Supplementary information for:

Dual Functional Salts of Benzo[1.2.3]thiadiazole-7-carboxylates as a Highly Efficient Weapon Against Viral Plant Diseases

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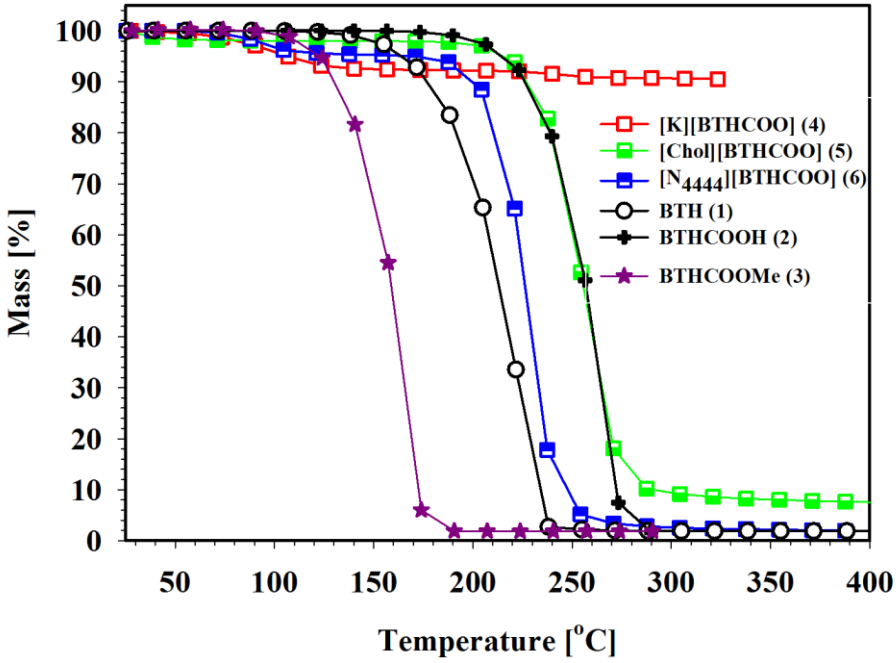


Figure. S1 Thermal stability of neutral BTH derivatives and salts of anionic derivatives with small counterion

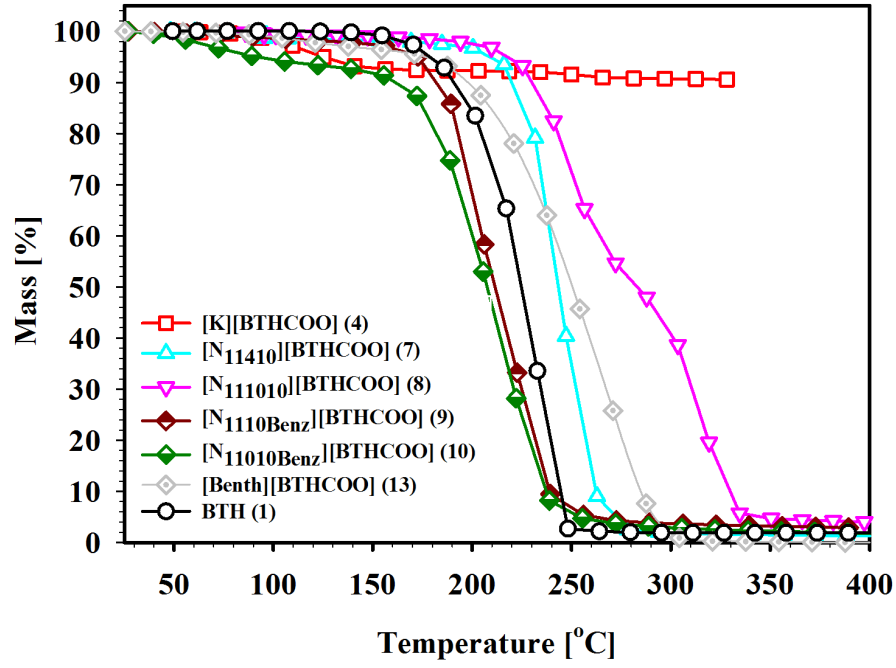


Figure S2 Thermal stability of salts with counterions based on non- aromatic ammonium cations with long alkyl chains

