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SYNTHESIS OF *N*-1-SKATYL URACIL DERIVATIVES

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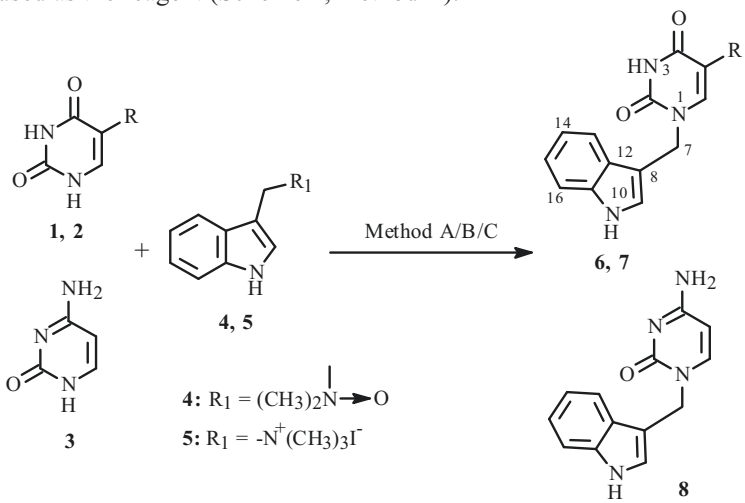
N-*1 skatyl derivatives were obtained in moderate yields via the reaction of uracil, 5-fluorouracyl, and cytosine with gramine* N-*oxide or its methyl iodide. Gramine* N-*oxide was converted into gramine in good yield upon microwave irradiation in DMF solution. Gramine was also formed in DMF solution in the presence of DBU from gramine methyl iodide although microwave irradiation did not affect the yield of gramine. Gramine did not form from its methyl iodide upon microwave irradiation without DBU.*

Keywords: uracil, fluorouracil, cytosine, gramine, gramine *N*-oxide, gramine methyl iodide, microwave irradiation.

Pyrimidine bases fulfill important functions in living systems. Several diseases develop if their metabolism is disrupted by various stressful situations. Obviously, many biologically active compounds derived from them act as antimetabolites because of their role in living systems [1–7].

Indoles are heterocyclic compounds that are broadly distributed in nature. The essential acid tryptophan occurs in the majority of proteins. Indole compounds are used in medicine because of their physiological activity [8]. Therefore, it seemed interesting to design hybrid drugs based on pyrimidine bases and an indole moiety.

A series of *N*-skatyl-5-substituted uracils were prepared by reacting gramine with the corresponding uracils in DMF at 160°C [9]. They included **6** and **7** in yields of 20 and 40%, respectively. The spectral data of the synthesized compounds were not reported. We synthesized derivatives **6**, **7**, and **8** by reacting uracil (**1**), fluorouracil (**2**), and cytosine (**3**) with gramine *N*-oxide (**4**) or its methyl iodide (**5**) in DMF at 130°C for 7 h. The reaction time was defined as the complete dissolution of the precipitate and the formation of a homogeneous solution. The yields of **6**, **7**, and **8** were 42, 55, and 30%, respectively, if gramine *N*-oxide (**4**) was used as the reagent (Scheme 1, method A).



1, 6: R = H; **2, 7:** R = F

A. **4** (1 eq.), DMF, 130°C, 7 h: **6** (42%), **7** (55%), **8** (30%); B. **5** (1 eq.), DMF, 130°C, 7 h: **6** (47%), **7** (59%), **8** (33%); C. **5** (1 eq.), DBU (0.37 eq.), DMF, 130°C, 7 h: **6** (60%), **7** (70%), **8** (46%)

Scheme 1

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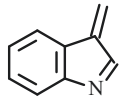
Alkylation of **1**–**3** by gramine methyl iodide (**5**) gave **6**–**8** in yields of 47, 59, and 33%, respectively (Scheme 1, method B). Doubling the amounts of **4** and **5** did not increase the yields of alkylation products.

The reaction mixture polymerized if the reactions in Scheme 1, methods A or B, were carried out at the solvent boiling point. Therefore, alkylation products were not isolated.

