

Synthesis and Fungicidal Activity of Lipophylic N- and O-Acyl Derivatives of β -Hydroxy DL- α -Amino Acids

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Synthesis of N- and O-acyl derivatives of DL-serine and threo-DL-phenylserine was accomplished by a regioselective acylation of the corresponding amino acid. The residues introduced into amino acid structure contain hydrophobic long chain or aromatic, namely lauroyl, myristoyl and phenylacetyl moieties. The fungicidal activity against six strains of fungi was studied. Several compounds were found to be effective against growth of fungi, and O-myristoyl-DL-serine **2** and N-phenylacetyl-threo-DL-phenylserine **8** completely inhibited the growth of the mycelium of the fungus *Verticillium dahliae*.

Key words: DL-(phenyl)Serines, N- and O-Acylation, Fungicidal Activity

Introduction

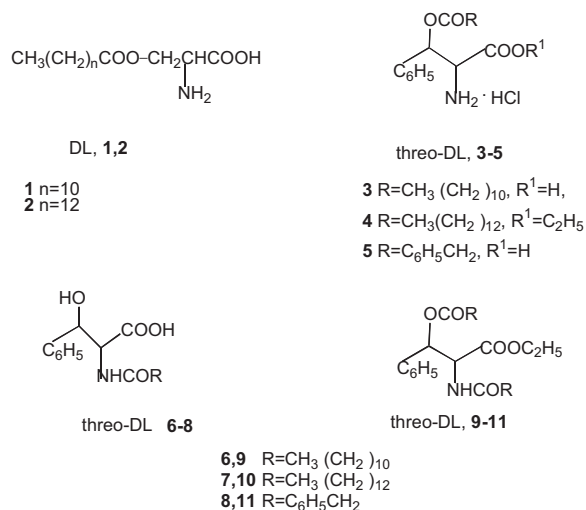
Development of new fungicides largely depends on random screening procedures and thousands of organic compounds of various classes were found as specific inhibitors of fungal growth (see, for example, Fisher *et al.*, 2001; Zou *et al.*, 2002). Amino acid derivatives (Strube *et al.*, 1985) and peptides (Rajasekaran *et al.*, 2001) were found to possess a wide fungicidal spectrum. Among the tested amino acid derivatives some N-acyl aliphatic amino acids showed protective activity against rice blast disease caused by the fungus *Pyricularia oryzae*, and the most persistent activity was exhibited by N-lauroyl-L-valine (Homma *et al.*, 1973; Shida *et al.*, 1975). In an earlier work threo-L-phenylserines have been found to increase the resistance of cucumber seedlings to *Cladosporium cucumerinum* (van Anandel, 1966). N-(2-cyano-2-methoximinoacetyl)amino acids and their ester derivatives were synthesised and tested for fungicidal activity against grape downy mildew (Smith *et al.*, 1995). Recently, several analogues of PMAP-23, a 23-mer peptide, were designed to increase the net hydrophobicity, and the results suggested that the increase of hydrophobicity of the peptides enhances the fungicidal activity (Lee *et al.*, 2002). Thus amino acid derivatives appear to be interesting in the search for new fungicides. Therefore in this

study derivatives of N- and O-acyl β -hydroxy amino acids, specifically DL-serine and threo-DL-phenylserine, containing hydrophobic chains were synthesized. These compounds were tested for fungicidal activity against six mycelial strains.

