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Synthesis and antibacterial activity of some new non-proteinogenic amino acids containing thiazole residues

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Summary. Some new thioamides and thiazoles have been synthesized using canavanine, S-cysteine, homo-S-cysteinesulfonamides and their N-ω-aminoethylated derivatives as adducts in order to investigate the structure-antimicrobial activity relationships. The compounds showed substantial antibacterial activity *in vitro* against various gram-positive (*Staphylococcus aureus, Bacillus cereus etc.*) and gram-negative (*Escherichia coli, Proteus vulgaris etc.*) bacteria. These findings indicate that the presence of the thiazole residue is an essential factor for the antibacterial effect.

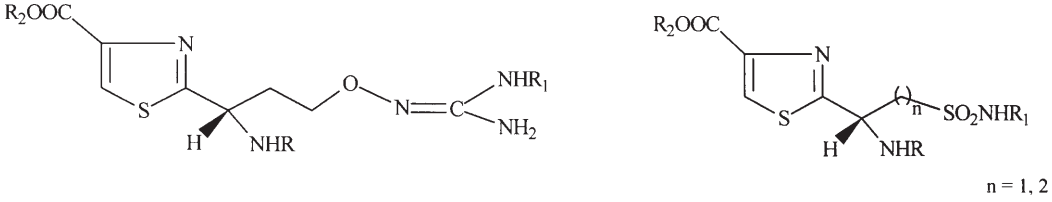
Keywords: Heterocyclic amino acids – Peptide mimetics – Thiazole – Antimicrobial activity – Canavanine – S-Cysteine- and homo-S-Cysteinesulfonamides – Alkaline protease

Abbreviations: Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; Bzl, benzyl; Tmob, 2,4,6-trimethoxybenzyl; $(Boc)_2O$, di-*tert*-butyl carbonate; DME, 1,2-dimethoxyethane; DMS, dimethyl sulfide; DCM, dichloromethane; DMF, N,N-dimethylformamide; MIC, minimal inhibitory concentrations; TFA, trifluoroacetic acid.

Introduction

In recent years many thiazole-containing secondary metabolites from marine sources have been isolated and characterized (Wipf et al., 1995). The interest in amino acid-derived thiazoles is growing because of their potential biological activity. For example, many of these compounds are potent antineoplastic (Pettit, 1987; Kobayashi et al., 1992) and antifungal (Northcote et al., 1991) agents. This has prompted many groups in the last years to develop general methods for the synthesis of this class of compounds in optically pure form.

The use of these modified amino acids as building blocks for the preparation of peptide analogues is well documented. These studies now comprise a large part of contemporary medicinal chemistry, protein engineering and molecular biology.



R= H, Boc, Z **R$_1$**= Boc-, Tmob-, BocHN(CH$_2$)$_2$- **R$_2$**= H, Et

Fig. 1

On the other hand, while there has been much research on the synthesis of amino acid-derived thiazoles, little has been published on the non-proteinogenic amino acids containing thiazole residue.

Following our current interest in application of unnatural amino acids in preparative peptide synthesis, we have synthesized some amino acid derivatives as structural analogues of arginine and canavanine (Pajpanova et al., 1997), asparagine and lysine (Videnov et al., 1993). Their effects on the growth of microorganisms, model plant systems, cultured tumor cells and their antitumor activity *in vivo* was evaluated (Pajpanova et al., 1992, Miersch et al., 1997). In addition amides and hydrazides of various proteinogenic amino acids as well as peptides containing heterocyclic backbone moieties have been studied extensively for their biological activity (M. Stanchev et al., 1998; 1999).

Our ongoing investigations of thiazole's antimicrobial activity are currently focused on the development of new analogues with lower toxicity and enhanced potency. This article describes synthetic methods for preparing a number of unnatural amino acids containing the thiazole residue (Fig. 1) and their growth inhibitory potency for various gram-negative and gram-positive bacteria.

Materials and methods

Melting points were determined on a Kofler melting point apparatus and are uncorrected. Thin layer chromatography was carried out on a precoated silica gel 60 F_{254} plates (Merck) and detection was performed using an UV lamp at 254 nm; solvent systems: A = $CHCl_3$/MeOH/H_2O (80/30/5); B = $CHCl_3$/MeOH/AcOH (95/5/3); C = $CHCl_3$/MeOH (9/1); D = EtOAc/*n*-hexane (1/1); E = *n*BuOH/AcOH/H_2O (4/1/1). Column chromatography was performed on silica gel 60 (70–230 mesh, Merck) using chloroform or ethyl acetate/*n*-hexane as solvent systems. Instrumentation and condition: HPLC was performed on a LC 1110 GBC chromatograph fitted with an ODS2 column (4.6 mm/250 mm). Optical rotation was measured with a Perkin-Elmer Model 141 polarimeter. IR spectra were recorded on Specord-71-IR (Zeiss) or Bruker 113 V spectrophotometers and spectral data are given as ν_{max} in cm^{-1}. ^1H NMR spectra were recorded on Bruker WM-250 and Avance DRX-250 (250 MHz) spectrometers using tetramethyl-silane as internal standard. All chemical shifts (δ) were recorded as ppm values; solution in $CDCl_3$ and [D_6]DMSO (c = 40 mg/ml^{-1}); chemical shifts referenced to the solvent peaks [δ (^{13}C $CDCl_3$) = 77, δ (^{13}C [D_6]DMSO) = 39.5 and δ (^1H[D_6]DMSO) = 2.49]. The electrospray mass spectra were

recorded on an API III triple quadruple mass spectrometer equipped with electrospray atmospheric pressure ion source (Sciex, Thronhill, Canada). The spectra were acquired in the positive mode. The samples for electrospray mass spectra were dissolved in 10% HCOOH/ methanol (1:1).

