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SYNTHESIS OF WATER SOLUBLE ISOXAZOL-3-YL(ISOTHIAZOL-3-YL) CARBOXAMIDES AND UREAS CONTAINING AMINO ACID RESIDUES – POTENTIAL ANTICANCER AGENTS

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ABSTRACT
By reaction of accessible 5-(*p*-tolyl)isoxazole-, and 4,5-dichloroisothiazole-3-carbonyl azides with 4-aminobutanioc and 6-aminohexanoic acids the corresponding substituted (1,2-azolyl)-3-carboxamides with amino acid residues were synthesized and transformed in water soluble Na salt forms. For the synthesis of isothiazolyl(isoxazolyl)ureas with amino acid residues the 5-(*p*-tolyl)isoxazole-, 4,5-dichloroisothiazole- and 5-(benzylthio)-4-chloroisothiazole-3-carbonyl azides were converted into corresponding (1,2-azol-3-yl)carbamates by action of phenol or 4-fluorophenol. Obtained carbamates were introduced in reaction with amino acids to form target substituted ureas, further transformed in water soluble salt forms. Some of the synthesized derivatives possess antitumor activity.

KEYWORDS
Carboxamides, ureas, isothiazoles, isoxazoles, amino acids.

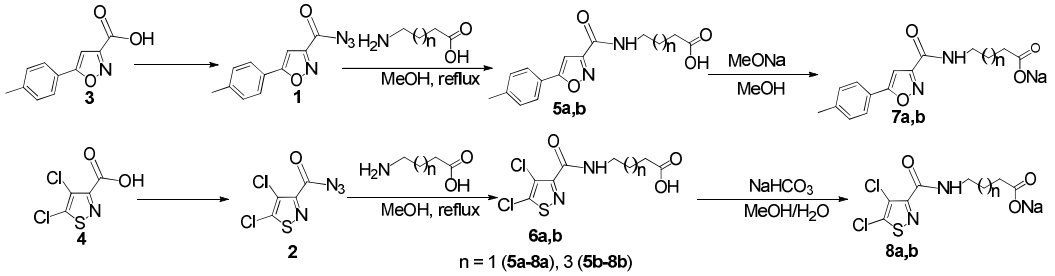
INTRODUCTION
Isothiazole and isoxazole are known to be fragments of wide range of biologically active compounds[i]. These 1,2-azoles often exhibit similar biological activity[ii]. For example, 2-amino-3-(3-hydroxy-5-methylisothiazol-4-yl)propionic acid as well as its isoxazole analogue blocks the activation of glutamate receptors which are crucial for different neurodegenerative diseases[iii]. Efficiency of the biological action of isothiazoles and isoxazoles is heavily regulated by functional ambience of 1,2-azole cycle. In this regard, isoxazolyl(isothiazolyl) carboxamides and carbamides (ureas) are very promising for the development of new targeted drugs. Some of the substituted isoxazole carboxamides are growth hormone secretagogue receptor (GHS-R) antagonists[iv]. One representative of (3-benzyloxy-4-carboxamidoisothiazol-3-yl)ureas is an effective inhibitor of tyrosine kinases and it is studied as a promising anticancer agent (CP-547.632)[v]. Among (isoxazol-3-yl)ureas a perspective Raf kinase inhibitor was discovered[vi].
Our purpose was to synthesize isoxazol-3-yl(isothiazol-3-yl) carboxamides and ureas with amino acid residues. It was expected that the presence of amino acid residue in the molecule

would increase the efficiency of biological action of the conjugate. Moreover, the amino acid moiety allows to obtain water soluble salt forms, which is important for biological activity and bioassay.

RESULTS AND DISCUSSION

There are many different approaches to the synthesis of substituted carboxamides and ureas. We chose the way based on the transformation of isoxazole(isothiazole)-3-carbonyl azides. As starting compounds the accessible 5-(*p*-tolyl)isoxazole-3-carbonyl and 4,5-dichloroisothiazole-3-carbonyl azides **1,2** were used. Recently we have reported their synthesis on the base of 5-(*p*-tolyl)isoxazole-3- and 4,5-dichloroisothiazole-3-caboxylic acids **3,4**[vii]. Of amino acids 4-aminobutanoic and 6-aminohexanoic acids were selected.

The general procedure for carboxamide synthesis included refluxing of isoxazole(isothiazole)-3-carbonyl azides **1,2** and amino acids in 80% aqueous ethanol (Scheme 1). The usage of azides allowed to carry out the acylation process selectively on amino group of amino acid and to avoid the formation of mixed anhydrides that was possible in case of acyl chlorides usage. The aqueous ethanol (optimal 80%) was necessary to ensure solubility of amino acids and homogeneity of reaction mixture. In these conditions the process was completed in 8 h, any by-products were not formed and target carboxamides **5,6** were synthesized in yield 86-97%. To obtain the water soluble forms which are preferred for biomedical study, the synthesized compounds were transformed in Na salts. Synthesis of sodium salts **7a,b** of isoxazolyl carboxamides was carried out by reaction with MeONa in MeOH. But this approach is not applicable in case of carboxamides with 4,5-dihlorisothiazole fragment **6a,b** because of competing reactions of nucleophile (MeO-) on chlorine atoms, so the synthesis of Na salts of isothiazolyl carboxamides **8a,b** was carried out by treating the amides with sodium bicarbonate (Scheme 1). The obtained compounds **5–8** were identified on basis of the data of IR, ^{1}H and ^{13}C NMR spectra.