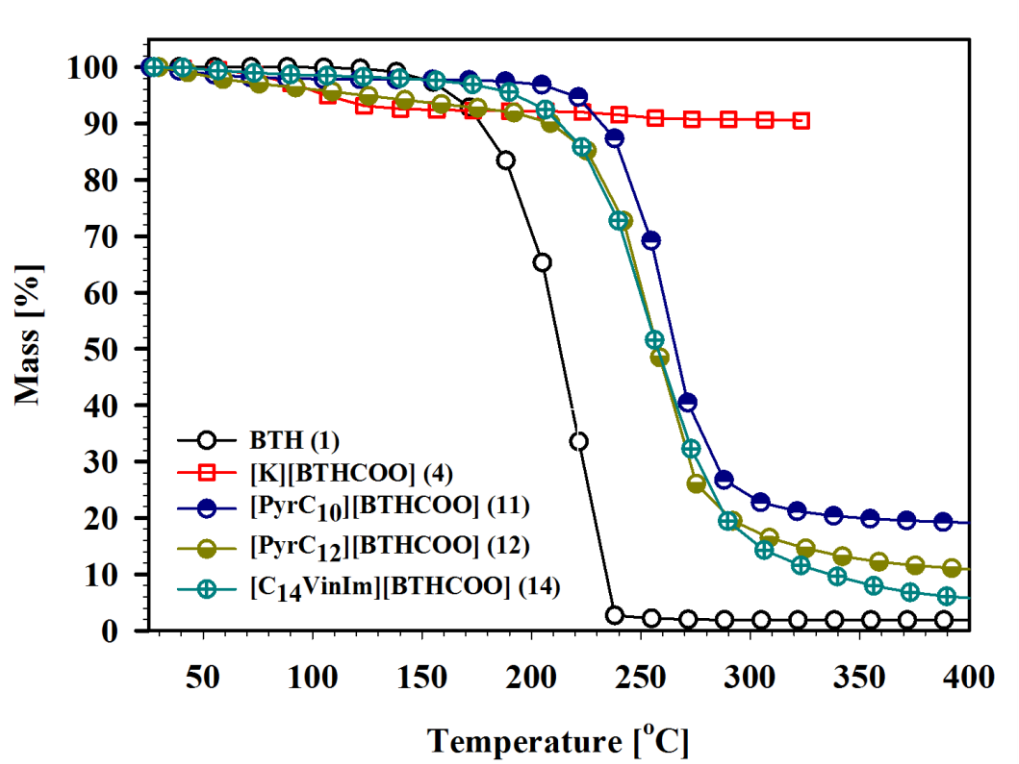


Figure S3 Thermal stability of neutral BTH derivatives and salts of anionic derivatives with aromatic ammonium cations with long alkyl chains

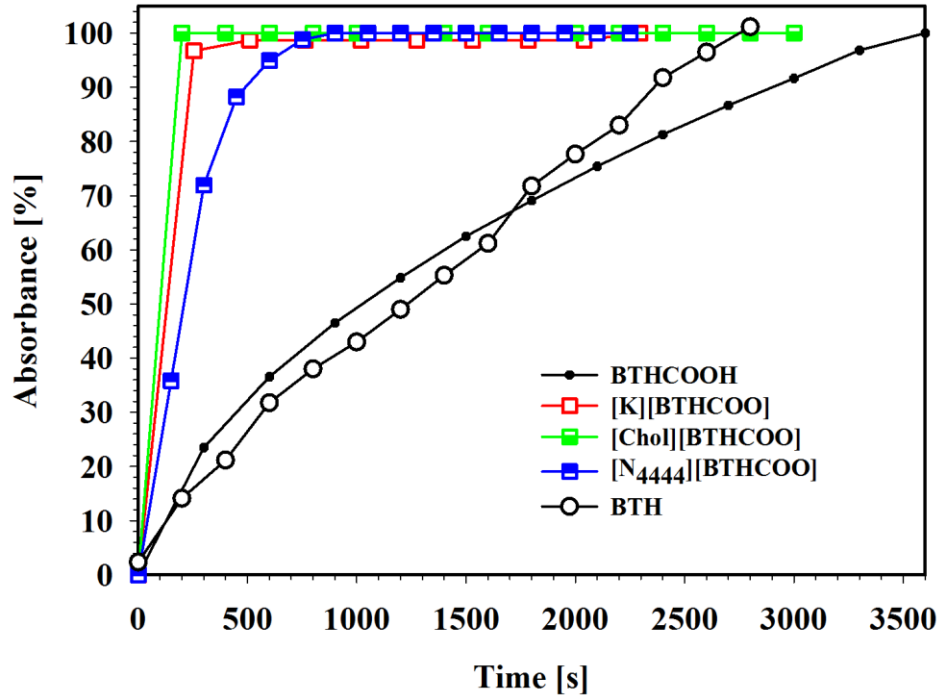


Figure S4 Dissolution curve of salts with small counterion

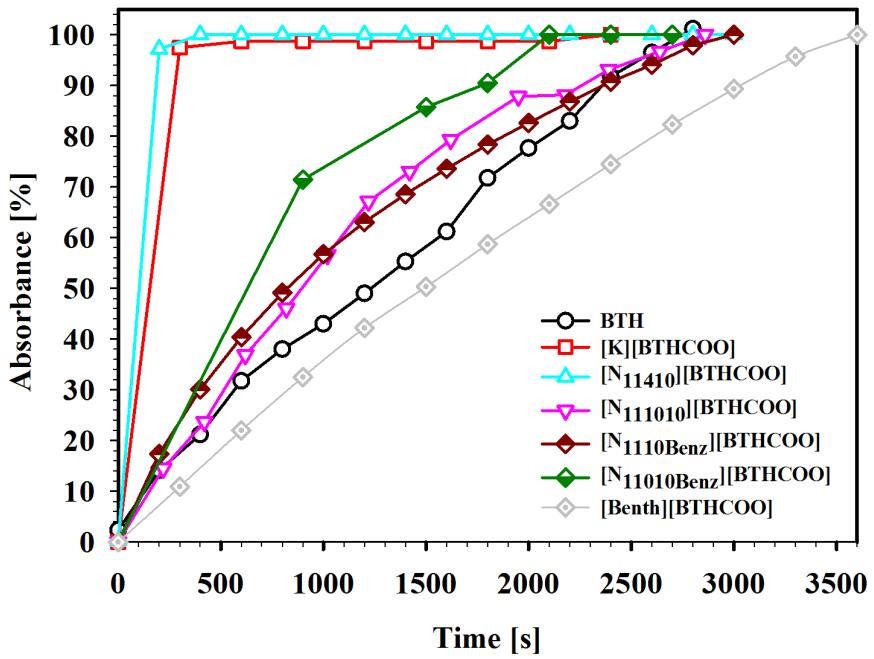


Figure S5 Dissolution curve of salts with counterions based on non-aromatic ammonium cations with long alkyl chains

