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2,4,5-Triphenylisothiazol-3(2*H*)-one 1,1-dioxides as inhibitors of human leukocyte elastase

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Abstract

A series of substituted 2,4,5-triphenylisothiazol-3(2*H*)-one 1,1-dioxides **9** was synthesized and investigated as inhibitors of human leukocyte elastase (HLE). All compounds were found to inhibit HLE in a time-dependent manner and most of them exhibited k_{obs}/[I] values $> 300\,M^{-1}s^{-1}$. The most potent 3-oxosultam of this series was **9l** (k_{obs}/[I] $= 2440\,M^{-1}s^{-1}$). Kinetic investigations performed with **9g** and different substrate concentrations did not allow to clearly distinguish between a competitive or noncompetitive mode of inhibition. A more complex interaction is supported by the failure of a linear dependency of k_{obs} values on the inhibitor concentration.

Keywords: *Human leukocyte elastase, 2,4,5-triphenylisothiazol-3(2H)-one 1,1-dioxides, sultams, inhibition*

Introduction

Human leukocyte elastase (HLE) is a serine protease implicated in several inflammatory diseases and represents a major target for the development of low-molecular weight inhibitors [1,2]. A number of elastase inhibitors have been progressing through the clinic within the last years [3,4]. The most advanced synthetic inhibitor is ONO-5046 (sivelestat) which has been launched in Japan (2002) as an injectable formulation for the treatment of acute lung injury associated with systemic inflammatory response syndrome (SIRS). Recently, results of clinical trials with ONO-5046 in patients with acute respiratory distress syndrome (ARDS) [5] and acute lung injury have been reported [6,7]. The thioester derivative MR-889 (midesteine) is in pre-registration for the treatment of chronic obstructive pulmonary disease in Italy, and the trifluoromethyl ketone inhibitor AE-3763 is in preclinical development in Japan [4].

Heterocyclic mechanism-based inhibitors of serine hydrolases are recognized by the target enzyme and form a covalent acyl-enzyme in the course of a ring-opening reaction. The modified inhibitor might be released hydrolytically in a slow deacylation step, and thus the interaction can be described as alternate substrate inhibition [8]. Examples of HLE inhibitors include 3,1-benzoxazin-4-ones [9,10] and thieno[1,3]oxazin-4-ones [11,12]. When a latent reactive group is unmasked by the catalytic action of the enzyme, covalent binding to an active site residue can lead to irreversible inhibition, *i.e.* inactivation. Such enzyme-activated inhibitors of HLE include saccharins, succinimides, and phthalimides [13,14]. *N*-Aryl and *N*-acyl saccharins **1** were shown to act by acylating the serine proteases [15,16]. It has been demonstrated by Groutas et al. that the incorporation of a leaving group (LG) into saccharin-based acylating agents **1** resulted in inactivators **2** with enhanced potency. Nucleophilic attack of the active-site serine, ring opening and expulsion of the leaving group produce a reactive intermediate with a $-N{=}CH_2$ moiety which can further react by cross-linking the enzyme's active site [17–19]. The potency of this class of inhibitors

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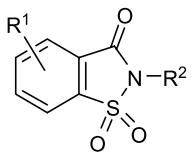
towards HLE has been shown to be dependent on the electronic properties of the leaving group and the substitution pattern of the saccharin unit. It has been predicted that substituents at position-4 interact with the S_1 specificity pocket of HLE and large lipophilic groups (in particular isopropyl) were examined to be highly favorable. Thus, Hlasta et al. have designed specific saccharin-based inactivators of HLE showing excellent potency and blood stability [20–22]. There are only a few reports on serine proteases being inhibited by 3-oxosultams that are not benzoanellated. Examples include the development of tetrahydrosaccharins and dihydroisothiazolone 1,1-dioxides as inactivators of HLE and cathepsin G [23,24].

