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Pseudosaccharin amine derivatives: synthesis and elastase inhibitory activity[1]

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Pseudosaccharin amines were synthesized from saccharin either by the reaction of pseudosaccharin chloride with amines, or via thiosaccharin which was treated with amines yielding thiosaccharinates, and their reaction with glacial acetic acid. This route gave lower yields than the first way. The synthesis of alkyl [(1,1-dioxo-benzo[*d*]isothiazol-3-yl)amino]alkanoates as possible Human Leukocyte Elastase (HLE) inhibitors was realized by the reaction between amino acid esters and pseudosaccharin chloride. Hydrolysis of the esters was possible under aqueous basic conditions. Selected compounds were screened for elastase inhibitory activity. Compounds **4k** and **4m** were found to be reversible inhibitors of HLE with K_i values of 45 μM and 60 μM.

1. Introduction

N-Substituted 1,2-benzo[*d*]isothiazol-3-ylamines (Structure **I**) (Grogen et al. 1955; Whitehead et al. 1960; Shapira et al. 1980; Singh 1959) showed slight hypotensive effects and their antihistaminic and bronchodilatory activity is reported (Grogen et al. 1955). Compounds of structure **II** (Scheme 1) are patented as antiinflammatory agents (Wade et al. 1979).

Human Leukocyte Elastase (HLE, EC 3.4.21.37) is a serine protease released by polymorphonuclear leukocytes in response to inflammatory stimuli. Excessive elastolytic activity has been implicated in the etiology of a number of diseases such as adult respiratory distress syndrome, cystic fibrosis and emphysema (Brown et al. 1994). Recently, it has been found that a serine elastase inhibitor reduces inflammation, fibrosis and preserve cardiac function after experimentally induced murine myocarditis (Lee et al. 1998). The highly specific and intravenously effective inhibitor PNO-5046 has been approved in Japan as an HLE inhibitor for the treatment of acute lung injury accompanying systemic inflammatory response syndrome (Nakayama et al. 2002). We have reported β-lactams as elastase inhibitors (Achilles et al. 2000a, 2000b; Venz et al. 2001). The elastase inhibitory mechanism was demonstrated by different authors by X-ray crystallographic studies of inhibitor-enzyme complex (Takahashi et al. 1989a; Bode et al. 1989; Navia et al. 1989). From these studies it has been deduced that the nucleophilic addition of the hydroxyl group of Ser-195 of the elastase on a carbonyl carbon of a scissile peptide bond or ester (Nakayama et al. 2002) is important.

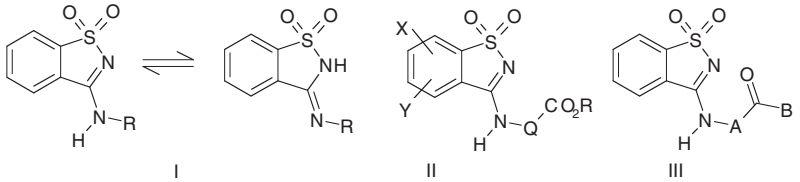
Here we report about our experiments to combine the two structural features, the pseudosaccharin amine skeleton as an antiinflammatory moiety and the carbonyl functionality as a centre for the hydroxyl attack by the elastase. Therefore, we synthezised compounds of the general structure **III**, and investigated whether these pseudosaccharin derivatives really possess elastase inhibitory effects.

2. Investigations, results and discussion

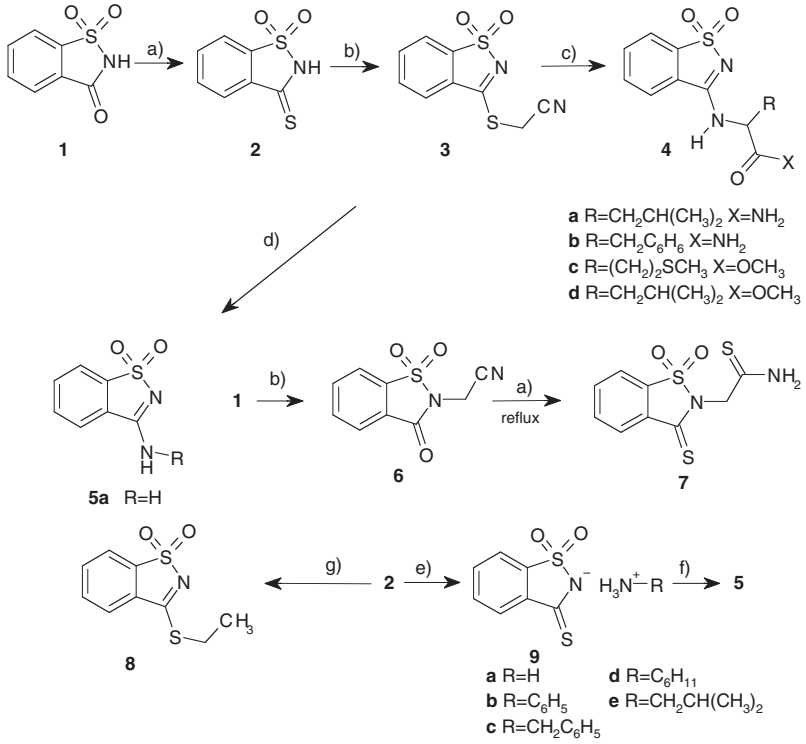
2.1. Chemistry

Synthesis of thiosaccharin (**2**) from saccharin (**1**) was carried out according to the literature (Meadow et al. 1951). A relatively low yield of thiosaccharin was obtained from the reaction of **1** with P_2S_5 in refluxing dioxan, while the

Scheme 1



Scheme 2



a) P_2S_5, heat, or P_2S_5, dioxan, reflux; b) NaH, DMF, $ClCH_2CN$, reflux; c) DMSO/MeOH/$CHCl_3$/CH_2Cl_2, amine, room temp.; d) NH_3, MeOH; e) amine, MeOH; f) AcOH, reflux; g) NaH, EtBr, DMF, reflux

reaction without a solvent below 185 °C resulted in higher yields. Amide proton abstraction by NaH followed by addition of chloroacetonitrile in DMF resulted in *S*-alkylation forming (1,1-dioxo-1*H*-λ^6-benzo[*d*]isothiazol-3-ylsulfanyl)-acetonitrile (**3**). The activation of the nitrile group of **3** by HCl in MeOH (over 1 week!) did not result in an imido ester formation, indicating that under these conditions the nitrile group was not attacked by nucleophiles. To optimize yields and conditions, reactions of different amino acid derivatives and ammonia with **3** were carried out in different solvents, and under different conditions, to give the N-substituted derivatives **4a**, **4b**, **4c**, **4d**, and **5a**. Best results were obtained from reactions in $CHCl_3$. (Scheme 2).

In HPLC experiments, compounds **4a**, **4b**, **4c** and **4d** gave only one peak when using a chiral column, indicating that no racemization had occurred during the reaction. Compounds **4** and **5** show tautomerism, as the exocyclic nitrogen atom is part of an amidino function, and they do not form hydrochlorides. In solution the inamine form seems to be preferred. In their ^{1}H NMR spectra a doublet is observed, at $\delta = 9.46$ ppm (**4a**), for the proton on the nitrogen atom.

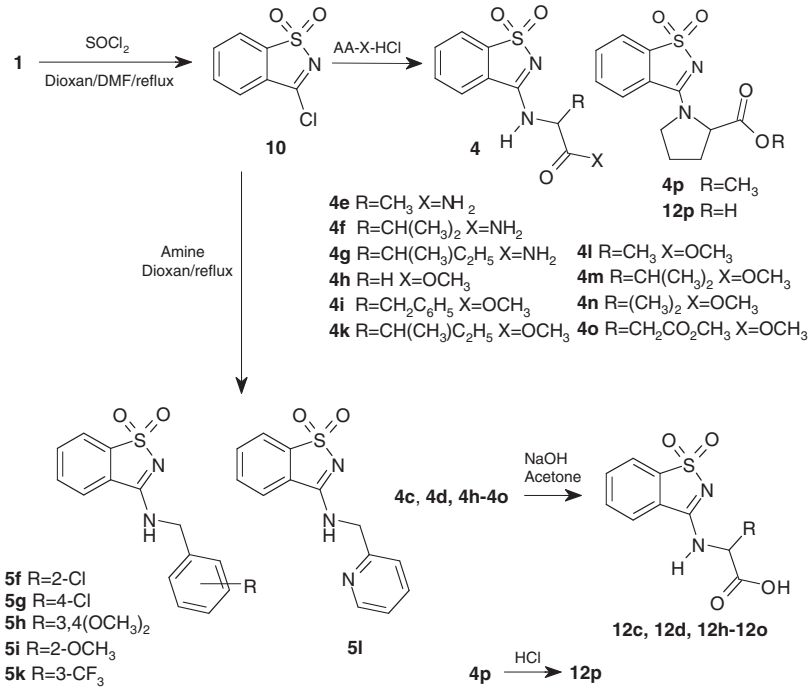
Additionally, we tried to obtain an isomer of **3**, thiosaccharin-2-acetonitrile, via the saccharin-2-acetonitrile (**6**), which was synthesized from the sodium salt of **1** (Scheme 2). But the reaction of **6** with P_2S_5 was not successful. Instead, the yellow compound **7** was isolated**,** indicating that the reaction had occurred at the carbonyl and the nitrile group of **6**. Using two equivalents of P_2S_5 the yield of **7** was slightly increased. Hence, even this route was not appropriate for the synthesis of thiosaccharin-2-acetonitrile from **6**. In the ^{13}C NMR spectrum of **7**, signals at $\delta = 196.42$ and 186.95 ppm were observed, and these peaks were absent in the DEPT spectrum, confirming the structure.

Furthermore, compounds **5a**–**5e** were successfully synthesized using the following route. When **2** was treated with amines in MeOH at room temperature, the corresponding salts, **9a**–**9e**, were obtained in good yields. In their ^{1}H NMR spectra the amine protons were identified by the proton exchange with D_2O. The thiosaccharinates **9** were refluxed in glacial AcOH for several hours (or days) and compounds **5** were obtained. This reaction may be explained by an equilibrium between **9**, aminium acetate, **2**, and free amine, where the free amine acting as a nucleophile attacks the C atom of the thiocarbonyl group in **2** forming **5**. The reaction was very slow and often required days, and yields were low, ranging from 20 to 41%. Under different conditions in DMF the sulfur atom is an appropriate nucleophile replacing halogene at C atoms as demonstrated by the reactions **2** → **3**, and **2** → **8**.

