SYNTHESIS AND EVALUATION OF NEW 2-AZOLYLINDOLES – DERIVATIVES OF INDOLE-2-CARBOXYLIC ACID

SINTEZA ȘI EVALUAREA NOILOR 2-AZOLILINDOLI – DERIVAȚI AI ACIDULUI INDOL-2-CARBOXILIC

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Summary. *Indole-2-carboxylic acid is a promising scaffold for the design of new compounds with application in biological studies and medicine practice. Its derivatives have shown a wide range of biological activity: antiviral, antifungal, antibacterial, anti-inflammatory. As part of the presented study, the method for preparation of alkyl derivatives of acetoacetic ester has been optimized. These derivatives are intermediates for the synthesis of indole-2-carboxylic acid esters according to the Japp-Klingemann/Fisher methodology. Several methods for the transformation of indole-2-carboxylic acid ester into previously undescribed 2-azolilindoles - derivatives of triazole, thiadiazole and oxadiazole have been proposed. Acylthiosemicarbazide on indole-2-carboxylic acid scaffold has been used for synthesis of corresponded azoles. Depending on the reaction conditions, indole-2-carboxylic acid acylthiosemicarbazide is capable of undergoing intramolecular cyclization into one of the target azoles.*

Rezumat. *Acidul indol-2-carboxilic este o schelă promițătoare pentru dezvoltarea compușilor care fac față nevoilor a biologiei și a medicinei. Derivații săi au demonstrat o gamă largă de activitate biologică: antivirală, antifungică, antibacteriană, antiinflamatore. Ca parte a studiului prezentat, a fost optimizată metoda de obținere a derivaților alchilici ai esterului acetoacetic. Acești derivați sunt intermediari pentru sinteza esterilor acidului indol-2-carboxilic conform metodologiei Japp-Klingemann/Fisher. Au fost propuse metode pentru transformarea esterului acidului indol-2-carboxilic în 2-azolilindoli anterior nedescriși - derivați ai triazolului,*

tiadiazolului şi oxadiazolului. Pentru obținerea azolilor, a fost folosită aciltiosemicarbazida acidului indol-2-carboxilic. În funcție de condițiile de reacție, aciltiosemicarbazida acidului indol-2-carboxilic este capabilă să fie supusă ciclizării intramoleculare într-unul dintre azolii țintă.

Keywords: *2-azolilindoles, indole-2-carboxylic acid, optimization, thiosemicarbasides, Japp-Klingemann method*

INTRODUCTION

The quality and length of human life significantly depend on the development of medicine. The searches and the evaluation of previously unknown biologically active substances are crucial in the modern chemistry and drug development. Indole-2-carboxylic acid derivatives should be hereby considered as perspective scaffold, due to their derivatives have antiviral and antifungal activities [1] and can inhibit several critical enzymes such as IDO1/TDO [2], HIV-1 integrase [3], and metallo-β-lactamases [4]. 2-Azolilindoles (pyrazole and triazole derivatives) are also of interest, exhibiting antitumor activity [5, 6]. Therefore, the development of new 2-azolylindoles and methods for their synthesis are important directions of the indole chemistry development. The purpose of this work is synthesis of new 2-azolilindoles - derivatives of triazole, thiadiazole and oxadiazole for further evaluation of biological activity and structure-activity relationship studies.

RESULTS AND DISCUSIONS

The cyclization of arylhydrazones in acidic conditions into 2,3-substituted indoles according to the Fischer method has found wide application in the synthesis of drugs [7]. Indole-2-carboxylic acid esters are synthetized from arylhydrazones of keto acids, obtained by the Japp-Klingemann reaction – via coupling of diazonium salts with 2-substituted acetoacetic esters [8].

Acetoacetic ester (**1**) alkylation with alkyl, allyl, benzyl and aryl halides in the presence of bases (NaH, K_2CO_3, NaOEt) usually proceeds in good yields (70-95%). However, during the work, difficulties were observed in the preparation of starting ethyl 2-(4-chlorobenzyl)-3-oxo-butanoate (**2**). The described procedure [9] was ineffective, however, its modification by an addition a phase transfer catalyst (TBAB) and potassium iodide lead to increasing of the yield of compound **2** up to 85%.

