Synthesis of 2-Phenylisothiazol-3(2*H*)-one 1,1-Dioxides: Inhibitors of Human Leukocyte Elastase

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Z. Naturforsch. **58b,** 111 – 120 (2003); received September 25, 2002

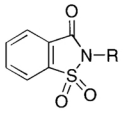
Professor Dr. P. Welzel on the occasion of his 65th birthday

A series of 2-phenylisothiazol-3(2*H*)-one 1,1-dioxides **14a – q** were synthesized by oxidation of isothiazolium perchlorates **12**. The inhibition of the serine proteases cathepsin G, chymotrypsin and human leukocyte elastase (HLE) by **14** was investigated. Some 4,5-diphenyl substituted derivatives (**14i – k**) were found to inhibit HLE in a time-dependent manner and exhibited k_{obs}/[I] values > 500 $M^{-1}s^{-1}$. **14k** (k_{obs}/[I] = 2400 $M^{-1}s^{-1}$), was the most potent HLE inhibitor of this series. Kinetic investigations led to the conclusion that 2-phenylisothiazol-3(2*H*)-one 1,1-dioxides interact with HLE at the active site as well as at another binding site, resulting in a complex type of inhibition.

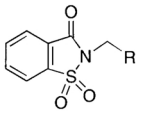
Key words: Sultams, Human Leukocyte Elastase, Enzyme Inhibition

Introduction

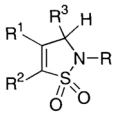
Isothiazole derivatives are known to exhibit a broad range of biological activities [1 – 3]. The ability of cyclic sulfonamides to serve as key functional groups in the development of pharmaceutically active compounds and fungicides has been described especially for the class of N-substituted 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxides **1 – 7** [1,2,4]. Series of saccharin **(1)** derivatives have been synthesized and evaluated for their inhibitory activity toward serine proteases such as human leukocyte elastase (HLE) and cathepsin G. Examples include N-acyl-**(2)** [5], N-aryl-(**3**) [6], N-triazolomethylsaccharins **(4)** [7], as well as 2-saccharinylmethyl carboxylates and sulfones **(5)** [8,9], 2,6-dichlorobenzoates **(6)** [10 – 16], phosphonates [17], phenylmercaptotetrazole [10 – 12] and O-heterocyclyl derivatives **7** [18]. Whereas **2** and **3** were shown to act by acylating the serine proteases, incorporation of a leaving group as present in **4 – 7** resulted in a new type of mechanism-based inhibitors. Nucleophilic attack of the active-site serine at C-3, ring opening and expulsion of the leaving group produce a reactive intermediate with a —N=CH_2 function which can further react by cross-linking the enzyme's active site [10,11,14]. Highly potent derivatives of **6** have a lipophilic substituent (*i*-Pr) at position 4 and a 6-alkoxy group; the isopropyl moiety is proposed to interact with the S_1 specificity pocket of serine proteases [12 – 14]. An efficient solid-phase synthesis of benzisothiazolone 1,1-dioxide-based serine protease inhibitors involving alkylation of carboxylic acids with N-(bromomethyl)benzisothiazolone 1,1-dioxide has been developed [19]. The first monocyclic 2,3-dihydroisothiazole 1,1-dioxides **8** (R^1 = NH_2) with anti-HIV-1 activity have recently been synthesized [20]. The sultams **8** (R^1 = NH_2 or OH, R^2 = aryl) can be used as herbicides, fungicides and pesticides [21]. Ring-closing metathesis (RCM) of vinylsulfonamide templates in the presence of Grubbs catalyst, providing the cyclic vinylsultams **8** (R^1 = R^2 = H), has also been described [22]. Isothiazol-3(2*H*)-one 1,1-dioxides **9** (R^2 = H, R = *t*-Bu, CH_2CO_2Et) are versatile dienophilic compounds in DielsAlder reactions [23]. The 3-oxosultams **9** are usually prepared by oxidation of isothiazol-3(2*H*)-ones [3] with m-CPBA [24,25], or by oxidation of 3-unsubstituted isothiazoles using H_2O_2 in glacial acetic acid (R = H) [26]. Recently, we have found a new approach to non-benzoannelated 2-phenyl-isothiazol-3(2*H*)-one 1,1-dioxides **14** (R^1 = R^2 = Me) in a one-step process by oxidation of the corresponding isothiazolium salts **12** [27].



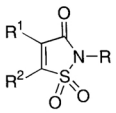
1: R=H
2: R=COR[1]
3: R=aryl



4: R=triazolo
5: R=OCOCH$_3$, O-styroyl, SO$_2$R[1]
6: R=OCO-2,6-Cl$_2$-C$_6$H$_3$
7: R=O-heterocyclyl



8: R=H, Me, CH$_2$C$_6$H$_5$
R[3]=Et, *i*-Pr



9: R=H, *t*-Bu, CH$_2$COOEt
R[2]=H, Me, Br

The development of serine protease inhibiting 3-oxosultams has been focussed on benzoannelated derivatives **2 – 7**. There are publications on HLE inhibition by tetrahydrosaccharins [15] and cathepsin G inhibiting dihydroisothiazolone 1,1-dioxides [28], but investigations on the inhibition of serine proteases by monocyclic isothiazolone 1,1-dioxides have not been reported thus far. Herein, we describe the preparation of isothiazol-3(2*H*)-one 1,1-dioxides **14** with stabilizing aryl substituents in 2-, 4- and/or 5-position and the evaluation of their inhibitory potential toward various serine proteases.

Results and Discussion

Syntheses

Isothiazolium salts **12** were prepared by a known procedure from thiocyanates **10** and ring-substituted anilines **11**. Compounds **12b – p** were prepared for the first time.

2-Phenylisothiazol-3-one 1,1-dioxides **14** were synthesized in moderate to good yield (20 – 65 %) by oxidation of isothiazolium salts **12** with H_2O_2 in glacial acetic acid at 80 °C (3 – 26 h, method A) forming the hydroperoxides **13** as intermediates. Merely in a few cases, we isolated a mixture of the hydroperoxides **13** and the corresponding 3-oxosultams **14**. The pure compounds **14** were then received simply by treatment of the mixtures with hot ethanol; thereby **13** underwent thermolysis to give **14d,l,p** (method B). The hydroperoxides **13** can be isolated as will be reported later.

The new 3-oxosultams **14b – p** were identified by spectroscopic methods, and the purity was determined by elemental analysis; compounds **14a** and **14q** have been described earlier [27,29].

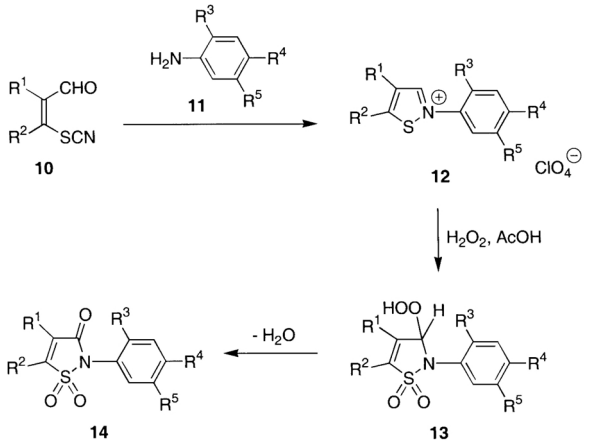
The typical symmetrical and antisymmetrical SO_2 absorption bands in the IR spectra of **14** were observed at 1143 – 1238 and 1290 – 1335 cm^{-1} and the CO absorption band at 1720 – 1743 cm^{-1}. In addition, the ^{13}C NMR signals for C-3 at 158.6 – 160.8 ppm, for C-4 at 129.7 – 134.0 ppm, and for C-5 at 142.2 – 145.2 ppm are characteristic for compounds **14**.