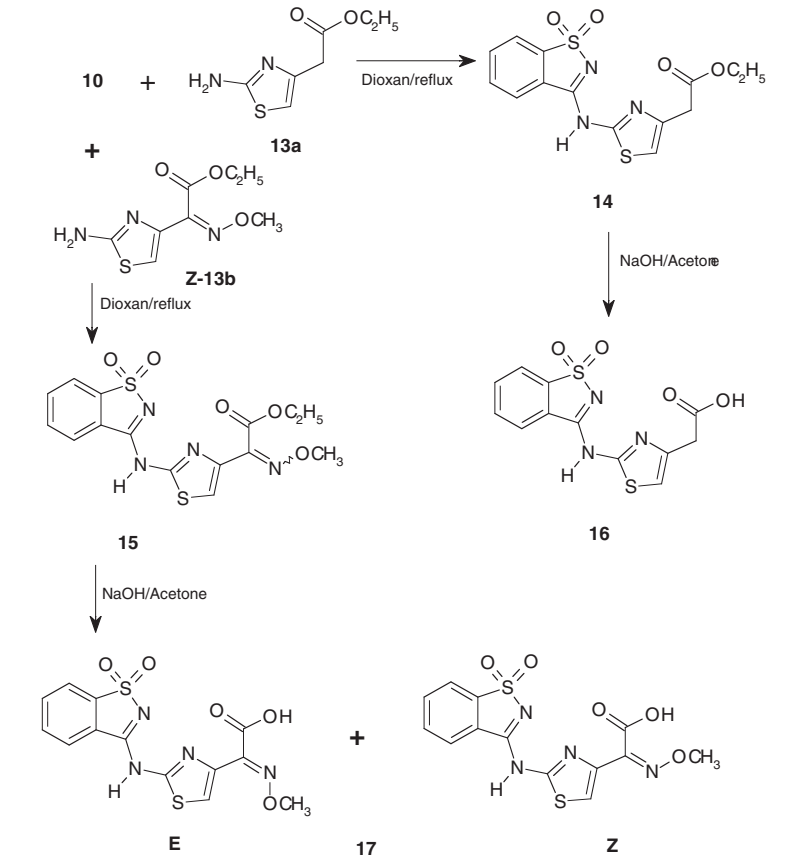
Finally, an effective synthesis for **4** and **5** was realized using the 3-chloro-benzo[*d*]isothiazole 1,1-dioxide (**10**), which was obtained from **1** by treatment with $SOCl_2$ as a relatively stable, colorless crystalline compound (Wade et al. 1979). When amines were refluxed with **10** in dioxan compounds **4** and **5** were obtained in moderate to good yields (Scheme 3). Compound **5l** was isolated as the hydrochloride. This compound dissolved in water and showed a very broad singlet at $\delta = 6.60$ ppm, and underwent D_2O exchange. The other compounds which were synthesized from different routes showed identical spectra.

The ester derivatives **4**, obtained as crystalline solids, were hydrolyzed with NaOH in acetone to the corresponding

Scheme 3



Scheme 4



acid derivatives **12**. Compound **4p** failed to be hydrolyzed under this condition but was successfully hydrolyzed by refluxing in 5% HCl.

Interesting results were observed in the reactions between **10** and the thiazol-2-amines **13a** and **13b** (Scheme 4). From **13a** the ester **14** was obtained with 50% yield as a crystalline compound which was hydrolyzed yielding the free acid **16** with 87% yield. The analoguous reaction between **10** and *Z*-**13b** yielded the ester **15**, yield 60%, and by hydrolysis **17** was obtained with 90% yield.

In the ^{1}H NMR spectra of **15** and **17** additional signals were detected indicating that isomerization had occurred during the reaction. This could be due to heat as the reaction mixture was refluxed in dioxan. In order to clarify the isomerization we compared their ^{1}H NMR spectra with that of the starting material, ethyl *syn*-(2-amino-thiazol-4-yl)-α-methoxyiminoacetate (*Z* form) (*Z*-**13b**). In [D_6]DMSO, the ^{1}H spectrum of *Z*-**13b** showed a broad singlet at $\delta = 7.25$ ppm for NH, a singlet at $\delta = 6.90$ ppm for 5-$H_{thiazol}$, a quartet at $\delta = 4.26$ ppm for CH_2, a singlet at $\delta = 3.87$ ppm for OCH_3, and a triplet at $\delta = 1.26$ ppm for CH_3. After irradiating *Z*-**13b** with UV light for 2 days, we observed in the ^{1}H NMR spectrum additional signals, a singlet at $\delta = 7.50$ ppm for 5-$H_{thiazol}$, a quartet at $\delta = 4.24$ ppm for CH_2, and a singlet at $\delta = 3.98$ ppm for OCH_3. These signals caused by the *anti* isomer (*E* form) indicate that the signals for the *E* form are shifted to higher δ values for 5-$H_{thiazol}$, and OCH_3 protons but to a lower δ value for CH_2. The ratio of isomers was found to be ≈2 : 8 (*E* : *Z*). A similar situation has been observed for **15**. Here we obtained two sets of signals representing the two different isomers. The dominant set showed the 5-$H_{thiazol}$ singlet at $\delta = 8.27$ ppm, a quartet at $\delta = 4.32$ ppm for CH_2, and for the OCH_3 protons a singlet at $\delta = 4.02$ ppm, whereas the minor set showed signals that are shifted to lower δ values for 5-$H_{thiazol}$, $\delta = 7.83$ ppm, and OCH_3, $\delta = 3.96$ ppm, but to a higher δ value for CH_2, $\delta = 4.37$ ppm. Assuming the chemical shift dependency on E/Z isomerism being equivalent to that for the starting material, we deduced the ratio to be 7 : 3 (*E* : *Z*). The similar situation was observed in the ^{1}H NMR spectra of **17**, where the ratio of isomers was 8.5 : 1.5 (*E* : *Z*). These results are in agreement with the HPLC analyses of the compounds.

All pseudosaccharin derivatives were characterized by their IR spectra. The absorption ≈3400–3300 cm^{-1} was caused by N–H, and absorption bands at 1140–1190 cm^{-1} and 1320–1390 cm^{-1} are due to the SO_2 group. A characteristic absorption between 1600 and 1640 cm^{-1} was attributed to the C=N moiety. The C=N band absorption for **5a** was shifted to a higher value, and was observed at 1679 cm^{-1}. The spectra of **4a**, **4b**, **4e**, **4f**, and **4g** showed two NH bands due to primary and secondary amides. In the spectra of the acid derivatives the band for OH and NH overlapped each other. The carbonyl absorptions were observed at 1675–1695, 1740–1760, and 1700–1760 cm^{-1} due to amide, ester and acid respectively. The ^{1}H NMR spectra were in full agreement with the structures. The ^{1}H NMR spectra of **9** were recorded in D_2O with sodium 2,2,3,3-d_4-3-(trimethylsilyl)propionate as internal standard. The amount of racemization in amino acid derivatives is estimated from ^{1}H NMR spectra to be less than 5%.

For the HPLC investigations of chiral compounds and of **15** and **17** a chiral OJ-R column was used. For all achiral compounds we used a RP-18 column. HPLC data were in full agreement with the results from spectroscopy.

2.2. Biochemical studies

Esters have been evaluated as acylating agents for their elastase inhibitory activity (Imaki et al. 1996). As reported, some of the ester derivatives have shown in vivo activity (Edwards and Bernstein 1994), but the challenge still remains to find a better agent. The strategy to find a better agent is based on the acylation power of the inhibitor and its slow deacylation from the enzyme. Therefore, an ideal inhibitor should have a very high acylation power and very slow deacylation from the elastase hence producing very high potency against it. We tested compounds **4**, **12c**, **12d**, **12h–p**, and **14–17** for their elastase inhibitory activity. Only compounds **4k** and **4m** were found to be HLE inhibitors but did not show any PPE inhibition. Both compounds showed a reversible inhibition. It is stated from the crystallographic studies of enzyme inhibitor complex that the valine (Warner et al. 1994) moiety occupies the S_1 pocket of the enzyme. Isoleucine (Takahashi et al. 1989b) containing inhibitors are known. Only **4k** and **4m** with an isoleucine or valine unit showed activity, while all other ester derivatives were inactive. Hence, possibly the isoleucine and valine moiety occupies the S_1 pocket of the enzyme and the hydroxyl group of the enzyme can attack the carbonyl of the ester producing inhibition. The inhibition of HLE by **4k** and **4m** at 0.5 M concentration was found to be 70 and 65% respectively. K_i was determined from the dixon plot (Dixon M 1953). A typical dixon plot for compound **4m** is shown in the figure. K_i was determined from the intersection of the three lines (each $R^2 > 0.98$).

3. Experimental

3.1. General

M.p.: PHMK 80/2747 (Küstner, Dresden) apparatus, not corrected. IR Spectra: Perkin-Elmer FTIR 1600; $\tilde{\nu}$ in KBr (cm^{-1}), if not noted otherwise. NMR Spectra: Bruker DPX 200 (200 MHz), for ^{1}H; Bruker DPX 200 (50 MHz) for ^{13}C; δ (ppm) rel. to TMS as internal standard, spectra in [D_6]DMSO, if not noted otherwise. Optical rotation: Polartronic D (Schmidt Haensch GmbH). Elementary analyses: Perkin-Elmer Elemental Analyzer 2400 CHN, Institute of Pharmacy at university of Greifswald. All results were in an acceptable range. TLC on Merck DC-Aluminiun plates, Silica Gel 60 F_{254}, Nr. 5554. CC with Silica Gel 60 Merck Nr. 7734 or 9385. HPLC with LaChrom apparatus series 7000 Merck Hitachi. Columns: LiChrospher 250-4, RP-18, 5 μm, and LiChroCART 250-4, Chiracel OJ-R, 5 μm.

PPE (EC 3.4.21.36, ≈200 U/mg) was purchased from Serva, Suc-$(Ala)_3$-pNA, and N-methoxysuccinyl-$(Ala)_2$-Pro-Val-pNA from Bachem and HLE (EC 3.4.21.37, ≈34 U/mg) from Serva. *syn*-Ethyl (2-amino-4-thiazolyl)-α-methoxyiminoacetate (Cat 28,015-1) and Ethyl (2-amino-4-thiazolyl)acetate (Cat 22, 055-8) were purchased from Aldrich. Solvents were purified according to literature procedures. Abbreviations: AcOEt = Ethyl acetate; CC = Column chromatography; DMF = Dimethylformamide; PE = Petroleum ether; ar = aromatic.

