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ORIGINAL ARTICLE / ÖZGÜN MAKALE

STUDY OF THE STRUCTURE OF PRODUCTS OF INTERACTION BETWEEN SOME NAPHTHOQUINONE DERIVATIVES AND PHARMACEUTICAL SUBSTANCES

BAZI NAFTOKİNON TÜREVLERİ İLE İLAÇ MADDELERİ ARASINDAKİ ETKİLEŞİM ÜRÜNLERİNİN YAPISININ İNCELENMESİ

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ABSTRACT

Objective: *The purpose of the present work is to detail a study of the interaction between naphthoquinone-based analytical reagents and such pharmacologically active ingredients as acetylcysteine, β-alanine and taurine (2-aminoethanesulfonic acid) including the isolation and structure elucidation of the formed products.*

Material and Method: *UV-visible spectrophotometry was applied to determine the stoichiometric coefficients of reactants in reactions between the pharmaceutical substances and naphthoquinone derivatives. 1H NMR-spectroscopy, IR spectroscopy, chromatomass-spectrometry were used to prove the structure of the reaction products.*

Result and Discussion: *The stoichiometric relationships of the reactants in the investigated medicinal substances reactions with quinone derivatives were determined by the methods of saturation and continuous changes. They are 1:1 in each case. According to the determined relationships of components reaction and optimal conditions for reactions path, the products of interaction of acetylcysteine with 2,3-dichloro-1,4-naphthoquinone and taurine, β-alanine with sodium 1,2-naphthoquinone-4-sulfonate were isolated and identified. In order to establish the structure of the compounds, the 1H NMR-spectroscopy, IR spectroscopy, chromatomass-spectrometry were used.*

Keywords: *Acetylcysteine, β-alanine, taurine, naphthoquinone derivatives, reaction products*

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ÖZ

Amaç: *Naftokinon esaslı analitik reaktiflerin asetilsistein, β-alanin ve taurin (2-aminoetanosülfonik asit) gibi farmasötik açıdan aktif maddelerle etkilişimin, oluşan ürünlerin ayırımı yapı oluşumu dahil olmak üzere detaylı incelemesidir.*

Gereç ve Yöntem: *Farmasötik maddeler ile naftokinon türevleri arasındaki reaksiyonlarda reaktanların stokiyometrik katsayılarını belirlemek için UV - Vis spektrofotometri yöntemi uygulandı. Reaksiyon ürünlerinin yapısını kanıtlamak için 1H NMR-spektroskopi, IR spektroskopi, kütle spektrometrisi kullanıldı.*

Sonuç ve Tartışma: *Kinon türevleri ile araştırılan tıbbi maddelerin reaksiyonlarında reaktanların stokiyometrik ilişkileri, doyurma ve sürekli değişim yöntemleri ile belirlenmiştir. İlişki her durumda 1: 1'dir. Bileşen reaksiyonu ve reaksiyon yolu için optimal koşulların belirlenen ilişkilerine göre, asetilsisteinin 2,3-dikloro-1,4-naftokinon ve taurin ile, β-alanin ile sodyum 1,2-naftokinon-4-sülfonat ile etkileşim ürünleri izole edilmiş ve tanımlanmıştır. Bileşiklerin yapısını oluşturmak için 1H NMR-spektroskopi, IR spektroskopisi, kütle spektrometrisi kullanılmıştır.*

Anahtar Kelimeler: *Asetilsistein, β-alanin, taurin, naftokinon türevleri, reaksiyon ürünleri*

