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# Synthesis and anti-cancer activity of naturally occurring 2,5-diketopiperazines

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ABSTRACT

Three naturally occurring oxyprenylated diketopiperazines were synthesized and preliminarily tested as growth inhibitory agents *in vitro* against various cancer cell lines. The compounds were tested on six human cancer cell lines with different sensitivity to proapoptotic stimuli using the MTT colorimetric assay. The data revealed that of the chemicals under study only deoxymicelianamide (**11**) displayed the highest activity, recording mean $IC_{50}$ growth inhibitory values ranging from 2 to 23 μM. A comparative study with the non-geranylated saturated derivative of (**11**) revealed the importance of the presence of the geranyloxy side chain and the exocyclic 2,5-DPK double bond moiety for the observed activity.



## 1. Introduction

2,5-Diketopiperazines (2,5-DKPs) represent cyclodipeptides deriving from the condensation of two amino acids. These products are quite abundant in nature occurring in several secondary metabolites. The 2,5 DKP subunit can be found as such or embedded in more structural complex architectures in a variety of products from fungi, bacteria, plants, and mammals and they are also often produced as the result of degradation of polypeptides in food and beverages. From a biological point of view the title compounds are able to bind to a wide range of receptors but they also have several features that make them interesting and valuable scaffolds for drug discovery having a rigid backbone. Containing constrained amino acids embedded within their structures without the unwanted physical and metabolitic properties of peptides, 2,5-DKPs can mimic a preferential peptide conformation. These chirally enriched molecules are easily synthesized from readily available amino acids using easy to handle chemistry and have been also used as scaffolds to build libraries in combinatorial chemistry. Recent progresses in solid-phase synthetic methodology have made this class of natural products even more attractive to combinatorial drug discovery effort. The phytochemistry and pharmacology of naturally occurring 2,5-DKPs and their semisynthetic derivatives have been recently exhaustively reviewed [1–3].

Very recently diketopiperazines have attracted the attention of researchers for their appreciable anti-cancer activity. A recent explicative example is plinabulin, a semi-synthetic derivative of naturally occurring phenylahistin, that was first designed as a vascular disrupting agent, and is now under phase II clinical trials as an anticancer drug behaving as a potent anti-microtubule agent with colchicine-like tubulin depolymerization activity [4]. In this context it has been found that the higher is the lipophylicity of the diketopiperazine the higher is the observed anti-cancer activity. In particular prenylation of natural and semisynthetic derivatives has been seen to largely

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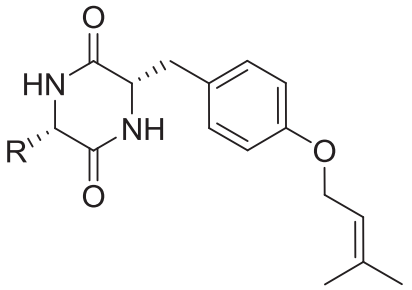
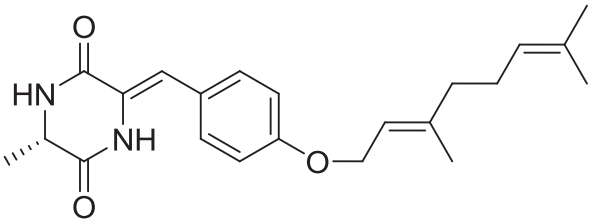


**3:** R = H
**6:** R = $CH_2OH$



**11**

**Fig. 1.** Chemical structures of naturally occurring diketopiperazines.

increase the *in vitro* growth inhibitory activity against cancer cells [5]. Thus the search for novel lipophilic products having a diketopiperazine ring as the central core is a field of current and growing interest.

In the context of isolation, synthesis, and structural characterization of 2,5-DKPs, only recently a very few number of products containing a terpenyl side chain attached to an oxygen atom of one or both amino acid residues, have been recently reported in the literature. These latter are represented by cyclo-(glycyl-L-tyrosyl)-*O*-methylbutenyl ether (**3**), obtained from *Gliocladium virens*, a mycoparasite of several other fungal species, phomamide (**6**), isolated from the Ascomycetes *Leptosphera maculans* and *Leptosphera biglobosa*, pathogenic fungi of plants belonging to the genus *Brassica* [6–11], and finally deoxymicelianamide (**11**), isolated from *Gliocladium* spp. [12,13] and *Penicillium janthinellum* [14] (Fig. 1).

Few biological data on these three secondary metabolites have been reported in the literature: to this regard deoxymicelianamide (**11**) was found to be cytotoxic *in vitro* against U373 (human glioblastoma) cell line with an $IC_{50}$ growth inhibitory concentration of 0.7 μg/mL [12].

As a continuation of our ongoing studies about the phytochemical and pharmacological properties of oxyprenylated secondary metabolites from plants, bacteria, and fungi we wish to report herein the total synthesis of the three above mentioned compounds from the parent commercially available amino acids. To the best of our knowledge, such synthetic schemes are reported herein for the first time, having been only described the process leading to racemic deoxymicelianamide (**11**) [15]. Moreover we characterized the *in vitro* $IC_{50}$ growth inhibitory concentration of compounds (**3**, **6**, and **11**) in a panel of six human cancer cell lines exhibiting different levels of resistance to pro-apoptotic stimuli using the MTT colorimetric assay.

