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Tetrahydro-2H-1,3,5- thiadiazin-2-thione Derivatives of The optical Isomers of Phenylalanine, Synthesis, Comparative Stability Study and Antifungal Activity

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Abstract

In order to investigate the effect of the optical properties of the 5-substituent on the stability and the antifungal activity of tetrahydro-2H-1,3,5-thiadizine-2-thione (THTT) moiety, optical isomers and racemic mixture of phenylalanine were incorporated in the 5th position of THTT to afford derivatives 2a-g. Chemical and enzymatic stability of these derivatives were studied *in vitro* in aqueous buffer solution of pH 7.4, physiological pH, and 80% human plasma at 37°C using HPLC. The chemical and enzymatic degradation rates of the tested compounds revealed that the optical properties of the 5-substituent of THTT moiety have no role on their stability at the investigated media. However, the chemical nature of the 3-substiuents has a significant effect on their chemical and enzymatic liability as 3-aralkyl derivatives, 2f and 2g , were the least stable compounds under the investigation conditions. Solid state stability of these derivatives was studied using Differential Scanning Calorimetry (DSC). In spite of the distinguished DSC curves of the racemic compounds from that of the corresponding single optical isomers no constant pattern of their thermal stability was observed. The antifungal activity of 2a-g, was investigated *in vitro* against *Candida albicans, C. parasilosis* and *C. stellatoidea* using tube dilution method. No role for the optical properties of the tested compounds on their antifungal activity was observed. The utmost antifungal activity revealed by compound 2g which has 3-phenethyl substituent. Moreover, 2g has the highest lipophilicity and the most susceptible compound for both chemical and enzymatic degradations.

Key words: Tetrahydro-2H-1,3,5-thiadizine-2-thione, Phenylalanine, Optical isomers, Degradation kinetics, Solid state stability, Antifungal activity.

1. Introduction

Compounds carrying the tetrahydro-2H-1,3,5-thiadizine-2-thione moiety (THTT) have been reported to exhibit antibacterial[1-8], antifungal[1,2,4-6,8-10], antiviral[1,2,11,12] and tuberculostatic activities[4,13,14]. Some 3,5-disubstituted-tetrahydro-2H-1,3,5-thiadizine-2-thione derivatives have found applications in

the treatment of dermatomycosis[15] and as soil fungicides[16]. The antimicrobial activity of these compounds has been suggested to be based on isothiocyanates and thiocarbamic acids which are formed by hydrolysis of the THTT ring[17,18] . Structure activity relationships which diverge from related compounds indicate that the polarity of the substituents at ring nitrogens determines the magnitude of antimicrobial action[15,17]. Optimum activities had been obtained in compounds bearing lipophilic groups at the 3^{ed} position and hydrophilic ones at the 5th position[3].

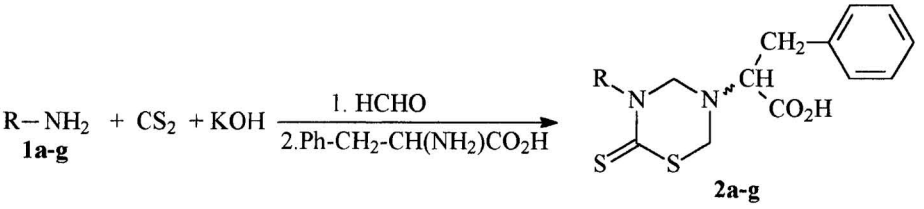
As a part of studies in progress at our laboratories to develop various types of bioreversible derivatives of chemical entities occurring in amino acids with potential antimicrobial activity the present work outlines the incorporation of the optical isomers and racemic mixture of phenylalanine at the 5th position of THTT moiety. This aimed to investigate the effect of optical activity of the substituents at the 5th position of THTT moiety on the chemical and enzymatic degradation, and on the antimicrobial activity. Moreover, the carboxylic group of the phenylalanine moiety at the 5th position of THTT may reduces the toxicity for human cells[19]. Additionally, investigation of the thermal (solid state) stability of these optical isomers seemed to be of interest.

2. Results and Discussion

2.1. Chemistry

The target compounds, 2a-g, were synthesized from appropriate amine, 1a-g, carbon disulfide, formaldehyde and proper optical isomer or racemic mixture of phenylalanine to provide the nitrogen atom at 5th position of thiazidiazine

ring via cyclocondensation reaction, scheme 1 and table 1. Cyclization to the anticipated products was verified on the bases of spectral and elemental methods of analyses.



R= a, C_2H_5; b, C_3H_7; c, iso-C_3H_7; d, C_4H_9; e, cyclo-C_6H_{12}; f, C_6H_5-CH_2; g, C_6H_5-CH_2-CH_2

Scheme 1

The UV spectra show two characteristic absorption maxima near 290 and 250 nm. The IR spectra displayed stretching bands for the carboxylic acid group at the range of 1721-1747 Cm^{-1} and 3450-3565 Cm^{-1} for the carbonyl and hydroxyl functionalities respectively. Furthermore, aliphatic C-H stretching around 2880-2990 Cm^{-1}, aromatic C-H stretching around 3010-3190 Cm^{-1} and C=S stretching around 1476-1515 Cm^{-1}.

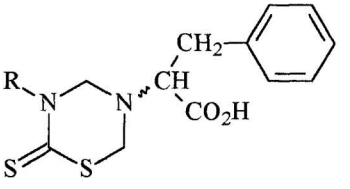
In ^{1}H-NMR spectra with the exception of the N^3-substituents of the THTT moiety the resonance of the remaining sites of the protons of the synthesized derivatives is almost superimposable.

