SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL 4-PHENYLDIAZENYL-4'-(4-CHLOROBENZYL)OXY]BIPHENYL DERIVATIVES AS ANTIBACTERIAL AGENTS

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ABSTRACT

A series of five new 4-phenyldiazenyl-4'-(4-chlorobenzyl)oxy]biphenyls have been synthesized by condensing different sodium salts of some 4'-phenyldiazenyl-biphenyl-4-ols with 1-chloro-4-(chloromethyl)benzene. These compounds have been characterized by elemental analysis (C, H, N) and electronic, IR, 1H NMR and mass spectrometry studies. The obtained compounds were assayed for their antibacterial activity against some bacteria by disk diffusion method.

Keywords: antibacterial activity, azomonoether, FTIR spectra, UV-Vis spectra, mass spectra, NMR spectra.

INTRODUCTION

Many synthetic compounds with antimicrobial activity have been discovered and are of considerable importance from the standpoint of research and practical applications: aminoglycosides, cephalosporins, lipopeptides, sulfonamides, macrodiles, oxazolidinones, quinolones, and pyrimidines derivatives. The development of new antimicrobial drugs is a very important objective not only from the rapidly developing drug resistance point of view, but also regarding the unsatisfactory status of present treatments of bacterial and fungal infections and drug side-effects. In recent years there has been a great deal of interest in exploiting multiple proximal functional groups in the design of novel structures capable of performing a variety of functions. Synthesis of molecules that are novel but still resemble known biologically active molecules by virtue of the presence of some critical structural features is an essential component of the search for new leads in drug design.

During the last few decades, considerable attention has been devoted to the synthesis of azoderivatives possessing such different types of bioactivities like: antibacterial, anti-inflammatory, antiviral and anti-HIV activities. In recent years, biphenyl derivatives are an extensively investigated class of compounds, which exhibits various biological activities, such as anti-tuberculosis, antibiotic, antifungal and antitumor. These observations place new emphasis on the synthesis of azoderivatives with a view to incorporation of a biphenyl fragment, for the evaluation of associated antibacterial activity.

As part of our continuous research in the synthesis of biologically active azocompounds, we have synthesized and their structures and antibacterial activities are reported in this paper.

EXPERIMENTAL

Materials

Aniline, p-toluidine, o-chloroaniline, p-chloroaniline, 3,4-dichloroaniline, 4-biphenyloxy and 1-chloro-4-(chloromethyl)benzene were purchased from Merck and were used without purification.

Methods

The melting point was determined using a Sanyo Gallenkamp melting point apparatus without correction. The analyses of carbon, hydrogen and nitrogen were performed with a Carlo Erba Erba 1108 analyzer. The UV-Vis measurements were carried out using a Uvicord Varian Cary-50 Bio spectrophotometer. FT-IR spectra of these compounds were recorded on a Brucker ATR zinc selenide spectrophotometer, within the range of 4000 - 550 cm⁻¹, at room temperature with a spectral resolution of 2 cm⁻¹. The 1H NMR spectra were registered on a Varian EM-360, 60 MHz spectrometer, using CDCl₃ as solvent and TMS as internal standard. Mass spectra were run with a HP GC-MS 5890 spectrometer at 70 eV and at 250 °C (the source temperature).

General procedure I for the synthesis of 2a-e

A solution of sodium nitrite (36 mmol) in 75 mL of water was slowly added under stirring to a solution of amine (36 mmol) dissolved in 150 mL of 2 mol L⁻¹ HCl solution, cooled at 0 – 5 °C. During the addition of the NaNO₂ solution, the reaction temperature was kept below 5 °C in order to stabilize diazonium ions. This solution was slowly added at 0 – 5 °C to 4-biphenyloxy (6.1 g, 36 mmol) in 10% NaOH solution and the pH was adjusted to 8 – 9 with concentrated NaOH solution. The obtained solution was stirred at room temperature for 6 h. The precipitate was collected by filtration, washed three times with distilled water, recrystallized in a mixture of 50 mL of ethanol and 100 mL of water and vacuum dried. Compounds 2a-e were obtained as orange crystalline solids.

