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Antibacterial Activity of Three Synthetic Cyclodidepsipeptides

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Abstract

Three 3,6-disubstituted morpholine-2,5-diones (3,6-dimethylmorpholine-2,5-dione 15a, 3-(1-methylpropyl)-6-(propan-2-yl)-morpholine-2,5-dione 15b and 3-phenyl-6-(propan-2-yl)-morpholine-2,5-dione 15c) were prepared by cyclization using *N*-(α-bromoacyl)-α-amino esters as starting reagents. The structures of the synthesized compounds were established by IR, NMR and theoretical methods. Antimicrobial activities of the studied compounds were evaluated against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*). Compounds 15a and 15c showed an antimicrobial effect against *B. subtilis* with MIC values of 2 mg/ml while compound 15b had no antibacterial effect.
Key words: cyclodidepsipeptides, morpholine-2,5-diones, synthesis, antimicrobial activity

Резюме

Три 3,6-дизаместениморфолин-2,5-диони (3,6-диметилморфолин-2,5-дион 15a, 3-(1-метилпропил)-6-(пропан-2-ил)-морфолин-2,5-дион 15b и 3-фенил-6-(пропан-2-ил)-мирфолин-2,5-дион 15c) бяха получени чрез циклизция, използвайки *N*-(α-бромоацил)-α-амино естерси като изходни реагенти. Структурите на синтезираните съединения бяха охарактеризирани чрез ИЧ, ЯМР и теоретични методи. Антимикробната активност на съединенията беше изследвана срещу два Gram-положителни щама бактерии (*Bacillus subtilis* и *Staphylococcus aureus*), три Gram-отрицателни щама бактерии (*Escherichia coli*, *Pseudomonas aeruginosa* и *Salmonella typhimurium*). Съединение 15a и 15c притежават антимикробна активност спрямо *B. subtilis* с MIC 2 mg/ml, съединение 15b няма антимикробен ефект.

Introduction

Cyclodidepsipeptides are small peptide lactones often isolated from natural sources along with larger cyclodepsipeptides (1-10, Scheme 1) (Woolley *et al*., 1955; Abe *et al*., 1959; Iijima *et al*., 1992; Hasumi *et al.,* 1993; Kagamizono *et al*., 1995; Kuo *et al*., 2002; Oh *et al*., 2002; Suntornchashwej *et al*., 2005; Pettit *et al*., 2010; Smelcerovic *et al*., 2011; Meng *et al*., 2011; Ola *et al.,* 2014; Smelcerovic *et al*., 2014; Huang *et al*., 2017). They have shown intriguing bioactivities (Iijima *et al*., 1992; Hasumi *et al*., 1993; Kagamizono *et al*., 1995; Kuo *et al.,* 2002; Oh *et al*., 2002; Suntornchashwej *et al.,* 2005; Pettit *et al*., 2010; Smelcerovic *et al*., 2011; Meng *et al.,* 2011; Ola *et al*., 2014; Smelcerovic *et al*., 2014; Huang *et al*., 2017) and additionally, they have been explored extensively as monomer units for preparation of synthetic biodegradable polymers (Jörres *et al*., 1998; Feng and Guo, 2009; Yu *et al*., 2012). The pharmacological

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potential of this class of compounds due to their antioxidant and immunomodulatory activities as well as inhibitory activities towards α-glucosidase, acyl-CoA:cholesterol acyltransferase, xanthine oxidase and platelet aggregation were reviewed recently in detail (Smelcerovic *et al*., 2014). Lateritin, denoted in some studies as (*3R,6R*)-bassiatin (**5**), isolated from the fruiting bodies of *Isaria japonica*, has been shown to induce apoptotic cell death of human leukemia cells (HL-60) in a dose-dependent manner (Pettit *et al.,* 2010). It inhibited proliferation and induced apoptosis also of MCF-7 breast cancer cells (Meng *et al*., 2011; Meng *et al*., 2012). Further it was shown that lateritin exhibits cytotoxicity against other human tumor cell lines as well (pancreas BXPC-3, breast MCF-7, CNS SF268, lung-NSC H460, colon KM20L2, and prostate DU-145) (Pettit *et al*., 2010). In a recent study, bassiatin **4** and lateritin **5** were screened for their cytotoxic activities against the human breast cancer cell line MDA-MB-435 and the human lung cancer cell line Calu3 (Huang *et al*., 2017). Interestingly, lateritin **5** exhibited distinguished cytotoxic activities against the cell lines MDA-MB-435 and Calu3, while bassiatin **4** did not show any distinct activity with IC values higher than 50 μM (Huang *et al.,* 2017).

Antimicrobial activity was found for cyclodepsipeptides **7** and **8** (Pavlovic *et al*., 2012), lateritin (Pettit *et al*., 2010) and a synthetic analogue derived from isovaleric acid and alanine **11** (Yancheva *et al*., 2012). The antimicrobial activity of the larger depsipeptides is well known (Sarabia *et al*., 2004; Lemmens-Gruber *et al*., 2009; Andavan and Lemmens-Gruber, 2010).

In the present contribution we report the synthesis and antimicrobial activity of three cyclodidepsipeptides, incorporating residues from lactic and α-hydroxy isovaleric acid and alanine, isoleucine and phenylglycine. Additionally, the physico-chemical properties of the synthesized compounds were calculated.

Materials and Methods

Chemistry

Materials

L-Alanine methyl ester hydrochloride, *L*-phenylglycine methyl ester hydrochloride and *L*-isoleucine methyl ester hydrochloride were purchased from Bachem AG. Dichloromethane and triethylamine were purchased from Sigma-Aldrich Co. (*R,S*)-2-bromopropanoyl chloride *R,S*)-2-bromo-3-methylbutanoyl chloride were obtained by reacting the corresponding acids with thionylchloride and *N*-bromosuccinimide using an earlier reported experimental protocol (Gleason and Harpp, 1970; Smelcerovic *et al*., 2011).

