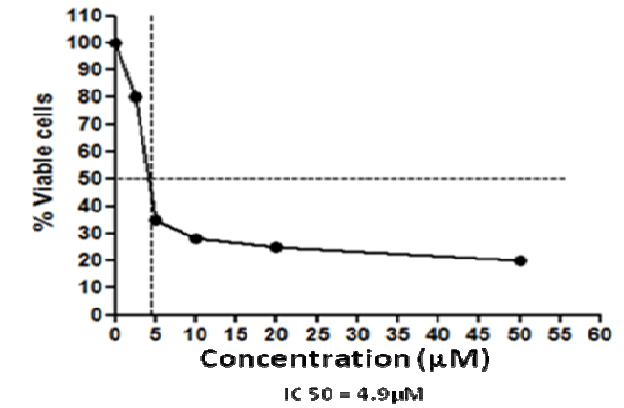


Figure 2 — The dose-response curve of compound **8c** measured by MTT assay on A549 lung carcinoma cells. X-axis shows the concentration of the compound, and Y-axis, the cell viability

Experimental Section

Melting points were determined in open capillaries on a Mel Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 2:0.5). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$ on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C NMR spectra were recorded in $CDCl_3$ on a Jeol JNM spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Jeol JMS-D 300 and Finnigan Mat 1210 B at 70 eV with an emission current of 100 μA. The microanalyses were performed on Perkin-Elmer 240C elemental analyzer. For cytotoxic activity the absorbance was estimated at 550 nm in ELISA plate reader (7520 Micro plate reader, Cambridge technologies, Inc). All chemicals and solvents were purchased from Merck and used without further purification. The synthetic intermediate 3-benzyl-5-chloro-1,2,4-thiadiazole **4** was prepared as per the literature procedure[16].

General procedure for the synthesis of 3-Benzyl-5-(phenylthio)-1,2,4-thiadiazole, 5a-c / 3-Benzyl-5-tosyl-1,2,4-thiadiazole, 6a-c

To a solution of 3-benzyl-5-chloro-1,2,4-thiadiazole **4** (1.0 mmol) in dry DMF (4 mL), benzenethiol / 4-methylbenzenesulfinate (1.1 mmol) followed by K_2CO_3 (2.03 mmol) were added and heated at 40°C for 3-4 h. The reaction mixture was cooled to RT, poured into ice water and extracted with CH_2Cl_2 (2×15 mL). Combined organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from 2-propanol.

3-Benzyl-5-(phenylthio)-1,2,4-thiadiazole, 5a: White solid, m.p. 123-125°C. ^1H NMR (400 MHz, $CDCl_3$): δ 3.91 (s, 2H, CH_2), 6.87-7.33 (m, 10H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.5, 111.8, 128.3, 130.0, 130.8, 132.4, 134.7, 135.0, 135.5, 142.0, 174.7; MS: *m/z* 284.40 [M^+]. Anal. Calcd for $C_{15}H_{12}N_2S_2$: C, 63.35; H, 4.25; N, 9.85. Found: C, 63.28; H, 4.20; N, 9.97%.

3-(*p*-Methylbenzyl)-5-(phenylthio)-1,2,4-thiadiazole, 5b: White solid, m.p. 142-144°C. ^1H NMR (400 MHz, $CDCl_3$): δ 2.24 (s, 3H, Ar-CH_3), 3.96 (s, 2H, CH_2), 6.73-7.10 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.4, 38.2, 111.4, 128.0, 129.4, 130.5, 132.7, 134.2, 134.8, 135.1, 141.7, 174.3; MS: (*m/z*) 298.43 [M^+]. Anal. Calcd for $C_{16}H_{14}N_2S_2$: C, 64.39; H, 4.73; N, 9.39. Found: C, 64.46; H, 4.67; N, 9.48%.

3-(4-Chlorobenzyl)-5-(phenylthio)-1,2,4-thiadiazole, 5c: White solid, m.p. 135-137°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.95 (s, 2H, CH_2), 7.03-7.48 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.6, 112.0, 175.0, 128.7, 130.4, 131.3, 132.7, 135.0, 135.2, 135.3, 142.1; MS: (*m/z)* 318.84 [M^+]. Anal. Calcd for $C_{15}H_{11}ClN_2S_2$: C, 56.50; H, 3.48; N, 8.79. Found: C, 56.57; H, 3.44; N, 9.09%.

