Synthesis and antimicrobial activity of new crown ethers of Schiff base type

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Abstract: New crown ether ligands of the Schiff base type (4a–d) were synthesized by the reaction of 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde with 6,7-dihydro-13H-dibenzo[e,h] [1,4]dioxin-2,11-diamine (3). The structures of ligands were investigated by elemental analysis as well as IR, UV–visible, 1H-NMR, 13C-NMR and MS spectroscopic data. The antimicrobial and anti-yeast activities of the ligands were screened in vitro against the organisms Escherichia coli ATCC 11230, Staphylococcus aureus ATCC 6538, Klebsiella pneumoniae UC57, Micrococcus luteus La 2971, Proteus vulgaris ATCC 8427, Pseudomonas aeruginosa ATCC 27853, Mycobacterium smegmatis CCM 2067, Bacillus cereus ATCC 7064, Listeria monocytogenes ATCC 15313, Candida albicans ATCC 10231, Kluyveromyces fragilis NRRL 2415, Rhodotorula rubra DSM 70403, Debaryomyces hansenii DSM 70238 and Hanseniaspora guilliermondii DSM 3432.

Keywords: crown ether, Schiff base, antimicrobial activity, spectroscopy.

INTRODUCTION

The study of macrocyclic ethers has attracted great interest in the last decades, not only from the synthetic and selective alkali and alkaline earth cation complexation properties point of view, but also with respect to their unusual characteristics.1,2 For the linkage of two crown ether units by means of aliphatic or aromatic chains, ester,3–5 amide,6,7 calixarene8–17 and Schiff base type18–20 precursors are commonly used. Macrocyclic polyethers form complexes with alkali metals, alkaline earth metals, and with some other metal ions, with oxonium ions, with protonated amines, including amino acids, and also with some neutral molecules such as acetonitrile. In addition to homonuclear mono- and ditopic complexes of bis(crown ethers) with alkali and alkaline earth metal ions, heteroatoms of the linkage unit be-

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tween the two crown ether rings can form complexes with transition metal cations\textsuperscript{21} to yield interesting heteronuclear oligotopic complexes of bis(crown ethers) in solution and in the solid state.\textsuperscript{1} Complexes of this type may be used as simple models for biological systems, such as metalloenzymes.\textsuperscript{22}

In this study, new macrocyclic polyether ligands of the Schiff base type were synthesized by the reaction of diamino crown ether 3 with 2-, 3- and 4-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde (Scheme 1).\textsuperscript{23} The structures of the synthesized Schiff bases were established by elemental analysis as well as IR, UV–visible, \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR and MS spectroscopy. The ligands were screened \textit{in vitro} against microorganisms by the disc diffusion method.\textsuperscript{24,25}

\begin{center}
\textbf{RESULTS AND DISCUSSION}
\end{center}

In the present study, dinitro-crown ether 2 was synthesized by the reaction of 2,2'-methylenebis[4-nitrophenol] (I) with 1,2-dibromoethane. Diamino crown ether 3 was synthesized by the reduction of the corresponding intermediate product, 2,11-dinitro-6,7-dihydro-13\textit{H}-dibenzo[e,h]1,4-dioxonin (2), with Pd–C (10 \%) / hydrazine hydrate in ethanol. The new crown ether ligands of the Schiff base type (4a–d) were synthesized by the reaction of diamino crown ether 3 with two equivalents of aromatic aldehyde. The structures of the synthesized Schiff bases were established by elemental analysis as well as IR, MS, UV–visible, \textsuperscript{1}H-NMR and