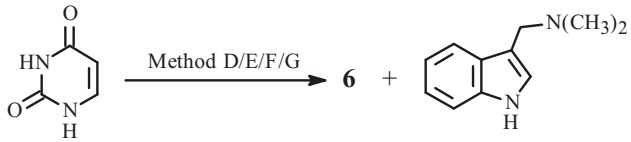
Alkylation of **1**, **2**, and **3** by **5** under analogous conditions in the presence of diazabicycloundecene (DBU) at a substrate–**5**–DBU ratio of 1:1:0.37 produced **6**, **7**, and **8** in yields of 60, 70, and 46%, respectively (Scheme 1, method C).

The increased yields of alkylation products **6**, **7**, and **8** if the reaction was performed with DBU confirmed the hypothesis [10] that the alkylation under certain conditions could pass through intermediate **5a**.



5a

Alkylation of uracil (**1**) by methyliodide (**5**) at 130°C in the presence of DBU in a **1**–**5**–DBU ratio of 1:1:1 reduced the yield of **6** to 40%. A second product (gramine, **9**) was obtained in 40% yield. Product **6** was not formed and **9** was isolated in 87% yield if the reaction was carried out at the solvent boiling point (~160°C) (Scheme 2, methods D and E, respectively).



1 **9**

D. **5** (1 eq.), DBU (1 eq.), DMF, 130°C, 7 h: **6** (40%) and **9** (40%); E. **5** (1 eq.), DBU (1 eq.), DMF, 160°C, 7 h: **9** (87%); F. **4** (1 eq.), DMF, 300 W, 160°C, 2 h: **6** (2%) and **9** (66%); G. **5** (1 eq.), DBU (0.37 eq.), DMF, 300 W, 160°C, 2 h: **6** (4%) and **9** (64%)

Scheme 2

An attempt to synthesize the *N*-skatyl derivative of uracil (**1**) using **4** and **5** with microwave irradiation (300 W) in DMF (~160°C) returned starting uracil **1** and a mixture of alkylation product **6** and gramine (**9**) (Scheme 2, methods F and G, respectively). A resinous product also formed although pure compounds could not be isolated from it.

Microwave irradiation (300 W) of gramine *N*-oxide (**4**) in DMF without **1** also gave **9** in 71% yield.

The formation of **9** from methyl iodide **5** may have been related to the presence of the strong base DBU in the reaction mixture and dealkylation of **5** to give **9** if enough of it was added to the reaction mixture. This process was enhanced if the temperature or amount of DBU was increased (Schemes 1 and 2) and did not depend on microwave irradiation. The formation of **9** via microwave irradiation of gramine *N*-oxide requires further research and may be related to traces of moisture in the reaction mixture.

The structures of the compounds were established using PMR and ^{13}C NMR spectroscopy and standard correlation methods H–H COSY, HSQC, HMBC, and NOESY. ^{13}C NMR spectra were recorded in DEPT-90 and DEPT-135 modes and with full proton suppression. Chemical shifts of methine, methylene, methyl, and quaternary C atoms were found. HSQC spectra gave chemical shifts of protons on the corresponding C atoms. Next, COSY and HMBC spectra confirmed couplings between protons and through-space coupling of protons with C atoms. HSQC and ^{15}N–^{1}H HMBC spectra were recorded for **6** and **8**.

Benzene proton resonances were easily assigned for **6** and included two doublets (H-13 and H-16) and two triplets (H-14 and H-15). These gave cross peaks for pairwise coupling in the COSY spectrum. Chemical shifts of C atoms were found from the HSQC spectrum. A singlet for H-9 that gave cross peaks in the HMBC spectrum with all β- and γ-C atoms allowed resonances in the ^{13}C NMR spectrum to be reliably assigned to C-8, C-12, C-11, and C-7. Proton H-9 in the ^{15}N–^{1}H HMBC spectrum gave cross peaks with N-10. The proton on this N atom (H-10) showed the corresponding couplings with C-9, C-11, and C-16 in the ^{15}N–^{1}H HMBC spectrum. In turn, the C-7 protons of doubled intensity gave cross peaks in the HMBC spectrum with C-8, C-9, C-12, C-2, and C-6 and with N-1 in the ^{15}N–^{1}H HMBC spectrum. Proton H-6 also coupled with this N atom and with H-5 according to the COSY spectrum. Proton H-5 gave a cross peak with N-3 in the ^{15}N–^{1}H HMBC spectrum. Its proton H-3 gave a cross peak with C-5 in the HMBC spectrum. The resonance of C-4 in the HMBC spectrum coupled with protons H-5 and H-6.