Enzyme inhibition

Compounds **14a – q** were investigated as inhibitors of the serine proteases cathepsin G, chymotrypsin, and HLE (Table 1). With the exception of **14j** and **14k**, none of the compounds showed inhibitory activity at 10 μM towards chymotrypsin. Both derivatives also led to weak inhibition of cathepsin G. Eight compounds of the present series were identified as inhibitors of HLE. Active compounds bear at least one phenyl rest at position 4 or 5 of the isothiazolone moiety. 4,5-Dimethyl substitution as well as the presence of a 4,5-tetramethylene moiety led to inactive compounds. When compared with both 4-methyl-5-phenyl and 4-phenyl-5-methyl derivatives, 4,5-diphenyl substitution resulted in an enhancement of inhibitory potency (**14i** *versus* **14e**, **14l**; **14j** *versus* **14f**, **14m**; **14k** *versus* **14h**, **14n**). Dichloro-4-isopropoxy substitution at the *N*-phenyl moiety was advantageous compared with mono- and dinitro substitution (**14h** *versus* **14e**, **14f**; **14n** *versus* **14l**, **14m**; **14k** *versus* **14i**, **14j**). Combination of the favoured residues led to a substitution pattern that is present in the most active HLE inhibitor of this series, **14k**.

Initially, all reactions to determine HLE activity were started by addition of the enzyme. Progress curves indicated time-dependent inhibition and were analyzed as first-order reactions. The second-order rate constants of inhibition, k_{obs}/[I], are outlined in Table 1, [I] = inhibitor concentration. These values are from experiments with an inhibitor concentration of 8 μM. Inhibition by isothiazolones **14d**, **14i**, and **14j** was also examined at four other concentrations in the range of 2 – 10 μ M. However, a significant linear correlation of k_{obs} *versus* [I] was not obtained. We address this to a more complex mode of inhibition.

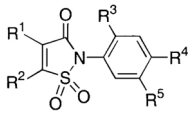
Preincubation of HLE and inhibitor **14i** (8 μM) for 10 min led to a stronger inhibition, and a reactivation of the enzyme after addition of the substrate did not occur (Fig. 1). Similar results were obtained in a preincubation experiment with **14i** at 6 μM (data not shown). The dependence of the HLE inhibition from the sub-



	R^1	R^2	R^3	R^4	R^5
a	Me	Me	H	NO_2	H
b	Me	Me	NO_2	NO_2	H
c	Me	Me	Cl	O-*i*-Pr	Cl
d	H	Ph	Cl	O-*i*-Pr	Cl
e	Me	Ph	H	NO_2	H
f	Me	Ph	NO_2	NO_2	H
g	Me	Ph	Cl	H	Cl
h	Me	Ph	Cl	O-*i*-Pr	Cl
i	Ph	Ph	H	NO_2	H
j	Ph	Ph	NO_2	NO_2	H
k	Ph	Ph	Cl	O-*i*-Pr	Cl
l	Ph	Me	H	NO_2	H
m	Ph	Me	NO_2	NO_2	H
n	Ph	Me	Cl	O-*i*-Pr	Cl
o	$(CH_2)_4$		NO_2	NO_2	H
p	$(CH_2)_4$		Cl	H	Cl
q	$(CH_2)_4$		Cl	O-*i*-Pr	Cl

Scheme 1. Pathway to 3-oxosultams.

Table 1. Inhibition of cathepsin G, chymotrypsin, and HLE.



14	R^1	R^2	R^3	R^4	R^5	Inhibition of cathepsin G[a] K_i	chymotrypsin[a] K_i	HLE[b] k_{obs}/[I] at 8 μM
a	Me	Me	H	NO_2	H	NI	NI	NI
b	Me	Me	NO_2	NO_2	H	NI	NI	NI
c	Me	Me	Cl	O-*i*-Pr	Cl	NI	NI	NI
d	H	Ph	Cl	O-*i*-Pr	Cl	NI	NI	340 $M^{-1}s^{-1}$
e	Me	Ph	H	NO_2	H	NI	NI	120 $M^{-1}s^{-1}$
f	Me	Ph	NO_2	NO_2	H	NI	NI	NI
g	Me	Ph	Cl	H	Cl	NI	NI	NI
h	Me	Ph	Cl	O-*i*-Pr	Cl	NI	NI	340 $M^{-1}s^{-1}$
i	Ph	Ph	H	NO_2	H	NI	NI	590 $M^{-1}s^{-1}$
j	Ph	Ph	NO_2	NO_2	H	17 μM[c]	0.35 $\pm$ 0.04 μM[d]	880 $M^{-1}s^{-1}$
k	Ph	Ph	Cl	O-*i*-Pr	Cl	25 μM[c]	2.0 μM[e]	2400 $M^{-1}s^{-1}$
l	Ph	Me	H	NO_2	H	NI	NI	NI
m	Ph	Me	NO_2	NO_2	H	NI	NI	120 $M^{-1}s^{-1}$
n	Ph	Me	Cl	O-*i*-Pr	Cl	NI	NI	320 $M^{-1}s^{-1}$
o	-$(CH_2)_4$-		NO_2	NO_2	H	NI	NI	NI
p	-$(CH_2)_4$-		Cl	H	Cl	NI	NI	NI
q	-$(CH_2)_4$-		Cl	O-*i*-Pr	Cl	NI	NI	NI

[a] NI, no inhibition, refers to an activity > 80 % at an inhibitor concentration of 10 μM compared to the control in the absence of inhibitor. Progress curves were analyzed by linear regression. [b] NI, no inhibition, refers to a value k_{obs}/[I] < 100 $M^{-1}s^{-1}$ at an inhibitor concentration of 8 μM. Progress curves were analyzed as first-order reactions. [c] Obtained from a duplicate determination at a single inhibitor concentration, [I] = 10 μM. Progress curves were analyzed by linear regression and K_i was calculated using equation $K_i = [I]/[(v_0/v_s) - 1]$, were v_0 and v_s are the reaction rates in the absence and presence of the inhibitor. [d] $k_{on} = 682 \pm 38$ $M^{-1}s^{-1}$, $k_{off} = 2.4 \times 10^{-4}$ s^{-1}. Progress curves at six different inhibitor concentrations (1–10 μM) were analyzed by using slow-binding kinetics [33]. Steady-state rates, v_s, were plotted against the inhibitor concentration, and non-linear regression according to equations $v_s = v_0/[([I]/K_i') + 1]$, $K_i = K_i'/(1 + [S]/K_m)$, gave K_i. Values k_{on} and k_{off}, respectively, were obtained as described [34]. [e] Obtained from a duplicate determination at a single inhibitor concentration, [I] = 10 μM. Progress curves were analyzed by using slow-binding kinetics [33]. k_s was calculated using equations $K_i' = [I]/[(v_0/v_s) - 1]$, $K_i = K_i'/(1 + [S]/K_m)$.

