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ORIGINAL PAPER

# Synthesis and antimicrobial properties of new 2-((4-ethylphenoxy)methyl)benzoylthioureas

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New acylthiourea derivatives, 2-((4-ethylphenoxy)methyl)-*N*-(phenylcarbamothioyl)benzamides, were tested by qualitative and quantitative methods on various bacterial and fungal strains and proved to be active at low concentrations against Gram-positive and Gram-negative bacteria as well as fungi. These compounds were prepared by the reaction of 2-((4-ethylphenoxy)methyl)benzoyl isothiocyanate with various primary aromatic amines, and were characterised by melting point and solubility. The structures were identified by elemental analysis, $^{1}$H and $^{13}$C NMR, and IR spectral data. The level of antimicrobial activity of the new 2-((4-ethylphenoxy)methyl)benzoylthiourea derivatives was dependent on the type, number and position of the substituent on the phenyl group attached to thiourea nitrogen. The iodine and nitro substituents favoured the antimicrobial activity against the Gram-negative bacterial strains, while the highest inhibitory effect against Gram-positive and fungal strains was exhibited by compounds with electron-donating substituents such as the methyl and ethyl groups.


**Keywords:** acylthiourea, 2-((4-ethylphenoxy)methyl)benzoic acid, $^{1}$H NMR, $^{13}$C NMR, antimicrobial activity

## Introduction

Increasing incidence of bacterial drug resistance requires an improvement to existing antimicrobial drugs and the development of new ones. Antimicrobial resistance of pathogenic microorganisms is spreading both in the community and in hospitals leading to an increased number of deaths, and increased illness and costs. Thiourea derivatives, and especially their *N*-acylated derivatives, are of great pharmacological importance, with proven antimicrobial activity (Cunha et al., 2007; Desai & Desai, 1989; Kapoor et al., 1991; Kurt et al., 2009; Saeed et al., 2008, 2009; Jia et al., 1993; Zhong et al., 2008). Some acylthiourea derivatives exhibit antiviral (Sun et al., 2006), antihelmintic (Chow, 1984; Zikán et al., 1988), and herbicidal (Ke & Xue, 2006) activities.

It is known that the thiourea derivatives containing both the carbonyl and thiocarbonyl groups are efficient ambidentate donor ligands to transition metal ions. Their antimicrobial activity can be explained by the chelating effect of some metal ions at the level of the thioureidic fraction; the molecular complex obtained can block the metal

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ions in certain enzymes and disturb the formation of some metalloenzymes. This is the premise behind our study of this series. In our previous papers, we presented the synthesis of some thioureides of 2-(phenoxymethyl)benzoic acid (Limban et al., 2000, 2004), 2-((4-chloro-phenoxy)methyl)benzoic acid (Limban et al., 2008a), 2-((4-methoxyphenoxy) methyl)benzoic acid (Limban et al., 2008b), 2-((4-methylphenoxy)methyl)benzoic acid (Limban et al., 2008c, 2008d), 2-((4-ethylphenoxy)methyl)benzoic acid (Limban et al., 2009a), 2-((4-fluorophenoxy) methyl)benzoic acid (Limban et al., 2009b) and their antimicrobial (Balotescu et al., 2007) and antiparasitic activity (Müller et al., 2009). Several transition metal complexes of these acylthioureas were also prepared (Nacea et al., 2005; Boşcencu et al., 2007).

As part of our interest in identifying a larger number of bioactive acylthioureas, we have synthesised new 2-((4-ethylphenoxy)methyl)benzoylthioureas by the addition of various primary aromatic amines to 2-((4-ethylphenoxy)methyl)benzoyl isothiocyanate. The structures were identified by spectral data and elemental analyses The antimicrobial activity of the synthesised compounds was performed by in vitro assay and compared with the reference and clinical multidrug resistant strains.

## Experimental

All chemicals were purchased (Merck, Fluka, Germany) and used as received, except for 4-ethylphenol which was distilled prior to its use. Acetone and ammonium thiocyanate were dried before use. Melting points were recorded with an Electrothermal 9100 apparatus and are uncorrected. Elemental analyses were carried out using a Perkin–Elmer CHNS/O Analyzer Series II 2400 apparatus and the results were within ±0.4 % of theoretical values.

The reaction progress was observed by thin layer chromatography performed on silica gel 60 $F_{254}$ (0.2 mm thick) plates (Merck, Germany) using chloroform/ethyl acetate ($\varphi_r = 4 : 6$) as a mobile phase with visualisation by ultraviolet light.

NMR spectra (in DMSO-$d_6$) were recorded using a Gemini 300BB instrument operating at 300 MHz for $^1$H and 75 MHz for $^{13}$C, and a Unity Inova 400 instrument, operating at 400 MHz for $^1$H and 100 MHz for $^{13}$C, respectively. TMS was used as the internal standard. IR spectra were recorded using a FT-IR Bruker Vertex 70 apparatus by ATR technique. The IR band intensities are denoted as w – weak; m – medium; s – strong; vs – very strong.

2-((4-Ethylphenoxy)methyl)benzoic acid (*I*) and 2-((4-ethylphenoxy)methyl)benzoyl chloride (*IV*) were prepared according to procedures described by Limban et al. (2009a).

Acylthioureas *Va–Vn* were synthesised according to the general method (Limban et al., 2009a) as follows: a solution of 2-((4-ethylphenoxy)methyl)benzoyl chloride (0.01 mol) in dry acetone (15 mL) was added to a solution of ammonium thiocyanate (0.76 g, 0.01 mol) in dry acetone (5 mL) and the reaction mixture was kept under reflux for 1 h. After cooling, primary aromatic amine (0.01 mol) in dry acetone (2 mL) was added to the reaction mixture upon stirring and kept under reflux for 1 h. After completion of the reaction (detected by TLC), the mixture was poured into cold water (500 mL). The precipitated thioureas were separated by filtration and recrystallised (after decolourisation by active carbon) from an appropriate solvent.

### *Antimicrobial activity assay*

Qualitative screening of the susceptibility spectra of various microbial strains to the compounds was performed by modified diffusion techniques, while the quantitative assay of minimal inhibitory concentration (MIC, μg mL$^{-1}$) was based on serial micro-dilutions with liquid medium. The compounds were solubilised in DMSO to a final concentration of 1000 μg mL$^{-1}$. The effects from in vitro biological screening were tested on a microbial inoculum of $\sim 1.5 \times 10^8$ CFU per cm$^3$, corresponding to 0.5 McFarland density, represented by bacteria, *Enterobacteriaceae* (*Escherichia coli* and *Klebsiella pneumoniae*), *Pseudomonadaceae* (*Pseudomonas aeruginosa*), *Micrococcaceae* (*Staphylococcus aureus)*, *Bacillaceae* (*Bacillus subtilis*), and fungi, *Candida albicans* and *Aspergillus niger*. The reference strains, *K. pneumoniae* IC 13420, *E. coli* IC 13529, *S. aureus* IC 13204, *P. aeruginosa* IC 13202, *B. subtilis* IC 12488, *C. albicans* IC 249, and *A. niger* IC 13534 from the Cantacuzino Institute Culture Collection Laboratory, were susceptible to all the antibiotics recommended by Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards, NCCLS) while the strains recently isolated from clinical samples exhibited multiresistance patterns (*S. aureus* 1263 – methicilin-resistant MRSA, *K. pneumoniae* 1204 and *E. coli* 13147 – producing an extended spectrum of $\beta$-lactamases, conferring the microorganisms with resistance to all $\beta$-lactams, including 3rd generation cephalosporins as well as other classes of antibiotics, such as quinolones and aminoglycosides), *P. aeruginosa* 1246 (multiresistant to all antibiotics except colistin) and *C. albicans* 101404, resistant to fluconazole. The microbial strains were identified using VITEK I automatic system. VITEK cards for the identification and susceptibility testing (GNS-522) were inoculated and incubated according to the manufacturer's recommendations. The results were interpreted using software version AMS R09.1.

