SYNTHESIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME NEW 1,3,4-OXADIAZOLE DERIVATIVES

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ABSTRACT

Novel oxadiazol derivatives from 2,2'-Thiodiacetic acid were synthesized by cyclization with Benzoic acid, Isonicotinic acid, P-Chlorobenzoic acid, and P-Aminobenzoic acid using the phosphoryl chloride (POCl₃). Analyses with 1H-NMR, IR, and MS spectrometry have confirmed the structures of synthesized compounds, which are evaluated as an antioxidant potential using the DPPH radical and Vitamin-C as a benchmark drug. The majority of the tested compounds exhibit a significant antioxidant activity.

Keywords: 1,3,4-Oxadiazole, 2,2'-Thiodiacetic Acid, Antioxidant Activity, DPPH

INTRODUCTION

Over the last years, Oxadiazoles have considerably interested many researchers, particularly in medicinal chemistry.¹ The methods of synthesizing heterocyclic molecules of this class have been developed due to their numerous applications in biology and in pharmacy for their agonist²-⁶, inhibitory of leukotriene receptors⁷,⁸, anti-inflammatory⁹,¹⁰, antibacterial, and antioxidant properties.¹¹-¹⁵ In addition to their complexing properties, oxadiazoles exhibit excellent protective properties against corrosion of steel in an acidic environment.¹⁶

Organosulfur compounds occupy an important place in the industry thanks to the presence of the sulfur atom, which plays an important biological role, particularly by its presence in common enzymes to all living cells and in amino-acids.¹⁷ Sulfuric acid, which is used in the lubricants industry as a component of extreme pressure (EP)¹⁸ and anti-wear (AW)¹⁹ additives, as well as in the fertilizer industry, up to the most complex organic molecules in the pharmaceutical industry such as cephalosporins, antibiotics related to penicillins or even in coenzyme A involved in many biochemical syntheses, including amino acids such as methionine, cystine, cysteine, and glutathione²⁰ Figure 1 used as a natural antioxidant due to the presence of the sulfur atom providing the antioxidant activity.²¹ Sulfur compounds are essential for life and are ubiquitous in our daily environment: living organisms, food, cosmetics, aroma drugs, polymers, resins, lubricants, detergents, phytosanitary products etc.

Taking into account the above-cited works and motivated by the oxadiazole's biological activities, the 1,3,4-oxadiazoles have been prepared by different methods. Currently, new preparation techniques and methodologies are rapidly being developed. The method used here is based on the dehydrating intramolecular cyclization of an N, N-diacylhydrazide derivative in the presence of phosphorus oxychloride with a base, a novel series of syntheses of Oxadiazole derivatives from 2,2'-Thiodiacetic acid have been performed through the present experiment in order to pursue the antioxidant activity.

EXPERIMENTAL

Materials and Methods

All chemicals products are provided by SIGMA. They are employed without purification. The determination of melting points was done in an open capillary tube method using GALLENKAMP
equipment. The IR spectra were obtained using a PerkinElmer FTIR 600 Fourier transform infrared spectrometer. All NMR was recorded with a Bruker NMR spectrometer at Es-senia, Oran University, Algeria. Mass spectral data were recorded on a Claruss GC interfaced to a Claruss MS equipped with an EI source and auto-injector. Turbo Mass software was used for data processing (PerkinElmer, USA). The antioxidant activity property was monitored using a PerkinElmer Lambda (25 UV-VIS) spectrophotometer.

General Procedure to Synthesize Dimethyl 2,2'-thiodiacetate (2)

2,2'-thiodiacetic acid (1) (2 g, 0.0133 mol), methanol (50 ml) and H₂SO₄ (1.5 ml) were refluxed for 5 h at 85°C. The synthesized ester (2) was obtained as an oil, yield (1.92 g; 81%), Rf Value: 0.9; FT-IR υmax (cm⁻¹): 1728 (C=O); 1H-NMR (ppm): 3.40-3.42 (d, 2H, -CH₂); 3.34-3.38 (d, 2H, -CH₂); 3.67 (s, 3H, COOMe).

General Procedure to Synthesize 2,2'-thiodiacetohydrazide (3)

A mixture of dimethyl 2,2'-thiodiacetate (2) (2 g, 0.0112 mol), absolute ethanol (30 ml) and hydrazine hydrate 80% (5 ml) was refluxed for 9 h. Iced water was used for washing the produced solid, which was filtered, and recrystallized with EtOH/H₂O. The synthesized (3) was obtained as a solid, product has the following characteristics: yield (1.78 g 89%), Rf Value: 0.15; FT-IR υmax (cm⁻¹): 3329.9 (NH, NH₂), 1607.05 (CO-N); 1H-NMR (ppm): 3.45-3.51 (d, 2H, -CH₂); 3.50-3.69 (d, 2H, -CH₂); 7.88 (s, 1H, NH), 2.10-2.02 (d, 2H, C-CONH-NH₂).

