

Diamides of squaric acid containing methioninamide fragment – synthesis, spectral and theoretical investigation

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Dedicated to Acad. Ivan Juchnovski on the occasion of his 80th birthday

Four novel diamides of squaric acid were synthesized in high yields using a condensation reaction of methioninamide esteramide of squaric acid with different amides of amino acids. Newly synthesized compounds were investigated by elemental analyses, IR and NMR. The molecular structures of the compounds were elucidated by a DFT method. The theoretical analysis showed that squaric fragment and NH-groups lied in one plane which was almost perpendicularly oriented to the plane of asymmetric C-atom.

Key words: diamide of squaric acid; methioninamide; IR; DFT calculations

INTRODUCTION

Methionine, one of the two sulfur-containing amino acids, is found in all living cells [1] and is critical for the synthesis of many proteins. It plays an important role in many biochemical processes involved in producing substances necessary for normal functioning of the cardiovascular, bone and nervous system [2]. Methionine is a major source of methyl groups in the body.

On the other hand, mono and diamides of squaric acid (H₂Sq) received much attention in the recent years. Their unique structural features: strained ring, two carbonyl groups and a C=C bond, two carbonyl acceptor and two NH donor groups are able to form hydrogen bonds [3] determined their specific reactivity and properties. These derivatives are important from pharmacological point of view due to the broad range of their biological activity. Many amides of H₂Sq are used as phosphates, carboxylate and amino acids isosters due to the similar structure [4-6], metal chelators for the inhibition of matrix metalloprotease enzymes [7,8] and ion receptors [3,9] in medicinal chemistry. Squaramide fragment is used as binding unit in bioconjugate chemistry [10,11]. Also squaramides have application as chiral ligands and catalyst in organometallic chemistry and organocatalysis [12,13]. For this reason, the synthesis of new mono- and diamides of squaric acid and the investigation of their properties are important for several chemistry fields.

Herein is presented the synthesis and spectral investigation of four novel methioninamide containing diamides of squaric acid. In order to get more information about their molecular structure the newly synthesized compounds were studied by theoretical methods.

MATERIALS AND METHODS

Amide of amino acids hydrochlorides were purchased from Bachem AG. Diethyl ester of squaric acid ethanol and triethylamine were purchased from Sigma-Aldrich.

The IR spectra were measured on a Bruker Tensor 27 FTIR spectrometer. In all cases the spectra were recorded at a resolution of 2 cm⁻¹ (64 scans). The NMR spectra were recorded on a Bruker DRX250 spectrometer in solvent DMSO-d₆ using TMS as internal standard. Standard Bruker pulse sequences and software were used to record and process the spectra. The elemental analyses were carried out according to the standard procedures for C and H (as CO₂, and H₂O) and N (by the Dumas method). The molecular structure and vibrational spectra of the compounds were studied by computational method. Thin layer chromatography (TLC) was performed on aluminum sheets pre-coated with Merck Kieselgel 60 F254 0.25 mm (Merck). All density functional theory (DFT) computations were performed with the Gaussian 09 program package [14] employing the B3LYP (Becke's three-parameter non-local exchange) [15,16] correlation functional and 6-311++G** basis set

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Synthesis of methionine containing diamides of squaric acid (**5a-d**)

Aqueous ethanolic solution (1:4) of corresponding amide of amino acids hydrochlorides (1mmol) was mixed with excess of Et₃N (5mmol) and the mixture was stirred for 10 minutes at room temperature. After that ethanol solution of methioninamide esteramide of H₂Sq (1 mmol) was added. The mixture was stirred at room temperature to complete the reaction. The products were isolated by precipitation and purified by recrystallization.