The qualitative screening was performed using the disc diffusion method. Petri dishes with Mueller Hinton (for bacterial strains) and YPG (Yeast Peptone Glucose) (for yeasts) media were inoculated with mi-

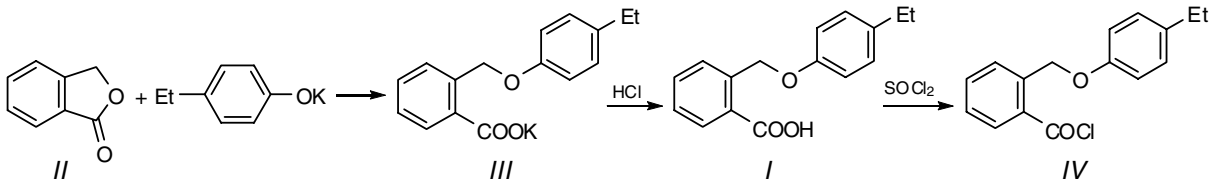


**Fig. 1.** Synthesis of 2-((4-ethylphenoxy)methyl)benzoic acid and its chloride.

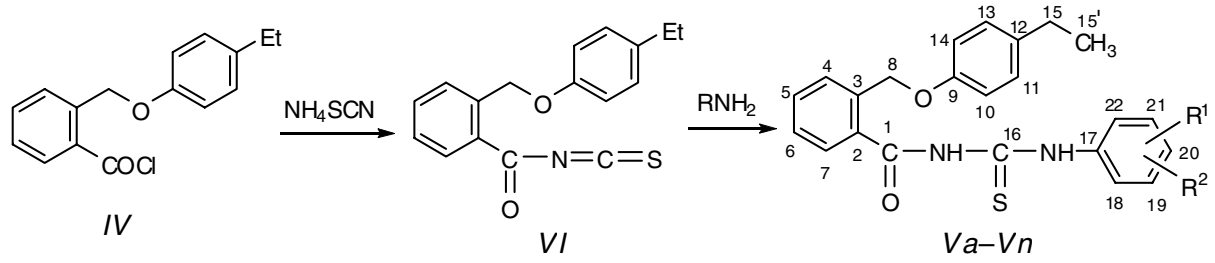


**Fig. 2.** Synthesis of new thioureides.

croorganisms as in the classical antibiotic susceptibility testing disc diffusion method (Kirby–Bauer); 6 mm diameter paper filter discs were placed on the inoculated medium at a distance of 30 mm. Subsequently, the discs were impregnated with 5 μL of the solution of the compound tested (1000 μg $mL^{-1}$ concentration). The plates were left at room temperature for 20–30 min and then incubated at 37℃ for 24 h. The positive results were read as the occurrence of an inhibition zone of microbial growth around the disc (Olar et al., 2005; Lazar et al., 2005; Balotescu et al., 2005).

The quantitative screening was performed using the binary micro-dilution method, in 96-well culture plates, in order to establish the MIC (CLSI, 2007; NCCLS, 2000). For this purpose, serial binary dilutions of the compounds tested (ranging from 1000 μg $mL^{-1}$ to 7.8 μg $mL^{-1}$) were performed in 200 μL of nutrient broth and each well was filled with 50 μL of microbial inoculum. The plates were incubated at 37℃ for 24 h, and MICs were read as the lowest concentration of the compound which inhibited the microbial growth. The results were interpreted using standard antibiotics (aminoglycosides – amikacin for the Gram-positive and ceftriaxone for the Gram-negative strains (CLSI, 2007), fluconazole for *Candida*, and voriconazole for *Aspergillus* (NCCLS, 2002) as controls.

## Results and discussion

### *Synthesis and spectral characterisation*

2-((4-Ethylphenoxy)methyl)benzoic acid (*I*) and 2-((4-ethylphenoxy)methyl)benzoyl chloride (*IV*) were prepared according to the reaction scheme shown in Fig. 1 (Limban et al., 2009a).

The new thioureides, *Va–Vn*, were prepared by heating the 2-((4-ethylphenoxy)methyl)benzoyl isothiocyanate (*VI*) (obtained from the corresponding chloride *IV*) with primary aromatic amines in dry acetone under reflux (Fig. 2, Limban et al., 2009a). All these acylthioureas (Table 1) are white or light-yellow crystalline solids, insoluble in water and soluble in acetone and chloroform at normal temperature and in short chain aliphatic alcohols, benzene, toluene, and xylene at higher temperatures. Their structures were identified by elemental analyses, IR, and NMR spectral data (Table 2).

In the $^1$H NMR spectra, the ethyl group exhibited a characteristic quartet at $\delta$ 2.48–2.50 and triplet at $\delta$ 1.07–1.19. The methylene group attached to the oxygen atom showed a singlet at $\delta$ 5.24–5.27. The signals of aromatic protons were observed in a range of $\delta$ 6.82–7.96, with the exception of *Vj*, where the signal for H-22 was shifted to $\delta$ 8.76 and *Vn* where a shift to $\delta$ 8.25 was recorded for H-19 (H-21). The NH protons exhibited two characteristic broad singlets at $\delta$ 11.74–12.04 and $\delta$ 11.86–12.70, respectively. In the $^{13}$C NMR spectra, the thiocarbonyl carbon showed a characteristic signal at $\delta$ 176.88–180.33, the carbonyl carbon at $\delta$ 169.67–170.49, the methylene carbon of $CH_2O$ group at $\delta$ 67.29–67.72, and the carbons of ethyl group at $\delta$ 26.98–27.34 ($CH_2$) and $\delta$ 13.84–15.94 ($CH_3$).

In the IR spectra, the bands (sharp peaks of weak intensity) due to stretching vibrations $\nu$(N—H) of the amide group were in the region of 3352–3238 $cm^{-1}$. The thioamide group showed a band of stretching vibrations at 3181–3144 $cm^{-1}$ and there is a high level of probability that the band situated at 1394–1344 $cm^{-1}$ can be attributed to the thioamide group. As to antisymmetric stretching vibrations, the methyl and methylene groups exhibited a saturated ($sp^3$) $\nu$(C—H) stretching at 2974–2954 $cm^{-1}$ and 2933–2918 $cm^{-1}$, respectively. These bands are typical of aromatic compounds containing some saturated carbon. These com-

**Table 1.** Characteristics of the compounds prepared

| Compound | $R^1$ | $R^2$ | Formula | $M_r$ | $w_i$(calc.)/% $w_i$(found)/% | | | | Yield | M.p. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | S | % | °C |
| *Va* | 2-$CH_3$ | 3-$CH_3$ | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.89 | 6.26<br>6.21 | 6.69<br>6.57 | 7.66<br>7.81 | 68 | 136–139 |
| *Vb* | 2-$CH_3$ | 4-$CH_3$ | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.67 | 6.26<br>6.30 | 6.69<br>6.89 | 7.66<br>7.69 | 70 | 134–136 |
| *Vc* | 2-$CH_3$ | 5-$CH_3$ | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.86 | 6.26<br>6.30 | 6.69<br>6.79 | 7.66<br>7.51 | 73 | 131–133 |
| *Vd* | 2-$CH_3$ | 6-$CH_3$ | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.66 | 6.26<br>6.25 | 6.69<br>6.51 | 7.66<br>7.80 | 65 | 149–150 |
| *Ve* | 3-$CH_3$ | 4-$CH_3$ | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.59 | 6.26<br>6.17 | 6.69<br>6.73 | 7.66<br>7.74 | 79 | 106–108 |
| *Vf* | 3-$CH_3$ | 5-$CH_3$ | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.43 | 6.26<br>6.19 | 6.69<br>6.70 | 7.66<br>7.75 | 82 | 139–140 |
| *Vg* | 2-$C_2H_5$ | H | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.48 | 6.26<br>6.29 | 6.69<br>6.81 | 7.66<br>7.83 | 71 | 90–91 |
| *Vh* | 3-$C_2H_5$ | H | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.94 | 6.26<br>6.21 | 6.69<br>6.56 | 7.66<br>7.60 | 81 | 91–92 |
| *Vi* | 4-$C_2H_5$ | H | $C_{25}H_{26}N_2O_2S$ | 418.55 | 71.74<br>71.71 | 6.26<br>6.15 | 6.69<br>6.78 | 7.66<br>7.62 | 85 | 136–138 |
| *Vj* | 2-$OC_2H_5$ | H | $C_{25}H_{26}N_2O_3S$ | 434.55 | 69.10<br>69.02 | 6.03<br>5.99 | 6.45<br>6.59 | 7.38<br>7.46 | 73 | 93–95 |
| *Vk* | 3-$OC_2H_5$ | H | $C_{25}H_{26}N_2O_3S$ | 434.55 | 69.10<br>68.97 | 6.03<br>6.14 | 6.45<br>6.39 | 7.38<br>7.47 | 79 | 123–125 |
| *Vl* | 4-$OC_2H_5$ | H | $C_{25}H_{26}N_2O_3S$ | 434.55 | 69.10<br>68.92 | 6.03<br>5.95 | 6.45<br>6.52 | 7.38<br>7.40 | 82 | 101–102 |
| *Vm* | 4-I | H | $C_{23}H_{21}N_2O_2SI$ | 516.40 | 53.49<br>53.39 | 4.10<br>4.02 | 5.43<br>5.49 | 6.21<br>6.23 | 71 | 123–126 |
| *Vn* | 4-$NO_2$ | H | $C_{23}H_{21}N_3O_4S$ | 435.49 | 63.43<br>63.59 | 4.86<br>4.78 | 9.65<br>9.67 | 7.36<br>7.31 | 68 | 173–176 |

