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# NOVEL PUTATIVE LIGANDS OF CANNABINOID RECEPTORS: SYNTHESIS AND EFFECTS ON CELL SIGNALING AND NEURONAL FUNCTIONS

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*Cannabinoid ligands are known to possess neuroprotective actions and may have utility in the treatment of neurodegeneration. The major targets for cannabinoids include the classical CB1 cannabinoid receptor, as well as the novel cannabinoid receptor GPR55 which binds to many synthetic cannabinoid ligands. In this study, novel thiopyranothiazoles **1**, **3**, **4**, **6**, and **7** were synthesized and their pharmacological activity as potential cannabinoid-like ligands was evaluated in glioblastoma cells, cultured cortical neurons, and cells of HEK293 line expressing GPR55. Stimulation of protein kinase ERK1/2, MAP-kinases and cAMP response element binding protein (CREB) was evaluated using Western-blot analysis. CREB activation was additionally monitored by means of confocal imaging of nuclear phospho-CREB labeling. Docking simulation confirmed the good affinity of the synthesized compounds to CB1 and CB2 receptors. Striking effects of the chromeno[4',3':4,5]thiopyrano[2,3-d][1,3]thiazol with ethylacetate moiety (**3**) and isothiochromeno[4a,4-d]thiazole with phenazone fragment (**7**) on pCREB activation as the indicator of stimulation of the pathway beneficial for neurons survival were observed.*

*K e y w o r d s: thiopyranothiazoles, putative cannabinoid ligands, CB1, CB2, cAMP response element binding protein (CREB), cultured cortical neurons.*

The term “cannabinoid” unites compounds of various structures and/or origins capable of interacting with cannabinoid receptors, particularly in the brain, and inducing effects similar to those caused by the Cannabis plant. According to a way of their production, cannabinoids are classified into phytocannabinoids, endocannabinoids, and synthetic cannabinoids. Synthetic cannabinoids include a big number of drugs mentioned in the EU Early Warning System and in the United Nations Office on Drugs and Crime [1]. At the same time, evidence is accumulating that cannabis plant-derived compounds (cannabinoids) can be used for the development of novel therapeutics [2]. These potent drugs act via the cannabinoid receptor 1 (CB1). New possibilities appeared after the discovering of the CB2 receptor and revealing endocannabinoids targeting this signaling system. However, real clinical success might be possible only after studying the endocannabinoidome and identifying of intracellular mediators and biochemical mechanisms of action of endocannabinoids, their specific receptors, and metabolism. The non-psychotropic cannabinoids have perspectives in the treatment of the neuropathic pain at multiple sclerosis. The purified plant cannabidiol is also explored in the treatment of paediatric epilepsy patients. Other neurological disorders, such as Parkinson disease, Alzheimer disease, Hunting-

ton disease, multiple sclerosis, amyotrophic lateral sclerosis, stroke, epilepsy, and glioblastoma wait for novel medicines involving endocannabinoids.

The role of endocannabinoids and their signaling in the development and functioning of the central nervous system stays poorly understood. Earlier, a member of our team reported that cannabinoid receptors CB1Rs participate in the regulation of the growth of axonal cones in GABAergic interneurons of rodent cortex in the gestation period [3]. The G protein-coupled receptors CB2 (CB2R) and GPR55 are overexpressed in cancer cells and cannabinoids affect functioning of these receptors [4]. GPR55 receptors were activated with both l-α-lysophosphatidylinositol (LPI) and certain cannabinoids [5]. In the submandibular acinar cells, cannabinoid receptors play a role of functional coupling system between electrolytes secretion with saliva fluid and $Ca^{2+}$ signalling [6]. Regulation of protein kinases in skeletal muscle cells was mediated via signalling by the cannabinoid receptor type 1 [7]. CB receptors are expressed not only in central nervous systems, but also in the immune system that suggests their role in the neurophysiological processes via their participation in the neuroinflammation processes [8].

Thiazolidinones are a class of small drug-like molecules revealing a wide range of pharmacological activities. The hypoglycemic, antibacterial, antivirus, anticancer, and anti-inflammatory are the best studied biological effects of thiazolidinones [9, 10]. These compounds have privileged scaffolds that are characteristic for drug-like molecular structures which provide a baseline affinity for a wide range of the biological targets [10]. Experiments in drug design permitted an evoking of multipoint interventions in molecular structures responsible for multiple mechanisms and different molecular targets [11]. Following this concept, the multi-target drugs dual or symbiotic drugs were synthesized [12]. 5-Ene-thiazolidinones showed an inhibitory activity towards glycogen synthase kinase-3 (GSK-3b) which has been emerging as a key therapeutic target not only in type-2 diabetes, but also at Alzheimer's disease, cancer, and chronic inflammation [13].

In this study, novel thiopyranothiazoles with the properties of endocanabinoids were synthesized. We hypothesized that the latter compounds are cyclic mimetics of the biologically active 5-ene-thiazolidinones [14]. Their capability of interacting with classical CB1 and CB2 cannabinoid receptors was demonstrated using the molecular docking analysis. Cell signaling regulators, such as protein kinase ERK1/2, MAP (mitogen-activated kinase) kinases, and cAMP response element binding protein (CREB), were measured in human glioblastoma cells of U271 line treated with the created cannabinoid ligands. Besides, the effect of these compounds was monitored with confocal imaging in cultured rat cortical neurons. While two of the studied compounds (**4**, **7**) are likely to be non-specific cannabinoid agonists, one of them (**4**) behaved like a potential antagonist of the CB1 receptors.

## Materials and Methods

*Chemistry.* The starting chromeno[4',3':4,5] thiopyrano[2,3-*d*]thiazol-2-ones **1** [15], **2** [16], isothiochromeno[4a,4-*d*]thiazol-2-one **5** [16] were synthesized in the Knoevenagel-*hetero*-Diels–Alder reaction. Ethyl rel-[(5aR,11bR)-10-bromo-2-oxo-5a,11b-dihydro-2*H*,5*H*-chromeno[4',3':4,5] thiopyrano[2,3-*d*][1,3]thiazol-3(6*H*)-yl]acetate **3** [15] was obtained in the reaction of compound **1** potassium salt with ethylchloroacetate.

