

## Synthesis of Dihydroxamic Acids from Dinitrones, Structural Characterization and Antimicrobial Activities

<sup>1,5</sup>Cherifa Mahieddine, <sup>1</sup>Said Zerkout, <sup>2</sup>Sidali Rayane, <sup>3</sup>Houssam Boulebd, <sup>6</sup>Affaf Laassami, <sup>\*4</sup>Salah Akkal  
<sup>1</sup>Département de chimie, Ecole Normale Supérieure de Kouba, B.P. 92, 16050 Vieux Kouba, Alger, Algérie.

<sup>2</sup>Laboratoire de Recherche en Didactique des Sciences, Ecole Normale Supérieure de Kouba,  
B.P. 92, 16050 Vieux Kouba, Alger, Algérie.

<sup>3</sup>Laboratoire de Synthèses de Molécules d'Intérêts Biologique,  
Université Mentouri de Constantine I, Constantine, Algérie.

<sup>4</sup>Unit for the Valorization of Natural Resources, Bioactive Molecules and Physico-Chemical and Biological  
Analyzes, Faculty of Exact Sciences, Department of Chemistry,  
Mentouri University of Constantine I- Route de Aïn El Bey, 25000, Constantine, Algeria.

<sup>5</sup>Laboratoire d'Etude et de Développement des Techniques de Traitement et d'Épuration des Eaux et de  
Gestion Environnementale, Ecole Normale Supérieure de Kouba, B.P. 92, 16050 Vieux Kouba, Alger, Algérie.

<sup>6</sup>Laboratoire de Biologie Des Système microbiens, Ecole Normale Supérieure de Kouba,  
B.P. 92, 16050 Vieux Kouba, Alger, Algérie.

[salah4dz@yahoo.fr](mailto:salah4dz@yahoo.fr)\*

(Received on 23<sup>rd</sup> April 2021, accepted in revised form 4<sup>th</sup> October 2021)

**Summary:** The benefits of dinitrones (N,N'-Diphenyl-2,5-thenylenedinitrone, N,N'-Dimethyl-1,4-phenylenedinitrone and N,N'-Dibenzyl-1,4-phenylenedinitrone) in the synthesis were chosen as simple process for synthesis of the purified dihydroxamic acid compounds in a shorter time with a good yield and high purity without need to purification. The most stable conformers of the synthesized compounds and their electronic properties have been determined using DFT/B3LYP calculations. Antimicrobial activity assessment of dihydroxamic acids showed their good efficiency.

**Keywords:** Synthesis, Dinitrone, Dihydroxamic Acid, Antimicrobial Activity.

### Introduction

Several studies have reported [1-3] the synthesis of hydroxamic acid from ester, carboxylic acid or acid chloride by reaction with hydroxylamine salt, and from aldehyde through Angeli-Rimini reaction [4, 5], The similarity of the functional groups components between hydroxamic acid and nitrone has enabled to produce hydroxamic acids from nitrones, such as the oxidation of cyclic nitrones by iron (III) chloride [6] and the reaction of the cyclic C-methoxy nitrones with nucleophilic reagent [7]. The C-aryl nitrones were synthesized via condensation of N-substituted hydroxylamine and aldehydes under suitable conditions as a directly method [8, 9]. Simultaneously, diphenylnitron oxidation via lead tetraacetate in dry benzene, to yield N-acetoxy-N-benzoylaniline was exhibited as an exothermic reaction [10]. And the acetoxyl group was eliminated with a catalytic amount of mineral acid, and nitrenium ions were trapped by water to form N-alkoxy hydroxamic acid [11].

As far as we know, hydroxamic acids are distinguished for their capacity to inhibit a variety of enzymes [12, 13] and microbes [14, 15]. At the same time, nitrones have been employed as synthetic

intermediates [16] and as antimicrobials [17].

### Experimental

#### Materials and Methods

Reactors, reagents and solutions were used as received, except the lead(IV) acetate was dried in vacuo over phosphorus pentoxide for three days. Infrared spectra were recorded with KBr pellet on an FT-IR-8300 spectrophotometer Shimadzu. The NMR spectra were taken on a BRUKER (400 MHz for <sup>1</sup>H, 100 MHz for <sup>13</sup>C) instrument in DMSO-d<sub>6</sub> with TMS as an internal standard. Density functional theory (DFT) calculations have been carried out using Gaussian09 software [18]. The B3LYP functional [19] and the 6-311G (d, p) basis set have been used for all calculations. This approach has been used successfully by several research groups and good agreement between theory and experiment was found [20]. All the ground states were confirmed by vibrational frequency analysis (no imaginary frequency).

#### Synthesis of dinitrones

Method (i): A mixture of dialdehyde (0.5eq.)