pounds were characterised by the $\nu$(C═O) vibrations in the region of 1690–1669 $cm^{-1}$. The medium intensity band of $\nu$(C═O) vibration of all the compounds appeared at 1690–1669 $cm^{-1}$ which was lower than the intensity of an ordinary carbonyl absorption (1730 $cm^{-1}$). The formation of intramolecular H-bonds led to an increase in their polarity decreasing the strength of their double bond and moving the absorption to a lower wavenumber. A very intense band of $\nu$(N—H) stretching was observed at 1518–1506 $cm^{-1}$. These compounds also showed a typical band corresponding to alkylaryl ether: at 1254–1224 $cm^{-1}$ for the antisymmetric vibration and at 1042–1019 $cm^{-1}$ for the symmetric vibration. The bands of $\nu$(C═S) stretching vibrations were in the range of 1178–1148 $cm^{-1}$, which is in agreement with the literature data. The presence of iodine at the aryl ring was indicated by the band of stretching vibration $\nu$(C—I) at 498 $cm^{-1}$.

### *Antimicrobial activity*

The MIC values were read by observation: in the first wells containing high concentrations of the compounds tested, the growth of microorganisms was completely suppressed, as they were killed or inhibited by the compound tested. At lower concentrations of the compounds tested, the microbial culture became visible. The lowest concentration which inhibited the visible microbial growth was considered to be the MIC (μg $mL^{-1}$) value of the compound tested. In the adjacent wells, containing the standard culture growth control wells, the medium became cloudy as a result of microbial growth. In the sterility control wells, the culture medium had to remain clear for an obvious reason. From the last well without any visible microbial growth and from the first with a microbial growth, Gram-stained smears were performed to confirm the results. The solvent used for evaluation of the compounds exhibited no antimicrobial activity. This property represented a practical advantage for the antimicrobial evaluation of these water-insoluble compounds. The in vitro screening of the antimicrobial properties of the new compounds was performed by an modified diffusion method, followed by the broth micro-dilution method, in order to establish the MIC for Gram-positive (*S. aureus*, *B. subtilis*), Gram-negative bacteria (*P. aeruginosa*, *E. coli*, *K. pneumoniae*), as well as fungi (*C. albicans* and *A. niger*), using both reference and clinical, multidrug resistant strains. Our results showed that the com-

