J Surfact Deterg (2020)
DOI 10.1002/jsde.12439

ORIGINAL ARTICLE

Synthesis, Surface Activity, and Biological Activities of Phosphonium and Metronidazole Salts

Miloš Lukáč[1] · Martin Pisárčik[1] · Mária Garajová[2] · Martin Mrva[2] · Aneta Dušeková[2] · Andrej Vrták[1] · Renáta Horáková[1] · Branislav Horváth[3] · Ferdinand Devínsky[4]

Received: 14 August 2019 / Revised: 20 February 2020 / Accepted: 10 April 2020

Abstract A series of phosphonium amphiphilic compounds was synthesized. Cationic parts of molecules contain triphenylphosphonium moieties. Lipophilic parts of compounds are represented by straight alkyl chain or the alkyl chains which are ornamented by benzyl or metronidazole. The physicochemical properties of phosphonium amphiphilic compounds were investigated by the measurements of surface tension and conductivity. The critical micelle concentration (cmc), the surface tension value at the cmc (γ_{cmc}), the surface area at the surface saturation per head group (A_{cmc}) were determined. The lowest cmc value was determined for phosphonium salts with straight dodecyl alkyl chain. Its value was 1.5×10^{-3} mol dm^{-3}. Surface tension at the cmc decreases with the addition of bulky moieties (benzyl, radical from metronidazol) at the end of alkyl chains. Biological activities of compounds were studied on human erythrocytes and strains of *Acanthamoeba lugdunensis* and *Acanthamoeba quina*. Dodecyltriphenylphosphonium bromide showed the highest activity against *Acanthamoeba*. To the best of our knowledge, it is the first compound of the group of phosphonium amphiphiles, which exhibited high activity against *Acanthamoeba*. The determined structure–activity relationship indicated nonspecific trophocidal and hemolytic activity that depends on physicochemical properties of the studied compounds.

Keywords *Acanthamoeba* · Critical micelle concentration · Erythrocytes · Metronidazole · Phosphonium amphiphilic compounds

J Surfact Deterg (2020).

Introduction

Metronidazole is an antibiotic used in therapy of infection caused by bacteria and parasites growing in anaerobic conditions. It was introduced to treat chronic trichomonad infections. Besides *Trichomonas vaginalis*, *Entamoeba histolytica* or *Gardia lamblia* are sensitive to this chemotherapeutic drug (Samuelson, 1999). These parasites do not have mitochondria. The oxidative-reduction processes are performed in hydrogenosomes or mitosomes (Kamkowska et al., 2016). Metronidazole is activated by reduction of its nitro group. It is occurred only under strongly reducing conditions (Samuelson, 1999).

The genus Acanthamoeba belongs to opportunistic amoebae, which causes amebic keratitis (AK) and granulomatous amoebic encephalitis (GAE) in humans and animals. Treatments of these diseases are difficult and many times without any success. Many patients had to undergo transplantation of cornea (Lorenzo-Morales et al., 2015) or the GAE is fatal for them (Kot et al., 2018). Metronidazole has been also infrequently used in therapy (Taravaud et al., 2017) but its effect is disputable. No benefit for the

✉ Miloš Lukáč
lukac@fpharm.uniba.sk

1 Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University, Kalinčiakova 8, Bratislava, SK-83232, Slovakia

2 Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina Ilkovičova 6, Bratislava, SK-84215, Slovakia

3 NMR laboratory, Faculty of Pharmacy, Comenius University, Odbojárov 10, Bratislava, SK-83232, Slovakia

4 Faculty of Pharmacy, Comenius University, Odbojárov 10, Bratislava, SK-83232, Slovakia

patients was observed when they were treated with combinations of drugs containing metronidazole (Gunawan et al., 2016; Rodríguez-Pérez et al., 2017). Although it was noted that *A. polyphaga* is sensitive to the action of metronidazole (Ondarza et al., 2006), this is not the case of *A. castellani* (Taravaud et al., 2017).

Cationic amphiphiles are used as antimicrobial compounds (Block, 2000; Falk, 2019). Their molecules are composed of lipophilic and hydrophilic parts. Lipophilic parts are mainly represented by alkyl chains, which can be modified by various groups. Hydrophilic parts are mainly represented by aminium cations connected with alkyl/aryl groups or they are parts of cationic heterocycles. This type of surfactants is denoted as quaternary aminium compounds (QAC). They are the most investigated cationic surfactants. The replacement of a nitrogen by a phosphorus in the structure of QAC leads to the formation of quaternary phosphonium compounds (QPC) (Devínsky et al., 2017). QPC are compounds with the biological activities studied only recently. Many of them contain lipophilic triphenylphosphonium (triarylphosphonium) cation. Their preparation is easier than that of trialkylphosphonium salts. Trialkylphosphines are more sensitive to oxidation than triarylphosphines (Lukáč et al., 2017). As with QAC, they also show antimicrobial activities (Bittner Fialová et al., 2019; Lukáč et al., 2017; Tatarinov et al., 2018). However, they are intensively studied for their unique ability to target compounds to mitochondria (Zielonka et al., 2017). The difference in membrane potential in mitochondria is greater than elsewhere in the cell, which causes the cations to selectively accumulate in mitochondria. Polar cations require a specific transmitter so that they can pass the lipid bilayer into the mitochondria. However, lipophilic cations pass easily through the phospholipid bilayer and may accumulate in the mitochondrial matrix (Sandoval-Acuña et al., 2016; Subramanian et al., 2010). Many drugs and bioactive compounds have been modified with a lipophilic phosphonium cation. The activities of mitochondrially targeted bioactive compounds are higher than the activities of drugs, which are used as templates in the synthesis. (Devínsky et al., 2017; Zielonka et al., 2017)

The aim of this study was the preparation, characterization, and investigation of the phosphonium amphiphilic compounds. The connection of metronidazole, benzyl group, and a straight alkyl chain with a lipophilic phosphonium cation was performed. The physicochemical properties, hemolytic and anti-*Acanthamoeba* activities of prepared compounds were investigated. Our main goal is the study of the influence of triphenylphosphonium cation on the antiprotozoal activity of metronidazole. In particular, we investigated the possible increase in antiprotozoal activity upon the transformation of metronidazole into a compound with the potential mitochondria targeting.