INTRODUCTION

Naphthoquinones are known as high reactive compounds, that intensively studied as promising bioactive agents [1, 2], mediators in oxidation processes [3] and dyes [4]. Lately, the abovementioned compounds repeatedly described as reagents used for the colorimetric quantitative determination of various pharmacologically active ingredients and biologically significant compounds. Among substituted naphthoquinones dichlone (2,3-dichloro-1,4-naphthoquinone) and 1,2-naphthoquinone-4-sulphonic acid sodium salt proved to be the most widely used colorizing reagents. Thus, dichlone was proposed as an effective reagent for quantitative determination of primary amines [5]. The spectrophotometric estimation of piperazine in dosage forms using dichlone and acetaldehyde as reagents was described as one of the possible modifications of elaborated before analytical methods [6]. Charge–transfer reaction of 2,3-dichloro-1,4-naphthoquinone with crizotinib was used for development of microwell assay for its quantitative determination in capsules [7]. Authors substantiated the nature of the abovementioned reaction products by computation approaches [8]. Also, 2,3-dichloro-1,4-naphthoquinone found to be an effective colorizing reagent for spectrophotometric determination of isoniazid in presence of its hydrazones.

Data related to the application of 1,2-naphthoquinone-4-sulphonic acid sodium salt for the spectrophotometric quantitative determination of amino-group containing pharmaceuticals in various objects were generalized and critically evaluated in the comprehensive review by Elbashir A.A. et al [9]. It was shown that usage of 1,2-naphthoquinone-4-sulphonic acid sodium salt was reasonable for quantitative determination of analytes as in dosage forms so in biological material (urine and plasma). The authors also summarized the information about optimal conditions of derivatization processes.

After publication of the abovementioned review 1,2-naphthoquinone-4-sulphonic acid sodium salt was described as valued colorizing reagents for spectrophotometric determination of cefotaxime [10] and other cephalosporins [11], finasteride [12], folic acid [13], rimantadine and memantine [14]

etc. 1,2-Naphthoquinone-4-sulphonate reagent also may be used for continuous-flow spectrophotometric determination of amino acids [15]. Some methods of pharmacologically active ingredients quantification require the usage of 1,2-naphthoquinone-4-sulphonic acid sodium salt together with the auxiliary reagent. Thus, application of tetradecylbenzyldimethylammonium chloride for sensitivity improvement of spectrophotometric determination of dopamine hydrochloride using sodium 1,2-naphthoquinone-4-sulfonate as chromogenic reagent was studied [16]. The method of isoniazid spectrophotometric quantification using combination of 1,2-naphthoquinone-4-sulphonic acid sodium salt and cetyltrimethyl ammonium bromide as colorizing reagents was elaborated as well [17]. It should be, noted that despite the wide usage of naphthoquinone derivatives as colorizing reagents the nature of products formed by the interaction of abovementioned compounds and analytes is a controversial issue. Some of the authors made assumptions about structure of the products of interactions between naphthoquinone colorizing reagents and analytes. As usual, their suppositions were based on data about stoichiometric coefficients of reactants, described chemical modification of naphthoquinone derivatives [4, 18] and computation approaches [19]. However, structures of the major and minor products formed in conditions of quantitative determination were not evaluated using appropriate physicochemical methods. Obviously understanding of the nature of processes that take place during the analytical reaction and structure of the products are essential for the improving of quantifying methods.

The present work was aimed at the detailed study of interaction between naphthoquinone-based analytical reagents and such pharmacologically active ingredients as acetylcysteine, β-alanine and taurine (2-aminoethanesulfonic acid) [20, 21] including the isolation and structure elucidation of the formed products.

MATERIAL AND METHOD

Reagents and chemicals

Analytical grade chemicals: 2,3-Dichloro-1,4-napthoquinone, sodium 1,2-naphthoquinone-4-sulfonate, sodium hydroxide, DMF, 2-propanol, 1,4-dioxane, ethanol and HPLC grade solvents: acetonitrile and water were obtained from commercial sources. The reference standards of acetylcysteine, taurine and β-alanine at 99,9 % purity were provided by the chemical pharmaceutical enterprises (Ukraine).

Instrumentation

Analytic Jena UV-visible spectrophotometer model Specord 200 with 1 cm matched quartz cells were used for UV-Vis studies. Kern electronic scales ABT-120-5DM was used measuring of the weight. Water bath Memmert WNB 7-45 was used for heating of reaction mixtures.

