SYNTHESES, STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF MONO-SQUARAMIDES

E. Cherneva[1*], Ts. Kolev[2]

[1] Department of Chemistry, Faculty of Pharmacy, Medical University - Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria
[2] Institute of Molecular Biology, Bulgarian Academy of Sciences, 21, G. Bontchev Str., Sofia 1113, Bulgaria
* To whom all correspondence should be sent: E-mail: e.d.cherneva@gmail.com

Abstract. In the present study we describe a simple synthesis of three mono-squaramides with amino acid amides residue. The structure of the compounds is elucidated by means of FTIR, Raman, NMR and elemental analysis. The structural and spectral analysis of the synthesized compounds are supported by theoretical analysis using DFT (B3LYP/6-311++G**) calculations. The simulated spectra satisfactorily coincide with the experimental ones.

Keywords: amino acid amides, squaric acid, IR, Raman, DFT calculations;

Introduction

Amine derivatives of squaric acid (H_2Sq), known as squaramides, are unique with their electronic and structural properties: aromatic strained ring, two carbonyl groups, C=C bond, donor–acceptor hydrogen bonding ability [1-3], restricted rotation around the C–N bonds [4]. These unique properties have aroused a great interest in several chemistry fields.

Squaramide fragment has been used as a coupling reagent in the structure of oligosaharides and sugar-protein biopolymers, which found application as vaccines for infection diseases and anticancer therapy [5-11]. Anthracycline antibiotics containing squaramide group has been used for anticancer therapies too, emphasizing their low cardiotoxic side effects [12,13].

Squaric fragment of H_2Sq and its derivatives are good electrostatic mimetics of phosphates and phosphoester group which motivated the incorporation of this fragment as a part of oligodesocsinucleotides [5,14-15]. Furthermore, Sq fragment is similar to carboxylate and sulphonic acids by its highly polarized electronic structure as well as strong acidity [2,16]. Squaramide derivates have also structural similarity with hydroxamic acid in respect of chelatation, which is important for inhibition of metalloproteases [2,17]. Squaramides are better transmembrane chloride and bicarbonate transporters than the thiourea and urea analogues [18,19].

As acid group Sq fragment has been included in the structure of amino acids and peptides. It is known that most of mammalian peptide hormones possess C-α-terminal amide and in many cases C-α-amides are much more bioactivie in comparison with the corresponding C-α-terminal free acids [20-22]. Amino acid amides have more rigid structure than the corresponding amino acids. On the other hand, amino acid amides participate in different interactions with neighboring molecules which leads to different hydrogen bonding [23,24]. In continuation of our previous studies on amino acid amides coupled to squaric acid fragment [23,24,25], we have synthesized three novel derivatives of isoleucine, tyrosine and glutamine. In the present contribution we report their spectral and structural characterization based on FTIR, Raman, NMR and theoretical methods. Several parameters such as lipophilicity, flexibility, hydrogen bonding ability, molecular size and volume are predicted by Molinspiration tool as preliminary screening on their bioavailability.

Materials and Methods

General Information

Hydrochlorides of the amino acid amides 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 600 spectrometer in solvent DMSO–d_6 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 CO_2, and H_2O) and N (by the Dumas method). Thin layer chromatography (TLC) was performed on aluminum sheets pre-coated with Merck silica gel 60 F254 0.25 mm (Merck). All density functional theory (DFT) computations were performed with the Gaussian 09 program package [26] employing the B3LYP (Becke's three-parameter non-local exchange correlation functional) [27,28] and 6-311++G** basis set. Molinspiration software [29] was used to calculated parameters such as miLogP, topological polar surface area (TPSA), hydrogen bond donors and acceptors, rotatable bonds, number of atoms, molecular weight, and violations of Lipinski's rule of five.

Synthesis of mono-squaramides

Aqueous ethanolic solution (1:4) of corresponding hydrochloride of amino acid amide (1 mmol) was mixed with of Et_3N (1 mmol) and the mixture was stirred for 10 minutes at room temperature. After that ethanol solution of diethylsquarate (1 mmol) was added. The mixture was stirred at room temperature to complete the reaction. The crude products were isolated by filtration and purified by recrystallization in mixture of methanol: water (4:1).

