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ORIGINAL PAPER

Synthesis of 5-heptadecyl- and 5-heptadec-8-enyl substituted 4-amino-1,2,4-triazole-3-thiol and 1,3,4-oxadiazole-2-thione from (*Z*)-octadec-9-enoic acid: preparation of Palladium(II) complexes and evaluation of their antimicrobial activity

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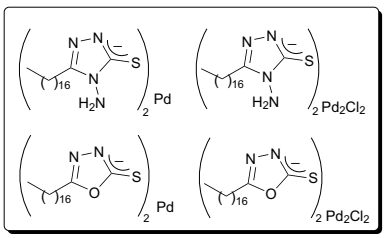
Abstract

Two 4-amino-1,2,4-triazoles and two 1,3,4-oxadiazoles are obtained in a common synthetic route including hydrogenation-hydrazidation of (*Z*)-methyl octadec-9-enoate to octadecanoic hydrazide under atmospheric air. Preservation of olefinic bond in heptadec-8-enyl group is achieved by carrying out hydrazidation reaction under the presence of an argon atmosphere. The disappearance of the olefinic bond is detected by physical data, IR, ^{1}H, and^{13}C NMR spectroscopy. New palladium complexes derived from 4-amino-5-heptadecyl-1,2,4-triazole-3-thiol and 5-heptadecyl-1,3,4-oxadiazole-2(3*H*)-thione are obtained and characterized by elemental analysis (solid state), IR, ^{1}H, ^{13}C NMR spectroscopy, XRD, and XPS. These resulting metallic entities are also identified in solution based in mass spectrometry (MS-ESI) experiments. Most compounds and their palladium(II) complexes are tested in vitro against Gram-positive, Gram-negative bacteria, and fungi, some of them showed variable activity.

Graphic abstract

ANTIBACTERIAL AGENTS



Keywords (*Z*)-Octadec-9-enoic acid · 1,3,4-Oxadiazole · Amino-1,2,4-triazole · Organometallic complexes · Antimicrobial activity

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Introduction

Saturated and unsaturated fats and their saturated and unsaturated fatty acids have been the concern of many chemists and biologists since a long time because of their advantages and disadvantages to the health of human beings via his dietary [1–6]. The importance of fatty acids in membrane function has been reinforced by observations that their deficiencies in brain and retinal tissues can result in compromised visual acuity [7], learning ability and even in neurologic problems [4, 8]. Concerning the importance of protein function, each fatty acid has the disadvantage of involving non-physiological conditions of making it difficult to assess their functional significance. However, many review articles recommended a diet low in saturated fat and argue it will lower risks of cardiovascular diseases, diabetes, or death [9, 10].

Fatty acids have also industrial uses as fuels, surfactants [11, 12], and catalysts [12]. Fatty acid derivatives such as branched chain-fatty acids and cyclic fatty acids have antibacterial effects [13]. Heterocyclic derivatives form suitable precursors for nucleoside, nucleotides formations [14] and showed high tendency to form complexes with various metals. Some of these complexes have been used as efficient antibiotics [15] and as catalysts in many reactions such as Tsuji–Trost and Mizoroki–Heck [16].

Mobile oxidation or/and hydrogenation of unsaturated bond have been also reported [17, 18] as a case that involves a serious danger to human health, caused by the food industry and synthetic works [19, 20]. This observation of mobile reactional changes from unsaturated to saturated fatty acid prompted us to investigate a similar work under atmosphere of air and argon to preserve the double bond.