**Table 2.** Spectral characterisation of newly prepared compounds

| Compound | Spectral data |
|---|---|
| *Va* | IR, $\tilde{\nu}$/cm$^{-1}$: 436 (w), 546 (w), 568 (w), 614 (w), 654 (w), 692 (w), 726 (m), 763 (m), 791 (w), 828 (m), 858 (w), 890 (w), 951 (w), 1021 (m), 1071 (w), 1091 (w), 1169 (s), 1235 (s), 1300 (w), 1333 (w), 1389 (m), 1461 (s), 1508 (vs), 1584 (w), 1608 (w), 1673 (m), 2871 (w), 2929 (m), 2965 (m), 3028 (w), 3155 (m), 3325 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.14 (t, 3H, H-15′, $J$ = 7.5 Hz), 2.01 (s, 3H, $CH_3$), 2.25 (s, 3H, $CH_3$), 2.50 (q, 2H, H-15, $J$ = 7.5 Hz), 5.27 (s, 2H, H-8), 6.90 (d, 2H, H-10, H-14, $J$ = 8.1 Hz), 7.10–7.12 (m, 4H, H-11, H-13, H-20, H-22), 7.21 (t, 1H, H-21, $J$ = 6.2 Hz), 7.46 (td, 1H, H-6, $J$ = 1.7 Hz, $J$ = 7.9 Hz,), 7.54 (td, 1H, H-5, $J$ = 1.7 Hz, $J$ = 7.9 Hz), 7.57 (dd, 1H, H-4, $J$ = 1.7 Hz, $J$ = 7.9 Hz), 7.63 (dd, 1H, H-7, $J$ = 1.7 Hz, $J$ = 7.9 Hz), 11.87 (br, s, 1H, NH), 12.05 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 13.84 (C-15′), 15.52 ($CH_3$), 20.01 ($CH_3$), 27.34 (C-15), 67.60 (C-8), 114.55 (C-10, C-14), 124.74, 125.37, 127.83, 128.53, 128.53, 128.53, 128.69 (C-11, C-13), 130.91, 132.40, 133.67, 135.72, 136.22, 136.87, 137.20, 156.33 (C-9), 170.37 (C-1), 180.14 (C-16) |
| *Vb* | IR, $\tilde{\nu}$/cm$^{-1}$: 442 (w), 541 (w), 627 (w), 663 (w), 735 (m), 824 (m), 852 (w), 919 (w), 954 (w), 1042 (w), 1089 (w), 1121 (w), 1150 (s), 1231 (s), 1253 (m), 1267 (w), 1301 (w), 1340 (m), 1391 (w), 1449 (w), 1507 (vs), 1533 (s), 1584 (w), 1605 (w), 1671 (m), 2870 (w), 2924 (w), 2961 (w), 3011 (w), 3149 (m), 3329 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.13 (t, 3H, H-15′, $J$ = 7.5 Hz), 2.08 (s, 3H, $CH_3$), 2.27 (s, 3H, $CH_3$), 2.50 (q, 2H, H-15, $J$ = 7.5 Hz), 5.26 (s, 2H, H-8), 6.89 (d, 2H, H-10, H-14, $J$ = 8.1 Hz), 7.03 (m, 2H, H-19, H-21), 7.09 (d, 2H, H-11, H-13, $J$ = 8.1 Hz), 7.35–7.63 (m, 5H, H-4, H-5, H-6, H-7, H-22), 11.85 (br, s, 1H, NH), 12.06 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.52 (C-15′), 17.09 ($CH_3$), 20.25 ($CH_3$), 26.98 (C-15), 67.31 (C-8), 114.26 (C-10, C-14), 125.93, 126.24, 127.46, 128.16, 128.23, 128.30 (C-11, C-13), 130.54, 130.56, 132.67, 133.28, 133.94, 135.39, 135.87, 135.92, 156.01 (C-9), 169.93 (C-1), 179.62 (C-16) |
| *Vc* | IR, $\tilde{\nu}$/cm$^{-1}$: 435 (w), 470 (w), 544 (w), 576 (w), 617 (w), 649 (w), 664 (w), 695 (w), 742 (m), 827 (w), 862 (w), 901 (w), 956 (w), 1019 (m), 1072 (w), 1089 (w), 1147 (m), 1172 (m), 1239 (s), 1268 (s), 1300 (m), 1336 (w), 1381 (w), 1460 (m), 1509 (vs), 1583 (w), 1606 (w), 1679 (m), 2872 (w), 2928 (w), 2966 (w), 3027 (w), 3157 (m), 3326 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.13 (t, 3H, H-15′, $J$ = 7.5 Hz), 2.08 (s, 3H, $CH_3$), 2.27 (s, 3H, $CH_3$), 2.50 (q, 2H, H-15, $J$ = 7.5 Hz), 5.26 (s, 2H, H-8), 6.89 (d, 2H, H-10, H-14, $J$ = 8.1 Hz), 7.01 (dd, 1H, H-20, $J$ = 1.6 Hz, $J$ = 8.0 Hz), 7.10 (d, 2H, H-11, H-13, $J$ = 8.1 Hz), 7.14 (d, 1H, H-19, $J$ = 8.0 Hz), 7.31 (dd, 1H, H-22, $J$ = 1.6 Hz, $J$ = 8.4 Hz), 7.46 (td, 1H, H-6, $J$ = 1.5 Hz, $J$ = 8.4 Hz), 7.57 (td, 1H, H-5, $J$ = 1.5 Hz, $J$ = 8.4 Hz), 7.58 (dd, 1H, H-4, $J$ = 1.6 Hz, $J$ = 8.4 Hz), 7.62 (dd, 1H, H-7, $J$ = 1.5 Hz, $J$ = 8.4 Hz), 11.85 (br, s, 1H, NH), 12.06 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.49 (C-15′), 16.76 ($CH_3$), 20.18 ($CH_3$), 27.00 (C-15), 67.35 (C-8), 114.25 (C-10, C-14), 126.48, 127.34, 127.48, 128.14, 128.22, 128.30 (C-11, C-13), 129.88, 130.57, 133.07, 133.29, 134.86, 135.37, 135.89, 136.26, 156.03 (C-9), 170.00 (C-1), 179.53 (C-16) |
| *Vd* | IR, $\tilde{\nu}$/cm$^{-1}$: 439 (w), 546 (w), 615 (w), 653 (w), 754 (m), 830 (w), 869 (w), 951 (w), 1014 (m), 1031 (w), 1053 (w), 1075 (w), 1089 (w), 1126 (m), 1151 (m), 1167 (m), 1215 (m), 1234 (s), 1297 (w), 1334 (w), 1377 (w), 1507 (vs), 1602 (m), 1685 (m), 2928 (w), 2966 (w), 3028 (w), 3160 (m), 3325 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.14 (t, 3H, H-15′, $J$ = 7.6 Hz), 2.11 (s, 6H, $CH_3$), 2.50 (q, 2H, H-15, $J$ = 7.6 Hz), 5.27 (s, 2H, H-8), 6.90 (d, 2H, H-10, H-14, $J$ = 8.6 Hz), 7.10 (d, 2H, H-11, H-13, $J$ = 8.6 Hz), 7.12 (d, 2H, H-19, H-21), 7.15 (t, 1H, H-20), 7.47 (td, 1H, H-6, $J$ = 1.4 Hz, $J$ = 7.5 Hz), 7.55 (td, 1H, H-5, $J$ = 1.6 Hz, $J$ = 7.5 Hz), 7.59 (dd, 1H, H-4, $J$ = 1.4 Hz, $J$ = 7.5 Hz), 7.63 (dd, 1H, H-7, $J$ = 1.6 Hz, $J$ = 7.5 Hz), 11.74 (br, s, 1H, NH), 11.86 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.94 (C-15′), 17.80 ($CH_3$), 27.32 (C-15), 67.57 (C-8), 114.58 (C-10, C-14), 127.38, 127.84, 128.59, 128.62, 128.64 (C-11, C-13), 128.72, 130.91, 133.75, 135.11 (C-18, C-22), 135.63, 136.02, 136.19, 156.27 (C-9), 170.06 (C-1), 180.18 (C-16) |
| *Ve* | IR, $\tilde{\nu}$/cm$^{-1}$: 424 (w), 442 (w), 520 (w), 545 (w), 617 (w), 668 (w), 728 (m), 826 (w), 854 (w), 874 (w), 902 (w), 1033 (m), 1049 (w), 1125 (m), 1151 (s), 1668 (m), 1233 (s), 1303 (w), 1330 (m), 1382 (m), 1448 (s), 1508 (vs), 1531 (vs), 1583 (m), 1606 (w), 1671 (m), 2868 (w), 2918 (m), 2963 (s), 3032 (w), 3063 (w), 3151 (m)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.12 (t, 3H, H-15′, $J$ = 6.1 Hz), 2.22 (s, 6H, $CH_3$), 2.50 (q, 2H, H-15, $J$ = 6.1 Hz), 5.26 (s, 2H, H-8), 6.89 (d, 2H, H-10, H-14, $J$ = 8.8 Hz), 7.09 (d, 2H, H-11, H-13, $J$ = 8.8 Hz), 7.15 (d, 1H, H-21, $J$ = 8.0 Hz), 7.28 (d, 1H, H-18, $J$ = 2.1 Hz), 7.35 (dd, 1H, H-22, $J$ = 2.1 Hz, $J$ = 8.0 Hz), 7.46 (td, 1H, H-6, $J$ = 7.2 Hz, $J$ = 1.9 Hz), 7.55 (td, 1H, H-5, $J$ = 7.2 Hz, $J$ = 1.3 Hz), 7.58 (dd, 1H, H-4, $J$ = 1.9 Hz, $J$ = 7.2 Hz), 7.61 (dd, 1H, H-7, $J$ = 7.2 Hz, $J$ = 1.3 Hz), 11.79 (br, s, 1H, NH), 12.33 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.84 (C-15′), 18.95 ($CH_3$), 19.39 ($CH_3$), 27.30 (C-15), 67.55 (C-8), 114.60 (C-10, C-14), 125.06, 125.14, 127.71, 128.27, 128.50, 128.64 (C-11, C-13), 129.46, 130.94, 133.48, 134.28, 135.81, 136.19, 136.53, 139.47, 156.32 (C-9), 170.30 (C-1), 178.81 (C-16) |
| *Vf* | IR, $\tilde{\nu}$/cm$^{-1}$: 435 (w), 489 (w), 540 (w), 580 (w), 607 (w), 659 (w), 673 (m), 708 (w), 742 (w), 759 (w), 803 (w), 822 (w), 851 (w), 895 (w), 961 (w), 1040 (m), 1122 (m), 1150 (m), 1180 (s), 1247 (w), 1305 (m), 1326 (w), 1388 (m), 1443 (vs), 1511 (vs), 1528 (w), 1582 (w), 1612 (w), 1669 (m), 2867 (w), 2925 (w), 2959 (m), 3030 (w), 3144 (m), 3327 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.12 (t, 3H, H-15′, $J$ = 7.6 Hz), 2.27 (s, 6H, $CH_3$), 2.50 (q, 2H, H-15, $J$ = 7.6 Hz), 5.26 (s, 2H, H-8), 6.89 (d, 2H, H-10, H-14, $J$ = 8.8 Hz), 7.10 (d, 2H, H-11, H-13, $J$ = 8.8 Hz), 7.21 (d, 2H, H-18, H-22, $J$ = 1.4 Hz), 7.46 (td, 1H, H-6, $J$ = 1.7 Hz, $J$ = 7.5 Hz), 7.55 (td, 1H, H-5, $J$ = 1.2 Hz, $J$ = 7.5 Hz), 7.58 (dd, 1H, H-4, $J$ = 1.7 Hz, $J$ = 7.5 Hz), 7.61 (dd, 1H, H-7, $J$ = 1.2 Hz, $J$ = 7.2 Hz), 11.81 (br, s, 1H, NH), 12.35 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.83 (C-15′), 20.87 ($CH_3$), 27.31 (C-15), 67.58 (C-8), 114.59 (C-10, C-14), 121.71, 127.62, 127.75, 128.36, 128.50, 128.65 (C-11, C-13), 130.96, 133.48, 135.81, 136.19, 137.63, 137.82 (C-19, C-21), 156.35 (C-9), 170.36 (C-1), 178.79 (C-16) |