Synthesis of 4'-phenyldiazenylbiphenyl-4-ol (2a)

Compound (2a) was obtained using the general procedure I with 3.35 g of aniline (36 mmol). Yield 84.3%; m.p.112 °C; Anal. Calcd. for C₁₈H₁₄N₂O (γ): C 78.83, H 5.10, N 10.21; Found (γ): C 78.69, H 4.97, N 10.15; IR (powder; cm⁻¹): 1610 (N=N), 1424 (N=N), 3033 (C=O), 1155 (Ar-Cl), 1H NMR (60 MHz, CDCl₃, δ/ppm): 6.4 (m, 13H, aromatic), 5.3 (s, 1H, OH), UV-Vis (dioxane) (ε max / nm (ε max / L mol⁻¹ cm⁻¹)): 227 (13790), 264 (24395), 335 (22690), 421 (6955).

Synthesis of 4'-[4-(methyl-phenyldiazenyl)]biphenyl-4-ol (2b)

Compound (2b) was synthesized using the above procedure with 3.85 g of p-toluolidine as aromatic amine. Yield 82.8%; m.p. 115 °C; Anal. Calcd. for C₁₈H₁₆N₂O (γ): C 79.16, H 5.55, N 9.72; Found (γ): C 79.01, H 5.63, N 9.64; IR (powder; cm⁻¹): 1608 (N=N), 1455 (N=N), 3019 (C=O), 1165 (Ar-N), 1H NMR (60 MHz, CDCl₃, δ/ppm): 6.6 (m, 12H, aromatic), 5.3 (s, 1H, OH), 2.4 (s, 3H, CH₃), UV-Vis (dioxiane) (δ max / nm (ε max / L mol⁻¹ cm⁻¹)): 227 (13790), 264 (24395), 335 (22690), 421 (6955).

Synthesis of 4'-[4-(chloro-phenyldiazenyl)]biphenyl-4-ol (2c)

Compound (2c) was synthesized using the above procedure with 4.6 g of p-chloroaniline as aromatic amine. Yield: 78.7%; m.p. 126 °C; Anal. Calcd. for C₁₈H₁₃ClN₂O (γ): C 70.01, H 4.21, N 9.07; Found (γ): C 69.96, H 4.17, N 9.15; IR (powder; cm⁻¹): 1580 (N=N), 1447 (N=N), 3029 (C=O), 1165 (Ar-Cl), 593 (Ar-Cl), 1H NMR (60 MHz, CDCl₃, δ/ppm): 6.8 (m, 12H, aromatic), 5.4 (s, 1H, OH), UV-Vis (dioxiane) (δ max / nm (ε max / L mol⁻¹ cm⁻¹)): 228 (12272), 264 (24395), 335 (22690), 421 (6955).

Synthesis of 4'-[2-(chloro-phenyldiazenyl)]biphenyl-4-ol (2d)

Compound (2d) was synthesized using the above procedure with 4.6 g of o-chloroaniline as aromatic amine. Yield: 84.2%; m.p. 111 °C; Anal. Calcd. for C₁₈H₁₄ClN₂O (γ): C 70.01, H 4.21, N 9.07; Found (γ): C 69.96, H 4.17, N 9.15; IR (powder; cm⁻¹): 1593 (N=N), 1451 (N=N), 3026 (C=O), 1161 (Ar-N), 696 (Ar-Cl), 1H NMR (60 MHz, CDCl₃, δ/ppm): 7.2 (m, 12H, aromatic), 5.6 (s, 1H, OH), UV-Vis (dioxiane) (δ max / nm (ε max / L mol⁻¹ cm⁻¹)): 221 (4717), 262 (13722), 334 (10657), 433 (3287).