strate concentration, [S], was investigated with **14i** at a concentration of 8 μM and six different concentrations of MeOSuc-Ala-Ala-Pro-Val-pNA in the range of 25–250 μM (Fig. 2 and 3). Progress curves were analyzed as first-order reactions and k_{obs} values as well as values for product formation at infinite time, [P_∞], were determined. A plot of $1/k_{obs}$ *versus* [S] gave a straight line indicating competitive inhibition (Fig. 2). On the

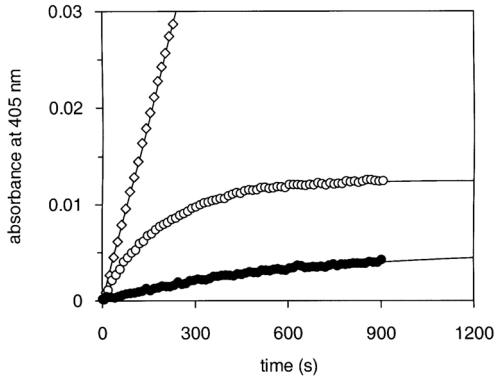


Fig. 1. Inhibition of human leukocyte elastase by compound **14i** (8 μM) in 50 mM sodium phosphate buffer, 500 mM NaCl, pH 7.8, 25 °C. The substrate was MeOSuc-Ala-Ala-Pro-Val-pNA. Open squares, control reaction in the absence of inhibitor without preincubation; open circles, progress curve of the reaction that was started by addition of the enzyme; full circles, progress curve of the reaction that was started by addition of the substrate after preincubation for 10 min.

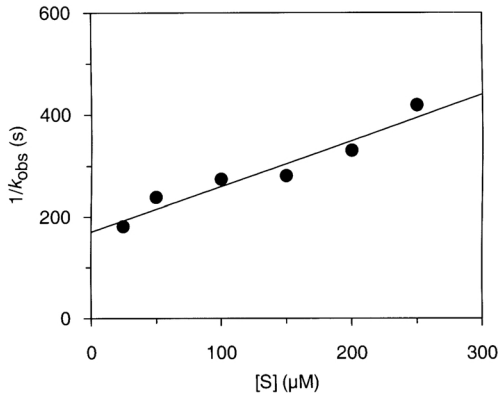


Fig. 2. Plot of reciprocal k_{obs} values *versus* concentrations of MeOSuc-Ala-Ala-Pro-Val-pNA. Reactions of **14i** (8 μM) with HLE in the presence of different substrate concentrations were analyzed as first-order reactions. Data are average values of triplicate experiments.

other hand, $1/[P_\infty]$ was plotted against 1/[S] (Fig. 3). The linear dependence as well as the vertical intercept indicated noncompetitive inhibition. The same interdependencies were found by using an inhibitor concentration of 6 μM (data not shown). The methodology to distinguish types of enzyme modification by using kinetic parameters was described by Tian and Tsou [30]. Therefore, it might be concluded that the isothiazolones interact with HLE at the active site as well as at another binding site. Further studies are needed to provide an insight into this complex interaction.

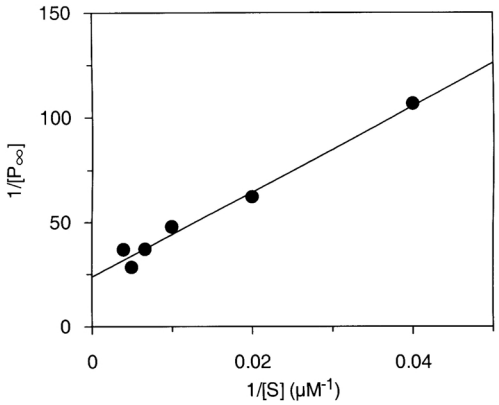


Fig. 3. Reaction of **14i** with HLE. Plot of reciprocal $[P_\infty]$ values *versus* reciprocal substrate concentrations. Data were obtained as noted for Fig. 2.

Experimental Section

General M. p.: Boetius micro-melting-point apparatus; corrected. IR spectra: Genisis FTIR Unicam Analytical System (ATI Mattson); KBr pellets; values in cm^{-1}. UV/vis spectra: Beckman DU-650. ^{1}H NMR: Varian Gemini-200 and Varian Unity-400; δ in ppm rel. to TMS as internal standard, *J* in Hz. ^{13}C NMR spectra: 50 or 100 MHz, recorded on the named spectrometers. MS: Quadrupol-MS VG 12-250; 70 eV. Elemental analyses: Heraeus CHNO Rapid Analyzer.

*2-Arylisothiazolium perchlorates (**12**)*

General method: **11** (1 mmol) was added to a solution of **10** (1 mmol) in glacial acetic acid (2 ml). Then perchloric acid (0.4 ml, 70 %) was dropped into the reaction mixture. After addition of ether, the precipitate was isolated by filtration and recrystallized from ethanol. Data of the salts **12 a**, **q** have been published in [27, 29].

*4,5-Dimethyl-2-(2,4-dinitrophenyl)-isothiazolium perchlorate (**12b**)*

Yield: 91 %. M. p. 223 – 226 °C. – IR (KBr): ν = 1091 s (O—Cl—O), 1346 s (NO_2), 1542 s (NO_2) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 2.42 (s, 3H, 4-CH_3), 2.92 (s, 3H, 5-CH_3), 8.37 (d, J = 8.4 Hz, 1H, arom. H), 8.91 (dd, ^3J = 8.4 Hz, ^4J = 2.3 Hz, 1H, arom. H), 9.11 (d, J = 2.3 Hz, 1H, arom. H), 9.45 (s,1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 11.5 (4-CH_3), 14.3 (5-CH_3), 122.6, 127.5, 133.4, 133.5, 134.9 (C-4), 145.2 (*o*-C), 149.8 (*p*-C), 157.7 (C-3), 173.0 (C-5). – EI-MS: *m/z* = 279 ([M-$HClO_4$]$^{+\cdot}$). – $C_{11}H_{10}ClN_3O_8S$ (379.73): calcd. C 34.79, H 2.65, N 11.07, O 33.71, S 8.44; found C 34.51, H 2.78, N 11.31, O 33.50, S 8.25.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-4,5-dimethyl-isothiazolium perchlorate (**12c**)*

Yield: 62 %. M. p. 168 – 170 °C. – IR (KBr): ν = 1090 s (O—Cl—O) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 1.37 (d, J = 6.0 Hz, 6H, 2 CH_3), 2.36 (s, 3H, 4-CH_3), 2.84 (s, 3H, 5-CH_3), 4.96 (m, 1H, O-CH), 7.71 (s, 1H, arom. H), 8.18 (s, 1H, arom. H), 9.35 (s, 1H, 3-CH). – EI-MS: *m/z* = 316 ([M-$HClO_4$]$^{+\cdot}$). – $C_{14}H_{16}Cl_3NO_5S$ (416.70): calcd. C 40.35, H 3.87, N 3.36, O 19.20, S 7.70; found C 40.22, H 3.61, N 3.21, O 19.01, S 7.62.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-5-phenyl-isothiazolium perchlorate (**12d**)*