Experimental

Material and Methods

Benzyl alcohol was purchased from AFT (Bratislava, Slovakia) and was of p.a. purity. p-Toluenesulfonic acid was obtained from LACHEMA (Brno, Czech Republic) and was of p.a. purity. The chemicals 11-bromoundecanoic acid, 4-dimethylaminopyridine, and 1-bromododecane were purchased from Sigma Aldrich (Saint Louis, MO, USA) and their purities were 99%, ≥ 98%, and 98%, respectively. Triphenylphosphine and metronidazole were obtained from Alfa Aesar (Kandel, Germany). The purities of chemicals were 99%. Dicyclocarbodiimide with the purity 99% was purchased from Fluka (Buchs, Switzerland). Sodium hydrogen carbonate, anhydrous magnesium sulfate, and solvents were obtained from CentralChem (Bratislava, Slovakia) and were of p.a. purity. NMR spectra were measured on a MERCURY plus spectrometer (Varian, Palo Alto, CA, USA). ^{1}H-, ^{13}C, and ^{31}P NMR spectra were measured at frequencies of 300, 75, and 121.5 MHz, respectively. Decoupling against protons was used in the measurements of ^{13}C and ^{31}P NMR spectra. The spectra were measured in deuterated chloroform and deuterated methanol. The chemical shifts were referenced with respect to an internal TMS (δ 1H = 0, δ 13C = 0) or 85% H_3PO_4 (δ ^{31}P = 0 for $\in$ ^{31}P = 40.4807420 MHz) signal. The IR spectra were recorded on Nicolet FT IR 6700 spectrophotometer (Thermo Scientific). Elemental analysis was performed on Flash 2000 Elemental Analyzer (Thermo Scientific). Melting points were determined using Kofler block and are reported uncorrected.

Benzyl 11-Bromoundecanoate

The ester was prepared in the Dean-Stark apparatus. Benzyl alcohol (0.14 mol, 12.26 g), 11-bromoundecanoic acid (0.095 mol, 25.1 g), and p-toluenesulfonic acid (0.01 mol, 1.8 g) were dissolved in toluene (150 mL). The reaction mixture was heated to 150 °C for 6 h. The solution was extracted with solution of sodium hydrogen carbonate and brine after reaction. The solvent was evaporated under reduced pressure after drying with anhydrous magnesium sulfate. The product was distilled under reduced pressure, b.p. 205–207°C, p = 40 Pa. Yield: 31 g (92.3%). ^{1}H NMR ($CDCl_3$, TMS) δ: 1.23–1.45 (m, 12H), 1.64 (t, J = 9 Hz, 2H), 1.80–1.87 (m, 2H), 2.36 (t, J = 9 Hz, 2H), 3.40 (t, J = 9 Hz, 2H), 5.11 (s, 2H), 7.25–7.38 (m, 5H); ^{13}C NMR: ($CDCl_3$, TMS) δ: 24.9, 28.1, 28.7, 29.1, 29.2, 29.3, 29.3, 32.3, 34.0, 34.3, 66.1, 128.1, 128.5, 136.1, 173.7; IR(ν_{max}/cm^{-1}): 2926, 2853, 1736, 1455, 1162, 735, 697.

2-(2-methyl-5-nitro-1*H*-Imidazol-1-Yl)Ethyl 11-Bromoundecanoate

Metronidazole (0.855 g, 5 mmol), 11-bromoundecanoic acid (1.328 g, 5 mmol), dicyclocarbodiimide (0.611 g, 5 mmol) and 4-dimethylaminopyridine (1.135 g, 5.5 mmol) were dissolved chloroform (40 mL). The reaction mixture was stirred at r.t. and formation of product was monitored by TLC. The dicyclourea formed was filtered off. The solution was extracted by water (3 × 50 mL), followed by 5% solution of acetic acid (3 × 50 mL), water (3 × 50 mL), dried over anhydrous Na_2SO_4, filtered, evaporated *in vacuo*, and purified by silica gel chromatography ($CHCl_3$/EtOAc, 95/5, v/v) to afford ester (1.7 g, 81.3%) as slightly yellow viscous liquid which solidified in refrigerator; m.p. = 38–40°C. ^{1}H NMR ($CDCl_3$, TMS) δ: 1.21–1.62 (m, 10H), 1.75–1.89 (m, 6H), 2.26 (t, J = 7.5 Hz, 2H), 2.52 (s, 3H), 3.41 (t, J = 6.9 Hz, 2H), 4.41 (t, J = 5 Hz, 2H), 4.60 (t, J = 5.3 Hz, 2H), 7.96 (s,1H); ^{13}C NMR: ($CDCl_3$, TMS) δ: 14.4, 24.6, 24.9, 25.6, 28.1, 28.7, 29.0, 29.1 29.3, 29.3, 32.8, 34.0, 45.0, 133.1, 150.1, 173.0; IR(ν_{max}/cm^{-1}): 2915, 2849, 1730, 1522, 1462, 1369, 1266, 1186, 825, 746, 681, 547.

