

Synthesis, characterization and antibacterial studies of dichlorodiazadienes

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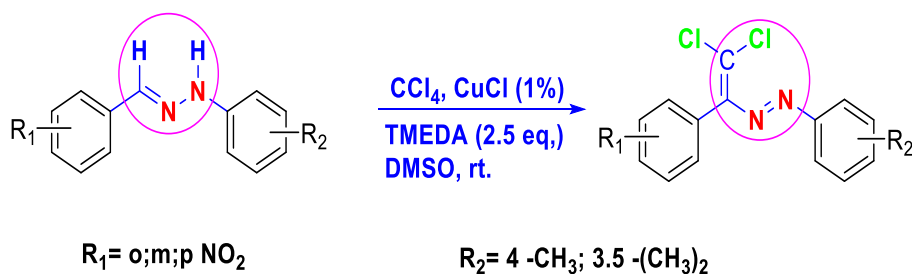
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Abstract

The synthesis of dichlorodiazadienes containing nitro and methyl groups was performed starting from phenylhydrazones. The role of functional groups in the crystal design was investigated by X-ray crystallographic analysis. Pnicogen (N...Cl), halogen (Cl...O) and halogen-halogen (Cl...Cl) bonds were found in the synthesized compounds. Antibacterial studies were performed against five bacterial strains and potent antibacterial activity was detected against *A.baumannii* BDU32 and *S.aureus* BDU23.

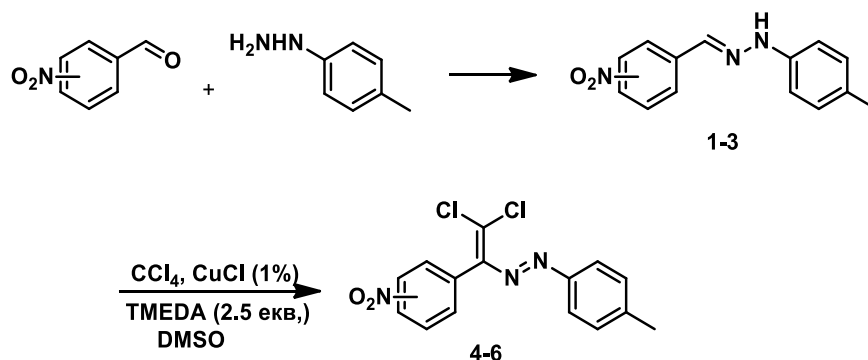


Keywords: Dichlorodiazadienes, phenylhydrazones, catalytic olefinization, antibacterial activity, pnicogen and halogen non-covalent bonds

Introduction

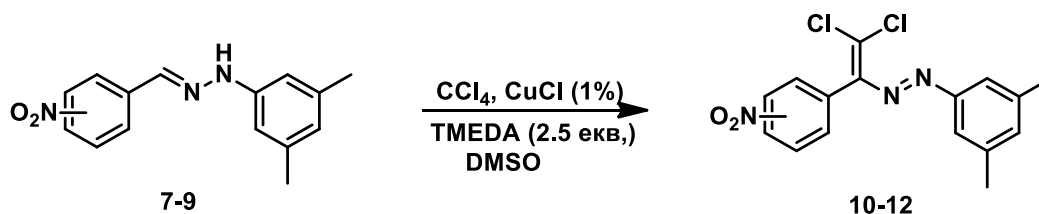
1,1-Dichlorodiazadienes are a valuable class of electrophiles. These compounds can be prepared using the reaction of phenylhydrazones of aldehydes in the presence of CuCl catalyst.¹⁻⁶ Recently we demonstrated that these compounds are interesting diazodyes.^{7,8} In addition, some compounds of this type exhibit antimicrobial properties.^{9,10}

The nitro group is one of the most fundamental functional groups due to its strong electron-withdrawing properties. It is a key component of some drugs, dyes, explosive substances and other high-energy materials.¹¹ Nitroaromatic compounds have been found in a number of natural products, including bacteria, fungi, and plants.¹¹ This paper describes the synthesis of a family of dichlorodiazadienes containing a nitrophenyl component. The methodology used is based on the copper-catalyzed reaction of carbon tetrachloride with arylhydrazones derived from *o*-, *m*-, and *p*-nitrobenzaldehyde and 4-methylphenylhydrazine (Scheme 1). The target products were prepared in up to 80% isolated yields.



Scheme 1. The synthesis of (*E*)-1-(2,2-dichloro-1-(nitrophenyl))-2-(*p*-tolyl) diazene.

To develop a broader family of dichlorodiazadienes, we also replaced 4-methylphenylhydrazine with 3,5-dimethylphenylhydrazine, keeping the aldehydes the same (Scheme 2). As a result, a new set of diazadienes was synthesized in up to 65% yield.



Scheme 2. The synthesis of (*E*)-1-(2,2-dichloro-1-(nitrophenyl) vinyl)-2-(3,5-dimethylphenyl) diazenes.

