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Novel Synthetic Routes to Quaternary Pyridinium Salts and their Antifungal Activity

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Abstract: Eleven pyridine derivatives were prepared by quaternization reactions by different synthetic routes: conventional, microwave, and ultrasound. Since acetone and other solvents used in conventional quaternization reactions are harmful, attempts were made to replace the organic solvents with more environmentally friendly alternative - deep eutectic solvents. The reactions were carried out using pyridine-3-aldoxime, pyridine-4-aldoxime, isonicotinamide and nicotinamide as nucleophiles and three different dihaloalkanes as electrophiles: diiodopropane, dibromopropane and diiodohexane. The results showed that the microwave method using acetone as solvent was significantly faster and gave higher yields than the conventional method. In contrast, synthesis in the eutectic solvents choline chloride : urea gave significantly lower yields. The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The antifungal activity of all compounds was tested at two different concentrations (10 and 100 μg mL^{-1}) against *Botrytis cinerea, Fusarium culmorum, Macrophomina phaseolina* and *Sclerotinia sclerotiorum in vitro*. All tested compounds showed excellent inhibitory activity against the studied phytopathogenic fungal species at a concentration of 100 μg mL^{-1}.

Keywords: antifungal activity, dihaloalkanes, deep eutectic solvents, microwave synthesis, quaternization, pyridine derivatives, ultrasound synthesis.

INTRODUCTION

PYRIDINIUM salts are the main topic of scientific research due to their physicochemical properties and various biological activities. Their antimicrobial,[1–4] antimalarial,[5,6] and antileishmanial[7–9] activities have been reported in the literature. Pyridinium-based oxime compounds are used worldwide as antidotes following exposure to anticholinesterase agents.[10,11] Bioassay results have shown that some of them have excellent antifungal,[12,13] insecticidal and herbicidal activities.[14] They are involved in a wide variety of synthetically useful reactions in organic chemistry and serve as important intermediates for the preparation of pharmacologically active heterocycles.[15–17]

Numerous reviews on quaternary pyridinium salts have described the synthetic pathways, reactivity, and importance of pyridinium compounds as antimicrobial, antimalarial, anticancer, and anticholinergic inhibitors.[18–24]

Since the compounds are of huge importance to industry, various routes of their synthesis need to be considered. In today's world, where the chemical industry produces a lot of waste, it has never been more important that chemical processes be performed in a more environmentally friendly manner. Therefore, methods for the production of pyridinium salts must be in line with green chemistry methods. The classical synthetic route for the preparation of quaternary pyridinium salts involves the quaternization reaction of pyridine with organic halides. The aim of our work was to carry out the quaternization reaction with different pyridine-aldoximes, nicotinamide and isonicotinamide as nucleophiles, and three different dihaloalkanes as electrophiles: diiodopropane, dibromopropane and diiodohexane. For this purpose, several methods for the preparation of quaternary pyridinium salts were investigated: conventional method, microwave method, ultrasound method. Acetonitrile,[25] anhydrous benzene,[26]

acetone and anhydrous dimethylformamide[27] are used as organic solvents for the conventional quaternization reactions of pyridinium salts. Inspired by our previous research[28] in which we successfully substituted classical organic solvents with eutectic solvents in the quaternization reaction, this study presents different synthetic routes to quaternary pyridinium salts and an attempt of quaternization in a deep eutectic solvent, choline-chloride (ChCl) : urea. Since the quaternary pyridinium salts have shown antifungal activity in our previous study,[13] in this paper we investigated whether the obtained products with the quaternary nitrogen atom and the hydrophobic tail are effective fungicides compared to commercial agricultural ones.

