Study of the Biological Activity of Alkyl Derivatives of Tetrahydroisoquinolines

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

A row of 1-alkyne (C6-C17) derivatives against tetrahydroisoquinoline have been synthesized. 1-alkyne (C6-C17) derivatives cytotoxicity against three lines of cancer cells and two lines of normal cells was studied. It was found that 1-tridecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline exhibits a high cytotoxic effect with low toxicity to healthy cells. Antimicrobial activity of 1-alkyne (C6-C17) derivatives against 5 strains of bacteria and fungus was studied. It has been found that 1-nonyl-6,7-dimethoxy-1,3,4-tetrahydroisoquinoline exhibits strong antibacterial and antifungal effects.

Keywords: Tetrahydroisoquinoline derivatives; cytotoxicity; cancer and normal cell lines; antibacterial and antifungal activity.
1. INTRODUCTION

The chemistry of nitrogen-containing heterocycles occupies one of the leading positions in pharmacology due to the presence of many substances that can be active and potential drugs. In particular, derivatives of tetrahydroisoquinolines are of interest in this point of view because they contain several reaction centers, which makes them very promising as new pharmacological drugs. So, isoquinolines have a wide spectrum of biological properties [1-4], including both antitumor and antimicrobial effects [5-7].

The literature provides information about the synthesis and activity of individual representatives of 1-alkyl-tetrahydroisoquinoline (1-methyl-, -ethyl, -propyl, -butyl) [8]; however, there is no information on the synthesis of 1-alkyl (C6-C17) -derivatives of tetrahydroisoquinoline and the dependence of their biological activity on the length of the hydrocarbon chain. Based on the foregoing, it is obvious that the targeted synthesis of a number of tetrahydroisoquinoline derivatives and the study of their cytotoxicity, antibacterial and antifungal activity is expedient from the point of view of the development of new and effective biologically active compounds.

2. MATERIALS AND METHODS

In this work, we used primary cultures of healthy cells - hepatocytes, fibroblasts; transplantable cultures of cancer cells, strains of gram-positive and gram-negative bacteria and a fungal strain. Epithelial carcinoma of the cervix HeLa, adenocarcinoma of the breast HBL-100 (ATCC NTV 124) and adenocarcinoma of the larynx Hep-2 (ATCC: CCL-23) were obtained by us from the Central Bank of the Collection of Cell Cultures of the Russian Science Cultural Research Center of the Russian Academy of Sciences, melanoma of the mouse skin KML - from the Institute of Bioorganic Chemistry of the AS RUz.RKM Uz strains - B. subtilis, S. aureus, P. auragenosa, E. coli, C. albicans were obtained from the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.

2.1 Synthesis of Tetrahydroisoquinolines 4a-4i

IR spectra were recorded on an FTIR system 2000 (Perkin-Elmer) in KBr tablets; 1H NMR spectra were recorded on a UNITY-400 + Varian (400 MHz) (solvent CDCl3, internal standard-HMDS). The Rf values were determined on silica gel plates LS 5/40 (Czechoslovakia) using a chloroform: methanol solvent system.

The melting points of all synthesized substances were determined on a BOETIUS microtable.

2.2 Reaction of Homoveratrylamine with Monobasic Acids

Amides were obtained by heating in an oil bath a mixture of 0.012 mol of homoveratrylamine (1) (the amine was taken in excess) and 0.01 mol of the corresponding acid for 2–4 h at a temperature of 178 °C. The reaction mixture was dissolved in 100 mL of chloroform and washed with 3% hydrochloric acid solution, 2% NaOH solution, and water until neutral. Chloroform was distilled off on a rotary evaporator. The residue was crystallized from acetone or hexane. The crystals obtained were filtered off. Purity was checked by chromatography on silica gel [9].

N-(3,4-Dimethoxy-β-phenethyl) heptanamide (3a). Prepared from 1.84 g (0.0102 mol) of homoveratrylamine and 1.32 g (0.0101 mol) of heptanoic acid (7:0). Yield 84% (2.47 g, mp. 55-57 °C (hexane), Rf 0.54 (system 6).

IR spectrum (KBr, ν, cm⁻¹): 3310 (NH), 2933 (Ar), 1645 (N-C=O). 1H NMR spectrum (400 MHz, CDCl3, δ, ppm, J/Hz): 0.81 (3H, t, J = 7.3, CH3); 1.21 (6H, br.s, 3CH2); 1.52 (2H, m, H-2'); 2.05 (2H, t, J = 7.3, H-1'); 2.70 (2H, t, J = 7.3, H-1'); 3.43 (2H, q, J=7, Hβ); 3.795 (3H, s, OCH3); 6.67 (1H, H-2'); 6.67 (1H, H-6); 6.74 (1H, H-5).

N-(3,4-Dimethoxy-β-phenethyl)octanamide (3b). Prepared from 5.18 g (0.028 mmol) of homoveratrylamine and 5.2 g (0.03 mmol) of octanoic acid (8:0). Yield 84% (7.0 g, mp. 71-72°C, Rf 0.84 (chloroform-methanol, 8:1, system 1).