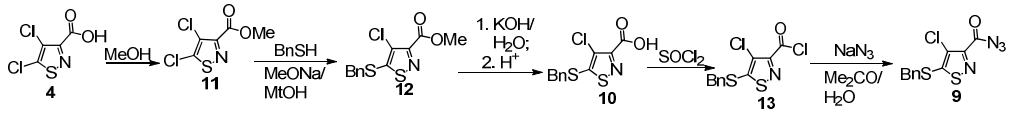


Scheme 1 Preparation of 5-(*p*-tolyl)isoxazole- and 4,5-dichloroisothiazole-3-carbonyl carboxamides with amino acid mojeties **5,6** and their Na salts **7,8**

Next step of our work was the synthesis of substituted isoxazol-3-yl(isothiazol-3-yl) ureas containing aminoacid residues. The general method based on acylcarbamates synthesis through Curtius rearrangement and their subsequent reaction with various amines appears to be most preferable for the synthesis of target compounds[viii]. As starting compounds for acylcarbamates synthesis we used 5-(*p-tolyl*)isoxazole-3-carbonyl and 4,5-dichloroisothiazole-3-carbonyl azides **1,2**. Along with the dichlorosubstitited azide **2** we also used 5-benzylthio-4-chlorizotiazol-3-carbonyl azide **9**. The presence of benzylthio group in 5-th position of isothiazole heterocycle allowed to count on obtaining urea isosteric analogue that is structurally more similar to the substance CP 547.632.

In the synthesis of azide **9** 5-benzylthio-4-chloroisothiazole-3-carboxylic acid **10** is the key compound. Recently we have reported the synthesis of acid **10** on base of 4,5-dichloroisothiazole-3-caboxylic acid **4**[ix]. The preparation procedure includes the reaction of

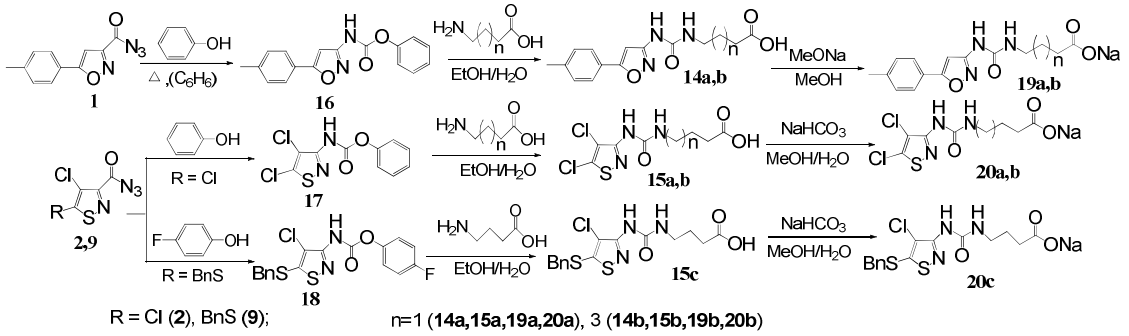
acid **4** with phenylmethanethiol in the presence of pyridine and leads to the acid **10** with 65% yield. We optimized this method. Our new approach involves selective substitution of the Cl-5 atom in methyl 4,5-dichloroisothiazole carboxylate **11** by BnSNa action and one-pot hydrolysis of the formed methyl 5-benzylthio-4-chloroisothiazole carboxylate **12** leading to the acid **10** with yield 95%. The starting ester **11** can be easily obtained by esterification of the acid **4** as previously described[x]. The 5-benzylthio-4-chloroisothiazol-3-carbonyl azide **9** was further synthesized by reaction of 5-benzylthio-4-chloroisothiazole-3-carbonyl chloride **13** with NaN_3 (Scheme 2).



Scheme 2 Preparation of 5-benzylthio-4-chlorizotiazol-3-carbonyl azide **9**

General procedure for the synthesis of target 5-(*p-tolyl*)isoxazole and 4,5-dichloroisothiazole containing ureas with amino acid residues **14,15a,b** included initial synthesis of corresponding (1,2-azol-3-yl)carbamates **16,17** by refluxing azides **1,2** with phenol in benzene media. Substituted (1,2-azol-3-yl)ureas **14,15a,b** were further obtained by reaction of formed (1,2-azol-3-yl)carbamates **16,17** with amino acids in 90% aqueous ethanol.

But we found significant features concerning 5-benzylthio substituted derivatives. It turned out that to obtain the (5-(benzylthio)-4-chloroisothiazol-3-yl)urea **15c** it was preferable to use *p*-fluorophenol instead of phenol on the stage of carbamate **18** synthesis because phenyl carbamate have a low solubility, which prevented its subsequent transformation into urea, as the reaction proceeded slowly with low conversion, and the resulting products required further purification. Furthermore, *p*-fluorophenol was more reactive in the reaction with azides compared with phenol, so (*p*-fluoro)phenolic carbamate **18** formation was completed within 30 min, whereas the reaction with phenol proceeded 4 h.



Scheme 3 Preparation of substituted isoxazol-3-yl(isothiazol-3-yl) ureas with amino acids moieties and their Na salts

Finally we obtained a series of substituted isoxazol-3-yl- and isothiazol-3-yl ureas **14,15** with the residues of 4-aminobutanoic and 6-aminohexanoic acids with yields 81-93% (Scheme 3). We transformed the obtained compounds into water soluble sodium salts by reaction with sodium methoxide in case of isoxazole derivatives **14** or sodium bicarbonate in case of isothiazole derivatives **15**. The structure of compounds **14–20** was confirmed by spectral data.