2.2. Lipophilicity

Lipophilicity of the synthesized derivatives, 2a-g, is expressed in the term of their log P values. These values, table 1, were computed with a routine method

called calculated log P (Clog P) contained in a PC-software package described in experimental section. Computation of the log P is based on the fragment method developed by Leo[20]. The enhanced lipophilicity of the synthesized compound may be rendering them more capable of penetrating various biomembrane consequently improving their permeation properties toward microbial cell membrane [21-23].

Table 1: Physicochemical constants of 5-[α-(benzyl)carboxymethyl]-3-substituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione, 2a-g, derivatives.



Compd. No.	R	Molecular Formula [a]	Yield (%)[b]	M.P. (°C)	Clog P [c]
D-2a			72	140	
L-2a	C_2H_5	$C_{14}H_{18}N_2S_2O_2$	75	143	2.519
DL-2a			77	150	
D-2b			70	146	
L-2b	C_3H_7	$C_{15}H_{20}N_2S_2O_2$	68	144-6	3.048
D-2c			65	137	
L-2c	*iso*-C_3H_7	$C_{15}H_{20}N_2S_2O_2$	66	139	2.828
DL-2c			60	141	
D-2d			62	210	
L-2d	C_4H_9	$C_{16}H_{22}N_2S_2O_2$	65	211	3.577
DL-2d			68	214-5	
D-2e			54	155	
L-2e	*Cyclo*-C_6H_{11}	$C_{18}H_{24}N_2S_2O_2$	50	155	3.931
DL-2e			56	157-8	
D-2f			78	138	
L-2f	C_6H_5-CH_2	$C_{19}H_{20}N_2S_2O_2$	75	140	3.630
DL-2f			75	142	
D-2g			70	130	
L-2g	C_6H_5-$(CH_2)_2$	$C_{20}H_{22}N_2S_2O_2$	67	131	3.937
DL-2g			70	135	

[a] Elemental analyses were satisfactory within ± 0.5% of the calculated values.
[b] Crude product.
[c] Clog P value for phenylalanine is −1.556 and the reported log P value is −1.520.[20]

2.3. Kinetic Measurements

It has been reported that the magnitude of activity of THTT derivatives may be attributed to the ease of hydrolysis of the ring system to furnish the isothiocyanates[24]. Accordingly, the degradation kinetics of the synthesized derivatives, 2a-g, were studied in aqueous buffer solution of pH 7.4, physiological pH, at 37°C. At constant temperature disappearance of the tested compounds displayed strict first order kinetic reactions over several half-lives and all reactions proceeded to completion. The rate data obtained for the various derivatives, table 2, revealed that as a general pattern the rate of hydrolysis is unaffected by the optical activity. However, the chemical degradation rates of the synthesized derivatives affected by the variation of substituents on N-3 of the THTT moiety. THTT derivatives bearing phenethyl group at position 3 is the most susceptible for chemical hydrolysis.

The degradation rates of the tested compounds were also investigated in 80% human plasma at 37°C in order to get information about the susceptibility of the synthesized derivatives toward enzymatic metabolism. Strict first order kinetic reactions were also observed with the enzyme system used under the investigated conditions. The enzymatic degradation data, table 2, showed that the liability of the investigated THTT derivatives, 2a-g, toward human plasma enzymes is also unaffected by their optical activity and have the same pattern of the previously reported analogous[14,25,26]. Furthermore, the 3-aralkyl derivatives were more susceptible to the enzymatic degradation.

Table 2: Degradation Kinetic data of the synthesized THTT derivatives, 2a-g, in isotonic physiological media (pH 7.4) and 80% human plasma at 37°C.

Compd. No.	Kx 10^3 min^{-1} ($t_{½}$ h)	
	pH 7.4	plasma
D-2a	1.2527 (09.2)	0.6379 (18.1)
L-2a	1.1441 (10.1)	0.6336 (18.2)
DL-2a	1.1949 (09.7)	0.6476 (17.8)
D-2b	1.0086 (11.5)	0.6043 (19.1)
L-2b	0.9347 (12.4)	0.6224 (18.6)
D-2c	1.0717 (10.8)	0.6379 (18.1)
L-2c	0.9650 (11.9)	0.6263 (18.4)
DL-2c	1.1227 (10.3)	0.6598 (17.5)
D-2d	1.0559 (10.9)	0.8029 (14.4)
L-2d	1.0276 (11.2)	0.7927 (14.6)
DL-2d	1.0732 (10.8)	0.8272 (14.0)
D-2e	0.6879 (16.8)	0.4402 (26.2)
L-2e	0.5833 (19.8)	0.4199 (27.5)
DL-2e	0.7708 (15.0)	0.4887 (23.6)
D-2f	0.9391 (12.3)	1.0817 (10.7)
L-2f	1.0799 (10.7)	0.9600 (12.0)
DL-2f	0.9466 (12.2)	0.9081 (12.7)
D-2g	1.4048 (08.2)	1.3053 (08.8)
L-2g	1.2378 (09.3)	1.3131 (08.8)
DL-2g	1.3344 (08.7)	0.8503 (13.6)

2.4. Solid State Stability Study:

Differential Scanning calorimetry (DSC) can be used for distinguishing optical isomers from the racemic mixtures in the solid state as well as investigation of solid state stability [27]. Accordingly, Solid state stability of the optical isomers and the corresponding racemic mixtures of the derivatives

2a,2c,2f and 2g was studied using DSC. Fig. 1 shows DSC curves as printouts from the instrument at heating rate 10°C/min for the tested compounds. As clear from the fig. racemic compounds have distinctive DSC curves which are characterized from that of the corresponding optical isomers. The melting point of (DL)-compounds is higher than that of single isomers D or L as appeared in Fig. 1. This may be attributed to the efficient packing of the crystals of (DL)-derivatives compared to the corresponding homochiral crystals [28].