IR spectrum (KBr, ν, cm⁻¹): 3310 (NH), 2933 (Ar). 1H NMR spectrum (400 MHz, CDCl3, ppm, J/Hz): 0.81 (3H, t, J = 7.3, CH3); 1.21 (8H, br.s, 4CH2); 1.52 (2H, m, H-2'); 2.05 (1H, m, H-1'); 2.70 (2H, t, J = 7.3, H-1'); 3.43 (2H, k, J=7, Hβ); 3.69 (3H, s, OCH3); 3.73 (3H, s, OCH3); 3.75 (3H, s, OCH3); 5.45 (1H, m, NH); 6.65 (1H, H-2'); 6.67 (1H, H-6); 6.74 (1H, H-5).
**N-(3,4-Dimethoxy-β-phenethyl)nonanamide (3c).** Prepared from homoveratrylamine (2 g, 0.011 mol) and nonanoic acid (9.0, 1.32 g, 0.008 mol). Yield 92% (2.47 g), HPLC yield 96%, mp. 69-71°C (hexane), Rf 0.6 (system1).

IR spectrum (KBr, v cm⁻¹): 3310 (NH), 2925 (Ar-CH), 1639 (N=C=O).¹H NMR spectrum (400 MHz, CDCl₃, δ ppm, J/Hz): 0.81 (3H, t, J = 7.5, CH₃); 1.20 (10H, br.s, 5CH₂); 1.52 (2H, m, H-2'); 2.05 (2H, t, J = 7.5, H-1'); 3.43 (2H, q, J = 7, H₂β); 3.799 (3H, s, OCH₃); 3.805 (3H, s, OCH₃); 5.37 (1H, m, NH); 6.68 (1H, H-2'); 6.70 (1H, H-6); 6.76 (1H, H-5).

**N-(3,4-Dimethoxy-β-phenethyl)decananamide (3d).** Prepared from 0.28 g (1.5 mmol) of homoveratrylamine and 0.27 g (15 mmol) of decanoic acid (10:0). Yield 83% (0.44 g), HPLC yield 92%, mp. 81-82°C (acetone), Rf 0.8 (system1).

IR spectrum (KBr, v cm⁻¹): 3318 (NH), 2923 (Ar-CH), 1638 (N=C=O).¹H NMR spectrum (400 MHz, CDCl₃, δ ppm, J/Hz): 0.81 (3H, t, J = 7.7, CH₃); 1.19 (12H, br.s, 6CH₂); 1.53 (2H, m, H-2'); 2.05 (2H, t, J = 7.7, H-1'); 2.70 (2H, t, J = 7, H₂α); 3.43 (2H, q, J = 7, H₂β); 3.80 (3H, s, OCH₃); 3.81 (3H, s, OCH₃); 5.36 (1H, m, NH); 6.65 (1H, H-2'); 6.70 (1H, H-6); 6.76 (1H, H-5).

**N-(3,4-Dimethoxy-β-phenethyl)decanamide (3e).** Prepared from 0.2 g (1.1 mmol) of homoveratrylamine and 0.18 g (0.9 mmol) of decanoic acid. Yield 88% (0.29 g), mp. 88-90°C (acetone), Rf 0.88 (system1).

**N-(3,4-Dimethoxy-β-phenethyl)tetradecanamide (3f).** Prepared from 0.16 g (0.9 mmol) of homoveratrylamine and 0.14 g (0.6 mmol) of tetradecanoic acid (14:0). Yield 71% (0.17 g), HPLC yield 90%, mp. 94-95°C (acetone), Rf 0.9 (system1).

**N-(3,4-Dimethoxy-β-phenethyl)octadecanamide (3i).** Prepared from 0.6 g (3.3 mmol) of homoveratrylamine and 0.75 g (3 mmol) of hexadecanoic acid (16:0). Yield 84.5% (1.04 g), HPLC yield 89%, mp. 102-103°C (acetone), Rf 0.9 (system2, chloroform: methanol, 4:1).

**N-(3,4-Dimethoxy-β-phenethyl)heptadecanamide (3h).** Prepared from 1.4 g (7.7 mmol) of homoveratrylamine and 2 g (7.4 mmol) of heptadecanoic acid (17:0). Yield 82% (2.82 g), HPLC yield 90%, mp. 99-101°C (acetone), Rf 0.8 (system2).

**N-(3,4-Dimethoxy-β-phenethyl)octadecanamide (3i).** Prepared from 0.4 g (2.2 mmol) of homoveratrylamine and 0.61 g (2 mmol) of tetradecanoic acid (14:0). Yield 82% (0.79 g), HPLC yield 86%, mp. 103-104°C (acetone), Rf 0.88 (system2).

