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Kinetics of solid state stability of glycine derivatives as a model for peptides using differential scanning calorimetry

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Abstract

Kinetics of solid state stability of seven derivatives of 3,5-disubstituted tetrahydro-2H-1,3,5-thiadiazine-2-thione (THTT) of glycine as a model for amino acids and peptide drugs were studied using differential scanning calorimetry (DSC). Each DSC curve for each derivative showed an endothermic peak followed by an exothermic one, which could be attributed to the melting and decomposition, respectively. The decomposition activation energy of each derivative was calculated using the Augis and Bennet, Kissinger equations and Mahadevan approximation. Also, the melting activation energies as well as the thermodynamic parameter (enthalpy) for the investigated derivatives were evaluated. The relative stability of the derivatives in the solid state according to the calculated values of the decomposition activation energy, frequency factors and half-life for each derivative could be determined.

Keywords: Organic crystals; Chemical synthesis; Calorimetry; Thermal analysis

1. Introduction

Development of peptide drugs is presently a major area in drug research and in recent years, several biologically active peptides have been introduced. Proteins and peptides have become an important class of potent therapeutic drugs. The application of these peptides as clinically useful drugs is still seriously hampered due to substantial delivery problems. Most peptides are rapidly metabolized by proteolysis at most routes of administration, they are non-lipophilic showing poor member penetration and they posess a short biological half-life [1–4]. As a part of the studies started in our laboratory, the moiety, tetrahydro-thiadiazine-2-thione (THTT), was synthesized and used as a drug delivery system for peptide drugs. The prepared peptide derivatives were readily bioreversible, lipophilic and more stable against peptidase enzymes in comparison with the parent peptide [5,6].

Differential scanning calorimetry (DSC) has a long and extensive application in the pharmaceutical industry as a method for the investigation of

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purity, glass transition, stability, compatibility, etc. [7,8]. DSC offers a useful means of predicting the solid state stability of drugs [9]. The DSC experiment can be used to compute the Arrhenius pre-exponential factor, activation energy and order of the reaction [10].

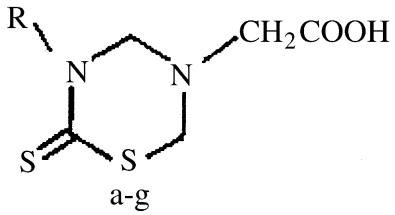
In this work, DSC was selected to study the solid state stability of seven derivatives of tetrahydrothiadiazine-2-thione (THTT) of glycine. Also, the objective was concerned with the evaluation of the parameters of decomposition kinetics applying the Augis and Bennet, Kissinger equations and Mahadevan approximation.

2. Experimental

The seven derivatives of tetrahydrothiadiazine-2-thione of glycine were prepared as mentioned previously in our publications [5,6]. The DSC curves of the samples were obtained using a Shimadzu DSC-50 connected with a TA-50I and TASYS programmed computer. Samples of the derivatives (2–4 mg) in non-hermetically crimped aluminum pans were heated at three different heating rates (α), namely 5, 10, and 20 °C/min under nitrogen purge at 40 ml/min. The peak temperatures of melting (T_m) and of decomposition (T_d) for each sample were measured from DSC traces. The temperature energy calibrations of the instrument were performed using the well-known melting temperature and melting enthalpy of high purity indium supplied with the instrument. The calorimetric sensitivity is 10 μW/cm and the temperature precision is $\pm$0.1 K. Best fits for the correlation between T_m, T_d and α were calculated by the least-squares method. The arithmetic mean as well as the standard deviation were calculated for the activation energies.

3. Results and discussion

Scheme 1 shows the chemical structures of the seven (a–g) synthesized derivatives of 3,5-disubstituted tetrahydro-2H-1,3,5-thiadiazine-2-thione (THTT). According to the substitution in position three, these derivatives can be classified into three classes, namely N-3 alkyl substituent (methyl gm, ethyl ge, propyl gp, and butyl gb), N-3 cycloalkyl



a-g

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 = $C_6H_5CH_2CH_2$.

substituent (cyclohexyl gc) and N-3 aralkyl substituents (benzyl gbz and phenethyl g). This moiety, THTT, was synthesized and found to be a promising prodrug approach of the drug delivery system for peptide drugs [5,6]. The DSC traces of these derivatives were taken at a heating rate (α) of 5, 10 and 20 °C/min. Fig. 1 shows the typical DSC curves of g, ge and gc derivatives representing the three classes at heating rate of 20 °C/min. Each DSC trace has two characteristic phenomena, an endothermic peak followed by an exothermic one, in the studied temperature range. The first one corresponds to the peak temperature of the melting (T_m) and the second one to the peak temperature of the decomposition(T_d) of each compound. The exothermic peaks of g, ge and gc derivatives at heating rate (α) of 5, 10 and 20 °C/min are shown on their DSC curves in Fig. 2. From these curves, it was clearly the shifting of the exothermic peak to a higher temperature by increasing the heating rate for each derivative. Generally, it is found that the two characteristic temperatures of each derivative increase with increasing the heating rate as shown in Table 1.

