

SYNTHESIS AND SPECTRAL CHARACTERIZATION OF NEW SALVADORICINE ANALOGUES

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Abstract

*Salvadoricine is an indole alkaloid isolated from the leaves of *Salvadora persica* Linn. This natural product has interesting and diverse biological profile. An effective one-pot synthesis of Salvadoricine analogues via multicomponent reaction of α -amidoalkylation is proposed. The conditions for the reaction of benzothiazole, alkyl chloroformates and 3-acetylindole were found. The structure of the obtained in good yields analogues of natural alkaloid - Salvadoricine was proved by spectral methods.*

Keywords: Salvadoricine, Benzothiazole derivatives, α -amidoalkylation, multicomponent reactions

1. INTRODUCTION

Salvadora Persica Linn (Miswak) is an evergreen tree one of the most frequently mentioned plants in Islamic literature [1] due to the diverse profile of the biologically active components contained therein [2, 3]. The chemical analysis of *S. persica* shows the content of a total of 19 natural substances which are applicable in dentistry [4]. Aqueous and alcoholic extracts from different parts of the plant exhibit a variety of biological activities including antimicrobial, antioxidant, antitumor, anticonvulsant, antihyperglycemic, anti-inflammatory etc. [5, 6].

Indole alkaloid *Salvadoricine* (Fig. 1) was first isolated in 1987 from the leaves of the tree *Salvadora Persica* Linn by initial extraction with 1% sulfuric acid in ethanol and subsequent treatment of the distillate with ethyl acetate [7]. *Salvadoricine* - (2-acetyl-3-methyl indole) shows activity in the treatment of various dental diseases, scurvy, rheumatism, etc. It has been found to exhibit antibacterial activity against several pathogenic cariogenic bacteria of the teeth [8]. The preparation of the alkaloid *in vitro* was described in 2011 [4].

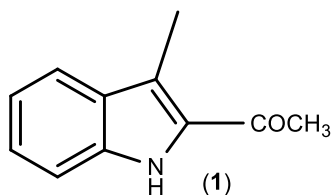


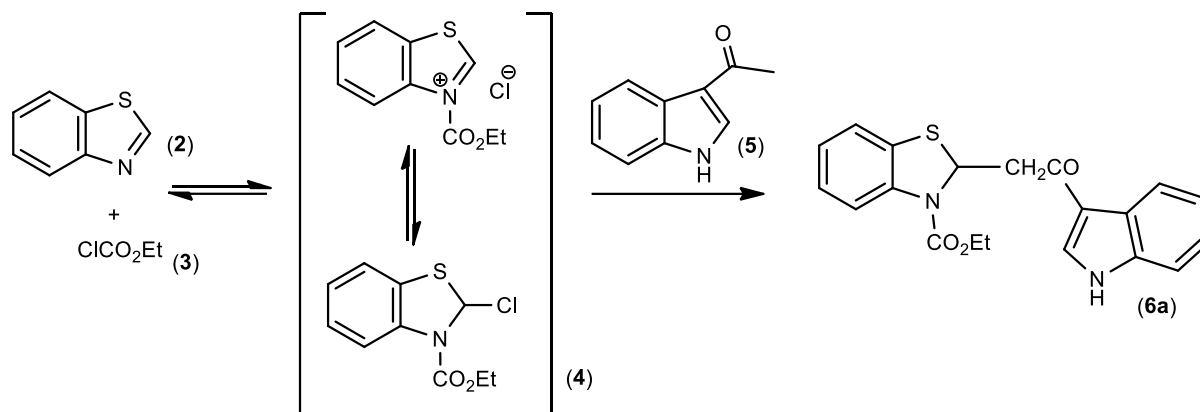
Fig. 1. *Salvadoricine*

Several methods for the synthesis of *Salvadoricine* are described in the literature including decarboxylation of 2-acylindol-3-acetic acid [9], direct Friedel-Crafts acylation of 3-methylindole in the presence of Lewis acids [10, 11] and intramolecular McMurry cyclization [12].

In connection with our interest in the synthesis of new indole derivatives via multicomponent reactions of α -amidoalkylation [13], a new approach for the synthesis of structural analogues of this alkaloid is proposed.