3-Benzyl-5-tosyl-1,2,4-thiadiazole, 6a: White solid, m.p. 123-125°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 3H, CH_3), 4.02 (s, 2H, CH_2), 6.95-7.70 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.3, 38.0, 128.1, 128.3, 128.5, 130.1, 130.6, 133.3, 133.9, 146.1, 165.1, 176.1; MS: (*m/z)* 330.42 [M^+]. Anal. Calcd for $C_{16}H_{14}N_2O_2S_2$: C, 56.94; H, 3.82; N, 8.85. Found: C, 57.03; H, 3.78; N, 8.95%.

3-(4-Methylbenzyl)-5-tosyl-1,2,4-thiadiazole, 6b: White solid, m.p. 142-144°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22 (s, 3H, CH_3), 2.31 (s, 3H, Ar-CH_3), 6.88-7.61 (m, 8H, Ar-H), 3.97 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.0, 23.4, 37.4, 126.3, 128.0, 128.3, 129.4, 129.6, 133.1, 133.2, 145.6, 165.7, 175.7; MS: (*m/z*) 344.45 [M^+]. Anal. Calcd for $C_{17}H_{16}N_2O_2S_2$: C, 58.16; H, 4.27; N, 8.48. Found: C, 58.24; H, 4.23; N, 8.59%.

3-(4-Chlorobenzyl)-5-tosyl-1,2,4-thiadiazole, 6c: White solid, m.p. 135-137°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 4.06 (s, 2H, CH_2), 7.01-7.79 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.7, 38.3, 128.6, 129.0, 129.1, 130.2, 130.3, 134.1, 134.3, 146.5, 165.4, 176.4; MS: (*m/z*) 364.87 [M^+]. Anal. Calcd for $C_{16}H_{13}ClN_2O_2S_2$: C, 52.67; H, 3.59; N, 7.68. Found: C, 52.62; H, 3.54; N, 7.75%.

General procedure for the synthesis of 5-(1-Methyl-1*H*-imidazol-2-ylthio)-3-benzyl-1,2,4-thiadiazole, 7a-c / 2-(3-Benzyl-1,2,4-thiadiazol-5-ylthio)-1*H*-benzimidazole, 8a-c

To a solution of 3-benzyl-5-chloro-1,2,4-thiadiazole **4** (1.0 mmol) in dry DMF (4 mL), 1-methyl-1*H*-imidazole-2-thiol/1*H*-benzo[*d*] imidazole-2-thiol (1.1 mmol) followed by K_2CO_3 (2.03 mmol) were added and heated at 40°C for 3-4 h. The reaction mixture was cooled to RT, poured into ice water and extracted with CH_2Cl_2 (2×15 mL). Combined organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from EtOH.

5-(1-Methyl-1*H*-imidazol-2-ylthio)-3-benzyl-1,2,4-thiadiazole, 7a: White solid, m.p. 69-71°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (s, 3H, N-CH_3), 4.21 (s, 2H, CH_2), 7.07-7.22 (m, 7H, Ar-H & Imd-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.1, 38.4, 124.1, 124.5, 127.2, 130.8, 132.3, 132.8, 135.7, 174.0, 186.0; MS: (*m/z*) 288.39 [M^+]. Anal. Calcd for $C_{13}H_{12}N_4S_2$: C, 54.14; H, 4.19; N, 19.43. Found: C, 54.20; H, 4.25; N, 19.35%.

5-(1-Methyl-1*H*-imidazol-2-ylthio)-3-(4-methylbenzyl)-1,2,4-thiadiazole, 7b: White solid, m.p. 72-74°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (s, 3H, Ar-CH_3), 3.74 (s, 3H, N-CH_3), 4.22 (s, 2H, CH_2), 6.93-7.17 (m, 6H, Ar-H & Imd-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.5, 32.6, 38.7, 123.8, 124.5, 128.0, 129.5, 131.6, 132.7, 134.6, 173.7, 185.0; MS: (*m/z*) 302.42 [M^+]. Anal. Calcd for $C_{14}H_{14}N_4S_2$: C, 55.60; H, 4.67; N, 18.53. Found: C, 55.67; H, 4.63; N, 18.64%.

