Synthesis and Antimicrobial Activity of Some Taurinamide Derivatives

Özlem Akgül, Ismail Öztürk, Abdurrahman Aygül, Şafak Ermertcan

ABSTRACT
In this study, a series of taurinamide derivatives were synthesized and screened for their antimicrobial activity. The structures of the newly synthesized compounds 11-15 were evaluated by 1H NMR, IR, MS-APCI and elemental analysis. Compounds (1-15) were tested against standard strains of Gram(+) and Gram(-) bacteria and fungi by using disc diffusion and broth microdilution methods. Although disc diffusion method did not show any comparable results due to the solubility properties of the compounds, microdilution method results indicated that title compounds showed from poor to good activity against only Enterococcus faecalis. It can be concluded that phtalimido moiety, secondary aryl sulphonamide group and electron-donating group substitution on phenyl ring are essential for the antibacterial activity. Among the tested compounds, para substituted methyl and methoxy derivatives of N-phenyl-2-phthalimidotaurinamide (4, 7), displayed equipotent activity compared to standard drug gentamicin.

Key Words: Taurinamide, N-substituted-2-phthalimidoethanesulfonamide, antibacterial, Enterococcus faecalis, microdilution.

Introduction
Infectious diseases are a challenging area for drug development studies as bacteria have variable resistant mechanisms to existing antimicrobials. It had been reported as the major public health problem by World Health Organization (WHO) in 2014 (1). In fact, there is a lack in development of new drugs and mortality rates are increasing due to bacterial infections. As a consequence, antibacterial drug development studies become an urgent area in medicinal chemistry (2, 3). The strategies for developing new compounds, mostly based on synthetic modifications of natural antibiotics and synthetic antibacterials like sulfonamides (4). Among these methods, molecular hybridization is known to be one of the most useful one for manipulating the pharmaceutical, pharmacokinetic or pharmacodynamic properties of the parent compound (5).

Phtalimide moiety (I) is a versatile structure that enable different variation of substitutions while amino acid
substituted derivatives from nitrogen known to possess strong antibacterial activity (6). Although the clear interaction mechanism is not known, it is considered to interact with host's mRNA (7). On the other hand, it is known for its anticonvulsant (8), antitubercular (9), antitumor (10) and anti-inflammatory effects (11).

Taurinamide (II) is lipophilic derivative of taurine and known to possess antibacterial (12), histamine H₄ receptor inverse agonists (13) and anticonvulsant (14) activities which indicate its potential for generating pharmacologically active compounds.

Sulfonamide scaffold is a building-block which creates antidiabetic (15), carbonic anhydrase inhibitor (16), anti-inflammatory (17) and antitumor (18) drug molecules. Moreover, the most prominent effect is antibacterial activity as various sulfonamide (III) derivatives are in clinical use for decades (19).

In the literature, introduction of electron withdrawing (i.e. chloro) and donating (i.e. methoxy, methyl) groups on different positions of the phenyl moiety considered to have diverse effects on biological activities (20). On the other hand, morpholine is the mostly used scaffold in drug design studies (21) and plays an important role in exerting antifungal activity by inhibiting the biotransformation of sterols (22).

According to above considerations, we designed and synthesized a group of taurinamide (II) derivative for evaluating their antimicrobial activity. The title compounds 1-13 have phthalimide scaffold tailored with taurine and functionalized as sulfonamide from its sulfonic acid part with ammonium hydroxide, morpholine and nonsubstituted/substituted aniline derivatives. On the other hand, deprotection of phthalimido moiety from nonsubstituted aniline and morpholine derivatives furnished compounds 14 and 15.

The title compounds 1-10 were reported in our previous study whereas 11-15 were reported in different studies as intermediates of diverse compounds (12, 13, 23). None of the compounds have been tested for their antimicrobial activity before though taurine derivatives known to posses antimicrobial activity (12, 24). Literature survey indicates that there is a need for elucidating the structural requirements that mediate their pharmacological activity (25). This study will provide us to compare the alteration on antimicrobial activity due to aliphatic, aromatic and phthalimide substitution on taurinamide structure.

Results and Discussion

1. Chemistry

The target molecules 1-10 were synthesized as outlined in Figure 1. Taurine and phthalic anhydride were condensed to give 2-phthalimidoethanesulfonic acid potassium salt A and this compound was converted to its chlorinated derivative B with phosphorus pentachloride. Compound B was then reacted with corresponding aniline derivatives to yield the title compounds 1-10. The detailed synthetic procedure and their characterizations by ¹H NMR, IR, mass spectral data and elemental analysis were reported in our previous study (23).