Table 3 shows the temperatures of endothermic peaks of the tested racemic compounds and their corresponding isomers at different heating rates. It is obvious that by increasing the heating rate, the peak temperature of endotherms of each compound was increased. The activation energies for the transition of each compound were calculated from the slope of a plot of $\ln(\alpha/T_m^2)$ versus 1/Tm (Kissinger plot)[29], and are listed in Table 3. The Kissinger plot showed a good linear correlation for each compound as r-value ranged between 0.99 and 0.9999. As seen from the data of activation energy there is no constant pattern for the solid state stability of the single optical isomers or racemic mixtures. It is worthy to note that with the exception of DL-2g derivatives of 3-aralkyl substituents are more stable for thermal degradation compared with the other tested ones.

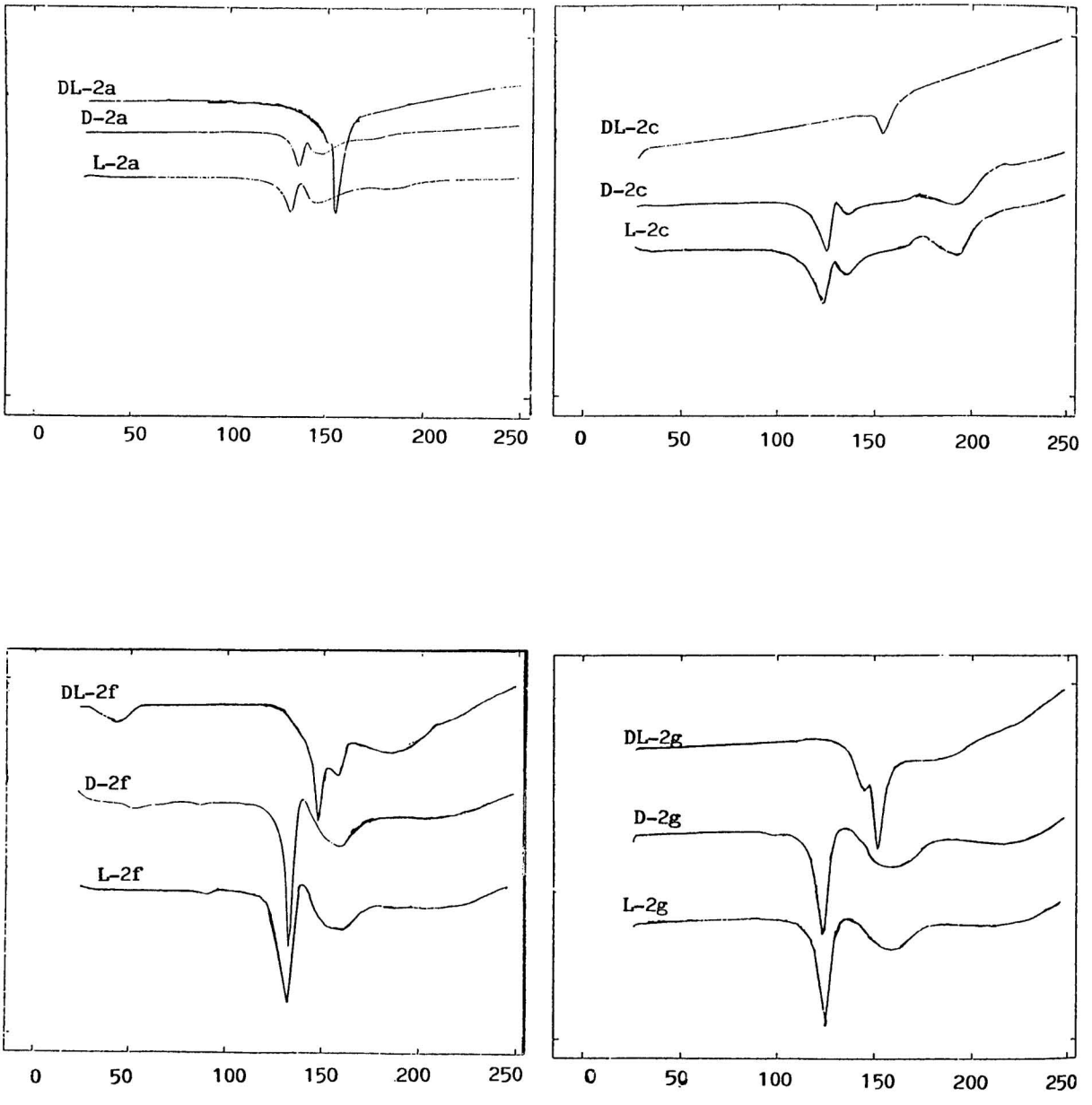


Fig. 1: Representatives for the DSC curves of the tested compounds at heating rate of 10°C/min.

Table 3: The temperatures of endothermic peaks of THTT derivatives at different heating rates.