3.2. Benz[d]isothiazol-3(2H)-thione 1,1-dioxide (**2**)

a) A mixture from **1** (5.0 g, 27 mmol) and P_2S_5 (6.67 g, 30 mmol) was heated in a small round-bottom flask in an oil bath. The bath temperature was al-

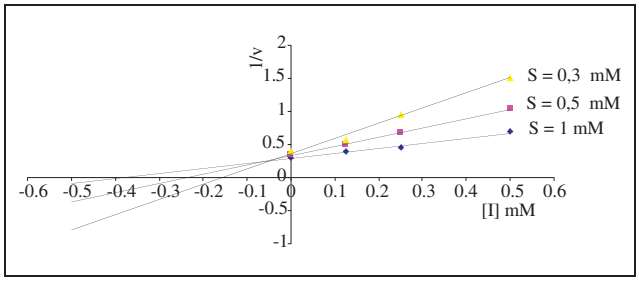


Fig.: Inhibition of HLE by compound **4m** (Dixon plot)

lowed to rise slowly from 50 to 155 °C over 45 min. Heating was continued for 15 min while the temperature rose to 170 °C. The mixture was extracted with toluene (1 l). The extract was concentrated and left for 12 h at room temp. Then, the crystals were separated and dried. Yield: 4.1 g (75%).
b) **1** (2.0 g, 11 mmol) dissolved in dioxan, and P_2S_5 (4.85 g, 22 mmol) were refluxed for 6 h. After cooling to room temperature, the precipitate was separated, the filtrate was concentrated to ca. 50%, poured into ice cold water and left for 12 h. Work-up as a). Yield: 0.8 g (37%); M.p. 178–180 °C (Toluene, 178–178.5 °C, Meadow et al. 1951); R_f: 0.6 (MeOH/CH_2Cl_2, 1 : 3); IR: $\tilde{\nu}$ = 3338 (NH), 1368, 1150 (SO_2), 1120 (C=S); ^{1}H NMR ($CDCl_3$): δ = 8.25–8.17, 7.96–7.77 (2 m, 4 ar H); HPLC: k′= −0.214, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_7H_5NO_2S_2$ (199.3)

3.3. (1,1-Dioxobenzo[d]isothiazol-3-ylsulfanyl)acetonitrile (**3**)

At 0 °C, and under N_2, NaH (1.56 g, 65.0 mmol) was slowly added with stirring to an ice-cold solution of **2** (6.0 g, 50.18 mmol) in DMF (100 ml), stirring was continued for 30 min, then, chloroacetonitrile (5.1 ml, 67.29 mmol) was added. Stirring was continued, and progress of reaction was monitored by TLC. After 10 h, the mixture was poured into water, and the precipitate was separated. Yield: 6.0 g (50%); M.p. 222–223 °C (Acetonitrile/acetone, 234.5-235.5 Katsuhiko et al. 1981); R_f: 0.5 (AcOEt/PE 6 : 4); IR: $\tilde{\nu}$ = 2991 (CH), 2256 (CN), 1616 (C=N), 1322, 1171 (SO_2), 1170 (C=S); ^{1}H NMR: δ = 8.18–8.12, 8.01–7.84 (2 m, 4 ar H), 4.52 (s, CH_2); HPLC: k′ = 1.41, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_9H_6N_2O_2S_2$ (238.3)

3.4. General procedure for the synthesis of 4 and 5 from 10

A solution of the amine in dioxan was added to a solution of the equivalent amount of **10** in dioxan, the mixture was refluxed for 4–5 h, evaporated in vacuo, the residue was washed with cold water (except for **5l**), and crystallized.
For using amino acid derivatives-HCl, the base was liberated with Et_3N before addition of **10**. Syntheses of amino acid methyl ester-HCl were done following literature procedures using $SOCl_2$ in MeOH. See Bodtke (2002), Venz et al. (2001) and lit. cited therein.

3.4.1. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentanamide (**4a**)

a) A mixture from **3** (0.3 g, 1.2 mmol) dissolved in DMSO (6 ml), and L-Leu-NH_2 (0.16 g, 1.2 mmol) in DMSO (3 ml) was stirred at room temperature for 2 h, then it was pored into H_2O and extracted with AcOEt, the organic layer was washed with brine, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by CC (MeOH/CH_2Cl_2 3 : 7). Yield: 0.05 g (13%). b) L-Leu-NH_2 (0.22 g, 1.68 mmol), and **3** (0.40 g, 1.67 mmol) in MeOH (10 ml) were stirred at room temperature for 24 h, then the solvent was evaporated in vacuo, and the residue was purified by CC (AcOEt/PE 8 : 2). Yield: 0.18 g (36%). c) L-Leu-NH_2 (0.16 g, 1.2 mmol), and **3** (0.30 g, 1.2 mmol) in $CHCl_3$ were stirred at room temperature for 6 d, the solvent was evaporated in vacuo, and the residue was recrystallized. Yield: 0.25 g (68%). d) From **10** (3.00 g, 14.88 mmol), and L-Leu-NH_2 (1.92 g, 14.88 mmol) in 60 ml dioxan, see **3.4**. Yield: 3.02 g (69%); M.p. 254–256 °C (MeOH); R_f: 0.66 (MeOH/CH_2Cl_2 1.5 : 8.5); $[\alpha]_D^{20}$ = +18.33 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3450, 3325 (NH), 3067, 2958, 2929 (CH), 1686 (CO), 1628 (C=N), 1370, 1165 (SO_2); ^{1}H NMR: δ = 9.46 (d, J = 8.28 Hz, NH), 8.44–8.32, 8.03–7.92, 7.89–7.81 (3 m, 4 ar H), 7.78 (bs, NH) 7.20 (bs, NH), 4.63–4.66 (m, 1 H, CH), 1.93–1.55 (m, 3 H, CH_2–CH), 0.93 (d, J = 5.96 Hz, Me) 0.90 (d, J = 5.81 Hz, Me); HPLC: k′ = 1.47, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O 2 : 8).
$C_{13}H_{17}N_3O_3S$ (295.4)

3.4.2. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-phenylpropanamide (**4b**)

a) From L-Phe-NH_2 (0.21 g, 1.2 mmol), and **3** (0.3 g, 1.2 mmol) in DMSO (9 ml) as described for **4a**. Yield: 0.09 g (21%). b) From L-Phe-NH_2 (0.25 g, 1.5 mmol), and **3** (0.37 g, 1.5 mmol) in MeOH (10 ml) for 5 h as described for **4a**. Yield: 0.08 g (16%). c) From L-Phe-NH_2 (0.21 g, 1.2 mmol), and **3** (0.3 g, 1.2 mmol) in $CHCl_3$ (25 ml) for 11 days. Yield: 0.25 g (60%). d) From **10** (1.0 g, 4.96 mmol), and L-Phe-NH_2 (0.81 g, 4.96 mmol) in 30 ml dioxan. Yield: 0.95 g (58%); M.p. 214 °C (AcOEt/PE); R_f: 0.66 (MeOH/CH_2Cl_2 1.5 : 8.5); $[\alpha]_D^{20}$ = −27.5 (c = 2, dioxan); IR: $\tilde{\nu}$ = 3444, 3311 (NH), 3100, 2934 (CH), 1691 (CO), 1618 (C=N), 1360, 1164 (SO_2); ^{1}H NMR: δ = 9.62 (d, J = 8.20 Hz, NH), 8.38–8.28, 7.98-7.25 (2 m, 4 ar H), 7.42–7.10 [m, 5 ar H (Phe)], 4.80–4.65 (m, 1 CH), 3.30–3.00 (m, CH_2); HPLC: k′ = 3.33, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O 2 : 8).
$C_{16}H_{15}N_3O_3S$ (329.4)

3.4.3. Methyl (S)-2 (1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methylsulfanylbutanoate (**4c**)

a) A mixture of L-Met-OMe-HCl (0.42 g, 2.09 mmol), and Et_3N (0.32 ml, 2.30 mmol) in CH_2Cl_2 (5–10 ml) was stirred for 10 min, then **3** (0.5 g, 2.09 mmol) in CH_2Cl_2 (5 ml) was added, and stirring was continued 2 h. After evaporation of the solvent the residue was purified by CC (AcOEt/PE 8 : 2). Yield: 0.12 g (17%). b) From **10** (7.0 g, 34.72 mmol), L-Met-OMe-HCl (6.93 g, 34.72 mmol), and Et_3N (11.2 ml, 80.50 mmol) in 150 ml dioxan. Yield: 3.5 g (31%); M.p. 166 °C (MeOH/H_2O); R_f = 0.56 (AcOEt/PE 7 : 3); $[\alpha]_D^{20}$ = −43.50 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3329 (NH), 3101, 2953 (CH), 1755 (CO), 1613 (C=N), 1372, 1158 (SO_2); ^{1}H NMR: δ = 9.68 (d, J = 7.60 Hz, 1 NH), 8.35–8.26, 8.04–7.97, 7.92–7.82 (3 m, 4 ar H), 4.76 (q, J = 7.4 Hz, CH), 3.73 (s, OMe), 2.69–2.56 (m, CH_2), 2.25–2.11 (q, J = 7.3 Hz, CH_2), 2.07 (s, SMe); HPLC: k′ = 2.39, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3 : 7).
$C_{13}H_{16}N_2O_4S_2$ (328.4)

3.4.4. Methyl (S)-2 (1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentanoate (**4d**)

a) From L-Leu-OMe-HCl (0.17 g, 0.83 mmol) as **4c**. Yield: 0.08 g (31%). b) From **10** (7.0 g, 34.72 mmol), L-Leu-OMe-HCl (6.3 g, 34.72 mmol), and Et_3N (11.2 ml, 80.50 mmol) in 150 ml dioxan. Yield: 6.0 g (55%); M.p. 172 °C (MeOH/H_2O); R_f = 0.68 (AcOEt/PE 7 : 3); $[\alpha]_D^{20}$ = −48.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3300 (NH), 2958, 2871 (CH), 1753 (CO), 1615 (C=N), 1369, 1155 (SO_2); ^{1}H NMR: δ = 9.69 (d, J = 7.77 Hz, NH), 8.40–8.27, 8.06–7.95, 7.93–7.81 (3 m, 4 ar H), 4.72–4.55 (m, CH), 3.71 (s, OMe), 1.97–1.61 (m, CH_2–CH), 0.95 (d, J = 6.2 Hz, Me), 0.91 (d, J = 6.2 Hz, Me); HPLC: k′ = 3.26, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3 : 7).
$C_{14}H_{18}N_2O_4S$ (310.4)

