UDC 547.945

DOI: 10.15372/CSD2020214

Salsolidine and 1-Aryltetrahydroisoquinolines in the Aminomethylation of 6-Methyluracil and Their Biological Activity

SH. N. ZHURAKULOV, E. O. TERENTEVA, U. B. KHAMIDOVA, M. G. LEVKOVICH, Z. S. KHASHIMOVA, SH. S. AZIMOVA, V. I. VINOGRADOVA

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan

E-mail: j.sherzod.78@mail.ru

(Received July 19, 2019; revised November 28, 2019)

Abstract

Mannich reaction of 6-methyluracil with salsolidine and substituted 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines was studied. The use of equimolecular amounts of reagents led to the production of new conjugates – 5-(1-(aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dions with 61–84 % yields. When studying the effect of these conjugates on the growth and viability of tumour cell lines (HeLa, HEp-2), it was found that the formation of tetrahydroisoquinoline derivatives with 6-methyluracil molecule does not contribute to the manifestation of cytotoxic effect.

Keywords: 6-methyluracil, 1-aryltetrahydroisoquinolines, salsolidine, conjugates, cytotoxicity

INTRODUCTION

Uracil derivatives are efficient medicinal agents widely used in medicine as preparations. For example, the potassium salt of uracil-6-carboxylic acid serves as a metabolic stimulator; methyluracil is applied to treat diseases caused by thyroid gland disturbance; fluorouracil is a cytostatic agent used to treat a number of malignant tumours [1, 2].

The derivatives of pyrimidin combine several kinds of biological activity: immunostimulating, anti-inflammatory, antiviral, antifungal, antitumour and many others [3].

Not less promising in this area are isoquinoline alkaloids exhibiting cytotoxic properties [4, 5] and possessing low toxicity to healthy cells and tissues of the organism [6, 7].

No compounds containing the fragments of 6-methyluracil and isoquinoline were found in nature, so the development of new synthetic derivatives containing principally different pharmacophore centres retains substantial theoretical and practical importance.

As a continuation of the work aimed at search for potential biologically active compounds and at the revelation of their cytotoxic properties [4, 5, 8], we synthesized conjugates containing tetrahydroisoquinoline and uracil fragments.

EXPERIMENTAL

Methods of investigation

Mass spectra were recorded with a chromatograph – mass spectrometer within a high-performance liquid chromatograph Agilent 1200 Infinity (Agilent Technologies, USA) and a mass detector 6420 Triple Quad LC/MS (Agilent Technologies, USA) (ionization by electrospraying +ESI TIC Scan).

IR spectra were recorded with the help of a FTIR System 2000 spectrometer (PerkinElmer, USA) in tablets with KBr.

NMR ^{1}H spectra were recorded using a Unity-400 spectrometer (Varian, USA, solvents: $CDCl_3$, DMSO-d_6, internal standard: HMDS). The assignment of NMR ^{13}C spectra was carried out through structure modeling in MestReNova software. The mobility values (R_f) were determined by means of thin layer chromatography with plates coated with silica gel LS 5/40, using solvent system $CHCl_3$/MeOH = 4 : 1 (I), 6 : 1 (II). Melting points of all synthesized substances were determined with a BOETIUS microtable.

Salsolidine alkaloid was extracted from *Salsola richteri* Karel plant, 1-aryltetrahydroisoquinolines were obtained according to the procedure described in [9, 10].

Procedures for the synthesis and characterization of compounds

Synthesis of 6-methyluracil (2) [11]. The reaction mixture was prepared in a porcelain bowl by mixing 240 mL of acetoacetic ester, 100 g of carbamide, 160 mL of ethanol and 5 mL of concentrated (34 %) hydrochloric acid. The mixture was heated to 30 °C, then cooled and placed in vacuum in a desiccator above H_2SO_4 (93 %). After keeping for 3 days with periodic mixing, the mixture was transferred into a conical flask, a solution of 100 g KOH in 1200 mL of water was added, and the mixture was heated on a heating ring up to 90 °C to achieve dissolution. After dissolution, the mixture was immediately cooled to 50 °C, and 34 % hydrochloric acid was added to pH 6. The mixture was left overnight. The precipitate was separated, washed with water, then with a 2 % solution of acetic acid, water, and ethanol. The product was obtained in the amount of 43.31 g (21 %), its melting point was 318–321 °C.