Title compounds 11-15 were synthesized starting from 2-phthalimidoethanesulfonyl chloride B which was prepared according to procedure described before (12, 13). The chlorinated derivative B was reacted with sulfanilamide in pyridine to give the title compound 11 as a white solid (Figure 2). ¹H NMR spectrum of the compound 11 displayed two singlets at 10.50 ppm and 7.31 ppm indicating the existence of secondary and primary sulfonamide groups respectively. Three N-H stretching bands between 3361 and 3245 cm⁻¹, two SO₂ asymmetric (1304, 1319 cm⁻¹) and SO₂ symmetric stretching bands (1141, 1160 cm⁻¹), were confirmative for the title compound's structure in IR spectrum.

Reaction of B with ammonium hydroxide solution furnished the title compound 12 (Figure 2). The structural confirmation was provided by the existence of broad singlet shown at 7.06 ppm in ¹H NMR spectra. It was indicative for primary sulfonamide protons as their chemical shift were observed at the upfield compared to the aromatic sulfonamide protons in compound 11. On the other hand; two NH stretching, one SO₂ asymmetric and SO₂ symmetric stretching bands (1141, 1160 cm⁻¹), were confirmative for the title compound's structure in IR spectrum.

The title compound 13 was obtained by the reaction of B with morpholine and TEA in DCM solution at room temperature (Figure 2). Disappearance of SO₂NH proton signal from aromatic field, appearance of the 2 triplets at 3.66 and 3.19 ppm in ¹H NMR spectrum and absence of the NH stretching bands in IR spectrum supported the substition of morpholine for compound 13.
Deprotection of the compounds 1 and 13 were performed according to Ing-Manske procedure (26) (Figure 1-2). The absence of the carbonyl stretching bands at 1700 cm⁻¹ region, existence of the broad NH stretching bands at 2600, 2800 cm⁻¹ in IR spectra and disappearance of phthalimide protons from aromatic region in ¹H NMR spectra were confirmative findings for the structures of the title compounds 14-15.

Mass spectra of compounds 11, 12 and 13 displayed two main fragmentation patterns as m/z 238 ion [C₁₀H₈NO₄S]+ and m/z 174 ion [C₁₀H₈NO₂]+ which were in agreement with the previously reported derivatives 1-10. On the other hand [M+1]+ ion peaks were observed for the deprotected derivatives 14-15.

The compounds 11-15 were reported earlier though were not fully characterized by spectral data.

2. Antimicrobial Activity

All the title compounds were screened for antibacterial activity against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 (Gram positive); *Escherichia coli* ATCC 25922 and *Pseudomonas Aeruginosa*.
ATCC 27853 (Gram negative) bacterial strains in addition to their antifungal activities against *Candida Albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019. Disc diffusion and broth microdilution methods were used to evaluate the antimicrobial activity in a qualitative and quantitative way respectively.

Preliminary screening tests was performed by using disc diffusion method for detecting the susceptibility of microorganisms to the title compounds (Table 1). The results showed that *Staphylococcus aureus* (*S. aureus*) is susceptible only to compound 9, while *Enterococcus faecalis* (*E. faecalis*) is susceptible to the title compounds 9, 11, 12 with about 8 mm inhibition zone which is not closely relative to standard drugs gentamicin and co-trimoxazole. Since the literature survey indicates that the solubility of the compounds is a limiting factor on the diffusion rate through the agar medium, we decided to employ broth microdilution method for overcoming this problem (27). Disc diffusion and broth microdilution methods did not show any comparable results which can be explained by the poor solubility and polar character of the title compounds (28).

Broth microdilution method results showed that none of the title compounds displayed significant antimicrobial activity against tested microorganisms except *E. faecalis* (Table 2). The cause of poor activity can be explained by wide range of resistant mechanism of *S. aureus* and different cell wall structures of Gram-negative bacteria and fungi (22,29). For this reason, the following structure activity relationship (SAR) will be discussed only for *E. faecalis* (Table 2):

1. Phtalimidotaurinamide [2-(1,3-dioxoisindolin-2-yl) ethanesulfonamide] 12 and its N-phenyl substituted derivative 1 exhibited equal activity by 256 µg/ml (MIC value) whereas removal of the phtalimide moiety from 1 yielded in a 4 fold decreased activity by 1024 µg/ml as MIC value.

2. Compound 13, which has morpholine substituted tertiary sulfonamide group, yielded in a poor antibacterial activity with 1024 µg/ml (MIC value) compared to all other substituted phtalimidotaurinamide derivatives 1-12. Moreover the antibacterial activity diminished with its deprotected derivative 15.

