



Cite this: *New J. Chem.*, 2019, 43, 5805

# Plant growth promoting *N*-alkyltropinium bromides enhance seed germination, biomass accumulation and photosynthesis parameters of maize (*Zea mays*)†

Anna Parus,<sup>ib</sup>\*<sup>a</sup> Grzegorz Framski,<sup>ib</sup>\*<sup>b</sup> Wojciech Rypniewski,<sup>ib</sup><sup>d</sup> Katarzyna Panasiewicz,<sup>c</sup> Piotr Szulc,<sup>c</sup> Kamila Myszk,<sup>ib</sup><sup>d</sup> Agnieszka Zgoła-Grześkowiak,<sup>a</sup> Łukasz Ławniczak<sup>ib</sup><sup>a</sup> and Łukasz Chrzanowski<sup>ib</sup><sup>a</sup>

Tropane alkaloids have attracted increasing scientific attention as natural compounds which display interesting biological properties. Tropane, a common constituent of tropane alkaloids, is a relatively inexpensive and commonly available compound which can be transformed into the form of quaternary salt in order to increase its uptake by plants. The aim of this study was to assess the properties of quaternary *N*-alkyltropinium bromides with various alkyl substituents and their impact on a model crop plant (maize) in order to evaluate their applicability as potential plant growth regulators. The products were obtained with high yield (81–96%) and their structures were confirmed using NMR. Some of the obtained salts exhibited surface active properties (CMC ranging from  $5.12 \times 10^{-2}$  to  $2.33 \times 10^{-3}$  mg L<sup>-1</sup>). The conducted experiments confirmed the positive effect of the synthesized salts on maize, such as increased seed germination (up to 60%), higher fresh/dry biomass yield (up to 20%), enhanced electron transfer rate (up to 40%) as well as improved total chlorophyll content (up to 60%). Further studies revealed that the properties of *N*-alkyltropinium salts depended on the structure of the substituent introduced during quaternization. The substituent can be adjusted to either ensure rapid biodegradability (100% in case of ethylacetyl substituent) or antimicrobial properties (MIC ranging from 2 to 20 mg L<sup>-1</sup>). The obtained result suggest that *N*-alkyltropinium bromides potentially constitute a promising group of novel plant growth promoting agents.

Received 13th December 2018,  
Accepted 25th February 2019

DOI: 10.1039/c8nj06298f

rsc.li/njc

## Introduction

The increasing food demand imposes the necessity to develop more efficient agricultural practices. The use of different agrochemicals is currently considered as a fundamental tool to maintain high yield and quality of crops.<sup>1,2</sup> Such practices mainly focus on the use of stimulants to enhance the growth of crops and the utilization of herbicides to reduce or eliminate the negative impact of weeds. Nevertheless, the majority of commercially available products are based on synthetic compounds, which often exhibit a negative environmental impact.<sup>3</sup> In order to adhere to the principles of sustainable development and

green chemistry, there is a need to develop novel agrochemicals based on natural compounds.<sup>4</sup>

In recent years, there has been a growing interest in tropane alkaloids, a group of natural bioactive compounds which are produced by plants.<sup>5</sup> This group of bicyclic alkaloids includes a tropane ring in their structure and is mainly produced by the *Solanaceae* family of plants.<sup>6</sup> Tropane alkaloids exhibit a variety of interesting properties, including anticholinergics (*e.g.* atropine, hyoscyamine, scopolamine) and stimulating (*e.g.* cocaine, ecgoine, hydroxytropacocaine) effects, which correspond to numerous medical applications.<sup>7</sup> Among several tropane alkaloids atropine has attracted much attention due to its use in the treatment of poisoning with organophosphate compounds, including nerve gasses such as tabun, sarin or novichok.<sup>8</sup> Moreover, it should be highlighted that, in addition to its other uses, atropine is suggested as a means of treatment in case of poisoning with pesticides, which may be crucial for reducing the health hazards associated with the use of potentially harmful agrochemicals. In addition to its biochemical characteristics, atropine is also a relatively cheap and commonly available natural compound, which occurs mainly in the nightshade family of plants.<sup>9</sup>

<sup>a</sup> Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland. E-mail: anna.parus@put.poznan.pl; Tel: +48-61-6653716

<sup>b</sup> Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland. E-mail: framski@ibch.poznan.pl

<sup>c</sup> University of Life Sciences in Poznan, Department of Agronomy, Dojazd 11, 60-632 Poznan, Poland

<sup>d</sup> University of Life Sciences in Poznan, Department of Biotechnology and Food Microbiology, Wojska Polskiego 48, 60-627 Poznan, Poland

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8nj06298f

The properties of atropine result from the fact that it functions as a muscarinic acetylcholine receptor antagonist, which is best studied in human and animal organisms. Muscarinic receptors are also present in plant cells and acetylcholine (being a neurotransmitter) influences several crucial cell functions in plants, particularly the regulation of photosynthesis and water balance. Nevertheless, the number of reports which describe the actual effect of atropine on plants is very limited. The hypothesis behind this research is that the introduction of tropine, which is a component of atropine, may influence the biochemistry of plant cells and potentially lead to increased crop quality. Furthermore, the structural modification of tropine *via* quaternisation may contribute to its increased uptake by plants and further enhance its action. Numerous studies indicate that quaternary tropinium salts exhibit surface activity<sup>10</sup> and may be used for extraction of other alkaloids,<sup>11</sup> separation of metal ions<sup>12</sup> and aminoacids,<sup>13</sup> phase-transition phenomena<sup>14</sup> and applications in chromatography.<sup>15</sup> Despite the potential of tropine-based compounds, there has been a lack of reports regarding their application in the agricultural sector to date.