Compounds **1-12** were synthesized according to the reported method.¹ The antibacterial activity of several synthesized compounds (**4**, **5**, **6**, **10**, **11**, and **12**) was studied by agar well diffusion method.¹² Moreover, the minimum inhibitory concentration (MIC) of the compounds was determined by the two-fold microdilution method.¹³⁻¹⁵

Results and Discussion

Structures of the synthesized compounds have been studied by a number of analytical methods, one of which was X-ray crystallographic method. The molecular structures of compounds **4**, **5**, **6**, **10** and **11** are presented in Figure 1. Crystallographic data for the structural analysis have been deposited in the Cambridge Crystallographic Data Center (CCDC 1841658 for **4**, 1897671 for **5**, 1897670 for **6**, 1898374 for **10**, and 1898098 for **11**). Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: (+44) 1223–336033; Email: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk/data_request/cif). The crystallographic-structure data of the substances are presented in Table 1.

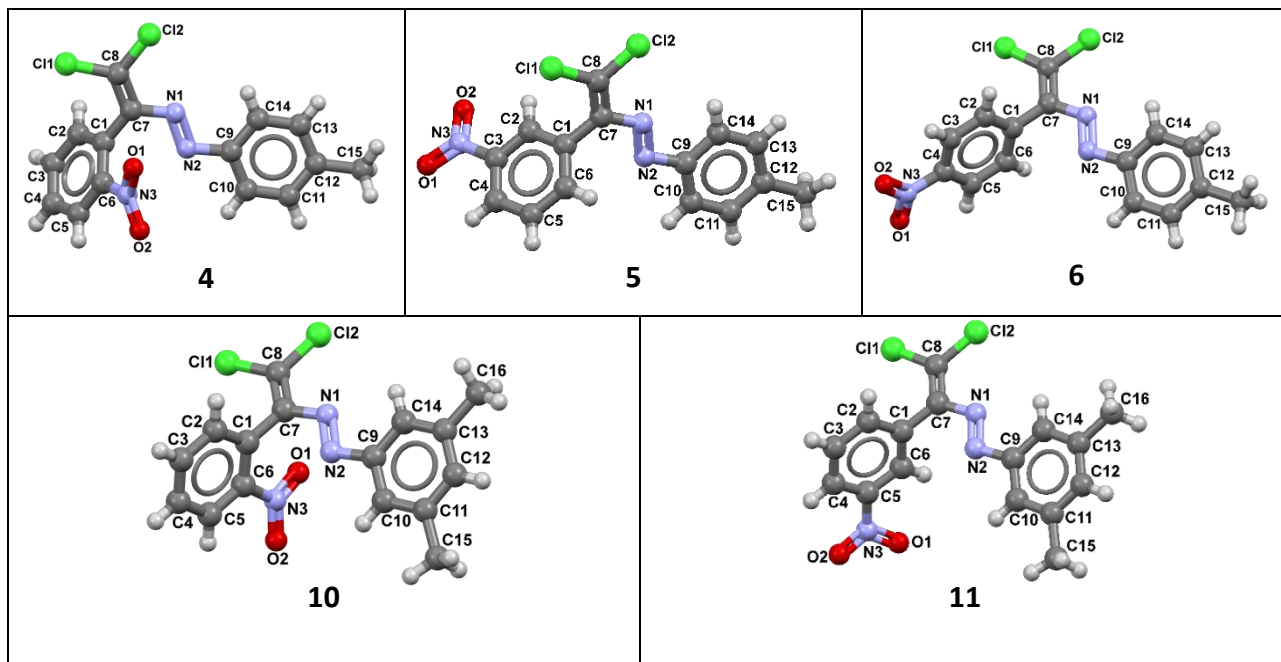


Figure 1. X-ray crystallographic structures of dichlorodiazadienes **4**, **5**, **6**, **10** and **11**.

Table 1. X-ray crystallographic data for **4**, **5**, **6**, **10** and **11**

	4	5	6	10	11
Empirical formula	C ₁₅ H ₁₁ Cl ₂ N ₃ O ₂	C ₁₅ H ₁₁ Cl ₂ N ₃ O ₂	C ₁₅ H ₁₁ Cl ₂ N ₃ O ₂	C ₁₆ H ₁₃ Cl ₂ N ₃ O ₂	C ₁₆ H ₁₃ Cl ₂ N ₃ O ₂
<i>F</i> _w	336.17	336.17	336.17	350.19	350.19
<i>Cryst. Syst.</i>	Triclinic	Monoclinic	Orthorhombic	Monoclinic	Triclinic
<i>Space group</i>	P-1	P2 ₁ /c	Pna2 ₁	P2 ₁ /c	P-1
<i>a</i> (Å)	8.3927(14)	7.971(3)	13.904(2)	14.8374(2)	8.0857(6)
<i>b</i> (Å)	14.112(3)	29.176(9)	13.443(2)	8.50440(10)	10.0229(7)
<i>c</i> (Å)	14.251(3)	7.083(2)	8.4016(17)	13.37370(10)	11.9447(9)
α , °	72.648(5)	90	90	90	114.722(2)
β , °	89.988(6)	111.510(12)	90	104.7220(10)	94.057(3)
γ , °	80.342(6)	90	90	90	102.360(3)
<i>V</i> , (Å ³)	1586.0(5)	1532.4(8)	1570.3(5)	1632.13(3)	844.80(11)
ρ_{calc} (g cm ⁻³)	1.408	1.457	1.422	1.425	1.377
μ (Mo <i>K</i> α) (mm ⁻¹)	0.418	0.433	0.423	3.689	0.396
<i>Z</i>	4	4	4	4	2
<i>R</i> 1 ^a (<i>I</i> ≥ 2 σ)	0.0720	0.0656	0.0325	0.0395	0.0439
<i>wR</i> 2 ^b (<i>I</i> ≥ 2 σ)	0.1559	0.1638	0.0809	0.1095	0.1132
<i>GOOF</i>	1.015	1.087	1.038	1.044	1.039