The antitumor activity of some synthesized compounds was studied. The experiments were carried out on primary and linear cultures of neuroepithelial tumors. It was found out that antitumor activity of isoxazoles and isothiazoles conjugates with 4-aminobutanoic acid (carboxamides and ureas) significantly exceeded the effect of the corresponding 1,2-azolyl carboxylic acids. In addition, death of over 40% of cells and decrease in proliferation index

($p \leq 0.05$) were recorded in 24 h of observation after the application of 1M solution of compounds on glioma cells C6 culture in the experiments *in vitro*. The observed phenomena are the basis for further screening of isoxazoles and isothiazoles conjugates with different amino acid moieties in order to identify the maximum antitumor effect of these compounds.

CONCLUSION

In conclusion, we have developed a convenient method for synthesis of new type of water soluble carboxamides and urea containing isoxazole or isothiazole fragment and amino acid residue in one molecule. The procedures are operationally simple and allow to synthesized pure target products in good overall yields. Some of the synthesized compounds possess antitumor activity and are promising for further study as new antitumor agents.

EXPERIMENTAL SECTION

All reagents were of analytical grade and used as purchased without further purification. Melting points were determined on Boetius heating table. The IR spectra were recorded on a Nicolet Protégé spectrometer, using KBr discs. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-500 spectrometer at 500 and 125.7 MHz, respectively. Chemical shifts were measured relative to the residual solvent signal. (1,2-Azolyl)-3-carbonyl azides **1,2** were synthesized according to the previously described procedures[vii].

5-(*p*-tolyl)isoxazole- and 4,5-dichloroisothiazole-3-carboxamides with amino acid moieties (5,6) (typical procedure).

A solution of corresponding azide **1,2** (5 mmol) and 5 mmol 4-aminobutanoic (or 6-aminohexanoic) acid in 30 mL 80% aqueous EtOH was refluxed for 8 h. After cooling to r.t. the reaction mixture was diluted with H_2O (250 mL), the precipitate was filtered, washed with H_2O (3 x 50 mL), dried in vacuo, and recrystallized from acetone.

4-(5-(*p*-tolyl)isoxazole-3-carboxamido) butanoic acid (5a).

Yield: 1.40 g (97%); white solid; mp 171–174 °C.IR (KBr ν(cm^{-1})) 3324, 3130, 3063, 3048, 3033, 2973, 2934, 2879, 2858, 1716, 1677, 1613, 1595, 1551, 1509, 1444, 1405, 1318, 1272, 1255, 1214, 935, 907, 886, 819, 781, 606, 496. δ_H (500 MHz, DMSO-d_6) 12.09 (s, 1H, COOH), 8.82 (t, *J* 5.8 Hz, 1H, NH), 7.80 (d, *J* 8 Hz, $2H_{arom}$), 7.34 (d, *J* 8Hz, $2H_{arom}$), 7.26 (s, $1H_{isoxazole}$), 3.29 (q, *J* 6.8 Hz, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.28 (t, *J* 7.4 Hz, 2H, CH_2), 1.77 (quint, *J* 7.2 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 174.78 (C=O), 171.06 (C=O), 160.25, 159.21, 141.38, 124.32 ($4C_q$), 130.45 ($2CH_{arom}$), 126.32 ($2CH_{arom}$), 99.81 ($CH_{isoxazole}$), 38.94 (CH_2), 31.66 (CH_2), 24.93 (CH_2), 21.63 (CH_3).

6-(5-(*p*-tolyl)isoxazole-3-carboxamido)hexanoic acid (5b).

Yield: 1.52 g (96%); white solid; mp 157–159 °C. IR (KBr νC(cm^{-1})) 3343, 3118, 3048, 3033, 2971, 2943, 2864, 1708, 1667, 1615, 1594, 1553, 1510, 1446, 1430, 1406, 1256, 1242, 948, 825, 613, 506. δ_H (500 MHz, DMSO-d_6) 12.01 (br.s, 1H, COOH), 8.77 (t, *J* 5.7 Hz, 1H, NH), 7.79 (d, *J* 8 Hz, $2H_{arom}$), 7.33 (d, *J* 8 Hz, $2H_{arom}$), 7.25 (s, $1H_{isoxazole}$), 3.25 (q, *J* 6.6 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.20 (t, *J* 7.3 Hz, 2H, CH_2), 1.59–1.44 (m, 4H, $2CH_2$), 1.30 (quint, *J* 7.4 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 175.12 (C=O), 171.07 (C=O), 160.33, 159.07, 141.38, 124.35 ($4C_q$), 130.46 ($2CH_{arom}$), 126.33 ($2CH_{arom}$), 99.82 ($CH_{isoxazole}$), 39.36 (CH_2), 34.23 (CH_2), 29.28 (CH_2), 26.58 (CH_2), 24.85 (CH_2), 21.64 (CH_3).

4-(4,5-Dichloroisothiazole-3-carboxamido) butanoic acid (6a).

Yield: 1.22 g (86%); white solid; mp 145–148.5 °C. IR (KBr ν (cm^{-1})) 3353, 2955, 2905, 2882, 1722, 1710, 1664, 1545, 1472, 1459, 1404, 1348, 1259, 1192, 1170, 1107, 1098, 1071, 965, 884, 851, 824, 755, 681, 628, 516, 424. δ_H (500 MHz, DMSO-d_6) 12.08 (br.s, 1H, COOH), 8.82 (s, 1H, NH), 3.26 (q, *J* 6.5 Hz, 2H, CH_2), 2.27 (t, *J* 7.3 Hz, 2H, CH_2), 1.73 (quint, *J* 7.0 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 174.73 (C=O), 160.22 (C=O), 159.27, 149.80, 123.23 ($3C_q$), 38.89 (CH_2), 31.60 (CH_2), 24.89 (CH_2).

