Synthesis and Antifungal Evaluation of Quaternary Ammonium Salts Derivatives of Dialkylaminoethyl Methacrylate Bearing 1,3,4-Oxadiazoles Moieties

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Abstract: Starting from lauric acid two novel quaternary ammonium salts containing 1,3,4-Oxadiazoles nucleus derivative from N,N-Diethylaminoethyl Methacrylate (DEAEMA) and N,N-Dimethylaminoethyl Methacrylate (DMAEMA) was successfully synthesized and characterized by IR, ¹H and ¹³C NMR. All the synthesized compounds were evaluated for their preliminary in vitro antifungal activity against three fungal strains such as *Fusarium oxysporum*, *Fusarium commune* and *Fusarium rodelens*. The synthesized compounds showed promising antifungal potential against the phytopathogenic test fungi.

1. Introduction

The phytopathogenic fungal pose serious problems worldwide in the cultivation of economically important plants because induction of wilt disease in a wide range of host plants such as, for example, cereals, tomatoes, potatoes, bananas and watermelons [1-5]. The search for new antifungal agents will consequently always remain as an important and challenging task for organic chemists; natural products are often used as starting points in the drug discovery for a variety of purposes [6]. In recent years, Fatty acids have attracted much attention due to their wide range of their biological activities [7-8]. Lauric acid or dodecanoic acid (C12) is a saturated fatty acid has antimicrobial activity [9] and antifungal properties against plant pathogenic fungi [10]. 1,3,4-oxadiazoles is a versatile heterocyclic nucleus have exhibited a wide range of biological properties [11], such as anti-bacterial [12], anti-viral [13], anti-fungal [14], anti-cancer [15-16], anti-tumor [17], anti-inflammatory [18], anti-hypertensive [19], anti-convulsant [20] and anti-diabetic properties [21]. Compounds containing 1,3,4-oxadiazole is an important moiety for development of new drugs [22-24]. Quaternary ammonium salts (QAS) have multiple applications in industry [25], in clinical medicine [26] and in the household [27]. These compounds are used as disinfectants [28], surfactants [29] and wood preservatives [30]. The mechanism of the biocidal action of QAS compounds is based on the adsorption of positively charged alkyl ammonium salt moiety on the negatively charged cell wall and its penetration by alkyl chain which leads to a leak of low molecular components of cell [31]. In a consequence the microorganism cell dies. Literature revealed that QAS compounds containing long chain n-alkyl act good Antimicrobial [32-33] and fungicidal properties [34]. In continuation to extend our research on fungicidal compounds, we focused our attention on the synthesis of novel 1,3,4-oxadiazoles containing quaternary ammonium moiety derivatives from lauric acid and investigated their antifungal activity against three phytopathogenic fungal.

2. Experimental

Material and method

All solvents and reagents used in this study were obtained from Sigma Aldrich and BIOCHEM. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel F254 supplied by MERCK, using mixture of different polar and nonpolar solvents in varying proportions and spots were observed using iodine as visualizing agent. All Melting points were determined in open capillary tubes on a BÜCHI 540 melting point apparatus and are uncorrected. The Infrared spectra of reactants and product in the range of 4000-400 cm^{-1} were recorded as potassium bromide discs on a Shimadzu FTIR-8300 Fourier Transform infrared spectrophotometer. The ^1H and ^{13}C NMR Spectra were measured in Chloroform-d (CDCl_3) on Bruker AM 300 MHZ Spectrometer (University of Oran, Essenia), relative to the internal standard tetramethylsilane (TMS), and chemical shift values are expressed in parts per million (δ , ppm).

Preparation of ethyl laurate (2)

This ester was prepared following the standard procedure reported in the literature [36]. Lauric acid (5g, 0.025mol) was dissolved in excess of ethanol (200 mL) with 5 mL of concentrated sulfuric acid and the mixture was refluxed at 80°C in an oil bath for 5-6 h, the progress of the reaction was monitored by TLC. The excess of acid was neutralized with sodium bicarbonate then the solvent was evaporated and the product was collected.