3a, 2-(2-ethoxy-3,4-dioxo-cyclobutyl-1-enamino)-3-methyl-pentanamide
Mr = 254.3; Anal. Calcd. for $C_{12}H_{18}N_2O_4$ (%), C 56.6, H 7.1, N 11.0; Found C 56.3, H 6.9, N 11.0;
^{1}H NMR (DMSO – d_6, 600 MHz), δ (ppm): 8.69 (dd, J=8.6, 9.16 Hz, 1H, NH), 7.48 (d, J=37.8 Hz, 1H, NH_2), 7.24 (d, J=25.7 Hz, 1H, NH_2), 4.65 (m, 2H, CH_2O), 4.43 (m, 1H, CH), 1.85 (s, 1H, CH-CH_3), 1.36 (q, 1H, CH_3), 1.05 (2H, CH_2), 0.84 (m, 6H, 2CH_3).
^{13}C NMR (DMSO – d_6, 600 MHz), δ (ppm): 189.17, 188.58, 182.72, 182.36, 177.22, 176.44, 172.96, 172.15, 171.86, 171.53, 68.93, 68.84, 62.49, 60.98, 56.17, 56.06, 36.91, 36.82, 23.91, 23.73, 15.62, 15.25, 11.03, 10.95.

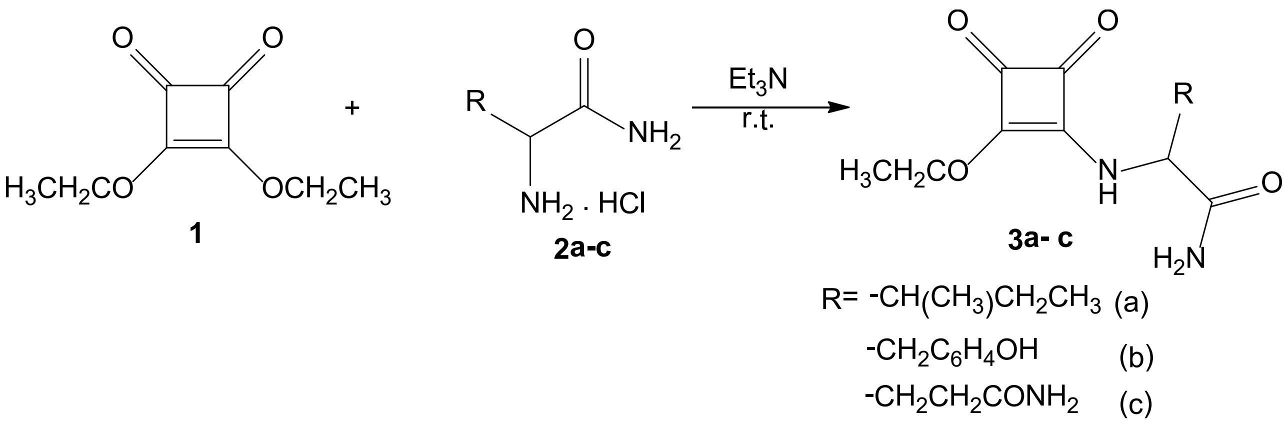
3b, 2-(2-ethoxy-3,4-dioxo-cyclobutyl-1-enamino)-3-(4-hydroxy-phenyl)- propionamide
Mr = 304.3; Anal. Calcd. for $C_{15}H_{16}N_2O_5$ (%), C 58.8, H 5.4, N 9.2; Found C 58.5, H 5.4, N 9.2;
^{1}H NMR (CD_3OD, 400 MHz), δ (ppm): 7.03 (d, J=8.4 Hz, 2H, Ar), 6.66 (br. s, 2H, Ar), 4.63 (d, J=6.8, 2H, OCH_2), 4.53 (m, 1H, CH), 3.15 (dd, J=4.4, 13.8, 2H, CH_2), 1.32 (m, 3H, CH_3).
^{13}C NMR (CD_3OD, 400 MHz), δ (ppm): 189.23, 188.53, 182.39, 182.24, 177.19, 176.42, 173.13, 172.25, 171.30, 156.01, 130.42, 126.65, 114.98, 69.20, 59.59, 30.70, 30.59, 14.50.

3c, 2-(2-ethoxy-3,4-dioxo-cyclobutyl-1-enamino)-pentandiamide
Mr = 269.3; Anal. Calcd. for $C_{11}H_{15}N_3O_5$, *(%)* C 49.1, H 5.6, N 15.6; Found C 49.0, H 5.4, N 15.9;
^{13}C NMR (DMSO – d_6, 600 MHz), δ (ppm): 188.47, 180.76, 173.78,173.66, 173.33, 173.12, 55.47, 55.08, 31.31, 30.11, 28.34, 27.73.

The spectral and structural characterizations of the synthesized compounds were established by IR, Raman, NMR spectra and elemental analyses. The results were consistent with the assigned structures.

