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Synthesis and Physicochemical Properties of Hydrazides of Malonic Acid with Different Substitutes

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Abstract

Shown that the rout of reaction of 2-ethoxycarbonyl-4,4-disubstituted-4-butanolide with hydrazine hydrate at soft conditions (45–50 °C) strongly depends on the substituents on position 2 of butanolide cycle. The effect of substituents on the mechanism of above-mentioned reaction and yields of products has been investigated. Obvious that, dihydrazides of substituted malonic acid are produced when R^3=H, whereas in the case of alkyl substituted butanolides intramolecular cyclization of intermediate occurs at the same conditions and as a result 4,4-disubstituted pyrazolidine-3,5-diones are produced. We studied physicochemical properties including antioxidant activity of the prepared compounds. The UV-Vis absorption spectroscopic studies reveal the effect of substituents on both absorption wavelength and molar absorption coefficient. From the spectra the transition type was determined. From dynamic light scattering measurements the mean hydrodynamic diameters of synthesized molecules is also determined. The antioxidant activity of malonic acid derivatives is studied using competitive reaction between hydroxyl radicals and free radical target p-nitroso-N,N-dimethylaniline. The rate constant between derivatives of malonic acid and hydroxyl radicals was determined and the results were compared with those of vitamin C.

1. Introduction

Dibasic organic acids and their derivatives have wide applications in industry as a plastificators and solvents as well as in the area of fine organic synthesis. In recent works [1–4] it was shown that different derivatives of substituted glutaric acid exhibit various types of biological activity. Particularly, dihydrazides 1 possess high hypotensing action, mixed esters 2 reveal anti-inflammatory and psychotropic influence, allylglutaric acidimides 3 act as anti-inflammatory agents, monosodium salt of glutaric acid amide 4 possesses antimineralocorticoid activity (Scheme 1).

Thus, from the comparison of the illustrated derivatives of dicarbonic acids one can conclude that the type of biological activity strongly depends on the structure of compounds. The present study is focused on the preparation, characterization and investigation of the hydrazides of malonic acid that contain different substituents. The main attention of our study is to investigate the relationship between structural changes, physicochemical properties and biological activity of the prepared compounds. The size determination of synthesized compounds in solutions was studied by the dynamic light scattering technique. Measurement of the size of novel small molecules and micelles is currently of great interest in many applications [5–7]. The size of molecules can be used as a quality control criterion for newly synthesized molecules with biological activity and antioxidant properties [8–10]. However, there are very few available methods for measuring the size of small molecules and particles in solutions. One such modern method is the method of dynamic light scattering, which measures the hydrodynamic diameter of a particle [11–12]. In this work, the measurements of the size and distribution of molecules for the above mentioned compounds (2a-e) in aqueous solutions were performed.

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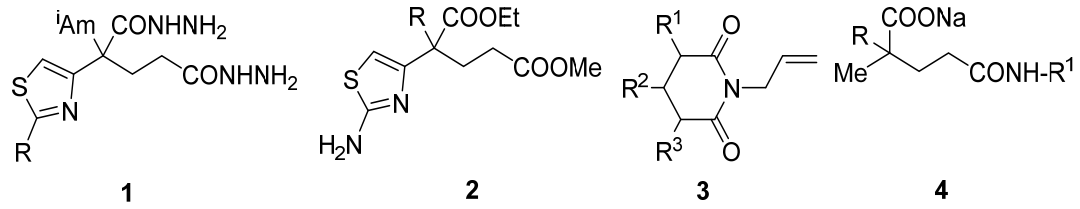


Scheme 1. Structure of some derivatives of dibasic acids.