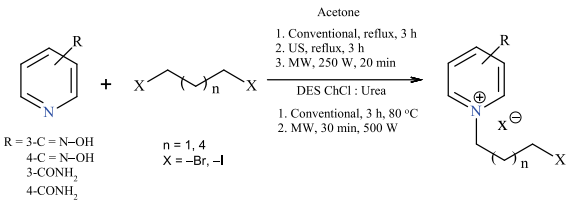
EXPERIMENTAL

Synthesis and Analysis of Quaternary Salts

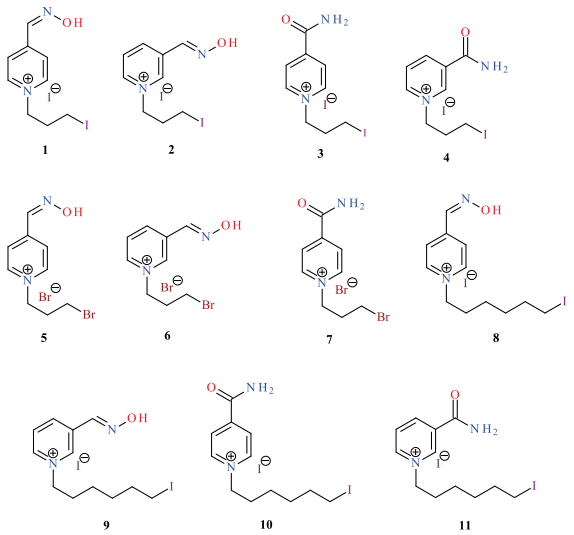
Microwave-assisted synthesis (MW) was performed in Milestone microwave system (Milestone, Srl, Sorisole (BG), Italy). Ultrasound (US) bath: BANDELIN GmbH & Co, DT 510 H, frequency 35 Hz, nominal output 160 W, temperature 20–80 °C, power 400 W. The purity of the synthesized compounds was tested by thin layer chromatography on fluorescent silica gel plates F254 (Merc, Darmstadt, Germany) under UV light (254 and 365 nm) using chloroform : methanol (6 : 2). Melting points were determined in electrothermal melting point apparatus SMP^3 (Mettler Toledo, Croatia). The following commercially available chemicals were used in synthesis: pyridine-4-aldoxime, pyridine-3-aldoxime, nicotinamide, isonicotinamide, 1,3-diiodopropane, 1,3-dibromopropane, 1,6-diiodohexane, choline chloride and urea manufactured by Sigma Aldrich (Poole, UK). Organic solvent used in this work were: chloroform, methanol, diethyl ether, acetone, ethanol and ethyl acetate.

All NMR spectra were recorded using a Bruker AV600 spectrometer (Rheinstetten, Germany) with a 5 mm RT probe in methanol-d_4. Used spectrometer operates at 600.130 MHz for the ^{1}H, and 150.903 MHz for the ^{13}C nucleus. Chemical shifts in ppm refer to the solvent peak at 3.31 ppm in the ^{1}H and 49.15 ppm in the ^{13}C NMR spectra. All NMR signals were assigned by two-dimensional homo- and heteronuclear correlation experiments using standard Bruker parameters: ^{1}H-^{1}H Correlation Spectroscopy (COSY), ^{1}H-^{13}C Heteronuclear Multiple Quantum Coherence (HMQC) and ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation (HMBC).

The general scheme of the quaternary pyridinium salts synthesis is shown in Scheme 1, and the products studied are shown in Scheme 2.



Scheme 1. General scheme of the quaternary pyridinium salts synthesis.



Scheme 2. Series of studied quaternary pyridinium salts.

CONVENTIONAL METHOD

The quaternization reactions of pyridine derivatives (pyridine-4-aldoxime, pyridine-3-aldoxime, nicotinamide, isonicotinamide) with an appropriate dihaloalkane reagents took place in acetone. In pyridine derivative solution (0.04 g, 4 mmol) dissolved in 10 mL of acetone, the corresponding dihaloalkane (20 mmol) was added in small portions. The dihaloalkanes were added in a molar ratio of 5:1 to the heterocyclic compound. The reaction mixture was heated under reflux for 3 h and then cooled to room temperature. The progress of the reaction was monitored by thin layer chromatography (TLC) in the mobile phase consisting of the solvent mixtures chloroform and methanol in the ratio 6 : 2. Slow cooling of the reaction mixture promoted the formation of crystals. The crude product was washed with diethyl ether to remove the residual dihaloalkane and with acetone to remove the starting pyridine derivatives, and recrystallized from the mixture (ethyl acetate : ethanol = 1 : 1) to obtain the pure product.

US SYNTHESIS

To a pyridine derivative solution (0.12 g, 1 mmol) dissolved in 5 mL of acetone, dihaloalkane (5 mmol) was added. The reaction mixture was subjected to US irradiation, heated at reflux for 3 h and then cooled to room temperature. Slow cooling of the reaction mixture promoted the formation of crystals. The crude product was purified by column chromatography using the solvent system chloroform : methanol = 6 : 1.

MW SYNTHESIS IN ACETONE

Pyridinium starting compound (0.12 g, 1 mmol) was dissolved in 5 mL of acetone. Dihaloalkane (5 mmol) was added in solution. The reaction mixture was subjected to MW irradiation (20 minutes at 250 W). Slow cooling of the reaction mixture promoted the formation of crystals. The crude product was purified by column chromatography (chloroform : methanol = 6 :1).

SYNTHESIS IN DES

Preparation of DES ChCl : urea

DES was prepared by mixing choline chloride (5 g), previously dried at 65 °C for 24 h, with urea in a 1:2 molar ratio at 80 °C on a magnetic stirrer for 3 h. Stable, homogeneous solutions were cooled and used without further purification.