In a previous publication we reported on the inhibitory activities of a series of tetrahydro-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxides **3** and monocyclic isothiazol-3(2*H*)-one 1,1-dioxides **4** towards chymotrypsin, cathepsin G and HLE [25]. Whereas most of these compounds were inactive against chymotrypsin and cathepsin G, some derivatives exhibited inhibitory activity against HLE. At least one phenyl ring present at the 4- or 5-position (*i.e.* R^2 or R^3) of **4** was necessary for HLE inhibition, and 4,5-dimethyl substitution of **4** or the presence of a 4,5-tetramethylene moiety in **3** led to inactive compounds. A comparison of both 4-methyl-5-phenyl- and 4-phenyl-5-methyl derivatives with 4,5-diphenyl substituted **4** revealed an enhanced inhibitory potency in the latter case. Therefore, we have prepared a new series of isothiazol-3(2*H*)-one 1,1-dioxides exclusively with three (substituted) phenyl groups at positions-2, -4 and -5 and analyzed their inhibition of HLE.



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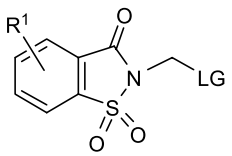

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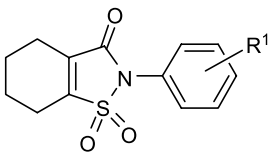

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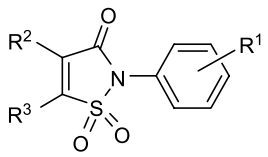

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Material and methods

Chemistry

Melting points were determined on a Boetius micro-melting-point apparatus and are corrected. Infrared spectra (IR) are expressed in cm^{-1} and were obtained on a Genisis FTIR Unicam Analytical System (ATI Mattson) using KBr pellets. ^{1}H Nuclear Magnetic Resonance spectra (NMR) were recorded on Varian Gemini-200 (200 MHz) and Varian Unity-400 (400 MHz) spectrometers. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane as internal standard. ^{19}F NMR spectra (282 MHz) were recorded on the named spectrometers. Electron Impact Mass Spectra (EIMS) were recorded on a Quadrupol-MS VG 12-250, 70 eV.

2-(4-Trifluoromethylphenyl)-4,5-diphenylisothiazolium perchlorate (**7b**) was prepared using the reported procedure [26]. 2-(2,4-Dinitrophenyl)-4,5-diphenylisothiazol-3(2*H*)-one 1,1-dioxide (**9e)** was prepared as reported [25]. The new 2,4,5-triphenylisothiazolium perchlorates **7a**, **c**, **d**, **f-l** were synthesized according to the literature procedure [27]. Yields and melting points are as follows. **7a**: 53%, 183–186°C; **7c**: 58%, 244–248°C; **7d**: 57%, 273–277°C; **7f**: 71%, 172–177°C; **7g**: 68%, 228–234°C; **7h**: 36%, 134–138°C; **7i**: 67%, 208–212°C; **7k**: 57%, 212–218°C; **7l**: 61%, 151–155°C.

*General procedure for the synthesis of 2,4,5-triphenylisothiazol-3(2H)-one 1,1-dioxides (**9**).* Hydrogen peroxide (0.7 ml, 30%) was added to a suspension of **7a–c**, **f–l** (0.26 mmol) in AcOH (0.7 ml). The solution was stirred for 8–72 h at 80°C. After cooling, the 3-oxosultams **9** were isolated and recrystallized from ethanol.

*2-(3-Nitrophenyl)-4,5-diphenylisothiazol-3(2H)-one 1,1-dioxide (**9a**).* Yield: 32%; m.p. 115–118°C; IR ν 1151, 1275 (SO_2), 1340, 1533 (NO_2), 1732 (CO); ^{1}H NMR δ 7.44–7.61 (m, 10H), 7.97 (t, $\mathcal{J} = 8.3$ Hz, 1H), 8.12 (dd, $^3\mathcal{J} = 8.4$, $^4\mathcal{J} = 1.6$ Hz, 1H), 8.46 (dd, $^3\mathcal{J} = 8.3$, $^4\mathcal{J} = 1.5$ Hz, 1H), 8.57 (s, 1H); $C_{21}H_{14}N_2O_5S$ (406.41); EIMS 406 ($M^+\cdot$).

