

SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF SOME PYRIDINIUM SALTS

BAZI PİRİDİNYUM TUZLARININ SENTEZ VE ANTİMİKROBİYAL ETKİLERİ

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ABSTRACT

*Some pyridinium oxime ether derivatives comprising naphthyl, phthalimido, 2,6-dichlorophenyl and cyclohexyl rings linked to the ether function by a methylene bridge were synthesized and screened for possible antibacterial and antifungal activities using the microdilution method. Propyl and 3-phenylpropyl chains were chosen as side chains attached to the pyridinium nitrogen. Among these derivatives, NF-MFE containing naphthyl ring and 3-phenylpropyl chain exhibited highest antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans* in 312.5, 39.1, 9.8, 9.8 and 19.5 µg/ml concentrations respectively. The antimicrobial test results indicated that all the compounds have mild antibacterial activity against both Gram negative and Gram positive bacterial species. It can be hypothesized that bioactivity may be affected by electron density of pyridinium nitrogen atom and size of planar ring structure.*

Key words: *Antimicrobial activities / Pyridinium salts / oxime ethers*

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ÖZET

*Eter grubuna metilen köprüsü ile bağlı naftil, ftalimido, 2,6- diklorofenil ve sikloheksil halkalarını içeren bazı piridinyum oksim eter türevleri sentez edildi ve mikrodilüsyon yöntemini kullanarak olası antibakteriyal ve antifungal aktiviteleri tarandı. Piridinyum azotuna bağlı yan zincirler olarak propil ve 3-fenilpropil zincirleri seçildi. Bu türevler arasında naftil halkası ve 3-fenilpropil zinciri taşıyan bileşik NF-MFE *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*'a karşı sırasıyla 312.5, 39.1, 9.8, 9.8 and 19.5 µg/ml konsantrasyonlarda en yüksek antimikrobiyal aktiviteyi sergiledi. Antimikrobiyal test sonuçları tüm bileşiklerin Gram pozitif ve Gram negatif bakterilere karşı orta derecede antibakteriyal aktiviteye sahip olduklarını gösterdi. Biyoaktivitenin piridinyum azotunun elektron yoğunluğundan ve düzlemsel halka boyutundan etkilendiği hipotezi ileri sürülebilir.*

Anahtar kelimeler: Antimikrobiyal aktivite / Piridinyum tuzları / oksim eterler

INTRODUCTION

The shortage of new antibacterial drugs and increasing resistance of bacteria to antimicrobial agents has been of some concern. Previously, antimicrobial properties were reported for quaternized amine derivatives (1-4). Several quaternary ammonium compounds including pyridinium salts with various alkyl chain lengths exert antibacterial activity against both Gram positive and Gram negative bacteria, as well as against some pathogenic species of fungi and protozoa (1). A series of mono- and bis-quaternary derivatives of dipyriddylenes and dipyriddylenes exhibit structure-dependent antimicrobial action on both Gram-negative (*E. coli*) and Gram-positive (*St. aureus*) bacterial species. Monoquaternized dipyriddylene and dipyriddylene derivatives show a higher antimicrobial activity than compounds with two onium nitrogen atoms (2). A class of N-alkylnicotinamides were synthesized (3) and correlated with their reduction potentials and reactivities (4). Also, it was reported that the quaternary ammonium compounds act on the cell wall and have a direct or indirect lethal effect on the cell (5). In this study, a series of bis(1-alkylpyridinium bromide)s were evaluated by their activities against the test microbes and the majority of the quaternary ammonium compounds displayed high activity against a broad spectrum of Gram-negative and Gram-positive bacteria, yeasts and some molds.

Besides, it is known that pyridinium halides as quaternary ammonium compounds have antimicrobial properties and adsorption on negatively charged solids. Polar heads are a cationic pyridinium, argued particularly concerning their antimicrobial activities and characteristics (6-11). The antimicrobial activity of 1-alkylpyridinium salts depends on the adsorptive activities on the

surface of bacterial cells and as a consequence their destruction (12) and pKa values of the corresponding pyridines (13). In addition, it was proved that the factors which control their antimicrobial activity, are molecular hydrophobicity (14, 15), adsorbability (16), surface activity (8), electron density (17,18) of the ammonium nitrogen atom.