Synthesis of 6-methyl-5-tetrahydroisoquinolino-methyluracil derivatives (3a–h). General procedure. Tetrahydroisoquinoline in the amount of 5.18 mmol was added to a boiling solution of 5.16 mmol of 6-methyluracil in 30 mL of ethanol, and then 30 % formalin solution (d = 1.092) 0.65 mL (5.95 mmol) was added in portions. The reaction mixture was boiled for 12–18 h. After the completion of the reaction, more than a half of the solvent was distilled, and the formed precipitate was separated.

5-((6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3a). $C_{17}H_{21}N_3O_4$. Synthesized from 0.65 g (5.16 mmol) of 6-methyluracil (**2**), 1.0 g (5.18 mmol) of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1a**) and 0.65 mL of formalin. Yield: 1.26 r (73 %), m.p. 214–216 °C (C_2H_5OH), R_f 0.33 (system **II**).

Mass spectrum (+ESI TIC Scan) m/z: 332 $[M+H]^+$, 194 $[M-138]^+$, 141 $[M-191]^+$.

NMR ^{1}H spectrum (400 MHz, CF_3COOD + + CD_3COOD), δ, ppm (J, Hz): 1.98 (3H, s, CH_3-11), 2.77 (1H, dt, J = 5.4, 18.0, H_a-4), 2.89 (1H, dt, J = 6.5, 17.7, H_b-4), 3.22 (1H, m, H_a-3), 3.46 (3H, s, 7-OCH_3), 3.49 (3H, s, 6-OCH_3), 3.50 (1H, signal overlapped, H_b-3), 3.93 (1H, td, J = 2.9, 14.9, H_a-9), 3.99 (2H, br. s, J = 14.9, H-1), 4.20 (1H, d, J = 15.0, H_b-9), 6.28 (1H, s, H-8), 6.42 (1H, s, H-5).

5-((1-Methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3b). $C_{18}H_{23}N_3O_4$. Synthesized from 0.30 g (2.41 mmol) of 6-methyluracil (**2**), 0.50 r (2.41 mmol) of 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1b**) and 0.22 mL of formalin. Yield: 0.69 g (82 %), m.p. 179–181 °C (ethylacetate), R_f 0.2 (system **I**).

Mass spectrum (+ESI TIC Scan) m/z: 346 $[M+H]^+$, 208 $[M-138]^+$, 141 $[M-205]^+$.

NMR ^{1}H spectrum (400 MHz, CF_3COOD + + CD_3COOD), δ, ppm (J, Hz): 1.36 (3H, d, J = 6.2, CH_3-1), 2.06 (3H, s, CH_3-11), 2.80 (2H, m, H-4), 3.27 (2H, m, H-3), 3.49 (3H, s, 7-OCH_3), 3.51 (3H, s, 6-OCH_3), 3.91 (2H, br. s, H-9), 4.16 (1H, m, H-1), 6.30 (1H, s, H-8), 6.45 (1H, s H-5).

5-(1-(3-Hydroxy-4-methoxyphenyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3c). $C_{24}H_{27}N_3O_6$. Synthesized from 0.40 g (3.17 mmol) of 6-methyluracil (**2**), 1.0 g (3.17 mmol) of 1-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1c**) and 0.29 mL of formalin. Yield: 1.21 g (84 %), m.p. 171–173 °C (C_2H_5OH), R_f 0.41 (system **I**).

Mass spectrum (+ESI TIC Scan) m/z: 454 $[M+H]^+$, 316 $[M-138]^+$, 141 $[M-313]^+$.

IR spectrum (KBr, ν_{max}, cm^{-1}): 3554, 3477, 3413 (NH), 3239, 2935 (CH_2), 1728, 1638 (C=O), 1618 (C=N), 1512 (C=C), 1444 (C–H), 1353 (C–N), 1250, 1217 (C–O), 1120, 1027, 989, 813, 626.

