Journal of Molecular Structure 1016 (2012) 147–154

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

# Synthesis, structure and antimicrobial activity of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione

Denitsa Yancheva [a,*], Lalka Daskalova [a], Emiliya Cherneva [a], Bozhanka Mikhova [a], Aleksandra Djordjevic [b], Zaklina Smelcerovic [c], Andrija Smelcerovic [d]

[a] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Build. 9, 1113 Sofia, Bulgaria
[b] Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia
[c] Center for Biomedicinal Science, Faculty of Medicine, University of Niš, Bulevar Dr. Zorana Djindjica 81, 18000 Niš, Serbia
[d] Department of Chemistry, Faculty of Medicine, University of Niš, Bulevar Dr. Zorana Djindjica 81, 18000 Niš, Serbia

ARTICLE INFO

*Article history:*
Received 9 December 2011
Received in revised form 21 February 2012
Accepted 22 February 2012
Available online 1 March 2012

*Keywords:*
Cyclodidepsipeptide
6-(Propan-2-yl)-3-methyl-morpholine-2,5-dione
Tautomerism
Anion
Antimicrobial activity

ABSTRACT

Cyclodepsipeptides are known to exhibit a broad spectrum of biological activities and present a great potential for pharmacological application. A novel didepsipeptide member of the family, 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione, was synthesized and its structure was confirmed by IR, $^{1}H$ and $^{13}C$ NMR spectral data. The structure and relative stability of the diastereoisomers, tautomers and anionic derivatives of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione were studied by DFT. Different conditions were considered by calculations in gas phase at the B3LYP/6-311++G** level and in polar medium utilizing PCM methods at the same level of theory. In all cases the keto forms were found to be more stable and this form should be expected to exist in real systems. Experimental evidence for this statement was found by the IR spectra measured in KBr, polar and nonpolar solvent. The IR spectroscopy was employed to monitor the formation of anion derivative of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione. The corresponding spectral and structural changes, accompanying the molecule → anion conversion were studied by IR spectra and DFT calculations. The 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione showed antimicrobial activity against four of five tested bacterial strains, being the most effective against *Escherichia coli*.



## 1. Introduction

Cyclodepsipeptides are known to exhibit a broad spectrum of biological activities [1–3]. In this way, they present a great potential for pharmacological application [3,4] yet demonstrated by the clinical trials of Kahalalide [5,6], Romidepsin [7,8], Aplidin [9], PF1022A and Emodepside [10,11], and the laboratory testing of many other compounds [1–4].

Among the large family of cyclodepsipeptides, the simplest members are the cyclodidepsipeptides which have an ester group and an amide group in the same 6-membered ring. They contain only one residue of amino acid and one residue of lactic, α-hydroxyisovaleric or other α-hydroxy acid. Some reports on their immunomodulating [12], anticoagulant [13], and inhibitory activity towards acyl-CoA:cholesterol acyltransferase [14] and α-glucosidase [15–17], were published. However, an extensive study on their structure and biological activity was not carried out until now. In this regard, detailed studies on the conformation and size of the depsipeptide cycle, electronic structure properties, tautomerism, hydrogen bonding and their effect on the biological activities of cyclodidepsipeptide will help to characterize these compounds in view of their pharmacological application.

Many studies relate the mechanism of the biological action of cyclodepsipeptides to their ionophoric properties *i.e.* to the interaction with metal ions [18–23]. The ability to bind to metal ions is important also in view of the interaction of the bioactive compounds with catalytic active sites of metallo-enzymes [24,25], and DNA coordination [26]. On the other side, biological activity of heterocycles depends on prototropic tautomerization [27–30]. All these points should be considered in the characterization of cyclodepsipeptides in order to gain better insight into their biological action and the molecular features governing the biochemical processes involved.

In our previous paper, we reported that three cyclodidepsipeptides containing residues of α-hydroxyisovaleric acid and valine, leucine and isoleucine were found for the first time in natural products, as potential precursors of enniatin B in the pathogenic fungi *Fusarium sporotrichioides*, isolated from the stem of fresh *Hypericum barbatum* Jacq. For identification and confirmation, those compounds were synthesized and studied by IR and DFT methods [31].

* Corresponding author. Tel.: +359 2 9606106; fax: +359 2 8700225.
*E-mail address:* deni@orgchm.bas.bg (D. Yancheva).

0022-2860/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.molstruc.2012.02.057

The present work is focused on the synthesis, structure and antimicrobial activity of a novel cyclodidepsipeptide, 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione (**5a–6d**, Scheme 1), containing an alanine moiety. It is structurally related to the three abovementioned cyclodidepsipeptides, isolated from *Fusarium sporotrichioides*. Its structure was confirmed by IR, $^{1}H$ and $^{13}C$ NMR spectral data. DFT computational methods and experimental IR spectral techniques were employed to investigate the preferred conformations of different diastereomeric structures and the prototropic tautomerism of the compound. The interaction of the cyclodidepsipeptide with sodium ion resulting in the formation of respective anion was monitored by IR spectra in solution. The changes arising from the ion formation were described in terms of structural variations, electron charge distribution over molecular fragments, and IR frequency shifting based on the experimental and theoretical data. The *in vitro* antimicrobial activity of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione was tested against five bacterial strains.

## 2. Experimental

### 2.1. Synthesis

The title compound was synthesized according to the general procedure reported by us earlier [31]. The reaction conditions were slightly modified (Scheme 1) which allowed us to isolate and characterize the noncyclic precursor **3**, as well as to obtain the cyclodidepsipeptide product **5** with higher yields.

The structures of both compounds were confirmed by IR, $^{1}H$ and $^{13}C$ NMR spectral data. The $^{1}H$ spectra indicated the presence of a mixture of two diastereomes in a 3:2 ratio for **3** and in a 4:3 ratio for **5**, respectively. It was previously reported that synthesis of optically active morpholine-2,5-diones, via N-(α-halogenoacyl)-α-amino acids, may result in racemic product [32, and references therein]. During cyclization the stereogenic center adjacent to the halogen atom is involved leading to racemization at $C^{6}$ of the heterocycle [32]. In our case, diastereomeric mixture is present for both the 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5** and its noncyclic precursor **3**. It is an indication that the racemization occurred in a preceding synthetic step – the introduction of the bromine atom in the isovaleryl chloride.