Results and Discussion

Mono-squaramides (**3a-c**) containing isoleucine, tyrosine and glutamine residue were obtained in good yields by treating diethyl ester of H_2Sq (**1**) with corresponding hydrochlorides of amino acid amides (**2a-c**) in the presence of Et_3N at room temperature. The synthesis of mono-squaramides (**3a-c**) is shown in Scheme 1.



Scheme 1. Synthesis of mono-squaramides (**3a-c**)

The products were isolated by filtration from the reaction mixture and purified by recrystalization in high yields (Table 1).

Table1. Mono-squaramides obtained according to Scheme 1.

3	R	Reaction conditions *	Yields (%)	Mp (°C)
a	$-CH(CH_3)CH_2CH_3$ (Ile)	r.t., 15 h., EtOH/H_2O	85	200-204
b	$-CH_2C_6H_4OH$ (Tyr)	r.t., 24 ч., EtOH/H_2O	65	181-185
c	$-CH_2CH_2CONH_2$ (Gln)	r.t., 19 h., EtOH/H_2O	75	182-185

*temperature, reaction time, solvent

Vibrational analysis

Characteristic stretching vibrations of the squaric fragment are observed in the range 1810-1600 cm^{-1}. In the all cases, the bands belonging to ν^{s}C=O, ν^{as}C=O, νC=C appear around 1808 cm^{-1}, 1700 cm^{-1} and 1617-1600 cm^{-1} (Tables1-3). The last one has the highest intensity in IR spectra, while in the Raman spectra it is with moderate intensity.

Table 1. Selected IR and Raman vibrational frequencies (ν in cm^{-1}) of **3a**

№	Assignment[a]	ν		
		B3LYP[c]	*IR*	*Raman*
1.	ν^{as}_{NH2}	3548	3413	3415
2.	ν_{NH}	3460	3183	3186
3.	ν^{s}_{NH2}	3408	3153; 3070	3150; 3071
4.	$\nu^{s}_{C=O\ (Sq)}$	1796	1809	1808
5.	$\nu^{as}_{C=O\ (Sq)}$	1727	1700	1703
6.	$\nu_{C=O}$ (Amid I)	1703	1683	-
7.	δ_{NH2} (Amide II)	1586	1668	1668
8.	$\nu_{C=C\ (Sq)}$	1608	1608	1611
9.	δ_{NH}	1443	1506	1506
10.	$\delta^{as}_{CH3(Sq)}$	1438	1478	1470
11.	$\delta^{s}_{CH3\ (Sq)}$	1375	1384	1387

[a]Vibrational modes: ν, stretching; δ, bending
[b]Scaled by 0.9686

In the case of **3b**, the strong lowering of the frequency and the high intensity of the 3428 cm^{-1} band corresponding to the stretching vibration of phenolic OH group show OH-group polymer association in the crystalline state. The band is very weak in the Raman spectrum (Table 2).

Table 2. Selected IR and Raman vibrational frequencies (ν in cm^{-1}) of **3b**

№	Assignment[a]	ν		
		B3LYP[c]	*IR*	*Raman*
1.	ν_{OH}	3713	3428	3432
2.	ν^{as}_{NH2}	3582	3342,3303	3306
3.	ν_{NH}	3460	3212	-
3.	ν^{s}_{NH2}	3419	3141,3064	3137,3067
4.	***2***	3055	3050	3050
5.	$\nu^{s}_{C=O\ (Sq)}$	1798	1806	1807
6.	$\nu^{as}_{C=O\ (Sq)}$	1731	1693	1695
7.	$\nu_{C=O}$ (Amide I)	1698	1677	1671
8.	δ_{NH2} (Amide II)	1577	1616	1621
9.	$\nu_{C=C\ (Sq)}$	1612	1602	1594
10.	δ_{NH}	1443	1511	1510
11.	***19a***	1495	1479	–
12.	***19b***	1419	1446	1450
13.	ρ_{NH2}	1071	1091	1095
14.	***10a***	936	850	853
15.	***11***	829	811	813
16.	***4***	695	703	–

[a]Vibrational modes: ν, stretching; δ, bending; ρ, rocking
[b]Scaled by 0.9686

Vibrational frequency of ν^{as}_{NH2} is strongly affected by the differences in molecular structure and the corresponding band is shifted by 60 cm^{-1} to lower frequency (3342 cm^{-1}). In addition, the band is split and the second peak is observed at 3303 cm^{-1}. In the Raman spectrum only one band at 3306 cm^{-1} appears. The benzene C-C stretching vibrations **8a**, **8b**, **19a** and **19b**, according Wilson's notation [30] are observed in the region 1700-1400 cm^{-1}. Vibrations modes **8a** and **8b** are overlapped by the highly intensive band at 1602 cm^{-1}. In contrast to the IR spectrum in which bands for **19a** and **19b** are observed at 1479 cm^{-1} and 1446 cm^{-1}, respectively, in the Raman spectrum there is only a band of **19b** at 1450 cm^{-1} (Table 2).