6-(4,5-Dichloroisothiazole-3-carboxamido)hexanoic acid (6b).
Yield: 1.38 g (89%); white solid; mp 61.5–63 °C. IR (KBr ν (cm^{-1})) 3302, 3088, 3038, 2935, 2924, 2862, 1704, 1658, 1550, 1466, 1433, 1416, 1357, 1315, 1305, 1263, 1237, 1204, 1169, 947, 869, 657. δ_H (500 MHz, DMSO-d_6) 11.97 (br.s, 1H, COOH), 8.78 (t, *J* 5.2 Hz, 1H, NH), 3.22 (q, *J* 6.6 Hz, 2H, CH_2), 2.19 (t, *J* 7.3 Hz, 2H, CH_2), 1.61–1.41 (m, 4H, 2 CH_2), 1.33–1.25 (m, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 175.11 (C=O), 160.14 (C=O), 159.43, 149.79, 123.21 ($3C_q$), 39.31 (CH_2), 34.25 (CH_2), 29.20 (CH_2), 26.53 (CH_2), 24.82 (CH_2).

One-pot synthesis of 5-(benzylthio)-4-chloroisothiazole-3-carboxylic acid (10) (optimized procedure).
To a solution of MeONa (1.35 g, 25 mmol) in anhyd MeOH (50 ml) phenylmethanethiol (5.85 g, 47 mmol) was added and the mixture was stirred for 15 min. Then a solution of methyl 4,5-dichlorizotiazol-3-carboxylate **11** (5 g, 23 mmol) in anhyd methanol (30 mL) was added and the reaction mixture was refluxed for 2 h. In boiling reaction mixture solution of 5 g of KOH in 10 mL water was added and heating was continued for 5 min. The reaction mixture was cooled to r.t. and quenched with 15 mL of concentrated hydrochloric acid. The precipitate formed was filtered, washed with water, hexane and crystallized from chloroform; yield 6.25 g (95%); white solid*;* m.p. 127-129 ºC. IR (KBr ν (cm^{-1})) 3061, 3029, 2924, 2854, 1700, 1660, 1490, 1473, 1448, 1406, 1375, 1340, 1246, 1080, 968, 916, 843, 714, 703. δ_H (500 MHz, DMSO-d_6) 13.76 (br.s, 1H, COOH), 7.42 (d, *J* 7.2 Hz, $2H_{arom}$), 7.34 (t, *J* 7.2 Hz, $2H_{arom}$), 7.29 (t, *J* 7.2 Hz, 1 H_{arom}), 4.43 (s, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 161.46 (C=O), 159.24, 155.95, 136.26, 122.46, ($4C_q$), 129.70 ($2CH_{arom}$), 129.32 ($2CH_{arom}$), 128.59 (CH_{arom}), 38.47 (CH_2).

5-(Benzylthio)-4-chloroisothiazole-3-carbonyl chloride (13).
Two drops of DMF was added to the mixture of 1.00 g (3.5 mmol) *of* acid **10** and 0.62 g (5.2 mmol) thionyl chloride and then refluxed for 4 h. Excess of $SOCl_2$ was removed in vacuo and solid product recrystallized from hexane; yield: 0.87 g (82%); white solid*;* m.p. 69–72ºC. IR (KBr ν(cm^{-1})) 3084, 3060, 3029, 3009, 2920, 2854, 1771, 1493, 1455, 1443, 1342, 1315, 1240, 1111, 1070, 861, 775, 731, 703, 693, 503, 486. δ_H (500 MHz, $CDCl_3$) 7.37–7.28 (m, $5H_{arom}$), 4.24 (s, 2H, CH_2); δ_C (125 MHz, $CDCl_3$) 161.04 (C=O), 160.59, 154.16, 134.72, 125.16 ($4C_q$), 129.19 ($2CH_{arom}$), 129.16 ($2CH_{arom}$), 128.67 (CH_{arom}), 39.33 (CH_2).

5-Benzylthio-4-chloroisothiazol-3-carbonyl azide (9).
0.23 g (3.5 mmol) *of* sodium azide in 5 ml *of* water was added dropwise at 0 °C to the stirred solution 1.00 g (3.3 mmol) of 5-(benzylthio)-4-chloroisothiazole-3-*carbonyl chloride **13** in* 30 mL of acetone. After the addition is complete, the reaction mixture was stirred at r.t. for 30 min. Then mixture was diluted with 200 mL of H_2O, precipitate was filtered, washed with water (3 x 25 mL) and dried in vacuo; yield: 0.84 g (82%); white solid; m.p. 34–38 °C. IR (KBr ν(cm^{-1})) 3086, 3065, 3031, 3008, 2923, 2856, 2255, 2219, 2147, 1683, 1494, 1454, 1406, 1344, 1244, 1191, 1136, 1068, 1031, 947, 869, 826, 744, 707, 696. δ_H (500 MHz, $CDCl_3$) 7.36–7.28 (m, $5H_{arom}$), 4.21 (s, 2 H, CH_2); δ_C (125 MHz, $CDCl_3$) 165.50 (C=O), 159.45, 153.68, 134.87, 125.64 (4 C_q), 129.07 (4 CH_{arom}), 128.50 (CH_{arom}), 39.33 (CH_2).