NMR ^{1}H spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.91 (3H, s, CH_3-11), 2.44 (1H, td, J = 3.3, 10.9, H_a-4), 2.65 (1H, dt, J = 16.1, 3.5, H_a-3), 2.82 (1H, dt, J = 10.9, 4.9, H_b-4), 2.92 (1H, m, H_b-3), 3.20 (1H, d, J = 13.1, H_a-9), 3.24 (1H, d, J = 13.0, H_b-9), 3.53 (3H, s, 7-OCH_3), 3.77 (3H, s, 6-OCH_3), 3.81 (3H, s, 4′-OCH_3), 4.25 (1H, s, H-1), 6.11 (1H, s,

H-8), 6.51 (1H, s, H-5), 6.68 (1H, dd, J = 1.8, 8.3, H-6′), 6.71 (1H, d, J = 1.8, H-2′), 6.72 (1H, d, J = 8.3, H-5′).

5-(1-(3,4-Dimethoxyphenyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3d). $C_{25}H_{29}N_3O_6$. Synthesized from 0.38 g (3.01 mmol) of 6-methyluracil (**2**), 1.0 g (3.03 mmol) of 1-(3,4-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1d**) and 0.28 mL of formalin. Yield: 1.17 g (82 %), m.p. 174–176 °C (C_2H_5OH), R_f 0.3 (system **I**).

Mass spectrum (+ESI TIC Scan) m/z: 468 $[M+H]^+$, 328 $[M-140]^+$, 141 $[M-327]^+$.

NMR ^1H spectrum (400 MHz, CF_3COOD + + CD_3COOD), δ, ppm (J, Hz): 1.85 (3H, s, CH_3-11), 2.90 (2H, m, H-4), 3.15 (1H, m, H_a-3), 3.33 (3H, s, 7-OCH_3), 3.43 (3H, s, 6-OCH_3), 3.45 (1H, m, H_b-3), 3.53 (3H, s, 3′-OCH_3), 3.55 (3H, s, 4′-OCH_3), 3.92 (1H, m, H_a-9), 4.04 (1H, m, H_b-9), 5.21 (1H, s, H-1), 6.09 (1H, s, H-8), 6.44 (2H, d, J = 2.1, H-2′), 6.48 (1H, br. d. J = 8.5, H-6′), 6.51 (1H, s, H-5), 6.64 (1H, d, J = 8.3, H-5′).

5-(1-(3,4-Methylenedioxyphenyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3e). $C_{24}H_{25}N_3O_6$. Synthesized from 0.40 g (3.17 mmol) of 6-methyluracil (**2**), 1.0 g (3.19 mmol) of 1-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1e**) and 0.23 mL of formalin. Yield: 0.88 g (63 %), m.p. 165–167 °C (C_2H_5OH), R_f 0.34 (system **I**).

Mass spectrum (+ESI TIC Scan) m/z: 452 $[M+H]^+$, 314 $[M-138]^+$, 141 $[M-311]^+$.

IR spectrum (KBr, ν_{max}, cm^{-1}): 3411 (NH), 2936, 2831 (CH_2), 1719, 1638 (C=O), 1626 (C=N), 1513 (C=C), 1484, 1440 (C–H), 1383, 1335 (C–N), 1239, 1216 (C–O), 1226, 1036, 929, 863, 818, 774, 619, 468.

NMR ^1H spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.97 (3H, s, CH_3-11), 2.43 (1H, td, J = 11.5, 4.2, H_a-4), 2.62 (1H, br. d, J = 15.9, H_a-3), 2.83 (1H, m, H_b-4), 2.89 (1H, m, H_b-3), 3.20 (1H, d, J = 12.9, H_a-9), 3.29 (1H, d, J = 12.9, H_b-9), 3.56 (3H, s, 7-OCH_3), 3.77 (3H, s, 6-OCH_3), 4.28 (1H, s, H-1), 5.84, 5.86 (each 1H, d, J = 1.4, 3′-OCH_2O-4′), 6.10 (1H, s, H-8), 6.50 (1H, s, H-5), 6.64 (1H, d, J = 1.5, H-2′), 6.66 (1H, d, J = 7.8, H-5′), 6.71 (1H, dd, J = 1.5, 7.9, H-6′), 9.33 (NH-12), 10.14 (NH-14).