### Table 1. *In vitro* anti-bacterial and anti-fungal activities of compounds 1-15 by disc diffusion method

<table>
<thead>
<tr>
<th>Comp. No</th>
<th>Gram(+)</th>
<th>Gram(-)</th>
<th>C. albicans</th>
<th>C. parapsilosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td><em>E. faecalis</em></td>
<td><em>E. coli</em></td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>8±0</td>
<td>7.33±0.58</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>8±1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>8±1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamisin</td>
<td>26±0</td>
<td>13±1</td>
<td>23.33±115</td>
<td>20.33±0.58</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>30.67±1.15</td>
<td>24.67±1.15</td>
<td>27±1</td>
<td>nt</td>
</tr>
<tr>
<td>Fluconazol</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
</tbody>
</table>

* zone of inhibition in mm: <10: weak; >10:moderate; >16: Significant
- Lack of activity
nt: not tested
3. For compounds 1-11 which have different substitution pattern on phenyl ring:

a. para-Substitution of the phenyl ring with methoxyl 4 and methyl 7 increased the activity 64 fold corresponding to nonsubstituted phenyl derivative 1. Also they showed equipotent activity with standard drug gentamicin with 4 µg/ml MIC value.

b. orto- and meta-substitution of the phenyl ring with methoxy (compound 2, 3) moiety increased the activity 4 fold while orto, meta methyl substituted phenyl ring increased the activity 2 fold compared to the nonsubstituted phenyl derivative 1.

c. MIC values obtained from orto-, meta- and para-substitution of the phenyl ring with chloro (compound 8, 9, 10) resulted in a gradually increasing pattern with 128, 64 and 32 µg/ml respectively.

d. para-Substitution of the phenyl moiety with chloro and sulfonamide (compound 10, 11) yielded 8 and 2 fold more active compounds corresponding to nonsubstituted derivative 1 respectively.

Our previous crystallographic study which was accomplished with compound 8, 9 and 10 indicated that all these positional isomers have different conformational structure and their aromaticity changes in the order of 8<10<9 by the means of torsion angles and highest occupied molecular orbital (HOMA) calculations (30). As their MIC values follow the 8<9<10 order, conformational changes and electronic effects may be considered together as significant parameters for antimicrobial activity.

On the other hand, literature survey indicated that lipophilicity of the compounds may have an impact on antibacterial activity (31). Though, in this study, there wasn't any correlation observed between calculated log P and MIC values (Table 2).

Moreover, in one of our study, title compounds 2, 3, 5, 8, 9, 10 were evaluated for their interaction with DNA and suggested to have different binding affinities which are not consistent with MIC values (32). Therefore, the antibacterial activity may not be suggested as a result of DNA interaction.

<table>
<thead>
<tr>
<th>Compound No</th>
<th>Gram (+)</th>
<th>Gram (-)</th>
<th>Fungal strains</th>
<th>Calc. Log P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.aureus</td>
<td>E. faecalis</td>
<td>E. coli</td>
<td>Paeruginosa</td>
</tr>
<tr>
<td>1</td>
<td>2048</td>
<td>256</td>
<td>1024</td>
<td>2048</td>
</tr>
<tr>
<td>2</td>
<td>1024</td>
<td>64</td>
<td>2048</td>
<td>1024</td>
</tr>
<tr>
<td>3</td>
<td>1024</td>
<td>64</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>4</td>
<td>&gt;2048</td>
<td>4</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>5</td>
<td>2048</td>
<td>128</td>
<td>&gt;2048</td>
<td>2048</td>
</tr>
<tr>
<td>6</td>
<td>2048</td>
<td>128</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>7</td>
<td>&gt;2048</td>
<td>4</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>8</td>
<td>1024</td>
<td>128</td>
<td>&gt;2048</td>
<td>&gt;2048</td>
</tr>
<tr>
<td>9</td>
<td>1024</td>
<td>64</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>10</td>
<td>2048</td>
<td>32</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>11</td>
<td>2048</td>
<td>128</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>12</td>
<td>2048</td>
<td>256</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>13</td>
<td>&gt;2048</td>
<td>1024</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>14</td>
<td>&gt;2048</td>
<td>1024</td>
<td>2048</td>
<td>1024</td>
</tr>
<tr>
<td>15</td>
<td>&gt;2048</td>
<td>2048</td>
<td>2048</td>
<td>2048</td>
</tr>
<tr>
<td>Gentamisin</td>
<td>0.5</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Amfoterisin B</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
</tbody>
</table>

* Calculated by the methodology developed by Molinspiration

nt: not tested

Bold indicating for significant activity
In conclusion, the structure activity relationship showed that phtalimido moiety, secondary aryl sulphonamide group and electron-donating group substitution on phenyl ring are essential for selective antibacterial activity. Moreover, para substituted methyl and methoxy derivatives of N-phenyl-2-phthalimidotaurinamide (4, 7), displayed equipotent activity compared to standard drug gentamicin against only E. faecalis. As most of the serious hospitalized infections caused from this bacteria, the title compounds 4 and 7 may be evaluated as promising core structures for developing drugs against infections caused by E. faecalis type (33, 34).