*2-(4-Trifluoromethylphenyl)-4,5-diphenylisothiazol-3(2H)-one 1,1-dioxide (**9b**).* Yield: 60%; m.p. 197–202°C; IR ν 1134, 1290 (SO_2), 1730 (CO); ^{1}H NMR δ 7.40–7.61 (m, 10H), 7.91, 8.01 (2 d, $\mathcal{J}_{AB} = 8.8$ Hz, 4H); ^{19}F NMR δ -63.8; $C_{22}H_{14}F_3NO_3S$ (429.41); EIMS 429 ($M^+\cdot$).

*2-(2-Trifluoromethyl-4-nitrophenyl)-4,5-diphenylisothiazol-3(2H)-one 1,1-dioxide (**9c**).* Yield: 49%; m.p. 176–180°C; IR ν 1182, 1298 (SO_2), 1348, 1539 (NO_2), 1740 (CO); ^{1}H NMR δ 7.44–7.65 (m, 10H), 8.18 (d, $\mathcal{J} = 8.4$ Hz, 1H), 8.74–8.83 (m, 2H); ^{19}F NMR δ -61.3; $C_{22}H_{13}F_3N_2O_5S$ (474.41); EIMS 474 ($M^+\cdot$).

*2-(2-Chloro-4-nitrophenyl)-4,5-diphenylisothiazol-3(2H)-one 1,1-dioxide (**9d**).* By following the General procedure, a mixture containing **9d** and the respective hydroperoxide **8d** was isolated in this case. The mixture was dissolved in ethanol (4 ml) and conc.

HCl (0.3 ml) was added. It was refluxed for 8 h. After cooling, the corresponding 3-oxosultam **9d** was isolated by filtration and recrystallized from ethanol. Yield: 58%; m.p. 180–185°C; IR ν 1151, 1302 (SO_2), 1344, 1531 (NO_2), 1736 (CO); ^{1}H NMR δ 7.50–7.70 (m, 10H), 8.10 (d, $\mathcal{J} = 8.7$ Hz, 1H), 8.52 (dd, $^3\mathcal{J} = 8.6$, $^4\mathcal{J} = 2.4$ Hz, 1H), 8.65 (d, $\mathcal{J} = 2.4$ Hz, 1H); $C_{21}H_{13}ClN_2O_5S$ (440.86); EIMS 440 ($M^+\cdot$).

*5-(4-Methoxyphenyl)-2-(3-nitrophenyl)-4-phenylisothiazol-3(2H)-one 1,1-dioxide (**9f**)*. Yield: 53%; m.p. 146–150°C; IR ν 1147, 1263 (SO_2), 1338, 1531 (NO_2), 1728 (CO); ^{1}H NMR δ 3.86 (s, 3H) 7.02, 7.07 (2 d, $\mathcal{J}_{AB} = 9.2$ Hz, 4H), 7.45–7.57 (m, 5H), 7.95 (t, $\mathcal{J} = 8.0$ Hz, 1H), 8.10 (d, $\mathcal{J} = 8.0$ Hz, 1H), 8.45 (d, $\mathcal{J} = 8.0$ Hz, 1H), 8.55 (s, 1H); $C_{22}H_{16}N_2O_6S$ (436.44); EIMS 436 ($M^+\cdot$).

*5-(4-Methoxyphenyl)-2-(4-nitrophenyl)-4-phenylisothiazol-3(2H)-one 1,1-dioxide (**9g**)*. Yield: 13%; m.p. 79–82°C; IR ν 1142, 1292 (SO_2), 1340, 1520 (NO_2), 1730 (CO); ^{1}H NMR δ 3.92 (s, 3H), 7.09, 7.11 (2 d, $\mathcal{J}_{AB} = 9.2$ Hz, 4H), 7.48–7.64 (m, 5H), 8.04, 8.55 (2 d, $\mathcal{J}_{AB} = 8.3$ Hz, 4H); $C_{22}H_{16}N_2O_6S$ (436.44); EIMS 436 ($M^+\cdot$).

*2-(4-Trifluoromethylphenyl)-5-(4-methoxyphenyl)-4-phenylisothiazol-3(2H)-one 1,1-dioxide (**9h**)*. Yield: 72%; m.p. 75–80°C; IR ν 1134, 1294 (SO_2), 1723 (CO); ^{1}H NMR δ 3.88 (s, 3H), 7.04, 7.07 (2 d, $\mathcal{J}_{AB} = 9.1$ Hz, 4H), 7.46–7.60 (m, 5H), 7.92, 8.01 (2 d, $\mathcal{J}_{AB} = 8.8$ Hz, 4H); ^{19}F NMR δ -63.8; $C_{23}H_{16}F_3NO_4S$ (459.44); EIMS 459 ($M^+\cdot$).