Yield: 48 %. M. p. 206 – 209 °C. – IR (KBr): ν = 1103 s (O—Cl—O) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 1.38 (d, J = 6.0 Hz, 6H, 2 CH_3), 4.99 (m, 1H, O-CH), 7.74 (m, 3H, arom. H), 7.76 (s, 1H, arom. H), 8.07 (d, J = 7.9 Hz, 2H, o-H), 8.29 (s, 1H, arom. H), 8.52 (d, J = 2.9 Hz, 1H, 4-CH), 9.68 (d, J = 2.9 Hz, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 21.8 (2 CH_3), 73.1 (O-CH), 116.1, 120.8, 121.7, 126.3, 126.8, 128.8, 130.1, 130.3, 130.7, 134.2, 156.3 (*p*-C), 162.5 (C-3), 174.3 (C-5). – EI-MS: *m/z* = 364 ([M-$HClO_4$]$^{+\cdot}$). – $C_{18}H_{16}Cl_3NO_5S$ (464.75): calcd. C 46.52, H 3.47, N 3.01, O 17.21, S 6.90; found C 46.59, H 3.26, N 3.35, O 17.38, S 6.71.

*4-Methyl-2-(4-nitrophenyl)-5-phenyl-isothiazolium perchlorate (**12e**)*

Yield: 72 %. M. p. 181 – 183 °C. – IR (KBr): ν = 1089 s (O—Cl—O), 1349 s (NO_2), 1529 s (NO_2) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 2.52 (s, 3H, 4-CH_3), 7.73-7.84 (m, 5H, arom. H), 8.22, 8.61 (J_{AB} = 6.0 Hz, 4H, arom. H), 9.90 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 12.1, 124.5, 126.0, 126.2, 129.6, 129.8, 132.3, 132.4, 140.9, 148.5 (*p*-C), 158.6 (C-3), 167.9 (C-5). – EI-MS: *m/z* = 296 ([M-$HClO_4$]$^{+\cdot}$). – $C_{16}H_{13}ClN_2O_6S$ (396.80): calcd. C 48.43, H 3.30, Cl 8.93, N 7.06, O 24.19, S 8.08; found C 48.30, H 2.98, Cl 9.10, N 6.96, O 24.29, S 7.62.

*4-Methyl-2-(2,4-dinitrophenyl)-5-phenyl-isothiazolium perchlorate (**12f**)*

Yield: 30 %. M. p. 172 – 173 °C. – IR (KBr): ν = 1097 s (O—Cl—O), 1346 s (NO_2), 1540 s (NO_2) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 2.45 (s, 3H, 4-CH_3), 7.67-7.78 (m, 5 H, arom. H), 8.37 (d, J = 8.6 Hz, 1H, arom. H), 8.90 (dd, ^3J = 8.4 Hz, ^4J = 2.6 Hz, 1H, arom. H), 9.05 (d, J = 2.2 Hz, 1H, arom. H), 9.58 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 12.6, 122.2, 126.7, 130.3, 130.4, 131.8, 133.0, 133.5, 134.6 (C-4), 144.9 (*o*-C), 149.8 (*p*-C), 162.4 (C-3), 171.1 (C-5). – EI-MS: *m/z* = 341 ([M-$HClO_4$]$^{+\cdot}$). – $C_{16}H_{12}ClN_3O_8S$ (441.80): calcd. C 43.50, H 2.74, N 9.51, O 28.97, S 7.26; found C 43.68, H 2.63, N 9.42, O 29.07, S 7.39.

*2-(2,5-Dichlorophenyl)-4-methyl-5-phenyl-isothiazolium perchlorate (**12g**)*

Yield: 16 %. M. p. 168 – 170 °C. – IR (KBr): ν = 1090 s (O—Cl—O) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 2.49 (s, 3H, 4-CH_3), 7.70-7.94 (m, 7H, arom. H), 8.25 (d, J = 2.2 Hz, 1H, arom. H), 9.63 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 11.8, 125.9, 128.4, 129.4, 129.5, 129.6, 131.3, 132.1, 132.2, 132.6, 133.2, 134.5, 161.7 (C-3), 169.5 (C-5). – EI-MS: *m/z* = 320 ([M-$HClO_4$]$^{+\cdot}$). – $C_{16}H_{12}Cl_3NO_4S$ (420.69): calcd. C 45.68, H 2.88, N 3.33, O 15.21, S 7.62; found C 45.51, H 2.65, N 3.49, O 15.41, S 7.45.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-4-methyl-5-phenyl-isothiazolium perchlorate (**12h**)*

Yield: 31 %. M. p. 195 – 198 °C. – IR (KBr): ν = 1095 s (O—Cl—O) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 1.32 (d, J = 6.2 Hz, 6H, 2 CH_3), 2.46 (s, 3H, CH_3), 4.93 (m, 1H, O-CH), 7.65-7.77 (m, 6H, arom. H), 8.20 (s, 1H, arom. H), 9.52 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 12.6 (4-CH_3), 22.3 (2 CH_3), 73.5 (O-CH), 116.5, 122.1, 126.8, 130.1, 130.3, 130.4, 131.0, 131.9, 132.9, 156.5 (*p*-C), 162.6 (C-3), 166.1, 169.7 (C-5). – EI-MS: *m/z* = 378 ([M-$HClO_4$]$^{+\cdot}$). – $C_{19}H_{18}Cl_3NO_5S$ (478.77): calcd. C 47.66, H 3.79, N 2.93, O 16.71, S 6.70; found C 47.85, H 3.91, N 2.99, O 16.49, S 6.58.

*2-(4-Nitrophenyl)-4,5-diphenyl-isothiazolium perchlorate (**12i**)*

Yield: 42 %. M. p. 237 – 238 °C. – IR (KBr): ν = 1100 s (O—Cl—O), 1345 s (NO_2), 1535 s (NO_2) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 7.51-7.70 (m, 10H, arom. H), 8.33, 8.64 (J_{AB} = 9.0 Hz, 4H, arom. H), 10.17 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 121.6, 124.7, 125.6, 128.5, 129.0, 129.2, 129.5, 129.5, 132.2, 135.5, 140.7, 148.6 (*p*-C), 158.0 (C-3), 167.0 (C-5). – EI-MS: *m/z* = 358 ([M-$HClO_4$]$^{+\cdot}$). – $C_{21}H_{15}ClN_2O_6S$ (458.87): calcd. C 54.97, H 3.29, N 6.10, S 6.99; found C 55.12, H 3.39, N 6.27, S 6.71.