General Procedure for Preparation of Phosphonium Salts

Bromoalkanes (1-bromododecane 24.9 g, 0.1 mol; benzyl 11-bromoundecanoate 2.84 g, 8 mmol or 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 11-bromoundecanoate 1.25 g, 3 mmol) and triphenylphosphine (26.2 g, 0.1 mol; 2.36 g, 9 mmol resp. 0.918 g, 3.5 mmol) were dissolved in acetonitrile (50 mL resp. 15 mL). The mixture was heated at 81 °C and stirred for 48 h. The reaction mixture was cooled down to the room temperature. Solvent was evaporated in rotary evaporator. Dodecyltriphenylphosphonium bromide was purified by crystallization from mixture aceton/diethyl ether. [11-(benzyloxy)-11-oxoundecyl] triphenylphosphonium bromide was purified by column chromatography over silica gel (ethyl acetate/petrolether, 95/5, v/v). {11-[2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy]-11-oxoundecyl}triphenylphosphonium bromide was purified by column chromatography over silica gel ($CHCl_3$/MeOH, 95/5, v/v).

Dodecyltriphenylphosphonium Bromide

White powder; yield 26.6 g (52%); m. p. = 96–97°C; ^{1}H NMR ($CDCl_3$, TMS) δ: 0.86 (t, J = 7.5 Hz, 3H); 1.20–1.28 (m, 16H); 1.63–1.64 (m, 4H); 3.63–3.76 (m, 2H); 7.69–7.88 (m, 15H); ^{13}C NMR ($CDCl_3$, TMS) δ: 14.1, 22.6(0) (d, J = 5,9 Hz), 22.6(3), 22.7 (d, J = 49.6 Hz), 29.2, 29.3, 29.5, 29.6, 30.4 (d, J = 15.4 Hz), 31.8, 118.2 (d, J = 85.5 Hz), 130.6 (d, J = 12.4 Hz), 133.6 (d, J = 9.9 Hz), 135.1 (d, J = 2.9 Hz); ^{31}P NMR ($CDCl_3$) δ: 24.1. Elemental analysis calcd (%) for $C_{30}H_{40}BrP \times 0.25\ H_2O$: C 69.83, H 7.91; found (%): C 69.97, H 7.59.

[11-(Benzyloxy)-11-Oxoundecyl]Triphenylphosphonium Bromide

Viscous liquid; yield 1.51 g (30.8%); ^{1}H NMR ($CDCl_3$, TMS) δ: 1.15–1.38 (m, 10H), 1.55–1.76 (m, 6H), 2.33 (t, J = 7.8 Hz, 2H), 3.78–3.91 (m, 2H), 5.1 (s, 2H), 7.30–7.37 (m, 5H), 7.66–7.91 (m, 15H); ^{13}C NMR: ($CDCl_3$, TMS) δ: 22,5 (d, J = 56.9 Hz) 22,6 (d, J = 4.8 Hz), 24.9, 29.0 29.1, 29.2, 30.3, 30.5, 66.0, 118.3 (d, J = 85.5 Hz), 128.1, 128.2, 128.5, 130.5 (d, J = 12.7 Hz), 133.6 (d, J = 10.5 Hz), 135.1 (d, J = 3.0 Hz) 136.1, 173.7; ^{31}P NMR: ($CDCl_3$) δ: 24.49; IR(ν_{max}/cm^{-1}): 2927, 2855, 1731, 1438, 1164, 1113, 746, 723, 691. Elemental analysis calcd (%) for $C_{36}H_{42}BrO_2P \times 1.5\ H_2O$: C 67.08, H 7.04; found (%): C 67.47, H 6.64.

*{11-[2-(2-methyl-5-nitro-1*H*-Imidazol-1-Yl)Ethoxy]-11-Oxoundecyl}Triphenylphosphonium Bromide*

Viscous liquid; yield 1.0 g (49.2%); ^{1}H NMR (CD_3OD, TMS) δ: 1.16–1.35 (m, 10H), 1.45–1.71 (m, 6H), 2.24 (t, J = 7.4 Hz, 2H), 2.51 (s, 3H), 3.32–3.38 (m, 2H), 4.43 (t, J = 4.6 Hz, 2H), 4.68 (t, J = 5.1 Hz, 2H), 4.85 (s, 1H), 7.75–7.91(m, 15H); ^{13}C NMR: (CD_3OD, TMS) δ: 12.7, 21.3 (d, J = 57.8 Hz), 22.1 (d, J = 4.4 Hz), 24.3, 28.4, 28.5, 28.7, 28.8, 30.0, 30.2, 33.3, 44.8, 61.9, 118.6 (d, J = 85.7 Hz), 130.1 (d, J = 12.6 Hz), 131.3, 133.4 (d, J = 9.8 Hz), 134.9 (d, J = 3.0 Hz), 151.2, 173.2; ^{31}P NMR: (CD_3OD) δ: 23.8; IR(ν_{max}/cm^{-1}): 2928, 2855, 1735, 1528, 1464, 1437, 1363, 1262, 1188, 1113, 824, 744, 723, 691. Elemental analysis calcd (%) for $C_{35}H_{43}BrN_3O_4P \times 1.5\ H_2O$: C 59.41, H 6.55, N 5.94; found (%): C 59.49, H 6.20, N 5.82.