NMR ^{13}C spectrum (100 MHz, $CDCl_3$), δ, ppm (J, Hz): 23.84 (C-16), 47.01 (C-4), 47.16 (C-3), 52.32 (C-9), 56.69 (6-OCH_3), 56.76 (7-OCH_3), 69.34 (C-1), 103.68 (C-7′), 104.74 (C-10), 110.53 (C-2′), 110.63 (C-5′), 112.56 (C-8), 112.72 (C-5), 121.99 (C-6′), 124.93 (C-4a), 126.10 (C-8a), 129.83 (C-1′), 147.90 (C-7), 150.01 (C-6), 150.67 (C-3′), 151.18 (C-4′), 151.54 (C-11), 154.43 (C-13), 167.91 (C-15).

5-(1-(3,4-Methylenedioxyphenyl-6,7-Methylenedioxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3f). $C_{23}H_{21}N_3O_6$. Synthesized from 0.30 g (2.38 mmol) of 6-methyluracil (**2**), 0.71 g (2.39 mmol) of 1-(3,4-methylenedioxyphenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (**1f**) and 0.22 mL of formalin. Yield: 0.69 g (67 %), m.p. >360 °C (C_2H_5OH), R_f 0.15 (system **I**).

Mass spectrum (+ESI TIC Scan) m/z: 436 $[M+H]^+$, 298 $[M-138]^+$, 141 $[M-295]^+$.

IR spectrum (KBr, ν_{max}, cm^{-1}): 3555, 3478, 3416 (NH), 3226, 2935 (CH_2), 1713, 1638 (C=O), 1618 (C=N), 1504 (C=C), 1486, 1454, 1423 (C–H), 1379, 1339 (C–N), 1298, 1233 (C–O), 1159, 1094, 1036, 873, 815, 623.

NMR ^1H spectrum (400 MHz, CF_3COOD + + CD_3COOD), δ, ppm (J, Hz): 1.85 (3H, s, CH_3-11), 2.85 (2H, m, H-4), 3.14 (1H, m, H_a-3), 3.44 (1H, m, H_b-3), 3.99 (2H, m, H-9), 5.09 (1H, s, H-1), 5.56 (4H, m, 6,7-OCH_2O-3′,4′), 5.97 (1H, s, H-8), 6.24 (1H, d, J = 1.6, H-2′), 6.35 (1H, dd, J = 8.0, 1.6, H-6′), 6.38 (1H, s, H-5), 6.45 (1H, d, J = 8.0, H-5′).

NMR ^{13}C spectrum (100 MHz, CF_3COOD + CD_3COOD), δ, ppm (J, Hz): 17.63 (C-16), 24.48 (C-4), 47.41 (C-3), 51.14 (C-9), 69.20 (C-1), 102.11 (C-7'), 103.44 (C-6a), 103.72 (C-10), 109.49 (C-2′), 109.84 (C-5′), 110.49 (C-8), 110.69 (C-5), 122.44 (C-6′), 125.49 (C-4a), 126.24 (C-8a), 129.76 (C-1′), 149.62 (C-7), 150.73 (C-6), 150.81 (C-3′), 151.59 (C-4′), 153.93 (C-11), 159.80 (C-13), 168.21 (C-15).

5-(1-(4-Nitrophenyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3g). $C_{23}H_{24}N_4O_6$. Synthesized from 0.40 g (3.17 mmol) of 6-methyluracil (**2**), 1.0 g (3.18 mmol) of 1-(4-nitrophenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (**1g**) and 0.29 mL of formalin. Yield: 0.98 g (68 %), m.p. 212–214 °C (C_2H_5OH), R_f 0.37 (system **I**).

Mass spectrum (+ESI TIC Scan) m/z: 453 $[M+H]^+$, 298 $[M-138]^+$, 141 $[M-312]^+$.