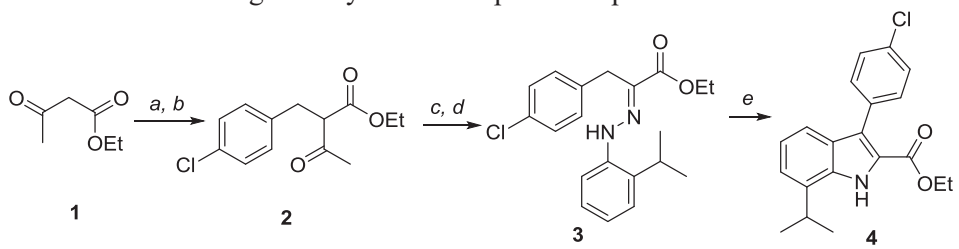

Figure 1. Synthesis of ethyl indole-2-carboxylate: a) 60% NaH, THF_{anh}, -10 °C; b) 4-chlorobenzyl chloride, TBAB, KI, THF_{anh}, rt; c) 2-isopropylaniline, $NaNO_2$, 36% HCl, -5 °C; d) KOH, EtOH, H_2O, rt; e) AcCl, $EtOH_{anh}$, 80 °C.

The preparation of arylhydrazones by the Japp-Klingemann method is performed by coupling of diazonium salts with acetoacetic ester derivatives in an acidic medium. However, the optimal pH value depends on the structure of the substrate: for 2-alkyl-substituted compounds the highest yields are achieved at pH 6.5, while for 2-benzyl-substituted - at pH 1-2 [8]. Thus, by reaction of ester **2** with 2-isopropylphenyldiazonium chloride (obtained by diazotization of 2-isopropylaniline) in an acidic condition, arylhydrazone **3** was synthesized and used in the further stage without any additional purification (**Figure 1**). Generally, the cyclization of arylhydrazones into indoles is carried out under anhydrous conditions in the presence of acids. The target ethyl 3-(4-chlorophenyl)-7-isopropyl-1H-indole-2-carboxylate (**4**) was obtained by refluxing arylhydrazone **3** in an $AcCl/EtOH_{(abs)}$ mixture in 60% yield.

The reaction of ester **4** with hydrazine hydrate in ethanol yielded indole-2-carboxylic acid hydrazide **5**, which under reflux in ethanol with phenyl isothiocyanate led to thiosemicarbazide **6**. Derivatives of triazole, thiadiazole and oxadiazole can be obtained by cyclization of thiosemicarbazide under different conditions (**Figure 2**) [10]. Thus, a treatment of thiosemicarbazide **6** with dilute sodium hydroxide solution under reflux led to triazole **7** in 86% yield. The common approach to the synthesis of thiadiazoles is based on heating thiosemicarbazides in the presence of mineral acids. However, during heating of the compound **6** in concentrated sulfuric acid, degradation of the indole ring occurred. Additionally, cyclization of compound **6** did not proceed after heating in phosphorous acid. Nevertheless, under optimized conditions, the treatment of thiosemicarbazide **6** with phosphorus oxychloride in chlorobenzene, led to the cyclization into target thiadiazole **8** in 81% yield.

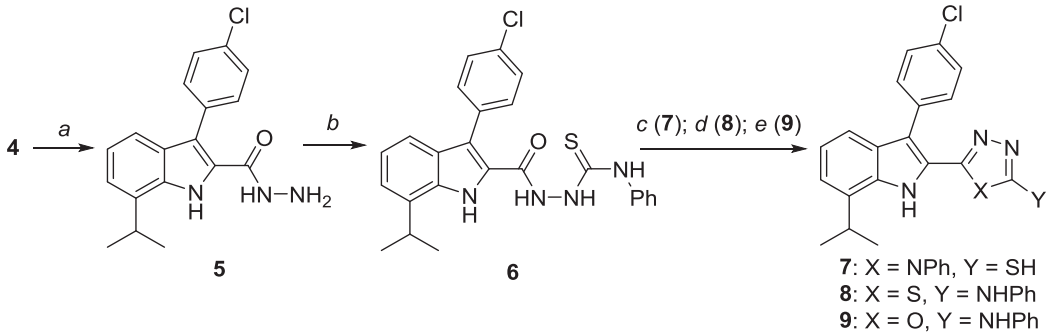

Figure 2. Synthesis of 2-azolylindoles. a) 51% N_2H_4, EtOH, 80 °C; b) PhNCS, EtOH, 80 °C; c) 2M NaOH, EtOH, H_2O, 80 °C; d) $POCl_3$, PhCl, 60 °C; e) DCC, PhH, DMF, 80 °C.