*2-(2-Chloro-4-nitrophenyl)-5-(4-methoxyphenyl)-4-phenylisothiazol-3(2H)-one 1,1-dioxide (**9i**)*. Yield: 35%; m.p. 141–143°C; IR ν 1180, 1302 (SO_2), 1344, 1529 (NO_2), 1734 (CO); ^{1}H NMR δ 3.93 (s, 3H), 7.10, 7.12 (2 d, $\mathcal{J}_{AB} = 9.0$ Hz, 4H), 7.50–7.64 (m, 5H), 8.09 (d, $\mathcal{J} = 8.4$ Hz, 1H), 8.52 (dd, $^3\mathcal{J} = 8.4$, $^4\mathcal{J} = 2.4$ Hz, 1H), 8.64 (d, $\mathcal{J} = 2.4$ Hz, 1H); $C_{22}H_{15}ClN_2O_6S$ (470.88); EIMS 470 ($M^+\cdot$).

*5-(4-Methoxyphenyl)-2-(2,4-dinitrophenyl)-4-phenylisothiazol-3(2H)-one 1,1-dioxide (**9k**)*. Yield: 37%; m.p. 98–105°C; IR ν 1153, 1300 (SO_2), 1346, 1541 (NO_2), 1734 (CO); ^{1}H NMR δ 3.89 (s, 3H), 7.05, 7.11 (2 d, $\mathcal{J}_{AB} = 9.0$ Hz, 4H), 7.41–7.60 (m, 5H), 8.26 (d, $\mathcal{J} = 8.7$ Hz, 1H), 8.89 (dd, $^3\mathcal{J} = 8.9$, $^4\mathcal{J} = 2.6$ Hz, 1H), 9.08 (d, $\mathcal{J} = 2.7$ Hz, 1H); $C_{22}H_{15}N_3O_8S$ (481.44); EIMS 481 ($M^+\cdot$).

*2-(2,5-Dichloro-4-isopropyloxyphenyl)-5-(4-methoxyphenyl)-4-phenylisothiazol-3(2H)-one 1,1-dioxide (**9l**)*. Yield: 53%; m.p. 88–92°C; IR ν 1180, 1305 (SO_2), 1732 (CO); ^{1}H NMR δ 1.47 (d, $\mathcal{J} = 6.0$ Hz, 6H), 3.88 (s, 3H), 4.97 (sept, $\mathcal{J} = 6.0$ Hz, 1H), 7.09, 7.12 (2 d, $\mathcal{J}_{AB} = 9.0$ Hz, 4H), 7.42–7.62 (m, 5H), 7.76 (s, 1H), 7.79 (s, 1H); $C_{25}H_{21}Cl_2NO_5S$ (518.41); EIMS 518 ($M^+\cdot$).

Enzyme inhibition assay

Human leukocyte elastase (HLE) prepared from human leukocytes and purified by affinity chromatography using an immobilized synthetic inhibitor [28] was available from a previous study [29]. MeOSuc-Ala-Ala-Pro-Val-pNA was purchased from Bachem, Heidelberg, Germany.

Inhibition of HLE by compounds **9a**–**l** was assayed spectrophotometrically on a Varian Cary 100 spectrophotometer with a multi-cell holder at 25°C. Inhibitor stock solutions were prepared in DMSO. A stock solution of the chromogenic substrate was prepared in DMSO and diluted with assay buffer. Assay buffer was 50 mM sodium phosphate buffer, 500 mM NaCl, pH 7.8. The entire volume of the assays was 1 ml containing 1.5% DMSO. Final concentration of the chromogenic substrate MeOSuc-Ala-Ala-Pro-Val-pNA was 100 μM = 1.9 × K_m, unless stated otherwise. Assays were performed with a final HLE concentration of 50 ng/ml, which corresponded to an initial rate of 0.6 μM/min. Reactions were initiated by addition of 50 μl of an HLE solution and monitored over 20 min at 405 nm.