Antimicrobial assessment

Antimicrobial activity *in vitro* was studied by determination of the minimal inhibitory concentrations of the compounds (MIC), using the two-fold broth dilution method (Reiner et al., 1980). The compounds were dissolved in small amounts of DMF and were subsequently diluted with the respective nutrient media (i.e. Nutrient broth, Difco or Sabouraud Dextrose broth) to give final concentrations of 1.62 to 0.05 mM. The DMF content was less than 5%. Inoculation was carried out with overnight cultures of the strains, to a final inoculum size of 1.10^5 cfu/ml for the bacteria and 1.10^4 cfu/ml for the yeast strains. MIC were read after 24 hours incubation at 37°C (bacteria) and 48 hours at 30°C for the yeast and yeast-like fungi and were taken equal to the lowest concentration of the compounds that allowed no visually observable growth. The tests were repeated three times and the values in Table 4 are the arithmetic mean of the three results.

*General procedure 1 for the synthesis of N-protected α-amino carboxamides (**2a–e**)*

To a stirred solution of protected acid **1a–e** (5 mmol), pyridine (0.25 ml) and $(Boc)_2O$ (7 mmol) in CH_3CN (50 ml) ammonium hydrogencarbonate (6.3 mmol) was added. The mixture was stirred for 4 hours at room temperature. The volatile components were removed *in vacuo*. The residue was suspended in EtOAc or in mixture chloroform/ 10% *n*-propanol and washed successively with 5% H_2SO_4 and water. The organic phase was dried over (Na_2SO_4), filtered and evaporated. After evaporation the solid **2a–e** was trituated with diethyl ether (Pozdnev et al., 1995).

*General procedure 2 for the synthesis of N-protected α-amino thiocarboxamides (**3a–e**)*

Laweson reagent (75 mmol) and a solution of amides **2a–e** (100 mmol) in dimethoxyethane (400 ml) was stirred at room temperature until **2a–e** was consumed. The solvent was removed *in vacuo*. A solution of the residue in EtOAc was extracted with 10% $NaHCO_3$, and the aqueous phase reextracted with EtOAc (2x). The combined organic phases were washed with brine, dried and evaporated. The residue **3a–e** was chromatographed on a silicagel column (eluent, $CHCl_3$/EtOAc, 9:1, v:v).

*General procedure 3 for the synthesis of 2-[(N-Protected)-(1-aminoalkyl)-thiazole-4-carboxylic esters (**4a–e**)*

A heterogeneous mixture of thioamide **2** (1 mmol) and powdered $KHCO_3$ (10 mmol) in 1,2-dimethoxyethane (DME) was vigorously stirred for 5 min under N_2, then ethyl bromopyruvate (1.5 mmol) was added. After 1 min the suspension was cooled to 0°C and a solution of $(CF_3CO)_2O$ (7 mmol) and pyridine (9 mmol) in DME (5 ml) was added. The reaction mixture was allowed to reach ambient temperature, and the volatile components were removed *in vacuo*. The crude product was suspended in EtOAc and washed successively with 10% $NaHCO_3$, 10% NaCl and 10% $NaHSO_4$. The organic phase was dried

(Na_2SO_4), filtered and evaporated. The residue was purified on a silicagel column (eluent, $CHCl_3$/EtOAc, 9:1, v:v) to furnish HPLC pure ethyl 2-(1-aminoalkyl)-thiazole-4-carboxylate **4** (Bredenkamp et al., 1990).

*General procedure 4 for the preparation of D and L enantiomers from DL-S-cysteine, homo-S-cysteine thiazole sulfonamides and their N-w-aminoethylated racemates (**4a–d**)*

10 mmol of **4a–e** was dissolved in a mixture of dioxane (20 ml) and water (70 ml) containing 20 mmol of $NaHCO_3$. Alkaline protease from *Bacillus subtilis* strain DY (100 mg) was added, and the mixture was stirred for 12 hours at 37°C. After removal of the dioxane *in vacuo* the suspension was extracted with EtOAc (3 × 70 ml). The combined organic phases were washed with water, dried with Na_2SO_4, and the solvent was evaporated *in vacuo*. The remaining D ester was crystallized from appropriate solvent. The aqueous phase was acidified with 5% $NaHSO_4$ to pH 3 and extracted with EtOAc (3 × 70 ml). The combined organic phases were washed with water, dried with Na_2SO_4, and the solvent was evaporated *in vacuo*. The resulting L-acid was crystallized from the appropriate solvent (Aleksiev et al., 1981).

*Na,NG-Di-tert.-butyloxycarbonyl-canavanine-amide Boc-Cav(Boc)-$CONH_2$ (**2e**)*

The amide **2e** was prepared according to general procedure **1** by adding ammonium hydrogencarbonate (1 g, 12.6 mmol) to a solution of **1e** (3.7 g, 10 mmol), pyridine (0.5 ml) and $(Boc)_2O$ (3 g, 13 mmol) in CH_3CN (100 ml). **2e** was crystallized from diethyl ether. Yield 2.96 g (80%); m.p. 110°C; homogeneous (TLC system A and C); $[\alpha]_D^{23} = -10.2$ (c = 1, DMF); IR (KBr): $\tilde{\nu}$ = 3420 (N-H), 3290, 2934, 2840, 1717 (C = N), 1650 (C = O), 1529, 1359; ^{1}H NMR ($CDCl_3$): δ = 1.40 (s, 18 H, 9 CH_3 − 2Boc), 2.03 (m, 2 H, β CH_2), 3.75 (m, 2 H, γ CH_2), 3.9 (m, 1 H, α CH), 5.70 (s, 2 H, amide-NH_2), 6.15 (s br, 3 H, guanidino-NH), 7.10 (d, 1 H, NH); -$C_{15}H_{30}N_5O_6$ (376.44).