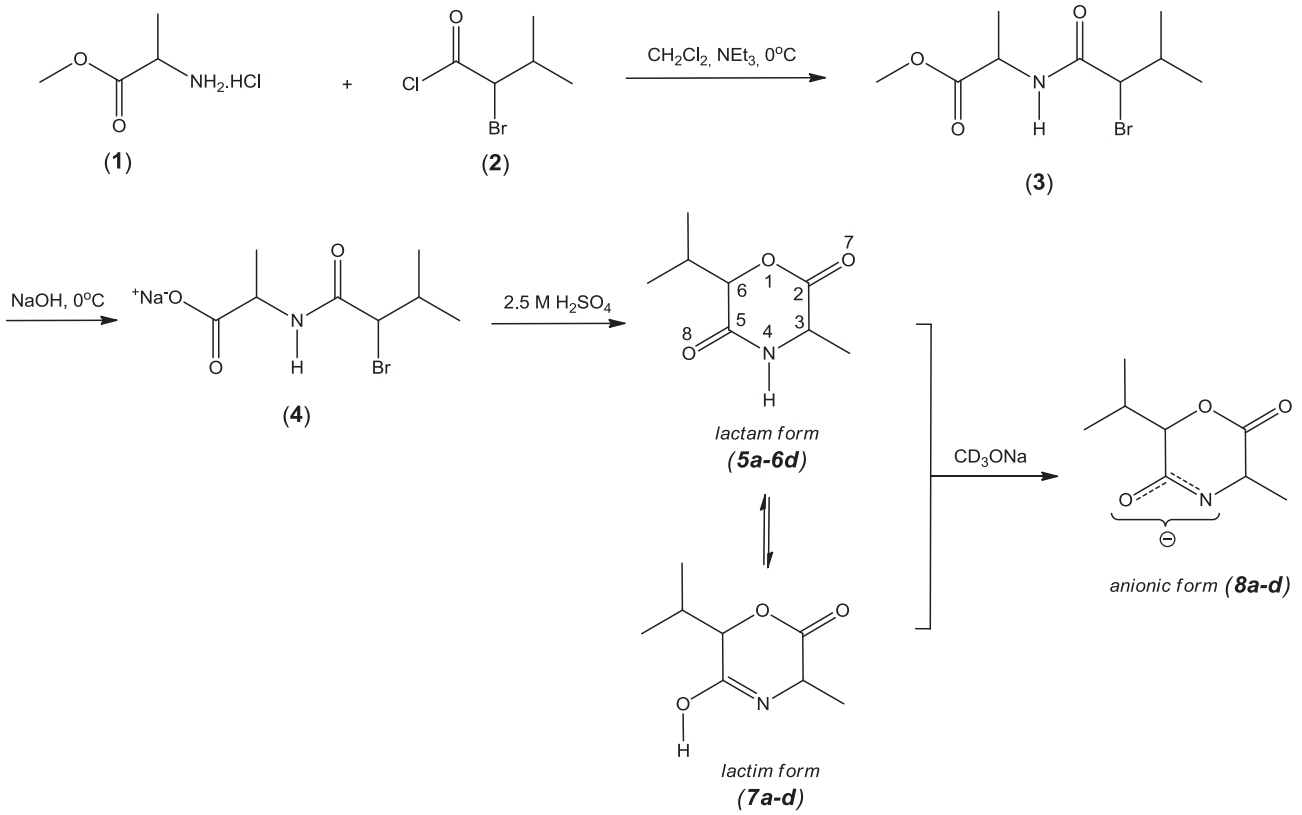
#### 2.1.1. Synthesis of noncyclic methyl 2-(2-bromo-3-methylbutanamido)propanoate (**3**)

L-Alanine methyl ester hydrochloride **1** (0.002 mol) was dissolved in 25 ml dry dichloromethane and 0.006 mol of triethylamine was added. The solution was cooled in ice bath, and 0.003 mol of 2-bromoisovaleryl chloride **2** was added dropwise. The mixture was stirred for 2 h, and then the temperature was allowed to rise to RT. The reaction mixture is washed by 0.5 M HCl, 10% $NaHCO_3$ and brine. The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude methyl 2-(2-bromo-3-methylbutanamido)propanoate **3** was recrystallized from water–methanol mixture (1:4) and light yellow crystals as needles were obtained.

*Methyl 2-(2-bromo-3-methylbutanamido)propanoate* (**3**): $C_9H_{16}BrNO_3$, $M$ = 266.13; yield = 75%; m.p. 58–59 °C; IR (KBr), $cm^{-1}$: 3294, 3076, 2994, 2967, 2935, 2874, 1745, 1652, 1549, 1452, 1437, 1380, 1342, 1320, 1283, 1226, 1191, 1149, 1115, 1072, 1057, 999, 979, 932, 859, 773, 767, 731, 622, 521.

The 1H spectra indicated the presence of a mixture of two diastereomes in a 3:2 ratio.

*2.1.1.1. Major isomer.* $^{1}H$ NMR (250 MHz, $CDCl_3$): $\delta_H$ = 7.10 (1H, br s, NH); 4.58 (1H, m, NCH); 4.33 (1H, d, $J$ = 5.2, CHBr); 3.78 (3H, s, $OCH_3$); 2.41 (1H, m, $CHMe_2$); 1.48 (3H, d, $J$ = 7.2, $NCHC\underline{H}_3$); 1.09




**Scheme 1.** Synthesis of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione and its anion.

(3H, d, $J$ = 6.8, CHC<u>H</u>$_3$); 1.03 (3H, d, $J$ = 6.8 Hz, CHC<u>H</u>$_3$). $^{13}$C NMR (62.8 MHz, $CDCl_3$): $\delta_C$ = 172.8 (COO); 167.9(CON); 60.9 (CHBr); 52.5 ($OCH_3$); 48.6 (NCH); 32.5 (C<u>Me</u>$_2$); 20.84, 18.3 (C<u>Me</u>$_2$); 17.9 (C<u>Me</u>).

*2.1.1.2. Minor isomer.* $^1$H NMR (250 MHz, $CDCl_3$): $\delta_H$ = 7.00 (1H, br s, NH); 4.59 (1H, m, NCH); 4.31 (1H, d, $J$ = 5.2, CHBr); 3.80 (3H, s, $OCH_3$); 2.40 (1H, m, CHO); 1.46 (3H, d, $J$ = 7.2, NCHC<u>H</u>$_3$); 1.08 (3H, d, $J$ = 6.8, CHC<u>H</u>$_3$); 1.01 (3H, d, $J$ = 6.8, CHC<u>H</u>$_3$). $^{13}$C NMR (62.8 MHz, $CDCl_3$): $\delta_C$ = 172.9 (COO); 167.8(CON); 60.6 (CHBr), 52.6 ($OCH_3$), 48.7 (NCH); 32.4 (C<u>Me</u>$_2$); 20.79, 18.4 (C<u>Me</u>$_2$); 18.2 (C<u>Me</u>).

