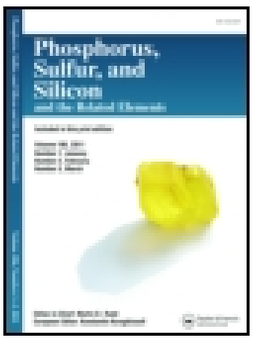

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Convenient synthesis and antibacterial activity of novel 5-phenyldiazenyl-1,3,4-thiadiazole derivatives

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ABSTRACT

Reaction of *N*,2-diaryldiazene-1-carbohydrazonoyl chlorides **1, 2** with 2-((methylthio)-carbonthioyl)-hydrazones **7, 12** and **18–21** in absolute ethanol at room temperature in the presence of triethylamine afforded the corresponding 1,3,4-thiadiazole derivatives **10, 11, 13, 14** and **22–29**. Stirring of *N*,2-diaryldiazene-1-carbohydrazonoyl chlorides **1–3** with thioanilides **31A–E** in acetonitrile at room temperature in the presence of triethylamine gave the corresponding 1,3,4-thiadiazol-2(3*H*)-ylidene derivatives **34–36**. The structures of all new compounds **10, 11, 13, 14, 22–29** and **34–36** were identified by elemental analysis and spectral data. Some new synthesized compounds were studied against *Staphylococcus aureus* and *Escherichia coli* and the most potent compounds were 3-phenyl-5-(phenyldiazenyl)-2-((1-(pyridin-2-yl)ethylidene)hydrazono)-2,3-dihydro-1,3,4-thiadiazole (**13c**), 2-((1-(pyridin-2-yl)ethylidene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole (**14c**) and 2-(benzo[*d*]thiazol-2-yl)-2-(3-(p-tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-acetonitrile (**35C**).

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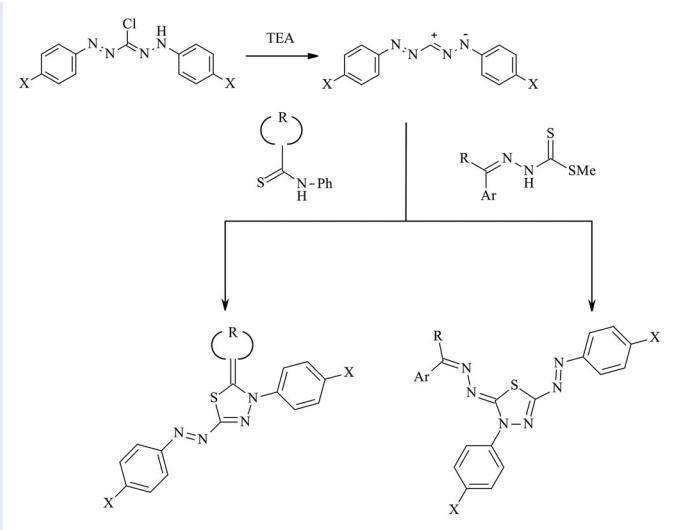
KEYWORDS
Chloroformazans; methyl hydrazinecarbodithioate; cycloaddition reaction; thioanilides; nitrileimines and antimicrobial activity

GRAPHICAL ABSTRACT



Introduction

Despite a large amount of literature[1–11] on formazans, little attention has been reported on the use of chloroformazans in heterocyclic synthesis of sulfur containing compounds.[3–5] It is well known that thiadiazole derivatives were formed *via* reactions of hydrazonoyl halides with potassium thiocyanate[12–16] or thiosemicarbazide and its aryl derivatives[17] or carbon disulfide,[18] and from reaction of *N*-benzylidenebenzohydrazonoyl chloride with potassium ethyl xanthate,[19] *N*-phenylbenzohydrazonoyl chloride with phenylisothiocyanate,[20–22] in addition to coupling of aroyldimethylsulfonium bromides with *N*-nitroso-*N*-arylacetamide.[23,24] Many substituted 1,3,4-thiadiazole derivatives exhibit wide range of biological activities such as antimicrobial, antituberculosis, antiviral, anti-inflammatory, anticancer, antidiabetic and anticonvulsant activities.[25] Also, 2-arylazo-1,3,4-thiadiazolines were prepared from reaction of chloroformazans with potassium thiocyanate.[3] In continuation of our work concerning the synthesis of heterocyclic compounds containing sulfur. Here, we wish to report a convenient synthetic procedure for the synthesis of 5-

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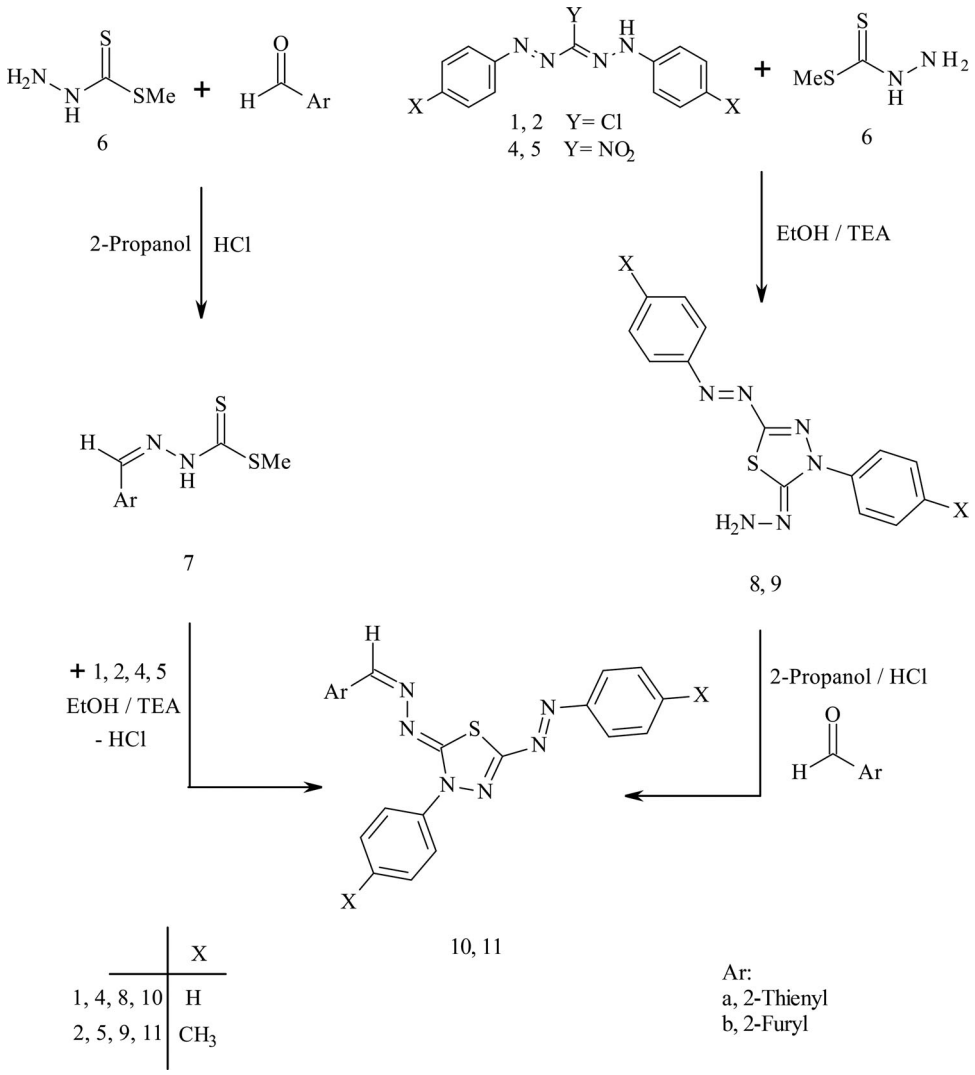


Scheme 1. Synthesis of 1,3,4-thiadiazoles **10,11**.

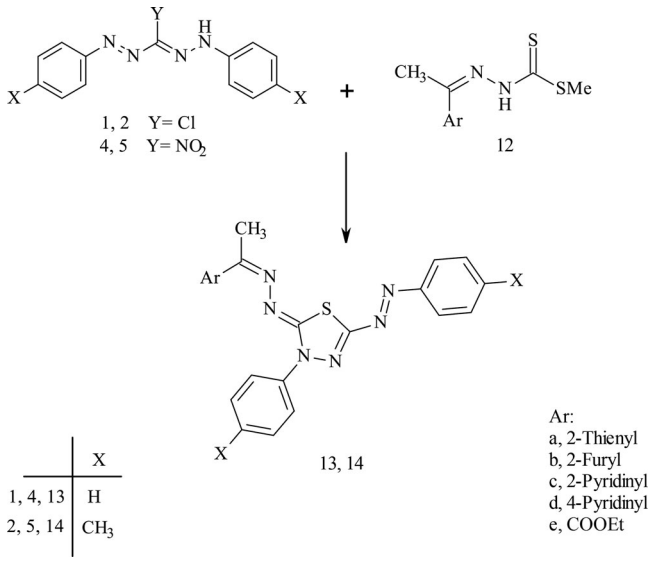
phenyldiazenyl-1,3,4-thiadiazole derivatives *via* reaction of chloroformazans with methyl hydrazinecarbodithioate or with thioanilides. To our knowledge, the latter products have not yet been reported in literature.

Results and discussion

Reaction of 2-((methylthio)carbonthioyl)hydrazones **7, 12**[26–30] with *N*,2-diaryldiazene-1-carbohydrazonoyl chlorides **1, 2** at room temperature in absolute ethanol in the presence of triethylamine afforded the corresponding 1,3,4-thiadiazole derivatives **10, 11, 13, 14** (Schemes 1 and 2). The latter products **10, 11, 13, 14** were prepared by alternative methods *via* treatment of 2-hydrazono-3-aryl-5-(aryldiazenyl)-2,3-dihydro-1,3,4-thiadiazoles **8, 9** with the appropriate aldehydes or ketones in 2-propanol in the presence of few drops of hydrochloric acid at reflux (Schemes 1 and 2).