## 2. Experimental

### 2.1. General

The identities of the compounds were confirmed by $^1H$ NMR spectra recorded on a Varian inova 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA). Chemical shifts are reported in parts per million (δ) downfield from the internal standard tetramethylsilane ($Me_4Si$). Homogeneity was confirmed by TLC on silica gel Merck 60F254 (Merck, Germany). Solutions were routinely dried over anhydrous $Na_2SO_4$ prior to evaporation. Chromatographic purifications were performed by Merck 60 70–230 mesh silica gel column. All other chemicals used were of the highest purity commercially available.

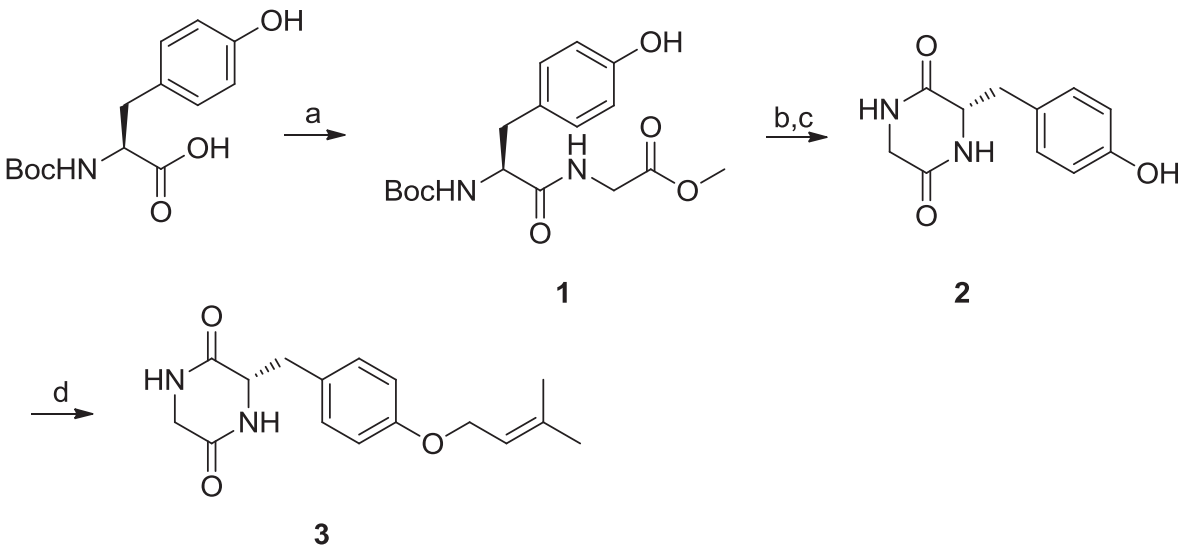
### 2.2. Chemistry

All linear dipeptides and cyclopeptides were synthesized by solution phase peptide synthesis using Boc strategy following well established reported procedures [16,17]. Diketopiperazines (**3**) and (**6**) were straightforward synthesized starting from the dipeptide Boc–Tyr–Gly–OMe (**1**) and Boc–Tyr–Ser–OMe (**4**) as outlined in Schemes 1 and 2.

$N^{\alpha}$–Boc deprotection was carried out by TFA treatment and cyclization following the procedures previously reported [18]. *O*-alkylation was carried out directly on the DKPs. Product (**11**), which contains a $\Delta^z$–tyrosine, was synthesized following the already reported procedures [19,20], as outlined in Schemes 3 and 4.

#### 2.2.1. Coupling reaction

To an ice-cooled mixture containing *N*-protected amino acid (1.1 mmol) in DMF, EDC · HCl (1.1 mmol), HOBt (1.1

