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ORIGINAL PAPER

Eco-friendly quaternization of nicotinamide and 2-bromoacetophenones in deep eutectic solvents. Antifungal activity of the products

Valentina Bušić[1] · Sunčica Roca[2] · Dražen Vikić-Topić[2,3] · Karolina Vrandečić[4] · Jasenka Ćosić[4] · Maja Molnar[1] · Dajana Gašo-Sokač[1]

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Abstract
Most organic solvents used in quaternization reactions are volatile, hazardous, toxic and form by-products, thus inducing health issues and pollution. Deep eutectic solvents are greener alternatives, but they have not been tested yet in the quaternization reaction. Here we propose eutectic solvents in the quaternization reaction of nicotinamide with substituted 2-bromoacetophenones. The reaction was performed at 80 °C by three synthetic approaches: conventional during 2–6 h, microwave during 20 min and ultrasonic during 3 h. The highest yields of about 98% were obtained by microwave. The most suitable eutectic solvents were choline chloride with either urea, oxalic or levulinic acid. The use of deep eutectic solvents has several advantages: environmental benignity, biodegradability, easy purification and simple preparation. All tested compounds showed antifungal activities against *Botrytis cinerea*, *Colletotrichum acutatum*, *Alternaria radicina* and *Fusarium graminearum* at 10 and 100 µg/mL.

Keywords Nicotinamide salts · Deep eutectic solvents · Microwave synthesis · Ultrasonic irradiation · Antifungal activity

Introduction

Quaternization of pyridine with alkyl halides or related compounds is an example of the Menschutkin reaction, the most common route for preparing quaternary salts. The most commonly used organic solvents in quaternization reactions of pyridinium salts are acetonitrile (Marek et al. 2015), anhydrous benzene (Pidlypnyi et al. 2014), acetone and anhydrous dimethylformamide (Pernak and Rogoża 2000). All solvents were classified by the European Commission's INDEX strategy as the priority pollutants associated with health risk. Ecological concerns have prompted the scientists to modify synthetic approaches by replacing the traditional organic solvents with more suitable ones. Deep eutectic solvents (DES) are touted as green, because of its notable advantages: biocompatible, readily available starting materials, which enable easier preparation with high purity, lower costs and no purification problems. Their application could reduce or eliminate the generation of hazardous substances. Design and development of ecological safe, green solvents in the quaternization reaction is a real challenge, since there is no scientific reference to confirm that the quaternization reaction was performed in the eutectic solvents.

Due to the importance of using eco-friendly solvents, herein, for the first time, we report an efficient and sustainable quaternization reactions of nicotinamide in choline chloride-based DES. It is a mild, fast and efficient method with several advantages: use environmentally benign and biodegradable solvent, easy purification process and simple methodology. Quaternary salts, generally, show biological activity: antimicrobial (Bušić et al. 2017), antifungal (Wu et al. 2012), insecticidal and herbicidal (Wu et al. 2017), cytotoxic (Peng et al. 2017) and

✉ Dajana Gašo-Sokač
dgaso@ptfos.hr

1 Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, F. Kuhača 20, 31000 Osijek, Croatia

2 NMR Centre, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

3 Department of Natural and Health Sciences, Juraj Dobrila University of Pula, Zagrebačka 30, 52100 Pula, Croatia

4 Faculty of Agrobiotechnical Sciences, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia

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antiangiogenesis (Choi et al. 2011). In our previous work (Siber et al. 2019), we confirmed the antifungal activity of nicotinamide derivatives against *Macrophomina phaseolina*, *Fusarium culmorum*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. In this study, we investigated in vitro effect of prepared compounds against four additional phytopathogenic fungi: *Botrytis cinerea*, *Colletotrichum* sp., *Alternaria radicina* and *Fusarium graminearum.*

Experimental

Microwave synthesis was performed on Milestone flexiWAVE (Milestone Srl, Italy). Sonorex digitec DT510H (BANDELIN, Germany) was used as ultrasonic bath. Thin-layer chromatography (TLC) was performed with fluorescent silica gel plates F254 (Merc, Germany) checked under UV light (254 and 365 nm) using chloroform/methanol (6:1.5).

The structure of tested compounds (**1–9**, Fig. 1A) has been determined on the basis of elemental analyses, NMR and IR spectra. Yields depend of used solvent as well as of the synthesis method (Fig. 1B).