Moreover, the 1,3,4-thiadiazole products **10, 11, 13, 14** were obtained *via* reaction of 3-nitro-1,5-diarylformazans **4, 5**[31] with 2-((methylthio)carbonthioyl)hydrazones **7, 12** in absolute ethanol in the presence of triethylamine (Schemes 1 and 2). The structures of isolated products were established by their spectral and elemental analyses (see Experimental). For example, ^{1}H NMR spectrum of compound **14c** showed signals: three singlet at δ 2.43, 2.46 (2CH_3 groups) and 2.60 (CH_3 group) in addition to multiplet signal at δ 7.26–8.64 corresponding to 12 aromatic protons. Its ^{13}C NMR spectrum showed 19 signals for asymmetric carbon atoms. Also, its mass spectrum showed the molecular ion peak at $m/z = 427$. The reaction involved initially the formation of the intermediate cycloadduct **17** which underwent *in situ* elimination of methyl mercaptan to give **10, 11, 13, 14** as end products (Scheme 3). Alternatively, the reaction involved first the formation of the thiohydrazonate ester **16** which underwent cyclization followed by elimination of methyl mercaptan (Scheme 3).

Analogously, methyl cyclocarbodithioates **18–21**[32,33] were reacted with *N*,2-diaryldiazene-1-carbohydrazonoyl chlorides **1, 2** or 3-nitro-1,5-diarylformazans **4, 5** in absolute ethanol at room temperature in the presence of triethylamine afforded the corresponding 1,3,4-thiadiazole derivatives **22–29** (Scheme 4). Elemental and spectral data verified the structures of the latter products (see Experimental). For example ^{1}H NMR spectrum of compound **29** showed signals: two singlet at δ 2.43, 2.47



Scheme 2. Synthesis of 1,3,4-thiadiazoles **13,14**.

($2CH_3$ groups) and two triplet at δ 2.84, 2.97 ($2CH_2$ groups) in addition to multiplet signals at δ 1.94–1.98 and 7.17–8.37 corresponding to CH_2 group of tetralone moiety and 12 aromatic protons, respectively. Its ^{13}C NMR spectrum showed 22 signals for asymmetric carbon atoms. Also, its mass spectrum showed the molecular ion peak at $m/z = 452$.

Stirring of thioanilides **31A–E**[34–40] with *N*,2-diaryldiazene-1-carbohydrazonoyl chlorides **1–3** in acetonitrile at room temperature in the presence of triethylamine gave, in each case, one isolable product (Scheme 5). The structures of the isolated products were proved to be **34–36** on the basis of both elemental and spectral data (MS, IR, NMR) (see Experimental). For example, IR spectrum of compound **35A** showed characteristic band at ν 1668 cm^{-1} assignable to C = O stretching frequency. Its ^1H NMR spectrum showed signals: four singlet at δ 0.98 ($2CH_3$ groups), 2.30 ($2CH_2$ groups), 2.50 and 2.52 ($2CH_3$ groups) in addition to multiplet signal at δ 7.27–7.95 corresponding to 8 aromatic protons. Also, its ^{13}C NMR spectrum showed 17 signals for asymmetric carbon atoms.

The reaction involved initially the formation of the intermediate cycloadduct **33** which underwent *in situ* elimination of aniline molecule to give **34–36** as end products (Scheme 5). Alternatively, the reaction involved first the formation of the thiohydrazonate ester **32** which underwent cyclization followed by elimination of aniline molecule (Scheme 5). These two suggested pathways were consistent with other literature reports on reactions of hydrazonoyl halide with thioanilides.[38–40]

Antibacterial screening

The antibacterial activities of new synthesized compounds were studied by the disk diffusion method. The antibacterial activities were done on the following pathogenic organisms; the gram-positive bacteria *Staphylococcus aureus*, the gram negative bacteria *Escherichia coli*. The synthesized compounds were used at the concentration of 20 mg/mL using DMSO as a solvent. The Ampicillin and Gentamicin 10 μL/disk was used as a standard antibacterial agent. All tested compounds showed no antibacterial activities except compounds 3-phenyl-5-(phenyldiazenyl)-2-((1-(pyridin-2-yl)ethylidene)hydrazono)-2,3-dihydro-1,3,4-thiadiazole (**13c**), 2-((1-(pyridin-2-yl)ethylidene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole (**14c**) and 2-(benzo[*d*]thiazol-2-yl)-2-(3-(p-tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-acetonitrile (**35C**) (Table S1 Supporting Information). Compound (**14c**) showed high potentiality in growth inhibition of tested microorganism (*S. aureus*) with relative activity of standard antibiotic of 137.7%.

In vitro susceptibility tests were performed to evaluate minimum inhibitory concentration (MIC) was measured by a broth dilution method.[41–43] MIC values were determined for the highly efficient antibacterial compounds using the most sensitive microorganisms. Compound **14c** achieved the lowest MIC values (high efficient derivative) against the sensitive bacterial strain *S.aureus* with MIC value 125 µg/mL, followed by compound **13c** with MIC value 250 µg/mL.

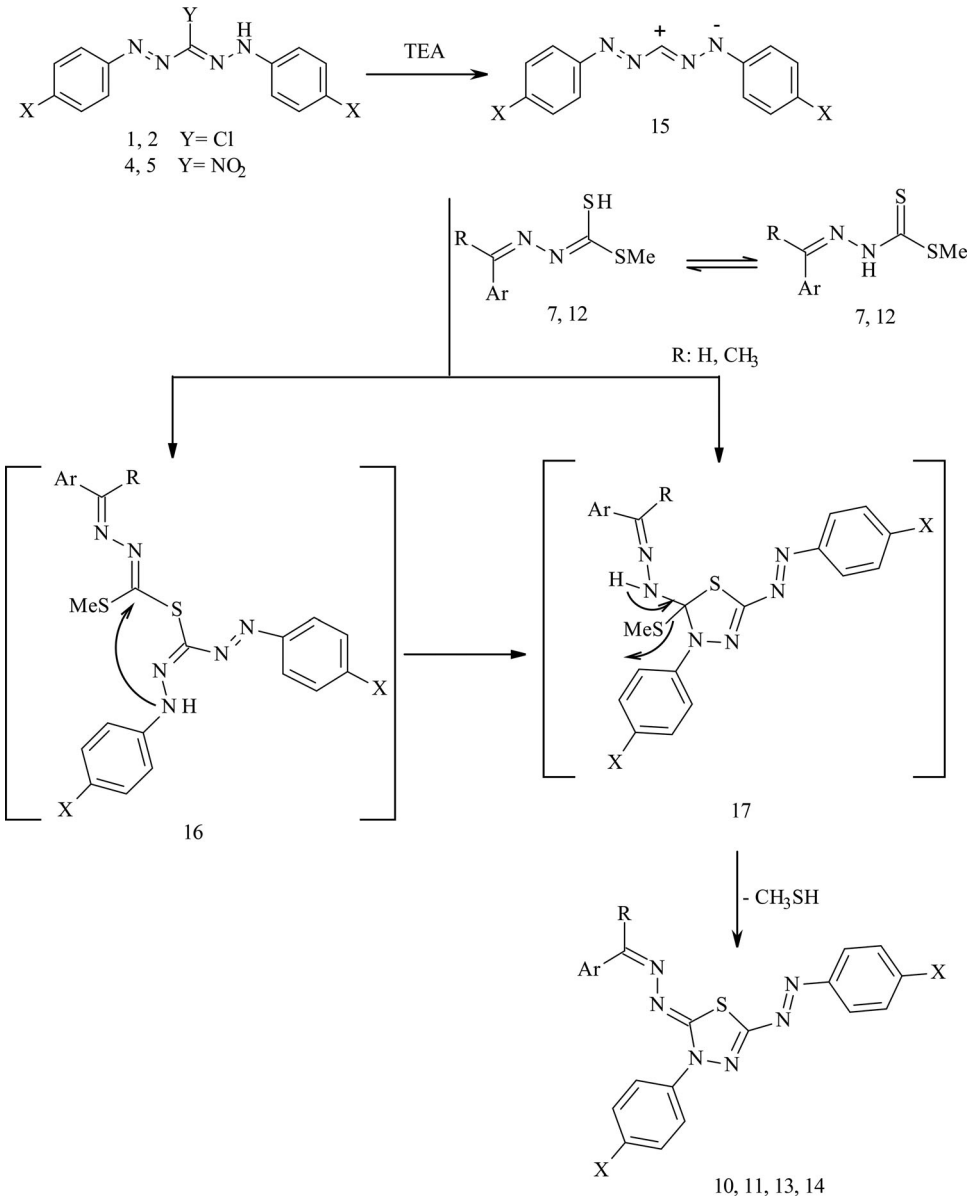
Experimental

Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using a FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 300 MHz and 75 MHz, respectively, using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70 eV) model. The elemental analyses and the *in vitro* antimicrobial testing were performed at the Microanalytical Center, Cairo University. The agar disk diffusion method and a panel of standard strains (*S.* aureus ATCC 6538, *E.* coli ATCC 9637) were employed (Table S1 Supporting Information). The 2-((methylthio)carbon-thioyl)hydrazones **7, 12**[28–30] and **18–21**[32,33] and thioanilides **31A–E**[34–37] were prepared using the reported procedures. The Supporting Information contains sample ^1H and ^{13}C NMR spectra for the products (Supporting Information Figures S1–S20).

Synthesis of 3-aryl-5-(aryldiazenyl)-2,3-dihydro-1,3,4-thiadiazoles (10, 11, 13, 14 and 22–29)

Method A. A mixture of *N*,2-diaryldiazene-1-carbohydrazonoyl chlorides **1, 2** (5 mmol) and the appropriate 2-((methylthio)carbonthioyl)hydrazones **7, 12** and **18–21** (5 mmol) was dissolved in absolute ethanol (50 mL). To the resulting solution triethylamine (2 mL) was added and reaction mixture was stirred for 6 h at room temperature. The resulting solid product that precipitated was collected, washed with ethanol and crystallized from a suitable solvent to give the corresponding 1,3,4-thiadiazole derivatives **10, 11, 13, 14** and **22–29**.