*L-4-(tert.-Butyloxycarbonylguanidinooxy)-2-(tert.-butyloxycarbonylamino) thiobutylamide Boc-Cav(Boc)-$CSNH_2$ (**3e**)*

The compound **3e** was prepared according to general procedure **2** by adding Lawesson's reagent (3.0 g, 7.5 mmol) to a solution of **2e** (3.7 g, 10 mmol) in 1,2-dimethoxyethane (100 ml). **2d** was crystallized from EtOAc/*n*-hexane. Yield 3.12 g (80%); m.p. 135°C; homogeneous (TLC system A and C); ^{1}H NMR ($CDCl_3$): δ = 1.40 (s, 18 H, 9 CH_3 − 2Boc), 2.03 (m, 2 H, β CH_2), 3.75 (m, 2 H, γ CH_2), 3.9 (m, 1 H, α CH), 6.15 (s br, 3 H, guanidino-NH), 7.10 (d, 1 H, NH), 9.0, 9.66 (br s, 1 H, $CSNH_2$); MS-ESI; *m/z* (%): 392.3 (100) [M^+], 358 (30) [M^+ − S], 336.2 (10) [M^+ − $(CH_3)_3C$], 302 (10), 292.1 (10) [M^+ − Boc], 246.1 (10), 214.1 (10). – $C_{15}H_{29}N_5O_5S_1$ (391.425).

*L-2-[3-(tert.-Butoxycarbonylguanidinooxy)-1-(tert.-butoxycarbonylamino) propyl]thiazole-4-carboxylic acid Boc-L-[cav(Boc)]Thz-COOH (**5e**)*

To solution of canavanine thioamide **3e** (1.95 g, 5 mmol) in 40 ml EtOH and $CaCO_3$ (1.6 g, 16 mmol) was added bromopyruvic acid (1.16 g, 7 mmol) under N_2 and stirred for 4 hours. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in EtOAc/H_2O acidified to pH 3, extracted 3 times with EtOAc. The collected extracts were washed with H_2O, dried under Na_2SO_4 and evaporated *in vacuo*. Crystallization was from EtOAc/*n*-hexane. Yield 0.86 g (98%); m.p. 90°C; homogeneous (TLC system A and C); $[\alpha]_D^{23} = -32.1$ (c = 1, MeOH); IR (KBr): $\tilde{\nu}$ = 3420 cm^{-1} (N-H), 3290, 2934, 2840, 1717 (C = N), 1650 (C = O), 1529, 1359, 1235 (C-H,

thz); ^{1}H NMR ($CDCl_3$); δ = 1.40 (s, 18 H, 6 CH_3-Boc), 2.03 (m, 2 H, β CH_2), 3.75 (m, 2 H, γ CH_2), 3.9 (m, 1 H, α CH), 6.15 (s br, 3 H, guanidino-NH), 7.10 (d, ^3JHα = 5.8 Hz, 1 H, NH), 8.01 (s, 1 H, CH_{Thz}); ^{13}C NMR ($CDCl_3$): δ 28.28 (Boc-CH_3), 32.60 (β CH_2), 55.70 (α CH), 72.10 (CH_2O) 80.0 (Boc-Cq), 125.8 (C^5_{Thz}), 148.0 (C^4_{Thz}), 154.9 (Boc-CO), 161.2 (C = N), 163.1 (C^2_{Thz}), 177.7 (COOH). – MS-ESI; *m/z* (%): 460 (100) [M^+], 360 (20) [M^+ − Boc], 245 (20). – $C_{18}H_{29}N_5O_7S_1$ (459.526).

*L-2-[3-(Guanidinooxy)-2-(aminopropyl)]thiazole-4-carboxylic acid H-(cav)Thz-COOH (**6e**)*

To solution of Boc-L-Cav(Boc)Thz-OH **5e** (0.5 g 1.5 mmol) in 10 ml EtOAc 3N HCl/EtOAc (4 ml) was added and stirred for 1 h. The resulting product was lyophylized. Yield 0.6 g (97%). – $C_8H_{14}N_5O_3S_1$ (260.3).

*Ethyl DL-2-[2-(Trimethoxybenzylaminosulfonyl)-1-(benzyloxycarbonyl-amino)ethyl]thiazole-4-carboxylate Z-DL-[cys(SO_2NHTmob)]Thz-COOEt (**4a**)*

The compound **4a** was prepared according to general procedure **3** by adding ethyl bromopyruvate (0.93 ml, 7.5 mmol) to a solution of **3a** (2.48 g, 5 mmol) in 1,2-dimethoxyethane (100 ml). **4a** was chromatographed on silicagel column (eluent, $CHCl_3$/EtOAc, 9:1, v:v). After evaporation the product **4a** was crystallized from EtOAc/*n*-hexane. Yield 2.37 g (80%); homogeneous (TLC system A and C).

*Ethyl DL-2-[3-(Trimethoxybenzylaminosulfonyl)-1-(benzyloxycarbonyl-amino)propyl]thiazole-4-carboxylate Z-DL-[hcys(SO_2NHTmob)]Thz-COOEt (**4b**)*

The compound **4b** was prepared according to general procedure **3** by adding ethyl bromopyruvate (0.93 ml, 7.5 mmol) to a solution of **3b** (2.56 g, 5 mmol) in 1,2-dimethoxyethane (100 ml). The compound **4b** was chromatographed on silicagel column (eluent, $CHCl_3$/EtOAc, 9:1, v:v). After evaporation the product **4b** was crystallized from EtOAc/*n*-hexane. Yield 2.49 g (89%); homogeneous (TLC system A and C).

*Ethyl DL-2-[2-(tert.-Butyloxycarbonylaminoethylaminosulfonyl)-1-(benzyl-oxycarbonylamino)ethyl]thiazole-4-carboxylate Z-DL-{cys[$SO_2NH(CH_2)_2$NHBoc]} Thz-COOEt (**4c**)*

The compound **4c** was prepared according to general procedure **3** by adding ethyl bromopyruvate (0.93 ml, 7.5 mmol) to a solution of **3c** (2.3 g, 5 mmol) in 1,2-dimethoxyethane (100 ml). **4c** was chromatographed on silicagel column (eluent, $CHCl_3$/EtOAc, 9:1, v:v). After evaporation the product **4c** was crystallized from EtOAc/*n*-hexane. Yield 2.17 g (78%); homogeneous (TLC system A and C).