The aim of this study was to assess the properties of quaternary *N*-alkyltropinium bromides with various alkyl substituents and their impact on a model crop plant (maize) in order to evaluate their applicability as potential plant growth regulators. The investigation included the chemical characterization of the synthesized salts and their effect on the germination index, biomass and chlorophyll fluorescence parameters of maize. Furthermore, the surface activity, biodegradability and toxicity towards common bacterial species was determined.

## Results and discussion

### Synthesis and purification of quaternary *N*-alkyltropinium salts

Quaternization of tropine was carried out using a series of straight chain alkyl bromides (C10–C18), benzyl bromide and ethylacetyl bromide. It was expected that the transformation of tropine (T) into a quaternary salt will result in its increased uptake by plants and potential enhancement of its effect. An additional advantage is associated with the fact that the use of different substituents may change the properties of tropine and allow for regulation of its solubility (and thus mobility in soil and availability for plants), surface activity, biodegradability and toxicity. Seven quaternary tropinium salts (QTS) were obtained with high yields ranging from 81 to 90% (Table 1). Among the synthesized compounds three salts (QTS-Benz, QTS-Ethy, QTS-C18) are novel and have not been previously described in the literature. After the reaction and subsequent purification the molecular structures of the obtained salts were confirmed by means of <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra (data presented in the ESI†). The quaternization of the nitrogen atom in tropine contributed to an increased surface activity of the obtained salts, which was confirmed by determination of their CMC values. Elongation of the alkyl substituent resulted in lower CMC values, which ranged from  $5.12 \times 10^{-2}$  mg L<sup>-1</sup> for QTS-C10 to  $2.33 \times 10^{-3}$  mg L<sup>-1</sup> for QTS-C14, however the use of

C16 and C18 alkyl chains decreased the water solubility of the corresponding salts and prevented the measurement.

### Effect of quaternary *N*-alkyltropinium bromides on the early development of maize (hybrid *Zea mays* Drim, FAO 220)

#### Effect of *N*-alkyltropinium bromides on seed germination.

The effect of tropine and the synthesized quaternary tropinium salts on the germination index of maize seeds was studied at three concentrations: 25, 50 and 100 mg L<sup>-1</sup>. In general, the introduction of the obtained salts increased the germination index of maize seeds. The effect depended on the type of salt as well as its concentration. The GI values ranged from 70 to 160% at 25 mg L<sup>-1</sup>, 80 to 130% at 50 mg L<sup>-1</sup> and 40 to 160% at 100 mg L<sup>-1</sup> (Fig. 1).

The best results were obtained at the lowest studied concentration (25 mg L<sup>-1</sup>) for QTS-C12 and at the highest studied concentration (100 mg L<sup>-1</sup>) in case of QTS-Ethy (in both cases the GI was increased by approx. 60% relative to control). In case of tropinium salts comprising straight chain alkyl (QTS-C10–C18) and benzyl (QTS-Benz) substituents the GI decreased with increasing concentration. At the highest studied concentration (100 mg L<sup>-1</sup>) these salts inhibited the GI by 20 (in case of QTS-C18) to 60% (in case of QTS-C10 and QTS-C16). In contrast, QTS-Ethy decreased the GI by 25% at the lowest studied concentration, whereas a notable stimulation occurred at the highest concentration. The observed contrasting effect may be associated with the structural difference of the ethylacetyl substituent which is less hydrophobic in comparison to alkyl substituents. High hydrophobicity of long-chain alkyl groups may potentially result in a better interaction with the seeds and confer a stimulating effect at lower concentrations, whereas their surface activity may damage the seedling when higher concentrations are applied. In order to eliminate the negative effects of the studied salts, further studies were carried out using the concentration of 50 mg L<sup>-1</sup>.

The presence of quaternary ammonium salts may result in inhibition of seed germination. For example, Bubalo *et al.*<sup>16</sup> studied the effect of different anions and alkyl chains lengths in quaternary imidazolium salts on the development of barley seedlings. They reported that the inhibitory effect depended on the concentration as well as the chemical structure of the studied salt. Inhibition of germination ranged from 20 to 70% in the concentration range up to 100 mg L<sup>-1</sup>. The most toxic compound contained 10 carbon atoms in the chain, whereas compounds comprising shorter chains were less toxic. The toxic effect was most likely associated with the disruption of membrane physiological functions.<sup>17,18</sup> In contrast, the quaternary tropinium salts synthesized in the framework of this study were less toxic and contributed to stimulation of seed germination at lower concentrations.

**Effect on fresh and dry weight of maize plants.** Evaluation of fresh and dry biomass of maize seedlings grown in the greenhouse also confirmed a stimulating effect of the synthesized salts (Table 2). The fresh and dry weight of the plants were increased relative to control in case of all the studied compounds. The highest increases of fresh and dry (by approx. 20% in both cases) were observed in cases of QTS-C12 and QTS-C14. The lowest values, which were similar to control, were noted in

Table 1 Structures of the synthesized *N*-alkyltropinium bromides

Abbreviation	Structures	Name	M.W.	Yield [%]	CMC [mg L <sup>-1</sup> ]	Melting point [°C]
T		Tropine	141.21	—	—	—
QTS-Benz		<i>N</i> -Benzyltropinium bromide	312.25	95	No surface active properties	243–245
QTS-Ethy		<i>N</i> -Ethylacetiltropinium bromide	302.21	96	No surface active properties	195
QTS-C10 <sup>a</sup>		<i>N</i> -Decyltropinium bromide	362.39	95	5.12 × 10 <sup>-2</sup>	215–216
QTS-C12 <sup>a</sup>		<i>N</i> -Dodecyltropinium bromide	390.44	92	1.44 × 10 <sup>-2</sup>	228–229
QTS-C14 <sup>a</sup>		<i>N</i> -Tetradecyltropinium bromide	418.50	89	2.33 × 10 <sup>-3</sup>	244–245
QTS-C16 <sup>a</sup>		<i>N</i> -Hexadecyltropinium bromide	446.54	85	Not measured due to limited solubility	241–242
QTS-C18		<i>N</i> -Octadecyltropinium bromide	474.60	81	Not measured due to limited solubility	238–239