Measurements of Physicochemical Properties

The measurements of equilibrium surface tension and electrical conductivity were carried out according to the previously described procedure (Lukáč et al., 2017).

In vitro Amoebicidal Activity Assay

The trophocidal activities against acanthamoebae were tested according to the previously described procedure (Garajová et al., 2014). *Acanthamoeba lugdunensis* (strain AcaVNAK02, T4 genotype) and *A. quina* (strain AcaVNAK03, T4 genotype) represented clinical isolates of

free-living amoebae, which were isolated from the corneas of two patients who suffered from *Acanthamoeba* keratitis.

In vitro Hemolytic Activity Assay

Human blood was collected into plastic tubes with volume 10 mL which contained di-potassium EDTA as anticoagulant. The blood was added to glass centrifuge tube and the cells were washed three times with phosphate-buffer saline (PBS—Na_2HPO_4 c = 22.2×10^{-3} mol dm^{-3}, KH_2PO_4 c = 5.6×10^{-3} mol dm^{-3}, NaCl c = 123.3×10^{-3} mol dm^{-3}). Erythrocytes were collected by centrifugation at 3000*g* for 10 min. The cells were then suspended in the PBS. The density of erythrocytes in the stock suspension was 7.6×10^8 cells mL^{-1}. The erythrocyte suspension was stored at 4°C for 48 h. The cell suspension (500 µL) was pipetted to 500 µL of buffer containing different concentrations of the tested compounds. Final erythrocyte concentrations were 3.8×10^8 cells mL^{-1}. The mixtures were incubated at 37 °C for 1 h. They were gently shaken during the incubation. The mixtures were then centrifugated at 3000 g for 10 min. The degree of hemolysis was determined by comparing the absorbance (542 nm) of dilutions of the supernatant 400 µL to 10 mL deionized water with that of control samples. The control samples were prepared by hemolysis of erythrocytes in deionized water (the stock solution of erythrocytes 25–200 µL were added to 10 mL of deionized water).

Results and Discussion

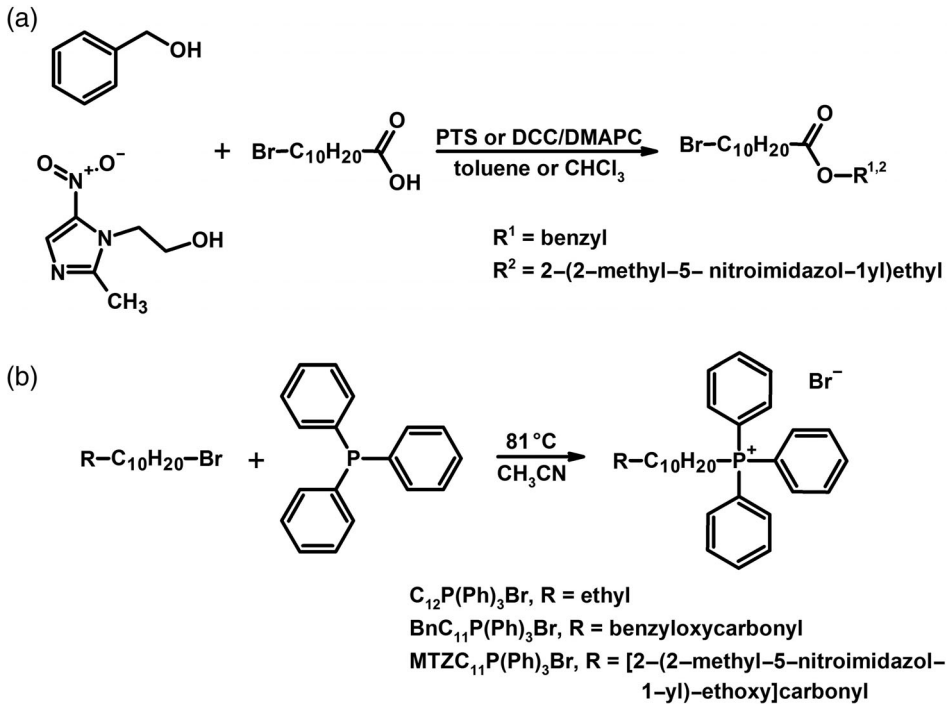
Phosphonium salts were prepared according the Scheme 1. One compound contains straight alkyl chain **$C_{12}P(Ph)_3Br$**. Two compounds are represented by phosphonium salts which alkyl chains contain ester groups. The connection of benzyl alcohol (**$BnC_{11}P(Ph)_3Br$**) and metronidazole (**$MTZC_{11}P(Ph)_3Br$**) on the alkyl chain was performed by this group. Ester is easily cleavable. The cleavable groups on the alkyl chain can be released after having been targeted to the place of action. Metronidazol represents antiinfective drugs and benzyl is a group without specific activities. **$BnC_{11}P(Ph)_3Br$** was prepared as a compound for the comparison with the metronidazole derivative. All phosphonium salts were prepared by quarternization of triphenylphosphine with 1-bromoalkanes. Substituted 1-bromoalkanes needed for preparation of compounds **$BnC_{11}P(Ph)_3Br$** and **$MTZC_{11}P(Ph)_3Br$** were prepared by the esterification of 11-bromoundecanoic acid with benzyl alcohol and metronidazole, respectively. The first esterification was performed as the Fischer esterification, the second one as the Steglich esterification. We also tried to prepare metronidazole derivatives using the Mitsunobu reaction, however, with no success.