*2-(2,4-Dinitrophenyl)-4,5-diphenyl-isothiazolium perchlorate (**12j**)*

Yield: 51 %. M. p. 278 – 280 °C. – IR (KBr): ν = 1089 s (O—Cl—O), 1346 s (NO_2), 1540 s (NO_2) cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 7.50-7.68 (m, 10 H, arom. H), 8.52 (d, J = 5.8 Hz, 1H, arom. H), 8.99 (dd, ^3J = 5.8 Hz, ^4J = 1.6 Hz, 1 H, arom. H), 9.15 (d, J = 1.6 Hz, 1 H, arom. H), 9.99 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 121.6, 125.7, 128.4, 129.2, 129.3, 129.6, 129.7, 129.8, 132.4, 133.1, 134.1,

134.5 (C-4), 144.2 (*o*-C), 149.3 (*p*-C), 161.7 (C-3), 169.6 (C-5). – EI-MS: *m/z* = 403 ($[M\text{-}HClO_4]^{+\cdot}$). – $C_{21}H_{14}ClN_3O_8S$ (503.87): calcd. C 50.06, H 2.80, N 8.34, S 6.36; found C 50.33, H 2.95, N 8.48, S 6.51.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-4,5-diphenyl-isothiazolium perchlorate (**12k**)*

Yield: 39 %. M. p. 136 – 140 °C. – IR (KBr): ν = 1090 s (O—Cl—O) cm^{-1}. – ^1H NMR (DMSO-d_6): δ = 1.38 (d, J = 6 Hz, 6H, 2 CH_3), 5.00 (m, 1H, O—CH), 7.27 – 7.68 (m, 10H, arom. H), 7.79 (s, 1H, arom. H), 8.34 (s, 1H, arom. H), 9.90 (s, 1H, 3-CH). – EI-MS: *m/z* = 440 ($[M\text{-}HClO_4]^{+\cdot}$). – $C_{24}H_{20}Cl_3NO_5S$ (540.84): calcd. C 53.30, H 3.73, N 2.59, S 5.93; found C 53.51, H 3.61, N 2.68, S 6.06.

*5-Methyl-2-(4-nitrophenyl)-4-phenyl-isothiazolium perchlorate (**12l**)*

Yield: 88 %. M. p. 156 – 158 °C. – IR (KBr): ν = 1095 s (O—Cl—O), 1353 s (NO_2), 1529 s (NO_2) cm^{-1}. – ^1H NMR (DMSO-d_6): δ = 2.94 (s, 3H, 5-CH_3), 7.59 – 7.70 (m, 5H, arom. H), 8.23, 8.56 (J_{AB} = 8.8 Hz, 4H, arom. H), 9.97 (s, 1H, 3-CH).– ^{13}C NMR (DMSO-d_6): δ = 14.3 (5-CH_3), 112.3, 124.5, 125.6, 129.0, 129.1, 129.5, 137.0, 140.9, 148.2 (*p*-C), 156.3 (C-3), 168.3 (C-5). – EI-MS: *m/z* = 296 ($[M\text{-}HClO_4]^{+\cdot}$). – $C_{16}H_{13}ClN_2O_6S$ (396.80): calcd. C 48.43, H 3.30, Cl 8.93, N 7.06, O 24.19, S 8.08; found C 48.10, H 3.01, Cl 9.49, N 6.88, O 24.00, S 8.18.

*5-Methyl-2-(2,4-dinitrophenyl)-4-phenyl-isothiazolium perchlorate (**12m**)*

Yield: 88 %. M. p. 214 – 217 °C. – IR (KBr): ν = 1108 s (O—Cl—O), 1346 s (NO_2), 1542 s (NO_2) cm^{-1}. – ^1H NMR (DMSO-d_6): δ = 2.94 (s, 3H, 5-CH_3), 7.56 – 7.61 (m, 5H, arom. H), 8.37 (d, J = 8.8 Hz, 1H, arom. H), 8.87 (dd, ^3J = 8.8 Hz, ^4J = 2.6 Hz, 1H, arom. H), 9.05 (d, J = 2.2 Hz, 1H, arom. H), 9.76 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 14.5, 119.8, 121.6, 123.5, 129.2, 129.4, 129.8, 133.0, 134.1, 136.2, 144.3, 149.2 (*p*-C), 159.9 (C-3), 171.0 (C-5). – EI-MS: *m/z* = 341 [M-$HClO_4$]. – $C_{16}H_{12}ClN_3O_8S$ (441.80): calcd. C 43.50, H 2.74, Cl 8.02, N 9.51, O 28.97, S 7.26; found C 43.20, H 2.02, Cl 8.50, N 9.32, O 29.20, S 7.79.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-5-methyl-4-phenyl-isothiazolium perchlorate (**12n**)*

Yield: 48 %. M. p. 182 – 185 °C. – IR (KBr): ν = 1091 s (O—Cl—O) cm^{-1}. – ^1H NMR (DMSO-d_6): δ = 1.38 (d, J = 4.2 Hz, 6H, 2 CH_3), 2.96 (s, 3H, 5-CH_3), 4.98 (m, 1H, O—CH), 7.61 – 7.72 (m, 5H, arom. H), 7.74 (s, 1H, arom. H), 8.28 (s, 1H, arom. H), 9.74 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 14.3 (5-CH_3), 21.5, 21.8, 72.8, 115.9, 121.5, 126.3, 128.8, 129.2, 129.3, 129.5, 129.6, 130.4, 136.1, 155.8 (*p*-C), 159.8 (C-3), 169.5 (C-5). – EI-MS: *m/z* = 378 ($[M\text{-}HClO_4]^{+\cdot}$). – $C_{19}H_{18}Cl_3NO_5S$ (478.77): calcd. C 47.66, H 3.79, N 2.93, O 16.71, S 6.70; found C 47.10, H 3.38, N 3.01, O 16.82, S 6.85.

*2-(2,4-Dinitrophenyl)-4,5,6,7-tetrahydro-1,2-benziso-thiazolium perchlorate (**12o**)*

Yield: 84 %. M. p. 225 – 227 °C. – IR (KBr): ν = 1095 s (O—Cl—O), 1346 s (NO_2), 1542 s (NO_2) cm^{-1}. – ^1H NMR (DMSO-d_6): δ = 1.90 (m, 4H, 2 CH_2), 2.86 (m, 2H, CH_2), 3.25 (m, 2H, CH_2), 8.32 (d, J = 8.1 Hz, 1H, arom. H), 8.86 (dd, ^3J = 8.2 Hz,^4J = 2.1 Hz, 1H, arom. H), 9.06 (d, 1H, arom. H), 9.41 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 20.4, 21.2, 22.3, 25.9, 121.5, 129.5, 132.6, 133.7, 134.2 (C-3a), 144.3, 148.9 (*p*-C), 158.9 (C-3), 172.9 (C-7a). – EI-MS: *m/z* = 305 ($[M\text{-}HClO_4]^{+\cdot}$). – $C_{13}H_{12}ClN_3O_8S$ (405.77): calcd. C 38.48, H 2.98, N 10.36, S 7.90; found C 38.61, H 2.81, N 10.47, S 8.05.