3.4.5. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)propanamide (**4e**)

From **10** (1.0 g, 4.96 mmol), and L-Ala-NH_2-HCl (0.62 g, 4.96 mmol), and Et_3N (1.38 ml, 9.92 mmol) in 30 ml dioxan. Yield: 0.60 g (55%); M.p. 236 °C (acetone); R_f = 0.50 (MeOH/CH_2Cl_2 1.5 : 8.5); $[\alpha]_D^{20}$ = +42.16 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3432, 3337 (NH), 3096, 3056, 2941 (CH), 1684 (CO), 1620 (C=N), 1361, 1160 (SO_2); ^{1}H NMR: δ = 9.49 (bs, NH), 8.40–8.30, 8.03–7.92, 7.90–7.78 (3 m, 4 ar H), 7.68 (bs, NH), 7.20 (bs, NH), 4.63–4.44 (m, CH), 1.46 (d, J = 7.16 Hz, Me); HPLC: k′ = 0.20, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O, 2 : 8).
$C_{10}H_{11}N_3O_3S$ (253.3)

3.4.6. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylbutanamide (**4f**)

From **10** (4.0 g, 19.85 mmol), L-Val-NH_2-HCl (3.1 g, 20.33 mmol), and Et_3N (6.9 ml, 39.7 mol) in 60 ml dioxan, CC (AcOEt/PE 9 : 1). Yield: 2.24 g (40%); M.p. 225 °C; R_f = 0.56 (MeOH/CH_2Cl_2 1.5 : 8.5); $[\alpha]_D^{20}$ = +12 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3523, 3399 (NH), 3093, 3058, 2967, 2937 (CH), 1675 (CO), 1619 (C=N), 1362, 1162 (SO_2); ^{1}H NMR: δ = 9.36 (d, J = 8.18 Hz, NH), 8.60–8.45, 8.05–7.94, 7.90–7.74 (3 m, 4 ar H), 7.31 (bs, NH), 4.36 (t, J = 8.18 Hz, CH), 2.32–2.11 (m, CH), 0.99 (d, J = 6.43 Hz, 2 Me); HPLC: k′ = 0.72, t_0 = 2.37 (Chiralcel OJ-R, MeCN/H_2O, 2 : 8).
$C_{12}H_{15}N_3O_3S$ (281.3)

3.4.7. (2S,3S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanamide (**4g**)

From **10** (1.0 g, 4.96 mmol), L-Ile-NH_2-HCl (0.83 g, 4.96 mmol), and Et_3N (1.38 ml, 9.92 mmol) in 30 ml dioxan. Yield: 1.07 g (72%); M.p. 247 °C (acetone); R_f = 0.60 (MeOH/CH_2Cl_2 1.5 : 8.5); $[\alpha]_D^{20}$ = −10.16 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3418, 3361 (NH), 2978, 2934, 2877 (CH), 1681 (CO), 1610 (C=N), 1366, 1163 (SO_2); ^{1}H NMR: δ = 9.39 (d, J = 8.62 Hz, NH), 8.58–8.44, 8.04–7.93, 7.90–7.77 (3 m, 4 ar H), 7.30 (bs, NH), 4.39 (t, J = 8.62 Hz, CH), 2.09-1.90 (m, CH), 1.66–1.42, 1.36–1.11 (2 m, CH_2), 0.96 (d, J = 6.87 Hz, Me), 0.86 (t, J = 7.16 Hz, Me); HPLC: k′ = 1.45, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O, 2 : 8).
$C_{13}H_{17}N_3O_3S$ (295.4)

3.4.8. Methyl (1,1-dioxobenzo[d]isothiazol-3-ylamino)acetate (**4h**)

From **10** (10.0 g, 49.58 mmol), and Gly-OMe-HCl (8.7 g, 69.42 mmol), and Et_3N (16.5 ml, 119.04 mmol) in 200 ml dioxan. Yield: 4.3 g (34%); M.p. 214 °C (MeOH/H_2O); R_f = 0.45 (AcOEt/PE 7 : 3); IR: $\tilde{\nu}$ = 3343 (NH), 3051, 2959 (CH), 1759 (CO), 1625 (C=N), 1375, 1158 (SO_2); ^{1}H NMR: δ = 9.93 (t, J = 5.70 Hz, NH), 8.25–8.16, 8.04–7.96, 7.90–7.82 (3 m, 4 ar H), 4.32 (d, J = 5.70 Hz, CH_2), 3.72 (s, OMe); HPLC: k′ = 1.42, t_0 = 1.81 (RP-18, MeCN/H_2O, 3 : 7).
$C_{10}H_{10}N_2O_4S$ (254.3)

3.4.9. Methyl (S)-2(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-phenylpropanoate (**4i**)

From **10** (7.0 g, 34.7 mmol), L-Phe-OMe-HCl (7.49 g, 34.7 mmol), and Et_3N (11.2 ml, 80.50 mmol) in 150 ml dioxan. Yield: 9.1 g (76%); M.p. 154 °C (MeOH/H_2O); R_f = 0.66 (AcOEt/PE 7 : 3); $[\alpha]_D^{20}$ = −51.66 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3307 (NH), 3059, 3027, 2952 (CH), 1740 (CO), 1616 (C=N), 1372, 1157 (SO_2); ^{1}H NMR: δ = 9.80 (d, J = 7.71 Hz, NH), 8.30-

8.22, 8.00–7.92, 7.88–7.80 (3 m, 4 ar H), 7.36–7.16 [m, 5 ar H(Phe)], 4.90–4.75 (m, CH), 3.69 (s, OMe), 3.27, 3.23 (dd, J = 4.20, 5.75 Hz, CH_2); HPLC: k′ = 6.23, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3 : 7).
$C_{17}H_{16}N_2O_4S$ (344.4)

3.4.10. Methyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanoate (**4k**)

From **10** (7.0 g, 34.72 mmol), L-Ile-OMe-HCl (6.3 g, 34.72 mmol), and Et_3N (11.2 ml, 80.50 mmol) in 150 ml dioxan. Yield: 4.8 g (43%); M.p. 168 °C (MeOH/H_2O); R_f = 0.68 (AcOEt/PE 7 : 3); $[\alpha]_D^{20}$ = −30.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3309 (NH), 3097, 3050, 2969, 2879 (CH), 1756 (CO), 1612 (C=N), 1365, 1157 (SO_2); ^{1}H NMR: δ = 9.57 (d, J = 7.81 Hz, NH), 8.52–8.42, 8.04–7.94, 7.91–7.81 (3 m, 4 ar H), 4.49 (d, J = 7.81 Hz, CH), 3.71 (s, OMe), 2.15–1.97 (m, CH), 1.63–1.11 (m, CH_2), 0.96 (d, J = 6.91 Hz, Me), 0.89 (t, J = 7.30 Hz, Me); HPLC: k′ = 3.60, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3 : 7).
$C_{14}H_{18}N_2O_4S$ (310.4)

3.4.11. Methyl (S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)propionate (**4l**)

From **10** (10.0 g, 49.58 mmol), L-Ala-OMe-HCl (9.68 g, 69.42 mmol), and Et_3N (16.56 ml, 119.04 mmol) in 200 ml dioxan. Yield: 5.5 g (41%); M.p. 232–234 °C (MeOH/Acetone/H_2O); R_f = 0.43 (AcOEt/PE 7 : 3); $[\alpha]_D^{20}$ = −9.66 (c = 2, Acetone); IR: $\tilde{\nu}$ = 3329 (NH), 3107, 3050, 2994, 2958 (CH), 1752 (CO), 1615 (C=N), 1368, 1158 (SO_2); ^{1}H NMR: δ = 9.73 (d, J = 7.25, NH), 8.34–8.25, 8.04–7.95, 7.91–7.82 (3 m, 4 ar H), 4.76–4.58 (quintet, J = 7.25 Hz, CH), 3.70 (s, OMe), 1.52 (d, J = 7.25 Hz, Me); HPLC: k′ = 1.73, t_0 = 2.39 (Chiralcel OJ-R, MeCN/H_2O, 2 : 8).
$C_{11}H_{12}N_2O_4S$ (268.3)

3.4.12. Methyl (S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylbutanoate (**4m**)

From **10** (20.0 g, 99.20 mmol), L-Val-OMe-HCl (18.2 g, 109.12 mmol), and Et_3N (34.6 ml, 248.01 mmol) in 400 ml dioxan, 2 h reflux. Yield: 18.7 g (61%); M.p. 238 °C (Acetone/H_2O); R_f = 0.65 (AcOEt/PE 7 : 3); $[\alpha]_D^{20}$ = −38.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3332 (NH), 3102, 3036, 2966, 2937 (CH), 1748 (CO), 1608 (C=N), 1359, 1155 (SO_2); ^{1}H NMR: δ = 9.55 (d, J = 7.01 Hz, NH), 8.55–8.43, 8.05–7.96, 7.94–7.82 (3 m, 4 ar H), 4.44 (t, J = 7.01 Hz, CH), 3.73 (s, OMe), 2.34–2.07 (m, CH), 1.04 (d, J = 6.9 Hz, Me), 0.99 (d, J = 6.6 Hz, Me); HPLC: k′ = 2.05, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3 : 7).
$C_{13}H_{16}N_2O_4S$ (296.4)

3.4.13. Methyl 2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-2-methylpropionate (**4n**)

From **10** (5.0 g, 24.80 mmol), α-Me-Ala-OMe-HCl (3.80 g, 24.8 mmol), and Et_3N (8.62 ml, 62.0 mmol) in 60 ml dioxan, reflux for 2 h. Yield: 2.75 g (39%); M.p. 235–238 °C (acetone); R_f = 0.45 (AcOEt/PE 8.5 : 1.5); IR: $\tilde{\nu}$ = 3301 (NH), 3100, 3043, 2991, 2951 (CH), 1753 (CO), 1615 (C=N), 1356, 1157 (SO_2); ^{1}H NMR: δ = 9.40 (bs, NH), 8.40–8.30, 8.00–7.95, 7.90–7.80 (3 m, 4 ar H), 3.61 (s, OMe), 1.60 (s, 2 Me); HPLC: k′ = 1.04, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{12}H_{14}N_2O_4S$ (382.3)