In addition it was postulated that certain compounds containing oxime and oxime ether groups such as 1-(2-naphtyl)-2-(1,2,4-triazol-1-il)etanon oxime ether derivatives might also have antifungal–antibacterial properties (19).

On the other hand, the quaternary salts having large planar rings such as phenanthridinium and acridinium ring systems bind nucleic acids via intercalative mode and cause major changes to DNA and RNA structures (20).

Insertion of the compounds between adjacent base pairs causes stretch of double helical structure. The binding of these compounds to double-stranded DNA inhibits a wide range of biologically important processes, such as DNA synthesis and gene transcription and translation (21). It has been reported that they show significant antitumor (22) and anti-viral properties (23)

The base pairs above and below the drug 'buckle' in conformation to afford a distorted DNA helix thereby preventing association with the DNA helicase, DNA topoisomerase (24, 25) and polymerase families of enzymes to initiate DNA replication for RNA synthesis, protein formation and thereby cell division.

Another promising effect of positively charged pyridinium ring resulted in an obvious loss in inhibitory activity toward AChE. It is known that one of the most prominent features of Alzheimer's disease (AD) is a significant deficit in cholinergic transmission in certain brain areas (26-30). Concentrations of acetylcholine (ACh) decrease can be observed by nearly 90% in patients with AD. The current focus of AD treatment is the use of agents that increase the availability of intrinsic ACh by inhibiting the enzyme acetylcholinesterase (AChE).

In our study, 1-alkyl-3-oxime ether pyridinium salts with two different alkyl groups which are propyl and 3-phenylpropyl chains (**Figure 1**) were synthesised in order to investigate the effects of such structural modifications of quaternary ammonium salts on the anticipated antimicrobial activity. These compounds possess one hydrophobic alkyl chain and one hydrophilic quaternary nitrogen ion group in the same molecule which provide greater surface activity and more excellent antimicrobial potency than conventional antimicrobials (31). Naphtyl, phthalimido, 2,6-dichlorophenyl and cyclohexyl rings with different electronic properties introduced to molecules by methylen bridge. Additionally, another purpose of the present investigation is to observe

relationship between antimicrobial activity and ring size of the compounds. Because the binding of these large planar ring systems to double-stranded DNA inhibits a wide range of biologically important processes, such as DNA synthesis and gene transcription and translation (32, 33).

EXPERIMENTAL

Melting points were determined with a Büchi 510 melting point apparatus (Büchi, Switzerland) and are not corrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 instrument (^1H 400.132 MHz; ^{13}C 100.613 MHz). Abbreviations for data quoted are: s, singlet; d, doublet; t, triplet; quin, quintet; dd, doublet of doublets; m, multiplet; br s, broad signal. The centers of the peaks of DMSO- d_6 were used as internal references. IR spectra of compounds were recorded as potassium bromide disk on a Jasco FT/IR-400 spectrometer. Dry solvents were used throughout the synthesis. The electrospray ionization (ESI) mass spectra were measured on an Agilent 1100 LC/MSD Trap. The conditions of the spray chamber were as follows: ion polarity, positive; drying gas temperature, 300 °C; nebulizer pressure, 10 psi; drying gas flow, 5.00 L min $^{-1}$. Reagents used for synthesis were purchased from Aldrich, Fluka, and Merck companies. Organic solvents were purchased from Merck Company. Thin-layer chromatographies were done on pre-coated silica gel 60 F $_{254}$ plates (Merck). The spots were visualized with UV light or iodine. Column chromatography was performed on silica gel 60 30–70 mesh (Merck).

Naphthylmethyl, dichlorobenzyl, cyclohexyl and phthalimidomethyl oxime ethers (NF, D, CYC and FE) were synthesized according to Botero Cid et al.(34) and Bejeuhr et al (35). NF, D and FE were reported before as acetylcholinesterase inhibitors in the literature (36), CYC is being reported for the first time by this study.

General Procedure for Synthesis of the Final Compounds

Corresponding dichlorobenzyl, naphthylmethyl or phthalimidomethyl oxime ethers and bromopropyl derivatives were heated in acetonitrile (60 ml) at reflux for 80 h. In case of incomplete conversion, remaining (NF, D, CYC and FE) could be removed by addition of water, extraction with diethyl ether, and evaporation of water. Otherwise, after the mixture was cooled to room temperature, the solvent was removed in vacuo and the obtained oil or solid was crystallized using the solvent mixtures of methanol/diethyl ether.