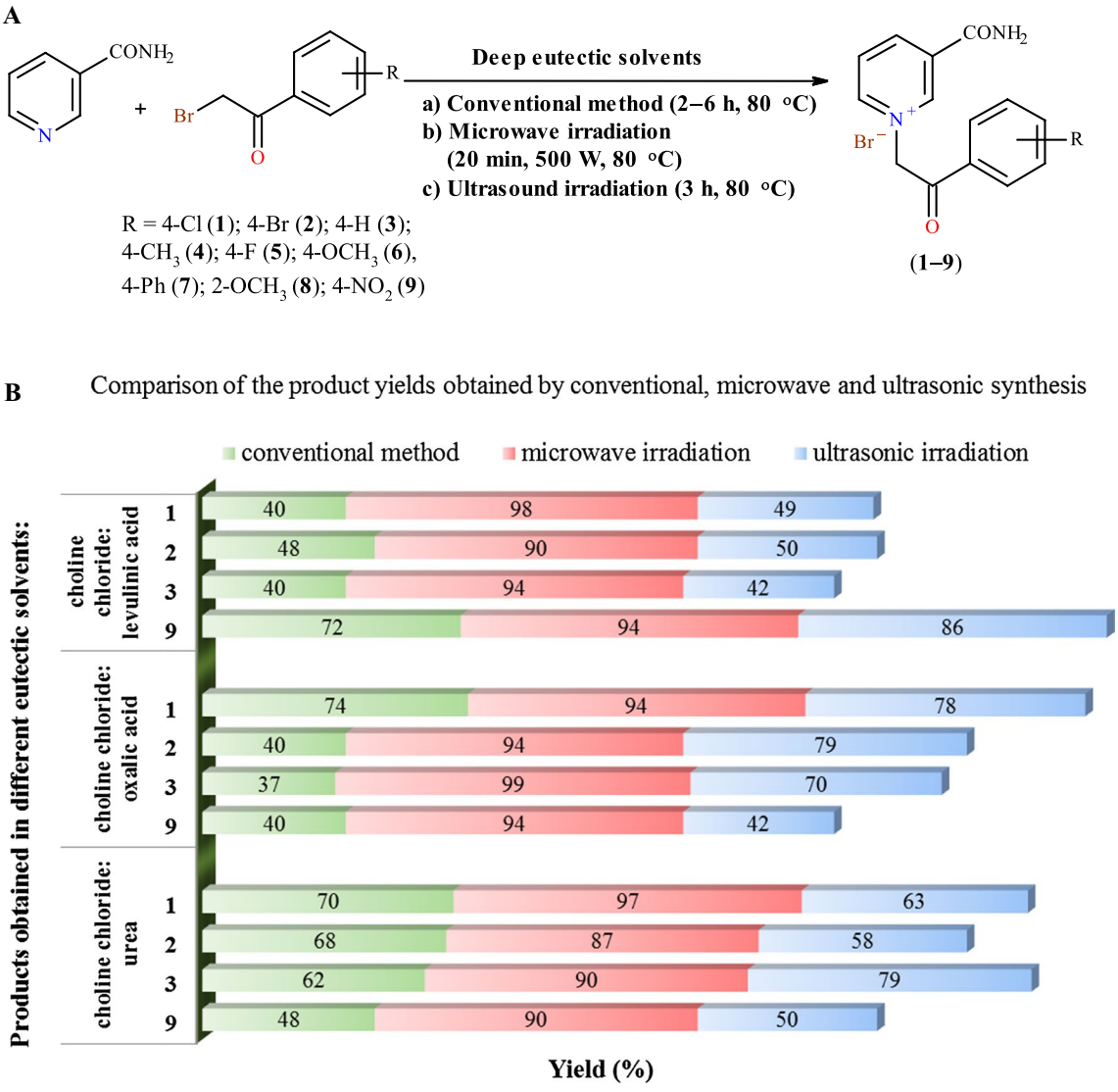

Fig. 1 **A** The quaternization reaction of nicotinamide with substituted 2-bromoacetophenones in deep eutectic solvents by three different methods (a–c). **B** Yields (%) of the compounds **1**, **2**, **3** and **9** obtained by conventional (green), microwave (red) and ultrasonic (blue) method in three eutectic solvents (choline chloride/levulinic acid, choline chloride/oxalic acid, choline chloride/urea) shown at the left-hand edge. Note that yields depend on the chosen solvent, but a significant increase was noticed when microwave irradiation was used

General procedure

Preparation of solvents

Deep eutectic solvents (DES) were prepared by mixing the choline chloride, previously dried at 65 °C for 24 h, with a hydrogen bonds donor at 80 °C on a magnetic stirrer. Mixing time was dependable of the hydrogen bonds donor. DES with glycerol was prepared by heating it to 80 °C, and choline chloride was added later. Choline chloride/glucose/fructose was performed according to Hayyan et al. (2012). Stable, homogeneous solutions were cooled down and used without further purification.

