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Bradykinin antagonists and thiazolidinone derivatives as new potential anti-cancer compounds

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ABSTRACT

Glioblastoma (GB), the most aggressive brain tumour, and mantle cell lymphoma (MCL), a rare but very aggressive type of lymphoma, are highly resistant to chemotherapy. GB and MCL chemotherapy gives very modest results, the vast majority of patients experience recurrent disease. To find out the new treatment modality for drug-resistant GB and MCL cells, combining of bradykinin (BK) antagonists with conventional temozolomide (TMZ) treatment, and screening of thiazolidinones derivatives were the main objectives of this work. As it was revealed here, BKM-570 was the lead compound among BK antagonists under investigation (IC_{50} was 3.3 μM) in human GB cells. It strongly suppressed extracellular signal-regulated kinases 1/2 (ERK1/2) and protein kinase B (AKT) phosphorylation. BK antagonists did not decrease the viability of MCL cells, thus showing the cell-specific mode, while thiazolidinone derivatives, a novel group of promising anti-tumour compounds inhibited proliferation of MCL cells: IC_{50} of ID 4526 and ID 4527 compounds were 0.27 μM and 0.16 μM, correspondingly. However, single agents are often not effective in clinic due to activation of collateral pathways in tumour cells. We demonstrated a strong synergistic effect after combinatorial treatment by BKM-570 together with TMZ that drastically increased cytotoxic action of this drug in rat and human glioma cells. Small proportion of cells was still viable after such treatment that could be explained by presence of TMZ-resistant cells in the population.

It is possible to expect that the combined therapy aimed simultaneously at different elements of tumourigenesis will be more effective with lower drug concentrations than the first-line drug temozolomide used alone in clinics.

1. Introduction

The tumours of central nervous system constitute from 2% to 5% of all human cancers, about half of them are the brain glial tumours of different malignancy grades of which glioblastoma (GB), the most malignant glioma, occupies up to 50%.[1] Mantle cell lymphoma (MCL) is a subtype of B-cell non-Hodgkin lymphoma characterized cytogenetically by the t(11;14)(q13;32) resulting in overexpression of cyclin D1.[2] GB and MCL patients exhibit an aggressive clinical course however, first-line cytotoxic chemotherapy is unable to induce long-term remission in the majority of patients, both GB and MCL are known to be highly therapy resistant and have the poor prognosis. The development of new treatment modalities, especially those based on multitargeted therapy, is desperately needed for these diseases.

The nonapeptide bradykinin (BK) has been shown to act as a growth factor in human lung, prostate, gastrointestinal, breast, and ovarian cancers.[3,4] Previously, Gera et al.[5,6] developed and synthesized a series of potent and metabolism-resistant BK antagonists, among which BKM-570 caused an impressive inhibition of human small cell lung cancer (SCLC) cell growth in vitro. Injection of this compound in nude mice displayed even higher inhibitory effects than the vascular endothelial growth factor receptor (VEGFR) inhibitor Semaxanib (SU5416) or the conventional chemotherapeutic drug cisplatin.[7] Among other BK antagonists, the inexpensive BKM-1800 derivative of BKM-570 had also valuable anti-cancer properties.[7] Interestingly, that orally active antagonist

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B10238 showed poor potency against cancer in vitro, however, was highly active in vivo.[5]

Last years, evaluation of anti-tumour activity appeared to be actual and promising for the thiazolidinone derivatives.[8,9] Several subgroups of these compounds, namely 5-arylidene-2-amino-4-thiazolones and pyrazoline substituted thiazolones, were evaluated for anti-cancer activity in vitro in a standard National Cancer Institute 60 cancer cell line assay. The majority of the tested substances have shown significant anti-tumour effects at the micromolar and submicromolar level.[10,11]

However, most tumours are driven by multiple molecular aberrations that cannot be controlled by a single targeted agent. The creation of smart schemes of multitargeted therapy aimed simultaneously at different elements of tumour formation mechanisms should be an effective strategy for cancer treatment.[12] The main purpose of this manuscript is to identify novel anti-cancer compounds and to develop modern approaches to complex glioma therapy based on the newly developed compounds and conventional chemotherapeutics used in clinics.

In this paper, we demonstrated that BKM-570 is the lead cytotoxic substance for glioma cells, while thiazolidinones ID 4526 and ID 4664 appeared to be efficient suppressors of MCL cell viability. Decreased viability of cells after BKM-570 treatment was probably associated with inhibition of ERK1/2 and AKT1 phosphorylation. Combination of BKM-570 with first-line drug temozolomide (TMZ) was much more effective even in much less concentration than anti-GB TMZ used alone.

2. Results and discussion

2.1. Compounds under investigation

Compounds synthesized for the first time for this investigation are described in the experimental section. Cytotoxic properties of tested substances are summarized in the Table 1. Chemical structures of compounds used in this study are depicted in Figure 1.

Design and synthesis of peptide B10238, non-peptide small mimetic BKM-570 and non-peptide small mimetic BKM-1800 were described previously.[5–7] B10238 is F5c-D-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-D-Igl-Oic-Arg, where F5c is (*E*)-1,2,3,4,5,6-penta-fluo-rocinnamoyl, Hyp is *trans*-4-hydroxyprolyl, Igl is α-(2-indanyl)glycyl, and Oic is octahydroindole-2-carbonyl); BKM-570 is N^{α}-[(*E*)-2,3,4,5,6-penta-fluorocin-namoyl]-*N*-(2,2,6,6-tetramethylpiperidin-4-yl)-*O*-(2,6-dichlorbenzyl)-L-tyrosin-amide; BKM-1800 is Fmoc-OC2Y-Atmp, where Fmoc is *N*-(fluorenyl-9-methoxycarbonyl), OC2Y is (*O*-2,6-dichlorobenzyl)tyrosyl, and Atmp is 4-amino-2,2,6,6-tetramethyl-piperidine. Compounds were prepared using solution or solid phase methods, purified by HPLC and characterized by thin layer chromatography (TLC) and laser desorption mass spectrometry (LDMS).