The stability of selected inhibitors under assay conditions was assessed. Compounds **9g** and **9l** were incubated at 25°C in assay buffer, pH 7.8, 1.5% DMSO. UV spectra were recorded at 5 min intervals over 75 min. A decrease in absorbance at 369 nm (i.e. at the absorption maximum of **9g** and **9l**) was not observed and spectra remained unchanged (data not shown).

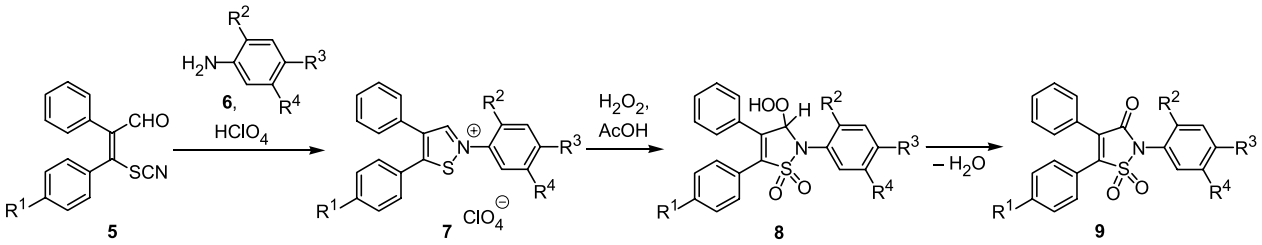
Results and discussion

Synthesis

The synthetic route to isothiazol-3(2*H*)-one dioxides **9** is shown in Scheme 1, and detailed information on these reactions are given elsewhere [27,30,31]. Isothiazolium salts **7** were obtained from the reaction of thiocyanates **5** with the substituted anilines **6**. Perchlorates **7** were oxidized with hydrogen peroxide in glacial acetic acid at 80°C to give the 3-oxosultams **9** *via* the intermediate hydroperoxides **8**. In one case, a mixture of the corresponding hydroperoxide (**8d**) and the 3-oxosultam (**9d**) was isolated and subsequently converted to **9d**.

Enzyme inhibiting activities

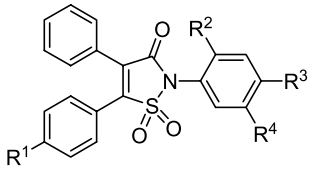
The newly synthesized compounds **9** were investigated as inhibitors of HLE. The enzyme-catalyzed cleavage of the chromogenic substrate MeOSuc-Ala-Ala-Pro-Val-pNA (initial concentration 100 μM) was followed by monitoring the release of *p*-nitroaniline. Progress curves indicated time-dependent inhibition and were analyzed as first-order reactions. The second-order rate constants of inhibition, k_{obs}/[I], are given in Table I. The values were obtained from quadruplicate



Scheme 1. General synthesis of (**9**).

measurements at a single inhibitor concentration of 2 μM. Within the group of 5-*p*-methoxyphenyl derivatives (**9f-l**), 2,5-dichloro-4-isopropyloxy substitution at the *N*-phenyl moiety (in compound **9l**) was advantageous compared to the substitution pattern of **9f**–**k**. Among all the compounds of this series, **9l** showed the strongest inhibition of HLE. The analogously substituted isothiazol−3-one 1,1-dioxide with a phenyl ring at position 5 (**9**, $R^1 = H$, $R^2 = R^4 = Cl$, $R^3 = O$-*i*-Pr) was the most effective inhibitor of HLE from a series of 3-oxosultames reported recently. This compound exhibited a $k_{obs}/[I]$ value of 2400 $M^{-1}s^{-1}$ determined at an inhibitor concentration of 8 μM [25]. The data presented herein confirm our initial conclusions on the structure–activity relationships of 2-aryl-isothiazol-3(2*H*)-one 1,1-dioxides insofar as (i) 4,5-diaryl substitution always led to at least moderately active compounds, and (ii) 2,5-dichloro-4-isopropyloxy substitution at the 2-phenyl ring was advantageous. However, introduction of the methoxy group (R^1) into the 5-phenyl ring did not remarkably change inhibitory potency (**9a** *versus* **9f**, **9b** *versus* **9h**, **9d** *versus* **9i, 9e** *versus* **9k**).