**N-(3,4-Dimethoxy-β-phenethyl)tetradecanamide (3f).** Prepared from 0.16 g (0.9 mmol) of homoveratrylamine and 0.14 g (0.6 mmol) of tetradecanoic acid (14:0). Yield 71% (0.17 g), HPLC yield 90%, mp. 94-95°C (acetone), Rf 0.9 (system1).
was refluxed for 1 h. The course of the reaction was monitored by TLC. The benzene and POCl₃ were distilled off, the residue was dissolved in 30 ml of methanol. To the resulting solution at a temperature of 0–5°C, 0.02 mol of NaBH₄ was added in portions. Methanol was distilled off. The residue was dissolved in H₂O and extracted with CHCl₃. After removal of CHCl₃, isoquinolines 4a-i were crystallized from acetone or methanol.

1-Hexyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4a). C₁₁H₂₁NO₂. Prepared from 1 g (3.4 mmol) of amide 3a and 0.7 ml of POCl₃. Yield 74% (0.7 g), HPLC yield 88%, mp. hydrochloride 188-191°C (acetone), R₇ 0.6 (system 2).

IR spectrum (KBr, υ, cm⁻¹): 3450, 2930, 1611, 1519, 1450, 1264. ¹H NMR spectrum (400 MHz, CD₂OD, δ, ppm, J/Hz): 0.86 (3H, t, J = 6.7, CH₃); 1.30 (6H, d, J=6, 3CH₂); 1.38 and 1.44 (each 1H, m, CH₃) 1.84 and 2.05 (each 1H, m, H-1'); 2.96 (2H, m, H-4); 3.47 (2H, q, J=6, H-3); 3.76 (6H, s, 2OCH₃); 4.40 (1H, m, H-1); 6.74 (2H, s, H-5, 8).

1-Heptyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4b). C₁₂H₂₂NO₂. Prepared from 5.8 g (0.018 mol) of amide 3b and 3.9 ml of POCl₃. Yield 76% (4.7 g), HPLC yield 88%, mp. hydrochloride 179-181°C (acetone), R₇ 0.6 (system 3, chloroform: methanol).

IR spectrum (KBr, υ, cm⁻¹): 3450, 2930, 1611, 1519, 1450, 1264. ¹H NMR spectrum (400 MHz, CD₂OD, δ, ppm, J/Hz): 0.84 (3H, t, J = 6.7, CH₃); 1.35 (10H, m, 5CH₂); 1.88 (1H, m, H-1'); 2.01 (1H, m, H-1'); 2.96 (1H, dt, J = 5.9, 17.2, H-4); 3.03 (1H, dt, J = 6.0, 18.4, H₄-2); 3.27 (1H, m, N₃); 3.49 (1H, dt, J = 6.8, 12.8, H-3); 3.75 (6H, s, 6,7-OCH₃); 4.39 (1H, dd, J = 4.4, 8.4, H-1); 6.74 (1H, s, H-8); 6.75 (1H, s, H-5).

1-Octyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4c). Prepared from 1.3 g (4 mmol) of amide 3c and 0.8 ml of POCl₃. Yield 71% (0.87 g), HPLC yield 91%, mp. hydrochloride 177-180°C (acetone), R₇ 0.6 (system 3).

IR spectrum (KBr, υ, cm⁻¹): 3611, 2928, 1611, 1519, 1460, 1264. ¹H NMR spectrum (400 MHz, CDCl₃, δ, ppm, J/Hz): 0.81 (3H, t, J = 7.0, CH₃); 1.20 (10H, brs, 5CH₂); 1.57 (2H, q, J = 7.4, H-1', 2'); 2.98 and 3.09 (each 1H, t, J = 6, H-4); 3.26 and 3.50 (each 1H, q, J = 6.4, H-3); 3.78 (3H, s, OCH₃); 3.79 (3H, s, OCH₃); 4.35 (1H, t, J = 6.6, H-1); 6.50 (1H, s, H-8); 6.53 (1H, s, H-5).

1-Nonyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4d). Prepared from 0.26 g (0.8 mmol) of amide 3d and 0.25 ml of POCl₃. Yield 80% (0.2 g), HPLC yield 86%, mp. hydrochloride 173-176°C (acetone), R₇ 0.55 (system 3).

IR spectrum (KBr, υ, cm⁻¹): 3749, 2926, 1611, 1519, 1449, 1265. ¹H NMR spectrum (400 MHz, CDCl₃, δ, ppm, J/Hz): 0.81 (3H, t, J = 7.0, CH₃); 1.20 (12H, br.s, 6CH₂); 1.57 (4H, q, J = 8, H-1', 2'); 2.99-3.10 (each 1H, t, J = 6, H-4); 3.26 (1H, q, J = 6, H-3a); 3.50 (1H, q, J = 6, H-3c); 3.78 (3H, s, OCH₃); 3.79 (3H, s, OCH₃); 4.35 (1H, t, J = 6, H-1); 6.50 (1H, s, H-8); 6.53 (1H, s, H-5).

