Supplementary Information

Synthesis, Radical Scavenging Activity and Structure-Activity Relationship of Uric Acid Analogs.

Daisuke Yasuda,∗ Kyoko Takahashi,∗ Tomohiro Kakinoki,∗ Yoko Tanaka,∗ Tomoyuki Ohe, Shigeo Nakamura ∗ and Tadahiko Mashino ∗

Contents

1. Preparation of Uric acid analogs
2. Scanned copies of the 1H and 13C NMR spectra
3. DPPH radical scavenging activity
4. Prediction of pKa values
5. Determination of cytotoxicity.

1. Preparation of Uric acid analogs

1.1. General

1H-NMR spectra (500 MHz) were measured on a JEOL JNM-A500 FT-NMR spectrometer with tetramethylsilane as an internal standard (δ = 0.00) in CD3OD or DMSO-d6. 13C-NMR spectra (125 MHz) were obtained on the same spectrometer and the chemical shifts were referenced to the signals of CD3OD (δ = 33.3) or DMSO-d6 (δ = 39.5). Melting points were determined using a Yanagimoto MP-J3 micro-melting point apparatus and uncorrected. Column chromatography was performed using Merck Silica gel 60. 6-Hydroxy-2-benzoxazolinone 4, 5-indanol 7 were purchased from Sigma-Aldrich Chemical Co., 5-hydroxyoxindole 3a was purchased from Apin Chemical Co. 5-hydroxyindole 5, 6-hydroxyindole 6, Benzimidazole 8 and 2-indanone 9 were purchased from Kanto Chemical Co., 1,1-diphenyl-2-picrylhydrazyl, hydroquinone, p-aminophenol and uric acid were purchased from Tokyo Kasei Kogyo Co. The regioisomers of 2a and 3a were synthesized according to the method reported by R. J. S. Beer et al (J. chem. Soc., 1948, 1605-1609) with some suitable modification.

1-2 Preparation of 1,3-dihydro-7-methoxy-2H-imidazo[4,5-b]pyridine-2-one (1b)
To solution of urea (0.45 g, 7.5 mmol) and o-6-methoxy-2,3-pyridinediamine (1.59 g, 7.5 mmol) in N, N-dimethylformamide (12 mL) was added ZnO (442.5 mg, 5.44 mmol) and reacted for 5 hr under microwave irradiation condition (120°C, 150 W). Whole the reaction mixture was diluted with H2O, extracted with ethyl acetate twice. The organic layer was washed with sat. NaCl solution, dried over anhydrous MgSO4, and concentrated under reduced pressure. The residue was recrystallized from CHCl3:MeOH to brown crystal (459.5 mg, 37 %).

$^1$H-NMR (DMSO-d6, 500MHz) δ 3.78 (s, 3H, -OCH3), 6.36 (d, 1H, J = 8.50 Hz, 8-H), 7.21 (d, 1H, J = 8.00 Hz, 9-H), 10.54 (brs, 1H, -NH-), 11.20 (brs, 1H, -NH-).

$^{13}$C-NMR (DMSO-d6, 125 MHz) δ 53 (-OCH3), 101 (8C), 117 (4C), 119 (9C), 142 (5C), 154 (7C), 159 (2C).

FAB-MS: m/z=166 [M+H]+ (84 %), 165 [M]+ (64 %), 138 (40 %), 137 (77 %), 136 (100 %), 107 (29 %), 89 (24 %), 77 (21 %).

FAB-HRMS: calcd. for C7H8N3O2 166.0617, found 166.0633.

1-3. Preparation of 5-methoxy-2-benzimidazolinone 2b

To solution of urea (0.55 g, 9.19 mmol) and 4-methoxy-1,2-phenylenediamine (1.94 g, 9.19 mmol) in N, N-dimethylformamide (20 mL) was added ZnO (540.0 mg, 6.63 mmol) and reacted for 10 hr under microwave irradiation condition (120°C, 150 W). Whole the reaction mixture was diluted with H2O, extracted with ethyl acetate twice. The organic layer was washed with sat. NaCl solution, dried over anhydrous MgSO4, and concentrated under reduced pressure. The residue was recrystallized from CHCl3:MeOH to give yellow crystal (560 mg, 37 %).

$^1$H-NMR (CD3OD, 500 MHz) δ 3.69 (s, 3H, -OCH3), 6.55 (dd, 1H, J = 8.50 Hz, 2.14 Hz, 6-H), 6.59 (d, 1H, J = 2.14 Hz, 4-H), 6.85 (d, 1H, J = 8.54 Hz, 7-H).

$^{13}$C-NMR (CD3OD, 125 MHz) δ 56 (-OCH3), 108 (4C), 110 (6C), 124 (7C), 131 (9C), 157 (5C), 158
1-4. Preparation of 2-benzimidazolinone 2c

![Chemical structure of 2c](image)

To solution of urea (0.20 g, 3.33 mmol) and o-phenylenediamine (0.36 g, 3.33 mmol) in N,N-dimethylformamide (8 mL) was added ZnO (196.7 mg, 2.42 mmol) and reacted for 6 hr under microwave irradiation condition (120°C, 150 W). Whole the reaction mixture was diluted with H₂O, extracted with ethyl acetate twice. The organic layer was washed with sat. NaCl solution, dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was recrystallized from CH₂Cl₂:MeOH to give black crystal (37.3 mg, 8%).

