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In Vitro Effect of 8-Prenylnaringenin and Naringenin on Fibroblasts and Glioblastoma Cells—Cellular Accumulation and Cytotoxicity

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Abstract: Gliomas are one of the most aggressive and treatment-resistant types of human cancer. One of the most promising field in gliomas cancer therapy is identification and evaluation of anticancer properties of compounds found in plants i.a. naringenin (N) and 8-prenylnaringenin (8PN). The prenyl group seem to be crucial to the anticancer activity of flavones, which may lead to enhanced cell membrane targeting and thus increased intracellular activity. Unfortunately, 8PN content in hop cones is from 10 to 100 times lower compared to other flavonoids i.e. xanthohumol. In this study we used a simple method for the synthesis of 8PN from isoxanthohumol, *via O*-demethylation with high, 97% of the isolated yield. Cellular accumulation and cytotoxicity of naringenin and 8-prenylnaringenin in normal (BJ) and cancer cells (U-118 MG) were also examined. Obtained data indicated that 8-prenylnaringenin exhibited higher toxicity against used cell lines than naringenin and both flavones inhibited stronger glioblastoma U-118 MG cells than normal fibroblasts. The anticancer properties of 8PN correlated with its significantly greater (37%), accumulation in glioblastoma cells than in normal fibroblasts. Additionally, naringenin indicated higher selectivity for glioblastoma as it was over 6 times more toxic for cancer than normal cells. Our results provide evidence that examined prenylated and non-prenylated flavanones have different biological activity against normal and cancer cell lines and this phenomenon may be useful in clinical practice to construct new, anticancer drugs for glioblastoma.

1. Introduction

A wide spectrum of biologically active flavonoids, including compounds from hop (*Humulus lupulus* L., *Cannabaceae*), encourage researchers to evaluate their possible application in therapies for various diseases. This activity is based on molecular mechanisms involving mainly inhibition of cyclooxygenase (COX), and

lipooxygenase (LOX) [1]. Common hop contains over 1000 of various chemical substances and it is the main dietary source of prenylated flavonoids. The most important hop flavonoids include xanthohumol, isoxanthohumol, naringenin, 8-prenylnaringenin and their derivatives.

Flavonoids have positive effect on nervous system, improving blood flow to the brain. It helps in formation of new blood vessels and growth of hippocampal neurons, which improve the memory processes. Such properties help to develop cognitive skills, which may be important for example in Alzheimer disease therapy. In research with the use of hippocampal cells, naringenin promotes neurogenesis and stimulates damaged neurons to growth [2]. Although plants have been used for a long time in therapy of various diseases, it is important to know the activity of individual compounds. According to the research by Kuete et al. (2014), isolated naringenin was more efficient than the plant extract itself [3].

Due to their pro-healthy properties, hop flavonoids and their synthetic derivatives have been thoroughly studied. Xanthohumol, the most important chalcone, which amounts up to 1% of the hop-cone dry weight, has many biological properties. Apart from strong antioxidant activity, it was proved to have antiviral [4], antimicrobial [5] and anti-inflammatory action [6]. Moreover, *in vitro* study proved that xanthohumol inhibits formation of new blood vessels during carcinogenesis and it has antiproliferative properties against human cancer cell lines: breast (MCF-6, MCF-7, T47-D), colon (HT-29), ovarian (A-2780) and prostate ones [7 - 12].

There is some evidence that consumption of flavonoids considerably reduces the risk of some types of cancers - breast, colon, lung, prostate, and pancreas [13]. The mechanism of blocking DNA replication by inhibiting the activity of such enzymes as DNA polymerase II and topoisomerases I and II is known. Flavonoid compounds are also involved in cell cycle arrest, which results in blocking proliferation and inducing apoptosis of cancer cells. They are also capable of preventing oncogene activation by interactions with metabolic enzymes, for example by inhibition of cytochrome P450, such as CYP1A1 and CYP1A2 [14].

In recent years there is also a great interest in 8-prenylnaringenin, the compound which content in hop cones is from 10 to 100 times lower compared to xanthohumol [10]. 8-Prenylnaringenin, a potential anticancer drug, demonstrates *in vitro* stronger affinity for the estrogen receptor ERα than coumestrol and genistein, which have been considered as the most active flavonoids, so far [15]. Movement of the prenyl substituent from C-8 to C-6 results in a loss of estrogenic activity. Additionally, other prenyl derivatives of naringenin present in hops, such as 6-prenylnaringenin, may play an important role in post-menopausal hormone-replacement therapy, among others being catalysts of regioselective estrogene hydroxylation, which reduces the risk of breast cancer [16].