1-Undecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4e). Prepared from 0.14 g (0.4 mmol) of amide 3 e and 0.10 ml of POCl₃. Yield 77% (0.1 g), HPLC yield 82%, mp. hydrochloride 165-168°C (acetone), R₇ 0.62 (system 3).

IR spectrum (KBr, υ, cm⁻¹): 3450, 2925, 1612, 1519, 1460, 1263. ¹H NMR spectrum (400 MHz, CDCl₃, δ, ppm, J/Hz): 0.81 (3H, t, J = 7.0, CH₃); 1.19 (16H, br.s, 8CH₂); 1.56 (4H, q, J = 7.4, H-1', 2'); 2.98 and 3.10 (each 1H, t, J = 6, H-4); 3.26 (1H, q, J = 6, H-3a); 3.50 (1H, q, J = 6, H-3c); 3.78 (3H, s, OCH₃); 3.79 (3H, s, OCH₃); 4.35 (1H, t, J = 6, H-1); 6.50 (1H, s, H-8); 6.53 (1H, s, H-5).

1-Tridecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4f). Prepared from 0.15 g (0.4 mmol) of amide 3f and 0.10 ml of POCl₃. Yield 71% (0.1 g), HPLC yield 86%, mp. hydrochloride 155-158°C (acetone), R₇ 0.44 (system 3).

IR spectrum (KBr, υ, cm⁻¹): 3608, 2923, 1612, 1519, 1460, 1264. ¹H NMR spectrum (400 MHz, CDCl₃, δ, ppm, J/Hz): 0.81 (3H, t, J = 7.0, CH₃); 1.19 (20H, br.s, 10CH₂); 1.57 (4H, q, J = 7.4, H-1', 2'); 2.98 and 3.09 (each 1H, t, J = 6, H-4); 3.26 (1H, q, J = 6.6, H-3a); 3.50 (1H, q, J = 6.6, H-3c); 3.78 (3H, s, OCH₃); 3.79 (3H, s, OCH₃); 4.35 (1H, t, J = 6, H-1); 6.50 (1H, s, H-8); 6.53 (1H, s, H-5).

1-Pentadecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4e). Prepared from 0.9 g (0.002 mol) of amide 3e and 0.4 ml of POCl₃. Yield 88% (0.76 g), HPLC yield 94%, mp. hydrochloride 155-158°C (acetone), R₇ 0.62 (system 1).
IR spectrum (KBr, ν, cm⁻¹): 3608, 2921, 1612, 1519, 1469, 1264. ¹H NMR spectrum (400 MHz, CDCl₃, δ, ppm, J/Hz): 0.80 (3H, t, J = 7.0, CH₃); 1.16 (26H, br.s, 13CH₂); 1.57 (2H, m, H-2’); 2.34 (1H, m, H-4a); 2.58 (2H, br.s, H-1’); 2.77 (1H, m, H-4c); 3.04 (1H, m, H-3a); 3.48 (1H, q, J = 6.6, H-3c); 3.73 (6H, s, 2OCH₃); 4.35 (1H, q, J = 5.6, H-1’); 6.43 (1H, s, H-8); 6.47 (1H, s, H-5).

1-Hexadecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4h). Prepared from 1 g (2.3 mmol) of amide 4h and 0.5 ml of POCl₃. Yield 90% (0.86 g), mp. hydrochloride 154-156°C (acetone), Rf 0.42 (system 2).

IR spectrum (KBr, ν, cm⁻¹): 3450, 2920, 1612, 1519, 1470, 1264. ¹H NMR spectrum (400 MHz, CDCl₃, δ, ppm, J/Hz): 0.81 (3H, t, J = 6.7, CH₃); 1.19 (26H, br.s, 13CH₂); 1.44 (2H, q, J = 8, H-2’); 1.92 (2H, q, J = 8.5, H-1’); 2.65 and 2.73 (each 1H, t, J = 6.4, H-4); 2.95 (1H, q, J = 6, H-3a); 3.21 (1H, J = 6, H-3c); 3.78 (6H, s, OCH₃); 3.92 (1H, q, J = 6, H-1’); 6.50 (1H, s, H-8); 6.54 (1H, s, H-5).

1-Heptadecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4i). Prepared from 0.75 g (1.7 mmol) of amide 3i and 0.4 ml of POCl₃. Yield 70% (0.5 g), mp. hydrochloride 143-146°C (acetone), Rf 0.57 (system 2).

IR spectrum (KBr, ν, cm⁻¹): 3450, 2920, 1612, 1519, 1470, 1264. ¹H NMR spectrum (400 MHz, CDCl₃, δ, ppm, J/Hz); 0.81 (3H, t, J = 7, CH₃); 1.18 (30H, br.s, 15CH₂); 1.57 (4H, q, J = 7.5, H-1’; 2’); 2.99 (1H, t, J = 6.17, H-4a); 3.10 (1H, t, J = 6.17, H-4c); 3.33 (1H, q, J = 6, 12.5, H-3a); 3.50 (1H, q, J = 6, 12.5, H-3c); 3.78 (3H, s, OCH₃); 3.79 (3H, s, OCH₃); 4.35 (1H, t, J = 6.4, H-1’); 6.50 (1H, s, H-8); 6.53 (1H, s, H-5).