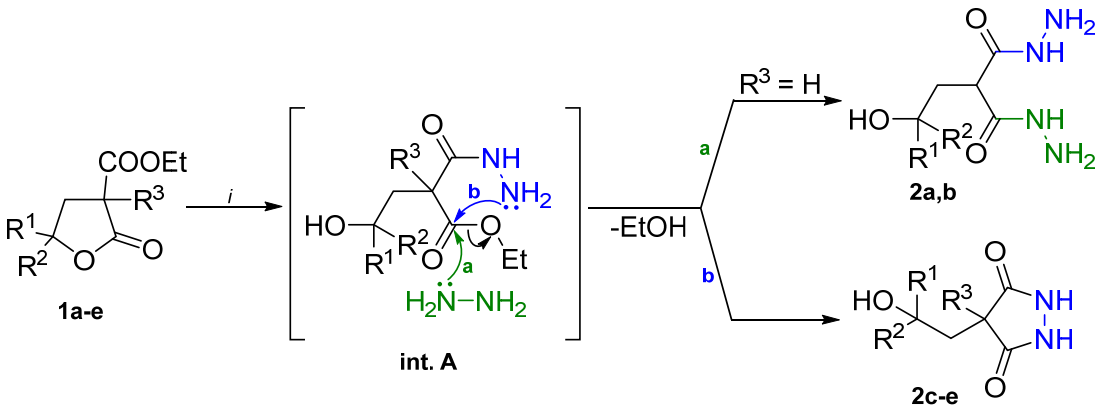
Another important ability of these compounds is their antioxidant activity. The compounds that penetrate into an organism from surroundings may effect human health and lifetime [13–15]. Worsening environmental conditions increases the risk of oxidative stress. Oxidative stress is a result of the accumulation of free radicals in organisms, which causes cardiovascular and neurological disorders, the development of hematological and respiratory diseases as well as eye disorders. In physiological systems, the high reactivity of free radicals increases the rate of oxidative processes causing degradation of the molecular basis of the cell, and as a result, various pathological conditions are developed. Antioxidants are compounds that can reduce the amount of free radicals and thus protect the macromolecules of living cells [16–20]. Therefore, the interaction between antioxidants and free radicals such as hydroxyl radicals is in the focus of researchers from both biomedical and environmental viewpoints [21–24].

It is known that one approach of synthesizing the derivatives of dibasic acids is the reaction of substituted 4-butanolides with various nucleophiles, e.g. hydrazine hydrate. From the literature data that in the case when butanolide is bearing the ester group at the beginning of the reaction the butanolide ring is opening and results in ester of hydrazide. In the next step, the interaction of hydrazine with the ester group leads to the formation of the corresponding dihydrazide [25].

In the present work, the influence of substituents on the mechanism of above mentioned reaction and yields of products has been investigated. For this goal 2-ethoxycarbonyl-4,4-disubstituted-4-butanolide was chosen as starting material and hydrazine hydrate as a nucleophile (Scheme 2 and Table 1).

In the case when R^3=H, dihydrazides of substituted malonic acid are produced, whereas in the case of 2-alkyl substituted butanolides, at the same conditions, intramolecular cyclization of intermediate occurs and as a result 4,4-disubstituted pyrazolidine-3,5-diones are produced. To explain such behavior for 2-ethoxycarbonyl-2-alkyl-4-butanolides we suggest the following mechanism: due to hydrophobic interaction between alkyl and hydroxopropyl groups located at 2 positions the distance between ester and amide groups decreases. As a result, the intramolecular reaction of hydrazinocarbonyl with ester predominates. Thus during the reaction a more stable cyclic structure of pyrazolidine-3,5-diones is formed.

All molecules were chemically characterized by ^{1}H-NMR, ^{13}C-NMR, UV-vis, LC-MS, agreeing well with their prospective structures.



Scheme 2. Synthesis of derivatives of malonic acid. Reagents and conditions: (i) N_2H_4(aq), EtOH, 45-50 °C 2 h.

Table 1. Synthesis of derivatives of malonic acid 2a-e

#	R^1	R^2	R^3	Yield of comp. 2, %
a	$^{i}BuOCH_2$	H	H	85
b	Me	H	H	90
c	$^{i}BuOCH_2$	H	Bu	79
d	$PrOCH_2$	H	Bu	83
e	Me	Me	iAm	88

Our study is focused on the effect of substituted alkyl groups on the synthesis process, the physicochemical properties, and the antioxidant activity of the resulting compounds as well. Similar studies on these systems have not been described in the literature. UV-vis spectra of compounds (2a–e) in aqueous solutions reveal the effect of structural changes on the spectral characteristics of these compounds (Fig. 1 and Table 2).