Based on diverse biological activity of flavonoids, their derivatives from hops may be potential therapeutic agents for treatment of glioblastomas. Epidemiological

studies have shown that the most common malignant glioblastoma multiforme is resistant tumor of the central nervous system in humans and effective strategies to inhibit its progression are especially needed. The major problem in the treatment of glioblastomas is low penetration of anticancer drugs through the blood-brain barrier and their low toxicity against cancer cells [17]. This phenomenon is caused by presence of multidrug resistance proteins e.g. p-glycoprotein in blood-brain barrier (BBB) and in many glioblastoma cell lines [18,19].

A large number of studies have indicated that many flavonoids have an inhibitory activity on P-glycoprotein mediated efflux [20]. Di Pietro et al. showed that prenylation of the flavonoids increased their affinity for P-glycoprotein and their intracellular accumulation [21]. Although naringenin is able to cross the BBB *in vitro* and *in vivo* model [22] and naringenin-induced apoptosis in glioma cells has been reported [23], the effect of 8-prenylnaringenin on glioma cells remains unclear. In current study, we for the first time examined in detail cellular accumulation, distribution and cytotoxicity of naringenin and 8-prenylnaringenin in U-118 MG cells compared with normal human cells - fibroblasts.

2. Results and discussion

2.1. Synthesis of 8-prenylnaringenin

The most potent known phytoestrogen contained in hop cones (*Humulus lupulus*), and also in such plants as *Marshalia grandiflora* and *Sophora tomentosa*, is 8-prenylnaringenin. Because of its interesting biological properties, the methods of obtaining 8-prenylnaringenin are being developed in addition to isolation protocols from natural sources. One of them is a four-step synthesis starting from naringenin, using an europium (III) complex and prenyl bromide in the Claisen-Cope rearrangement. However, this is a multi-step process, requiring protection of the 7-OH and 4'-OH hydroxyl groups and therefore the reaction yield was moderate (48%) [24]. An alternative method described by Wilhelm and Wessjohann [25] involves demethylation of isoxanthohumol. The authors tested various demethylation agents and achieved the yield of 92%.

In our study isoxanthohumol, obtained by alkaline isomerization of xanthohumol isolated from hops, was subjected to *O*-demethylation with ethereal solution of MgI_2 to give 8-Prenylnaringenin with 97% of the isolated yield. The obtained product was characterized spectroscopically. In the ^{13}C NMR the signals we ascribed to carbon atoms using NMR decoupling spectra and correlation 2D NMR spectra.

The analysis of the product spectral data (^1H NMR, ^{13}C NMR, IR, MS and UV) led to the conclusion that we have obtained the isoxanthohumol derivative without the methoxy group at C-5. In the HR ESI-MS spectrum we observed the $[M-H]^-$ ion of m/z = 339.1235, which is in accordance with the mass of $[C_{20}H_{20}O_5-H]^-$ (339.1232). In the IR spectrum there is no absorption band at 1275 cm^{-1}, which was observed for the substrate ($-OCH_3$ group).