Phenyl (4,5-dichloroisothiazol-3-yl)carbamate (17).
A solution of 4,5-dichloroisothiazol-3-carbonyl azide **2** (2.23 g, 10.0 mmol) and phenol (1.13 g, 12.0 mmol) in 40 mL of anhyd benzene was refluxed for 4 h. The solvent was removed in quarter the original volume, the mixture was cooled to 10 °C, and precipitate was filtered, washed with cooled benzene and dried in vacuo; yield: 2.31 g (80%); white solid; m.p. 125–127 °C. IR (KBr ν(cm^{-1})) 3198, 3080, 3044, 3032, 2979, 2924, 1746, 1717, 1553, 1520, 1494, 1483, 1456, 1433, 1293, 1233, 1198, 1167, 1124, 1074, 1032, 995, 896, 780, 7561, 740, 687, 647, 519, 501. δ_H (500 MHz, $CDCl_3$) 7.66 (s, 1H, NH), 7.42–7.36 (m, $2CH_{arom}$), 7.27–7.19 (m, $3CH_{arom}$); δ_C (125 MHz, $CDCl_3$) 151.24 (C=O), 150.29, 149.81, 148.28, 114.11 ($4C_q$), 129.62 ($2CH_{arom}$), 126.24 (CH_{arom}), 121.46 ($2CH_{arom}$).

4-Fluorophenyl (5-(benzylthio)-4-chloroisothiazol-3-yl)carbamate (18).
A solution of 5-benzylthio-4-chloroisothiazol-3-carbonyl azide **9** (6.21 g, 20.0 mmol) and carefully pre dried 4-fluorophenole (2.36 g, 21.0 mmol) in 20 mL of anhyd toluene was refluxed for 30 min. The solvent was removed in quarter the original volume, the mixture was cooled to 10 ^{o}C, and precipitate was filtered, washed with cooled toluene and dried in vacuo; yield: 7.73 g (98%); white solid; m.p. 133–137°C. IR (KBr ν(cm^{-1})) 3263, 3061, 3032, 2924, 2851, 1736, 1597, 1536, 1496, 1455, 1435, 1402, 1227, 1189, 1152, 1113, 1026, 1003, 892, 851, 826, 770, 709, 696, 633, 513, 486. δ_H (500 MHz, $CDCl_3$) 7.60 (s, 1H, NH), 7.38–7.25 (m, 5H$_{arom}$), 7.20–7.12 (m, 2H$_{arom}$), 7.06 (t, *J* 8.5 Hz, 2H$_{arom}$), 4.19 (s, 2H, CH_2); δ_C (125 MHz, $CDCl_3$) 161.36 (C=O), 159.41, 155.64, 151.43, 149.96, 146.18, 135.17 (6C$_q$), 129.12 (2CH$_{arom}$), 129.00 (2CH$_{arom}$), 128.37 (CH$_{arom}$), 123.01 (CH$_{arom}$), 122.94 (CH$_{arom}$), 116.29 (CH$_{arom}$), 116.11 (CH$_{arom}$), 39.24 (CH_2).
Phenyl (5-(*p*-tolyl)isoxazol-3-yl)carbamate (**16**) was synthesized as we recently described. [7]
Substituted isoxazol-3-yl(isothiazol-3-yl) ureas with amino acid moieties (14,15) (typical procedure).
A solution of corresponding (1,2-azol-3-yl)carbamate **16-18** (5 mmol) and 5 mmol 4-aminobutanoic (or 6 aminohaxanoic) acid in 25 mL of 90% aqueous ethanol was refluxed for 12 h. The reaction mixture was cooled to 10 ^{o}C, precipitate was filtered, washed with H_2O (3 x 50 mL) and dried in vacuo.
4-(3-(5-(*p-tolyl*)isoxazol-3-yl)ureido)butanoic acid (14a).
Yield: 1.23 g (81%); white solid; mp 179–182 °C. IR (KBr ν (cm^{-1})) 3330, 3235, 3167, 3033, 3007, 2959, 2922, 2858, 1706, 1660, 1621, 1569, 1529, 1504, 1488, 1426, 1379, 1320, 1214, 1098, 946, 824, 776, 756, 560, 501. δ_H(500 MHz, DMSO-d_6) 12.12 (s, 1H, COOH), 9.44 (br.s, 1H, NH), 7.70 (d, *J* 8 Hz, 2H$_{arom}$), 7.30 (d, *J* 8 Hz, 2H$_{arom}$), 7.05 (s, 1H$_{isoxazole}$), 6.57 (t, *J* 5.8 Hz, 1H, NH), 3.15 (q, *J* 6.4 Hz, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.49 (t, *J* 7.4 Hz, 2H, CH_2), 1.68 (quint, *J* 7.1 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 174.23 (C=O), 168.16 (C=O), 159.69, 153.86, 140.24, 124.33 (4C$_q$), 129.75 (2CH$_{arom}$), 125.36 (2CH$_{arom}$), 93.05 (CH$_{isoxazole}$), 38.66 (CH_2), 30.99 (CH_2), 25.12 (CH_2), 20.99 (CH_3).
6-(3-(5-(*p*-tolyl)isoxazol-3-yl)ureido)hexanoic acid (14b).
Yield: 1.34 g (81%); white solid; mp 196–200 °C. IR (KBr ν (cm^{-1})) 3332, 3228, 3130, 3069, 3007, 2949, 2929, 2867, 1690, 1629, 1609, 1568, 1530, 1479, 1453, 1411, 1387, 1369, 1314, 1295, 1206, 1185, 1068, 969, 945, 816, 776, 761, 549. δ_H (500 MHz, DMSO-d_6) 12.02 (s, 1H, COOH), 9.41 (s, 1H, NH), 7.71 (d, *J* 7.9 Hz, 2H$_{arom}$), 7.31 (d, *J* 7.9 Hz, 2H$_{arom}$), 7.05 (s, 1H$_{isoxazole}$), 6.51 (t, *J* 5.1 Hz, 1H, NH), 3.11 (q, *J* 6.3 Hz, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.21 (t, *J* 7.3 Hz, 2H, CH_2), 1.51 (quint, *J* 7.4 Hz, 2 H, CH_2), 1.44 (quint, *J* 7.1 Hz, 2H, CH_2), 1.28 (quint, *J* 7.4 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 174.43 (C=O), 168.09 (C=O), 159.66, 153.72, 140.18, 124.31 (4C$_q$), 129.69 (2CH$_{arom}$), 125.32 (2CH$_{arom}$), 92.98 (CH$_{isoxazole}$), 39.09 (CH_2), 33.57 (CH_2), 29.31 (CH_2), 25.86 (CH_2), 24.20 (CH_2),20.95 (CH_3).
4-(3-(4,5-Dichloroisothiazol-3-yl)ureido)butanoic acid (15a).
Yield: 1.34 g (90%); white solid; mp 158–161 °C. IR (KBr ν (cm^{-1})) 3296, 3127, 2959, 2926, 1721, 1664, 1571, 1532, 1489, 1468, 1449, 1427, 1354, 1322, 1302, 1265, 1212, 1029, 582. δ_H (500 MHz, DMSO-d_6) 12.02 (br.s, 1H, COOH), 9.29 (br.s, 1H, NH), 7.53 (br.s, 1H, NH), 3.17 (q, *J* 6.6 Hz, 2H, CH_2), 2.24 (t, *J* 7.4 Hz, 2H, CH_2), 1.68 (quint, *J* 7.1 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 174.81 (C=O), 155.07 (C=O), 153.85, 146.73, 114.32 (3C$_q$), 39.40 (CH_2), 31.68 (CH_2), 25.59 (CH_2).
6-(3-(4,5-Dichloroisothiazol-3-yl)ureido)hexanoic acid (15b).
Yield: 1.45 g (89%); white solid; mp 53–56 °C. IR (KBr ν (cm^{-1})) 3298, 3216, 3113, 2950, 2901, 2854, 1725, 1678, 1566, 1535, 1489, 1470, 1440, 1421, 1361, 1327, 1280, 1240, 1199, 1114, 1021, 894, 698, 688, 568. δ_H (500 MHz, DMSO-d_6) 12.00 (br.s, 1H, COOH), 9.27 (s, 1H, NH), 7.50 (s, 1H, NH), 3.13 (q, *J* 6.5 Hz, 2H, CH_2), 2.20 (t, *J* 7.3 Hz, 2H, CH_2), 1.50