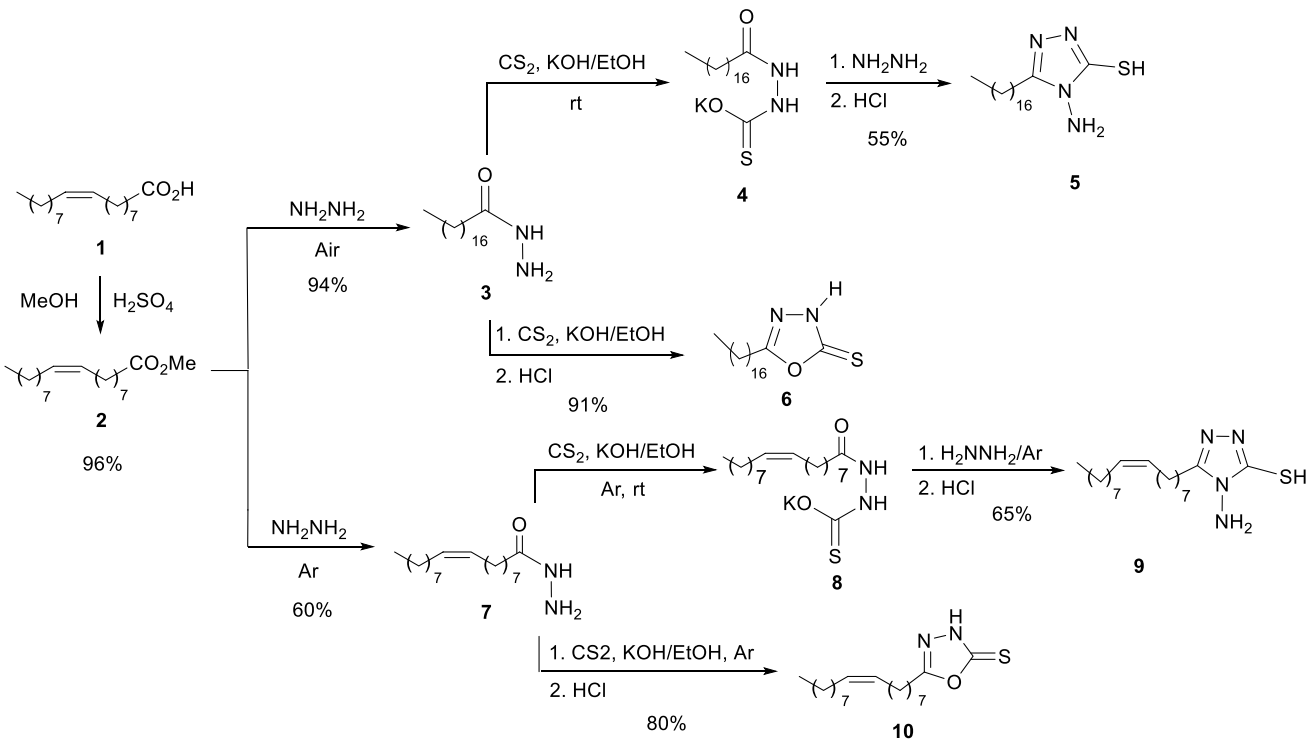
In this article, we studied the behavior of the employment of saturated and unsaturated fatty acids during the process to prepare two 4-amino-1,2,4-triazoles and two 1,3,4-oxadiazoles through a common synthetic route. Also, the preparation of palladium complexes from saturated amino-1,2,4-triazole and 1,3,4-oxadiazole were considered. Biological activity was tested upon saturated heterocyclic products, their intermediates, and their palladium(II) complexes.

Results and discussion

Synthesis of heterocyclic compounds 5, 6, 9, and 10

Ester **2** of (*Z*)-octadec-9-enoic acid (**1**, oleic acid) have been prepared and identified by classical methods [16]. When ester **2** was treated with hydrazine hydrate (85%), under atmospheric oxygen, a non-catalytic hydrogenation–hydrazination process took place (see Scheme 1) and octadecanoic hydrazide **3** was exclusively obtained. The ^{1}H NMR spectrum did not show the olefinic protons indicating

Scheme 1



that the double bond was reduced by the hydrazine hydrate under atmospheric oxygen. This behavior was documented in the literature [21, 22] (Scheme 2). Hydrazide **3** was transformed into 4-amino-5-heptadecyl-1,2,4-triazole-3-thiol (**5**) and 5-heptadecyl-(3*H*)-1,3,4-oxadiazole-2-thione (**6**) [16] as illustrated in Scheme 1. Structures of intermediates and heterocyclic products **5** and **6** were determined by IR, ^{1}H NMR, ^{13}C NMR, and MS. The selection of these heterocycles as suitable ligands of palladium(II) salts was carried out with the idea of improving the biological activity of the pure isolated heterocycles.

However, when ester **2** was treated with hydrazine hydrate (99%) under the atmosphere of argon (using freezing-pump conditions before the mixture of the reagents) (*Z*)-octadec-9-ene hydrazide **7** was isolated. IR spectrum showed absorption at 1535 cm^{-1} indicating the presence of the olefinic bond, which was confirmed by ^{1}H NMR (multiplet at 5.35 ppm) and ^{13}C NMR (signal at 129.84 ppm) (see Figs. 1, 2, basic lines). The salt **8**, (*Z*)-4-amino-5-heptadec-8-enyl-1,2,4-triazole-3-thiol (**9**), and (*Z*)-5-heptadec-8-enyl-1,3,4-oxadiazole-2(3H)-thione (**10**) were obtained by the known method [16] under argon atmosphere. Structural proof for **8**–**10** were provided by IR, ^{1}H NMR, ^{13}C NMR, and MS data.