*Synthesis of noncyclic dipeptides (**14a-c**)*

General procedure: Amino acid methyl ester hydrochloride (0.002 mol) was dissolved in 25 mL of dry dichloromethane and 0.006 mol of triethylamine was added. The solution was cooled in an ice bath, and 0.003 mol of (*R,S*)-2-bromo-acyl chloride was added dropwise. The mixture was stirred for 2 h, and then the temperature was allowed to rise to room temperature. The reaction mixture was 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.

methyl-2-(2-bromopropanamido)-propanoate (**14a**)

$C_7H_{12}BrNO_3$, M = 238.08; yield = 25 %; m.p. 90-91 °C; IR (KBr), cm^{-1}: 3317, 2966, 2937, 2879, 1843, 1657, 1535, 1460, 1437, 1377, 1338, 1255, 1205, 1149, 1070, 1016, 987, 941, 874, 758, 665, 615, 542; ^{1}H-NMR (250 MHz, DMSO-d_6): δ (ppm) 8.70 (2H, overl., 2NH), 4.55 (1H, m, H-6_{major}) 4.52 (1H, d, J = 9.0 Hz, H-6_{minor}), 4.25 (2H, m, H-3_{major}, H-3_{minor}), 3.63 (3H, s, OCH_3-10_{minor}), 3.62 (3H, s, OCH_3-10_{major}), 1.64 (3H, d, J = 6.5 Hz, CH_3-11_{minor}), 1.63 (3H, d, J = 6.5 Hz, CH_3-11_{major}), 1.28 (6H, d, J = 6.5 Hz, $2CH_3$-12). Mixture of *two diastereomers* in an approximate proportion of *4:1* as determined from the integrated intensity of the signals. ^{13}C-NMR (62.5 MHz, DMSO-d_6): δ (ppm) 172.8 (C-2_{major}), 172.7 (C-2_{minor}), 169.0 (C-5_{major}), 168.9 (C-5_{minor}), 52.1 (2C-10), 48.0 (C-3_{major}), 47.9 (C-3_{minor}), 43.2 (2C, C-6), 21.6 (2C, C-11), 16.8 (C-12_{minor}), 16.7 (C-12_{major}).

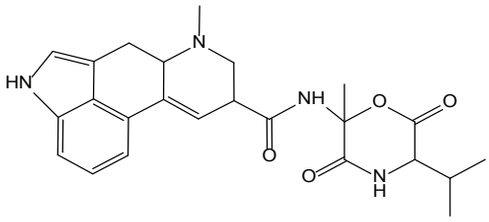
methyl 2-(-2 bromo-3-methylbutanamido)-pentanoate (**14b**)

After multiple recrystallization from methanol/water (4:1) mixture, white solid. $C_{12}H_{22}BrNO_3$; M = 308.21; yield = 62%; m.p. 170-171^{o}C; IR (KBr), cm^{-1}: 3292, 2968, 2936, 2878, 1745, 1654, 1555, 1462, 1424, 1386, 1371, 1336, 1301, 1248, 1212, 1194, 1146, 1115, 1055, 1029, 1010, 947, 851, 782, 743;

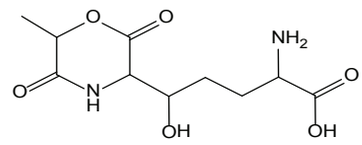
methyl 2-(2-bromo-3-methylbutanamido)-2-phenyl acetate (**14c**): synthetic details are reported in [25].

*Synthesis of 3,6-dimethylmorpholine-2,5-diones (**15a-c**)*

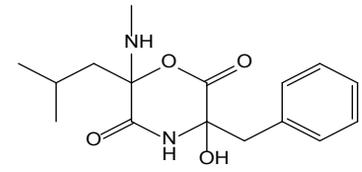
General procedure: 0.002 mol of N-(α-bromoacyl)-α-amino esters (**14a-c**), dissolved in 2 ml of abs. ethanol, and 3 ml of 0.5 M NaOH were mixed and cooled in ice. 0.3 ml of 5 M NaOH



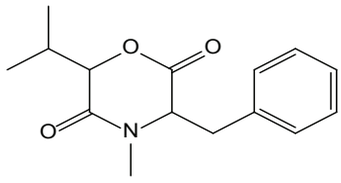
1, Ergosecalinine from *Claviceps purpurea* (Abe *et al*., 1959)



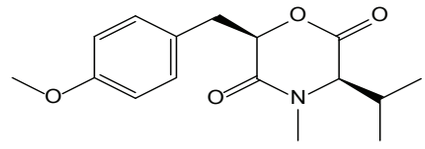
2, from *Pseudomonas tabaci* (Woolley *et al*., 1955)



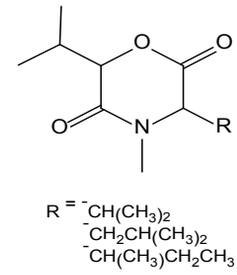
3, Metacytofilin from a fungus *Metarhizium* sp. TA2759 (Iijima *et al*., 1992)



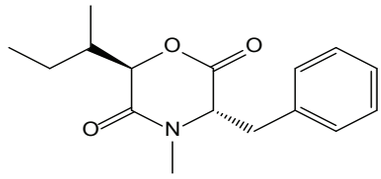
4, (3S,6R)-Bassiatin the broth of *Beauveria bassiana* K-717 (Hasumi *et al*., 1993)
5, Lateritin (Kagamizono *et al.,* 1995, Ola *et al*., 2014), (3R,6R)-Bassiatin (Oh *et al*., 2002; Pettit *et al*., 2010)



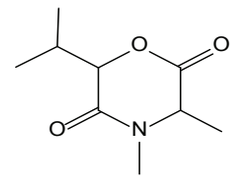
6, from the Thai sea hare, *Bursatella leachii* (Suntornchashwej *et al*., 2005)



7-9, from *Fusarium sporotrichioides*, isolated from the stem of fresh *Hypericum barbatum* Jacq. (Smelcerovic *et al*., 2011)



10, Bassiatin A from *Cordyceps cicada*e Shing is a parasitic fungus on the larvae of *Cicada flammata* Dist.