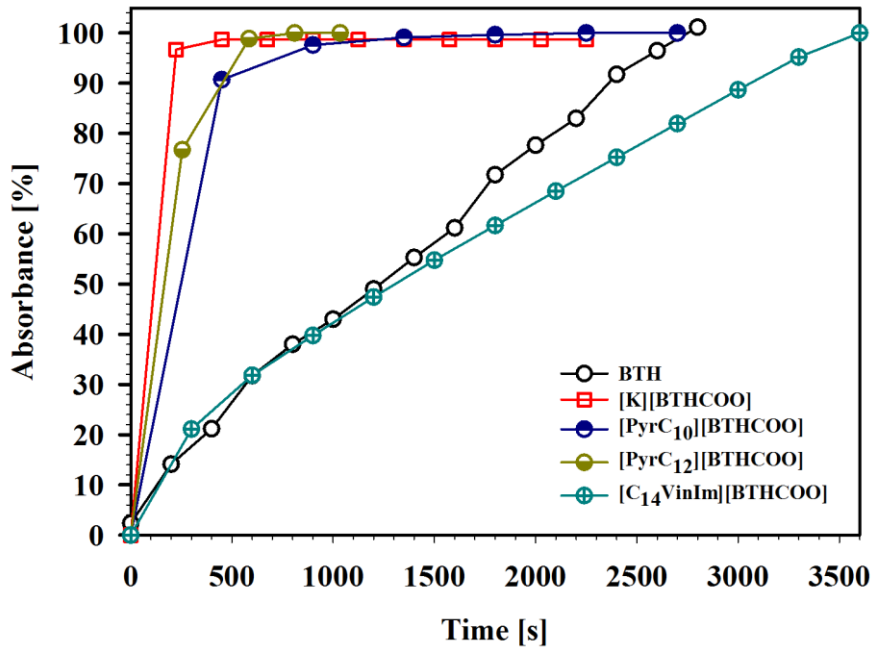


Figure S6 Dissolution curve of salts with counterions based on aromatic ammonium cations with long alkyl chains

Table S1 Average gene expression of defense-related genes *PR-1* and *PAL* at three time points.

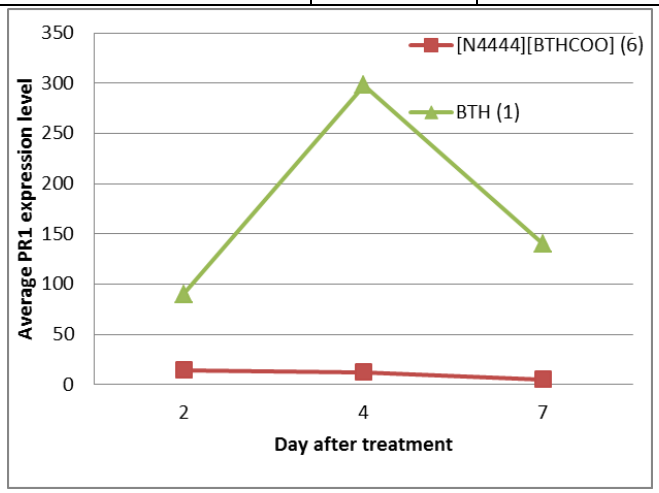
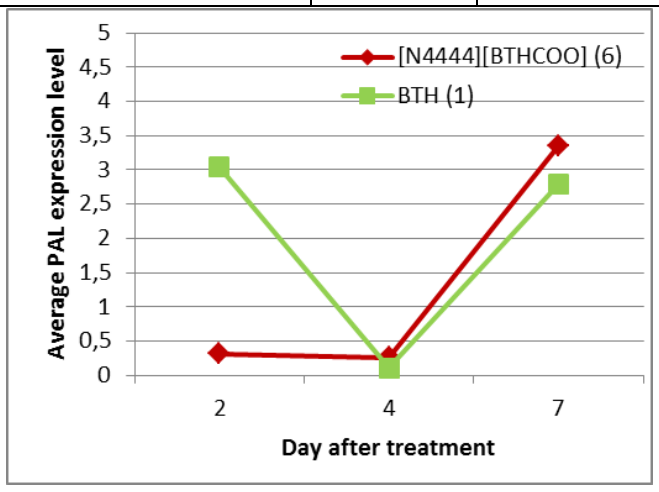
Day after treatment	2	4	7
Gene PR1			
$[N_{4444}][BTHCOO]$ (6) average expression	14.402	12.554	4.922
p-value	0.003	0	0.038
BTH (1) average expression	89.688	297.944	139.862
p-value	0	0	0
			
Gene PAL			
$[N_{4444}][BTHCOO]$ (6) average expression	0.313	0.263	3.351
p-value	0.026	0.001	0.1
BTH (1) average expression	3.041	0.111	2.792
p-value	0.04	0.001	0.191
			

Table S2 MIC and MBC values (at concentrations in [mg/L] of the tested compounds)

Strain/Compound		**C**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Clavibacter michiganensis ss. *michiganensis*	MIC	60	-	-	-	-	-	-	250	2	60	30	120	60	16	250
	MBC	120	-	-	-	-	-	-	250	4	120	60	250	60	16	250
Staphyllococcus epidermidis	MIC	60	-	-	-	-	-	-	250	2	60	8	120	60	16	120
	MBC	120	-	-	-	-	-	-	500	4	120	16	250	60	16	250
Pantoea ananatis	MIC	60	-	-	-	-	-	-	250	2	60	30	250	120	16	250
	MBC	120	-	-	-	-	-	-	500	4	120	60	250	250	16	250
Enterobacter cloaceae subsp. dissolvens	MIC	60	-	-	-	-	-	-	250	4	120	60	250	60	16	250
	MBC	120	-	-	-	-	-	-	500	4	120	60	500	120	16	250
Escherichia coli	MIC	250	-	-	-	-	-	-	500	30	250	250	500	250	60	250
	MBC	250	-	-	-	-	-	-	500	60	250	500	500	250	120	250
Pseudomonas aeruginosa	MIC	120	-	-	-	-	-	-	250	16	120	16	250	120	60	250
	MBC	250	-	-	-	-	-	-	500	30	250	30	500	250	60	500
Pseudomonas fluorescens	MIC	120	-	-	-	-	-	-	500	2	60	60	120	60	30	250
	MBC	120	-	-	-	-	-	-	500	2	120	60	250	60	30	250
Serratia marescens	MIC	250	-	-	-	-	-	-	500	8	250	250	500	120	60	250
	MBC	250	-	-	-	-	-	-	-	16	500	250	500	250	120	250
Proteus vulgaris	MIC	250	-	-	-	-	-	-	500	16	250	250	250	250	60	250
	MBC	250	-	-	-	-	-	-	500	16	250	500	500	250	60	250
Bacillus subtilis	MIC	120	-	-	-	-	-	-	500	8	250	250	250	120	30	250
	MBC	250	-	-	-	-	-	-	500	16	250	500	500	120	30	250

