Synthesis of quaternary ammonium salts based on quinuclidin-3-ol and pyridine-4-aldoxime with alkyl chains

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Aim: In search of a new class of potential antimicrobial agents, novel ammonium salts based on quinuclidine-3-ol and pyridine-4-aldoxime with different lengths of alkyl chains were synthesized and analyzed. In addition to their potential biological application, newly synthesized salts containing terminal bromine could possibly be used for the synthesis of more potent bisquaternary salts.

Methods: The commercially available quinuclidine-3-ol and pyridine-4-aldoxime were used for the synthesis of new derivatives with the appropriate alkyl chains. The purity of the synthesized salts was tested by thin-layer chromatography on an aluminum plate where aluminum oxide was used as a stationary phase. Melting points were determined in open capillaries using an instrument for melting point determination and the obtained values were left uncorrected. The FTIR spectra were recorded with a spectrometer and the data were analyzed in cm⁻¹.

Results: All the synthesized compounds, which contained heterocyclic moiety and alkyl chains (with or without a terminal bromine atom), were obtained in very good yields under the simple quaternization conditions. The better product yields were observed in reactions with quinuclidine-3-ol (46-95%) compared to those with pyridine-4-aldoxime (49-59%). The obtained products were analyzed and confirmed by the thin-layer chromatography, the melting point measurement and the FTIR spectra.

Conclusion: The observed difference in product yields could be explained by the different basicity of the nitrogen atom of quinuclidine-3-ol than that of pyridine-4-aldoxime. All prepared salts have a positively charged nitrogen atom as a part of their polar “head” and a long hydrocarbon chain as the non-polar “tail”. Such a structure allows electrostatic interaction with the negatively charged bacterial membrane and causes it to be disrupted. The structure of the prepared salts containing long alkyl chains could be crucial for their antimicrobial activity.

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**Introduction**

Quaternary ammonium salts have a wide range of applications across a variety of industries; due to their pronounced antimicrobial activity, they can be used as ingredients in surfactants, such as cationic surfactants, or incorporated into drugs, such as antibiotics [1]. The structure of these heterocyclic compounds features a nitrogen atom, most commonly as part of a cyclic structure, and may occasionally contain other heteroatoms. These compounds also play a major biological role as participants in various metabolic processes and as building blocks of some of the most important molecules in nature, such as DNA and RNA [2]. Heterocyclic compounds are present in the structure of naturally occurring bioactive compounds, but they are also commonly found in synthesized compounds that can be used to research biological properties. Pyridine and quinuclidine are among the various heterocyclic compounds whose derivatives, for example pyridinium oximes or 3-substituted quinuclidine derivatives, have a widespread application in the pharmaceutical industry [3].

Commercially available quaternary ammonium salts, such as benzalkonium chloride (BAC), cetrimonium bromide (CTAB), cetylpyridinium chloride (CPC) and dodecyldimethylammonium chloride (DDAC), are weakly reactive, resulting in their accumulation in the environment. Consequently, due to heavy exposure to these compounds, bacteria may develop resistance to commercially available antimicrobial agents [4, 5]. With technological advancement, attempts have been made to produce more effective alternatives to said agents so as to overcome bacterial resistance. Previously synthesized quinuclidin-3-ol-based quaternary ammonium salts have exhibited good antimicrobial activity against Gram-positive and Gram-negative bacteria in combination with various aryl reagents [6]. The aim of this paper is to present a synthesis of new quaternary ammonium salts, which had not been described in the literature. For this purpose, we used quaternization reactions of quinuclidin-3-ol and pyridine-4-aldoxime with alkyl chains containing three to eight carbon atoms and one or two terminal bromine atoms.