Physicochemical properties of the compounds were investigated by the measurements of surface tension and conductivity of their aqueous solutions. The high critical micelle concentration of **$MTZC_{11}P(Ph)_3Br$** allowed to determine its physiochemical properties only by the surface tension method. (The conductivity measurement would have required high amount of phosphonium compound.). Figure 1 depicts the surface tension *vs* log surfactant concentration dependence. The results of the measurements are summarized in Table 1. The cmc values were obtained from the breakpoints of the curves. The values for each compound obtained from the surface tension and conductivity are in a good agreement (Fig. 2, Table 1). However, the cmc values obtained from the conductivity measurements are somewhat higher than the cmcs obtained from surface tension measurements.

The cmc of **$C_{12}P(Ph)_3Br$** corresponds well with the published data (Prasad et al., 2004). This compound has cmc smaller than **$BnC_{11}P(Ph)_3Br$** and **$MTZC_{11}P(Ph)_3Br$**. The insertion of ester moiety at the end of alkyl chains decreases hydrophobicity of the lipophylic part of molecules. A similar behavior was observed by De et al. (2010). The insertion of 1,4-dioxyphenylene unit into the hydrophobic part of QAC resulted in the cmc increase. The position of the units also had the influence on the lipophilicity of surfactants. Increasing the distances between the polar head group and 1,4-dioxyphenylene unit resulted in the cmc increase. The replacement of benzyl in **$BnC_{11}P(Ph)_3Br$** with metronidazole in **$MTZC_{11}P(Ph)_3Br$** caused an additional increase in cmc. He et al., 2019 observed a similar behavior when phenyl was replaced with a heterocycle (thiazole, pyrimidine) in the polar head group of the investigated surfactants. Heterocyclic surfactants had higher values of cmc than the hydrocarbon analogues.

Surface tension at cmc (γ_{cmc}) decreases with the addition of bulky moieties (benzyl, MTZ) at the end of alkyl chains. The density increase of surfactant-tail layer at the air/water interface resulted in the γ_{cmc} decrease (Czajka et al., 2015). We observed a similar influence on the surface activity in the case of alkylphosphocholines. Branching of alkyl chains or replacing hydrogens with fluorine atoms in alkyl chains decreased the value of γ_{cmc} (Lukáč et al., 2012, 2014).

The values of area per head group (A_{cmc}) show that **$C_{12}P(Ph)_3Br$** and **$MTZC_{11}P(Ph)_3Br$** molecules are packed more densely at the air/water interface than **$BnC_{11}P(Ph)_3Br$** molecules. The A_{cmc} value of **$MTZC_{11}P(Ph)_3Br$** is lower than that of **$BnC_{11}P(Ph)_3Br$.** This can be caused by stronger π-stacking of aromatic parts of molecules in the case of the metronidazole derivative. Imidazole ring is substituted with two types of groups. One is represented by



Scheme 1 Synthesis of amphiphilic phosphonium salts: (**a**) preparation of esters, (**b**) quarternisation of triphenylphospine with bromoalkanes

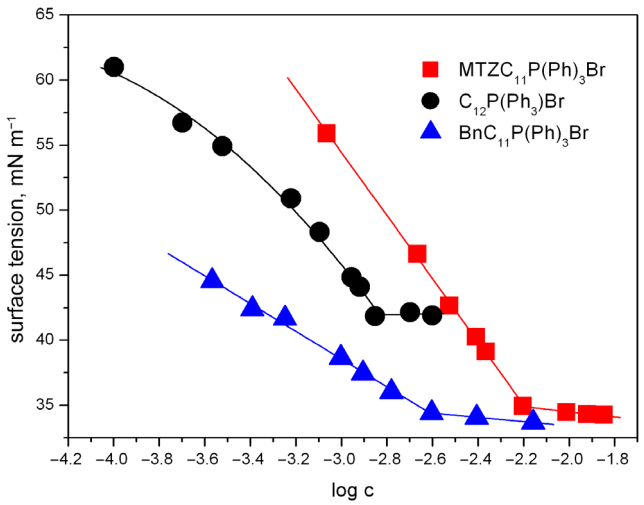
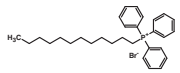
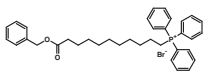
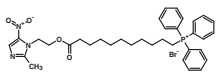


Fig. 1 Dependence of surface tension on log surfactant concentration for phosphonium salts

alkyls. They are bonded in positions 1 and 2 of the heterocycle. Especially, the methyl group represents a weak electron-donating group. The second type of the substituent is represented by the nitro group. It is a strong electron-withdrawing group. The presence of electron-donating and withdrawing substituents and electron-withdrawing nitrogen atoms in the ring causes the polarization of imidazole skeleton. Unequal distribution of electrons in the heterocycle causes stronger π-π interactions between the aromates. Denser packing of molecules of **$MTZC_{11}P(Ph)_3Br$** in comparison with **$BnC_{11}P(Ph)_3Br$** can be supported by the presence of hydrogen bonds utilizing nitrogen and oxygen atoms as acceptors. These two van der Waals interactions were considered as the factors, which have influenced the crystal packing of 1-alkyl-2-methyl-4-nitroimidazoles (Kubicki, 2005).