<sup>a</sup> Indicates that the compound has been previously synthesized.<sup>19</sup>

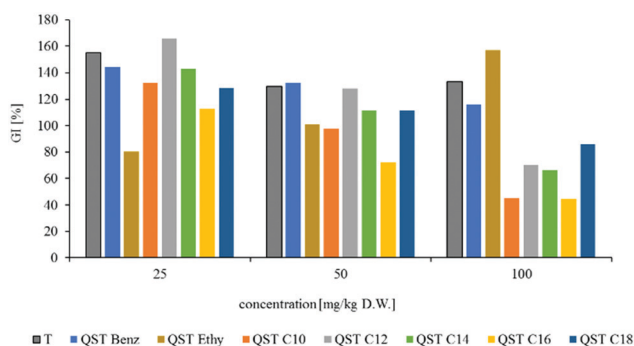


Fig. 1 The effect of *N*-alkyltropinium bromides concentration on the germination index of maize.

case of QTS-C10. The obtained results confirmed that the introduction of the synthesized salts at 50 mg L<sup>-1</sup> did not lead to any phytotoxic effects towards maize after the germination stage and that enhancement of seedling growth was achieved.

Table 2 Effect of *N*-alkyltropinium bromides on fresh and dry weight of aboveground parts of maize (hybrid *Zea mays* drim, FAO 220)

Compounds	Fresh weight of 1 plant [g]	Dry weight of 1 plant [g]	% of dry weight relative to the fresh weight
Control	25.50 ± 2.26	3.22 ± 0.31	12.61
T	30.37 ± 1.81	3.78 ± 0.19	12.49
QTS-Benz	29.43 ± 1.39	3.53 ± 0.09	12.02
QTS-Ethy	29.15 ± 1.29	3.55 ± 0.02	12.18
QTS-C10	26.73 ± 1.15	3.42 ± 0.29	12.78
QTS-C12	31.20 ± 1.15	3.85 ± 0.09	12.35
QTS-C14	31.45 ± 1.56	3.87 ± 0.12	12.30
QTS-C16	30.15 ± 1.39	3.60 ± 0.19	11.94
QTS-C18	29.95 ± 1.91	3.83 ± 0.19	12.81

**Parameters of chlorophyll fluorescence.** The effect of the synthesized salts on the growth of maize was further investigated by analysis of chlorophyll fluorescence parameters which were conducted at the stage of 6/7 leaves (Table 3).

**Table 3** Parameters of chlorophyll fluorescence in maize (hybrid *Zea mays* drim, FAO 220) leaves

Compounds	<i>Y</i> , efficiency photosystem	ETR	<i>F</i> <sub>o</sub> , minima fluorescence	<i>F</i> <sub>m</sub> , maxima fluorescence	<i>F</i> <sub>v</sub> , variable fluorescence	Chlorophyll
Control	0.654 ± 0.010	35.25 ± 4.03	268.00 ± 1.41	1040.50 ± 2.12	806.00 ± 1.41	7.85 ± 0.49
T	0.634 ± 0.040	44.30 ± 1.90	255.00 ± 11.31	1043.50 ± 103.94	838.50 ± 21.92	11.75 ± 2.76
QTS-Benz	0.645 ± 0.010	39.00 ± 1.56	255.50 ± 19.09	1041.50 ± 13.89	846.00 ± 33.94	11.40 ± 1.84
QTS-Ethy	0.659 ± 0.050	55.90 ± 2.55	277.50 ± 23.33	1133.01 ± 76.37	880.50 ± 20.51	12.20 ± 0.99
QTS-C10	0.643 ± 0.001	53.90 ± 4.67	267.50 ± 4.95	1123.50 ± 6.36	846.00 ± 1.41	9.35 ± 0.07
QTS-C12	0.636 ± 0.001	57.90 ± 0.42	269.00 ± 7.07	1050.00 ± 51.91	861.00 ± 12.73	11.10 ± 2.55
QTS-C14	0.667 ± 0.001	46.35 ± 1.20	259.50 ± 6.36	1157.00 ± 31.11	867.50 ± 4.95	12.75 ± 1.06
QTS-C16	0.657 ± 0.001	47.60 ± 2.69	258.00 ± 12.73	1080.50 ± 13.44	822.50 ± 0.71	11.45 ± 0.49
QTS-C18	0.659 ± 0.010	53.00 ± 4.53	263.50 ± 10.41	1074.00 ± 124.45	860.50 ± 23.33	11.55 ± 1.63

The obtained data indicated that the studied compounds did not contribute to any negative effects on the plants' photosystem and in some cases an enhancement could be observed.

The positive impact of quaternary tropinium salts was clearly visible in case of the electron transfer rate (ETR) and chlorophyll content values. In case of QTS-Ethy and QTS-C12 the ETR values were increased by approx. 40% relative to control, whereas in case of the remaining compounds the increase ranged from 10 to 20% (the lowest results were obtained for QTS-Benz). The highest chlorophyll content was observed in case of QTS-C14 and QTS-Ethy (increase by 60 and 55% relative to control, accordingly), whereas the lowest values were noted in case of QTS-C10 (increase by 20% relative to control). It should also be emphasized that in all cases the maximum quantum efficiency (FV/FM) remained at approx. 0.8, which is typical for an intact photosystem II in higher plants.<sup>20</sup>

Estimation of stress factors is the quantification of environmental effects on plants. When plants are subjected to non-ideal growing conditions, they are considered to be under stress. Stress factors can affect growth, survival and crop yields. The estimation of plant stress can involve visual assessments of plant vitality; however, the focus has moved to the use of instruments and protocols that reveal the response of particular processes within the plant (photosynthetic capacity, plant cell signaling, plant secondary metabolism, post-translational and post-transcriptional gene regulation). Different environmental factors, also including chemical compounds, might cause a considerable reduction in contents of important photosynthetic pigments, such as chlorophylls. This reduction may occur due to stress-induced impairment in pigment biosynthetic pathways or in pigment degradation. This may lead to the impairment in electron transport and hence reduced photosynthetic capacity in plants.<sup>21</sup> The comparison of pigment level and fluorescence parameters with the values obtained for control samples indicated that no negative effect of the studied compounds on the model plant occurred. On the contrary, the changes of chlorophyll fluorescence parameters suggested a positive, stimulating effect on plant growth.