Synthesis of 4'-[3,4-dichloro-phenyldiazenyl]biphenyl-4-ol (2e)

Compound (2e) was synthesized using the above procedure with 5.83 g of 3,4-dichloroaniline as aromatic amine. Yield: 91.5%; m.p. 147 °C; Anal. Calcd. for C₁₈H₁₂Cl₃N₂O (γ): C 62.97, H 3.50, N 8.16; Found (γ): C 62.83, H 3.42, N 8.03; IR (powder; cm⁻¹): 1574 (N=N), 1451 (N=N), 3030 (C=O), 1161 (Ar-N), 692 (Ar-Cl), 1H NMR (60 MHz, CDCl₃, δ/ppm): 7.2 (m, 11H, aromatic), 5.67 (s, 1H, OH), UV-Vis (dioxiane) (δ max / nm (ε max / L mol⁻¹ cm⁻¹)): 228 (31975), 260 (9167), 338 (7340), 432 (16067).
The sodium salts were obtained from the corresponding 4'-phenyldiazenyl-biphenyl-4-ols dissolved in an ethanol-benzene mixture (1:1, in volumes) and sodium hydroxide. These salts are obtained in anhydrous state by azeotropic distillation of the benzene-ethanol-water mixture.

**RESULTS AND DISCUSSION**

The new azomonoethers were prepared using the etherification of the corresponding sodium salts of substituted 4'-phenyldiazenyl-biphenyl-4-ols with 1-chloro-4-(chloromethyl)benzene in alkaline medium (Scheme 1) employing Williamson method. The sodium salts were obtained from the corresponding 4'-phenyldiazenyl-biphenyl-4-ols dissolved in an ethanol-benzene mixture (1:1, in volumes) and sodium hydroxide. These salts are obtained in anhydrous state by azeotropic distillation of the benzene-ethanol-water mixture.

**Scheme 1: Synthesis of 4-(4-phenyldiazenyl)-4'-(4-chlorobenzyloxy)biphenyl 3a-e**

The composition and purity of synthesized azoethers was confirmed by elemental analyses (see experimental sections). The obtained compounds were crystalline yellow-orange powders. They are stable at room temperature and all compounds are insoluble in water.

**Spectral study**

The structure of these compounds has been investigated on the basis of UV-visible, IR, 'H NMR and mass spectra.

The electronic spectra, recorded in dioxan, exhibit a R-band due to azo-group at 432 - 452 nm, a high intensity K-band due to the conjugated system aromatic rings at 234 - 263 nm which are in agreement with earlier reports.

The infrared spectra confirm the presence of azo and ether groups in the structure of compounds 3a-e. The vibration frequency of the N=N stretches appears at 1400 - 1413 cm\(^{-1}\).

The proofs of the etherification reaction between the hydroxyl group of azophenol and the 1-chloro-4-(chloromethyl)benzene are:

- absence in the IR spectra of the bands characteristic for the hydroxyl group;
- presence of absorption bands of the C-O-C newly formed group; thus spectrum contains an intense absorption band at 1260 - 1290 cm\(^{-1}\) which can be assigned to the antisymmetrical valence vibrations of the C-O-C group and a moderate absorption band due to the symmetrical valence vibrations of the C-O-C group at 1013 - 1014 cm\(^{-1}\).

The 'H NMR spectra of all compounds show that the signal of the CH\(_2\) group appears like a singlet at a values between \(\delta = 5.0 - 5.5\) ppm. The aromatic protons from the four substituted benzene rings came into resonance as a multiplet at \(\delta = 7.2 - 7.7\) ppm. For 4-(4-methylphenyl)diazenyl-4'-(4-chlorobenzyloxy)biphenyl 3b an additional singlet is present at \(\delta = 2.2\) ppm, corresponding to the methyl group protons.
The fragmentation pattern described in Scheme 2 for 4-phenyldiazenyl-4’-[(4-chlorobenzyl)oxy]biphenyl 3a, is characteristic for all compounds 3a-e:

\[
\text{Scheme 2: Fragmentation of 3a under electron impact ionization}
\]

According to the literature, scheme 2 shows a major fragmentation of 4-phenyldiazenyl-4’-[(4-chlorobenzyl)oxy]biphenyl in which the molecular ion peak at m/z 398 is abundant and the molecule tends to undergo a cleavage of the O-CH bond to give the base peak.6

**Antibacterial activity**

The antibacterial activity of the investigated 4-phenyldiazenyl-4’-[(4-chlorobenzyl)oxy]biphenyls 3a-e was done by microdiscs paper diffusion against three gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*) and four gram-negative bacteria (*Klebsiella pneumonia*, *Salmonella paratyphae*, *Proteus vulgaris* and *Escherichia coli*). Chloramphenicol was used as standard drug and methanol served as control.