Results and Discussion

Synthesis

The regioselective acylation of hydrophobic derivatives of DL-serine and threo-DL-phenylserine containing aromatic and long chain fatty acids residues was achieved under elaborated reaction conditions in present work. Structures of the synthesized compounds are presented in Scheme 1. The O-acylation of DL-serine and threo-DL-phenylserine and its ethyl ester hydrochloride was accomplished by a reaction with acid chlorides, *i.e.* myristoyl, lauroyl and phenylacetyl chlorides in anhydrous trifluoroacetic acid. The reaction proceeded smoothly and regioselectively to yield the respective O-acylated derivatives **1–5**. Interestingly to note that the corresponding hydrochlorides formed in the reaction mixture were isolated as a free base upon crystallization from a methanol-water mixture. The selective N-acylation of threo-DL-phenylserine was performed by an interaction of the lauroyl, myristoyl and phenyl-

Scheme 1. Structures of synthesized compounds **1–11**.

acetyl chlorides with threo-DL-phenylserine in aqueous alkaline solution at 0 °C to give the derivatives **6–8**, respectively. The reaction of the above indicated fatty acid chlorides at 80 °C afforded the corresponding N,O-diacyl derivatives **9–11**. The structure of the synthesized compounds was proved by spectroscopic and elemental analysis data. The chemical shift of the methine proton signals at α - and β -carbon atoms in 1H NMR spectra and the respective carbon atom signals in ^{13}C the spectra are indicative to assign the position of acyl group in the amino acid derivative.

Fungicidal activity

The results of fungicidal activity of synthesized compounds **1–11** on the six strains of mycelial fungi are presented in Table I. These compounds exhibited the most pronounced activity against the mycelium of *Verticillium dahliae*. O-myristoyl-DL-serine **2** completely stops the growth of the latter micella as does the reference fungicide thiram (IUPAC name bis(dimethylthiocarbamoyl)disulfide).

The same hydrophobic chain containing O-myristoyl-threo-DL-phenylserine retarded the growth of this fungus by 80%, while other O-acylderivatives, namely **1**, **3**, and **5** exhibited insignificant fungicidal effect. N-phenylacetyl-threo-DL-phenylserine **8** contrary to its O-phenylacetyl analogue **5** completely inhibited the growth of *Verticillium dahliae*. Compounds **6** and **7** retarded the growth of this micelle to 50 and 40%, respectively. The N,O-diacyl derivatives **9–11** stopped multiplication of this fungus strain by 30–50%. Two synthesized derivatives, *i.e.* N-phenylacetyl-threo-DL-phenylserine **8** and N,O-dimyristoyl-threo-DL-phenylserine **10** slowed down the growth of micella *Xanthomonas malvacearum* by 50%, while compounds **1–7**, **9** and **11** were inactive or showed insignificant effect on this fungus strain. Similar undistinguished effect (10–27%) all synthesized compounds possessed against the rest of investigated fungi, *i.e.* *Aspergillus niger*, *Botrytis cinerea*, *Fusarium moniliforme*, *Venturia inaequalis*.

Table I. Fungicidal activity of compounds **1–11**.

Compound	Concentration μM	Inhibition of fungal growth (%)					
		<i>Aspergillus niger</i>	<i>Botrytis cinerea</i>	<i>Fusarium moniliforme</i>	<i>Venturia inaequalis</i>	<i>Verticillium dahliae</i>	<i>Xanthomonas malvacearum</i>
1	100	10	25	20	18	40	25
2	95	10	20	20	24	100	0
3	82	10	27	20	24	20	15
4	65	10	27	28	26	80	37
5	100	10	25	23	24	20	0
6	82	10	25	23	16	50	15
7	76	10	27	15	24	40	17
8	100	10	27	23	18	100	50
9	52	15	25	20	24	50	15
10	47	15	27	23	18	50	50
11	67	10	20	20	18	30	12
Thiram	125	100	100	100	100	100	100

In conclusion two of the tested compounds, namely O-myristoyl-DL-serine **2** and N-phenylacetyl-threo-DL-phenylserine **8** completely inhibited the growth of *Verticillium dahliae* fungus. Thus the length of the alkyl group plays a role in eliciting the fungicidal activity which depends on the hydrophobic part of the molecules in accordance with recent results (Kubo *et al.*, 2002). These findings may induce further search of synthetic fungicides among acyl derivatives of β -hydroxy DL- α -amino acids containing hydrophobic chains.