11, synthetic derivative (Yancheva *et al*., 2012)

Scheme 1. Cyclodidepsipeptides isolated from natural sources and synthetic derivatives.

were added and the mixture was stirred for 2 h at 0°C. The solution was acidified with an equimolar amount of 2.5 M H_2SO_4 (pH 2), and stirred for another 30 min. The mixture was diluted with 25 ml of water and extracted with 4 portions of 20 ml dichloromethane. The combined organic layers were washed by brine, dried over sodium sulfate and the solvent was removed under reduced pressure. The evaporation yielded a small amount of light yellow oil which crystallized upon storage. The crude products were purified by multiple recrystallization from a methanol/water (4:1) mixture.

3,6-dimethylmorpholine-2,5-dione (**15a**)

$C_6H_9NO_3$, M = 143.14; yield = 34 %; m.p. 169-170 °C; IR (KBr), cm^{-1}: 3305, 3066, 2987, 1728, 1546, 1457, 1377, 1342, 1285, 1242, 1195,

1166, 1110, 1085, 1066, 1047, 987, 926, 842, 771, 737, 672, 651, 612, 529.

^{1}H-NMR (250.13 MHz, DMSO-d_6): δ (ppm); 8.54 (2H, d, J = 7.0 Hz, NH), 4.55 (2H, m, 2H-6), 4.18 (2H, m, 2H-3), 1.63 (3H, dd , J = 6.5, 1.5 Hz, CH_3-9_{major}), 1.62 (3H, dd , J = 6.5, 1.5 Hz, CH_3-9_{minor}), 1.27 (6H, d, J = 7.0 Hz, $2CH_3$-10). Mixture of two diastereomers in an approximate proportion of 1.3:1 determined from the integrated intensity of the signals. ^{13}C-NMR (62.5 MHz, DMSO-d_6): δ (ppm) 173.8 (C-2_{minor}), 173.7 (C-2_{major}), 168.9 (C-5_{minor}), 168.6 (C-5_{major}) 47.9 (C-3_{minor}), 47.8 (C-3_{major}), 43.52 (C-6_{minor}), 43.50 (C-6_{major}), 21.8 (C-10_{major}), 21.7 (C-10_{minor}), 17.2 (C-9_{major}), 17.0 (C-9_{minor}).

3-(1-methylpropyl)-6-(propan-2-yl)-morpholine-2,5-dione **(15b):** $C_{11}H_{19}NO_3$; M = 213.27; yield = 55 %; m.p. 88-89 °C; IR (KBr), cm^{-1}: 3341, 2968, 2936, 2878, 1724, 1640, 1548, 1462, 1424, 1386, 1371, 1336, 1288, 1258, 1215, 1194, 1169, 1143, 1115, 1055, 1029, 1010, 947, 929, 851, 782, 743;

^{1}H NMR (250 MHz, DMSO-d_6): δ_H = 8.4 (1H, d, J = 7.0, NH), 8.3 (1H, d, J = 7.0, NH); 4.33 (2H, d, J = 8.0, CHO); 4.2 (2H, m, NCH); 2.08 (2H, m, C<u>H</u>Me_2); 1.8 (2H, m, CH_2C<u>H</u>CH_3); 1.4- 1.1(4H, m, C<u>H</u>$_2$CHCH_3); 1.02 (6H, d, J = 7.0, CH_2CHC<u>H</u>$_3$); 0.90 (6H, d, J = 7.0, CH<u>Me</u>$_2$); 0.87 (6H, d, J = 7.0, CH<u>Me</u>$_2$); 0.84 (6H, t, J = 7.0, CH_2C<u>H</u>$_3$).

Mixture of two diastereomers in an approximate proportion of 1:1.

^{13}C NMR (62.8 MHz, DMSO-d_6): δ_C = 173.11, 173.07 (COO); 169.0, 168.5 (CON); 57.7, 57.4 (CHO); 56.9, 56.8 (NCH); 32.4 (CMe2), 32.4 (CH_2C<u>H</u>CH_3); 24.5 (2xC<u>H</u>$_2$CHCH_3); 20.8,20.7 (CH<u>Me</u>$_2$); 16.1, 16.0 (CH_2CHC<u>H</u>$_3$); 11.7, 11.6 (CH_2C<u>H</u>$_3$).

3-phenyl-6-(propan-2-yl)-morpholine-2,5-dione **(15c):** $C_{13}H_{15}NO_3$; M = 233.26; yield = 60 %; m.p. 112-113 °C; IR (KBr), cm^{-1}: 3332, 3282, 3089, 3065, 3034, 2965, 2931, 2874, 2858, 1723, 1648, 1587, 1543, 1497, 1468, 1456, 1422, 1388, 1371, 1322, 1289, 1255, 1232, 1214, 1183, 1114, 1072, 1030, 1003, 932, 919, 850, 836, 721, 697, 648, 617, 519, 489;

^{1}H NMR (250 MHz, DMSO-d_6): δ_H = 9.04 (1H, d, J = 8.0, NH-major); 8.97 (1H, d, J = 8.0, NH-minor); 7.4 - 7.3 (10H, overlap,Ph,); 5.36 (1H, d, J = 7.5, CHO-major); 5.28 (1H, d, J = 7.5, CHO-minor); 4.39 (1H, d, J = 8.0, NCH-major); 4.37 (1H, d, J = 8.0, NCH-minor); 2.06 (1H, m, C<u>H</u>Me_2-major); 2.11 (1H, m, C<u>H</u>Me_2-minor); 1.00 (3H, d, J = 7.0, CH<u>Me</u>$_2$ -major), 1.05 (3H, d, J = 7.0, CH<u>Me</u>$_2$-minor), 0.82 (3H, d, J = 7.0, CH<u>Me</u>$_2$ -major), 0.97 (3H, d, J = 7.0, CH<u>Me</u>$_2$-minor)

Mixture of two diastereomers in an approximate proportion of 3:2.