*Ethyl DL-2-[3-(tert.-Butyloxycarbonylaminoethylaminosulfonyl)-2-(benzyl-oxycarbonylamino)propyl)]thiazole-4-carboxylate Z-DL-{hcys[$SO_2NH(CH_2)_2$NH Boc]}Thz-COOEt (**4d**)*

The compound **4d** was prepared according to general procedure **3** by adding ethyl bromopyruvate (0.93 ml, 7.5 mmol) to a solution of **3d** (2.37 g, 5 mmol) in 1,2-dimethoxyethane (100 ml). **4d** was chromatographed on silicagel column (eluent, $CHCl_3$/

EtOAc, 9:1, v:v). After evaporation the product **4b** was crystallized from EtOAc/*n*-hexane. Yield 2.28 g (80%); homogeneous (TLC system A and C).

*L-2-[2-(Trimethoxybenzylaminosulfonyl)-1-(benzyloxycarbonylamino)ethyl]thiazole-4-carboxylic acid Z-L-[cys(SO_2NHTmob)]Thz-COOH (**L-10a**)*

The main product was prepared by treating **DL-4a** (2.3 g, 3.8 mmol) with alkaline protease from *Bacillus subtilis* strain DY according to the general procedure **4**; reaction time 12 hours, temperature 37°C. The product **L-10a** solidified upon treatment with *n*-hexane. Yield 1.1 g (93%); m.p. 140°C; homogeneous (TLC system A and C); $[\alpha]_D^{23} = -20.1$ (c = 1, MeOH); MS-ESI; *m/z* (%): 587 (20) $[M + Na]^+$, 557 (100) $[M^+]$; $C_{24}H_{27}N_3O_9S_2$ (556.673).

*Ethyl D-2-[2-(Trimethoxybenzylaminosulfonyl)-1-(benzyloxycarbonylamino)ethyl]thiazole-4-carboxylate (**D-7a**) Z-D-[cys(SO_2NHTmob)]Thz-COOEt*

According to the general procedure **4** compound **DL-4a** (2.3 g, 3.8 mmol) was treated with alkaline protease from *Bacillus subtilis* strain DY for 12 hours at 37°C to afford ester **D-7a**. Yield 1.2 g (98%); m.p. 130°C; homogeneous (TLC system A and C); $[\alpha]_D^{23} = +10.6$ (c = 1, MeOH).

*L-2-[3-(Trimethoxybenzylaminosulfonyl)-1-(benzyloxycarbonylamino)propyl]thiazole-4-carboxylic acid Z-L-[hcys(SO_2NHTmob)]Thz-COOH (**L-10b**)*

The main product was prepared by treatment of **DL-4b** (2.3 g, 3.8 mmol) with alkaline protease from *Bacillus subtilis* strain DY for 12 hours at 37°C according to the general procedure **4**. **L-10b** solidified on treatment with *n*-hexane. Yield 1.1 g (96%); m.p. 150°C; homogeneous (TLC system A and C); $[\alpha]_D^{23} = -25$ (c = 1, MeOH); MS-ESI; *m/z* (%): 601 (20) $[M + Na]^+$, 580 (100) $[M^+] - C_{25}H_{29}N_3O_9S_2$ (579.663).

*D-2-[3-(Trimethoxybenzylaminosulfonyl)-1-(benzyloxycarbonylamino)propyl]thiazole-4-carboxylate Z-D-[hcys(SO_2NHTmob)Thz-COOEt (**D-7b**)*

According to the general procedure **4** compound **DL-4b** (2.3 g, 3.8 mmol) was treated with alkaline protease from *Bacillus subtilis* strain DY for 12 hours at 37°C to afford ester **D-7a**. Yield 1.07 g (93%); homogeneous (TLC system A and C); $[\alpha]_D^{23} = +8.7$ (c = 1, MeOH).

*Ethyl D-2-[2-(tert.-Butyloxycarbonylaminoethylaminosulfonyl)-1-(benzyloxycarbonylamino)ethyl]thiazole-4-carboxylate Z-D-{cys[$SO_2NH(CH_2)_2NHBoc$]}Thz-COOEt (**7c**)*

According to the general procedure **4** compound **DL-4c** (2.2 g, 4 mmol) was treated with alkaline protease from *Bacillus subtilis* strain DY for 12 hours at 37°C to afford ester **D-7a**. Yield 1.1 g (98%); homogeneous (TLC system A and C); $[\alpha]_D^{23} = +15.1$ (c = 1, MeOH).

*L-2-[2-(tert.-Butyloxycarbonylaminoethylaminosulfonyl)-1-(benzyloxycarbonylamino)ethyl)thiazole-4-carboxylic acid Z-L-{cys[$SO_2NH(CH_2)_2NHBoc$]}Thz-COOH (**10c**)*

The main product was prepared by treating of **DL-4c** (2.2 g, 4 mmol) with alkaline protease from *Bacillus subtilis* strain DY according to the general procedure **4**; reaction time 12 hours, temperature 37°C. **10c** was solidified by treatment with *n*-hexane. Yield 1.1 g (96%); m.p. 110°C; homogeneous (TLC system A and C); $[\alpha]_D^{23} = -23.1$ (c = 1, MeOH); MS-ESI; *m/z* (%): 550 (20) $[M + Na]^+$, 529 (100) $[M^+]$, 427 (30) $[M^+ - Boc]$. $-C_{21}H_{28}N_4O_8S_2$ (528.619).

*Ethyl D-2-[3-(tert.-Butyloxycarbonylaminoethylaminosulfonyl)-1-(benzyloxycarbonylaminopropyl)]thiazole-4-carboxylate Z-D-{hcys[$SO_2NH(CH_2)_2NHBoc$]}Thz-COOEt (**7d**)*

According to the general procedure 4 compound **DL-4d** (2.1 g, 3.8 mmol) was treated with alkaline protease from *Bacillus subtilis* strain DY for 12 hours at 37°C to afford ester **D-7a**. Yield 1.0 g (95%); homogeneous (TLC system A and C). $[\alpha]_D^{23} = +17.5$ (c = 1, MeOH).