Experimental Section

General remarks

M.p.'s were determined in open capillaries and are uncorrected. IR spectra were recorded on a Specord 75 instrument (Carl Zeiss, Jena, Germany) in KBr pellets, ^1H NMR spectra – on a Hitachi R-22 spectrometer (90 MHz, Japan) and ^{13}C spectra – on a Tesla BS-587A (Brno, Czech Republic) (20 MHz) instrument using HMDS as an internal reference ($\delta = 0.05$ ppm to TMS) in DMSO- d_6 solution. Chemical shifts δ are reported in ppm, coupling constants (J) are given in Hz. Multiplicity of signals is expressed as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet).

DL-serine and threo-DL-phenylserine were purchased from Chemapol (Prague, Czech Republic).

Preparation of O-acyl-DL-serines (**1,2**)

General procedure

To a vigorously stirred solution of 0.01 mol DL-serine in 5.0 ml of anhydrous trifluoroacetic acid, 0.013 mol of lauroyl or myristoyl chloride was slowly added dropwise at 0 °C. After the complete addition the reaction mixture was stirred for 1 h at room temperature and 1.0 ml of anhydrous ethanol and ether was added. The precipitate of the corresponding hydrochloride was filtered off and recrystallized from a methanol-water mixture. The free amino acids were obtained dissolving the product in methanol under stirring.

O-Lauroyl-DL-serine (**1**)

Yield 2.2 g (71%); m.p. 148–149 °C (dec.). – IR: $\nu = 1590$ (COO^-) and 1733 (C=O) cm^{-1} . – ^1H NMR (CF_3COOH): $\delta = 0.49$ (m, 3H, CH_3), 0.9–

1.0 (m, 16H, $(\text{CH}_2)_8$), 1.24 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}$), 2.10 (t, $J = 7.0$, 2H, CH_2CO), 4.30–4.40 (m, 3H, CH_2CH), 7.25 (m, 3H, NH_2). – $\text{C}_{15}\text{H}_{29}\text{NO}_4$ (287.39): calcd C 62.69, H 10.17, N 4.87; found C 62.76, H 10.30, N 4.47.

O-Myristoyl-DL-serine (**2**)

Yield 2.1 g (67%); m.p. 136–138 °C. – IR: $\nu = 1589$ (COO^-) and 1736 (C=O) cm^{-1} . – ^1H NMR (CF_3COOH): $\delta = 0.47$ (m, 3H, CH_3), 0.9–1.0 (m, 20H, $(\text{CH}_2)_{10}$), 1.25 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}$), 2.05 (t, $J = 7.0$, 2H, CH_2CO), 4.20–4.40 (m, 3H, CH_2CH), 7.25 (m, 3H, NH_3^+). – $\text{C}_{17}\text{H}_{33}\text{NO}_4$ (315.45): calcd C 64.73, H 10.54, N 4.44; found C 64.76, H 10.30, N 4.37.

Preparation of O-acyl-threo-DL-phenylserines (**3,4,5**)

General procedure

To a vigorously stirred solution of 0.02 mol threo-DL-phenylserine or the corresponding ethyl ester hydrochloride in 7.0 ml of anhydrous trifluoroacetic acid a slight excess (0.026 mol) of lauroyl or myristoyl chloride, respectively, was slowly added dropwise at 0 °C. The solution was filtered and the formed precipitate was recrystallized from methanol-ether mixture.