2.4 Obtaining of Fibroblasts and Hepatocytes

It is particular importance to study the toxicity of compounds not only on cancer cell lines, but also on healthy cells. In this regard, the cultures of liver cells and fibroblasts as the most common cells in the body (the liver is the main filtrate of blood from toxins, fibroblasts make up the inner layer of all organs of the body) are the most important healthy cells for screening biologically active substances.

We have investigated methods for obtaining primary cells and optimized the method for obtaining cell cultures. Thus, the primary hepatocytes were obtained from liver perfusion in rats [10]. After washing the cell suspension and counting them, hepatocytes were plated into 96-well plates in DMEM medium and 10% FBS, 1% antimycotic antibiotic, and 2 mM glutamine.

Fibroblast cells were obtained by the method [11] with some modifications. The epidermal layer of the skin of newborn (3–4-day-old) rats was removed and kept in a 1% antibiotic-antifungal solution for 4–5 min. Then the skin fragments were washed with PBS solution (phosphate-salt buffer, pH 7.2) and crushed to a size of 1×1 mm. Then 5 mL of 5% trypsin (Samson-Med, Russia) - EDTA (Himedia, India) solution was shaken for 25 minutes at 37°C. The resulting mass was filtered through a Teflon filter with a size of 0.2 μm. The filtrate was centrifuged at 800 rpm for 8 min. After centrifugation, it was evident that the fibroblast cells separated from the skin biopsies had sunk to the bottom of the test tube. After the supernatant was spilled, the precipitated cells were mixed with a 20% FBS (Gibco, USA) DMEM/F-12 nutrient medium and placed on a culture mattress. The obtained fibroblast cells were observed to be transparent, spherical in shape when viewed under a microscope (Leica, Germany). Fibroblast cells were placed in an CO₂ incubator at 5% CO₂, 37°C (SHEL LAB, USA). After 48 h, the nutrient medium of the cells was changed. After 72 hours, the fibroblast cells formed a monolayer and were used in the study.

2.5 Cytotoxicity

Cytotoxic properties of the compounds were determined in vitro by using MTT method in 96-well plates [12]. HeLa and HEP-2 cells (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 were applied in the concentrations of 10 and 1 mmol, after preliminary dissolution in DMSO (not more than 0.8% of the volume of the nutrient medium) and left in a CO₂-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 CysplatinNaprod (India), containing cisplatin as the active component.

2.6 Antimicrobial Activity

A suspension of bacterial cells was prepared from a daily subculture of the corresponding strain, with 1×10⁵ colonies in 1 ml. Sterile Nutrient Agar (25 g agar/L diss. water) were inoculated with bacterial cells (200μl of bacterial cells in 2 mL of 0.9% NaCl suspension and 20 mL of medium) and added to Petri dishes to obtain a solid phase. Candida albicans (1×10⁵ CFU/mL) was inoculated into sterile Mueller-Hinton agar in accordance with CLS and DIN E 58940-3 for agar-disk diffusion methods [13,14]. Test materials in an amount of 40 μL (0.2 mg of individual compounds) were dissolved in DMSO and applied to sterile paper discs (6 mm diameter). Ampicillin, Ceftriaxone, and Fluconazole were used as positive controls. The corresponding solvent used as a negative control. DMSO was evaporated in a stream of air at room temperature.

The discs were deposited on the surface of inoculated agar plates and incubated for 2 h in the refrigerator to pre-diffusion the substances in the agar. Plates with bacteria were incubated at 37°C for 24 h, with fungi at 26°C for 48 h. The zone of inhibition (including the diameter of the disc) was measured and recorded after the incubation time. Average inhibition values were calculated after 3 replication.

The analysis and statistical processing of the obtained data were carried out using the OriginPro 8.6 software. We considered the results to be reliable with a value of ≤0.05.

3. RESULTS AND DISCUSSION

It was established, that tetrahydroisoquinoline derivatives exhibited antitumor activity against gliomas, breast cancer, cervical and colon cancer, leukemia [15,16] and overcome multiple drug resistance of tumor cells [17-19]. For example, an experimental antitumor drug Pixantrone, containing isoquinoline derivative 6,9-bis (2-aminoethylamino)benzisoquinoline-5,10-dione (Cell Therapeutics, USA), has been developed, which exhibits a slight toxic effect on heart tissue. The mechanism of the cytotoxic action of Pixantrone is inhibition of topoisomerase II and DNA intercalation. The cytotoxicity of the drug is not accompanied by oxidative stress.

In connection with the above, the identification of compounds among new tetrahydroisoquinoline derivatives exhibiting cytotoxic activity against tumor cells, with low toxicity for normal cells, is an urgent and promising direction.

The cytotoxicity results are shown in Table 1.