Compd No.	Endothermic peaks at heating rate (°C/min) 5	10	20	Activation energy Ea (KJ/mol)	Kissinger plot and Correlation coefficient (r)
D-2a	130.0	135.3	141.1	167.60	y = 31.64 -20.16x (0.99998)
L-2a	126.0	132.0	137.5	157.91	y = 37.22 -18.99x (0.99994)
DL-2a	148.4	155.0	161.7	150.57	y = 32.49 -18.11x (0.99997)
D-2c	119.5	124.8	129.4	175.26	y = 43.36 -21.08x (0.9987)
L-2c	117.0	123.2	129.4	139.01	y = 32.55 -16.72x (0.99997)
DL-2c	146.4	153.8	158.6	159.40	y = 35.22 -19.17x (0.99)
D-2f	129.0	133.0	136.0	265.76	y = 69.10 -31.95x (0.9964)
L-2f	127.7	132.4	137.8	179.17	y = 43.42 -21.55x (0.9993)
DL-2f	143.0	148.0	152.0	213.30	y = 51.20 -25.66x (0.9978)
D-2g	118.6	123.0	128.0	185.61	y = 46.70 -22.33x (0.9998)
L-2g	119.6	124.6	128.0	208.67	y = 53.55 -25.10x (0.9924)
DL-2g	144.0	151.4	159.3	128.33	y = 26.56 -15.44x (0.99997)

2.5. Antifungal Activity

The antifungal activity of the synthesized compounds, 2a-g, were tested *in vitro* against *Candida albicans*, *C. parasilosis* and *C. stellatoidea* using tube dilution method[30]. Results of the *in vitro* antifungal investigation, table 4, revealed that the antifungal activity of the tested compounds is independent on their optical properties. It is worthy notice that **2g** is the most active compound

has the highest lipophilicity among the synthesized derivatives, and the most susceptible compound for both chemical and enzymatic degradation. This data is not only consistent with the structural requirements for the antimicrobial activity but also a strong evidence to that the activity attributed to the released isothiocyanates.

Table 4: *In vitro* Antifungal activity of the synthesized THTT derivatives.

Compd. No.	MIC [a] (μM/ml)		
	C. albicans	*C. tropicalis*	*C. stellatoidea*
D-2c	50	25	50
L-2c	50	25	--
DL-2c	100	100	100
L-2d	--	50	--
DL-2d	--	50	--
D-2e	50	--	50
L-2e	50	100	--
DL-2e	25	100	100
D-2f	25	100	100
L-2f	--	100	--
DL-2f	50	100	100
D-2g	25	50	25
L-2g	25	25	25
DL-2g	25	100	25

[a] Minimum Inhibitory Concentration

3. Experimental

3.1. Materials and equipment

Phenylalanine optical isomers and racemic mixture were obtained from Sigma Chemical Co. All other chemicals were of commercial grade except the HPLC solvents and the buffer reagents were of analytical grade.

Melting points were determined on an electrothermal melting point apparatus [Fa. Sturat Scientific, England], and were uncorrected.

Precoated silica gel plates (kiesel gel 0.25 mm, 60G F254, Merk) were used for thin layer chromatography. Developing solvent system of chloroform/ methanol (8:2) was used and the spots were detected by ultraviolet light.

UV spectra of ethanolic solutions of the synthesized compounds were measured on UVP-1601 Shimadzu spectrometer, Japan.

IR spectra (KBr disc) were recorded on IR-470 Shimadzu spectrometer, Japan.

^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60MHZ) USA. Chemical shifts are expressed in δ(ppm) relative to TMS as an internal standard.

Perkin Elmer Polarimetre 341, Germany, was used for measurement of optical rotation. The samples dissolved in DMF and their optical activities were measured using sodium lamp (589 nm) at room temperature.

Elemental analyses were performed at the Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt.

pH Values were recorded on a Chekit micro pH meter (England) at room temperature.

HPLC system is consisting of a pump [KNAUER HPLC pump 64, Germany], a variable-wavelength detector [KNAUER], a reversed-phase HPLC column [stainless steel (25x0.5 cm i.d.) C-18 Eurospher 80] connected with a cartidge guard column, a Shimadzu C-R 6A chromatopac recording integrator, and a

20-μl injection loop was used. Mobile phase systems of methanol, water and 1% triethylamine were used. The ratio of methanol : water was adjusted in order to give a retention time of 5~7 minutes for the synthesized derivatives. The column effluent was monitored at 254 nm and the flow rate was 1 ml/min. Quantification of the eluted compounds was done from peak area measurements in relation to those of standards chromatographed under the same conditions.

DSC were determined using Shimadzu DSC-50 connected with a data station TA-50I. The DSC apparatus was calibrated with indium (peak maximum at 156.6°C).

Antifungal activity was performed at the Department of Microbiology, Faculty of Medicine, Assiut University, Assiut, Egypt.

3.2. Synthesis of5-[α-(benzyl)carboxymethyl]-3-substituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione, 2a-g, derivatives.

Carbon disulfide (10 mmol) was added portion wise to a stirred mixture of the appropriate alkyl or aralkylamine, 1a-g, (10 mmol) and potassium hydroxide (20%, 10 mmol) in water (5 ml), stirring was continued at ambient temperature for 4 hours. Formaldehyde solution (35%, 22 mmol) was added to the mixture and the stirring was continued for further one hour. The mixture was filtered and the resulting clear solution was added dropwise to a solution of appropriate optical isomer or racemic mixture of phenylalanine (10 mmol) in a mixture of phosphate buffer (pH 7.8, 5 ml) and ethanol (5 ml). After stirring for 2 hours at ambient temperature, dilute hydrochloric acid (~ 5 ml) was added and the stirring was continued for further 1 hour. The formed precipitate, 2a-g,

were collected by filtration, washed with aqueous methanol, dried and crystallized from methanol. Yields, melting points and physical data are given in table 1.