Quaternization reaction

The equimolar mixture of nicotinamide (1.19 mmol) and substituted 2-bromoacetophenone was dissolved in DES (reactant/choline chloride = 1:10) and subjected to three synthetic routes:

Conventional method In the reaction mixture, mixed on a magnetic stirrer for 2–6 h at 80 °C, absolute ethanol was added and the product was precipitated in the next 24 h. The crude product was filtered off and recrystallized from the methanol/ethanol/ethyl acetate = 1:1. The reaction progress was monitored by thinl layer chromatography (TLC).

Microwave method The reaction mixture was irradiated at 80 °C for 20 min with 500 W. Absolute ethanol was added into the reaction mixture, and precipitated product was filtered off and recrystallized from the appropriate solvent.

Ultrasonic method The reaction mixture was sonicated for 3 h at 80 °C. After cooling at room temperature, the product was precipitated from absolute ethanol. The crude product was filtered off and recrystallized from the appropriate solvent.

Antifungal assay

The fungicidal activity of **1**–**9** at concentrations of 10 and 100 µg/mL was tested against *Botrytis cinerea*, *Colletotrichum acutatum*, *Alternaria radicina* and *Fusarium graminearum* according to Siber et al. (2019). Commercially, agricultural fungicides were used as positive control: for *B. cinerea* and *C. acutatum* copper oxychloride; for *A. radicina* and *F. graminearum* the fungicide from the triazoles group with tebuconazole and difenoconazole as active ingredients. Inhibition rate of the synthetic compounds on the fungi species was calculated by the antifungal index (*I*/%) (Bušić et al. 2019). Statistical analysis was performed using factorial analysis of variance ANOVA by grouping the data depending on concentration and inhibition rate. For estimating statistical significance of differences between synthetic compounds at different concentrations, Fisher's least significant difference test was applied by using SAS 9.2 Statistical Package (2008). Mean values were considered significantly different when $p \leq 0.05$.

Results and discussion

16 choline chloride-based deep eutectic solvents (DES) were used for quaternization reactions performed by conventional (Table 1), microwave and ultrasound method (Table 2). The lowest product yields were obtained by conventional method: 55% of compounds had a 3–40% yield, 19% have 41–60%, and only 10% have 61–74% yield. The highest yields obtained by conventional methods were for choline chloride/urea, of 42–70%, and choline chloride/oxalic acid (32–74%).

A half of the products obtained by ultrasound method had a 30–60% yield, and 21% of them had yields higher than 60%. The best yields were for choline chloride/glycerol (50–86%) and choline chloride/oxalic acid (40–82%). The quaternization under ultrasound irradiation was first performed 24 h at room temperature, but desired products did not appear. Then, the reactions were carried out at 80 °C, and after 3 h the products were visible in all tested DES. Ultrasound method was extensively adopted as a promising green pathway in several organic transformations (Cella and Stefani 2009).

The microwave method showed the best results: a half of obtained compounds had a yield 60–98% (fifteen compounds ≥ 90%), and 35% of them had a 30–59% yield. The most appropriate DES were the choline chloride/levulinic acid (71–98%), choline chloride/urea (60–97%) and choline chloride/oxalic acid (50–95%). The reaction optimization for microwave method was performed using nicotinamide and 2-bromoacetophenone.

Used DES proved to be very good alternative for the quaternization reaction, except those based on sugar (glucose, fructose) and their alcohol (xylitol, sorbitol). In most cases in sugar-based DES, it was not possible to isolate the obtained product, but we identified them by TLC.

