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Laboratory note

New prodrug approach for amino acids and amino-acid-like drugs

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Summary — A series of disubstituted tetrahydro-2*H*-1,3,5-thiadiazine-2-thione (THTT) derivatives **4a–g** were prepared and found to be promising prodrugs for amino acids and similar compounds. The pH profile for the degradation of the THTT derivatives in aqueous buffer solutions was determined using HPLC and was accounted for in terms of specific base-catalyzed reactions. The compounds, however, showed high acid stability. Enzymatic hydrolysis (human serum) of the derivatives offered an advantageous range of $t_{1/2}$ values, which may be useful in controlling the onset and the duration of action of drugs.

tetrahydro-2*H*-1,3,5-thiadiazine-2-thione / primary amine / amino acid / glycine / prodrug / lipophilicity / drug delivery / first-order hydrolysis / human serum / half life

Introduction

In an attempt to explore new, generally applicable, biolabile prodrugs as drug delivery systems possessing a high lipid solubility and enzymatic rate of hydrolysis, we recently developed the model tetrahydrothiadiazine-2-thione (THTT) [1], which is suitable for primary-amine-containing drugs. Amino acids and related drugs have previously been included in different forms of prodrug targets especially as esters and amides [2–13]. The chemical stability and enzymatic lability of those targets have been studied by measuring the rates of hydrolysis at different pHs of the prodrug forms and by enzymes. The purpose of the present work was to show that the new THTT target is a good model for amino acids and related drugs. Besides being remarkably lipophilic, THTT is highly vulnerable for both chemical and enzymatic hydrolysis. An HPLC study evidenced the concomitant appearance and the complete liberation of glycine, the amino acid used in the model targets. The different alkyl-, cycloalkyl-, and aralkylamines used in the model afforded a wide range of $t_{1/2}$ values which helps the choice of one or more in a mixture of targets for the required onset and duration of action of drugs.

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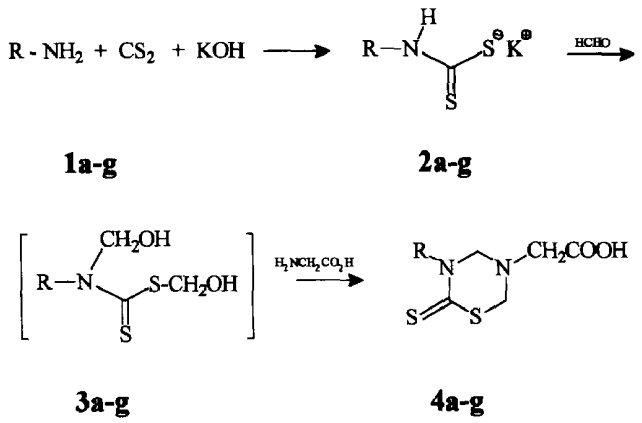
Abbreviations: THTT: tetrahydro-2*H*-1,3,5-thiadiazine-2-thione; HPLC: high performance liquid chromatography; DDS: drug delivery system; RP: reversed phase; SGF: simulated gastric fluid.

Results and discussion

Chemistry

Primary amines **1a–g** were allowed to react with carbon disulfide in the presence of KOH to form their corresponding potassium dithiocarbamate derivatives **2a–g**. Addition of formalin to the formed dithiocarbamates resulted in the formation of compounds **3a–g** (in situ), which were allowed to react with the amino group of glycine in a slightly alkaline medium (phosphate buffer, pH 7.8) to form the THTT derivatives **4a–g** (scheme 1, table I). The structures of the prepared compounds were verified on the basis of elemental analyses and spectroscopic methods. In the IR spectra, **4a–g** showed the characteristic stretching absorption of COOH in the range 3480–3340 cm^{-1} (free and hydrogen-bonded OH), of C=O at 1710–1720 cm^{-1} and of C=S at 1150–1155 cm^{-1}. In the ^{1}H-NMR spectra, the synthesized derivatives showed the C-4 and C-6 methylenes as one singlet integrating for four protons in derivatives **4a–e**, but separated as two singlets each of two protons in **4f,g**. There was no interaction between the C-2 and C-4 protons. In addition, there was no restricted rotation of the substituents about the N3-R sigma bond evidenced by the