The ligands were screened in vitro against microorganisms by the disc diffusion method. The infrared spectra of compounds 4a, 4b, 4c and 4d show bands at 3448, 3393, 3364 and 3402 cm⁻¹, respectively, for v (O–H), 1619, 1624, 1605 and 1623 cm⁻¹, respectively, for v (C=O), indicating the formation of Schiff bases (4a–d). The aryl and alkyl C–H stretching frequencies of compounds 4a–d were observed at 3066, 3060, 3070 and 3050 cm⁻¹ and 2933–2878, 2937–2873, 2928–2870 and 2927 cm⁻¹ and v(C–O–C) bands are observed at 1249–1188–1151, 1243–1172–1068, 1239–1162–1167 and 1248–1176–1087 cm⁻¹, respectively. The v(C–O) vibrations appear at 1364, 1376, 1379 and 1320 cm⁻¹, respectively, in these ligands (4a, 4b, 4c and 4d). The compounds 4a, 4b and 4c with a strong band at 1282, 1287 and 1284 cm⁻¹, respectively, possess the highest percentage of enol-imino tautomer, due to the stabilization of the phenolic C–O bond.26

The UV–visible spectra of compounds 4a–d were studied in polar (DMSO, ethanol and chloroform) and non-polar (benzene) solvents. The Schiff bases show absorption in the range above 400 nm in polar and non-polar solvents. It should be pointed out that the new band belongs to the keto-amine form of the Schiff bases with the OH group in the ortho position to the imino group in polar and nonpolar solvents in both acidic and basic media.27–32 The compounds 4a–c do not show any absorption in the range >400 nm in DMSO, ethanol, chloroform and benzene. For compound 4d, a new band is observed at >400 nm in the same solvents. Only the phenol-imine tautomer is dominant in DMSO, ethanol, chloroform and benzene for 4a–c. On the other hand, compound 4d is in tautomeric equilibria (phenol-imine, O–H...N ⇌ keto-amine, O...H–N forms) in DMSO, et-

Fig. 1. Solvent effect on compound 4d. DMSO (–––), ethanol (– ·– ·–) CHCl₃ (-------), benzene (– – –).
anol, chloroform and benzene (Fig. 1). This tautomer is observed in DMSO for 4d as supported by the UV–visible data, while it is not observed in DMSO by 1H-NMR spectroscopy. The calculated percentage of keto-amine form is given in Table I.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Table I. The UV-Vis spectra of the compounds 4a-d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Keto-amine tautomer/%</td>
</tr>
<tr>
<td></td>
<td>4a</td>
</tr>
<tr>
<td>DMSO</td>
<td>–</td>
</tr>
<tr>
<td>EtOH</td>
<td>–</td>
</tr>
<tr>
<td>CHCl3</td>
<td>–</td>
</tr>
<tr>
<td>Benzene</td>
<td>–</td>
</tr>
</tbody>
</table>

The 1H-NMR spectra of the compounds 4a–d show that the tautomeric equilibrium favors the phenol-imine in DMSO. The OH protons are observed as signals at 13.27, 10.78, 10.06 and 15.95 ppm for ligands 4a, 4b, 4c and 4d, respectively. The azomethine protons are observed as singlets at 8.96, 8.48, 8.55 and 9.69 ppm for 4a–d, respectively. The phenyl protons are observed as multiplet at $\delta = 6.51$–8.51 ppm for compounds 4a–d. The proton of the etheric group Ar–OCH$_2$ also gave a triplet at $\delta = 4.24$ and 4.31 ppm for 4b and 4d, respectively, and a singlet at $\delta = 4.38$ and 4.34 for 4a and 4c, respectively, and Ar–CH$_2$–Ar gave a singlet at $\delta = 4.18$, 4.15, 4.20 and 4.08 ppm for 4a–d, respectively.

The 13C-NMR spectra of compounds 4a–d were analyzed (Scheme 2), and it was concluded that the structures in solution are symmetrical. According to the 13C-NMR spectra, compounds 4a, 4b, 4c and 4d have 15, 15, 13 and 19 signals, respectively.