5-[(D)-α-(Benzyl)carboxymethyl]-3-ethyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (D-2a)

$[\alpha]_D^{22}$: +14 (0.60, DMF)
UV λ_{max} (nm): 288, 256
IR spectra (Cm^{-1}): 1514 (C=S); 1722 (C=O); 2800, 3030, 3105 (C-H); 3450 (O-H).
^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 1.20(3H, t, J= 7.8Hz, N^3-$CH_2\underline{CH_3}$), 3.13 (2H, d, J=5.4 Hz, Ph-$\underline{CH_2}$-CH-), 3.97 (2H, m, N^3-$\underline{CH_2}CH_3$), 3.99(1H, m, N^5-$\underline{CH}$-CO_2H), 4.51(4H, bs, $4CH_2$ & 6-CH_2), 7.30(5H, s, $\underline{Ph}CH_2$-).

5-[(L)-α-(Benzyl)carboxymethyl]-3-ethyl--tetrahydro-2H-1,3,5-thiadiazine-2-thione (L-2a)

$[\alpha]_D^{22}$: -14.2 (0.53, DMF)
UV λ_{max} (nm): 288, 255
IR spectra (Cm^{-1}): 1515 (C=S); 1722 (C=O); 2990, 3035, 3105 (C-H); 3530 (O-H).
^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 1.21(3H, t, J= 7.6Hz, N^3-$CH_2\underline{CH_3}$), 3.11 (2H, d, J=5.6 Hz, Ph-$\underline{CH_2}$-CH-), 3.98 (2H, m, N^3-$\underline{CH_2}CH_3$), 4.00(1H, m, N^5-$\underline{CH}$-CO_2H), 4.50(4H, bs, $4CH_2$ & 6-CH_2), 7.30(5H, s, $\underline{Ph}CH_2$-).

5-[(DL)-α-(Benzyl)carboxy-methyl]-3-ethyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (DL-2a)

UV λ_{max} (nm): 289, 253
IR spectra (Cm^{-1}): 1491 (C=S); 1747 (C=O); 2865, 3030, 3120 (C-H); 3450 (O-H).
^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 1.18(3H, t, J= 7.5Hz, N^3-$CH_2\underline{CH_3}$), 3.11 (2H, d, J=5.4 Hz, Ph-$\underline{CH_2}$-CH-), 3.96(2H, m, N^3-$\underline{CH_2}CH_3$), 3.97(1H, m, N^5-$\underline{CH}$-CO_2H), 4.51(4H, bs, $4CH_2$ & 6-CH_2), 7.33(5H, s, $\underline{Ph}CH_2$-).

5-[(D)-α-(Benzyl)carboxymethyl]-3-propyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (D-2b)

$[\alpha]_D^{22}$: +6.5(0.22 ,DMF)
UV λ_{max} (nm): 287, 252
IR spectra (Cm^{-1}): 1480 (C=S); 1721 (C=O); 2995, 3010, 3120 (C-H); 3505 (O-H).
^{1}H-NMR (DMSO-d_6): δ 0.89(3H, t, J= 7.3Hz, N^3-$(CH_2)_2\underline{CH_3}$), 1.71(2H, m, N^3-CH_2-$\underline{CH_2}CH_3$), 3.28 (2H, d, J=7.2 Hz, Ph-$\underline{CH_2}$-CH-), 3.95 (2H, m, N^3-$\underline{CH_2}$-CH_2CH_3), 3.97(1H, m, N^5-$\underline{CH}$-CO_2H), 4.53(4H, bs, $4CH_2$ & 6-CH_2), 7.33(5H, s, $\underline{Ph}CH_2$-).

5-[(L)-α-(Benzyl)carboxymethyl]-3-propyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (L-2b)

$[\alpha]_D^{22}$: -4.0 (0.29 ,DMF)

UV λ_{max} (nm): 288, 252
IR spectra (Cm^{-1}): 1485 (C=S); 1725 (C=O); 2960, 3030, 3085 (C-H); 3495 (O-H).
^{1}H-NMR (DMSO-d_6): δ 0.90(3H, t, J= 7.1Hz, N^3-$(CH_2)_2\underline{CH_3}$), 1.73(2H, m, N^3-CH_2-$\underline{CH_2}CH_3$), 3.30 (2H, d, J=7.0 Hz, Ph$\underline{CH_2}$-CH-), 3.96 (2H, m, N^3-$\underline{CH_2}$-CH_2CH_3), 3.98(1H, m, N^5-$\underline{CH}$-CO_2H), 4.55(4H, bs, $4CH_2$ & 6-CH_2), 7.33(5H, s, $\underline{Ph}CH_2$-).

5-[(D)-α-(Benzyl)carboxymethyl]-3-isopropyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (D-2c)

$[\alpha]_D^{22}$: +8.8 (0.66, DMF)
UV λ_{max} (nm): 288, 253
IR spectra (Cm^{-1}): 1491 (C=S); 1722 (C=O); 2887, 3035, 3150 (C-H); 3505 (O-H).
^{1}H-NMR ($CDCl_3$): δ 1.24(6H, d, J= 7.6Hz, N^3-$CH(\underline{CH_3})_2$, 3.31 (2H, d, J=7.9 Hz, Ph$\underline{CH_2}$-CH-), 4.01(1H, t, J=7.1Hz, N^5-$\underline{CH}$-CO_2H), 4.62(4H, bs, $4CH_2$ & 6-CH_2), 6.35 (H, m, N^3-$\underline{CH}(CH_3)_2$) 7.33(5H, s, $\underline{Ph}CH_2$-).