*2.1.2. Cyclization of **3** to 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione (**5**)*

0.002 mol of methyl 2-(2-bromo-3-methylbutanamido)propanoate **3**, dissolved in 2 ml abs. ethanol, and 3 ml 0.5 N NaOH were mixed and cooled in ice. 0.3 ml 5 N NaOH were added and the mixture was stirred for 2 h at 0 °C. The solution was acidified with equimolar amount of 2.5 M $H_2SO_4$ (pH ~ 2), stirred for another 30 min, and extracted with 30 ml dichloromethane (3 portions of 10 ml). The combined organic layers were washed by brine, dried over sodium sulfate and the solvent was removed under reduced pressure. The evaporation yielded small amount of light yellow oil which crystallized on being kept. The crude 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5** was purified by multiple recrystallization from water–ethanol mixture (1:4).

*6-(Propan-2-yl)-3-methyl-morpholine-2,5-dione (**5**)*: $C_8H_{13}NO_3$, $M$ = 171.19; yield = 60%; m.p. 101–102 °C; IR (KBr), cm$^{-1}$: 3288, 3083, 2966, 2924, 2874, 2852, 1718, 1650, 1547, 1457, 1421, 1376, 1338, 1313, 1284, 1231, 1194, 1165, 1119, 1049, 927, 853, 646, 566.

The 1H spectra indicated the presence of a mixture of two diastereomes in a 4:3 ratio.

*2.1.2.1. Major isomer.* $^1$H NMR (250 MHz, $CDCl_3$): $\delta_H$ = 7.33 (1H, d, $J$ = 8.2, NH); 4.59(1H, m, NCH); 4.31 (1H, d, $J$ = 4.3, CHO); 2.36 (1H, m, CHMe$_2$); 1.47 (3H, d, $J$ = 7.2, NCHC<u>H</u>$_3$); 1.05 (3H, d, $J$ = 6.5, OCHC<u>H</u>$_3$); 1.00 (3H, d, $J$ = 6.5, OCHC<u>H</u>$_3$). $^{13}$C NMR (62.8 MHz, $CDCl_3$): $\delta_C$ = 175.6 (COO); 168.7(CON); 59.7 (CHO); 48.65 (NCH); 32.3 (C<u>Me</u>$_2$), 20.57, 18.51 (C<u>Me</u>$_2$); 17.8 (C<u>Me</u>).

*2.1.2.2. Minor isomer.* $^1$H NMR (250 MHz, $CDCl_3$): $\delta_H$ = 7.22 (1H, d, $J$ = 8.2, NH); 4.58 (1H, m, NCH); 4.33 (1H, d, $J$ = 4.3, CHO); 2.37 (1H, m, C<u>H</u>Me$_2$); 1.50 (3H, d, $J$ = 7.2, NCHC<u>H</u>$_3$); 1.06 (3H, d, $J$ = 6.5, OCHC<u>H</u>$_3$); 1.01 (3H, d, $J$ = 6.5, OCHC<u>H</u>$_3$). $^{13}$C NMR (62.8 MHz, $CDCl_3$): $\delta_C$ = 175.7 (COO); 168.8(CON); 60.0 (CHO); 48.60 (NCH); 32.4 (C<u>Me</u>$_2$); 20.60, 18.46 (C<u>Me</u>$_2$); 17.6 (C<u>Me</u>).

*2.1.3. Conversion of 3-methyl-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione into anion (**8**)*

The corresponding anion **8** was obtained by adding a DMSO-$d_6$ solution of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione to excess of dry $CD_3ONa$ ($CD_3ONa$ was preliminary prepared by reacting $CD_3OD$ (Merck, 99% at. enrichment) with Na). The reaction mixture was filtered to remove the excess of solid $CD_3ONa$. The filtered solution was put immediately into a spectroscopic cell and the IR spectra were recorded.

*2.2. IR spectra measurements*

Commercially available spectral quality tetrachlormethane, chloroform, and DMSO-$d_6$, were employed as solvents. The following sample cells were used: 0.6 mm NaCl for tetraclormethane (0.1 M) solution; and 0.125 mm $CaF_2$ for DMSO-$d_6$ (0.1 M) solutions. All IR spectra were recorded on a Brucker Tensor 27 FT spectrometer at a resolution of 2 cm$^{-1}$ and 64 scans. The FT-IR spectra in solid state (in KBr) of **3** and **5** were also measured.

*2.3. NMR spectra measurements*

The NMR spectra were recorded on a Bruker DRX250 spectrometer in solvent $CDCl_3$ using TMS as internal standard. The structures of the investigated compounds were elucidated with the help of 1D and 2D (COSY, HMQC, HMBC) spectra. Standard Bruker pulse sequences and software were used to record and process the spectra.

*2.4. Computations*

All theoretical calculations were performed using the Gaussian 09 package [33] of programs. Geometry and vibrational frequencies of species studied were performed by analytical based gradient technique without any symmetry constraint. All the results were obtained using the density functional theory (DFT), employing the B3LYP (Becke's three-parameter non-local exchange [34] and Lee et al. correlation [33]) potentials. In order to determine the preferred geometry of the compound studied, a large number of probable geometries of the neutral compound in keto form were constructed taking into account the flexibility of the ring system and the change-over to chair- and boat-conformations. For each boat or chair ring conformation, all relevant combinations of axial and equatorial positions of the 3- and 6-alkyl groups were studied. In this way, four diastereoisomeric structures (3R,6R), (3R,6S), (3S,6S), and (3S,6R), were optimized for each ring conformation. Then the same procedure was applied for selecting the most probable geometries for the enol form and anionic derivative. For a better correspondence between experimental and calculated wave values, we modified the results using the empirical scaling factors (0.9688 for B3LYP/6-311++G$^{**}$), reported by Merrick and Radom [35]. To establish the stability order for the neutral diastereoisomers and anions in water and DMSO solution we used the Polarizable Continuum Model (PCM) [36] on the same level of theory.

*2.5. Assay for in vitro antibacterial activity*

The *in vitro* antimicrobial activity of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione sample was tested against a panel of laboratory control strains belonging to the American Type Culture Collection Maryland, USA (except one, belonging to National Collection of Type Cultures, see below). Antibacterial activity was evaluated against two Gram-positive and three Gram-negative bacteria. Gram-positive bacteria used were: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538 while Gram-negative bacteria utilized in the assay were: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017.