All H and C resonances of **7** and **8** were assigned in the same manner as for **6**.

Thus, it was shown that the yields of *N*-1-skatyl derivatives of uracil, fluorouracil, and cytosine were higher if gramine *N*-oxide and methyl iodide were used instead of gramine. Treatment of gramine *N*-oxide with microwave radiation produced gramine, which was also formed from gramine methyl iodide in the presence of DBU. Increasing the temperature enhanced this process.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded on a Bruker Avance-III (500 MHz) pulsed spectrometer at operating frequency 500.13 MHz for ^{1}H, 125.47 MHz for ^{13}C, and 50.58 MHz for ^{15}N using a 5-mm probe with PABBO *Z*-gradient at constant temperature (298 K). Chemical shifts in ^{13}C and PMR spectra were given in ppm with solvent resonances as internal standards. Chemical shifts in ^{15}N NMR spectra were obtained from F_1-projections in ^{1}H–^{15}N HMBC spectra with values given on the ammonia scale. ^{13}C NMR spectra with proton suppression were recorded with spectral window 29.8 kHz, 64k points, 3.2 μs exciting pulse (30°), 2 s relaxation delay, and 512–2048 scans. ^{13}C NMR spectra were adjusted based on DEPT-90 and DEPT-135 experiments. Two-dimensional spectra were recorded in standard multi-pulse sequences of the instrument software. The gsCOSY spectrum was recorded using a 4k matrix from 512 experiments and spectral window 5.0 kHz and was processed using a sinusoidal–bell-shaped weighting function for the F_1 and F_2 projections (ssb = 2). gsHSQC spectra were recorded with a delay optimized for J_{CH} = 145 Hz and J_{NH} = 80 Hz and matrix size 2 k from 256 experiments. gsHMBC spectra were recorded with delayed evolution of small constants 71.4 ms for ^{1}H–^{13}C and 142.8 ms for ^{1}H–^{15}N HMBC with a 2k matrix from 256 experiments. Elemental analysis was performed on a EURO-3000 instrument.

A modified multi-modal microwave system based on a SAM-OM75S-(31) magnetron was used for the reactions with microwave irradiation. The irradiation frequency was $2.45 \cdot 10^9$ Hz with maximum used power 300 W. Microwave radiation generated by the system was fed into the reaction zone (quartz reactor, 50-mL volume). The mechanical stirrer drive and reflux condenser were removed from the microwave irradiation zone.

1-(1*H*-Indol-3-ylmethyl)pyrimidine-2,4(1*H*,3*H*)-dione (6). Method A. Uracil (**1**, 0.20 g, 1.80 mmol) and gramine *N*-oxide (**4**, 0.34 g, 1.80 mmol) in DMF (2 mL) were stirred and refluxed at 130°C for 7 h. The homogeneous reaction mixture was cooled and poured into cold distilled H_2O (~20 mL). The resulting precipitate was filtered off, rinsed with distilled H_2O, and dried to afford **6** (0.18 g, 42%) as an amorphous compound.

Method B. Uracil (**1**, 0.20 g, 1.80 mmol) and gramine methyl iodide (**5**, 0.57 g, 1.80 mmol) gave **6** (0.20 g, 47%).

Method C. Uracil (**1**, 0.20 g, 1.80 mmol) and gramine methyl iodide (**5**, 0.57 g, 1.80 mmol) in the presence of DBU (0.10 mL, 0.67 mmol) gave **6** (0.26 g, 60%).