3.4.14. Dimethyl (S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)succinate (**4o**)

From **10** (5.0 g, 24.8 mmol), L-Asp(OMe)$_2$-HCl (4.9 g, 24.8 mmol), and Et_3N (6.9 ml, 50 mmol) in 100 ml dioxan, reflux for 2 h. Yield: 3.0 g (37%); M.p. 62 °C (Acetone/MeOH/H_2O); R_f = 0.51 (AcOEt/PE 7 : 3); $[\alpha]_D^{20}$ = −24.16 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3333 (NH), 3098, 2955 (CH), 1741 (CO), 1618 (C=N), 1370, 1164 (SO_2); ^{1}H NMR: δ = 9.81 (bs, NH), 8.27–8.16, 8.07–7.95, 7.92–7.80 (3 m, 4 ar H), 4.99 (bs, CH), 3.71 (d, J = 1.12 Hz, OMe), 3.65 (d, J = 1.12 Hz, OMe), 3.09–2.99 (m, CH_2); HPLC: k′ = 2.17, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O, 2 : 8).
$C_{13}H_{14}N_2O_6S$ (326.3)

3.4.15. Methyl (S)-1-(1,1-dioxobenzo[d]isothiazol-3-yl)pyrrolidine-2-carboxylate (**4p**)

From **10** (10.0 g, 49.58 mmol), L-Pro-OMe-HCl (8.1 g, 49.58 mmol), and Et_3N (13.8 ml, 99.2 mmol) in 200 ml dioxan, reflux for 1.5 h. Yield: 8.5 g (61%); M.p. 185–187 °C (Acetone); R_f = 0.27 (AcOEt/PE 8.5 : 1.5); $[\alpha]_D^{20}$ = −65.55 (c = 0.6, MeOH); IR: $\tilde{\nu}$ = 3462 (OH), 3003, 2959 (CH), 1746 (CO), 1605 (C=N), 1350, 1157 (SO_2); ^{1}H NMR: δ = 8.23-8.14, 8.07–7.98, 7.93–7.78 (3 m, 4 ar H), 4.75 (dd, J = 9.00, 4.00 Hz, CH), 4.30–4.12 (m, CH_2), 3.69 (s, OMe), 2.46–2.25 (m, CH), 2.23–1.95 (m, CH-CH_2); HPLC: k′ = 3.41, t_0 = 1.92 (Chiralcel OJ-R, MeCN/H_2O, 3 : 7).
$C_{13}H_{14}N_2O_4S$ (294.3)

3.5. General procedure for the synthesis of 5 from 9

The thiosaccharinate **9** was refluxed in glacial AcOH (20 ml) for 3–5 d. After cooling to room temperature, the precipitate was separated, washed with H_2O, and dried.

3.5.1. Benzo[d]isothiazol-3-ylamine 1,1-dioxide (**5a**)

a) **3** (0.3 g, 1.2 mmol) in methanolic ammonia (15 ml) was stirred for 0.5 h. Then, the solution was evaporated in vacuo. Yield: 0.11 g (48%). b) From **9a** (0.3 g, 1.38 mmol) see ***3.5***. Yield: 0.05 g (20%). c) From **10** (1.0 g, 4.96 mmol), and 20 ml of methanolic ammonia, see ***3.4***. Yield: 0.84 g (93%); M.p. 307 °C (308 °C, Shapira et al. 1980); R_f: 0.55 (MeOH/CH_2Cl_2 1.5 : 8.5); IR: $\tilde{\nu}$ = 3340, 3282 (NH), 1679 (C=N), 1289, 1164 (SO_2); ^{1}H NMR: δ = 8.96 (bs, NH_2), 8.20–8.11, 8.02–7.93, 7.88–7.78 (3 m, 4 ar H); HPLC: k′ = 0.29, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_7H_6N_2O_2S$ (182.2)

3.5.2. N-(1,1-Dioxobenzo[d]isothiazol-3-yl)aniline (**5b**)

a) From **9b** (0.4 g, 1.36 mmol), 3 d. Yield: 0.12 g (33%). b) From **10** (1.0 g, 4.96 mmol), and aniline (0.46 ml, 4.96 mmol) in 20 ml dioxan, see ***3.4***. Yield: 0.62 g (69%); M.p. 315 °C (315–317 °C, Mannessier 1935); R_f = 0.75 (MeOH/CH_2Cl_2 1.5 : 8.5); IR: $\tilde{\nu}$ = 3326 (NH), 3099, 3058 (CH), 1618 (C=N), 1353, 1155 (SO_2); ^{1}H NMR: δ = 8.54–8.46 (m, 1 ar H), 8.12–8.04 (m, 1 ar H), 7.95–7.82 (m, 4 ar H), 7.55–7.44 (m, 2 ar H), 7.32–7.23 (m, 1 ar H); HPLC: k′ = 1.76, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{13}H_{10}N_2O_2S$ (258.3)

3.5.3. N-(1,1-Dioxobenzo[d]isothiazol-3-yl)benzylamine (**5c**)

From **9c** (0.3 g, 0.98 mmol), 3 d. Yield: 0.11 g (41%); M.p. 202 °C; R_f = 0.4 (CH_2Cl_2); IR: $\tilde{\nu}$ = 3312 (NH), 3091, 3052 (CH), 1623 (C=N), 1350, 1151 (SO_2); ^{1}H NMR: δ = 8.27–8.19, 8.02–7.94, 7.88–7.79 (3 m, 4 ar H), 7.43–7.29 [m, 5 ar H (Phe)], 4.70 (s, CH_2); HPLC: k′ = 1.89, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{14}H_{12}N_2O_2S$ (272.3)

3.5.4. N-(1,1-Dioxobenzo[d]isothiazol-3-yl)cyclohexylamine (**5d**)

From **9d** (0.4 g, 1.34 mmol), 3 d. Yield: 0.09 g (25%); M.p. 252 °C; R_f = 0.56 (CH_2Cl_2); IR: $\tilde{\nu}$ = 3299 (NH), 3107, 3047, 2938 (CH), 1618 (C=N), 1350, 1154 (SO_2); ^{1}H NMR: δ = 9.15 (d, J = 7.63 Hz, NH), 8.23–8.20, 8.00–7.91, 7.86–7.77 (3 m, 4 ar H), 3.93–3.73, 2.04–1.91, 1.83–1.57, 1.48–1.11 (4 m, 11 $H_{cyclohexyl}$); HPLC: k′ = 2.55, t_0 = 1.85 (RP-18, MeCN/H_2O, 1 : 1).
$C_{13}H_{16}N_2O_2S$ (364.4)

3.5.5. N-(1,1-Dioxobenzo[d]isothiazol-3-yl)isobutylamine (**5e**)

From **9e** (0.3 g, 1.09 mmol), 5 d. Yield: 0.08 g (31%); M.p. 216–217 °C; R_f = 0.40 (CH_2Cl_2); IR: $\tilde{\nu}$ = 3314 (NH), 3045, 2960 (CH), 1621 (C=N), 1351, 1152 (SO_2); ^{1}H NMR: δ = 9.42 (bs, NH), 8.30–8.18, 8.02–7.92, 7.88–7.78 (3 m, 4 ar H), 3.31 (t, J = 7.02 Hz, CH_2), 2.09–1.92 (m, CH), 0.95 (d, J = 6.62 Hz, 2 Me); HPLC: k′ = 0.88, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{11}H_{14}N_2O_2S$ (238.3)

3.5.6. 2-Chloro-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine (**5f**)

From **10** (2.0 g, 9.90 mmol), and 2-chlorobenzylamine (1.4 g, 9.90 mmol) in 30 ml dioxan, see 3.4. Yield: 1.68 g (55%); M.p. 244 °C (acetone); R_f = 0.577 (MeOH/CH_2Cl_2 1.5 : 8.5); IR: $\tilde{\nu}$ = 3300 (NH), 3062, 2926 (CH), 1622 (C=N), 1345, 1158 (SO_2); ^{1}H NMR: δ = 9.88 (s, NH), 8.32–8.21, 8.06–7.95, 7.91–7.80 (3 m, 4 ar H), 7.58–7.45, 7.43–7.33 (2 m, 4 ar H), 4.77 (s, CH_2); HPLC: k′ = 2.35, t_0 = 1.85 (RP-18, MeCN/H_2O, 1 : 1).
$C_{14}H_{11}ClN_2O_2S$ (306.8)

3.5.7. 4-Chloro-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine (**5g**)

From **10** (2.0 g, 9.90 mmol), and 4-chlorobenzylamine (1.4 g, 9.90 mmol) in 30 ml dioxan, see 3.4. Yield: 2.45 g (81%); M.p. 256 °C (acetone); R_f = 0.70 (MeOH/CH_2Cl_2 1.5 : 8.5); IR: $\tilde{\nu}$ = 3302 (NH), 3064, 2917 (CH), 1623 (C=N), 1349, 1153 (SO_2); ^{1}H NMR: δ = 9.93 (bs, NH), 8.26–8.16, 8.02–7.94, 7.89–7.80 (3 m, 4 ar H), 7.50–7.38 (m, 4 ar H), 4.69 (s, CH_2); HPLC: k′ = 2.73, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{14}H_{11}ClN_2O_2S$ (306.8)

3.5.8. 3,4-Dimethoxy-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine (**5h**)

From **10** (2.0 g, 9.90 mmol), and 3,4-dimethoxybenzylamine (1.66 g, 9.90 mmol) in 30 ml dioxan, see 3.4. Yield: 1.23 g (37%); M.p. 301–303 °C; R_f = 0.62 (MeOH/CH_2Cl_2 1.5 : 8.5); IR: $\tilde{\nu}$ = 3341 (NH), 3103, 2959, 2939 (CH), 1622 (C=N), 1359, 1155 (SO_2); ^{1}H NMR: δ = 9.86 (t,