Spectral data of methionine containing diamides of H₂Sq **5a-d**:

5a: 2-[2-(1-Carbamoyl-3-methylsulfanyl-propylamino)-3,4-dioxo-cyclobut-1-enylamino]-3-methyl-butryamide

M = 342.41; Elemental analysis: C₁₄H₂₂N₄O₄S (Met/Val) (%), Calcd. C 49.1, H 6.4, N 16.3, S 9.3; Found C 49.0, H 6.4, N 16.4, S 9.3;

¹H NMR (DMSO- d₆, 250 MHz), δ (ppm): 8.00 (dd, J=9.0, 14.5 Hz, 2H, 2NH), 7.74 (d, J=5.9, 2H, NH₂), 7.28 (d, J=16.9 Hz, 2H, NH₂), 4.65 (dd, J=7.2, 13.1 Hz, 1H, CH), 4.53 (dd, J=5.1, 9.4 Hz, 1H, CH), 2.43 (m, 2H, CH₂), 2.05 (s, 3H, SCH₃), 1.89 (m, 2H, CH₂), 0.86 (dd, J=6.8, 11.4 Hz, 6H, 2CH₃);

¹³C NMR (DMSO - d₆, 250 MHz), δ (ppm): 182.41, 182.36, 172.06, 167.67, 166.75, 60.67, 55.26, 34.58, 32.08, 28.69, 18.80, 16.78, 14.66

+ MS, m/e (relative intensity): 343.22 (M⁺);

5b: 2-[2-(1-Carbamoyl-3-methylsulfanyl-propylamino)-3,4-dioxo-cyclobut-1-enylamino]-3-methyl-pentanoic acid amide

M = 356.44; Elemental analysis C₁₅H₂₄N₄O₄S (Met/Ile) (%), Calcd. C 50.5, H 6.7, N 15.7, S 9.0; Found C 50.4, H 6.8, N 8.8; S 9.0

¹H NMR (DMSO- d₆, 250 MHz), δ (ppm): 7.98 (dd, J=9.0, 13.0 Hz, 2H, 2NH), 7.73 (d, J=8.7 Hz, 2H, NH₂), 7.28 (d, J=16.8 Hz, 2H, NH₂), 4.65 (dd, J=6.3, 11.2 Hz, 1H, CH), 4.53 (dd, J=5.3, 9.0 Hz, 1H, CH), 2.44 (m, 2H, CH₂), 2.03 (s, 3H, SCH₃), 1.85 (m, 2H, CH₂), 1.04 (m, 2H, CH₂), 0.85 (dd, J=6.8, 11.4 Hz, 6H, 2CH₃)

¹³C NMR (DMSO- d₆, 250 MHz), δ (ppm): 182.40, 182.32, 172.14, 172.06, 167.56, 166.77, 60.47, 55.27, 34.58, 28.70, 23.50, 15.13, 14.67, 11.46

+ MS, m/e (relative intensity): 357.18 (M⁺);

5c: 2-[2-(1-Carbamoyl-2-hydroxy-ethylamino)-3,4-dioxo-cyclobut-1-enylamino]-4-methylsulfanyl-butryamide

M = 330.36; Elemental analyses: C₁₂H₁₈N₄O₅S (Met/Ser) (%), Calcd. C 43.6, H 5.4, N 16.9, S 9.7;

Found C 43.6, H 6.2, N 16.7, S 9.6

¹³C NMR (DMSO- d₆, 250 MHz), δ (ppm): 182.49, 182.30, 172.20, 172.11, 167.30, 166.42, 60.01, 55.80, 55.53, 33.24, 28.72, 14.48

+ MS, m/e (relative intensity): 331.66 (M⁺);

5d: 2-[2-[1-Carbamoyl-2-(4-hydroxy-phenyl)-ethylamino]-3,4-dioxo-cyclobut-1-enylamino]-4-methylsulfanyl-butryamide

M = 406.5; Elemental analyses C₁₈H₂₂N₄O₅S (Met/Tyr) (%), Calcd. C 53.1, H 5.4, N 13.7, S 7.8; Found C 52.7, H 4.9, N 13.5, S 7.9;