Biological activities of the compounds were tested against two strains of *Acanthamoeba*. The most active compound was **$C_{12}P(Ph)_3Br$,** a phosphonium salt with straight alkyl chain (Table 2). After 24 h of compounds activity, EC_{50} (effective concentration of tested compound that reduces the survival of amoebae by 50%) and MTC (minimal trophocidal concentration) values against both strains (*A. lugdunensis*, *A. quina*) were very low. These activities are comparable with those of previously tested CTAB, benzalkoniumbromide with hexadecyl alkyl chain, and cetylpyridinium bromide. Their values of MTC are the same (Lukáč et al., 2013). Other phosphonium salts with benzyl and metronidazol moiety indicate lower anti-*Acanthamoeba* activities. Figure 3 shows the dependence of anti-*Acanthamoeba* activity on critical micelle concentration. The increase in cmc resulted in the decrease of biological activities. **$BnC_{11}P(Ph)_3$** show higher activity than **$MTZC_{11}P(Ph)_3$** which contains the drug moiety. This is the proof of a nonspecific action of the prepared phosphonium salt. The activities of salts depend on physicochemical properties.

Table 1 Structure and physicochemical properties of phosphonium salts

No.	Compound	Structure	cmc (mol dm^{-3}) surface tension	cmc (mol dm^{-3}) conductivity	γ_{cmc} (mN m^{-1})	A_{cmc} (nm^2)
1	**$C_{12}P(Ph)_3Br$**		1.5×10^{-3}	1.8×10^{-3}	41.9	0.83
2	**$BnC_{11}P(Ph)_3Br$**		2.5×10^{-3}	3.3×10^{-3}	34.8	1.77
3	**$MTZC_{11}P(Ph)_3Br$**		6.0×10^{-3}	—	34.9	0.75

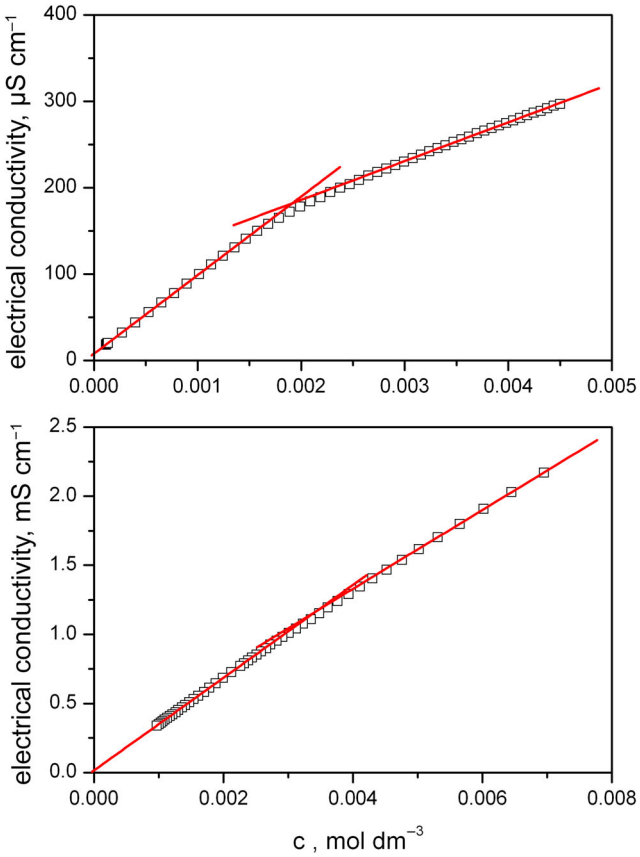


Fig. 2 Dependence of electrical conductivity on surfactant concentration for phosphonium salts

Interesting dependences of the aggregation properties of phosphonium amphiphilic compounds on biological activities of unicellular organisms were observed by Ryzhkina et al. (2019). They investigated the influence of tributylhexadecylphosphonium bromide and hexadecyltriphenylphosphonium bromide on the biological activity of infusoria (*Parametium caudatum*) and one single-celled algae (*Chlorella vulgaris*). Protozoa *Parametium caudatum* was sensitive to phosphonium salt in a similar way to our case of *Acanthamoeba*. The minimum protocidal concentration was 1×10^{-5} mol dm^{-3}. An interesting influence of phosphonium salts was observed in the case of algae. Micromolar concentrations have no effect on *Chlorella vulgaris*. However, the concentrations of salts in range 1×10^{-15} to 1×10^{-6} mol dm^{-3} have a harmful or even toxic effect. The authors explained it by the formation of the nanoassociates at low concentrations of phosphonium salts in aqueous solutions.

Metronidazol, a compound without amphiphilic character, showed no biological activity against *Acanthamoeba*. Both strains are insusceptible to the action of metronidazole. This observation corresponds with the investigations of Taravaud et al. (2017) who used the strain of *A. castellanii*. This species is included in the group II according to the classification of Pussard and Pons (1977) which is similar to our investigated strains of *A. lugdunensis* and *A. quina*. It is interesting to note that *A. polyphaga* also belongs in group II but it was noted susceptible to the action of metronidazole (Ondarza et al., 2006).