$$^a R1 = \sum ||F_o| - |F_c|| / \sum |F_o|.$$

$$^b wR2 = [\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]]^{1/2}.$$

Non-covalent interactions were detected between molecules by X-ray diffraction. In recent years, extensive research has been carried out for studying the role of non-covalent bonds in the design and synthesis of new compounds. Different types of non-covalent bonds were observed depending on the position of the nitro-group as was demonstrated using compounds **10** and **11**. In these examples, there are non-covalent intermolecular and intramolecular bonds in the the crystal structure (Figure 2). Intermolecular Cl...Cl bonds [3.485 Å] and intramolecular Cl ••• N pnicoen bonds [2.930 Å] were observed in compound **10**. These distances between atoms are shorter than the sum of the Van der Vaals radius (Cl + Cl = 1.75 + 1.75 = 3.5 Å), (N + Cl = 1.55 + 1.75 = 3.30 Å). At the same time, for compound **11**, along with intramolecular pnicoen Cl...N bonds [2.926 Å], intermolecular halogen Cl...O bonds [2.984, 3.301 Å] were found, which were smaller than the sum of the Van der Vaals radius (Cl + O = 1.75 + 1.52 = 3.27 Å). Thus, both intermolecular and intramolecular non-covalent bonds play an important role in the formation of the crystalline structures of compounds **10** and **11**.

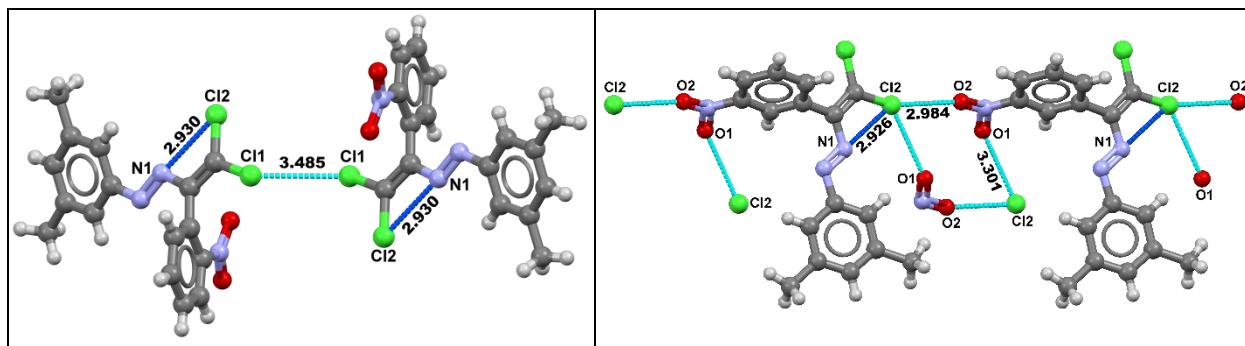


Figure 2. Non-covalent bonds are shown with fractured lines.

Next, antibacterial activity of the prepared compounds was screened against *Escherichia coli* BDU12, *Acenitobacter baumannii* BDU32, *Pseudomonas aeruginosa* BDU49, *Klebsiella pneumonia* BDU44 and *Staphylococcus aureus* BDU23. The compounds were evaluated at a concentration of 0.2% in DMSO by agar well diffusion method¹². The zone of inhibition was measured and the results obtained are shown in Table 2. Compound **5** showed moderate activity against bacterial strains and better activity was detected only against *P.aeruginosa* BDU49. From the results, it was revealed that the antibacterial activity of compound **11** was better against the various bacterial strains than the other compounds. As shown in Table 2, minimal activity against *A.baumannii* BDU32 and *S.aureus* BDU23 bacterial strains was detected in the case of compound **5**, whereas, compound **11** showed better activity when tested against *A.baumannii* BDU32 and *S.aureus* BDU23. Maximum and minimal antibacterial effects of compounds **4** and **10** were found against *K.pneumoniae* BDU44 and *S.aureus* BDU23, respectively. The screening results revealed that activity of compound **6** was weaker than other tested compounds. Moreover, compound **6** was inactive against *A.baumannii* BDU32 and *S.aureus* BDU23, and its maximum activity was observed against *K.pneumoniae* BDU44. Compound **12** has better activity towards *A.baumannii* BDU32 and *S.aureus* BDU23 as compared with compound **6**, whereas **6** is more active in the case of *P.aeruginosa* BDU49. In the case of *P.aeruginosa* BDU49, we can order the antibacterial activity of the compounds shown below as **11**>**12**>**5**>**4**>**10**>**6**.