Method B. Stirring of methyl hydrazinecarbodithioate **6** (0.61 g, 5 mmol) with *N*,2-diaryldiazene-1-carbohydrazonoyl chlorides **1, 2** (5 mmol) in absolute ethanol (50 mL) in the



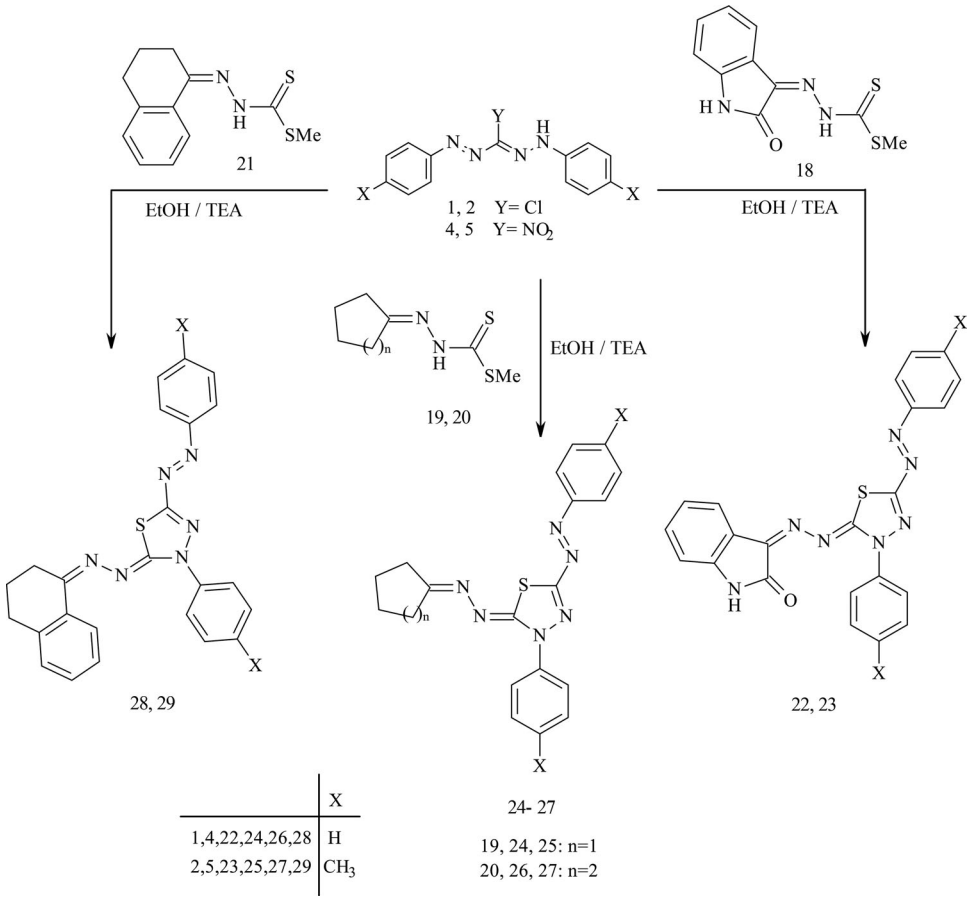
Scheme 3. A plausible mechanism for the formation 1,3,4-thiadiazole derivatives **10,11,13,14**.

presence of triethylamine (2 mL) at room temperature. The solid product of 2-hydrazono-3-aryl-5-(aryldiazenyl)-2,3-dihydro-1,3,4-thiadiazoles **8, 9** was collected, washed with ethanol and crystallized from a suitable solvent. Refluxing of the appropriate aldehydes or ketones (5 mmol) with the resulted compounds **8, 9** (5 mmol) in 2-propanol in the presence of few drops of hydrochloric acid for 4 h. The resulting solid was collected, washed with ethanol and crystallized from a suitable solvent to give the corresponding 1,3,4-thiadiazole derivatives **10, 11, 13, 14** and **22–29**.

Method C. A mixture of 3-nitro-1,5-diarylformazans **4, 5** (5 mmol) and the appropriate 2-((methylthio)carbonthioyl)hydrazones **7, 12** and **18–21** (5 mmol) was dissolved in absolute ethanol (50 mL). To the resulting solution triethylamine (2 mL) was added and reaction mixture was stirred for 6 h at room temperature. The resulting solid product that precipitated was collected, washed with ethanol and crystallized from a suitable solvent to give the corresponding 1,3,4-thiadiazole derivatives **10, 11, 13, 14** and **22–29**.

2-Hydrazono-3-phenyl-5-(phenyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(8)** Black crystals, mp 200–202 °C (DMF), Yield (72%); IR (ν_{max}, cm^{-1}) ν 3420, 3483(NH_2); ^1H NMR (300 MHz, $CDCl_3$) δ 6.01 (s, 2H, NH_2) and 7.14–7.93 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 120.9, 122.6, 123.5, 128.8, 128.9, 129.7, 138.5, 147.6, 149.6, 166.7; MS (EI, 70 eV) *m/z* (%): 296 (M^+, 0.14), 105 (19.01), 77 (100), 51 (16.67); Anal. Calcd. for $C_{14}H_{12}N_6S$ (296.4): C, 56.74; H, 4.08; N, 28.36; S, 10.82. Found: C, 56.67; H, 4.16; N, 28.29; S, 10.75.

2-Hydrazono-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(9)** Dark brown crystals, mp 284–286 °C (DMF), Yield (67%); IR (ν_{max}, cm^{-1}) ν 3418, 3480 (NH_2); ^1H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.01 (s, 2H, NH_2) and 7.29–7.80 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 21.3, 121.5, 128.6, 129.2, 129.9, 131.4, 135.6, 138.6, 144.8, 149.5, 166.6; MS (EI, 70 eV) *m/z* (%): 324 (M^+, 16.24), 119 (13.62), 91 (100), 65 (14.57);



Scheme 4. Synthesis of 1,3,4-thiadiazoles **22–29**.

Anal. Calcd. for $C_{16}H_{16}N_6S$ (324.4): C, 59.24; H, 4.97; N, 25.91; S, 9.88. Found: C, 59.36; H, 4.91; N, 25.97; S, 9.82.

3-Phenyl-5-(phenyldiazenyl)-2-((thiophen-2-ylmethylene) hydrazono)-2,3-dihydro-1,3,4-thiadiazole **(10a)** Dark red crystals, mp 200–202 °C (DMF), Yield (80%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11–7.96 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 120.7, 122.6, 123.3, 126.1, 127.3, 128.6, 128.7, 128.9, 129.6, 138.4, 138.5, 142.3, 147.8, 158.5, 166.6; MS (EI, 70 eV) *m/z* (%): 390 (M^+, 31.3), 105 (25.1), 96 (21.1), 77 (100), 51 (10.7); Anal. Calcd. for $C_{19}H_{14}N_6S_2$ (390.5): C, 58.44; H, 3.61; N, 21.52; S, 16.42. Found: C, 58.36; H, 3.54; N, 21.43; S, 16.35.

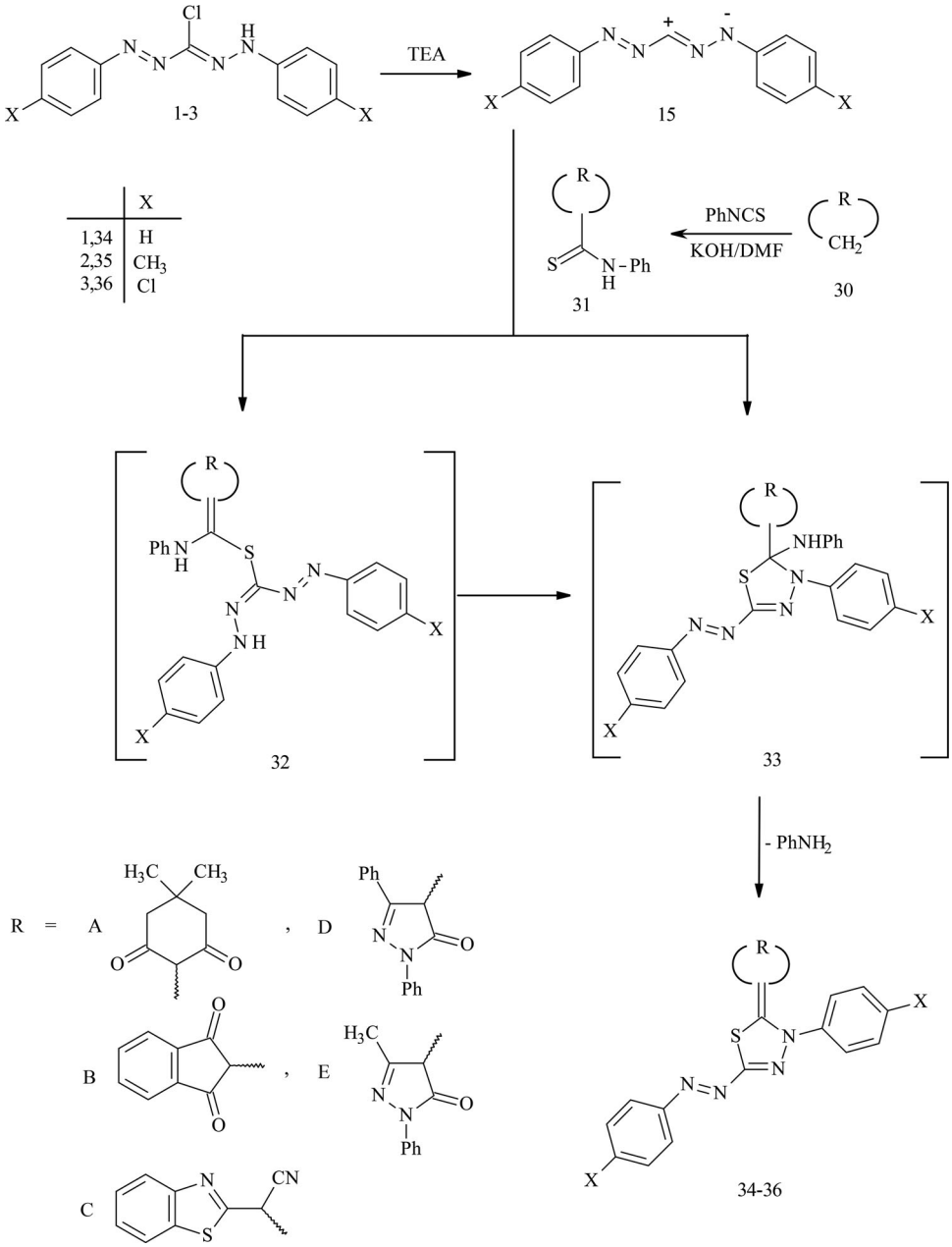
2-((Furan-2-ylmethylene)hydrazono)-3-phenyl-5-(phenyl-diazenyl)-2,3-dihydro-1,3,4-thiadiazole **(10b)** Dark red crystals, mp 190–192 °C (CH_3CN), Yield (71%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.62–8.21 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 112.8, 118.9, 120.8, 122.4, 123.2, 128.7, 128.9, 129.5, 138.4, 144.4, 147.9, 149.1, 158.3, 163.7, 166.4; MS (EI, 70 eV) *m/z* (%): 374 (M^+, 34.9), 105 (11.6), 77 (100), 52 (23.0); Anal. Calcd. for $C_{19}H_{14}N_6OS$ (374.4): C, 60.95; H, 3.77; N, 22.45; S, 8.56. Found: C, 60.88; H, 3.69; N, 22.53; S, 8.49.