Due to the presence of a second amide group in the glutamine residue of **3c**, more bands for N-H vibrations should be expected in the region 3500-3000 cm^{-1} (Table 3). Compound **3c** is characterized by five absorbtions in the mentioned region. The bands at 3397 and 3323 cm^{-1} are attributed to the ν^{as}_{NH2}. The symmetric NH stretching vibrations occurs at 3278 and 3201cm^{-1}. The last one is strong intensive band of large half-width.

Table 3. Selected IR and Raman vibrational frequencies (ν in cm^{-1}) of **3c**

№	Assignment[a]	ν		
		B3LYP[c]	*IR*	*Raman*
1.	ν^{as}_{NH2}	3606;3555	3397;3323	3398
2.	ν^{s}_{NH2}	3470;3408	3278;3201	3275;3200
3.	ν_{NH}	3450	3098sh	3100
4.	$\nu^{s}_{C=O\ (Sq)}$	1795	1805	1804
5.	$\nu^{as}_{C=O\ (Sq)}$	1728	1704	1706
6.	$\nu_{C=O}$ (Amid I)	1709;1705	1697sh; 1675	1697;1676
7.	δ_{NH2} (Amide II)	1582;1568	1637;1581	1643;1578
8.	$\nu_{C=C\ (Sq)}$	1610	1623	–
9.	δ_{NH}	1454	1535	1535
10.	δ_{CH}	1327	1332	1335
11.	ρ_{NH2}	1085	1130	1129
12.	$\rho_{NH2(Gln)}$	1055	1099	1102
13.	τ_{NH2}	627	617	607

[a]Vibrational modes: ν, stretching; δ, bending; ρ, rocking
[b]Scaled by 0.9686

The Amide I bands of **3c** are very intensive and the higher frequency one of them appears as a shoulder of the band at 1704 cm^{-1} ($\nu^{as}_{C=O\ (Sq)}$). The δ_{NH2} modes occur as two bands. The vibration (Amide II) of the amide group connected with the asymmetric C-atom is characterized by a very intensive band at 1637 cm^{-1} (IR) and 1643 cm^{-1} (Raman). The corresponding vibration of the second amide group from the glutamate residue is occurring at lower frequencies as a very strong band at 1581 cm^{-1} (IR) and 1578 cm^{-1} (Raman).

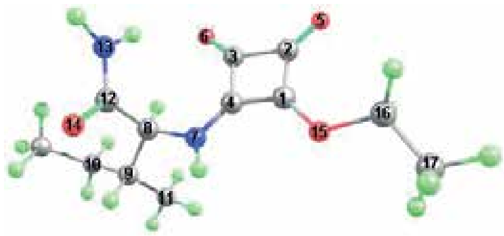
NMR analysis

The signals for the C-atoms of the OCH_2 and CH_3 groups are typically found at 68-69 ppm and 14-15 ppm, respectively. In the ^{1}H-NMR spectra the corresponding signals appear at 4.6 ppm (OCH_2) and 1.3-1.4 ppm (CH_3). The asymmetric carbon atoms of the studied compound resonate around 59-64 ppm. The proton signals of the asymmetric carbon atoms in the amino acid residues are observed at 4.3 ppm.

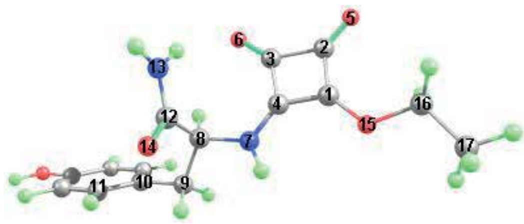
The signals in the ^{13}C-NMR spectra at 180-188 ppm and 171 ppm confirm the presence of a cyclobutene ring. Signals for the carbonyl carbon atoms of the amide fragment appear around 172-173 ppm.