The inhibitory effect of tested compounds on mycelium growth was significant at higher concentration (Table 3). At 100 µg/mL, all tested compounds show statistical significant inhibition rate against *B. cinerea*. Against *C. acutatum*, **2**, **5**, **7** and **8** showed very good inhibitory activity at 100 µg/

Table 1 Yields (%) and total reaction times (h) in conventional method synthesis of nicotinamide derivatives (**1**–**9**) in choline chloride-based deep eutectic solvents

Entry	Deep eutectic solvent (choline chloride: hydrogen bond donor)		Reaction time (h)	Yield (%)								
	Hydrogen bond donor	Molar ratio		**1** 4-Cl	**2** 4-Br	**3** 4-H	**4** 4-CH_3	**5** 4-F	**6** 4-OCH_3	**7** 4-Ph	**8** 2-OCH_3	**9** 4-NO_2
1	Urea	1:2	3	70	68	62	54	42	65	42	43	48
2	*N*-Methylurea	1:3	3	66	34	–	33	46	35	41	32	24
3	Thiourea	1:2	4	23	38	33	32	34	25	36	30	24
4	Glucose	1:1	6	–[a]	–	–	27	–	–	–	–	–
5	Fructose	1:1	6	–	–	–	38	–	–	–	–	–
6	Xylitol	1:1	5	18	20	22	23	31	26	33	38	39
7	Sorbitol	1:1	5	45	31	32	48	23	30	29	30	33
8	Glycerol	1:2	3	68	37	50	45	48	72	40	32	60
9	Acetamide	1:2	3	61	48	45	67	68	15	36	31	66
10	Mallic acid	1:1	2	54	37	37	28	47	37	65	34	36
11	Citric acid	1:2	2	56	29	3	38	35	28	36	37	–
12	Malonic acid	1:1	2	59	35	41	25	34	33	34	22	–
13	Oxalic acid	1: 1	2	74	40	37	55	44	69	74	32	40
14	Lactic acid	1: 2	2	32	32	–	18	16	14	18	21	34
15	Levulinic acid	1: 2	2	40	39	44	43	49	21	45	32	72
16	*trans*-Cinnamic acid	1: 1	2	15	30	34	15	44	13	14	8	–

[a]Note that time for the preparation of compounds was long (2–6 h). Only ten prepared compounds showed the best yield ca. 70%. A half of them have a yield ≤ 40% and twenty-one were obtained in traces

mL, while **7** and **9** were also effective at 10 µg/mL. Mycelial growth of *A. radicina* was the least sensitive and only at the highest concentration of **9** showed moderate inhibition. The **8** and **9** had the highest inhibitory effect against the *F. graminearum* for both tested concentrations.

Conclusion

For the first time, quaternary salts of nicotinamide were prepared in choline chloride-based DES. Used methodology has proved to be complete in terms of eco-sustainability, eco-toxicity and economics. The high reaction yields, the easy environment-friendly solvents and short reaction times are in agreement with principles of green chemistry, making microwave method as a new approach for preparation of quaternary salts in DES. All compounds showed weak inhibition of mycelium growth at 10 µg/mL, and moderate inhibition against all phytopathogenic fungi at 100 µg/mL.

Table 2 Yields (%) by microwave (MW; 500 W, 20 min) and ultrasonic irradiation synthesis (US; 3 h) of nicotinamide derivatives in choline chloride-based deep eutectic solvents

Entry	Deep eutectic solvent (choline chloride: hydrogen bond donor)		Yield (%)																	
			1 4-Cl		**2** 4-Br		**3** 4-H		**4** 4-CH_3		**5** 4-F		**6** 4-OCH_3		**7** 4-Ph		**8** 2-OCH_3		**9** 4-NO_2	
	Hydrogen bond donor	Molar ratio	MW	US	MW	US	MW	US	MW	US	MW	US	MW	US	MW	US	MW	US	MW	US
1	Urea	1:2	97	63	87	58	90	79	64	72	80	57	60	76	69	64	66	38	90	50
2	*N*-Methylurea	1:3	72	37	67	50	78	66	46	62	52	34	58	61	67	66	69	10	70	6
3	Thiourea	1:2	37	42	70	47	70	45	39	54	56	45	38	44	59	46	45	11	69	12
4	Glucose	1:1	–[a]	23	23	11	35	15	–	25	15	12	–	20	13	15	33	12	12	22
5	Fructose	1:1	–	26	28	10	46	11	–	40	15	16	–	23	32	11	45	12	13	13
6	Xylitol	1:1	68	35	46	21	41	19	64	47	25	21	66	28	37	30	29	–[b]	21	15
7	Sorbitol	1:1	70	35	46	30	50	37	68	50	63	23	70	58	56	48	61	–	19	20
8	Glycerol	1:2	88	78	70	80	66	86	68	84	55	78	58	80	67	81	66	58	46	50
9	Acetamide	1:2	21	22	67	67	87	65	23	21	58	67	24	23	78	25	56	65	90	60
10	Mallic acid	1:1	74	56	87	57	89	45	88	55	67	54	82	52	87	55	60	–	42	58
11	Citric acid	1:2	86	50	82	45	74	50	81	59	57	43	82	54	56	47	40	–	63	–
12	Malonic acid	1:1	86	63	83	52	80	62	64	42	55	46	62	41	78	48	59	–	62	60
13	Oxalic acid	1:1	94	78	94	79	99	70	98	82	70	79	91	68	87	76	50	40	94	42
14	Lactic acid	1:2	57	40	42	48	38	59	47	38	49	33	34	33	36	30	31	43	35	60
15	Levulinic acid	1:2	98	49	67	49	93	47	93	51	97	46	91	40	76	48	71	60	94	86
16	*trans*-Cinnamic acid	1:1	26	23	30	38	34	30	30	18	41	18	31	19	42	20	32	–	48	32