IR (KBr, ν , cm^{-1}): 1743.5 ($\text{C}=\text{O}$), 1170.7 ($\text{C}-\text{O}-\text{C}$).

Preparation of lauric acid hydrazide (3)

To a solution of lauric methyl ester **2** (3.5g, 0.016mol) in ethanol (100 mL), hydrazine hydrate (98%; 5ml) was added and heated for 5h on oil bath. The progress of the reaction was monitored by TLC, the reaction mixture was cooled, and the excess of ethanol was distilled off under reduced pressure. The crude product was filtered, washed with water and evaporated to dryness to give the products.

IR (KBr, ν , cm^{-1}): 3315.4 (NHNH_2), 1631.7 ($\text{C}=\text{O}$).

^1H -NMR (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 7.281 (3H, $\text{O}=\text{C}-\text{NH}-\text{NH}_2$), 2.326 (2H, $\text{CH}_2-\text{C}=\text{O}$), 0.879 (3H, CH_3-CH_2-). ^{13}C -NMR (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 174.913 ($\text{O}=\text{C}-\text{NH}-\text{NH}_2$), 34.294 ($\text{CH}_2-\text{C}=\text{O}$), 14.628 (CH_3-CH_2-).

Synthesis of 2-(bromomethyl)-5-undecyl-1,3,4-oxadiazole (4)

A mixture of hydrazide **3** (2g, 0.0094mol), bromoacetic acid (1.31g, 0.0094mol) and phosphoryl chloride (5ml) was refluxed on a oil-bath for 6-8h. The progress of the reaction was monitored by TLC. After cooling to room temperature, the resulting precipitate was filtered, washed with water and dried.

IR (KBr, ν , cm^{-1}): 1598.9 ($\text{C}=\text{N}$), 1002.9 ($\text{C}-\text{O}-\text{C}$), 495.7 ($\text{C}-\text{Br}$).

^1H -NMR (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 0.899 (3H, CH_3-CH_2-), 2.320 (2H, $\text{CH}_2-\text{C}=\text{N}$), 3.684 (2H, $\text{N}=\text{C}-\text{CH}_2-\text{Br}$). ^{13}C -NMR (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 179.126 ($-\text{CH}_2-\text{C}=\text{N}$), 154.257 ($\text{N}=\text{C}-\text{CH}_2-\text{Br}$), 29.043 ($-\text{CH}_2-\text{C}=\text{N}$), 15.902 ($\text{N}=\text{C}-\text{CH}_2-\text{Br}$), 14.083 (CH_3-CH_2-).

Synthesis of 2-(bromomethyl)-5-undecyl-1,3,4-oxadiazole-DEAEMA (5a)

An equimolar quantity of DEAEMA (0.29g, 0.0015mol) and 2-(bromomethyl)-5-undecyl-1,3,4-oxadiazole (**4**) (0.5g, 0.0015mol) in dry acetone (50 ml) and a small amount of hydroquinone was added, This Mixture was gently refluxed for 8 hours. TLC analysis was used to monitor the progress of the reaction. After that, the white solid that appeared on cooling was filtered and the excess solvent was removed by vacuum evaporation. The residue was washed several times with cold diethyl ether and allowed to dry.

IR (KBr, ν , cm^{-1}): 1724.2 ($\text{C}=\text{O}$), 1596.9 ($\text{C}=\text{N}$), 1461.9 ($\text{C}=\text{C}$), 1018.3 ($\text{C}-\text{O}-\text{C}$).