^{13}C NMR (62.8 MHz, DMSO-d_6): δ_C = 171.9 (COO-major), 172.0 (COO-minor); 168.2 (CON-major), 168.6 (CON-minor); 137.3 (2C1-Ph); 129.0 (2C2-Ph-major), 129.0 (2C2-Ph-major); 128.5 (2C3-Ph-major), 129.7 (2C3-Ph-major); 127.9 (C4-Ph-major), 128.2 (C4-Ph-major); 57.28 (CHO-major), 57.3 (CHO-minor); 56.8 (2C, NCH); 32.2 (2CMe2); 20.7, 19.8 (CH<u>Me</u>$_2$ -major), 20.8, 20.7 (CH<u>Me</u>$_2$-minor).

IR spectra measurements

The FT-IR spectra were measured in solid state (in KBr) on a Brucker Tensor 27 FT spectrometer at a resolution of 2 cm^{-1} and 64 scans.

NMR spectra measurements

The NMR spectra were recorded on a Bruker DRX 250 spectrometer in solvent DMSO-d_6 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.

Antimicrobial activity

The *in vitro* antimicrobial activity of **15a-c** was tested against a panel of laboratory control strains belonging to American Type Culture Collection Maryland, USA. Antimicrobial activity was evaluated against two Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhimurium* ATCC 14028). The minimal inhibitory concentration (MIC) of samples against the tested bacteria was determined by using a broth microdilution method (National Committee for Clinical Laboratory Standard, 2003). After overnight cultivation, microbial suspensions were made in Mueller Hinton broth and their turbidity was standardised to 0.5 McFarland. Dimethyl sulfoxide (10 %, v/v aqueous solution) was used to dissolve and to dilute the samples. A serial double dilution of the samples was prepared in 96 well microtiter plates, using the method of Sarker *et al.* (Sarker *et al.,* 2007). The lowest concentration of the sample that inhibited visible growth was taken as the MIC value. One row 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 bacteria while the other row contained the solvent as a negative control. Tests were carried out in triplicate.

Geometry optimization

All theoretical calculations were performed using the Gaussian 09 package (Frisch *et al.,* 2009) of programs. Geometry and vibrational frequencies of the species studied were performed by analytical gradient-based 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 (Stephens et al., 1994) and Lee *at al*. correlation (Frisch et al., 2009) potentials and 6-311++G** basis set. 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.

Results and Discussion

Synthesis

The preparation of the cyclodidepsipeptide 15a containing lactic acid and alanine residues was first described by in't Veld *et al*. (1990; 1992) by cyclization of *N*-(α-bromopropionyl)alanine. Two other methods were described by Nakamura *et al.* (1995) and Jörres *et al.* (1998) applying the corresponding *N*-(α-hydroxypropionyl) alanine and its ethyl ester.

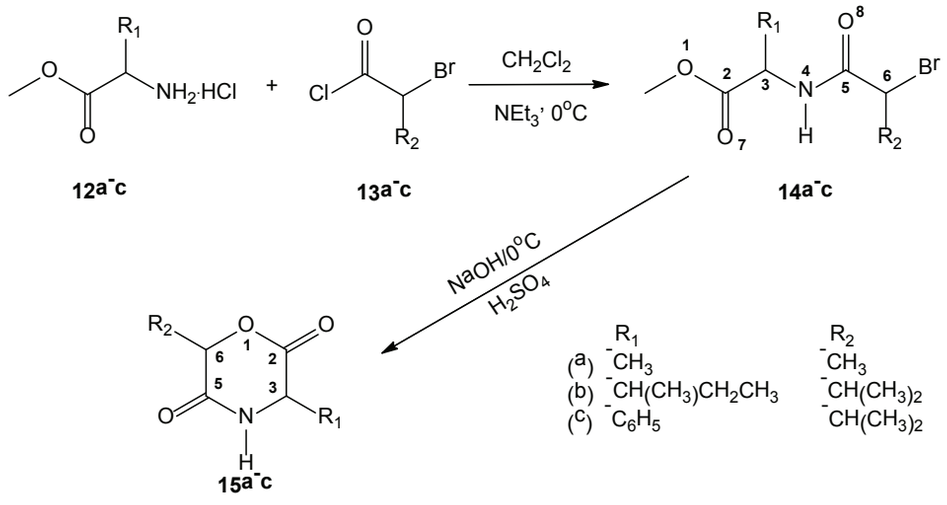
Cyclodidepsipeptide **15c** was prepared previously by a multistep reaction from α-hydroxy isovaleric acid and DL-phenylglycin-trimethylsilylester (Hartwigand Schoellkopf, 1982).

Herein we have applied a synthetic approach route based upon condensation of α-bromo acylchorides with α-amino esters to corresponding *N*-(α-bromoacyl)-α-amino esters and subsequent cyclization to cyclodidepsipeptides. The synthesis of 15a-c is depicted in Scheme 2.