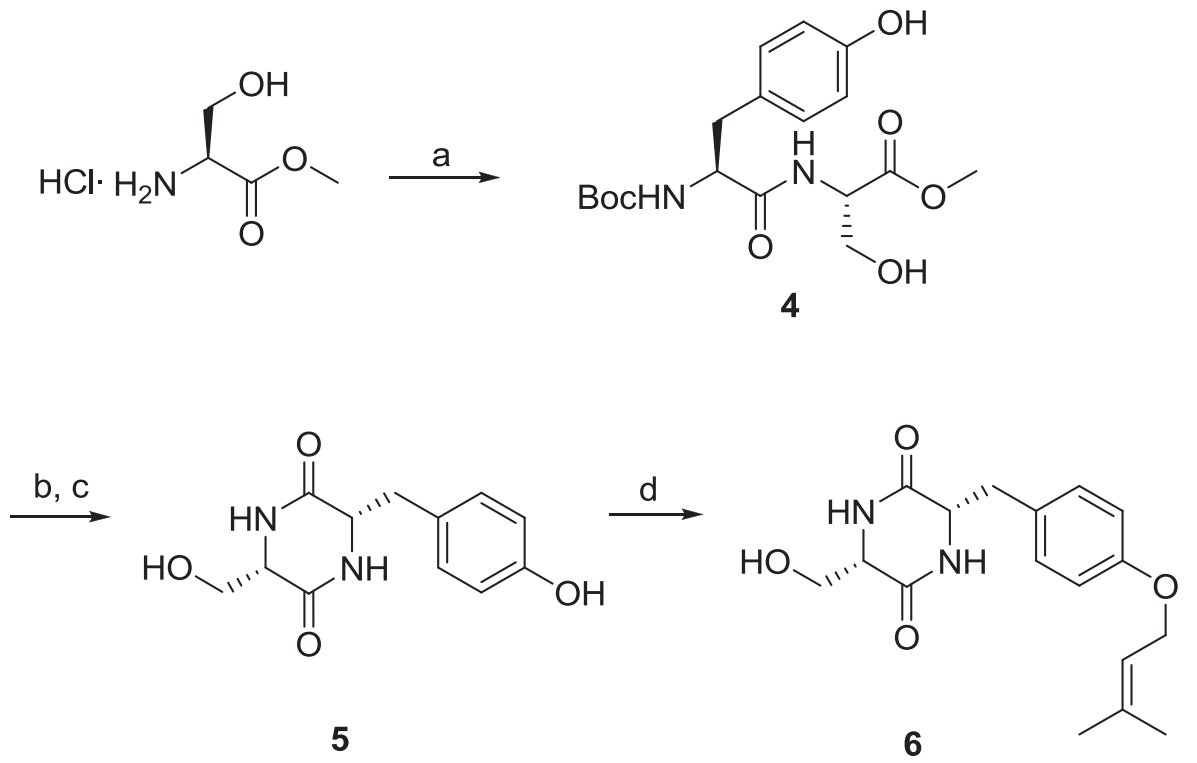



**Scheme 1.** Reagents and conditions: a) HCl · Gly–OMe, EDC · HCl, NMM, HOBt, DMF, r.t., overnight; b) TFA/$CH_2Cl_2$ 1:1, 1 h, r.t., under $N_2$; c) AcOH 0.1 M/2-butanol, NMM, 120 °C, 3 h; d) Dal–Br, $K_2CO_3$, DMF, r.t., overnight.

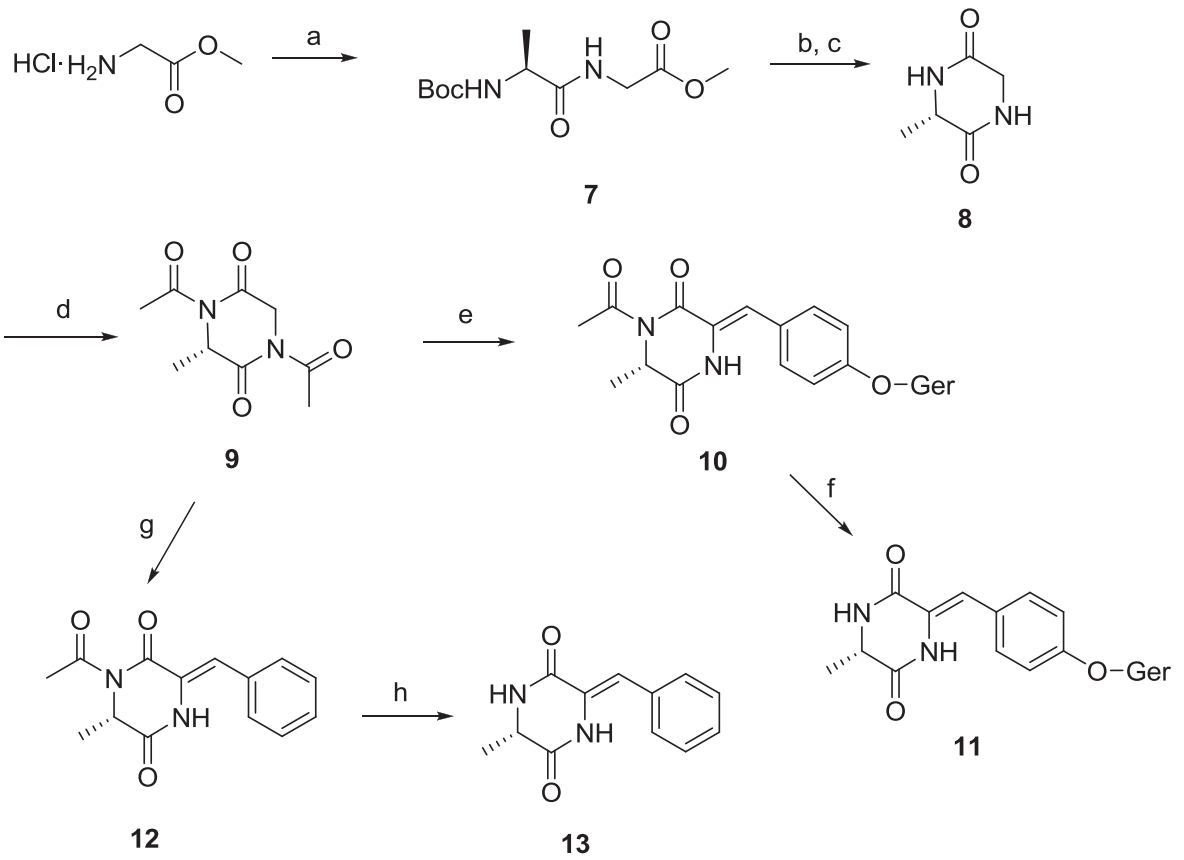
mmol), DIPEA (3.3 mmol) and the required *C*-protected amino acid (1.0 mmol) were added. The reaction mixture was allowed to warm at r.t., stirred overnight under $N_2$ atmosphere, and evaporated under reduced pressure. The residue was then dissolved in EtOAc and washed with three portions of 5% citric acid, saturated solution of $NaHCO_3$ and brine. The organic phases were collected and dried on $Na_2SO_4$, and the solvent evaporated under reduced pressure to give the desired product.