**Table 2.** (continued)

| Compound | Spectral data |
|---|---|
| *Vg* | IR, $\tilde{\nu}$/cm$^{-1}$: 436 (w), 546 (w), 610 (w), 657 (w), 727 (m), 762 (w), 790 (w), 827 (m), 846 (w), 869 (w), 887 (w), 948 (w), 1019 (m), 1050 (w), 1089 (w), 1161 (s), 1240 (s), 1300 (w), 1333 (w), 1382 (m), 1458 (s), 1509 (vs), 1604 (m), 1676 (m), 2875 (w), 2933 (w), 2964 (m), 3027 (w), 3158 (m), 3325 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.04 (t, 3H, $CH_2C\underline{H}_3$, $J$ = 7.5 Hz), 1.13 (t, 3H, H-15′, $J$ = 7.6 Hz), 2.50 (m, 4H, H-15, $C\underline{H}_2CH_3$), 5.26 (s, 2H, H-8), 6.90 (d, 2H, H-10, H-14, $J$ = 8.8 Hz), 7.10 (d, 2H, H-11, H-13, $J$ = 8.8 Hz), 7.20–7.30 (m, 3H, H-19, H-20, H-21), 7.40–7.60 (m, 4H, H-4, H-5, H-6, H-22), 7.63 (dd, 1H, H-7, $J$ = 1.2 Hz, $J$ = 7.2 Hz), 11.92 (br, s, 1H, NH), 12.17 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 14.26 ($CH_2\underline{C}H_3$), 15.90 (C-15′), 23.97 ($\underline{C}H_2CH_3$), 27.32 (C-15), 67.60 (C-8), 114.47 (C-10, C-14), 126.01, 127.20, 127.54, 127.87, 128.52, 128.64 (C-11, C-13), 130.94, 133.54, 135.65, 136.19, 137.18, 138.98, 156.33 (C-9), 170.49 (C-1), 180.33 (C-16) |
| *Vh* | IR, $\tilde{\nu}$/cm$^{-1}$: 446 (w), 543 (w), 566 (w), 613 (w), 684 (m), 711 (m), 744 (w), 778 (w), 797 (m), 823 (w), 856 (w), 886 (w), 1040 (m), 1120 (m), 1151 (s), 1168 (m), 1237 (s), 1309 (m), 1348 (s), 1386 (w), 1448 (s), 1511 (vs), 1529 (vs), 1566 (s), 1591 (m), 1612 (m), 1672 (m), 2869 (m), 2929 (m), 2961 (s), 3032 (m), 3238 (m)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.12 (t, 3H, $CH_2C\underline{H}_3$, $J$ = 7.6 Hz), 1.18 (t, 3H, H-15′, $J$ = 7.6 Hz), 2.50 (q, 2H, H-15, $J$ = 7.6 Hz), 2.61 (q, 2H, $C\underline{H}_2CH_3$, $J$ = 7.6 Hz), 5.26 (s, 2H, H-8), 6.90 (d, 2H, H-10, H-14, $J$ = 8.8 Hz), 6.98 (dd, 1H, H-20, $J$ = 1.7 Hz, $J$ = 7.8 Hz), 7.09 (d, 2H, H-11, H-13, $J$ = 8.8 Hz), 7.10 (dd, 1H, H-22, $J$ = 1.7 Hz, $J$ = 7.8 Hz), 7.30 (t, 1H, H-21, $J$ = 7.8 Hz), 7.39 (t, 1H, H-18, $J$ = 1.7 Hz), 7.46 (td, 1H, H-6, $J$ = 1.8 Hz, $J$ = 7.2 Hz), 7.56 (td, 1H, H-5, $J$ = 1.2 Hz, $J$ = 7.2 Hz), 7.58 (dd, 1H, H-4, $J$ = 1.8 Hz, $J$ = 7.2 Hz), 7.61 (dd, 1H, H-7, $J$ = 1.2 Hz, $J$ = 7.2 Hz), 11.83 (br, s, 1H, NH), 12.39 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.46 ($CH_2\underline{C}H_3$), 15.82 (C-15′), 27.30 (C-15), 28.01 ($\underline{C}H_2CH_3$), 67.57 (C-8), 114.60 (C-10, C-14), 121.58, 123.46, 125.73, 127.74, 128.35, 128.42, 128.50, 128.64 (C-11, C-13), 130.97, 133.45, 135.81, 136.19, 137.88, 144.39 (C-19), 156.33 (C-9), 170.34 (C-1), 178.89 (C-16) |
| *Vi* | IR, $\tilde{\nu}$/cm$^{-1}$: 541 (w), 604 (w), 638 (w), 666 (w), 737 (m), 808 (w), 833 (m), 852 (w), 885 (w), 959 (w), 1031 (m), 1048 (w), 1066 (w), 1121 (m), 1145 (s), 1178 (w), 1224 (s), 1263 (w), 1302 (m), 1344 (m), 1453 (m), 1508 (vs), 1555 (m), 1600 (m), 1671 (m), 2870 (w), 2928 (m), 2963 (m), 3039 (w), 3352 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.12 (t, 3H, $CH_2C\underline{H}_3$, $J$ = 7.6 Hz), 1.19 (t, 3H, H-15′, $J$ = 7.6 Hz), 2.50 (q, 2H, H-15, $J$ = 7.6 Hz), 2.61 (q, 2H, $C\underline{H}_2CH_3$, $J$ = 7.6 Hz), 5.27 (s, 2H, H-8), 6.90 (d, 2H, H-10, H-14, $J$ = 8.6 Hz), 7.09 (d, 2H, H-11, H-13, $J$ = 8.6 Hz), 7.23 (d, 2H, H-19, H-21, $J$ = 8.2 Hz), 7.46 (td, 1H, H-6, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.49 (d, 2H, H-18, H-22, $J$ = 8.2 Hz), 7.55 (td, 1H, H-5, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 7.58 (dd, 1H, H-4, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.62 (dd, 1H, H-7, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 11.81 (br, s, 1H, NH), 12.37 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.54 ($CH_2\underline{C}H_3$), 15.85 (C-15′), 27.31 (C-15), 27.74 ($\underline{C}H_2CH_3$), 67.53 (C-8), 114.59 (C-10, C-14), 124.23, 127.73, 127.87, 128.34, 128.52, 128.64 (C-11, C-13), 130.99, 133.41, 135.84, 136.20, 144.39 (C-20), 156.32 (C-9), 170.22 (C-1), 178.90 (C-16) |
| *Vj* | IR, $\tilde{\nu}$/cm$^{-1}$: 431 (w), 458 (w), 481 (w), 507 (w), 536 (w), 602 (m), 640 (m), 674 (w), 746 (s), 794 (w), 807 (w), 827 (m), 852 (m), 920 (m), 946 (w), 1031 (m), 1117 (m), 1148 (m), 1254 (s), 1289 (m), 1306 (m), 1335 (m), 1378 (w), 1439 (s), 1454 (s), 1507 (vs), 1541 (m), 1681 (m), 2874 (w), 2929 (w), 2963 (m), 3030 (w), 3163 (w), 3415 (s), 3485 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.09 (t, 3H, H-15′, $J$ = 7.5 Hz), 1.29 (t, 3H, $OCH_2C\underline{H}_3$, $J$ = 7.0 Hz), 2.49 (q, 2H, H-15, $J$ = 7.4 Hz), 4.05 (q, 2H, $OC\underline{H}_2CH_3$, $J$ = 7.0 Hz), 5.25 (s, 2H, H-8), 6.86 (d, 2H, H-10, H-14, $J$ = 8.6 Hz), 6.97 (m, 1H, H-21), 7.03 (d, 2H, H-11, H-13, $J$ = 8.6 Hz), 7.08 (dd, 1H, H-19, $J$ = 1.4 Hz, $J$ = 8.2 Hz), 7.18 (m, 1H, H-20), 7.46 (td, 1H, H-6, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.55 (td, 1H, H-5, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 7.58 (dd, 1H, H-4, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.62 (dd, 1H, H-7, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 8.76 (dd, 1H, H-22, $J$ = 1.7 Hz, $J$ = 8.2 Hz), 11.78 (br, s, 2H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 14.09 ($OCH_2\underline{C}H_3$), 15.43 (C-15′), 26.93 (C-15), 64.11 ($O\underline{C}H_2CH_3$), 67.29 (C-8), 111.98 (C-19), 114.31 (C-10, C-14), 119.40 (C-21), 121.67, 125.86, 127.13, 127.44, 128.18 (C-11, C-13), 133.04, 135.46, 135.85, 149.10 (C-18), 155.96 (C-9), 169.67 (C-1), 176.88 (C-16) |
| *Vk* | IR, $\tilde{\nu}$/cm$^{-1}$: 440 (w), 481 (w), 545 (w), 607 (w), 676 (w), 738 (m), 778 (m), 836 (m), 866 (w), 929 (w), 960 (w), 1027 (m), 1049 (m), 1142 (s), 1165 (m), 1189 (w), 1226 (s), 1251 (m), 1293 (m), 1313 (m), 1347 (w), 1387 (w), 1455 (m), 1506 (vs), 1559 (s), 1593 (m), 1675 (m), 2867 (w), 2926 (w), 2956 (w), 2978 (w), 3031 (w), 3339 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.11 (t, 3H, H-15′, $J$ = 7.5 Hz), 1.33 (t, 3H, $OCH_2C\underline{H}_3$, $J$ = 7.0 Hz), 2.50 (q, 2H, H-15, $J$ = 7.4 Hz), 4.02 (q, 2H, $OC\underline{H}_2CH_3$, $J$ = 7.0 Hz), 5.26 (s, 2H, H-8), 6.82 (dd, 1H, H-20, $J$ = 2.0 Hz, $J$ = 8.2 Hz), 6.89 (d, 2H, H-10, H-14, $J$ = 8.6 Hz), 7.08 (d, 2H, H-11, H-13, $J$ = 8.6 Hz), 7.09 (m, 1H, H-22), 7.28 (t, 1H, H-21, $J$ = 8.2 Hz), 7.35 (t, 1H, H-18, $J$ = 2.0 Hz), 7.46 (td, 1H, H-6, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.55 (td, 1H, H-5, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 7.58 (dd, 1H, H-4, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.62 (dd, 1H, H-7, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 11.83 (br, s, 1H, NH), 12.27 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 14.60 ($OCH_2\underline{C}H_3$), 15.80 (C-15′), 27.28 (C-15), 63.19 ($O\underline{C}H_2CH_3$), 67.53 (C-8), 114.20 (C-20), 114.58 (C-10, C-14), 116.12 (C-18), 125.91, 127.75, 128.37, 128.51, 128.64 (C-11, C-13), 129.45, 131.02, 133.32, 135.83, 136.21, 138.90, 156.31 (C-9), 158.54 (C-19), 170.18 (C-1), 178.70 (C-16) |