Table I. Inhibition of human leukocyte elastase by 2,4,5-triphenylisothiazol-3(2*H*)-one 1,1-dioxides **9**.



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Compound	R^1	R^2	R^3	R^4	$k_{obs}/[I]$ at 2 μM ($M^{-1}s^{-1}$)⋆
9a	H	H	H	NO_2	324
9b	H	H	CF_3	H	191
9c	H	CF_3	NO_2	H	243
9d	H	Cl	NO_2	H	428
9e	H	NO_2	NO_2	H	465
9f	OMe	H	H	NO_2	725
9g	OMe	H	NO_2	H	349
9h	OMe	H	CF_3	H	225
9i	OMe	Cl	NO_2	H	603
9k	OMe	NO_2	NO_2	H	556
9l	OMe	Cl	O-*i*-Pr	Cl	2440

⋆ Progress curves were analyzed as first-order reactions in the presence of an inhibitor concentration [I] of 2 μM. k_{obs} is the-first-order rate constant of inhibition. The values are means of quadruplicate determinations. For compound **9e** at 8 μM, a $k_{obs}/[I]$ values of 880 $M^{-1}s^{-1}$ was obtained [25].

Kinetic investigations

In a recent report, we have observed that a linear correlation of the first-order rate constant of inhibition, k_{obs}, *versus* the inhibitor concentration, [I], was not attained in the case of trisubstituted isothiazol-3-one 1,1-dioxides [25]. This was attributed to a complex mode of interaction and prompted us to perform additional kinetic measurements. The dependence of the HLE inhibition on the substrate concentration, [S], was investigated with compound **9g** as example. Six different concentrations of MeOSuc-Ala-Ala-Pro-Val-pNA in the range of 25–250 μM and three different concentrations of **9g** were used. Progress curves were analyzed as first-order reactions to obtain k_{obs} values, as well as the concentration of product formed at time approaching infinity, $[P_\infty]$. At inhibitor concentrations of 2 μM as well as 4 μM, a significant dependence of the k_{obs} values on the substrate concentration was not found. With an inhibitor concentration of 6 μM, k_{obs} values decreased with increasing substrate concentrations. A plot of reciprocal k_{obs} values *versus* [S] is depicted in Figure 1. For enzyme modifiers it has been described by Tian and Tsou [32] that the type of inhibition can be distinguished on the basis of the effects of different substrate concentrations on the inhibition rate constant and on the product concentration at time approaching infinity. A kinetic model from this report [32] was simplified according to irreversible inhibition (Scheme 2) and used for the derivation of Equations (1) and (2).

The apparent inhibition rate constant, k, is a second-order rate constant defined by Equation (1),

$$k = \{k_0 + ([S]k_0'/K_m)\}/\{1 + [S]/K_m\} \qquad (1)$$

where k_0 is the second-order rate constant of the irreversible reaction of the enzyme with the inhibitor, k_0' is the second-order rate constant of the irreversible reaction of the Michaelis complex, ES, with the

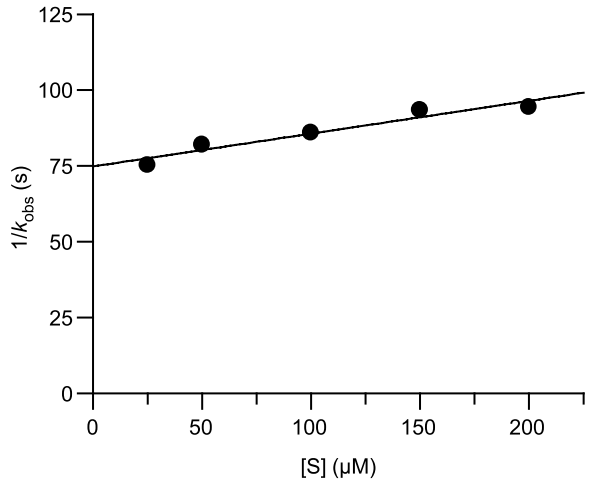


Figure 1. Plot of reciprocal k_{obs} values *versus* concentrations of MeOSuc–Ala–Ala–Pro–Val–pNA. HLE-catalyzed hydrolyses in the presence of **9g** (6 μM) and different substrate concentrations, [S], were analyzed as first-order reactions. Data are mean values of duplicate experiments.

inhibitor, and K_m is the Michaelis-Menten constant. The relevant relationship for the product concentrations at time approaching infinity, $[P_\infty]$, is given by Equation (2),

$$[P_\infty] = V[S]/\{k_0[I]K_m + k_0'[I][S]\} \quad (2)$$

where V is the maximum velocity.