General Procedure to Synthesize 1,3,4-oxadiazoles: 4. (a-d)

(1.782 g, 0.01 mol) of 2,2'-thiodiacetohydrazide (3), (0.01 mol) of aromatic acids and (10 mL) of phosphorous oxychloride were mixed and refluxed for 15 hrs. The product was made basic with NaHCO₃. Then, the product obtained was and recrystallized with alcohol. Table-1 illustrates the physical properties of the compounds 4(a-d).

| Compounds | Various Aromatic Acid | MP (°C) | Yield (%) |
|-----------|-----------------------|---------|-----------|
| 4.a       | Benzoic acid          | 125.7-126.9 | 70.0      |
| 4.b       | Isonicotinic acid     | 175.2-180.9 | 65.8      |
| 4.c       | p-Chlorobenzoic acid  | 198.6-200.5 | 60.7      |
| 4.d       | p-Aminobenzoic acid   | 205.6-207.3 | 69.0      |

Benzoic Oxadiazol Derivative (4a)

FT-IR υmax (cm⁻¹): 3000-2907 (CH) Aromatic; 1681.5 (C=N); 1639.1590.9,1570.6 (C=C) Aromatic; 1115.3 (C-O-C). 910(C-Cl); 1H-NMR (ppm): 7.35-7.29 (d, 2H, Ar-CH), 3.51-3.73 (d, 2H, -CH₂); 7.45-7.52 (d, 2H, Ar-CH), 3.48-3.60 (d, 2H, -CH₂), 7.40-7.46 (d, 2H, Ar-CH), MS m/z: 351[M⁺].

Isonicotin Oxadiazol Derivative (4b)

Obtained from hydrazide (3) and isonicotinic acid (Pry) in (4.b, 2.60 g, 65.8%), white powder, Mp. FT-IR υmax (cm⁻¹): 3038.8-2943.3 (CH) Aromatic; 1682.2 (C=N); 1635.5-1512.9 (C=C) Aromatic; 1115.3 (C-O-C). 1H-NMR (ppm): 8.52-8.46 (d, 2H, Ar-CH), 3.50-3.76 (d, 2H, -CH₂); 7.90-7.85 (d, 2H, Ar-CH), 7.93-7.82 (d, 2H, Ar-CH), 3.50-3.58 (d, 2H, -CH₂), MS m/z: 353[M⁺].

p-Chlorobenzoic Oxadiazol Derivative (4c)

FT-IR υmax (cm⁻¹): 3038.8-2943.3 (CH) Aromatic; 1682.2 (C=N); 1635.5-1512.9 (C=C) Aromatic; 1115.3 (C-O-C). 1H-NMR (ppm): 8.52-8.46 (d, 2H, Ar-CH), 3.50-3.76 (d, 2H, -CH₂); 7.90-7.85 (d, 2H, Ar-CH), 7.93-7.82 (d, 2H, Ar-CH), 3.50-3.58 (d, 2H, -CH₂), MS m/z: 353[M⁺].

p-Aminobenzoic Oxadiazol Derivative (4d)

FT-IR υmax (cm⁻¹): 3038.8-2943.3 (CH) Aromatic; 1682.2 (C=N); 1635.5-1512.9 (C=C) Aromatic; 1115.3 (C-O-C). 1H-NMR (ppm): 6.30-6.22 (d, 2H, Ar-NH₂), 3.58-3.59 (d, 2H, -CH₂); 7.95-7.88 (d, 2H,
RESULTS AND DISCUSSION

Chemistry

Alkyl 2,2′-thiodiacetate: dimethyl 2,2′-thiodiacetate (2a) was prepared by esterification of 2,2′-thiodiacetic acid (1) with methanol. IR, 1H-NMR spectra were correct for 2(a, b)- see experimental-. Both thioesters reacted with hydrazine hydrate 64% gave the same hydrazide 3 as indicated by spectroscopy. The tri-1,3,4-oxadiazole derivative of 2,2′-thiodiacetic acid was obtained by treating hydrazide (3) with POCl₃ cyclization with different substituted aromatic acids. Structural determination was done by IR, 1H-NMR spectra²²-²⁴ and confirmed by mass spectrometry. The synthesis route of the products is shown in Figure 1.