2. RESULTS AND DISCUSSION

Our previous publication back from the year 2010 describes the results of studies on α -amidoalkylation of methyl ketones with acyliminium reagents derived from nitrogen-containing heterocycles and ethyl chloroformate. Conducting benzothiazole reactions by boiling the reaction mixture in anhydrous 1,2-dichloroethane leads to low yields of the products [14].



Scheme 1

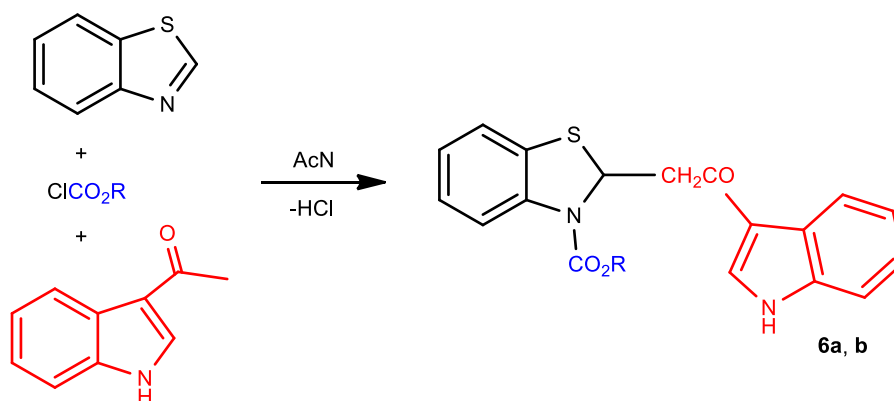
The high reactivity of the N-acyliminium reagents studied by us, obtained by reacting benzothiazole and acyl chlorides to indole [13] under the conditions of a multicomponent α -amidoalkylation reaction provoked the need for revision of the earlier studies. To conduct the reaction of amidoalkylation of ketones at heating probably impacts negatively on the equilibrium reaction between benzothiazole and acyl chloride (Scheme 1). The working hypothesis was that extending the reaction time at lower temperatures would lead to an increase in extraction rate, while obtaining the product of the amidoalkylation will move the equilibrium to the right. To confirm the hypothesis and to synthesize *Salvadoricine* analogues containing a benzothiazole fragment, a multicomponent amidoalkylation reaction of 3-acetylindole (**5**) with benzothiazole, ethyl and methyl chloroformate was applied (Scheme 2).

In search of optimum conditions for obtaining compounds (**6a**, **b**, Scheme 2), the solvent, the reaction temperature, the ratio of the quantities of the starting substances, and the order of their mixing were varied. In testing the three-component reactions of α -amidoalkylation, we found that in the interaction of two equivalents of benzothiazole and alkyl chloroformate with one equivalent of 3-acetylindole in anhydrous dichloroethane and heating (60 °C at R=Me, 80 °C at R=Et) for 5 hours, the reactions proceeded with the obtaining of two products. Unreacted 3-acetylindole was also observed in the reaction mixture. After chromatographic separation (preparative column with fixed-phase neutral alumina and a mobile-phase petroleum ether/diethyl ether in various ratios), both products were isolated (55 % for **6a** and 55 % for **6b**, Scheme 2) as well as some quantities of unreacted 3-acetylindole (**5**) (24 % in the reaction with ethyl chloroformate and 55 % - with methyl chloroformate).

During the reactions (at the same proportion of reactants) in acetonitrile solvent, at room temperature, for 80 hours, we succeeded in significantly increasing the yields of the isolated products (74 % for **6a** and 53 % for **6b** after column chromatography purification) (Table 1). In confirming the hypothesis made, it was found that at room temperature the prolongation of the reaction time resulted in a greater depletion of the starting reagents, and production of the target products. Thus, during one week of reactions at room temperature in acetonitrile, we observed crystallization of the products in the reaction medium. This has helped to isolate the products by filtration, and without the need for a chromatographic column. The yields obtained under these conditions are satisfactory (80 % for **6a** and

65 % for **6b**). Further prolongation of time (up to 2 weeks) results in minimal increase in yields of 2 % and 5 %, respectively.

The results of the reactions performed and the optimized conditions are illustrated in Table 1. Accordingly, two analogues of alkaloid *Salvadoricine* (**1**, Fig.1) have been successfully synthesized with good yields.