From the data in the table it follows that 1-Tridecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4f) with a chain length of 13 methylene fragments showed the greatest cytotoxic effect against all cancer cell lines: suppression of melanoma cell growth skin accounted for 88.3% compared with the control (hereinafter in parentheses: under the same conditions, cisplatin - 56.0%, laryngeal adenocarcinomas - 87.5% (82.5%), cervical carcinomas - 77.0% (31.7%), breast adenocarcinomas - 91.0% (36.0%). At the same time, the toxicity of the sample (4f) on hepatocytes was identical to that of cisplatin (Table 1). Selective cytotoxicity was also shown by 4d and 4e samples (9 and 11 fragments -CH₂-) against HEp-2 and HBL-100 cell lines, as well as the derivative with (-CH₂)₁₅ in relation to the HEp-2 cells.

With a decrease in the concentration of substances to 1 μM, the cytotoxicity of the compounds was markedly reduced (Table 2).

Thus, among compounds 4e, 4f and 4g, activity were retained only against laryngeal adenocarcinoma cells - 48.5, 50.1 and 52.7% of HEp-2 cell death, respectively, which turned out to be higher than the values of the cisplatin. At the same time, no toxicity of the derivatives were observed on healthy cells.

Thus, it was found that the sequential elongation of the methylene chain from 7 or more fragments causes a gradual increase in the cytotoxic effect of the compounds against all five lines of cancer cells, reaching its peak at (-CH₂)₁₃ and (-CH₂)₁₅. Further lengthening of the methylene chain to 17-19 fragments leads to a decrease in the cytotoxicity of the molecule, and to 18-20 fragments - to the disappearance of solubility, and, consequently, the impossibility of carrying out the reaction.
Table 1. Cytotoxicity of tetrahydroisoquinoline derivatives at 10 μM

| substances | HEp-2 | HeLa | KML | HBL-100 | hepatocytes | fibroblasts |
|------------|-------|------|-----|---------|-------------|-------------|
| 4b         | 34.9 ± 6.2 | 19.4 ± 1.1 | 20.4 ± 6.3 | 25.0 ± 3.9 | 9.2 ± 2.4 | 9.9 ± 3.0 |
| 4c         | 58.9 ± 7.7 | 14.3 ± 1.6 | 22.6 ± 3.8 | 35.7 ± 3.3 | 7.6 ± 1.8 | 3.0 ± 0.8 |
| 4d         | **70.9 ± 9.6** | 28.9 ± 9.5 | 28.1 ± 7.8 | **73.6 ± 4.8** | **11.7 ± 3.0** | **7.4 ± 1.3** |
| 4e         | 74.5 ± 6.1 | 47.2 ± 3.7 | 35.3 ± 2.2 | 74.5 ± 1.6 | 21.1 ± 3.9 | 27.5 ± 3.6 |
| 4f         | **87.5 ± 4.8** | **77.0 ± 5.9** | **88.3 ± 9.4** | **91.0 ± 7.7** | **0.0 ± 0.0** | **33.3 ± 4.3** |
| 4g         | 66.0 ± 5.2 | 55.8 ± 5.2 | 55.5 ± 6.9 | 40.8 ± 7.7 | 14.1 ± 3.4 | 36.7 ± 5.2 |
| 4h         | 54.6 ± 1.1 | 44.6 ± 4.6 | 17.6 ± 5.2 | 35.6 ± 7.6 | 11.2 ± 2.8 | 25.8 ± 2.7 |
| 4i         | 39.7 ± 6.6 | 26.9 ± 2.4 | 12.6 ± 3.4 | 25.1 ± 3.5 | 0.0 ± 0.0 | 14.2 ± 1.6 |
| cisplatin  | 82.5 ± 17.0 | 31.7 ± 3.3 | 56.0 ± 4.1 | 36.0 ± 4.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |

Table 2. Cytotoxicity of alkyl tetrahydroisoquinoline derivatives at 1 μM

| substances | HEp-2 | HeLa | KML | HBL-100 | hepatocytes | fibroblasts |
|------------|-------|------|-----|---------|-------------|-------------|
| 4b         | 37.0 ± 6.3 | 4.4 ± 1.3 | 28.5 ± 3.6 | 12.6 ± 1.6 | 6.5 ± 1.2 | 2.7 ± 0.0 |
| 4c         | 29.2 ± 6.0 | 9.7 ± 4.9 | 19.2 ± 1.5 | 18.0 ± 7.9 | 8.4 ± 1.8 | 2.6 ± 1.3 |
| 4d         | 40.4 ± 0.6 | 10.9 ± 2.2 | 15.9 ± 1.6 | 23.7 ± 5.9 | 9.8 ± 2.2 | 4.3 ± 0.5 |
| 4e         | 48.5 ± 3.8 | 18.4 ± 3.0 | 22.0 ± 2.6 | 20.0 ± 7.7 | 11.2 ± 1.4 | 1.5 ± 0.1 |
| 4f         | **50.1 ± 5.7** | 11.3 ± 3.4 | 19.6 ± 3.7 | 2.8 ± 1.9 | **1.3 ± 0.3** | **0.3 ± 0.0** |
| 4g         | 52.7 ± 3.8 | 7.9 ± 3.2 | 19.8 ± 2.3 | 21.1 ± 2.0 | **7.0 ± 1.8** | **0.0 ± 0.0** |
| 4h         | 26.3 ± 1.2 | 1.3 ± 2.2 | 0.0 ± 0.0 | 25.0 ± 3.7 | 3.6 ± 0.1 | 3.1 ± 1.3 |
| 4i         | 21.6 ± 8.7 | 9.5 ± 8.5 | 0.3 ± 0.5 | 9.0 ± 2.0 | 0.2 ± 0.0 | 0.0 ± 0.0 |
| cisplatin  | 47.9 ± 9.2 | 4.4 ± 4.7 | 45.3 ± 27.3 | 29.7 ± 5.7 | 0.0 ± 0.0 | 0.0 ± 0.0 |