The minimal inhibitory concentration (MIC) of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione, against tested bacteria was determined by using a broth microdilution method in 96 multi-well microtitre plates [37]. After overnight cultivation, bacterial suspensions were made in Mueller Hinton broth and their turbidity was standardized to 0.5 McFarland. Dimethyl sulphoxide (10%, v/v aqueous solution) was used to dissolve and to dilute sample. A serial double dilution of the sample was prepared in 96 well microtitre plates, using method of Sarker et al. [38]. A stock concentration was 33 mg/ml. The lowest concentration of the sample that inhibited visible growth was taken as the MIC value. To determine minimal bactericidal concentration (MBC), broth was taken from each well without visible growth and inoculated in Mueller Hinton agar for 24 h at 37 °C. The lowest concentration of the tested sample that killed 99.9% of bacterial cells was evaluated as the MBC value. Two columns in each plate were used

as controls. One column was used as a positive control and contained a broad-spectrum antibiotic (doxycycline in a serial dilution of 200–0.05 μg/ml) to determine the sensitivity of Gram-negative and Gram-positive bacterial species. The other column contained the solvent as negative control. Tests were carried out in triplicate.

## 3. Result and discussion

### 3.1. Energy analysis

Being one of the smallest cyclodepsipeptides, 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione contains in its molecule the functional groups characteristic for those compounds *i.e.* an ester group and a secondary amide function which make possible the formation of lactam (keto) and lactim (enol) tautomeric forms (Scheme 1). The relative stability of the different tautomeric forms is of fundamental importance for the prediction of the probability of mutations in biomechanism [39].

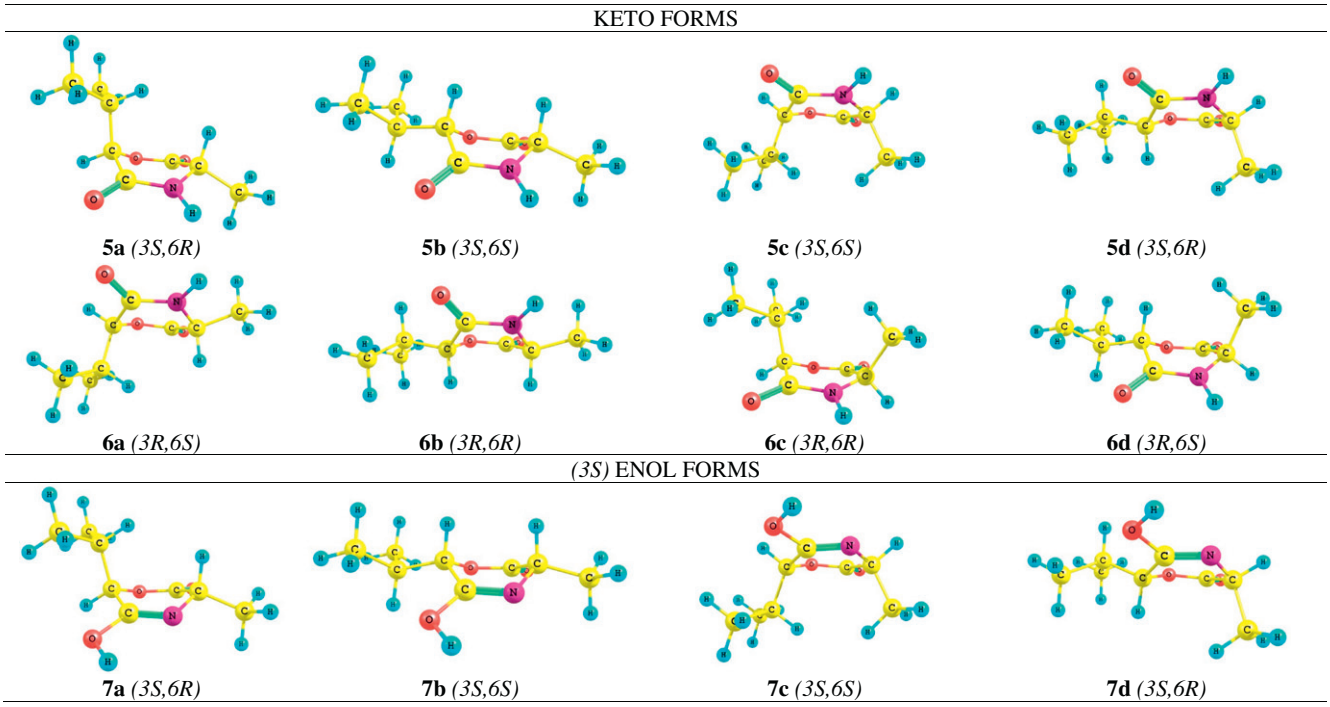
On the other hand, the conformational flexibility of heterocycle and the presence of two stereogenic centers ($C^3$ and $C^6$, for numbering see Scheme 1) give rise to a wide number of possible diastereoisomers and enantiomers. In order to determine the preferred geometry we constructed the most probable structures of keto and enol forms and then optimized their geometries at the B3LYP/6-311++G** level of theory. The optimized species describe all relevant combinations of boat and chair cycle conformations as well as (*R*) and (*S*) configuration of both stereogenic centers. In this way, 16 keto isomers and 16 enol isomers were studied and we found that in all cases the morpholinedione ring adopts boat conformation. Thus the possible isomers were reduced to 8 keto and 8 enol forms. The optimized geometries of the eight keto forms are presented in Scheme 2. They correspond to four enantiomeric pairs of molecules (**5a–d** and **6a–d**).

For illustration of their relative stability, we examined the molecules with (*3S*) configuration. Their total ($E_{tot}$) and relative ($\Delta E$) energies are summarized in Table 1. As it could be seen from the values listed in Table 1, the stability of the keto diastereoisomers (No. 1–4) in gas phase decreases in the following order: **5a** > **5b** > **5c** > **5d**. The energy difference between the most stable and the least stable diastereoisomers is in the range 1.23–6.53 kJ mol$^{-1}$. The molecular structures of **5a–d** were studied also by PCM calculations in water, which is the common medium in real bio-chemical processes, and in DMSO. It was found that the higher polarity of the medium does not influence the stability order of **5a–d**, and the relative energies are in the same range.

Similarly to the keto forms, the most stable among the four corresponding enol diastereoisomers (**7a–d**, Scheme 2) is the one with axial 3-methyl group and equatorial 6-isopropyl group. The relative energies of enol forms **7a–d** calculated at the same level of theory are given in Table 1 (No. 5–8). In all cases the keto form is more stable with 47–56.66 kJ mol$^{-1}$ than the enol configuration. According to Minkin et al. [40] prototropic conversions are probable in case when the energy differences between the initial and the final structure do not exceed 25 kJ mol$^{-1}$, with activation barrier not higher than 105 kJ mol$^{-1}$. The energy difference is larger in the present case and convinces that only keto form should be expected to exist in real systems. However, since the tautomeric equilibria in heterocyclic compounds depend strongly on the medium polarity [41,42], optimization of molecular structures of **7a–d** was also carried out in polar medium. The respective $E_{tot.}$ in water and DMSO show unambiguously that enol structures are highly energetically unfavored even in polar medium. This conclusion is supported by the experimentally measured IR spectra, described in Section 3.2.