A plausible mechanism for the non-catalytic hydrogenation of the carbon–carbon double bond can be observed in Scheme 2. The easy oxidation and disproportionation of the hydrazine could contribute to the hydrogen transfer from the diimide to the olefin with the subsequent extrusion of nitrogen. This process is very fast and the presence of oxygen was crucial to enhance it.

Stability of the olefinic bond in (*Z*)-octadec-9-enehydrazide (oleic hydrazide) 7

Such as it was mentioned earlier, (*Z*)-octadec-9-enehydrazide **7**, prepared under the atmosphere of argon, showed ^{1}H NMR spectrum (basic line in Fig. 1) illustrating clearly the presence of olefinic bond at 5.35 ppm with an integration of two protons and about twenty paraffinic protons at 1.28 ppm. In addition, its ^{13}C NMR spectra in Fig. 2 showed a signal at 129.84 ppm for (C=C) (basic line in Fig. 2).

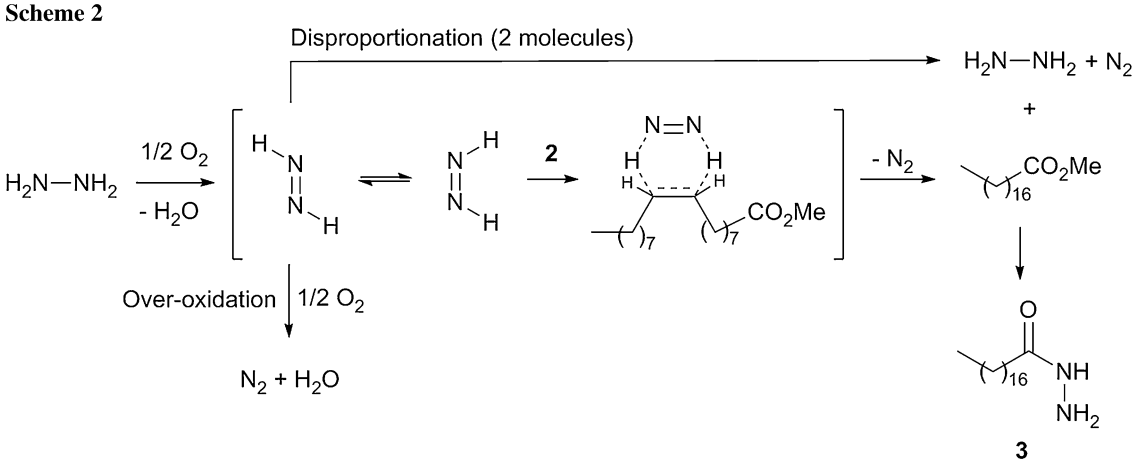
After the exposure to air, the olefinic and allylic protons diminished as illustrated at chemical shift positions at 5.35, 2.01 ppm, while the integration of paraffinic protons at 1.29 ppm were increased by two protons (see upper line in Fig. 1). Also, ^{13}C NMR spectra in Fig. 2 showed the disappearance of the signal at 129.84 ppm for (C=C).

When the hydrazide **7** was kept for almost 1 year under the atmosphere of argon, its ^{1}H NMR of **7** remained unaltered, but immediately diminished within few hours when compound **7** was exposed to air. Therefore, due to sensitivity of the olefinic bond in (*Z*)-octadec-9-ene hydrazide **7** and other products to air, no further application reactions neither complex formation nor biological activity test were carried out with these unsaturated derivatives.