Melting points were measured in open capillary tubes on a BUCHI B-545 melting point apparatus and were uncorrected. The elemental analyses (C, H, N) were performed using the Perkin–Elmer 2400 CHN analyzer and were within 0.4% of the theoretical values. The 1H NMR spectra were recorded on Varian Gemini 400 MHz or Bruker 125 MHz for frequencies 100 MHz in DMSO-$d_6$ using tetramethylsilane as an internal standard. Chemical shifts are reported in ppm units with the use of the *d* scale.

***Synthesis of ethyl rel-[(5aR,11bR)-10-bromo-2-oxo-5a,11b-dihydro-2H,5H-chromeno[4',3':4,5] thiopyrano[2,3-d][1,3]thiazol-3(6H)-yl]acetate (3).*** Synthetic protocol, spectral and analytical data are described [15].

***Synthesis of rel-N-(3-trifluoromethylphenyl)-2-(2-oxo-5a-methyl-(5aR,11bS)-3,5a,6,11b-tetrahydro-2H,5H-chromeno[4',3':4,5]thiopyrano[2,3-d]thiazol-3-yl)-acetamide (4).*** 0.01 Mol of compound **2** was stirred in 30 ml of ethanol. To the obtained suspension ethanol solution of potassium hydroxide (0.011 mol) was added. The reaction mixture was stirred at room temperature for 1 h. Obtained potassium salt was filtered, washed by ethanol and diethyl ether, and used without additional purification. Then a mixture of obtained potassium salt (0.01 mol), N-(3-fluoromethyl)-chloroacetamide (0.01 mol), and catalytic amounts of potassium iodate in the medium

of DMF (5 ml) and ethanol (15 ml) was refluxed for 6 h (TLC control of the reaction progress). After cooling, the reaction mixture obtained solid product was collected by filtration and recrystallized from DMFA/ethanol mixture.

Yield: 80%, mp 204-205°C. $^1$H NMR (400 MHz, DMSO-$d_6$): 0.82 (s, 3H, $CH_3$), 2.90 (d, 1H, J = 11.4 Hz, 5-H), 3.07 (d, 1H, J = 11.4 Hz, 5-H), 3.94 (d, 1H, J = 10.4 Hz, 6-H), 4.11 (s, 1H, 11b-H), 4.17 (d, 1H, J = 10.4 Hz, 6-H), 4.58 (s, 2H, $CH_2$), 6.90 (d, 1H, J = 8.0 Hz, 8-H), 7.01 (t, 1H, J = 7.2 Hz, 10-H), 7.22 (t, 1H, J = 7.6 Hz, 9-H), 7.44 (t, 2H, J = 7.3 Hz, arom.), 7.58 (t, 1H, J = 7.9 Hz, arom.), 7.75 (d, 1H, J = 7.9 Hz, arom.), 8.07 (s, 1H, arom.), 10.75 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 16.2, 33.9, 35.4, 41.1, 45.8, 73.2, 104.1, 115.2 (d, J = 3.3 Hz), 117.0, 120.0 (d, J = 3.4 Hz), 120.8, 121.0, 123.0, 123.7 (q, J = 249.6 Hz), 125.1, 127.7, 128.4, 129.6 (q, J = 31.4 Hz), 130.2, 139.3, 153.7, 165.1, 169.2. LCMS (ESI): m/z 493 (100%, $[M+H]^+$). Anal. Calcd for $C_{23}H_{19}F_3N_2O_3S_2$, %: C, 56.09; H, 3.89; N, 5.69. Found, % C, 56.20; H, 3.80; N, 5.60.

***Synthesis of rel-3-(2-oxo-(5aR,8R,9aR)-5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d]thiazol-3-yl)-propionitrile (6).*** To the 0.01 mol of compound **5**, a mixture of pyridine (50 ml) and water (10 ml) containing 3 ml of acrylonitrile was added. The reaction mixture was refluxed for 6 h, the solid product was formed by adding water. The obtained precipitate was filtered and recrystallized from ethanol.

Yield: 76%, mp 96-98 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): 0.90 (d, 3H, J = 6.4 Hz, $CH_3$), 0.94-1.09 (m, 3H), 1.29 (s, 3H, $CH_3$), 1.36 (s, 3H, $CH_3$), 1.51 (br.s, 1H), 1.66 (t, 1H, J = 10.8 Hz), 1.76 (d, 1H, J = 12.4 Hz), 1.82-1.92 (m, 2H), 2.33 (t, 1H, J = 11.6 Hz), 2.85 (t, 2H, J = 6.4 Hz, $CH_2$), 3.79 (t, 2H, J = 6.4 Hz, $CH_2$). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 17.2, 22.3, 23.3, 25.9, 27.6, 31.9, 34.8, 36.7, 38.9, 42.3, 48.8, 50.7, 107.1, 118.2, 121.2, 170.1. LCMS (ESI): m/z 323 (100%, $[M+H]^+$). Anal. Calcd for $C_{16}H_{22}N_2OS_2$, %: C, 59.59; H, 6.88; N, 8.69. Found, % C, 59.40; H, 6.80; N, 8.60.

***Synthesis of rel-N1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-4-pyrazolyl)-2-(2-oxo-(5aR,8R,9aR)-5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d] thiazol-3-yl)-acetamide (7).*** To the suspension of compound **5** (0.01 mol) in ethanol medium, an ethanol solution of potassium hydroxide (0.011 mol) and catalytic amounts of potassium iodate was added. After stirring the reaction mixture 2-chloro-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-acetamide (0.011 mol) was added. The mixture was refluxed for 5 h (TLC control of the reaction progress) and precipitated by the addition of water. The obtained precipitate was filtered and recrystallized from ethanol.

Yield: 74%, mp 176-178°C. $^1$H NMR (400 MHz, DMSO-$d_6$): 0.91 (d, 3H, J = 6.4 Hz, $CH_3$), 0.95-1.00 (m, 3H), 1.29 (s, 3H, $CH_3$), 1.30 (s, 3H, $CH_3$), 1.48-1.55 (m, 1H), 1.59 (t, 1H, J = 11.0 Hz), 1.69 (d, 1H, J = 11.0 Hz), 1.87 (m, 2H), 2.13 (s, 3H, $CH_3$), 2.29 (t, 1H, J = 9.4 Hz), 3.06 (s, 3H, $CH_3$), 4.33 (d, 1H, J = 17.6 Hz, $CH_2$), 4.37 (d, 1H, J = 17.6 Hz, $CH_2$), 7.30-7.40 (m, 3H, arom.), 7.52 (t, 2H, J = 7.2 Hz, arom.), 9.53 (s, 1H, NH). LCMS (ESI): m/z 513 (100.0%, $[M+H]^+$). Anal. Calcd for $C_{26}H_{32}N_4O_3S_2$, %: C, 60.91; H, 6.29; N, 10.93. Found, % C, 60.93; H,6.31; N, 10.90.