Method D. Uracil (**1**, 0.20 g, 1.80 mmol) and gramine methyl iodide (**5**, 0.57 g, 1.80 mmol) in the presence of DBU (0.27 mL, 1.80 mmol) gave **6** (0.17 g, 40%). The aqueous layer was extracted with $CHCl_3$ (3 × 15 mL). The combined organic fraction was washed with distilled H_2O (20 mL) and dried over Na_2SO_4. The solvent was evaporated to afford **9** (0.13 g, 40%). The spectral data agreed with those published [11].

Method E. Uracil (**1**, 0.20 g, 1.80 mmol) and gramine methyl iodide (**5**, 0.57 g, 1.80 mmol) in the presence of DBU (0.27 mL, 1.80 mmol) in DMF (2 mL) were stirred and refluxed at 160°C for 7 h. Subsequent extraction of the aqueous layer with $CHCl_3$ (3 × 15 mL) afforded **9** (0.27 g, 87%). The spectral data agreed with those published [11]. The aqueous layer was evaporated to dryness to afford **1** (0.20 g). The spectral data agreed with those published [12].

Method F. Uracil (**1**, 0.20 g, 1.80 mmol) and gramine *N*-oxide (**4**, 0.34 g, 1.80 mmol) in DMF (2 mL) were irradiated with microwaves at 300 W for 2 h. The reaction mixture boiled during the treatment. The homogeneous mixture was cooled and poured into cold distilled H_2O (~20 mL). The resulting precipitate was filtered off, rinsed with distilled H_2O, and dried to afford **6** (0.01 g, 2%). The aqueous layer was extracted with $CHCl_3$ (3 × 15 mL). The combined organic fraction was washed with distilled H_2O (20 mL) and dried over Na_2SO_4. The solvent was evaporated to afford **9** (0.20 g, 66%). The spectral data agreed with those published [11]. The aqueous layer was evaporated to dryness. The resulting precipitate was rinsed with MeOH to afford **1** (0.17 g). The spectral data agreed with those published [12]. An unidentified resinous product (0.18 g) was isolated from the MeOH.

Method G. Uracil (**1**, 0.20 g, 1.80 mmol) and gramine methyl iodide (**5**, 0.57 g, 1.80 mmol) in the presence of DBU (0.10 mL, 0.67 mmol) were irradiated with microwaves to produce **6** (0.02 g, 4%), **9** (0.20 g, 64%) (spectral data agreed with those published [11]), **1** (0.18 g) (spectral data agreed with those published [12]), and an unidentified resinous product (0.19 g).

^{1}H NMR spectrum (500.13 MHz, DMSO-d_6, δ, ppm, J/Hz): 5.05 (2H, s, H-7), 5.55 (1H, d, J = 7.8, H-5), 7.05 (1H, t, J = 7.6, H-14), 7.13 (1H, t, J = 7.6, H-15), 7.43 (1H, d, J = 7.6, H-16), 7.50 (1H, s, H-9), 7.67 (1H, d, J = 7.6, H-13), 7.69 (1H, d, J = 7.8, H-6), 11.20 (1H, s, N-10), 11.50 (1H, s, N-3). ^{13}C NMR spectrum (125.47 MHz, DMSO-d_6, δ, ppm): 42.07 (t, C-7), 101.29 (d, C-5), 109.80 (s, C-8), 111.92 (d, C-16), 118.66 (d, C-13), 119.35 (d, C-14), 121.77 (d, C-15), 126.00 (d, C-9), 126.23 (s, C-12), 136.52 (s, C-11), 145.05 (d, C-6), 151.28 (s, C-2), 163.89 (s, C-4). ^{15}N NMR spectrum (50.58 MHz, DMSO-d_6): 132.95 (N-10), 140.78 (N-1), 157.65 (N-3). $C_{13}H_{11}N_3O_2$.

1-(1*H*-Indol-3-ylmethyl)-5-fluoropyrimidine-2,4(1*H*,3*H*)-dione (7). Method A. 5-Fluorouracil (**2**, 0.20 g, 1.50 mmol) and gramine *N*-oxide (**4**, 0.29 g, 1.50 mmol) produced **7** (0.22 g, 55%) as an amorphous compound.

Method B. 5-Fluorouracil (**2**, 0.20 g, 1.50 mmol) and gramine methyl iodide (**5**, 0.47 g, 1.50 mmol) gave **7** (0.23 g, 59%).