*2.2.2. Boc deprotection*

The $N^{\alpha}$ terminal Boc-protected peptides were deprotected by a 50% mixture of TFA in $CH_2Cl_2$ at r.t for 1 h. The intermediate




**Scheme 2.** Reagents and conditions: a) Boc–Tyr–OH, EDC · HCl, HOBt, DMF, r.t., overnight; b) TFA/$CH_2Cl_2$ 1:1, r.t. 1 h, under $N_2$; c) AcOH 0.1 M/2-butanol, NMM, 120 °C, 3 h; d) $K_2CO_3$, Dal–Br; DMF, r.t., overnight.




**Scheme 3.** Reactions and conditions: a) Boc–Ala–OH, EDC · HCl, HOBt, NMM, DMF, r.t., overnight; b) TFA/$CH_2Cl_2$ 1:1, r.t., 1 h, under $N_2$; c) AcOH/2-butanol, NMM, 120 °C, 3 h; d) $Ac_2O$, reflux, overnight; e) **14**, tBuOK, DMF, r.t., 4 h; f) $NH_2$–$NH_2$ · $H_2O$, MeOH, 2 h; g) PhCOH, tBuOK, DMF, r.t., 4 h; h) $NH_2$–$NH_2$ · $H_2O$, MeOH, 2 h.

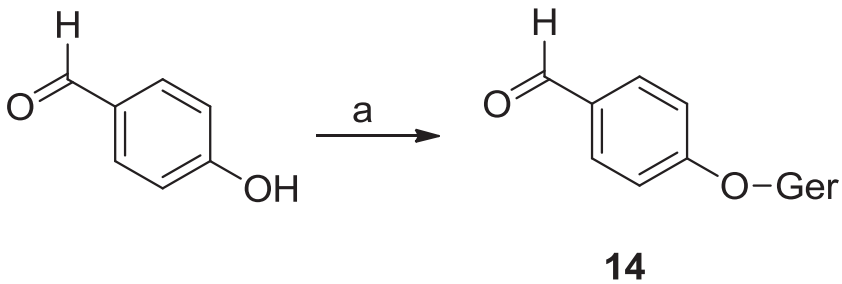
TFA salts were used for subsequent reactions without further purification.

*2.2.3. Diketopiperazine ring formation*

TFA · dipeptide methyl ester (1 mmol) was dissolved in 0.1 M AcOH-2-butanol (1.5 eq.), and NMM (1 eq.) was added. The resulting weakly acidic solution was refluxed in an oil bath (120 °C) for 4 h. The diketopiperazine began to crystallize out after cooling the solution. After concentration of the reaction mixture to a small volume, the product was collected by filtration on paper, washed with small amounts of cold $H_2O$ and recrystallized from a mixture of $H_2O$/2-butanol to afford the pure product.

*2.2.4. O-alkylation*

Products (**2**), (**5**) or *p*-OH-benzaldeyde (1 mmol), respectively, were dissolved in DMF then $K_2CO_3$ (2 eq.) and 3,3-dimethylallylbromide (Dal–Br) — in case of product (**3**) or geranyl bromide (Ger–Br) (1.2 eq.) in case of products (**6**) and (**14**) were added to the stirring solution. The mixture was reacted overnight, then filtered and the residue concentrated under vacuum. The crude residue was taken up in $CHCl_3$/*i*PrOH 3:1 and washed with a 1 N HCl solution and brine. The organic




**Scheme 4.** Reagents and conditions: a) Ger–Br, $K_2CO_3$, DMF, r.t., overnight.

layer was dried over $Na_2SO_4$, filtered and dried under vacuum. The crude residue was treated with $Et_2O$ to give the acylated products (**3**), (**6**), and (**14**).