Formally both tautomeric forms could be ionized and would produce corresponding azanion or oxanion. In fact, according to Pauling's resonance theory the two structures are canonical forms of the resonance hybrid **8** (Scheme 1), rather than tautomeric forms.

As it can be seen from Table 1, the most stable among the anionic diasereoisomers **8a–d** (No. 9–12) is the one with equatorial configuration of both alkyl substituents. Following the same computational scheme as employed with neutral 6-(propan-2-yl)-3-




**Scheme 2.** Diastereoisomeric keto (**5a–d** and **6a–d**) and enol (**7a–d**) forms of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione.

**Table 1**
Calculated ZPVE-corrected total energies (B3LYP/6-311++G(d,p)) of the species studied. For their structures see Scheme 2.

| No. | Species | Gas phase | | Water medium | | DMSO | |
|---|---|---|---|---|---|---|---|
| | | $E_{tot}$ (Hartree) | $\Delta E$ (kJ mol$^{-1}$) | $E_{tot}$ (Hartree) | $\Delta E$ (kJ mol$^{-1}$) | $E_{tot}$ (Hartree) | $\Delta E$ (kJ mol$^{-1}$) |
| *Keto forms* | | | | | | | |
| 1 | **5a** | −593.095578 | 0.00 | −593.130809 | 0.00 | −593.108487 | 0.00 |
| 2 | **5b** | −593.095108 | 1.23[a] | −593.130108 | 1.84[a] | −593.108144 | 0.90[a] |
| 3 | **5c** | −593.093806 | 4.65[a] | −593.128883 | 5.06[a] | −593.107246 | 3.26[a] |
| 4 | **5d** | −593.093090 | 6.53[a] | −593.126946 | 10.14[a] | −593.105764 | 7.15[a] |
| *Enol forms* | | | | | | | |
| 5 | **7a** | −593.077677 | 47.00[b] | −593.110431 | 53.50[c] | −593.088855 | 51.54[d] |
| 6 | **7b** | −593.075493 | 52.73[b] | −593.109157 | 55.01[c] | −593.087111 | 55.22[d] |
| 7 | **7c** | −593.076704 | 49.55[b] | −593.107764 | 55.45[c] | −593.087575 | 51.65[d] |
| 8 | **7d** | −593.073999 | 56.66[b] | −593.104678 | 58.46[c] | −593.084294 | 56.37[d] |
| *Anionic forms* | | | | | | | |
| 9 | **8a** | −592.547037 | 2.03[e] | −592.652463 | 0.42[e] | −592.629746 | 0.00 |
| 10 | **8b** | −592.547810 | 0.00 | −592.652624 | 0.00 | −592.628400 | 3.53[e] |
| 11 | **8c** | −592.544596 | 8.44[e] | −592.648620 | 10.51[e] | −592.629035 | 1.87[e] |
| 12 | **8d** | −592.544281 | 9.27[e] | −592.646049 | 17.26[e] | −592.628344 | 3.68[e] |

[a] $\Delta E = E_{n(\text{diastereoisomer})} - E_{1(\text{diastereoismer})}$.
[b] $\Delta E = E_n^{\text{gas}_{(\text{enol})}} - E_1^{\text{gas}_{(\text{keto})}}$.
[c] $\Delta E = E_n^{\text{water}_{(\text{enol})}} - E_1^{\text{water}_{(\text{keto})}}$.
[d] $\Delta E = E_n^{\text{DSMO}_{(\text{enol})}} - E_1^{\text{DSMO}_{(\text{keto})}}$.
[e] $\Delta E = E_{n(\text{anion})} - E_{1(\text{anion})}$.

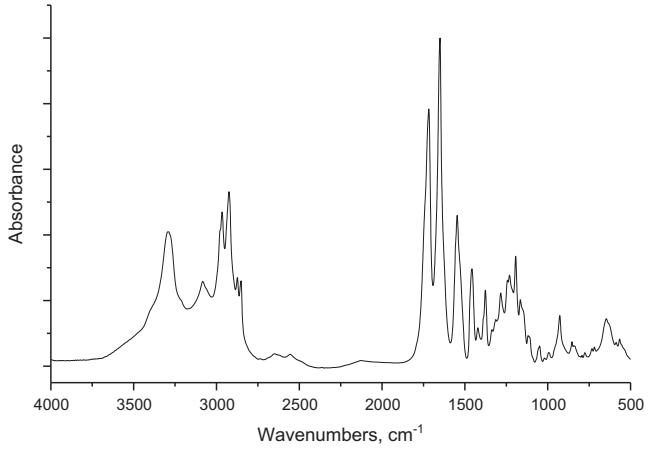



**Fig. 1.** Experimental IR spectrum (4000–500 cm$^{-1}$) of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione in KBr.

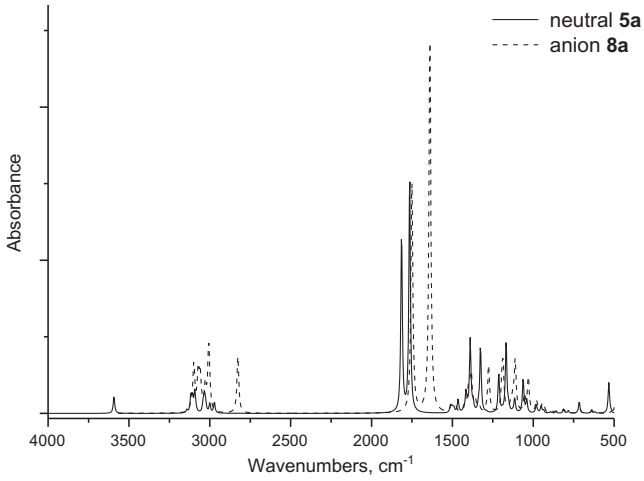



**Fig. 2.** Theoretical (B3LYP/6-311++G$^{**}$) IR spectrum (4000–500 cm$^{-1}$) of the anion **8a** and neutral 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5a** in gas phase.

methyl-morpholine-2,5-dione, we optimized the structures of the anions in water and DMSO. In water medium the stability order does not differ from this one in gas phase, while in DMSO the most stable is diastereisomer **8a**.