IR spectrum (KBr, ν_{max}, cm^{-1}): 3553, 3409, 3478 (NH), 3236, 3002, 2946 (CH_2), 1713, 1638 (C=O), 1617 (C=N), 1518 (C=C), 1446 (C–H), 1348 (C–N), 1250, 1220 (C–O), 1124, 1011, 980, 873, 829, 777, 620, 523, 484.

NMR ^1H spectrum (400 MHz, CD_3COOD), δ, ppm (J, Hz): 1.85 (3H, s, CH_3-11), 2.96 (2H, m, H-4), 3.25 (1H, m, H_a-3), 3.32 (3H, s, 7-OCH_3), 3.35 (1H, m, H_b-3), 3.57 (3H, s, 6-OCH_3), 4.06 (2H, m, H-9), 5.43 (1H, s, H-1), 6.05 (1H, s, H-8), 6.57 (1H,

s, H-5), 7.11 (2H, d, J = 8.2, H-2′,6′), 7.93 (1H, d, J = 8.2, H-3′,5′).

NMR ^{13}C spectrum (100 MHz, CF_3COOH + + CD_3COOD), δ, ppm (J, Hz): 17.67 (C-16), 23.72 (C-4), 47.44 (C-3), 51.60 (C-9), 56.90 (6-OCH_3), 56.96 (7-OCH_3), 67.26 (C-1), 101.69 (C-10), 112.43 (C-8), 113.06 (C-5), 120.30 (C-3′), 125.13 (C-5′), 126.41 (C-4a), 132.74 (C-8a), 143.12 (C-2′), 150.58 (C-6′), 150.83 (C-1′), 150.58 (C-7), 150.83 (C-6), 151.51 (C-4′), 153.88 (C-11), 160.28 (C-13), 168.38 (C-15).

5-(1-(3-Nitrophenyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidin-2,4(1*H*,3*H*)-dion (3h). $C_{23}H_{24}N_4O_6$. Synthesized from 0.40 g (3.17 mmol) of 6-methyluracil (**2**), 1.0 g (3.18 mmol) of 1-(3-nitrophenyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (**1h**) and 0.30 mL of formalin. Yield: 1.07 g (74 %), m.p. >360 °C (C_2H_5OH), R_f 0.39 (system **I**).

Mass spectrum (+ESI TIC Scan) m/z: 453 $[M+H]^+$, 298 $[M-138]^+$, 141 $[M-312]^+$.

IR spectrum (KBr, ν_{max}, cm^{-1}): 3554, 3419, 3467 (NH), 3235, 2929, 2823 (CH_2), 1709, 1637 (C=O), 1618 (C=N), 1526 (C=C), 1454 (C−H), 1349 (C−N), 1249, 1222 (C−O), 1126, 1007, 981, 861, 829, 735, 624.

NMR ^{1}H spectrum (400 MHz, CD_3COOD), δ, ppm (J, Hz): 1.86 (3H, s, CH_3-11), 2.98 (2H, m, H-4), 3.26 (1H, m, H_a-3), 3.32 (3H, s, 7-OCH_3), 3.40 (1H, m, H_b-3), 3.57 (3H, s, 6-OCH_3), 4.07 (2H, m, H-9), 5.48 (1H, s, H-1), 6.08 (1H, s, H-8), 7.26 (2H, dt, J = 7.8, 7.8, H-4′, 5), 7.81 (1H, s, H-5′), 7.97 (2H, d, J = 8.0, H-2′,6′).

NMR ^{13}C (100 MHz, CF_3COOH + CD_3COOD), δ, ppm (J, Hz): 17.84 (C-16), 23.79 (C-4), 47.56 (C-3), 51.55 (C-9), 56.87 (6-OCH_3), 57.02 (7-OCH_3), 67.41 (C-1), 101.88 (C-10), 112.52 (C-8), 113.08 (C-5), 125.30 (C-4′), 126.44 (C-2′), 127.44 (C-4a), 132.81 (C-8a), 137.95 (C-5′), 138.40 (C-6′), 150.29 (C-1′), 150.51 (C-7), 151.83 (C-6), 153.85 (C-3′), 153.85 (C-11), 160.28 (C-13), 168.38 (C-15).