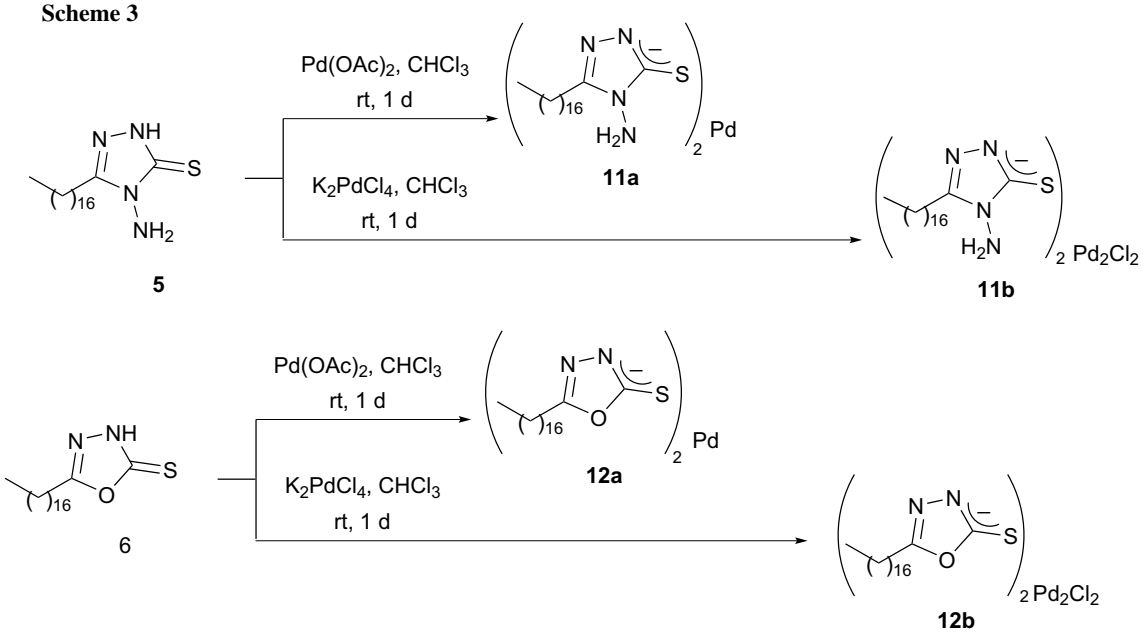
Preparation of palladium(II) complexes 11a, 11b, 12a, 12b

Compounds 4-amino-5-heptadecyl-1,2,4-triazole-3-thiol (**5**) and 5-heptadecyl-1,3,4-oxadiazole-2(3*H*)-thione (**6**) were treated individually with palladium salts, $Pd(OAc)_2$ and K_2PdCl_4 at room temperature. In all cases, orange color solutions were obtained. The possible structures of complexes **11a**, **11b**, **12a**, **12b** were very difficult to determine despite many spectroscopic data and microanalysis performed. For example, FT-IR results showed that the disappearance of N–H in **11a**, **11b**, and **12a** and the delocalization of C=N absorption in IR is a clear evidence for the formation metallic palladium(II) complexes. Similarly, the differences in ^{1}H NMR and ^{13}C NMR between ligands **5** and **6** and their Pd-complexes **11a**, **11b**, **12a**, and **12b** supported that structural arrangement occurred.