The decomposition activation energy of the derivatives can be calculated using the following equation proposed by Augis and Bennet [11]:

$$\ln(\alpha) = -E/RT + \ln k_0, \quad (1)$$

where R is the gas constant and k_0 is the frequency factor. This equation is used specifically for non-isothermal crystallization [11]. It can be applied here, for the first time, to obtain the decomposition kinetics of the derivatives of THTT. In this case, E represents the activation energy for the decomposition, E_d, and T depicts the decomposition peak

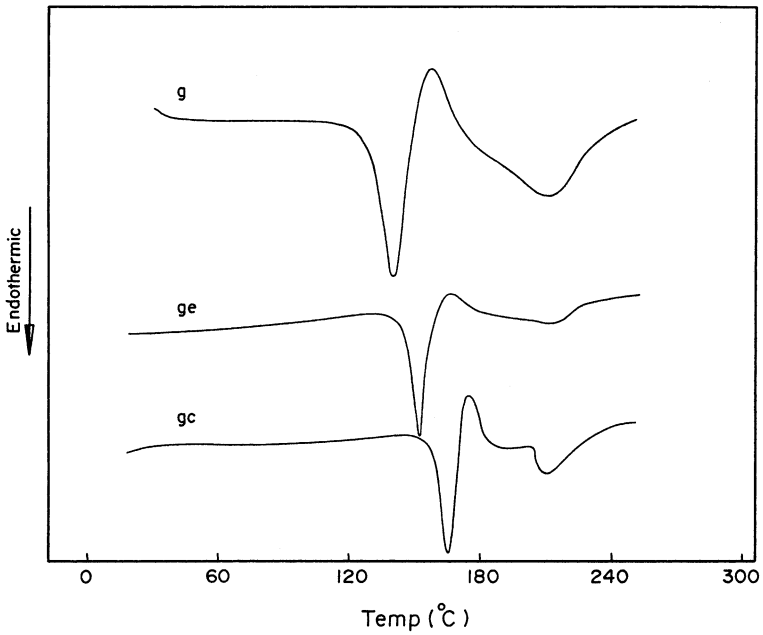


Fig. 1. Typical DSC traces of g, ge and gc derivatives of THTT at a heating rate of 20 °C/min.

temperature, T_d. The constant k_0 measures the probability of molecular collisions effective for the formation of the activated complexes. The relation between $\ln(\alpha/T_d)$ and $(1/T_d)$ obtained from the thermograms of a given derivative is linear and indicates the validity of this relationship for all derivatives (Fig. 3). The decomposition activation energies are calculated from the slopes of this function fitted to the data. In addition, the frequency factor (k_0) for a given derivative is calculated from the original intercept of the straight line with vertical axis and given together with the E_d in Table 2. The results in this table show a correspondence between the values of E_d and k_0 for a specific derivative. According to the definition of k_0, this correspondence seems to be reasonable.

The interpretation of the DSC data is provided by the formal theory of transformation kinetics as the decomposition rate constant (K) is usually assumed to be an Arrhenian temperature dependence

$$K = k_0 \exp(-E_d/RT), \quad (2)$$

where T is any temperature in the entire region of exothermic peak. The constant K describes the decomposition reaction rate at any temperature, T. Substituting the obtained values of E_d and k_0 from Eq. (1) for each derivative, one can calculate K as a function of T for each heating rate. The calculated values of $K(T)$ for three derivatives representing the three classes of the derivatives at a constant heating rate ($\alpha = 10$ °C/min) are plotted as $\ln K$ vs. $1/T$ and the straight lines are shown in Fig. 4. The validity of Eq. (2) for each derivative confirms the results obtained for E_d and k_0. From Fig. 4, it can be noticed that the ge derivative thermally decomposes before the derivative g which in turn decomposes before gc. In other words, gc is the most thermally stable derivative among the selected ones followed by g then ge. This result was confirmed by the obtained values of E_d which relates to the stability for these derivatives as shown in Table 2.