(quint, *J* 7.0 Hz, 2H, CH_2), 1.45 (quint, *J* 6.7 Hz, 2H, CH_2), 1.28 (quint, *J* 7.6 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 175.06 (C=O), 155.07 (C=O), 153.72, 146.71, 114.18 ($3C_q$), 39.81 (CH_2), 34.22 (CH_2), 29.80 (CH_2), 26.52 (CH_2), 24.83 (CH_2).

4-(3-(5-(Benzylthio)-4-chloroisothiazol-3-yl)ureido)butanoic acid (15c).

Yield: 1.79 g (93%); white solid; mp 159–160 °C. IR (KBr ν (cm^{-1})) 3296, 3221, 3121, 3085, 3030, 2956, 2888, 2766, 2692, 2607, 2548, 1706, 1663, 1569, 1514, 1453, 1414, 1357, 1323, 1265, 1218, 713, 696, 600. δ_H (500 MHz, DMSO-d_6) 12.08 (br.s, 1H, COOH), 9.06 (s, 1H, NH), 7.66 (br.s, 1H, NH), 7.41 (d, *J* 7.3 Hz, $2H_{arom}$), 7.34 (t, *J* 7.3 Hz, $2H_{arom}$), 7.29 (t, *J* 7.3 Hz, $1H_{arom}$), 4.36 (s, 2H, SCH_2), 3.17 (q, *J* 6.4 Hz, 2H, CH_2), 2.23 (t, *J* 7.3 Hz, 2H, CH_2), 1.68 (quint, *J* 7.4 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 174.78 (C=O), 155.22 (C=O), 154.91, 153.98, 136.55, 111.47 ($4C_q$), 129.65 ($2CH_{arom}$), 129.25 ($2CH_{arom}$), 128.48 ($1CH_{arom}$), 39.36 (CH_2), 38.10 (CH_2), 31.66 (CH_2), 25.60 (CH_2).

Sodium salts of 5-(*p*-tolyl)isoxazol-3-yl carboxamides (7) and ureas (19) (typical procedure).

To a suspension of the corresponding carboxamide **5** or urea **14** (5 mmol) in 20 mL ahyd MeOH was added a solution of MeONa (5 mmol) in 20 mL ahyd MeOH and the mixture was stirred at r.t. for 1 h. The solvent was removed under reduced pressure evaporation in half the original volume, 40 mL anhyd Et_2O was added, the precipitate was filtered and dried in vacuo.

Sodium 4-(5-(*p*-tolyl)isoxazole-3-carboxamido)butanoate (7a).