¹H NMR (DMSO- d₆, 250 MHz), δ (ppm): 9.20 (s, 1H, OH), 7.95 (m, 2H, NH), 7.70 (s, 2H, NH₂), 7.27 (d, J=12.0 Hz, 2H, NH₂), 6.96 (d, J=8.4, 2H, Ar), 6.63 (d, J=8.4, 2H, Ar), 4.76 (d, J=7.1, 1H, CH), 4.63 (d, J=5.7, 1H, CH), 2.78-2.98 (m, 2H, CH₂), 2.40 (m, 2H, CH₂), 2.02 (s, 3H, SCH₃), 1.86 (m, 2H, CH₂)

¹³C NMR (DMSO- d₆, 250 MHz), δ (ppm): 182.42, 172.02, 167.20, 166.93, 162.38, 156.01, 130.42, 126.65, 114.98, 57.32, 55.24, 35.85, 34.58, 30.83, 28.72, 14.69

+ MS, m/e (relative intensity): 407.46 (M⁺).

RESULTS AND DISCUSSION

Synthesis

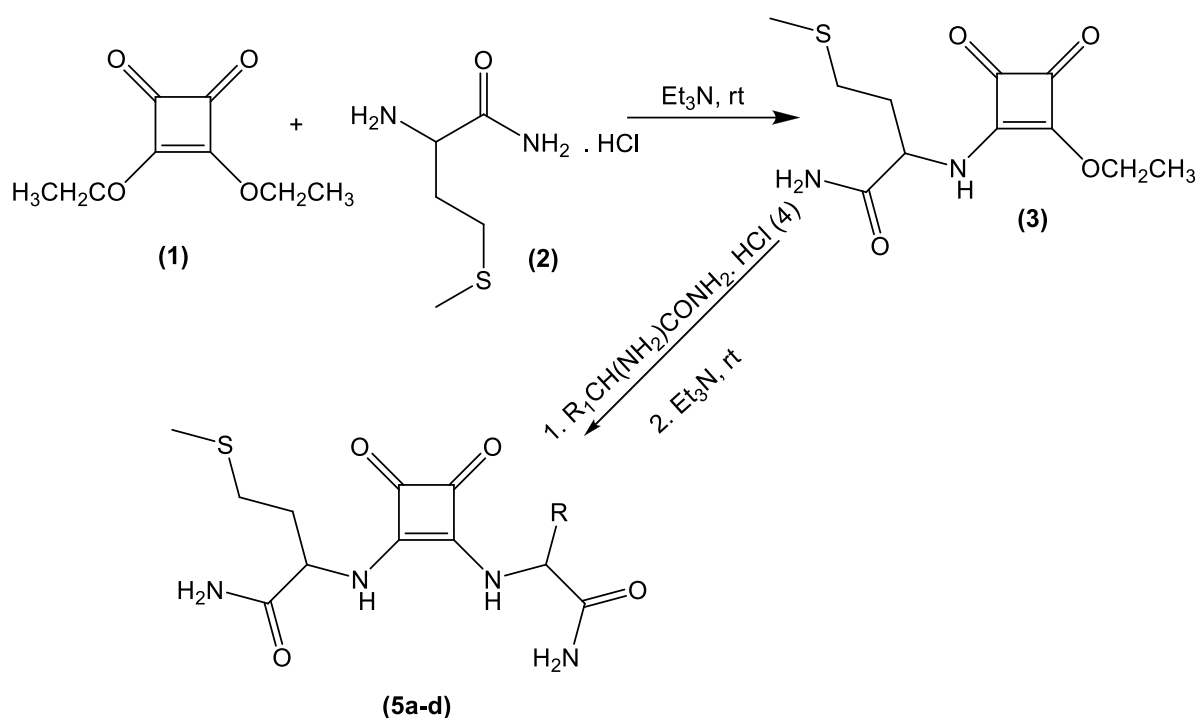
Nonsymmetric diamides of H₂Sq **5a-d** were prepared in two steps *via* condensation of amino acid amides and diethyl squarate (**1**), using as a catalyst Et₃N in ethanol at room temperature to methioninamide esteramide of H₂Sq (**3**) and condensation of monoamide to diamides. Synthetic pathway to obtain the new derivatives is illustrated on the Scheme 1. The synthesis and properties of methioninamide esteramide of squaric acid were described in our previously published work [17].