IR spectra (4000–600 cm−1) were recorded on a Bruker ALPHA FT-IR spectrometer using a module ATR eco ZnSe. 1H NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometers with TMS as internal standard in DMSO-d6 solution.

Mass spectra were recorded on a high performance liquid chromatograph «Agilent 1100 Series» (Agilent, Palo Alto, CA, USA) equipped with DAD, ELSD and MSD «Agilent LC/MSD SL».

Chromatographic conditions

Column «Zorbax SB-C18» —1.8 μm, 4.6 mm x 15 mm. Eluent: A — CH3CN—H2O (95:5), 0.1 % HCOOH, B — H2O (0.1 % HCOOH). Gradient: 0 min – 0% A, 0.01 min – 0% A, 1.5 min – 100% A, 1.7 min – 100% A, 1.71 min – 0% A. The injection volume 1.0 μL. The column temperature: 40 °C. DAD: 215, 254 nm.

Mass-spectrometry conditions

Ion Source: API-ES. Scan. Mass Range: m/z 70-600. Fragmentor voltage: 100V. Positive and negative polarity.

Procedure for interaction of acetylcysteine with 2,3-dichloro-1,4-naphthoquinone

To the solution of 0.66 g (4 mmol) of acetylcysteine in 10 ml of (DMF) solution of 0.91 g (mmol) of 2,3-dichloro-1,4-naphthoquinone in 10 ml of DMF was added. The formed mixture was shaken and heated on a water bath within 30 min at the 95°C. Then the reaction mixture was cooled and poured into the purified water. For ^{1}H NMR analysis compound **3** was recrystallized from 2-propanol. The isolated product of the reaction is yellow-brown crystals soluble in DMF, acetonitrile, 1,4-dioxane, slightly soluble in water, ethanol, 2-propanol. The yield of the obtained chemical compound is 1.0 g.

2,3-dichloro-1,4-napththoquinone (**1**) IR (cm^{-1}): 1678, 1530, 950, 985; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 8.20-8.01 (m, 2H, naphthalene H-5,8), 7.97-7.76 (m, 2H, naphthalene H-6,7); LC-MS (APCI): *m/z*= 227

Acetylcysteine (**2**) IR (cm^{-1}): 3300, 2550, 1712, 1680, 1530, 950, 985; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 12.71 (s, 1H, COOH), 8.16 (d, 1H, NH) 4.37 (td, 1H, CH) 2.90-2.79 (m, 1H, CH_2), 2.76-2.62 (m, 1H, CH_2), 2.41 (bs, 1H, SH), 1.87 (s, 3H, CH_3) ; LC-MS (APCI): *m/z*= 164.

N-acetyl-S-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)cysteine (**3**). IR (cm^{-1}): 1679, 1662, 1557, 910, 1273, 1137, 820, 705, 639; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 8.34 (d, 1H, NH), 8.06-7.97 (m, 2H, naphthalene H-5,8), 7.88-7.85 (m, 2H, naphthalene H-6,7), 4.57-4.45 (m, 1H, CH), 3.91-3.75 (m, 1H, CH_2), 3.55-3.43 (m, 1H, CH_2),1.69 (s, 3H, CH_3); LC-MS (APCI): *m/z*= 354

Procedure for interaction of taurine with sodium 1,2-naphthoquinone-4-sulfonate

To the solution of 3.1 g of taurine (25 mmol) in 50 ml of purified water solution of 6.5 g (25 mmol) of sodium 1,2-naphthoquinone-4-sulfonate in 50 ml of purified water was added. A mixture was shaken, then 1.0 g of crystalline NaOH was added. The reaction mixture was heated on a water bath

during 5 min at 95ºC. The obtained mixture was cooled. The solvent was removed under vacuum using rotary evaporator. Yield of the obtained compound is 8.0 g (75.47%).