Scheme 1. In formulae: a) R = CH_3; b) R = CH_3CH_2; c) R = $CH_3CH_2CH_2$; d) R = *n*-C_4H_9; e) R = cyclo-C_6H_{11}; f) R = $C_6H_5CH_2$; g) R = $C_6H_5CH_2CH_2$.

typical ethylenic coupling in **4g** of N3-β-CH_2 and N3-α-CH_2 as shown by ^{1}H-NMR (table II).

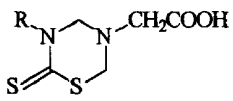
Estimation of the lipophilicity

The use of R_M values from reversed-phase thin-layer chromatography (RP-TLC) as an alternative lipophilicity parameter had been extensively reviewed [14–16]. The experimental determination of R_M values offers several advantages over the measurement of partition coefficients. The RP-TLC technique is rapid and accurate, withstands the use of impure substances, and does not require extensive laboratory experience; it is therefore a highly suitable technique for obtaining high quality correlations [17]. Accordingly, the lipophilicity of the target derivatives was estimated, and the obtained results generally showed a significant improvement in the lipophilicity of the synthesized compounds compared with the parent (table I). A strong linear relationship was observed between the measured R_M and the calculated log P values of these derivatives according to an equation prosposed by Moriguchi et al [18] (r = 0.933, n = 7). Such a relationship is slightly changed for glycine in the linear equation (r = 0.919, n = 8). The slopes, however, were 5.160 and 2.204, respectively, which indicates that the lipophilicity is greatly affected by the presence of THTT moiety or, in other words, reflects the enhanced effect of the THTT moiety on the lipophilicity of the parent drug.

Kinetics of hydrolysis

The kinetics of hydrolysis of the synthesized compounds were studied in aqueous buffer solutions at 37 °C over the pH values of 1.2, 7.4 and 9.0 (μ = 0.5). At constant pH and temperature, the disappearance of THTT derivatives displayed strict first-order kinetics over several half-lives (table III). Representative examples of first-order plots are shown in figure 1. The influence of pH on the hydrolysis rate is shown in figure 2, where the logarithms of the observed pseudo-first-order rate constants (K_{obs}) are plotted against pH. The shape of the pH–rate profile indicates that the hydrolysis can be described in terms of specific base-catalyzed reactions. The rate data obtained for the various derivatives also showed that these

Table I. Physicochemical data of the synthesized THTT derivatives.



Compound	*R*	*Yield (%)*	*Mp (°C)*	*Formula*	*Elemental analysis*[a]	*RM*	*Calculated log P*
4a	CH_3	45	124	$C_6H_{10}N_2O_2S_2$	C, H, N, S	–0.327	–2.331
4b	C_2H_5	66	132	$C_7H_{12}N_2O_2S_2$	C, H, N, S	–0.231	–1.998
4c	C_3H_7	70	145	$C_8H_{14}N_2O_2S_2$	C, H, N, S	–0.140	–1.644
4d	C_4H_9	72	127	$C_9H_{16}N_2O_2S_2$	C, H, N, S	–0.126	–1.327
4e	C_6H_{12}	81	153	$C_{11}H_{18}N_2O_2S_2$	C, H, N, S	–0.105	–1.124
4f	$C_6H_5CH_2$	88	142	$C_{12}H_{14}N_2O_2S_2$	C, H, N, S	–0.158	–1.358
4g	$C_6H_5C_2H_4$	85	139	$C_{13}H_{16}N_2O_2S_2$	C, H, N, S	–0.122	–1.274

The R_M value of glycine was found at –0.95 under the same experimental conditions, and its calculated log P = –3.128. [a]All elemental analyses (C, H, N, S) were satisfactory within ± 0.5% of the calculated values.