2-((Thiophen-2-ylmethylene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(11a)** Dark red crystals, mp 186–188 °C (DMF), Yield (71%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 3H, CH_3), 2.34 (s, 3H, CH_3) and 7.23–7.95 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 21.4, 121.3, 126.2, 127.2, 128.5, 128.7, 129.0, 129.8, 131.2, 135.4, 138.3, 138.4, 142.2, 144.9, 158.3, 166.4; MS (EI, 70 eV) *m/z* (%): 418 (M^+, 75.9), 119 (9.2), 91 (100), 65 (28.1); Anal. Calcd. for $C_{21}H_{18}N_6S_2$ (418.5): C, 60.26; H, 4.34; N, 20.08; S, 15.32. Found: C, 60.32; H, 4.25; N, 20.15; S, 15.24.

2-((Furan-2-ylmethylene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(11b)** Dark red crystals, mp 205–207 °C (CH_3CN), Yield (73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3), 2.47 (s, 3H, CH_3) and 6.51–8.38 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.9, 21.6, 111.8, 112.6, 113.7, 122.9, 123.8, 123.9, 129.3, 129.9, 136.9, 140.2, 144.1, 144.7, 149.8, 150.0, 164.7; Anal. Calcd. for $C_{21}H_{18}N_6OS$ (402.5): C, 62.67; H, 4.51; N, 20.88; S, 7.97. Found: C, 62.60; H, 4.45; N, 20.80; S, 7.89.

3-Phenyl-5-(phenyldiazenyl)-2-((1-(thiophen-2-yl)ethylidene) hydrazono)-2,3-dihydro-1,3,4-thiadiazole **(13a)** Black crystals, mp 188–190 °C (DMF), Yield (70%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.51 (s, 3H, CH_3) and 7.14–7.99 (m, 13H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.1, 122.7, 124.0, 127.7, 128.4, 129.3, 129.6, 130.1, 130.4, 134.2, 139.4, 143.2, 151.7, 157.2, 161.6, 162.7; Anal. Calcd. for $C_{20}H_{16}N_6S_2$ (404.5): C, 59.39; H, 3.99; N, 20.78; S, 15.85. Found: C, 59.29; H, 3.90; N, 20.71; S, 15.79.

2-((1-(Furan-2-yl)ethylidene)hydrazono)-3-phenyl-5-(phenyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(13b)** Dark red crystals, mp 192–194 °C (DMF), Yield (68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.84 (s, 3H, CH_3) and 6.50–8.05 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.8, 109.3, 109.9, 120.6, 122.6, 123.4, 128.7, 128.8, 129.3, 138.7, 141.9, 142.3, 147.7, 158.1, 164.7, 166.8; MS (EI, 70 eV) *m/z* (%): 388 (M^+, 29.3), 105 (23.2), 94 (11.4), 77 (100), 66 (17.3); Anal. Calcd. for



Scheme 5. Synthesis of 1,3,4-thiadiazol-2(*3H*)-ylidene derivatives **34–36**.

$C_{20}H_{16}N_6OS$ (388.4): C, 61.84; H, 4.15; N, 21.64; S, 8.25. Found: C, 61.77; H, 4.07; N, 21.58; S, 8.19.

3-Phenyl-5-(phenyldiazenyl)-2-((1-(pyridin-2-yl)ethylidene)hydrazono)-2,3-dihydro-1,3,4-thiadiazole **(13c)** Dark red crystals, mp 206-208 °C (DMF), Yield (64%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.62 (s, 3H, CH_3) and 7.27–8.65 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 121.3, 122.1, 123.9, 126.8, 128.7, 128.8, 129.4, 133.0, 136.1, 139.5, 148.6, 151.7, 155.7, 158.5, 161.9, 164.8; Anal. Calcd. for $C_{21}H_{17}N_7S$ (399.5): C, 63.14; H, 4.29; N, 24.54; S, 8.03. Found: C, 63.07; H, 4.20; N, 24.46; S, 8.12.

3-Phenyl-5-(phenyldiazenyl)-2-((1-(pyridin-4-yl)ethylidene)hydrazono)-2,3-dihydro-1,3,4-thiadiazole **(13d)** Dark red crystals, mp 222–224 °C (DMF), Yield (76%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 (s, 3H, CH_3) and 7.12–8.68 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 120.5, 122.3, 123.4, 124.0, 127.4, 128.7, 128.9, 129.6, 138.3, 138.4, 147.8, 149.5, 158.4, 166.5; MS (EI, 70 eV) *m/z* (%): 399 (M^+, 29.6), 105 (25.5), 77 (100); Anal. Calcd. for $C_{21}H_{17}N_7S$ (399.5): C, 63.13; H, 4.29; N, 24.54; S, 8.03. Found: C, 63.04; H, 4.21; N, 24.48; S, 8.11.

Ethyl 2-((3-phenyl-5-(phenyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)propanoate **(13e)** Red crystals, mp 148–150 °C (CH_3CN), Yield (63%); IR (ν_{max}, cm^{-1}) ν 1705 (CO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (t, 3H, CH_3), 2.30 (s, 3H, CH_3), 4.27 (q, 2H, CH_2) and 7.44–8.07 (m, 10H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6, 15.3, 61.6, 123.2, 124.2, 128.2, 129.6, 130.5, 134.6, 139.0, 151.6, 154.0, 162.7, 164.6, 167.6; Anal. Calcd. for $C_{19}H_{18}N_6O_2S$ (394.5): C, 57.85; H, 4.60; N, 21.31; S, 8.13. Found: C, 57.79; H, 4.54; N, 21.23; S, 8.05.

2-(((1-(Thiophen-2-yl)ethylidene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(14a)** Black crystals, mp 190–192 °C (DMF), Yield (70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.40 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.48 (s, 3H, CH_3) and 7.07–8.06 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 20.9, 21.3, 121.7, 124.0, 125.7, 127.4, 128.4, 129.1, 129.6, 131.3, 134.5, 138.7, 143.9, 151.7, 157.8, 161.4, 163.7; MS (EI, 70 eV) *m/z* (%): 432 (M^+, 28.8), 119 (15.3), 110 (13.1), 91 (100), 65 (15.4); Anal. Calcd. for $C_{22}H_{20}N_6S_2$ (432.6): C, 61.09; H, 4.66; N, 19.43; S, 14.82. Found: C, 61.16; H, 4.60; N, 19.34; S, 14.76.

2-((1-(Furan-2-yl)ethylidene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(14b)** Black crystals, mp 168–170 °C (CH_3CN), Yield (79%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.84 (s, 3H, CH_3) and 6.50–8.05 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.2, 21.1, 21.3, 109.3, 109.8, 120.6, 121.6, 128.8, 129.1, 129.8, 135.5, 138.6, 141.8, 142.4, 144.7, 158.2, 164.7, 166.9; MS (EI, 70 eV) *m/z* (%): 416 (M^+, 100), 91 (67.5), 65 (19.0); Anal. Calcd. for $C_{22}H_{20}N_6OS$ (416.5): C, 63.44; H, 4.84; N, 20.18; S, 7.70. Found: C, 63.38; H, 4.91; N, 20.23; S, 7.79.

2-((1-(Pyridin-2-yl)ethylidene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(14c)** Dark red crystals, mp 192–194 °C (DMF), Yield (68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.60 (s, 3H, CH_3) and 7.26–8.64 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 21.0, 21.7, 121.2, 122.0, 123.9, 125.8, 129.0, 130.0, 135.0, 136.6, 137.1, 144.2, 148.6, 149.9, 155.8, 161.5, 162.2, 164.7; MS (EI, 70 eV) *m/z* (%): 427 (M^+, 62.2), 91 (100), 78 (26.8), 65 (25.5); Anal. Calcd. for $C_{23}H_{21}N_7S$ (427.5): C, 64.61; H, 4.92; N, 22.93; S, 7.50. Found: C, 64.52; H, 4.84; N, 22.85; S, 7.56.

2-((1-(Pyridin-4-yl)ethylidene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(14d)** Dark red crystals, mp 220–222 °C (DMF), Yield (76%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.46 (s, 3H, CH_3) and 7.27–8.67 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 21.2, 21.5, 121.3, 124.2, 127.3, 128.7, 128.9, 129.1, 129.7, 135.6, 138.2, 138.3, 144.7, 149.7, 158.5, 166.8; Anal. Calcd. for $C_{23}H_{21}N_7S$ (427.5): C, 64.62; H, 4.95; N, 22.93; S, 7.50. Found: C, 64.56; H, 4.89; N, 22.88; S, 7.57.

