Prostate gland cancer is one of the most widespread malignant diseases in men. More than 600,000 new cases of this disease are registered annually [1]. Medical associates attribute this with improvement in methodology of screening, aimed at detecting prostate cancer on early stages, which are mandatory at certain age [2]. Important factors of disease risk are age (nearly 80% of cases are in men older than 60), stress, which weaken immune system, excessively high-calorie foods, hereditary causes. Lack of testosterone and vitamin D plays an important role too [3]. There are references in literature about significant ethnic variability in cases of prostate cancer in the world. Interesting, that tendency in values persists in men of different skin colors on all continents [4].

Methods of treatment depend on factors, such as stage of disease, tumor growth intensity, presence of metastases and possible complications, etc. However, plans of prostate cancer treatment in the world practice change, as consequence of new medicines addition on the pharmaceutical market, such as arbitaron acetate, docetaxel, enzalumatin [4].

In last 10 years in Ukraine there is observed upward trend in number of prostate cancer cases among male population. Disease progresses slowly and symptoms appear on 3–4 stages. Lethal cases are 41 on 100 thousands of people [5]. This is why creation of new locally produced antitumor agents is an urgent task of pharmacy.

Agent from group of antagonists, 5-fluorouracil, is used as a standard for comparison of antitumor activity of new perspective compounds. It inhibits the process of cancer cells division by blocking DNA synthesis.

Increase in effectiveness of chemotherapy treatment for tumor disease is related to creation of new, effective antitumor medical agents. Particularly, based on azulenes derivatives [6–11].

The aim of this study – to synthesize novel derivatives of 1-(4\textsuperscript{1}-isopropylphenyl)-4-(4\textsuperscript{2}-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid arylamides and to evaluate their activity against PC-3 prostate cancer cells as compared with known antitumor compound 5-fluorouracil (Fig. 1).

Materials and methods. Test compounds – 1-(4\textsuperscript{1}-isopropylphenyl)-4-(4\textsuperscript{2}-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid arylamides (8 a-i) were synthesized in department of medical chemistry, SI «Institute of Pharmacology and Toxicology NAMS of Ukraine» (Fig. 2).
2-Methoxy-3,4,5,6-tetrahydro-7H-azepine 1 was obtained by alkylating caprolactam with dimethyl sulfate using the method [12]. Chlorohydrate $\alpha$-amino-4-chloroacetophenone 2 was obtained [13] by condensation of $\alpha$-bromo-4-chloroacetophenone with hexamethylenetetramine. 3-(4-Chlorophenyl)-6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepine 3 was obtained by methods [14, 15].

$^1$H-NMR spectra were recorded on the Bruker VXR-500 (Germany), the working frequency of 500.13 MHz, in DMSO-d$_6$ + CCl$_4$ (1:3) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with use of the $\delta$ scale. Purity control of novel compounds was conducted by thin-layer chromatography in the system chloroform – methanol 9:1. The melting points were measured on a small-sized heating table with the observation device RNMK 05 (VEB Analytik, Dresden). Lipophilicity (LogP) of compounds 6 and 8 a-i was calculated using the program ACD LogP.

**Synthesis of 1-(4$^1$-isopropylphenyl)-4-(4$^2$-chlorophenyl)-5,6,7,8-tetrahydro-2,4$\alpha$-diazacyclopenta[cd]azulene 6.** To solution of 4.93 g (0.02 M) 3-(4-chlorophenyl)-6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepine 3 in 150 ml ethylacetate 4.82 g (0.02 M) $\alpha$-bromo-4-isopropylacetophenone 4 was added. The reaction mixture was refluxed for 1 hour. After cooling the solid 5 was filtered, washed with ethylacetate, then dried on air. To suspension of 1-[2-(4$^1$-isopropylphenyl)-2-oxoethyl]-3-(4$^2$-chlorophenyl)-6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepin-1-ium bromide 5 in 40 ml water 5% NaOH in 20 ml was added. The reaction mixture was refluxed for 3 hours. After
cooling the solid 6 was filtered, washed with water, then dried on air and recrystallized from benzene. Yield 3.35 g (43 %). Anal. Calc. for C_{18}H_{18}ClN_{2}, %: N 7.20. Found, %: N 7.32. 1H NMR (500 MHz, DMSO-d6), δ (ppm): 1.33 (d, 6H, CH(CH_{3})_{2}), 1.89 (m, 2H, CH_{2}), 2.05 (m, 2H, CH_{2}), 2.53 (m, 2H, CH_{2}), 3.06 (m, 1H, CH(CH_{3})_{2}), 3.95 (m, 2H, CH_{2}), 6.85 (s, 1H, 2-CH), 7.40 and 7.52 (d-d, 4H, C_{6}H_{4}, J=7.8 Hz), 7.49 (s, 1H, 3-CH), 7.57 and 7.63 (d-d, 4H, C_{6}H_{4}, J=8.7 Hz). Log P = 7.88 ± 0.88.

**Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid (2-methoxyphenyl)-amide 8 a.** The mixture 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.745 g (0.005 M) of 2-methoxyphenylisocyanate 7 a was refluxed in 100 ml of dried benzene during 2 hours. After cooling the solid 8 a was filtered, washed with benzene, then dried on air and recrystallized from ethanol. Yield 1.86 g (69 %). M.p.= 205–206 °C. Anal. Calc. for C_{38}H_{32}ClN_{2}O_{2}, %: Cl 6.60; N 7.81. Found, %: Cl 6.77; N 7.98. 1H NMR (500 MHz, DMSO-d6), δ (ppm): 1.31 (d, 6H, CH(CH_{3})_{2}), 1.86 (m, 2H, CH_{2}), 2.02 (m, 2H, CH_{2}), 2.41 (m, 2H, CH_{2}), 3.03 (m, 1H, CH(CH_{3})_{2}), 3.42 (s, 3H, OCH_{3}), 3.93 (m, 2H, CH_{2}), 7.69 (s, 1H, NH), 6.80–8.36 (m, 4H, o-C_{6}H_{4}), 7.36 and 7.45 (d-d, 4H, C_{6}H_{4}, J=8.4 Hz), 7.59 and 7.66 (d-d, 4H, C_{6}H_{4}, J=8.4 Hz), 8.01 (s, 1H, 3-CH). Log P = 7.98 ± 1.17.

**Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid (3-methylphenyl)-amide 8 b was obtained as amide 8 a from 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.745 g (0.005 M) of 3-methoxyphenylisocyanate 7 b.** Yield 1.93 g (77 %). M.p.= 220–222 °C. Anal. Calc. for C_{38}H_{32}ClN_{2}O_{2}, %: Cl 6.60; N 7.81. Found, %: Cl 6.74; N 7.95. 1H NMR (500 MHz, DMSO-d6), δ (ppm): 1.31 (d, 6H, CH(CH_{3})_{2}), 1.88 (m, 2H, CH_{2}), 2.05 (m, 2H, CH_{2}), 2.53 (m, 2H, CH_{2}), 3.04 (m, 1H, CH(CH_{3})_{2}), 3.65 (s, 3H, OCH_{3}), 3.94 (m, 2H, CH_{2}), 6.39–7.04 (m, 4H, C_{6}H_{4}), 6.83 (s, 1H, NH), 7.41 and 7.49 (d-d, 4H, C_{6}H_{4}, J=7.8 Hz), 7.58 and 7.65 (d-d, 4H, C_{6}H_{4}, J=8.7 Hz), 7.94 (s, 1H, 3-CH). Log P = 8.25 ± 1.17.

**Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid (4-methoxyphenyl)-amide 8 c was obtained as amide 8 a from 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.745 g (0.005 M) of 4-methoxyphenylisocyanate 7 c.** Yield 2.15 g (80 %). M.p.= 182–183 °C. Anal. Calc. for C_{38}H_{32}ClN_{2}O_{2}, %: Cl 6.60; N 7.81. Found, %: Cl 6.51; N 7.95. 1H NMR (500 MHz, DMSO-d6), δ (ppm): 1.30 (d, 6H, CH(CH_{3})_{2}), 1.88 (m, 2H, CH_{2}), 2.03 (m, 2H, CH_{2}), 2.51 (m, 2H, CH_{2}), 3.03 (m, 1H, CH(CH_{3})_{2}), 3.67 (s, 3H, OCH_{3}), 3.93 (m, 2H, CH_{2}), 6.76 and 6.97 (d-d, 4H, C_{6}H_{4}, J=9.0 Hz), 6.95 (s, 1H, NH), 7.40 and 7.47 (d-d, 4H, C_{6}H_{4}, J=8.1 Hz), 7.58 and 7.65 (d-d, 4H, C_{6}H_{4}, J=8.4 Hz), 7.93 (s, 1H, 3-CH). Log P = 8.04 ± 1.17.

**Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid (3-methylphenyl)-amide 8 d was obtained as amide 8 a from 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.67 g (0.005 M) of 3-methylphenylisocyanate 7 d.** Yield 1.93 g (74 %). M.p.= 204–205 °C. Anal. Calc. for C_{38}H_{32}ClN_{2}O_{2}, %: Cl 6.80; N 8.04. Found, %: Cl 6.69; N 8.21. 1H NMR (500 MHz, DMSO-d6), δ (ppm): 1.32 (d, 6H, CH(CH_{3})_{2}), 1.89 (m, 2H, CH_{2}), 2.04 (m, 2H, CH_{2}), 2.16 (s, 3H, CH_{3}), 2.51 (m, 2H, CH_{2}), 3.05 (m, 1H, CH(CH_{3})_{2}), 3.93 (m, 2H, CH_{2}), 6.56 (s, 1H, NH), 6.71–7.07 (m, 4H, C_{6}H_{4}), 7.42 and 7.50 (d-d, 4H, C_{6}H_{4}, J=7.8 Hz), 7.59 and 7.66 (d-d, 4H, C_{6}H_{4}, J=8.7 Hz), 7.95 (s, 1H, 3-CH). Log P = 8.55 ± 1.16.

**Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid (4-methylphenyl)-amide 8 e was obtained as amide 8 a from 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.67 g (0.005 M) of
Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid (2-chlorophenyl)-amide 8h was obtained as amide 8a from 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.77 g (0.005 M) of 4-chlorophenylicosyanate 7h. Yield 2.25 g (83 %). M.p. = 214–215 °C. Anal. Calc. for C32H32Cl2N2O: %: Cl 13.1; N 7.75. Found, %: Cl 12.9; N 7.64. 

Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.77 g (0.005 M) of 3-chlorophenylisocyanate was obtained as amide 8a from 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6. Yield 1.98 g (76 %). M.p. = 207–208 °C. Anal. Calc. for C33H32ClN2O: %: Cl 6.80; N 8.04. Found, %: Cl 6.93; N 8.04. 

H NMR (500 MHz, DMSO-d6), δ (ppm): 1.31 (d, 6H, CH(CH3)2), 1.88 (m, 2H, CH2), 2.02 (m, 2H, CH2), 2.18 (s, 3H, CH3), 2.49 (m, 2H, CH2), 3.03 (m, 1H, CH2), 0.94 (m, 2H, CH2), 0.94 (m, 1H, CH(CH3)), 3.94 (m, 2H, CH2), 3.95 (m, 2H, CH2), 7.16 (s, 1H, NH), 6.91–7.45 (m, 4H, CHH), 7.39 and 7.46 (d-d, 4H, CHH, J=8.1 Hz), 7.57 and 7.65 (d-d, 4H, CHH, J=8.7 Hz), 7.93 (s, 1H, 3-CH). Log P = 8.59 ± 1.17.

Synthesis of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene-2-carboxylic acid (3,4-dichlorophenyl)-amide 8i was obtained as amide 8a from 1.94 g (0.005 M) of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclopenta[cd]azulene 6 and 0.77 g (0.005 M) of 3,4-dichlorophenylisocyanate 7i. Yield 2.45 g (85 %). M.p. = 240–241 °C. Anal. Calc. for C32H28Cl2N2O: %: Cl 18.5; N 7.28. Found, %: Cl 18.3; N 7.35. 

H NMR (500 MHz, DMSO-d6), δ (ppm): 1.31 (d, 6H, CH(CH3)2), 1.89 (m, 2H, CH2), 2.04 (m, 2H, CH2), 2.17 (s, 3H, CH3), 2.51 (m, 2H, CH2), 3.02 (m, 1H, CH(CH3)), 3.94 (m, 2H, CH2), 7.15 (s, 1H, NH), 6.93–7.27 (m, 4H, CHH), 7.41 and 7.45 (d-d, 4H, CHH, J=7.7 Hz), 7.57 and 7.65 (d-d, 4H, CHH, J=8.7 Hz), 7.90 (s, 1H, 3-CH). Log P = 9.08 ± 1.17.

Antitumor activity in vitro against PC-3 prostate cancer cells was studied in National Cancer Institute of Health USA, according to Development Therapeutic Program by standard procedure [16]. For pharmacological screening compounds 8b,d were picked, which contain electron-donor substituents in meta position of heterocyclic system, according to the results of molecular modeling [17]. Identification of antitumor activity was conducted with highly sensitive fluorimetry method by fluorescence intensity.
The results obtained showed, that compounds 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclo[cd]azulene-2-carboxylic acid (3-methoxyphenyl)-amide 8 b and 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclo[cd]azulene-2-carboxylic acid (3-methylphenyl)-amide 8 d demonstrate the ability to suppress PC-3 prostate cancer cells growth in concentration of 10^-5 M.