*L-2-[3-(tert.-Butyloxycarbonylaminoethylaminosulfonyl)-1-(benzyloxycarbonylamino)propyl)]thiazole-4-carboxylic acid Z-L-{hcys[$SO_2NH(CH_2)_2NHBoc$]}Thz-COOH (**10d**)*

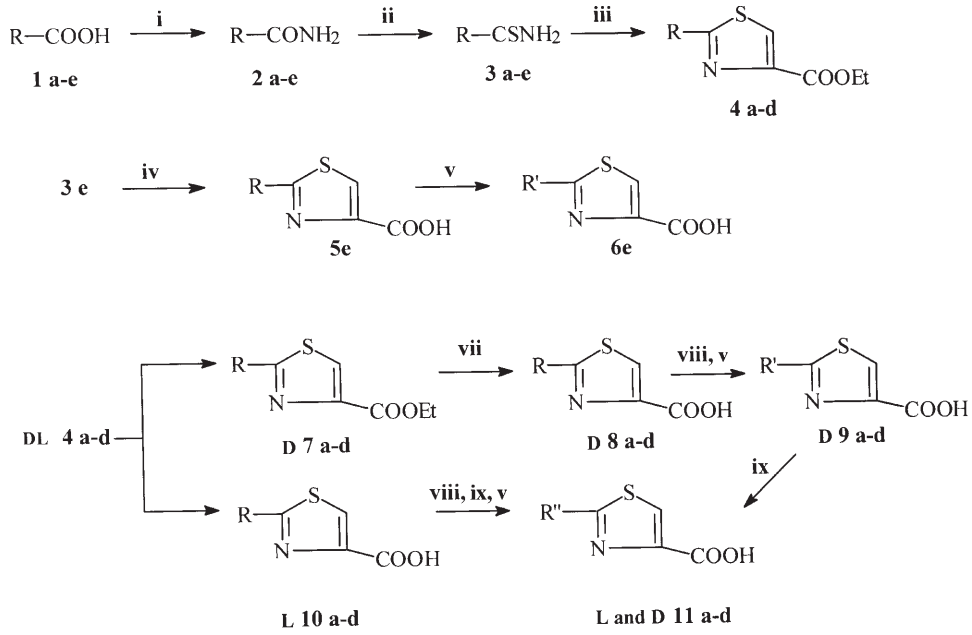
The main product was prepared by treatment of **DL-4d** (2.1 g, 3.8 mmol) with alkaline protease from *Bacillus subtilis* strain DY for 12 hours at 37°C according to the general procedure **4**. **10d** was solidified by treatment with *n*-hexane. Yield 0.9 g (97%); m.p. 170°C; homogeneous (TLC system A and C); $[\alpha]_D^{23} = -23.5$ (c = 1, MeOH); MS-ESI; *m/z* (%): 564 (20) $[M + Na]^+$, 543.6 (100) $[M^+]$, 441 (30) $[M^+ - Boc]$. $-C_{22}H_{30}N_4O_8S_2$ (542.646).

*D-2-[3-(Trimethoxybenzylaminosulfonyl)-1-(benzyloxycarbonylamino)propyl]thiazole-carboxylic acid Z-D-[cys($SO_2NHTmob$)]Thz-COOH (**D-8a**)*

1N NaOH (6 ml) was added to solution of ester **7a** (1.9 g, 3.3 mmol) in dioxane/H_2O (1:3, v:v) and 2 drops thymolphthaleine. After 2 hours stirring at room temperature the solution was neutralized with 10% $KHCO_3$ to pH 6. Removal of the dioxane *in vacuo* was followed by acidification to pH 3 and extraction with EtOAc (3x). The extract was dried and the solvent removed *in vacuo*. Crystallization from EtOAc/*n*-hexane followed. Yield 1.86 g (93%); homogeneous (TLC system B); −MS-ESI; *m/z* (%): 604 (10) $[M + K]^+$, 587.6 (45) $[M + Na]^+$, 566 (100) $[M^+]$; $-C_{24}H_{27}N_3O_9S_2$ (565.636).

*D-2-[3-(Trimethoxybenzylaminosulfonyl)-1-(aminopropyl)thiazole-4-carboxylic acid H-D-[cys($SO_2NHTmob$)]Thz-COOH (**D-9a**)*

D-8a (0.94 g, 1.67 mmol) was heated in methanol (30 ml) in the presence of ammonium formiate (8.36 mmol), 10% Pd-C (0.5 g) for 5 min at 65°C. The mixture was filtered and condensed *in vacuo*. The residue was crystallized from MeOH/H_2O. Yield 0.72 g (96%); m.p. 105°C; homogeneous (TLC system B); −MS-ESI; *m/z* (%): 470 (10) $[M + K]^+$, 453.1 (45) $[M + Na]^+$, 432.5 (100) $[M^+]$. $-C_{16}H_{21}N_3O_7S_2$ (431.5).



1-10 : **a :** R= (TmobNHSO2)-CH2-CH(NH-Z)-

b : R= (TmobNHSO2)-(CH2)2-CH(NH-Z)-

c : R= [Boc-HN(CH2)2NHSO2]-CH2-CH(NH-Boc)

d : R= [Boc-HN(CH2)2NHSO2]-(CH2)2-CH(NH-Boc)

e : R= [Boc-HN-C(NH2)=N]-O-(CH2)2-CH(NH-Boc)

9 **a :** R' = (TmobNHSO2)-CH2-CH(NH2)-

11 **a :** R"= (NH2SO2)-(CH2)2-CH(NH2)-

b : R"= (NH2SO2)-(CH2)2-CH(NH2)-

c : R"= [H2N(CH2)2NHSO2]-CH2-CH(NH2)

d : R"= [H2N(CH2)2NHSO2]-(CH2)2-CH(NH2)

e : R"= [H2N-C(NH2)=N]-O-(CH2)2-CH(NH2)