1-(3-Phenylpropyl)-3-[[[(naphthyl-1-yl)methoxy]imino)methyl]pyridinium bromide (NF-MFE) Yield 47% mp 179°C (36).

1-(Propyl)-3-[(1-naphthyl-1-yl)methoxy]imino)methyl]pyridinium bromide (NF-MPRO)

Yield 58% mp 192°C (36).

1-(3-Phenylpropyl)-3-[(1-(2,6-dichlorophenyl)methoxy]imino)methyl]pyridinium bromide (D-MFE)

Yield 60% mp 120°C (36).

1-(Propyl)-3-[(1-(2,6-dichlorophenyl)methoxy]imino)methyl]pyridinium bromide (D-MPRO)

Yield 42% mp 219°C (36).

1-(3-Phenylpropyl)-3-[(1-phthalimidomethoxy]imino)methyl]pyridinium bromide (FT-MFE)

Yield 41% mp 193–195 °C (36).

1-(Propyl)-3-[(1-phthalimidomethoxy]imino)methyl]pyridinium bromide (FT-MPRO)

Yield 58% mp 225–228°C (36).

1-(3-Phenylpropyl)-3-[(1-cyclohexylmethoxy]imino)methyl]pyridinium bromide (CYC-MFE)

Yield 38% mp 153–157°C. IR (KBr) ν (cm⁻¹) 1639, 1606, 1502, 1041, 748, 673. ¹H NMR (DMSO-*d*₆): δ ppm 2.28 (2H, quin, $J = 7.2$ Hz, N⁺-CH₂-CH₂-CH₂-Ar), 2.66 (2H, t, $J = 7.2$ Hz, Ar-CH₂), 4.03 (2H, d, $J = 7.2$ OCH₂), 4.67 (2H, t, $J = 6.4$ Hz, N⁺-CH₂), 7.19–7.28 (5H, m, Ar-H), 8.13 (1H, dd, $J = 6.4/8.4$ Hz, H-5), 8.42 (1H, s, N=CH), 8.68 (1H, d, $J = 8.0$ Hz, H-4), 9.08 (1H, d, $J = 6.4$ Hz, H-6) 9.26 (1H, br s, H-2). ¹³C NMR (DMSO-*d*₆): δ ppm 25.87, 26.89, 29.72, 32.28, 32.52, 37.67, 61.70, 80.67, 126.82, 128.87, 128.91, 129.10, 133.14, 140.97, 142.50, 143.64, 144.26, 145.52.

Determination of Antimicrobial Activities

The minimal inhibitory concentration (MIC) were determined by broth microdilution methods according to NCCLS standards (37). The in vitro antimicrobial activity of the compounds was evaluated against standart strains; *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 90028. The antibacterial and antifungal assay were performed in Mueller–Hinton broth and Sabouraud dextrose broth respectively. All the synthesized compounds were weighed (20 mg) and dissolved in DMSO (250 μ l) and diluted with water (750 μ l) to prepare the stock solutions of 20 mg /ml. The serial dilution from 5000 to 10 μ g /ml were made in a 96-wells plate. To each well 50 μ l of a bacterial suspension, obtained from a 24 h culture, containing $\sim 10^6$ cfu/mL was added. The plate was incubated at 35° C for 24 h. For quality control of the method streptomycin was tested as antimicrobial agent. These experiments were carried out in duplicate.

RESULTS AND DISCUSSION

Cyclohexyl oxime ether was synthesized as described in the experimental part. The synthesized compound was determined on the basis of IR, ¹H NMR, ¹³C NMR and its purity was confirmed by thin layer chromatography (TLC) and melting point. These results showed that the compound synthesized have the purposed structure.

The previously synthesized compounds of naphthyl (NF), dichlorobenzyl (D), phthalimidomethyl (FT) and newly synthesized cyclohexyl (CYC) oxime ether derivatives with the pyridinium nitrogen in position 3 was evaluated for antimicrobial activity toward the Gram positive and Gram negative bacteria and a type of fungus (**Figure 1**). Both consisted of pyridinium-propyl and pyridinium-3-phenylpropyl moieties at the opposite end of the molecule. The bacterial strains represent important Gram positive and Gram negative species which are *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Antibacterial activity of the compounds was assessed by measuring minimum inhibitory concentration (MIC) using standard broth dilution assay (**Table 1**).