Table II. ^{1}H-NMR spectral data of 3,5-disubstituted tetrahydro-2*H*-1,3,5-thiadiazine-2-thione derivatives.

Compound	*R*	*Chemical shifts (δ-values, ppm) in DMSO-d_6, J (Hz)*				
		4-CH_2	*4-CH_2 and 6-CH_2*	*6-CH_2*	*N^5-CH_2CO*	*N^3-R*
4a	CH_3		4.66 (4H, br, s)		3.70 (2H, s)	3.52 (3H, s, CH_3)
4b	C_2H_5		4.68 (4H, br, s)		3.62 (2H, s)	1.25 (3H, t, J = 7.6, 7.8, CH_2-CH_3), 4.08 (2H, q, J = 7.5, CH_2 CH_3)
4c	*n*-C_3H_7		4.63 (4H, br, s)		3.69 (2H, s)	1.00 (3H, t, J = 7.5, propyl CH_3), 1.75 (2H, m, N^3-$CH_2CH_2CH_3$), 3.98 (2H, t, J = 7.5, N^3-CH_2-CH_2CH_3)
4d	*n*-C_4H_9		4.58 (4H, br, s)		3.60 (2H, s)	0.88 (3H, t, J = 7.4, butyl CH_3), 1.51 (4H, br, m, N^3-CH_2CH_2-CH_2CH_3), 3.96 (2H, t, J = 7.6, N^3-CH_2)
4e	cyclo-C_6H_{11}		4.54 (4H, br, s)		3.62 (2H, s)	1.20–2.10 (10H, br, m, cyclohexyl methylene groups), 5.39 (1H, br, m, N^3-CH methine)
4f	$C_6H_5CH_2$	4.68 (2H, s)		4.56 (2H, s)	3.52 (2H, s)	5.40 (2H, s, N^3-$CH_2C_6H_5$), 7.47 (5H, s, benzyl C_6H_5)
4g	$C_6H_5(CH_2)_2$	4.53 (2H, s)		4.51 (2H, s)	3.56 (2H, s)	3.00 (2H, t, J = 8.3, N^3-β-CH_2), 4.21 (2H, t, J = 8.4, N^3-α-CH_2), 7.49 (5H, s, phenethyl C_6H_5)

s: singlet, t: triplet, m: multiplet, br: broad.

compounds are quite stable in acidic media and the stability is significantly reversed in alkaline solutions. Reversion of glycine as a drug molecule from these targets was confirmed using HPLC by matching the retention time of the reaction products at different pHs with that of the authentic glycine at 218 nm. The susceptibility of THTT (**4a–g**) to undergo enzymatic

Table III. Rate data for the hydrolysis of the synthesized THTT in aqueous buffer solutions and in 80% human plasma (pH 7.4) at 37 °C.

Compound	$t_{1/2}$			
	pH			*Plasma*
	1.2	*7.4*	*9*	
4a	13.9 h	19.4 min	8.9 min	41.3 min
4b	16.5 h	69.3 min	16.6 min	1.8 h
4c	18.1 h	72.2 min	19.1 min	2.4 h
4d	19.4 h	99.8 min	28.0 min	2.4 h
4e	6.0 h	48.8 min	38.2 min	56.1 min
4f	17.8 h	33.4 min	7.5 min	41.4 min
4g	20.3 h	43.0 min	13.8 min	42.4 min