*2-(2,5-Dichlorophenyl)-4,5,6,7-tetrahydro-1,2-benziso-thiazolium perchlorate (**12p**)*

Yield: 85 %. M. p. 234 – 238 °C. – IR (KBr): ν = 1100 s (O—Cl—O) cm^{-1}. – ^1H NMR (DMSO-d_6): δ = 1.88 (m, 4H, 2 CH_2), 2.85 (t, 2H, CH_2), 3.24 (t, 2H, CH_2), 7.85 (m, 2H, arom. H), 8.16 (m, 1H, arom. H), 9.40 (s, 1H, 3-CH). – ^{13}C NMR (DMSO-d_6): δ = 20.8, 21.5, 22.6, 26.1, 128.8, 129.8, 132.3, 132.9, 133.3, 134.0, 135.2 (C-3a), 159.2 (C-3), 172.1 (C-7a). – EI-MS: *m/z* = 284 ($[M\text{-}HClO_4]^{+\cdot}$). – $C_{13}H_{12}C_{l3}NO_4S$ (384.66): calcd. C 40.59, H 3.14, N 3.64, S 8.34; found C 40.71, H 3.25, N 3.51, S 8.21.

*2-Aryl-2,3-dihydro-isothiazol-3-one 1,1-dioxides (**14b – p**)*

General Procedures: Method A: H_2O_2 (0.5 ml, 30 %) was added to a suspension of **12** (0.15 mmol) in AcOH (0.7 ml). The solution was stirred for 4 – 25 h at 80 °C. After cooling, the 3-oxosultams **14** were isolated and recrystallized from ethanol. Methode B: By following procedure A, a mixture containing **14** and the respective hydroperoxide **13** was isolated in a few cases. This mixture was dissolved in ethanol (4 ml) and conc. HCl (0.3 ml) was added. It was refluxed for 2 h. After cooling, the corresponding 3-oxosultam **14** was isolated by filtration and recrystallized from ethanol. Data of **14a** are given in [27], those of **14q** in [29].

*4,5-Dimethyl-2-(2,4-dinitrophenyl)-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14b**)*

Yield: 27 %, (A). M. p. 170 – 172 °C. – IR (KBr): ν = 1182 s (SO_2), 1335 s (SO_2), 1349 s (NO_2), 1542 s (NO_2), 1743 s (CO). – UV (ethanol): λ_{max} (lg ε) 278.5 nm (3.10). – ^1H NMR (acetone-d_6): δ = 2.16 (s, 3H, 4-CH_3), 2.42 (s, 3H, 5-CH_3), 8.12 (d, J = 8.5 Hz, H, arom. H), 8.77 (dd, ^3J =

8.6 Hz, ^4J = 2.5 Hz, 1H, arom. H), 8.94 (d, J = 2.5 Hz, 1H, arom. H). – EI-MS: *m/z* = 327 ($M^{+\cdot}$). – $C_{11}H_9N_3O_7S$ (327.27): calcd. C 40.37, H 2.77, N 12.84, S 9.80; found C 40.55, H 2.85, N 12.71, S 9.97.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-4,5-dimethyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14c**)*

Yield: 48 %, (A). M. p. 167 – 170 °C. – IR (KBr): ν = 1178 s (SO_2), 1332 s (SO_2), 1737 s (CO). – UV (ethanol): λ_{max} (lg ε) 284.5 nm (3.23). – ^{1}H NMR (DMSO-d_6): δ = 1.35 (d, J = 5.9 Hz, 6H, 2 CH_3), 2.07 (s, 3H, 4-CH_3), 2.33 (s, 3H, 5-CH_3), 4.89 (m, 1H, O-CH), 7.61 (s, 1H, arom. H), 7.66 (s, 1H, arom. H). – ^{13}C NMR (DMSO-d_6): δ = 7.9 (5-CH_3), 8.9 (4-CH_3), 21.6 (2 CH_3), 72.4 (O-CH), 116.2, 117.9, 121.3, 132.6, 133.1, 134.0 (C-4), 143.3 (C-5), 155.2 (*C*-O-*i*-Pr), 159.6 (C=O). – EI-MS: *m/z* = 364 ($M^{+\cdot}$). – $C_{14}H_{15}Cl_2NO_4S$ (364.24): calcd. C 48.28, H 4.34, N 4.02, S 9.21; found C 48.41, H 4.38, N 4.16, S 9.44.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-5-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14d**)*

Yield: 20 %, (B). M. p. 150 – 152 °C. – IR (KBr): ν = 1162 s (SO_2), 1245 s, 1340 s (SO_2), 1729 s (CO). – UV (ethanol): λ_{max} (lg ε) 285.3 nm (3.54). – ^{1}H NMR (DMSO-d_6): δ = 1.37 (d, J = 6.0 Hz, 6H, 2 CH_3), 4.88 (m, 1H, O-CH), 7.58 (s, 1H, arom. H), 7.69 (s, 1H, arom. H), 7.82 (m, 5H, arom. H), 6.93 (s, 1H, CH-4). – EI-MS: *m/z* = 412 ($M^{+\cdot}$). – $C_{18}H_{15}Cl_2NO_4S$ (412.29): calcd. C 52.44, H 3.67, N 3.40, S 7.78; found C 52.57, H 3.78, N 3.25, S 7.85.

*4-Methyl-2-(4-nitrophenyl)-5-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14e**)*

Yield: 63 %, (A). M. p. 224 – 225 °C. – IR (KBr): ν = 1170 s (SO_2), 1310 s (SO_2), 1321 s (NO_2), 1527 s (NO_2), 1725 s (CO). – UV (ethanol): λ_{max} (lg ε) 277.5 nm (3.88). – ^{1}H NMR (DMSO-d_6): δ = 2.24 (s, 3H, CH_3), 7.68-7.79 (m, 5H, arom. H), 7.91, 8.51 (J_{AB} = 10.8 Hz, 4H, arom. H). – ^{13}C NMR (DMSO-d_6): δ = 10.2 (CH_3), 114.4, 125.1, 127.6, 128.8, 129.5, 131.5, 133.9, 135.2, 142.2 (C-5), 147.3 (*p*-C), 159.5 (C=O). – EI-MS: *m/z* = 344 ($M^{+\cdot}$). – $C_{16}H_{12}N_2O_5S$ (344.34): calcd. C 55.81, H 3.51, N 8.14, S 9.31; found C 55.93, H 3.42, N 8.21, S 9.45.

*4-Methyl-2-(2,4-dinitrophenyl)-5-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14f**)*

Yield: 36 %, (A). M. p. 215 – 217 °C. – IR (KBr): ν = 1172 s (SO_2), 1305 s (SO_2), 1349 s (NO_2), 1532 s (NO_2), 1720 s (CO). – UV (ethanol): λ_{max} (lg ε) 287.5 nm (3.74). – ^{1}H NMR (DMSO-d_6): δ = 2.09 (s, 3H, 4-CH_3), 7.64-7.79 (m, 5H, arom. H), 8.12 (d, J = 8.6 Hz, 1H, arom. H), 8.77 (dd, ^3J = 8.8 Hz, ^4J = 2.6 Hz, 1H, arom. H), 8.94 (d, J = 2.6 Hz, 1H, arom. H). – ^{13}C NMR (DMSO-d_6): δ = 10.4 (CH_3), 122.1, 123.8, 126.4, 128.9, 129.6, 129.7, 131.9, 133.0, 133.5, 143.7 (C-5), 146.5 (*o*-C), 148.4 (*p*-C), 159.5 (C=O). – EI-MS: *m/z* = 389 ($M^{+\cdot}$). – $C_{16}H_{11}N_3O_7S$ (389.34): calcd. C 49.36, H 2.85, N 10.79, S 8.24; found C 49.21, H 2.96, N 10.61, S 8.36.