Procedure for interaction of β-alanine with sodium 1,2-naphthoquinone-4-sulfonate

To the solution of 2.23 g of β-alanine (25 mmol) in 50 ml of purified water solution of 6.5 g (25 mmol) of sodium 1,2-naphthoquinone-4-sulfonate in 50 ml of purified water was added. A mixture was shaken, then 1.0 g of crystalline NaOH was added. The reaction mixture was heated on a water bath during 10 min at 60ºC. The obtained mixture was cooled. The solvent was removed under vacuum using rotary evaporator. The yield of the obtained product is 8.6 g.

LC-MS (APCI): compound **7**: tR = 0.834 min, *m/z*= 282; compound **8**: tR = 0.766 min, *m/z*= 246; compound **9**: tR = 1.077 min, *m/z*= 389; compound **10**: tR = 0.991 min, *m/z*= 317; compound **11**: tR = 0.678 min, *m/z*=563; compound **12**: tR = 0.871 min, *m/z*=491.

RESULT AND DISCUSSION

Determination of stoichiometric coefficients of reactants in reactions

In order to determine the stoichiometric coefficients of reactants in reactions between the selected pharmaceutical substances and some naphthoquinone derivatives, the most common methods were used: method of continuous changes (method of isomolar series) and saturation method (method of molar ratios). Method of continuous changes is based on the determination of relations of isomolar concentrations of reacting substances, which corresponds to the maximum yield of the product formed. We cite the principle of determination of stoichiometric coefficients between taurine and sodium 1,2-naphthquinone-4-sulfonate as an example. For carrying out the chemical analysis, solutions of the reagent and the pharmaceutical substance under study of the same molar concentration (0.005 M) were prepared and mixed in inverse ratios (from 1:9 to 9:1), leaving the total volume of the solution unchanged. The reaction was carried out in accordance with the methodology developed. Absorption of the solutions formed was measured at the selected analytic wavelength. A diagram of dependence of the absorption on the relation between the volumes of components of isomolar series was constructed based on the data obtained (Figure 1).

Method of molar ratios is a widely used method of study of compounds. The principle of the method is to define the dependence on the concentration of one of the components at the steady-state concentration of another and vice versa. A break point of the curve corresponds to the ratio of stoichiometric coefficients, which is equal to the equivalent concentration of components (Figure 2).

As is clear from figures 1 and 2, stoichiometric ratios of reactants “taurine – sodium 1,2-naphthoquinone-4-sulfonate”, obtained by the methods of continuous changes and the saturation method, fully correlate with one another as the 1:1 ratio.