Results revealed that in general, all tested compounds possessed good antibacterial activity against three gram-negative bacteria (*Salmonella paratyphae*, *Proteus vulgaris* and *Escherichia coli*). The best efficiency at the tested concentrations was exhibited by 4-(2-chloro-phenyldiazenyl)-4’-[(4-chlorobenzyl)oxy]biphenyl (3d) against *Escherichia Coli* and by 4-phenyldiazenyl-4’-[(4-chlorobenzyl)oxy]biphenyl (3a) against *Proteus vulgaris* (Table 1). The compounds 3a-e exhibited moderate activity against *Klebsiella pneumonia*. Interpretation of antibacterial screening data revealed that all the tested compound 3a-e showed good inhibition on the growth of *Bacillus subtilis* (Table 1). All tested 4-phenyldiazenyl-4’-[(4-chlorobenzyl)oxy]biphenyls are inactive against *Streptococcus pyogenes* and *Staphylococcus aureus*.

The results of antibacterial activity of compounds 3a-e were compared with the standard drug for evaluating their relative percentages of inhibition (Table 2). The maximum relative percentage of inhibition was exhibited by 3d against *Escherichia coli* (100%), followed by 3a against *Proteus vulgaris* (97.29%).

<table>
<thead>
<tr>
<th>Name of organisms</th>
<th>Mean zone of inhibition / mm</th>
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<tbody>
<tr>
<td><em>Salmonella paratyphae</em></td>
<td>21.66 20 22.33 18.66 19.33 24 0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>21.33 24.66 28 30 25.33 30 0</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>23.33 26 24.33 27.66 27.33 30 0</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>10 11.33 12 11.66 12.66 28 0</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>24.66 22.33 18 16.66 17 25 0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>- - - - - - 20 0</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>- - - - - 21 0</td>
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<table>
<thead>
<tr>
<th>Name of organisms</th>
<th>Relative percentage of inhibition / %</th>
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</thead>
<tbody>
<tr>
<td>3a</td>
<td>54.43 75.11 65.77 85 83</td>
</tr>
<tr>
<td>3b</td>
<td>97.29 72.79 51.84 44.40 46.24</td>
</tr>
<tr>
<td>3c</td>
<td>81.45 69.44 86.56 60.45 64.87</td>
</tr>
<tr>
<td>3d</td>
<td>50.55 67.56 87.11 100 71.29</td>
</tr>
<tr>
<td>3e</td>
<td>12.75 16.37 18.36 17.34 20.44</td>
</tr>
</tbody>
</table>

It can be concluded that a combination of biphenyl fragment with azo group shown promising antibacterial activity and hence they are ideally suited for further modifications to obtain more efficacious antimicrobial compounds, in near future. The properties of new microbial substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed.

**CONCLUSIONS**

This paper presents the synthesis of five new 4-phenyldiazenyl-4’-[(4-chlorobenzyl)oxy]biphenyls under Williamson conditions, using the condensation of different sodium salts of some 4’-phenyldiazenyl-biphenyl-4-ols with 1-chloro-4-(chloromethyl)benzene.

The formation of azomonoethers was confirmed by the disappearance of the signal at 3019 - 3030 cm⁻¹ in IR spectra which is typical for hydroxyl group of azophenols and by the appearance of an intensive absorption band at 1260 - 1280 cm⁻¹ which can be assigned to the antisymmetrical valence vibrations of the C-O-C group and a moderate absorption band due to the symmetrical valence vibrations of the C-O-C group at 1013 - 1014 cm⁻¹.

All compounds were subjected to antibacterial activity tests and it can be concluded that 3a and 3d are microbiological active against *Proteus vulgaris* and *Escherichia Coli*, respectively, and deserve further studies.

**REFERENCES**