5-(1-Methyl-1*H*-imidazol-2-ylthio)-3-(4-chlorobenzyl)-1,2,4-thiadiazole, 7c: White solid, m.p. 67-69°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75 (s, 3H, N-CH_3), 4.25 (s, 2H, CH_2), 7.18-7.30 (m, 6H, Ar-H & Imd-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.6 (N-CH_3), 38.5 (CH_2), 124.5, 124.7, 128.6, 131.0, 132.6, 133.9, 135.1, 174.2, 185.6; MS: (*m/z*) 322.84 [M^+]. Anal. Calcd for $C_{13}H_{11}ClN_4S_2$: C, 48.36; H, 3.43; N, 17.36. Found: C, 48.41; H, 3.40; N, 17.19%.

2-((3-Benzyl)-1,2,4-thiadiazol-5-ylthio)-1*H*-benzo[*d*]imidazole, 8a: White solid, m.p. 159-161°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.30 (s, 2H, CH_2), 7.18-7.60 (m, 9H, Ar-H), 7.75 (bs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.4, 114.0, 122.6, 127.5, 129.6, 131.1, 132.4, 134.5, 142.0, 172.2, 180.1; MS: (*m/z*) 324.42 [M^+]. Anal. Calcd for $C_{16}H_{12}N_4S_2$: C, 59.24; H, 3.73; N, 17.27. Found: C, 59.33; H, 3.71; N, 17.39%.

2-(3-(4-Methylbenzyl)-1,2,4-thiadiazol-5-ylthio)-1*H*-benzo[*d*]imidazole, 8b: White solid, m.p. 163-165°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.26 (s, 3H, CH_3), 4.34 (s, 2H, CH_2), 7.16-7.49 (m, 8H, Ar-H), 7.70 (bs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.0, 37.1, 113.7, 122.0, 127.1, 129.2, 129.6, 132.1, 134.0, 142.0, 171.2, 180.0; MS: (*m/z*) 338.45 [M^+]. Anal. Calcd for $C_{17}H_{14}N_4S_2$: C, 60.33; H, 4.17; N, 16.55. Found: C, 60.25; H, 4.14; N, 16.63%.

2-(3-(4-Chlorobenzyl)-1,2,4-thiadiazol-5-ylthio)-1*H*-benzo[*d*]imidazole, 8c: White solid, m.p.175−177°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.29 (s, 2H, CH_2), 7.24-7.31 (m, 8H, Ar-H), 7.77 (bs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.8, 114.5, 122.3, 128.0, 130.0, 131.7, 133.3, 135.3, 142.3, 171.8,

180.5; MS: (*m/z*) 358.87 [M^+]. Anal. Calcd for $C_{16}H_{11}ClN_4S_2$: C, 53.55; H, 3.09; N, 15.61. Found: C, 53.47; H, 3.04; N, 15.72.

General procedure for the synthesis of 2-(3-Benzyl-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-oxadiazole, 9a-c / 2-(3-Benzyl-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-thiadiazole, 10a-c

To a solution of 3-benzyl-5-chloro-1,2,4-thiadiazole **4** (1.0 mmol) in dry DMF (4 mL), 5-phenyl-1,3,4-oxadiazole-2-thiol **/** 5-phenyl-1,3,4-thiadiazole-2-thiol (1.1 mmol) followed by K_2CO_3 (2.03 mmol) were added and heated at 40°C for 3-4 h. The reaction mixture was cooled to RT and poured into ice water, extracted with CH_2Cl_2 (2×15 mL). Combined organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude product was purified by recrystallization from EtOH.

2-(3-Benzyl-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-oxadiazole, 9a: White solid, m.p. 105-107°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.04 (s, 2H, CH_2), 6.96-7.73 (m, 10H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.6, 111.5, 122.0, 126.2, 127.6, 128.0, 128.5, 129.7, 131.7, 134.1, 134.6, 173.7, 174.2; MS: (*m/z*) 352.43 [M^+]. Anal. Calcd for $C_{17}H_{12}N_4OS_2$: C, 57.93; H, 3.43; N, 15.90. Found: C, 57.87; H, 3.40; N, 15.98%.

2-(3-(4-Methylbenzyl)-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-oxadiazole, 9b: White solid, m.p. 99-101°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.30 (s, 3H, Ar-CH_3), 4.01 (s, 2H, CH_2), 6.85-7.57 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.7, 37.1, 111.0, 121.4, 126.5, 127.3, 127.8, 128.1, 129.3, 131.1, 132.5, 133.7, 172.6, 173.5; MS: (*m/z*) 366.46 [M^+]. Anal. Calcd for $C_{18}H_{14}N_4OS_2$: C, 58.99; H, 3.85; N, 15.29. Found: C, 58.90; H, 3.89; N, 15.19%.