Oxadiazole derivatives can be obtained by reaction of carbodiimides with thiosemicarbazides. However, due to the side urea derivative, which is formed in the reaction, labor-intensive purification is required to isolate the target products. In order to obtain oxadiazole **9** from thiosemicarbazide **6**, the conditions were optimized and a benzene/DMF mixture (1:1) was used as solvent for the reaction. The addition of DMF lead to

homogenization of the reaction mixture (thiosemicarbazide **6** is poorly soluble in benzene) and simplified the further purification - addition of water and washing the precipitate with water made it possible to obtain product **9** in 90% yield and with 99.7% purity.

The structure of the obtained compounds was confirmed by spectral methods (^{1}H and ^{13}C NMR, HRMS-ESI). The purity of the obtained compounds (>95%) was determined by HPLC.

MATERIALS AND METHODS

Materials:

Syntheses were performed using reagents purchased from Alfa Aesar, Sigma-Aldrich, and Reahim without additional purification. Several reagents (2-isopropylaniline, acetoacetic ester, solvents: DMF, THF, ethyl alcohol) are pre-distilled and absolutized. Spectral (^{1}H and ^{13}C NMR, HRMS-ESI) and chromatographic (HPLC) studies were performed for structural and purity analysis of the obtained compounds.

Synthesis of ethyl 2-(4-chlorobenzyl)- 3-oxo-butanoate (1)

While vigorous stirring, 60% sodium hydride (8.5 g, 0.21 mol) is dissolved in absolute THF (70,0 ml) with cooling (-10^{0}C). Freshly distilled acetoacetic ester (24.3 ml, 0.19 mol) is added dropwise to the resulting mixture so that the temperature of the mixture does not exceed -5^{0}C. After hydrogen forming has ceased, TBAB (2.5 g, 7.70 mmol), potassium iodide (1.3 g, 7.70 mmol) and a solution of 4-chlorobenzyl chloride (27.8 g, 0.17 mol) in absolute THF (15,0 ml) were added. The reaction mixture is heated to 60^{0}C. The reaction is monitored by TLC. The reaction mixture was poured on ice with NH_4Cl and extracted with ethyl acetate. The organic layer was successively washed with a brine solution and water, and dried over sodium sulfate. The solution is concentrated under reduced pressure. The resulting dark yellow oil is distilled in vacuum (6 mm Hg), with the fraction boiling in the range of 158-162^{0}C collected. The yield of the eater **2** was 41.4 g (85%). Colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (dd, J = 48.5, 8.3 Hz, 4H), 4.25 – 4.04 (m, 2H), 3.74 (t,
J = 7.6 Hz, 1H), 3.12 (dd, J = 7.6, 3.3 Hz, 2H), 2.20 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H).

Synthesis of ethyl 3-(4-chlorophenyl)-7-isopropyl-1H-indole-2-carboxylate (4)

2-Isopropylaniline (162 mg, 2,10 mmol) is dissolved in hydrochloric acid (36%, 1,1 ml, 13,20 mmol) and a solution of sodium nitrite (168 mg, 2,31 mmol) in water (2,0 ml) is added dropwise to vigorously stirred mixture, so that the temperature of the mixture does not exceed -5^{0}C. While cooling (-10^{0}C), a mixture of potassium hydroxide (412 mg, 7,35 mmol), ethanol (4,0 ml), water (4,0 ml) and ester **2** (530 mg, 2,10 mmol) was prepared. Anteriorly prepared solution of diazonium salt was then dropwise added, so that the temperature of the mixture does not exceed -5^{0}C. After addition is complete, the mixture is stirred at room temperature 2-4 h. The reaction is monitored by TLC. The reaction