*2-(2,5-Dichlorophenyl)-4-methyl-5-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14g**)*

Yield: 31 %, (A). M. p. 176 – 178 °C. – IR (KBr): ν = 1170 s (SO_2), 1336 s (SO_2), 1733 s (CO). – UV (ethanol): λ_{max} (lg ε) 285.5 nm (3.71). – ^{1}H NMR (DMSO-d_6): δ = 2.15 (s, 3H, 4-CH_3), 7.65-7.92 (m, 7H, arom. H), 8.01 (s, 1H, arom. H). – EI-MS: *m/z* = 368 ($M^{+\cdot}$). – $C_{16}H_{11}Cl_2NO_3S$ (368.23): calcd. C 52.19, H 3.01, N 3.80, S 8.71; found C 52.25, H 3.15, N 3.88, S 8.79.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-4-methyl-5-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14h**)*

Yield: 57 %, (A). M. p. 161 – 162 °C. – IR (KBr): ν = 1172 s (SO_2), 1242 s, 1338 s (SO_2), 1733 s (CO). – UV (ethanol): λ_{max} (lg ε) 286.0 (3.72) nm. – ^{1}H NMR (acetone-d_6): δ = 1.42 (d, J = 6, 0, 6H, 2 CH_3), 2.29 (s, 3H, 4-CH_3), 4.90 (m, 1H, O-CH), 7.48 (s, 1H, arom. H), 7.65 (s, 1H, arom. H), 7.64-7.86 (m, 5H, arom. H). – EI-MS: *m/z* = 426 ($M^{+\cdot}$). – $C_{19}H_{17}Cl_2NO_4S$ (426.31): calcd. C 53.53, H 4.02, N 3.29, S 7.52; found C 53.71, H 4.15, N 3.33, S 7.43.

*2-(4-Nitrophenyl)-4,5-diphenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14i**)*

Yield: 65 %, (A). M. p. 212 – 214 °C. – IR (KBr): ν = 1143 m (SO_2), 1290 s (SO_2), 1338 s (NO_2), 1517 s (NO_2), 1729 m (CO). – UV (ethanol): λ_{max} (lg ε) 282.0 nm (4.14). – ^{1}H NMR (DMSO-d_6): δ = 7.43-7.55 (m, 10H, arom. H), 7.97, 8.52 (J_{AB} = 8.4 Hz, 4H, arom. H). – EI-MS: *m/z* = 406 ($M^{+\cdot}$). – $C_{21}H_{14}N_2O_5S$ (406.41): calcd. C 62.06, H 3.47, N 6.89, S 7.89; found C 62.21, H 3.59, N 6.63, S 7.96.

*2-(2,4-Dinitrophenyl)-4,5-diphenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14j**)*

Yield: 38 %, (A). M. p. 161 – 163 °C. – IR (KBr): ν = 1147 m (SO_2), 1295 s (SO_2), 1340 s (NO_2), 1536 s (NO_2), 1737 m (CO). – UV (ethanol): λ_{max} (lg ε) 222.0 nm (4.37), 315 nm (4.12). – ^{1}H NMR (DMSO-d_6): δ = 7.28-7.55 (m, 10H, arom. H), 8.20 (d, J = 8.4 Hz, 1H, arom. H), 8.80 (dd, ^3J = 8.4 Hz, ^4J = 2.8 Hz, 1H, arom. H), 8.96 (d, J = 2.8 Hz, 1H, arom. H). – ^{13}C NMR (DMSO-d_6): δ = 122.2, 122.9, 123.7, 126.2, 126.3, 128.2, 128.8, 129.2, 129.7, 130.1, 130.7, 132.0, 133.2, 143.8 (C-5), 146.5 (*o*-C), 148.5 (*p*-C), 158.6 (C=O). – EI-MS: *m/z* = 451 ($M^{+\cdot}$). – $C_{21}H_{13}N_3O_7S$ (451.41): calcd.

C 55.87, H 2.90, N 9.31, S 7.10; found C 55.94, H 2.81, N 9.46, S 7.23.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-4,5-diphenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14k**)*

Yield: 32 %, (A). M. p. 88 – 90 °C. – IR (KBr): ν = 1149 s (SO_2), 1336 s (SO_2), 1729 s (CO). – UV (ethanol): λ_{max} (lg ε) 238.0 nm (4.32), 282.5 nm (3.94). – ^{1}H NMR (acetone-d_6): δ = 1.42 (d, J = 6.2 Hz, 6H, 2 CH_3), 4.91 (m, 1H, O-CH), 7.41-7.57 (m, 10H, arom. H), 7.59 (s, 1H, arom. H), 7.73 (s, 1 H, arom. H). – EI-MS: *m/z* = 488 ($M^{+\cdot}$). – $C_{24}H_{19}Cl_2NO_4S$ (488.38): calcd. C 59.02, H 3.92, N 2.87, S 6.57; found C 59.29, H 3.82, N 2.98, S 6.66.

*5-Methyl-2-(4-nitrophenyl)-4-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14l**)*

Yield: 91 % (B). M. p. 155 – 156 °C. – IR (KBr): ν = 1735 s (CO), 1523 s (NO_2), 1342 s (NO_2), 1284 s (SO_2), 1147 s (SO_2). – UV (ethanol): λ_{max} (lg ε) 273.5 nm (3.35). – ^{1}H NMR (DMSO-d_6): δ = 2.41 (s, 3H, 5-CH_3), 7.53-7.60 (m, 3H, arom. H), 7.67-7.70 (m, 2H, arom. H), 7.86, 8.42 (J_{AB} = 6.0 Hz, 4H, arom. H). – ^{13}C NMR (DMSO-d_6): δ = 10.0, 125.9, 128.1, 127.2, 129.0, 130.7, 131.1, 134.8 (C-4), 136.0 (i-C), 143.3 (C-5), 148.7 (*p*-C), 159.5 (C=O). – EI-MS: *m/z* = 359 ($M^{+\cdot}$). – $C_{16}H_{12}N_2O_5S$ (344.34): calcd. C 55.81, H 3.51, N 8.14, O 23.23, S 9.31; found C 54.40, H 3.16, N 8.74, O 25.40, S 8.51.