**Table 2.** (continued)

| Compound | Spectral data |
|---|---|
| *Vl* | IR, $\tilde{\nu}$/cm$^{-1}$: 444 (w), 492 (w), 522 (w), 539 (w), 563 (w), 620 (w), 645 (w), 673 (w), 735 (w), 766 (s), 823 (m), 859 (w), 921 (w), 952 (w), 1042 (s), 1072 (m), 1116 (s), 1164 (s), 1236 (vs), 1270 (m), 1299 (m), 1334 (m), 1394 (m), 1461 (m), 1508 (vs), 1536 (s), 1592 (m), 1670 (m), 2868 (w), 2974 (m), 3039 (m), 3154 (m)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.12 (t, 3H, H-15′, $J$ = 7.4 Hz), 1.33 (t, 3H, $OCH_2C\underline{H}_3$, $J$ = 7.0 Hz), 2.50 (q, 2H, H-15, $J$ = 7.4 Hz), 4.03 (q, 2H, $OC\underline{H}_2CH_3$, $J$ = 7.0 Hz), 5.26 (s, 2H, H-8), 6.89 (d, 2H, H-10, H-14, $J$ = 8.6 Hz), 6.93 (d, 2H, H-19, H-21, $J$ = 9.0 Hz), 7.10 (d, 2H, H-11, H-13, $J$ = 8.6 Hz), 7.45 (d, 2H, H-18, H-22, $J$ = 9.0 Hz), 7.46 (td, 1H, H-6, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.55 (td, 1H, H-5, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 7.58 (dd, 1H, H-4, $J$ = 2.0 Hz, $J$ = 7.4 Hz), 7.61 (dd, 1H, H-7, $J$ = 1.4 Hz, $J$ = 7.4 Hz), 11.78 (br, s, 1H, NH), 12.27 (br, s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 14.40 ($OCH_2\underline{C}H_3$), 15.56 (C-15′), 27.05 (C-15), 63.04 ($O\underline{C}H_2CH_3$), 67.33 (C-8), 114.02 (C-19, C-21), 114.41 (C-10, C-14), 125.62 (C-18, C-22), 127.49, 128.13, 128.22, 128.39 (C-11, C-13), 130.40, 130.74, 133.17, 135.58, 136.01, 156.09 (C-20), 156.49 (C-9), 169.88 (C-1), 178.78 (C-16) |
| *Vm* | IR, $\tilde{\nu}$/cm$^{-1}$: 498 (w), 544 (w), 613 (w), 649 (w), 739 (m), 818 (w), 872 (w), 944 (w), 1002 (m), 1028 (m), 1054 (w), 1078 (w), 1117 (m), 1163 (m), 1241 (s), 1324 (w), 1390 (w), 1451 (m), 1481 (m), 1518 (vs), 1584 (w), 1607 (w), 1688 (m), 1876 (m), 2864 (w), 2922 (w), 2954 (w), 3024 (w), 3175 (m)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.10 (t, 3H, H-15′, $J$ = 7.5 Hz), 2.49 (q, 2H, H-15, $J$ = 7.5 Hz), 5.24 (s, 2H, H-8), 6.87 (d, 2H, H-10, H-14, $J$ = 8.5 Hz), 7.07 (d, 2H, H-11, H-13, $J$ = 8.5 Hz), 7.40 (d, 2H, H-18, H-22, $J$ = 8.7 Hz), 7.39–7.60 (m, H-4, H-7), 7.73 (d, 2H, H-19, H-21, $J$ = 8.7 Hz), 11.87 (s, 1H, NH), 12.36 (s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.73 (C-15′), 27.30 (C-15), 67.72 (C-8), 90.87 (C-20), 114.74 (C-10, C-14), 126.35 (C-18, C-22), 126.47 (C-4), 127.87 (C-6), 128.46 (C-7), 128.53 (C-11, C-13), 131.14 (C-5), 131.27 (C-2), 135.88 (C-12), 137.10 (C-17), 137.41 (C-19, C-21), 143.32 (C-3), 156.37 (C-9), 170.13 (C-1), 178.74 (C-16) |
| *Vn* | IR, $\tilde{\nu}$/cm$^{-1}$: 546 (w), 611 (w), 652 (w), 708 (m), 737 (m), 828 (w), 851 (w), 879 (w), 950 (w), 1031 (w), 1078 (w), 1111 (m), 1161 (m), 1235 (s), 1314 (w), 1337 (w), 1394 (w), 1452 (s), 1513 (vs), 1607 (m), 1690 (m), 1894 (m), 2868 (w), 2923 (w), 2956 (w), 3019 (w), 3181 (w)<br>$^1$H NMR (DMSO-$d_6$), $\delta$: 1.07 (t, 3H, H-15′, $J$ = 7.4 Hz), 2.48 (q, 2H, H-15, $J$ = 7.4 Hz), 5.26 (s, 2H, H-8), 6.87 (d, 2H, H-10, H-14, $J$ = 8.7 Hz), 7.05 (d, 2H, H-11, H-13, $J$ = 8.7 Hz), 7.42–7.65 (m, H-4, H-7), 7.96 (d, 2H, H-18, H-22, $J$ = 9.2 Hz), 8.25 (d, 2H, H-19, H-21, $J$ = 9.2 Hz), 12.04 (s, 1H, NH), 12.70 (s, 1H, NH)<br>$^{13}$C NMR (DMSO-$d_6$), $\delta$: 15.73 (C-15′) 27.29 (C-15), 67.65 (C-8), 114.68 (C-10, C-14), 123.90 (C-18, C-22), 124.27 (C-20), 127.88 (C-6), 128.52 (C-7), 128.66 (C-11, C-13), 128.71 (C-4), 131.25 (C-5), 133.20 (C-2), 135.98 (C-12), 136.38 (C-17), 143.00 (C-19, C-21), 143.91 (C-3), 156.34 (C-9), 170.15 (C-1), 179.15 (C-16) |