Ethyl-2-((3-(p-tolyl)-5-((p-tolyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)propanoate **(14e)** Red crystals, mp 176–178 °C (CH_3CN), Yield (85%); IR (ν_{max}, cm^{-1}) ν 1707 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 4.34 (q, 2H, CH_2) and 7.27–8.00 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 15.1, 21.0, 21.3, 61.4, 121.3, 128.7, 129.0, 129.8, 131.6, 135.2, 138.5, 144.6, 154.0, 158.7, 163.4, 166.6; MS (EI, 70 eV) *m/z* (%): 422 (M^+, 24.9), 119 (17.3), 91 (100), 65 (13.2); Anal. Calcd. for $C_{21}H_{22}N_6O_2S$ (422.5): C, 59.70; H, 5.25; N, 19.89; S, 7.59. Found: C, 59.79; H, 5.17; N, 19.80; S, 7.51.

3-((3-Phenyl-5-(phenyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)indolin-2-one **(22)** Dark red crystals, mp 338–340 °C (DMF), Yield (73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12–7.91 (m, 14H) and 10.03 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 117.5, 119.3, 120.7, 122.2, 123.5, 124.6, 128.8, 128.9, 129.1, 129.3, 131.4, 138.1, 138.5, 141.4, 147.7, 158.5, 166.2, 169.2; MS (EI, 70 eV) *m/z* (%): 425 (M^+, 33.7), 131 (10.5), 105 (22.7), 77 (100); Anal. Calcd. for $C_{22}H_{15}N_7OS$ (425.5): C, 62.11; H, 3.55; N, 23.04; S, 7.54. Found: C, 62.20; H, 3.47; N, 23.12; S, 7.48.

3-((3-(p-Tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)indolin-2-one **(23)** Dark red crystals, mp 282–284 °C (DMF), Yield (80%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.29–7.92 (m, 12H) and 10.02 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 21.5, 117.6, 119.7, 120.5, 121.5, 123.4, 124.8, 128.3, 129.2, 129.4, 131.3, 135.6, 138.1, 138.3, 141.3, 144.7, 158.2, 166.5, 169.1; Anal. Calcd. for $C_{24}H_{19}N_7OS$ (453.5): C, 63.56; H, 4.22; N, 21.62; S, 7.07. Found: C, 63.49; H, 4.16; N, 21.55; S, 7.14.

2-(Cyclopentylidenehydrazono)-3-phenyl-5-(phenyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(24)** Dark red crystals, mp 196–198 °C (DMF), Yield (73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.69–1.83 (m, 4H, $2CH_2$), 2.58 (t, 4H, $2CH_2$) and 7.27–8.21 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.7, 32.9, 38.8, 120.5, 122.6, 123.4, 128.5, 129.4, 138.1, 147.6, 158.2, 164.8, 166.4; MS (EI, 70 eV) *m/z* (%): 362 (M^+, 100), 105 (12.4), 77 (89.6); 51 (11.2); Anal. Calcd. for $C_{19}H_{18}N_6S$ (362.5): C, 62.96; H, 5.01; N, 23.19; S, 8.85. Found: C, 62.89; H, 5.10; N, 23.11; S, 8.78.

2-(Cyclopentylidenehydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(25)** Red crystals, mp 190–192 °C (DMF), Yield (75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82–1.87 (m, 4H, $2CH_2$), 2.40 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.61 (t, 4H, $2CH_2$) and 7.26–8.00 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8, 21.2, 24.9, 32.6, 121.5, 128.4, 129.4, 129.9, 131.0, 135.3, 138.3, 144.6, 158.2, 164.8, 166.3; MS (EI, 70 eV) *m/z* (%): 390 (M^+, 21.5), 119 (16.4), 91 (100), 65 (12.8); Anal. Calcd. for $C_{21}H_{22}N_6S$ (390.5): C, 64.59; H, 5.68; N, 21.52; S, 8.21. Found: C, 64.51; H, 5.61; N, 21.60; S, 8.15.

2-(Cyclohexylidenehydrazono)-3-phenyl-5-(phenyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(26)** Red crystals, mp 148–150 °C (CH_3CN), Yield (70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.69–1.78 (m, 6H, $3CH_2$), 2.46 (t, 4H, $2CH_2$) and 7.27–8.18 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 26.6, 27.5, 29.5, 121.9, 123.8, 126.4, 128.8, 129.3, 132.8, 139.7, 140.2, 151.8, 169.8; Anal. Calcd. for $C_{20}H_{20}N_6S$ (376.5): C, 63.81; H, 5.35; N, 22.32; S, 8.52. Found: C, 63.89; H, 5.28; N, 22.24; S, 8.45.

2-(Cyclohexylidenehydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-dihydro-1,3,4-thiadiazole **(27)** Red crystals, mp 160–162 °C (CH_3CN), Yield (67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72-1.86 (m, 6H, $3CH_2$), 2.44 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.70 (t, 4H, $2CH_2$) and 7.25–7.90 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0, 21.2, 25.8, 27.3, 28.8, 121.5, 128.4, 129.2, 129.9, 131.8, 135.7, 138.9, 144.2, 159.1, 161.7, 166.8; Anal. Calcd. for $C_{22}H_{24}N_6S$ (404.5): C, 65.32; H, 5.98; N, 20.77; S, 7.93. Found: C, 65.26; H, 5.91; N, 20.69; S, 7.87.

2-((3,4-Dihydronaphthalen-1(2H)-ylidene)hydrazono)-3-phenyl-5-(phenyldiazenyl)-2,3-di-hydro-1,3,4-thiadiazole **(28)** Dark red crystals, mp 180–182 °C (DMF + CH_3CN), Yield (81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96–1.99 (m,

2H, CH_2), 2.85 (t, 2H, CH_2), 2.98 (t, 2H, CH_2) and 7.16–8.38 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8, 24.3, 29.6, 120.5, 122.5, 123.3, 124.4, 126.2, 128.1, 128.4, 128.5, 128.7, 129.7, 131.4, 138.5, 138.9, 147.8, 158.7, 164.7, 166.6; Anal. Calcd. for $C_{24}H_{20}N_6S$ (424.5): C, 67.90; H, 4.75; N, 19.80; S, 7.55. Found: C, 67.97; H, 4.68; N, 19.87; S, 7.59.

2-((3,4-Dihydronaphthalen-1(2H)-ylidene)hydrazono)-3-(p-tolyl)-5-(p-tolyldiazenyl)-2,3-di-hydro-1,3,4-thiadiazole **(29)** Dark red crystals, mp 198–200 °C (DMF), Yield (81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94–1.98 (m, 2H, CH_2), 2.43 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.84 (t, 2H, CH_2), 2.97 (t, 2H, CH_2) and 7.17–8.37 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 21.7, 22.2, 28.1, 30.0, 122.0, 122.3, 123.9, 125.8, 126.4, 128.6, 129.9, 130.1, 132.2, 136.5, 137.2, 140.7, 144.1, 150.0, 160.8, 162.2, 163.4; MS (EI, 70 eV) *m/z* (%): 452 (M^+, 51.4), 91 (100), 65 (19.5); Anal. Calcd. for $C_{26}H_{24}N_6S$ (452.6): C, 69.00; H, 5.35; N, 18.57; S, 7.08. Found: C, 69.08; H, 5.41; N, 18.49; S, 7.16.

Synthesis of 1,3,4-thiadiazol-2(3H)-ylidene derivatives (34–36)

These compounds were prepared by the same procedure described for the preparation of thiadiazole derivatives **10, 11, 13, 14** and **22–29** (Method A) using thioanilides **31A–E** (5 mmol) in place of 2-((methylthio)carbonthioyl)hydrazones **7, 12** and **18–21** in acetonitrile (50 mL). The resulting solid product that precipitated was collected, washed with ethanol and crystallized from a suitable solvent to afford the corresponding 1,3,4-thiadiazole derivatives **34–36**. The products **34–36** prepared together with their physical constants are listed below:

5,5-Dimethyl-2-(3-phenyl-5-(phenyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)cyclohexane-1,3-dione **(34A)** Red crystals, mp 188–190 °C (EtOH), yield (70%); IR (ν_{max}, cm^{-1}) ν 1664 (CO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.97 (s, 6H, $2CH_3$), 2.30 (s, 4H, $2CH_2$) and 7.20–7.95 (m, 10H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.9, 30.7, 50.9, 105.3, 123.2, 124.6, 129.7, 131.3, 138.5, 141.4, 146.8, 149.5, 162.6, 167.4, 192.6; MS (EI, 70 eV) *m/z* (%): 404 (M^+,9.9),105 (47.0), 77 (100); Anal. Calcd. for $C_{22}H_{20}N_4O_2S$ (404.5): C, 65.33; H, 4.98; N, 13.85; S, 7.93. Found: C, 65.42; H, 4.87; N, 13.92; S, 8.02.

2-(3-Phenyl-5-(phenyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-1H-indene-1,3(2H)-dione **(34B)** Red crystals, mp 284–286 °C (DMF), yield (82%); IR (ν_{max}, cm^{-1}) ν 1648 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–8.07 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 112.3, 119.6, 120.6, 122.7, 127.1, 128.6, 128.9, 129.8, 135.3, 140.2, 146.4, 147.7, 149.6, 166.7, 190.6; MS (EI, 70 eV) *m/z* (%): 410 (M^+, 27.7), 248 (3.2), 105 (28.19), 77 (100); Anal. Calcd. for $C_{23}H_{14}N_4O_2S$ (410.5): C, 67.30; H, 3.44; N, 13.65; S, 7.81. Found: C, 67.22; H, 3.35; N, 13.56; S, 7.70.

2-(Benzo[d]thiazol-2-yl)-2-(3-phenyl-5-(phenyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-acetonitrile **(34C)** Dark brown crystals, mp 340–342 °C (DMF), yield (81%); IR (ν_{max}, cm^{-1}) ν 2186 (CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12–8.14 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 83.3, 118.9, 119.6, 120.5, 121.4, 122.7, 124.6, 124.7, 125.1, 128.7, 128.8, 129.9, 136.4, 146.5, 147.7, 149.6, 153.7, 160.6, 166.8; MS (EI, 70 eV) *m/z* (%): 438 (M^+, 13.9), 105 (24.35), 77 (100); Anal. Calcd. for $C_{23}H_{14}N_6S_2$ (438.5): C, 63.00; H, 3.22; N, 19.16; S, 14.62. Found: C, 63.09; H, 3.16; N, 19.08; S, 14.55.