Table 2. Antibacterial activity of compounds [diameter of inhibition zone (mm)] against bacterial strains

Compound	Bacterial strains				
	<i>A.baumannii</i> BDU32	<i>E.coli</i> BDU12	<i>K.pneumoniae</i> BDU44	<i>P.aeruginosa</i> BDU49	<i>S.aureus</i> BDU23
5	11.0 ± 0.5	15.5 ± 0.6	14.5 ± 0.6	17.0 ± 0.8	11.0 ± 0.5
11	19.5 ± 0.7	16.7 ± 0.8	17.6 ± 0.8	18.3 ± 1.0	20.3 ± 0.7
4	16.3 ± 0.3	14.3 ± 0.7	16.7 ± 0.5	15.7 ± 0.7	13.3 ± 0.4
10	16.0 ± 0.5	16.3 ± 0.3	16.7 ± 0.4	15.3 ± 0.5	13.7 ± 0.5
6	-	12.3 ± 0.4	15.3 ± 0.6	12.7 ± 0.6	-
12	15.3 ± 0.7	16.0 ± 0.5	16.3 ± 0.7	18.7 ± 0.6	14.7 ± 0.6

After initial screening, the antibacterial activity of the compounds using the minimum inhibitory concentration (MIC) was tested by the two-fold microdilution method¹⁵⁻¹⁷. As shown in Table 3, the bacterial strains were more susceptible to compound **11** than the other compounds. Compound **11** had the highest inhibitory effect against *A.baumannii* BDU32 and *S.aureus* BDU23 in (value of 31.25 µg/mL).

Table 3. Minimum inhibitory concentration of compounds ($\mu\text{g/mL}$)

Compound	Bacterial strains				
	<i>A.baumannii</i> BDU32	<i>E.coli</i> BDU12	<i>K.pneumoniae</i> BDU44	<i>P.aeruginosa</i> BDU49	<i>S.aureus</i> BDU23
5	500	1000	2000	500	1000
11	31.25	1000	1000	500	31.25
4	500	1000	2000	500	2000
10	1000	1000	2000	500	2000
6	-	1000	1000	500	-
12	500	2000	2000	500	2000

Moreover, compound **11** presented a MIC of 500 $\mu\text{g/mL}$ for *P.aeruginosa* BDU49, half the value obtained with *E.coli* BDU12 and *K.pneumoniae* BDU44. No inhibition was detected for compound **6** against *S.aureus* BDU23 and *A.baumannii* BDU32. The MIC values of compound **6** ranged from 500-1000 $\mu\text{g/mL}$ for *P.aeruginosa* BDU49, *E.coli* BDU12, and *K.pneumoniae* BDU44, respectively. *E. coli* BDU12 was more susceptible against all of the compounds (1000 $\mu\text{g/mL}$) except compound **12** (2000 $\mu\text{g/mL}$). All compounds reached the similar inhibitory effect (500 $\mu\text{g/mL}$) against *P.aeruginosa* BDU49. The MIC values of compounds **4**, **10** and **12** were identical (2000 $\mu\text{g/mL}$) in the case of *S.aureus* BDU23, and the MIC of compound **5** was two times lower than this value (1000 $\mu\text{g/mL}$). All tested compounds presented MIC values in the range of 1000 – 2000 $\mu\text{g/mL}$ for *K.pneumoniae* BDU44. The MIC value of compounds **4**, **5** and **12** was 500 $\mu\text{g/mL}$ when tested with *A.baumannii* BDU32, while the value was 1000 $\mu\text{g/mL}$ in the case of compound **10**.

Conclusions

A family of nitro-substituted 1,1-dichlorodiazadienes was prepared using the copper-catalysed (CuCl) reaction of phenylhydrazones of aldehydes with CCl_4 . Crystal structures of diazadienes were studied using X-ray crystallography which demonstrated intermolecular and intramolecular non-covalent interactions in the crystals. Some of the prepared 1,1-dichlorodiazadienes exhibited pronounced antibacterial activity.

Experimental Section

General. The syntheses of compounds were carried out at the Chemistry Department of Baku State University (Azerbaijan). The X-ray analyses of compounds **4**, **5**, **6**, **10** and **11** were carried out using the Bruker APEX II CCD (T 296 K, $\lambda\text{MoK } \alpha$ - radiation, graphite monochromator, ϕ - and ω -scan) diffractometer. NMR ^1H and ^{13}C spectra were recorded using a Bruker Avance 300 (working frequency is 300 MHz) using CDCl_3 and DMSO solvents, respectively. SiMe_4 (TMS) was used as an internal standard. Elemental Analysis was performed on a Carlo Erba 1108 Analyzer. Thin-layer chromatography (TLC) was performed on silhouette plate UB-254 and acidified KMnO_4 solution; UV lamp rays were used to make spots visible. Column chromatography was performed on silica gel of Merk firm (63-200). All compounds were crystallized from a methylene chloride and hexane (1:3) solvent system.

General procedure for synthesis of phenylhydrazones. Schiff bases **1–3** and **7–9** were synthesized according to the reported method.^{1–4} A mixture of (2-nitrophenyl) hydrazine (10.2 mmol), CH₃COONa (0.82 g), ethanol (50 mL) and the corresponding 4-substituted aldehyde (10 mmol) was refluxed at 80 °C with stirring for 2 h. The reaction mixture was cooled to room temperature and water (50 mL) was added to give a precipitate of crude product, which was filtered off, washed with diluted ethanol (1:1 with water) and dried under vacuum of a rotary evaporator.