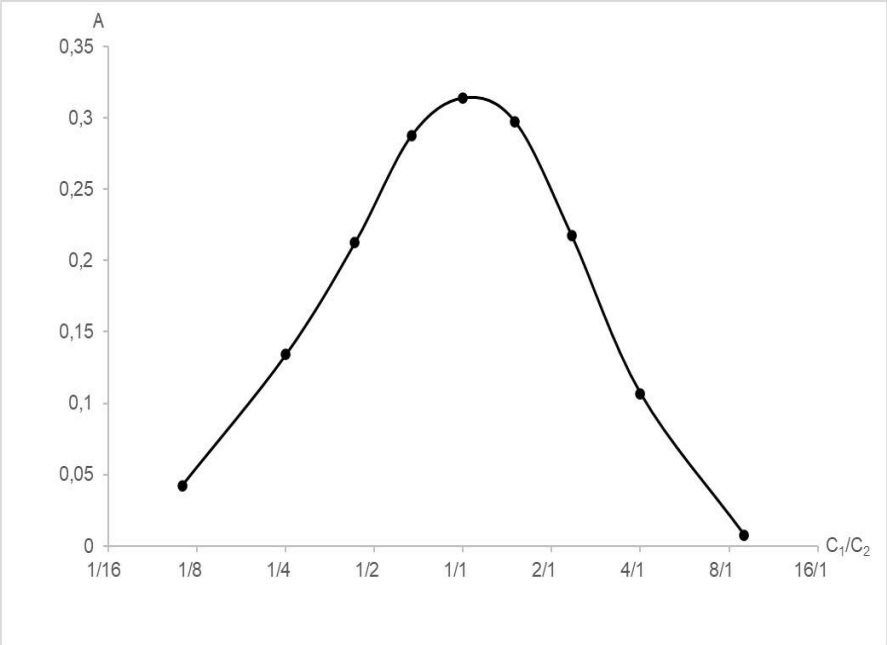

Figure 1. The graph of the absorbance values as a function of isomolar solution composition (C_1 – 0.005 M NQS solution, C_2 – 0.005 M taurine solution)

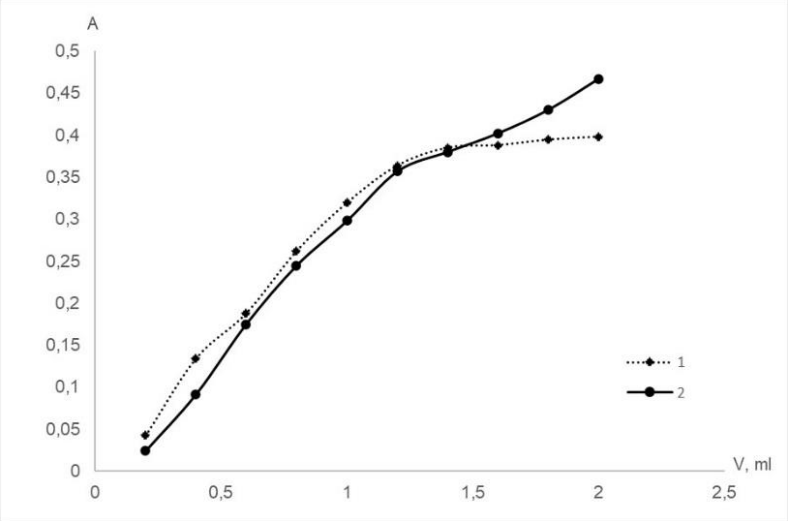

Figure 2. The saturation curves: 1 – sodium 1,2-naphthoquinone-4-sulfonate at constant concentration of taurine (1 ml 0.005 M solution); 2 – taurine at constant concentration of sodium 1,2-naphthoquinone-4-sulfonate (1 ml 0.005 M solution)

Using the results of the above-mentioned methods, we have determined stoichiometric ratios of reactants “pharmaceutical substance – reagent”, which clearly correlate with one another (Table 1).

Table 1. Stoichiometric ratios of reactants «pharmaceutical substance – reagent»

Pharmaceutical substance – reagent	Methods	
	Method of isomolar series	Method of molar ratios
Acetylcysteine – 2,3-dichloro-1,4-napththoquinone	1:1	1:1
Taurine – sodium 1,2-naphthoquinone-4-sulfonate	1:1	1:1
β-Alanine – sodium 1,2-naphthoquinone-4-sulfonate	1:1	1:1

Isolation and structural elucidation of products of interaction between the investigative pharmaceutical substances and naphthoquinone derivatives

Colored product of interaction of acetylcysteine with 2,3-dichloro-1,4-naphthoquinone was isolated and identified using LC-MS and ^{1}H NMR methods. LC-MS spectra showed that interaction between abovementioned reagents resulted single product with m/z = 227.

^{1}H NMR-spectroscopy was used for confirmation of the structure of the formed products of the reaction. Comparing of characteristic protons chemical shifts in ^{1}H NMR spectra of acetylcysteine (**2**), 2,3-dichloro-1,4-naphthoquinone (**1**) and product (**3)** as well as LC-MS data analysis allowed to identify a product as *N*-acetyl-*S*-(3-chloro-1,4-dioxo-1,4-dihydronaphthalene-2-yl)cysteine. Thus, nucleophilic substitution of halogen in 2,3-dichloro-1,4-naphthoquinone by mercapto-group of acetylcysteine occurred (Figure 3).