Scheme 1. i) NH_4HCO_3, $(Boc)_2O$; **ii)** Lawesson's reagent: **iii)** Ethyl bromopyruvate/$(CF_3CO)_2O$/pyridine **iv)** 3-bromo-2-oxo-propionic acid [1]; **v)** HCl/EtOAc; **vi)** Bac. subtilis. DY strain, 37°C, dioxane/H_2O (1:3) **vii)** NaOH; **viii)** Pd-C, MeOH; **ix)** TFA/DCM/DMS

*D-2-[3-(Aminosulfonyl)-1-(aminopropyl)thiazole-4-carboxylic acid H-D-[cys(SO_2NH_2)]Thz-COOH (**11a**)*

Compound **D-9a** (0.72 g, 1.67 mmol) was treated with TFA/DCM/DMS (9.5:9.5:1 v/v) for 45 min at room temperature according to (Hudson et al., 1988). After evaporation of the organic solvent, the residue was dissolved in ethyl acetate (70 ml) and the solution neutralized with triethylamine. It was subsequently washed with 1N HC1 and water, dried, and the solvent was evaporated. The residue was crystallized from DCM/n-hexane. Yield 0.39 g (90%); m.p. 89°C; homogeneous (TLC system B); −MS-ESI; *m/z* (%): 290 (10) $[M + K]^+$, 273.2 (45) $[M + Na]^+$, 252 (100) $[M^+]$. $-C_6H_9N_3O_4S_2$ (251.294).

Results and discussion

The synthetic route chosen for preparation of the required compounds is illustrated in (Scheme 1).

For the synthesis of (Cav)Thz **5e**, ($CySO_2$ NH_2)Thz **4a** and ($HCySO_2$ NH_2)Thz **4b** suitable protected amino acid derivatives prepared by previously described procedures were used. Thus, Boc-Cav(Boc)-OH **1e** was prepared according to Pajpanova et al., (1997), **1a–d** were synthesized as described by Videnov et al. (1993).

As reported earlier amide **2e** can be easily prepared by common procedures using aqueous solution of 25% NH_3 in MeOH. $(Boc)_2O$ treatment of **1a–e** in pyridine/CH_3CN solution at room temperature according (Pozdnev et al., 1995) afforded the amide **2a–e** in high yields also (Table 1). As the next step, thionation was easily achieved using Lawesson's reagent in high yields without loss of optical purity (Table 2). The following cyclization of the thioamide to give the thiazole was achieved by using ethyl bromopyruvate according to the modified Hantzsch reaction (Table 3). The reaction of ethyl bromopyruvate with protected thioamides **3a–e** in 1,2-dimethoxyethane (Bredenkamp et al., 1990) lead to the formation of hydroxythiazoline derivative. Without intermediate isolation, suitable activation of the hydroxy group of this compound with trifluoroacetic acid anhydride in pyridine results in immediate aromatization to furnish the thiazole amino acid derivatives **4a–d** with complete retention of configuration. The reaction of bromopyruvic acid with thioamide **3e** (Pettit et al., 1986) furnish the thiazole amino acid with 74% yield.

Therefore we investigated the applicability of the enzyme catalyzed saponification of various esters – a well known method for deprotection of carboxylic group used in contemporary peptide chemistry (Aleksiev et al., 1981). For resolution of the racemates **4a–d** the saponification of ethyl esters of thiazole containing amino acids catalyzed by alkaline protease from *Bacillus subtilis* DY strain offered the best results.

Cleavage of the protecting Boc-group was achieved by ethyl acetate saturated with anhydrous HCl (1.5–4N HCl/EtOAc) or TFA/anisole (9:1) in 98% yield. Removal of the Tmob-protecting group was carried out by TFA/DCM/DMS using Hudson procedure (Hudson et al., 1988). The Z-protecting group was removed by hydrogenolysis with 10% Palladium on charcoal in methanol using formic acid as a hydrogen donor.

Finally, the compounds were purified by column chromatography on Kiselgel 60 ($CHCl_3$/EtOAc, 9:1) which allowed the products to be simply recovered by lyophylization.

In the case of canavanine resulted derivatives, the compounds could be readily isolated from reaction mixture by simple washing techniques in good yields and purified by recrystalization.

The antibacterial activity of the compounds studied was determined *in vitro* against a large number of microorganisms. MIC values of the compounds are presented in (Table 4). The compounds showed antibacterial activity *in vitro* at concentrations of 0.75–3 mM except for the thioamides **3a, 3b** of S-cysteine and homo-S-cysteinesulfonamides. Thioamides **3a** and **3b** showed maximum activity (MIC 0.04 mM) against *Staphylococcus aureus, Klebsiella pneumoniae 450, Escherichia coli* and (MIC 0.18 mM and 0.37 mM) against *Proteus vulgaris*. The activity depended on the amino acid moiety

Table 1. Amides **2a–d** of S-cysteine- and homo-S-cysteinesulfonamides and their N ϖ-aminoethylated derivatives