Table 1
Compounds under investigation

	IC_{50} (μM)						
	U373	C6	293_*CHI3L1*	Granta	Jeko	UPN1	Mino
Bradykinin antagonists							
BKM-570	3.3	4	3.8	63.3	49.3	*>100*	—[b]
BKM-1800	20	15	25.8	41.2	52.3	*>100*	—[b]
B10238	*>100*[a]	37	67.9	*>100*	50.5	*>100*	—[b]
Thiazolidinones							
ID 11	—[b]	—[b]	—[b]	*>100*	*>100*	*>100*	—[b]
ID 28	—[b]	—[b]	—[b]	*>100*	*>100*	*>100*	61.2
ID 28car*	15	4	0.13	—[b]	—[b]	—[b]	—[b]
ID 3166	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	55.3
ID 3643	—[b]	—[b]	—[b]	*>100*	*>100*	*>100*	—[b]
ID 4132*	—[b]	—[b]	—[b]	*>100*	*>100*	*>100*	—[b]
ID 4522*	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	68.2
ID 4523*	—[b]	1.22	—[b]	—[b]	—[b]	—[b]	80.7
ID 4523car.*	—[b]	0.13	—[b]	—[b]	—[b]	—[b]	1.58
ID 4524*	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	0.73
ID 4525*	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	10
ID 4526*	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	0.27
ID 4527*	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	7.4
ID 4664*	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	0.16

* Newly synthesized compounds are marked by asterisk. Lead compounds are given in *italics* and underlined.
[a] Compound did not reach the IC_{50} value in corresponding cell line.
[b] Compound was not tested in corresponding cell line.

ID 28 and ID 3166 (Scheme 1) were synthesized starting from 2-(4-hydroxyphenylamino)thiazol-4-one **1** and 3-(benzothiazol-2-ylamino)-2-thioxo-thiazolidin-4-one **2**, respectively, using standard Knoevenagel reaction procedure (medium: acetic acid, catalyst: fused sodium acetate) and structurally analyzed as described elsewhere.[10,13]

The pyrazoline–thiazolidinone–isatin conjugates ID 3643, ID 4522, ID 4523, ID 4524, ID 4525, ID 4526, ID 4527, and ID 4664 were synthesized as described here by one-pot methodology involving reaction of 3,5-diaryl-1-thiocarbamoyl-2-pyrazolines **3** with chloroacetic acid and isatins in the presence of fused sodium acetate in refluxing acetic acid (Scheme 2).[14]

The oxadiazole–thiazolidinone hybrid ID 4132 (Scheme 3) was synthesized following the reaction of generated in situ potassium salts of 5-(4-dimethylamino-benzylidene)-thiazolidine-2,4-dione **4** with 2-chloro-*N*-[5-(4-methoxyphenyl)-[1,3,4]oxadiazol-2-yl]-acetamide.[15]

Synthesized compounds were characterized by NMR spectra, which are presented in the experimental part. ^{1}H NMR spectra of compounds ID 4522, ID 4523, ID 4524, ID 4525, ID 4526, ID 4527, and ID 4664 show characteristic patterns of the AMX system for CH_2–CH protons of pyrazoline fragment. The chemical shifts of the protons H_A, H_M, and H_X have been assigned to about δ ~3.47–3.52, δ ~4.18–4.20, and δ ~5.85–6.03 with coupling constants of J_{AM} = 18.0–18.6, J_{MX} = 11.0–11.5, and J_{AX} = 3.5–3.9 Hz, respectively. The chemical shift for the CH(4) of indoline cycle is insignificantly displaced in weak magnetic field (δ = 8.95–9.14) and clearly indicated that only *Z*-isomers were obtained. The protons of the methylene group (CH_2CO) of ID 4132 appear as broad singlet at δ = 4.60 ppm.

Compounds ID 28 and ID 4523, immobilized on a PEGylated carrier (PEGylated olygoelectrolyte polymers),[16] were kindly provided by Dr. A. Zaichenko (Lviv Polytechnic National University).

2.2. Cytotoxicity of synthesized compounds in cancer cell lines

Increased proliferation rate is a common feature of cancer cells. To analyse the anti-viability effects of synthesized compounds, we used several different cancer cell lines, namely rat glioma C6 cells, human GB U373 cells, 293 cells, overexpressing *CHI3L1* oncogene (293_*CHI3L1*),[17] MCL cell lines Granta, Jeko, UPN1, and Mino.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-based) cell viability assay revealed significant dose-dependent cytotoxicity of BKM-570 and BKM-1800 in 293_*CHI3L1* and U373 cells 72 h after addition to the culture medium (Fig. 2, panels A and B). IC_{50} (molar concentration of compound leading to 50% cell death compared to control group treated with DMSO alone) for BKM-570 and BKM-1800 in 293_*CHI3L1* cells was 3.8 μM and 25.8 μM, respectively, and 3.3 μM and 20 μM, respectively, in U373 cells. B10238 did not suppress U373 cell viability at all and had only a partial cytotoxic activity in 293_*CHI3L1* cells, with IC_{50} 100 μM. In C6 rat glioma cells, BKM-570 was the most effective of BK antagonists under study, with IC_{50} 4 μM. The IC_{50} values for BKM-1800 and B10238 in C6 cells were 15 μM and 37 μM, respectively (Fig. 2, panel C). In our study we revealed very