Experimental

1. General remarks

Aniline, morpholine and sulfanilamide were purchased from Merck (Darmstadt, Germany). All other reagents and solvents were obtained from Sigma Chemical Co. (St. Louis, MO). All solvents used in this study were of analytical grade. Compound A and B was prepared as described previously (13, 23). 1H NMR spectra were scanned on a Varian AS 400 Mercury Plus NMR spectrometer using DMSO-d6 as solvent. Chemical shifts were reported in parts per million (ppm) and the coupling constants (J) were expressed in Hertz (Hz). Splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet. Infrared spectra were run on a Perkin Elmer Spectrum 100 FT-IR equipped with a Universal ATR Sampling Accessory and the frequencies were expressed in cm⁻¹. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Melting points were taken with a Stuart SMP 30 melting point apparatus in open capillary tubes and are uncorrected. Elemental analyses (C, H, N and S) were performed using a Leco TruSpec CHNS Micro analyzer (Leco Corporation, St. Joseph, MI, USA) and results were within ±0.4% of calculated values. clog P values were calculated by the methodology developed by Molinspiration (35).

2. Chemistry


A solution of 2-phtalimidoethanesulfonyl chloride (1 equiv.) in pyridine (5 ml) was treated with p-aminobenzenesulfonamide (1 equiv.) and heated at 60 °C until the starting material was consumed as evidenced by TLC. The reaction was quenched with water and the precipitation was filtered. The crude product crystallized from acetic acid:H2O to give the 4-(2-(1,3-dioxoisooindolin-2-yl)ethylsulfonylamido)benzenesulfonamide 11 as a white solid.

2.2. Synthesis of 2-(1,3-dioxoisooindolin-2-yl)ethanesulfonamide 12.

Concentrated ammonium hydroxide solution (5 ml) was added dropwise to the stirring 2-phtalimidoethanesulfonyl chloride (1 equiv.) in an ice bath. The reaction mixture was first dissolved and then a white precipitation was formed. After observing the consumption of the starting materials by TLC, the solvent was evaporated under vacuo, treated with 60% acetic acid solution to give a white precipitate, which was then filtered and crystallized from EtOH:H2O to furnish the final product 2-(1,3-dioxoisooindolin-2-yl)ethanesulfonamide 12 as a white solid.

2.3. Synthesis of 2-(morpulinosulfonyl)ethylisoidoline-1,3-dione 13.

A solution of 2-phtalimidoethanesulfonyl chloride (1 equiv.) in DCM (5 ml) was treated with morpholine (1 equiv.) and TEA (3 equiv.) in an ice bath. The reaction was stirred at room temperature until the starting material was...
consumed as evidenced by TLC. The solvent was evaporated under \textit{vacuo} and treated with water. The white precipitate was filtered and crystallized from ethanol to give the 2-(2-(morpholinosulfonyl)ethyl)isoindoline-1,3-dione 13 as white solid.

2-(2-(Morpholinosulfonyl)ethyl)isoindoline-1,3-dione (13): Yield 55%, silica gel TLC Rf 0.4 (EtOAc:Hex 50% v/v); mp 167-168 °C (found), 178-180 °C (reported) (39). IR \textit{max} (cm\(^{-1}\)); \textit{\nu} J 4.02 (2H, t, J: 6.7 Hz, -CH\(_2\)CH\(_2\)SO\(_2\)NH-), 3.47 (2H, t, J: 5.7 Hz, -CH\(_2\)CH\(_2\)SO\(_2\)NH-), 4.02 (2H, t, J: 6.7 Hz, -CH\(_2\)CH\(_2\)SO\(_2\)NH-), 3.79-9.88 (4H, m, phtalimide-H) (13). MS-APCI m/z (% intensity) 325 (1, [M+1]+), 238 (67), 174 (100). Anal. Calculated for C\(_{14}\)H\(_{16}\)N\(_2\)O\(_5\)S (MW = 324.35): C, 51.84; H, 4.97; N, 8.64; O, 21.69; S, 13.91. Found: C, 51.57; H, 5.28; N, 8.37; S, 9.84.