2,5-Diphenyl-4-(3-phenyl-5-(phenyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-2,4-dihydro-3H-pyrazol-3-one **(34D)** Black crystals, mp 229–231 °C (DMF), yield (80%); IR (ν_{max}, cm^{-1}) ν 1636 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–8.19 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 104.3, 118.7, 119.4, 120.9, 122.1, 128.1, 128.5, 128.6, 128.7, 128.9, 129.4, 129.7, 131.2, 137.8, 140.4, 142.3, 146.4, 147.7, 155.4, 165.6, 166.7; MS (EI, 70 eV) *m/z* (%): 500 (M^+, 23.8), 337 (14.0), 105 (15.2), 91 (15.4), 77 (100); Anal. Calcd. for $C_{29}H_{20}N_6OS$ (500.6): C, 69.58; H, 4.03; N, 16.79; S, 6.40. Found: C, 69.49; H, 3.93; N, 16.70; S, 6.32.

5-Methyl-2-phenyl-4-(3-phenyl-5-(phenyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-2,4-dihydro-3H-pyrazol-3-one **(34E)** Black crystals, mp 178–180 °C (DMF), yield (82%); IR (ν_{max}, cm^{-1}) ν 1633 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3) and 7.15–8.07 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 104.3, 118.7, 119.5, 120.9, 122.6, 128.2, 128.6, 128.7, 128.9, 129.7, 140.5, 142.3, 146.4, 147.7, 147.9, 165.7, 166.5; MS (EI, 70 eV) *m/z* (%): 438 (M^+, 30.7), 275 (11.2), 105 (14.6), 91 (13.5), 77 (100); Anal. Calcd. for $C_{24}H_{18}N_6OS$ (438.5): C, 65.74; H, 4.14; N, 19.17; S, 7.31. Found: C, 65.64; H, 4.07; N, 19.08; S, 7.23.

5,5-Dimethyl-2-(3-(p-tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)cyclohexane-1,3-dione **(35A)** Red crystals, mp 216–218 °C (CH_3CN), yield (74%); IR (ν_{max}, cm^{-1}) ν 1668 (CO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.98 (s, 6H, $2CH_3$), 2.30 (s, 4H, $2CH_2$), 2.50 (s, 3H, CH_3), 2.52 (s, 3H, CH_3) and 7.27–7.95 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.2, 21.9, 28.5, 30.9, 51.1, 105.1, 123.1, 124.5, 129.8, 131.1, 138.3, 141.1, 146.5, 149.9, 162.0, 168.4, 191.5; Anal. Calcd. for $C_{24}H_{24}N_4O_2S$ (432.5): C, 66.64; H, 5.59; N, 12.95; S, 7.41. Found: C, 66.73; H, 5.68; N, 13.04; S, 7.51.

2-(3-(p-Tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-1H-indene-1,3(2H)-dione **(35B)** Red crystals, mp 296–298 °C (DMF), yield (80%); IR (ν_{max}, cm^{-1}) ν 1650 (CO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 2.52 (s, 3H, CH_3) and 7.34–7.99 (m, 12H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.2, 21.4, 112.1, 119.5, 121.6, 126.9, 128.5, 129.2, 129.9, 131.3, 135.2, 138.2, 143.4, 144.8, 149.4, 166.6, 190.5; MS (EI, 70 eV) *m/z* (%): 438 (M^+, 10.7), 119 (9.9), 91 (100), 65 (22.1); Anal. Calcd. for $C_{25}H_{18}N_4O_2S$ (438.5): C, 68.48; H, 4.14; N, 12.78; S, 7.31. Found: C, 68.39; H, 4.03; N, 12.69; S, 7.22.

2-(Benzo[d]thiazol-2-yl)-2-(3-(p-tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-acetonitrile **(35C)** Black crystals, mp 310–312 °C (DMF), yield (78%); IR (ν_{max}, cm^{-1}) ν 2189 (CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (s, 3H, CH_3), 2.52 (s, 3H, CH_3) and 7.27–8.02 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8, 21.2, 83.6, 118.8, 121.4, 121.7, 124.4, 124.5, 125.5, 128.5, 128.9, 129.2, 129.9, 136.4, 138.6, 143.3, 145.1, 149.5, 153.6, 160.7, 166.5; MS (EI, 70 eV) *m/z* (%): 466 (M^+, 56.1), 119 (12.5), 91 (100), 65 (37.4); Anal. Calcd. for $C_{25}H_{18}N_6S_2$ (466.6): C, 64.36; H, 3.89; N, 18.01; S, 13.74. Found: C, 64.30; H, 3.81; N, 18.10; S, 13.67.

2,5-Diphenyl-4-(3-(p-tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-2,4-dihydro-3H-pyrazol-3-one **(35D)** Black crystals, mp 300–302 °C (DMF), yield (78%); IR (ν_{max}, cm^{-1}) ν 1634 (CO); ^1H NMR (300 MHz, $CDCl_3$) δ 2.21 (s, 3H, CH_3), 2.49 (s, 3H, CH_3) and 6.80–8.18 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 21.3, 104.3, 118.7, 121.5, 128.2, 128.6, 128.8, 128.9, 129.1, 129.4, 129.7, 131.2, 131.3, 137.8, 138.6, 140.4, 142.3, 143.4, 144.8, 155.5, 165.6, 166.6; Anal. Calcd. for $C_{31}H_{24}N_6OS$ (528.6): C, 70.43; H, 4.58; N, 15.90; S, 6.06. Found: C, 70.34; H, 4.49; N, 15.82; S, 5.96.

5-Methyl-2-phenyl-4-(3-(p-tolyl)-5-(p-tolyldiazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-2,4-di-hydro-3H-pyrazol-3-one **(35E)** Black crystals, mp 270–272 °C (DMF), yield (76%); IR (ν_{max}, cm^{-1}) ν 1632 (CO); ^1H NMR (300 MHz, DMSO-d_6) δ 2.47 (s, 3H, N = C-CH_3), 2.50 (s, 3H, CH_3), 2.52 (s, 3H, CH_3) and 7.13–7.98 (m, 13H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.4, 21.2, 21.4, 104.2, 118.6, 121.5, 128.2, 128.8, 128.9, 129.2, 129.9, 138.6, 140.4, 142.2, 143.3, 145.1, 147.7, 147.9, 165.7, 166.4; Anal. Calcd. for $C_{26}H_{22}N_6OS$ (466.6): C, 66.93; H, 4.75; N, 18.01; S, 6.87. Found: C, 66.85; H, 4.65; N, 17.91; S, 6.79.

2-(3-(4-Chlorophenyl)-5-((4-chlorophenyl)diazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-5,5-di-methylcyclohexane-1,3-dione **(36A)** Pale red crystals, mp 266–268 °C (DMF + EtOH), yield (71%); IR (ν_{max}, cm^{-1}) ν 1665 (CO); ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (s, 6H, $2CH_3$), 2.30 (s, 4H, $2CH_2$) and 7.20–7.95 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.7, 30.6, 50.7, 105.4, 123.5, 124.7, 129.4, 131.5, 138.2, 141.5, 146.4, 149.9, 162.9, 167.7, 192.2; MS (EI, 70 eV) *m/z* (%): 472 (M^+,10.04), 349 (24.9), 252 (26.4), 137 (29.3),109 (100), 69 (44.5), 55 (88.8); Anal. Calcd. for $C_{22}H_{18}Cl_2N_4O_2S$ (473.4): C, 55.82; H, 3.83; Cl, 14.98; N, 11.84; S, 6.77. Found: C, 55.69; H, 3.78; Cl, 15.10; N, 11.91; S, 6.85.

2-(3-(4-Chlorophenyl)-5-((4-chlorophenyl)diazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-1H-indene-1,3(2H)-dione **(36B)** Dark red crystals, mp 332–334 °C (DMF), yield (77%); IR (ν_{max}, cm^{-1}) ν 1649 (CO); ^1H NMR (300 MHz, DMSO-d_6) δ 7.22–7.79 (m, 12H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 112.4, 121.6, 126.9, 127.9, 128.7, 129.5, 130.3, 134.5, 135.3, 140.2, 144.4, 146.3, 149.6, 166.7, 190.4; MS (EI, 70 eV) *m/z* (%): 479 (M^+, 25.4), 481 (M^++2, 17.1), 483 (M^++4, 3.5), 478 (90.7), 139 (29.5), 113 (30.8), 111 (100), 75 (43.0); Anal. Calcd. for $C_{23}H_{12}Cl_2N_4O_2S$ (479.3): C, 57.63; H, 2.52; Cl, 14.79; N, 11.69; S, 6.69. Found: C, 57.54; H, 2.43; Cl, 14.68; N, 11.60; S, 6.60.

2-(Benzo[d]thiazol-2-yl)-2-(3-(4-chlorophenyl)-5-((4-chlorophenyl)diazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)acetonitrile **(36C)** Black crystals, mp 338–340 °C (DMF), yield (79%); IR (ν_{max}, cm^{-1}) ν 2185 (CN); ^1H NMR (300 MHz, DMSO-d_6) δ 7.21–8.17 (m, 12H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 83.7, 118.5, 121.1, 121.9, 124.5, 124.7, 125.2, 127.4, 128.6, 129.4, 130.4, 134.6, 136.5, 144.6, 146.2, 149.2, 153.7, 160.8, 166.7; MS (EI, 70 eV) *m/z* (%): 506 (M^+, 10.8), 508 (M^++2, 8.5), 510 (M^++4, 2.2), 139 (28.4), 113 (31.8), 111 (100), 75 (38.6); Anal. Calcd. for $C_{23}H_{12}Cl_2N_6S_2$ (507.4): C, 54.44; H, 2.38; Cl, 13.97; N, 16.56; S, 12.64. Found: C, 54.37; H, 2.33; Cl, 13.92; N, 16.51; S, 12.58.