These compounds were more effective in suppressing of cancer cells growth compared to control – 5-fluorouracil.

Results and discussion. The results were expressed in percentage of tumor cell growth percentage in vitro under influence of tested compound. The data obtained allow to consider these biologically active compounds as basis for creation of new, effective antitumor agents for prostate cancer treatment.

Conclusion

1. New derivatives of 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclo[cd]azulene-2-carboxylic acid arylamides inhibit growth of PC-3 prostate cancer cells more effective compared with standard – 5-fluorouracil under experimental condition in vitro.

2. 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclo[cd]azulene-2-carboxylic acid arylamides show antitumor activity against PC-3 prostate cancer cells in vitro. The compound 8 d in concentration of 10^-3 M exceeds an antitumor activity of 5-fluorouracil against PC-3 prostate cancer cells by 52.32%.

Therefore, it can be concluded, that 1-(4'-isopropylphenyl)-4-(4'-chlorophenyl)-5,6,7,8-tetrahydro-2,4a-diazacyclo[cd]azulene-2 carboxylic acid arylamides inhibit growth of PC-3 prostate cancer cells more effective compared with standard – 5-fluorouracil under experimental condition in vitro. The data obtained allow to consider these biologically active compounds as basis for creation of new, effective antitumor agents for prostate cancer treatment.


6. Патент на корисну модель № 117545 Україна, МПК (2017.01) C07D 487/00, A61P 35/00. 1-Феніл-4-арил-5,6,7,8-тетрагідро-2,2а,8а-триазациклопента-[cd]азулен з гідратом, що мають протипухлинну активність. Демченко С. А., Колосникова О. В., Демченко А. М., Бобкова Л. С. № u 2017 01 123; Заявл. 07.02.2017; Опубл. 26.06.2017, Бюл. № 12.


Anticancer drug development guide: preclinical screening, clinical.

The Derivatives of the 2a,4a-Diazacyclopen-

ta[c,d]azulen-2-carboxylic acid arylamides as new, potential antitumor medicines for prostate cancer treatment. 1-(4-chlorophenyl)-5,6,7,8-tetrahydro-2,4а-diazacyclopenta[c,d]azulen-2-carboxylic acid, that have protipuhalmin activity. Demchenko S. A., Kolosnikov O. V., Demchenko A. M., Bobkova L. S. № 1 2017 01124; Заявл. 07.02.2017; Опубл. 11.12.2017, Бюл. № 23.

The Patents on the Priority Model № 135600 Ukraine, МПК (2006) C07D 487/06 (2006.01), A61K 31/343 (2006.01), A61P 35/00, C07C 13/52 (2006.01). 1-Фенил-4-арил-5,6,7,8-тетрагидро-2,2а,8а-триазацикло-


The Patents on the Priority Model № 135600 Ukraine, МПК (2006) С07D 487/06 (2006.01), A61K 31/343 (2006.01), A61P 35/00, C07C 13/52 (2006.01). 1-Фенил-4-арил-5,6,7,8-тетрагидро-2,2а,8а-триазацикло-


С. А. Демченко, Ю. А. Федченкова, Т. А. Бухтиарова, Л. С. Бобкова, В. В. Суховеев, А. М. Демченко

Синтез и противоопухолевая активность ариламидов 1-(4H-изопропилифенил)-4-(4H-хлорфенил)-5,6,7,8-тетрагидро-2,4а-диазациклопента[cd]азулен-2-карбовочной кислоты по отношению к клеткам РС-3 рака простаты

Фармакотерапия рака простаты является важной составляющей в борьбе с онкологическими заболеваниями. Это является очень актуальным, так как рак простаты является причиной 10 % смертей среди всех видов раковых заболеваний у мужчин. Мета дослідження – синтезувати та вивчити протипухлинну активність ариламідів 1-(4H-изопропилифенил)-4-(4H-хлорфенил)-5,6,7,8-тетрагидро-2,4а-диазациклопента[cd]азулен-2-карбовочної кислоти як нових протипухлинних засобів для лікування раку простати.

Ключові слова: протипухлинна активність, 5-фторурацил, похідні 1-(4H-изопропилифенил)-4-(4H-хлорфенил)-5,6,7,8-тетрагидро-2,4а-диазациклопента[cd]азулен-2-карбовочної кислоти, клетки РС-3 раку простати