***Biological experiments.*** MTT assay. *In vitro* screening of anticancer activity of the synthesized compounds and doxorubicin used as a reference drug control towards cancer cell lines was measured using the MTT assay [17]. Tumor cells were seeded for 24 h in 96-well microtiter plates at a concentration of 2,000 substrate-dependent cells/well or 10,000 suspensions cells/well (100 μl/well). After that, cells were incubated for 72 h with various additions of the synthesized compounds (0; 0.01; 0.1; 0.5; 1; 10 μM). MTT which is converted to dark blue, water-insoluble MTT formazan by the mitochondrial dehydrogenases was used to determine viable cells according to the manufacturer's protocol (Sigma-Aldrich, USA). The $IC_{50}$ of the tested compounds was calculated as a concentration of drug killing 50% of cells in comparison with an untreated culture.

*Western-blot analysis.* Stimulation of protein kinase (ERK1/2) mitogen-activated kinase MAP-kinases and cAMP response element binding protein (CREB). After 30 min exposure to the tested compound, cellular proteins were isolated, resolved by SDS/PAGE, and transferred onto a polyvinylidene difluoride (PVDF) membrane for Western-blotting, as described [18]. The following antibodies (Cell Signaling Technology, USA) were used at a 1:1,000 dilution: anti-p38, anti-ERK ½, anti-DARPP-32, anti-phospho-DARPP-32, anti-pSTAT3 (Tyr 705), anti-phospho-CREB, anti-JNK (sc-571). Equal loading of protein on each lane was evaluated by the immunoblotting of the same membrane with anti-beta-actin monoclonal mouse AC-15 (Sigma-Aldrich, USA).

All secondary peroxidase-labeled antibodies (Cell Signaling, USA) were used at a working dilution of 1:5,000.

*Culture of cortical neurons.* Primary cortical neurons were prepared from brain of 1-day-old Wistar rats and maintained in a neurobasal medium (Gibco BRL, Paisley, U.K.). Rats were decapitated (Danylo Halytsky Lviv National Medical University Ethical Committee Approval No 9 21/12/2018), the cerebral cortices were dissected, then incubated in phosphate-buffered saline (PBS) with trypsin (0.25 μg·ml$^{-1}$) for 25 min at 37°C. Then, the cortical tissue was triturated in PBS containing soybean trypsin inhibitor (0.2 μg·ml$^{-1}$) and DNAse (0.2 mg·ml$^{-1}$) and gently filtered through a sterile mesh filter (40 μm). The suspension was centrifuged at 2,000 × g for 3 min at 20°C and the pellet was resuspended in a warm neurobasal medium, supplemented with heat-inactivated horse serum (10%), penicillin (100 U·ml$^{-1}$), streptomycin (100 U·ml$^{-1}$) and glutamax (2 mm). Suspended cells were plated at a density of 0.25×10$^6$ cells on circular 10 mm diameter coverslips, coated with poly-l-lysine (60 μg·ml$^{-1}$), and incubated in a humidified atmosphere containing 5% $CO_2$: 95% air at 37°C for 2 h before being flooded with the prewarmed neurobasal medium. After 48 h, 5 ng·ml$^{-1}$ cytosinearabinofuranoside was added to the culture medium to suppress the proliferation of non-neuronal cells. The medium was exchanged for 3 days and cells were grown in culture for up to 14 days.

*Confocal microscopy and pCREB immunolabelling.* Cortical neurons cultured on coverslips were treated with ligands for 30 min at 37°C. Cells were then fixed using 100% methanol at -20°C for 10 min. Cells were then treated with a mouse antibody against pCREB (1:250; Millipore, Billerica, USA) for 60 min at room temperature, followed by Alexa-fluor 488 donkey-anti-mouse secondary antibody (1:500; Molecular Probes, Life Technologies, Paisley, UK) applied for 30 min. Then, the coverslips were mounted onto slides and images were obtained using a laser scanning confocal microscope imaging system (Zeiss LSM510 Meta; Carl Zeiss Microscopy Ltd, Cambridge UK).

*Molecular docking.* The molecular docking simulations were performed to assess the affinity of the synthesized compounds to CB1 and CB2 receptors. 2D structures of the synthesized ligand molecules were drawn by Biovia Draw v.21.1 and converted to energy-minimized 3D structures by Hyperchem 7.5 [19]. The X-ray crystallographic structures of the CB1 (PDB code: 5XRA) [20, 21] and CB2 (PDB code: 6PT0) were retrieved from the protein data bank. Molecular docking simulations using the ligand molecules were conducted using the Autodock 4.2.6 docking suite by employing the Lamarckian genetic algorithm [22, 23]. The preparations of proteins and further processing of ligand data were done in the AutoDock Tools suite. Before docking, the protein crystal structures were cleaned by removing the water molecules and other inclusions. Polar H-atoms were added to these target proteins for correct ionization and tautomeric states, and non-polar H-atoms were merged. Docked ligands were set as flexible and the Gasteiger charges were added to them. The grid maps representing the center of active site pockets for the ligand were calculated using the Autogrid Tools. The grid dimensions for CB1 and CB2 were 60*60*60 points with a spacing of 0.375 Å between the grid points. Validations of the docking parameters were made by the redocking technique. During those procedures, ligands were removed from the binding sites and docked again. Overlaying the crystallographic on the theoretically docked structure enabled the RMSD to be calculated. Obtained RMSD values of <2.0 Å indicated that the methodology was appropriate for relevant data (Fig. 1). 100 poses docking conformations were generated and the best-docked conformation was selected based on the free binding energy (kcal/mol) and Inhibition Constant ($K_i$) for further analysis. Data obtained from the redocking procedures were used for quantitative estimating of