For deprotection of the phtalimido moiety; \beta\-phtalimido-N-phenylethanesulfonamide (1 eqv.) was suspended in 4 ml ethanol and treated with 80% hydrazine hydrate (0.87 eqv.) solution according to ing-manske procedure. The reaction mixture was refluxed for three hours and evaporated under \textit{vacuo}. The crude product dissolved in water and acidified with 1 eqv. conc. HCl solution. The precipitate was filtered, evaporated under \textit{vacuo} and crystallized from isopropanol to give 2-amino-N-phenylethanesulfonamide HCl salt 14 as white solid.

2-Amino-N-phenylethanesulfonamide HCl salt (14): Yield 37%, silica gel TLC Rf 0.27 (MeOH/DCM 17% v/v); mp 173-175 °C (found), 178-180 °C (reported) (39). IR \textit{max} (cm\(^{-1}\)): 314 (SO\(_2\)\textit{sym}); 2624 (NH\(_3\)) (39). MS-APCI m/z (intensity%): 205 (100, [M+1]+), 87 (81). Anal. Calculated for C\(_{10}\)H\(_{11}\)ClN\(_2\)O\(_3\)S (0.1 H\(_2\)O) (MW = 230.71): C, 30.99; H, 6.59; N, 12.05; S, 13.79. Found: C, 30.80; H, 6.84; N, 12.16; S, 13.62.

3. Antimicrobial activity

In vitro antimicrobial activities of the compounds 1-15 were evaluated by disc diffusion and broth microdilution methods against various bacterial and fungal strains.

3.1. Microorganisms

The following bacterial and fungal strains were used: \textit{Staphylococcus aureus} (ATCC 25923), \textit{Staphylococcus aureus} (ATCC 29213), \textit{Enterococcus faecalis} (ATCC 29212), \textit{Escherichia coli} (ATCC 25922), \textit{Pseudomonas aeruginosa} (ATCC 27853), \textit{Candida albicans} (ATCC 90028) and \textit{Candida parapsilosis} (ATCC 22019). All strains were stored in Brain-Heart Infusion Broth (Merck, Germany) with 10% glycerol at -80 °C. \textit{Müller-Hinton} agar (MHA) and \textit{Sabouraud} dextrose agar (SDA) (Oxoid, UK) were used for bacteria and yeasts, respectively.

3.2. Disc diffusion test

Inhibition zone diameters of the strains were assayed by Clinical and Laboratory Standards Institute (CLSI) recommendations with minor modifications (40, 41). The compounds were prepared solving in dimethyl sulfoxide (DMSO). Sterile empty discs (6 mm diameter) (Oxoid, UK) impregnated with 10 µl of each formulation (10 mg/ml DMSO) were placed on the inoculated MHA and SDA plates and the plates were incubated at 37 °C 24 h for bacteria and 48 h for yeasts. All solutions were sterilized with 0.22
µm pore size filters. Gentamisin (Oxoid, Germany) and trimethoprim/sulfamethoxazole (co-trimoxazole) (Oxoid, Germany) discs were used as reference for bacteria and fluconazole (Sigma-Aldrich, Germany) were used as reference for yeasts. DMSO was also tested separately and each sample was tested in triplicate. After incubation periods of 16-20 hours for bacteria and 48 hours for yeasts, the diameters of the growth inhibition zones were measured and means ± standard deviations (SD) were reported.

3.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of the compounds were evaluated for each strain using the broth microdilution method as described by Kotmakçı et al. (42) with minor modification. Mueller-Hinton II broth (cation adjusted) (MHIIB) (Merck, Germany) and Sabouraud dextrose broth (SDB) (Oxoid, UK) were used for broth microdilution for bacteria and yeasts, respectively. The experiments were performed in 96-well microtitration plates using 50 µl inoculum of all strains with ½ serial dilutions of the compounds. The final concentrations of the compounds were ranged from 2048 to 1 µg/ml. The lowest concentrations that have no visible microbial growth is determined as MIC. Gentamicin (I.E. Ulugay, Turkey) and fluconazole were used as reference agents for antibacterial and antifungal activities, respectively. Quality control ranges were evaluated according to CLSI (40). Each sample was tested in triplicate and DMSO was also tested separately for antibacterial and antifungal activity.

ACKNOWLEDGMENTS

This work was supported by research grant from Ege University (Project Number: 12/Ecz/24). The authors gratefully acknowledge Pharmaceutical Sciences Research Center (FABAL) of the Faculty of Pharmacy, Ege University for providing spectral analysis.

The authors have declared no conflict of interest.

REFERENCES


41. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.