2-(3-(4-Chlorobenzyl)-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-oxadiazole, 9c: White solid, m.p. 110-112°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.09 (s, 2H, CH_2), 7.09-7.88 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.1, 111.9, 122.3, 126.7, 128.4, 128.5, 129.0, 130.2, 132.3, 134.8, 135.9, 173.4, 174.8; MS: (*m/z*) 386.88 [M^+]. Anal. Calcd for $C_{17}H_{11}ClN_4OS_2$: C, 52.78; H, 2.87; N, 14.48. Found: C, 52.84; H, 2.90; N, 14.39%.

2-(3-Benzyl-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-thiadiazole, 10a: White solid, m.p. 135-137°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.24 (s, 2H, CH_2), 7.12-7.67 (m, 10H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.1, 110.0, 124.5, 127.6, 127.9, 129.0, 129.3, 130.1, 130.7, 162.6, 167.0, 169.8, 174.4 ppm; MS: (*m/z*) 368.50 [M^+]. Anal. Calcd for $C_{17}H_{12}N_4S_3$: C, 55.41; H, 3.28; N, 15.20. Found: C, 55.49; H, 3.24; N, 15.32%.

2-(3-(4-Methylbenzyl)-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-thiadiazole, 10b: White solid, m.p. 146-148°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, Ar-CH_3), 4.22 (s, 2H, CH_2), 7.01-7.55 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 37.6, 109.1, 124.0, 127.1, 127.3, 128.0, 128.5, 129.0, 129.5, 161.2, 166.6, 168.3, 174.0; MS: (*m/z*) 382.53 [M^+]. Anal. Calcd for $C_{18}H_{14}N_4S_3$: C, 56.52; H, 3.69; N, 14.65. Found: C, 56.47; H, 3.75; N, 14.73%.

2-(3-(4-Chlorobenzyl)-1,2,4-thiadiazol-5-ylthio)-5-phenyl-1,3,4-thiadiazole, 10c: White solid, m.p. 168-170°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.29 (s, 2H, CH_2), 7.26-7.97 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.5, 110.8, 125.2, 128.0, 128.7, 129.4, 129.8, 130.5, 131.1, 162.2, 175.0, 169.3, ppm; MS: (*m/z*) 402.94 [M^+]. *Anal.* Calcd. for $C_{17}H_{11}ClN_4S_3$: C, 50.67; H, 2.75; N, 13.90; Found: C, 50.58; H, 2.71; N, 13.97%.

Cytotoxic Activity

The compounds **5-10** were subjected to MTT assay to determine cytotoxic capability.

Cells

Compounds **5-10** were dissolved in DMSO at different concentrations of 12.5-50 mm. A549 lung adenocarcinoma cells were maintained in DMEM (Dulbecco's Modified Eagle's Medium) medium substituted with 10% fetal bovine serum and 1% Penicillin and Streptomycin. The cells were plated in T25 tissue culture flask and incubated at 37°C in a 5% CO_2 atmosphere with 90% humidity.

MTT assay for cell viability

The cytotoxicity of the compounds was tested using A549 lung carcinoma cells. 5×10^4 cells were plated in each well of a 96-well tissue culture cluster (Nunc Inc Germany) and incubated at 37°C in a medium containing DMEM, 10% fetal bovine serum and antibiotics (Invitrogen, USA), in 5% CO_2 atmosphere[17,18]. Compounds were dissolved in DMSO. Serial dilutions were made for the compounds **5-10** from stock solution 10 mg/mL in DMSO. After attachment of the cells (usually 3-4 h),

different concentrations of dilutions were added to cells in 96 well plate and incubated for 20 h. MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) solution (20 mL of 5 mg/mL) was added to each well and the incubation was continued for additional 3 h. The dark blue formazan crystals formed within the healthy cells were solubilized with DMSO and the absorbance was estimated in ELISA plate reader (7520 Micro plate reader, Cambridge technologies, Inc) at 550 nm and correlated with the cell number. Experiments were performed in triplicates and the values are the average of three (n=3) independent experiments. The inhibitory concentration (IC_{50}) of the compound was assessed by Graph Pad Prism software.

Conclusion

In conclusion, a variety of sulfur linked mono/bis heterocycles were prepared and studied their cytotoxic activity. It was observed that chloro substituted imidazolyl and benzimidazolyl thiadiazoles (**7c, 8c**) displayed good cytotoxic activity against A549 lung adenocarcinoma cells.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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