**Table 2**
Selected experimental and theoretical IR data for 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5a** and its anion **8a**.

| Neutral compound | | | | | | Approximate description[e] | Anion | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Experimental | | | | B3LYP/6-311++G$^{**}$ | | | Experimental | | B3LYP/6-311++G$^{**}$ | |
| $\nu$[a] (KBr) | $\nu$[a] ($CCl_4$) | $\nu$[a](DMSO-$d_6$) | $A_{rel.}$[b](DMSO-$d_6$) | $\nu$[c] | $A$[d] | | $\nu$[a] (DMSO-$d_6$) | $A_{rel.}$[b] (DMSO-$d_6$) | $\nu$[c] | $A$[d] |
| – | 3406 | – | m | 3406 | 121.6 | $\nu_{NH}$(free) | – | – | – | – |
| 3288 | – | 3267 | s | – | – | $\nu_{NH}$(assoc.) | – | – | – | – |
| 3083 | – | 3055 | w | – | – | Combination of $\delta_{CNH}$ and $\nu_{CO}^{amid.}$ | – | – | – | – |
| 1717 | 1722 | 1722 | s | 1729 | 499.0 | $\nu_{CO}^{ester.}$ | 1669 | vs | 1687 | 651.8 |
| 1650 | 1672 | 1672 | vs | 1674 | 850.1 | $\nu_{CO}^{amid.}$ | 1610 | vs | 1543 | 1125.7 |
| 1546 | 1514 | 1547 | s | 1424 | 35.8 | $\delta_{CNH} + \nu_{CN} + \delta_{CNC}$ | – | – | – | – |

[a] Frequencies in cm$^{-1}$.
[b] Relative intensities: vs very strong; s, strong; m, moderate; w, weak.
[c] Frequencies in cm$^{-1}$, scaled according to [37].
[d] Integrated intensities in km mol$^{-1}$.
[e] Vibration modes: $\nu$, stretching; $\delta$, in-plane bending.

**Table 3**
Selected geometrical parameters (B3LYP/6-311++G(d,p)) of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5a** and its anion **8a**.

| Parameter[a] | Keto form | Anion | $\Delta$[b] |
|---|---|---|---|
| *Bondh length*[c] | | | |
| $C^5N^4$ | 1.359 | 1.317 | −0.042 |
| $C^5O^8$ | 1.219 | 1.256 | 0.037 |
| $C^3H$ | 1.100 | 1.112 | 0.012 |
| $C^2O^7$ | 1.201 | 1.214 | 0.013 |
| $C^6O^1$ | 1.455 | 1.466 | 0.011 |
| $C^5C^6$ | 1.527 | 1.551 | 0.024 |
| *Angle*[d] | | | |
| $C^5N^4C^3$ | 124.54 | 117.20 | −7.34 |
| $O^8C^5N^4$ | 123.81 | 126.94 | 3.13 |
| $N^4C^3C^2$ | 110.62 | 114.25 | 3.63 |

[a] See Scheme 1 for numbering of atoms.
[b] $\Delta$ = Anion-molecule.
[c] In angstroms.
[d] In degrees.

### 3.2. Spectral analysis

The possibility of prototropic tautomerism of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5** was studied by measuring IR spectra in solid state (KBr disc), polar DMSO-$d_6$ and nonpolar $CCl_4$ solvent. The IR spectrum of **5** in KBr recorded in our laboratory is reported in Fig. 1. Selected experimentally observed and the theoretically calculated data are presented in Table 2. It can be seen, there is a good agreement between experimental and scaled theoretical frequencies. An exception is found for $\delta_{CNH}$ vibrations in KBr and DMSO-$d_6$. This deviation is obviously due to the participation of the NH-group in hydrogen bonds.

The spectrum of **5** in KBr shows that the molecule exists in keto form in solid state. The evidences for this statement are:

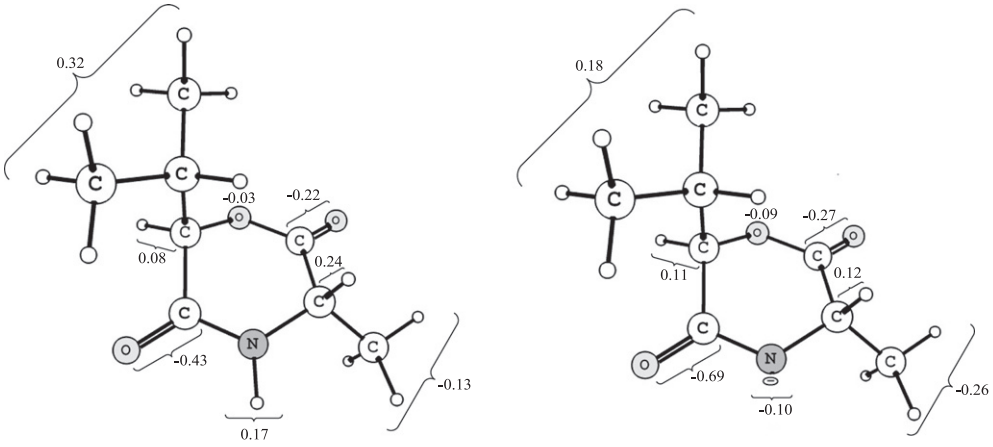
- The absence of the band for $\nu_{OH}$ stretching vibration in the 3600–3500 $cm^{-1}$ region.
- The presence of two intensive bands in 1700–1600 $cm^{-1}$ region, corresponding to $\nu_{CO}^{ester}$ and $\nu_{CO}^{amide}$ stretching vibrations.

The IR spectrum of molecule **5** in DMSO-$d_6$ solution (as in KBr) shows very broad band near 3267 $cm^{-1}$ (3288 $cm^{-1}$ in KBr), that corresponds to hydrogen-bonded NH-groups. Single $\nu_{NH}$ band of free NH-groups appears under other conditions: 3406 $cm^{-1}$ in $CCl_4$ solution. The bands for $\nu_{CO}^{ester}$ and $\nu_{CO}^{amide}$ are observed in DMSO-$d_6$ and $CCl_4$ at identical positions, slightly shifted in comparison to the solid state spectrum. The examination of the IR spectra in DMSO-$d_6$ and $CCl_4$ revealed no evidences for enol formation neither in polar nor in nonpolar solvent.

We used the IR spectroscopy to monitor the formation of the anion of **5** in DMSO-$d_6$ solution. The spectral changes expected, caused by the conversion of the neutral **5** into anion **8**, are illustrated in Fig. 2 by theoretical spectra of species studied in gas phase.

The experimental IR data for the anion as well as the corresponding calculated frequencies and intensities are summarized in Table 2. The bands of the C–H vibrations do not undergo changes. This result is reasonable, as the CH, $CH_2$, and $CH_3$ groups cannot be involved in strong interactions with anionic center. Therefore these vibrations are not included in Table 2.