2,3-Dimethoxy-5,6,8,9-tetrahydro-13b*H*-dibenzo[*a*,*h*]quinolyzidin-9-chroman (6). $C_{18}H_{19}NO_3$. Synthesized from 0.44 g (3.49 mmol) of 6-methyluracil (**2**), 1.0 g (3.49 mmol) of 1-(2′-hydroxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1i**) and 0.32 mL (3.50 mmol) of formalin. Yield: 0.85 g (82 %), m.p. 201−203 °C (C_2H_5OH), R_f 0.54 (system **II**).

Mass spectrum (+ESI) m/z: 298 $[M+H]^+$.

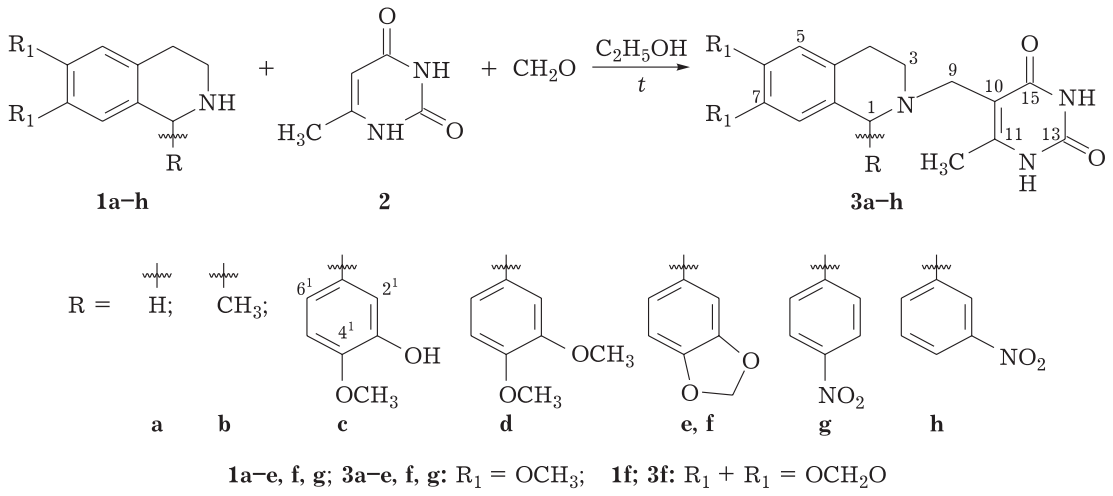
NMR ^{1}H spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 2.62 (1H, m, H_e-5), 3.00 (2H, m, H-6), 3.13 (1H, m, H_a-5), 3.80 (3H, s, 2-OCH_3), 3.88 (3H, s, 3-OCH_3), 4.89, 5.26 (each 1H, d, J = 11.0, H-8), 5.27 (1H, s, H-13b), 6.59 (1H, s, H-1), 6.64 (2H, m, H-10,11), 6.74 (1H, s, H-4), 7.03 (2H, m, H-12,13).

Cytotoxic properties of the compounds were determined *in vitro* with the help of MTT method in 96-well plates [12]. Cells of HeLa and HEp-2 lines (ATCC:CCL-23; Institute of Cytology, RAS, RF) were cultivated in the RPMI-1640 and DMEM/12F media (Himedia, India), containing 10 % fetal bovine serum, glutamine 2 mmol/L (Himedia, India) and antibiotics (penicillin, streptomycin) for 24 h. Then the compounds under investigation were applied in the concentrations of 10 and 1 μg/mL, after preliminary dissolution in DMSO (not more than 0.8 % of the volume of the nutrient medium) and left in a CO_2-incubator (SHELLAB, USA) for 24 h. After incubation, MTT reagent was added in the samples and the optical density was determined at 620 nm with the subtraction of the measured background absorption with the help of the plate analyzer 2300 EnSpire® Multimode Plate Reader (PerkinElmer, USA). The data were obtained in three independent experiments and expressed as an average value over four measurements for each concentration ± the standard error of the mean with respect to the reference values (cells without the introduction of the substances under test). The effect was compared with that from Cysplatin-Naprod cytostatic (India) containing cysplatin as the active component.