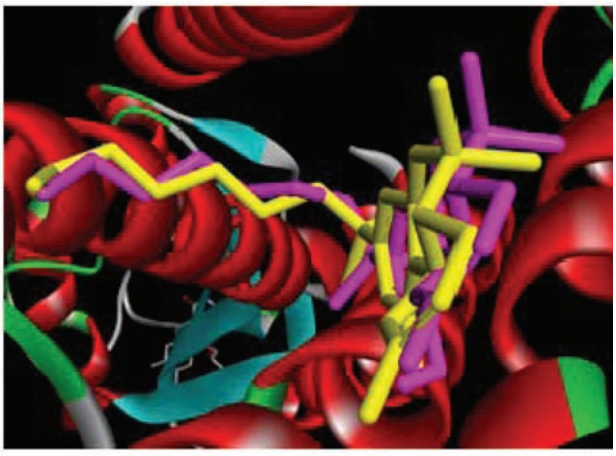


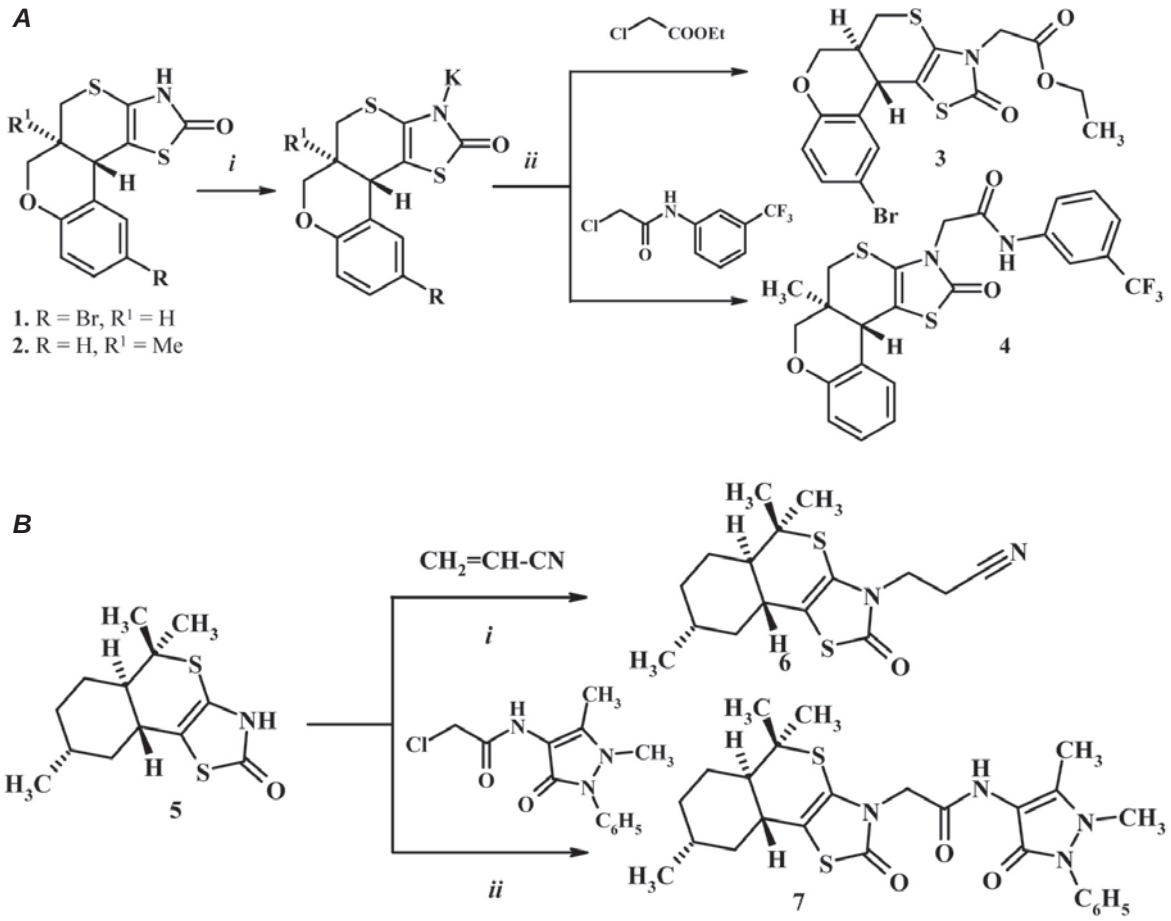
*Fig. 1. Real (yellow-colored) and predicted (pink-colored) positions of AM11542 inside CB1 receptor (PDB code: 5XRA, RMSD = 0.79)*

ligands affinity to our targets and compared to established agonists. Finally, the conformations with the most favorable free binding energy were selected for analyzing the interactions between the target cannabinoid receptors and ligands by Discovery Studio Visualizer v 21.1.0.20298 and AutoDock Tools suite.

## Results

In this study, we used a range of functional assays to compare the pharmacological activity of selected potential cannabinoid-like ligands **1**, **3**, **4**, **6**, and **7**, as well as endogenous lysophospholipid L-alysophosphatidylinositol (LPI) as positive control. Target compounds were synthesized according to multi-step protocol [15, 16] as summarized in Scheme 1 (ESI). *rel*-N-(3-Trifluoromethylphenyl)-2-(2-oxo-5a-methyl-(5a*R*,11b*S*)-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4',3':4,5]thiopyrano[2,3-*d*] thiazol-3-yl)-acetamide **4** was synthesized in the alkylation reaction of compound **2** potassium salt by 3-trifluoromethylphenylacetamide in the presence of catalytic amounts of potassium iodide. 2-(2-Oxoisothiochromeno[4a,4-*d*]thiazol-3-yl)acetamide **7** was synthesized using the same synthetic procedure, as for compound **4**. To obtain propionitrile **6** cyanoethylation reaction of compound **5** with acrylonitrile in the pyridine medium was performed.

To investigate the biological actions of these substances, we evaluated their effects on glioblastoma cells, cultured cortical neurons, and cells of HEK293 line expressing GPR55 (ESI). Initially, the effects of compounds on glioblastoma proliferation and viability were established, with no evidence of toxicity observed up to 10 μM (Fig. 2). Next, various