Scheme 2



Scheme 3



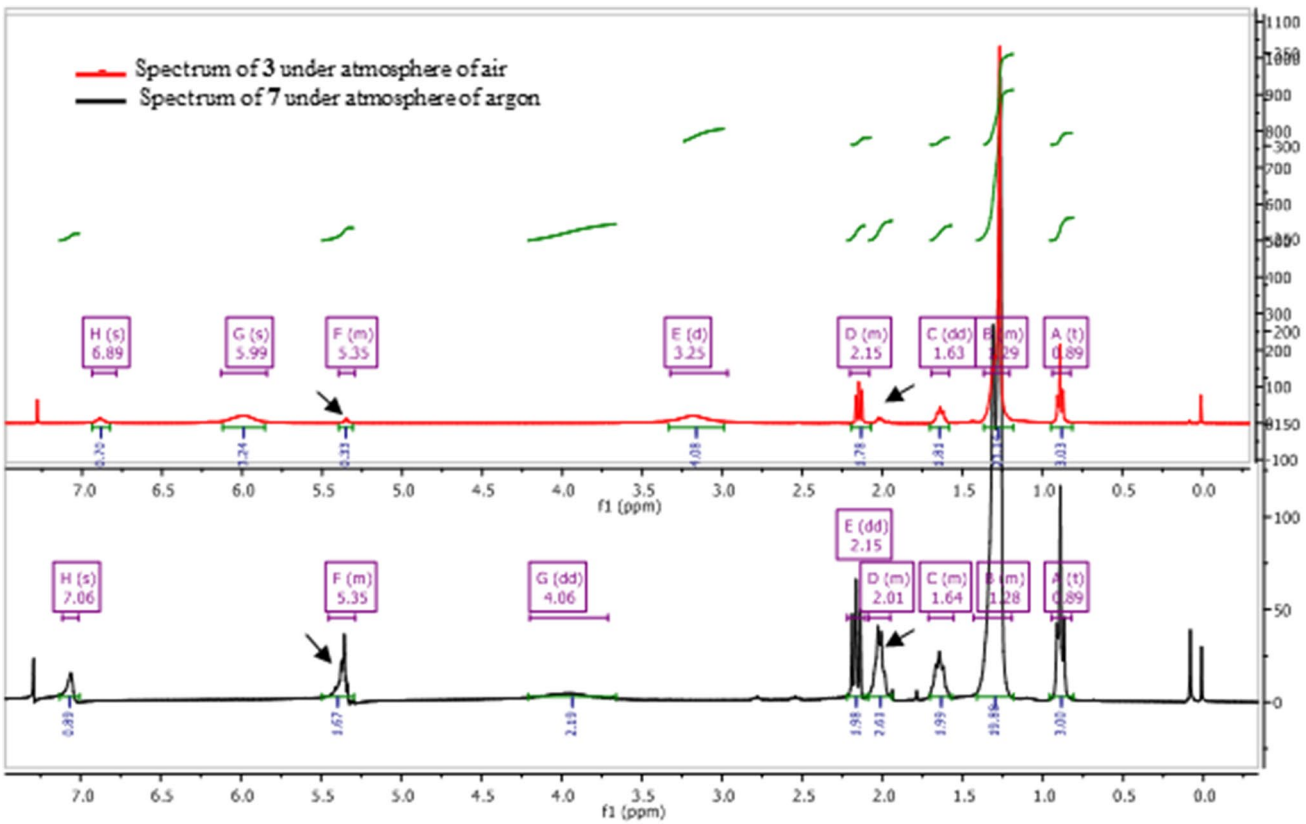


Fig. 1 ^{1}H NMR spectra of oleic hydrazide **7** synthesized under the atmosphere of argon (basic line) and after exposure to air (upper line)