The electron impact MS spectrum of compounds 4a, 4b, 4c and 4d showed a well-defined parent ion at $m/z$ 464, 464, 464, and 564 (28, 20, 18 and 25 %) with the expected isotope pattern. The peaks at $m/z$ values of 334, 230, 213 and 108 correspond to the loss of (CHC$_6$H$_4$OH + CH$_2$CH$_2$), (CHC$_6$H$_4$OH + CH$_2$CH$_2$ + CHC$_6$H$_4$OH), (CHC$_6$H$_4$OH + CH$_2$CH$_2$ + CHC$_6$H$_4$OH + CH$_2$), and (CHC$_6$H$_4$OH + CH$_2$CH$_2$OC$_6$H$_3$CH$_2$N–CHC$_6$H$_4$OH) groups for 4a. The fragmentation patterns of 4b and 4c were similar to that of 4a. The peaks at $m/z$ values of 360, 334, 212, 120 and 108 correspond to the loss of (CHC$_{10}$H$_6$OH + CH$_2$O), (CHC$_{10}$H$_6$OH + OCH$_2$CH$_2$OH), (CHC$_{10}$H$_6$OH + CH$_2$ + CHC$_{10}$H$_6$OH), (CH$_2$OC$_6$H$_3$CH$_2$N–CHC$_{10}$H$_6$OH) and (CHC$_{10}$H$_6$OH + CH$_2$CH$_2$OC$_6$H$_3$CH$_2$N–CHC$_{10}$H$_6$OH) for 4d. The OCH$_3$H$_2$NH$_2$ system in the compounds is stable (dominant ion: $m/z$ 108) during the fragmentation, which indicates first the loss of aldehyde and ethylene fragments.

The antimicrobial activities of the compounds are shown in Table II, in which the inhibition zones formed by standard antibiotic discs are also indicated. As can be clearly seen from Table II, the compounds showed antibacterial activity against both Gram-positive and Gram-negative bacteria and the yeast cultures. In classifying antibacterial activity as Gram-positive or Gram-negative, it would generally be...
expected that a much greater number would be active against Gram-positive than against Gram-negative bacteria. However, in this study, the compounds were active against both Gram-positive and Gram-negative bacteria and the yeast cultures. Notably, the compounds have stronger antimicrobial activities against the yeast cultures than against bacteria used in this study.

When the obtained results are compared to those of the standard antibiotics, it can be seen that compound 4d is the most effective compound against bacteria. The compounds have no antibacterial effect against the acid-resistant bacterium *Mycobacterium smegmatis* and *Listeria monocytogenes*. *Staphylococcus aureus* is more susceptible to the compound, as compared to the standard antibiotics except for OFX5 and TE30. Notably, compound 4d has stronger antibacterial effects than those of the others. Against *Bacillus cereus*, only the compound 4d has antibacterial activity but its effect is equivalent to the standard SAM20. The compounds have weaker antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae* than those of the standard antibiotics, except for SAM20 and CTX30. Similarly, they show weak effects against *Micrococcus luteus*. All the compounds have stronger antibacterial activity than those of the standard antibiotics P10, SAM20 and VA30 against *Pseudomonas aeruginosa*. *Proteus vulgaris* is the most sensitive bacterium towards the compounds, especially 4d, having zone diameters of 15–21 mm. In general, all compounds show high anti-yeast activity against the yeast culture used in this study. While compound 4d is the most effectual compound against the yeast cultures, *Kluyveromyces fragilis* is the most sensitive microorganism,
<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Compounds</th>
<th>Inhibition zone/mm</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4a</td>
<td>4b</td>
<td>4c</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>10</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>13</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>12</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>15</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mycobacterium smegmatis</td>
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<tr>
<td>Listeria monocytogenes</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Micrococcus luteus</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>13</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Kluyveromyces fragilis</td>
<td>15</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Rhodotorula rubra</td>
<td>14</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Hanseniaspora guilliermondii</td>
<td>12</td>
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<td>18</td>
</tr>
<tr>
<td>Debaryomyces hansenii</td>
<td>14</td>
<td>16</td>
<td>20</td>
</tr>
</tbody>
</table>

P10: Penicillin G (10 Units), SAM20: Ampicillin 10 μg, CTX30: Cefotaxime 30 μg, V30: Vancomycin 30 μg, OFX 5: Ofloxacin 5 μg, TE30: Tetracyclin 30 μg, N100: Nystatin 100 μg, KETO20: Ketoconazole 20 μg, CLT10: Clotrimazole 10 μg
having zone diameters of 15–25 mm. *Candida albicans* is more susceptible to compound 4d, as compared to the standard antibiotics CLT10. Compound 4d has a stronger anti-yeast activity against *Rhodotorula rubra* than all the tested standard antifungal agents. Notably, compounds 4c and 4d have higher anti-yeast activity against *Debaryomyces Hansenii* than the standard antibiotics. Also *Hanseniaspora guilliermondii* is influenced at different levels.