Scheme 2

Table 1. Reaction conditions and yields of products **6a, b**

6	R	Time, h / T, °C	Yield, (%)	Time, h / T, °C	Yield, (%)	mp, °C
a	-Et	80 h / 25 °C	74	1 week / 25 °C	80	210 – 212
b	-Me	80 h / 25 °C	53	1 week / 25 °C	65	228 – 230

The data from the spectroscopic methods were used to demonstrate the structure of compounds (**6a, b**). The position of the newly-formed C-C bond in the molecule of the starting 3-acetylindole is proved by NMR-spectra. In the ¹H-spectra of compounds (**6a, b**) captured in DMSO-d₆ at 80 °C (600 MHz) a distinct set of doublets of doublets (dd) was observed in the range δ = 3.53 – 3.63 ppm, corresponding to the protons of CH₂ group linked to the newly formed asymmetric centre at C-2 in the benzothiazole molecule (Figure 2).

Characteristically split are also the signals corresponding to the protons at the asymmetric carbon atoms - “dd” 6.30 ppm (at **6a**) and 6.23 ppm (at **6b**). This characteristic split is due to the newly formed C-C bond, and the presence of the two protons from the methylene group. In the ¹H-NMR spectra of the two products was clearly observed the presence of the singlet corresponding to the C-2 proton of the indole fragment - at 8.30 ppm (**6a**) and 8.24 ppm (**6b**). The presence of these singlets rejects the possibility of an α-position substitution reaction in the indole heterocycle molecule. These ¹H-NMR spectra are sufficient evidence of the location of the occurred reaction of α-amidoalkylation in the process of obtaining of the two products (**6a, b**), namely in the α-position relative to the carbonyl group.