Legend: Symbol "-" means that at maximal tested concentration of the ionic liquid showed no antimicrobial activity, hence determination of MIC and MBC at this concentration rate was not possible., "C" is a reference substance (Benzalkonium Chloride (BAC)).

EXPERIMENTAL

Synthesis

All of reactions, unless otherwise stated, were conducted on commercially available pure solvents (abcr GmbH & Co. KG (Germany), POCh S.A. (Poland), P.P.H. STANLAB Sp. J. (Poland), CHEMPUR (Poland)) without drying or further purification. Benzethonium chloride, neutral amines (tributylamine, didecylmethylamine, decyldimethylamine, piridine, 1-vinylimidazole) and alkylating agents (decylchloride, butylbromide, dimethylsulfate, benzylchloride, and tetradecylbromide) were commercially available and used as received (abcr GmbH & Co. KG (Germany), Iolitech GmbH (Germany), SIGMA-ALDRICH Corporation (USA), POCh S.A. (Poland)). Quaternary ammonium salts for ionic exchange such as tetrabutylammonium bromide, didecyldimethylammonium methylsulfate,

benzyldecyldimethylammonium chloride, benzyldidecylmethylammonium chloride, N-decylpiridinium bromide, N-dodecylpyridinium bromide and 3-tetradecyl-1-vinylimidazolium bromide were synthesized by alkylation reactions from tertiary amines via general procedures known from literature.[1]

BTH used for further derivatization was extracted from commercially available BION™ 50WG (Syngenta) plant protection agent. Method of BTH **(1)** extraction and synthesis of compounds [K][BTHCOO] **(4)**, [Chol][BTHCOO] **(5)** and [N_{11410}][BTHCOO] **(7)** were previously reported. All of other compounds were prepared through methods described below.

Purification

Presented synthetic pathways were generally based on extraction or precipitation methods, where mostly inorganic side products were washed out or filtered from organic phase with product.[2] In the stage, when impurities could contain halides, purity was determined by reaction with acidic solution of $AgNO_3$ in order to define their concentration at level below 500 ppm. Next, solvents were evaporated from purified salts and obtained solids or liquids were dried under high vacuum (0.01 mBar) for 24 to 48 hours for the purification from water traces. Finally, of all of synthesized compounds were directed to NMR analysis for determination of exact ions ratio and lack of traces of organic impurities. Because of ionic character of prepared compounds and their melting points often below 100 ^{o}C, samples were not crystalline, rather unstructured solids, waxes or even oils. Other impurities have not been analysed because of the lack of significance for biological tests, especially in used concentration.

Synthesis of benzo[1.2.3]thiadiazole-7-carboxylic acid, BTHCOOH, (2). 1 equivalent of benzo[1,2,3]thiadiazole-7-carboxylic-S-methyl ester **(1)** (1.400×10^{-2} mol, 2.944g) was dissolved in 200 mL of toluene and added to 2 equivalents of potassium hydroxide (2.800×10^{-2} mol, 1.571g) dissolved in 150 mL of water. Mixture was heated for 24 hours under reflux and stirred

with magnetic stirrer. After this time reaction was cooled down to room temperature and phases were separated. Toluene phase was washed several times with water, a water phases were combined and evaporated to the half of volume (for removal of water soluble gaseous methanethiol). Remaining water solution was acidified by adding dropwise 2 equivalents of hydrochloric acid (2.800×10^{-2} mol, 1.022g) at room temperature. White precipitate of amorphous benzo[1.2.3]thiadiazole-7-carboxylic acid **(2)** was obtained. Precipitate was filtered under vacuum and washed with water until neutral pH of filtrate. Organic acid was dried at 50^{o}C in vacuum (100 mbar) dryer (Yield 92.0%, 1.300×10^{-2} mol, 2.342g).

1H NMR (300 MHz, DMSO, TMS) δ/ppm = 14.25 (1H, s, COOH), 8.99 (1H, d, C(6)H), 8.39 (1H, d, C(6)H), 7.91(1H, t, C(6)H).

13C NMR (75 MHz, DMSO) δ/ppm = 166.58, 158.88, 139.96, 131.40, 128.75, 128.74, 124.30.

Synthesis of benzo[1.2.3]thiadiazole-7-carboxylic methyl ester, BTHCOOMe, (3). Transestrification reaction of BTH **(1)** (4.756×10^{-2} mol, 10.000g) was carried out in methanol (1000 mL) in alkaline conditions with catalytic amounts of potassium hydroxide (approx. 0.010g). Mixture was heated under reflux for 24 hours. After this time the solvent was evaporated and obtained solid residue was suspended in water, filtered and washed several times with water to separate pro-duct from residues. White powder was obtained (Yield 85.0%, 4.042×10^{-2} mol, 7.850g).

1H NMR (300 MHz, DMSO, TMS) δ/ppm = δ 9.05 (1H, d, C(6)H), 8.46 (1H, d, C(6)H), 7.96 (1H, t, C(6)H), 4.02 (3H, s, OCH_3).

13C NMR (75 MHz, DMSO) δ/ppm = 164.95, 158.46, 139.43, 130.99, 128.78, 128.37, 122.50, 53.37.

Synthesis of tetrabutylammonium benzo[1.2.3]thiadiazole-7-carboxylate, [N_{4444}][BTHCOO], (6). 1 equivalent of methanol solution of tetrabutylammonium bromide

(1.188 x 10^{-3} mol, 0.383g in 100 mL of MeOH) was added to 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (1.188 x 10^{-3} mol, 0.259g) dissolved in 100 mL in methanol. Solvent was then evaporated and to the dried residue acetone was added. Part of sediment did not dissolve (inorganic side product) and after cooling the solid was filtered off from the solution. Organic phase was then evaporated and yellow amorphous powder was obtained (Yield = 91.0%, 1.074 x 10^{-3} mol, 0.453g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 8.58 (1H, d, C(6)H), 8.09 (1H, d, C(6)H), 7.70 (1H, t, C(6)H), 3.17 (8H, t, N-CH_2-), 1.56 (8H, p, -CH_2-), 1.29 (8H, m, -CH_2-), 0.91 (12H, t, CH_3)

13C NMR (75 MHz, DMSO) δ/ppm = 165.62, 157.22, 140.01, 133.87, 127.75, 127.28, 123.42, 57.51, 23.05, 19.17, 13.44.

Synthesis of didecyldimethylammonium benzo[1.2.3]thiadiazole-7-carboxylate, [N_{111010}][BTHCOO], (8). 1 equivalent of didecyldimethylammonium methylsulfate (9.899 x 10^{-4} mol, 0.430g) dissolved in 200 mL of water was mixed with 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (9.899 x 10^{-4} mol, 0.216g) also dissolved in 200 mL of water. While stirring, the mixture transformed into colloid form. After 30 min of reaction at room temperature the mixture was cooled to 0^{o}C and then centrifuged (3000 rpm, 20 min). Yellow oil was formed at a bottom and was separated from water phase and washed two times with fresh water (40 mL). Separated water phase was washed with dichloromethane in order to extract any remaining product and then dichloromethane phase was combined with yellow oil phase separated earlier and evaporated under high vacuum what giving yellow wax (Yield 86.6%, 8.573 x 10^{-4} mol, 0.434g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 8.69 (1H, d, C(6)H), 8.18 (1H, d, C(6)H), 7.76 (1H, t, C(6)H), 3.21 (4H, t, N-CH_2-), 2.98 (6H, s, N-CH_3), 1.62 (4H, p, -CH_2-), 1.23 (28H, m, -CH_2-), 0.85 (6H, t, CH_3)

13C NMR (75 MHz, DMSO) δ/ppm = 166.71, 157.82, 140.12, 131.71, 128.83, 127.74, 124.87, 63.02, 53.09, 31.50, 29.11, 29.02, 28.89, 28.68, 25.97, 22.31, 21.90, 14.12

Synthesis of benzyldecyldimethylammonium benzo[1.2.3]thiadiazole-7-carboxylate, [$N_{1110Benz}$][BTHCOO], (9). 1 equivalent of of benzyldecyldimethylammonium chloride (1.100 x 10^{-3} mol, 0.343g) and 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (1.100 x 10^{-3} mol, 0.240g) were added into the mixture of dichloromethane and water (1:1 v/v) and stirred for 12 hours. After stirring both phases were moved into separator funnel and separated. Organic phase was washed two times with 20 mL of water, than all water phases were washed once with 40 mL of dichloromethane. Organic phases were combined, evaporated and dried under high vacuum. Yellow oil was obtained (Yield = 90.0%, 9.897 x 10^{-4} mol, 0.451g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 8.61 (1H, d, C(6)H), 8.11 (1H, d, C(6)H), 7.72 (1H, t, C(6)H), 7.54 (5H, m, C(Benz)H), 4.53 (2H,s, Benz-CH_2-N), 3.24 (2H, t, N-CH_2-), 2.96 (6H, s, N-CH_3), 1.77 (2H, p, -CH_2-), 1.24 (14H, m, -CH_2-), 0.85 (3H, t, -CH_3)

13C NMR (75 MHz, DMSO) δ/ppm = 166.08, 157.79, 140.45, 133.63, 133.39, 130.70, 129.36, 128.63, 128.46, 127.83, 124.26, 66.56, 63.98, 49.57, 31.73, 29.34, 29.24, 29.11, 28.96, 26.29, 22.56, 22.25, 14.41

Synthesis of benzyldidecylmethylammonium benzo[1.2.3]thiadiazole-7-carboxylate, [$N_{11010Benz}$][BTHCOO], (10). 1 equivalent of benzyldidecylmethylammonium chloride (1.188 x 10^{-3} mol, 0.521g) and 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (1.188 x 10^{-3} mol, 0.259g) were added into the mixture of dichloromethane and water (1:1 v/v) and stirred for 12 hours. After stirring both phases were moved into separator funnel and separated. Organic

phase was washed two times with 20 mL of water, than all water phases were washed once with 40 mL of dichloromethane. Organic phases were combined and solvent was evaporated and dried under high vacuum. Yellow oil was obtained (Yield = 88.0%, 1.045 x 10^{-3} mol, 0.608g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 8.65 (1H, d, C(6)H), 8.15 (1H, d, C(6)H), 7.74 (1H, t, C(6)H), 7.54 (2H, d, C(Benz)H), 7.52 (3H, t,t, C(Benz)H), 4.55 (2H,s, Benz-CH_2-N), 3.18 (2H, t, N-CH_2-), 3.14 (2H, t, N-CH_2-), 2.90 (3H, s, N-CH_3), 1.71 (4H, m, -CH_2-), 1.24 (28H, m, -CH_2-), 0.85 (6H, t, -CH_3)