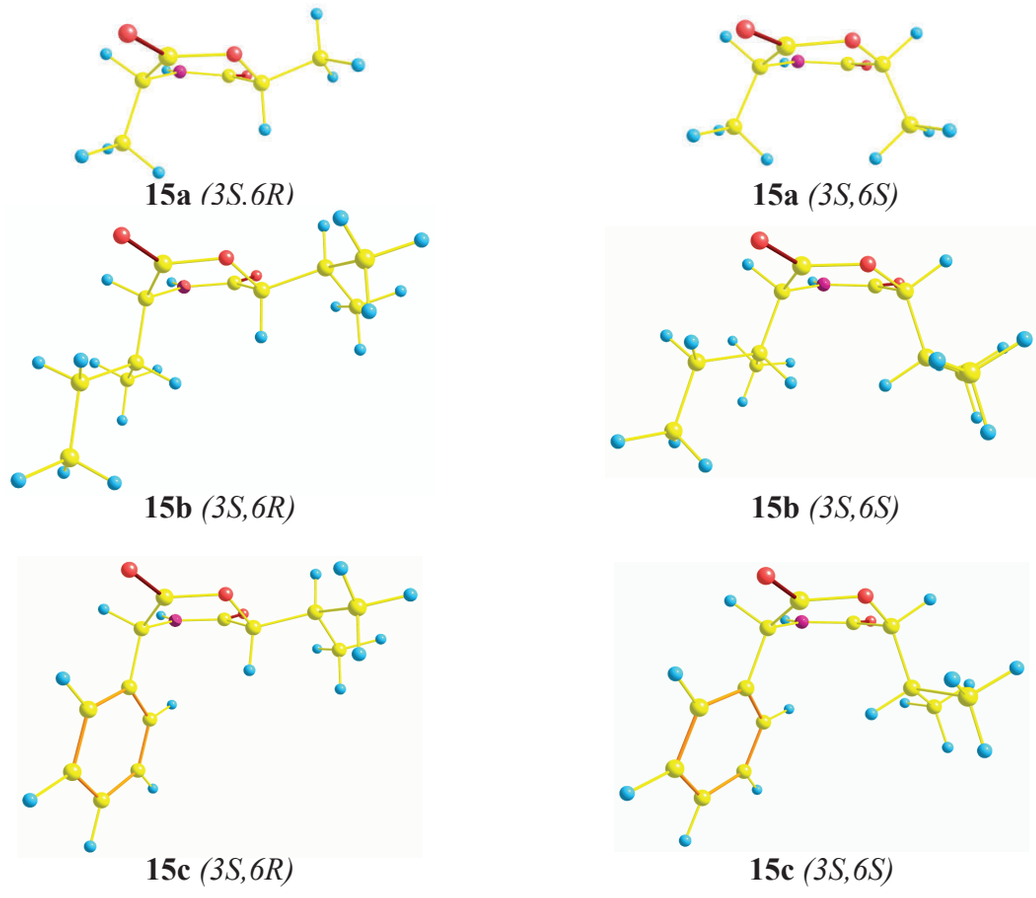
The structures of 14a-c and 15a-c were confirmed with the help of IR, ^{1}H and ^{13}C NMR spectra. The formation of 14a-c was confirmed by the characteristic bands for N-H stretching vibration of the amide groups between 3280 and 3320 cm^{-1}, amide C=O stretching vibration (Amide I) in the interval 1654 - 1657 cm^{-1}; N-H deformation vibration (Amide II) in the interval 1535-1555 cm^{-1}. The stretching vibration of the C-N bond of the amide group occur around 1250 cm^{-1} (Amide III). The ester group is characterized by a very strong band at 1743 cm^{-1} for the carbonyl stretching vibration.

The cyclic products 15a-c were identified as lactam forms, in accordance with the structure given in Scheme 1, based on the presence of two very strong bands at 1728 cm^{-1} (estrer C=O group) and 1652 cm^{-1} (amide C=O group, Amide I), and an intense band at 1548 cm^{-1} (N-H bending vibrations, Amide II). In polar DMSO-d_6 and nonpolar $CDCl_3$ solvent, the compounds were present also in a lactam form.

The ^{1}H and ^{13}C spectra indicated the presence of a mixture of two diastereoisomers for all compounds. Having in mind that the starting amino acid ester has (*S*) configuration and the mechanism of the



Scheme 2. Synthesis of 3,6-dimethylmorpholine-2,5-dione (15a), 3-(1-methylpropyl)-6-(propan-2-yl)-morpholine-2,5-dione (15b) and 3-phenyl-6-(propan-2-yl)-morpholine-2,5-dione (15c).



Scheme 3. Stability of the diastereoisomers optimized at B3LYP/6-311++G** level: (3S,6R) > (3S,6S).

amide group formation does not involve conversion of the stereo configuration of the amino acid residues, it is expected that the stereogenic center C3 retains its initial (*S*) configuration while the stereo configuration of C6, coming from the (*R,S*)-2-bromoacyl chloride, could be either *(R)* or *(S)*. The two forms are expected to differ by the stereo configuration of C6, coming from the (*R,S*)-2-bromoacyl chloride, while the stereogenic center C3, coming from the amino acid ester, is expected to retain its initial (*S*) configuration.

The NMR data of 15a and 15c have been already published (Stephens *et al.,* 1994; Jörres *et al.,* 1998). However, after assigning the 1- and 2D spectra, we believed that some of the data for 15a should be corrected and those for 15c should be complemented.

In the ^{1}H NMR spectra the signals around 8.54-9.00 ppm are assigned to the NH groups. In the range 5.5-3.7 ppm two doublet signals for CH-O and for CH-N were observed, corresponding to the two diastereoisomers. The structure and the presence of diastereoisomeric mixtures were confirmed by ^{13}C NMR spectra, where the signals for the ester and amide carbonyl groups are doubled.

Molecular Structure

The geometry of the diastereoisomers of cyclodidepsipeptides 15a-c was modeled by taking into account *(R)* and *(S)* stereo configuration at C3 and C6 and optimizing the respective forms at B3LYP/6-311++G** level of theory. Based on previous X-ray (Bolte and Egert, 1994; Chisholm *et al*., 2006) and computational (Smelcerovic *et al*., 2011, Yancheva *et al*., 2012) studies, it is known that the morpholine-2,5-dione ring preferably adopts a boat conformation, so in the present study chair conformations of the heterocycle were not taken into account. The optimized geometries of *(3S,6R)* and *(3S,6S)* diastereoisomers of 15a-c, present in their diastereoisomeric mixtures, are shown in Scheme 3.

