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MINIREVIEW ARTICLE

Cyclodidepsipeptides with a promising scaffold in medicinal chemistry

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Abstract Among the large family of cyclodepsipeptides, the simplest members are the cyclodidepsipeptides which have an ester group and an amide group in the same six-membered ring. To point out the pharmacological potential of this class of compounds, the present article reviews structure, isolation, synthesis and biological properties of the known cyclodidepsipeptides. Synthesis of cyclodidepsipeptides is achieved by two general approaches—by initial formation of the amide bond, or initial formation of the ester bond; and subsequent intermolecular cyclization to cyclodidepsipeptide structure. It is closely related to the condensation and ring-closure strategies applied in the preparation of the larger members of the cyclodepsipeptide family. However, due to synthesis of the smaller heretocycles it allows for the use of more versatile building blocks. There are data on antimicrobial, antioxidant and immunomodulatory activities of cyclodidepsipeptides as well as their inhibitory activities toward α-glucosidase, acyl-CoA:cholesterol acyltransferase, xanthine oxidase and platelet aggregation. Because we have recently found that two 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones, as novel non-purine xanthine oxidase inhibitors, may give promise to be used in the treatment of gout, in this review we have included a study of molecular interactions of the selected cyclodidepsipeptides with xanthine oxidase using idTarget web server. Cyclodidepsipeptides showed promising pharmacological activities and meet all criteria for good solubility and permeability. However, further research of their medical application is necessary. In addition to this, the diversity of natural cyclodidepsipeptides, simplicity for synthesis and convenience for rational drug design indicate the cyclodidepsipeptide as promising scaffold in medicinal chemistry.

Keywords Biological activities · Cyclodidepsipeptides · Docking · Drug-likeness · Structure–activity relationships · Synthesis

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Introduction

The family of cyclodepsipeptides comprises natural products and synthetic peptide lactones with at least one ester bond in their skeleton. Biological activity of cyclodepsipeptides is well documented by numerous studies which were reported in several review articles (Sarabia et al. 2004; Lemmens-Gruber et al. 2009; Bagavananthem Andavan and Lemmens-Gruber 2010). In brief, cyclodepsipeptides display a variety of biological effects, such as immunosuppressant, antibiotic, antifungal, antiinflammatory and antitumoral activities (Sarabia et al. 2004;

Lemmens-Gruber et al. 2009; Bagavananthem Andavan and Lemmens-Gruber 2010).

Cyclodidepsipeptides are the simplest among cyclodepsipeptides family containing one residue of amino acid and one residue of lactic, α-hydroxyisovaleric or other α-hydroxy acid. There are reports on their antimicrobial (Pavlovic et al. 2012a; Yancheva et al. 2012) and immunomodulatory (Iijima et al. 1992; Pavlovic et al. 2012a, b) activities. Moreover, cyclodidepsipeptides exhibit inhibitory activities toward acyl-CoA:cholesterol acyltransferase (ACAT) (Hasumi et al. 1993), α-glucosidase (Arcelli et al. 2004, 2005, 2007), xanthine oxidase (Smelcerovic et al. 2013) and also platelet aggregation (Kagamizono et al. 1995).

The aim of this article is to review the literature on chemistry and pharmacological activities of cyclodidepsipeptides. It will focus on the chemical structure, producing organisms, isolation and synthesis of cyclodidepsipeptides, followed by a detailed description of their biological activities. Because two 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones, as novel non-purine xanthine oxidase inhibitors, may give promise to be used in the treatment of gout (Smelcerovic et al. 2013), in this review we have included a study of molecular interactions of the selected cyclodidepsipeptides with xanthine oxidase using idTarget web server. The obtained results are in good correlation with the experimental results (Smelcerovic et al. 2013; Yancheva Pantaleeva et al. 2013). Physico-chemical properties of cyclodidepsipeptides will be calculated using Molinspiration tool (Molinspiration Cheminformatics 2013). Finally, promising pharmacological applications as well as probable future trends in chemistry and pharmacology of cyclodidepsipeptides will be discussed. To the best of our knowledge, this is the first review on chemistry and pharmacology of cyclodidepsipeptides.

Chemistry of cyclodidepsipeptides

Cyclodidepsipeptides from natural sources

Three cyclodidepsipeptides, 3,6-di(propan-2-yl)-4-methyl-morpholine-2,5-dione (**1**), 3-(2-methylpropyl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione (**2**) and 3-(butan-2-yl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione (**3**) (Fig. 1) were found for the first time in the 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 density functional theory calculations and infrared spectroscopy (Smelcerovic et al. 2011).

Metacytofilin (**4**) was isolated from a fungus *Metarhizium* sp. TA2759 in a soil sample which was collected in Enzan city, Yamanashi Prefecture, Japan. Its structure was determined using X-ray crystallographic analysis to be 3α-hydroxy-6β-methylamino-6α-(2-methylpropyl)-3β-phenylmethyl-4H-2,3,5,6-tetrahydro-l,4-oxazine-2,5-dione (Iijima et al. 1992).

Bassiatin (**5a**) was isolated from the broth of *Beauveria bassiana* K-717 found in a soil sample from Yunnan province, China. Its structure was determined using NMR, X-ray crystallographic analysis and chemical synthesis to be (3*S*,6*R*)-4-methyl-6-(l-methylethyl)-3-phenylmethyl-l,4-perhydrooxazine-2,5-dione (Kagamizono et al. 1995). *Cordyceps cicadae* Shing is a parasitic fungus on the larvae of *Cicada flammata* Dist. Both the ascocarps and the insect-body portions are named Chan-hua. Bassiatin (**5a**) and bassiatin A (**6**) have been isolated from the methanol extract of the insect-body portions of Chan-hua (Kuo et al. 2002).

Lateritin (**7**) was isolated from the mycelial cake of *Gibberella lateritium* IFO 7188 (Hasumi et al. 1993), while *syn*-3-isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione (**8**) was isolated from ethyl acetate extract from the Thai sea hare, *Bursatella leachii* (Suntornchashwej et al. 2005). Ergosecalinine (**9**), ergot alkaloid with cyclodidepsipeptide structure, was found in *Claviceps purpurea* (Abe et al. 1959). Moreover, a phytopathogenic toxin (**10**) was isolated from *Pseudomonas tabaci* (Woolley et al. 1955).

Synthesis of cyclodidepsipeptides

The methods used for the synthesis of cyclodidepsipeptides are based on two general approaches (Scheme 1): I—initial formation of the amide bond, or II—initial formation of the ester bond; and subsequent intermolecular cyclization to cyclodidepsipeptide structure. Synthetic protocols and advantages of the different methods are here summarized (Jörres et al. 1998; Vinsova 2001; Feng and Guo 2009).

Following these two approaches, diverse cyclodidepsipeptides were obtained starting from α-amino acids and their α-hydroxy or α-halogeno acid analogs. Many of them were used as monomers in ring-opening polymerization reactions for the preparation of biodegradable medicinal polymers. Usually these cyclodidepsipeptides are not substituted at the N-atom and contain small alkyl substituents at C-6 position of the heterocycle. Certain attempts for the solid phase synthesis of cyclodepsipeptides by multi-component Ugi reaction were also reported (Szardenings et al. 1997) and yielded cyclodidepsipeptides with bulky N-substituents.

The present review focuses on the synthetic routes for preparation of naturally occurring and pharmacologically