### Biodegradation of *N*-alkyltropinium bromides

The next experimental stage was focused on the evaluation of primary biodegradability of the synthesized salts (Table 4).

The majority of the studied compounds were characterized by limited susceptibility to biodegradation processes. The sole exception was QTS-Ethy which was completely biodegraded.

**Table 4** Efficiency of biodegradation of *N*-alkyltropinium bromides

Abbreviation	Biodegradation efficiency [%]
T	9.50 ± 0.61
QTS-Benz	19.33 ± 0.73
QTS-Ethy	99.85 ± 0.82
QTS-C10	0.63 ± 0.15
QTS-C12	3.00 ± 0.53
QTS-C14	2.58 ± 0.47
QTS-C16	3.00 ± 0.68
QTS-C18	3.53 ± 0.33
Paclobutrazol	60% <sup>a</sup>
Chlormequat chloride	50% <sup>a</sup>
Uniconazole	Not readily biodegradable <sup>a</sup>

<sup>a</sup> Data from material safety data sheets of commercial products.

QTS-Benz could be classified as inherently biodegradable (dissipation of 20% during the test duration), whereas the salts comprising long-chain alkyl substituents were resistant to biodegradation (dissipation <5%). Taking into account that plant growth regulators are introduced directly into the environment, the rapid biodegradability of QTS-Ethy is of importance. This salt exhibited higher biodegradability compared to other biodegradable plant protection regulators (*e.g.* paclobutrazol and chlormequat chloride). Commercial growth regulators are often characterized by low biodegradability (*e.g.* uniconazole) similar to the synthesized tropinium homologues comprising alkyl chains. Furthermore, the number of studies focused on the biodegradability of tropine and its derivatives is very limited to date. To the best of the authors' knowledge, this is the first report regarding the biodegradation of quaternary tropinium salts.

### Antimicrobial activity

In order to further explore the reason for the low biodegradability of the studied salts, their toxicity to model microbial species was evaluated (Table 5).

It was established that the presence of a long-chain alkyl substituent in QTS-C10–C18 contributed to notable toxicity towards the studied microorganisms, as expressed by MIC values which were one or two orders of magnitude lower compared to tropine, QTS-Benz and QTS-Ethy. The toxicity increased with increasing carbon chain length up to QTS-C16, then a decrease was observed for QTS-C18. The toxic effect of quaternary ammonium salts is commonly attributed to their surface-active properties and disruption of cell membranes. The decrease of toxicity in case of QTS-C18 may be attributed to its low solubility in water or increased spherical hindrance



Table 5 Minimum inhibitory concentrations of the synthesized *N*-alkyltropinium bromides against model microorganisms

Microorganism	MIC [mg L <sup>-1</sup> ]									
	T	QTS Benz	QTS Ethy	QTS C10	QTS C12	QTS C14	QTS C16	QTS C18	DDAC	BAC
<i>Staphylococcus aureus</i>	150	>150	>300	9.38	9.38	2.34	2.34	9.38	9.38	18.75
<i>Pseudomonas aeruginosa</i>	150	>150	>300	9.38	9.38	2.34	2.34	9.38	18.75	18.75
<i>Escherichia coli</i>	>300	>150	>300	150	150	18.75	18.75	>300	1.17	2.34
<i>Bacillus cereus</i>	>300	>150	>300	75	37.50	13.3	6.65	9.38	9.38	9.38
<i>Candida albicans</i>	150	>150	>300	9.38	9.38	2.34	2.34	37.50	18.75	18.75

compared to shorter alkyl chains. The MIC values of QTS-C10–C18 salts were at a similar level compared to model cationic surfactants (didecyldimethylammonium chloride or benzalkonium chloride). The highest susceptibility to the synthesized salts was observed in case of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* (MIC ≤ 10 mg L<sup>-1</sup>). The obtained results indicate that the low biodegradability of long-chain alkyl salts may be associated with their high toxicity.

Quaternary ammonium salts display the ability to inhibit the growth of bacteria,<sup>22–25</sup> fungal cells,<sup>26</sup> protozoa<sup>27</sup> or to increase the efficacy of antimicrobial agents.<sup>28</sup> The mechanism associated with the toxic effect may result in disruption of plasma membrane continuity<sup>29</sup> and oxidative stress in bacterial cells.<sup>30</sup> This effect was also reported for quaternary tropinium salts. For example, Corte *et al.*<sup>19</sup> and Tiecco *et al.*<sup>31</sup> studied structurally different quaternary ammonium salts, including *N*-alkyltropinium bromides, and reported that *N*-tetradecyltropinium bromide displayed antimicrobial activity against *Escherichia coli*, *Listeria innocua*, and *Listeria monocytogenes*, as well as fungi *Saccharomyces cerevisiae* and *Candida albicans*. Many authors reported that the toxicity of quaternary salts increased with the increase of the length of the side chain.<sup>32–40</sup> The presence of a long alkyl chain contributes to increased surface activity and, in consequence, disruption of membrane permeability.<sup>41,42</sup> It was also established that the compounds with a more complex molecular structure had a greater tendency to inhibit growth of the tested organisms compared to the low molecular weight compounds with relatively simple structures.