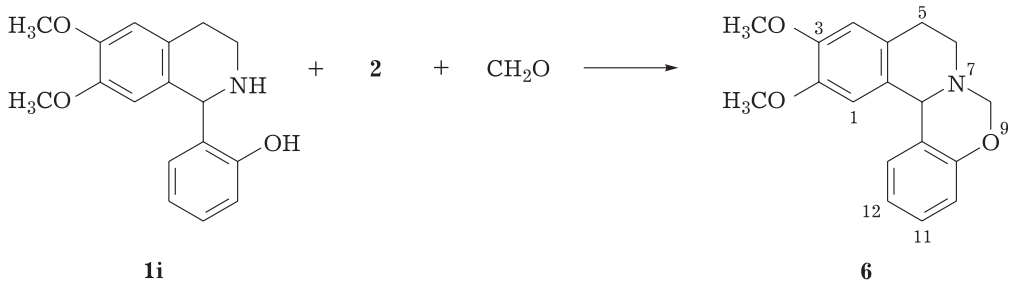
RESULTS AND DISCUSSION

Previously we demonstrated that the amination of dihydroquercetin with 1-aryltetrahydroisoquinoline compounds (**1a−h**) occurs at 20−25 °C in isopropanol [13, 14]. Continuing tetrahydroisoquinoline modification, in the present work we studied Mannich reaction between 6-methyluracil (**2**) and a series of isoquinolines: salsolidine (**1b**) and substituted tetrahydroisoquinoline compounds (**1a, 1c−h**) in ethanol, because 6-methyluracil is insoluble in isopropanol. The use of equimolar amounts of reagents for boiling in ethanol has led to the formation of 6-methyl-5-tetrahydroisoquinolinouracil compounds **3a−h** with the yield of 61−84 % (Scheme 1). The use of a two-fold excess of formaldehyde and isoquinoline promoted the formation of an additional amount of N-hydroxymethyl derivatives in addition to the target product, which brought complications into purification and caused a decrease in the yield of products **3a−h**.

The structures of the synthesized substances were confirmed by spectral data. The IR spectra



Scheme 1. Synthesis of 6-methyl-5-tetrahydroisoquinolineuracil compounds.



Scheme 2. Synthesis of 2,3-dimethoxy-5,6,8,9-tetrahydro-13b*H*-dibenzo[*a*,*h*]quinolisidin-9-chroman.

of conjugates **3a–h** contain intense absorption bands related to NH (3411–3555 cm^{-1}) and carbonyl groups (1728–1637 cm^{-1}) of 6-methyluracil fragment. NMR ^1H spectra contain the signals from methyl protons CH_3-11 in the region of 1.85–2.06 ppm and the protons of bridging CH_2 group resonating at 3.20–4.07 ppm in the form of doublets or multiplets, while the signals from H-5 of 6-methyluracil are absent. The protons of methoxy groups and aromatic protons H-5 and H-8 of the tetrahydroisoquinoline part of the molecule manifest themselves as singlets at 3.32–3.77, 6.38–6.57 and 5.97–6.30 ppm, respectively.

It is known [4, 15] that halogen-containing 1-aryltetrahydroisoquinolines exhibit a pronounced inhibiting effect against tumour cells of larynx cancer and cervical carcinoma. For instance, 1-(2′-chloro-4′,5′-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**4**) in the concentration of 10 μg/mL caused the death of 56–85 % of cells (Table 1). Less active agent against cancer cells turned out to be 1-(2′-bromo-4′,5′-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**5**): cell death in the presence of this compound did not exceed 43.9 % in comparison with the reference. Under the same conditions, reference preparation Cysplatin exhibited cytotoxicity only at a level of 50–70 % as average in comparison with the control value.

Because of this, it appeared very interesting to obtain the conjugates of 6-methyluracil with compounds **4** and **5**. However, 6-methyluracil did not enter Mannich reaction with halogenated tetrahydroisoquinolines **4** and **5**. Under the above-described conditions, mainly N-oxymethyl derivatives of tetrahydroisoquinolines are formed.