The products were isolated by precipitation from the corresponding reaction mixture in good to excellent yields and high purity (Table 1).

Vibrational analysis

For the investigated diamides of squaric acid the most important vibrational frequencies were stretching vibrations of amide NH₂ groups, secondary amino groups, ν^{as}_{C=O(Sq)}, ν^s_{C=O(Sq)}, ν_{C=C(Sq)}, Amide I (ν_{C=O}), amide II (δ_{NH₂}) and deformation vibrations of NH groups (δ_{NH}).

The accurate assignment of the main experimental frequencies of compounds **5a-d** to the corresponding normal modes was supported by B3LYP/6-311++G ** calculations.



Scheme 1. Synthesis of methionine containing diamides of squaric acid (**5a-d**).

Table 1. Diamides of squaric acid obtained according to the Scheme 1.

5	R		Reaction conditions*	Yield (%)	M.p. (°C)
a	-CH(CH ₃) ₂	(Val)	r.t., 17 h., EtOH/H ₂ O	75	325-334
b	-CH(CH ₃)CH ₂ CH	(Ile)	r.t., 20 h., EtOH/H ₂ O	86	330-349
c	-CH ₂ OH	(Ser)	r.t., 24 h., EtOH/H ₂ O	80	330-351
d	-CH ₂ C ₆ H ₄ OH	(Tyr)	r.t., 24 h., EtOH/H ₂ O	85	210-266

*Temperature, reaction time, solvent

Selected experimental and calculated vibrations are summarized in Tables 2 and 3. Predicted frequency of the stretching vibrations of the amide groups and secondary amino groups occur at higher frequencies in comparison with the experimental data. This discrepancy is due to the fact that the calculations were performed in the gas phase, where there are no intramolecular hydrogen bonds, while IR spectra were measured in the solid state, where in the formation of hydrogen bonds is possible.

The vibrations of cyclobutene fragment appear as a two bands for the C=O bonds (weak symmetric, strong asymmetric) and a very intensive band for C=C bond. The band for the stretching vibration of double bond in diamides of H₂Sq was shifted to lower frequency, while the bending vibrations of the amino groups were shifted to high

frequency in comparison with esteramides.

As should be expected, in the case of **5c** and **5d** the ν_{OH} vibration was influenced by hydrogen bonding and its assignment to a separate band in the spectrum was difficult. The bands for ν_{OH} and $\nu_{NH_2}^{as}$ were overlapped, the first one appeared as a shoulder of the second one.

NMR analysis

The NMR spectra of compounds showed protons signals for the secondary amino groups around 8.02-7.94 ppm. The protons of primary amino groups were observed as a doublet in the 7.32-7.25 ppm and 7.76-7.72 ppm. In the case of the compound **5d** signals appeared as a singlet at 7.71 ppm and a doublet at 7.27 ppm ($J=12.0$ Hz).

Table 2. Selected theoretical and experimental vibrational frequencies (ν in cm^{-1}) of **5a** and **5b**.

Assignment ^a	5a		5b	
	<i>B3LYP</i> ^b	IR	<i>B3LYP</i> ^b	IR
$\nu^{\text{as}}_{\text{NH}_2}$	3544; 3541	3353, 3333	3544, 3542	3356; 3332
ν_{NH}	3465; 3458	3272, 3248	3466, 3455	3278; 3250
$\nu^{\text{s}}_{\text{NH}_2}$	3397; 3379	3201, 3166	3401, 3378	3170; 3141
$\nu^{\text{s}}_{\text{C=O}}$ (Sq)	1785	1805	1785	1803
$\nu^{\text{as}}_{\text{C=O}}$ (Sq)	1693	1702	1692	1700
$\nu_{\text{C=O}}$ (Amide I)	1711; 1706	1670, 1660	1711; 1706	1670; 1658
δ_{NH_2} (Amide II)	1588; 1587	1647, 1636	1587; 1585	1648sh; 1638
$\nu_{\text{C=C}}$ (Sq)	1597	1590	1596	1595
δ_{NH}	1476	1538, 1527	1475	1538; 1529
$\nu_{\text{C-S(CH}_3)}$	683	721	684	721

^aVibrational modes: ν , stretching; δ , bending^bScaled by 0.9686 [18]**Table 3.** Selected theoretical and experimental vibrational frequencies (ν in cm^{-1}) of **5c** and **5d**.