The compounds differ significantly in their activity against the tested microorganisms. These differences may be attributed to fact that the cell wall in Gram-positive bacteria is single layered, whereas the Gram-negative cell wall is a multi-layered structure and the yeast cell wall is quite complex.34

**EXPERIMENTAL**

The 1H- and 13C-NMR spectra were recorded on a Bruker DPX FT-NMR spectrometer operating at 400 and 101.6 MHz, respectively. The 1H and 13C chemical shifts were measured using SiMe4 as the internal standard. The infrared absorption spectra were recorded on a Perkin Elmer BX II spectrometer in KBr discs. The UV–Vis spectra were recorded on a Shimadzu 1201 series spectrometer. Carbon, hydrogen and nitrogen analyses were performed on a Leco CHNS-932 C-, H-, N- analyzer. The melting points were measured on an Electro Thermal IA 9100 apparatus using a capillary tube. THF, EtOH, DMSO, DMF, CHCl3, H2SO4, NaOH, K2CO3, benzene, formaldehyde, 4-nitrophenol, hydrazine hydrate, 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde and Pd–C (10 %) were purchased from Merck (Germany).

**Synthesis of 2,2'-methylenebis[4-nitrophenol] (I)**35,36

To formaldehyde (10 mL, 40 %) and H2O (6 mL) was added H2SO4 (24 mL, 98 %). The mixture was stirred and heated at 80 °C. The hot mixture was added rapidly with stirring and heating to a solution of p-nitrophenol (38.0 g, 0.273 mol) in H2O (5 mL) at 75 °C. The mixture was heated under stirring at 130 °C for 1 h and then dissolved in NaOH (4 %). The solution was filtered and acidified with HCl, whereby the product separated. It was crystallized from acetic acid. The analytical and experimental data are summarized in Table III.

**Synthesis of 2,11-dinitro-6,7-dihydro-13H-dibenzo[e,h][1,4]dioxonin (2)**

To 2,2'-methylenebis [4-nitrophenol] (1) (3.718 g, 0.01113 mol) dissolved in DMF (150 mL, 99 %) was added 1,2-dibromoethane (2.081 g, 0.0113 mol). The mixture was stirred at reflux temperature for 24 h and then filtered. The solvent was removed under reduced pressure and the remaining yellow product was washed with water. The crude product was recrystallized from methanol. The analytical and experimental data are summarized in Table III.

**Synthesis of 6,7-dihydro-13H-dibenzo[e,h][1,4]dioxonin-2,11 diamine (3)**

To 2,11-dinitro-6,7-dihydro-13H-dibenzo[e,h][1,4]dioxonin (2) (0.5412 g, 0.00171 mol) and Pd/C (0.032 g, 10 %) dissolved in ethanol (150 mL, 99 %) was added dropwise hydrazine monohydrate (2.081 g, 0.0113 mol). The mixture was stirred at reflux temperature for 3 h and then filtered through a pad of celite to remove the catalyst. The solvent and excess hydrazine were removed in vacuo to give the crude product 3, which was recrystallized from ethanol. The analytical and experimental data are summarized in Table III.

**Synthesis of Schiff bases**

7,13-Dihydro-6H-dibenzo[e,h][1,4]dioxonin-2,11-diamine (3) (0.0790 g, 0.0003 mol) was added to a dry THF (100 mL) solution of 2-hydroxybenzaldehyde (0.0763 g, 0.0006 mol). The
mixture was heated under stirring for 3 h. Compound 2,2'-(1E,1'E)-[(6,7-dihydro-13H-dibenzo[e,h][1,4]-dioxin-2, 11-diyl]bis[nitrilomethylidyne])diphenol (4a) was obtained by evaporation of the THF. It was crystallized from CHCl3:n-hexane (3:1).