Table 2. UV-vis data of compounds (2a–e) in aqueous solutions

#	λ_{max}, nm	ε, l · mol^{-1} · cm^{-1}
2a	241	1819
2b	247 (shoulder)	1514
2c	253	3532
2d	252	3661
2e	261 (shoulder)	528

The values of absorption maxima of these compounds in aqueous solutions and their corresponding molar extinction coefficient values, which are given in Table 2, are characteristic of n→π* transitions and are determined by the carbonyl groups present in these compounds. As it can be seen from the data the hydrazide group on one hand and pyrazolidine cycle on the other hand have certain influence on the spectral characteristics. The absorptions, whose maxima have been registered on 252 and 261 nm, belong to substituted pyrazolidine-3,5-diones, whereas the absorptions at lower wavelengths at 241 and 247 nm ascribing hydrazide group containing compounds.

The DLS equipment measures the time-dependent fluctuation in the intensity of light scattered by the particles in solution at a fixed scattering angle. The selection of the measurement angle is based on the transmission. The latter is continuously measured for all samples by the same equipment.

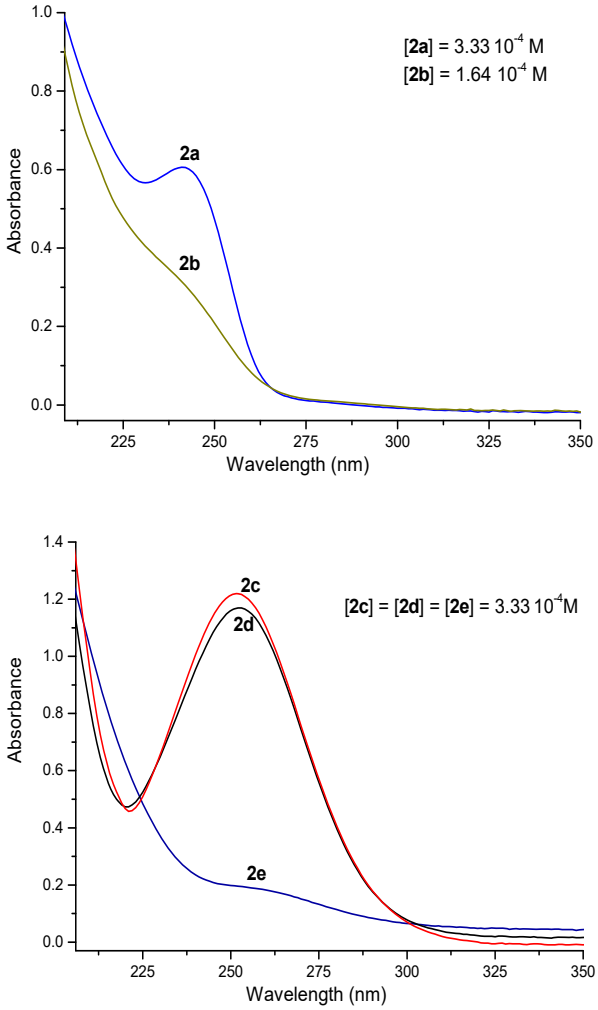


Fig. 1. UV-Vis spectra for compounds 2a-e.

The hydrodynamic diameter (d_H) of the particles is defined using the Stokes-Einstein equation:

$$d_H = \frac{k_B T}{6\pi\eta D} \quad (1)$$

where k_B is the Boltzmann constant, η is the shear viscosity, T is the thermodynamic temperature, and D is the translational self-diffusion coefficient. The latter is evaluated from the autocorrelation function of the time-dependent intensity fluctuations.

The mean hydrodynamic diameters of the molecules of compounds (2a–e) were measured at optimal concentrations. The automatic measurement angle selection in software Kalliope was employed. The equipment implicitly chose side scattering (90°) in all conducted measurements.