13C NMR (75 MHz, DMSO) δ/ppm = 166.34, 157.32, 140.40, 133.41, 132.66, 130.65, 129.35, 128.78, 128.52, 127.90, 124.73, 66.95, 60.61, 47.50, 31.75, 29.34, 29.22, 29.13, 28.90, 26.23, 22.56, 21.93, 14.40

Synthesis of N-decylpiridinium benzo[1.2.3]thiadiazole-7-carboxylate, [PyrC_{10}][BTHCOO], (11). 1 equivalent of methanol solution of N-decylpiridinium bromide (5.000 x 10^{-3} mol, 2.00g in 100 mL of MeOH) was added to 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (5.000 x 10^{-3} mol, 1.091g in 100 mL of MeOH). Solvent was then evaporated and to the dried solid residue acetone (200 mL) was added. Most of white residue dissolved in boiling acetone. Even then part of sediment did not dissolve (inorganic side product) and after cooling it was filtered from the solution. Organic phase was then evaporated and white crystals were obtained (Yield = 58.0%, 2.900 x 10^{-3} mol, 1.159g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 9.10 (2H, d, C(pyr)H), 8.61 (1H, d, C(pyr)H), 8.59 (1H, d, C(6)H), 8.16 (2H, t, C(pyr)H), 8.10 (1H, d, C(6)H), 7.72 (1H, t, C(6)H), 4.59 (2H, t, NCH_2), 1.90 (2H, p, -CH_2-), 1.23 (14H, m, -CH_2-), 0.85 (3H, t, CH_3)

13C NMR (75 MHz, DMSO) δ/ppm = 166.43, 157.59, 145.68, 145.06, 140.22, 133.29, 128.33, 128.31, 127.62, 124.12, 60.92, 31.46, 31.01, 29.15, 28.98, 28.84, 28.58, 25.61, 22.29, 14.15

Synthesis of N-dodecylpiridinium benzo[1.2.3]thiadiazole-7-carboxylate, [PyrC$_{12}$][BTHCOO], (12). 1 equivalent of methanol solution of N-dodecylpyridinium bromide (4.687 x 10^{-3} mol, 1.539g in 100 mL of MeOH) was added to 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (4.687 x 10^{-3} mol, 1.023g in 100 mL of MeOH). Next, solvent was evaporated and to the obtained and dried residue dichloromethane (150 mL) was added. Dichloromethane phase was then extracted several times with water in order to remove inorganic byproducts. After extraction organic phases were evaporated and yellow oil was obtained (Yield = 92.0%, 4.312 x 10^{-3} mol, 1.844g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 9.26 (2H, d, C(pyr)H), 8.61 (1H, d, C(pyr)H), 8.59 (1H, d, C(6)H), 8.16 (2H, t, C(pyr)H), 8.14 (1H, d, C(6)H), 7.70 (1H, t, C(6)H), 4.65 (2H, t, NCH_2), 1.85 (2H, p, -CH_2-), 1.12 (18H, m, -CH_2-), 0.77 (3H, t, CH_3)

13C NMR (75 MHz, DMSO) δ/ppm = 166.53, 157.63, 145.65, 145.13, 140.22, 133.09, 128.39, 128.30, 127.59, 124.19, 60.89, 31.49, 31.10, 29.19, 29.09, 28.98, 28.91, 28.61, 25.63, 22.29, 14.12

Synthesis of benzethonium benzo[1.2.3]thiadiazole-7-carboxylate, [Benth][BTHCOO], (13). 1 equivalent of methanol solution of benzethonium chloride (8.446 x 10^{-4} mol, 0.378g in 100 mL of MeOH) was added to 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (8.446 x 10^{-4} mol, 0.184g) dissolved in 100 mL of methanol. Solvent was then evaporated and to the dried residue acetone was added. Part of sediment did not dissolve (inorganic side product). Amount of sediment was very small thus the solution after cooling was centrifuged and more solid was decanted. Organic phase was filtered, evaporated and yellow viscous oil was obtained (Yield = 96.0%, 8.108 x 10^{-4} mol, 0.480g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 8.56 (1H, d, C(6)H), 8.06 (1H, d, C(6)H), 7.69 (1H, t, C(6)H), 7.56 (2H, d, C(6)H), 7.51(3H, t, C(6)H), 7.25 (2H, d, C(6)H), 6,82 (2H, d,

C(6)H), 4.59 (2H, s, C(Benz-CH_2)H), 4.11 (2H, t, C(-O-CH_2)H), 3.99 (2H, t, C(-O-CH_2)H), 3.82 (2H, t, C(-O-CH_2)H), 2.42 (2H, t, C(-N-CH_2)H), 3.01 (6H, s, C(-N-CH_3)H), 1.67 (2H, s, C(-CH_2-)H), 1.28 (6H, s, C(-C-CH_3)H), 0.66 (9H, s, C(-CH_2-CH_3)H).

13C NMR (75 MHz, DMSO) δ/ppm = 165.43, 157.20, 155.93, 141.56, 140.04, 134.07, 133.11, 130.27, 128.87, 128.03, 127.70, 127.27, 126.92, 123.34, 113.54, 68.85, 67.48, 66.55, 63.90, 62.63, 56.28, 49.78, 37.54, 31.98, 31.52.