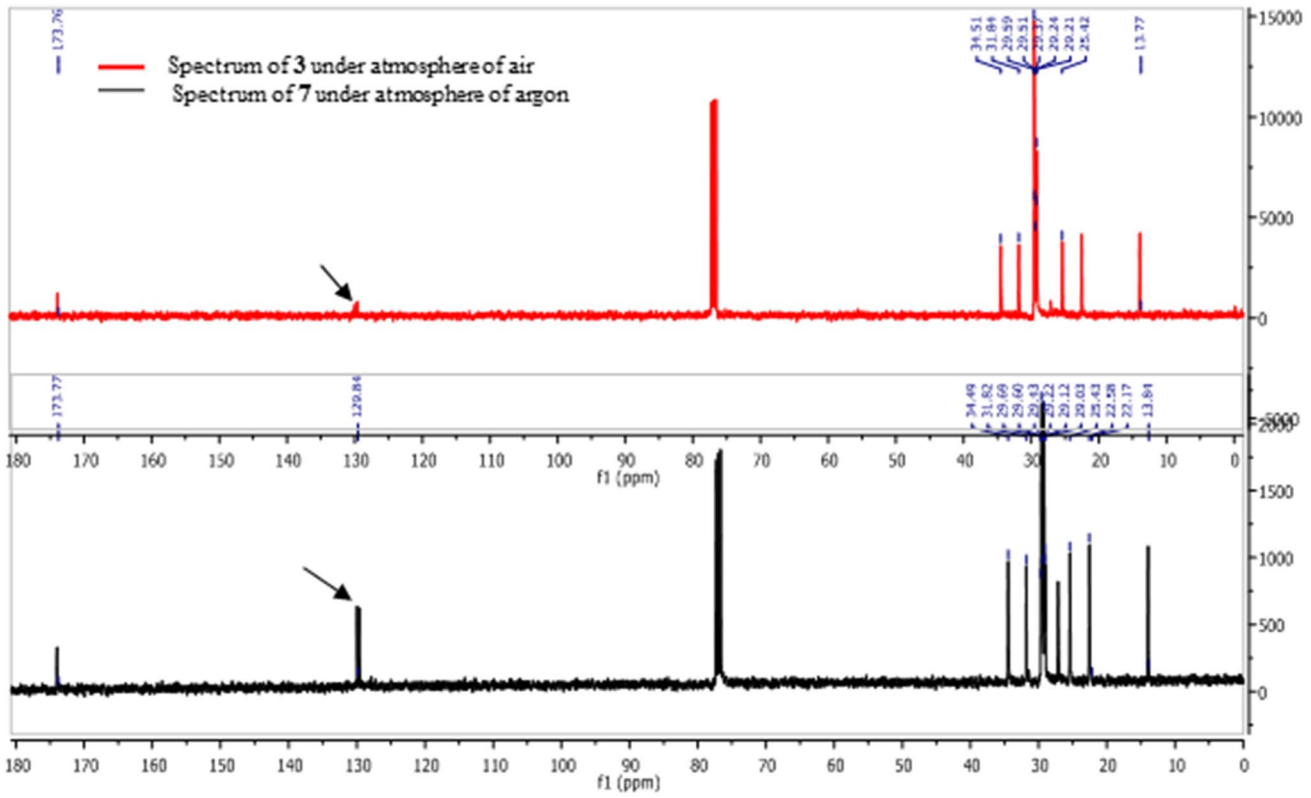


Fig. 2 ^{13}C NMR spectra of oleic hydrazide **7** under the atmosphere of argon (in black line) and after exposure to air (in red line)

XPS and XRD analysis were another instrumental techniques used for the characterization of these complexes (see SI). Mass spectra analysis also confirmed the presence of the dinuclear/dimeric structures using ESI, which was not determining (Scheme 3).

We have studied X-ray powder diffraction (XRD) of two resulting complexes (**11b** and **12a**) using K_2PdCl_4 and $Pd(OAc)_2$ palladium sources (see SI). Resulting spectra showed intense Bragg's reflections related to the palladium which are very similar to $PdCl_2$ or $(NH)_4PdCl_2$ pattern [23, 24] confirming the presence of palladium(II) species in complexes structure.

The reported X-ray diffraction analysis of similar triazol-palladium(II) complexes did not provide an uniform trend for these complexes and are not helpful to determine the exact nature of complexes **11** and **12**. Thus, a dinuclear structure with strong S–Pd coordination [25], a dinuclear paddle-wheel structure incorporating phosphines as ligands [26], mono- and binuclear complexes with S–Pd–N coordination [27], etc. In similar way, oxadiazole-2-thiones formed complexes with palladium(II) salts and stabilizing phosphines to give mononuclear complexes with two units of the heterocyclic moiety [28], mononuclear species with two heterocyclic ligands [29], homobinuclear complexes [30], etc.

Palladium(II) complexes for ligands **9** and **10** had not been performed due to the fact that mobility of the olefinic bond in them may be affected by Pd ions and atmospheric oxygen during synthetic and structural determination times.

Antimicrobial activity

(*Z*)-Octadec-9-enoic acid (oleic acid) (**1**), the ester **2**, octadecanehydrazide (**3**), 4-amino-5-heptadecyl-1,2,4-triazole-3-thiol (**5**), and 5-heptadecyl-1,3,4-oxadiazole-2(3*H*)-thione (**6**) and palladium(II) complexes **11a**, **11b**, **12a**, **12b**, were tested in vitro against eight strains, Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* laboratory isolate, *Enterococcus faecalis* (ATCC 29212), Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and fungus *Aspergillus niger*, *Candida albicans*, *Trichosporon.* Vancomycin, colistin were used as positive references and DMSO as negative reference. Results are summarized in Tables 1, 2, and 3. Table 1 showed that 4-amino triazole **5** had significant effect upon Gram-positive, Gram-negative bacteria, and one fungus

Table 1 Antimicrobial activity of synthesized compounds (10 mg cm^{-3}), zone inhibition in mm

Comp.	G(+) bacteria			G(−) bacteria		Fungi		
	i	ii	iii	iv	v	vi	vii	viii
1	–	8	–	–	–	–	–	–
2	7	9	–	7	–	–	–	–
3	–	–	–	–	–	–	–	–
5	29	15	19	22	34	–	10	–
11a	–	–	–	–	–	–	–	–
11b	–	–	–	–	–	–	–	10
6	12	14	9	–	–	–	–	–
12a	–	–	–	–	7	–	–	–
12b	7	9	–	7	11	–	–	–
Control DMSO	–	–	–	–	–	–	–	–
Vancomycin	18	30	23	–	–	–	–	–
Colistin	–	–	–	14	11	–	–	–

(-): Inactive, no inhibition zone

i: Staphylococcus aureus (ATCC 25923), ii: Staphylococcus aureus laboratory isolate, iii: Enterococcus faecalis (ATCC 29212), iv: Escherichia coli (ATCC 25922), v: Pseudomonas aeruginosa (ATCC 27853), vi: Aspergillusniger, vii: Candida albicans, viii: Trichosporon