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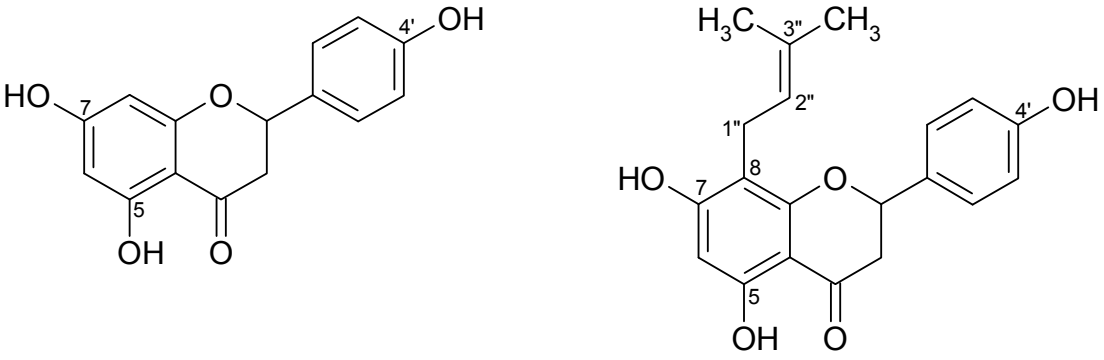
In the low-field region of the ^{1}H NMR spectrum there is a one-proton singlet at δ = 12.14 ppm of free 5-OH group. The demethylation in the aliphatic region of the ^{1}H NMR of the product was evidenced by absence of three-proton singlet at δ = 3.73 ppm, present in the spectrum of isoxanthohumol. Due to the presence of the C-2 atom in 8-Prenylnaringeninin one can see in the ^{1}H NMR spectrum also the signals of the geminally coupled hydrogen atoms at C-3 (J = 17.0 Hz). In the DEPT 135° spectrum of the product there are no signals of C=O (δ = 198.6 ppm), C-8 (δ = 109.3 ppm), C-9 (δ = 165.9 ppm), C-10 (δ = 104.3 ppm) and C-1′ (δ = 132.1 ppm), which indicate that they are the quaternary carbon atoms. Whereas the signals of methylene carbon atoms C-1″ and C-3 (δ = 23.2 ppm and δ = 44.4 ppm) were negative. The absorption band in the UV spectrum was observed at λ_{max} = 293.6 nm.

2.2. Cytotoxicity of naringenin and 8-prenylnaringenin

Prenylated hop compounds may play an important role in therapy of brain tumors. The major hop chalcone – xanthohumol reduced cell viability and induced apoptosis in the U87 glioma cells and T98G cells of human glioblastoma multiforme [17].

In this study we compared the cytotoxicity of naringenin and 8-prenylnaringenin (Figure 1) against normal fibroblasts and U-118 MG glioma cells due to reports that substitution of the prenyl group increases the hydrophobicity of flavonoids and may led to its higher cellular accumulation [26] and in consequence higher toxicity against cancer cells [27].



N
naringenin

8PN
8-prenylnaringenin

Figure 1. The chemical structures of tested compounds.

Neutral red assay showed that both flavonoids exerted stronger inhibitory effect on the cancer cell line as compared with normal cells (Figure 2). At concentration above 150 μM the 8PN was significantly more toxic than naringenin for both cell lines and its higher toxicity was seen stronger in U-118 MG glioblastoma

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cells than in normal fibroblasts (Figure 2). Estimated IC_{50} for glioblastoma was found to be approximately 138 and 211 μM for 8-prenylnaringenin and naringenin, respectively, whereas in normal fibroblasts IC_{50} was 172 and 1090 μM for 8PN and N, respectively. It has to be pointed out that naringenin was more selective for glioblastoma as it was over 6 times more toxic for cancer than normal cells, while higher inhibitory activity in of 8-prenylnaringenin for both cell types was evident.