### X-ray crystallography

Three salts which were characterized by different biodegradability and toxicity (QTS-Ethy, QTS-Benz and QTS-C10) were selected for studies using X-ray crystallography (Fig. 2). The crystallographic parameters of the tested compounds are summarized in the ESI.† Each of the three crystal structures contain in the asymmetric unit one molecule of the respective *N*-alkyltropinium derivative and one bromide anion to balance the electrostatic charge. In addition, the QTS-C10 structure contained in the asymmetric unit a well-ordered toluene molecule (Fig. 2D).

Although the unit cell parameters differ between the three crystal structures, the molecular packing shows an interesting similarity. Each bromide ion is surrounded by five tropinium moieties in such a way that the nitrogen atoms form approximately a right square pyramid with the anion in the center of its base (Fig. 3). Distances from Br<sup>-</sup> to the N atoms that form the base of the pyramid are in the range 4.2–6.0 Å, while the distance to N at the apex is 6.5–6.6 Å. Angles formed by adjacent

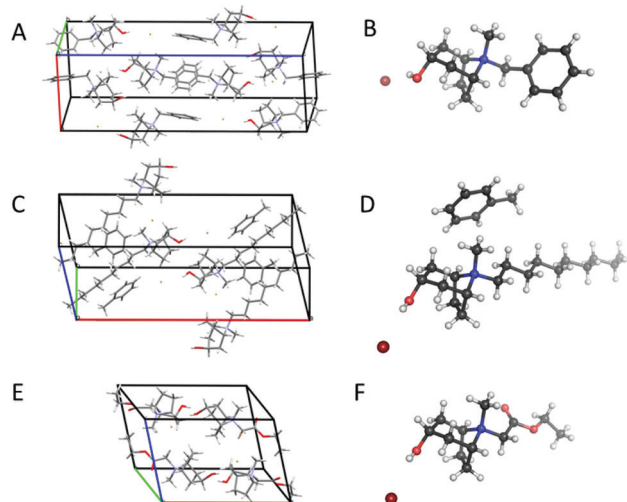


Fig. 2 The crystal structure of tropinium salts: QTS-Benz (A and B), QTS-C10 (C and D) and QTS-Ethy (E and F).

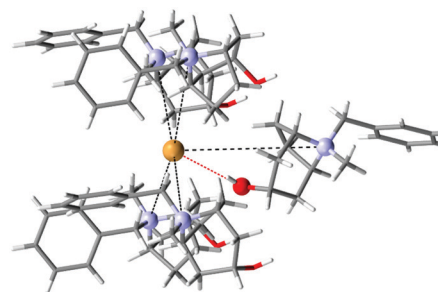


Fig. 3 Arrangement of five QTS-Benz molecules around a bromide anion (yellow sphere). Distances to nitrogen atoms (blue spheres) are marked with black dashed lines. Hydrogen bond between the bromide and the hydroxyl group of the tropinium moiety is marked with a red dashed line. Similar arrangements are observed in the other two crystal structures.

vertices with the bromide are in the range 82–103°. In addition, there is a hydrogen bond between the bromide and the hydroxyl group of the tropinium moiety located at the vertex of the 'pyramid' (O–Br<sup>-</sup> distance is 3.3–3.4 Å). There is no sixth 'ligand' that would complete an octahedral geometry around the bromide, the corresponding space in all three crystal structures is occupied by the packed *N*-substituents of the tropinium derivatives or toluene solvent molecules, in the case of QTS-C10.

Thus, one side of the cluster formed around the bromide ion formed a surface open for interactions with other molecules.

The surface is polar in the middle, due to the bromide, while the edge of the binding area is formed by the substituents, therefore its nature depends on the type of tropinium derivative used. It is plausible that this feature could define some properties of the studied compounds observed in the framework of this study. The chemical shifts are given in ppm relative to the residual solvent signal. The X-ray data and molecular models were summarized in the ESI.†

## Conclusions

This manuscript presents the results of a case study focused on the effect of *N*-alkyltropinium homologues on maize. It was observed that the synthesized salts enhanced the germination of seeds, contributed to higher fresh and dry biomass gain, and improved chlorophyll fluorescence parameters (most notably the electron transfer rate and total chlorophyll content). The best results were observed for salts comprising ethylacetyl, dodecyl and teteradecyl substituents. Further studies indicated that the biodegradability of the synthesized compounds depended on the type of substituent (complete biodegradation in case of the ethylacetyl substituent and low biodegradation in case of long alkyl chains). The low biodegradability may be attributed to the high surface activity of compounds with long alkyl substituents, which most likely resulted in the disruption of cell membranes. The data from crystallographic analyzes suggested that arrangement of molecules allows for interaction between the substituents and the cell membrane. This is further supported by the low MIC values obtained for such compounds during the evaluation of antimicrobial activity. Based on the obtained results, it can be established that the *N*-alkyltropinium salts may potentially be used as novel and efficient plant growth promoting agents, and that substituent can be adjusted to add a desired secondary property: rapid biodegradability or antimicrobial properties. Future studies should be focused on evaluation of the plant growth promoting effect using a broad range of crop plants and introduction of different functional properties *via* modification of the substituent.

## Experimental

### Synthesis of quaternary *N*-alkyltropinium salts

The quaternary tropinium salts were synthesized by quaternization of tropine with the corresponding *n*-alkyl bromide. Tropine (0.007 mol), dry acetone (50 mL) and alkyl bromide (0.007 mol) were added to a round-bottom flask equipped with a reflux condenser. The mixture was stirred for 5 days at 60 °C in argon atmosphere. After 5 days a white precipitate was separated from the solution. Recrystallization from acetone resulted in pure (98%) white crystals. All reagents were from Sigma Aldrich with a purity > 97%.

### Plants

The phytotoxicity tests were carried out with seeds of *Zea mays* (maize – Hybrid Drim, FAO 220) provided by the Department of

Plant Physiology at the Poznan University of Life Sciences, Poland.