Table 2 The minimal inhibitory concentrations (MIC/mg cm^{-3}) data

Comp.	G(+) bacteria			G(−) bacteria	
	i	ii	iii	iv	v
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
5	C/2	C/2		C/4	C/4
11a	–	–	–	–	–
11b	–	–	–	–	–
6	C/4	C/4	–	–	–
12a	–	–	–	–	–
12b	–	–	–	–	–

C/2 = 5 mg, C/4 = 2.5 mg, C/8 = 1.25 mg, C/16 = 0.625 mg

Table 3 Bacteriostatic and bactericidal tests

Comp.	i	ii	iii	iv	v
11	Bacteriostatic	Bacteriostatic	–	Bacteriostatic	Bacteriostatic
12	Bacteriostatic	Bacteriostatic	–	–	–

Candida albicans. There are cases when ligand **5** against Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922), exceeded the references, whilst oxadiazole **6** was effective against Gram-positive bacteria only. Pd complexes of aminotriazole **11a** and **11b** and Pd complex of oxadiazole **12a** were almost ineffective against Gram-positive, Gram-negative bacteria nor fungus due to the powerful attachment of ligand-Pd [22]. Only compounds active in this primary screening were further tested in a second set of dilution 5 μg cm^{-3} and downward against all microorganisms as shown in Table 3.

Conclusions

During our attempt to synthesize diazole derivatives from unsaturated fatty acid, (*Z*)-octadec-9-enoic acid (**1**, oleic acid) was noticed that during hydrazination step, under normal conditions of the atmosphere of air, the olefinic bond had been subjected to internal hydrogenation. When the same hydrazination reaction was repeated under atmospheric pressure of argon, the olefinic bond kept intact. It was found also that the double bond was kept as it for a long time-up to 1 year-under storage under the atmosphere of argon and gradually diminished after the exposition to air at any stage of reaction after hydrazination. Therefore, the synthesis of 4-amino-5-heptadecyl-1,2,4-triazole-3-thiol (**5**) and 5-heptadecyl-1,3,4-oxadiazole-2(3*H*)-thione (**6**) were carried out under atmosphere of air and for (*Z*)-4-amino-5-heptadec-8-enyl-1,2,4-triazole-3-thiol (**9**) and (*Z*)-5-heptadec-8-enyl-1,3,4-oxadiazole-2(3*H*)-thione (**10**) were achieved under atmosphere of argon. Ligands **5** and **6** showed a tendency to form stable Pd complexes **11a**, **11b**, **12a**, and **12b** possessing a weak biological activity.

Experimental

All chemicals were purchased from Acros, Alpha, or Sigma-Aldrich and used without further purification. Methyl (*Z*)-heptadec-9-enoate (methyl oleate, **2**) [32], stearohydrazide (**3**) [31a], 4-amino-5-heptadecyl-4*H*-1,2,4-triazole-3-thiol (**5**) [33], 5-heptadecyl-1,3,4-oxadiazole-2(3*H*)-thione (**6**) [31a], oleohydrazide (**7**) [34], (*Z*)-4-amino-5-(heptadec-8-en-1-yl)-4*H*-1,2,4-triazole-3-thiol (**9**) [31b], and (*Z*)-5-(heptadec-8-en-1-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**10**) [31b] are known compounds. Melting points were determined with a Reichert Thermowar hot plate apparatus. Only the structurally most important peaks of the IR spectra (recorded with a FT-IR 4100LE (JASCO) (PIKE MIRacle ATR) are listed. ^{1}H NMR (300, 400, or 500 MHz) and ^{13}C NMR (75, 101, or 126 MHz) spectra were recorded using Bruker AV300, Bruker AV400, and Bruker ADVANCE DRX500, with $CDCl_3$ as solvent and TMS as internal standard and chemical shifts are given in ppm. Low-resolution electron impact (GC-EI) mass spectra were obtained at 70 eV using an Agilent 6890 N Network GC system and Agilent 5973Network Mass Selective Detector. Microanalyses were performed in a Thermo Finnigan Flash 1112 series. TLC was performed on Schleicher and Schuell F1400/LS 254 silica gel plates and the spots were visualized under UV light ($\lambda = 254$ nm). Merck silica gel 60 (0.040–0.063 mm) was used for flash chromatography.