(E)-1-(2-nitrobenzylidene)-2-(p-tolyl)hydrazine (1). Red solid (90%); mp 141 °C. ¹H NMR (300 MHz, DMSO): δ 10.82 (s, 1H, -NH), 8.22 (s, 1H, -CH), 8.17 (d, *J* 9 Hz, 1H, arom), 7.97 (d, *J* 6 Hz, 1H, arom), 7.72 (t, *J* 9 Hz, 1H, arom), 7.50 (t, *J* 9 Hz, 1H, arom), 7.08 (d, *J* 9 Hz, 2H, arom), 7.02 (d, *J* 9 Hz, 2H, arom), 2.50 (s, 3H, -CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 162.3, 147.0, 142.7, 133.6, 130.5, 130.3, 128.9, 128.4, 127.3, 125.0, 112.9, 20.7. Anal. Calcd for C₁₄H₁₃N₃O₂ (*M* 241.25); Calculated %: C, 65.87; H, 5.13; N, 16.46. Found %: C, 65.84; H, 5.15; N, 16.44.

(E)-1-(3-nitrobenzylidene)-2-(p-tolyl)hydrazine (2). Yellow solid, (90%), mp 148 °C. ¹H NMR (300 MHz, DMSO) δ 10.59 (s, 1H, NH), 8.41 (s, 1H, CH), 8.09–8.05 (m, 2H, arom), 7.92 (s, 1H, arom), 7.64 (t, 1H, *J* 9.1 Hz, arom), 7.08–7.00 (m, 4H, arom), 2.22 (s, 3H, CH₃). ¹³C NMR (75 MHz, DMSO) δ 148.8, 142.9, 138.5, 133.5, 131.8, 130.5, 130.1, 128.5, 122.2, 119.8, 112.7, 20.7. Anal. Calcd for C₁₄H₁₃N₃O₂ (*M* 241.25); Calculated %: C, 65.87; H, 5.13; N, 16.46. Found %: C, 65.85; H, 5.17; N, 16.47.

(E)-1-(4-nitrobenzylidene)-2-(p-tolyl)hydrazine (3). Orange solid (87%), mp 152 °C. ¹H NMR (300 MHz, DMSO) δ 10.82 (s, 1H, NH), 8.21 (d, 2H, *J* 6.2 Hz, arom), 7.88 (d, 2H, *J* 6.2 Hz, arom), 7.84 (s, 1H, CH), 7.06 (s, 4H, arom), 2.23 (s, 3H, CH₃). ¹³C NMR (75 MHz, DMSO) δ 162.3, 146.3, 143.2, 142.6, 133.3, 130.1, 129.1, 126.3, 124.5, 113.0, 20.8. Anal. Calcd for C₁₄H₁₃N₃O₂ (*M* 241.25); Calculated %: C, 65.87; H, 5.13; N, 16.46. Found %: C, 65.84; H, 5.16; N, 16.42.

(E)-1-(3,5-dimethylphenyl)-2-(2-nitrobenzylidene)hydrazine (7). Purple solid (92%), mp 147 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.74 (s, 1H, NH), 8.20 (s, 1H, CH-), 8.13 (d, *J* 7.9 Hz, 1H, arom), 7.93 (d, *J* 9.0 Hz, 1H, arom), 7.68 (t, *J* 6.2 Hz, 1H, arom), 7.45 (t, *J* 7.6 Hz, 1H, arom), 2.21 (s, 6H, -CH₃). ¹³C NMR (75 MHz, DMSO) δ 147.0, 144.8, 138.7, 133.6, 130.6, 130.4, 128.5, 127.3, 124.9, 122.2, 110.7, 21.6. Anal. Calcd for C₁₄H₁₃N₃O₂ (*M* 269.30); Calculated %: C, 66.90; H, 5.61; N, 15.60. Found %: C, 66.93; H, 5.63; N, 15.64.

(E)-1-(3,5-dimethylphenyl)-2-(3-nitrobenzylidene)hydrazine (8). Orange solid (87%), mp 169 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.55 (s, 1H, NH), 8.39 (s, 1H, CH), 8.09 (d, *J* 9.6 Hz, 2H, arom), 7.93 (s, 1H, arom), 7.65 (t, *J* 8.0 Hz, 1H, arom), 6.73 (s, 2H, arom), 6.44 (s, 1H, arom), 2.23 (s, 6H, -CH₃). ¹³C NMR (75 MHz, DMSO) δ 162.3, 148.8, 145.1, 138.6, 138.4, 133.8, 131.8, 130.6, 122.3, 121.8, 120.0, 110.6, 21.7. Anal. Calcd for C₁₄H₁₃N₃O₂ (*M* 269.30); Calculated %: C, 66.90; H, 5.61; N, 15.60. Found %: C, 66.92; H, 5.60; N, 15.62.