**Materials and methods**

The following commercially available chemicals were used in the synthesis process: quinuclidin-3-ol (C\textsubscript{7}H\textsubscript{13}NO), pyridine-4-aldoxime (C\textsubscript{6}H\textsubscript{6}N\textsubscript{2}O), 1-bromopropane (C\textsubscript{3}H\textsubscript{7}Br), 1-bromobutane (C\textsubscript{4}H\textsubscript{9}Br), 1-bromohexane (C\textsubscript{6}H\textsubscript{13}Br), 1,3-dibromopropane (C\textsubscript{3}H\textsubscript{6}Br\textsubscript{2}), 1,4-dibromobutane (C\textsubscript{4}H\textsubscript{8}Br\textsubscript{2}), 1,6-dibromohexane (C\textsubscript{6}H\textsubscript{12}Br\textsubscript{2}), 1,8-dibromooctane (C\textsubscript{8}H\textsubscript{16}Br\textsubscript{2}), manufactured by Sigma Aldrich (Poole, UK). The following organic solvents were used in the synthesis: chloroform (CHCl\textsubscript{3}) (Lachner, Zagreb, Croatia), methanol (CH\textsubscript{3}OH) (Kemika, Zagreb, Croatia), diethyl ether (CH\textsubscript{3}CH\textsubscript{2}OCH\textsubscript{2}CH\textsubscript{3}) (VWR Chemicals), acetone (C\textsubscript{3}H\textsubscript{6}O) (Kemika) and ethanol 96% (C\textsubscript{2}H\textsubscript{5}OH) (Carlo Erba). For the quaternization reactions of the two precursors, quinuclidin-3-ol and pyridine-4-aldoxime, we used purified and anhydrous solvents, in this case “dry” diethyl ether and “dry” acetone; therefore, commercially available solvents had to be purified beforehand by distillation and drying with sodium (diethyl ether) or silicagel (aceton) [7].
Quaternization reactions of quinuclidin-3-ol and pyridine-4-aldoxime

The quaternization reactions of quinuclidin-3-ol and pyridine-4-aldoxime with appropriate alkyl reagents took place in anhydrous acetone. Quinuclidin-3-ol or pyridine-4-aldoxime, respectively, were dissolved in a minimum volume of acetone. An appropriate alkyl reagent was then added directly into the solution in small portions. Alkyl bromide was added in equimolar ratio (1:1) and alkyl dibromide in double excess with respect to the heterocyclic compound. The reaction mixture was left for two or three days in a dark place on a magnetic stirrer in an air-tight environment.

Purity testing of newly synthesized quaternary salts

After synthesis, the purity of the produced quaternary ammonium salts was tested by thin-layer chromatography, where aluminum oxide on an aluminum plate was used as a stationary phase (Merck, Darmstadt, Germany) and the mobile phase consisted of the solvent mixtures of chloroform and methanol in the 9:1 and/or 5:1 ratio. Detection was performed using a UV lamp for all synthesized compounds and pyridine-4-oxime. Treatment with iodine vapor was applied to quinuclidine-3-ol as the structure of this compound lacks conjugated double bonds. The melting point was determined for each newly synthesized solid using the Melting Point SMP1 (Stuart Scientific) device. IR spectra were recorded using the Shimadzu FTIR-8400S spectrophotometer, with wave numbers \( \nu \) expressed in cm\(^{-1}\).

Results

Synthesis of quinuclidin-3-ol-based quaternary salts

30.5 mg of quinuclidin-3-ol was weighed using an analytical balance (\( M = 127.18 \) g mol\(^{-1}\); \( n = 0.2398 \) mmol) in a penicillin vial and dissolved in 900 \( \mu \)L of acetone. An equimolar amount of an appropriate alkyl reagent (1-bromopropane, 1-bromobutane, 1-bromohexane, 1-bromoocotane) was added directly into the prepared solution, whereas 1,3-dibromopropane was added in double excess. The reaction mixture was left in the dark for 48–72 hours at
room temperature until the formation of white crystals, which were washed several times with ether (Figure 1). Thin-layer chromatography determined a $R_f$ value corresponding to the formation of a quaternary ammonium salt which, due to the high polarity, did not deviate from the starting value, unlike the reactants.