The mixture of pyridine derivatives (1 mmol) and dihaloalkanes (5 mmol) was dissolved in DES ChCl : urea (reactant : choline chloride = 1:10). The reaction mixture was heated to 80 °C and stirred for 3 h on a magnetic stirrer. Absolute ethanol was then added and the product was precipitated over the next 24 h. The crude product was filtered off and washed with diethyl ether to remove the residual dihaloalkane. The crude product was purified by column chromatography carried out with the solvent system chloroform : methanol = 6 : 1.

The optimization of the quaternization reaction in DES ChCl: urea was carried out on the model reaction of nicotinamide and diiodopropane by the conventional method at 40, 60 and 80 °C for 1 h in the solvent ChCl: urea (Table 2). Then, optimization was performed at a temperature of 80 °C for 30 min, 1 h, 2 h, 3 h and 4 h to determine the reaction time which gave the highest yield (Table 3).

The experimental parameters for compounds **1**, **3** and **7** were already published in our paper Bušić et al.[3] In this work, they were synthesized from acetone and deep eutectic solvents, and their antifungal activity was also studied.

3-hydroxyiminomethyl-*N*-(3-iodopropyl)pyridinium iodide (2)

m.p. 130 – 133 °C. ^{1}H (600 MHz, MeOD-d_4): δ = 9.25 (1H, s, H-2), 9.00 (1H, dt, J = 6.08; 1.32 Hz, H-6), 8.79 (1H, dt, J = 8.20; 1.32 Hz, H-4), 8.31 (1H, s, H-10), 8.13 (1H, dd, J = 8.09; 7.95 Hz, H-5), 4.79 (2H, t, J = 7.27 Hz, H-7), 3.33 – 3.29 (2H, m, H-9), 2.59 (2H, quint, J = 7.07 Hz, H-8) ppm. ^{13}C (150 MHz, MeOD-d_4): δ = 145.7 (1C, C-6), 144.1 (1C, C-2), 143.8 (1C, C-10), 143.3 (1C, C-4), 136.5 (1C, C-3), 129.7 (1C, C-5), 63.7 (1C, C-7), 35.6 (1C, C-8), –0.5 (1C, C-9) ppm. MS m / z: 290.71 (M^+, 100 %). *Anal*. Calcd. mass fractions of elements, w / %, for $C_9H_{12}N_2OI_2$ (M_r = 418.01) are: C 25.86, H 2.89, N 6.70; found: C 25.93, H 2.87, N 6.72.

3-carbamoyl-*N*-(3-iodopropyl)pyridinium iodide (4)

m.p. 175 – 180 °C. ^{1}H (600 MHz, MeOD-d_4): δ = 9.47 (1H, s, H-2), 9.16 (1H, dt, J = 6.16; 1.17 Hz, H-6), 8.98 (1H, dt, J = 8.19; 1.48 Hz, H-4), 8.24 (1H, dd, J = 8.13; 7.84 Hz, H-5), 4.81 (2H, t, J = 7.34 Hz, H-7), 3.32 – 3.29 (2H, m , H-9), 2.59 (2H, quint, J = 6.87 Hz, H-8) ppm. ^{13}C (150 MHz, MeOD-d_4): δ = 165.3 (1C, C-10), 148.0 (1C, C-6), 146.5 (1C, C-2), 145.8 (1C, C-4), 136.3 (1C, C-3), 129.7 (1C, C-5), 63.9 (1C, C-7), 35.5 (1C, C-8), –0.8 (1C, C-9) ppm. MS m / z: 290.84 (M^+, 100 %). *Anal.* Calcd. mass fractions of elements, w / %, for $C_9H_{12}N_2OI_2$ (M_r = 418.01) are: C 25.86, H 2.89, N 6.70; found: C 26.75, H 2.80, N 6.70.

***N*-(3-bromo)propyl-4-hydroxyiminomethylpyridinium bromide (5)**

m.p. 132 – 135 °C. ^{1}H (600 MHz, MeOD-d_4): δ = 9.00 (2H, d, J = 6.59 Hz, H-2 and H-6), 8.35 (1H, s, H-10), 8.25 (2H, d, J = 6.59 Hz, H-3 and H-5), 4.81 (2H, t, J = 7.30 Hz, H-7), 3.56 (2H, t, J = 6.29 Hz, H-9), 2.61 (2H, quint, J = 6.80 Hz, H-8) ppm. ^{13}C (150 MHz, MeOD-d_4): δ = 151.6 (1C, C-4), 146.4 (2C, C-2 and C-6), 145.8 (1C, C-10), 125.7 (2C, C-3 and C-5), 61.0 (1C, C-7), 34.7 (1C, C-8), 29.5 (1C, C-9) ppm. MS m / z: 242.89 (M^+, 100 %). *Anal.* Calcd. mass fractions of elements, w / %, for $C_9H_{12}Br_2N_2O$ (M_r = 321.93) are: C 33.36, H 3.73, N 8.65; found: C 33.53, H 3.80, N 8.60.