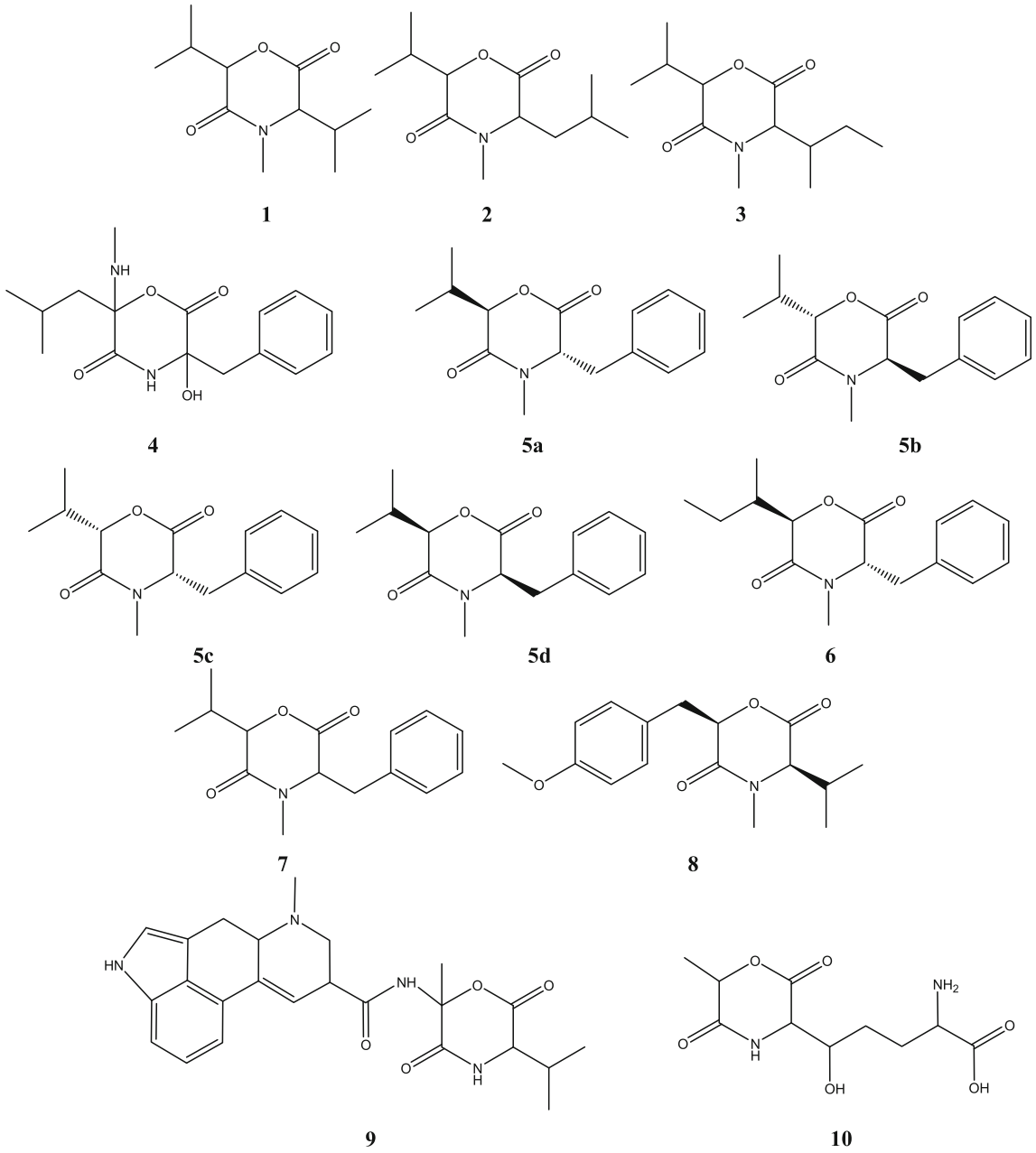
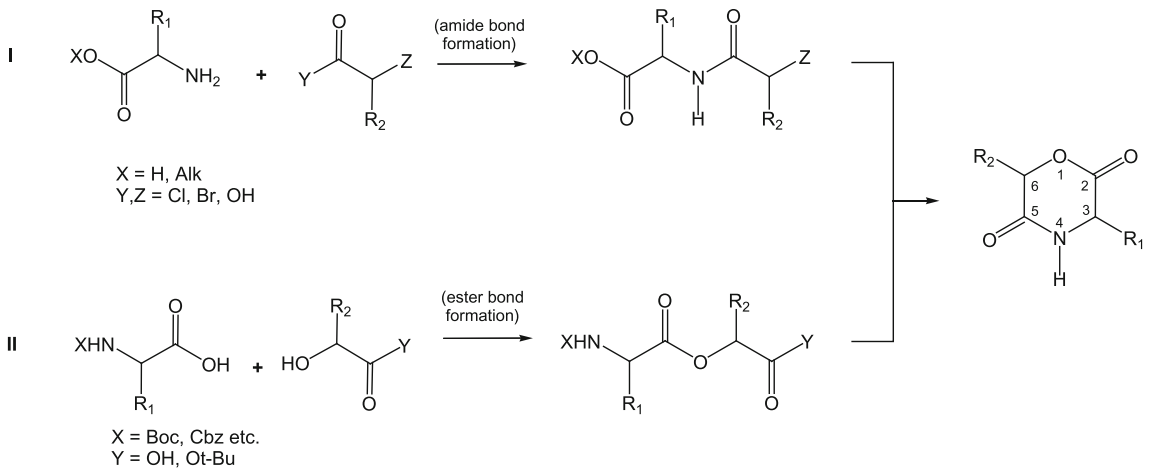


Fig. 1 Structures of cyclodidepsipeptides from natural sources

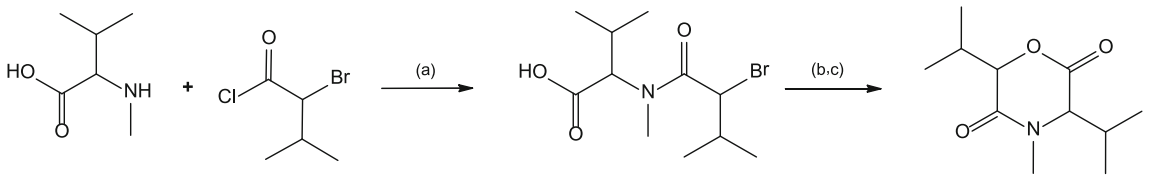
interesting cyclodidepsipeptides. Most of the cyclodidepsipeptides isolated from natural sources contain *N*-methyl group and C-3 and C-6 substituents originating from amino acids and α-hydroxy carboxylic acids.

The synthesis of compound **1** was first reported by Cook and Cox (1949) as a coupling of *N*-methylvaline and 2-bromo-3-methylbutanoyl chloride in dry chloroform cooled in ice (Scheme 2). The linear precursor was isolated after treatment with sodium carbonate, acidification, and extraction procedures. The cyclization was carried out in pyridine/water mixture under reflux.

More recently these synthetic conditions were adjusted and used to obtain compounds **1–3** without isolation of the linear *N*-(α-bromoacyl)-α-amino acids (Smelcerovic et al. 2011). The amidation of 2-bromo-3-methylbutanoyl chloride with large excess of the *N*-methyl amino acids was done according to the original procedure of Cook and Cox. The respective 2-(2-bromo-3-methylbutanoyl(methyl)amino) acids were converted into K^+ salts and after acidification, the corresponding 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones (**1–3**) were directly obtained.



Scheme 1 General approaches for preparation of cyclodidepsipeptides



Scheme 2 **a** Ice bath, dry $CHCl_3$; **b** extraction with Na_2CO_3, acidification, extraction with ether; **c** reflux in pyridine/water

As part of the identification of *Depsilairdin*, a phytotoxin produced by the plant pathogenic fungus *Leptosphaeria maculans*/*Phoma lingam*, Pedras et al. (2004) also synthesized (3*S*,6*R*)-3,6-di(propan-2-yl)-4-methylmorpholine-2,5-dione and its (3*R*,6*S*) and (3*R*,6*R*) stereoisomers. The cyclodidepsipeptides were prepared by initial formation of ester and intramolecular cyclization of the *O*-(α-aminoacyl)-α-hydroxycarboxylic acids (Scheme 3).

Esterification of the *N*-protected *N*-methylvaline with (*R*)- or (*L*)-2-hydroxy-3-methylbutyric acid was assisted by *N*,*N*′-carbonyldiimidazole as a coupling agent in THF at room temperature under argon atmosphere. After removing the protecting group by catalytic hydrogenation, the cyclization was achieved by treatment with 2-chloro-1-methylpyridinium iodide and Et_3N.

In the synthesis of **5a** Kagamizono et al. (1995) used both strategies (formation of either amide or ester) to synthesize the different diastereoisomers (Scheme 4). The formation of ester led to very low yields.

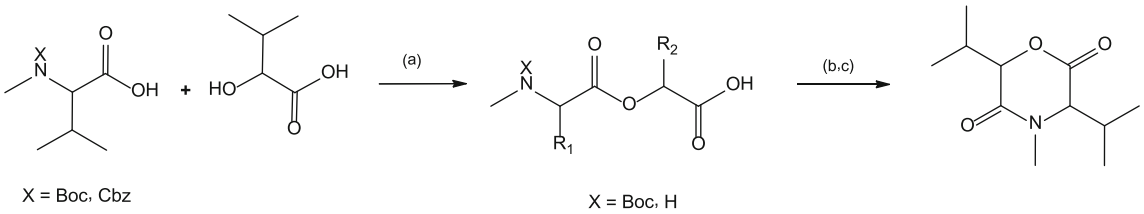
The amide bond was formed by coupling *p*-toluenesulfonyl salt of D-phenylalanine benzyl ester with 2-hydroxy-3-methylbutanoic acid in the presence of *N*-hydroxysuccinimide, water soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and *N*-methylmorpholine in THF. Deprotection of the benzyl esters group was carried out with H_2/Pd–C in EtOH and then the linear product was subjected to cyclization via treatment with *p*-TsOH in benzene. The last step to obtain **5a** was methylation of the cyclic product using CH_3I and NaH.