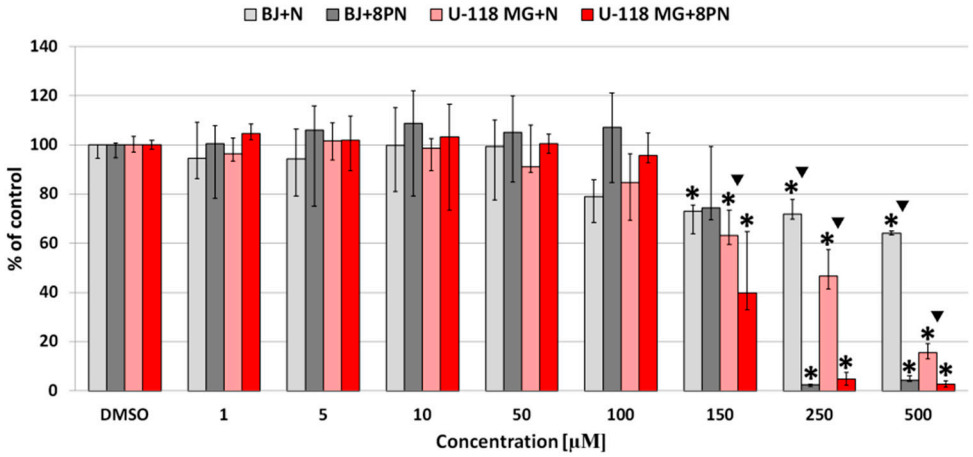

Figure 2. Effects of N or 8PN on the viability of normal human fibroblasts (BJ) and glioblastoma cells (U-118 MG) after 24 hours treatment with flavonoids. Results are presented as median of triplicate assays from three independent experiments and expressed as a % of the non-treated controls. The whiskers are lower (25%) and upper (75%) quartile ranges. *P>0.05; Kruskal–Wallis test (against non-treated control), ▼P<0.05; Mann–Whitney U-test (N against 8PN).

Similar results were obtained by other researchers. Tundis and coworkers [28] tested antiproliferative effect of naringenin with nine cancer and one normal cell lines after 48 hours exposure. This flavonoid exhibited strong inhibitory effect against all cancer lines (IC_{50} from 0.6 to 48.4 μg/mL) and very week activity in normal human skin fibroblasts (142BR) (IC_{50}>50 μg/mL). Our results indicated that IC_{50} naringenin values for BJ and U-118 MG cell lines were equal to 297 and 57 μg/mL respectively. Ayob et al. [29] estimated naringenin IC_{50} values for basal-B mammary carcinoma (MDA-MB-231), basal-A mammary carcinoma (MDA-MB-468) and Chinese hamster ovary (CHO) in similar range of values as 238, 70 and 21 μg/mL after 72 hours treatment using MTT assay.

Anticancer properties of 8-prenylnaringenin after 24 hours treatment were measured with colon cancer cell line (Caco-2) by Allsopp et al. [30]. They demonstrated that this prenylflavonoid showed no significant cytotoxicity against Caco-2 cells at concentrations up to 20 μM with the 25% reduction of cell viability at 40 μM concentration. Toxicity of 8-Prenylnaringenin was also evaluated by Tokalov et al. [31] with the resazurin-based assay. 8PN had no significant inhibitory effect for human promyeloid leukemia cell line (HL-60) and breast cancer cell line (MCF-7) up to 50 μM concentration after 24 hour incubation, but MCF-7 cells were clearly more

resistant. This is in accordance with results of our experiment. Our data confirmed, that cytotoxicity of investigated flavonoid compounds N and 8PN depended on cell type (normal/cancer), with 8PN being always more inhibitory than N.

2.3. Cellular accumulation of naringenin and 8-prenylnaringenin

The degree of cellular accumulation of N and 8PN was examined with the use of confocal microscopy. Mukai et al. [32] and Sudo et al. [33] reported fluorescence microscopy examined flavonoids due to their autofluorescence. Confocal images of BJ and U-118 MG cells were obtained using 80% maximum energy laser excitation and high 80% gain because of weak autofluorescence of both compounds. Also for this reason cells were incubated with high, but non-toxic 10 μM concentrations of both investigated flavonoids N and 8PN. Figure 3 demonstrate subcellular localization of both compounds after 24 hours incubation with a moderate penetration into nuclei.