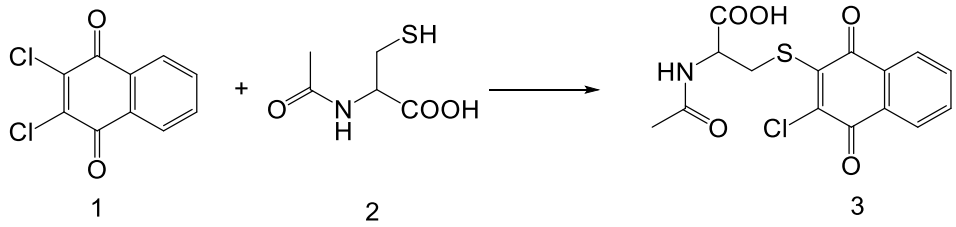

Figure 3. Reaction of acetylcysteine with 2,3-dichloro-1,4-naphthoquinone

The following spectral characteristics confirm the structure of the above-mentioned compound **3:** the disappearance of a characteristic signal of – SH-group proton (2.41 ppm) in the spectrum; a slight paramagnetic shift (by 0.2 ppm) and a change of multiplicity (from triplet doublet to multiplet) of a classifying signal of a proton of the asymmetrical -CH-group. A singlet of the *NH*-group also has a paramagnetic shift (by 0.18 ppm), one-proton multiplet signals of the -CH_2-group at the chiral center – by 0.9-0.8 ppm, and a triple-proton singlet of a methyl group of an acyl residue - by 0.2. ppm. Adding acetylcysteine residue to 2,3-dichloro-1,4-naphthoquinone, as expected, causes a diamagnetic shift of aromatic protons of positions 5, 6, 7 and 8 by 0.14 ppm.

IR-spectra of the reaction product **3** additionally proved the structure of obtained compounds. Comparing of IR-spectra of the reaction product (**3**) with the spectra of acetylcysteine (**2**) and 2,3-dichloro-1,4-napththoquinone (**1**) revealed that main bands of absorption of initial reagents had also been observed in the spectrum of the reaction product (**3**). However, as expected, stretching vibrations of the NH- and SH-groups in the spectrum of the compound **3** disappear at 3300 cm^{-1} and 2550 cm^{-1}, respectively. There must be a certain bathochromic shift (by 20-30 cm^{-1}) of, stretching vibrations of the CO-group of carboxylic acids (resonates together with $\nu_{C=O}$ dichlone at 1679 cm^{-1}) and ν_{CO-} ("Amide I"). Moreover, adding a substituent with donor properties to a molecule leads to a significant reduction of the strength of stretching vibrations –C=C-bond in the aromatic system. The above-mentioned facts clearly substantiate the reaction of nucleophilic substitution between acetylcysteine and 2,3-dichloro-1,4-naphthoquinone and the formation of a new reaction product.

Further, the interactions of sodium 1,2-naphthoquinone-4-sulfonate(**4**) with taurine (**5**) and alanine (**6**) were studied (Figure 4). LC-MS-spectra revealed that both reactions resulted mixtures that consist of two major and traces of one minor products. Considering *m/z* values and literature data [18] one of the major components were identified as products of sulfo-group substitution (compound **7** or **8** with *m/z* = 282 and *m/z* = 246 correspondingly). The second major products of the reaction had the doubled relative to the compounds **7** or **8** value of *m/z* and probably were the dimers of abovementioned compounds. In view of the nature and position of reaction centers in molecules of compounds **7** and **8**, it was proposed that their dimers were formed as result of addition of nucleophilic amino-group to the double bond of oxo-fragment (compounds **11** and **12** with *m/z* values 563 and 491 respectively). We emphasize that formation of the products like compounds **11** and **12** were not previously reported. The minor products had *m/z* values that allowed to identify them as products of nucleophilic addition followed by elimination reaction of compounds **7**, **8** and corresponding analytes (compounds **9** and **10**). It should be noted that nature and content of the products completely agreed with evaluated stoichiometric ratios.

