DEVELOPMENT OF BIOCONJUGATES AND THEIR MODUL CONSTRUCTS FOR TARGETED THERAPY OF CANCERS WITH HIGH MORTALITY

Excerption from the results obtained in frame of the grant

NVKP_16-1-2016-0036

supported by the National Research, Development and Innovation Office

ISBN 978-963-489-286-1

Budapest, 2020

In vitro antitumor effect and structure–activity relationships of ferrocene-containing impiridone hybrids

Rita Oláh-Szabó[1], Péter Bárány[2], Imre Kovács[2], Tamás Czuczi[2], Szilvia Bősze[1], Antal Csámpai[2]

[1]MTA-ELTE Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University, Budapest, Hungary

[2]Institute of Chemistry, Eötvös L. University, Budapest, Hungary

Introduction

ONC201 (TIC10, TRAIL-inducing compound 10) is an effective small molecular antitumor agent that is able to induce apoptosis in tumor cells (Figure 1).

The mechanism of action is based on activating TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand. It was explored that TIC10 and its derivative, ONC212 inhibit Akt and Erk signaling, in this way induce translocation of the transcription factor Foxo3a into the nucleus (Figure 1 and Figure 2). The transcription factor Foxo3a enhance the TRAIL gene expression and stimulates TRAIL and death receptor-5 (DR5) transcription, finally activating TRAIL-mediated apoptosis pathway.[1,2]

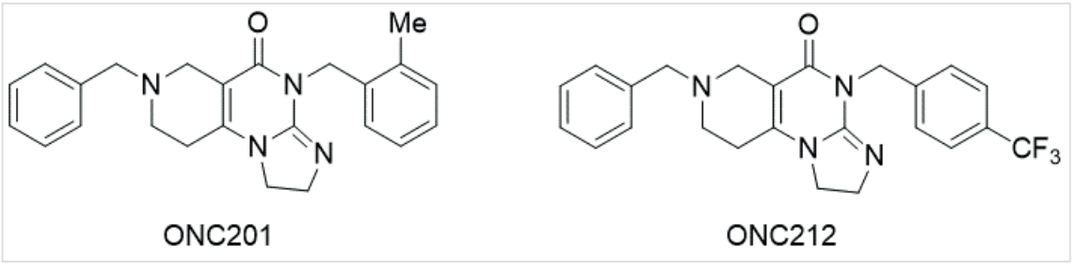

Figure 1. Structure of ONC201 (TIC10) and ONC212

Apoptotic effect of ONC201 was demonstrated on hepatocellular carcinoma, including HepG2 cells,[3] whereas ONC212 showed an anti-tumor effect via blocking Akt/Erk pathway in a glioblastoma cell line[2] and its apoptosis induction was demonstrated on pancreatic tumor cell lines and xenografts as well.[4]

The organometallic ferrocene (*bis*(cyclopentadeinyl)iron) is also known as an antitumor agent; several ferrocene derivatives proved to be effective against HL-60 human leukemia cells[5-7] and cisplatin-resistant ovarian cancer cells.[8] Ferrocene can cause cell death via different mechanisms*: e.g. via* inhibition of COX-2 isoenzyme that is frequently

overexpressed in various tumor cells, [9] but topoisomerase II inhibition of different ferrocene derivatives was also described.[5]

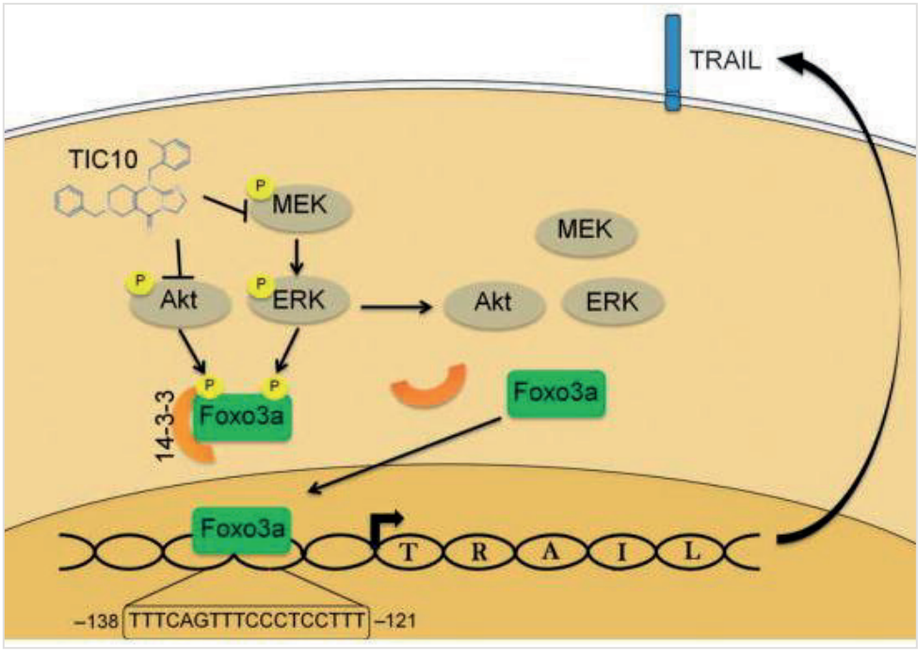

Figure 2. Mechanism of action of ONC201 and ONC212 initiating cell death

In these studies, we investigated antitumor effect of novel ONC201 hybrids with impiridone core and one or two differently positioned ferrocenylalkyl groups or halogenated benzyl groups on different human tumor cell lines, including melanoma, epithelial skin cancer, glioma, colon carcinoma and hepatocellular carcinoma.[10]

Results

Synthesis of the compounds was described in details by Bárány *et al.*[10] Novel compounds were characterized by ^{1}H- and ^{13}C-NMR methods.

In vitro cytostatic effect of the compounds was measured by MTT assay[11] on A2058 human melanoma, A431 human epithelial skin carcinoma, U87 human glioblastoma, HT-29 human colon carcinoma and HepG2 human hepatocellular carcinoma cell lines. Cells were treated with the compounds at 0.8-100 µM, 0.4-50 µM or 0.2-25 µM concentrations. Highest concentration for the treatment was determined with a preliminary solubility probe in serum free medium (RPMI-1640 or DMEM). Highest concentration for the treatment was determined with a preliminary solubility probe in serum-free medium (RPMI-1640 or DMEM). Cells were incubated with the compounds overnight, then the agents were removed and after several washing steps, cells were cultured for further 72 hours at 37 °C. The cytostatic effect was calculated with the following formula:

$$Cytostasis\ [\%] = (1-A_{treated}/A_{control})\times 100$$

IC_{50} values were calculated from sigmoidal curves fitted on the cytostasis data using Origin2018 software.

First, we tested a set of new compounds on A2058 human melanoma and HT-29 human colon carcinoma cell lines. We found 10 compounds that showed a moderate cytostatic effect ($8.82 < IC_{50} < 69.43$) including three ferrocene containing derivatives. Two compounds, however, proved to be markedly effective, the hydrochloride salt of 7-benzyl-4-(4-iodophenylmethyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1*H*)-one (NZS-009·HCl, IC_{50} = 2.7±1.1 μM on HT-29 colon carcinoma cells) (Table 1, Figure 3)

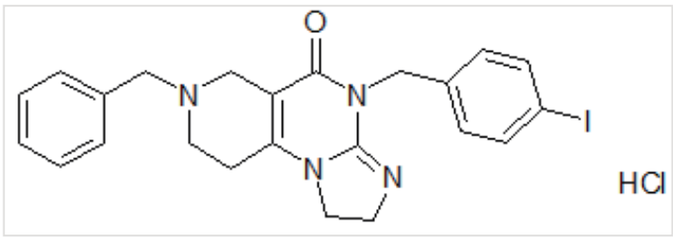

Figure 3. Chemical structure of the most effective compound, 7-benzyl-4-(4-iodophenylmethyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one

The second group of compounds was tested on A431 human skin carcinoma and U87 glioblastoma cells (Table 2). Of these substances, several compounds showed significant anti-tumor effect and two compounds induced extraordinary activity on 431 cells (IC_{50} < 0.2 μM). For the most active compounds a patent application have been submitted.[12]

A third group of compounds was tested on four tumor cell lines, including the HepG2 human hepatocarcinoma cell line, A2058 human melanoma, A431 human skin carcinoma, and U87 human glioblastoma cells (Table 3). The results show that HepG2 cells were found to be much more sensitive to the compounds than the other cell lines, which is in consistence with the effect described for of ONC201.[3] Among the highly cytostatic compounds we found azide, fluoro- and iodine-substituted derivatives as well as impiridone-gemcitabine hybrids (reference compound gemcitabine, CT-059G also showed a remarkable antitumor effect on three cell lines, except for U87 glioblastoma). Compound TBP-274 (7-(4-azidobenzyl)-4-(4-iodophenylmethyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one) showed an outstanding effect on A2058 cells. (Table 3, Figure 4.).

Table 1. Cytostatic effect of impiridone hybrids on HT-29 human colon carcinoma and A2058 human melanoma cells

code	HT-29		A2058	
	IC_{50} [μM]	±SD	IC_{50} [μM]	±SD
NZS-009 B·HCl	**16.9**	**8.6**	**5.28**	**0.86**
NZS-009·HCl	**2.7**	**1.1**	**8.82**	**7.14**
TBP-038·HCl	>100	-	>100	-
TBP-039·HCl	>100	-	>100	-
TBP-040·HCl	>100	-	>100	-
KIM-011·HCl	>100	-	>100	-
TBP-052·HCl	>100	-	47.365	23.50
TBP-053·HCl	>100	-	>100	-
TBP-054·HCl	**33.0**	**23.3**	**20.91**	**12.46**
TBP-055·HCl	**34.0**	**21.9**	**17.64**	**6.65**
NZS-009 B	>100	-	27.57	33.88
NZS-009	>100	-	>100	-
TBP-038	>100	-	>100	-
TBP-039	58.6	18.6	>100	-
TBP-040	>100	-	>100	-
KIM-011	>100	-	>100	-
TBP-052	35.6	0.5	31.22	8.98
TBP-053	**11.6**	**3.5**	**13.51**	**8.49**
TBP-054	>100	-	>100	-
TBP-055	>100	-	>100	-
NZS-033	22.08	9.43	**9.53**	**8.14**
NZS-034	>100	-	>100	-
NZS-036	>100	-	>100	-
TBP-116	45.35	38.56	35.12	30.64
TBP-117	69.43	7.83	>100	-
TBP-118	**14.33**	**7.81**	**14.16**	**8.05**
TBP-125	40.16	5.32	28.73	11.92
TBP-126	>100	-	49.84	13.56
TBP-127	39.86	16.50	32.33	22.82
TBP-128	>100	-	57.23	20.24
TBP-129	40.87	2.37	28.12	13.31
TBP-130	>100	-	42.00	26.85
CZT-016	55.68	3.77	45.4	2.60
CZT-016·HCl	54.69	9.21	43.6	2.97
CZT-018	72.40	13.02	52.0	1.12
CZT-018·HCl	45.97	3.12	45.0	0.98
NZS-022	26.52	15.25	36.3	13.35
TBP-029	24.82	4.46	>100	-
NZS-033	22.1	9.4	**9.5**	**8.1**
NZS-034	>100	-	>100	-
NZS-036	>100	-	>100	-

Table 2. Cytostatic effect of impiridone hybrids on A431 human skin carcinoma and U87 human glioblastoma cells.

code	IC_{50} [μM]	
	A431	**U87**
TBP-072	>25	>25
TBP-073	>25	>25
TBP-098·HCl	>25	>25
TBP-098	>25	>25
TBP-109	>25	>25
TBP-134	**3.55**	>25
TBP-135	7.29	>25
TBP-136	>25	>25
TBP-161	>25	>25
TBP-162	>25	>25
TBP-163	>25	>25
TBP-183	>25	>25
KIM-074/E	**3.63**	**3.51**
KIM-075/E	**8.95**	**5.99**
KIM-056/E	**1.39**	**6.02**
KIM-061/E	>25	>25
KIM-072/E	>25	>25
KIM-073/E	**2.66**	17.44
TBP-149	<0.2	**4.48**
TBP-150	**6.16**	>25
CZT-021	**<0.2**	**4.16**
CZT-022	**<0.2**	>25

Table 3. Cytostatic effect of impiridone hybrids on HepG2 human hepatocarcinoma cell line, A2058 human melanoma, A431 human skin carcinoma, and U87 human glioblastoma cells.

Code	IC_{50} [μM]			
	U87	**A2058**	**A431**	**HepG2**
TBP-272	6.1	**1.25**	11.1	**<0.2**
TBP-274	>25	**0.7**	>25	**<0.2**
CZT-054	>25	7.5	>25	**0.6**
CZT-059	>25	>25	>25	**2.6**
CZT-059G	>25	**<0.2**	**2.7**	**<0.2**
CZT-061	>25	>25	>25	**0.6**
CZT-069	>25	>25	>25	**0.7**
CZT-091	>25	>25	8.26	**3.8**
CZT-092	>25	7.25	*4.4*	**3.4**
CZT-097	10.6	**1.6**	9.3	**0.8**
CZT-099	>25	**4.65**	>25	8.1
CZT-100	22.3	**1.7**	>25	**0.9**
CZT-102	>25	**6.3**	>25	**4.0**

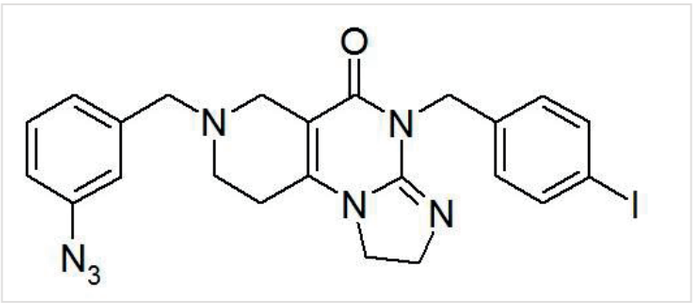

Figure 4. Structure of TBP-274 (7-(4-azidobenzyl)-4-(4-iodophenylmethyl)-2,4,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(1H)-one)

References

1. