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Spectroscopy, molecular modeling and anti-oxidant activity studies on novel conjugates containing indole and uracil moiety

Weronika Kozanecka-Okupnik [a], Beata Jasiewicz [a, *], Tomasz Pospieszny [a], Renata Jastrząb [a], Monika Skrobańska [a], Lucyna Mrówczyńska [b, **]

[a] Faculty of Chemistry, A. Mickiewicz University, Umultowska 89b, 61-614 Poznań, Poland
[b] Faculty of Biology, Department of Cell Biology, A. Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland

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ABSTRACT

Herein we describe our results on the synthesis and molecular modeling of new conjugates containing indole and uracil moiety. This group of compounds has been synthesized by the reaction of indole alkaloid - gramine with appropriate uracil (uracil, 2-thiouracil, 6-methyl-2-thiouracil, thymine, 6-methyluracil and barbituric acid) using DMF as solvent. The structures of all products were confirmed by spectroscopic (^{1}H NMR, ^{13}C NMR, and FT-IR) analysis, mass spectrometry (EI) and PM5 semiempirical methods. Moreover the protonation constants for investigated compounds were determined. The obtained conjugates were screened *in vitro* for antioxidant and haemolytic activities, as well as for the protective effects against 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH)-induced oxidative haemolysis of human erythrocytes.

1. Introduction

Recently, compounds containing an indole moiety have received attention due their broad application as antibacterial, antiviral and cytotoxic agents: indolyl triazoles have anticancer activity [1], melatonin has antioxidant activity [2] and indolyl thiohydantoins have *anti*-HIV activity [3]. Furthermore, indole is found to reduce *cis* platin-induced reactive oxygen species formation [4] and scavenge hydroxyl radical directly [5]. Alkaloid gramine, 3-(dimethylaminomethyl)indole (**1**), the best known indole derivative is often used as initial compounds in the synthesis of various biologically active substituted indoles. Some gramine derivatives are identified as highly effective anticancer agents [6], benzylgramines are capable of blocking serotonin activity [7]. Gramine can also modify the membrane perturbing activity of steroids [8]. Moreover, it is among the substances most frequently used in the synthesis of L-tryptophan and its derivatives.

Uracil belong to a class of compounds important in drug discovery with a wide range of biological activities e.g. uracil and its modified derivatives exhibit anti-viral and anti-cancer properties [9–12]. An important roles in the structure of these compounds play the prototropic tautomerism. The tautomeric equilibrium of uracil, 2-thiouracil and barbituric acid determines their chemo- and regioselectivity. The prototropic tautomerism depends on the temperature, pH as well as nature of solvent [13–15].

In view of the significant role of gramine in the synthesis of indoles and the role of uracil as important scaffolds in drug discovery this paper reports the synthesis, spectroscopic analysis, determination of protonation constants and molecular modeling of new mono- and bis-gramine-uracil conjugates. Consequently, the aims of present study was to estimate *in vitro* the antioxidant potential of new derivatives in the cell-free assays as well as the cytoprotective effects against 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH)-induced oxidative damage of human erythrocytes. Moreover, the effect of new derivatives on the human erythrocytes shape and membrane permeability was also investigated.

* Corresponding author.
** Corresponding author.
E-mail addresses: beatakoz@amu.edu.pl, beata.jasiewicz@amu.edu.pl (B. Jasiewicz), lumro@amu.edu.pl (L. Mrówczyńska).

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2. Experimental

2.1. General methods

The melting points (mp) were obtained with a Büchi SMP-20 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Warian 300/400 spectrometer with DMSO-d_6 as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. EI mass spectra were measured on Bruker 320MS/450 GC mass spectrometer. FT-IR spectra were recorded on Nicolet iS 5 (KBr pellets). TLC analysis was used using Sigma-Aldrich silica gel 60 plates with fluorescent indicator (254 nm) and visualized with UV or Dragendorff's Reagent. All chemicals or reagents used for syntheses were commercially available.

2.2. Synthesis

2.2.1. General procedure for the synthesis of compounds **2**–**8**

New gramine-uracil conjugates were obtained following a procedure already reported [16] with some modification. A mixture of gramine (1 mmol) and appropriate uracil (1.0 mmol) in dry DMF (5 mL) was refluxed for 5–6 h (9 h for compound **5**) and the progress was monitored by using TLC. On completion of the reaction, the mixture was extracted with ethyl acetate (3 × 20 mL, compounds **2**–**6**) or with diethyl ether (3 × 20 mL, compounds **7**, **8**). The combined organic layer was washed with water (100 mL) and brine (50 mL), dired (Na_2SO_4) and concentrated under reduced pressure yielding compounds **2**–**8** in good yields. New compounds were recrystallized from DMF.