J = 5.70 Hz, NH), 8.27–8.18, 8.04–7.95, 7.88–7.77 (3 m, 4 ar H), 7.07 (d, J = 1.17 Hz, 1 ar H), 6.98–6.88 (m, 2 ar H), 4.62 (d, J = 5.70 Hz, CH_2), 3.75, 3.74 (2s, 2 OMe); HPLC: k′ = 1.15, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{16}H_{16}N_2O_4S$ (332.4)

3.5.9. 2-Methoxy-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine (**5i**)

From **10** (2.0 g, 9.90 mmol), and 2-methoxybenzylamine (1.36 g, 9.90 mmol) in 30 ml dioxan, see ***3.4.*** Yield: 2.0 g (67%); M.p. 219–221 °C (acetone); R_f = 0.70 (MeOH/CH_2Cl_2 1.5 : 8.5); IR: $\tilde{\nu}$ = 3328 (NH), 3108, 2927 (CH), 1622 (C=N), 1366, 1155 (SO_2); ^{1}H NMR: δ = 9.77 (t, J = 5.41 Hz, NH), 8.33–8.24, 8.05–7.94, 7.89–7.77 (3 m, 4 ar H), 7.38–2.26, 7.09–6.90 (2 m, 4 ar H), 4.66 (d, J = 5.41 Hz, CH_2), 3.85 (s, OMe); HPLC: k′ = 2.01, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{15}H_{14}N_2O_3S$ (302.4)

3.5.10. 3-(Trifluoromethyl)-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine (**5k**)

From **10** (2.0 g, 9.90 mmol), and 3-(trifluoromethyl)benzylamine (1.74 g, 9.90 mmol) in 30 ml dioxan, see ***3.4.*** Yield: 2.86 g (85%); R_f = 0.78 (MeOH/CH_2Cl_2 1 : 9); M.p. 254-256 °C (Acetone); IR: $\tilde{\nu}$ = 3343 (NH), 3103, 2944 (CH), 1621 (C=N), 1348, 1160 (SO_2); ^{1}H NMR: δ = 10.00 (bs, NH), 8.28–8.18, 8.05–7.97 (2 m, 2 ar H), 7.91–7.78 (m, 3 ar H), 7.78–7.58 (m, 3 ar H), 4.83 (s, CH_2); HPLC: k′ = 3.41 t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{15}H_{11}F_3N_2O_2S$ (340.3)

3.5.11. N-(1,1-Dioxobenzo[d]isothiazol-3-yl)-2-pyridylmethylamine hydrochloride (**5l**)

From **10** (2.0 g, 9.90 mmol), and 2-(aminomethyl)pyridine (1.07 g, 9.90 mmol) in 30 ml dioxan, see ***3.4.*** Yield: 1.53 g (50%); M.p. 260 °C (dec., acetone); R_f = 0.55 (MeOH/CH_2Cl_2 1.5 : 8.5); IR: $\tilde{\nu}$ = 3440 (NH), 3097, 3050, 2995 (CH), 1626 (C=N), 1354, 1163 (SO_2); ^{1}H NMR: δ = 10.59 (t, J = 5.70 Hz, NH), 8.84–8.75, 8.46–8.26, 8.03–7.94 (3 m, 4 ar H), 7.92–7.72 (m, 4 ar H), 6.60 (bs, HCl), 4.99 (d, J = 5.70 Hz, CH_2); HPLC: k′ = 0.73 t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_{13}H_{12}ClN_3O_2S$ (309.8)

3.6. (1,2-Dihydro-3-oxobenzo[d]isothiazol-2-yl)acetonitrile 1,1-dioxide (**6**)

Chloroacetonitrile (17 ml, 260 mmol) was added to a solution of saccharin sodium (20.0 g, 98 mmol) in DMF (50 ml), and the mixture was heated at 100 °C for 4 h, then it was poured into cold water, and the precipitate was separated. Yield: 17.0 g (79%); M.p. 138–140 °C (AcOEt, 140 °C, Shapira et al. 1993); R_f = 0.45 (AcOEt/PE 4 : 6); IR: $\tilde{\nu}$ = 3088, 2996 (CH), 2257(CN), 1746 (CO), 1338, 1170 (SO_2); ^{1}H NMR: δ = 8.43–8.37, 8.22–8.00 (2 m, 4 ar H), 5.10 (s, CH_2); HPLC: k′ = 1.43, t_0 = 1.76 (RP-18, MeCN/H_2O, 1 : 1).
$C_9H_6N_2O_3S$ (222.2)

3.7. 1,2-Dihydro-3-thioxobenzo[d]isothiazole-2-thioacetamide 1,1-dioxide (**7**)

The mixture from P_2S_5 (2 g, 9 mmol), and a solution of **6** (1.0 g, 4.50 mmol) in dioxan (20 ml) was refluxed for 4 h, cooled and filtered. The filtrate was concentrated to dryness, and extracted with AcOEt. The extract was concentrated *in vacuo*, and the residue was purified by CC (AcOEt/PE 0.5 : 9.5 with increasing polarity). Yield: 0.3 g (24%); yellow solid; M.p. 190–192 °C; R_f = 0.6 (AcOEt/PE 4 : 6); IR: $\tilde{\nu}$ = 3372, 3299 (NH), 3148 (CH), 1637 (C=N), 1328, 1186 (SO_2), 1138 (C=S); ^{1}H NMR: δ = 9.93 (s, NH), 9.95 (s, NH), 8.36–8.22, 8.14–7.96 (2 m, 4 ar H), 4.95 (s, CH_2); ^{13}C NMR: δ = 196.42 (C=S), 186.95 (C=S), 135.86, 135.76, 130.96, 130.44, 126.96, 121.82 (ar C), 50.39 (CH_2): DEPT: 135.86, 135.76, 126.96, 121.82 (ar CH), 50.39 (CH_2); HPLC: k′ = 1.75, t_0 = 1.77 (RP-18, MeCN/H_2O, 1 : 1).
$C_9H_8N_2O_2S_3$ (272.4)

3.8. 3-(Ethylsulfanyl)benzo[d]isothiazol 1,1-dioxide (**8**)

From **2** (1.0 g, 5.02 mmol), EtBr (0.53 ml, 7.03 mmol), and NaH (0.3 g, 7.5 mmol), as described for **3**. Yield: 0.3 g (26%); M.p. 183 °C (EtOH, 182.5–183 °C Meadow and Cavagnol 1951); R_f = 0.92 (AcOEt); IR: $\tilde{\nu}$ = 3085, 2987 (CH), 1616 (C=N), 1321, 1161 (SO_2); ^{1}H NMR ($CDCl_3$): δ = 7.90–7.62 (2 m, 4 ar H), 3.38 (quartet, J = 7.6 Hz, CH_2), 1.51 (t, J = 7.5 Hz, Me); HPLC: k′ = 2.84, t_0 = 1.77 (RP-18, MeCN/H_2O 1 : 1).
$C_9H_9NO_2S_2$ (227.3)

3.9. General procedure for the synthesis of thiosaccharinates (**9**)

Compound **2** (0.7 g, 3.51 mmol), and the equivalent amount of the amine in MeOH (20 ml) were stirred at room temperature for 2 h. Then, the solvent was removed *in vacuo*, and the residue was crystallized as noted.

3.9.1. Ammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id (**9a**)

With 1 ml saturated solution of ammonia in MeOH. Yield: 0.58 g (76%); M.p. 209–211 °C (AcOEt/MeOH); R_f = 0.36 (MeOH/CH_2Cl_2 2 : 8); IR: $\tilde{\nu}$ = 3426 (NH), 1339, 1153 (SO_2), 1110 (C=S); ^{1}H NMR (D_2O): δ = 8.13–7.92, 7.26–7.57 (2 m, 4 ar H); HPLC: k′ = 0.92, t_0 = 2.00 (RP-18, MeCN/0.02m KH_2PO_4, 2 : 8, pH: 2.72).
$C_7H_8N_2O_2S_2$ (216.3)

3.9.2. Anilinium 1,1-dioxobenz[d]isothiazol-3-thion-2-id (**9b**)

From aniline (0.37 ml, 3.51 mmol). Yield: 0.80 g (78%); M.p. 305–308 °C (AcOEt, 290–300 °C, Mannessier 1935); R_f = 0.28 (MeOH/CH_2Cl_2 2 : 8); IR: $\tilde{\nu}$ = 3433 (NH), 1336, 1142, (SO_2), 1116 (C=S); ^{1}H NMR (D_2O): δ = 8.12-8.02, 7.90–7.73, 7.50–7.36, 7.32–7.15 (4 m, 9 ar H); HPLC: k′ = 0.89, t_0 = 2.00 (RP-18, MeCN/0.02m KH_2PO_4, 2 : 8, pH: 2.72).
$C_{13}H_{12}N_2O_2S_2$ (292.4)

3.9.3. Benzylammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id (**9c**)

From benzylamine (0.38 g, 3.51 mmol). Yield: 0.88 g (82%); M.p. 178 °C (AcOEt); R_f = 0.48 (MeOH/CH_2Cl_2 1 : 9); IR: $\tilde{\nu}$ = 3432 (NH), 3046 (CH), 1339, 1157, (SO_2), 1119 (C=S); ^{1}H NMR (D_2O): δ = 8.12–8.00, 7.90–7.72 (2 m, 4 ar H), 7.51–7.40 [m, 5 ar H (Phe)], 4.21–4.13 (m, CH_2); HPLC: k′ = 0.89, t_0 = 2.00 (RP-18, MeCN/0.02m KH_2PO_4, 2 : 8, pH: 2.72).
$C_{14}H_{14}N_2O_2S_2$ (306.4)

3.9.4. Cyclohexylammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id (**9d**)

From cyclohexylamine (0.35 g, 3.51 mmol). Yield: 0.78 g (74%); M.p. 172 °C (AcOEt); R_f = 0.60 (MeOH/CH_2Cl_2 1 : 9); IR: $\tilde{\nu}$ = 3432 (NH), 3010, 2938 (CH), 1339, 1145, (SO_2), 1118 C=S); ^{1}H NMR (D_2O): δ = 8.13–8.02, 7.90–7.73 (2 m, 4 ar H), 3.23–3.03, 2.07–1.90, 1.84–1.69, 1.68–1.54, 1.46–1.06 (5 m, CH, 5 CH_2); HPLC: k′ = 0.90, t_0 = 2.00 (RP-18, MeCN/0.02m KH_2PO_4, 2 : 8, pH: 2.72).
$C_{13}H_{18}N_2O_2S_2$ (298.4)