Strong changes after ionization of the molecule **1** are observed in the range 1720–1540 $cm^{-1}$. The formation of anion caused decrease in both $\nu_{CO}^{ester}$ and $\nu_{CO}^{amide}$ stretching vibrations. The predicted frequency shift for $\nu_{CO}^{ester}$ is 42 $cm^{-1}$, experimentally measured –




**Scheme 3.** B3LYP/6-311++G** Mulliken net electric charges of the fragments of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione **5a** and its anion **8a**.

**Table 4**
Minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations of the 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione.

| Microorganisms | Tested sample (**5**) | | Doxycycline (μg/ml) | DMSO (10%) |
|---|---|---|---|---|
| | MIC (mg/ml) | MBC (mg/ml) | MIC | |
| *B. subtilis* | n.a. | n.a. | 1.56 | n.a. |
| *S. aureus* | 8.25 | 16.50 | 0.78 | n.a. |
| *P. aeruginosa* | 8.25 | 16.50 | 12.50 | n.a. |
| *S. abony* | 8.25 | 16.50 | 6.25 | n.a. |
| *E. coli* | 4.125 | 8.25 | 0.78 | n.a. |

n.a. – not active.

53 $cm^{-1}$, and for $\nu_{CO}^{amide}$: 131 $cm^{-1}$, measured 62 $cm^{-1}$. The formation of anion is also demonstrated by disappearance of the band for $\delta_{CNH}$ in the ion spectrum.

After ionization the rest of the frequencies remain at unchanged positions in the IR spectrum.

### 3.3. Structural analysis

#### 3.3.1. Steric structure

In view of the fact that single crystals of **5** or the respective anionic derivative **8** could not be obtained and analyzed by X-ray analysis, no experimental geometrical data for them are available. In this case the quantum chemical calculations are the best tools to study the structural features of the molecule and anion and the changes of the geometrical parameters caused by the conversion molecule → anion. For this purpose we have performed full geometry optimization of the studied species. The steric parameters (in gas phase) that are the most sensitive to this conversion are given in Table 3. We can state the following comments on the results in this table:

- The strongest bond length changes take place at and next to the azanionic center. They are: 0.042 Å shortening of the $C^5$–$N^4$ bond and 0.037 Å lengthening of the $C^5$=$O^8$ bond. These effects are obviously due to the strong resonance between the azanionic center and carbonyl group.
- The conversion into anion does not lead to significant changes in the bond angles.

#### 3.3.2. Electronic structure

It is known, that charge distribution play an important role to biological activity. That is why, the next step in this study was to determine the charge rearrangement of the species studied after ionization. The Mulliken electronic charges $q_i$ on fragments of the species studied can be seen in Scheme 3.

The charges *changes* $\Delta q_i = q_i(\text{anion}) - q_i(\text{molecule})$ are usually quite informative to show the distribution of the new charge over the corresponding anion [43,44] (and references therein). So, according to the calculations (Scheme 3), 0.52$e^-$ of the anionic charge remained localized at the amide group (0.25$e^-$ of them are localized over the carbonyl group and 0.27$e^-$ at the N atom), and 0.48$e^-$ are delocalized over the rest part of the anion.

### 3.4. Antimicrobial activity

The antimicrobial activity of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione was evaluated against 5 bacterial strains and the results are listed in Table 4. In general, the sample exhibited activity against all bacterial strains (except one – *B. subtilis*), with MIC values ranging from 4.125 to 8.25 mg/ml and MBC from 8.25 to 16.50 mg/ml. Both inhibitory and microbicidal concentrations of the tested sample were the most prominent against *E. coli*, with values two times lower, in comparison with the other bacteria tested (Table 4). The assayed sample was less effective than the antibiotic used as a referent standard (Table 4).

Recently we have shown that two cyclodidepsipeptides, 3,6-di(propan-2-yl)-4-methyl-morpholine-2,5-dione and 3-(2-methylpropyl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione, do not induce the toxicity and mitochondrial membrane potential decrease in rat thymocytes and, for the first time for this group of compounds, do not trigger the significant intracellular reactive oxygen species production and exhibited antibacterial activity. On the other hand, higher concentrations of two studied cyclodidepsipeptides were able to stimulate proliferative activity of thymocytes, with mechanisms not yet known, indicating potential stimulatory effect on the cells of the immune system [45]. In this way, cyclodidepsipeptides may give a promise to be used as antibacterial agents.

## 4. Conclusions

A novel cyclodidepsipeptide, 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione, was synthesized and its structure was confirmed by IR, $^1H$ and $^{13}C$ NMR spectral data. The cyclodidepsipeptide was studied by using Infrared Spectroscopy and Density Functional Theory, and the following conclusions were reached:

(i) The most stable molecular structure of species studied in gas phase and polar medium is **5a** (3S,6R) *i.e.* this one with axial 3-alkyl group and equatorial 6-isopropyl group. The species studied exist in lactam form both in polar and nonpolar medium.
(ii) In DMSO the most stable diastereoisomeric structure of the ion, formed as a result of the interaction with $CD_3ONa$, is similar to the one of the neutral compound. In gas phase and water medium is those with equatorial configuration of both alkyl substituents.
(iii) Ionization of the cyclodidepsipeptide studied leads to shortening of the C–N bond and lengthening of the C=O bond of the amide function. 0.52$e^-$ of the anionic charge are localized at the amide group and 0.48$e^-$ are delocalized over the rest part of the anion.
(iv) The structural changes occurring as a result of the ionization are characterized by strong decrease of the stretching vibrations of both carbonyl groups and by disappearance of the N–H deformation vibration.

6-(Propan-2-yl)-3-methyl-morpholine-2,5-dione displays antimicrobial activity against four bacterial strains, being the most effective against *E. coli*.

## Acknowledgments

The financial support of this work by National Science Fund of Bulgaria (Young Researchers Project DMU-03/66 and Contract RNF01/0110) and Ministry of Education and Science of the Republic of Serbia (Project OI 172044) is gratefully acknowledged.