\*To whom all correspondence should be addressed.

and triethylamine (0.6eq.) solute in THF was poured gradually to a stirred solution of N-substituted hydroxylamine hydrochloride **1** (1.0eq.) solute in THF, and the stirring was maintained at room temperature for 10 min. The precipitated salt was filtered off. After an hour of stirring the precipitated dinitrone (powder or crystal) was filtered [21].

Method (ii):  $\beta$ -phenylhydroxylamine **1'** was synthesized from the reduction of nitrobenzene (1.0eq.) by zinc dust (1.14eq.) in a warm aqueous solution of ammonium chloride (0.5eq.). The mixture of  $\beta$ -phenylhydroxylamine **1'** (1.0eq.) and dialdehyde (0.5eq.) was refluxed in 20mL ethanol for two hours. After overnight the precipitated dinitrone (crystal or powder) was filtered under vacuum [15.22].

The crude product from the two methods was washed in place once to afford the pure dinitrone.

#### Oxidation of dinitrones

Dry LTA (2eq.) was added partially to a stirred solution of N,N'-diphenyl-2,5-thienylenedinitrone **3a** (1eq.) in benzene 30mL at 20°C (laboratory temperature). The mixture was allowed to stir for two hours. After over night the brown precipitate of lead(II) acetate was filtered off and the solvent was removed by a rotary evaporator. The crude product **3a** was purified by crystallization in butanol to yield a buff powder of N,N'-diacetoxy N,N'-Diphenyl -2,5-thiophenedicarboxamide [10].

#### Hydrolysis of diacetoxy dicarboxamide

N,N'-diacetoxy N,N'-Diphenyl -2,5-thiophenedicarboxamide **3a** was added to a dilute sulfuric acid (4N). The reaction mixture was heated at 50°C for an hour. The mixture was filtered and the crude product of **3a** was added to 10% sodium carbonate solution [11]. The obtained mixture was treated with a diluted Hydrochloric acid (10%). The hydrolysis product N,N'-dihydroxy N,N'-Diphenyl-2,5-thiophene dicarboxamide **3a** was extracted from the aqueous solution by ether diethyl without the need of purification.

N,N'-Dimethyl-1,4-phenylenedinitrone (**2a**) The compound was prepared using 1,4-terephthalaldehyde (0.45g, 0.0033mol) and N-methylhydroxylamine hydrochloride (0.56g, 0.0067mol) in the presence of triethylamine

(0.873mL, 0.01mol) in THF. Yield 70.1%, mp 165°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 860.73 (1,4-disubstituted aryl), 1163.60 (N $\rightarrow$ O), 1410.16 (-CH<sub>3</sub>), 1579.28 (C=N), 2988.34 and 2942.09 (-CH, aryl). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ : 3.79(s, 6H, Me), 7.85 (m, 4H, Ar), 8.23 (s, 2H, N=CH). <sup>13</sup>C NMR (100MHz, DMSO-d<sub>6</sub>)  $\delta$ : 54.70 (N-CH<sub>3</sub>), 127.99 (N=CH-C-CH, Ar<sub>central</sub>), 132.10 (N=CH-C, Ar<sub>central</sub>), 133.98(N=CH).

-1,4-phenylenedinitrone (**2c**) The compound was prepared using 1,4-terephthalaldehyde (0.378g, 0.0028mol) and N-benzylhydroxylamine hydrochloride (0.9g, 0.0056mol) in the presence of triethylamine (0.46mL, 0.0056mol) in THF. Yield 87.5%, mp 185°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 855.12 (1,4-disubstituted aryl), 1149.18(N $\rightarrow$ O), 1458.54 (-CH<sub>2</sub>-), 1571.38 (C=N), 2973.90 and 2937.81 (-CH, aryl). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ : 5.08 (s, 4H, -CH<sub>2</sub>-), 7.37 (d, J= 6.6 Hz, 8H, Ar), 7.49 (d, J= 6.5 Hz, 2H, Ar), 8.05 (s, 2H, N=CH), .823 (s, 4H, Ar). <sup>13</sup>C NMR (100MHz, DMSO-d<sub>6</sub>)  $\delta$ : 70.65(N-CH<sub>2</sub>-C), 128.20 (N=CH-C-CH, Ar<sub>central</sub>), 128.80 (CH=N-C-CH-CH, Ar<sub>lateral</sub>), 128.89 (CH=N-C-CH, Ar<sub>lateral</sub>), 129.45(CH=N-C-CH-CH-CH, Ar<sub>lateral</sub>), 132.36 (N=CH-C, Ar<sub>central</sub>), 133.39 (N=CH), 135.11(CH=N-C, Ar<sub>lateral</sub>).