The data of mean hydrodynamic diameters, d_H, and polydispersity indexes (PDIs) of the sizes obtained for all solutions of compounds (2a-e) at 298 K are presented in Table 3.

Table 3. The hydrodynamic diameters, d_H, and polydispersity indexes (PDIs) of the sizes of solutions of compounds (2a–e) at 298 K

#	Solvent	d_H, nm	PDIs, %
2a	EtOH	1.08±0.04	41.0
2b	EtOH	1.15±0.15	52.0
2c	EtOH	0.83±0.13	43.1
2d	H_2O	1.05±0.03	34.0
2e	H_2O	0.96±0.05	64.0

The intensity-weighted size distributions for some compounds are presented in the Supplementary material (Fig. S-11 – Fig. S-13).

The antioxidant activity of organic compounds (2a-e) was determined by the study of competitive reaction between hydroxyl radicals and free radical target p-nitroso-N,N-dimethyl aniline (PNDMA). The reaction to free radicals causes de-colorization of PNDMA and, by measuring the absorption at 440 nm it is possible to calculate the rate of reaction. The dependences of absorption of PNDMA from the period of radiation of hydrogen peroxide in the absence and presence of compounds (2a-e) are depicted in the Supplementary material (Fig. S-14 – Fig. S-18). With the increase in concentrations of compounds (2a-e) the rate of competitive hydroxyl radical – PNDMA reaction decreases significantly, showing that these compounds have pronounced antioxidant properties.

It turned out that an increase in its concentration results in a decrease in the rate of decolorization of PNDMA, so compounds (2a-e) react with hydroxyl radicals and as a result, the competitive PNDMA-hydroxyl radical reaction becomes slower. From the slopes of the plots for different contents of compounds (2a-e) the rate of hydroxyl radical-PNDMA reaction is determined and the plots of slopes versus molar concentration of compounds (2a-e) are depicted in Fig. 2.

The rate constants of compounds (2a-e) – hydroxyl radicals reaction are determined from the slopes of the plots of the rate of the reaction versus concentration using Eq. (2).

$$k_{OH+antioxidant} = 1.25 \cdot 10^{10} \cdot \frac{[PNDMA]}{[Antioxidant]} \cdot \left(\frac{W_1}{W_2} - 1 \right) \qquad (2)$$

where [Antioxidant] and [PNDMA] are molar concentrations of antioxidant and PNDMA, respectively, $1.25 \cdot 10^{10}$ is the rate constant of reaction between hydroxyl radical and PNDMA (*l mol*$^{-1}$ *sec*$^{-1}$), W_1 and W_2 are the slopes of plots of PNDMA absorption versus time of hydrogen peroxide radiation in the absence and presence of antioxidant, respectively.

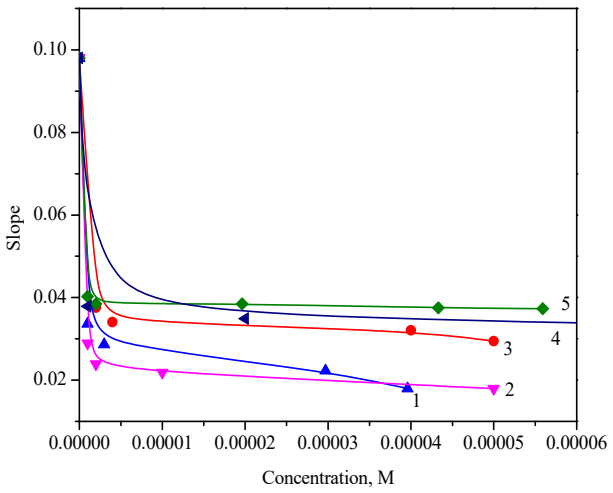


Fig. 2. Dependence of slopes of plots presented in supporting materials from the molar concentration: 1 – (2c); 2 – (2d); 3 – (2a); 4 – (2b); 5 – (2e).