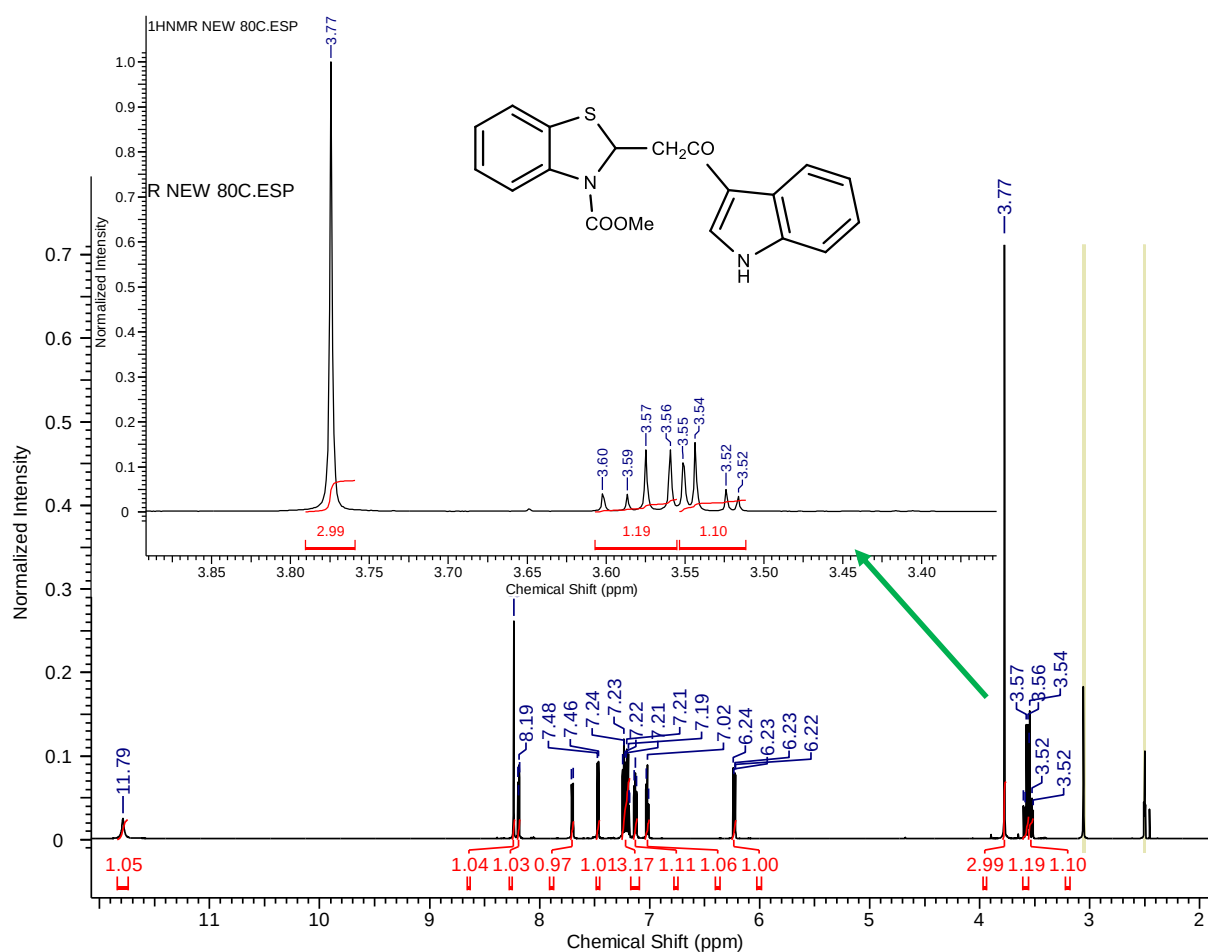


Fig. 2. ¹H-NMR spectra of (**6b**) - DMSO-d₆ - 80 °C (600 MHz)

In the ¹³C-NMR spectra were observed characteristic signals for the carbonyl carbon atoms at 152.3 ppm, 191.5 ppm for **6a** and at 152.8 ppm, 191.6 ppm for **6b** (Figure 3). The presence of these signals confirms the combination of benzothiazole and 3-acetyl indole fragments.

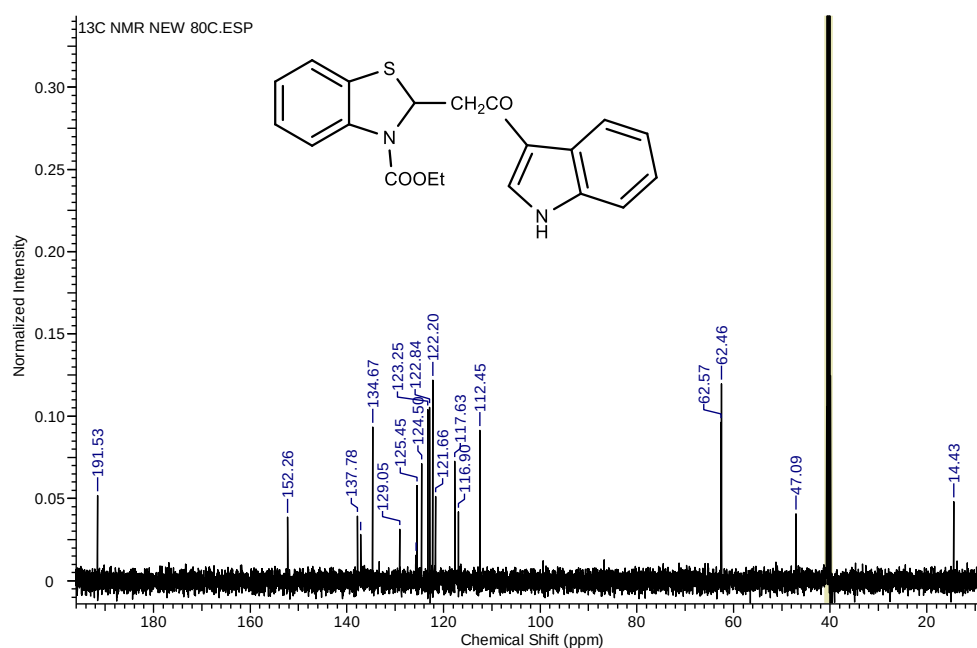


Fig. 3. ¹³C-NMR spectra of (**6a**) - DMSO-d₆ - 80 °C (150 MHz)

In the ESI-HRMS spectra were observed basic peaks m/z $[M+Na]^+$ (389.0934, **6a**) and (375.0774, **6b**) corresponding to the theoretically calculated molecular weights of the expected products.

In the IR spectra of the products the bands for $\nu(N-H)$ - 3291cm^{-1} (**6a**), 3279cm^{-1} (**6b**) and $\nu(C=O)$ - 1720cm^{-1} (**6a**), 1729cm^{-1} (**6b**) were observed (Fig. 4).