***N*-(3-bromo)propyl-3-hydroxyiminomethylpyridinium bromide (6)**

m.p. 68 – 88 °C. ^{1}H (600 MHz, MeOD-d_4): δ = 9.27 (1H, s, H-2), 9.02 (1H, d, J = 6.10 Hz, H-6), 8.79 (1H, d, J = 8.30 Hz, H-4), 8.31 (1H, s, H-10), 8.13 (1H, dd, J = 8.09; 8.03 Hz, H-5), 4.86 (2H, t, J = 7.31 Hz, H-7), 3.57 (2H, t, J = 6.68 Hz, H-9), 2.64 (2H, quint, J = 6.91 Hz, H-8) ppm. ^{13}C (150 MHz, MeOD-d_4): δ = 145.8 (1C, C-6), 144.1 (1C, C-2), 143.8 (1C, C-10), 143.3 (1C, C-4), 136.5 (1C, C-3), 129.7 (1C, C-5), 61.9 (1C, C-7), 34.7 (1C, C-8), 29.5 (1C, C-9) ppm. MS m / z: 242.96 (M^+, 100 %). *Anal.* Calcd. mass fractions of elements, w / %, for $C_9H_{12}Br_2N_2O$ (M_r = 321.93) are: C 33.36, H 3.73, N 8.65; found: C 33.46, H 3.69, N 8.71.

4-(hydoxiimino)methyl-*N*-(6-iodohexyl)pyridinium iodide (8)

m.p. 168 – 170 °C. ^{1}H (600 MHz, MeOD-d_4): δ = 8.97 (2H, d, J = 7.09 Hz, H-2 and H-6), 8.33 (1H, s, H-10), 8.23 (2H, d, J = 6.65 Hz, H-3 and H-5), 4.63 (2H, t, J = 7.55 Hz, H-7), 3.25

(2H, t, *J* = 6.89 Hz, H-12), 2.05 (2H, quint, *J* = 7.44 Hz, H-8), 1.84 (2H, quint, *J* = 7.00 Hz, H-11), 1.54 – 1.41 (4H, m, H-9 and H-10) ppm. ^{13}C (150 MHz, MeOD-d$_4$): δ = 151.3 (1C, C-4), 146.1 (2C, C-2 and C-6), 145.7 (1C, C-13), 125.6 (2C, C-3 and C-5), 62.5 (1C, C-7), 34.4 (1C, C-11), 32.3 (1C, C-8), 30.9 (1C, C-10), 26.1 (1C, C-9), 6.89 (1C, C-12) ppm. MS *m / z*: 332.79 (M$^+$, 100 %). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_9H_{12}N_2OI_2$ (M_r = 459.95) are: C 31.33, H 3.94, N 6.09; found: C 31.26, H 3.98, N 6.14.

3-(hydoxiimino)methyl-*N*-(6-iodohexyl)pyridinium iodide (9)
m.p. 137 – 143 °C. ^{1}H (600 MHz, MeOD-d$_4$): δ = 9.23 (1H, s, H-2), 8.99 (1H, d, *J* = 5.97 Hz, H-6), 8.77 (1H, d, *J* = 8.20 Hz, H-4), 8.30 (1H, s, H-13), 8.11 (1H, dd, *J* = 7.89 Hz, H-5), 4.68 (2H, t, *J* = 7.59 Hz, H-7), 3.25 (2H, t, *J* = 6.84 Hz, H-12), 2.08 (2H, quint, *J* = 7.59 Hz, H-8), 1.84 (2H, quint, *J* = 7.14 Hz, H-11), 1.55 – 1.42 (4H, m, H-9 and H-10) ppm. ^{13}C (150 MHz, MeOD-d$_4$): δ = 145.5 (1C, C-6), 143.8 (2C, C-2 and C-13), 143.0 (1C, C-4), 136.4 (1C, C-3), 129.6 (1C, C-5), 63.4 (1C, C-7), 34.4 (1C, C-11), 32.3 (1C, C-8), 30.9 (1C, C-10), 26.2 (1C, C-9), 6.8 (1C, C-12) ppm. MS *m / z*: 332.79 (M$^+$, 100 %). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_9H_{12}N_2OI_2$ (M_r = 459.95) are C 31.33, H 3.94, N 6.09; found: C 31.40, H 3.88, N 6.10.