Alternatively, *N*-carbobenzoxy-*N*-methylphenylalanine was used as a starting material to prepare the (3*S*,6*S*)- and (3*R*,6*R*)-isomers. The esterification was assisted by DCC as a coupling reagent and catalyzed by DMAP. The reaction conditions during the intramolecular cyclization were similar to those applied for the formation of amide in the first approach (Scheme 4).

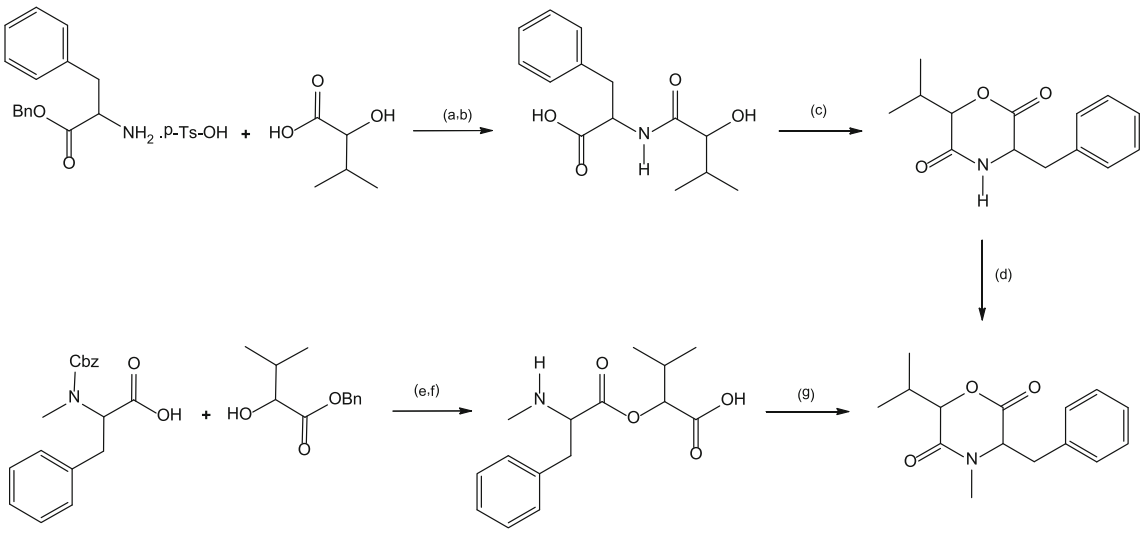
Hughes and Sleebs (2005) also obtained and studied all Bassiatin's diastereoisomers. Their synthetic path included conversion of (*R*)- and (*S*)-valine into the corresponding α-acetoxy acids by diazotization and coupling of the α-acetoxy acids with amino acid esters, as shown in Scheme 5. Further the synthesis proceeded through Mitsunobu cyclization with retention of the configuration.

Cyclodidepsipeptides with larger *N*-substituents were synthesized by Porzi and Sandri (1996) as new α-glucosidase inhibitors, according to a modified reaction scheme (Porzi and Sandri 1996). In this case, the authors explored step-wise condensation of (*S*)-1-phenylethylamine with ethylbromoacetate and 2-chloro propionyl chloride (Scheme 6) to prepare the noncyclic intermediates. The cyclization was achieved by treatment with NaOH and 1 N HCl. The (6*S*) and (6*R*) products of the reaction were further alkylated, epoxidized and converted to dioles to introduce optically active longer hydroxyl containing substituents at 3-position in the heterocycle.

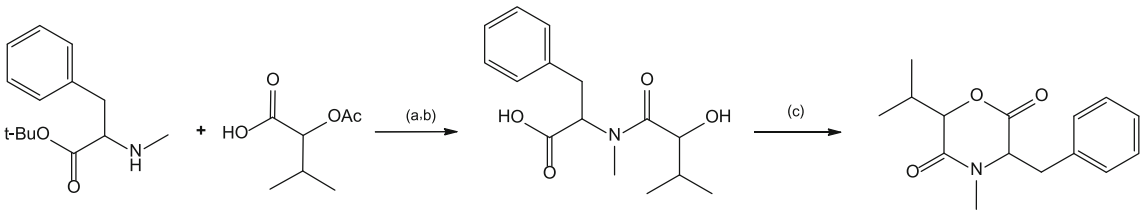
The synthesis of the cyclodidepsipeptides is closely related to the condensation and ring-closure strategies applied in the preparation of the larger members of the cyclodepsipeptide family (Hamada and Shioiri 2005; Stolze and Kaiser 2013). It presents the same challenges and strategies for overcoming the difficulties. In this way, finding efficient methodologies for the ester and amide bond formation by studying the smaller morpholine-2,5-dione systems would contribute to the development of total synthesis of larger pharmacologically active cyclodepsipeptides. Along with the methods, typical for the peptide chemistry, the smaller heterocycles allow synthesis from more versatile building blocks as applied by Porzi and Sandri (1996).



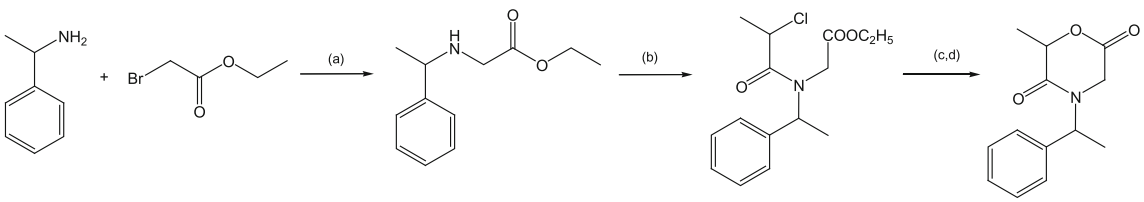
Scheme 3 **a** THF, CDI; **b** deprotection by H_2/Pd–C or HCOOH (excess); **c** reflux in CH_2Cl_2, Et_3N, CMPI



Scheme 4 **a** HOSu, WSC.HCl, NMM in THF; **b** deprotection by H_2/Pd–C in EtOH; **c** p-TsOH.H_2O in benzene; **d** CH_3I, NaH in DMF; **e** DMAP, DCC in CH_2Cl_2; **f** deprotection by H_2/Pd–C in EtOH; **g** HOSu, WSC.HCl in DMF



Scheme 5 **a** HATU, DIPEA, 0 °C in THF; **b** dioxane, HCl; **c** Ph_2PPy, DEAD in THF



Scheme 6 **a** Et_3N in CH_2Cl_2; **b** 2-chloro propionyl chloride, Na_2CO_3, 0 °C; **c** NaOH in water/EtOH; **d** HCl

Structure of cyclodidepsipeptides

The cyclodidepsipeptides possess conformationally flexible heterocycle with two stereogenic centers (C-3 and C-6, for numbering see Scheme 1) which could give rise to a wide number of possible diastereoisomers and enantiomers. On the other hand, the presence of ester group and a secondary amide function makes possible the formation of lactam (keto) and lactim (enol) tautomeric forms.

The relative stability of different conformations of the heterocycle, diastereoisomers resulting from the (*R*)- and (*S*)-configuration at C-3 and C-6 and possibility of prototropic tautomerism were studied by DFT methods with compounds **1–3** (Smelcerovic et al. 2011) and a related compound, 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione (**11**, Fig. 2) (Yancheva et al. 2012). Pursuant to the density functional theory calculations and IR spectral studies in solution, keto forms are proposed to be dominant molecular forms in real systems because they were determined to be more stable. This statement was confirmed using IR spectroscopy (Yancheva et al. 2012). No evidences for enol formation were found neither in polar nor in nonpolar medium.

According to the DFT calculations, in all cases the morpholine-2,5-dione ring adopts boat conformation and the most favorable orientation of the larger 3- and 6-substituents is equatorial/axial and axial/equatorial. Indeed X-ray studies on morpholine-2,5-dione and its derivatives (Martínez-Palau et al. 2006; Bolte and Egert 1994; Linden et al. 2001; Chisholm et al. 2006; Kuang et al. 2004; Mawad et al. 2010; Kagamizono et al. 1995; Kuo et al. 2002; Iijima et al. 1992) report boat conformations of the heterocycle with the sp^3 C3 and C6 atoms displaced by 25°–35° out of the plane formed by O-1, C-2, N-4 and C-5. A X-ray study on the structure of **1** shows much flatter envelop conformation—C-2, C-3, N-4, C-5 and C-6 lying in one plane and only O-1 being displaced by 18° out of the plane formed by the other atoms (Zhukhlistova and Tishchenko 1980). However, this should be regarded rather as an exception since all other X-ray studies on related morpholine-2,5-diones report boat conformations regardless of the nature and size of the 3-, 4- and 6-substituents.