5-[(L)-α-(Benzyl)carboxymethyl]-3-isopropyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (L-2c)

$[\alpha]_D^{22}$: -10.2 (0.47, DMF)
UV λ_{max} (nm): 288, 254
IR spectra (Cm^{-1}): 1495 (C=S); 1721 (C=O); 2930, 3065, 3135 (C-H); 3495 (O-H).
^{1}H-NMR ($CDCl_3$): δ 1.26(6H, d, J= 7.9Hz, N^3-$CH(\underline{CH_3})_2$, 3.33 (2H, d, J=7.9 Hz, Ph-$\underline{CH_2}$-CH-), 4.01(1H, t, J=7.2Hz, N^5-$\underline{CH}$-CO_2H), 4.63(4H, bs, $4CH_2$ & 6-CH_2), 6.35 (H, m, N^3-$\underline{CH}(CH_3)_2$) 7.33(5H, s, $\underline{Ph}CH_2$-).

5-[(DL)-α-(Benzyl)carboxymethyl]-3-isopropyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (DL-2c)

UV λ_{max} (nm): 288, 253
IR spectra (Cm^{-1}): 1478 (C=S); 1738 (C=O); 2960, 3030, 3125 (C-H); 3490 (O-H).
^{1}H-NMR ($CDCl_3$): δ 1.24(6H, d, J= 7.8Hz, N^3-$CH(\underline{CH_3})_2$, 3.33 (2H, d, J=7.7 Hz, Ph-$\underline{CH_2}$-CH-), 4.00(1H, t, J=7.0Hz, N^5-$\underline{CH}$-CO_2H), 4.63(4H, bs, $4CH_2$ & 6-CH_2), 6.36 (H, m, N^3-$\underline{CH}(CH_3)_2$), 7.31(5H, s, $\underline{Ph}CH_2$-).

5-[(D)-α-(Benzyl)carboxymethyl]-3-butyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (D-2d)

$[\alpha]_D^{22}$: +9.6(0.44, DMF)
UV λ_{max} (nm): 287, 253
IR spectra (Cm^{-1}): 1491 (C=S); 1726 (C=O); 2956, 3025, 3135 (C-H); 3500 (O-H).
^{1}H-NMR (DMSO-d_6): δ 0.96(3H, t, J= 5.9Hz, N^3-$(CH_2)_3\underline{CH_3}$), 1.49 (4H, m, N^3-CH_2 $(\underline{CH_2})_2CH_3$), 3.13 (2H, d, J=7.2 Hz, Ph-$\underline{CH_2}$-CH-), 4.13 (2H, m, N^3-$\underline{CH_2}$-$(CH_2)_2CH_3$,), 4.13(1H, m, N^5-$\underline{CH}$-CO_2H), 4.50(2H, bs, $4CH_2$), 4.66(2H, bs, 6-CH_2) 7.33(5H, s, $\underline{Ph}CH_2$-).

5-[(L)-α-(Benzyl)carboxymethyl]-3-butyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (L-2d)

$[\alpha]_D^{22}$: -5.9 (0.23, DMF)
UV λ_{max} (nm): 288, 250
IR spectra (Cm^{-1}): 1493 (C=S); 1725 (C=O); 2965, 3015, 3115 (C-H); 3505 (O-H).
^{1}H-NMR (DMSO-d_6): δ 0.97(3H, t, J= 6.0Hz, N^3-$(CH_2)_3\underline{CH_3}$), 1.50 (4H, m, N^3-CH_2 $\underline{(CH_2)_2}CH_3$), 3.15 (2H, d, J=7.0 Hz, Ph-$\underline{CH_2}$-CH-), 4.13 (2H, m, N^3-$\underline{CH_2}$ $(CH_2)_2CH_3$,), 4.13(1H, m, N^5-$\underline{CH}$-CO_2H), 4.52(2H, bs, $4CH_2$), 4.65(2H, bs, 6-CH_2) 7.33(5H, s, $\underline{Ph}CH_2$-).

5-[(DL)-α-(Benzyl)carboxymethyl]-3-butyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (DL-2d)

UV λ_{max} (nm): 288, 253
IR spectra (Cm^{-1}): 1497 (C=S); 1726 (C=O); 2960, 3030, 3130 (C-H); 3505 (O-H).
^{1}H-NMR (DMSO-d_6): δ 0.96(3H, t, J= 5.9Hz, N^3-$(CH_2)_3\underline{CH_3}$, 1.49 (4H, m, N^3-CH_2 $\underline{(CH_2)_2}CH_3$), 3.14 (2H, d, J=7.1 Hz, Ph-$\underline{CH_2}$-CH-), 4.12 (2H, m, N^3-$\underline{CH_2}$ $(CH_2)_2CH_3$,), 4.12(1H, m, N^5-$\underline{CH}$-CO_2H), 4.48(2H, bs, $4CH_2$), 4.64(2H, bs, 6-CH_2) 7.31(5H, s, $\underline{Ph}CH_2$-).