Yield: 1.53 g (99%); white solid; mp >250 °C. IR (KBr ν (cm^{-1})) 3305, 3128, 3038, 3027, 2961, 2923, 2868, 1667, 1614, 1566, 1508, 1446, 1432, 1416, 1377, 1330, 1263, 1230, 1185, 947, 815, 699, 671, 613, 496, 423. δ_H (500 MHz, D_2O) 6.98 (d, *J* 8 Hz, $2H_{arom}$), 6.64 (d, *J* 8 Hz, $2H_{arom}$), 6.36 (s, $1H_{isoxazole}$), 3.29 (t, *J* 7.4 Hz, 2H, CH_2), 2.21 (t, *J* 7.6 Hz, 2H, CH_2), 1.86 (s, 3H, CH_3), 1.77 (quint, *J* 7.5 Hz, 2H, CH_2); δ_C (125 MHz, D_2O) 180.11 (C=O), 168.92 (C=O), 157.76, 156.17, 138.66, 120.76 ($4C_q$), 127.04 ($2CH_{arom}$), 123.03 ($2CH_{arom}$), 95.74 ($CH_{isoxazole}$), 37.35 (CH_2), 32.80 (CH_2), 23.33 (CH_2), 18.42 (CH_3).

Sodium 6-(5-(*p*-tolyl)isoxazole-3-carboxamido)hexanoate (7b).

Yield: 1.67 g (99%); white solid; mp >250 °C. IR (KBr ν(cm^{-1})) 3320, 3127, 3040, 3030, 2962, 2925, 2855, 1667, 1618, 1564, 1509, 1446, 1421, 1378, 1312, 1274, 1261, 1237, 1168, 948, 817, 701, 672, 614, 500. δ_H (500 MHz, DMSO-d_6) 7.24 (d, *J* 8 Hz, $2H_{arom}$), 6.97 (d, *J* 8 Hz, $2H_{arom}$), 6.47 (s, $1H_{isoxazole}$), 3.21 (t, *J* 7.2 Hz, 2H, CH_2), 2.17 (t, *J* 7.5 Hz, 2H, CH_2), 2.14 (s, 3H, CH_3), 1.61–1.45 (m, 4H, 2 CH_2), 1.30 (quint, *J* 7.6 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 183.72 (C=O), 171.37 (C=O), 160.12, 158.37, 141.44, 122.97 ($4C_q$), 129.49 ($2CH_{arom}$), 125.41 ($2CH_{arom}$), 97.89 ($CH_{isoxazole}$), 39.66 (CH_2), 37.60 (CH_2), 28.26 (CH_2), 26.30 (CH_2), 25.65 (CH_2), 20.68 (CH_3).

Sodium 4-(3-(5-(*p*-tolyl)isoxazol-3-yl)ureido)butanoate (19a).

Yield: 1.50 g (92%); white solid; mp >250 °C. IR (KBr ν (cm^{-1})) 3276, 3243, 3172, 3057, 3026, 2949, 2926, 2869, 2811, 1703, 1631, 1606, 1579, 1565, 1511, 1492, 1456, 1422, 1401, 1317, 1229, 945, 819, 792, 761, 498. δ_H (500 MHz, D_2O) 6.88 (d, *J* 8 Hz, $2H_{arom}$), 6.53 (d, *J* 8 Hz, $2H_{arom}$), 6.04 (s, $1H_{isoxazole}$), 2.93 (t, *J* 7.2 Hz, 2H, CH_2), 2.01 (t, *J* 7.6 Hz, 2H, CH_2), 1.71 (s, 3H, CH_3), 1.54 (quint, *J* 7.4 Hz, 2H, CH_2); δ_C (125 MHz, D_2O) 182.80 (C=O), 168.99 (C=O), 158.89, 155.43, 140.56, 123.54 ($4C_q$), 129.18 ($2CH_{arom}$), 125.13 ($2CH_{arom}$), 92.13 ($CH_{isoxazole}$), 39.86 (CH_2), 35.00 (CH_2), 26.23 (CH_2), 20.61 (CH_3).

Sodium 6-(3-(5-(*p*-tolyl)isoxazol-3-yl)ureido)hexanoate (19b).

Yield: 1.75 g (99%); white solid; mp 211–213 °C. IR (KBr ν (cm^{-1})) 3300, 3163, 3096, 3038, 2935, 2890, 2857, 1675, 1626, 1563, 1524, 1467, 1436, 1415, 1271, 1248, 1209, 1199, 1184, 1109, 1057, 1019, 946, 821, 795, 699, 685, 499. δ_H (500 MHz, D_2O) 7.10 (d, *J* 7.9 Hz, $2H_{arom}$), 6.65 (d, *J* 7.9 Hz, $2H_{arom}$), 6.29 (s, $1H_{isoxazole}$), 3.01 (t, *J* 6.9 Hz, 2H, CH_2), 2.12 (t, *J* 7.5 Hz, 2H, CH_2), 1.80 (s, 3H, CH_3), 1.47 (quint, *J* 7.6 Hz, 2H, CH_2), 1.34 (quint, *J* 7.3 Hz,

2H, CH_2), 1.19 (quint, *J* 7.5 Hz, 2H, CH_2); δ_C (125 MHz, D_2O) 183.50 (C=O), 168.80 (C=O), 158.91, 155.32, 140.22, 123.71 ($4C_q$), 129.13 ($2CH_{arom}$), 125.13 ($2CH_{arom}$), 92.08 ($CH_{isoxazole}$), 40.03 (CH_2), 37.68 (CH_2), 29.07 (CH_2), 26.37 (CH_2), 25.72 (CH_2), 20.65 (CH_3).

Sodium salts of isothiazol-3-yl carboxamides (8) and ureas (20) (typical procedure).

To a solution of the corresponding carboxamide **6** or urea **15** (5 mmol) in 70 mL 90% aqueous MeOH was added $NaHCO_3$ (5 mmol) and the mixture was refluxed for 1 h. *The solvent was removed* under reduced pressure evaporation, 5 mL anhyd EtOH and 30 mL anhyd benzene was added and mixture was stirred for 10 min. After removal of the solvents by rotary evaporation the solid residue dried in vacuo.