The other Schiff bases 3,3'-(1E,1'E)-[(6,7-dihydro-13H-dibenzo[e,h][1,4]-dioxin-2, 11-diyl]bis[nitrilomethylidyne])diphenol (4b), 4,4'-(1E,1'E)-[(6,7-dihydro13H-dibenzo[e,h][1,4]-dioxo-

4,4'-diyldibenzene)]diphenol (4c) and 1,1'-(1E,1'E)-[(6,7-dihydro-13H-dibenzo[e,h][1,4]-dioxin-2,11-diyl]bis[nitrilomethylidyne])diphenol (4d) were obtained by the same method. The analytical and experimental details for all compounds are given in Table III.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>M.p. °C</th>
<th>Color</th>
<th>Yield %</th>
<th>C analysis%</th>
<th>H analysis%</th>
<th>N analysis%</th>
</tr>
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<td>95</td>
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<td>75</td>
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</table>

Antimicrobial test

Escherichia coli ATCC 11230, Staphylococcus aureus ATCC 6538, Klebsiella pneumoniae UC57, Micrococcus luteus La 2971, Proteus vulgaris ATCC 8427, Pseudomonas aeruginosa ATCC 27853, Mycobacterium smegmatis CCM 2067, Bacillus cereus ATCC 7064, Listeria monocytogenes ATCC 15313, Candida albicans ATCC 10231, Kluyveromyces fragilis NRRL 2415, Rhodotorula rubra DSM 70403, Debaryomyces hansenii DSM 70238 and Hanseniaspora guilliermondii DSM 3432 were used as bacteria and yeast cultures. The compounds were dissolved in DMSO to a final concentration of 30 μg/mL. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Schull No 2668, Germany) were each impregnated with 20 μL of solution. All the bacteria above-mentioned were incubated at 30±0.1 °C for 24 h by inoculation into Nutrient Broth (Difco), and the studied yeasts were incubated in Malt Extract Broth (Difco) for 48 h. An inoculum containing 10⁶ bacterial cells or 10⁸ yeast cells / mL was spread on Mueller–Hinton Agar (Oxoid) plates (1 mL inoculum / plate). The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35 °C (24 h) for the bacteria, and at 25 °C (72 h) for the yeasts. On each plate, an appropriate reference antibiotic disc was applied depending on the test microorganisms.24,25 The data reported in Table II are the average data of three experiments.

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ИЗВОД

СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ НОВИХ КРУНА-ЕТАРАТИПА ШИФОВЕ БАЗЕ

МУСТАФА ЈИЛЂИЗ1, АŞКИН КИРАЗ2 и БАŞАРАН ДУЛЂЕР3

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Нови крна-етар лиганди типа Шифове базе (4a–d) синтетизовани су у реакцији 2-хидроксизбензалдейхида, 3-хидроксизбензалдейхида, 4-хидроксизбензалдейхида и 2-хидрокси-1-нафталдейхида са 6,7-дихидро-13H-дибензо[е,г] [1,4]диоксинонин-2,11-димином (3). Структура лиганда испитивана је елементалном анализом, као и UV-видљивом, 1H-NMR, 13C-NMR и масном спектрометријом. Антибактеријска активност и активност према гљивичним културалама проверавана је према микроорганизмима: Escherichia coli ATCC 11230, Staphylococcus aureus ATCC 6538, Klebsiella pneumoniae La 2971, Proteus vulgaris ATCC 8427, Pseudomonas aeruginosa ATCC 27853, Mycobacterium smegmatis CCM 2067, Bacillus cereus ATCC 7064 и Listeria monocytogenes ATCC 15313; Candida albicans ATCC 10231, Kluyveromyces fragilis NRRL 2415, Rhodotorula rubra DSM 70403, Debaryomyces hansenii DSM 70238 и Hanseniaspora guilliermondii DSM 3432.

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REFERENCES