Product	Yield [%]	m.p. [°C]	Molecular formula	^{13}C NMR ($CDCl_3$) δ [ppm]	MS-ESI m/z (%)
(DL)-2a	85	150 (DMF)	$C_{21}H_{27}N_3O_8S_1$ (481.537)	35.9 (Tmob CH_2), 49.0 (Cys-SO_2NH_2, C α), 52.5 (Cys-SO_2NH_2, C β), 55.3, 55.7 (Tmob-3 × OCH_3), 66.39 (Phe, CH_2), 90.5 (C^3_{Tmob}, C^5_{Tmob}), 105.4 (C^1_{Tmob}), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 158.8 (C^4_{Tmob}), 161.4 (C^2_{Tmob}, C^6_{Tmob}), 174.5 (Cys-SO_2NH_2, $CONH_2$)	503.5 (50) $[M + Na]^+$, 482.3 (100) $[M^+]$, 300.2 (10) $[M^+ - Tmob]$, 181 (20) $[Tmob]^+$
(DL)-2b	82	140 (DMF)	$C_{22}H_{29}N_3O_8S_1$ (495.558)	26.3 (HCys-SO_2NH_2, C β), 35.9 (Tmob CH_2), 49.0 (HCys-SO_2NH_2, C α), 52.5 (HCys-SO_2NH_2, C γ), 55.3, 55.7 (Tmob-3 × OCH_3), 66.39 (Phe, CH_2), 90.5 (C^3_{Tmob}, C^5_{Tmob}), 105.4 (C^1_{Tmob}), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 158.8 (C^4_{Tmob}), 161.4 (C^2_{Tmob}, C^6_{Tmob}), 174.5 (HCys-SO_2NH_2, $CONH_2$)	534.5 (35) $[M + K]^+$, 517.5 (20) $[M + Na]^+$, 496 (100) $[M^+]$, 315.2 (20) $[M^+ - Tmob]$, 181 (20) $[Tmob]^+$
(DL)-2c	75	180 (MeOH)	$C_{18}H_{28}N_4O_7S_1$ (444.52)	42.0, 43.1 ($NHCH_2CH_2NH$), 50.0 (Cys-SO_2NH_2, C α), 53.0 (Cys-SO_2NH_2, C β), 66.39 (Phe, CH_2), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 174.5 (Cys-SO_2NH_2, $CONH_2$)	465 (20) $[M + Na]^+$, 445 (100) $[M^+]$, 343 (30) $[M^+ - Boc]$
(DL)-2d	75	180 (MeOH)	$C_{19}H_{29}N_5O_5S_1$ (458.541)	42.0, 43.1 ($NHCH_2CH_2NH$), 50.0 (HCys-SO_2NH_2, C α), 52.4 (HCys-SO_2NH_2, C γ), 53.0 (HCys-SO_2NH_2, C β), 66.39 (Phe, CH_2), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 174.5 (HCys-SO_2NH_2, $CONH_2$)	497(20) $[M + K]^+$, 480.5 (20) $[M + Na]^+$, 459.5 (100) $[M^+]$, 358 (30) $[M^+ - Boc]$

Table 2. Thioamides **3a–d** of S-cysteine- and homo-S-cysteinesulfonamides and their N ϖ-aminoethylated derivatives

Product	Yield [%]	m.p. [°C]	Molecular formula	^{13}C NMR ($CDCl_3$) δ [ppm]	MS-ESI m/z (%)
(DL)-3a	89	110 (DMF)	$C_{21}H_{27}N_3O_7S_2$ (497.558)	35.9 (Tmob CH_2), 49.0 (Cys-SO_2NH_2, C α), 52.5 (Cys-SO_2NH_2, C β), 55.3, 55.7 (Tmob-3 × OCH_3), 66.39 (Phe, CH_2), 90.5 (C^3_{Tmob}, C^5_{Tmob}) 105.4 (C^1_{Tmob}), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 158.8 (C^4_{Tmob}), 161.4 (C^2_{Tmob}, C^6_{Tmob}), 204 (Cys-SO_2NH_2, $CSNH_2$)	519 (20) $[M + Na]^+$, 498 (100) $[M^+]$, 317.2 (20) $[M^+ - Tmob]$, 181 (20) $[Tmob]^+$
(DL)-3b	85	110 (DMF)	$C_{22}H_{29}N_3O_7S_2$ (511.63)	35.9 (Tmob CH_2), 49.0 (HCys-SO_2NH_2, C α), 50.5 (HCys-SO_2NH_2, C β), 52.5 (HCys-SO_2NH_2, C γ), 55.3, 55.7 (Tmob-3 × OCH_3), 66.39 (Phe, CH_2), 90.5 (C^3_{Tmob}, C^5_{Tmob}), 105.4 (C^1_{Tmob}), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 158.8 (C^4_{Tmob}), 161.4 (C^2_{Tmob}, C^6_{Tmob}), 204.1 (HCys-SO_2NH_2, $CSNH_2$)	533 (20) $[M + Na]^+$, 512 (100) $[M^+]$
(DL)-3c	85	140 (MeOH)	$C_{18}H_{28}N_4O_6S_2$ (460.52)	42.0, 43.1 ($NHCH_2CH_2NH$), 50.0 (Cys-SO_2NH_2, C α), 53.0 (Cys-SO_2NH_2, C β), 66.39 (Phe, CH_2), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 207.1 (Cys-SO_2NH_2, $CSNH_2$)	482.1 (20) $[M + Na]^+$, 461.0 (100) $[M^+]$, 359 (30) $[M^+ - Boc]$
(DL)-3d	80	140 (DMF)	$C_{19}H_{30}N_4O_6S_2$ (474.52)	42.0, 43.1 ($NHCH_2CH_2NH$), 50.0 (HCys-SO_2NH_2, C α), 52.5 (HCys-SO_2NH_2, C γ), 53.0 (HCys-SO_2NH_2, C β), 66.39 (Phe, CH_2), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 155.4 (Z-OCO), 205.2 (HCys-SO_2NH_2, $CSNH_2$)	496.3 (20) $[M + Na]^+$, 475 (100) $[M^+]$, 373 (30) $[M^+ - Boc]$

Table 3. Thiazoles **4a–d** of S-cysteine- and homo-S-cysteinesulfonamides and their N ω-aminoethylated derivatives