*Scheme.* ***(A)*** *Synthesis of 3,5a,6,11b-tetrahydro-2H,5H-chromeno[4',3':4,5]thiopyrano[2,3-d]thiazoles* ***3*** *and* ***4****. Reagents and conditions: i) KOH, EtOH, r.t., 1h; ii) chloroacetic acid derivative, DMF:EtOH, KI (cat.), reflux, 5-6 h.* ***(B)*** *Synthesis of isothiochromeno[4a,4-d]thiazoles* ***6*** *and* ***7****. Reagents and conditions: i) acrylonitrile, pyridine, $H_2O$, reflux, 6 h; ii) 2-chloro-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-acetamide, KOH, EtOH, KI (cat.), $K_2CO_3$, reflux, 5 h*

signaling readouts were evaluated in the glioblastoma cells. In these experiments, cells were incubated for 30 min with the following substances: 1 – control (untreated cells), 2 – LPI (positive control), 0.3 μM, 3 – LPI, 3 μM, 4 – compound **3**, 1 μM, 5 – compound **3**, 10 μM, 6 – compound **7**, 1 μM, 7 – compound **7**, 10 μM. The phosphorylation of ERK1/2 protein has been reported as one of the main signaling pathways initiated upon stimulation of the GPR55 receptor. It was found that there was a modest MAPK 42/44 phosphorylation in cells treated with LPI and the experimental compounds **3** and **7**. Likewise, using analysis of pCREB, LPI and compounds **3** and **7** showed an increased activity (Fig. 2). DARPP (dopamine and cyclic AMP-regulated phosphoprotein) is the protein that accumulates in neurons [24]. It is a bifunctional signaling molecule that controls serine/threonine kinase and serine/threonine phosphatase activity [25]. DARPP is activated by cAMP or dopamine. Cyclic AMP induces phosphorylation of DARPP in position 34 of threonine, and this leads inhibition of protein phosphatase-1 [26]. The control of the activity of protein phosphatase-1 by DARPP plays an important role in neuronal excitability [24]. In experimental studies, pDARPP-32 protein levels also increased following the treatment with LPI and compounds **3** and **7** (Fig. 3).

Next, we investigated the effects of LPI and synthetic cannabinoids on pCREB immunoreactivity in cultured cortical neurons (ESI). Increased pCREB labeling was detected within the nucleus for cells treated with LPI and compounds **3** and **7** (Fig. 3). Taking into account that GPR55 might activate pCREB, we evaluated more directly the effects of these compounds on GPR55 receptor trafficking in HEK293 cells which expressed HA-tagged GPR55 (Fig. 4). It was known from published articles that GPR55 agonists induced receptor internalization [27], thus, the receptor antagonists should block this internalization. The internalization of the FITC-labeled fluorescence by AM251 in the presence of the GPR55 antagonist compounds **1** and **3** respectively were shown to be much more pronounced than with the antagonist treatment alone. No clear

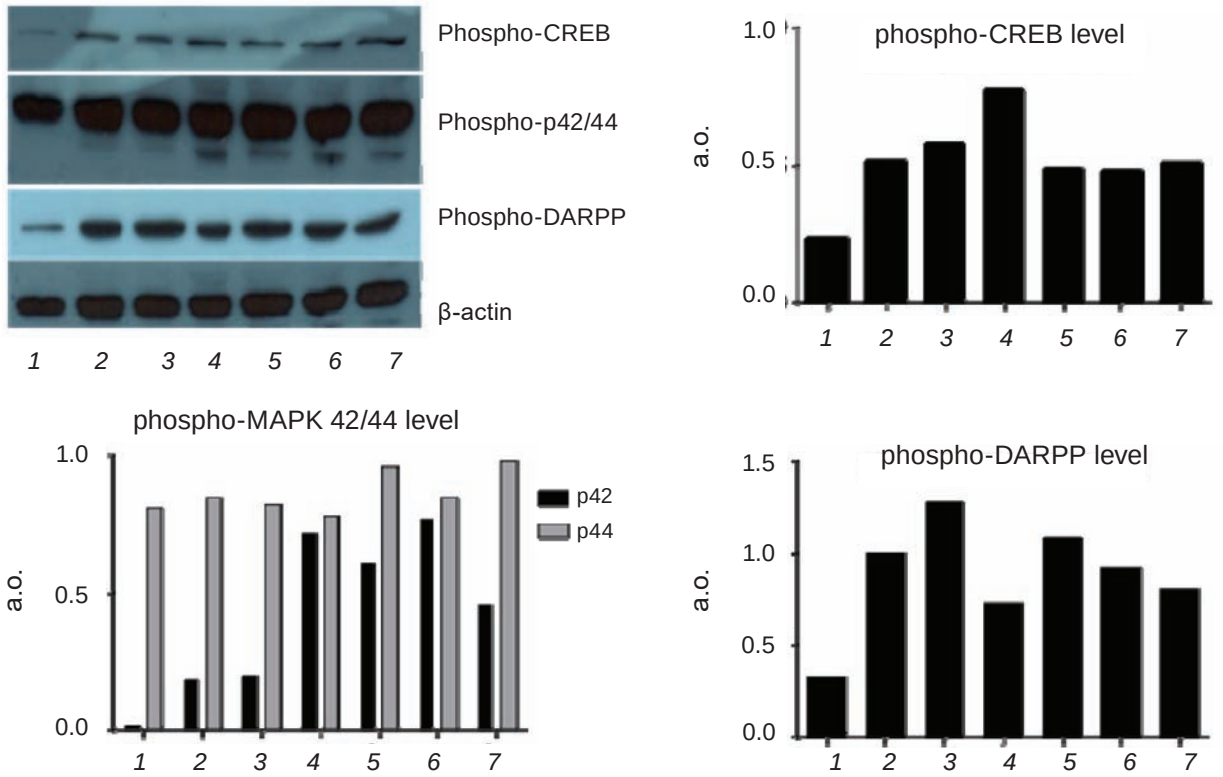



*Fig. 2. Levels of protein kinase (ERK1/2) mitogen-activated kinase MAP-kinases and cAMP response element binding protein (CREB) were measured by Western blot analysis in U271 glioblastoma cells (1 – control; 2 – LPI, 0.3 μM; 3 – LPI, 3 μM; 4 – compound **3**, 1 μM; 5 – compound **3**, 10 μM; 6 – compound **7**, 1 μM; 7 – compound **7**, 10 μM)*

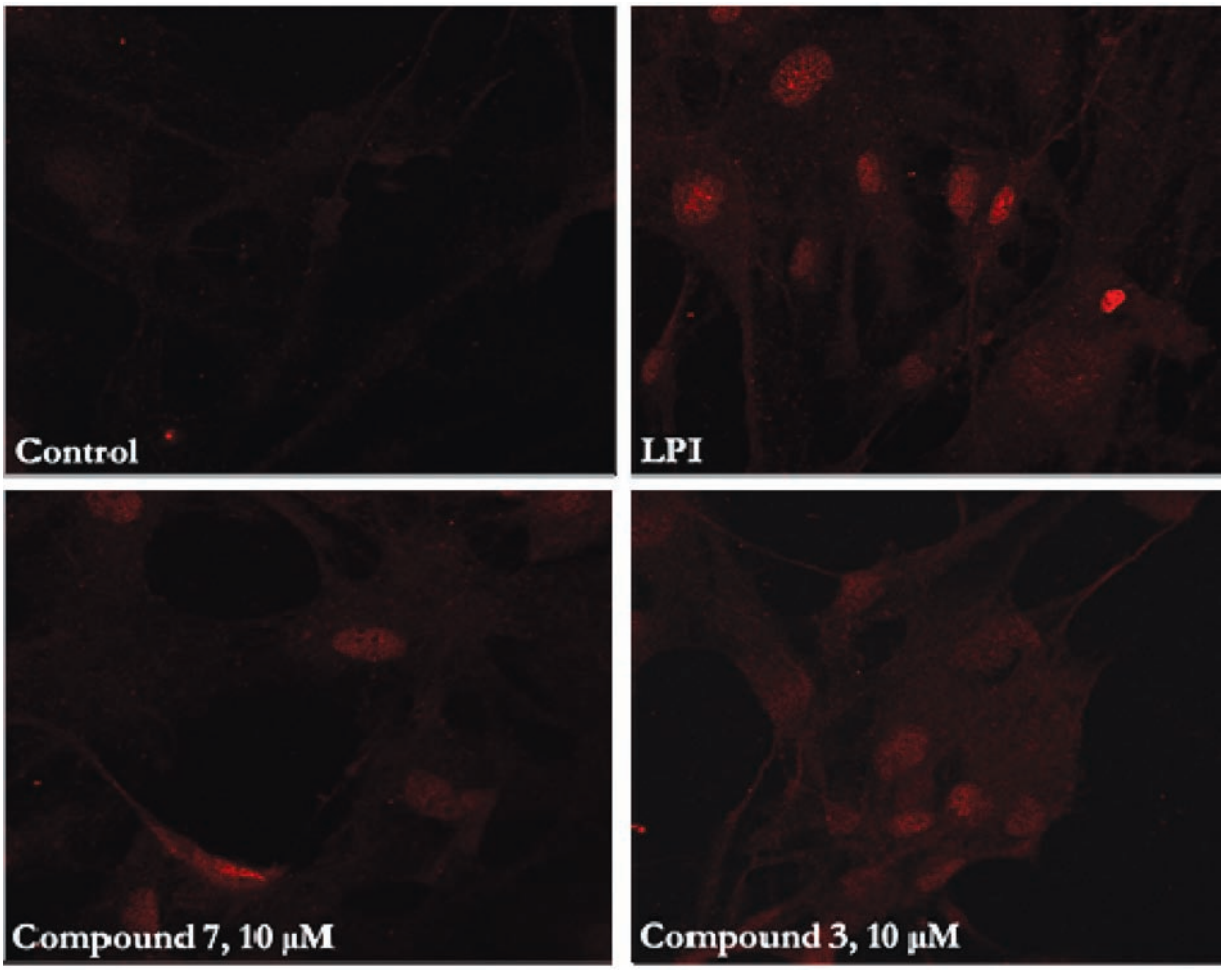



*Fig. 3. Level of pCREB labeling in cultured cortical neurons. Confocal microscopy images of pCREB labeling (red) following the treatment with DMSO control, LPI (1 μM), compound **7** (10 μM), and compound **3** (10 μM). Cells were stained with a mouse antibody against pCREB (1:250; Millipore, Billerica, USA) for 60 min at room temperature, followed by 30 min staining with Alexa-fluor 488 donkey-anti-mouse secondary antibody (1:500; Molecular Probes, Life Technologies, Paisley, UK)*

effects of compounds **1** and **3** were observed, suggesting that these compounds may not target this receptor. However, effects on GPR55 signaling remain to be studied.

To gain insight into the putative binding mode of our compounds with CB1 and CB2, they were docked into a structure of human CB1 receptor and human CB2 receptor (ESI). Docking simulation confirmed the good affinity of the synthesized compounds to CB1 and CB2 receptors. The most potent compound **7** demonstrated excellent results either to CB1 (-14.08 kcal/mol) or CB2 (-12.27 kcal/mol) receptors. In addition, compound **4** possesses binding energy with CB1 receptors higher than reported agonist AM11542, which is a tetrahydrocannabinol derivative. Also, derivative 4 shows good affinity to CB2 approximate to reference ligand Win 55 (-10.68 kcal/mol for 4 and -11.43 kcal/mol for Win 55). According to the in silico simulations, all compounds are rather nonselective with a moderate prevalence to CB1 receptors (Table).

Compound **7** forms necessary interactions with key residues Phe268, Ile 267 of CB1, and especially Ser383, which is a significant point for cannabinoid-like agonists because its mutation greatly reduces the agonist activity of AM11542 (Figure 1, *A*) [21]. Inside CB2 derivative 4 forms a Pi-Pi stacked interaction with one of the key residues Phe117. Additionally, the molecule connects to the CB2 by three hydrogen bonds with Lys278, Ser 285, and His 95, respectively. CF3 group forms bonds with the Glu181 and Leu182 (Fig. 5, *B*). Also, compound **4** locates far from Trp258, which is crucial for an antagonistic activity for binding ligands [28].