Microorganisms in this study were supplied and identified by the laboratory of microbiology by the university hospital of Oran. The Mueller–Hinton medium was supplied by (Difco).

Synthesis of the palladium(II) complexes 11 and 12

The complexes were prepared individually by adding 0.011 mol of ligands **5** or **6** in 10 cm^3 of chloroform to a solution of palladium salt ($Pd(OAc)_2$ or K_2PdCl_4, 0.01 mol) in 10 cm^3 of the same solvent and stirring the solutions at room temperature for 24 h; orange color solutions were obtained. Complexes were collected after, the solvents were evaporated, washed with methanol, and dried under vacuum.

11a ($C_{38}H_{74}N_8PdS_2$) Brown powder; m.p.: > 300 °C (methanol/water); IR: $\bar{\nu} = 1609$, 1313 cm^{-1}; ^{1}H NMR: $\delta = 0.87$ (t, 6H, 2xCH_3), 1.48 (m, 60H, 30xCH_2), 2.04 (m, 4H, 2xNH_2), 2.68 (m, 4H, 2xCH_2CN) ppm; ^{13}C NMR: $\delta = 13.8$ (2xCH_3), 22.8 (m, 32xCH_2), 156.1 (2xNC=O), 168.1 (2xCS) ppm; MS-ESI: $m/z = 965$ [$L_2Pd_2H_2O(MeCN)_2 + 1$].

11b ($C_{38}H_{74}Cl_2N_8Pd_2S_2$) Brown powder; m.p.: 274–279 °C (methanol/water); IR: $\bar{\nu} = 1606$, 1213 cm^{-1}; ^{1}H NMR: $\delta = 0.87$ (t, 6H, 2xCH_3), 1.27 (m, 60H, 30xCH_2), 1.67 (m, 4H, 2xNH_2), 2.70 (m, 4H, 2xCH_2CN) ppm; ^{13}C NMR: $\delta = 14.1$ (2xCH_3), 31.0 (m, 32xCH_2), 158.0 (2xNC=O), 165.3 (2xCS) ppm; MS-ESI: $m/z = 712.5$ ($L_2Pd_2Cl_2$·MeCN-$(CH_2)_{16}$-$CH_3 + 1$).

12a ($C_{38}H_{70}N_4O_2PdS_2$) Brown sticky powder; m.p.: > 300 °C (methanol/water); IR: $\bar{\nu} = 1597$, 1185 cm^{-1}; ^{1}H NMR: $\delta = 0.87$ (t, 6H, 2xCH_3), 1.25 (s, 56H, 28CH_2), 1.68 (m, 4H, 2xCH_2CH_2CN), 2.65 (m, 4H, 2xCH_2CN) ppm; ^{13}C NMR: $\delta = 14.1$ (2xCH_3), 30.0 (m, 32xCH_2), 168.2 (2xNC=O), 178.7 (2xC=S) ppm; MS-ESI: $m/z = 993$ [$L_2Pd_2(H_2O)(MeCN)_2 + 1$].

12b ($C_{38}H_{70}Cl_2N_4O_2Pd_2S_2$) Brown sticky solid; m.p.: > 300 °C; IR: $\bar{\nu} = 3219$, 1678, 1185 cm^{-1}; ^{1}H NMR: $\delta = 0.88$ (t, 6H, 2xCH_3), 1.29 (m, 56H, 28xCH_2), 1.67 (m, 4H, 2xCH_2CH_2), 2.66 (m, 4H, 2xCH_2) ppm; ^{13}C NMR: $\delta = 14.1$ (2xCH_3), 28.5 (32xCH_2), 168.2 (2xNC=O), 179.5 (2xC=S) ppm; MS-ESI: $m/z = 960$ ($L_2Pd_2Cl_2$).

Antibacterial and antifungal activity

The respective different strain was spread separately on the Mueller–Hinton for antibacterial activity (CLSI 2004; CLSI 2012). Then the test organism suspension was added and incubated at 37 °C for 24 h for bacteria studies. The drugs colistin and vancomycin were taken as standard drug to compare the results and dimethylsulfoxide (DMSO) was taken as blank [35]. Bacteriostatic or bactericide test was determined as follows: a small sample was taken from each well where there was no visible growth, using an inoculation loop, which was then spread on GN plates and incubated overnight at 37 °C [36]. The minimum inhibitory concentration (MIC) was the lowest concentration of test compound that inhibit the visible growth of the organism and was determined in triplicates [37].

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