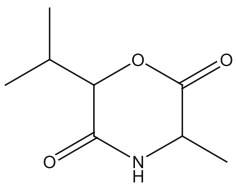
The relative stereochemistry at C3 and C6 in the structure of **5a** and **6** isolated from natural sources is determined by X-ray as *S*/*R* or *R*/*S* (Kagamizono et al. 1995; Kuo et al. 2002). These stereostructures are consistent with the possible mechanism of their biosynthesis. The mechanism of biosynthesis of enniatins was described (Hornbogen et al. 2002) as step-wise condensation of dipeptidol blocks, terminated by cyclization reaction of the formed linear hexapeptide. The cyclodidepsipeptides **5a** and **6** are most probably synthesized by the microorganisms as side products of cyclization in this process using similar nonribosomal biosynthetic mechanism. According to the suggested scheme (Hornbogen et al. 2002) the main precursors for the formation of enniatins would be a dipeptide block, containing *N*-methyl-L-amino acids and D-α-hydroxy-carboxilic acids.

On the other hand, the structurally similar 3-isopropyl-6-(4-methoxybenzyl)-4-methylmorpholine-2,5-dione (**8**) from Thai sea hare was established as *syn*, with methoxyl benzyl and isopropyl substituents in the pseudoaxial orientation and H-6 and H-3 in the pseudoequatorial orientation. The authors suggest that from biogenetic point of view, the compound could be derived by condensation of 2-hydroxy-3-(4-methoxyphenyl)propionate, a product of the Shikimate pathway, and *N*-methylvaline, which is often found as an amino acid component in cyanobacterial metabolites (Suntornchashwej et al. 2005).

Stereochemistry of the structure proposed to be lateritin (**7**) was not considered (Hasumi et al. 1993). The 1H and 13C NMR spectra of bassiatin (**5a**) significantly differ in comparison with 1H and 13C NMR spectra of lateritin. Furthermore, the difference between the physico-chemical characteristics of synthetic compounds **5b, 5c** and **5d** and **7** is obvious. As a conclusion, the published paper (Hasumi et al. 1993) may have proposed inaccurate chemical structure of **7** (Kagamizono et al. 1995).

The stereochemistry of synthetic cyclodidepsipeptides depends primarily on the stereo configuration of starting reagents. However, it is known that in the preparation of optically active cyclodidepsipeptides by initial formation of amide bond, the C6 chiral center sometimes suffers of partial racemization (Jörres et al. 1998; Vinsova 2001; Feng and Guo 2009). In the case of intermediate *N*-(α-haloacyl)-α-amino acids, racemization was observed during the cyclization which preferentially takes place via inversion of the configuration of C6 and could produce diastereomeric mixture (In 't Veld et al. 1990; Feng and Guo 2009). In the case of *N*-(α-hydroxy acyl)-α-amino acids and esters, a low degree of racemization for the hydroxyacyl moiety (C6) is reported for both the condensation and cyclization steps (Jörres et al. 1998). The preparation of optically active cyclodidepsipeptides by initial formation of ester bond is not affecting any of the stereogenic centers (Feng and Guo 2009).

Fig. 2 Structure of 6-(propan-2-yl)-3-methyl-morpholine-2,5-dione



11

Pharmacological activities of cyclodidepsipeptides

Antimicrobial activity

Antimicrobial activity of compounds **1**, **2** and **11** was tested in vitro against Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017) (Pavlovic et al. 2012a; Yancheva et al. 2012). Minimal inhibitory concentration (MIC) against the above-mentioned bacteria ranged between 2 and 25 mg/ml. Compound **1** showed stronger activity against all tested strains than other two 6-(propan-2-yl)-morpholine-2,5-diones. Compound **1** at the dose of 2 mg/ml displays equal activity against Gram-positive and Gram-negative bacteria (Pavlovic et al. 2012a). Compound **2** showed 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). Compound **11** showed activity against all bacteria except for *B. subtilis*, with MIC values from 4.125 to 8.25 mg/ml and minimal bactericidal concentration from 8.25 to 16.50 mg/ml. The investigated compound was most active against *E. coli* in comparison with other strains (Yancheva et al. 2012). The presence of methyl group in position 4 or/and the length of alkyl chain in position 3 of 6-(propan-2-yl)-morpholine-2,5-dione core play a role for the obtained differences between the antibacterial activities of **1**, **2** and **11**.

Metacytofilin (**4**) did not show antimicrobial activity against bacteria and fungi (it was stated in general without any Latin name of strains that were tested) (Iijima et al. 1992).

Antioxidant activity

The antioxidant activity of compounds **1** and **2** was investigated in vitro. According to DPPH-radical scavenging and total reducing power assays, two studied compounds display moderate antioxidant potential. Moreover, it was noticed a high correlation between DPPH-radical scavenging capacity and total reducing power. Pursuant to the density functional theory calculations, hydrogen atom abstraction from the activated C–H group at 3-position in the morpholine-2,5-dione ring is the most probable mechanism of antioxidant action (Stankov-Jovanovic et al. 2012).

Immunomodulatory activity

Immunomodulatory activity of compounds **1**, **2** and **11** was examined on rat thymocytes under identical experimental conditions (Pavlovic et al. 2012a, b). Rat thymocytes were cultivated with increasing concentrations of **1**, **2** and **11** (0.1, 1, 10 μg/well), for 24 h, and evaluated for proliferative activity, viability, reactive oxygen species and mitochondrial membrane potential. Increasing concentration of **1** and **2** was not able to display cytotoxic effect on rat thymocytes as well as decreased mitochondrial membrane potential. In addition to this, increase of concentration of **1** and **2** did not induce statistically significant reactive oxygen species production in rat thymocytes. Considering the effect of **1** and **2** on proliferative activity, it was noticed that 0.1 and 1 μg/well show no significant influence on proliferative activity. On the other hand, proliferative activity that significantly increased was observed with 10 μg of **1** ($p < 0.001$) and **2** ($p < 0.05$) in comparison with their respective controls. It seems that high concentration of **1** and **2** may have stimulatory effect on the thymocytes (Pavlovic et al. 2012a). As a conclusion, the two compounds may represent important natural immunostimulants.

The higher concentrations of the compound **11** (1 and 10 μg/well) inhibited proliferative activity of thymocytes mainly by inducing oxidative stress which resulted in increased cytotoxicity but without alteration of mitochondrial membrane potential (Pavlovic et al. 2012b). In a previous study it has been shown that some cyclodepsipeptides inhibit an enzyme involved in the opening of the mitochondrial permeability transition pore, which is a critical event in some forms of necrotic and apoptotic cell death (Clarke et al. 2002). Since increased cytotoxicity was not followed by altered mitochondrial membrane potential in cells, it seems that this alteration is not crucial for **11**-induced cytotoxicity but rather leads to low intracellular energy levels resulting in impaired immune function (Pavlovic et al. 2012b).

An obvious difference in biological responses between **1**, **2** and **11** is attributed to the differences in the chemical structure. Namely, compound **11** does not contain the methyl group in position 4 of 6-(propan-2-yl)-morpholine-2,5-dione core, but 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones (**1** and **2**) contain the methyl group in the same position. Moreover, compound **11** contains a methyl group in position 3 of 6-(propan-2-yl)-morpholine-2,5-dione core, contrary to the two previously investigated 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones (**1** and **2**) which contain longer alkyl chains (isopropyl and isobutyl, respectively) in the same position.

Metacytofilin (**4**) displays an immunosuppressive effect on the mixed lymphocyte culture reaction (IC_{50} was equal to 1 μg/ml). Moreover, metacytofilin that was administered intraperitoneally suppressed delayed-type hypersensitivity (at the dose of 25–100 mg/kg) and significantly suppressed antibody formation (at the dose of 100 mg/kg) to sheep red blood cells in CDF_1 mice, indicating the different immune

response regarding the concentrations used in the study. However, it did not display toxicity at the dose of 200 mg/kg when it was administered intraperitoneally to ICR mice. In addition to this, cytotoxicity was not observed at 100 μg/ml against carcinoma cell lines (L1210, EL-4 and IMC) (Iijima et al. 1992).