The order of the reaction rate constant was found to be a first order reaction, and from which the half-life for each derivative could be calculated by a simple relation: $t_{1/2} = 0.693/K$ [12]. Here $t_{1/2}$ is the period of time required for a drug to

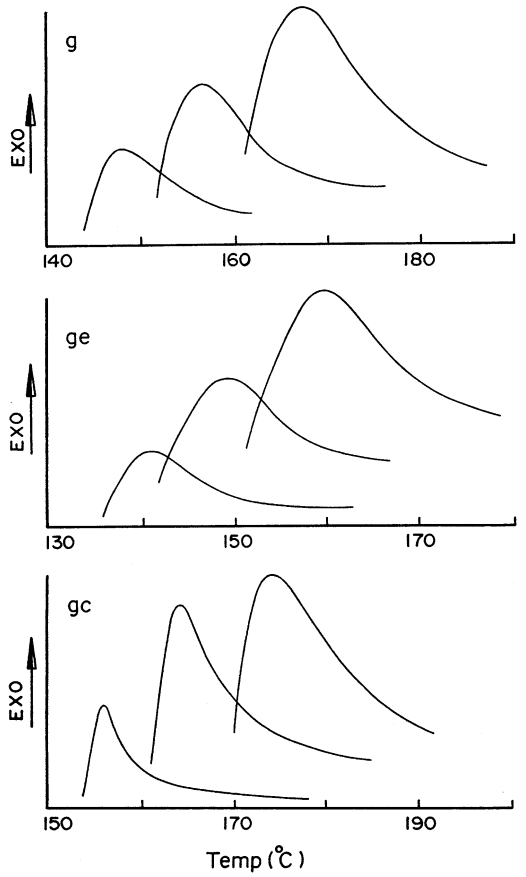


Fig. 2. Exothermic traces of g, ge and gc derivatives of THTT at heating rates of 5, 10 and 20 °C/min.

decompose to one-half the original concentration. The half-lives of the THTT derivatives for glycine were calculated at 25 °C and presented in Table 3. In the case of N-3 alkyl substituents, the methyl group decreased the half-life to 0.2 month, i.e. increased the decomposition rate, whereas chain elongation (ethyl, propyl) was accompanied by increasing the half-life to 25 and 40 months, respectively. The solid state stability seemed to be affected by substituents at N-3 of THTT moiety. However, the N-3 aralkyl substitution, benzyl group gave a higher half-life (57 months) than that of the phenethyl group (23 months). Cycloalkyl substituent, on the other hand, revealed a varied pattern that gave the highest value of half-life (200 months), i.e. the least decomposition rate

Table 1
The peak temperatures of endothermic and exothermic peak of THTT derivatives

Derivative	Endothermic peak (°C) at heating rate (°C/min)			Exothermic peak (°C) at heating rate (°C/min)		
	5	10	20	5	10	20
gm	122.7	128.2	143.3	148.8	163	122.7
ge	131	135	141	141	150	160
gp	144	149	155	150	158	169
gb	128	131	137	140	149	155
gc	151	157	164	156	164	174
gbz	142	148	154	149	158	176.5
g	141	146	152.4	148.7	157.2	167.5

constant and the most stable derivative in the solid state. The mechanism of the hydrolysis of THTT in solution state to liberate the parent drug glycine, was previously reported to occur via ring cleavage at N5 of the THTT moiety [5].

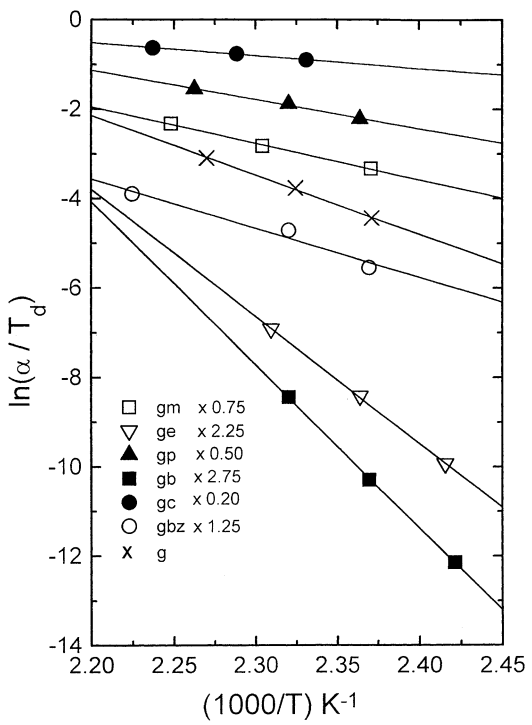


Fig. 3. Augis and Bennet plots: $\ln(\alpha/T_d)$ vs. $(1/T_d)$ for the investigated seven derivatives of THTT.