Assignment ^a	5c		5d	
	<i>B3LYP</i> ^b	IR	<i>B3LYP</i> ^b	IR
ν_{OH}	3701	3471sh	3714	3438sh
$\nu^{\text{as}}_{\text{NH}_2}$	3554; 3543	3374sh; 3340	3549; 3543	3400sh
ν_{NH}	3466; 3450	3273; 3250sh	3466; 3464	3272; 3167
$\nu^{\text{s}}_{\text{NH}_2}$	3419; 3384	3172; 3140	3395; 3381	3370; 3325
$\nu^{\text{s}}_{\text{C=O}}$ (Sq)	1787	1809	1786	1809
$\nu^{\text{as}}_{\text{C=O}}$ (Sq)	1697	1705	1693	1700
$\nu_{\text{C=O}}$ (Amide I)	1717; 1710	1676; 1666	1712; 1705	1673; 1653
δ_{NH_2} (Amide II)	1587; 1586	1646sh; 1623	1588; 1586	1612; 1606
$\nu_{\text{C=C}}$ (Sq)	1598	1593	1598	1597
δ_{NH}	1476	1539; 1520	1475	1533; 1521
$\nu_{\text{C-S(CH}_3)}$	684	721	683	721

^aVibrational modes: ν , stretching; δ , bending^bScaled by 0.9686 [18]

In the experimental ^{13}C NMR spectra the signals of the carbon atoms of the cyclobutene moiety appeared at a slightly higher field (167 ppm and 182 ppm) than that of the methioninamide esteramide of squaric acids (170 ppm and 188 ppm). The amide carbons were detected at 172–173 ppm in the ^{13}C NMR spectra. The carbon atoms from C=C bond in diamides are shifted to higher fields than those of esteramides.

Computational analysis

Due to the difficulties to obtain crystals suitable for X-ray analysis we studied the structure of the diamides of H_2Sq mainly by theoretical methods. All the structures were optimized at the DFT level/*B3LYP* functional and the 6-311++ G^{**} basis set. The calculations were used to obtain important information about the structural characteristics and spectroscopic properties of synthesized compounds (**5a-d**). The optimized structures of the compounds (**5a-d**) are presented in Figure 1.

Since all investigated compounds contain Sq fragment and methioninamide residue, the theoretically found structural parameters for this part of the molecule were compared with the corresponding experimental data from the X-ray analysis for methioninamide and valinamide esteramides of H_2Sq [17,19]. Squaric fragment and NH-groups lied in one plane which was almost perpendicularly oriented to the plane of asymmetric C-atom and amides groups (76–77°). By comparing the theoretical data of the studied methioninamide containing diamides of H_2Sq with experimental data of the methioninamide esteramide, it becomes apparent that the theoretical method predicts with particularly good accuracy the lengths of the C=O (theor. 1.21 Å, exp. 1.21 Å) and C=C (theor. 1.40 Å, exp. 1.39 Å) bonds in the cyclobutene ring. In contrast to methioninamide esteramide, due to the presence of two structurally analogous substituents on the cyclobutanedione ring, the lengths of the carbonyl bonds of the diamides H_2Sq were the identical.

CONCLUSION

In summary, an efficient and simple method was used to prepare a novel methioninamide containing diamides of squaric acid. The structures of the synthesized compounds were confirmed by IR, ^1H NMR, ^{13}C NMR spectra and elemental analyses. We determined the frequency range of the most characteristic vibrations - $\nu^{\text{as}}_{\text{C}=\text{O}(\text{Sq})}$, $\nu^{\text{s}}_{\text{C}=\text{O}(\text{Sq})}$, $\nu_{\text{C}=\text{C}(\text{Sq})}$, Amide I ($\nu_{\text{C}=\text{O}}$), Amide II (δ_{NH_2}) and δ_{NH} . The molecular optimization showed that the NH-groups lie in plane of squaric fragment which was almost perpendicularly oriented to the plane of asymmetric C-atom and amides groups.