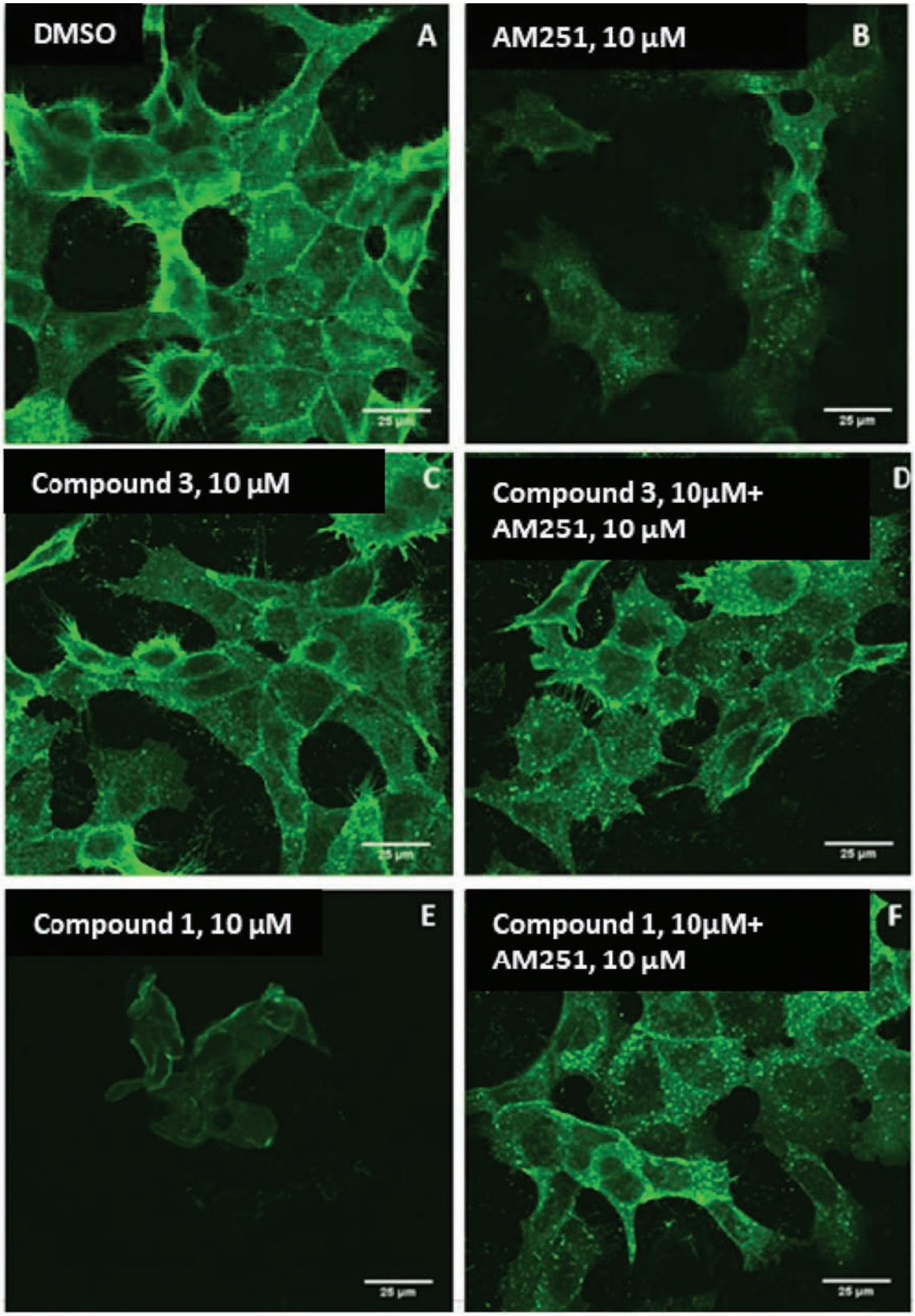



*Fig. 4. Compound **3** (10 uM, GPR55 ligand), compound **1** (10 uM) and GSK71747A (10 uM) do not inhibit AM251 (10 uM)-induced internalization of cannabinoid receptor. Labeling of receptor trafficking in hGPR55-HEK293 cells treated with: (**A**) DMSO; (**B**) AM251; (**C**) compound **3;** (**D**) compound **3** plus AM251; (**E**) compound **1**; (F) compound **1** plus AM251. **A**-**F**, n = 3. Scale bar = 25 µm*

*T a b l e. Docking results of the compounds with CB1 and CB2*

| Comp. | Binding energy | | Inhibition concentration | | H-bonds connection | | Length (A) | |
|---|---|---|---|---|---|---|---|---|
| | CB1 | CB2 | CB1 | CB2 | CB1 | CB2 | CB1 | CB2 |
| **1** | -8.13 | -7.89 | 1.64 uM | 1.09 uM | – | His95 | 2.57 | – |
| **3** | -8.75 | -8.91 | 292.97 nM | 384.27 nM | Ser383 | Trh114 | 1.81 | 1.83 |
| **4** | -11.58 | -10.68 | 14.77 nM | 3.25 nM | Ser383 | His95<br>Ser285<br>Lys278 | 1.89<br>2.02<br>2.61 | 2.09 |
| **6** | -9.19 | -8.41 | 681.70 nM | 182.70 nM | His178<br>Ser383 | Ser90 | 1.92 | 2.02<br>2.98 |
| **7** | -14.08 | -12.27 | 1.02 nM | 47.48 pM | Ser383 | – | – | 2.03 |
| AM11542 | -9.79 | | 66.89 | – | | Ser383 | 2.08 | |
| Win 55 | – | -11.43 | – | 4.17 nM | – | – | – | – |

## Discussion

It was shown that the endocannabinoid system (eCBs) plays an important role as a therapeutic target in AD treatment [29,30]. The eCBs include receptors that are present on cells of the central nervous system (CNS). The best-known cannabinoid receptors are CB1 and CB2, however novel receptors (GPR55, GPR118, and GPR19) have been described recently [27, 31]. CB1 receptors are activated by cannabinoids and are classically coupled with GI/O proteins, which cause inhibition of adenylate cyclase and decrease the concentration level of cAMP (Cyclic adenosine monophosphate). At the same time, it causes an increased concentration level of MAPK (Mitogen-activated protein kinase) [32]. GPCRs that coupe with GS protein can increase the levels of cAMP, via the stimulation of adenylate cyclase [32, 33]. cAMP is a second messenger and it activates different kinds of protein kinases or other proteins (ERK, DARPP) and the transcription factor CREB (cAMP response element-binding protein). CREB can also respond to many different signaling pathways including calcium/CaM kinase and MAP kinase. It regulates a variety of biological functions, including long-term memory [34], and neuronal activity [35]. The mechanism via which CREB regulates long-term memory is not yet known [36]. It can activate or depress synaptic strength or model new synaptic connections [37]. ERK (extracellular-signal-regulated kinases) belong to the classical group of MAP kinases. ERK classically regulates cell proliferation and differentiation via downstream phosphorylation of nuclear targets. Interestingly, ERK can phosphorylate RSK [38] and RSK proteins are serine-threonine kinases, which can phosphorylate and activate CREB [38-40]. In the current study, all the ligands tested were able to activate ERK and CREB. Both of these pathways are downstream of GPR55, however, the effects of the putative cannabinoid compounds on GPR55 activity remain to be established.

*Conclusions.* We have evaluated a new series of cannabinoid ligands that have clear effects on signaling in glioblastoma cells and neurons. In particular, activation of pCREB might be beneficial concerning neurodegeneration, as this pathway can influence cell survival. Performed molecular docking studies had shown good affinity of the synthesized compounds to CB1 and CB2 receptors with the most potent derivative **7**, demonstrating excellent scoring functions to CB1 and CB2. However, the precise molecular targets for these ligands are currently under evaluation.