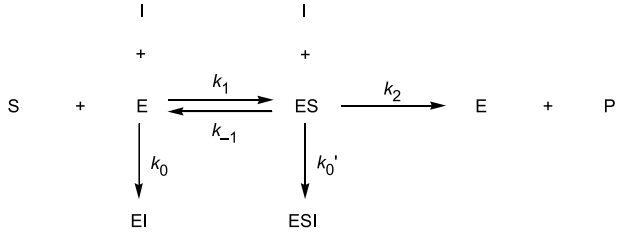
Considering Equation (1) and $k = k_{obs}/[I]$, for competitive inhibition where $k_0' = 0$, a plot of reciprocal k_{obs} values *versus* [S] would be a straight line with a vertical intercept of $1/(k_0[I])$. For noncompetitive inhibition it was defined that $k_0 = k_0'$, and reciprocal k_{obs} values would then be independent of [S]. Our data obtained with compound **9g** at a concentration of 6 μM therefore refers to a competitive mode of inhibition.

However, a more complex interaction could be concluded from the plots of reciprocal $[P_\infty]$ values *versus* reciprocal substrate concentrations, based on Equation (2). This plot can also be used to ascertain the type of inhibition in the presence of a modifying agent [32]. In the case of competitive inhibition, a straight line would pass through the origin, whereas in the case of a noncompetitive inhibition, the plot would be a straight line with a vertical intercept of $(k_0[I])/V$. Using inhibitor **9g** at concentrations of 2 μM, 4 μM (data not shown), and 6 μM (Figure 2), a linear dependence arose, and vertical intercepts indicated a noncompetitive mode of inhibition. Comparable results were obtained with **9** ($R^1 = R^2 = R^4 = H$, $R^3 = NO_2$) [25]. However it should be noted, that the kinetic model applied here is based on the assumption of only two modified enzyme complexes. These complexes are defined to be generated from the reaction of the inhibitor with either the free enzyme or the Michaelis-Menten complex, ES, with constants, k_0 and k_0', respectively. A mixed type of inhibition would result from different rate constants and, moreover, binding of the inhibitor at one binding site might affect the binding at a postulated second site.

The interdependence of k_{obs} values and the concentration of inhibitor **9g** is given in Figure 3. An equation was used that governs the transition of the exponential dependency of k_{obs} and [I] to a linear one;

$$k_{obs} = k[I]\{1 - \exp(-a[I])\} \quad (3)$$

In Equation (3), k is the second-order rate constant for inhibition, as it can be taken from the linear part of the plot, and a is an exponent to correlate the dependency in the exponential part of the plot. Nonlinear regression according to Equation (3) gave $k = 2100\ M^{-1}s^{-1}$, a result that was explicitly different from the value ($349\ M^{-1}s^{-s1}$) obtained with the single inhibitor concentration of 2 μM (Table I). It can easily be seen, that a linear dependency was only achieved when higher inhibitor concentrations were used. A similar interdependency of k_{obs} on [I] was determined for compound **9e** and is illustrated in



Scheme 2. Kinetic model for inhibition of HLE by (**9**).