Table 2 Biological activities of phosphonium compounds and metronidazole

Compound	*A. lugdunensis*		*A. quina*		Haemolytic activity EC_{50} (μM)
	EC_{50} (μM)	MTC (μM)	EC_{50} (μM)	MTC (μM)	
$C_{12}P(Ph)_3Br$	5 ± 1.6	15.6	7.9 ± 1.4	15.6	51
$BnC_{11}P(Ph)_3$	44 ± 4	62.5	39 ± 5	62.5	72
$MTZC_{11}P(Ph)_3$	144 ± 12	250	72 ± 7	250	205
MTZ	> 500	> 500	> 500	> 500	> 1000

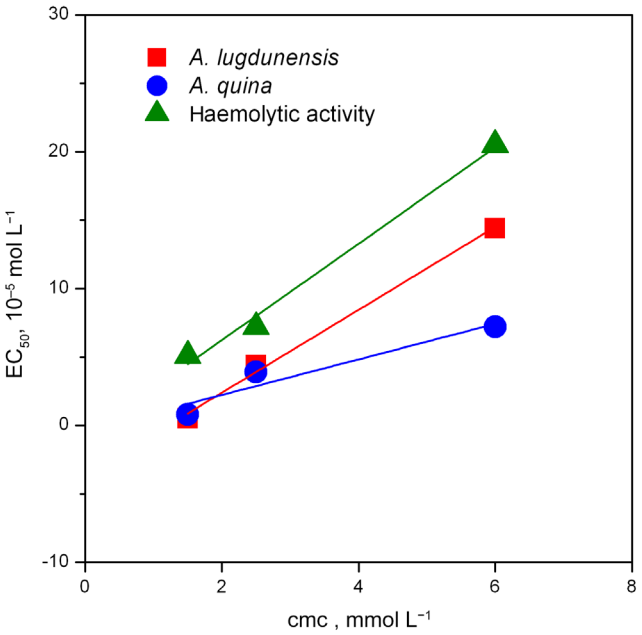


Fig. 3 Dependence of EC_{50} on cmc for phosphonium salts

A similar relationship between the activity and cmc of the compounds was observed in the case of hemolytic activities. The activity of the compounds increased with their lipophilicity which is obvious in the plot depicted in Fig. 3. It means that the amoebicidally more active compounds were also more toxic to erythrocytes. Only MTZ was hemolytically inactive up to concentration 1 mM. The ratio $EC_{50(amoebicidal)}/EC_{50(hemolytic)}$ for compounds **$MTZC_{11}P(Ph)_3$** and **$BnC_{11}P(Ph)_3$** have values 1.5–2.5. The best ratio was observed in the case of **$C_{12}P(Ph)_3Br$** reaching the value of 6.5 for *A. quina* and even 10 for *A. lugdunensis*.

Conclusion

The present study provides an information about synthesis, physicochemical properties, and biological activities of several phosphonium amphiphilic compounds with different lipophilic parts of molecules. Metronidazole derivative was synthetized with the aim of obtaining a potentially mitochondrially targeted compound. However, the activity of **$MTZC_{11}P(Ph)_3$** is nonspecific, depending only on the solubilization of membrane of trophozoites and erythrocytes. We observed that the activity of **$C_{12}P(Ph)_3Br$** was comparable with the activity of quaternary aminium salts used as disinfectants. To the best of our knowledge, **$C_{12}P(Ph)_3Br$** is the first compound from the group of phosphonium amphiphiles which exhibited high activity against *Acanthamoeba*. This is the indication of the possible application of phosphonium salts as promising candidates for the drugs against *Acanthamoeba* infections, the therapy of which remains still difficult.

Acknowledgements The authors thank to Andrej Slivka, MSc. for assistance in obtaining of human blood. This research was funded by the Grant Agency of Ministry of Education and Academy of Science of Slovak republic (VEGA), Project No. VEGA 1/0298/16 and VEGA 1/0054/19, by the Slovak Research and Development Agency Grant No. APVV-17-0373. This work utilizes the results of the research funded by the Slovak Research and Development Agency Grant No. APVV-0516-12 and by the CEBV project, ITMS: 26240120034. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0123. Further, it was supported with grants VEGA 1/0365/16, VEGA 1/0389/19 and KEGA 013TTU-4/2019.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Bittner Fialová, S., Kello, M., Čoma, M., Slobodníková, L., Drobná, E., Holková, I., … Lukáč, M. (2019) Derivatization of rosmarinic acid enhances its in vitro antitumor, antimicrobial and antiprotozoal properties. *Molecules*, **24**:24061078.

Block, S. S. (2000) *Disinfection, sterilization, and preservation* (5th ed., p. 1504). Philadelphia, PA: Lippincott Williams and Wilkins.

Czajka, A., Hazell, G., & Eastoe, J. (2015) Surfactants at the design limit. *Langmuir*, **31**:8205–8217.

De, S., Aswal, V. K., & Ramakrishnan, S. (2010) Phenyl-ring-bearing cationic surfactants: Effect of ring location on the micellar structure. *Langmuir*, **26**:17882–17889.

Devínsky, F., Pisárčik, M., & Lukáč, M. (2017) *Cationic amphiphiles. Self-assembling systems for biomedicine and biopharmacy* (p. 295). New York, NY: Nova Science Publishers.

Falk, N. A. (2019) Surfactants as antimicrobials: A brief overview of microbial interfacial chemistry and surfactant antimicrobial activity. *Journal of Surfactants and Detergents*, **22**:1119–1127.

Garajová, M., Mrva, M., Timko, L., Lukáč, M., & Ondriska, F. (2014) Cytomorphological changes and susceptibility of clinical isolates of *Acanthamoeba* spp. to heterocyclic alkylphosphocholines. *Experimental Parasitology*, **145**:102–110.

Gunawan, P. I., Idarto, A., & Saharso, D. (2016) *Acanthamoeba* infection in a drowning child. *Ethiopian Journal of Health Science*, **26**:289–292.

He, X., Wang, L., Wu, J., Yang, J., Ma, W., Bai, L., … Song, B. (2019) The effects of amide bonds and aromatic rings on the surface properties and antimicrobial activity of cationic surfactants. *Journal of Surfactants and Detergents*, **22**:315–325.