Computational analysis

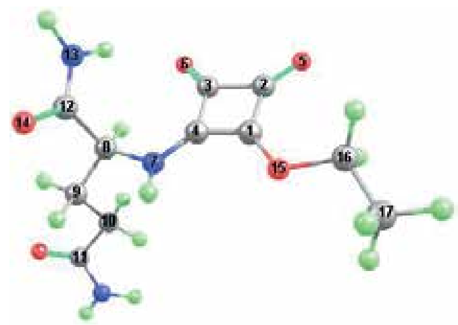
Since X-ray analysis of the crystal structure was not possible due to difficulties to obtain a suitable single crystal, computational analysis was used to obtain important information about structural characteristics and spectroscopic properties of the newly synthesized mono-squaramides. DFT computational studies carried out in gas phase at B3LYP/6-311++G** level of theory. The optimized structures of the compounds **(3a-c)** are presented in Figure 3. Computed geometrical parameters of the squaramide fragment of compounds **3a-c** were compared with the experimental data from the X-ray analysis for another mono-squaramides [23,24]. The theoretical method predicts with very good accuracy the bond lengths and bond angle values in the cyclobutene ring: C=O (theor. around 1.20 Å, exp. 1.21Å) and C=C (theor. 1.39 Å, exp. 1.39 Å). The ester C_1-O_{15} bond length in all compounds is 1.32 Å and squaramide bond C_4-N_7 is around 1.33 Å which are also in good agreement with the experimentally repored bond lengths (exp. around 1.30 and 1.31 Å) [23,24]. Variation of the side chain type does not lead to a change in the C_4-N_7 bond lengths. Predicted bond lengths of the amide C=O (1.226 Å for **3a** and **3c**) groups in the amino acid residues are close to the experimentally found for starting H-Gln–NH_2 (1.220 Å) and slightly longer from this for H-Ile–NH^2 (1.211 Å) [31].



3a



3b



3c

Figure 3. Molecular structure of mono-squaramides, optimized at DFT B3LYP/6-311++G** level

The secondary amino group lies in the plane of cyclobutene ring which is oriented almost perpendicularly to the plane of asymmetric C-atom and the amides groups.

Physico-chemical properties

Preliminary screening of molecular physico-chemical properties such as lipophilicity, flexibility and presence of hydrogen-donor and acceptors, molecular size and a good understanding of these properties are crucial for a successful drug discovery. For this reason, physico-chemical properties of synthezed compounds **3a-c** and other two mono-squaramides are calculated using Molinspiration tool and summarized in Table 3. Molinspiration methodology is very robust and is able to process practically all organic and organometalic compounds[32,33]. It enables the design of lots of compounds that can be screened to determine the most capable ones [34-36].

Table 3. Physico-chemical properties of synthesized compounds **3a-c** and valinamide and prolinamide esteramides of squaric acid (ValSqOEt, ProSqOEt), calculated using Molinspiration tool

Compound	LogP < 5	MW <500	N_{ON}[a] <10	N_{OHNH}[b] <5	N_{rotb}[c]	N_{viol}[d]	Vol.[e]	TPSA
3a	1.64	254.2	6	3	7	0	235.7	98.5
3b	1.34	304.3	7	4	7	0	265.2	118.7
3c	-0.71	269.2	8	5	8	0	232.9	141.5
ValSqOEt[f]	1.14	240.2	6	3	6	0	218.9	98.5
ProSqOEt[g]	-0.01	238.2	6	2	4	0	237.3	89.7

[a]number of hydrogen-bond acceptors (O and N atoms); [b]number of hydrogen-bond donors (OH and NH groups); [c]number of rotatable bonds; [d] Number of "Rule of five" violations; [e] molecular volume; TPSA- topological surface area; [f] ValSqOEt [23]; [g] ProSqOEt [25]

Lipophilicity (LogP) together with polar surface area (TPSA) is the main physico-chemical parameters linked with oral bioavailability. The calculated LogP values for the investigated compounds are in the range of -0.71 to 1.64 therefore the compounds are expected to present good bioavailability. LogP increases with increasing the size of the alkyl chains and the presence of phenyl ring. Total polar surface area (TPSA) represents the hydrogen bonding capacity of the molecules. The presented compounds show a TPSA of less than 140 Å², indicating a good intestinal absorption. TPSA value is not influence by the size of the alkyl chains.

Conclusion

We have synthesized a small series of mono-squaramides containing isoleucine, tyrosine and glutamine residue. The synthetic procedure advantages were mild reaction conditions, easy purification of the compounds and high product yields. The structures of the compounds are elucidated by means of FTIR, Raman, NMR and elemental analysis. The structures and spectral properties of the synthesized compounds are supported by theoretical analysis using DFT (B3LYP/6-311++G**).

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

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Corresponding author:
Emiliya Cherneva, PhD
Department of Chemistry, Faculty of Pharmacy,
Medical University of Sofia,
2 Dunav Str., Sofia 1000, Bulgaria,
Tel: +359 2 9236 513;
Fax: +359 2 9879 874;
E-mail: e.d.cherneva@gmail.com