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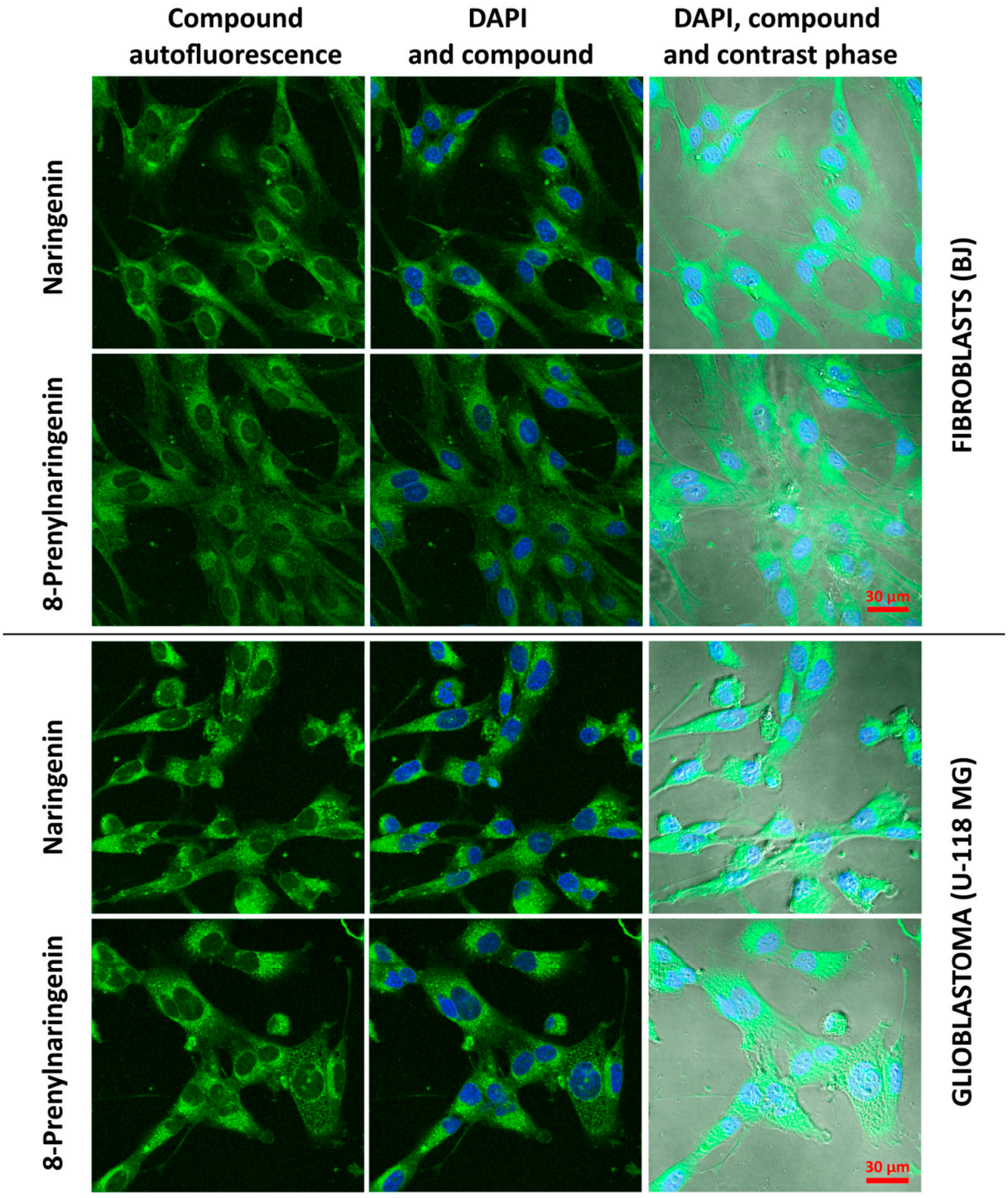

Figure 3. Accumulation and distribution of 10 µM naringenin and 8-prenylnaringenin in BJ and U-118 MG cells after 24 hours incubation. Green signal was derived from flavonoid auto fluorescence, blue from DAPI stained nuclei. Scale bar 30 µm.

N and 8PN were distributed evenly in fibroblasts' subcellular area compared with astrocytoma cells, inside of which numerous vesicles with flavonoids were visible. This suggests, that large part of examined flavonoids may be accumulated

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inside subcellular organelles, such as lysosomes, GA, ER or mitochondria. It was reported that another flavonoid, quercetin was binding to intracellular components and accumulated inside mitochondria [34]. Differences in subcellular distribution of flavonoids and efficiency of accumulation/exclusion processes may lead to alterations in cell lines response.

Accumulation and distribution of each of compound in investigated cell lines were presented on Figure 4.