*2-(2,4-Dinitrophenyl)-5-methyl-4-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14m**)*

Yield: 48 %, (A). M. p. 155 – 157 °C. – IR (KBr): ν = 1157 s (SO_2), 1297 s (SO_2), 1344 s (NO_2), 1540 s (NO_2), 1739 s (CO). – UV (ethanol): λ_{max} (lg ε) 218.5 nm (4.35). – ^{1}H NMR (acetone-d_6): δ = 2.44 (s, 3H, 5-CH_3), 7.48-7.53 (m, 3H, arom. H), 7.53-7.54 (m, 2H, arom. H), 8.10 (d, J = 2.9 Hz, 1H, arom. H), 8.75 (dd, ^3J = 2.9 Hz, ^4J = 0.9 Hz, 1H, arom. H), 8.94 (d, J = 0.9 Hz, 1H, arom. H). – ^{13}C NMR (acetone-d_6): δ = 8.7, 122.3, 126.7, 127.9, 128.9, 129.4, 130.1, 130.7, 133.0, 134.4, 145.2 (C-5), 148.9 (*p*-C), 159.2 (C=O). – EI-MS: *m/z* = 389 ($M^{+\cdot}$). – $C_{16}H_{11}N_3O_7S$ (389.34): calcd. C 49.36, H 2.85, N 10.79, S 8.24; found C 49.50, H 2.91, N 10.93, S 8.55.

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-5-methyl-4-phenyl-2,3-dihydro-isothiazol-3-one 1,1-dioxide (**14n**)*

Yield: 82 %, (A). M. p. 169 – 170 °C. – IR (KBr): ν = 1157 s (SO_2), 1238 s (SO_2), 1739 s (CO).– UV (ethanol): λ_{max} (lg ε) 239.0 nm (4.25), 283.5 nm (3.97) – ^{1}H NMR (DMSO-d_6): δ = 1.39 (d, J = 6.1 Hz, 6H, 2 CH_3), 2.49 (s, 3H, 5-CH_3) 4.92 (m, 1H, OCH), 7.48 (s, 1H, arom. H), 7.59 (s, 1H, arom. H) 7.56 – 7.66 (m, 5H, arom. H). – ^{13}C NMR (DMSO-d_6): δ = 9.25 (5-CH_3), 22.0 (2 CH_3), 73.6 (OCH), 117.1, 119.8, 123.0, 127.9, 129.5, 130.9, 131.2, 133.6, 134.9, 135.3, 145.2 (C-5), 156.7 (C-O-*i*-Pr), 159.8 (C=O) – EI-MS: *m/z* = 425 ($M^{+\cdot}$). – $C_{19}H_{17}Cl_2NO_4S$ (426.31): calcd. C 53.53, H 4.02, Cl 16.63, N 3.29, O 15.01, S 7.52; found C 53.50, H 4.15, Cl 16.43, N 3.36, S 7.78.

*2-(2,4-Dinitrophenyl)-2,3,4,5,6,7-hexahydro-1,2-benz-isothiazol-3-one 1,1-dioxide (**14o**)*

Yield: 32 %, (A). M. p. 183 – 185 °C. – IR (KBr): ν = 1180 s (SO_2), 1297 s (SO_2), 1349 s (NO_2), 1538 s (NO_2), 1743 s (CO). – UV (ethanol): λ_{max} (lg ε) 291 nm (3.28). – ^{1}H NMR (acetone-d_6): δ = 1.92 (m, 4H, 2 CH_2), 2.55 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 8.11 (d, J = 8.7 Hz, 1H, arom. H), 8.81 (dd, ^3J = 8.7 Hz, ^4J = 2.6 Hz, 1H, arom. H), 9.01 (d, J = 2.6 Hz, 1H, arom. H). – ^{13}C NMR (acetone-d_6): δ = 19.8, 21.1, 21.1, 21.5, 115.6, 122.7, 122.9, 129.9, 133.6, 137.3 (C-3a), 148.8 (C-7a), 149.1 (*p*-C), 160.1 (C=O). – EI-MS: *m/z* = 353 ($M^{+\cdot}$). – $C_{13}H_{11}N_3O_7S$ (353.31): calcd. C 44.19, H 3.14, N 11.89, S 9.08; found C 44.28, H 3.25, N 11.78, S 9.24.

*2-(2,5-Dichlorophenyl)-2,3,4,5,6,7-hexahydro-1,2-benz-isothiazol-3-one 1,1-dioxide (**14p**)*

Yield: 40 %, (B). M. p. 159 – 160 °C. – IR (KBr): ν = 1159 s (SO_2), 1305 s (SO_2), 1732 s (CO). – UV (ethanol): λ_{max} (lg ε) 277.5 nm (3.11), 285.0 nm (3.05). – ^{1}H NMR (acetone-d_6): δ = 1.92 (m, 4H, 2 CH_2), 2.53 (m, 2H, CH_2), 2.69 (m, 2H, CH_2), 7.62 – 7.73 (m, 3H, arom. H). – EI-MS: *m/z* = 332 ($M^{+\cdot}$). – $C_{13}H_{11}Cl_2NO_3S$ (332.20): calcd. C 47.00, H 3.34, N 4.22, S 9.65; found C 47.27, H 3.51, N 4.31, S 9.73.

Enzyme Inhibition Assays

HLE prepared from human leukocytes and purified by affinity chromatography using an immobilized synthetic inhibitor [31] was available from a previous study [32]. Human cathepsin G was purchased from Calbiochem, Bad Soden, Germany. Chymotrypsin (bovine pancreas) was purchased from Merck, Darmstadt, Germany. Suc-Ala-Ala-Pro-Phe-pNA and MeOSuc-Ala-Ala-Pro-Val-pNA were purchased from Bachem, Heidelberg, Germany.

Inhibition of serine proteases by compounds **14a – q** was assayed spectrophotometrically on a Varian Cary 50 spectrophotometer with a multi-cell holder at 25 °C. Inhibitor stock solutions were prepared in DMSO. Stock solutions of the chromogenic substrates were prepared in DMSO and diluted with the corresponding assay buffer. Assay buffers were as follows: 50 mM sodium phosphate buffer, 500 mM NaCl, pH 7.0 for cathepsin G, 50 mM HEPES, 500 mM NaCl, pH 7.0 for chymotrypsin, 50 mM sodium phosphate buffer, 500 mM NaCl, pH 7.8 for HLE. The following substrates

were used: Suc-Ala-Ala-Pro-Phe-pNA for cathepsin G (final concentration 500 μM $\ll K_m$) and chymotrypsin (final concentration 200μM $= 3.46 \times K_m$), and MeOSuc-Ala-Ala-Pro-Val-pNA for HLE (final concentration 100μM $= 1.9 \times K_m$). Reactions were initiated by addition of 50 μl of an enzyme solution and monitored over 50 min (chymotrypsin, cathepsin G) and 10 – 15 min (HLE), respectively. The entire volume of the assays was 1 ml containing 6 % DMSO (cathepsin G, chymotrypsin) and 1.5 % DMSO (HLE), respectively. Assays were performed with final enzyme concentrations of cathepsin G (1.25 mU/ml), chymotrypsin (12.5 ng/ml), and HLE (125 ng/ml). Unless noted otherwise, a single inhibitor concentration of 10 μM (cathepsin G, chymotrypsin) or 8 μM (HLE) was used to test for enzyme inhibition.

Acknowledgements

The work was supported by the Graduiertenkolleg 378 „Mechanistische und Anwendungsaspekte nichtkonventioneller Oxidationsreaktionen“ and the Graduiertenkolleg 677/1 „Struktur und molekulare Interaktion als Basis der Arzneimittelwirkung“.

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