Antibacterial activity

According to literature data (Sartoratto *et al*., 2004), a compound is considered as a weak antimicrobial agent when its MIC value is above 1.50 mg/ml. Therefore, we decided to examine compounds 15a-c as potential antimicrobial agents, starting with a concentration of 2 mg/ml. Compounds 15a and 15c showed an antimicrobial effect against *B.*

Table 1. Calculated physico-chemical properties of compounds 15a-c, 3 and 5.

Compound	LogP < 5	MW <500	N_{ON} [a] <10	N_{OHNH} [b] <5	N_{rotb} [c]	N_{viol} [d]	Vol.[e]	TPSA
15a	-0.16	143.1	4	1	0	0	127.92	55.40
15b	1.86	213.28	4	1	3	0	211.5	55.40
15c	1.83	233.27	4	5	2	0	216.1	55.40
3[f]	1.84	306.3	6	3	5	0	286.1	87.6
5[g]	2.29	261.3	4	0	3	0	249.9	46.6

[a]number of hydrogen-bond acceptors (O and N atoms); [b]number of hydrogen-bond donors (OH and NH groups); [c]number of rotatable bonds; [d] Number of "Rule of five" violations; [e] molecular volume; TPSA- topological surface area; [f] Metacytofilin (Smelcerovic et al., 2014); [g] Denoted as bassiatin in Smelcerovic et al. (2014).

subtilis with MIC values of 2 mg/ml. They were less effective than the antibiotic used as a reference standard (MIC for doxycycline against *B. subtilis* was 1.56 μg/ml). Compound 15b had no antibacterial effect.

The antibacterial activity of compound 15a is in accordance with our two recent studies (Pavlovic *et al*., 2012, Yancheva *et al*., 2012), in which the activity of three cyclodidepsipeptides, 7, 8 and 11 was tested *in vitro* against the same five bacteria strains. MIC values of the above-mentioned cyclodidepsipeptides ranged between 2 and 25 mg/ml (Pavlovic *et al*., 2012, Yancheva *et al*., 2012). Compound **7** at the dose of 2 mg/ml displayed equal activity against Gram-positive and Gram-negative bacteria (Pavlovic *et al*., 2012). Compound 8 showed a slightly better activity against Gram-positive bacteria in comparison with Gram-negative bacteria, being the most susceptible against *S. aureus* (MIC = 12.50 mg/ml) (Pavlovic *et al*., 2012). The synthetic derivative 11 showed activity against all bacteria except for *B. subtilis*, with minimal inibitory concentration (MIC) values from 4.125 to 8.25 mg/ml and minimal bactericidal concentration (MBC) from 8.25 to 16.50 mg/ml (Yancheva *et al*., 2012). Compound 11 was most active against *E. coli* in comparision with other strains (Yancheva *et al*., 2012).The noncyclic *N*-(α-bromoacyl)-α-amino esters, precursors of compound 15a and 15c, showed no activity against the same five bacteria strains in concentrations 1-2 mg/ml (Yancheva *et al.,* 2015), which indicates that the cyclic didepsipeptide structure is essential for antibacterial activity. Metacytofilin 3, another member of the cyclodidepsipeptide family, did not show any antimicrobial activity against bacteria and fungi at 100 μg/ml (Iijima *et al*., 1992). On the other hand, lateritin **5** isolated from a Mixed Fungal Culture, was found effective against gram-positive bacteria and fungi with MICs ranging between 2 and 16 μg/ml: *Candida albicans* ATCC 90028, 4-8 μg/ml; *Micrococcus luteus* Presque Isle 456, 2-4 μg/mL; *Staphylococcus aureus* ATCC 29213, 4-8 μg/ml; *Enterococcus faecalis* ATCC 29212, 8 μg/ml; and *Streptococcus pneumoniae* ATCC 6303, 8-16 μg/ml. At 64 μg/ml, lateritin did not inhibit the growth of *Cryptococcus neoformans* ATCC 90112, *Stenotrophomonas maltophilia* ATCC 13637, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, or *Neisseria gonorrhoeae* ATCC 49226 (Pettit *et al*., 2010).

The physico-chemical properties of the synthesized compounds (15a-c) and compounds 3, 5 were calculated using Molinspiration tool (Molinspiration Cheminformatics, 2016) (Table 1). The method is very robust and is able to process practically all organic and organometallic compounds.

LogP values and total polar surface area (TPSA) are very important parameters for the prediction of oral bioavailability (Veber *et al*., 2002), drug transport properties such as intestinal absorption (Prasanna *et al.,* 2009) and blood brain barrier penetration. Compounds 15a-c and 3 show LogP values lower than *N*-methylated compound 5. This is in accordance with the known fact that *N*-methylation in peptides leads to increased membrane permeability, proteolytic resistance (Turker *et al*., 1972; Haviv *et al*., 1993; Fairlie *et al*., 1995), and conformational rigidity (Fairlie *et al*., 1995; Cody *et al*., 1997). This better membrane permeability of compound 5 might be connected to the observed differences in antibacterial activity of 15a-c and 3.

On the other hand, in contrast to most of the cyclodidepsipeptides isolated from natural sources which contain *N*-methyl group and C-3 and C-6 substituents originating from amino acids and α-hydroxy carboxylic acids, lateritin 5 has R configuration at both chiral C-atoms. Therefore, it could be inferred that not only the anticancer activ-

ity depends crucially on the stereo configuration of cyclodidepsipeptides (Pettit *et al*., 2010; Ola *et al*., 2014), but also the antimicrobial activity is sensitive to this feature.