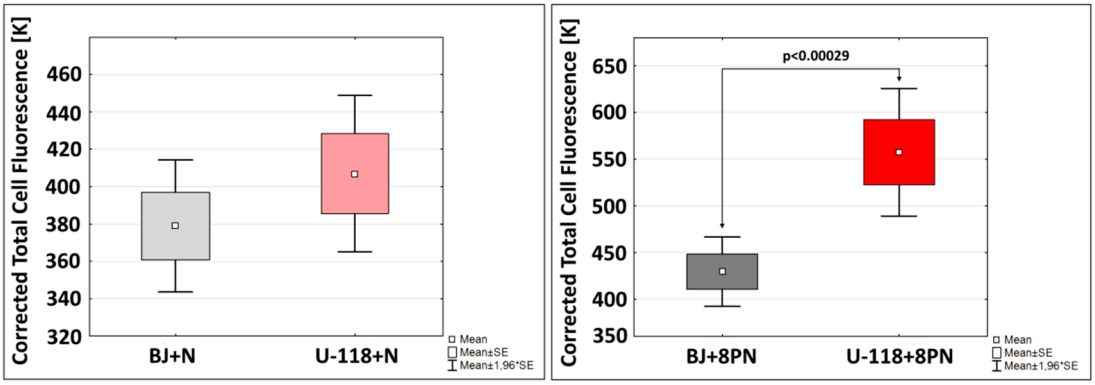

Figure 4. Accumulation of 10 μM naringenin or 8-prenylnaringenin in normal fibroblasts (BJ) and astrocytoma cells (U-118 MG) after 24 hours treatment. Data presented as mean of Corrected Total Cell Fluorescence. Error bars represent mean±1.96*SE. Statistically significant differences are marked as arrows for P<0.05 (t-Student test).

Content of naringenin in normal fibroblasts and glioblastoma cells was similar (Figure 4) whereas the accumulation of 8-prenylnaringenin in cancer cells was 37% higher than in normal cells. This phenomenon may be due to naringenin prenylation, which may generate its higher uptake and accumulation in various cell types [35]. Obtained results are consistent with cytotoxicity pattern, showing the 8-prenylnaringenin as more toxic for glioblastoma cells than for fibroblasts. This phenomenon may be of greater importance at higher, toxic concentrations of both compounds. Moreover, the time of cells' incubation with examined compounds amounted to only 24 hours. *In vivo* studies have shown that prenylflavonoids may circulate in the blood stream for much longer periods than non-prenylated, so higher accumulation to the target tissue may be achieved by efficient cellular uptake [35]. Mukai et al. (2013) suggested that the biological potential of prenyl flavonoids could be due to their greater absorption by the body and efficient accumulation in target tissues [36]. Therefore, cellular accumulation and selective toxicity against cancer cells *in vivo* may be stronger than *in vitro*, and this phenomenon requires further study *in vivo*. However, our *in vitro* data revealed that the presence of the isoprenyl group increases the biological activity of 8-Prenylnaringenin, compared to non-substituted naringenin. This results indicates that an addition of the prenyl group may increase cellular uptake of 8PN and its anticancer activity in U-118 MG cells.

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3. Materials and methods

3.1. Reagents

Naringenin and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The organic solvents were dried and purified according to the standard procedures.

3.1.2. 8-Prenylnaringenin

Isolation of xanthohumol from spent hops and chemical cyclization of xanthohumol into isoxanthohumol were performed as described earlier [24]. 8-Prenylnaringenin was obtained by demethylation of isoxanthohumol in a two-step process, carried out under nitrogen atmosphere. At first ethereal MgI_2 solution was prepared: in a 100 mL round-bottom flask 0.33 g of magnesium was weighed and the flask was placed on a magnetic stirrer. Then crystalline iodine (1.73 g, 3.39 mmoL) dissolved in anhydrous diethyl ether (60 ml) was added portionwise. The flask was tightly closed and the mixture was stirred in the dark for 4 hours, until the colour disappeared.

In the second step 800.3 mg (2.26 mmoL) of isoxanthohumol was weighed to a 250 mL 2-neck round bottom flask, which was immersed in a glycerin bath and placed on a magnetic stirrer and 160 mL of THF was added to it. Then the clear solution of magnesium iodide etherate was added, through a long needle. The reaction was conducted at 65-72°C, in the dark, for 20 h. The progress of the reaction was monitored by TLC, using chloroform–metanol 98:10 (v/v) as eluent. When the substrate was fully consumed, the solvent was evaporated to the volume of 10 mL and then sat. NH_4Cl (200 mL) was added. The resulting mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (3 ×100 mL). The combined organic layers were dried over Na_2SO_4, then filtered and evaporated off. The crude product was purified by flask chromatography using silica gel (eluent: $CHCl_3$: MeOH 99:4 (v/v) to give the pure product as yellow crystals.