N,N'-Diphenyl-2,5-thienylenedinitrone (**3a**) The compound was prepared using 2,5-thiophenedicarboxaldehyde (0.578g, 0.0041mol) and  $\beta$ -phenylhydroxylamine (0.9g, 0.0082mol) in ethanol. Yield 95%, mp 154°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 690.5(C-S), 763.8 (2,5-disubstituted aryl), 1172.6(N $\rightarrow$ O), 1546.8(C=N), 2927.7 and 2812.0 (-CH, aryl). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ :7.50-7.58 (m, 8H, Ar), 7.83 (s, 2H, N=CH), 7.99-8.01 (d, J= 4.0Hz, 2H,Ar), 9.19 (s, 2H, Ar). <sup>13</sup>C NMR (100MHz, DMSO-d<sub>6</sub>)  $\delta$ :121.19 (N=CH-C-CH, Ar<sub>central</sub>), 129.70 (CH=N-C-CH-CH, Ar<sub>lateral</sub>), 129.88 (CH=N-C-CH, Ar<sub>lateral</sub>), 130.33 (CH=N-C-CH-CH-CH, Ar<sub>lateral</sub>), 131.35 (N=CH), 135.66 (N=CH-C, Ar<sub>central</sub>), 146.40 (CH=N-C, Ar<sub>lateral</sub>).

N,N'-diacetoxy N,N'-Dimethyl-1,4-benzenedicarboxamide (**2a'**) The compound was prepared using dinitrone **2a** (0.31g, 0.0016mol) and LTA (1.43g, 0.0032mol) in benzene. Yield 89%, mp 135°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 860.2 (1,4-disubstituted aryl), 1168.8 (N-O), 1411.8 (-CH<sub>3</sub>), 1577.7 (C=O), 1678.0 (-OAc). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ :2.08 (s, 6H, Me, -OAc), 3.89 (s, 6H, Me), 7.39 (s, H, Ar), 7.53 (s, H, Ar), 8.2 (s, 2H, Ar).

*N,N'*-diacetoxy *N,N'*-Dibenzyl-1,4-benzenedicarboxamide (**2c'**) The compound was prepared using dinitrone **2c** (0.52g, 0.0015mol) and LTA (1.34g, 0.003mol) in benzene. Yield 82%, mp 155°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 855.12 (1,4-disubstituted aryl), 1292.2 (N-O), 1326.9 (-CH<sub>2</sub>-), 1573.8 (C=O), 1678.5 (-OAc). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ : 2.14 (s, 6H, Me, -OAc), 5.08 (s, 4H, -CH<sub>2</sub>-), 7.37-7.61 (m, 8H, Ar), 8.10-8.23 (s, 6H, Ar).

*N,N'*-diacetoxy *N,N'*-Diphenyl-2,5-thiophenedicarboxamide (**3a'**) The compound was prepared using dinitrone **3a** (0.24g, 0.74mmol) and LTA (0.66g, 1.48mmol) in benzene Yield 84.6 % , mp 140°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 744.5 (1,4-disubstituted aryl), 1180.4 (N-O), 1681.8 (C=O), 1789.8 (-OAc). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ : 2.25 (s, 6H, Me, -OAc), 7.10 (s, 2H, Ar), 7.40-7.48 (m, 10H, Ar). <sup>13</sup>C NMR (100MHz, DMSO-d<sub>6</sub>)  $\delta$ : 18.17(-CH<sub>3</sub>, -OAc), 126.78 (N=CH-C-CH, Ar<sub>central</sub>), 129.67-129.56 (Ar<sub>lateral</sub>), 132.85 (N-CO-C, Ar<sub>central</sub>), 138.86-138.82 (Ar<sub>lateral</sub>), 158.23(-CO-N-C), 167.99 (CO, -OAc).

*N,N'*-dihydroxy-*N,N'*-Dimethyl-1,4-benzenedicarbohydroxamic acid (**2a''**) The compound was prepared from diamide **2a** by hydrolysis with (4eq.) of (H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O). Yield 86%, pale brown granular, mp 80°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 860.2 (1,4-disubstituted aryl), 1199.6 (N-O), 1388.7(-CH<sub>3</sub>), 1693.4 (C=O), 3255.6 (O-H). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ : 2.12-2.19 (s, 6H, Me), 7.16-7.19 (t, H, Ar), 7.67-7.69 (t, H, Ar), 7.75-7.79 (m, H, Ar), 9.70-9.77 (s, 2H, -OH).