(E)-1-(3,5-dimethylphenyl)-2-(4-nitrobenzylidene)hydrazine (9). Red solid (93%) mp 150 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.74 (s, 1H), 8.18 (d, *J* 8.5 Hz, 2H), 7.88 (s, 1H), 7.83 (d, *J* 8.5 Hz, 2H), 6.77 (s, 2H), 6.46 (s, 1H), 2.23 (s, 6H). ¹³C NMR (75 MHz, DMSO) δ 146.3, 144.8, 143.1, 138.7, 133.6, 126.3, 124.4, 122.3, 110.9, 31.2, 21.6. Anal. Calcd for C₁₄H₁₃N₃O₂ (*M* 269.30); Calculated %: C, 66.90; H, 5.61; N, 15.60. Found %: C, 66.97; H, 5.62; N, 15.58.

General procedure for synthesis of dichlorodiazadienes. Diazenes **4–6** and **10–12** were synthesized according to the reported method.^{1–4} A 20-mL screw neck vial was charged with DMSO (10 mL), compounds **1–3**, and **7–9** (1 mmol), respectively, tetramethylethylenediamine (TMEDA) (295 mg, 2.5 mmol), CuCl (2 mg, 0.02 mmol) and CCl₄ (20 mmol, 10 equiv). After 1–3 hours (until TLC analysis showed complete consumption of the corresponding Schiff base), the reaction mixture was poured into an ~0.01 M solution of HCl (100 mL, ~pH 2–3), and extracted with dichloromethane (3x20 mL). The combined organic phase was washed with water (3x50 mL), followed by brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated in vacuo by rotary evaporator. The residue was

purified by column chromatography on silica gel using appropriate mixtures of hexane and dichloromethane (3/1-1/1), and the corresponding diazenes were obtained. IR spectra of all of the diazadienes contained adsorption bands at 1350-1360 cm^{-1} and 1520-1590 cm^{-1} .

Single crystals of the dichlorodiazadienes were prepared from a mixture of n-hexane and CH_2Cl_2 solvents by slow evaporation.

(E)-1-(2,2-dichloro-1-(2-nitrophenyl) vinyl)-2-(p-tolyl)diazene (4). Orange solid (74%); mp 92°C. ^1H NMR (300 MHz, CDCl_3): δ 8.22 (d, J 6 Hz, 1H, arom), 7.68 - 7.22 (m, 7H, arom), 2.38 (s, 3H, $-\text{CH}_3$). ^{13}C NMR (75 MHz, CDCl_3): δ 150.6, 150.5, 148.2, 142.9, 134.4, 133.8, 132.3, 130.2, 129.8, 128.5, 124.5, 123.5, 21.7. Anal. Calcd for $\text{C}_{15}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2$ (M 336.17). Calculated %: C, 53.59; H, 3.30; Cl, 21.09; N, 12.50. Found %: C, 53.55; H, 3.37; Cl, 21.04; N, 12.56.

(E)-1-(2,2-dichloro-1-(3-nitrophenyl) vinyl)-2-(p-tolyl)diazene (5). Red solid (69%), mp 141 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.30 (d, 1H, J 9.1 Hz, arom), 8.11 (d, 1H, J 9.1 Hz, arom), 7.68 (d, 2H, J 6.1 Hz, arom), 7.62 (d, 1H, J 6.1 Hz, arom), 7.53 (d, 1H, J 6.1 Hz, arom), 7.27 (d, 2H, J 9.1 Hz, arom), 2.42 (s, 3H, CH_3). ^{13}C NMR (75 MHz, CDCl_3) δ 161.7, 150.7, 150.2, 148.1, 143.0, 136.4, 134.4, 129.9, 129.2, 125.4, 123.7, 123.4, 21.6. Anal Calcd for $\text{C}_{15}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2$ (M 336.17); Calculated %: C, 53.59; H, 3.30; Cl, 21.09; N, 12.50. Found %: C, 53.52; H, 3.35; Cl, 21.00; N, 12.54.

(E)-1-(2,2-dichloro-1-(4-nitrophenyl) vinyl)-2-(p-tolyl)diazene (6). Red solid (80%), mp 140°C. ^1H NMR (300 MHz, CDCl_3) δ 8.28 (d, 2H, J 9.1 Hz, arom), 7.70 (d, 2H, J 9.1 Hz, arom), 7.37 (d, 2H, J 9.1 Hz, arom), 7.26 (d, 2H, J 9.1 Hz, arom), 2.42 (s, 3H, CH_3). ^{13}C NMR (75 MHz, CDCl_3) δ 150.8, 150.5, 147.9, 143.1, 139.7, 135.4, 131.3, 129.9, 123.4, 123.3, 21.6. Anal Calcd for $\text{C}_{15}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2$ (M 336.17); Calculated %: C, 53.59; H, 3.30; Cl, 21.09; N, 12.50. Found %: C, 53.57; H, 3.31; Cl, 21.06; N, 12.48.