4-carbamoyl- *N*-(6-iodohexyl)pyridinium iodide (10)
m.p. 192 – 195 °C. ^{1}H (600 MHz, MeOD-d$_4$): δ = 9.18 (2H, d, *J* = 6.73 Hz, H-2 and H-6), 8.44 (2H, d, *J* = 6.33 Hz, H-3 and H-5), 4.72 (2H, t, *J* = 7.69 Hz, H-7), 3.25 (2H, t, *J* = 6.96 Hz, H-12), 2.08 (2H, quint, *J* = 7.82 Hz, H-8), 1.84 (2H, quint, *J* = 6.99 Hz, H-11), 1.55 – 1.42 (2H, m, H-9 and H-10) ppm. ^{13}C (150 MHz, MeOD-d$_4$): 166.0 (1C, C-13), 150.6 (1C, C-4), 147.1 (2C, C-2 and C-6), 127.7 (2C, C-3 and C-5), 63.3 (1C, C-7), 34.4 (1C, C-11), 32.4 (1C, C-8), 30.9 (1C, C-10), 26.2 (1C, C-9), 6.7 (1C, C-12) ppm. MS *m / z*: 332.98 (M$^+$, 100 %). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_9H_{12}N_2OI_2$ (M_r = 459.95) are C 31.33, H 3.94, N 6.09; found: C 31.33, H 3.90, N 6.13.

3-carbamoyl-*N*-(6-iodohexyl)pyridinium iodide (11)
m.p. 113 – 115 °C. ^{1}H (600 MHz, MeOD-d$_4$): δ = 9.49 (1H, s, H-2), 9.21 (1H, dt, *J* = 6.20; 1.21 Hz, H-6), 8.98 (1H, dt, *J* = 8.16; 1.36 Hz, H-4), 8.24 (1H, dd, *J* = 7.85 Hz, H-5), 4.75 (2H, t, *J* = 7.64 Hz, H-7), 3.25 (2H, t, *J* = 6.69 Hz, H-12), 2.10 (2H, quint, *J* = 7.04 Hz, H-8), 1.85 (2H, quint, *J* = 7.04 Hz, H-11), 1.55 – 1.44 (2H, m, H-9 and H-10) ppm. ^{13}C (150 MHz, MeOD-d$_4$): δ = 165.3 (1C, C-13), 147.8 (1C, C-6), 146.1 (1C, C-2), 145 2 (1C, C-4), 136.0 (1C, C-3), 129.6 (1C, C-5), 63.6 (1C, C-7), 34.4 (1C, C-11), 32.3 (1C, C-8), 30.9 (1C, C-10), 26.2 (1C, C-9), 6.88 (1C, C-12) ppm. MS *m / z*: 332.79 (M$^+$, 100 %). *Anal.* Calcd. mass fractions of elements, *w* / %, for $C_9H_{12}N_2OI_2$ (M_r = 459.95) are C 31.33, H 3.94, N 6.09; found: C 31.30, H 3.87, N 6.06.

ANTIFUNGAL TEST

The antifungal test was performed on four cultures of phytopathogenic fungi (*Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *Fusarium culmorum*, and *Botrytis cinerea*) from the culture collections of the Department of Phytopathology, Faculty of Agrobiotechnical Sciences Osijek, University of Osijek. The fungicidal activity of eleven synthetic compounds at concentrations of 10 μg mL^{-1} and 100 μg mL^{-1} was tested. For the antifungal tests, the method of Bušić et al.[28] was used. Each treatment was performed in four replicates for each fungal species. An untreated PDA was used as a control. The commercial fungicide was used as a positive control. For *B. cinerea*, the fungicide from the hydroxynalide group with the active ingredient fenhexaminet was used. For *M. phaseolina*, *S. sclerotiorum* and *F. culmorum*, the fungicide from the tebuconazole group with the active ingredient difenconazole was used. The fungicides were mixed with PDA at the recommended concentration. The first diameter of mycelial growth of each culture was measured 48 h after inoculation. Measurements were taken for up to 168 h (depending on the fungal species) in two directions to determine the average colony growth in millimeters. Based on the measured mycelial growth, the inhibition rate was calculated. The inhibition rate of the synthetic compounds on the four fungal species was calculated based on the inhibition index (*I,* %):

$$I\ /\ \% = [(C - S) / (C - 0.4)] \times 100$$

C = diameter of the fungal growth on untreated PDA, *S*= diameter of fungal growth on PDA treated with synthetic compounds.