*N,N'*-dihydroxy-*N,N'*-Dibenzyl-1,4-benzenedicarbohydroxamic acid (**2c''**) The compound was prepared from diamide **2c** by hydrolysis with (4eq.) of (H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O). Yield 90%, mp 130°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 860.2 (1,4-disubstituted aryl), 1245.9 (-CH<sub>2</sub>-), 1288.4 (N-O), 1685.7 (C=O), 2923.9 (O-H). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ : 5.11 (s, 4H, -CH<sub>2</sub>-), 7.55-7.71 (m, 8H, Ar), 8.00-8.14 (s, 6H, Ar), 10.10 (s, 2H, -OH).

*N,N'*-dihydroxy-*N,N'*-Diphenyl-2,5-thiophenedicarbohydroxamic acid (**3a''**) The compound was prepared using diamide **3a** by hydrolysis with (4eq.) of (H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O). Yield 80%, mp 145°C. IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 752.2 (2,5-disubstituted aryl), 1161.1 (N-O), 1569.9 (C=O), 3062.7 (O-H). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>)  $\delta$ : 7.24-7.29 (s, 2H, Ar), 7.43-7.49 (s, 4H, Ar), 7.74-7.76 (s, 4H, Ar), 7.98 (s, 2H, Ar), 11.50 (s, 2H, -OH).

<sup>13</sup>C NMR (100MHz, DMSO-d<sub>6</sub>)  $\delta$ : 123.27(NOH-CO-C-CH, Ar<sub>central</sub>), 125.66 (CH-NOH-C-CH, Ar<sub>lateral</sub>), 128.57 (CO-NOH-C-CH-CH, Ar<sub>lateral</sub>), 133.38 (CO-NOH-C-CH-CH-CH, Ar<sub>lateral</sub>), 140.00 (C<sub>1</sub>, Ar<sub>central</sub>), 141.32 (C<sub>1'</sub>, Ar<sub>lateral</sub>), 159.88(CO-NOH).

## Results and Discussion

In the course of our study on the synthesis of the acyclic hydroxamic acids from the synthesized nitrones, we report herein, the condensation of 1,4-terephthalaldehyde or 2,5-thiophenedicarboxaldehyde with N-substituted hydroxylamine salts **1** in the presence of the reagent triethylamine [21], or  $\beta$ -phenylhydroxylamine **1'** in organic solvent without make it under nitrogen gas to produce symmetric bis-substituted nitrones (Fig.1). If THF was used the longer time of reaction and the solvent warm-up were not needed to procure the best results. Instead, the same results were obtained when the reaction mixture was placed in boiling ethanol. Further, the central nucleus di-substituted (2,5-thenylene or 1,4-phenylene) in the molecular entity of reactant imposes a positive mesomeric due to  $\pi$ -conjugated system, which increases the stability of the formed dinitrone. To might be justified the high purity of the yielded dinitrones.

The oxidation of the synthesized dinitrones was accomplished by lead(IV) acetate (LTA) in benzene solution under laboratory conditions without heating or cooling (Fig. 2). The reaction mixture was left over night in the dark at room temperature. The brown precipitate of lead(II) acetate was filtered off, the solvent was removed via rotary evaporation and the crude oily products were recrystallized once from petroleum ether or butanol to obtain *N,N'*-diacetoxy-*N,N'*-R-diamides. The acyclic dinitrones oxidation was recorded in this study as non-thermal reaction in benzene without purification, unlike aryl nitrones requires cooling the reaction mixture and dry benzene [10].

The hydrolysis of *N,N'*-diacetoxy-*N,N'*-R-diamide compounds was achieved in enough quantity of a dilute sulfuric acid (4N) with light heating, because the amount of the catalyst matter was insufficient to remove acetoxyl group in the form of acetic acid. Also, the formed nitrenium ions were trapped by water to receive dihydroxamic acids in good yield as shown in Experimental.

Spectral data obvious the expected molecular structure of the synthesized compounds, which were in good agreement [22, 23]. The aromatic protons (thiophene and benzene nuclei) appear at

7.35-9.19 ppm in all three spectrums (shown in Fig. 5). Two nitron protons exhibit singlet signal at 7.83ppm in spectrum of N,N'-Diphenyl-2,5-thienylenedinitrone **3a**, which disappears after oxidation. The spectrum of oxidation produce **3a'** contains a signal at 2.25ppm due to three protons of acetoxy group. The nmr spectrum for N,N'-dihydroxy N,N'-Diphenyl -2,5-thiophenedicarboxamide **3a''** show the apparition of singlet peak at 11.50 ppm corresponding to the protons of the OH groups (Fig. 6). Likewise, the  $^{13}\text{C}$  NMR spectra of compounds **3a**, **3a'** and **3a''** registered similar carbon chemical shifts each others, with a slightly different depending on the change of functional group. As it is known the aromatic carbon chemicals shifts cover a range of 100-150 ppm [22]. And the shielding of the carbon nucleus will be affected by electronegativity of the attached atoms. So, the azomethine carbon atom gives signal at 131.35 ppm. The quaternary aromatic carbon atoms attached to N-O group and

functional carbon atom **3a** give a signal at, respectively, 146.40 and 135.66 ppm. Other aromatic carbon atoms give NMR signal at the region of 130.33-121.19 ppm. Carbonyl carbon in dicarboxamide appeared at 158.23 ppm and acetoxy carbon showed two chemical shift values at 167.99ppm and 18.17 ppm. Also, carbonyl carbon of hydroxamic acid **3a''** gives a signal at 159.88 ppm.