Table 3. Antimicrobial activity of isoquinoline derivatives

| substances | B. subtilis | i. aureus | E. coli | P. aeruginosa | C. albicans |
|------------|-------------|----------|---------|---------------|-------------|
| 4b         | **20.0 ± 0.2** | 13.0 ± 0.15 | 15.0 ± 0.11 | 7.0 ± 0.1 | 12.0 ± 0.11 |
| 4c         | 21.0 ± 0.21 | 15.0 ± 0.13 | 15.0 ± 0.1 | 8.0 ± 0.11 | 20.0 ± 0.11 |
| 4d         | **22.0 ± 0.19** | 15.0 ± 0.11 | 16.0 ± 0.1 | 8.0 ± 0.11 | **21.0 ± 0.22** |
| 4e         | 10.0 ± 0.11 | 10.0 ± 0.11 | 7.0 ± 0.1 | 6.0 ± 0.1 | na |
| 4f         | 7.0 ± 0.1 | 8.0 ± 0.1 | 6.0 ± 0.0 | na | na |
| 4g         | 6.0 ± 0.09 | 6.0 ± 0.09 | na | na | na |
| 4h         | na | na | na | na | na |
| 4i         | na | na | na | na | na |
| Ampicillin (10 µg/disc) | 28.04 ± 0.10 | 27.08 ± 0.12 | 2.0 ± 0.15 | nt | nt |
| Ceftriaxone (30 µg/disc) | nt | nt | 3.0 ± 0.1 | 28.12 ± 0.1 | nt |
| Fluconazole (25 µg/disc) | nt | nt | nt | 30.04 ± 0.1 | nt |

Literature data indicate a high antibacterial activity of tetrahydroisoquinolines. Synthetic derivatives with high selectivity are active against Moraxella catarrhalis, Campylobacter jejuni with
The antibacterial and antifungal activity of new synthesized compounds were tested by a modified disk diffusion method on agar [13,14]. The test results are given in the Table 3.

As can be seen from Table 3, a gradual increase in the length of the methylene chain in the tetrahydroisoquinoline molecule first leads to an increase in its antimicrobial activity, and after overcoming a certain length - to its gradual decrease. So, the greatest antibacterial and antifungal activity is shown by 4d - 1-Nonyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline with a methylene chain length of 8 fragments. It is interesting to note that the strain of gram-positive bacteria Bacillus subtilis -22.0±0.19 mm and Candida albicans - 21.0±0.22 mm. However, already in the following derivatives, in which the methylene chain length is increased by 2 or more fragments (samples 4e-4i), the antifungal activity disappears, and starting from a molecule with 12 or more (-CH2-) substituents - 4g-4i - the antimicrobial properties are reduced to zero.

7. CONCLUSIONS

It was established that tetrahydroisoquinoline 4d was cytotoxic only against lines HEP-2 and HBL-100, and 1-Tridecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4f), which differs from the first in the length of the methylene chain - (-CH2-)13 per 4 fragments, showed cytotoxicity against all 5 types of cancer cells, but, on the contrary, did not show antimicrobial effect. Probably, such different effects in the activity of compounds with a similar chemical structure are observed due to differences in the structure of plasma membranes and walls of microbial and cancer cells.