(E)-1-(2,2-dichloro-1-(2-nitrophenyl) vinyl)-2-(3,5-dimethylphenyl) diazene (10). Orange solid, (60%), mp 186 °C. ^1H NMR (300 MHz, CDCl_3 -d) δ 8.23 (dd, J 8.0, 1.5 Hz, 1H, arom), 7.67 (td, J 8.0, 1.4 Hz, 2H, arom), 7.34 (dd, J 5.7, 1.6 Hz, 3H, arom), 7.09 (s, 1H, arom), 2.35 (s, 6H, CH_3). ^{13}C NMR (75 MHz, CDCl_3) δ 152.6, 150.4, 148.1, 138.7, 134.6, 133.7, 132.2, 130.0, 128.7, 124.5, 121.2, 31.6, 22.7, 21.1, 14.1. Anal Calcd for $\text{C}_{15}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2$ (M 350.20). Calculated %: C, 54.87; H, 3.74; Cl, 20.25; N, 12.00. Found %: C, 54.81; H, 3.75; Cl, 20.28; N, 12.04.

(E)-1-(2,2-dichloro-1-(3-nitrophenyl) vinyl)-2-(3,5-dimethylphenyl) diazene (11). Red solid (64%), mp 165 °C. ^1H NMR (300 MHz, CDCl_3 -d) δ 8.30 (d, J 7.6 Hz, 1H, arom), 8.10 (s, 1H, arom), 7.64 (t, J 7.6 Hz, 1H, arom), 7.52 (d, J 7.6 Hz, 1H, arom), 7.39 (s, 2H, arom), 7.13 (s, 1H, arom), 2.38 (s, 6H, CH_3). ^{13}C NMR (75 MHz, CDCl_3) δ 162.3, 152.7, 150.3, 148.1, 138.9, 136.4, 134.4, 133.9, 129.2, 125.4, 123.7, 121.15, 21.18. Anal Calcd for $\text{C}_{15}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2$ (M 350.20), Calculated %: C, 54.87; H, 3.74; Cl, 20.25; N, 12.00. Found %: C, 54.85; H, 3.76; Cl, 20.20; N, 12.03.

(E)-1-(2,2-dichloro-1-(4-nitrophenyl) vinyl)-2-(3,5-dimethylphenyl) diazene (12). Red solid, (65%), mp 138°C. ^1H NMR (300 MHz, CDCl_3 -d) δ 8.30 (d, J 8.8 Hz, 2H, arom), 7.37 (dd, J 6.8, 1.9 Hz, 4H, arom), 7.13 (s, 1H, arom), 2.38 (s, 6H, CH_3). ^{13}C NMR (75 MHz, CDCl_3) δ 152.8, 150.6, 147.9, 139.8, 138.9, 133.9, 131.3, 123.4, 121.1, 100.0, 21.2. Anal Calcd for $\text{C}_{15}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2$ (M 350.20). Calculated %: C, 54.82; H, 3.74; Cl, 20.25; N, 12.00. Found % C, 54.89; H, 3.72; Cl, 20.27; N, 12.09.

Antibacterial activity. Initial *in vitro* antibacterial activity of compounds **4**, **5**, **6**, **10**, **11**, and **12** was screened against gram-positive *Staphylococcus aureus* BDU23 and gram-negative *Escherichia coli* BDU12, *Acinetobacter baumannii* BDU32, *Pseudomonas aeruginosa* BDU49, *Klebsiella pneumonia* BDU44 by the agar well diffusion method as described elsewhere.⁵ Bacterial strains were taken from culture collections of the Department of Microbiology of Baku State University (Azerbaijan). Firstly, the bacterial strains were grown in Muller Hinton broth overnight at 37 °C. The optical density of the overnight bacterial cultures was adjusted to 0.5 McFarland (1.5×10^8 cfu/mL) by diluting in saline solution (0.8% NaCl). Then 0.1 mL of this bacterial culture suspension was

spread on sterile Muller Hinton Agar (MHA) plates and the plates were kept about 10 min. After adsorption, the wells of 8 mm diameter were punched with the sterile tips or glass cork borer. The solution of test compounds (0.2%), which were prepared freshly by dissolving of the chemicals in DMSO, were incorporated into the wells, and the plates were incubated at 37°C for 24 h. After incubation, the antibacterial properties of test compounds were evaluated according to the diameter of the inhibition zone (mm). Control experiments were carried out using DMSO solvent and no activity was detected against the studied bacterial strains.⁵

Determination of minimum inhibitory concentration (MIC). Minimum inhibitory concentrations of six compounds were determined based on CLSI by the two-fold microdilution method using 96-well microtiter plates (U-bottom).¹⁵⁻¹⁷ The tested compounds were prepared according to CLSI guidelines and diluted in microplates which contained Muller Hinton broth. At the end of microdilution, the compounds' concentrations ranged from 2000 to 15.625 µg/mL. The inoculum was prepared freshly on Muller Hinton broth (MHB) ("Liofilchem") and bacterial strains [10^5 colony forming units (CFU)] were inoculated to each well of the microplate and kept for incubation at 37 °C for 24 h. After incubation, the growth of the bacterial strains was determined by the resazurin method. So, 30 µl of resazurin solution (0.01%) ("Sigma Aldrich") were added to each well and the microplates were left for incubation at 37 °C for about 4 h. MIC is the lowest concentration of the tested compounds that inhibits the growth of the bacteria. In our assay, MIC was determined as the lowest concentration of the compounds which prevented the change in color from blue to pink since the color change from blue to pink indicated the growth of bacteria.¹⁸