In the same way, N-O stretching vibration at  $1172.60\text{ cm}^{-1}$  and C=N vibration at  $1546.80\text{ cm}^{-1}$  were detected in IR spectrum of dinitrone **3a**. Instead, a strong signals of N-O stretching vibration at  $1180.4\text{ cm}^{-1}$ , C=O vibration at  $1681.8\text{ cm}^{-1}$  and  $-\text{OC}(=\text{O})\text{CH}_3$  vibration at  $1789.8\text{ cm}^{-1}$  of amide function appeared. Moreover, IR spectrum of hydroxamic acid **3a''** appears C=O vibration at  $1600.8\text{ cm}^{-1}$  and strong broad signal of O-H stretching vibration at  $3062.7\text{ cm}^{-1}$ .

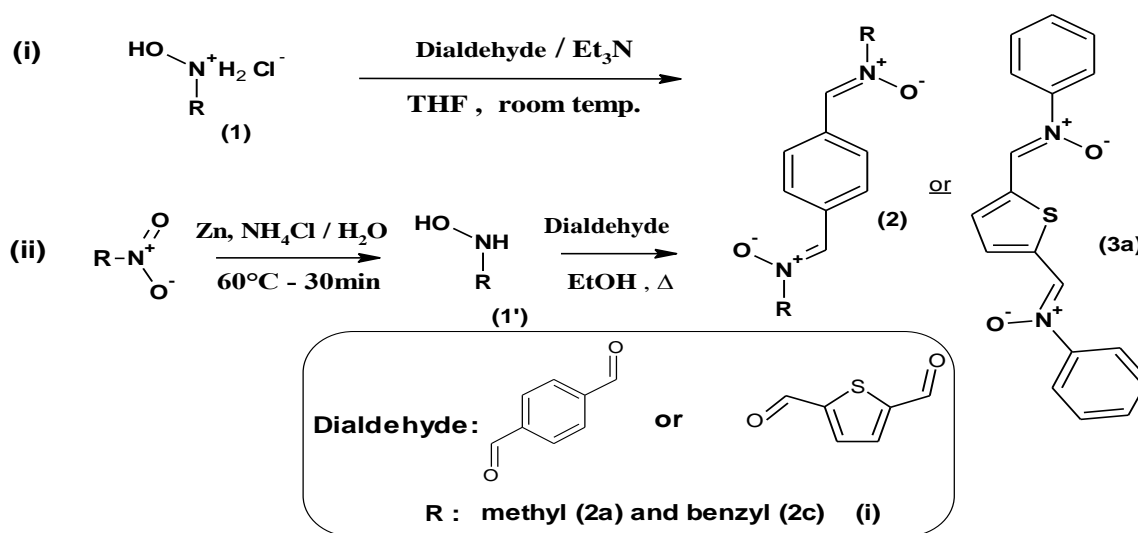


Fig. 1: Dinitrones synthesis.

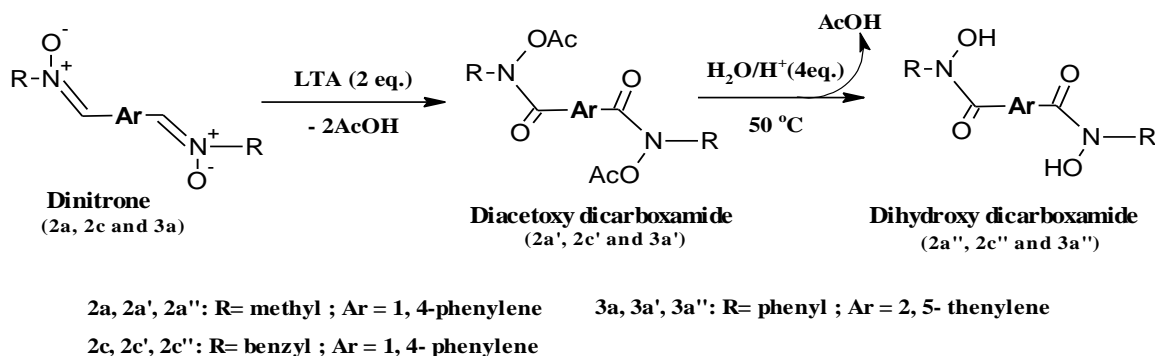


Fig. 2: Oxidation and hydrolysis steps.