3.9.5. Isobutylammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id (**9e**)

From isobutylamine (0.26 g, 3.51 mmol). Yield: 0.77 g (79%); M.p. 212 °C (AcOEt); R_f = 0.60 (MeOH/CH_2Cl_2 1 : 9); IR: $\tilde{\nu}$ = 3432 (NH), 3055, 2964 (CH), 1352, 1142, (SO_2), 1117 (C=S); ^{1}H NMR (D_2O): δ = 8.13–8.05, 7.90–7.77 (2 m, 4 ar H), 2.85 (d, J = 6.97 Hz, CH_2), 1.94 (heptet, J = 6.97 Hz, CH), 0.94 (d, J = 6.97 Hz, 2 Me); HPLC: k′ = 0.78, t_0 = 2.00 (RP-18, MeCN/0.02m KH_2PO_4, 2 : 8, pH: 2.72).
$C_{11}H_{16}N_2O_2S_2$ (272.4)

3.10. 3-Chlorobenzo[d]isothiazol 1,1-dioxide (**10**)

From saccharin (**1**) (50.0 g, 272.3 mmol), $SOCl_2$, and DMF in dioxan (200 ml) as described (Wade et al. 1979). Yield: 42.0 g (76%); M.p. 143–147 °C (Toluene, 140–145 °C Wade et al. 1979); R_f = 0.63 (AcOEt/PE 4 : 6); IR: $\tilde{\nu}$ = 1605 (C=N), 1346, 1175 (SO_2); ^{1}H NMR ($CDCl_3$): δ = 8.15–7.85 (m, 4 ar H); HPLC: k′ = 2.06, t_0 = 2.00 (RP-18, MeCN/H_2O, 1 : 1).
$C_7H_4ClNO_2S$ (201.6)

3.11. General procedure for the synthesis of acid derivatives (**12**)

With stirring and cooled with an ice-water bath, an 1N aq. NaOH solution (except for **12p**) was added dropwise to a solution of the ester in acetone over 5 min. Then the mixture was left without stirring for 6 h, acetone was removed under reduced pressure, the remaining aqueous layer was cooled to 0 °C, and acidified with 1N HCl to pH = 1–2. The precipitate was separated, washed with cold H_2O, recrystallized, and dried over NaOH pellets.

3.11.1. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-4-methylsulfanyl-butyric acid (**12c**)

From **4c** (3.5 g, 10.67 mmol) in 80 ml acetone, and 26.63 ml 1N NaOH. Yield: 2.48 g (67%); M.p. 162 °C (Acetone/H_2O); R_f = 0.50 (MeOH/CH_2Cl_2 2.5 : 7.5); $[\alpha]_D^{20}$ = −29.6 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3304 (NH, OH), 3112, 2918 (CH), 1722 (CO), 1614 (C=N), 1363, 1154 (SO_2); ^{1}H NMR: δ = 9.58 (d, J = 7.30 Hz, NH), 8.35–8.27, 8.02–7.95, 7.89–7.82 (3 m, 4 ar H), 4.64 (quartet, J = 7.30 Hz, CH), 2.70–2.55 (m, CH_2), 2.24–2.10 (m, CH_2), 2.07 (s, SMe); HPLC: k′ = 0.38, t_0 = 2.34 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.95).
$C_{12}H_{14}N_2O_4S_2$ (314.4)

3.11.2. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentanoic acid (**12d**)

From **4d** (3.0 g, 9.67 mmol) in 80 ml acetone, and 25 ml 1N NaOH. Yield: 1.5 g (52%); M.p. 229 °C (AcOEt/PE); R_f = 0.62 (MeOH/CH_2Cl_2

2.5 : 7.5); $[\alpha]_D^{20} = -14.00$ (c = 1, Dioxan); IR: $\tilde{\nu} = 3300$ (NH, OH), 3103, 2961 (CH), 1731 (CO), 1614 (C=N), 1365, 1154 (SO_2); ^{1}H NMR: δ = 9.56 (d, J = 7.77 Hz, NH), 8.37–8.26, 8.02–7.94, 7.90–7.81 (3m, 4 ar H), 4.61-4.45 (m, CH), 1.98-1.63 (m, CH_2–CH), 0.94 (d, J = 5.8 Hz, Me), 0.91 (d, J = 5.8 Hz, Me); HPLC: k′ = 0.74, t_0 = 2.34 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.95)
$C_{13}H_{16}N_2O_4S$ (296.4)

3.11.3. 1,1-Dioxobenzo[d]isothiazol-3-ylaminoacetic acid (**12h**)

From **4h** (4.0 g, 15.74 mmol) in 100 ml acetone, and 39.38 ml 1N NaOH. Yield: 1.50 g (40%); M.p. 255–257 °C (MeOH/H_2O; 259–260 °C Wade et al. 1979); $R_f = 0.20$ (MeOH/CH_2Cl_2 2.5 : 7.5); IR: $\tilde{\nu}$ = 3289 (NH, OH), 3109, 3061, 2937 (CH), 1705 (CO), 1620 (C=N), 1361, 1156 (SO_2); ^{1}H NMR: δ = 13.03 (bs, CO_2H), 9.84 (t, J = 6.00 Hz, NH), 8.25–8.17, 8.03–7.94, 7.91–7.80 (3 m, 4 ar H), 4.21 (d, J = 6.00 Hz, CH_2); HPLC: k′ = 0.39, t_0 = 1.92 (RP-18, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.88).
$C_9H_8N_2O_4S$ (240.2)

3.11.4. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-phenylpropionic acid (**12i**)

From **4i** (9.1 g, 26.42 mmol) in 200 ml acetone, and 67 ml 1N NaOH. Yield: 5.61 g (64%); M.p. 234 °C (AcOEt); $R_f = 0.53$ (MeOH/CH_2Cl_2 2.5 : 7.5); $[\alpha]_D^{20} = -32.33$ (c = 2, acetone); IR: $\tilde{\nu}$ = 3327 (NH, OH), 3080, 2945 (CH), 1733 (CO), 1617 (C=N), 1360, 1151 (SO_2); ^{1}H NMR: δ = 13.25 (bs, CO_2H), 9.70 (d, J = 7.95 Hz, NH), 8.31–8.21, 8.00–7.90), 7.88–7.78 (3 m, 4 ar H), 7.38–7.14 [m, 5 ar H (Phe)], 4.81–4.65 (m, CH), 3.30 (dd, J = 13.8, 4.8 Hz, 1 H, CH_2), 3.18 (dd, J = 13.8, 10.4 Hz, 1 H, CH_2); HPLC: k′ = 0.93, t_0 = 2.34 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.95)
$C_{16}H_{14}N_2O_4S$ (330.4)

3.11.5. (2S,3S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanoic acid (**12k**)

From **4k** (4.0 g, 12.90 mmol) in 100 ml acetone, and 33 ml 1N NaOH. Yield: 2.92 g (76%); M.p. 211 °C (AcOEt/PE); $R_f = 0.62$ (MeOH/CH_2Cl_2 2.5 : 7.5); $[\alpha]_D^{20} = +11.5$ (c = 2, Acetone); IR: $\tilde{\nu}$ = 3280 (NH, OH), 3109, 2965 (CH), 1719 (CO), 1619 (C=N), 1361, 1157 (SO_2); ^{1}H NMR: δ = 9.43 (d, J = 7.77 Hz, NH), 8.53–8.44, 8.02–7.94, 7.90–7.80 (3 m, 4 ar H), 4.42 (t, J = 7.77 Hz, CH), 2.15–1.96 (m, CH), 1.64–1.44 (m, CH), 1.41–1.16 (m, CH), 0.98 (d, J = 6.71 Hz, Me), 0.89 (t, J = 7.24 Hz, Me); HPLC: k′ = 0.72, t_0 = 2.34 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.95)
$C_{13}H_{16}N_2O_4S$ (296.4)

3.11.6. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)propionic acid (**12l**)

From **4l** (5.0 g, 18.65 mmol) in 200 ml acetone, and 47 ml 1N NaOH. Yield: 2.90 g (61%); M.p. 277 °C (dec.); $R_f = 0.23$ (MeOH/CH_2Cl_2 2.5 : 7.5); $[\alpha]_D^{20} = -3.00$ (c = 2, DMSO); IR: $\tilde{\nu}$ = 3321 (NH, OH), 3100 (CH), 1760 (CO), 1614 (C=N), 1359, 1161 (SO_2); ^{1}H NMR: δ = 9.61 (d, J = 7.24, NH), 8.37–8.25, 8.02–7.94, 7.90–7.79 (3 m, 4 ar H), 4.76–4.48 (quintet, J = 7.24 Hz, CH), 1.52 (d, J = 7.24 Hz, Me); HPLC: k′ = 0.16, t_0 = 1.87 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.88).
$C_{10}H_{10}N_2O_4S$ (254.3)

3.11.7. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylbutyric acid (**12m**)

From **4m** (18.0 g, 54.87 mmol) in 200 ml acetone, and 137 ml 1N NaOH, 2 d. Yield: 6.0 g (35%); M.p. 238 °C (AcOEt/PE); $R_f = 0.62$ (MeOH/CH_2Cl_2 2.5 : 7.5); $[\alpha]_D^{20} = -16.16$ (c = 2, DMSO); IR: $\tilde{\nu}$ = 3318 (NH, OH), 3095, 2970, 2934 (CH), 1745 (CO), 1611 (C=N), 1357, 1157 (SO_2); ^{1}H NMR: δ = 9.41 (d, J = 7.95 Hz, NH), 8.54–8.46, 8.02–7.91, 7.90–7.81 (3 m, 4 ar H), 4.37 (t, J = 7.95 Hz, CH), 2.38–2.20 (m, CH), 1.04 (d, J = 6.70 Hz, Me), 1.01 (d, J = 6.70 Hz, Me); HPLC: k′ = 0.52, t_0 = 2.57 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.85).
$C_{12}H_{14}N_2O_4S$ (282.3)

3.11.8. 2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-2-methylpropionic acid (**12n**)