Thus, the most promising compound for further study is 1-Tridecyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4f), which showed a high cytotoxic effect, and 1-Nonyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (4d), which has antibacterial and antifungal properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Demerson C.A., Philipp A.H., Humber L.G., Kraml M.J., Charest M.P., Tom H. et al. Pyrrolo[4,3,2-de]isoquinolines with central nervous system and antihypertensive activities. J. Med. Chem. 1974;17:1140–1145
2. Alagumuthu M., Sathiyanarayanan K.I., Arumugam S. Molecular docking, design, synthesis, in vitro antioxidant and anti-inflammatory evaluations of new isoquinoline derivatives. Int. J Pharm. Sci. 2015;7:200–208
3. Manikandan A., Sivakumar A. Analgesic, anti-inflammatory and antipyretic evaluations of new isoquinoline derivatives. J. Pharm. Sci. 2016;8:339–343
4. Kubota H., Watanabe T., Kakufuda A., Masuda N., Wada K., Ishii N. et al. Synthesis and pharmacological evaluation of piperidinoalkanoyl-1,2,3,4-tetrahydroisoquinoline derivatives as novel specific bradycardic agents. Bioorg Med Chem Lett, 2004:14:3049-3052. DOI: 10.1016/j.bmcl.2004.04.031
5. Scott J.D., Williams R.M. Chemistry and biology of the tetrahydroisoquinoline antitumor antibiotics. Chem Rev. 2002;102(5):1669-730. DOI: 10.1021/cr010212u
6. Galán A., Moreno L., Párraga J., Serrano A., Sanz M.J., Cortes D. et al. Novel isoquinoline derivatives as antimicrobial agents. Bioorg. Med. Chem. 2013:21:3221-3230. DOI: 10.1016/j.bmc.2013.03.042
7. Cushnie T.P., Cushnie B.T., Lamb A.J. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. International Journal of Antimicrobial Agents. 2014:44:377–386. DOI: 10.1016/j.ijantimicag.2014.06.001
8. Adams R. Organic Reactions, coll. 6, ed. 1953:98.
9. Saidov A.S.N., Levkovich M.G., Vinogradova V.I. Synthesis of 1-Alkyltetrahydroisoquinolines. Chemistry of Nat. Comp. 2013;49:897–90.
10. Cabral F., Miller C.M., Kudrna K.M., Hass B.E., Daubendiek J.G., Kellar B.M. et. al. Purification of hepatocytes and sinusoidal endothelial cells from mouse liver perfusion. Journal of Visualized Experiments. 2018:2:12. DOI: 10.3791/56993
11. Kruglov E.A., Jain D., Dranoff J.A. Isolation of primary rat liver fibroblasts. Journal of investigative medicine. 2002;50(3):179-84. DOI: 10.2310/6650.2002.33431
12. Niks M., Otto M. Towards an optimized MTT assay. Journal of Immunological Methods. 1990;130:149
13. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI document M02. 13th Edition. PA, USA; 2018.
14. DIN, Deutsche Institution für Normung e.V., DIN Taschenbuch 222, Medizinische Mikrobiologie und Immunologie. Beuth-Verlag, Berlin 2004.
15. Patil R., Hosni-Ahmed A., Jones T.S., Patil S.A., Asres L.B., Wang X., Yates R.C., Geisert E.E., Miller D.D. et. al. Synthesis and in vitro evaluation of novel 1,2,3,4-tetrahydroisoquinoline derivatives as potent antiangioma agents. Anticancer Agents Med Chem. 2014:14(3):473-482
16. Lin Y., Shi R., Wang X., Shen H.M. Luteolin, a flavonoid with potentials for cancer prevention and therapy. Curr Cancer Drug Targets. 2008;8(7):634-646. DOI: 10.2174/156800908786241050
17. Fang W., Li Y., Cai Y., Kang K., Yan F., Liu G. et. al. Substituted tetrahydroisoquinoline compounds B3 inhibited P glycoprotein-mediated multidrug resistance in vitro and in vivo. J Pharm Pharmacol. 2007;59:1649-1655
18. Li Y., Zhang H.B., Huang W.L., Li Y.M. Design and synthesis of tetrahydroisoquinoline derivatives as potential multidrug resistance reversal agents in cancer. Bioorg Med Chem Lett. 2008:18:3652-3655
19. Yan F., Jiang Y., Li Y-M., Zhen X., Chen J., Fang W-R. Reversal of P-glycoprotein and multidrug resistance-associated protein 1-mediated multidrug resistance in cancer cells by HZ08 isomers, tetrahydroquinoline derivatives. Biol Pharm Bull. 2008:31:1258-1264
20. Bieławski K, Leszczyńska K, Kaluża Z, Bieławska A., Michalak O, Daniūk T., et. al. Synthesis and antimicrobial activity of chiral quaternary N-spiro ammonium bromides with 3',4'-dihydro-1'H-spiro[isoindoline-2,2'-isoquinoline] skeleton. Drug Des Devel Ther. 2017:11:2015-2028. DOI: 10.2147/DDDT.S133250
21. Guzman J.D., Pesnot T., Barrera D.A., Davies H.M., McMahon E., Evangelopoulos D., et. al. Tetrahydroisoquinolines affect the whole-cell phenotype of Mycobacterium tuberculosis by inhibiting the ATP-dependent MurE ligase. J Antimicrob Chemother. 2015;70(6):1691-1703. DOI: 10.1093/jac/dkv010
22. Panchaud P., Bruyère T., Blumstein A.C., Bur D., Chambovey A., Ertel E.A. et. al. Discovery and Optimization of Isoquinoline Ethyl Ureas as Antibacterial Agents. J Med Chem. 2017:60(9):3755-3775. DOI: 10.1021/acs.jmedchem.6b01834

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246