Table 1. The antimicrobial activity of the synthesized compounds (MIC in µg/ml)

| | FT-MPRO | NF-MPRO | D-MPRO | FT-MFE | CYC-MFE | NF-MFE | D-MFE | Streptomycin |
|----------------------|---------|---------|--------|--------|---------|--------|-------|--------------|
| <i>E.coli</i> | 5000 | 312.5 | 312.5 | 2500 | 78.1 | 39.1 | 78.1 | ≤ 2 |
| <i>P. aeruginosa</i> | 5000 | 5000 | 5000 | 5000 | 625 | 312.5 | 625 | ≤ 2 |
| <i>S. aureus</i> | 5000 | 39.1 | 78.1 | 1250 | 9.8 | 9.8 | 9.8 | ≤ 2 |
| <i>E. faecalis</i> | 5000 | 312.5 | 312.5 | 2500 | 39.1 | 9.8 | 19.5 | ≤ 2 |
| <i>C. albicans</i> | 2500 | 156.3 | 312.5 | 2500 | 39.1 | 19.5 | 39.1 | - |

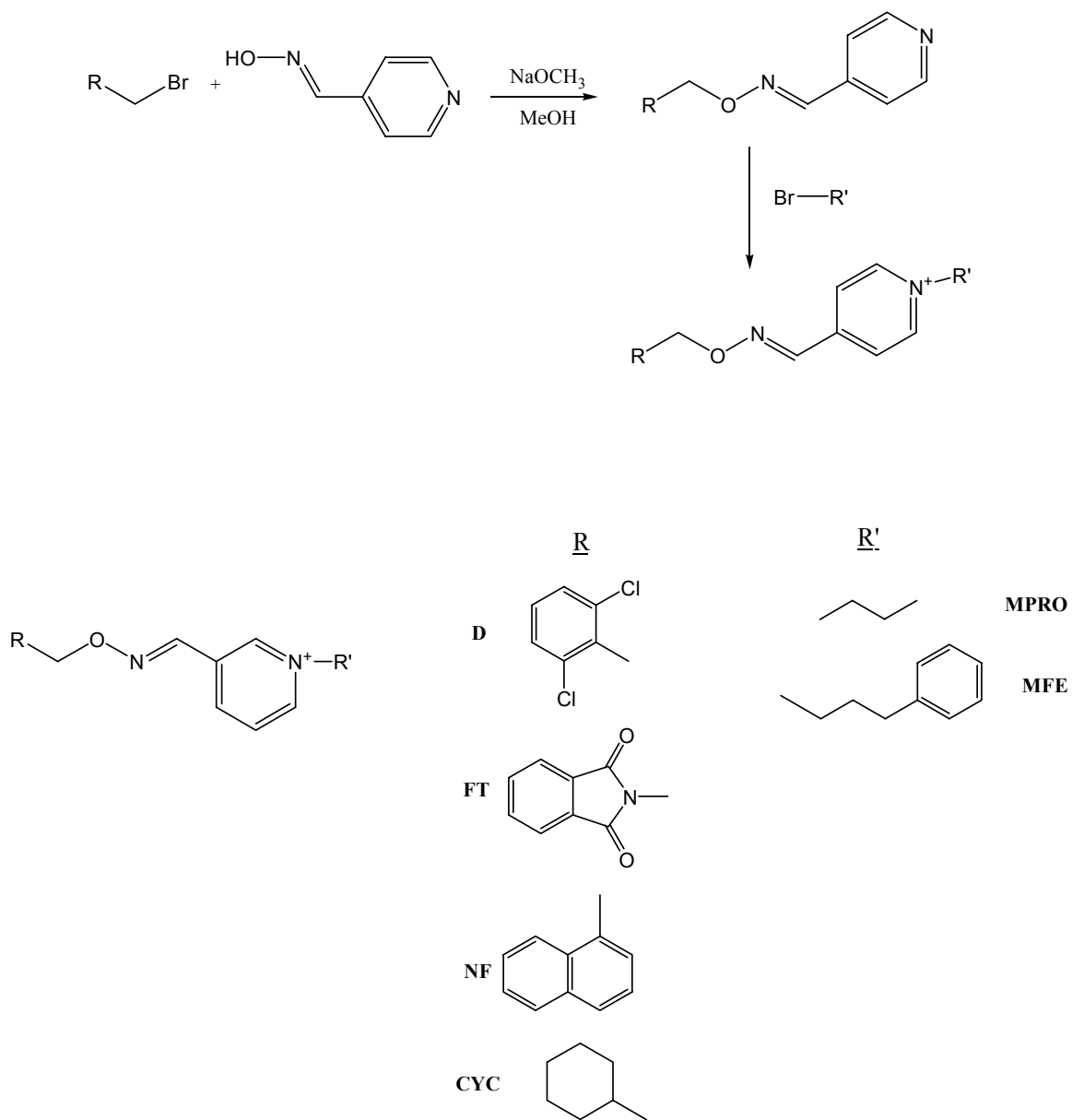


Figure 1. Synthesis pathway and structures of pyridinium-type compounds presented in this study

Comparison of antibacterial activities of the compounds showed that phenylpropyl substituted series are more active than those of propyl substituted derivatives. In the phenylpropyl substituted series, the antimicrobial activity of the naphthyl derivative NF-MFE is followed by the dichlorobenzyl derivative D-MFE, cyclohexyl derivative CYC-MFE and phthalimido derivative

FT-MFE respectively. In fact no meaningful difference was observed in antibacterial activities of D-MFE and CYC-MFE. Both of the compounds have same MIC values against test microorganisms except against *Enterococcus faecalis*. D-MFE exhibited antibacterial activity in 19.5 µg/ml concentration, whereas the antibacterial activity of CYC-MFE was observed in 39.1 µg/ml MIC value against *Enterococcus faecalis*. In another word, antimicrobial activities of both compounds were not affected significantly by the change of phenyl and its saturated form cyclohexyl rings which are connected to oxime ether *via* methylen bridge. Among the propyl substituted compounds, dichlorobenzyl derivative D-MPRO is more active than that of phthalimidomethyl derivative FT-MPRO. Indeed, FT-MPRO had highest MIC values compared with the other pyridinium salts in this work. It was proved that all the compounds have stronger antibacterial activity against Gram positive bacteria when compared with Gram negative bacteria. The