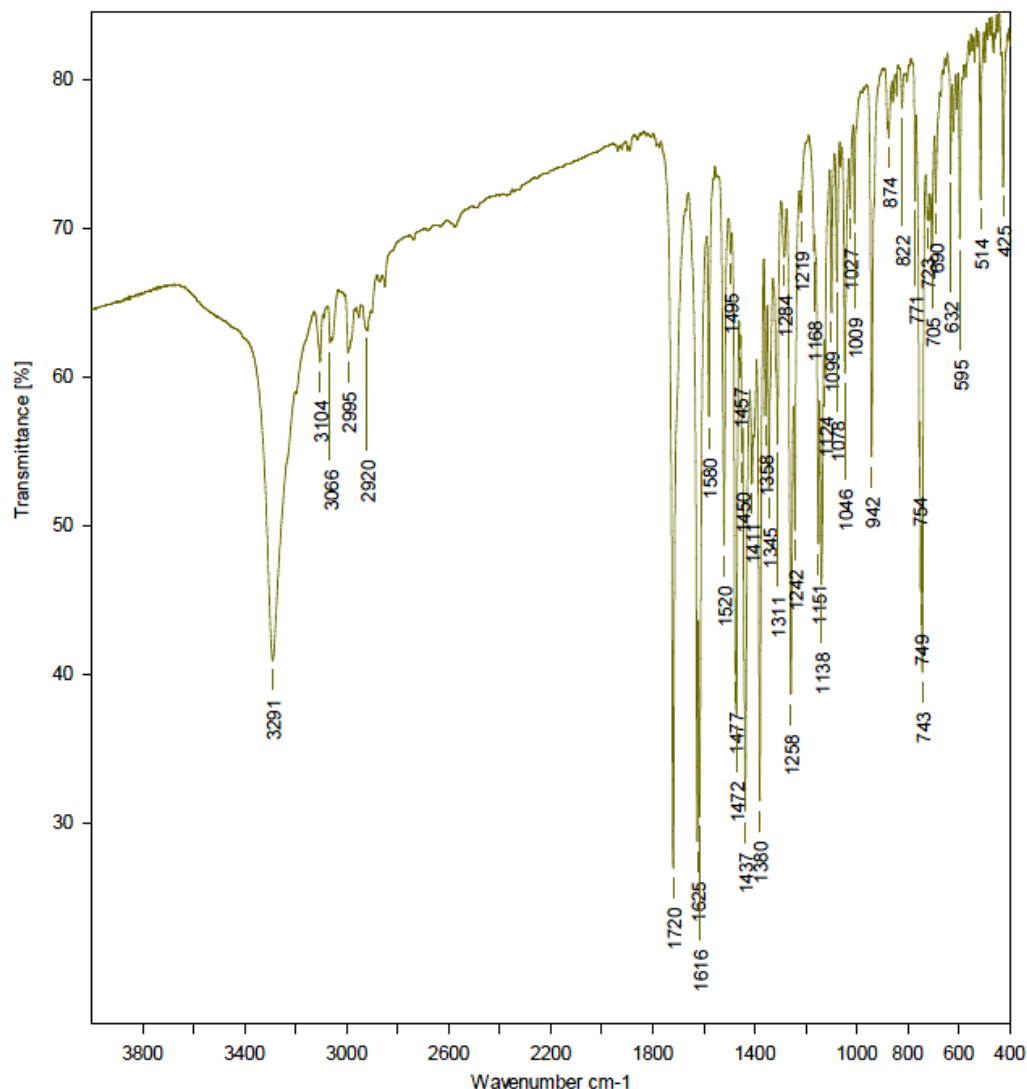


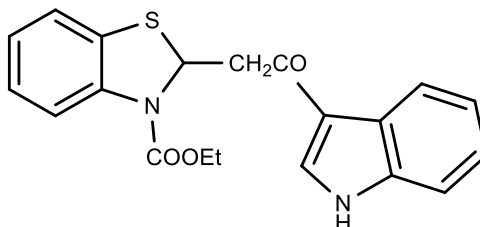
Fig. 4. IR spectra of (**6a**), KBr-tablet

3. EXPERIMENTAL

General information: Commercial solvents and reagents, such as benzothiazole, alkyl chloroformates and 3-acetylindole were purchased from Merck and were used without further purification. Melting points were determined on a Boetius PHMKO5 hot stage apparatus and are uncorrected. IR spectra were measured on Perkin Elmer 1750 Furie Transform spectrometer. Mass spectral measurements were performed on a Thermo Scientific Q Exactive hybrid quadrupole-orbitrap mass spectrometer. ^1H -NMR, ^{13}C -NMR spectra were measured on Bruker Avance AV600 spectrometers and chemical shifts (δ , ppm) are downfield from TMS. NMR spectra were taken at r.t. and 80°C in DMSO-d_6 . TLC was done on precoated 0.2 mm Merck silica gel 60 plates. Neutral alumina were used for column chromatographic separation.