## Methods

### Analysis of the synthesized salts

The synthesized quaternary tropinium bromides were characterized by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. <sup>1</sup>H NMR spectra were recorded using a Bruker AvanceII 400 MHz Ultra Shield Plus spectrometer operating at 400 MHz with tetramethylsilane as the internal standard. <sup>13</sup>C NMR spectra were obtained with the same instrument at 100 MHz. DMSO was used as a solvent. The melting point values for each synthesized compound were determined using a Mettler Toledo MP90 Melting Point System apparatus. The MS spectra were obtained using liquid chromatography UltiMate 3000 Dionex (USA) conjugated with mass spectrometer API 4000 QTRAP Applied Biosystems (MSD Sciex (USA)).

### CMC measurement

Air–water surface tension ( $\gamma$ ) was measured at 20 °C by using an Attension Theta Optical Tensiometer (Biolin Scientific, Gothenburg, Sweden). The surface tension was measured using freshly prepared aqueous solutions of the quaternary tropinium salts (QTS) in a concentration range from  $2 \times 10^{-3}$  to  $10 \times 10^3$  mg L<sup>-1</sup> at 20 °C. The surface tension was determined using the pendant drop method.<sup>43</sup> This method consists of fitting the Young–Laplace equation to the digitized shape of a drop suspended from the end of a capillary tube. The image of the drop (6  $\mu$ L) was taken from a charge couple device camera. The critical micellar concentration (CMC) was determined by extrapolation of the two straight trends in low and high concentration regions in surface tension curves (the  $\gamma$  *versus* log *C* plot). Apparent surface tensions were measured three times for each sample.

### Germination tests

The germination tests were carried out in Petri dishes and 50 maize seeds were sown per dish on a wet filter paper. After application of tropine and *N*-alkyltropinium bromides, the Petri dishes were incubated at 20 °C ( $\pm 1$  °C) for 7 days. The concentration of compounds was at 25, 50 and 100 mg L<sup>-1</sup>. A control sample was prepared with distilled water. This experiment was conducted in three independent runs and each sample was prepared in four replicates. The germination index was calculated according to the formula:<sup>44</sup>

$$GI = \left( \frac{L_x}{L_c} \right) \times 100\%$$

where  $G_x$  and  $G_c$  are the number of seeds germinated in the sample and control, respectively whereas  $L_x$  and  $L_c$  are the length of root in the sample and control, respectively.

During experiments in the greenhouse, maize seeds were placed on the soil in flower pots (5 seeds per pot). For the experiment, the soil with the following elemental composition was used: 81 mg of P kg<sup>-1</sup> soil, 88 mg of K kg<sup>-1</sup> soil, 69 mg of Mg kg<sup>-1</sup> soil, pH 5.92, the content of organic C 1.01% [10.1 g kg<sup>-1</sup> soil]. After application

of tropine derivatives, the samples were placed in the greenhouse at 20/17 °C ( $\pm 3$  °C) and a 16/8 h photoperiod. The concentration of tropine and *N*-alkyltropinium bromides was at 50 mg L<sup>-1</sup>. After emergence, the plants were thinned to 1 plant in each pot. Biometric measurements of fresh weight and fluorescence parameters were carried out at the 6–7 leaf stage (BBCH 16/17).

**Determination of fluorescence parameters.** The chlorophyll fluorescence emitted by plant leaves reflects the state of the photosynthetic systems. Measurement of the fluorescence parameters was performed using the OS5P – OPTI Science fluorometer (USA). The quantitative chlorophyll content (CCI) and four chlorophyll fluorescence parameters, namely initial fluorescence ( $F_0$ ), maximal fluorescence ( $F_m$ ), variable fluorescence ( $F_v$ ) and maximum quantum efficiency of PSII ( $F_v/F_m$ ) being related to the activity of the photosynthetic apparatus, were measured after application of the *N*-alkyltropinium quaternary salts.

### Biodegradation of quaternary tropinium bromides

**Preparation of biodegradation tests.** The bacterial consortium used throughout the studies was isolated from soil collected in Gorlice, Poland, according to the procedure described by Owsianiak *et al.*<sup>42</sup> Bacterial species were identified according to procedure described by Wyrwas *et al.*,<sup>45</sup> by sequencing genes coding for 16S rRNA. The obtained sequences were submitted to GenBank. The following microorganisms were identified in the isolated consortium: *Pseudomonas stutzeri*, *Alcaligenes xylosoxidans*, *Sphingobacterium* sp., *Comamonadaceae* bacterium, *Citrobacter freundii*, *Sphingobacterium kitahiroshimense* and *Pseudomonas* sp. The sequencing of 16S rRNA genes was performed in the Department of Biotechnology and Microbiology of Food at the Poznan University of Life Sciences.

The biodegradation tests were carried out in 100 mL SIMAX bottles. The bottles were filled with 50 mL of mineral medium at pH 7 (7.0 g L<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 2.8 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.5 g L<sup>-1</sup> NaCl, 1.0 g L<sup>-1</sup> NH<sub>4</sub>Cl, 50.0 g L<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.18 g L<sup>-1</sup> CoSO<sub>4</sub>·7H<sub>2</sub>O, 5.0 g L<sup>-1</sup> FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.18 g L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O, 2.5 g L<sup>-1</sup> MnSO<sub>4</sub>·4H<sub>2</sub>O, 3.25 g L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>, 3.2 g L<sup>-1</sup> ZnCl<sub>2</sub>, 5.0 g L<sup>-1</sup> EDTA, CaCl<sub>2</sub>·H<sub>2</sub>O, 73 mL HCl (37%)) at 30 °C, in glass bottles that were shaken in a water bath, at 180 rpm. Tropine and each quaternary tropinium salt were introduced concentration of 50 mg L<sup>-1</sup>. Control samples (inoculated media without addition of the compounds) and investigated samples including media with 20 mg L<sup>-1</sup> tropine or *N*-alkyltropinium bromides were also prepared. Three replicates of the control and tested samples were kept at 25 °C  $\pm$  3 °C. The biodegradation efficiency for each sample was calculated based on the initial concentration of tropine or quaternary tropinium bromide.