Conclusion

3,6-Dimethylmorpholine-2,5-dione (15a), 3-(1-methylpropyl)-6-(propan-2-yl)-morpholine-2,5-dione (15b) and 3-phenyl-6-(propan-2-yl)-morpholine-2,5-dione (15c) were synthesized by cyclization reaction of corresponding *N*-(α-bromoacyl)-α-amino esters. The studied compounds were characterized by spectral techniques such as IR, ^{1}H and ^{13}C NMR / experimental and theoretical methods. Compounds 15a and 15c showed an antimicrobial effect against *B. subtilis* with MIC values of 2 mg/ml while compound 15b had no antibacterial effect.

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References

Abe, M., T. Yamano, S. Yamatodani, Y. Kozu, M. Kosumoto, H. Komatsu, S. Yamada (1959). On the, ergosecaline new peptide-type ergot alkaloids and ergosecalinine. *Bull. Agric. Chem. Soc. Jpn.* **23**: 246-248.

Andavan, G. S., R. Lemmens-Gruber (2010). Cyclodepsipeptides from marine sponges: natural agents for drug research. *Mar. Drugs* **8**: 810-834.

Bolte, M., E. Egert (1994). A 2,5-diketomorpholine. *Acta Crystallograph.* **C50**: 1117-1118.

Chisholm, M. H., J. Galucci, C. Krempner, C. Wiggenhorn (2006). Comments on the ring-opening polymerization of morpholine-2,5-dione derivatives by various metal catalysts and characterization of the products formed in the reactions involving R2 SnX2,whereX= OPr I and R = Bun, Ph and p-Me2NC6H4. *Dalton Trans*. 846–851.

Cody, W. L., J. X. He, M. D. Reily, S. J. Haleen, D. M. Walker, E. L. Reyner, B. H. Stewart, A. M. Doherty (1997). Design of a potent combined pseudopeptide Endothelin-A/Endothelin-B receptor antagonist, Ac-dBhg16-Leu-Asp-Ile-[NMe]Ile-Trp21 (PD 156252): Examination of its pharmacokinetic and spectral properties. *J. Med. Chem*. **40**: 2228-2240.

Feng, Y., J. Guo (2009). Biodegradable polydepsipeptides. *Int. J. Mol. Sci.* **10(2):** 589–615.

Frisch, M. J., 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 (2009). Gaussian 09, Revision A1, Gaussian Inc., Wallingford, CT.

Fairlie, D. P., G. Abbenante, D. R. March (1995). Macrocyclic peptidomimetics: forcing peptides into bioactive conformations. *Curr. Med. Chem*. **2**: 654-686.

Hasumi, K., C. Shinohara, T. Iwanaga, A. Endo (1993). Lateritin, a new inhibitor of acyl-CoA : cholesterol acyltransferase produced by *Gibberella lateritium* IFO 7188 K, *J. Antibiot.* **46**: 1782-1787.

Huang, L. H., Y. X. Chen, J. C. Yu, J. Yuan, H. J. Li, W. Z. Ma, R. Watanapokasin, K. C. Hu, S. I. Niaz, D. P. Yang, W. J. Lan (2017). Secondary metabolites from the marine-derived fungus *Dichotomomyces* sp. L-8 and their cytotoxic activity. *Molecules* **22**, pii: E444.

Hartwig, W., U. Schoellkopf (1982). Asymmetrische synthesen über heterocyclische zwischenstufen, XVI. Enantioselektive synthese von α-alkyl-α-phenylglycinen durch alkylieren von an C-6 chiral substituierten 3,6-dihydro-3-phenyl-2H-1,4-oxazin-2-onen. *Liebigs Ann. Chem*. 1952-1970.

Haviv, F., T. D. Fitzpatrick, R. E. Swenson, C. J. Nichols, N. A. Mort, E. U. Bush, G. Diaz, G. Bammert, A. Nguyen, H. N. Nellans, D. J. Hoffman, E.S. Johnson, J. Greer (1993). Effect of N-methyl substitution of the peptide bonds in luteinizing hormone-releasing hormone agonists. *J. Med. Chem*. **36**: 363-369.

Iijima, M., T. Masuda, H. Nakamura, H. Naganawa, S. Kurasawa, Y. Okami, M. Ishizuka, T. Takeuchi, Y. Iitaka (1992). Metacytofilin, a novel immunomodulator produced by Metarhrizium sp. TA2759. *J. Antibiot*. **45**: 1553-1556.

Gleason, J. G., D. N. Harpp (1970). α-Bromination of acid halides. *Tetrahedron Lett.* **39**: 3431–3434.

Jörres, V., H. Keul, H. Höcker (1998). Polymerization of (3S,6S)-3-isopropyl-6-methyl-2,5- morpholinedione with tin octoate and tin acetylacetonate. *Macromol. Chem. Phys*. **199**: 835-843.

Jörres, V., H. Keul, H. Höcker (1998). Aminolysis of α-hydroxy acid esters with α-amino acid salts; first step in the synthesis of optically active 2,5-morpholinediones. *Macromol. Chem. Phys*. **199:** 825-833.

Kagamizono, T., E. Nishino, K. Matsumoto, A. Kawashima, M. Kishimoto, N. Sakai, B. M. He, Z. X. Chen, T. Adachi, S. Morimoto, K. Hanada (1995). Bassiatin, a new platelet aggregation inhibitor produced by Beauveria bassiana K-717. *J. Antibiot*. **48**: 1407-1412.

Kuo, Y-C., L-C. Lin, M-J. Don, H-F. Liao, Y-p. Tsai, G-H. Lee, C-J. Chou (2002). Cyclodesipeptide and dioxomorpholine derivatives isolated from the insectbody portion of the fungus cordyceps cicadae. *J. Chin. Med*. **13**: 209-219.

Lemmens-Gruber, R., M. R. Kamyar, R. Dornetshuber (2009). Cyclodepsipeptides - potential drugs and lead compounds in the drug development process. *Curr. Med. Chem*. **16**: 1122-1137.