Statistical analysis of the experimental results was performed using factorial analysis of variance ANOVA, in which the data were grouped according to the inhibition rate and the applied concentration. To estimate the statistical significance of the differences between the synthetic compounds at different concentrations, the Fisher LSD test was applied using the SAS 9.2 statistical package. Means were considered significantly different when $p \leq 0.05$.

RESULTS AND DISCUSSION

In an effort to find a more greener alternative for performing quaternization reactions, the reactions were performed in acetone using three methods: conventional, ultrasound and microwave and in a deep eutectic solvent (ChCl : urea = 1:2) using conventional and microwave methods. In this paper, 4 different pyridine derivatives (pyridine-3-aldoxime, pyridine-4-aldoxime, isonicotinamide and nicotinamide)

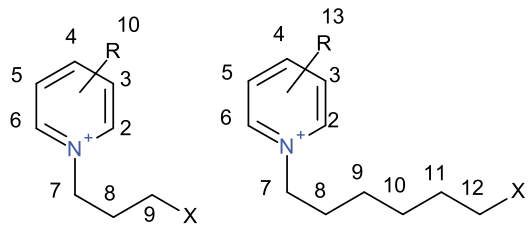


Figure 1. Enumeration scheme used for the assignment of the NMR spectra (X = Br, I; R = 3-$CONH_2$, 4-$CONH_2$, 3-CNOH, 4-CNOH).

were quaternized as nucleophiles with 3 different electrophiles: 1,3-diiodopropane, 1,3-dibromopropane and 1,6-diiodohexane.

The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The chemical shifts of the NMR signals in the spectra (δ = 9.30 – 8.10 and 4.70–1.40 ppm in the ^{1}H, and δ = 165 – 130 and 65 – (–0.5) ppm in the ^{13}C), their multiplicities (singlets, doublets, doublet of doublets, doublet of triplets, quintets and multiplets) and coupling constants (^3J = 8.80 – 5.90 Hz, ^4J = 1.50 – 1.20 Hz) confirm the structure of all newly synthesized compounds (**2**, **4–6**, **8–11**). The atom labels of the synthesized compounds used for assignment in the NMR spectra, as well as the ^{1}H and ^{13}C spectra of **11** with labeled signals are shown in Figures 1.

The results show a different reaction yield depending on the method and the reaction performed (Table 5). In acetone as solvent, the lowest yields were obtained by the conventional method.

The highest yield obtained by conventional method was achieved for product **1** (85 %) formed by the quaternization reaction of pyridinium-4-aldoxime and 1,3-diiodopropane. The lowest yield was obtained for product **7** obtained by the quaternization reaction of isonicotinamide and 1,3-dibromopropane. Iodides are generally found to be better nucleophiles than bromides.

Ultrasound method showed significantly higher yields than the conventional method, but not as high as with the microwave method. The highest yield by ultrasound method was also achieved for product **1** (91 %).

The optimization of the quaternization reaction under the influence of microwave irradiation was carried out on the model reaction of nicotinamide and diiodopropane for 2.5, 5, 10, 20, and 40 min and 250 and 500 W, respectively. The optimization showed that the most favorable reaction conditions where a time of 20 min and a power of 250 W (Table 1). The highest yields were obtained with this synthetic method. The quaternization reaction of pyridinium-4-aldoxime and diiodopropane gave compound **1** in almost quantitative yield (98 %).

To determine the reaction parameters for performing the quaternization reaction in the deep eutectic solvent ChCl : urea (1 : 2), the model reaction of nicotinamide and diiodopropane was performed at different temperatures and times. The optimization showed that the most suitable temperature for the quaternization reaction is 80 °C (Table 2) and a reaction time of 3 h for the conventional method (Table 3). To carry out microwave synthesis in DES, an optimization method based on time and power was obtained. From this study, the most suitable time in the MW method is 30 min at a power of 250 W (Table 4). Quaternary salts were formed in the eutectic solvent ChCl : urea. However,

Table 2. Yields in the model reaction of nicotinamide and diiodopropane at different temperatures in the deep eutectic solvent choline chloride : urea, conventional method.

Temperature / °C	Yield / %
40	6
60	38
80	41

Table 3. Optimization of the model reaction of nicotinamide and diiodopropane at 80 °C at different reaction times by conventional method in the deep eutectic solvent choline chloride : urea.

Time / h	Yield / %
0.5	26
1	41
2	44
3	62
4	56

Table 1. Optimization of reaction parameters in acetone as solvent on the model reaction of nicotinamide and diiodopropane by the microwave method.

Time / h	Yield / %	
	250 W	500 W
2.5	13	16
5	43	54
10	90	91
20	96	88
40	90	78

Table 4. Optimization of the model reaction of nicotinamide and diiodopropane at 80 °C at different reaction times in DES ChCl: Urea by microwave method.