**¹H-NMR (CD₃OD, 600 MHz)** δ 7.01 (s, 4H, 4,5,6,7-H).

**¹³C-NMR (CD₃OD, 125 MHz)** δ 110 (5C, 6C), 122 (4C, 7C), 130 (8C, 9C), 158 (2C).

1-5 Preparation of 1,3-dihydro-7-hydroxy-2H-imidazo[4,5-b]pyridine-2-one (1a)

![Chemical structure of 1a and 1b](image)

**1b** (100.0 mg, 0.61 mmol) was dissolved in 47 % aqueous HBr (1.0 mL) and stirred at 130 °C for 3 hr. After excess HBr and H₂O were removed by evaporation, the crude product (101.2 mg) was purified by column chromatography (SiO₂) to give white solid (10.0 mg, 11%).

**¹H-NMR (DMSO-d₆, 500MHz)** δ 6.17 (d, 1H, J = 8.30 Hz, 7-H), 7.11 (d, 1H, J = 8.06 Hz, 8-H), 10.04 (brs, 1H, -OH), 10.40 (s, 1H, -NH₂), 11.02 (s, 1H, -NH₂).

**¹³C-NMR (DMSO-d₆, 125 MHz)** δ 100 (8C), 116 (4C), 118 (9C), 142 (5C), 154 (7C), 158 (2C).

**FAB-MS**: m/z=152 [M+H]⁺ (39 %), 151 [M]⁺ (19 %), 138 (39 %), 137 (76 %), 136 (100 %), 107 (28 %), 89 (25 %), 77 (21 %).

**FAB-HRMS**: calcd. for C₆H₆N₃O₂ 152.0460, found 152.0472.

Mp. : over 300 °C (decomp.)
1-6. Preparation of 5-hydroxy-2-benzimidazolinone 2a

2b (80.6 mg, 0.49 mmol) was dissolved in 47% aqueous HBr (1.0 mL) and stirred at 130 °C for 3 hr. After excess HBr and H₂O were removed by evaporation, the crude product (81.5 mg) was purified by column chromatography (SiO₂) to give white solid (65.6 mg, 89%).

\[ \text{H}_3\text{CO} \xrightarrow{47\% \text{ HBr}} \xrightarrow{130 \degree \text{C}} \text{HO} \]

\[ \text{2b} \rightarrow \text{2a} \]

\[^1\text{H}-\text{NMR (CD}_3\text{OD, 500 MHz)} \delta 6.49 (dd, 1H, J = 8.50, 2.14 \text{ Hz, 6-H}), 6.53 (d, 1H, J = 1.83, 4-H), 6.83 (d, 1H, J = 8.28 \text{ Hz, 7-H}).\]

\[^{13}\text{C}-\text{NMR (CD}_3\text{OD, 125 MHz)} \delta 98 (4\text{C}), 109 (6\text{C}), 110 (1\text{C}), 123 (7\text{C}), 131 (3\text{C}), 153 (5\text{C}), 158 (2\text{C}).\]

M. p.: over 300 °C (decomp.)

2. Scanned copies of the \[^1\text{H}\] and \[^{13}\text{C}\] NMR spectra
3. DPPH radical scavenging activity

The measurement procedure is a modification of the method of Yamaji et al (Yamaji, K.; Sarker, K. P.; Maruyama, I.; Hizukuri, S. Planta Med., 2002, 60, 16). Sample (500 μM) and DPPH (50 μM) was mixed in a solution of MES buffer (pH 7.4) and ethanol (3:2) at 25 °C. The decrease in absorbance at 517 nm was recorded on a stopped-flow Rapid-Scan Spectrophotometer RSP-1000 (UNISOKU Co., Ltd.) for 60 sec. The second-order rate constant was calculated based on a decreasing curve fitting method (UNISOKU Spectroscopy & Kinetics, NISOKU Co., Ltd.).

4. Prediction of pKa values

Apparent pKa values of all UA analogs were theoretically calculated by using ACD/pKa DB (version 9.0) software.

4. Determination of cytotoxicity.

HL-60 cells (5x10^5 cells/mL) were plated onto a six-well multi-plate and incubated with the test compound in DMSO (0.5-100 μM) at 37 °C for 24 h under a 5% CO₂ atmosphere. Only the cells treated with DMSO were used as a non-treated control. The concentration of DMSO was set at 1 v/v %. The incubation mixture was centrifuged at 1,000 rpm for 5 min, and the pellet was suspended in 2 mL of PBS(-). The cells were stained with trypan blue, and the viable cells were counted by a Vi-CELL™ cell viability analyzer (Beckman Coulter Inc.). Cell viability was calculated by the following equation. Cell Viability (%) = (treated viable cells)/(non-treated control viable cells) x 100.