One-pot procedure for the synthesis of *Salvadoricine* analoges (6a, b):

To benzothiazole (4 mmol) dissolved in CH₃CN (8 mL) were added alkyl chloroformate (4.4 mmol) and 3-acetylindole (2 mmol). The reaction mixture is stirred for the time and temperature specified in Table 1. For 1 week at room temperature in CH₃CN, crystallization of the products (6a, b) was observed. The crystals were filtered, washed with acetonitrile and dried.



Ethyl 2-(2-(1H-indol-3-yl)-2-oxoethyl)benzo[d]thiazole-3(2H)-carboxylate (6a):

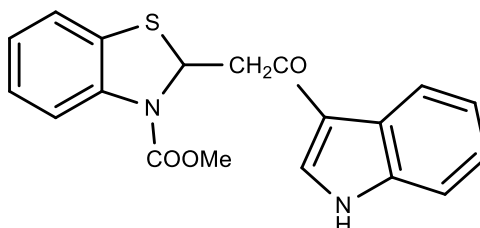
Yield: 80 %; 0.586 g, white crystalline powder; mp: 210 – 212 °C;

¹H-NMR (600 MHz, 80 °C, DMSO-d₆, δ ppm, *J* Hz): 1.30 (t, *J* = 7, 3H), 3.58 (dd, ²*J* = 15.8, ³*J* = 4.7, 1H), 3.63 (dd, ²*J* = 15.8, ³*J* = 8.8, 1H), 4.23 – 4.34 (m, 2H), 6.30 (dd, ²*J* = 9.4, ³*J* = 4.7, 1H), 7.08 (t, *J* = 7.6, 1H), 7.19 (t, *J* = 7.6, 1H), 7.24 – 7.31 (m, 3H), 7.53 (d, *J* = 7, 1H), 7.76 (d, *J* = 7.6, 1H), 8.25 (d, *J* = 7, 1H), 8.30 (s, 1H), 11.85 (s, 1H);

¹³C-NMR (150 MHz, 80 °C, DMSO-d₆, δ ppm): 14.4, 47.1, 62.5, 62.6, 112.5, 116.9, 117.6, 121.7, 122.2, 122.8, 123.3, 124.5, 125.5, 125.7, 129.1, 134.7, 137.1, 137.8, 152.3, 191.5;

IR (KBr, cm⁻¹): 3291, 3104, 2995, 1720, 1580, 1477, 1380, 1258, 749;

HRMS *m/z* (ESI): calcd for C₂₀H₁₈N₂NaO₃S⁺ [*M*+Na]⁺ 389.0930, found 389.0934;



Methyl 2-(2-(1H-indol-3-yl)-2-oxoethyl)benzo[d]thiazole-3(2H)-carboxylate (6b):

Yield: 65 %; 0.454 g, white crystalline powder; mp: 228 – 230 °C;

¹H-NMR (600 MHz, 80 °C, DMSO-d₆, δ ppm, *J* Hz): 3.53 (dd, ²*J* = 16.4, ³*J* = 4.7, 1H), 3.58 (dd, ²*J* = 16.4, ³*J* = 9.4, 1H), 3.77 (s, 3H), 6.23 (dd, ²*J* = 9.4, ³*J* = 4.7, 1H), 7.02 (t, *J* = 7.6, 1H), 7.13 (t, *J* = 7.6, 1H), 7.18 – 7.25 (m, 3H), 7.47 (d, *J* = 7, 1H), 7.70 (d, *J* = 8.2, 1H), 8.19 (d, *J* = 7, 1H), 8.24 (s, 1H), 11.79 (s, 1H);

¹³C-NMR (150 MHz, 80 °C, DMSO-d₆, δ ppm): 47.0, 53.5, 62.6, 112.5, 116.9, 117.6, 121.6, 122.2, 122.8, 123.3, 124.6, 125.5, 125.6, 129.0, 134.7, 137.1, 137.8, 152.8, 191.6;

IR (KBr, cm⁻¹): 3279, 3120, 2952, 1729, 1580, 1476, 1375, 1257, 748;

HRMS *m/z* (ESI): calcd for C₁₉H₁₆N₂NaO₃S⁺ [*M*+Na]⁺ 375.0774, found 375.0778;

4. CONCLUSIONS

An efficient method for multicomponent synthesis of two *Salvadoricine* analogues in high yields is demonstrated. The parameters of their spectral characterization have been determined. The presence of a rotameric effect was observed. NMR spectra were taken DMSO-d₆ at 80 °C. Under these conditions, correct spectra are recorded. Newly synthesized compounds reveal potential biological activity.