The data presented in Table 4 show that the rate of reaction between compounds (2a-e) and hydroxyl radicals is maximum with (2e) and it decreases in the following order: (2e)>(2a)>(2b)>(2c)>(2d). For the comparison, it should be noted that the rate constant of reaction between well-known antioxidant ascorbic acid and hydroxyl radicals is 9.45×10^9 L mol^{-1} sec^{-1}, which was determined using the same method [13].

Table 4. Rate constants of the reaction between (2a-e) and hydroxyl radicals

#	$k_{OH+antioxidant}$, L mol^{-1} sec^{-1}
2a	1.40×10^8
2b	0.73×10^8
2c	0.587×10^8
2d	0.344×10^8
2e	1.53×10^8

2. Experimental part

All reagents were of analytical grade and were used as such or distilled prior to the use. Starting 4-butanolides (1a-e) were prepared as previously reported [26,27].

^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury-300 MHz in DMSO-CCl_4 mixture (1:3). Chemical shifts (δ) in ppm are reported as quoted relative to the residual signals of DMSO-d_6 (2.5 for ^{1}H NMR and 39.5 for ^{13}C NMR) as internal references.

The coupling constants (J) are given in Hertz (figures are in the Supplementary material from Fig. S-1 to Fig. S-10). TLC analysis was performed on silica gel "Silufol UV-254" plates. Melting points were determined on "Boetius" micro-heating stage.

ESI-MS spectra were measured with a LC-MS 2020 Shimadzu. The UV-vis absorption spectra were recorded with the Specord 50PC spectrophotometer using Aspect Plus Software. The path length used in absorption experiments was 1 cm. All measurements were performed at a constant temperature, 25.0 ± 0.3 Celsius, using the thermostatic device (LAUDA A100) attached to the spectrophotometer.

The dynamic light scattering (DLS) measurements were performed using the Litesizer 500 (Anton Parr, Austria) equipped with the Kalliope software. The "General" analysis model is used for the determination of the particle size distribution chart and peak analysis. For the determination of hydrodynamic diameter and polydispersity index the "Advanced" cumulant model with CONTIN algorithm is used. The measurement series were performed in quartz cuvettes at 298.15 K with 20 repetitions to verify reproducibility.

The optimal measured angle, number of runs, focus position, and optical filter settings were used as recommended by the equipment. Every sample was equilibrated before the measurement to avoid temperature gradients. The DLS experiments were reported with an uncertainty of less than 5%. An important factor for making good DLS measurements is using the right concentration. The concentration of particles must be high enough to produce a good scattering intensity. On the other hand, if the concentration is too high, then multiple scattering may occur, which can produce an erroneous result.

Therefore, to find out optimal concentration in DLS measurements, the measurements of several concentrations for each solution have been done. The solutions of (2a-e) compounds were prepared at least in three concentrations (0.01, 0.05, and 0.1 mol/L) in the deionized water and ethanol. It should be noted that the solubility of (2a-c) in water was less than those for (2d,e) compounds. Therefore for compounds (2a-c) ethanol was used as a solvent to obtain the same concentration range as it was for compounds (2d,e). The solvents were filtered through the 20 nm pore-size syringe filter (Whatman Anotop-25) before preparing the solutions. As an extra check, the particle-size measurements were carried out for the samples of the solvents before adding them to (2a-e) compounds. The absence of unwanted particles was in this way ensured.

Antioxidant activity of (2a-e) compounds was measured by PNDMA assay. In this method, the antioxidant properties are estimated by the study of kinetics of competitive reaction between hydroxyl radicals and PNDMA. Hydrogen peroxide is radiated by UV light at 313 nm causing the formation of hydroxyl radicals that react with PNDMA and as a result dye is decolorized. The rate of the reaction between hydroxyl radicals and PNDMA is determined from the absorption at 440 nm using photometric method. Addition of antioxidant affects this reaction due to the competitive reaction between (2a-e) compounds and hydroxyl radicals resulting in slower decolorization of radical target PNDMA. By the examination of rate of decolorization of dye the rate constant of reaction between (2a-e) compounds and hydroxyl radical may be determined according to the Eq. (2) (the data are given in Table 4, and figures are in the Supplementary material: Fig. S-14. – Fig. S-18) [28–31].