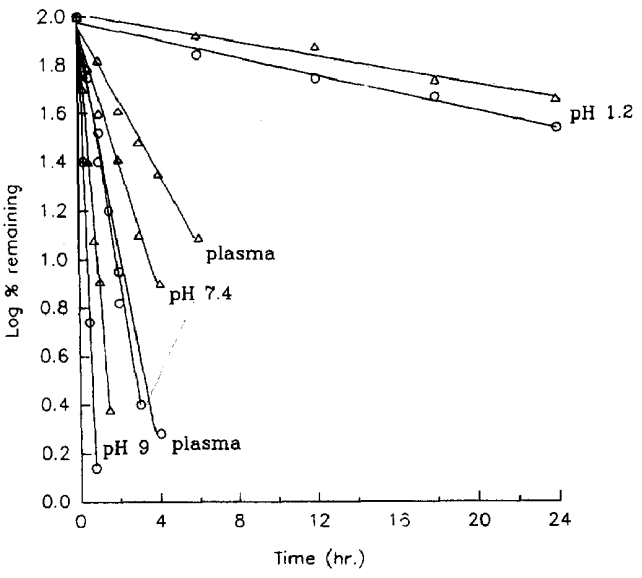


Fig 1. The apparent first-order kinetic plot of the degradation of **4c** (Δ) and **4f** (○) in aqueous buffer solution and in 80% human plasma at 37 °C.

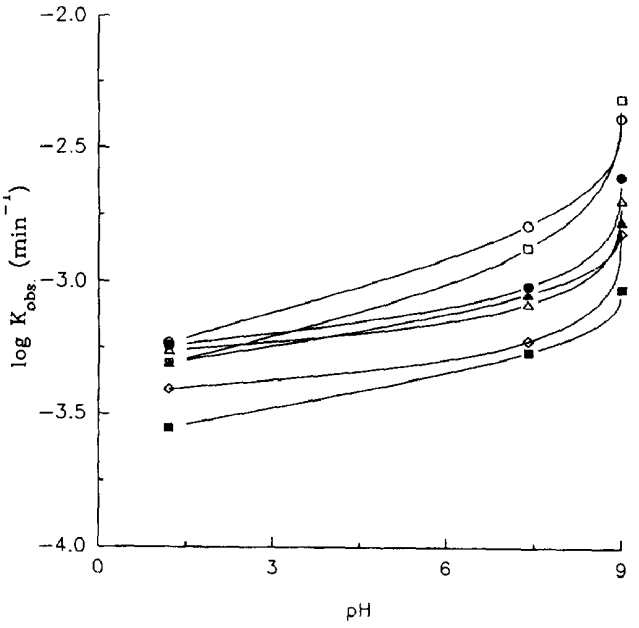


Fig 2. The pH rate profiles for the degradation of the synthesized compounds **4a** (○), **4b** (●), **4c** (Δ), **4d** (▲), **4e** (□), **4f** (■) and **4g** (◇) in aqueous buffer solutions at 37 °C.

hydrolysis was studied in 80% human plasma (pH 7.4) at 37 °C. Under these conditions strict first-order kinetics were observed (fig 1). The half-lives observed for the tested compounds were different from those in pH 7.4 buffer solution (table III) indicating the occurrence of plasma catalysis. However, they exhibited more tendency to chemical alkaline hydrolysis than enzymatic hydrolysis. Liberation of glycine (the drug representative) was also detected by enzymatic hydrolysis. The mechanism of the hydrolysis of THTT was previously reported to occur via ring cleavage at N5 of the THTT structure [1].

It can be concluded from these experiments that the kinetic data and the measured lipophilic parameters demonstrate a potential utility of the THTT model as a drug delivery system (DDS) for amino acids or amino-acid-like drugs, which enhances their cellular uptake by improving lipophilicity in the area where the drug molecule is released by the physiological and/or enzyme catalytic effects. Another advantage of THTT derivatives is their stability in simulated gastric fluid (SGF) (pH 1.2), which facilitate their stomach absorption in a less ionized form in the case of oral administration. Finally, the range of $t_{1/2}$ values offered by the supplied targets in enzymatic and chemical hydrolysis makes the drug release controllable. In other words, the onset and duration of the drugs can be controlled by the choice of the N3-substituent.

Experimental protocols

Precoated silica-gel 60 F-254 plates (Merck) were used for TLC spots were detected by ultraviolet light and/or staining with iodine vapor. Melting points were determined on an electrothermal melting point apparatus (Fa, Stuart Scientific, UK), and were uncorrected. ^{1}H-NMR spectra were determined on an EM-60 Varian spectrometer in DMSO-d_6, using TMS as an internal standard and the chemical shifts were given in δ ppm. Exchangeable protons were confirmed with D_2O. IR spectra were recorded (KBr discs) on a Shimadzu-408 spectrophotometer. Elemental analyses (C, H, N, and S) were performed at the Department of Chemistry, Faculty of Science, Assiut University.