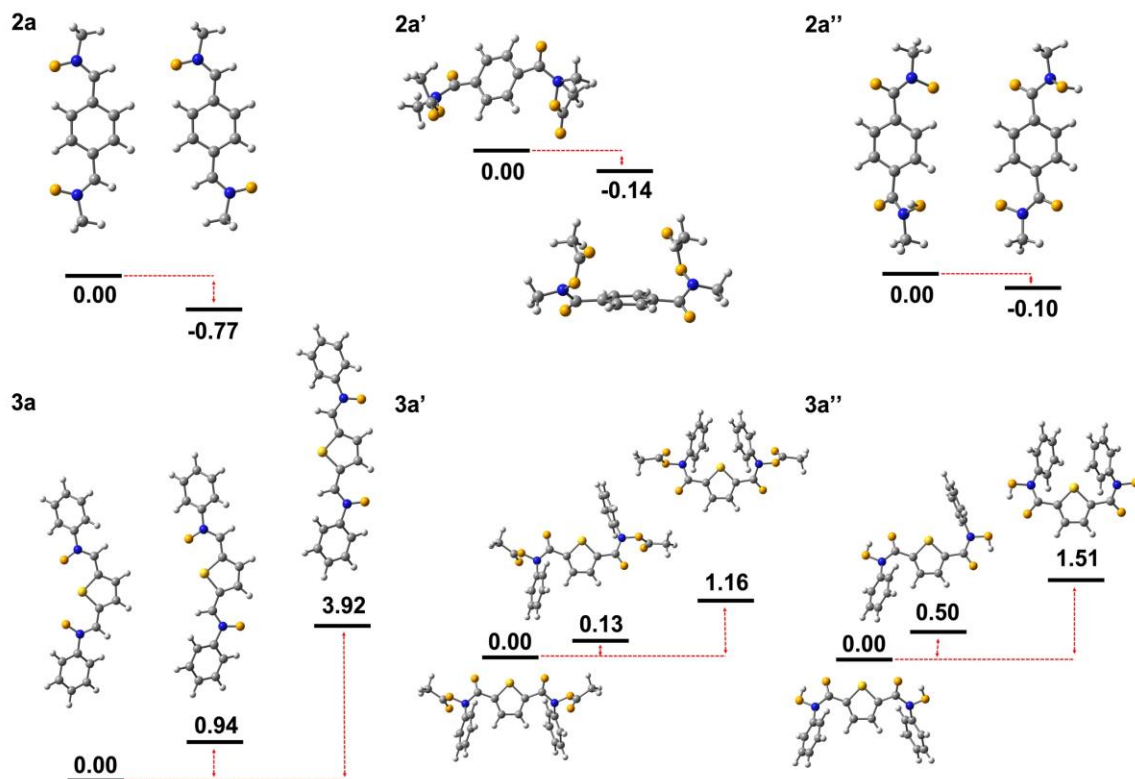


Fig. 3: Most stable conformers of compounds 2a-a'' and 3a-a'' and their relative energy in kcal/mol.