Inhibition of glucosidases

Glucosidase is a class of enzymes that participate in modifying process of carbohydrates and glycoconjugates (Arcelli et al. 2004). Since glucosidases are important for a variety of biological processes, glucosidase inhibitors as natural products or obtained via synthetic routes are promising agents against HIV, Gaucher's disease, hepatitis, cancer (Lillelund et al. 2002), diabetes (Gura 1998) and influenza (Hanessian et al. 2002).

D-gluconolactone is a good competitive inhibitor of glucosidases. However, taking into consideration the instability of D-gluconolactone and the structural similarity of its transition state to the heterocyclic ring of 1,4-morpholine-2,5-dione in the boat conformation, derivatives of optically active 1,4-morpholine-2,5-dione were proposed as good potential inhibitors of glucosidases and synthesized (Arcelli et al. 2004, 2005, 2007). According to the screening of activity against α-glucosidase from bakers yeast and β-glucosidase from almonds, compounds **12–20** (Fig. 3) display non-competitive mechanism of inhibition activity. Decreasing values of inhibition constants (K_i/mM) of compounds **12**, **13**, **14**, **15**, **16**, **17**, **18** and **19** (measured in HEPES buffer pH 6.85 at 37 °C) are 23.7, 4.8, 3.35, 2.38, 0.8, 0.7, 0.7 and 0.3, respectively. It is noticed that there is a difference between K_i values owing to structural dissimilarity between investigated compounds (differences in absolute configuration at C-3 and C-6 stereocenters as well as type of substituents at C-3 and N-4 position of morpholine-2,5-dione ring). None of the above-mentioned inhibitors of α-glucosidase display inhibition of β-glucosidase. The only compound that inhibits β-glucosidase is compound **20**. It was assumed that the moderate inhibition activities of the compounds stem from being unable to interact with the active site of the enzyme by hydrogen bonds or electrostatic interactions (Arcelli et al. 2004).

Considering assumption that optically active morpholine-2,5-dione that are more polar and more soluble in water would be able to interact with the enzyme by hydrogen bonds or electrostatic interactions, they were synthesized as well as their activity was tested against a variety of glucosidases (α-glucosidases from baker's yeast, β-glucosidases from almonds, α-glucosidases from *Bacillus stearothermophilus*, α-mannosidase from jack beans and α-galactosidase from green coffee beans). Inhibition constants (K_i/mM) of compounds **21**, **22**, **23**, **24**, **25**, **26**, **27**, **28**, **29** and **30** (Fig. 4) (measured in HEPES buffer pH 6.85 at 37 °C) are 3.35, 1.5, 1.02, 1.00, 0.96, 0.52, 0.18, 0.11, 0.11, and 0.05, respectively. Different K_i values of the investigated compounds may be result of differences in absolute configuration at C-3 (for all substrates) as well as at C-6 and C-2′ stereocenters (for epoxides and diols). All the investigated compounds behave as non-competitive inhibitors against α-glucosidase from baker's yeast and the only compound **26** is active against α-glucosidase from *B. stearothermophilus.* On the other hand, none of the investigated compounds show inhibition toward β-glucosidases from almonds, α-mannosidase from jack beans and α-galactosidase from green coffee beans. It is important to stress that the lactone function is essential to a compound to display inhibition toward α-glucosidase (reduction of the carbonyl function of α-glucosidase inhibitor (**31**) resulted in inactive **32**) (Arcelli et al. 2005).

In addition to this, the solvent effect on the inhibition ability of diols was evaluated. Increase of ethanol concentration (in buffer solution) leads to significant increase of activity of compound **15** toward α-glucosidase (and also decrease of K_i values). It is proposed that the diol interacted with the ethanol by hydrogen bonding and subsequently could gain an easy access in the closeness of the active site inducing change in the solvation which destabilizes the transition state. According to the second hypothesis, p*K*a of the carboxyl group which is in charge of catalysis is altered by ethanol (Arcelli et al. 2005).

Moreover, chiral morpholine-2,5-dione derivatives (compounds **33–40**, Fig. 5) with further elongated side-chains were submitted to the tests of inhibitory activities toward α-glucosidases from baker's yeast, α-glucosidases from *B. stearothermophilus*, β-glucosidases from almonds, α-mannosidase from jack beans and α-galactosidase from green coffee beans. All the substrates exhibited non-competitive inhibition solely against α-glucosidases. It is believed that the extension of the side chain at C-3 introducing CH_2OBn group resulted in increased biological activity. Compounds **35**, **36** and **39** are the most active of all the substrates: K_i/μM is 40 (from baker's yeast), 33 and 4 (from *B. stearothermophilus*), respectively. It seems that not only the stereochemistry at C-2′ and C-3′ is important for the biological activity, but also that at C-3 and C-6 stereocenters. In addition to this, corresponding triol obtained by debenzylation of **39** showed significantly smaller inhibitory activity against α-glucosidases from baker's yeast. Therefore, it can be concluded that benzyl group at C-3′ of the side chain favours interaction with hydrophobic sites of the enzyme (Arcelli et al. 2007).

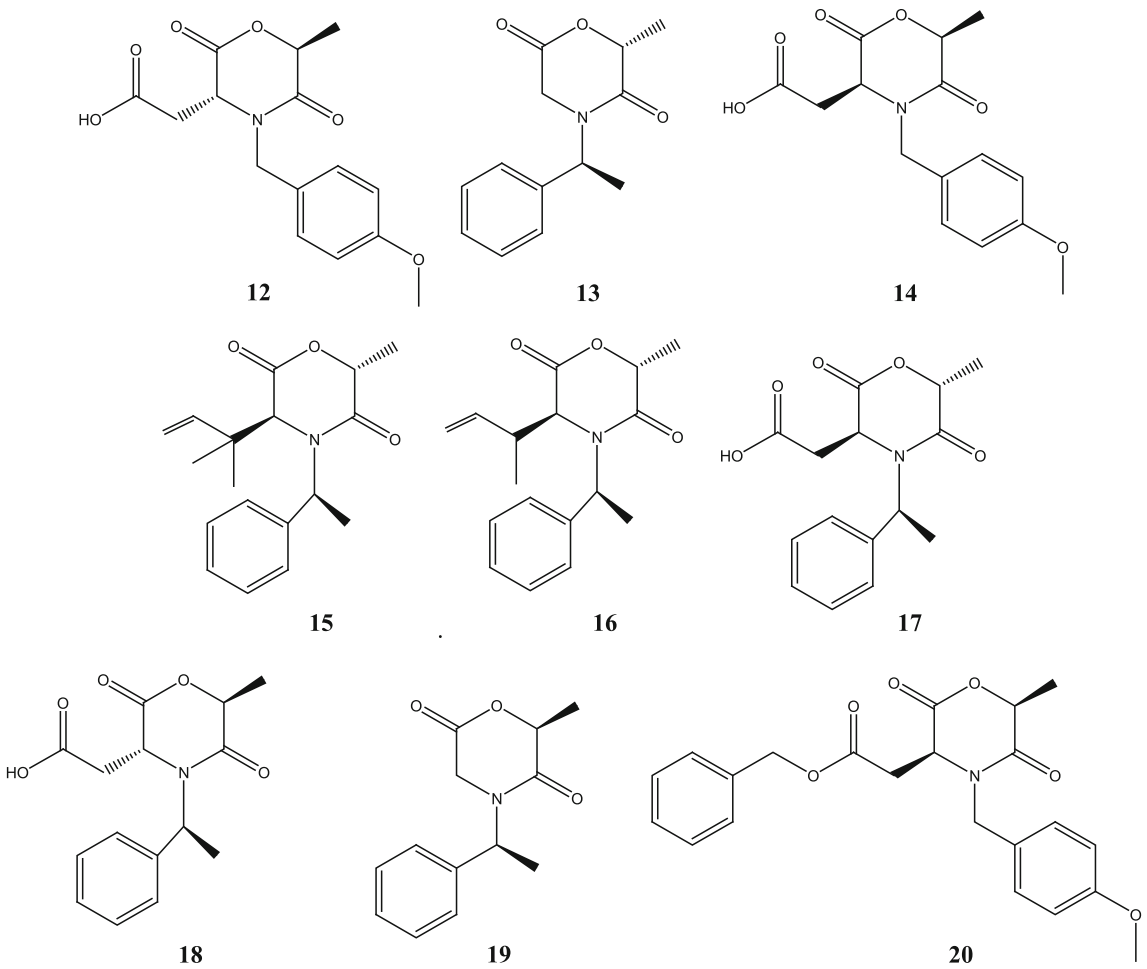


Fig. 3 Structures of glucosidases inhibitors (**12–20**)

Inhibition of Acyl-CoA:cholesterol acyltransferase

ACAT is an enzyme that catalyzes synthesis of cholesteryl esters from fatty acyl-CoA and cholesterol in macrophages. In case of hyperlipidemia, oxidized LDL lipoproteins are taken up and degraded by macrophages and subsequently cholesterol could be esterified again. As a result, cholesterol ester accumulates and macrophages are transformed into foam cells, which is a sign of the early stage of atherosclerosis (Hasumi et al. 1993; Kumar et al. 2010).