The quaternization reaction of quinuclidin-3-ol with an appropriate alkyl dibromide was performed using the same procedure, with the exception being that the amount of the appropriate alkyl reagent was doubled and pre-dissolved in a minimum volume of acetone (Figure 2). The product was a white solid.

Figure 2. Quaternization reaction of quinuclidin-3-ol with the appropriate alkyl dibromide.

Table 1 shows data on the newly synthesized quinuclidin-3-ol-based quaternary ammonium salts.

| Compound name                              | Mass, mg | Melting point, °C | Yield, % | FT-IR (KBr) $\text{v}/\text{cm}^{-1}$ |
|--------------------------------------------|----------|-------------------|----------|--------------------------------------|
| $N$-propyl-3-hydroxyquinuclidinium bromide | 43       | 151-153           | 72       | 3600-3060 (3315 max), 2995, 2946, 2922, 2853, 1630, 1461 |
| $N$-butyl-3-hydroxyquinuclidinium bromide  | 59       | 165-169           | 88       | 3600-3033 (3305 max), 2993, 2944, 2925, 2855, 1635, 1463 |
| $N$-hexyl-3-hydroxyquinuclidinium bromide  | 60       | 130-131           | 99       | 3600-3055 (3296 max), 2992, 2943, 2923, 2857, 1639, 1462 |
| $N$-octyl-3-hydroxyquinuclidinium bromide  | 70       | 88-90             | 46       | 3600-3040 (3300 max), 2991, 2945, 2930, 2855, 1640, 1464 |
| $N$-(3-bromo)propyl-3-hydroxyquinuclidinium bromide | 95     | 99-101            | 95       | 3600-3060 (3327 max), 2995, 2941, 2925, 2853, 1630, 1461, 621 |

Synthesis of pyridine-4-aldoxime-based quaternary salts

Fifty mg of pyridine-4-aldoxime was weighed using an analytical balance ($M = 122.12$ gmol$^{-1}$; $n = 0.4094$ mmol) in a penicillin vial and dissolved in 400 μL of acetone. Double that amount of an appropriate alkyl reagent was dissolved in a minimum volume of acetone. The penicillin vial containing the dissolved pyridine-4-aldoxime was placed on a magnetic
stirrer and the alkyl reagent solution was added to it dropwise in portions over the span of 15 minutes. The reaction mixture was left in the dark on a magnetic stirrer for 48–72 hours at room temperature until the formation of white crystals, which were washed with ether (Figure 3). Using thin layer chromatography, $R_f$ value corresponding to the formation of a quaternary ammonium salt was determined in this case as well, which, due to the high polarity, did not deviate from the starting value, unlike the reactants.

![Figure 3. Quaternary reaction of pyridine-4-aldoxime with the corresponding alkyl chains.](image)

Table 2 shows data on the newly synthesized pyridine-4-aldoxime-based quaternary ammonium salts.

| Compound name | Mass, mg | Melting point, °C | Yield, % | FT-IR (KBr) $\nu$/cm$^{-1}$ |
|---------------|----------|-------------------|---------|---------------------------|
| $N$-(3-bromo)propyl-4-hydroxyiminomethylpyridinium bromide | 65       | 131-133           | 49      | 3437-2607, 1604, 1410, 991, 538 |
| $N$-(4-bromo)butyl-4-hydroxyiminomethylpyridinium bromide | 74       | 150-152           | 53      | 3416-2719, 1639, 1436, 1000, 547 |
| $N$-(6-bromo)hexyl-4-hydroxyiminomethylpyridinium bromide | 85       | 153-155           | 57      | 3417-2719, 1641, 1437, 1003, 543 |
| $N$-(8-bromo)octyl-4-hydroxyiminomethylpyridinium bromide | 96       | 100-102           | 59      | 3410-2713, 1602, 1410, 999, 538 |

**Discussion**

The purpose of synthesizing quaternary ammonium salts as described in this paper was to test their biological properties – primarily, their antimicrobial activity – for the purpose of discovering new potential precursors in drug synthesis in response to the global issue of bacterial resistance. Recently, Odžak et al. demonstrated that the quaternization
of quinuclidin-3-ol that has a benzyl group with different substituents in the \textit{para}-position produced quaternary compounds with beneficial antimicrobial and antioxidant properties \cite{6}.