Sodium 4-(4,5-dichloroisothiazole-3-carboxamido)butanoate (8a).

Yield: 1.50 g (98%); white solid; mp >250 °C. IR (KBr ν(cm^{-1})) 3358, 2937, 2871, 1673, 1567, 1531, 1479, 1439, 1417, 1349, 1327, 1307, 1238, 1223, 1183, 1105, 1089, 1065, 963, 885, 849, 727, 678, 622, 577, 514, 433. δ_H (500 MHz, D_2O) 8.19 (s, 1H, NH), 4.18 (t, *J* 7.0 Hz, 2H, CH_2), 3.07 (t, *J* 7.6 Hz, 2H, CH_2), 2.66 (quint, *J* 7.2 Hz, 2H, CH_2); δ_C (125 MHz, D_2O) 182.46 (C=O), 161.85 (C=O), 156.77, 151.02, 123.32 ($3C_q$), 39.44 (CH_2), 34.83 (CH_2), 25.38 (CH_2).

Sodium 6-(4,5-dichloroisothiazole-3-carboxamido)hexanoate (8b).

Yield: 1.63 g (98%); white solid; mp 181–182 °C. IR (KBr ν (cm^{-1})) 3316, 3108, 2929, 2856, 1702, 1572, 1520, 1433, 1405, 1349, 1319, 1271, 1227, 1168, 1103, 1028, 997, 884, 755, 734, 697, 680, 596, 514. δ_H (500 MHz, DMSO-d_6) 8.88 (t, *J* 5.3 Hz, 1H, NH), 3.20 (q, *J* 6.8 Hz, 2H, CH_2), 1.88 (t, *J* 7.4 Hz, 2H, CH_2), 1.52–1.39 (m, 4H, 2 CH_2), 1.31–1.21 (m, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 178.05 (C=O), 160.11 (C=O), 159.60, 149.60, 123.08 ($3C_q$), 39.56 (CH_2), 38.67 (CH_2), 29.52 (CH_2), 27.35 (CH_2), 26.65 (CH_2).

Sodium 4-(3-(4,5-dichloroisothiazol-3-yl)ureido)butanoate (20a).

Yield: 1.58 g (99%); white solid; mp >250 °C. IR (KBr ν(cm^{-1})) 3301, 3108, 2953, 2921, 2878, 1699, 1594, 1563, 1530, 1437, 1405, 1323, 1270, 1231, 1144, 1110, 1085, 1029, 895, 752, 717, 698, 681, 568, 408. δ_H (500 MHz, DMSO-d_6) 10.10 (br.s, 1H, NH), 8.05 (br.s, 1H, NH), 3.08 (q, *J* 6.3 Hz, 2H, CH_2), 1.98 (t, *J* 7.2 Hz, 2H, CH_2), 1.62 (quint, *J* 7.0 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 177.63 (C=O), 155.83 (C=O), 154.38, 146.27, 115.51 ($3C_q$), 40.36 (CH_2), 35.67 (CH_2), 27.12 (CH_2).

Sodium 6-(3-(4,5-dichloroisothiazol-3-yl)ureido)hexanoate (20b).

Yield: 1.71 g (98%); white solid; mp 172–176 °C. IR (KBr ν(cm^{-1})) 3316, 2929, 2856, 1702, 1572, 1520, 1433, 1405, 1319, 1271, 1227, 1168, 1103, 997, 884, 697, 680, 514. δ_H (500 MHz, DMSO-d_6) 10.48 (br.s, 1H, NH), 8.27 (s, 1H, NH), 3.11–2.98 (m, 2H, CH_2), 1.92 (t, *J* 7.0 Hz, 2H, CH_2), 1.54–1.33 (m, 4H, 2 CH_2), 1.31–1.18 (m, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 178.46 (C=O), 156.18 (C=O), 154.88, 145.95, 115.50 ($3C_q$), 39.93 (CH_2), 38.76 (CH_2), 30.22 (CH_2), 27.37 (CH_2), 26.72 (CH_2).

Sodium 4-(3-(5-(benzylthio)-4-chloroisothiazol-3-yl)ureido)butanoate (20c).

Yield: 2.02 g (99%); white solid; mp >250°C. IR (KBr ν(cm^{-1})) 3303, 3084, 3060, 3028, 2924, 2865, 1691, 1565, 1512, 1452, 1403, 1314, 1271, 1231, 1101, 1070, 1027, 867, 760, 696, 601, 565, 523. δ_H (500 MHz, DMSO-d_6) 10.03 (s, 1H, NH) 8.21 (s, 1H, NH), 7.40 (d, *J* 7.2 Hz, $2H_{arom}$), 7.33 (t, *J* 7.2 Hz, $2H_{arom}$), 7.28 (t, *J* 7.2 Hz, $1H_{arom}$), 4.34 (s, 2H, SCH_2), 3.08 (q, *J* 6.2 Hz, 2H, CH_2), 1.97 (t, *J* 7.1 Hz, 2H, CH_2), 1.62 (quint, *J* 7.0 Hz, 2H, CH_2); δ_C (125 MHz, DMSO-d_6) 178.11 (C=O), 155.91 (C=O), 154.68, 154.30, 136.63, 112.83 ($4C_q$), 129.62 ($2CH_{arom}$), 129.24 ($2CH_{arom}$), 128.43 ($1CH_{arom}$), 40.36 (CH_2), 38.05 (CH_2), 35.98 (CH_2), 27.34 (CH_2).

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