Synthesis of 3-tetradecyl-1-vinylimidazolium benzo[1.2.3]thiadiazole-7-carboxylate, [C_{14}VinIm][BTHCOO], (14) 1 equivalent of methanol solution of 3-tetradecyl-1-vinylimidazolium bromide (2.362×10^{-3} mol, 0.877g in 100 mL of MeOH) was added to 1 equivalent of potassium benzo[1.2.3]thiadiazole-7-carboxylate (2.362×10^{-3} mol, 0.516g in 100 mL of MeOH). No sedimentation of byproduct occurred even after cooling. Methanol was evaporated and to the obtained and dried residue dichloromethane was added. Dichloromethane phase was then extracted several times with water in order to remove inorganic byproducts. After extraction organic phases were evaporated and yellow oil was obtained (Yield = 85.0%, 2.008×10^{-3} mol, 0.945g).

1H NMR (300 MHz, DMSO TMS) δ/ppm = 9.75 (1H,s, C(im)H), 8.59 (1H, d, C(6)H), 8.23 (1H, s, C(im)H), 8.11 (1H, d, C(6)H), 7.96 (1H, s, C(im)H), 7.70 (1H, t, C(6)H), 7.34 (1H, d,d, N-CH=CH_2), 5.99 (1H, d,d, N-CH=CH_2), 5.41 (1H, d,d, N-CH=CH_2), 4.20 (2H, t, NCH_2), 1.80 (2H, p, -CH_2-), 1.20 (22H, m, -CH_2-), 0.82 (3H, t, CH_3)

13C NMR (75 MHz, DMSO) δ/ppm = 167.14, 157.55, 140.26, 135.80, 131.70, 129.17, 128.86, 127.51, 123.89, 119.38, 108.74, 65.82, 49.42, 31.52, 29.35, 29.29, 29.27, 29.24, 29.16, 29.03, 28.94, 28.61, 25.73, 22.32, 14.12

Analytical methods

NMR. H^1 NMR spectra were recorded on a Varian XL 300 NMR (300 MHz) using d^6-DMSO as solvent with tetramethylsilane as the internal standard. Proton chemical shifts are shown in parts per million (δ ppm). C^{13} NMR spectra were obtained using the same instrument at 75 MHz.

Differential Scanning Calorymetry (DSC). Melting points were determined using DSC method in STARe System (Mettler Toledo). Samples were between 6 – 15 mg, closed in aluminum pans and stored under argon atmosphere (flow rate: 20 mL/min). In the first heating cycle the heating ramp was set from 25°C to 160°C with heating rate 10°C/min. At this temperature samples were held for 5 minutes in isotherm. In the next step samples were cooled from 160°C to -50°C with cooling rate of 10°C/min, and then were in constant temperature for 5 min at -50°C. The last step was heating sample from 50°C to 160°C with heating rate 10°C/min. Only in case of DSC analysis performed for BTHCOOH **(2)** the maximum temperature was set at 270°C.

Thermogravimetric Analysis (TGA). Thermogravimetrical analysis was performed on TGA Q50 Texas Instrument. Samples between 5 – 10 mg were heated form 25°C to 500°C with heating rate of 10°C/min with a 10 min isotherm at 80°C under nitrogen atmosphere. This isotherm step was intended to help in removing any remaining water and possible volatile impurities present in the samples. Temperatures reported for the decomposition profiles for all materials were established as the onset temperature for decomposition of the first 5% of the sample ($T_{5\%onset}$), and later as the regular onset temperature for decomposition (T_{onset}), either for the whole sample or for each of the consecutive steps in multistep decomposition.

Dissolution kinetics. Tests were performed in closed circuit system, by analysis of absorbance of light (wavelength specific for tested compounds) measured in spectrophotometer Merck Spectroquant® Pharo 300 with flow-through cell as a result of slow dissolution of the analyzed

substance in water. Solution was transported (25 mL/min) from dissolution reactor to spectrophotometer by peristaltic pump Heidolph PD 5101 and back to the reactor. All measurements were performed with unified parameters; besides wavelength unique for each compound. At first, system was washed with water, filled with fresh water and peristaltic pump was enabled. Continuous flow UV cell was placed in spectrophotometer, which baseline was set for solvent. Next each sample (between 5 to 10 mg) was placed in cellulose bags and immersed in continuous flow reactor and stirred magnetically (250 rpm) for up to 60 min for maximum saturation. All tests were performed at 25^{o}C.

Determination of LogP values via HPLC method. The LogP values were measured for all of reported compounds by using standard reverse phase HPLC method with OECD procedure. Samples were dissolved in mixture of HPLC grade methanol and water (75:25 v/v) in concentration 0.6 mg/mL. Prepared samples were analyzed using a Acquity UPLC BEH C18 1.7μm column by Waters with a mobile phase of methanol and water (75:25 v/v) with a flow rate 0.25 mL/min and detected by PDA detector. The standard curve was generated by using materials of known logP values, butanone (0.30), benzyl alcohol (1.10), methyl benzoate (2.10), ethyl benzoate (2.60) and naphthalene (3.60) as references. Retention times were collected in duplicate for each of analyzed salts. Retention times of reference compounds were adjusted for the T_0 (359 s) , and a standard curve of logP vs. retention time was generated (R^2 = 0.998). Values of logP for analyzed new salts, based on their the retention time, were calculated using equation of the calibration curve.

Biological tests

SAR properties. Plants of *N. tabacum var Xanthi*, at the stage of three-developed leaves, were watered with 50 mL or sprayed with 20 mL solutions of analysed salts in water at concentration 20 mg/L and the control sprayed or watered with distilled water that was used for the preparation

of solutions of ionic liquids. Seven days later, the treated plants were infected mechanically with *Tobacco mosaic virus* (TMV). After the next 4-5 days a local necrotic spots, as a result of a viral infection, were counted and compared between the number of spots on the leaves treated with salt solutions and distilled water (control). Reduction in the number of necrotic spots on the leaves treated with salts, in comparison with the control, show inhibition of viral infection by induction of plant resistance through the usage of new salts. Moreover aside from the reduction in the number of local necrotic spots, in tobacco plants treated with tested compounds, also their size reduction was observed. Data were collected in three replicates and statistically subjected to the one-way analysis of variance (ANOVA).