Lateritin (**7**) inhibited activity of ACAT from rat liver by 50 % at a concentration of 5.7 μM in an irreversible and time-dependent manner. Moreover, lateritin is an selective inhibitor of ACAT since it does not affect synthesis of triacylglycerols as well as taking up and degradation of oxidized LDL in macrophage (Hasumi et al. 1993).

Bassiatin (**5a**) showed inhibition of aggregation of rabbit platelets induced by ADP, collagen and arachidonic acid with IC_{50} values equal to 1.9×10^{-4} M, 3.8×10^{-4} M and 3.8×10^{-4} M, respectively. The data indicate that adenosine is slightly more active than bassiatin (**5a**) in inhibition of platelet aggregation. Compounds **5b**, **5c** and **5d** that are synthesised stereoisomers of bassiatin (**5a**) displayed no activity against platelet aggregation (Kagamizono et al. 1995).

None of the above-mentioned cyclodidepsipeptides (**5a, 5b, 5c** and **5d**) showed inhibition against ACAT from rabbit intestine at concentration levels less than 10 μM (Kagamizono et al. 1995). However, it has already been stated that cyclodidepsipeptide lateritin is an inhibitor of ACAT from rat liver (Hasumi et al. 1993).

According to the previously published paper (Tomoda et al. 1992), cyclodepsipeptides, beauvericin and seven distinct enniatins (A, A1, B, B1, D, E and F) exhibit inhibition of ACAT from rat liver. Beauvericin is more potent inhibitor of ACAT than the above-mentioned enniatins. Moreover, increase in hydrophobicity of the enniatins results in more potent inhibitory activity toward ACAT (Tomoda et al. 1992). As a conclusion, further investigations of bassiatin (**5a**) and its stereoisomers (**5b, 5c** and **5d**) as well as beauvericin and the seven enniatins are encouraged owing to the fact that they may have a

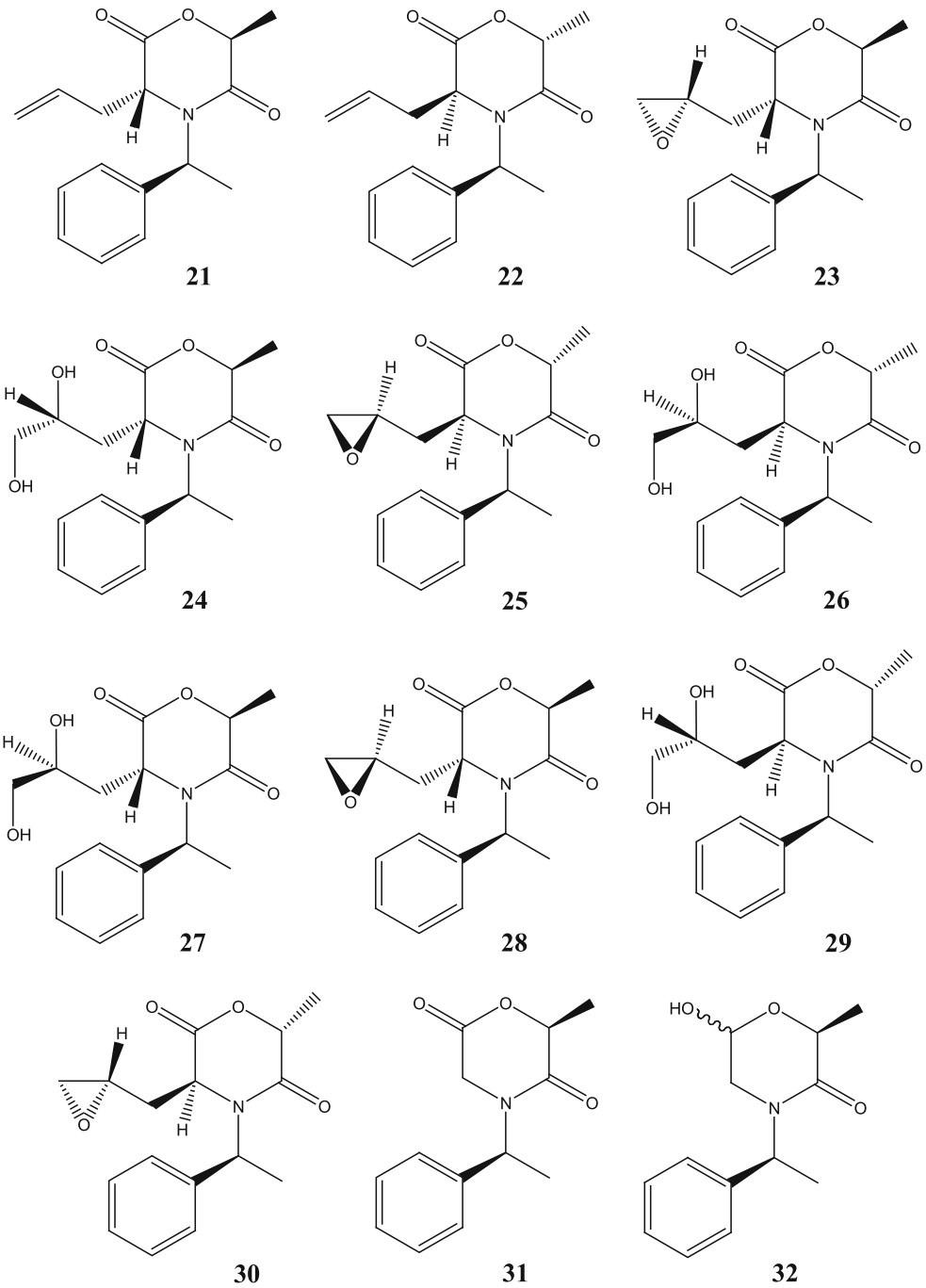


Fig. 4 Structures of glucosidases inhibitors (**21**–**32**)

potential to be used as a prophylactic agent against atherosclerosis.

Inhibition of xanthine oxidase

Recently, two cyclodidepsipeptides, **1** and **2**, were evaluated for inhibitory activity against commercial enzyme xanthine oxidase (XO) in vitro and XO in rat liver homogenate as well as anti-inflammatory response on human peripheral blood mononuclear cells. The two cyclodidepsipeptides were excellent inhibitors of XO and significantly suppressed the activation of nuclear factor κB. Based on molecular docking study, the binding modes of **1** and **2** with XO were clarified and recommendations for future structure-guided design of new morpholine-dione inhibitors of XO were drawn (Smelcerovic et al. 2013). **1** and **2** bind in the entrance of the narrow tunnel toward the dioxothiomolybdenum moiety of the active center of XO, blocking in this way the approach of the substrates toward the metal atom. Their molecular interactions resemble