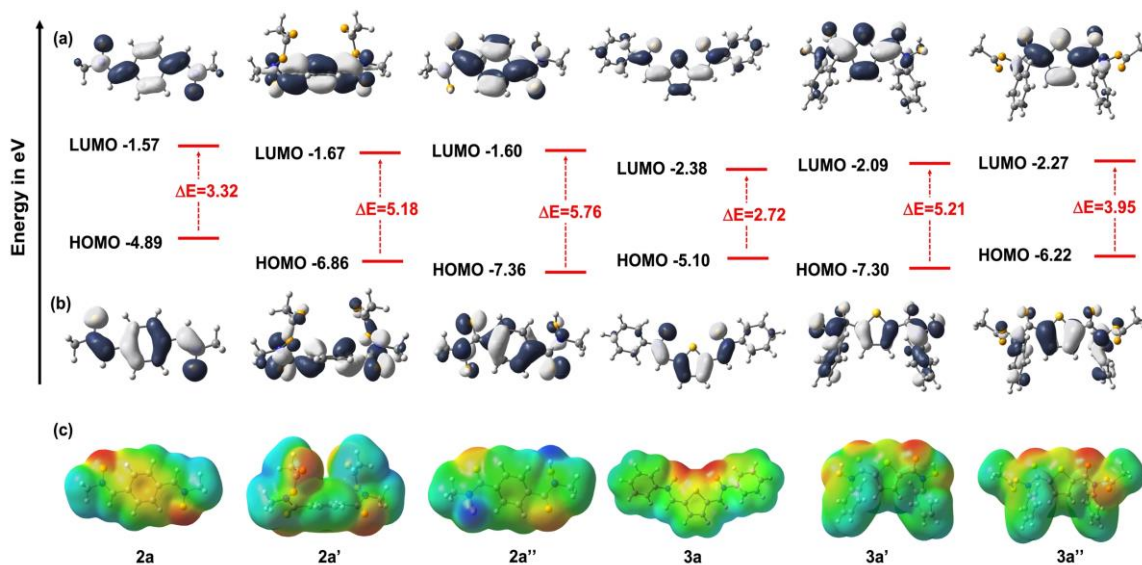


Fig. 4: HOMO (a) and LUMO (b) distributions and energies of compounds 2a-a'' and 3a-a'' as well as their molecular electrostatic potentials (c). For the ESP, a deep red color indicates an electron-rich site, whereas deep blue indicates an electron-deficient site.

To get insights into the molecular geometry and electronic properties of the synthesized compounds, Density functional theory calculations at

B3LYP/6-311G (d, p) level of theory has been performed for compounds 2a-a'' and 3a-a'' as representative molecules.

Firstly, a conformational analysis has been carried out in order to determine the most stable molecular geometry of the investigated compounds and the obtained results are reported in Fig.3. As shown, there are two conformers of each compound of the series **2a-a''** with relatively small energy difference of 0.1-0.7 kcal/mol. The most stable conformer was obtained when the  $\beta$ -oxygen atoms of compound **2a** or  $\alpha$ -oxygen atoms of compound **2a'** and **2a''** of the benzene ring are in *trans* configuration. Concerning compounds **3a-a''**, there are three conformers of each compound with relative energies ranging from 0.13 to 3.92 kcal/mol. Different from compounds **2a-a''**, the most stable conformer of compounds **3a-a''** is when  $\alpha$  or  $\beta$ -oxygen atoms of the thiophene ring are in *cis* configuration. This can be explained by the formation of favorable non-covalent intramolecular interactions between the sulfur atom and the neighboring oxygen atoms [24].

Using the most stable geometry of compounds **2a-a''** and **3a-a''**, we have calculated some of their electronic properties including frontier molecular orbitals (FMO) and electrostatic potentials (ESPs). FMO, i.e. highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), as well as ESPs are important parameters that characterizes the reactivity of organic compounds [25]. The energy of HOMO and LUMO indicates the ability of a molecule to donate or accept electrons, respectively. The distribution of HOMO also indicates the sites of nucleophilic attacks. ESPs represent a 3D view of charge distribution, which is indicative of sites of electrophilic and nucleophilic attacks. The frontier molecular orbitals energies and distributions and ESPs of compounds **2a-a''** and **3a-a''** have been computed at B3LYP/6-311G (d,p) level of theory, and the obtained results are shown in Fig. 4. As can be seen, all the investigated compounds have shown the typical  $\pi$ -like molecular orbitals. Both the HOMO and LUMO of compounds **2a-a''** and **3a-a''** are distributed over the entire molecule, with the exception of LUMOs of compounds **2a'** and **3a''** as well as the HOMOs of compound **3a**. This is an indication of the stability of the synthesized compounds. Examination of the HOMO energies reveals that the energy of the nitrone derivatives, i.e. compound **2a** and **3a**, is significantly higher than that of the other compounds. For example,

the HOMO energy of compound **2a** is higher by 1.97 eV and 2.47 eV than compounds **2a'** and **2a''**, respectively. This indicates that the electron donation ability of compounds **2a** and **3a** is better than that of the other compounds. As for the ESPs shown in Fig. 4, it is clearly observed that the negative charges are mainly located on the oxygen atoms (red color, Fig.4), while the positive charges are distributed around several hydrogen atoms, with the exception of compound **2a''**. For the latter the positive charge is mainly localized on the hydrogen atoms of the OH bonds. These results suggest that compound **2a''** is the most acidic compound in the series.