Summing up the above, we studied the interaction between naphthoquinone colorizing reagents and such analytes as acetylcysteine, taurine, and β-alanine. We evaluated the structure of the products of the reaction and showed that their nature corresponded to the found stoichiometric ratios. Also, we revealed the previously unknown process of products dimerization when quantifying taurine and β-alanine using sodium 1,2-naphthoquinone-4-sulfonate as colorizing reagent.

Conducted studies revealed that reaction of acetylcysteine with 2,3-dichloro-1,4-naphthoquinone proceeded unambiguously and yielded one product namely N-acetyl-S-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)cysteine. The reaction between sodium 1,2-naphthoquinone-4-sulfonate and β-alanine (or taurine) resulted the mixture wherein product of substitution of sulfonic group by amine moiety and its dimer were identified as main component. The structures of products

were proposed according to the *m/z* values and nature of reactional centers in interacting molecules. The obtained information is valuable for future development of spectrophotometric methods based on the usage of naphthoquinone colorizing agents.

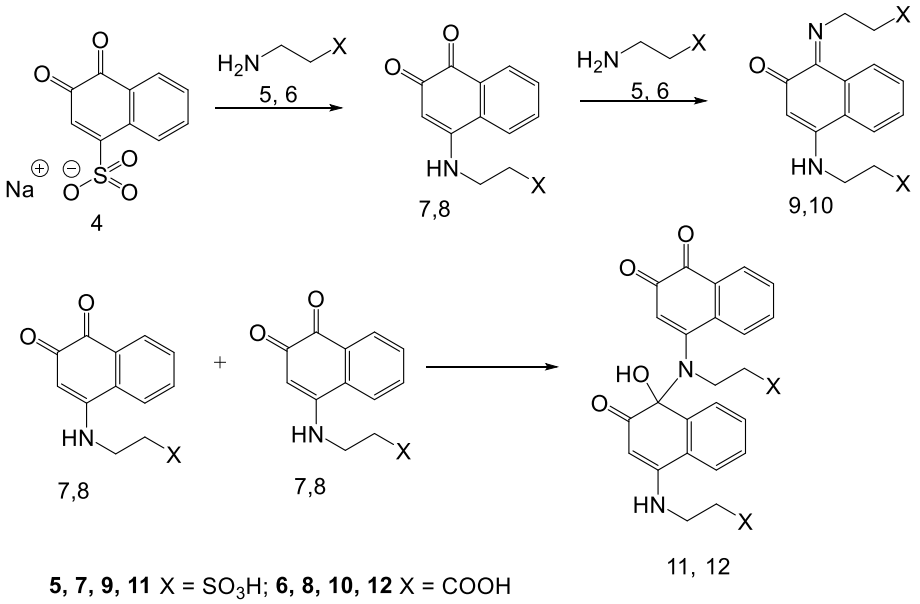

Figure 4. Reaction of sodium 1,2-naphthoquinone-4-sulfonate with taurine and β-alanine.

AUTHOR CONTRIBUTIONS

Conception: *A.D., K.M., O.V.*; Design: *S.V., S.K.*; Supervision: *A.D., K.M., O.V., S.V., S.K.*; Resources: *S.V., S.K.*; Materials: *S.V., S.K.*; Data collection and/or processing: *A.D., K.M., O.V.*; Analysis and/or interpretation: *A.D., K.M., O.V.*; Literature search: *O.V*; Writing manuscript: *A.D., K.M., O.V., S.V., S.K.*; Critical review: *A.D., K.M., O.V., S.V., S.K.*; Other: -

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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