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References

1. Nenajdenko, V.G.; Shastin, A.V.; Gorbachev, V.M.; Shorunov, S. V.; Muzalevskiy, V.M.; Lukianova, A.I.; Dorovatovskii, P.V. *ACS Catalysis*, **2017**, 7, 205-209.
<https://doi.org/10.1021/acscatal.6b03196>
2. Maharramov, A.M.; Suleymanova G.T.; Babayeva, G.V.; Ibragimova, Sh.A., Niyazova, A.A.; Shikhaliev, N.G.; Musayev, F.N.; Nenajdenko V.G. *Chemical Problems Journal*, **2018**, 2, 230-238.
<https://doi.org/10.32737/2221-8688-2018-2-230-238>
3. Shastin, A.V.; Tsyrenova, B.D.; Sergeev, P.G.; Roznyatovsky, V.A.; Smolyar, I.V.; Khrustalev, V.N.; Nenajdenko V.G. *Org. Lett.*, **2018**, 20 (24), 7803-7806.
<https://doi.org/10.1021/acs.orglett.8b03227>
4. Shastin, A.V.; Sergeev, P.G.; Lukianova, A.I.; Muzalevskiy, V.M.; Khrustalev, V.N.; Dorovatovskii, P.V.; Nenajdenko V.G. *Eur. J. Org. Chem.*, **2018**, 36, 4996-5006
<https://doi.org/10.1002/ejoc.201800441>
5. Maharramov, A.M.; Suleymanova, G.T.; Babayeva, G.V.; Niyazova, A.A.; Mamedov, I.G.; Hasanova, U.A.; Shikhaliev, N.G.; Nenajdenko V.G. *Journal of Low Dimensional Systems*, **2018**, 2 (1), 37-44.
6. Shikhaliyev, N. G.; Suleymanova, G.T.; Bagirova, Kh.N.; Asgerova, U.F.; Garazadeh, Kh.A.; Babayeva, G.V.; Ahmedova, N.E.; Nenajdenko, V.G. *Journal of Low Dimensional Systems*, **2018**, 2 (2), 24-29.

7. Shikhaliyev, N. Q. Ahmadova, N.E.; Gurbanov, A.V.; Maharramov, A.M.; Mammadova, G.Z.; Nenajdenko, V.G.; Zubkov, F.I.; Mahmudov, K.T.; Pombeiro A.J.L. *Dyes and Pigments*, **2018**, *150*, 377–381.
<https://doi.org/10.1016/j.dyepig.2017.12.033>
8. Maharramov, A.M.; Shikhaliyev, N.Q.; Suleymanova, G.T.; Gurbanov, A.V.; Babayeva, G.V.; Mammadova, G.Z.; Zubkov, F.I.; Nenajdenko, V.G.; Mahmudov, K.T.; Pombeiro, A.J.L. *Dyes and Pigments*, **2018**, *159*, 135-141.
<https://doi.org/10.1016/j.dyepig.2018.06.022>
9. Israyilova, A.; Mukhtarova, S.; Asgerova, U.; Shikhaliyev, N.; Ganbarov, Kh.; Maharramov, A. *IJIRSET*, **2017**, *6*, 21191-21195.
10. Maharramov, A.M.; Askerova, U.F.; Akhmedova, N.E.; Mukhtarova, S.Kh.; Garazadeh, Kh.A.; Shikhaliyev, N.G. *Journal of Low Dimensional Systems*, **2017**, *1*, 1-4.
11. Winkler, R.; Hertweck, C. *Chembiochem*, **2007**, *8*, 973-977.
<https://doi.org/10.1002/cbic.200700042>
12. Hoshino, K.; Sato, K.; Akahane, K.; Yoshida, A.; Hayakawa, I.; Sato, M.; Une, T.; Osada, Y. *Antimicrob. Agents Chemother.* **1991**, *35*(2), 309-12.
<https://doi.org/10.1128/AAC.35.2.309>
13. Grabarczyk, M.; Mączka, W.; Żarowska B.; Maciejewska G.; Anioł, M; *Journal of Saudi Chemical Society*, **2018**, *22* (3), 363-371.
<https://doi.org/10.1016/j.jscs.2016.04.004>
14. Tirkey, V.; Mishra, S.; Hirak, R.D.; Das, S.; Nayak, B.P.; Mobin, S.M.; Chatterjee, S. *Journal of Organometallic Chemistry*, **2013**, *732*, 122-129.
<https://doi.org/10.1016/j.jorganchem.2013.02.020>
15. CLSI-Clinical Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 11th edition, **2018**.
16. Martin, A.; Takiff, H.; Vandamme, P.; Swings, J.; Palomino, J.C.; Portaels, F. *J. Antimicrob Chemother.*, **2006**, *58*, 327-331.
<https://doi.org/10.1093/jac/dkl231>
17. Israyilova, A.; Buroni S.; Forneris, F.; Scoffone, V. C.; Shixaliyev, N.Q.; Riccardi, G.; Chiarelli, L.R. *PLos One*, **2016**, *11*, 1-17.
<https://doi.org/10.1371/journal.pone.0167350>