General method for preparing compounds (2a-e). 0.05 mol of the corresponding 2,2,5-trisubstituted-4-butanolide solved in 100 ml EtOH is placed in Erlen-meyer flask. Then 7.4 g (0.125 mol) 85% of hydrazine hydrate was added. After the solution was stirred and left for 2 h, then the reaction mixture was heated at 45–50 °C for 1 hour. The ethanol was evaporated in vacuum and the crude solid was left for crystallization. The precipitated crystals were washed with ether and dried.

2-(2-hydroxy-3-isobutoxypropyl)malonohydrazide (2a). Yield 11.3 g, 85%, m.p. 136–137 °C, R_f 0.49 (benzol: aceton: methanol - 1:5:15). ^{1}H NMR (300 MHz, DMSO/CCl_4 - 1/3), δ, p.p.m. (*J*, Hz): 8.77 br.s (1H, NH), 8.65 br.s (1H, NH), 4.64–3.54 m (5H, NH_2, OH), 3.53–3.40 m (1H, OCH), 3.31–3.20 m (3H, OCH_2, CH-C=O), 3.16 d (2H, OCH_2 $CHMe_2$, *J* 6.6), 2.01 ddd (1H, $HCCH_2CH$ J^1 13.3, J^2 9.9, J^3 3.0), 1.92–1.74 m (1H, $CHMe_2$), 1.56 ddd (1H, $HCCH_2CH$, J^1 14.1, J^2 9.9, J^3 4.6), 0.90 d (6H, CH_3, *J* 6.7). ^{13}C NMR (75 MHz, DMSO/CCl_4 - 1/3), δ: 168.93, 168.60, 77.34, 75.13, 66.73, 46.05, 33.25, 27.80, 19.00.

LCMS [M+Na] 285.

2-(2-hydroxypropyl)malonohydrazide (2b). Yield 8.5 g, 90%, m.p. 131–132 °C, R_f 0.57 (aceton: hexane - 2:3). ^{1}H NMR (300 MHz, DMSO/CCl_4 - 1/4), δ, p.p.m. (*J*, Hz): 8.80 br.s (1H, NH), 8.70 br.s (1H, NH), 4.13 s (4H, NH_2), 3.60–3.38 m (1H, OCH), 3.20 dd (1H, CH-C=O, J^1 9.4, J^2 5.2), 1.89 ddd (1H^a, CH_2, J^1 13.3, J^2 9.4, J^3 3.7), 1.62 ddd (1H^b, CH_2, J^1 13.9, J2 9.0, J^3 5.2), 1.07 d (3H, CH_3, *J* 6.1). ^{13}C NMR (75 MHz, DMSO/CCl_4 - 1/4) δ: 169.0, 168.8, 63.7, 46.6, 38.4, 23.5.

LCMS [M+1] 191, [M+Na] 213.

4-Butyl-4-(2-hydroxy-3-isobutoxypropyl)pyrazolidine-3,5-dione (2c). Yield 12 g, 83%, m.p. 146–147 °C, R_f 0.45 (benzol:aceton:methanol - 1:5:15). ^{1}H NMR (300 MHz, DMSO/CCl_4 - 1/3), δ, p.p.m. (*J*, Hz): 10.13 br.s (2H, NHC=O), 4.10 d (1H, OH, *J* 5.3), 3.74–3.60 m (1H, CHO), 3.23 dd (1H^a, CH_2O, J^1 13.4, J^2 9.5, J^3 5.6), 3.18–3.09 m (3H, CH_2O), 1.82 m (1H, $CHMe_2$), 1.72–1.63 m (2H, CH_2), 1.61–1.49 m (2H, CH_2), 1.30 m (2H, CH_2), 1.24–1.12 m (2H, CH_2), 0.95–0.86 m (9H, CH_3). ^{13}C NMR (75 MHz, DMSO/CCl_4 - 1/3) δ. 174.4, 173.4, 77.3, 75.0, 66.0, 49.6, 38.1, 35.2, 27.8, 25.4, 22.2, 19.0, 13.5.

LCMS [M+1] 287.