O-Lauroyl-threo-DL-phenylserine hydrochloride (**3**)

Yield: 79%; m.p. 134–137 °C. – IR: $\nu = 1600$, 1735, 1750 (C=O) cm^{-1} . – ^1H NMR: (CF_3COOH): $\delta = 0.45$ (m, 3H, CH_3), 0.90–1.0 (m, 16H, $(\text{CH}_2)_8$), 1.3 (m, 2H, CH_2), 2.18 (t, $J = 7.0$, 2H, CH_2CO), 4.37 (m, 1H, $\alpha\text{-CH}$), 6.30 (d, $J = 2.5$, 1H, $\beta\text{-CH}$), 6.9–7.3 (m, 8 H, aromatic protons and NH_3^+). – ^{13}C NMR (DMSO- d_6): $\delta = 13.4$, 22.1, 24.1, 28.3, 28.7, 28.9, 31.3, 33.3, 56.0 ($\alpha\text{-C}$), 72.8 ($\beta\text{-C}$), 128.7, 129.1, 134.9, 166.4 (C=O), 171.6 ($\beta\text{-CH-OCO}$). – $\text{C}_{21}\text{H}_{33}\text{NO}_4$ (363.49): calcd C 69.39, H 9.15, N 3.85; found C 69.23, H 8.90, N 3.75.

O-myristoyl-threo-DL-phenylserine ethyl ester hydrochloride (**4**)

Yield: 78%; m.p. 136–138 °C. – IR: $\nu = 1737$, 1754 (C=O) cm^{-1} . – ^1H NMR (CF_3COOH): $\delta = 0.44$ (t, 3H, CH_3), 0.78 (m, 3H, CH_3), 0.76–1.40 (m, 22H, $(\text{CH}_2)_{11}$), 2.20 (t, $J = 7.0$, 2H, COCH_2), 4.0 (q, $J = 7.0$, 2H, CH_2), 4.42 (t, $J = 5.0$, 1H, $\alpha\text{-}$

CH), 6.20 (d, $J = 5.0$, 1H, β -CH), 7.0–7.2 (m, 5H, aromatic protons), 7.25 (bs, 3H, NH_3^+). – ^{13}C NMR (DMSO- d_6) δ (ppm): 13.7, 14.1, 22.2, 24.3, 28.5, 28.7, 28.8, 29.1, 31.4, 33.5, 56.3 (α -C), 62.1, 73.0 (β -C), 127.1, 128.9, 129.3, 134.9, 166.6 (C=O), 171.8 (β -CH-OCO). – $\text{C}_{25}\text{H}_{42}\text{ClNO}_4$ (456.06): calcd C 65.84, H 9.28, Cl 7.77, N 3.07; found C 65.80, H 9.50, Cl 7.28, N 3.27.

O-Phenylacetyl-threo-DL-phenylserine (5)

Yield: 76%, m.p. 118–120° C. – IR: $\nu = 1600$, 1720 (C=O) cm^{-1} . – ^1H NMR (CD_3OD): $\delta = 3.5$ (s, 2H, CH_2Ph), 4.4 (d, $J = 3.0$, 1H, α -CH), 6.25 (d, $J = 3.0$, 1H, β -CH), 6.7–7.4 (m, 10 H, aromatic protons). – $\text{C}_{17}\text{H}_{17}\text{NO}_4$ (299.32): calcd C 68.21, H 5.72, N 4.68; found C 68.30, H 5.94, N 5.03.

Preparation of *N*-acyl derivatives of threo-DL-phenylserine (6–8)

General procedure

To a vigorously stirred solution of 20 mmol threo-DL-phenylserine in 40 ml 0.5 NaOH solution in water (20 mmol) at 0 °C simultaneously dropwise was added 23 mmol of the corresponding acyl chloride in 10 ml of 1,4-dioxane and 2 N NaOH in water in 15 min maintaining the alkaline reaction media. After stirring for 1 h at room temperature the reaction mixture was poured into crushed ice and acidified with 1 N HCl to pH 3. The product was filtered off, washed with water and recrystallized from ethanol-water.