Kamkowska, A., Vacek, V., Zubáčová, Z., Treitli, S. C., Petrželková, R., Eme, L., … Hampl, V. (2016) A eukaryote without a mitochondrial organelle. *Curent Biology*, **26**:1274–1284.

Kot, K., Łanocha-Arendarczyk, N. A., & Kosik-Bogacka, D. I. (2018) Amoebas from the genera *Acanthamoeba* and their pathogenic properties. *Annals of Parasitology*, **64**:299–308.

Kubicki, M. (2005) Crystal packing modes of two 1-CH_2R-2-methyl-4-nitroimidazoles: Packing with Z' = 2. *Journal of Molecular Structure*, **743**:209–215.

Lorenzo-Morales, J., Khan, N. A., & Walochnik, J. (2015) An update on *Acanthamoeba* keratitis: Diagnosis, pathogenesis and treatment. *Parasite*, **22**:10.

Lukáč, M., Devínsky, F., Pisárčik, M., Papapetropoulou, A., Bukovský, M., & Horváth, B. (2017) Novel phospholium-type cationic surfactants: Synthesis, aggregation properties and antimicrobial activity. *Journal of Surfactants and Detergents*, **20**:159–171.

Lukáč, M., Garajová, M., Mrva, M., Bukovský, M., Ondriska, F., Máriássy, E., … Lacko, I. (2012) Relationship between aggregation properties and antimicrobial activities of alkylphosphocholines with branched alkyl chains. *International Journal of Pharmaceutics*, **423**:247–256.

Lukáč, M., Garajová, M., Mrva, M., Devínsky, F., Ondriska, F., & Kubincová, J. (2014) Novel fluorinated dialkylphosphonatocholines: Synthesis, physicochemical properties and antiprotozoal activities against *Acanthamoeba* spp. *Journal of Fluorine Chemistry*, **164**: 10–17.

Lukáč, M., Mrva, M., Garajová, M., Mojžišová, G., Varinská, L., Mojžiš, J., … Devínsky, F. (2013) Synthesis, self-aggregation and biological properties of alkylphosphocholine and alkylphosphohomocholine derivatives of cetyltrimethylammonium bromide, cetylpyridinium bromide, benzalkonium bromide (C16) and benzethonium chloride. *European Journal of Medicinal Chemistry*, **66**:46–55.

Ondarza, R. N., Iturbe, A., & Hernández, E. (2006) In vitro antiproliferative effect of neuroleptics, antimycotics and antibiotics on the human pathogens *Acanthamoeba polyphaga* and *Naegleria fowleri*. *Archives of Medical Ressearch*, **37**:723–729.

Prasad, M., Moulik, S. P., MacDonald, A., & Palepu, R. (2004) Self-aggregation of alkyl (C10-, C12-, C14-, and C16-) triphenyl phosphonium bromides and their 1:1 molar mixtures in aqueous medium: A thermodynamic study. *Journal of Physical Chemistry B*, **108**:355–362.

Pussard, M., & Pons, R. (1977) Morphologies de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida). *Protistologica*, **13**:557–610.

Rodríguez-Pérez, E. G., Escandón-Vargas, K., & Ancer, A. (2017) Granulomatous amebic encephalitis caused by *Acanthamoeba* sp. in an immunocompetent Mexican adult. *Revista da Sociedade Brasileira de Medicina Tropical*, **50**:432.

Ryzhkina, I. S., Murtazina, L. I., Shevelev, M. D., Akhmetzyanova, L. R., Galkina, I. V., Kuznetsova, T. V., … Konovalov, A. I. (2019) Aqueous systems based on organophosphorous compounds in low concentrations: Interconnection of self-organization and biological properties. *Phosphorus, Sulfur and Silicon and the Related Elements*, **194**:497–501.

Samuelson, J. (1999) Why metronidazole is active against both bacteria and parasites. *Antimicrobial Agents and Chemotherapy*, **43**: 1533–1541.

Sandoval-Acuña, C., Fuentes-Retamal, S., Guzmán-Rivera, D., Peredo-Silva, L., Madrid-Rojas, M., Rebolledo, S., … Ferreira, J. (2016) Destabilization of mitochondrial functions as a target against breast cancer progression: Role of TPP+-linked-polyhydroxybenzoates. *Toxicology and Applied Pharmacology*, **309**: 2–14.

Subramanian, S., Kalyanaraman, B., & Migrino, R. Q. (2010) Mitochondrially targeted antioxidants for the treatment of cardiovascular diseases. *Recent Patents on Cardiovascular Drug Discovery*, **5**:54–65.

Taravaud, A., Loiseau, P. M., & Pomel, S. (2017) *In vitro* evaluation of antimicrobial agents on *Acanthamoeba* sp. and evidence of a natural resilience to amphotericine B. *International Journal for Parasitology: Dugs and Drug Resistance*, **7**:328–336.

Tatarinov, D. A., Terekhova, N. V., Voloshina, A. D., Sapunova, A. S., Lyubina, A. P., & Mironov, V. F. (2018) Synthesis and antimicrobial activity of new Dialkyl(diaryl)-2-(5-chloro-2-hydroxyphenyl)-2-(phenylethenyl)pentylphosphonium salts. *Russian Journal of General Chemistry*, **88**:1800–1805.

Zielonka, J., Joseph, J., Sikora, A., Hardy, M., Ouari, O., Vasquez-Vivar, J., … Kalyanaraman, B. (2017) Mitochondria-targeted triphenylphosphonium-based compounds: Syntheses, mechanisms of action, and therapeutic and diagnostic applications. *Chemical Reviews*, **117**:10043–10120.