2.2.2. N(1′)-(3-methylindole)uracil **(2)**

Mp 232–234 °C, lit 252–256 (DMF/H_2O) [16], yield 61%. ^{1}H NMR (403 MHz, DMSO-d_6, TMS, ppm): δ = 11.27 (s, 1H, NH), 11.15 (s, 1H, NH), 7.67 (d, J = 8.1 Hz, 1H, 6′-H), 7.63 (d, J = 8.0 Hz, 1H, 7-H), 7.46 (s, 1H, 2-H), 7.38 (d, J = 0.6 Hz, 1H, 4-H), 7.11 (t, J = 1.1 Hz, 1H, 6-H), 7.01 (t, J = 0.9 Hz, 1H, 5-H), 5.52 (dd, J = 8.1 Hz, 1H, 5′-H), 5.00 (s, 2H, 10-CH_2). ^{13}C NMR (101 MHz, DMSO-d_6, TMS, ppm): δ = 163.66, 151.05, 144.87, 136.28, 126.02, 125.83, 121.52, 119.10, 118.49, 111.70, 109.63, 101.08, 41.79. EI MS (m/z, % int.): 241 (M^+, 60). FT-IR (KBr) ν_{max}: 3326, 3046, 2886, 2834, 1674, 1476-1323, 1256-1239, 1256-1239, 1164, 1093, 748.

2.2.3. N(1′)-(3-methylindole)-2′-thiouracil **(3)**

Mp 103–104 °C, yield 64%. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ = 11.24 (s, 1H, NH), 11.03 (s, 1H, NH), 7.85 (d, J = 8.0 Hz, 1H, 6′-H), 7.78 (d, J = 8.0 Hz, 1H, 7-H), 7.44 (s, 1H, 2-H), 7.32 (d, J = 8.0 Hz, 1H, 4-H), 7.02 (m, 2H, 5-H, 6-H), 5.96 (d, J = 7.5 Hz, 1H, 5′-H), 5.64 (s, 2H, 10-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ = 176.44, 160.40, 144.76, 135.61, 126.64, 126.21, 120.99, 119.64, 118.56, 111.32, 109.18, 104.40, 47.92. EI MS (m/z, % int.): 257 (M^+, 100). FT-IR (KBr) ν_{max}: 3399, 2926, 1671, 1505-1430, 1384-1316, 1250-1097, 812, 745.

2.2.4. N(1′)-(3-methylindole)-6′-methyl-2′-thiouracil (**4**)

Mp 108–109 °C, yield 87%. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ = 12.13 (s, 1H, NH), 11.00 (s, 1H, NH), 7.85 (d, J = 7.7 Hz, 1H, 7-H), 7.42 (s, 1H, 2-H), 7.34–7.28 (m, 1H, 4-H), 7.07–6.78 (m, 2H, 5-H, 6-H), 5.83 (s, 1H, 5′-H), 5.63 (s, 2H, 10-CH_2), 2.06 (s, 3H, 7′-H). ^{13}C NMR (101 MHz, DMSO-d_6, ppm): δ = 176.23, 160.22, 151.81, 135.61, 126.78, 126.21, 120.87, 119.69, 118.68, 111.30, 109.49, 102.99, 41.06, 17.84. EI MS (m/z, % int.): 271 (M^+, 100). FT-IR (KBr) ν_{max}: 3404-2949, 1660, 1531, 1456-1428, 1340-1318, 1249-1096, 744.

2.2.5. N(1′),N(3′)-di-(3-methylindole)uracil **(5)**

Mp 114–120 °C, yield 20%. ^{1}H NMR (403 MHz, DMSO-d_6, TMS, ppm): δ = 8.23 (s, 1H, NH), 8.10 (s, 1H, N'H), 7.48 (d, J = 8.1 Hz, 1H, 6′-H), 7.47 (s, 2H, 2-H), 7.37 (m, 4H, 4-H/7-H), 7.09 (m, 4H, 5-H/6-H), 5.61 (dd, J = 8.1 Hz, 1H, 5′-H), 5.36 (s, 2H, 10′-H), 5.08 (s, 2H, 10-CH_2). EI MS (m/z, % int.): 370 (M^+, 80). FT-IR (KBr) ν_{max}: 3401, 1697, 1652, 1458, 1424, 1395, 1341. Anal. calcd. for $C_{22}H_{18}N_4O_2$: C, 71.35; H, 4.86; N, 15.14. Found: C, 70.98; H, 4.92; N, 15.21.