*2.2.5. Compound **1** (Boc–Tyr–Gly–OMe)*

The title Boc protected dipeptide was prepared following the general coupling procedure described above starting from Boc–Tyr–OH (0.986 g, 3.50 mmol). Usual work up gave a crude residue which was purified by treatment with $Et_2O$/petroleum ether, to afford pure Boc–Tyr–Gly–OMe (0.860 g, 3.18 mmol, 76.6% yield) as oil. $^1$H NMR ($CDCl_3$) δ: 1.34 (9H, s, Boc); 3.00 (2H, m, βCH$_2$–Tyr); 3.73 (3H, s, OMe); 3.95 (2H, m, CH$_2$–Gly); 4.34 (1H, m, αCH–Tyr); 5.10 (1H, br, NH–Tyr); 6.48 (1H, t, NH–Gly); 6.71–7.04 (4H, dd, aromatics). Rf = 0.6 ($CH_2Cl_2$/EtOAc 1:1).

*2.2.6. Compound **2** (c[Tyr–Gly])*

Dipeptide (**1**) (0.434 g, 1.23 mmol) was treated with a mixture of TFA/$CH_2Cl_2$ 1:1 (5 mL) following the general procedure described above to give the $N^{\alpha}$-deprotected dipeptide (quantitative yield) which was used in the next step without further purification.

Product **2** was obtained in 43% of yield following the general DKP preparation procedure.

Rf = 0.70 (EtOAc/EtOH/AcOH/$H_2O$ 7:1:1:1). $^1$H NMR (DMSO-$d_6$) δ: 2.85 (2H, m, βCH$_2$–Tyr); 3.25 (2H, m, CH$_2$–Gly); 3.94 (1H, m, αCH–Tyr); 6.62–6.85 (4H, dd, aromatics); 7.84 and 8.08 (2H, br, NH–Tyr and NH–Gly); 9.28 (1H, s, OH–Tyr).

*2.2.7. Compound **3** (c[Tyr(O-Dal)–Gly])*

DKP **2** (0.108 g, 0.5 mmol) was alkylated in DMF (5 mL), $K_2CO_3$ (0.339 mg) and 3,3-dimethylallyl-bromide (0.3 mL) by following the general *O*-alkylation procedure described above. After the work-up the crude residue was treated with $Et_2O$ to give the pure product **3** in 46% of yield. Rf = 0.2 ($CHCl_3$/MeOH 95:5). $^1$H NMR (DMSO-$d_6$) δ: 1.66 and 1.71 (6H, 2d, 2 × CH$_3$ Dal); 2.65–3.02 (4H, m, βCH$_2$ Tyr and CH$_2$ Gly); 4.00 (2H, d, α-CH Tyr); 4.45 (2H, d, O-CH$_2$ Dal); 5.39 (1H, t,=*CH*–CH$_2$–O); 6.81 and 7.15 (4H, 2d, aromatics); 7.87 and 8.11 (2H, br, NH Tyr and NH Gly).

*2.2.8. Compound **4** (Boc–Tyr–Ser–OMe)*

The title $N^{\alpha}$–Boc protected dipeptide was prepared following the general coupling procedure described above starting from Boc–Tyr–OH (0.497 g, 1.76 mmol). Usual work-up gave an oily residue which was purified by silica gel column chromatography ($CH_2Cl_2$/EtOAc from 8:2 to 100% EtOAc), to afford pure Boc–Tyr–Ser–OMe (0.550 g) 78% of yield as oil. Rf = 0.6 ($CH_2Cl_2$: EtOAc 1:1). $^1$H NMR ($CDCl_3$) δ: 1.40 (9H, s, Boc); 2.98 (2H, m, βCH$_2$–Tyr); 3,73 (3H, s, $OCH_3$); 3.88 (2H, m, βCH$_2$–Ser); 4.27 (1H, m, αCH–Tyr); 4.57 (1H, m, αCH–Ser); 5.18 (1H, br, NH–Tyr); 6.77 and 7.05 (4H, dd, aromatics); 6.83 (1H, t, NH–Ser).

*2.2.9. Compound **5** (c[Tyr–Ser])*

Boc–Tyr–Ser–OMe (**4**) (0.434 g, 1.23 mmol) was treated with a mixture of TFA/$CH_2Cl_2$ 1:1 (5 mL) following the general procedure described above to give the $N^{\alpha}$-deprotected dipeptide (quantitative yield) which was used in the next step without further purification.

Product (**5**) was obtained in 56% of yield following the general DKP preparation procedure. Rf = 0.76 (EtOAc/EtOH/AcOH/$H_2O$ 7:1:1:1). $^1$H NMR (DMSO-$d_6$) δ: 2.85 (2H, m, βCH$_2$–Tyr); 3.84 (2H, m, βCH$_2$–Ser); 3.96 (2H, m, αCH Tyr); 4.85 (1H, m, αCH Ser); 6.65 and 6.90 (4H, dd, aromatics); 7.83 and 7.95 (2H, br, NH–Tyr and NH–Ser); 8.10 (1H, t, OH Ser); 9.18 (1H, s, OH–Tyr).