**Evaluation of biodegradation efficiency.** The determination of biodegradation was carried out after 28 days using liquid chromatography UltiMate 3000 Dionex (USA) conjugated with mass spectrometer API 4000 QTRAP Applied Biosystems (MSD Sciex (USA)).<sup>45</sup> The chromatographic separation was conducted using a Hypersil GOLD column (Thermo Scientific, Waltham, MA, USA) size 100 mm  $\times$  2.1 mm, the graining fill 1.9  $\mu$ m with the filter 2.1 mm. The monitored samples were injected at a volume of 5  $\mu$ L each and the column was thermostated at 35 °C.

The mobile phase consisted of  $5 \times 10^{-3}$  mol ammonium ethanoate in water (A) and methanol (B). The analysis time was 8 min. In the beginning stage the gradient elution was 90% compounds A and 10% compound B. The content of compound B was increased every 2 min to 100% and next this composition was carried out by 6 min. The following parameters were used during sample analysis: shielding gas pressure of 20 psi, nebulizing gas pressure of 45 psi, auxiliary gas pressure 45 psi, temperature 300 °C, the voltage applied to electron 4500 V, and a collision gas or tertiary medium. The waiting time for each transition was set to 100 ms.

### Antimicrobial activity

The antimicrobial activity of the studied compounds was evaluated based on MIC values evaluated using the microdilution method according to the European Committee on Antimicrobial Susceptibility Testing. The following microorganisms were used during the tests: *Staphylococcus aureus* ATCC 4163, *Pseudomonas aeruginosa* ATCC 10145 and *Candida albicans* ATCC 10231. All the test species were stored at -70 °C. Prior to the test, the microbial cells were re-cultivated using a BHI medium (Brain Heart Infusion, BioMerieux, France) at 35 °C for 18 h.

The prepared solutions of the tested compounds were diluted twice in a sterile BHI medium (BioMerieux, France). Then, 100  $\mu$ L of each sample was introduced to 96-well plates (Kartell, Italy). Afterwards, 100  $\mu$ L of appropriate inoculum including microbial cells (optical density was set to a value which corresponded to approx. 10<sup>6</sup> cfu mL<sup>-1</sup>) was introduced into each test well. The plates were incubated at 35 °C for 18 h, then MIC values were established – minimal concentrations which contributed to the inhibition of microbial growth.

### X-ray crystallography

Crystals of QTS-Benz, QTS C-10 and QTS-Ethy were obtained by slow evaporation of the solutions of corresponding salts in ethanol (in case of QSTC-10 a droplet of toluene was added in order to improve the process). The X-ray diffraction data were collected on the beamline BL14.2 at the BESSY II electron storage ring using a Rayonix MX225 detector.

The crystals throughout the data collection were kept at the temperature of 100 K by a stream of cold nitrogen gas. The X-ray data were collected on each crystal to the resolution of 0.78–0.81 Å and processed using the HKL200 program suite.<sup>46</sup> The structures were solved with SHELXT<sup>47</sup> and refined with SHELXL.<sup>48</sup>

### Conflicts of interest

There are no conflicts to declare.

### Acknowledgements

This study was supported by the Polish Ministry of Science and Higher Education as project No. 03/32/DSMK/0464.