5-[(D)-α-(Benzyl)carboxymethyl]-3-cyclohexyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (D-2e)

$[\alpha]_D^{22}$: +5.0 (0.28, DMF)
UV λ_{max} (nm): 288, 255
IR spectra (Cm^{-1}): 1507 (C=S); 1722 (C=O); 2930, 3035, 3130 (C-H); 3500 (O-H).
^{1}H-NMR (DMSO-d_6): δ 1.13-2.10 (10H, m, N^3-CH($\underline{CH_2}$)$_5$), 3.26 (2H, d, J=6.9 Hz, Ph-$\underline{CH_2}$-CH-), 4.04(1H, t, J=7.9Hz, N^5-$\underline{CH}$-CO_2H), 4.62(4H, bs, $4CH_2$ & 6-CH_2), 5.56 (H, m, N^3-$\underline{CH}(CH_2)_5$) 7.53(5H, s, $\underline{Ph}CH_2$-).

5-[(L)-α-(Benzyl)carboxymethyl]-3-cyclohexyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (L-2e)

$[\alpha]_D^{22}$: -3.4(0.31, DMF)
UV λ_{max} (nm): 288, 256
IR spectra (Cm^{-1}): 1505 (C=S); 1722 (C=O); 2930, 3025, 3115 (C-H); 3505 (O-H).
^{1}H-NMR (DMSO-d_6): δ 1.12-2.14 (10H, m, N^3-CH($\underline{CH_2}$)$_5$), 3.27 (2H, d, J=7.0 Hz, Ph-$\underline{CH_2}$-CH-), 4.05(1H, t, J=8.0Hz, N^5-$\underline{CH}$-CO_2H), 4.63(4H, bs, $4CH_2$ & 6-CH_2), 5.60 (H, m, N^3-$\underline{CH}(CH_2)_5$) 7.51(5H, s, $\underline{Ph}CH_2$-).

5-[(DL)-α-(Benzyl)carboxymethyl]-3-cyclohexyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (DL-2e)

UV λ_{max} (nm): 289, 254
IR spectra (Cm^{-1}): 1503 (C=S); 1727 (C=O); 2935, 3055, 3170 (C-H); 3505 (O-H).
^{1}H-NMR (DMSO-d_6,): δ 1.13-2.10 (10H, m, N^3-CH($\underline{CH_2}$)$_5$), 3.24 (2H, d, J=6.7 Hz, Ph-$\underline{CH_2}$-CH-), 4.02(1H, t, J=7.9Hz, N^5-$\underline{CH}$-CO_2H), 4.60(4H, bs, $4CH_2$ & 6-CH_2), 5.54 (H, m, N^3-$\underline{CH}(CH_2)_5$) 7.53(5H, s, $\underline{Ph}CH_2$-).

3-Benzyl-5-[(D)-α-(benzyl)carboxymethyl]-tetrahydro-2H-1,3,5-thiadiazine-2-thione (D-2f)

$[\alpha]_D^{22}$: +10.7 (0.55, DMF)
UV λ_{max} (nm): 291, 253
IR spectra (Cm^{-1}): 1478 (C=S); 1741 (C=O); 2950, 3065, 3180 (C-H); 3475 (O-H).
^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 2.95 (2H, d, J=6.5 Hz, Ph-$\underline{CH_2}$-CH-), 3.89 (1H, t, J=9.2, N^5-$\underline{CH}$-CO_2H), 4.26 (2H, bs, 4-CH_2), 4.53(2H, bs, 6-CH_2), 5.33 (2H, s, N^3-$\underline{CH_2}$-Ph) , 7.23(5H, s, $\underline{Ph}CH_2$-N^3), 7.26(5H, s, $\underline{Ph}CH_2$-).

3-Benzyl-5-[(L)-α-(benzyl)carboxymethyl]-tetrahydro-2H-1,3,5-thiadiazine-2-thione (L-2f)

$[\alpha]_D^{22}$: -11.4 (0.55, DMF)
UV λ_{max} (nm): 291, 254
IR spectra (Cm^{-1}): 1491 (C=S); 1742 (C=O); 2910, 3030, 3190 (C-H); 3525 (O-H).
^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 2.96 (2H, d, J=6.3 Hz, $Ph\underline{CH_2}$-CH-), 3.90 (1H, t, J=9.0, N^5-$\underline{CH}$-CO_2H), 4.25 (2H, bs, 4-CH_2), 4.51(2H, bs, 6-CH_2), 5.33 (2H, s, N^3-$\underline{CH_2}$-Ph) , 7.24(5H, s, $\underline{Ph}$-CH_2-N^3), 7.26(5H, s, $\underline{Ph}$-CH_2-).

3-Benzyl-5-[(DL)-α-(benzyl)carboxymethyl]-tetrahydro-2H-1,3,5-thiadiazine-2-thione (DL-2f)

UV λ_{max} (nm): 290, 256
IR spectra (Cm^{-1}): 1490 (C=S); 1713 (C=O); 2990, 3110 (C-H); 3505 (O-H).
^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 2.93 (2H, d, J=6.2 Hz, $Ph\underline{CH_2}$-CH-), 3.87 (1H, t, J=9.1, N^5-$\underline{CH}$-CO_2H), 4.26 (2H, bs, 4-CH_2), 4.53(2H, bs, 6-CH_2), 5.33 (2H, s, N^3-$\underline{CH_2}$-Ph) , 7.22(5H, s, $\underline{Ph}CH_2$-N^3), 7.25(5H, s, $\underline{Ph}CH_2$-).