An HPLC system consisting of a pump (Knauer HPLC pump 64, Germany), a variable-wavelength detector (Knauer), a reversed-phase HPLC column (stainless steel (25 × 0.5 cm id) C-18 Eurospher 80), a Shimadzu C-R 6A chromatopac recording integrator, and a 20 μL injection loop were used. Mobile phase systems of acetonitrile, water and 1% phosphoric acid (85%) were used and the ratio of acetonitrile/water was adjusted in order to give a retention time of 3.5–5 min. The column effluent was monitored at 258 nm and the flow rate was 1 mL/min. All chemicals were of commercial grade except the HPLC solvents and the buffer reagents (analytical grade).

*5-(Carboxymethyl)-3-substituted tetrahydro-2H-1,3,5-thiadiazine-2-thione **4a–g**. General procedure*

Carbon disulfide (60 mmol) was added to a stirred mixture of the appropriate alkyl-, cycloalkyl- or aralkylamine **1a–g** (10 mmol) and potassium hydroxide (20%, 10 mmol) in ethanol (10 mL); stirring was continued for 3 h. To the reaction mixture, which now contained the dithiocarbamates **2a–g**, formaldehyde solution (35%, 22 mmol) was added and the stirring was continued for 1 h further. The resulting clear solution obtained (**3a–g**) was added portionwise over 15 min to a stirred solution of glycine (10 mmol) in phosphate buffer (pH 7.8, 20 mL). After stirring for 4 h at ambient temperature, the reaction mixture was acidified with dilute hydrochloric acid (5%, ~15–18 mL) to pH 2. Methylene chloride (50 mL) was added and stirring was continued for further 30 min. The precipitate formed was collected by filtration, washed with 0.5% hydrochloric acid and dried. The organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude solid collected was crystallized from ethanol/chloroform to afford **4a–g**. Yields, melting points, and physical and spectral data are given in tables I and II.

Determination of R_M values of the synthesized derivatives

Silica-gel TLC plates (20 × 20) were soaked for 5 h in acetone containing 3% *n*-octanol, and left to dry overnight. Three spots of 5 μL of each compound solution (1 mg/mL) were loaded at 1.5 cm intervals. The compounds were allowed to develop by an ascending technique in a chromatographic tank under conditions of equilibrium using a mobile phase of aqueous buffer phosphate solution and acetone (9:1) containing 3% *n*-octanol.

The plates were dried and the developed spots were localized under a UV lamp and/ or by iodine staining. The R_f values were determined for each compound as the average of three readings, and the corresponding R_M values were calculated using the following formula: $R_M = \log (1/R_f - 1)$. Data are given in table I.

Kinetic measurements

The degradation rates of the synthesized derivatives **4a–g** in aqueous solutions of isotonic phosphate buffer pH 7.4, SGF

without enzyme pH 1.2, and in glycine/NaOH buffer pH 9, were determined at 37 °C. The ionic strength of the prepared buffer solutions was adjusted to $\mu = 0.5$ with NaCl. The reactions were initiated by adding 25 μL of the stock methanolic solution of the derivatives (1 mg/mL) to 2.5 mL of preheated buffer solutions in screw-capped test tubes. At appropriate intervals, samples were taken and chromatographed. Pseudo-first-order rate constants for the degradation were obtained from the slopes of linear plots of the logarithm of residual derivative against time as the average of three experiments for each compound.

The degradation of these derivatives was also studied at 37 °C in isotonic buffer of pH 7.4 containing 80% human plasma. At appropriate times, samples of 50 μL were withdrawn and mixed with 50 μL of acetonitrile for deproteinization and centrifuged at 10 000 rpm for 5 min; 20 μL of the clear supernatant was analyzed by HPLC as described above. The resulting data are given in table III.

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