**Table 3.** Antimicrobial activity (MIC/(μg mL$^{-1}$)) of acylthioureas *Va*–*Vn*

| Microorganisms | Compound | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Va* | *Vb* | *Vc* | *Vd* | *Ve* | *Vf* | *Vg* | *Vh* | *Vi* | *Vj* | *Vk* | *Vl* | *Vm* | *Vn* | Standard |
| *S. aureus* 1263 | 62.5 | 125 | 125 | 125 | 125 | 62.5 | 125 | 500 | 125 | 500 | 500 | 500 | 125 | 125 | 256[a] |
| *S. aureus* IC 13204 | 62.5 | 125 | 125 | 125 | 125 | 125 | 125 | 500 | 500 | 500 | 500 | 500 | 250 | 250 | 16[a] |
| *B. subtilis* IC 12488 | 62.5 | 250 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 250 | 250 | 16[a] |
| *K. pneumoniae* 1204 | 1000 | 125 | 62.5 | 125 | 1000 | 125 | 125 | 1000 | 125 | 1000 | 125 | 1000 | 62.5 | 62.5 | 1024[b] |
| *K. pneumoniae* IC 13420 | 125 | 125 | 125 | 1000 | 1000 | 1000 | 125 | 125 | 1000 | 1000 | 1000 | 1000 | 62.5 | 62.5 | 8[b] |
| *E. coli* 13147 | 62.5 | 250 | 62.5 | 62.5 | 125 | 125 | 125 | 125 | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 1024[b] |
| *E. coli* IC 13529 | 62.5 | 125 | 62.5 | 62.5 | 125 | 125 | 125 | 125 | 62.5 | 125 | 125 | 125 | 31.2 | 31.2 | 8[b] |
| *P. aeruginosa* 1246 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 62.5 | 62.5 | 512[b] |
| *P. aeruginosa* IC 13202 | 125 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 125 | 1000 | 1000 | 1000 | 62.5 | 62.5 | 8[b] |
| *C. albicans* 101404 | 62.5 | 62.5 | 31.2 | 31.2 | 62.5 | 31.2 | 31.2 | 31.2 | 31.2 | 125 | 125 | 31.2 | 62.5 | 62.5 | 128[c] |
| *C. albicans* IC 249 | 62.5 | 62.5 | 7.8 | 62.5 | 31.2 | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 62.5 | 32[c] |
| *A. niger* IC 13534 | 15.6 | 15.6 | 31.2 | 31.2 | 31.2 | 31.2 | 62.5 | 62.5 | 15.6 | 15.6 | 31.2 | 31.2 | 62.5 | 62.5 | 5[d] |

*a*) Amikacin; *b*) ceftriaxone; *c*) fluconazole; *d*) voriconazole.

pounds tested exhibited specific antimicrobial activities as far as the microbial spectrum and the MIC value were concerned (Table 3).

Compared with the active-substance charge applied onto standard antibiotic discs (i.e. up to 30 μg per disc), it was considered that a low concentration of 7.8 μg mL$^{-1}$ represented a strong effect and a concentration of 250 μg mL$^{-1}$ represented a moderate effect. All the compounds inhibited bacterial growth at the MIC values ranging between 1000 μg mL$^{-1}$ and 31.2 μg mL$^{-1}$ and showed antifungal activity at the MIC in the range of 125–7.8 μg mL$^{-1}$.

The level of antimicrobial activity of the new 2-((4-ethylphenoxy)methyl)benzoylthiourea derivatives was dependent on the type, number and position of the substituent on the phenyl ring attached to the thiourea nitrogen which probably exhibited a steric and an electronic effect on the nitrogen's electron den-

sity, modifying its capacity to form intramolecular H-bonds, thus influencing the compound's ability to penetrate the microbial cell.

The lowest MIC values, thus the most evident antimicrobial activity against Gram-negative bacterial strains, were noticed for the compound *Vm* with iodine in position 4 and for *Vn* with the nitro group in position 4. These compounds are significantly more active than the antibiotic used as a standard on clinical strains, but they are less efficacious on standard strains. The presence of the iodine substituent in *Vm* produces inductive electron-withdrawing and resonance electron-donating effects, determining a decrease in the nitrogen's electron density. Similarly, the nitro group, by inductive and electron-withdrawing effects, reduces the nitrogen's electron density. These effects are responsible for a better internalisation of the compounds tested through the Gram-negative negatively charged outer membrane.

By contrast, the compounds with electron-donating substituents, like methyl and ethyl, are more active against Gram-positive strains (*S. aureus* and *B. subtilis*). The most efficient compound, exhibiting the lowest MIC values (62.5 μg $mL^{-1}$) against the Gram-positive bacterial strains, was *Va*, with two methyl substituents at positions 2 and 3 of the phenyl ring. Compared with amikacin, the activity of *Va* was better only on the isolated clinical strains.

As for the antifungal activity of the compounds tested, they exhibited higher MIC values against *A. niger*; in comparison with variconazole the most active compounds were *Va*, *Vb*, *Vi*, and *Vj* (MIC 15.6 μg $mL^{-1}$). All the compounds tested proved to be more active against *C. albicans* 101404 strain than fluconazole. With *C. albicans* IC249, only compounds *Vc* and *Ve* were more effective than fluconazole.

The results obtained indicated *Vc* with two methyl substituents as the most active antifungal compound. These data are consistent with previous results (Hammam et al., 2007); they show that electron-donating groups, such as alkyl and alkoxy groups, increase the fungicidal activity, On the other hand, the introduction of electron-withdrawing groups decreases this activity. The specific antifungal activity exhibited by the substances tested could be due to easier internalisation of the compound tested into the eukaryotic pathogens, as compared with the prokaryotic ones, leading to the intracellular accumulation of the antimicrobial substances resulting in an antifungal effect at low MIC values.

Compared with other 2-((4-ethylphenoxy)methyl) benzoylthioureas investigated previously (Limban et al., 2009a), the new compounds proved to be more active antifungals. The introduction of a methyl group into different positions of the benzene ring was not a major determinant in the activity against *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *B. subtilis* but represented an advantage in the activity against *S. aureus* and *C. albicans* IC 249 over the monomethyl derivatives. Replacement of the methoxy group with the ethoxy group increased the efficiency against *E. coli* and *B. subtilis*.

The 4-nitro or 4-iodo substituted compounds (*Vm*, *Vn*) showed improved antimicrobial effect on *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* clinical strains than their 2-((4-methoxyphenoxy)methyl)benzoic acid congeners (Balotescu et al., 2007).

A comparative study of the MIC values indicated that the 2-((4-ethylphenoxy)methyl)benzoic acid thioureides exhibited higher antimicrobial activity against *E. coli*, *B. subtilis*, and *C. albicans* than the 2-((4-chlorophenoxy)methyl)benzoic acid thioureide derivatives, but lower against *S. aureus* (Limban et al., 2008a). Analysis of the effect of the substituent position on the arylthiourea part showed that the *para* position favours the antimicrobial activity against *E. coli* in both series. These 2-((4-ethylphenoxy)methyl)benzoic acid thioureides present better antimicrobial activity when compared with those containing the fluorine atom in position 4 at the phenoxymethyl moiety. In general, the substitution in position 4 of the phenoxymethyl moiety with the ethyl group is better for the antibacterial effect than the substitution with the methyl, methoxy, chlorine or fluorine group. These compounds show an even higher antifungal activity and the same structure-activity relationship.

In the future, we intend to extend our research into this class of (phenoxy)methylbenzoylthioureas by synthesising new alkyl or alkoxy-substituted compounds and metal complexes with these ligands in order to obtain better antifungals and to better understand the SARs in this thioureide class.

## Conclusions

In order to obtain new compounds with improved antimicrobial activity, fourteen new 2-((4-ethylphenoxy)methyl)benzoylthioureas were synthesised. Their structures were identified by spectral (IR, NMR) data and by elemental analysis. The compounds tested exhibited specific antimicrobial activity against different bacterial and fungal strains, recently isolated from clinical samples and exhibiting resistance to conventional antibiotics, with MIC values in a range of 7.8–1000 μg $mL^{-1}$. The level of antimicrobial activity of the new 2-((4- ethylphenoxy)methyl)benzoylthiourea derivatives was dependent on the type, number and position of substituents on the phenyl ring attached to the thiourea nitrogen atom. The iodine and nitro substituents favoured the antimicrobial activity against Gram-negative bacterial strains, while the highest inhibitory effect against Gram-positive and fungal strains was exhibited by the compounds with electron-donating substituents, like methyl and ethyl.

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## References


Balotescu, M. C., Limban, C., Missir, A. V., Chirita, I. C., & Nitulescu, G. M. (2007). The synthesis and biological activities of some new 2-(4-methoxy-phenoxymethyl)benzoic acid thioureides. *Revista de Chimie (Bucureşti)*, *58*, 1064–1068.

Balotescu, M. C., Oprea, E., Petrache, L. M., Bleotu, C., & Lazar, V. (2005). Antibacterial, antifungal and cytotoxic activity of salvia officinalis essential oil and tinctures. *Romanian Biotechnological Letters*, *10*, 2481–2481.