4-Butyl-4-(2-hydroxy-3-propoxypropyl)pyrazolidine-3,5-dione (2d). Yield 10.7 g, 79%, m.p. 126–128 °C, R_f 0.45 (benzol:aceton:methanol - 1:5:15). ^{1}H NMR (300 MHz, DMSO/CCl_4 - 1/3) δ, p.p.m. (J, Hz): 10.13 br.s (2H, NH), 4.11 d (1H, OH, *J* 5.4), 3.74–3.60 m (1H, CHO), 3.34 t (2H, OCH_2, *J* 6.6), 3.23 dd (1H^a, $CHCH_2O$, J^1 9.5, J^2 5.7), 3.15 dd (1H^b, $CHCH_2O$, J^1 9.5, J^2 5.7), 1.74–1.65 m (2H, CH_2), 1.63–1.48 m (4H, CH_2), 1.37–1.24 m (2H, CH_2), 1.24–1.10 m (2H, CH_2), 0.91 t (3H, CH_3, *J* 7.3), 0.90 t (3H, CH_3, *J* 7.3). ^{13}C NMR (75 MHz, DMSO), δ. 174.5, 173.4, 74.9, 72.1, 66.1, 49.7, 38.2, 35.3, 25.4, 22.4, 22.2, 13.5, 10.3.

LCMS [M+1] 273, [M+Na] 295.

4-(2-Hydroxy-2-methylpropyl)-4-isopentylpyrazolidine-3,5-dione (2e). Yield 10.6 g, 88%, m.p. 87–88 °C, R_f 0.60 (aceton: hexane - 2:3). ^{1}H NMR (300 MHz, DMSO/CCl_4 - 1/3) δ, p.p.m. (J, Hz): 8.47 br.s (2H, NH), 4.10 br.s (1H, OH), 2.87 d (1H, CH_2, *J* 13.3), 1.98–1.80 m (2H, CH_2), 1.74–1.60 m (1H, CH_2), 1.59–1.40 m (4H, CH_2, CH_3), 1.32 s (3H, CH_3), 1.24–1.08 m (1H, CH_2), 1.08–0.94 m (1H, CH_2), 0.89 d (6H, CH_3, *J* 6.6). ^{13}C NMR (75 MHz, DMSO/CCl_4 - 1/3) δ, pp: 175.5, 167.5, 81.6, 55.5, 41.6, 35.0, 33.1, 29.1, 27.8, 27.5, 22.0, 22.0.

LCMS [M+1] 243, [M+Na] 265.

4. Conclusions

In summary, five new derivatives of hydrazides of malonic acid with different substitutes are synthesized. The reaction of 2-ethoxycarbonyl-4,4-disubstituted-4-butanolide with hydrazine hydrate occurs with formation of intermediante following further two ways transformations providing pyrazolidine-3,5-dione and dihydrazide of malonic acid. It was found, that the character of products depends on the type of substituents on position 2 of butanolide cycle. The hydrophobic interaction between alkyl groups of substitutes located in position 2 is responsible for the formation of pyrazolidine-3,5-dione.

The physicochemical and spectroscopic characteristics of malonic acid derivatives was studied using UV-Vis absorption spectroscopy and dynamic light scattering. From the UV-Vis absorption spectra the effect of substituent on both absorption wavelength and molar absorption coefficient was revealed. Moreover, it was found that the absorption arises due to n→π* transitions. From DLS measurements the mean hydrodynamic diameters of synthesized molecules is also determined. The antioxidant activity of malonic acid derivatives is studied using competitive reaction between hydroxyl radicals and free radical target PNDMA. The rate constant between derivatives of malonic acid and hydroxyl radicals was determined and the results were compared with those of well-known antioxidant vitamin C. A correlation between the size and polydispersity on one hand and antioxidant activity of synthesized compound on the other hand have been revealed. Particularly, the less is size of molecule and higher is polydispersity the more is the rate constant of the reaction of antioxidant molecule with hydroxyl radical

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Supplementary material

Supplementary data to this article can be found online at: https://doi.org/10.18321.ectj1644

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