8-Prenylnaringenin (yield 97%) mp = 192-193°C lit. 193°C [24] **IR** (KBr) cm^{-1}: 3262, 1639, 1603, 1518, 1439, 1343, 1170, 1071, 832. **^{1}H NMR** (500 MHz, acetone-d_6) δ: 12.14 (1H, s, 5-OH), 9.52 (1H, s, 7-OH or 4′-OH), 8.50 (1H, s, 7-OH or 4′-OH), 7.41 (2H, d, *J* = 8.3 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.3 Hz, H-3′, H-5′), 6.02 (1H, s, H-6), 5.45 (1H, dd, *J* = 12.7 Hz, *J* = 2.8 Hz, H-2), 5.18 (1H, t, *J* = 7.0 Hz, H-2′′), 3.22 (2H, d, *J* = 7.0 Hz, H-1′′), 3.14 (1H, dd, *J* = 17.0 Hz, *J* = 12.7 Hz, H-3 ax), 2.76 (1H, dd, *J* = 17.0 Hz, *J* = 2.8 Hz, H-3 eq), 1.60 (3H, s, H-4′′), 1.59 (3H, s, H-5′′). **^{13}C NMR** (150 MHz, acetone-d_6) δ: 198.6 (C=O), 165.9 (C-9), 163.9 (C-5), 162.1 (C-7), 159.6 (C-4′), 132.2 (C-3′′), 132.1 (C-1′), 129.8 (C-2′, C-6′), 124.7 (C-2′′), 117.1 (C-3′, C-5′), 109.3 (C-8), 104.3 (C-10), 97.4 (C-6), 80.7 (C-2), 44.4 (C-3), 26.9 (C-5′′), 23.2 (C-1′′), 18.9 (C-4′′). **HR ESI-MS** *m*/*z*: 339.1235 $[M-H]^-$; cald: 339.1232 $[C_{20}H_{20}O_5-H]^-$. **UV** (MeOH) λ_{max}: 338.7; 293.6 nm.

3.2. Cell culture

Human glioblastoma U-118 MG (ATCC HTB-15) and human normal fibroblast BJ (ATCC-CRL-2522) cell lines, Dulbecco's Modified Eagle's Medium (DMEM), Eagle's

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minimum essential medium (EMEM), fetal bovine serum (FBS), penicillin and streptomycin solution were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Tripsin-EDTA solution, phosphate-buffered saline (PBS) with and without magnesium and calcium ions, hydrocortisone, 0.33% neutral red solution (3-amino-m-dimethylamino-2-methyl-phenazine hydrochloride), 0.4% trypan blue solution were provided by Sigma-Aldrich (St Louis, MO, USA). DAPI (4′,6-diamidino-2-phenylindole, dihydrochloride) was purchased from Life Technologies (Carlsbad, CA, USA). Chamber culture slides were obtained from Nunc (Rochester, NY, USA) and all other cell culture sterile materials were purchased from Corning Incorporated (Corning, NY, USA).

Glioblastoma cells were cultured in DMEM and normal human skin fibroblasts in EMEM media, containing 10% heat-inactivated FBS,100 U/mL penicillin and 100 µg/mL streptomycin at 37ºC in 5% CO_2 and 95% humidity. Growth media were changed every 3 days and cells were passaged at 80% confluence after treatment with 0.25% trypsin-EDTA in PBS (calcium and magnesium free). Cell morphology was checked under Nikon TE2000S Inverted Microscope (Tokyo, Japan) with phase contrast. Number and viability of cells were estimated by trypan blue exclusion test using Automatic Cell Counter TC20™ (Bio-Rad Laboratories, Hercules, CA, USA). All assays were performed in triplicates in three independent experiments.

3.3. Analytical methods

The NMR spectra (^{1}H NMR, ^{13}C NMR, DEPT 135°, COSY, HMQC, HMBC) of 8-Prenylnarinenin were recorded at 500 MHz on Bruker Ultra Shield TM Plus instrument in acetone-d_6. UV spectra (Cintra 303 spectrofotometer; GBC, Braeside, Australia) were recorded in methanol. HR-ESI-mass spectrum was taken on a Bruker micrOTOF-Q spectrometer. IR spectra: Mattson IR 300 Thermo-Nicolet spectrophotometer (Mattson, Warszawa, Poland).