*2.2.10. Compound **6** (c[Tyr(O-Dal)–Ser])*

DKP (**5**) (0.05 g, 0.2 mmol) was alkylated in DMF (5 mL), $K_2CO_3$ (138 mg) and 3,3-dimethylallyl-bromide (0.03 mL) by following the general *O*-alkylation procedure described above. After the work-up the crude residue was purified by silica gel chromatography, using $CHCl_3$/MeOH 95:5 as eluent to give the pure product (**6**) in 35% of yield. Rf = 0.45 ($CHCl_3$/MeOH 95:5). $^1$H NMR (DMSO-$d_6$) δ: 1.66 and 1.71 (6H 2d, CH$_3$ Dal); 2.90 (4H, m, βCH$_2$ Tyr and CH$_2$ Gly); 3.62 and 3.90 (2H, m, αCH Tyr and αCH–Ser); 4.45 (2H, d, *O*-CH$_2$ Dal); 4.95 (1H, t, OH Ser); 5.39 (1H, t,=*CH*–CH$_2$–O); 6.81 and 7.05 (4H, 2d, aromatics); 7.87 and 7.96 (2H, br, NH Tyr and NH Gly).

*2.2.11. Compound **7** (Boc–Ala–Gly–OMe)*

The title *N*-Boc protected dipeptide was prepared following the general coupling procedure described above starting from Boc–Ala–OH (0.497 g, 2.63 mmol). Usual work-up gave an oily residue which was used for the next step without further purification (quantitative yield). Rf = 0.38 ($CH_2Cl_2$/EtOAc 8:2). $^1$H NMR ($CDCl_3$) δ: 1.37 (3H, d, $CH_3$Ala); 1.44 (9H, s, Boc); 3.75 (3H, s, $OCH_3$); 4.03 (2H, m, $CH_2$Gly); 4.22 (1H, m, CH–Ala); 4.99 (1H, br, NH–Boc); 7.05 (1H, br, NH–Gly).

*2.2.12. Compound **8** (c[Ala–Gly])*

Dipeptide (**7**) (0.622 g, 2.39 mmol) was treated with a mixture of TFA/$CH_2Cl_2$ 1:1 (5 mL) following the general procedure described above to give the *N*-deprotected dipeptide (quantitative yield) which was used in the next step without further purification.

Product (**8**) was obtained in 77% of yield following the general DKP preparation procedure. Rf = 0.55 (EtOAc/EtOH/AcOH/$H_2O$ 7:1:1:1).

*2.2.13. Compound **9** (N,N′-diacetyl-c[Ala–Gly])*

A mixture of DKP (**8**) (0.224 g, 1.75 mmol) in acetic anhydride (10 mL) was stirred under reflux for 7 h. The solvent was removed by azeotropic distillation with methanol and toluene under reduced pressure. The residue was crystallized from EtOAc/$Et_2O$ to yield (**9**) (91.5% yield) as a brown oil. $^1$H NMR ($CDCl_3$) δ: 1.50 (3H, d, $CH_3$ Ala); 2.52–2.54 (6H, 2 × s, $CH_3CO$); 4.02 and 5.10 (2H, dd, $CH_2$–Gly); 5.20 (1H, m, αCH–Ala).

*2.2.14. Compound **10** (c[Δ$^z$–Tyr(O-Ger)-N-acetyl-Ala])*

Compound (**9**) (0.054 g, 0.25 mmol) was dissolved in DMF, then (**14**) (0.164 g, 0.64 mmol) and tBuOK 0.5 M in tBuOH (1.2 eq.) were added at 0 °C. The mixture was allowed to warm to r.t. and stirred for 6 h. Then the reaction was quenched with aqueous $NH_4Cl$ solution and extracted three times with EtOAc. The combined organic layers were dried on $Na_2SO_4$, filtered and evaporated.

The obtained crude was purified on silica gel chromatography, using $CH_2Cl_2$/EtOAc 9:1 as eluent (73% yield). Rf = 0.65

($CH_2Cl_2$/EtOAc 9:1). $^1$H NMR ($CDCl_3$) δ: 1.48 (3H, d, $CH_3$ Ala); 1.60–1.65 (9H, 3 × s, 3 × $CH_3$ Ger); 2.07 (4H, m, $CH_2$–$CH_2$ Ger); 2.53 (3H, s, $CH_3$–CO); 4.52 (2H, d, $CH_2$–O Ger); 5.02 (1H, m,=C*H*–C($CH_3$)$_2$ Ger); 5.08 (1H, m, α-CH Ala); 5.41 (1H, m, O–$CH_2$–C*H*=Ger); 6.92 and 7.32 (4H, 2 × d, aromatics); 7.06 (1H, s, C*H*=Ar); 7.63 (1H, br, NH).