mixture extracted with ethyl acetate, the organic layer is successively washed with brine solution, water, and dried over sodium sulfate. The solution is concentrated under reduced pressure. The resulting dark red, viscous liquid (arylhydrazone **3**) is solved in a mixture of absolute ethanol and acetyl chloride (3:1, 1,8 ml) and refluxed for 30 min. The reaction is monitored by TLC. The mixture is then cooled and concentrated under reduced pressure. The resulting ester **4** is recrystallized from ethanol. The yield of the product **4** was 270 mg (60% in terms of aniline). White fluffy powder. M.p. 148-152 ^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 7.56 – 7.48 (m, 2H), 7.47 – 7.40 (m, 3H), 7.28 (s, 1H), 7.16 (t, J = 7.6 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.35 (p, J = 6.9 Hz, 1H), 1.44 (d, J = 6.9 Hz, 6H), 1.27 (t, J = 7.1 Hz, 3H).

Synthesis of 3-(4-chlorophenyl)- 7-isopropyl-1H-indole-2-carboxylic acid hydrazide (5)

A mixture of ester **4** (270 g, 0,79 mmol) and hydrazine hydrate (51%, 1,5 ml, 24,57 mmol) in ethanol (10,0 ml) was refluxed for 4 h. The reaction is monitored by TLC. Water is added into the solution, and the formed precipitate is filtered off and dried. The solid residue is recrystallized from an ethanol/water mixture (4:1). The yield of product **5** was 230 mg (89%). White powder. M.p. 248-251 ^{0}C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.39 (s, 1H), 9.33 (s, 1H), 7.46 (s, 4H), 7.33 (d, J = 7.9 Hz, 1H), 7.12 (d, J = 7.1 Hz, 1H), 7.05 (t, J = 7.6 Hz, 1H), 4.49 (s, 2H), 3.51 (p, J = 6.8 Hz, 1H), 1.30 (d, J = 6.8 Hz, 6H); *m/z* (ES) (calculated for $C_{18}H_{18}ClN_3O^+$: 328.1211; found: 328.1217); HPLC: t_R = 9.3 (94.7% rel. area).

Synthesis of 2-(3-(4-chlorophenyl)-7-isopropyl-1H-indole-2-carbonyl)-N-phenylhydrazine- carbothioamide (6)

Hydrazide **5** (230 mg, 0,70 mmol) was added into a boiling solution of phenylisothiocyanate (0,1 ml, 0,77 mmol) in ethanol (10,0 ml) and refluxed for 30 min. The reaction is monitored by TLC. The formed precipitate is filtered off and recrystallized from ethanol. The yield of the hydrazide derivative **6** was 300 mg (92%). Slightly greenish powder. M.p. 203-207 ^{0}C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (d, J = 42.8 Hz, 1H), 10.26 (s, 1H), 9.80 (s, 1H), 9.71 (s, 1H), 7.60 – 7.40 (m, 6H), 7.40 – 7.30 (m, 3H), 7.20 (d, J = 7.3 Hz, 1H), 7.16 (d, J = 7.0 Hz, 1H), 7.09 (t, J = 7.7 Hz, 1H), 3.48 (ddd, J = 20.1, 13.4, 5.4 Hz, 1H), 1.42 – 1.26 (d, 6H); *m/z* (ES) (calculated for $C_{25}H_{24}ClN_4OS^+$: 463.1354; found: 463.1488); HPLC: t_R = 16.6 (94.9% rel. area).

Synthesis of 5-(3-(4-chlorophenyl)-7-isopropyl-1H-indol-2-yl)-4-phenyl-4H-1,2,4-triazole-5(4H)-thione (7)

A mixture of thiosemicarbazide **6** (100 mg, 0,22 mmol), aqueous sodium hydroxide (2M, 5,0 ml, 10,0 mmol) and ethanol (5,0 ml) was refluxed for 2-4 h. The reaction is monitored by TLC. The solution was cooled and acidified with diluted hydrochloric acid to pH

7. The formed precipitate was filtered off and recrystallized from an ethanol/water mixture (6:1). The yield of triazole **7** was 86 mg (89%). Orange powder. M.p. 194-198 ^{0}C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 7.31 (dd, J = 16.0, 8.0 Hz, 6H), 7.11 (q, J = 6.9, 6.2 Hz, 3H), 7.06 (s, 1H), 7.05 (d, J = 1.7 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H), 6.90 – 6.78 (m, 1H), 3.39 (h, J = 6.4 Hz, 1H), 1.24 (d, J = 6.8 Hz, 6H); *m/z* (ES) (calculated for $C_{25}H_{22}ClN_4S^+$: 445.1278; found: 445.9885); HPLC: t_R = 18.3 (91.5% rel. area).