Table 2
The decomposition activation energies (E_d) for the investigated derivatives calculated by different methods and the correspondence frequency factors (k_0)

Derivative	Kissinger $\ln(\alpha/T_d^2)$ vs. $1/T_d$ E_d (kJ/mol)	Mahadevan $1/T_d$ vs. $\ln(\alpha)$ E_d (kJ/mol)	Augis and Bennet $\ln(\alpha/T)$ vs. $1/T_d$	
			E_d (kJ/mol)	k_0 (min^{-1})
gbz	69.68±1.70	79.50±0.021	73.32±1.69	$0.015\times10^{9}\pm50$
gm	86.61±0.56	94.03±0.088	90.85±0.56	$1.71\times10^{9}\pm4$
ge	101.70±0.23	108.77±0.001	105.22±0.23	$2.27\times10^{11}\pm2$
gp	105.55±1.07	113.44±0.004	109.15±1.07	$3.66\times10^{11}\pm12$
g	106.54±0.59	113.91±0.003	110.13±0.58	$5.23\times10^{11}\pm7$
gb	106.97±0.16	114.02±0.001	110.48±0.16	$11.25\times10^{11}\pm2$
gc	114.76±1.70	122.83±0.002	118.85±0.77	$34.92\times10^{11}\pm6$

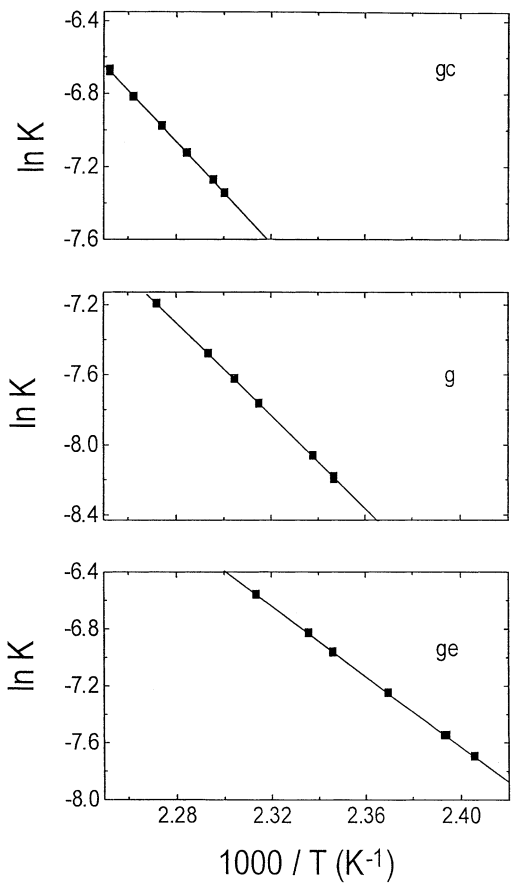


Fig. 4. Temperature dependence of the decomposition rate constant for gc, g and ge derivatives of THTT at a heating rate of 10 °C/min.

The activation energy for decomposition of the derivatives of THTT can also be calculated by the other two methods [13,14]. According to Kissinger's formula [13], the temperature of the exothermic peak depends on the heating rate by the following relation

$$\ln(\alpha/T^2) = -E/RT + \text{constant}. \quad (3)$$

This expression has been originally suggested for the studying of the crystallization kinetics in materials having amorphous–crystalline transformations. In which E is the activation energy for the crystallization and T is the crystallization peak temperature. However, it is possible to use this expression to study the decomposition kinetics of materials which undergo thermal disintegration [15]. In this case, E_d and T_d replace E and T. Based on Eq. (3), plotting $\ln(\alpha/T_d^2)$ vs. $(1/T_d)$ gives a linear relation. The experimental points show a good fit with the above relation, as shown in Fig. 5. The decomposition activation energies for the derivatives were calculated from the slopes of the straight lines and listed in ascending order in Table 2. The obtained values of E_d values indicate that the more stable derivative is gc in the solid state, which has the largest value of E_d (114.76±1.70 kJ/mol). In addition, gc showed