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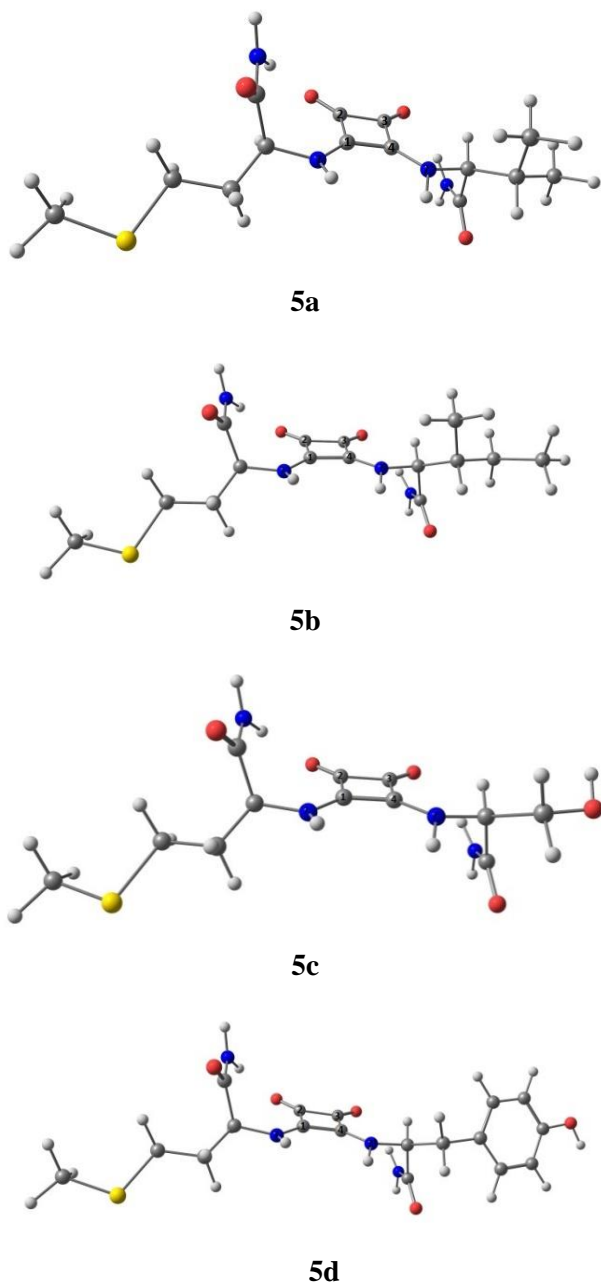


Fig. 1. Optimized structure of methioninamide containing diamides of H_2Sq , performed at DFT/B3LYP/6-311++G** level.

The $\text{C}_1\text{-N}_7$ and $\text{C}_4\text{-N}_7$ bonds that connect the Sq fragment with the amino acid residue were also similar and were in the range of 1.341-1.346 Å, independently of the side chains. In alkyl side chains, as in the experimentally studied squamarides, these bonds were shorter with 0.027-0.021 Å [20].

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ДИАМИДИ НА КВАДРАТНАТА КИСЕЛИНА, СЪДЪРЖАЩИ МЕТИОНИНАМИДОВ ОСТАТЪК – СИНТЕЗ, СПЕКТРАЛНО И ТЕОРЕТИЧНО ИЗСЛЕДВАНЕ

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(Резюме)

Синтезирани са четири нови диамида на квадратната киселина с висок добив, кондензирайки метионинамид естерамид на квадратната киселина с различни амиди на аминокиселини. Новосинтезираните съединения са охарактеризирани чрез елементарен анализ, ИЧ и ЯМР спектроскопия. Молекулната структура на съединенията беше изследвана чрез DFT метод. Теоретичният анализ показва, че сквартната част и HN-групите лежат в една равнина, която се пресича от равнините на асиметричните С-атоми.