Analytical TLC was carried out on silica gel G 60 F_{254} plates (Merck, Darmstadt, Germany) with mixture of CH_3Cl:MeOH in various ratios as developing systems. Compounds were detected by spraying the plates with 1% $Ce(SO_4)_2$ and 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4, followed by heating to 120-200ºC. The product was separated by column chromatography using silica gel (SiO_2, Kieselgel 60, 230-400 mesh, 40-63 µm, Merck).

3.4. Cytotoxicity Neutral Red (NR) assay

The cytotoxic effect of N and 8PN was estimated by neutral red assay (NR) which is based on the ability of normal cells to incorporate and bind neutral red within lysosomes. NR cell assay is more sensitive than other commonly applied tests (tetrazolium salts, enzyme leakage or protein contents) [37]. Both cell lines were seeded in flat-bottom 96-well plates at density $1x10^4$ cells/well and allowed to attach for 24 hours before treatment. Working solutions of naringenin or 8-prenylnaringenin (1-500 µM) were prepared in culture media. The DMSO concentration was adjusted to 0.1% in all samples, which had no significant effect on

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treated cell lines (not shown). After 24 hours exposure to naringenin or 8-prenylnaringenin, medium was replaced by 100 µL of NR solution (2% of the culture medium volume) and cells were incubated for 1hour (U-118 MG) or 2 hours (BJ), followed by washing (with PBS). 100 µL/well of fixative (50% ethanol, 49% H_2O, and 1% glacial acetic acid) was added and plates were shaken until complete dye dissolution (300 rpm. 15 minutes, RT). Absorbance was measured at 540/620 nm with microtiter plate reader (µQuant – BioTek, Winooski, Vermont, USA) against blank sample (fixative without cells).

3.5. Confocal microscopy

BJ and U-118 MG cells were seeded in 8-well chamber culture slides at density 4x104/well. After 24 hour incubation culture media were replaced with 10 µM solution of naringenin or 8-prenylnaringenin and cells were incubated for 24 hours, followed by rinsing with PBS (3 times). Cells were fixed with 3.7% formaldehyde solution, washed with PBS (3 times) and cell nuclei were stained with 300 nM DAPI in PBS for 15 minutes. The images were collected with a Olympus FV10i confocal microscope (Olympus, Tokyo, Japan) under 60× magnification using 60× water immersion lens in blue (DAPI) and green (FITC) channel with pinhole set to 1.0 confocal aperture in each channel (thickness of an optical section = 0.905 µm). Excitation and emission for flavonoids were set to 490 and 520 nm, according to Mukai et al. [32] results. Confocal images were processed using ImageJ software with bio-formats plugin.

3.6. Cellular accumulation of naringenin and 8-prenylnaringenin

Confocal microscopy images were analyzed using ImageJ software (US National Institute of Health, Bethesda, MD, USA). The total corrected cell fluorescence (TCCF) was measured according to procedure described by McCloy et al. [38]. Each cell was outlined and area; mean gray value and integrated density parameters were measured, including background. Then total corrected cell fluorescence was estimated according to the equation: TCCF = integrated density – (area of selected cell x mean fluorescence of background readings). Fluorescence of at least 90 cells was measured in each group.

3.7. Statistical analysis

To estimate the differences between flavonoids-treated and non-treated control samples a statistical analysis was performed using the nonparametric Kruskal–Wallis test and paired Mann–Whitney U-test to evaluate the differences between cells treated with naringenin and 8-Prenylnaringenin. To assess differences between degree of N and 8PN accumulation in cells *t*-Student test was executed. $P<0.05$ was considered as statistically significant. Calculations were performed using Statistica 12 software (StatSoft).

4. Conclusions

Our study revealed that isoxanthohumol derivative, 8-prenylnaringenin was more toxic against used cell lines than its structural analogue naringenin and

additionally both flavones inhibited stronger glioblastoma cells than normal fibroblasts. The evident anticancer properties of 8PN correlated with its significantly greater accumulation in U-118 MG cells than in BJ cells. Nevertheless, native naringenin indicated higher selectivity for glioblastoma as it was over 6 times more toxic for cancer than normal cells.

Our results provide evidence that prenylated and noprenylated isoflavones have different biological activity against normal and cancer cells, and this phenomenon may be useful in clinical practice to construct new, safe chemotherapeutic agents for glioblastoma.

Conflict of interest: No conflict of interest declared.

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