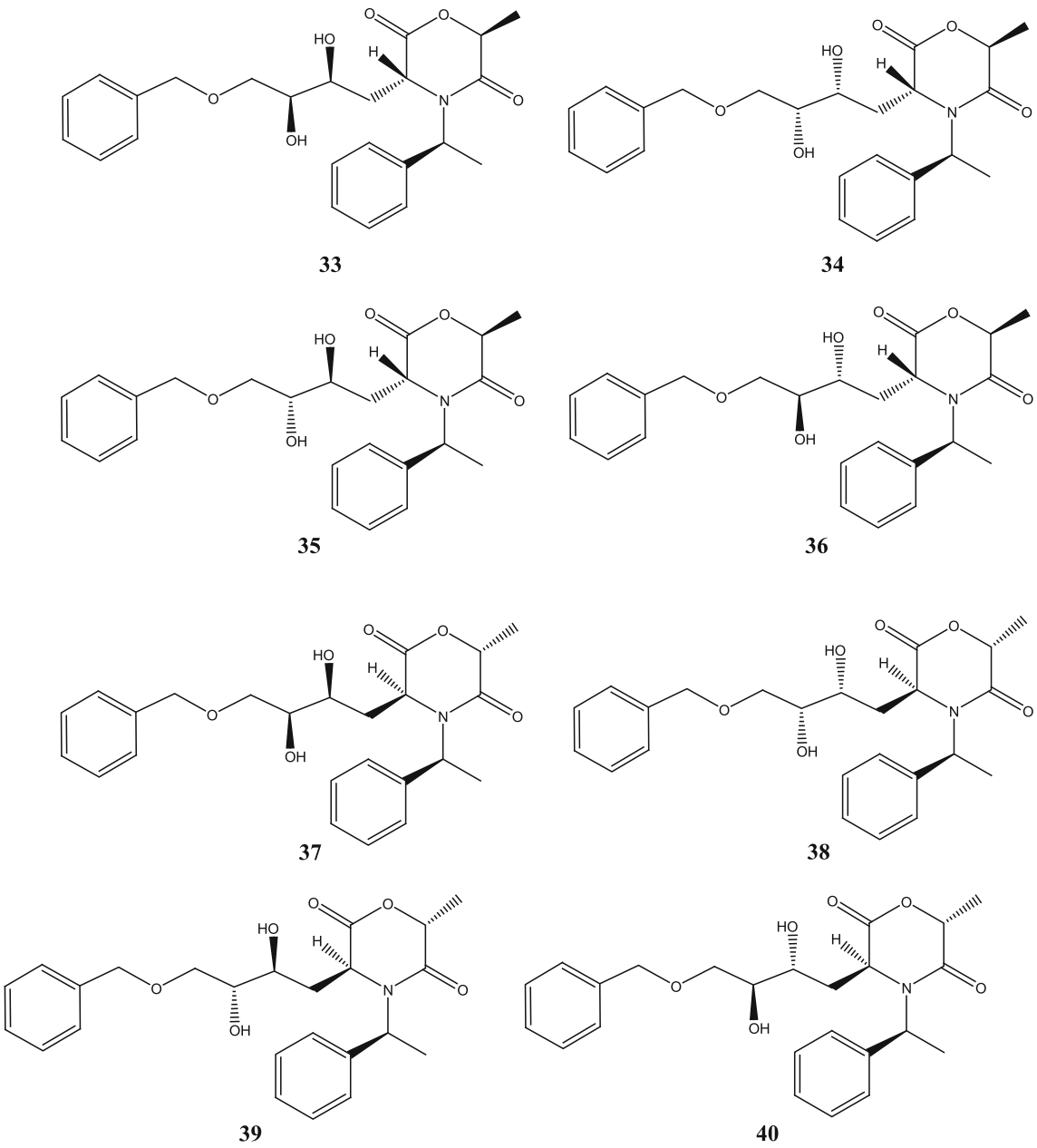


Fig. 5 Structures of glucosidases inhibitors (**33–40**)

those found by crystallographic studies on complexes of XO with other inhibitors not forming a covalent bond with the molybdenum atom such as salicylic acid and febuxostat. Introduction of protonodonor or acceptor groups in the side-chains of **1** and **2** could enhance the interactions with the enzyme via hydrogen bonding and thus provide better inhibitor potency. Both **1** and **2** were tested and confirmed as non-toxic (Pavlovic et al. 2012a) and also this is the first example of morpholine-diones derivatives exerting XO inhibition and anti-inflammatory effect. Therefore, **1** and **2** may give a promise to be used in the treatment of gout and other excessive uric acid production or inflammatory condition. More recently, it was found that compound **11** is also a potent inhibitor of XO in rat liver homogenate (Yancheva Pantaleeva et al. 2013).

We have investigated molecular interactions of compounds **1**, **2** and **11** with XO using idTarget web server. This server utilizes divide-and-conquer docking approach combined with scoring functions to predict affinity of a small molecule for a target protein. Docking energy (binding free energy, ΔG^{pred}), predicted inhibition constant ($K_i^{\text{pred.}}$) as well as *Z*-score for the above-mentioned compounds and XO (marked as 3BDJ in protein database bank) were calculated (Wang et al. 2012). Docking energies for compounds **1, 2** and **11** are −8.02,

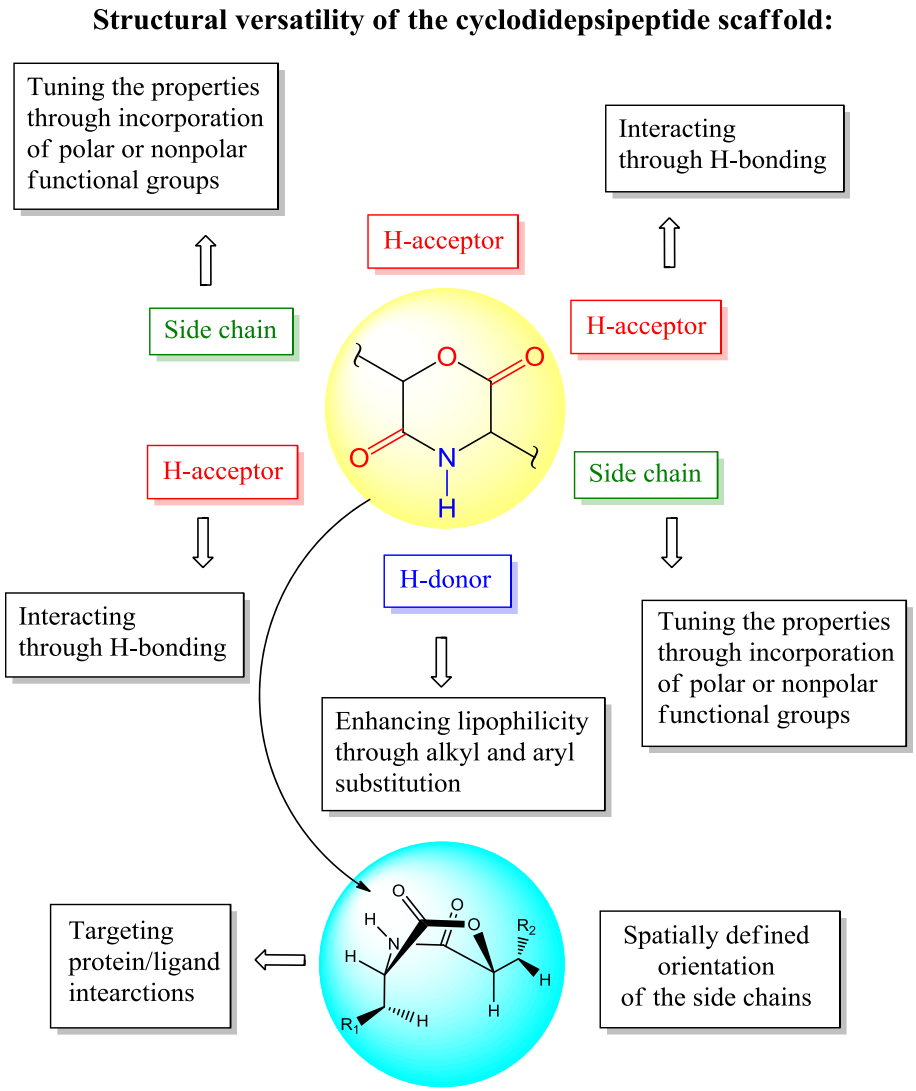


Fig. 6 Structural versatility of the cyclodidepsipeptide scaffold

−8.04, and −7.59 kcal/mol, predicted inhibition constants are 1.3, 1.3 and 2.7 μM, while *Z*-scores are 1.93, 1.91 and 2.21, respectively. A large negative value for *Z*-score signifies an important target of the query compound (Wang et al. 2012). Moreover, a low (negative) energy is indication of a more stable system (such as a complex of a small molecule and a target protein). According to the obtained results, it can be concluded that compounds **1** and **2** are able to exhibit more potent effects toward XO than compound **11**. Docking energy, predicted inhibition constant and *Z*-score for allopurinol are −8.47 kcal/mol, 0.62 μM and 1.63, respectively. Those results suggest that potency of compounds **1**, **2** and **11** is lower than that of allopurinol, a golden standard for the treatment of hyperuricemia complications in gout.

According to our experimentally obtained results (Yancheva Pantaleeva et al. 2013; Smelcerovic et al. 2013), the inhibitory activities of compounds **1**, **2** and **11** toward XO in rat liver homogenate were similar and are decreasing in the given order. Allopurinol showed stronger inhibitory effect on rat liver XO activity than the studied compounds, which is in agreement with the docking results. To conclude with, the idTarget docking results confirmed to be useful for preliminary assessment of inhibitory potential of new compounds toward XO.

Structural versatility of the scaffold and drug-likeness

Heterocyclic compounds with their unique chemical properties and wide-ranging biological activities are ever attracting attention in the discovery and optimization of new lead structures. In the particular case of the cyclodidepsipeptides, the main core—morpholine-2,5-dione ring, provides a relatively simple heterocyclic scaffold combining at the same time many versatile opportunities for structural modification. The advantageous structural parameters of the scaffold are illustrated in Fig. 6.