Antioxidant Study

The antioxidant activities of all compounds were assessed by DPPH protocol.²⁵-²⁷ The antioxidant capacity is reported relative to the ascorbic acid as a benchmark antioxidant. The stock solution was freshly prepared with 24 mg of DPPH in 100 ml of methanol. The ascorbic acid (Vit-C) was dissolved in methanol at a concentration of 0.0376 mg. mL⁻¹ (150 µmol. L⁻¹). The solution was then stored in the refrigerator. 3 mL of the DPPH stock solutions and 100 µl of the sample are added to a spectrophotometric cuvette to obtain a solution with a final concentration within the range of 20 - 100 µg. mL⁻¹. The cuvettes were placed in the dark at 25 °C for 15 minutes when the reaction was considered complete. The absorbance value in the presence of antioxidants was then recorded. An average of three spectrometric measurements was carried. At room temperature and wavelength of 515 nm, was calculated for each concentration and averaged. Antioxidant capacity is reported relative to ascorbic acid as a benchmark antioxidant according to the following formula:

\[
\text{Antioxidant activity} \% = 1 - \frac{\text{Abs (Ascorbic acid)}}{\text{Abs (methanol)}} \times 100
\]

The plotted curve presents an ascending linear line of the form: \(\text{AO} \% = a \times \text{[Ascorbic acid]}\). From this formula, it is possible to deduce the ascorbic acid concentration (Vit-C), allowing to trap 50% of the radicals present or to determine the IC₅₀ in mg / l (minimum inhibitory concentration at 50%) of the Ascorbic acid (Vit-C).

\[
\text{Ascorbic acid} = \frac{50}{a}
\]

"a" denotes the slope of the linear regression. The IC₅₀ of the compounds is based on the same calculation method as that described above.
The ascorbic acid standard range was obtained for the concentrations ranging from (62.5 μg/mL up to 1000 μg/mL), and the results are shown in Fig.-2.

![Fig.-2: DPPH Inhibition Rate as a Function of Ascorbic Acid Concentration](image)

The results obtained allowed us to plot the percentage inhibition plots as a function of the concentrations. From these curves (Fig.-2 and 3) we were able to determine the inhibitory value of 50% of the free radical DPPH (IC$_{50}$), the smaller this value, the more the sample has good antioxidant activity. The results of antioxidant activity are summarized in Table-2. Figure-3 shows that the synthesized compounds exhibit different antioxidant capacities. With respect to ascorbic acid (IC$_{50}$ = 282) the compounds 4b and 4c have a much more efficient antioxidant activity higher IC$_{50}$ than ascorbic acid, than compounds 4a and 4d have lower IC$_{50}$ than ascorbic acid. Compounds 4b and 4c exhibit weaker antioxidant activity against DPPH than ascorbic acid, the latter at an IC$_{50}$ of 281.8 μg / ml. The fraction of compound 4d exhibits a lower IC$_{50}$ of 99.1 μg / ml; therefore, it is the fraction that carries the molecules with the highest antioxidant power, followed by the fraction of compound 4a with IC$_{50}$: 109.5 μg / ml).

![Fig.-3: DPPH Inhibition Rate as a Function of Ascorbic Acid and tested Compounds Concentrations](image)

Table-2: Concentration of Compounds (4a-d) and Vit-C at DPPH Radical Scavenging Activity (IC$_{50}$)

| Compounds | DPPH IC50 (μg/mL) |
|-----------|-------------------|
| 4.a       | 109.5 ± 0.01      |
| 4.b       | 343.6 ± 0.02      |

Figure-4 and Table-3 show the inhibition rate of the DPPH radical for the compounds tested during 15 min, 30 min, 2 h, 24 h. 

![Fig.-4](image)

Table-3: Concentration of Compounds at DPPH Radical Scavenging Activity (IC$_{50}$)

| Compounds | Concentration (μg/mL) |
|-----------|-----------------------|
| 4.a       | 0, 20, 40, 60, 80, 100, 120 |
| 4.b       | 0, 20, 40, 60, 80, 100, 120 |
| 4.c       | 0, 20, 40, 60, 80, 100, 120 |
| 4.d       | 0, 20, 40, 60, 80, 100, 120 |

Figures and Tables are placeholders as the actual images and data are not available in the text.
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Table 3: Concentration of Compounds (4a-d) and Vit-C at DPPH Radical Scavenging Activity (IC\textsubscript{50})

| Ascorbic Acid Concentration | Time   | Inhibition Rate of Ascorbic Acid and all Compounds tested |
|-----------------------------|--------|---------------------------------------------------------|
| 62.5(\textmu g/mL)         | 00.25 h| 62.70 18.7 19.44 36.89 30.74                             |
|                             | 00.50 h| 60.97 24.45 24.03 41.17 31.74                             |

Fig. 4: Influence of Different Ascorbic Acid Concentrations (62.5 \textmu g/mL up to 1000 \textmu g/mL) Vt-C on the Inhibition Rate of Compounds (a-d) at each Monitoring Time
### CONCLUSION

A range of new 1,3,4-Oxadiazole derivatives from 2,2'-Thiodiacetic acid has been synthesized and their antioxidant activity has been tested. The physical and chemical analyses such as the melting point, FT-IR, and NMR spectroscopies were used for the characterization of the synthesized compounds. The results show that the Benzoic oxadiazol derivative (4a) and Aminobenzoic oxadiazol derivative (4d) have a great antioxidant activity than the Pyridin oxadiazol derivative (4b) and Chlorophenyl oxadiazol derivative (4c).

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