Boşcencu, R., Nacea, V., Limban, C., Missir, A., & Socoteanu, R. (2007). Combinaţii complexe ale unor metale tranziţionale cu noi tioureide $N,N'$-disubstituite. *Revista de Chimie (Bucureşti)*, *58*, 16–19.

Chow, A. W. (1984). U.S. Patent No. 4438135. Washington, D.C., USA: U.S. Patent and Trademark Office.

Clinical and Laboratory Standards Institute (2007). *Performance standards for antimicrobial susceptibility testing.* Seventeenth Informational Supplement M100-S17. Wayne, PA, USA: CLSI.

Cunha, S., Macedo, F. C., Jr., Costa, G. A. N., Rodrigues, M. T., Jr., Verde, R. B. V., de Souza Neta, L. C., Vencato, I., Lariucci, C., & Sá, F. P. (2007). Antimicrobial activity and structural study of disubstituted thiourea derivatives. *Monatshefte für Chemie*, *138*, 511–516. DOI: 10.1007/s00706-007-0600-y.

Desai, K. R., & Desai, P. H. (1989). Synthesis and antibacterial activity of 2,4-bis(4-methoxyanilino)-6-(N-arylthioureido-N'-aminoacyl)-s-triasine derivatives. *Journal of the Institution of Chemists (India)*, *61*, 19–20.

Hammam, A. S., Youssef, M. S. K., Atta, F. M., & Mohamed, T. A. (2007). Synthesis of new triphenodithiazine- and indolocarbazolediones of biological interest. *Chemical Papers*, *61*, 292–299. DOI: 10.2478/s11696-007-0036-1.

Jia, X.-S., Wang, Y.-L., Li, Y.-F., He, F.-S., & Shan, Y.-F. (1993). Synthesis of 1-aroyl-3-(4-benzosulfonamidopyrimidine)-thioureas. *Chinese Journal of Organic Chemistry*, *13*, 250–252.

Kapoor, K. K., Somal, P., Gupta, R., & Kachroo, P. L. (1991). Synthesis and antimicrobial activity of some 5-aryl-2-[N-(5-nitrofurfurylidene)amino]- and 5-aryl-2-(N-thiocarbamoylamino)-1,3,4-oxadiazoles. *Journal of the Indian Chemical Society*, *68*, 104–106.

Ke, S.-Y., & Xue, S.-J. (2006). Synthesis and herbicidal activity of *N*-(*o*-fluorophenoxyacetyl)thioureas derivatives and related fused heterocyclic compounds. *ARKIVOC*, *2006*(x), 63–68.

Kurt, G., Sevgi, F., & Mercimek, B. (2009). Synthesis, characterization, and antimicrobial activity of new benzoylthiourea ligands. *Chemical Papers*, *63*, 548–553. DOI: 10.2478/s11696-009-0055-1.

Lazar, V., Balotescu, M. C., Moldovan, L., Vasilescu, G., Petrache, L. M., Bulai, D., & Cernat, R. (2005). Comparative evaluation of qualitative and quantitative methods used in the study of antifungal and antibacterial activity of hydroalcoholic vegetal extracts. *Romanian Biotechnological Letters*, *10*, 2225–2232.

Limban, C., Balotescu Chifiriuc, M. C., Missir, A. V., Chiriţă, I., & Bleotu, C. (2008a). Antimicrobial activity of some new thioureides derived from 2-(4-chlorophenoxymethyl)benzoic acid. *Molecules*, *13*, 567–580. DOI: 10.3390/molecules13030567.

Limban, C., Missir, A., & Chirita, I. (2004). Noi tioureide ale acidului 2-(fenoximetil)-benzoic. Nota II. *Farmacia*, *52*, 7–12.

Limban, C., Missir, A., & Chirita, I. (2000). Noi tioureide ale acidului 2-(fenoximetil)-benzoic. Nota I. *Farmacia*, *48*, 73–78.

Limban, C., Missir, A. V., Chiriţă, I. C., Bădiceanu, C. D., Drăghici, C., Balotescu, M. C., & Stamatoiu, O. (2008b). New thioureides of 2-(4-methyl-phenoxymethyl)-benzoic and 2-(4-methoxy-phenoxymethyl)-benzoic acids with biological activity. *Revue Roumaine de Chimie*, *53*, 595–602.

Limban, C., Missir, A. V., Chiriţă, I. C., Drăghici, B., Chifiriuc, M. C., & Bleotu, C. (2009b). RO Patent No. 122638. Bucharest, Romania: Romanian State Office for Inventions and Trademarks.

Limban, C., Missir, A. V., Chirită, I. C., Ilie, C., & Căproiu, M. T. (2008c). Studies on synthesis of some novel thioureides of 2-(4-methyl-phenoxymethyl)benzoic acid with antimicrobial activity. *Revista de Chimie (Bucureşti)*, *59*, 1136–1139.

Limban, C., Missir, A., Chirita, I., Nitulescu, G. M., Caproiu, M. T., & Ilie, C. (2008d). The synthesis and characterization of some new thioureides of 2-(4-methyl-phenoxymethyl)benzoic acid with antimicrobial activity. *Revista de Chimie (Bucureşti)*, *59*, 1245–1248.

Limban, C., Missir, A. V., Chirita, I. C., Nitulescu, G. M., Ilie, C., & Caproiu, M. T. (2009a). Some new 2-(4-ethylphenoxymethyl)benzoic acid thioureides: Synthesis and spectral characterisation. *Revista de Chimie (Bucureşti)*, *60*, 657–661.

Müller, J., Limban, C., Stadelmann, B., Missir, A. V., Chirita, I. C., Chifiriuc, M. C., Nitulescu, G. M., & Hemphill, A. (2009). Thioureides of 2-(phenoxymethyl)benzoic acid 4-R substituted: A novel class of anti-parasitic compounds. *Parasitology International*, *58*, 128–135. DOI: 10.1016/j.parint.2008.12.003.

Nacea, V., Boscencu, R., Missir, A., Limban, C., & Barbuceanu, S. (2005). Sinteza şi caracterizarea unor combinaţii complexe ale Cu(I) şi Cu(II) cu noi tioureide $N,N'$-disubstituite. *Revista de Chimie (Bucureşti)*, *56*, 68–71.

National Committee for Clinical Laboratory Standards (2000). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (5th ed.). Approved standard M7-A5. Wayne, PA, USA: NCCLS.

National Committee for Clinical Laboratory Standards (2002). *Reference method for broth dilution antifungal susceptibility testing of filametous fungi.* Approved standard M38-A. Wayne, PA, USA: NCCLS.

Olar, R., Badea, M., Cristurean, E., Lazar, V., Cernat, R., & Balotescu, C. (2005). Thermal behavior, spectroscopic and biological characterization of Co(II), Zn(II) and Pt(II) complexes with N,N-dimethylbiguanide. *Journal of Thermal Analysis and Calorimetry*, *80*, 451–455. DOI: 10.1007/s10973-005-0676-8.

Saeed, A., Rafique, H., Hameed, A., & Rasheed, S. (2008). Synthesis and antibacterial activity of some new 1-aroyl-3-(substituted-2-benzothiazolyl)thioureas. *Pharmaceutical Chemistry Journal*, *42*, 191–195. DOI: 10.1007/s11094-008-0094-x.

Saeed, A., Shaheen, U., Hameed, A., & Naqvi, S. Z. H. (2009). Synthesis, characterization and antimicrobial activity of some new 1-(fluorobenzoyl)-3-(fluorophenyl)thioureas. *Journal of Fluorine Chemistry*, *130*, 1028–1034. DOI: 10.1016/j.jfluchem.2009.09.003.

Sun, C., Huang, H., Feng, M., Shi, X., Zhang, X., & Zhou, P. (2006). A novel class of potent influenza virus inhibitors: Polysubstituted acylthiourea and its fused heterocycle derivatives. *Bioorganic & Medicinal Chemistry Letters*, *16*, 162–166. DOI: 10.1016/j.bmcl.2005.09.033.

Zhong, Z., Xing, R., Liu, S., Wang, L., Cai, S., & Li, P. (2008). Synthesis of acyl thiourea derivatives of chitosan and their

antimicrobial activities in vitro. *Carbohydrate Research*, *343*, 566–570. DOI: 10.1016/j.carres.2007.11.024.

Zikán, V., Sluka, J., & Daněk, J. (1988). CS Patent No. 8800122. Prague, Czechoslovakia: Czechoslovak Office for Trademarks and Inventions.