4-(3-(4-Chlorophenyl)-5-(4-chlorophenyl)diazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-2,5-di-phenyl-2,4-dihydro-3H-pyrazol-3-one **(36D)** Black crystals, mp 298–300 °C (DMF), yield (85%); IR (ν_{max}, cm^{-1}) ν 1638 (CO); ^1H NMR (300 MHz, DMSO-d_6) δ 7.20–8.16 (m, 18H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 104.2, 118.6, 121.4, 128.1, 128.6, 128.7, 128.8, 129.3, 129.8, 130.3, 131.2, 134.4, 137.8, 138.6, 140.5, 142.3, 144.8, 146.2, 155.7, 165.6, 166.5; MS (EI, 70 eV) *m/z* (%): 568 (M^+, 7.11), 570 (M^++2, 5.4), 572 (M^++4, 1.3), 139 (31.2), 113 (30.9), 111 (100), 91 (34.7); Anal. Calcd. for $C_{29}H_{18}Cl_2N_6OS$ (569.5): C, 61.17; H, 3.19; Cl, 12.45; N, 14.76; S, 5.63. Found: C, 61.09; H, 3.09; Cl, 12.37; N, 14.68; S, 5.55.

4-(3-(4-Chlorophenyl)-5-((4-chlorophenyl)diazenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **(36E)** Black crystals, mp 264–266 °C (DMF), yield (74%); IR (ν_{max}, cm^{-1}) ν 1635 (CO); ^1H NMR (300 MHz, $CDCl_3$) δ 2.47 (s, 3H, N = C-CH_3), and 7.19–7.99 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 104.1, 118.7, 121.4, 127.9, 128.1, 128.6, 128.8, 129.8, 130.4, 134.5, 140.5, 142.3, 144.5, 146.2, 147.9, 165.6, 166.5; MS (EI, 70 eV) *m/z* (%): 506 (M^+, 5.0), 508 (M^++2, 3.7), 510 (M^++4, 0.85), 309 (4.1), 113 (31.0), 111 (100), 91 (30.5), 75 (25.9); Anal. Calcd. for $C_{24}H_{16}Cl_2N_6OS$ (507.4): C, 56.81; H, 3.18; Cl, 13.97; N, 16.56; S, 6.32. Found: C, 56.72; H, 3.09; Cl, 13.89; N, 16.56; S, 6.23.

Antibacterial activity evaluation

Antimicrobial activity of the tested compounds was determined using a modified Kirby–Bauer disk diffusion method.[44] Briefly, 100 µL of the test bacteria was grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL for bacteria.[45] Hundred microliter of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Of the many media available, NCCLS recommends Mueller–Hinton agar due to: it results in good batch-to-batch reproducibility. Disk diffusion method for yeasts developed by approved standard method (M44-P) by the (NCCLS, 2009).[46]

Minimum inhibitory concentration study

The MIC values were measured by the broth dilution method.[47] Five hundred micro-liter of a stock solution (10.24 mg/mL) of each tested compound in dimethyl sulfoxide (DMSO) was prepared and then diluted with Mueller–Hinton broth to 1024 μg/mL. The strains were grown briefly at 37 °C in Mueller–Hinton media. After 5 h of bacterial growth, the bacterial culture was diluted to obtain a concentration of 5×10^5 cells/mL. Then, 150 μL bacterial suspensions were added to each well of the flat-bottomed 96-well tissue culture plate. Twofold serial dilutions were carried out from the first well to the tenth well; the final concentrations of the compounds ranged from 1 to 512 μg/mL; and excess media (150 μL) were discarded from the last well. The plates were incubated at 37 °C for 24 h in an electro-heating standing temperature cultivator and were read visually. The MIC of the sample showing no turbidity was recorded as the lowest concentration of compound that inhibited bacterial growth completely. Each assay was run in triplicate.

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References

[1] Irving, H.; Bell, C. F. Studies with Dithizone. Part V. A Comparison of Routes for the Synthesis of [35 S] Dithizones. *J. Chem. Soc.* **1953**, 3538–3543. DOI: 10.1039/jr9530003538.
[2] Araki, S.; Hirose, S.; Konishi, Y.; Nogura, M.; Hirashita, T. Synthesis of 3-(Phenylazo)-1,2,4-Triazoles by a Nucleophilic Reaction of Primary Amines with 5-Chloro-2,3-Diphenyltetrazolium Salt *via* Mesoionic 2,3-Diphenyltetrazolium-5-Aminides. *Beilstein J. Org. Chem.* **2009**, *5*, 8. DOI: 10.3762/bjoc.5.8.
[3] Abdelhamid, A. O.; Abbas, I. M.; Abdallah, M. A.; Fahmi, A. A.; Shawali, A. S. Convenient Synthesis of 3-Arylazopyrazoles and 2-Arylazo-1,3,4-Δ2- Thiadiazoline Derivatives from 3-Nitroformazans. *J. Heterocycl. Chem.* **1985**, *22*, 813–816. DOI: 10.1002/jhet.5570220340.
[4] Shawali, A. S.; Mosselhi, M. A. N.; Farghaly, T. A. Site Selectivity in Reactions of Hydrazonoyl Halides with 2-Amino-3-Quinoxalinethiol: A New General Access to Functionalized 4H-1,3,4-Thiadiazino-[5,6-B]Quinoxalines. *Phosphorus Sulfur Silicon Relat. Elem.* **2005**, *180*, 2391–2402. DOI: 10.1080/104265090921100.
[5] Abdallah, M. A. Convenient Synthesis of 6H-[1,2,4,5]Tetrazino[3,2-B]Quinazolin-6-Ones. *Monatsh. Chem.* **2001**, *132*, 959–965. DOI: 10.1007/s007060170058.
[6] Riyadh, S. M. Novel Regioselective Synthesis and Biological Activity of 6-Phenylazo and 3,6-Bis(Arylazo)-7-Hydroxy-1H-[1,2,4]Triazolo[4,3-a]Pyrimidin-5(4H)-One. J. Chin. *Chem. Soc.* **2005**, *52*, 545–551. DOI: 10.1002/jccs.200500080.
[7] Shawali, A. S.; Sayed, A. R. A Convenient One-Pot Synthesis of 3-Arylazo Derivatives of Azino. *J. Chem. Res.* **2004**, *2004*, 399–401. DOI: 10.3184/0308234041423718.
[8] Shawali, A. S.; Sayed A. R. A Facile Entry for Synthesis of 3-Arylazo Derivatives of [1,2,4]Triazolo[4,3-a] Benzimidazole and [1,2,4]Triazolo[3,4-B]Quinazolin-5-One. *J. Chem. Res.* **2005**, (5) 285–288. DOI: 10.3184/0308234054324020.
[9] Shawali, A. S.; Abdelkhalek, A. A.; Sayed, A. R. Kinetics and Mechanism of Dehydrochlorination of 3-Chloro-1,5-Diarylformazans and Their Mass Spectra. J. Chin. *Chemical Soc.* **2001**, *48*, 693–699. DOI: 10.1002/jccs.200100099.
[10] Hooper, W. D. Recent Chemistry and Uses of Formazans and Tetrazolium Salts. *Rev. Pure Appl. Chem.* **1969**, *19*, 221–241.
[11] Nineham, A. W. The chemistry of formazans and tetrazolium salts. *Chem. Rev.* **1955**, *55*, 355–483. DOI: 10.1021/cr50002a004.
[12] Shawali, A. S.; Abdelhamid, A. O. New Routes to Aroylthiadiazolines and Arylazothiazoles from Phenylglyoxalyl Bromide Arylhydrazones and Phenacyl Thiocyanate. *J. Heterocycl. Chem.* **1976**, *13*, 45–49. DOI: 10.1002/jhet.5570130108.
[13] Shawali, A. S.; Hassaneen, H. M. Chemistry of Aliphatic Hydrazonyl Bromides. I. Reactions of N-(4-Nitrophenyl) Acetohydrazonyl Bromide with. *Indian J. Chem.* **1976**, *14*, 425.
[14] Hassaneen, H. M.; Mousa, H. A. H. C-(2-Thinyl)-N-Arylformohydrazidoyl Chlorides in the Synthesis of Selenadiazolines and Thiadiazolines. *Sulfur Lett.* **1988**, *7*, 93–102.
[15] Hassaneen, H. M.; Shawali, A. S.; Abdallah, T. A. One-Step Synthesis of 2, 3-Dihydrothiadiazole Derivatives from Hydrazonoyl Halides. *Sulfur Lett.* **1992**, *15*, 103–109.
[16] Pocar, D.; Rossi, L. M.; Stradi, R. Preparation of 1-Aryl-4,5- and 5,5-Diamino-4,5-Dihydropyrazoles and 4- and 5-Amino-1-Arylpyrazoles. *Synthesis (Stuttg)* **1976**, *1976*, 684–685. DOI: 10.1055/s-1976-24158.
[17] Quilico, A.; Rend, F. R. Sopra un problema di propagazione del calore. *1st. Lombardo. Sci. Lett.* **1936**, 439. DOI: 10.1002/fedr.19380440505..
[18] Huisgen, R.; Grashey, R.; Seidel, M.; Knupfer, H.; Schmidt, R. 1.3-Dipolare Additionen, III. Umsetzungen Des Diphenylnitrilimins Mit Carbonyl Und Thiocarbonyl-Verbindungen. *Justus Liebigs Ann. Chem.* **1962**, *658*, 169–180. DOI: 10.1002/jlac.19626580115.
[19] Flowers, W. T.; Robinson, J. F.; Taylor, D. R.; Tipping, A. E. Unsaturated Compounds Containing Nitrogen. Part 5. Reactions of 1, 4-Dichloro-1, 4-Diphenyl-2, 3-Diazabutadiene with Nucleophiles. *J. Chem. Soc. Perkin Trans. 1.* **1981**, 356–359. DOI: 10.1039/p19810000356.
[20] Wolkoff, P.; Nemeth, S. T.; Gibson, M. S. Reaction of Hydrazonyl Halides with Derivatives of Thiourea and Thiosemicarbazide; a New Source of C-Amino-and C-Hydrazino-1, 2, 4-Triazoles. *Can. J. Chem.* **1975**, *53*, 3211–3215. DOI: 10.1139/v75-458.
[21] Gomha, S. M.; Edrees, M. M.; Muhammad, Z. A.; El-Reedy, A. A. 5-(Thiophen-2-Yl)-1,3,4-Thiadiazole Derivatives: Synthesis, Molecular Docking and in Vitro Cytotoxicity Evaluation as Potential Anticancer Agents. *Drug Des. Devel. Ther.* **2018**, *12*, 1511–1522. DOI: 10.2147/DDDT.S165276.
[22] Saleh, F. M.; Abdelhamid, A. O.; Hassaneen, H. M. Synthesis and Antimicrobial Activity of New Thiazole and Thiadiazole Derivatives *via* Ethyl Pyruvate. *J. Sulfur Chem.* **2020**, *41*, 130–145. DOI: 10.1080/17415993.2019.1694678.
[23] Eweiss, N. F.; Osman, A. Synthesis of Heterocycles. Part II. New Routes to Acetylthiadiazolines and Alkylazothiazoles. *J. Heterocycl. Chem.* **1980**, *17*, 1713–1717. DOI: 10.1002/jhet.5570170814.
[24] Farag, A. M.; Hassaneen, H. M.; Abbas, I. M.; Shawali, A. S.; Algharib, M. S. Synthesis and Reactions of Some 2-Thienyl- and 2-Thenoyl-Derivatives of Thiazole and Thiadiazoline and Their Selenium Analogs. *Phosphorous Sulfur Relat. Elem.* **1988**, *40*, 243–249. DOI: 10.1080/03086648808072921.
[25] Shawali, A. S. 1,3,4-Thiadiazoles of Pharmacological Interest: Recent Trends in their Synthesis *via* Tandem 1,3-Dipolar Cycloaddition: Review. J. Adv. Res. **2014**, *5*, 1–17. DOI: 10.1016/j.jare.2013.01.004.
[26] Audrieth, L. F.; Scott, E. S.; Kippur, P. S. Hydrazine Derivatives of the Carbonic and Thiocarbonic Acids. I. The Preparation and Properties of Thiocarbohydrazide. *J. Org. Chem.* **1954**, *19*, 733–741. DOI: 10.1021/jo01370a006.
[27] Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. 2-Acetylpyridine Thiosemicarbazones. 1. A New Class of Potential Antimalarial Agents. J. Med. Chem. **1979**, *22*, 855–862. DOI: 10.1021/jm00193a020.
[28] Zohdi, H. F.; Emam, H. A.; Abdelhamid, A. O. Reactions with Hydrazonoyl Halides Xiv [1]. A Convenient Synthesis of 2, 3-Dihydro-1, 3, 4-Thiadiazole Derivatives. *Phosphorus Sulfur Silicon Relat. Elem.* **1997**, *129*, 147–154. DOI: 10.1080/10426509708031589.
[29] Emam, H. A.; Zohdi, H. F.; Abdelhamid, A. O. Reactions with Hydrazonoyl Halides. Part 15.1 A Synthetic Approach to 2,3-Dihydrothiadiazoles. *J. Chem. Res.* **1998**, ***1998***, 12–13. DOI: 10.1039/a705088g.
[30] Zohdi, H. F.; Rateb, N. M.; Sallam, M. M.; Abdelhamid, A. O. Reactions with Hydrazonoyl Halides. Part 20. 1 Synthesis of New Unsymmetrical Azines, Dihydro-1, 3, 4-Thiadiazoles and 5-Arylazothiazoles. *J. Chem. Res.* **1998**, 742–743. DOI: 10.1039/a802941e.
[31] Hutton, A. T. Studies on the Existence of a Tautomeric Equilibrium in Solutions of the Analytical Reagent Dithizone. *Polyhedron* **1987**, *6*, 13–23. DOI: 10.1016/S0277-5387(00)81233-4.
[32] Abdelhamid, A. O.; Mohamed, G. S. Reactions with Hydrazonoyl Halides XXIV [1]: Synthesis of Some New Unsymmetrical Azines and Dihydro-1, 3, 4-Thiadiazoles. *Phosphorus. Sulfur Silicon Relat. Elem.* **1999**, *152*, 115–128. DOI: 10.1080/10426509908031623.
[33] Mahapatra, M.; Kulandaivelu, U.; Saiko, P.; Graser, G.; Szekeres, T.; Andrei, G.; Snoeck, R.; Balzarini, J.; Jayaprakash, V. Methyl-2-Arylidene Hydrazinecarbodithioates: Synthesis and Biological Activity. *Chem. Pap.* **2013**, *67*, 650–656. DOI: 10.2478/s11696-013-0346-4..