N-Lauroyl-threo-DL-phenylserine (6)

Yield: 73%; m.p. 108–109° C (dec.). – IR: $\nu = 1654$, 1708, 1732 (C=O), 3320 (NH) cm^{-1} . – ^1H NMR (CD_3OD): $\delta = 0.76$ (m, 3 H, CH_3), 1.10–1.30 (m, 18 H, $(\text{CH}_2)_9$), 2.0 (t, $J = 7.0$, 2H, CH_2CO), 4.65 (d, $J = 2.0$, 1H, α -CH), 5.2 (d, $J = 2.0$, 1H, β -CH), 7.0–7.36 (m, 5 H, aromatic protons). – ^{13}C NMR (DMSO- d_6): $\delta = 13.9$, 22.1, 22.2, 25.3, 29.1, 31.4, 35.0, 57.9 (α -C), 72.3 (β -C), 126.2, 126.9, 127.7, 142.4, 171.9 (C=O), 172.5 (C=O). – $\text{C}_{21}\text{H}_{33}\text{NO}_4$ (363.49): calcd C 69.39, H 9.15, N 3.85; found C 69.50, H 8.90, N 3.76.

N-Myristoyl-threo-DL-phenylserine (7)

Yield: 83%; m.p. 103–104° C. – IR: $\nu = 1645$, 1708 and 1732 (C=O), 3320 (NH) cm^{-1} . – ^1H

NMR (CD_3OD): $\delta = 0.8$ (m, 3 H, CH_3), 1.18–1.30 (m, 22 H, $(\text{CH}_2)_{11}$), 2.0 (t, $J = 7.0$, 2H, CH_2CO), 4.7 (d, $J = 2.0$, 1H, α -CH), 5.27 (d, $J = 2.0$, 1H, β -CH), 7.1–7.45 (m, 5 H, aromatic protons). – ^{13}C NMR (DMSO- d_6): $\delta = 13.9$, 22.1, 25.3, 28.3, 29.1, 31.5, 35.0, 57.2 (α -C), 72.3 (β -C), 126.3, 126.9, 127.7, 142.3, 172.0 (C=O), 172.5 (C=O). – $\text{C}_{23}\text{H}_{37}\text{NO}_4$ (391.55): calcd C 70.55, H 9.52, N 3.58; found C 70.32, H 9.73, N 3.51.

N-Phenylacetyl-threo-DL-phenylserine (8)

Yield: 58%, m.p. 158–159° C. – IR: $\nu = 1650$, 1725 (C=O) cm^{-1} . – ^1H NMR (CD_3OD): $\delta = 3.4$ (s, 2H, CH_2Ph), 4.7 (d, $J = 3.0$, 1H, α -CH), 4.9 (s, 1H, OH), 5.25 (d, $J = 3.0$, 1H, β -CH), 6.9–7.34 (m, 10 H, aromatic protons). – ^{13}C NMR (DMSO- d_6): $\delta = 41.9$, 35.0, 58.3 (α -C), 72.3 (β -C), 127.2–142.3, 170.5 (C=O), 171.9 (C=O). – $\text{C}_{17}\text{H}_{17}\text{NO}_4$ (299.32): calcd C 68.21, H 5.72, N 4.68; found C 68.01, H 5.84, N 4.63.

Preparation of *N,O*-diacyl derivatives of threo-DL-phenylserine ethyl ester (9–11)

General procedure

A mixture of 20 mmol threo-DL-phenylserine ethyl ester hydrochloride and 60 mmol of acyl chloride was heated at 80 °C for 1 h. The reaction mixture was cooled to room temperature and diluted with 40 ml of anhydrous chloroform. 60 mmol of dry triethylamine was added dropwise to this solution over 10 min. The resultant mixture was refluxed for 1 h, cooled, washed with water, 5% NaHCO_3 solution, water, and organic layer was dried over anh. MgSO_4 . Solvent was evaporated *in vacuo* and the residue recrystallized from ether-pentane or ether-ethyl acetate.