$^1\text{H-NMR}$ (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 6.128-5.643 (2H, $\text{O=C-(CH}_3\text{)C=CH}_2$), 4.585 (2H, $^+\text{N-CH}_2\text{-CH}_2\text{O}$), 4.203 (2H, $\text{N=C-CH}_2\text{-N}^+$), 3.617 (2H, $^+\text{N-CH}_2\text{-CH}_2\text{O}$), 3.129 (2H, $^+\text{N-CH}_2\text{-CH}_3$), 2.265 (2H, $\text{CH}_2\text{-C=N}$), 1.940 (3H, $\text{O=C-(CH}_3\text{)C=CH}_2$), 1.326 (3H, $^+\text{N-CH}_2\text{-CH}_3$), 0.868 (3H, $\text{CH}_3\text{-CH}_2\text{-}$). $^{13}\text{C-NMR}$ (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 174.874 ($-\text{CH}_2\text{-C=N}$), 166.443 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 150.016 ($-\text{N=C-CH}_2\text{-N}^+$), 135.491 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 127.051 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 59.059 ($^+\text{N-CH}_2\text{-CH}_2\text{O}$), 58.113 ($^+\text{N-CH}_2\text{-CH}_2\text{O}$), 51.562 ($\text{N=C-CH}_2\text{-N}^+$), 49.665 ($^+\text{N-CH}_2\text{-CH}_3$), 29.474 ($\text{CH}_2\text{-C=N}$), 18.210 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 14.063 ($\text{CH}_3\text{-CH}_2\text{-}$), 8.858 ($^+\text{N-CH}_2\text{-CH}_3$).

Synthesis of 2-(bromomethyl)-5-undecyl-1,3,4-oxadiazole-DMAEMA (5b)

An equimolar quantity of compound (**4**) and the tertiary amine DMAEMA (0.24g, 0.0015mol) acid were dissolved in dry acetone (50 ml) with a small amount of hydroquinone. This mixture was refluxed for 8 hours. The reaction was monitored by TLC. After that, solvent was evaporated and the products collected by filtration, washed with cold diethyl ether and allowed to dry.

IR (KBr, ν , cm^{-1}): 1722.3 (C=O), 1687.7 (C=N), 1467.7 (C=C), 1161.1 (C-O-C).

$^1\text{H-NMR}$ (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 6.151-5.913 (2H, $\text{O=C-(CH}_3\text{)C=CH}_2$), 4.572 (2H, $^+\text{N-CH}_2\text{-CH}_2$), 4.117 (2H, $\text{N=C-CH}_2\text{-N}^+$), 3.627 (2H, $^+\text{N-CH}_2\text{-CH}_2$), 3.469 (3% H , $^+\text{N-CH}_3$), 2.871 (2H, $\text{CH}_2\text{-C=N}$), 1.905 (3H, $\text{O=C-(CH}_3\text{)C=CH}_2$), 0.816 (3H, $\text{CH}_3\text{-CH}_2\text{-}$). $^{13}\text{C-NMR}$ (300MHz, δ , $\text{CDCl}_3\text{-d}_6$) δ (ppm): 173.492 ($-\text{CH}_2\text{-C=N}$), 167.916 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 150.068 ($-\text{N=C-CH}_2\text{-N}^+$), 135.083 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 127.143 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 59.180 ($^+\text{N-CH}_2\text{-CH}_2$), 58.233 ($\text{N=C-CH}_2\text{-N}^+$), 52.612 ($^+\text{N-CH}_2\text{-CH}_2$), 43.700 ($^+\text{N-CH}_3$), 29.604 ($\text{CH}_2\text{-C=N}$), 18.848 ($\text{O=C-(CH}_3\text{)C=CH}_2$), 14.151 ($\text{CH}_3\text{-CH}_2\text{-}$).

3. Results and Discussion

The synthetic route designed for the compounds **5a** and **5b** is summarized in Scheme 1.

Scheme 1. Synthetic route to title compounds **5a** and **5b**.

Reagents and conditions: (i) $\text{C}_2\text{H}_5\text{OH}$, and conc. H_2SO_4 , reflux for 8h; (ii) $\text{NH}_2\text{NH}_2\text{-H}_2\text{O}$ and $\text{C}_2\text{H}_5\text{OH}$, reflux for 5 h; (iii) POCl_3 and BrCH_2COOH , Reflux (iv) DMAEMA, DEAEMA and acetone.