From **4n** (1.7 g, 6.02 mmol) in 35 ml acetone, and 15.0 ml 1N NaOH for 18 h. Yield: 0.85 g (53%); M.p. 279 °C; $R_f = 0.47$ (MeOH/CH_2Cl_2 2.5 : 7.5); IR: $\tilde{\nu}$ = 3281 (NH, OH), 3104, 3056, 2994, 2942 (CH), 1722 (CO), 1613 (C=N), 1356, 1156 (SO_2); ^{1}H NMR: δ = 9.18 (s, NH), 8.40-8.32, 8.00–7.93, 7.87–7.80 (3 m, 4 ar H), 1.60 (s, 2 Me); HPLC: k′ = 0.56, t_0 = 1.77 (RP-18, MeCN/0.02m KH_2PO_4, 3 : 7, pH: 2.95).
$C_{11}H_{12}N_2O_4S$ (268.3)

3.11.9. (S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)succinic acid (**12o**)

From **4o** (2.0 g, 6.12 mmol) in 200 ml acetone, and 30 ml 1N NaOH. Yield: 0.75 g (41%); M.p. 215 °C; $R_f = 0.84$ (MeOH/H_2O 1 : 1, Reverse Phase TLC); $[\alpha]_D^{20} = -31.00$ (c = 2, DMSO); IR: $\tilde{\nu}$ = 3372 (NH, OH), 3093, 2954 (CH), 1710 (CO), 1620 (C=N), 1363, 1151 (SO_2); ^{1}H NMR: δ = 9.69 (d, J = 7.92 Hz, NH), 8.28–8.19, 8.03–7.96, 7.90–7.18 (3 m, 4 ar H), 4.83 (td, J = 5.64, 7.92 Hz, CH), 3.05–2.78 (m, CH_2); HPLC: k′ = 0.52, t_0 = 2.57 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.85).
$C_{11}H_{10}N_2O_6S$ (298.3)

3.11.10. (S)-1-(1,1-Dioxobenzo[d]isothiazol-3-yl)pyrrolidine-2-carboxylic acid (**12p**)

Compound **4p** (2.0 g, 6.79 mmol) in 40 ml 5% HCl was refluxed for 8 h. After filtration the filtrate was left for 2 h, then the resulted solid crystals were separated, washed with a few ml H_2O, and dried. Yield: 0.64 g (34%); M.p. 253 °C; $R_f = 0.32$ (MeOH/CH_2Cl_2 2.5 : 7.5); $[\alpha]_D^{20} = -85.00$ (c = 2, DMSO); IR: $\tilde{\nu}$ = 3452 (NH, OH) 3006, 2988, 2962, 2889 (CH), 1741 (CO), 1606 (C=N), 1334, 1154 (SO_2); ^{1}H NMR: δ = 12.96 (bs, OH), 8.22–8.13, 8.06–7.98, 7.91–7.72 (3 m, 4 ar H), 4.65 (dd, J = 8.83, 3.89 Hz, CH), 4.29–4.09 (m, CH_2), 2.38–2.23 (m, CH), 2.20–1.95 (m, CH–CH_2); HPLC: k′ = 0.22, t_0 = 1.87 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.88).
$C_{12}H_{12}N_2O_4S$ (280.3)

3.12. Compounds (**13**)

3.12.1. Ethyl (Z)-(2-aminothiazol-4-yl)-α-methoxyiminoacetate (**13b**)

^{1}H NMR: δ = 7.25 (bs, NH_2), 6.90 (s, 5-$H_{thiazol}$), 4.26 (quartet, J = 7.14 Hz, CH_2), 3.87 (s, OMe), 1.26 (t, J = 7.14 Hz, Me); ^{13}C NMR: δ = 169.24 (C=N), 162.86 (CO), 147.38 (C-2′), 141.56 (C-4′), 109.02 (C-5′), 62.95 (OMe), 61.92 (CH_2), 14.43 (Me); HPLC: k′ = 1.07, t_0 = 1.81 (RP-18, MeCN/H_2O, 1 : 1).

3.12.2. Ethyl (EZ)-(2-aminothiazol-4-yl)-α-methoxyiminoacetate (**13b**)

Compound **13** (1.0 g) in dioxan was irradiated with UV light (λ = 254 nm) for 2 days; Yield: 1.0 g (100%); M.p.128-132 °C; $R_f = 0.48$ (MeOH/CH_2Cl_2 0.2 : 9.8); IR: $\tilde{\nu}$ = 3442 (NH), 3129, 2984, 2938 (CH), 1730 (CO), 1619 (C=N); ^{1}H NMR: δ = 7.50, 6.90 (2s, 5-$H_{thiazol}$), 7.25 (bs, NH_2), 4.28, 4.24 (2quartet, J = 7.14 Hz, CH_2), 3.98, 3.87 (2 s, OMe); ^{13}C NMR: δ = 169.24 (C=N), 167.30, 162.86 (CO), 147.37 (C-2′), 141.56 (C-4′), 116.62, 109.02 (C-5′), 63.55, 62.95 (OMe), 61.92 (OCH_2), 14.42 (Me); E : Z ≈ 2 : 8; HPLC: k′ = 1.06 and 1.40, t_0 = 1.81 (RP-18, MeCN/H_2O, 1 : 1).

3.13. Ethyl [2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]acetate (**14**)

From **10** (3.0 g, 14.88 mmol), and ethyl (2-aminothiazol-4-yl)acetate (2.77 g, 14.88 mmol) in 70 ml dioxan, reflux for 2 h, see 3.4. Yield: 2.6 g (50%); M.p. 214 °C (H_2O); $R_f = 0.26$ (MeOH/CH_2Cl_2 2.5 : 7.5); IR: $\tilde{\nu}$ = 3330 (NH), 3100, 2970, 2932 (CH), 1727 (CO), 1613 (C=N), 1350, 1157 (SO_2); ^{1}H NMR: δ = 8.27 (bs, NH), 8.10–8.00, 7.91–7.80 (2 m, 4 ar H), 7.30 (s, 5-$H_{thiazol}$), 4.13 (quartet, J = 7.1 Hz, CH_2), 3.83 (s, CH_2), 1.25 (t, J = 7.1 Hz, Me); HPLC: k′ = 3.90, t_0 = 1.85 (RP-18, MeCN/0.02m KH_2PO_4, 3 : 7, pH: 2.95).
$C_{14}H_{13}N_3O_4S_2$ (351.4)

3.14. Ethyl (EZ)-[2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]-α-methoxyiminoacetate (**15**)

From **10** (3.0 g, 14.88 mmol), and ethyl *syn*-(2-aminothiazol-4-yl)-α-methoxyiminoacetate (3.42 g, 14.88 mmol) in 100 ml dioxan, reflux for 2 h, see 3.4. Yield: 3.5 g (60%); M.p. 210–212 °C (acetone/H_2O); $R_f = 0.71$ (MeOH/CH_2Cl_2 2.5 : 7.5); IR: $\tilde{\nu}$ = 3477 (NH), 2983, 2941 (CH), 1727 (CO), 1614 (C=N), 1369, 1160 (SO_2); ^{1}H NMR: δ = 8.64–8.54 (m, 1 ar H), 8.27, 7.83 (2s, 5-$H_{thiazol}$), 8.18–8.08 (m, 1 ar H), 7.95–7.88 (m, 2 ar H), 4.37, 4.32 (2quartet, J = 7.1 Hz, CH_2), 4.02, 3.96 (2s, OMe); E : Z ≈ 7 : 3; HPLC: k′ = 8.64 and 10.64, t_0 = 2.03 (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH = 2.88).
$C_{15}H_{14}N_4O_5S_2$ (394.4)

3.15. [2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]acetic acid (**16**)

From **14** (0.5 g, 1.42 mmol) in 20 ml acetone, and 2.9 ml 1N NaOH, see 3.11. Yield: 0.40 g (87%); M.p. 294 °C (dec., AcOEt/MeOH/PE); $R_f = 0.23$ (MeOH/CH_2Cl_2 2.5 : 7.5); IR: $\tilde{\nu}$ = 3440 (NH, OH), 3129, 2920 (CH), 1715 (CO), 1613 (C=N), 1387, 1162 (SO_2); ^{1}H NMR: δ = 8.26-8.15, 8.00–7.98, 7.88–7.93 (3 m, 4 ar H), 7.25 (s, 5-$H_{thiazol}$), 3.79 (s, CH_2); HPLC: k' = 1.42, t_0 = 1.85 (RP-18, MeCN/0.02m KH_2PO_4, 3 : 7, pH: 2.85).
$C_{12}H_9N_3O_4S_2$ (323.4)

3.16. (EZ)-[2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]-α-methoxyiminoacetic acid (**17**)

From **15** (0.3 g, 0.76 mmol) in 20 ml acetone, and 1.9 ml 1 N NaOH, see 3.11. Yield: 0.25 g (90%); M.p. 238 °C; $R_f = 0.39$ (MeOH/CH_2Cl_2

2.5 : 7.5); IR: $\tilde{\nu} = 3453$ (NH, OH), 2947 (CH), 1708 (CO), 1600 (C=N), 1368, 1158 (SO_2); ^{1}H NMR: $\delta = 8.65–8.55$ (m, 1 ar H), 8.25, 7.77 (2 s, 5-$H_{thiazol}$), 8.16–8.07, 7.95–7.85 (2 m, 3 ar H), 4.07, 3.95 (2 s, OMe); E : Z ≈ 8.5 : 1.5; HPLC: $k' = -0.02$ and 0.12, $t_0 = 1.92$ (Chiralcel OJ-R, MeCN/0.01m KH_2PO_4, 2 : 8, pH: 2.88).
$C_{13}H_{10}N_4O_5S_2$ (366.4)

3.17. Elastase inhibition studies

To a thermostatted solution of 850 μL 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.5, was added DMSO (100 μL), Human leukocyte elastase (100 μL solution in 0.05 M sodium acetate buffer containing 0.4 M NaCl, pH 5.5, for a final enzyme concentration of 0.034 μM) and, finally, 50 μL methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide in DMSO for a final substrate concentration of 1 mM. The rate of substrate hydrolysis was determined by monitoring the absorption at 410 nm for 5 min. The experiment was repeated in the presence of varying amounts of the inhibitors **4k** and **4m** (final inhibitor concentration 0.125, 0.25, 0.5 mM) at a constant final concentration of DMSO (10%) and the rates of substrate hydrolysis were determined.
The series of experiments was repeated at two additional substrate concentrations (final substrate concentrations: 0.5 and 0.3 mM). All rates were determined in triplicate. The inverse of the average velocities was plotted against the final inhibitor concentration and $\mathbf{K_i}$ determined from the intersection of three lines (each $R^2 > 0.98$).

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