It should be stressed that 1-(2′-hydroxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1i**) containing an *ortho*-hydroxyl group in the C ring also did not enter Mannich reaction with 6-methyluracil; the latter was returned from the reaction mixture without changes. The product of this reaction with a 86 % yield is 2,3-dimethoxy-5,6,8,9-tetrahydro-13b*H*-dibenzo[*a*,*h*]quinolisidin-9-chroman (**6**) (scheme 2) [13].

The structure of compound **6** was proven on the basis of the data obtained by means of mass spectrometry and ^1H NMR spectroscopy.

TABLE 1

Cytotoxic effect of the synthesized compounds

Compound	Suppression of cell growth, % ($M\pm m$, n = 12)			
	HeLa	HeLa	HEp-2	HEp-2
	Concentration of the compound, mg/mL			
	10	1	10	1
1a*	13.5±2.6	10.0±1.4	**42.5±0.8**	6.9±3.4
3a	23.5±2.3	7.3±0.8	14.1±2.1	6.3±0.9
1b*	8.5±2.4	2.6±0.6	10.0±4.3	7.9±2.8
3b	8.1±1.0	3.7±0.6	14.2±2.5	3.7±0.5
1c	11.8±3.4	0.0±0.0	16.8±3.5	11.3±2.4
3c	16.4±2.8	7.3±0.8	11.7±2.3	9.3±3.6
1d*	18.4±1.3	0.0±0.5	13.5±1.7	7.7±1.1
3d	12.4±2.0	4.6±1.2	17.2±2.5	7.5±2.4
1e*	26.5±1.6	22.8±2.5	37.4±0.7	37.1±2.5
3e	34.2±5.1	7.9±2.0	11.3±3.4	2.1±0.6
1f*	25.9±0.8	15.3±0.5	9.7±0.2	1.2±0.0
3f	7.8±0.5	1.4±0.0	16.7±1.6	6.0±0.4
1g	22.6±3.6	6.5±2.2	16.5±0.8	13.0±3.0
3g	22.7±3.1	5.3±2.3	25.2±1.6	15.7±1.5
1h	21.4±0.9	10.3±3.0	7.7±1.5	8.0±1.1
3h	23.3±2.6	11.8±4.0	17.1±3.6	9.1±0.0
4*	**43.9±0.6**	**41.6±1.1**	37.0±1.7	33.6±2.0
5*	**85.0±2.7**	**46.1±0.8**	**56.2±0.9**	**52.4±2.2**
Cysplatin	70.0±1.4	51.9±1.7	51.1±1.6	20.8±2.1
Reference	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Note. Percentage of cell growth suppression corresponding to the compounds with pronounced cytotoxicity is marked with bold; M is the mean value, m is statistical error, n is the number of experiments.

* Previously published results [5].

The ability of compounds to affect the growth and vitality of cells was evaluated with the lines of epithelial cervical carcinoma HeLa and larynx adenocarcinoma HEp-2 after incubation for 24 hours (the time within which a cell passes the complete cycle of cell division). The results of the investigation are presented in Table 1.

One can see that the introduction of 6-methyluracil fragment into the structure of isoquinoline derivatives did not cause the enhancement of cytotoxic properties of the compounds.

CONCLUSION

Mannich reaction of 6-methyluracil with salsolidine and substituted 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline compounds was studied. New conjugates were obtained: 5-(1-(aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl)-2-methyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dions with the yield of 63-84 %. The presence of methylenedioxy and nitro groups in the phenyl ring of tetrahydroisoquinoline causes a decrease in the yield of uracil derivatives. Investigation of their cytotoxic properties with respect to tumour cell lines (HeLa, HEp-2) showed that the conjugation of tetrahydroisoquinolines with 6-methyluracil molecule does not promote an increase in cytotoxic effect.

Acknowledgements

The work was carried out with the financial support from the Ministry of Innovative Development at the Cabinet of Ministers of the Republic of Uzbekistan (projects MRU-FA-21/2017, VA-FA-F-6-009).

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