Table 3
The half-lives at 25 °C of the THTT derivatives

Derivative	gm	ge	gp	gb	gc	gbz	g
$t_{1/2}$ (months)	0.2	24.7	40.1	7.3	200.5	57.3	22.6

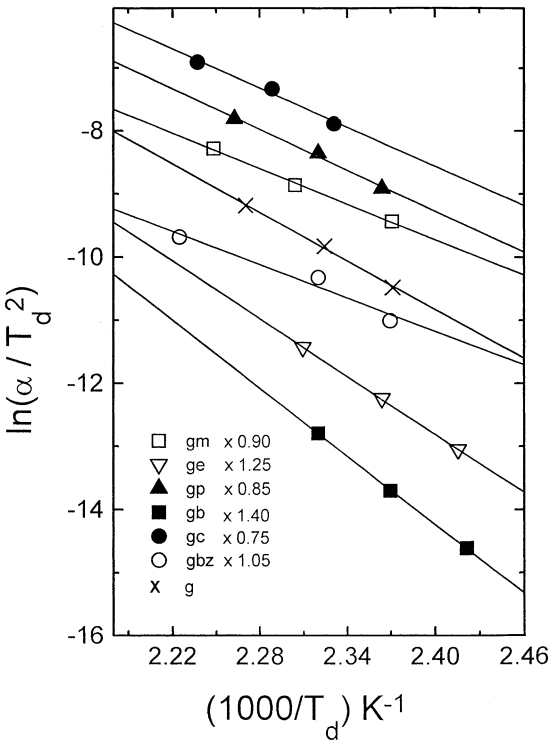


Fig. 5. Kissinger plots: $\ln(\alpha/T_d^2)$ vs. $1000/T_d$ for the investigated seven derivatives of THTT.

the longest $t_{1/2}$ and highest melting point. It was observed that the activation energy of decomposition of alkyl substituents was increased by elongation of alkyl chain from methyl to propyl groups (Table 2). These results are in agreement with the results of half-lives (Table 3) and their melting points (Table 1) which increased by the lengthening of the side chain from methyl to propyl in the three positions of THTT, due to progressive increasing of their intermolecular forces. On the other hand, the derivative gbz having the smallest values of both E_d (69.68 ± 1.70 kJ/mol) and $t_{1/2}$ (0.2 month) can be considered as the fast thermally decompose derivative.

The value of E_d can also be calculated by using the Mahadevan et al. approximation [14]. The variation of $\ln(1/T_d^2)$with $\ln(\alpha)$ is much slower than that of $(1/T_d)$ with $\ln(\alpha)$. Thus, the Kissinger equation can be rewritten in the form of Mahadevan et al. as follows:

$$\ln(\alpha) = -E_d/RT_d + \text{constant}. \quad (4)$$

This equation can be rearranged in the form

$$\frac{1}{T_d} = -\frac{R}{E_d}\ln(\alpha) + \text{constant}. \quad (5)$$

Plots of $(1/T_d)$ vs. $\ln(\alpha)$ for the seven derivatives gave straight lines, as shown in Fig. 6. The value of E_d for a given derivative was deduced from the slope of the correspondence straight line. The obtained E_d values of the derivatives of THTT are listed in Table 2. The results in this table show that the errors due to the fitting of Eq. (3) to the experimental data are much smaller than those due to the fitting using Eq. (5). Thus, one can conclude that the Mahadevan et al. approximation is more suitable in the determination of the E_d value and in the describing of the decomposition mechanism than the Kissinger equation.

The melting activation energies E_m values for the investigated derivatives of THTT can be evaluated using relations similar to those in Eqs. (3) and (5). However, in this case E_m and T_m replace

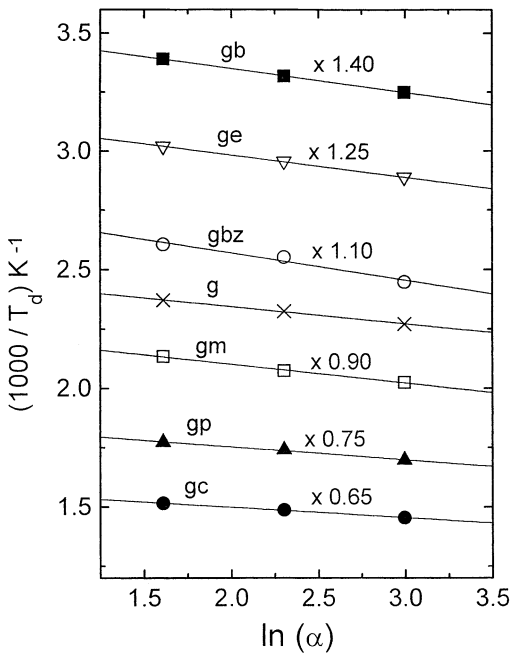


Fig. 6. Madahevan plots: $1000/T_d$ vs. $\ln(\alpha)$ for the investigated seven derivatives of THTT.