*2.2.15. Compound **11** (c[Δ$^z$–Tyr(O-Ger)–Ala])*

Compound (**10**) (39 mg, 0.11 mmol) was dissolved in MeOH (2 mL) and hydrazine monohydrate (2 eq.) was added. The product began to precipitate from the solution. After 2 h the solution was filtered and the pure final product was obtained as a white powder in quantitative yield. Rf = 0.92 (EtOAc/AcOH/n-BuOH/$H_2O$ 1:1:1:1). $^1$H NMR ($CD_3OD$) δ: 1.48 (3H, d, $CH_3$ Ala); 1.60–1.78 (9H, 3 × s, $CH_3$ Ger); 2.09 (4H, m, $CH_2$–$CH_2$ Ger); 4.20 (1H, m, CH Ala) 4.62 (2H, d, $CH_2$–O Ger); 5.09 (1H, m,=C*H*–C($CH_3$)$_2$ Ger); 5.44 (1H, m, O–$CH_2$–C*H*=Ger); 6.82 (1H, s, C*H*=Ar); 6.96 and 7.42 (4H, 2 × d, aromatics).

*2.2.16. Compound **12** (c[Δ$^z$–Phe–N-acetyl–Ala])*

Compound (**12**) was obtained from (**9**) (0.150 g, 0.71 mmol) following the same procedure used for compound (**10**). After the work-up the crude product was purified on silica gel chromatography, using $CH_2Cl_2$/EtOAc 95:5 as eluent to give the pure product (**12**) in 72% of yield. Rf = 0.45 ($CH_2Cl_2$/EtOAc 95:5). $^1$H NMR ($CDCl_3$) δ: 1.58 (3H, d, $CH_3$ Ala); 2.61 (3H, s, $CH_3$–CO); 5.12 (1H, m, α-CH Ala); 7.40–7.47 (5H, m, aromatics); 7.18 (1H, s, C*H*=Ar); 7.75 (1H, br, NH).

*2.2.17. Compound **13** (c[Δ$^z$–Phe–Ala])*

Compound (**12**) (131 mg, 0.50 mmol) was deacetylated following the procedure used for compound (**11**). After the filtration, compound (**15**) was obtained in quantitative yield. Rf = 0.88 (EtOAc/AcOH/n-BuOH/$H_2O$ 1:1:1:1). $^1$H NMR (DMSO-$d_6$) δ: 1.05 (3H, d, $CH_3$ Ala); 4.15 (1H, m, α-CH Ala); 7.06 (1H, s, C*H*=Ar); 7.12–7.28 (5H, m, aromatics); 8.00 and 8.11 (2H, 2 × s, 2 × NH).

*2.2.18. Compound **14** (O-Ger-4-OH-benzaldehyde)*

*p*-OH-Benzaldehyde (0.5 g, 4.05 mmol) was alkylated in DMF (10 mL), $K_2CO_3$ (4 eq.) and geranyl bromide (2 eq.) by following the general *O*-alkylation procedure described above. After the work-up the crude residue was purified on silica gel chromatography, using n-hexane/EtOAc 75:25 as eluent to give the pure product (**14**) in 94% of yield. Rf = 0.55 (n-hexane/EtOAc 8:2). $^1$H NMR ($CDCl_3$) δ: 1.60–1.75 (9H, 3 × s, 3 × $CH_3$ Ger); 2.07 (4H, m, $CH_2$–$CH_2$ Ger); 4.62 (2H, d, $CH_2$–O Ger); 5.08 (1H, m,=C*H*–C($CH_3$)$_2$ Ger); 5.51 (1H, m, O–$CH_2$–C*H*=Ger); 7.00 and 7.82 (4H, 2 × d, aromatics); 9.86 (1H, s, HCO).

*2.3. Pharmacology*

*2.3.1. Determination of cancer cell growth inhibition*

The human cancer cell lines studied were obtained either from the American Type Culture Collection (ATCC, Manassas, USA; see the legend to Table 1), the European Collection of Cell Culture (ECACC, Salisbury, UK; see the legend to Table 1) or the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ, Braunschweig, Germany; see the legend to Table 1). Six cancer cell lines were used as detailed in the legend of Table 1.

Cells were cultured in MEM (Invitrogen, Merelbeke, Belgium; the U373 cell line) and in RPMI (Invitrogen) media supplemented with 10% heat inactivated fetal calf serum (Invitrogen). All culture media were supplemented with 4 mM glutamine, 100 μg/mL gentamicin, and penicillin–streptomycin (200 U/mL and 200 μg/mL, respectively) (Invitrogen).

The overall growth level of each cell line was determined using the colorimetric MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide, Sigma, Belgium) assay as detailed previously [21–23]. Each experimental condition was carried out in six replicates.