Table 1 Calculated molecular properties of compounds **1–40** for assessment of the drug likeness

Compd. no.	miLogP[a]	TPSA[b]	N^{c}_{atoms}	MW[d]	N^{e}_{ON}	N^{f}_{OHNH}	$N^{g}_{viol.}$	$N^{h}_{rotb.}$	Vol[i]
"Rule of five"	≤5			<500	<10	<5			
1	1.60	46	15	213	4	0	0	2	211
2	2.13	46	16	227	4	0	0	3	228
3	2.10	46	16	227	4	0	0	3	228
4	1.84	87	22	306	6	3	0	5	286
5, 7	2.28	46	19	261	4	0	0	3	249
6	2.79	46	20	275	4	0	0	4	266
8	2.34	55	21	291	5	0	0	4	275
9	2.02	103	32	436	8	3	0	3	395
10	−3.95	138	18	260	8	5	0	5	224
11	0.58	55	12	171	4	1	0	1	161
12, 14	0.70	93	22	307	7	1	0	5	269
13, 19, 31	1.70	46	17	233	4	0	0	2	216
15	3.49	46	22	301	4	0	0	4	293
16	3.08	46	21	287	4	0	0	4	277
17, 18	1.21	83	21	291	6	1	0	4	260
20	2.61	82	29	397	7	0	0	8	358
21, 22	2.61	46	20	273	4	0	0	4	261
23, 25, 28, 30	1.79	59	21	289	5	0	0	4	265
24, 26, 27, 29	0.68	87	22	307	6	2	0	5	283
32	1.17	49	17	235	4	1	0	2	222
33–40	2.25	96	31	427	7	2	0	9	397
Morph[j]	−0.85	55	8	115	4	1	0	0	94

[a] Octanol–water partition coefficient

[b] Polar surface area

[c] Number of nonhydrogen atoms

[d] Molecular weight

[e] Number of hydrogen-bond acceptors (O and N atoms)

[f] Number of hydrogen-bond donors (OH and NH groups)

[g] Number of "Rule of five" violations

[h] Number of rotatable bonds

[i] Molecular volume

[j] Morph—unsubstituted morpholine-2,5-dione

The morpholine-2,5-dione ring contains three oxygen atoms able to act as hydrogen acceptors and also one N–H group which could act either as hydrogen bond donating site or hydrogen bond accepting site. In addition, the ring enables modifications in three positions—at the stereogenic centers C3 and C6, along with the amide N4 atom. Substitution in the side chains allows fine tuning of the desired pharmacological properties through incorporation of polar or nonpolar functional groups. Lipophilicity of cyclodidepsipeptide derivatives could be further controlled and enhanced through appropriate alkyl and aryl substitution at N4. As discussed in the above sections, a specific characteristic of the morpholine-2,5-dione moiety is the spatially defined orientation of the side chains. This may aid in the design of lead structures with specific steric and stereoelectronic requirements for targeting particular protein–ligand interactions.

The above sections demonstrate the various promising pharmacological activities of cyclodidepsipeptides. However, in view of future medical application, other important features should also be taken into account—favorable pharmacokinetical behavior in living organisms, providing the required bioavailability and transportation through different membranes to the site of action, optimal process of metabolization and elimination.

For this reason preliminary screening of molecular physico-chemical properties such as lipophilicity, molecular size, flexibility and presence of hydrogen-donors and

acceptors facilitates considerably the development of new pharmaceuticals and outlines the usefulness of newly emerging scaffolds in the medicinal chemistry.

Lipinski and coworkers established an effective methodology for estimation of potential drug solubility and permeability based on the calculation of molecular weight, octanol/water partition coefficient, number of H-bond donors and number of H-bond acceptors (Lipinski et al. 2012). Based on the analysis of a large number of drugs, a cut-off for each of the four physico-chemical properties was set into a "Rule of five": poor absorption or permeation are more likely to occur when the molecule has molecular weight more than 500, log P over 5, and contains more than 5 H-bond donors or 10 H-bond acceptors. More than one violation of the rule is the critical limit for acceptable drug-likeness.

Physico-chemical properties of cyclodidepsipeptides **1–40**, calculated using Molinspiration tool (Molinspiration Cheminformatics 2013), are shown in Table 1. Data indicate that none of the compounds violate the "Rule of five". Molinspiration methodology for calculation of miLogP implements fragment-based contributions and correlation factors which makes it robust and applicable to virtually all organic and organometallic compounds. The miLogP values of **1–40** are below 4 even for the lager derivatives such as **9** and **33–40**, having molecular weight over 400, therefore they process a favorable physico-chemical profiles for per oral bioavailability. Topological polar surface area (TPSA) is good descriptor for the oral bioavailability (Veber et al. 2002) and drug transport properties (Ertl et al. 2000; Prasanna and Doerksen 2009). TPSA is a sum of the surface areas occupied by the oxygen and nitrogen atoms and the hydrogens attached to them. It represents the hydrogen bonding capacity of the molecules. Molecules with TPSA <140 $Å^2$ are recognized to have good intestinal absorption, and those with TPSA <60 $Å^2$ show good blood–brain barrier penetration (Ertl et al. 2000; Prasanna and Doerksen 2009). As could be seen in Table 1, all presented cyclodidepsipeptides satisfy the criterion for good intestinal absorption and most of them have also TPSA indicating good blood–brain barrier penetration. Hydrogen bonding capacity of the cyclodepsipeptides is also expressed by the number of H-bond donors and acceptors. The compounds in the series show 4–8 H-bond acceptors. Cyclodidepsipeptides substituted at N4 position and containing alkyl side chains do not have any H-bond donors, while those with free amide function and more versatile functional groups in the side chains provide up to 5 H-bond donors.

The conformational flexibility of the molecules described by the number of rotatable bonds is important factor for oral bioavailability as well as the efficient bonding of receptors and channels (Veber et al. 2002). Sufficient oral bioavailability is expected for molecules with 10 rotatable bonds or fewer. The majority of cyclodidepsipeptides **1–40** show low number of rotatable bonds (5 and less). It should be noted that the morpholine-2,5-dione moiety itself as scaffold possess two rotatable bonds. Molecular volumes of the compounds in the series are <300 $Å^3$ with a few exceptions.

Summarizing the physico-chemical properties of cyclodidepsipeptides, we could conclude that they obey the "Rule of five" and meet all criteria for good solubility and permeability in such a way that they allow further structural modification for achieving desired pharmacological properties by introduction of particularly interesting structural motives. The morpholine-2,5-dione moiety is small and possess versatile character that makes it appropriate for incorporation of different functionalities.

Conclusions

This review provides an overview of isolation, synthesis and potential pharmaceutical application of cyclodidepsipeptides, with an emphasis on their biological activities.

Cyclodidepsipeptides from natural origin are mostly produced by different fungal strains, such as *F. sporotrichioides* isolated from *H. barbatum*. Since one of contemporary trends in natural products chemistry is search for bioactive compounds produced by endophytes and other microorganisms (from soil, seawater, thermal water etc.), isolation of new cyclodidepsipeptides from natural resources can be expected in the future.

The synthesis of cyclodidepsipeptides follows two general approaches—by initial formation of the amide bond, or initial formation of the ester bond; and subsequent intermolecular cyclization to cyclodidepsipeptide structure. It is closely related to the condensation and ring-closure strategies applied in the preparation of the larger members of the cyclodepsipeptide family. However, due to synthesis of the smaller heretocycles it allows for the use of more versatile building blocks.

Cyclodidepsipeptides represent a relatively small group of cyclodepsipeptide family that exhibit antimicrobial, antioxidant and immunomodulatory activities as well as inhibitory activities toward α-glucosidase, ACAT, xanthine oxidase and platelet aggregation. For example, there is the possibility of using two 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones as novel non-purine xanthine oxidase inhibitors in the treatment of gout. As a conclusion, cyclodidepsipeptides may give promise to be used as potential drugs in the future. Summarizing the physico-chemical properties of cyclodidepsipeptides, we could conclude that they obey the Lipinski "Rule of five" and meet all criteria for good solubility and permeability. The advantage of

cyclodidepsipeptides is that they are simpler for synthesis than larger cyclodepsipeptide members (for example, cyclohexadepsipeptides, cyclooctadepsipeptides, or 18-membered cyclodepsipeptides, the so-called enniatins). In addition to this, small molecules are more suitable for rational drug design. Therefore, cyclodidepsipeptides can be regarded as a promising scaffold for medicinal chemistry and we should expect more forthcoming papers on this topic as well as their application in medicine.

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Conflict of interest The authors declare that they have no conflict of interest.

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