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REFERENCES

1. Sheikh, DK, Dixit, AK, 2015, "Plants in Holy Quran: A Look", *World Journal Of Pharmacy And Pharmaceutical Sciences*, vol. 4, no. 8, pp. 715-738.
2. Khan, W, Atar, M, Shaikh, T, Tambe, R, Katekar, TS, Rub, RA, 2010, "Phytochemical and pharmacological profile of Miswak (*Salvadora persica* Linn - salvadoraceae): An Overview", *Pharmacologyonline - Newsletter*, vol. 2, pp. 534-548.
3. Darout, IA, 2015, "Review on chemical and biologically active components of the toothbrush tree (*Salvadora persica*)", *European Journal of Pharmaceutical and Medical Research*, vol. 2, no. 6, pp. 12-17.
4. Mathur, S, Batra, A, 2011, "Production of An Indole Alkaloid from Callus Cultures of *Salvadora Persica* L.", *International Journal of Chemical, Environmental and Pharmaceutical Research*, vol. 2, no. 2-3, pp. 141-145.
5. Ahmad, A, Ahamed, N, Dar, JM, Mohammad, UJ, 2012, "Ethnobotany, Pharmacology and Chemistry of *Salvadora persica* L. - A Review", *Research in Plant Biology*, vol. 2, no. 1, pp. 22-31.
6. Aumeeruddy, MZ, Zengin, G, Mahomoodally, MF, 2018, "A review of the traditional and modern uses of *Salvadora persica* L. (Miswak): Toothbrush tree of Prophet Muhammad", *Journal of Ethnopharmacology*, vol. 213, pp. 409-444.
7. Malik, S, Ahmad, SS, Haider, SI, Muzaffar, A, 1987, "Salvadoricine - A new indole alkaloid from the leaves of *Salvadora persica*", *Tetrahedron Letters*, vol. 28, no. 2, pp. 163-164.
8. Almas, K, al-Lafi, TR, 1995, "The natural toothbrush", *World Health Forum*, vol. 16, no. 2, pp. 206-210.
9. Pindor, U, Erfanian-Abdoust, H, 1992, "First transformations of pyrano[3,4-b]indol-3-ones to Salvadoricine and 2,3-diacylindoles", *Journal of Heterocyclic Chemistry*, vol. 29, no. 1, pp. 145-147.
10. Pal, M, Dakarapu, R, Padakanti, S, 2004, "A direct access to 3-(2-Oxoalkyl)indoles via aluminum chloride induced C-C bond formation", *The Journal of Organic Chemistry*, vol. 69, no. 8, pp. 2913-2916.
11. Vargas, DA, Méndez, LJ, Cánepa, AS, Bravo, RD, 2017, "Sulfated zirconia: An efficient and reusable heterogeneous catalyst in the Friedel-Crafts acylation reaction of 3-methylindole", *Catalysis Letters*, vol. 147, no. 6, pp. 1496-1502.
12. Fürstner, A, Jumbam, DN, 1993, "Reversed chemoselectivity in titanium-induced coupling reactions: syntheses of Salvadoricine and Diazepam", *Journal of the Chemical Society, Chemical Communications*, no. 2, pp. 211-212.
13. Stremski, Y, Statkova-Abeghe, S, Angelov, P, Ivanov, I, 2018, "Synthesis of Camalexin and related analogues", *Journal of Heterocyclic Chemistry*, vol. 55, no. 6, Accepted 11 May 2018
14. Статкова-Абегхе, Ст, Иванов, И, Стайкова, М, Николова, Ст, 2010, "Синтез на нови N-хетероциклени съединения чрез реакция на α -амидоалкилиране на хетероарилметилкетони", *Годишник на Шуменския университет „Константин Преславски“*, *Природни науки - Химия*, no. XXB2, pp. 151-156.