N,O-Dilauroyl-threo-DL-phenylserine ethyl ester (9)

Yield: 88%; m.p. 62–64° C. – IR: $\nu = 1670$, 1745 (C=O), 3330 (NH) cm^{-1} . – ^1H NMR (CF_3COOH): $\delta = 0.5$ (m, 6 H, $2 \times \text{CH}_3$), 0.9–1.20 (m, 35 H, $2 \times (\text{CH}_2)_8$ and CH_3), 2.05 (m, 4 H, $2 \times \text{CH}_2\text{CO}$), 3.87 (q, $J = 7.0$, 2 H, COOCH_2), 4.90 (d, $J = 5.0$, 1 H, α -CH), 5.95 (d, $J = 5.0$, 1 H, β -CH), 6.9–7.0 (m, 5 H, aromatic protons), 7.5 (bs, 1 H, NH). – $\text{C}_{35}\text{H}_{59}\text{NO}_5$ (573.86): calcd C 73.25, H 10.36, N 2.44; found C 73.15, H 10.10, N 2.39.

N,O-Dimyristoyl-threo-DL-phenylserine ethyl ester (**10**)

Yield: 76%; m.p. 75–77° C. – IR: ν = 1660, 1745 (C=O), 3320 (NH) cm^{-1} . – ^1H NMR (CF_3COOH): δ = 0.5 (t, J = 7.0, 3 H, CH_3), 0.9–1.10 (m, 43 H, $2 \times (\text{CH}_2)_{10}$ and CH_3), 1.28 (m, 4 H, $2 \times \text{CH}_2$), 2.14 (t, J = 6.5, 4 H, $2 \times \text{CH}_2\text{CO}$), 3.6 (q, 2 H, CH_2), 4.95 (d, J = 5.0, 1H, α -CH), 6.1 (d, J = 5.0, 1 H, β -CH), 7.0 (brs, 5 H, aromatic protons), 7.5 (d, 1 H, NH). – $\text{C}_{39}\text{H}_{67}\text{NO}_5$ (629.97): calcd C 74.35, H 10.72, N 2.23; found C 74.50, H 11.09, N 2.29.

N,O-Diphenylacetyl-threo-DL-phenylserine ethyl ester (**11**)

Yield: 66%; m.p. 131–133° C. – IR: ν = 1670, 1745 (C=O), 3280 (NH) cm^{-1} . – ^1H NMR (CF_3COOH): δ = 1.13 (t, J = 5.0, 3 H, CH_3), 3.44 (s, 4 H, CH_2CO), 3.9 (q, J = 5.0, 2 H, CH_2), 4.05 (q, J = 3.5, 1 H, α -CH), 4.9 (q, J = 3.5, 1 H, α -CH), 5.98 (d, J = 6, 1 H, NH), 6.22 (d, J = 3.5, 1H, β -CH), 6.9–7.50 (m, 15 H, aromatic protons). – ^{13}C NMR (DMSO-d_6): δ = 13.7, 39.8, 40.7, 56.2 (α -C), 61.3, 74.83 (β -C), 126.3–136.5, 170.0 (C=O), 169.1, 170.6 (C=O). – $\text{C}_{27}\text{H}_{27}\text{NO}_5$ (445.52): calcd C 72.79, H 6.10, N 3.17; found C 72.62, H 6.17, N 3.17.

Evaluation of fungicidal activity

Preparations **1**–**11** in acetone under sterile conditions were introduced into the melted potato dextrose agar and poured into a petri plate. The concentration of tested compounds and the reference compound thiram was 47–100 μM in acetone. After 18–20 h since pouring out and solidifying of the medium, the agar plates were inoculated by some mycelium of the corresponding fungus and incubated in a thermostat at 22–25° C for 4–5 days. Mycelial growth on the agar plates was evaluated by measuring the growth diameter of the mycelial colony in comparison with the growth in controls, *i.e.* untreated petridishes. Inhibition I in percentage of the growth of fungus was estimated according to the formula:

$$I = (c - a/c) 100,$$

where c is mycelial growth in control in the absence of tested compound, and a is mycelial growth in the presence of tested compound. The experiments were performed in duplicate.

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