Quaternary ammonium salts (QAS) were synthesized from two different heterocyclic compounds with different alkyl chains. Both used heterocyclic compounds are commercially available, as well as alkyl bromide or alkyl dibromide as reagents for quaternization. The precursors in the synthesis of new quaternary ammonium salts, quinuclidin-3-ol and pyridine-4-aldoxime, share an important property. Both heterocyclic compounds contain a nitrogen atom with an unshared pair of electrons that carries its basic properties. Therefore, in quaternization reactions, the nitrogen atom acts as an electron donor and easily reacts with alkyl bromides. Still, the quaternization reaction of aliphatic amine is different than the quaternization of aromatic amine. The nitrogen atom in an aliphatic amine such as quinuclidin-3-ol has an unshared pair of electrons in $sp^3$-hybridization and exhibits higher basicity and nucleophilicity than pyridine-4-aldoxime, where the nitrogen atom has $sp^2$-hybridization with delocalization of electrons in aromatic ring. Therefore, quaternization reactions with quinuclidin-3-ol were expected to exhibit greater recovery. This was confirmed by the quaternization reaction of quinuclidin-3-ol and pyridine-4-aldoxime with alkyl dibromide. Quaternization of pyridine4-aldoxime with propyl dibromide had lower yields than the quaternization of quinuclidine 3-ol with the same reagents (\textbf{Figure 4}).

Alkyl chains used as reagents for quaternization had a different number of carbon atoms (C3, C4, C6 and C8) in the form of alkyl bromides or alkyl dibromides. Quaternization of heterocyclic compounds with appropriate alkyl chains was achieved in acetone, an aprotic solvent in which both components, the polar bromide and the polar alcohol and/or oxime, could be dissolved. For the synthesis QAS without terminal bromine atom we used equimolar quantities of heterocyclic compound and appropriate alkyl chain, whereas for the synthesis QAS with terminal bromine atom we used double excess of the chain.

\textbf{Figure 4.} Mechanism and yield of the quaternization reaction of quinuclidin-3-ol and pyridine-4-aldoxime with alkyl dibromide.
The purity of synthesized compounds was determined by TLC chromatography and all compounds were obtained in very good yields. The quaternary bromides were identified by melting point and IR spectroscopy which shows characteristic vibration of functional groups heterocyclic compounds but also vibration characteristic for C-Br bond. The weakness of our study is the lack of a more detailed structure analysis of newly synthetized compounds using NMR or MS spectroscopies. FTIR spectroscopy and determination of melting point do not provide proof of the chemical structure and prior of biological testing the compounds will be sent for further structure analysis.

All the synthesized salts described in this paper featured a positively charged nitrogen atom as part of their polar “head” and a long hydrocarbon chain as the non-polar “tail”. Due to their surface-tension decreasing property and electrostatic attraction to a negatively charged bacterial cell wall, these compounds penetrate the bacterial membrane bilayer. The consequence of this interaction between the quaternary salt and the bacterial membrane is the disruption of the membrane and leakage of the cytoplasmic content, or the “lysis” of the bacterial cell (Figure 5). Therefore, these compounds are frequently used as ingredients of surfactants with membrane activity [8].

Moreover, we have shown that quaternization with long alkyl chains results in compounds with good antimicrobial activity, more specifically against ESKAPE human pathogens [6] for which reason, newly synthesized compounds could have industrial and medical application.

![Figure 5. Schematic representation of the mechanism of quaternary ammonium salts acting on the bacterial membrane. The diagram shows the interaction between the positively charged nitrogen atom in the structure of quaternary ammonium salts and the negatively charged bacterial wall.](image)
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