5-[(D)-α-(Benzyl)carboxymethyl]-3-phenethyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (D-2g)

$[\alpha]_D^{22}$: +13.2 (0.42, DMF)
UV λ_{max} (nm): 289, 250
IR spectra (Cm^{-1}): 1504 (C=S); 1730 (C=O); 2980, 3060, 3175 (C-H); 3565 (O-H).
^{1}H-NMR ($CDCl_3$): δ 3.23 (2H, d, J=7.9 Hz, $Ph\underline{CH_2}$-CH-), 3.92 (1H, m, N^5-$\underline{CH}$-CO_2H), 3.92-4.30 (4H, m, N^3-$(\underline{CH_2})_2$-Ph) , 4.13 (2H, bs, 4-CH_2), 4.26(2H, bs, 6-CH_2), 7.23(5H, s, $\underline{Ph}(CH_2)_2$-N^3), 7.26(5H, s, $\underline{Ph}CH_2$-).

5-[(L)-α-(Benzyl)carboxymethyl]-3-phenethyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (L-2g)

$[\alpha]_D^{22}$: -6.0 (0.30, DMF)
UV λ_{max} (nm): 288, 252
IR spectra (Cm^{-1}): 1476 (C=S); 1730 (C=O); 2995, 3060, 3190 (C-H); 3505 (O-H).
^{1}H-NMR ($CDCl_3$): δ 3.24 (2H, d, J=7.9 Hz, $Ph\underline{CH_2}$-CH-), 3.94 (1H, m, N^5-$\underline{CH}$-CO_2H), 3.93-4.30 (4H, m, N^3-$(\underline{CH_2})_2$-Ph) , 4.15 (2H, bs, 4-CH_2), 4.27(2H, bs, 6-CH_2), 7.24(5H, s, $\underline{Ph}(CH_2)_2$-N^3), 7.26(5H, s, $\underline{Ph}CH_2$-).

5-[(DL)-α-(Benzyl)carboxymethyl]-3-phenethyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione (DL-2g)

UV λ_{max} (nm): 290, 253

IR spectra (Cm^{-1}): 1488 (C=S); 1742 (C=O); 2880, 3030, 3155 (C-H); 3500 (O-H).

^{1}H-NMR ($CDCl_3$): δ 3.21 (2H, d, J=7.6 Hz, $PhCH_2$-CH-), 3.90 (1H, m, N^5-CH-CO_2H), 3.92-4.28 (4H, m, N^3-$(CH_2)_2$-Ph) , 4.11 (2H, bs, 4-CH_2), 4.25(2H, bs, 6-CH_2), 7.23(5H, s, $Ph(CH_2)_2$-N^3), 7.26(5H, s, $PhCH_2$-).

3.3. Calculation of log P values

The log P values of the synthesized derivatives were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (MacLogP 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where hydrogens are omitted, or 'suppressed' (SMILES notation), is entered into the program, which computes the log P based on the fragment method developed by Leo[20].

3.4. Kinetic Measurements

Degradation rates of the synthesized derivatives 2a-g in aqueous solution of isotonic phosphate buffer, pH 7.4, was determined at 37°C. The ionic strength of the prepared buffer solution was adjusted with KCl to $\mu = 0.5$.

The reactions were initiated by adding 250 μl of the stock ethanolic solution of each derivative (10^{-3} M) to 2.5 ml of preheated buffer solution in screw-capped test tubes. At appropriate intervals samples were taken and chromatographed. The residual concentrations displayed a pseudo first order rate of hydrolysis.

Degradation studies in 80% human plasma containing isotonic phosphate buffer of pH 7.4 at 37°C was done by adding appropriate amount of the stock

methanolic solution of derivatives to the plasma solution, initial concentration was 10^{-5} M. At appropriate times samples of 50 µl were withdrawn, mixed with 50 µl of acetonitrile for deproteinization and centrifuged at 10^4 rpm for 10 minutes. 20 µl of the clear supernatant was analyzed by HPLC as described above. Results of Kinetic Measurements are given in table 2.

3.5. DSC Measurements:

The DSC curves of the prepared samples were determined using Shimadzu DSC-50 connected with TA-50I. Accurate weight (1.5-3.0 mg) of each sample in open aluminum pan was heated under nitrogen purge at 40 ml/min, at different heating rates 5. 10, and 20°C/min. The peak temperature and the heat of fusion for each sample were obtained from DSC traces.

The activation energies were calculated independently using temperature-ramp DSC via the Kissinger equation (equation 1), which takes into account only the peak of temperature (T_m) of the endotherm response as a function of the heating rate[29].

$$\ln(\alpha/T_m^2) = -Ea/RT_m + \text{constant} \qquad \text{(equation 1)}$$

Where α is the heating rate, T_m is the temperature at peak maximum of the endotherm, Ea is the activation energy, and R is the gas constant.

Based on equation 1, plotting $\ln(\alpha/T_m^2)$ versus 1/Tm gives a linear relation, from its slope, the Ea can be calculated. Results are given in table 3.

3.6. Antifungal activity

Fungal species used in the present study are: *Candida albicans, C. tropicalis* and *C. stellatoidea.* These fungi were obtained from the culture collections of the Microbiology Dept., Faculty of Medicine, Assiut University.

Sabouraud dextrose agar and Sabouraud dextrose liquid medium were used as growing media for fungi. The tube dilution method was used for determination of antifungal activities[30]. The concentrations of the compounds in tube were 100, 50 and 25 μM/ml (DMSO). The final inoculum size was 10^{-5}-10^{-6} CFU/ml (CFU: colony forming unites). The solvent (DMSO) were served as control. Triplicate set were applied for each treatment and the MIC values are given in table 4.

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