#### Antimicrobial activity

The microbial activities of the yielded compounds were estimated by the disc diffusion method [26] against bacteria Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative (*Escherichia coli*), and fungi (*Umbelopsis ramanniana* and *Fusarium culmorum*). The quantity of sample per a disk was 0.25mg. Incubation of bacterial plate was 24h for bacteria and 48h for fungi at 30°C. Results are illustrated in Table 1. Dihydroxamic acid compounds (**2a''**, **2c''** and **3a''**) exhibit a good activity on contrary to their precursors: dinitrones and N,N'-diacetoxy N,N'-diamides. The compound **2a''** was active against *S. aureus* bacteria Gram-positive and *U. ramanniana* fungus (15mm clear). Likewise, the compound **2c''** was active against *S. aureus* bacteria Gram-positive (12mm), *E. coli* bacteria Gram-negative, *U. ramanniana* fungus and *F. culmorum* fungus. The activity of compound **3a''** was best against *S. aureus* bacteria Gram-positive (11mm) and *E. coli* bacteria Gram-negative. But not active against *B. subtilis* bacteria Gram-positive and *F. culmorum* fungus, which is good with **3a'**. The presence of specific functional groups and the permeability of compound through the microbial cell are essential for the effectiveness of an antimicrobial agent to influence the activity [27]. So probably dihydroxamic acid molecules inhibit deoxyribose nucleic acid (DNA) synthetic by impairing the activity of enzyme ribonucleotide reductase in microbial cells[13], which increase their activity. Instead the microbial activity of dinitrones and N,N'-diacetoxy N,N'-diamides was impaired.

Table-1: Antimicrobial activities of the efficient compounds.

| Microorganism        | Inhibition zone diameter (mm) |       |        |        |        |        |
|----------------------|-------------------------------|-------|--------|--------|--------|--------|
|                      | 2a                            | 3a    | 3a'    | 2a''   | 2c''   | 3a''   |
| <i>B. subtilis</i>   | 7±0.5                         | 9±0.4 | 13±0.4 | -      | -      | -      |
| <i>S. aureus</i>     | -                             | -     | 7±0.2  | 8±0.5  | 12±0.2 | 11±0.2 |
| <i>E. coli</i>       | -                             | -     | -      | -      | 10±0.4 | 6±0.3  |
| <i>U. ramanniana</i> | -                             | -     | -      | 15±0.2 | 7±0.3  | -      |
| <i>F. culmorum</i>   | -                             | -     | 7±0.2  | -      | 7±0.3  | -      |

- : no activity



- Antibacterial Properties, *Can. J. Chem.*, **48**, 2393 (1970).
16. R. C. F. Jones and J. N. Martin, Nitrones In Synthetic Application of 1,3-Dipolar Cyclo-addition Chemistry Towards Heterocycles and Natural Products, Padwa, A., Pearson, W. H., Ed., John Wiley & Sons, New York, 8 (2002).
  17. M. C. Ferraz, R. A. Mano, D. H. Oliveira, D. S. V. Maia, W. P. Silva, L. Savegnago, E. J. Lenardão and R. G. Jacob, Synthesis, Antimicrobial and Antioxidant Activities of Chalcogen-Containing Nitroene Derivatives from (R)-citronella, *Medicines*, **4**, 39 (2017).
  18. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, Gaussian 09, Revision E.01. Wallingford, CT, (2009).
  19. A. D. Becke, Density-functional exchange-energy approximation with correct asymptotic behavior, *Phys. Rev. A*, **38**, 3098 (1988).
  20. I. Y. Zhang, J. Wu and X. Xu, Extending the reliability and applicability of B3LYP, *Chem. Comm.*, **46** (18), 3057 (2010).
  21. K. L. Sorgi, Encyclopedia of Reagents for Organic Synthesis. Wiley, New York, (2001).
  22. H. Feuer, Nitril oxides, Nitrones, and Nitronate in organic synthesis- Novel Strategies in synthesis. John Wiley & Sons, New Jersey, (2008).
  23. D. Hadzi and D. Prevorsek, Infra-red absorption bands associated with the NH group—III<sup>1</sup>: Hydroxamic acids and derivatives, *Spectroc. Acta.*, **10**, 38 (1957).
  24. G. Conboy, H. J. Spencer, E. Angioni, A. L. Kanibolotsky, N. J. Findlay, S. J. Coles, C. Wilson, M. B. Pitak, C. Risko, V. Coropceanu, J. L. Brédas and P. J. Skabara, To bend or not to bend – are heteroatom interactions within conjugated molecules effective in dictating conformation and planarity, *Mater. Horiz.*, **3**, 333 (2016).
  25. K. Sadasivam, R. Kumaresan, Antioxidant behavior of mearnsetin and myricetin flavonoid compounds — A DFT study, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **79**, 282 (2011).
  26. A. W. Bauer, W. M. Kirby, J. C. Sherris and M. Turck, Antibiotic susceptibility testing by a standardized single disk method, *Am. J. Clin. Pathol.*, **45**, 493 (1966).
  27. A. Irshad, N. Khana, Y. Farina, N. Baloch, Samiullah, A. Ali, L. K. Mun, G. Murtaza, Attiqur-Rehman, Synthesis, Spectroscopic Characterization, X-ray Diffraction Studies and In-vitro Antibacterial Activities of Diorganotin(IV) Derivatives with N-methyl-4-bromobenzohydroxamic acid, *Inorg. Chim. Acta.*, **469**, 280 (2018).