Lauric ethyl ester **2** was synthesized using starting lauric acid **1** in ethanol and concentrated sulfuric acid. In the second step, the esters then converted to acid hydrazides **3** using Hydrazine hydrate in ethanol. This hydrazide was converted to give 2-(bromomethyl)-5-undecyl-1,3,4-oxadiazole **4** by ring-closing reactions using bromoacetic acid in the presence of phosphorus oxychloride. In the last step, compound **5a-b** was prepared by quaternization of the corresponding tertiary amine (DEAEMA, DMAEMA) with compound **4** to give the final products.

Table 1. Physical properties and analytical data of the synthesized compounds.

Compound	Color	%Yield	Melting Point (°C)	Rf Value	Solvent system
2	Colorless	84	liquid	0.33	CHCl ₃ :MeOH 1.5 :1
3	White	88	145-147	0.42	CHCl ₃ :MeOH 1.5 :1
4	White	92	194-195	0.35	CHCl ₃ :MeOH 1.5:1
5a	light yellow	87	semi-solide	0.38	CHCl ₃ :MeOH 9:1
5b	light yellow	90	semi-solide	0.46	CHCl ₃ :MeOH 9:1

3.1. Antifungal activity

The antifungal activities of the synthesized compounds were evaluated against three phytopathogenic fungi of tomato such as, *Fusarium oxysporum*, *Fusarium commune* and *Fusarium rodelens*, supplied from the fungal collection of the laboratory of Applied Microbiology Faculty of Nature Sciences and Life, Oran University, Algeria, by agar diffusion plate method [35]. In vitro Screening of Antifungal Activity of synthesized compounds was determined on the potato dextrose agar (PDA) as the growth medium for the tested fungi, and PDA was prepared by dissolving potato extract (200 g), D-glucose (20 g) and agar (15 g) in distilled water (1000 ml). Finally, the medium was transferred to a flask, sealed, sterilized by autoclaving at 121 °C for 30 min and cooled down. The compounds were tested at various concentrations of 50, 100, 150 and 200 µg/ml and have been incorporated into the PDA culture medium maintained molten at a temperature of 40 to 45°C. After the mixture flow and solidification; Mycelial implants of 6 mm diameter on the pathogen fungi are deposited in the center of the Petri dish containing PDA medium with 04 concentrations for each synthesized compounds to be studied. The Petri-dishes were incubated at 25°C for 4 days. Three replications were performed. After the completion of incubation period, the Relative inhibition rate of the circle mycelium compared to blank assay was calculated via the following equation:

$$\text{Relative inhibition rate (\%)} = [(T-C)/T] \times 100\%$$

Where T is the extended diameter of the circle mycelium during the blank assay; and C is the extended diameter of the circle mycelium during testing. The antifungal activity data are listed in Table 2.

Table 2. Antifungal activity for 4 days of the synthesized compounds

compounds	Inhibition Zone %											
	<i>Fusarium oxysporum</i>				<i>Fusarium commune</i>				<i>Fusarium rodelens</i>			
	concentration in µg/mL											
	50	100	150	200	50	100	150	200	50	100	150	200
3	+	++	+++	+++	++	+++	+++	+++	+	+	++	+++
4	+	+	++	++	++	++	++	+++	+	+	+	+++
5a	++	+++	+++	+++	+++	+++	+++	+++	+	++	++	+++
5b	++	++	+++	+++	+++	+++	+++	+++	+	+	+++	+++

Zone diameter of growth inhibition: (+) < 10 mm, (++) 10-15 mm, (+++) >16 mm

The evaluation of antifungal activity revealed that all the synthesized compounds have a significant biological activity, the best activity was observed at a concentration of 200 µg/mL against all the tested fungi, and moderate activity was observed at a concentration of 150 µg/mL. The compounds (**3**) and (**4**) displayed poor activities against *Fusarium oxysporum* and *Fusarium rodelens* at a concentration of 50 to 100 µg/mL.

4. Conclusion

New quaternary ammonium compounds containing in their structure 1,3,4-oxadiazole ring system were successfully synthesized and characterized by IR, ¹H and ¹³C NMR. The in vitro antifungal activity assay indicated that most of the compounds showed good to moderate antifungal activities against both systemic pathogenic fungi. These compounds can be considered as lead molecules for future investigations.

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