[a]The product yields by microwave irradiation are significantly the highest (35% of compounds shown yields 70–99%). Only six compounds were obtained in trace and in all cases glucose and fructose were used as hydrogen bond donors

[b]The results by ultrasonic irradiation shown that only 12% of all compounds had yields 70–89%. By this method, the compounds in choline chloride/glucose/fructose were also obtained. Here, the yields in traces are related mainly to compound **8**

Table 3 Antifungal activities of the tested compounds

Compounds	Inhibition rate (%) at 10 and 100 µg/mL							
	B. cinerea		*C. acutatum*		*A. radicina*		*F. graminearum*	
	10	100	10	100	10	100	10	100
1	35.4 ± 11.2	55.6 ± 6.4	8.8 ± 16.7	30.8 ± 8.4	–	7.8 ± 17.2	5.2 ± 4.9	20.7 ± 4.9
2	8.7 ± 10.8	57.1 ± 24.9	6.6 ± 7.2	39.6 ± 8.4	–	13.6 ± 13.5	12.9 ± 4.9	36.2 ± 7.7
3	10.1 ± 8.7	42.6 ± 11.2	11.0 ± 15.3	28.6 ± 11.4	–	–	7.7 ± 17.6	12.9 ± 4.9
4	21.7 ± 5.0	54.9 ± 5.3	2.2 ± 5.1	22.0 ± 4.4	3.9 ± 7.4	–	16.8 ± 5.2	31.0 ± 4.9
5	24.5 ± 12.6	57.1 ± 8.6	24.2 ± 0	46.2 ± 15.3	–	–	–	24.5 ± 17.4
6	23.1 ± 9.7	52.7 ± 11.9	17.6 ± 11.1	26.4 ± 4.4	–	–	12.9 ± 8.8	21.9 ± 10.7
7	21.7 ± 6.0	41.2 ± 7.9	37.4 ± 5.1	41.8 ± 0	3.9 ± 7.4	13.6 ± 4.5	36.2 ± 2.6	37.5 ± 5.2
8	19.5 ± 9.5	44.8 ± 1.7	13.2 ± 8.4	50.7 ± 0	–	15.6 ± 11.7	40.0 ± 4.2	40.0 ± 4.2
9	23.1 ± 4.7	49.1 ± 9.1	37.4 ± 23.3	37.4 ± 5.1	5.8 ± 13.5	33.1 ± 6.3	45.2 ± 12.6	40.0 ± 4.2
Untreated PDA	0 ± 4.1	0 ± 4.1	0 ± 4.4	0 ± 4.4	0 ± 7.4	0 ± 7.4	0 ± 18.1	0 ± 18.1
Copper oxychloride	4.3 ± 9.6	6.5 ± 5.5	–	7.7 ± 8.4	–	–	–	–
Tebuconazole	–	–	–	–	64.2 ± 8.9	64.2 ± 8.9	81.4 ± 0	81.4 ± 0
Fisher's least significant difference test	12.7	15.2	16.1	11.1	14.2	14.2	15.2	13.3

Inhibition rate (%) present average value of four replications

Mean values ± significant difference were determined from Fisher's test and considered significantly different when $p \leq 0.05$. The commercial fungicide based on copper and the triazoles was used for comparison

Note that antifungal effect of tested compounds was more significant at higher concentration (100 µg/mL)

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