## Notes and references

- H. Choudhary, J. Pernak, J. L. Shamshina, M. Niemczak, R. Giszter, Ł. Chrzanowski, T. Praczyk, K. Marcinkowska, O. A. Cojocar and R. D. Rogers, *ACS Sustainable Chem. Eng.*, 2017, **5**, 6261.
- A. Syguda, A. Gielnik, A. Borkowski, M. Woźniak-Karczewska, A. Parus, A. Piechalak, A. Olejnik, R. Marecik, Ł. Ławniczak and Ł. Chrzanowski, *New J. Chem.*, 2018, **42**, 981.
- R. Marecik, J. Wojtera-Kwiczor, Ł. Ławniczak, P. Cyplik, A. Szulc, A. Piotrowska-Cyplik and Ł. Chrzanowski, *Water, Air, Soil Pollut.*, 2012, **223**, 4275.
- J. Pernak, B. Łęgosz, F. Walkiewicz, T. Klejdysz, A. Borkowski and Ł. Chrzanowski, *RSC Adv.*, 2015, **5**, 6547.
- J. W. Medley and M. Movassaghi, *Chem. Commun.*, 2013, **49**, 10775.
- X. J. Yin, C. A. Geng, X. Y. Huang, H. Chen, Y. B. Ma, X. L. Chen, C. L. Sun, T. H. Yang, J. Zhou, X. M. Zhang and J. J. Chen, *RSC Adv.*, 2016, **6**(51), 45059.
- E. Aehle and B. Dräger, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2010, **878**, 1391.
- P. R. Chai, B. D. Hayes, T. B. Erickson and E. W. Boyer, *Toxicol. Commun.*, 2018, **2**, 45.
- R. J. Hamilton, *Tarascon pharmacopoeia*, ed. N. A. Duffy, D. Stone and A. Spencer, 2014, p. 386.
- J. Lu, H. Song, Y. Yang, G. Qian, L. Nie and S. Yao, *J. Mol. Liq.*, 2015, **209**, 648.
- B. Dong, J. Tang, A. Yonannes and S. Yao, *RSC Adv.*, 2018, **8**(1), 262.
- G. Qian, H. Song and S. Yao, *J. Chromatogr. A*, 2016, **1429**, 127.
- H. Wu, S. Yao, G. Qian, T. Yao and H. Song, *J. Chromatogr. A*, 2015, **1418**, 150.
- Y. Yang, S. Yao, J. Lu, X. Dai and H. Song, *J. Mol. Liq.*, 2016, **215**, 258.
- H. Wu, S. Yao, G. Qian and H. Song, *J. Chromatogr. A*, 2016, **1461**, 1.
- M. C. Bubalo, K. Hanousek, K. Radošević, G. V. Srček, T. Jakovljević and I. R. Redovniković, *Ecotoxicol. Environ. Saf.*, 2014, **101**, 116.
- K. Docherty and C. Kulpa, *Green Chem.*, 2005, **7**, 185.
- P. Stepnowski, A. C. Składanowski, A. Ludwiczak and E. Łaczyńska, *Hum. Exp. Toxicol.*, 2004, **23**, 513.
- L. Corte, M. Tiecco, L. Roscini, R. Germani and G. Cardinali, *Colloids Surf., B*, 2014, **116**, 761–771.
- O. Björkman and B. Demmig, *Planta*, 1987, **170**, 489.
- M. Ashraf and P. J. C. Harris, *Photosynthetica*, 2013, **51**, 163.
- A. Piotrowska, A. Syguda, B. Wyrwas, Ł. Chrzanowski and H. J. Heipieper, *Chemosphere*, 2017, **167**, 114–119.
- A. Borkowski, Ł. Ławniczak, T. Clapa, D. Narozna, M. Selwet, D. Peziak, B. Markiewicz and Ł. Chrzanowski, *Ecotoxicol. Environ. Saf.*, 2016, **130**, 54–64.
- Ł. Ławniczak, K. Materna, G. Framski, A. Szulc and A. Syguda, *Biodegradation*, 2015, **26**, 327–340.
- T. Wu, A. G. Xie, S. Z. Tan and X. Cai, *Colloids Surf., B*, 2011, **86**, 232–236.
- S. He, B. Wonga, H. Chen, C. Tang and Y. Feng, *ACS Appl. Mater. Interfaces*, 2012, **4**, 2116–2123.
- M. Tischer, G. Pradel, K. Ohlsen and U. Holzgrabe, *Chem-MedChem*, 2012, **7**(1), 22–31.
- N. Simonetti, F. D. D'Auria and V. Strippoli, *Chemotherapy*, 1991, **37**, 32–37.
- M. Nazari, M. Kurdi and H. Heerklotz, *Biophys. J.*, 2012, **10**, 498–506.
- K. Nakata, T. Tsuchido and Y. Matsumura, *J. Appl. Microbiol.*, 2011, **110**, 568–579.
- M. Tiecco, G. Cardinali, L. Roscini, R. Germani and L. Corte, *Colloids Surf., B*, 2013, **111**, 407–417.
- J. Ranke, K. Molter, F. Stock, U. Bottin-Weber, J. Poczobutt, J. Hoffmann, B. Ondruschka, J. Filser and B. Jastorff, *Ecotoxicol. Environ. Saf.*, 2004, **58**, 396–404.
- B. Jastorff, K. Mölter, P. Behrend, U. Bottin-Weber, J. Filser, A. Heimers, B. Ondruschka, J. Ranke, M. Schaefer, H. Schröder, A. Stark, P. Stepnowski, F. Stock, R. Strörmann, S. Stolte, U. Weltz-Biermann, S. Ziegert and J. Thöming, *Green Chem.*, 2005, **7**, 362–372.
- M. Matzke, S. Stolte, J. Arning, U. Uebers and J. Filser, *Ecotoxicology*, 2009, **18**, 197–203.
- S. Studzińska and B. Buszewski, *Anal. Bioanal. Chem.*, 2009, **393**(3), 983–990.
- B. Zhang, X. Li, D. Chen and J. Wang, *Protoplasma*, 2012, **250**, 103–110.
- K. Radošević, M. Cvjetko, N. Kopjar, R. Novak, J. Dumić and G. V. Srček, *Ecotoxicol. Environ. Saf.*, 2013, **92**, 112–118.
- B. Markiewicz, A. Sznajdrowska, Ł. Chrzanowski, Ł. Ławniczak, A. Zgoła-Grzeškowiak, K. Kubiak, J. Nawrot and J. Pernak, *New J. Chem.*, 2014, **38**, 3146.
- A. Piotrowska, A. Syguda, Ł. Chrzanowski and H. J. Heipieper, *Chemosphere*, 2016, **144**, 107.
- A. S. Well and V. T. Coombe, *Org. Process Res. Dev.*, 2006, **10**, 794–798.
- S. Stolte, J. Arning, U. Bottin-Weber, A. Müller, W. R. Pitner, U. Welz-Biermann, B. Jastorff, S. Studzińska and B. Buszewski, *Anal. Bioanal. Chem.*, 2008, **393**, 983–990.
- M. Owsianiak, A. Szulc, Ł. Chrzanowski, P. Cyplik, M. Bogacki, A. K. Olejnik-Schmidt and H. J. Heipieper, *Appl. Microbiol. Biotechnol.*, 2009, **84**, 545–553.
- J. J. Bikerman, *Foams: Theory and Industrial Application*, 1953, p. 38.
- K. P. M. Mosse, A. F. Patti, E. W. Christend and T. R. Cavagnaro, *J. Hazard. Mater.*, 2010, **180**, 63–70.
- B. Wyrwas, Z. Dymaczewski, A. Zgoła-Grzeškowiak, A. Szymański, M. Frańska, I. Kruszelnicka, D. Ginter-Kramarczyk, P. Cyplik, Ł. Ławniczak and Ł. Chrzanowski, *J. Environ. Manage.*, 2013, **128**, 292–299.
- Z. Otwinowski and W. Minor, *Methods Enzymol.*, 1997, **276**, 307–326.
- G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Adv.*, 2015, **71**, 3–8.
- G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, 2008, **64**, 112–122.