Phytotoxicity test on plants. The *N. tabacum var Xanthi* plants were watered with water solutions containing active substance in concentration of 100 mg/L. After 3-5 days visual effects of used compounds on the plants were analyzed. The symptoms of phytotoxicity were observed as necrotic spots, yellowing of leaves or their parts, retarded leaf growth or general plant growth inhibition.

Direct influence of BTH derivatives on virus. To asses direct impact of tested BTH derivatives on viruses, compounds were dissolved in water at concentration: 100 mg/L. Initially, TMV was incubated for about 30 minutes in solution of BTH derivatives, and then *N. tabacum var. Xanthi* leaves were mechanically infected. In the control, tobacco leaves were infected with a mixture of TMV which was previously incubated only in distilled water. 4 days after infection the necrotic spots on tobacco plants infected with virus treated before with solutions of tested salts were counted and compared to control.

Antibiocidal tests. Microorgansisms used: The reference strains of: *Bacillus subtilis* ATCC 6633, *Enterobacter cloaceae* subsp. *dissolvens* 23373, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 49134, *Pantoea ananatis* 33244, *Pseudomonas aeruginosa*

ATCC 10145, *Proteus vulgaris* NCTC 4635 and *Serratia marcescens* ATCC 8100 were supplied by the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. The strains of: *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) and *Pseudomonas fluorescens* (Pfl) came from the Institute of Plant Protection-National Research Institute`s own collection. Anti-microbial activity was determined by the broth microdilution method, according to Approved Standard for aerobic bacteria (CLSI document M07-A9). Nineteen ionic liquids were tested against their anti-microbial activity and compared to reference (Benzalkonium Chloride (BAC)). Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined and are given in table 5 S2. All antimicrobial agents were tested in a series of twofold dilutions from 0.500 to 0.001 mg/mL. Cultures of tested reference strains (24 hours) were suspended in Mueller-Hinton Broth (MHB) (Sigma-Aldrich. Co.) to concentration of 10^5 –10^6 cfu/mL.

Analysis of SAR marker genes expression

RNA isolation and cDNA synthesis, *N. tabacum* plants treated with **(1)**, **(6)**, in concentration 20 mg/L and water were harvested 2, 4 and 7 days after treatment. For each day and analyzed conditions five plants were tested. For each plant also three technical replicates were done. Total RNA was extracted from the first, developed leaves of collected plants using TRI-Reagent (ThermoFisher Scientific, Waltham, MA) according to producer's instruction. The pellet of total RNA was dissolved in 20-40 µl of RNase-free water and its quantity was measured spectrophotometrically. RNA samples were treated with RNase-free DNase I (ThermoFisher Scientific, Waltham, MA) to remove contaminating DNA in samples. Then, the cDNA synthesis was performed using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher). One µg of total RNA was combined with 200 ng of random hexamer primers and incubated at 65°C for 5 min. Then, the mixture was cooled on ice and 4 µl of 5x Reaction Buffer, 2 µl of 10 mM dNTPs,

20 U/µl RiboLock RNase Inhibitor and 200 U/µl of RevertAid M-MuLV Reverse Transcriptase (Thermo Scientific) were added. The reaction was carried out in a total volume of 20 µl using following thermal conditions: 25°C for 10 min, 60 min at 42°C followed by reaction termination at 70°C for 5 min. The cDNA samples were diluted in sterile RNase-free water in 2:1 ratio and then used as template for real-time PCR reactions.

Relative gene expression of *PR1* and *PAL*. The effectiveness of treatments with analyzed salt and BTH on the expression levels of defense related genes - *PR1a* and *PAL* (phenyl-alanine ammonia-lyase a key enzyme of a pathway that leads to SA synthesis) in relation to control plants (treated only with water) was determined by using real-time PCR approach. The *PR-1a* gene was amplified as described previously, with specific primers PR-1F 5′-CATAACACAGCTCGTGCAGATGTAG-3′ and PR-1R 5′-AACCACCTGAGTATAGTGTCCACAC-3′.[3] The gene of defense-related PAL enzyme was amplified with primers palF 5′-TGAGGCTGCTGCTATTATGG-3′, palR 5’-AGAGGATCCGTTTCGTGAAG-3′.[4] For normalization, the level of the *N. tabacum EF1a* gene transcription was used as a reference. The *EF1a* specific primers EF1aF 5′-TACCACCCCCAAGTATTCCA-3′, EF1R 5′-GGACAAAGGGGATCTTGTCA-3′ were designed using Primer3 on the basis of the sequences of elongation factor 1 *a* deposited in NCBI databank. The real-time PCR reaction was carried out in a Roche LightCycler 480 Real-Time PCR System. The experimental run protocol for *EF1a* gene amplification was composed of denaturation step (5 min 95˚C), amplification and quantification program repeated 40 times (15 s at 95˚C and 60 s at 60˚C) and for *PR1a* and *PAL* genes: denaturation step 5 min 95˚C, and then 40 cycles of 15 s at 95˚C, 20 s at 58˚C and 20 s at 72˚C with a single fluorescence measurement, followed by the melting curve program (72-99˚C with a heating rate 0.1˚C per s). The real-time PCR mastermix was prepared as follows: 1x iTaq™ universal SYBR Green supermix (Biorad)

with primers at the final concentration of 0.5μM each, 2μl diluted template and water to 20 μl of total volume. All reactions were performed for four biological replicates, and for each biological replicate three technical replicates were done. The expression level of the genes was normalized with the *EF1a* as a reference gene, and statistically analyzed. Statistical significant differences ($p<0.05$) were evaluated using REST 2009 software (Relative Expression Software Tool,www.qiagen.com/Support).

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