Method C. 5-Fluorouracil (**2**, 0.20 g, 1.50 mmol) and gramine methyl iodide (**5**, 0.47 g, 1.50 mmol) in the presence of DBU (0.08 mL, 0.56 mmol) gave **7** (0.28 g, 70%). ^{1}H NMR spectrum (500.13 MHz, DMSO-d_6, δ, ppm, J/Hz): 5.05 (2H, s, H-7), 7.05 (1H, t, J = 7.8, H-14), 7.11 (1H, t, J = 7.8, H-15), 7.40 (1H, d, J = 7.8, H-16), 7.59 (1H, s, H-9), 7.63 (1H, d, J = 7.8, H-13), 7.95 (1H, d, J = 6.0, H-6), 11.20 (1H, s, N-10), 11.50 (1H, s, N-3). ^{13}C NMR spectrum (125.47 MHz, DMSO-d_6, δ, ppm): 42.34 (t, C-7), 109.34 (s, C-8), 111.74 (d, C-16), 118.54 (d, C-13), 119.19 (d, C-14), 121.59 (d, C-15), 126.03 (d, C-9), 126.50 (s, C-12), 128.96, 129.21 (d, J = 32.3, C-6), 136.36 (s, C-11), 138.18, 140.00 (d, J = 228, C-5), 149.65 (s, C-2), 157.34, 157.54 (d, J = 26.3, C-4). $C_{13}H_{10}FN_3O_2$.

4-Amino-1-(1*H*-indol-3-ylmethyl)pyrimidin-2(1*H*)-one (8). Method A. Cytosine (**3**, 0.20 g, 1.80 mmol) and gramine *N*-oxide (**4**, 0.34 g, 1.80 mmol) gave **8** (0.13 g, 30%) as an amorphous compound.

Method B. Cytosine (**3**, 0.20 g, 1.80 mmol) and gramine methyl iodide (**5**, 0.57 g, 1.80 mmol) gave **8** (0.14 g, 33%).

Method C. Cytosine (**3**, 0.20 g, 1.80 mmol) and gramine methyl iodide (**5**, 0.57 g, 1.80 mmol) in the presence of DBU (0.10 mL, 0.67 mmol) gave **8** (0.20 g, 46%). ^{1}H NMR spectrum (500.13 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.73 (2H, s, NH_2), 5.05 (2H, s, H-7), 5.62 (1H, d, J = 7.2, H-5), 7.00 (1H, t, J = 7.8, H-14), 7.10 (1H, t, J = 7.8, H-15), 7.38 (1H, d, J = 7.8, H-16), 7.41 (1H, s, H-9), 7.58 (1H, d, J = 7.2, H-6), 7.68 (1H, d, J = 7.8, H-13), 11.20 (1H, s, N-10). ^{13}C NMR spectrum (125.47 MHz, DMSO-d_6, δ, ppm, J/Hz): 42.90 (t, C-7), 93.77 (d, C-5), 110.75 (s, C-8), 111.76 (d, C-16), 118.50 (d, C-14), 118.90 (d, C-13), 121.30 (d, C-15), 125.65 (d, C-9), 126.37 (s, C-12), 136.46 (s, C-11), 145.38 (d, C-6), 156.33 (s, C-2), 165.81 (s, C-4). ^{15}N NMR spectrum (50.58 MHz, DMSO-d_6, δ, ppm): 104.80 (NH_2), 132.68 (N-10), 150.80 (N-1), 177.20 (N-13). $C_{13}H_{12}N_4O$.

Microwave Irradiation of Gramine *N*-oxide (4). Gramine *N*-oxide (**4**, 0.20 g, 1.00 mmol) in DMF (2 mL) was irradiated with microwaves (300 W) for 2 h. The reaction mixture boiled during the treatment. The homogeneous mixture was cooled and poured into cold distilled H_2O (~20 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 15 mL). The combined organic fraction was washed with distilled H_2O (20 mL) and dried over Na_2SO_4. The solvent was evaporated to afford **9** (0.13 g, 71%). The spectral data agreed with those published [11].

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