## References

[1] C.E. Ballard, H. Yu, B. Wang, Curr. Med. Chem. 9 (2002) 471.
[2] J. Jimeno, G. Faircloth, J.M. Fernández Sousa-Faro, P. Scheuer, K. Rinehart, Mar. Drugs 2 (2004) 14.
[3] F. Sarabia, S. Chammaa, A.S. Ruiz, L.M. Ortiz, F.J.L. Herrera, Curr. Med. Chem. 11 (2004) 1309.
[4] R. Lemmens-Gruber, M.R. Kamyar, R. Dornetshuber, Curr. Med. Chem. 16 (2009) 1122.
[5] Y. Suárez, L. González, A. Cuadrado, M. Berciano, M. Lafarga, A. Munoz, F. Kahalalide, Mol. Cancer Ther. 2 (2003) 863.
[6] S.G. Gómez, J.A. Bueren, G. Faircloth, L. López-Lázaro, J. Jimeno, B. Albella, Exp. Hematol. 31 (2003) 1104.
[7] D.S. Schrump, M.R. Fischette, D.M. Ngyen, M. Zhao, X. Li, T.F. Kunst, A. Hancox, J.A. Hong, G.A. Chen, E. Kruchin, J.J. Wright, D.R. Rosing, A. Sparreboom, W.D. Figg, S.M. Steinberg, Clin. Cancer Res. 14 (2008) 188.
[8] S. Emanuele, M. Lauricella, G. Tesoriere, Int. J. Oncol. 33 (2008) 637.
[9] J. Caers, E. Menu, H. De Raeve, D. Lepage, E. Van Valckenborgh, B. Van Camp, E. Alvarez, K. Vanderkerken, Br. J. Cancer 98 (2008) 1966.
[10] G. von Samson-Himmelstjerna, A. Harder, N.C. Sangster, G.C. Coles, Parasitology 130 (2005) 343.
[11] G. Altreuther, J. Buch, S.D. Charles, W.L. Davis, K.J. Krieger, I. Radeloff, Parasitol. Res. 97 (2005) 58.
[12] M. Iijima, T. Masuda, H. Nakamura, H. Naganawa, S. Kurasawa, Y. Okami, M. Ishizuka, T. Takeuchi, J. Antibiot. 45 (1992) 1553.
[13] T. Kagamizono, E. Nishino, K. Matsumoto, A. Kawashima, M. Kishimoto, N. Sakai, B.M. He, Z.X. Chen, T. Adachi, S. Morimoto, K. Hanada, J. Antibiot. 48 (1995) 1407.
[14] K. Hasumi, C. Shinohara, T. Iwanaga, A. Endo, J. Antibiot. 46 (1993) 1782.

[15] A. Arcelli, D. Balducci, A. Grandi, G. Porzi, M. Sandri, S. Sandri, Monatsh. Chem. 135 (2004) 951.
[16] A. Arcelli, D. Balducci, A. Grandi, G. Porzi, M. Sandri, S. Sandri, Tetrahedron: Asymm. 16 (2005) 1495.
[17] A. Arcelli, D. Balducci, S. Neto, G. Porzi, M. Sandri, Tetrahedron: Asymm. 18 (2007) 562.
[18] P. Lauge, J. Membrane Biol. 57 (1980) 163.
[19] K. Kouri, M. Lemmens, R. Lemmens-Gruber, Biochim. Biophys. Acta 1609 (2003) 203.
[20] M. Kamyar, P. Rawnduzi, C.R. Studenik, K. Kouri, R. Lemmens-Gruber, Arch. Biochem. Biophys. 429 (2004) 215.
[21] G. Gessner, S. Meder, T. Rink, G. Boheim, A. Harder, P. Jeschke, J. Scherkenbeck, M. Londershausen, Pest. Sci. 48 (1996) 399.
[22] D.M. Ojcius, A. Zychlinsky, L.M. Zheng, J.D. Young, Exp. Cell Res. 197 (1991) 43.
[23] M. Hinaje, M. Ford, L. Banting, S. Arkle, B. Khambay, Arch. Biochem. Biophys. 405 (2002) 73.
[24] A.B. Hughes, in: A.B. Hughes (Ed.), Amino Acids, Peptides and Proteins in Organic Chemistry: Origins and Synthesis of Amino Acids, Wiley-VCH, Weinheim, 2009.
[25] R. Codd, Coord. Chem. Rev. 252 (2008) 1387.
[26] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA, 1994.
[27] M. Brent, J.S. Kwiatkowski, in: G. Szabo (Ed.), Theoretical Chemistry of Biological Systems, Elsevier, Amsterdam, 1986.
[28] L.I. Belenkii, V.N. Gramenitskaya, Yu.B. Evdokimenkova, Adv. Heterocycl. Chem. 102 (2011) 1.
[29] M.M. Shemyakin, V.K. Antonov, A.M. Shkrob, Yu.N. Sheinker, L.B. Senyavina, Tetrahedron Lett. 3 (1962) 701.
[30] Y. Podolyan, L. Gorb, J. Leszczynski, Int. J. Mol. Sci. 4 (2003) 410.
[31] A. Smelcerovic, D. Yancheva, E. Cherneva, Z. Petronijevic, M. Lamshoeft, D. Herebian, J. Mol. Struct. 985 (2011) 397.
[32] Y. Feng, J. Guo, Int. J. Mol. Sci. 10 (2009) 589.
[33] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A1, Gaussian Inc., Wallingford, CT, 2009.
[34] P.J. Stephens, F.J. Devlin, C.F. Chabalowski, M.J. Frisch, J. Phys. Chem. 98 (1994) 623.
[35] J. Merrick, D. Moran, L. Radom, J. Phys. Chem. A 111 (2007) 11683.
[36] J. Tomasi, M. Perisco, Chem. Rev. 94 (1994) 2027.
[37] National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Susceptibility Testing: Eleventh Informational Supplement, M100-S11, National Committee for Clinical Laboratory Standard, Wayne, PA, USA, 2003.
[38] S.A. Sarker, L. Nahar, Y. Kumarasamy, Methods 42 (2007) 321.
[39] K. Senthilkumar, P. Kolandaivel, J. Com-Aided Mol. Des. 16 (2002) 263.
[40] V.I. Minkin, B.Y. Simkin, R.M. Minyaev, Quantum Chemistry of Organic Compounds. Mechanisms of Reactions, Springer, Heidelberg, 1990.
[41] Y.V. Rubin, Y. Morozov, D. Venkateswarlu, J. Leszczynski, J. Phys. Chem. 102 (1998) 2194.
[42] J. Elguero, A.R. Katritzky, O.V. Denisko, Adv. Heterocycl. Chem. 76 (2000) 1.
[43] M.K. Georgieva, I.G. Binev, J. Mol. Struct. 752 (2005) 14.
[44] I.G. Binev, B.A. Stamboliyska, Y.I. Binev, E.A. Velcheva, J.A. Tsenov, J. Mol. Struct. 513 (1999) 231.
[45] V. Pavlovic, A. Djordjevic, E. Cherneva, D. Yancheva, A. Smelcerovic, Food Chem. Toxicol. 50 (2012) 761.