2.2.6. N(1′),N(3′)-di-(3-methylindole)-6′-methyluracil **(6)**

Mp 233–235 °C, yield 20%. ^{1}H NMR (403 MHz, DMSO-d_6, ppm): δ = 10.97 (s, 1H, NH), 10.72 (s, 1H, NH), 7.81 (d, J = 7.9 Hz, 1H, 7-H), 7.54 (d, J = 7.9 Hz, 1H, 7-H), 7.33, (s, 1H, 2-H), 7.27 (s, 1H, 2-H), 7.00 (m, 6H, 4-H, 5-H, 6-H), 5.46 (s, 1H, 5′-H), 5.14 (s, 2H, 10-CH_2), 5.05 (s, 2H, 10-CH_2), 2.05 (s, 3H, 7′-H). ^{13}C NMR (101 MHz, DMSO-d_6, ppm): δ = 163.22, 151.19, 150.64, 136.29, 135.77, 126.79, 126.58, 126.00, 125.32, 122.59, 120.92, 119.40, 118.80, 118.57, 118.12, 112.81, 111.27, 110.52, 108.15, 98.43, 34.71, 34.10, 15.97. EI MS (m/z, % int.): 384 (M^+, 17), (254, 100). FT-IR (KBr) ν_{max}: 3401, 3054, 2955, 1702-1641, 1455, 1340, 1093, 744. Anal. calcd. for $C_{23}H_{20}N_4O_2$: C, 71.88; H, 5.20; N, 14.59. Found: C, 71.90; H, 5.25; N, 14.64.

2.2.7. N(1′),N(3′)-di-(3-methylindole)thymine **(7)**

Mp 175–180 °C, yield 50%. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ = 10.98 (s, 1H, NH), 10.88 (s, 1H, NH), 7.84 (d, J = 7.9, 2H, 7-H), 7.79 (d, J = 7.9, 2H, 4-H), 7.48 (s, 1H, 2-H), 7.47 (s, 1H, 2-H), 7.34 (s, 1H, 6′-H), 713–6.89 (m, 4H, 2x5-H, 2x6-H), 5.10 (s, 2H, 10-CH_2), 4.97 (s, 2H, 10-H), 1.78 (s, 3H, 7′-H).^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ = 163.61, 151.19, 140.54, 136.17, 135.71, 126.60, 126.25, 126.03, 125.67, 121.41, 120.94, 119.31, 119.04, 118.70, 118.60, 111.30, 110.38, 108.76, 108.18, 107.32, 35.47, 34.53, 12.51. EI MS (m/z, % int.): 384 (M^+, 25), (254, 100). FT-IR (KBr) ν_{max}: 3405-3320, 3060, 2924, 1702-1626, 1456, 1385-1346, 1248-1212, 1098, 782-745. Anal. calcd. for $C_{23}H_{20}N_4O_2$: C, 71.88; H, 5.20; N, 14.59. Found: C, 71.14; H, 5.37; N, 14.62.

2.2.8. N(1′),N(3′)-di-(3-methylindole)barbituric acid **(8)**

Compound **8** was obtained as oil, yield 34%. ^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ = 10.95 (s, 2H, NH), 7.52 (d, J = 7.0 Hz, 2H, 7-H), 7.29 (d, J = 8.1 Hz, 2H, 4-H), 7.04 (t, J = 6.1 Hz, 2H, 6-H), 6.96 (m, 4H, 5-H, 2-H), 3.48 (s, 4H, 10-CH_2), 3.34 (s, 2H, 5′-H). ^{13}C NMR (151 MHz, DMSO-d_6, ppm): δ = 173.57, 149.34, 135.69, 126.84, 124.05, 121.02, 118.60, 118.44, 111.28, 108.31, 58.79, 34.16. EI MS (m/z, % int.): 386 (M^+, 20), (257, 27). FT-IR (KBr) ν_{max}: 3412, 3316, 3220, 3054, 2925, 2842, 1765-1700, 1456-1313, 1209, 1098, 1009, 818, 740. Anal. calcd. for $C_{22}H_{18}N_4O_3$: C, 68.39; H, 4.66; N, 14.51. Found: C, 69.02; H, 4.81; N, 14.17.

2.3. Equilibrium study

All experiments were prepared using DMSO: water 30: 70 (v/v) solution (demineralised CO_2-free water was used). The potentiometric studies were carried out using Titrino 702 SM Metrohm equipped with an autoburette with a MetrohmSolvotrode combination pH glass electrode specially designed for use with nonaqueous acid-base titrations. Prior to each series of measurements, the pH-meter was corrected using two standard buffer solutions of pH 4.002 (phthalate) and pH 9.225 (borax) and electrode was calibrated in terms of H^+ concentration [17]. All potentiometric titrations were made in atmosphere of neutral gas (helium 5.0) in the constant ionic strength (μ = 0.1 M $LiNO_3$), at 25±1 °C (titration dish placed in thermostatic bath set at this temperature), in the pH range from 2.5 to 10.5, using CO_2-free NaOH as a titrant at a concentration of 0.1823 M. The concentration of the ligand was $2 \cdot 10^{-3}$ M, acidified with HCl (1.5 mL; 0.1 M). Determined pKw for DMSO: water (30: 70) was 14.501 [18,19]. The calculations were performed using 150–350 points for each job. The model assumed