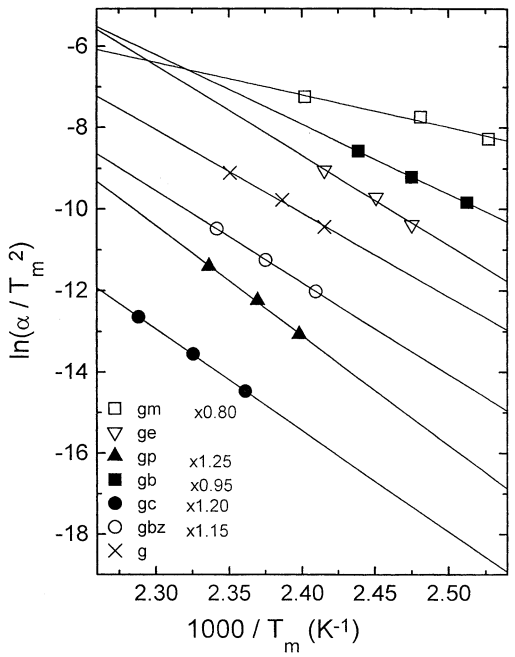


Fig. 7. Kissinger plots: $\ln(\alpha/T_m^2)$ vs. $1000/T_m$ for the investigated seven derivatives of THTT.

E_d and T_d, respectively. Fig. 7 shows a good fit between the experimental points and the straight lines according to Kissinger's equation. Similar results were obtained by applying Kissinger's equation for mercaptopurine dehydration [15]. The obtained E_m values using Kissinger's equation and Mahadevan approximation are presented in ascending order in Table 4.

The heat of fusion or enthalpy (ΔH), as a thermodynamic parameter, may be considered as the heat required to increase the interatomic or intermolecular distance in crystals, thus allowing melting to occur [12]. A derivative that is bound together by weak forces generally has a low heat of fusion and a low melting point, whereas one bound together by strong forces has a high heat of fusion and a high melting point. The enthalpy of the investigated derivatives were obtained from the computer program involved in the DSC apparatus and presented in Table 4. The value of ΔH was increased by increasing the length of side chain from methyl (gm) to propyl group (gp), as found to be 51.6–104.1 J/g, respectively. This could be attributed to the relationship between the heat of fusion and intermolecular forces, which increased by increasing the side chain in the N-3 position of THTT moiety. On the other hand, cycloalkyl (gc) and aralkyl (gbz and g) substituents gave their enthalpy a varied value independently.

4. Conclusion

From the results of DSC thermograms, it was found that the cycloalkyl derivative of THTT of glycine was the most stable prodrug as it has the highest activation energy and longest half-life among the tested derivatives. Moreover, in the case of the alkyl substituent, the stability was enhanced upon the lengthening of the side chain at position N-3. Generally, the solid state stability

Table 4
The melting activation energies (E_m) for the derivatives calculated by two different methods and their enthalpy (ΔH) at heating rate $\alpha = 10$ °/min

Derivative	Kissinger $\ln(\alpha/T_m^2)$ vs. $1/T_m$ E_m (kJ/mol)	Madahevan $1/T_m$ vs. $\ln(\alpha)$ E_m (kJ/mol)	Enthalpy at $\alpha = 10$ °C/min ΔH (J/g)
gm	83.22 ± 1.72	92.19 ± 0.0320	51.6
gb	149.35 ± 0.15	156.78 ± 0.0005	62.5
gc	150.87 ± 0.23	160.49 ± 0.0008	83.6
gbz	160.25 ± 0.16	170.24 ± 0.0004	77.8
g	170.43 ± 135	178.13 ± 0.0026	70.2
gp	179.66 ± 1.01	187.06 ± 0.0020	112.7
ge	183.78 ± 2.49	192.83 ± 0.0050	72.3

of such derivatives can be controlled by the choice of N-3 substituent. Finally, this work shed some light on the solid state stability of potentially useful promoieties THTT as a drug delivery system for amino acids and peptide drugs.

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