Meng, L., B. Feng, H. Tao, T. Yang, Y. Meng, W. Zhu, C. Huang (2011). A novel antioestrogen agent (3R,6R)-bassiatin inhibits cell proliferation and cell cycle progression by repressing cyclin D1 expression in 17b-oestradiol-treated MCF-7 cells. *Cell Biol. Int*. **35**: 599–605.

Meng, L., H. Tao, G. Dong, T. Yang, W. Zhang, W. Zhu, C. Huang (2012). ERK1/2 and Akt pathway activated during (3R,6R)-bassiatin(1)-induced apoptosis in MCF-7 cells. *Cell Biol. Int*. **36**: 345–348.

Molinspiration Cheminformatics (2016). www.molinspiration.com, Molinspiration property engine v2016.03

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.

Nakamura, C. E., R. D. Cosimo, J. R. Moran (1995). Process for the preparation of 3-, 6-substituted 2,5-morpholinediones. US Patent No. 5466801.

Oh, H., K. Taewan, G. S. Oh, H. O. Pae, K. H. Hong, K. Y. Chai, T. O. Kwon, H. T. Chung, H. S. Lee (2002). (3R,6R)-4-methyl-6-(1-methylethyl)-3-phenylmethyl-perhydro-1,4-oxazine-2,5-dione: an apoptosis-inducer from the fruiting bodies of *Isaria japonica*. *Planta Med*. **68**: 345–348.

Ola, A., A. Aly, W. Lin, V. Wray, A. Debbab (2014). Structural revision and absolute configuration of lateritin. *Tetrahedron Lett*. **55**: 6184–6187.

Pettit, R., G. Pettit, Jung-Ping Xu, C. Weber, L. Richert (2010). Isolation of human cancer cell growth inhibitory, antimicrobial lateritin from a mixed fungal culture. *Planta Med*. **76**(5): 500-501.

Pavlovic, V., A. Djordjevic, E. Cherneva, D. Yancheva, A. Smelcerovic (2012). Stimulatory effect on rat thymocytes proliferation and antimicrobial activity of two 6-(propan-2-yl)-4-methyl-morpholine-2,5-dione. *Food Chem. Toxicol.* **50**: 761-766.

Prasanna, S., R. J. Doerksen (2009). Topological polar surface area: a useful descriptor in 2DQSAR. *Curr. Med. Chem*. **16**: 21–41.

Suntornchashwej, S., N. Chaichit, M. Isobe, K. Suwanborirux (2005). Hectochlorin and Morpholine Derivatives from the Thai Sea Hare, Bursatella leachii. *J. Nat. Prod*. **68**: 951-955.

Smelcerovic, A., D. Yancheva, E. Cherneva, Z. Petronijevic, M. Lamshoeft, D. Herebian (2011). Identification and synthesis of three cyclodidepsipeptides as potential precursors of enniatin B in Fusarium sporotrichioides. *J. Mol. Struct*. **985**: 397-402.

Smelcerovic, A., P. Dzodic, V. Pavlovic, E. Cherneva, D. Yancheva (2014). Cyclodidepsipeptides with a promising scaffold in medicinal chemistry. *Amino Acids*. **46**: 825-840.

Sarabia, F., S. Chammaa, A. Sanchez, L. M. Ortiz, F. Herrera (2004). Chemistry and biology of cyclic depsipeptides of medicinal and biological interest. *Curr. Med. Chem*. **11**: 1309-1332.

Sarker, S. A., L. Nahar, Y. Kumarasamy (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods* **42:** 321-324.

Stephens, P. J., F. J. Devlin, C. F. Chabalowski, M. J. Frisch (1994). Ab initio calculation of vibrational absorption and circular dichroism spectra using density functional force fields. *J. Phys. Chem*. **98**: 11623-11627.

Sartoratto, A., A. Machado, C. Delarmelina, G. M. Figueira, M. C. T. Duarte, V. L. G. Rehder (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in brazil. *Braz. J. Microbiol*. **35**: 275–280.

Turker, R. K., M. M. Hall, M. Yamamoto, C. S. Sweet, F. M. Bumpus (1972). A new, long-lasting competitive inhibitor of angiotensin. *Science* 177: 1203-1205

Veld, P. J. A., P. J. Dijkstra, J. H. van Lochem, J. Feijen (1990). Synthesis of alternating polydepsipeptides by ring-opening polymerization of morpholine-2,5-dione derivatives. *Makromol. Chem.* **191:** 1813-1825.

Veld, P. J. A., P. J. Dijkstra, J. Feijen (1992). Synthesis of biodegradable polyes- teramides with pendant functional groups. *Makromol. Chem*. **193**: 2713- 2730.

Veber, D. F., S. R. Johnson, H-Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple (2002). Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **45:** 2615–2623.

Woolley, D. W., G. Schaffner, A. C. Braun (1955). Studies on the structure of the phytopathogenic toxin of Pseudomonas tabaci. *J. Biol. Chem.* **215:** 485-493.

Yu, Z., B. He, C. Long, R. Liu, M. Sheng, G. Wang, J. Z. Tang, Z. Gu (2012). Synthesis, characterization, and drug delivery of amphiphilic poly{(lactic acid)-co-[(glycolic acid)-alt-(L-glutamic acid)]}-g-poly(ethylene glycol). *Macromol. Res*. **20:** 250-258.

Yancheva, D., L. Daskalova, E. Cherneva, B. Mikhova, A. Djordjevic, Z. Smelcerovic, A. Smelcerovic (2012). Synthesis, structure and antimicrobial activity of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione. *J. Mol. Struct.* **1016:** 147-154.

Yancheva, D., E. Cherneva, M. Quick, B. Mikhova, B. Shivachev, R. Nikolova, A. Djordjevic, M. Untergehrer, G. Jürgenliemk, B. Kraus, A. Smelcerovic (2015). Synthesis, crystal structure and biological activity screening of novel N-(α-bromoacyl)-α-amino esters containing valyl moiety. Acta Chim. Slovenica **62:** 689-699.