## 3. Results and discussion

In the current study, we synthesized and characterized the *in vitro* growth inhibitory activity of three naturally occurring prenyloxy DKPs. As outlined in Schemes 1–4, briefly compounds (**3**) and (**6**) have been obtained starting from Boc–Tyr that has been coupled to Gly–OMe and Ser–OMe respectively in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), HOBt and *N*-methylmorpholine (NMM) in DMF at 0 °C with yields of 77% and 89%. The resulting dipeptides have been then deprotected with $CF_3COOH$ 50% in $CH_2Cl_2$ at r.t. and cyclized by treatment with AcOH 0.1 N, isobutanol and NMM to provide the desired diketopiperazines in 44% (R=H) and 56% (R=OH) yields respectively. Finally alkylation with 3,3-dimethylallyl bromide in the presence of $K_2CO_3$ in DMF gave (**3**) and (**6**) in 46% and 21% of yields. Deoxymycelianamide (**11**) has been obtained by the initial coupling and subsequent cyclization of Ala–Boc and Gly–OMe under the same experimental conditions as above to provide the corresponding diketopiperazine in 96% of yield. The latter has been acetylated (**9**) (yield 92%) and then coupled to geranylated p-OH-benzaldehyde (**14**) (yield 69%). Hydrazinolysis of the acetyl derivative in aqueous MeOH at r.t. yielded (**11**) in nearly quantitative yield.

The *in vitro* $IC_{50}$ growth inhibitory concentration was determined for each compound in a panel of six human cancer cell lines exhibiting different levels of resistance to pro-apoptotic stimuli using the MTT colorimetric assay as detailed in Table 1.

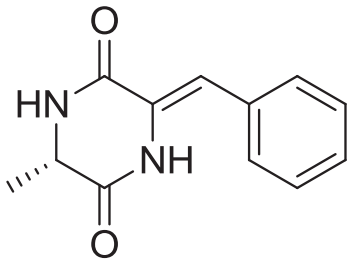
The data show that of the three phytochemicals under study, compounds (**3**) and (**6**) display weak or no ($IC_{50}$ > 100 μM) inhibitory activities, while deoxymycelianamide (**11**) was recorded as the only effective compound with a mean $IC_{50}$ value of 11 μM. This latter was seen to be particularly active against human Hs683 and U373 glioma, SKMEL-28 melanoma and A549 non-small-cell lung cancer cells. The presence of the shorter isopentenyloxy side chains led to largely decrease the growth inhibitory activity. Comparing the structures of compounds (**3**), (**6**), and (**11**), it can be hypothesized that not only the presence of an *O*-geranyl chain like in (**11**) is a determinant for the observed effects but also, the carbon–carbon double bond exocyclic to the DKP ring may play a key role to this regard. It has been already reported in fact how the presence of an exocyclic double bond in diketopiperazine enhances the inhibitory activity towards proliferating cells when compared to the saturated counterparts [24]. However also the presence of a geranyloxy chain seems to greatly enhance the observed growth inhibitory effects. Supporting this hypothesis, data recorded for compound **13** (Fig. 2), that we

**Table 1**
*In vitro* growth inhibitory activity of the compounds under study.

| $IC_{50}$ (μM)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|
| Compound | Hs683 | A549 | MCF-7 | SKMEL-28 | B16F10 | U373 | Mean ± SD |
| **3** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **6** | 96 | 45 | >100 | 94 | >100 | 33 | >78 ± 12 |
| **11** | 5 | 7 | 22 | 2 | 23 | 6 | 11 ± 4 |
| **13** | >100 | >100 | 58 | 85 | 87 | >100 | >86 ± 7 |

The cell lines include the U373 (code ECACC 08061901) and Hs683 (code ATCC HTB-138) human glioma, the human A549 (code DSMZ ACC107) non-small-cell lung cancer, the human SKMEL-28 (code ATCC HTB-72) melanoma, the human MCF-7 (code DSMZ ACC115) breast adenocarcinoma and the mouse B16F10 (code ATCC CRL-6475) melanoma cells.

[a] The $IC_{50}$ growth inhibitory concentrations were determined *in vitro* by the MTT colorimetric assay. The $IC_{50}$ value represents the concentration that reduces by 50% the growth of a given cancer cell population after having culturing it for 3 days with the compound of interest.




**13**

**Fig. 2.** Structure of compound **13**, a non-genarylated analog of compound **11**.

synthesized following a scheme very close to that described above, not having the geranyloxy chain, revealed no appreciable activity against all the six cancer cell lines under investigation as outlined in Table 1.

Prenylation is a common metabolic reaction in nature, most frequently occurring in bacteria, fungi, and plants. Very frequently, the addition of an isoprenoid chain renders the molecule pharmacologically more effective than the parent non-prenylated compound. One of the clearest examples of this phenomenon was recently reported by Kretzschmar and coworkers [24] who observed that prenylated genistein and naringenin, among the most common flavonoids extracted from plants, exerted stronger estrogenic activities than the respective parent compounds. In this context, data collected herein enforce the concept of how a prenyl side chain is crucial for the growth inhibitory activity on selected cancer cell lines by naturally occurring diketopiperazines. However studies to get further insights into the mechanism of action of deoxymycelianamide **11** as well as to investigate the effects as cancer cell growth inhibitory agents exerted by semisynthetic derivatives are needed. Such a research activity is now ongoing in our laboratories.

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