*Conflict of interest.* The authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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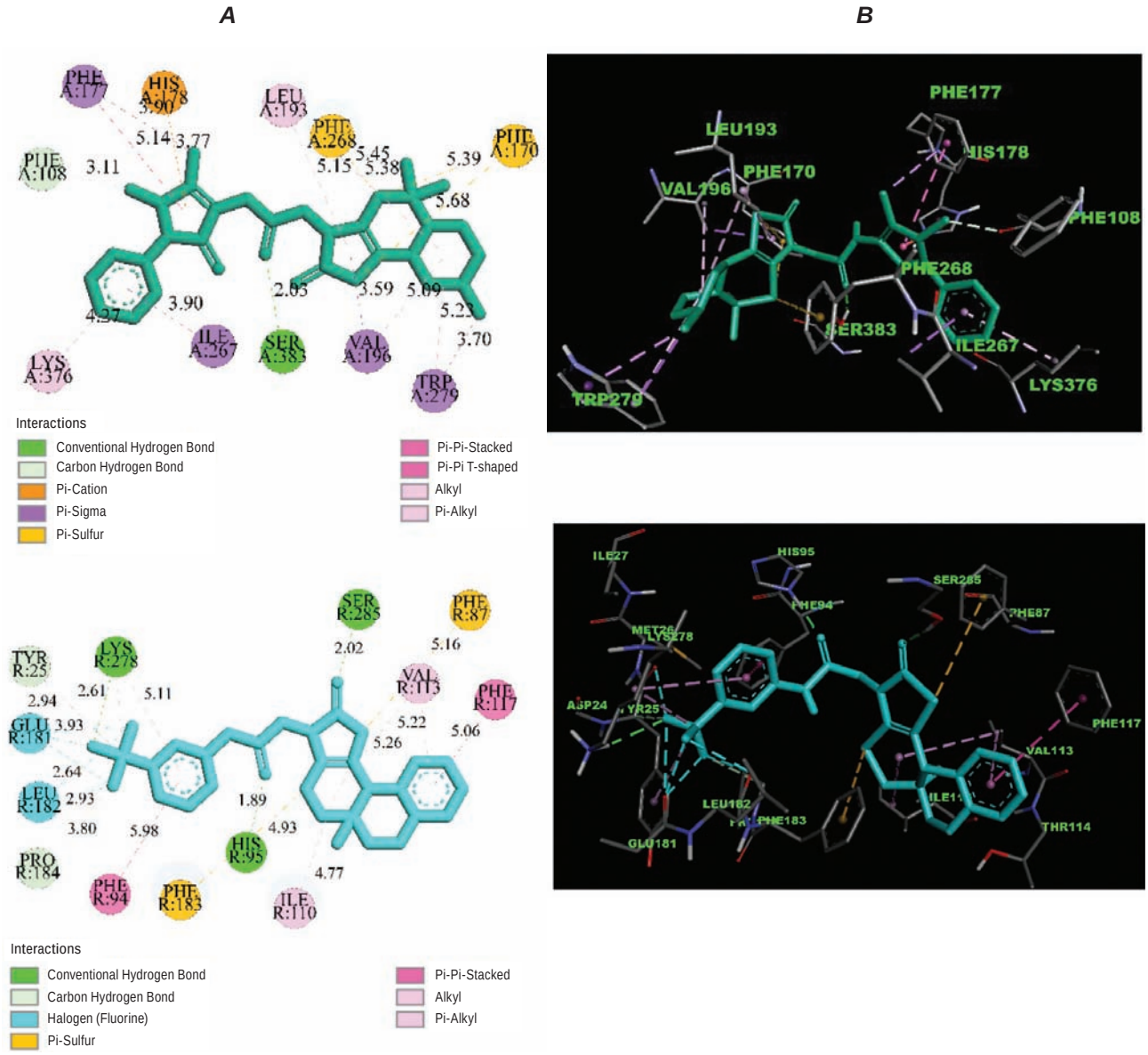


*Fig. 5. Overview of CB1 and CB2 receptor showing the location of the proposed binding sites for studied compounds **7** and **4** correspondingly. **A**. 2D & 3D interaction **7** with CB1 (PDB code: 5XRA). **B**. 2D & 3D interaction maps of 4 with CB2 (PDB code: 6PT0)*

## НОВІ ПОТЕНЦІЙНІ ЛІГАНДИ КАНАБІНОЇДНИХ РЕЦЕПТОРІВ: СИНТЕЗ І ВПЛИВ НА СИГНАЛЬНІ СИСТЕМИ КЛІТИНИ ТА ФУНКЦІЇ НЕЙРОНІВ

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Відомо, що канабіноїдні ліганди володіють нейропротекторною дією і можуть бути корисними у терапії нейродегенеративних захворювань. Основні мішені для канабіноїдів включають класичний канабіноїдний рецептор CB1, а також новий канабіноїдний рецептор GPR55, який зв'язується з багатьма синтетичними канабіноїдними лігандами. У цьому дослідженні було синтезовано нові тіопіранотіазоли **1**, **3**, **4**, **6** і **7**. Їхня фармакологічна активність як потенційних канабіноїдоподібних лігандів була оцінена в клітинах гліобластоми, культивованих кортикальних нейронах і клітинах лінії HEK293, що експресують GPR55. Стимуляцію протеїнкінази ERK1/2, MAP-кінази і протеїну, що зв'язує елемент відповіді cAMP (CREB), оцінювали за допомогою Вестерн-блот аналізу, активацію CREB додатково контролювали за допомогою конфокальної мікроскопії ядерного фосфо-CREB-мічення. Моделювання докінгу підтвердило хорошу спорідненість синтезованих сполук до рецепторів CB1 і CB2. Вражаючі ефекти хромено[4',3':4,5]тіопірано[2,3-*d*][1,3]тіазолу з фрагментом етилацетату **3** та ізотіохромено[4a,4-*d*]тіазолу з феназоновим фрагментом **7** спостерігали за активацією pCREB як індикатором стимуляції шляху, сприятливого для виживання нейронів.

Ключові слова: тіопіранотіазоли, G-протеїновий рецептор (GPCR), CB1, CB2, потенційні канабіноїдні ліганди, функції нейронів.

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