Product	Yield [%]	m.p. [°C]	Molecular formula	^{13}C NMR ($CDCl_3$) δ [ppm]	MS-ESI m/z (%)
(DL)-4a	80	110 (DMF)	$C_{26}H_{31}N_3O_9S_2$ (593.69)	35.9 (Tmob CH_2), 49.0 (Cys-SO_2NH_2, C α), 52.5 (Cys-SO_2NH_2, C β), 55.3, 55.7 (Tmob-3 × OCH_3), 66.39 (Phe, CH_2), 90.5 (C^3_{Tmob}, C^5_{Tmob}), 105.4 (C^1_{Tmob}), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 129.1 (C^1_{Thz}), 148 (C^4_{Thz}), 158.2 (Z-OCO), 158.8 (C^4_{Tmob}, 161.4 (C^2_{Tmob}, C^6_{Tmob}), 164 C^2_{Thz}), 172.7 (Cys-SO_2NH_2, COOEt)	615 (20) $[M + Na]^+$, 594 (100) $[M^+]$, 412 (20) $[M^+ - Tmob]$, 181 (20) $[Tmob]^+$
(DL)-4b	89	110 (DMF)	$C_{27}H_{33}N_3O_9S_2$ (607.717)	35.9 (Tmob CH_2), 49.0 (HCys-SO_2NH_2, C α), 52.5 (HCys-SO_2NH_2, C β), 52.5 (HCys-SO_2NH_2, C γ), 55.3, 55.7 (Tmob-3 × OCH_3), 66.39 (Phe, CH_2), 90.5 (C^3_{Tmob}, C^5_{Tmob}), 105.4 (C^1_{Tmob}), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 130 (C^5_{Thz}), 149 (C^4_{Thz}), 157.6 (Z-OCO), 158.8 (C^4_{Tmob}), 161.4 (C^2_{Tmob}, C^6_{Tmob}), 164 (C^2_{Thz}), 174.0 (HCys-SO_2NH_2, COOEt)	629 (20) $[M + Na]^+$, 608 (100) $[M^+]$, 426 (20) $[M^+ - Tmob]$, 181 (20) $[Tmob]^+$
(DL)-4c	78	110 (MeOH)	$C_{23}H_{32}N_4O_8S_2$ (556.673)	42.0, 43.1 ($NHCH_2CH_2NH$), 50.0 (Cys-SO_2NH_2, C α), 53.0 (Cys-SO_2NH_2, C β), 66.39 (Phe, CH_2), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 130 (C^5_{Thz}), 149 (C^4_{Thz}) 155.4 (Z-OCO), 164 (C^2_{Thz}), 174.0 (Cys-SO_2NH_2, COOEt)	579.1 (20) $[M + Na]^+$, 557.6 (100) $[M^+]$, 456 (30) $[M^+ - Boc]$
(DL)-4d	80	110 (DMF)	$C_{23}H_{32}N_4O_8S_2$ (556.673)	42.0, 43.1 ($NHCH_2CH_2NH$), 50.0 (HCys-SO_2NH_2, C α), 52.5 (HCys-SO_2NH_2, C γ), 53.0 (HCys-SO_2NH_2, C β), 66.39 (Phe, CH_2), 128.01 (*o* or *m*-Phe), 128.09 (*p*-Phe), 128.37 (*m* or *o*-Phe), 130 (C^5_{Thz}), 149 (C^4_{Thz}) 155.4 (Z-OCO), 164 (C^2_{Thz}), 174.0 (HCys-SO_2NH_2, COOEt)	592 (20) $[M + Na]^+$, 571.6 (100) $[M^+]$, 469 (30) $[M^+ - Boc]$

Table 4. Antibacterial activity of the thiazoles **2a–4e** and **6e**. Minimal inhibitory concentrations (MIC, $\mu g/ml^{-1}$)

Comp. No	*Staphylococcus aureus* (3 strains)	*Klebsiella pneumoniae 450*	*Escherichia coli* (2 strains)	*Salmonella Kauffmann* 1314	*Proteus vulgaris*	*Pseudomonas aeruginosa* 27853	*Bacillus cereus*	*Candida albicans clin. isol.* (7 strains)	*Saccharomyces cerevisiae* (3 strains)
2a	>1.5	>1.5	>1.5	>1.5	>1.5	>1.5	–	>1.5	>1.5
2b	>1.5	>1.5	>1.5	>1.5	>1.5	>1.5	–	>1.5	>1.5
2e	>5.0	>5.0	>5.0	>5.0	>5.0	–	>5.0	>5.0	>5.0
3e	>1.5	>3.0	>3.0	>3.0	>3.0	–	>3.0	>3.0	>3.0
3a	0.04	0.04	0.04	–	0.18	0.75	–	0.37	0.75
3b	0.04	0.04	0.04	–	0.37	0.18	–	0.37	0.75
4a	>1.5	>1.5	>0.75	>1.5	>1.5	>1.5	–	>1.5	1.5
4b	>1.5	>1.5	>0.75	>1.5	>3.0	>3.0	–	>1.5	1.5
4e	>3.0	>3.0	>3.0	–	3.0	>3.0	–	3.0	>3.0
6e	0.75	1.5	>1.5	1.5	0.37	1.5	–	0.37	>1.5

and thiazole and thioamide derivatized analogues were slightly more active.

Conclusion

In summary, we elaborated a practicable procedure for the preparation of a variety of unusual amino acid derivatives based on S-cysteine- and homo-S-cysteinesulfonamides, which are of potential interest in structure-activity studies, e.g. as analogs of asparagine and glutamine residues in biologically active peptides.

Thus a series of amino acids containing thiazole residue was synthesized. The method chosen allows to obtain the compounds with higher yields than previously described. The MIC values of the thiazole and thioacarboxamide amino acids derived from canavanine, S-cysteine and homo-S-cysteine analogues are lower than those of previously reported cyclic oligopeptides containing heterocyclic backbone modifications (Northcote et al., 1991) and other structurally related N-Tos-dipeptidylamino-2-thiazolines (El-Naggar et al., 1982). The MIC values of reported amino acid derivatives are higher than previously described thiazole derivatized analogues from proteinogenic amino acids (Ala, Val, Pro, Leu and Asp) (Stanchev et al., 1998). Gram-positive bacteria are generally more sensitive than the Gram-negative strains. *Saccharomyces cerevisiae* growth is inhibited at lower concentration of the compounds. *Candida albicans* is inhibited at generally lower concentrations than the bacteria, but they fall within the same order of magnitude.

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