increasing order of antimicrobial activity for NF-MFE according to test microorganisms is in sequence *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albican*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Antimicrobial activities of the compounds could be affected by the electron density of the pyridinium nitrogen in position 3. When compared NMR spectra of propyl and phenylpropyl derivatives, chemical shifts of methylene protons adjacent to pyridinium nitrogen linked to phenylpropyl chain changed to higher magnetic field as a result of electronic shielding. For example, the chemical shifts of methylen protons in phenylpropyl and propyl substituents of NF-MFE, NF-MPRO and D-MFE, D-MPRO derivatives are as follow. δ 5.60 ppm (NF-MPRO), δ 4.68 ppm (NF-MFE) and δ 5.14 ppm (D-MPRO), δ 5.04 ppm (D-MFE). Therefore, we assumed that there might be a relationship between the electron density of pyridinium nitrogen and antimicrobial activity. This result was confirmed by previous studies (17, 18) described as “the higher electron density is, the higher the anti bacterial activity of quaterner ammonium compounds.” Also, it can be hypothesized that high activity of naphthyl derivatives (NF-MFE and NF-MPRO) may be due to their large planar naphthyl ring system, since intercalation between naphthyl ring and DNA causes inhibition of the replication process as explained in Introduction.

In conclusion, naphthyl substituted compound (NF-MFE) is the most active compound found in these series (**Table 1**). It was shown that the 3-phenylpropyl substitution at the pyridinium nitrogen mediates the most active compounds of each of the series (NF-MFE, D-MFE and FT-MFE) in this study. We also discovered that all the compounds investigated in our work demonstrated mild anti-microbial activity against Gram positive and Gram negative bacteria and also a type of fungus.

ACKNOWLEDGMENTS

One of the authors (Vildan Alptuzun) thanks Prof. Dr. Ulrike Holzgrabe for some of the spectral analysis and giving opportunity to work in her laboratory, University of Wurzburg, Germany.

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Received: 19.02.2007

Accepted: 28.03.2007