[34] Görges, S.; Kristen, H.; Peseke, K.; Farkas, I. Synthesen Von C-Nucleosiden Und C-Nucleosidanaloga. I. Darstellung Von 1-(D-Galacto-2, 3, 4, 5, 6-Pentakisacetoxyhexanoyl)-3-Alkyl-Oder Arylamino-6, 6-Dimethyl-4, 5, 6, 7-Tetrahydro-Benzo [c] Thiophen-4-Onen. *J. Prakt. Chem.* **1983**, *325*, 143–147. DOI: 10.1002/prac.19833250117.

[35] Elwan, N. M.; Hassaneen, H. M.; Hassaneen, H. M. Synthesis and Reactions of Indane-1, 3-Dione-2-Thiocarboxanilides with Hydrazonoyl Halides and Active Chloromethylene Compounds. *Heteroatom Chem.* **2002**, *13*, 585–591. DOI: 10.1002/hc.10132.

[36] Hassaneen, H. M.; Shawali, A. S.; Abdallah, T. A.; Saleh, F. M. Synthesis and Cycloaddition Reactions of 4, 4-Dimethyl-2, 6-Dioxocyclohexane-Thiocarboxamides with Nitrilimines. *Arch. Org. Chem.* **2014**, 2014, 155–169. DOI: 10.3998/ark.5550190.p008.855..

[37] Hassaneen, H. M.; Elwan, N. M.; Abdelhadi, H. A. A. T. A. Reactions of Hydrazonoyl Halides with 3-Thioxo-1,2,4-Triazin-5-Ones. Synthesis of 1,2,4-Triazolo[4,3-b][1,2,4]Triazin-5-One. *Sulfur Lett.* **1995**, *18*, 121.

[38] Fadda, A. A.; Abdel-Latif, E.; El-Mekawy, R. E. Synthesis and Molluscicidal Activity of Some New Thiophene, Thiadiazole and Pyrazole Derivatives. *Eur. J. Med. Chem.* **2009**, *44*, 1250–1256. DOI: 10.1016/j.ejmech.2008.09.006.

[39] Hassaneen, H. M.; Hassaneen, H. M.; Mohammed, Y. S. Reactivity of 1-Methylisoquinoline Synthesis of Pyrazolyl Triazoloisoquinoline and Thia-Diazolyl Isoquinoline Derivatives. *Nat. Sci.* **2011**, *03*, 651–660. DOI: 10.4236/ns.2011.38089.

[40] Hassaneen, H. M.; Hassaneen, H. M.; Gomaa, Z. A. Reactivity of 3-Cyanoacetylindole Derivatives: Synthesisof 3-Hydrazonopyrazolyl and 3-Thiadiazolyl Indole Derivatives. *Int. J. Org. Chem.* **2011**, *01*, 97–104. DOI: 10.4236/ijoc.2011.13015.

[41] Geng, Z. Z.; Zhang, J. J.; Lin, J.; Huang, M. Y.; An, L. K.; Zhang, H. B.; Sun, P. H.; Ye, W. C.; Chen, W. M. Novel Cajaninstilbene Acid Derivatives as Antibacterial Agents. Eur. J. Med. Chem. **2015**, *100*, 235–245. DOI: 10.1016/j.ejmech.2015.06.008.

[42] Lei, X.; Li, J.; Liu, B.; Zhang, N.; Liu, H. Separation and Identification of Four New Compounds with Antibacterial Activity from *Portulaca oleracea* L. *Molecules* **2015**, *20*, 16375–16387. DOI: 10.3390/molecules200916375.

[43] Dawood, K. One-Pot Synthesis of Novel Polysubstituted Pyrazole and Pyrrolo [2, 1-B] Benzothiazole Derivatives. *J. Chem. Res.* **1998**, 128–129. DOI: 10.1039/a706107b.

[44] Bauer, A. W.; Kirby, W. M.; Sherris, J. C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Am. J. Clin. Pathol. **1966**, *45*, 493–496.

[45] Pfaller, M. A.; Burmeister, L.; Bartlett, M. S.; Rinaldi, M. G. Multicenter Evaluation of Four Methods of Yeast Inoculum Preparation. J. Clin. Microbiol. **1988**, *26*, 1437–1441. DOI: 10.1128/JCM.26.8.1437-1441.1988.

[46] Cossu, F.; Mastrangelo, E.; Milani, M.; Sorrentino, G.; Lecis, D.; Delia, D.; Manzoni, L.; Seneci, P.; Scolastico, C.; Bolognesi, M. Designing Smac-Mimetics as Antagonists of XIAP, cIAP1, and cIAP2. Biochem. Biophys. Res. Commun. **2009**, *378*, 162–167. DOI: 10.1016/j.bbrc.2008.10.139.

[47] Jordão, A. K.; Novais, J.; Leal, B.; Escobar, A. C.; dos Santos, H. M.; Castro, H. C.; Ferreira, V. F. Synthesis Using Microwave Irradiation and Antibacterial Evaluation of New N,O-Acetals and N,S-Acetals Derived from 2-Amino-1,4-Naphthoquinones. Eur. J. Med. Chem. **2013**, *63*, 196–201. DOI: DOI: 10.1016/j.ejmech.2013.01.010.