Synthesis of 5-(3-(4-chlorophenyl)-7-isopropyl-1H-indol-2-yl)-N-phenyl-1,3,4-thiadiazol-2-amine (8)

Thiosemicarbazide **6** (100 mg, 0,22 mmol) and phosphorus oxychloride (0,2 ml, 2.16 mmol) are dissolved in chlorobenzene (15,0 ml) and the mixture is heated to 60^{0}C. The reaction is monitored by TLC. The reaction mass is then treated with a 15% aqueous solution of sodium hydroxide to pH 10 and stirred for 1 hour. The mixture is extracted with dichloromethane, the organic layer is washed with water, dried and concentrated under reduced pressure. The solid residue is recrystallized from an ethanol/water mixture (5:1). The final product yield is 78 mg (81%). Bright yellow powder. M.p. 301-305 ^{0}C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s, 1H), 10.57 (s, 1H), 7.68 – 7.60 (m, 2H), 7.60 – 7.52 (m, 2H), 7.51 – 7.44 (m, 2H), 7.32 (t, J = 7.7 Hz, 2H), 7.17 (d, J = 7.9 Hz, 1H), 7.13 (d, J = 7.2 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.97 (t, J = 7.3 Hz, 1H), 3.74 (p, J = 6.8 Hz, 1H), 1.29 (d, J = 6.8 Hz, 6H); *m/z* (ES) (calculated for $C_{25}H_{20}ClN_4S^-$: 443.1103; found: 443.0639); HPLC: t_R = 22.5 (99.4% rel. area).

Synthesis of 5-(3-(4-chlorophenyl)-7-isopropyl-1H-indol-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine (9)

A mixture of thiosemicarbazide **6** (100 mg, 0,22 mmol) and 1,3-dicyclohexylcarbodiimide (68 mg, 0,32 mmol) was refluxed in benzene/DMF mixture (1:1, 10,00 ml). The reaction is monitored by TLC. The reaction mixture was treated with water and stirred for 30 min. The precipitate is filtered off and washed with water. The yield of oxadiazole **9** was 83 mg (90%). White powder. M.p. 292-296 ^{0}C.

^{1}H NMR (400 MHz, DMSO- d_6) δ 12.03 (s, 1H), 10.57 (s, 1H), 7.61 – 7.50 (m, 4H), 7.50 – 7.42 (m, 2H), 7.38 (d, J = 7.9 Hz, 1H), 7.31 (t, J = 7.8 Hz, 2H), 7.19 (d, J = 7.2 Hz, 1H), 7.11 (t, J = 7.6 Hz, 1H), 7.05 – 6.94 (m, 1H), 3.69 (p, J = 6.8 Hz, 1H), 1.32 (d, J = 6.7 Hz, 6H); *m/z* (ES) (calculated for $C_{25}H_{22}ClN_4O^+$: 429.1477; found: 429.1461); HPLC: t_R = 19.0 (99.7% rel. area).

CONCLUSIONS

Novel 2-azolylindoles based on indole-2-carboxylic acid scaffold have been synthesized. The procedure for the synthesis of the starting 2-benzyl derivative of acetoacetic ester was modified. This made it possible to increase the yield of the product in alkylation of acetoacetic ester. The synthesis of indole-2-carboxylic acid acylthiosemi-

carbazide was also carried out and procedures for its cyclizations into thiadiazole and oxazole derivatives were developed. The optimized reaction conditions made it possible to obtain the target compounds in high yields (> 80%) and acceptable purity (> 99%) as well as to simplify the procedures for their purification. The results of evaluation of biological activity of obtained 2-azolylindoles and studies of structure-activity relationship will be reported soon.

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