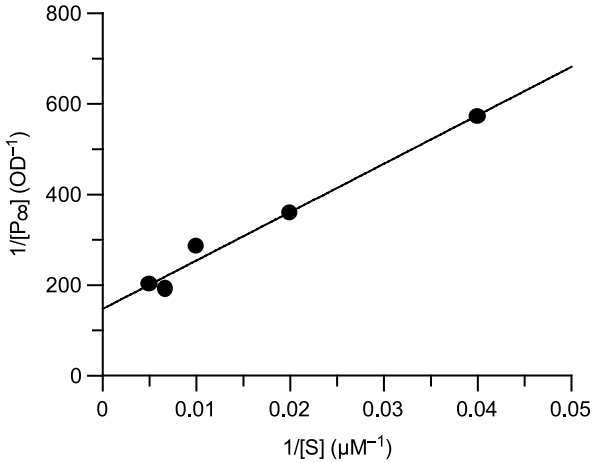


Figure 2. Plot of reciprocal $[P_\infty]$ values *versus* reciprocal concentrations of MeOSuc–Ala–Ala–Pro–Val–pNA. HLE-catalyzed hydrolyses in the presence of **9g** (6 μM) and different substrate concentrations, [S], were analyzed as first-order reactions. Data are mean values of duplicate experiments.

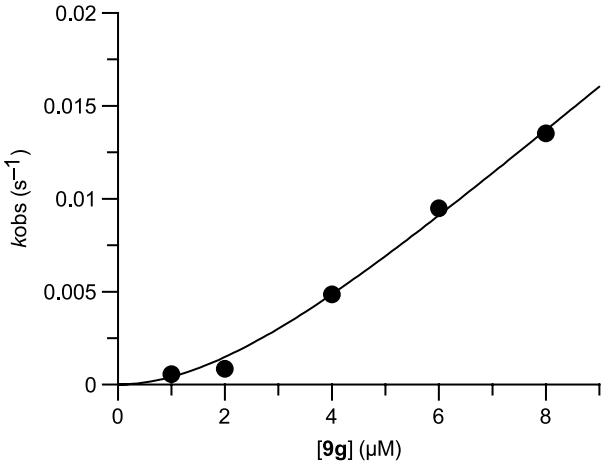


Figure 3. Plot of k_{obs} values *versus* concentrations of inhibitor **9g**. HLE-catalyzed hydrolyses of MeOSuc–Ala–Ala–Pro–Val–pNA (100 μM) were analyzed as first-order reactions. Data are mean values of sextuplicate experiments. Non-linear regression using Equation (3) gave $k = 0.0021\ \mu M^{-1}s^{-1}$ and $a = 0.27\ \mu M^{-1}$.

Figure 4. However, in reactions with irreversible inhibitors, measurements under such conditions (with high inhibitor concentrations) have sometimes been difficult, due to an inadequate number of data points in the initial part of the reaction.

In this study, the inhibitor **9g** was used as an example to characterize the interaction with HLE. The non-linear dependence between k_{obs} and [I] is assumed to be the reason for the failure of a clear interpretation of the kinetic results with different substrate concentrations. Further investigations are needed to ascertain whether this kinetic behavior probably reflects different binding sites of HLE for triarylisothiazol-3-one 1,1-dioxides.

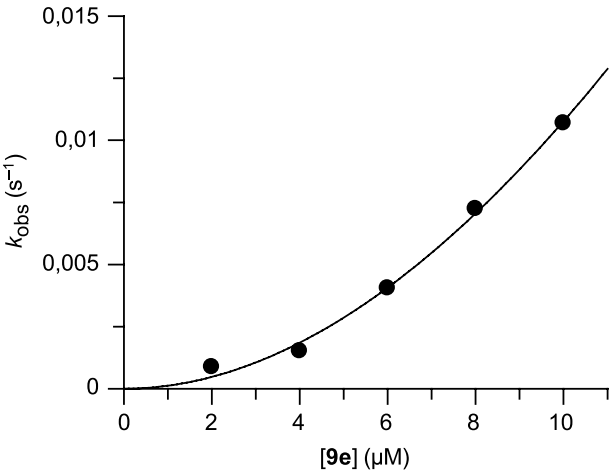


Figure 4. Plot of k_{obs} values *versus* concentrations of inhibitor **9e**. HLE-catalyzed hydrolyses of MeOSuc–Ala–Ala–Pro–Val–pNA (100 μM) were analyzed as first-order reactions. Data are mean values of duplicate experiments. Non-linear regression using Equation (3) gave $k = 0.0049\ \mu M^{-1}s^{-1}$ and $a = 0.025\ \mu M^{-1}$.

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