Time / h	Yield / %	
	250 W	500 W
15	27	26
30	71	34
60	62	58

the reaction yields were significantly lower than in acetone as solvent. It was also surprising that lower yields were obtained by the microwave method in the eutectic solvent than as opposed to the same in acetone. The highest yields for conventional and microwave synthesis in CHCl : urea were obtained for compound **1** (75 % and 80 %, respectively).

Antifungal Activity

The inhibitory activity of all eleven compounds at a concentration of 10 μg mL^{-1} (Table 6) showed very strong inhibition of the pathogen *S. sclerotiorum*. The inhibition of the growth of this pathogen ranged from 84.16 to 90.76 %, with compounds **5** and **2** showing the strongest inhibition. Compound **4** had the strongest inhibitory effect on the growth of the pathogen *B. cinerea* with an antifungal index of 83.43 %, while compound **11** showed the weakest effect (22.55 %). Compound **3** showed the strongest inhibitory effect on the pathogen *F. culmorum* with an inhibition

Table 5. Yields (%) in conventional, US, and MW synthesis in acetone and in the deep eutectic solvent ChCl : urea of pyridine derivatives **1–11**.

Compound	Yield / %				
	Acetone			Deep eutectic solvents	
	Conventional	US	MW	Conventional	MX
1	85	91	98	65	70
2	84	48	87	19	36
3	54	60	43	22	40
4	47	72	96	38	71
5	14	43	58	36	36
6	24	71	83	18	32
7	6	40	59	17	21
8	39	49	62	61	64
9	26	33	32	29	40
10	59	50	88	10	22
11	63	80	78	13	24

Table 6. Inhibition rate (%) of the different compounds at a concentration of 10 μg mL^{-1}, 48 h after inoculation.

Compounds concentration 10 μg mL^{-1}	Inhibition rate[a] / %				
	M. phaseolina	*S. sclerotiorum*	*F. culmorum*	*B. cinerea*	LSD 0.05
1	50.21 ± 29.67	84.98 ± 2.13	32.03 ± 3.56	46.79 ± 7.13	23.72
2	33.24 ± 6.11	89.52 ± 3.66	32.03 ± 2.05	27.06 ± 4.65	6.74
3	31.82 ± 3.65	89.11 ± 4.86	44.48 ± 7.69	24.80 ± 6.98	9.27
4	54.46 ± 25.77	88.28 ± 7.19	35.59 ± 3.56	83.43 ± 3.85	21.00
5	27.58 ± 3.27	90.76 ± 1.35	37.37 ± 0.00	24.80 ± 2.16	3.19
6	23.34 ± 7.12	88.70 ± 2.08	30.25 ± 2.91	47.91 ± 2.91	6.53
7	31.12 ± 9.63	84.16 ± 1.35	19.57 ± 5.03	49.04 ± 9.57	11.20
8	33.24 ± 16.17	85.81 ± 8.84	37.37 ± 2.91	27.62 ± 2.25	14.47
9	22.63 ± 11.17	86.22 ± 2.48	19.57 ± 8.72	45.10 ± 6.73	12.23
10	31.82 ± 14.33	86.22 ± 2.82	28.47 ± 4.59	54.68 ± 21.67	20.44
11	33.95 ± 7.44	84.98 ± 7.68	28.47 ± 4.59	22.55 ± 3.85	9.44
Untreated PDA	0.00 ± 2.71	0.00 ± 2.69	0.00 ± 6.08	0.00 ± 5.00	
Fenhexamine[b] Difenconazole[b]	91.88 ± 0.00	94.06 ± 0.00	86.30 ± 0.00	91.88 ± 0.00	
LSD 0.05	19.45	16.15	6.71	11.22	

[a] Calculate the antifungal index according to the formula given for four repetitions. Means ± SD determinated from Fisher's test, when these means were compared with controls, mean values were considered significantly different when $p \leq 0.05$.

[b] The commercial fungicide fenhexamine and difenconazole were used as positive controls for comparisons.

Table 7. Inhibition rate (%) of the different compounds at a concentration of 100 μg mL^{-1}, 48 h after inoculation.

	Inhibition rate[a] / %				
Compounds concentration 100 μg mL^{-1}	*M. phaseolina*	*S. sclerotiorum*	*F. culmorum*	*B. cinerea*	LSD 0.05
1	41.73 ± 10.07	89.11 ± 3.56	42.70 ± 6.08	47.35 ± 20.53	18.43
2	41.02 ± 11.4	87.05 ± 1.58	40.93 ± 6.74	47.91 ± 19.26	18.05
3	30.41 ± 14.97	87.05 ± 5.11	32.03 ± 14.64	38.90 ± 7.01	17.46
4	54.46 ± 25.77	88.70 ± 8.67	48.93 ± 4.59	86.25 ± 4.32	21.50
5	15.56 ± 7.07	87.87 ± 4.93	19.57 ± 3.41	46.22 ± 9.81	10.40
6	55.16 ± 23.82	87.87 ± 7.18	41.81 ± 2.05	86.81 ± 4.27	19.51
7	24.05 ± 10.17	79.62 ± 4.13	26.69 ± 5.34	46.79 ± 7.13	10.89
8	57.28 ± 27.57	90.76 ± 4.04	44.48 ± 4.48	86.81 ± 2.16	21.81
9	25.46 ± 16.39	89.52 ± 3.12	26.69 ± 14.05	36.64 ± 14.55	20.20
10	31.12 ± 13.14	89.93 ± 2.86	30.25 ± 10.63	39.46 ± 11.70	15.99
11	38.90 ± 31.25	86.22 ± 3.12	39.15 ± 6.08	47.91 ± 13.34	26.70
Untreated PDA	0.00 ± 2.71	0 ± 2.69	0.00 ± 6.08	0.00 ± 5.00	
Fenhexamine[b] Difenconazole[b]	91.88 ± 0.00	94.06 ± 0.00	86.30 ± 0.00	91.88 ± 0.00	
LSD 0.05	25.17	6.40	10.98	15.78	

[a] Calculate the antifungal index according to the given formula for four repetitions. Mean ± SD determinated from Fisher's test, when these means were compared with controls, mean values were considered significantly different when $p \leq 0.05$.

[b] The commercial fungicide fenhexamine and difenconazole were used as positive controls for comparisons.

index of 44.48 %, while compounds **7** and **9** showed the weakest inhibitory effect with 19.57 %. However, compared to the control variant, the inhibitory effect of these compounds was statistically significantly higher. Compound **4** with an inhibitory effect of 54.46 % and compound **1** with 50.21% had the strongest effect on the growth of the pathogen *M. phaseolina* at a concentration of 10 μg mL^{-1}. Compound **9** had the weakest inhibitory effect with 22.63 %.

Considering the highest and lowest inhibition index of each pathogen, it can be concluded that the effect of all compounds at a concentration of 10 μg mL^{-1} had the weakest inhibition effect on the pathogens *M. phaseolina* (22.63 – 54.46 %) and *F. culmorum* (19.57 % – 44.48 %).

All eleven compounds at a concentration of 100 μg mL^{-1} (Table 7) again showed very strong growth inhibition for the pathogen *S. sclerotiorum*. The growth inhibition for this pathogen ranged from 79.62 to 90.76 %. Compounds **8** and **10** showed the strongest inhibition with an inhibition index of 90.76 % and 89.93 %, respectively.

Compounds **4**, **6** and **8** showed the strongest inhibitory effect on the pathogen *B. cinerea*, while compound **9** showed the weakest effect with 36.64 %.

Compound **4** had a good inhibitory effect on the pathogen *F. culmorum* with an inhibition index of 48.93 %, while compound **5** had the least effect with 19.57 %. Compounds **8** and **6** had a very good inhibitory effect on the pathogens of *M. phaseolina* with 56.58 % and 55.16 %, respectively, while compounds **5** and **7** had the weakest effect with an inhibition index of 15.56 % and 24.05 %, respectively. Considering the highest and lowest inhibition index for each pathogen, we find that the compounds acting at a concentration of 100 μg mL^{-1} have the weakest inhibition effect on the same pathogens at both concentrations, *M. phaseolina* (15.56 – 57.28 %) and *F. culmorum* (19.57 – 48.93 %).

CONCLUSIONS

The studies showed that the quaternization reaction could be successfully carried out in acetone as solvent. The highest yields were obtained with the microwave method, slightly lower with the ultrasound method, while low to medium yields were obtained with the conventional method. When syntheses were carried out in eutectic solvents as an environmentally friendly alternative to conventional organic solvents, products were formed but the yields were lower. Therefore, the quaternization of pyridine compounds with dihaloalkanes in other choline chloride based deep eutectic solvents remains to be explored. From the obtained results, it can be concluded that three compounds (**4**, **6**, **8**) have excellent inhibitory activity on the studied phytopathogenic fungal species at a concentration of 100 μg mL^{-1}. However, it should be noted

that compound **5** has a very good inhibitory effect on *S. sclerotiorum* at both concentrations, while the same compound has the weakest effect on the pathogens *F. culmorum* and *M. phaseolina* at a concentration of 100 μg mL^{-1}. Thus, it can be said that the effect of a single compound depends on the pathogen species, but it can also have a stimulatory effect on them.

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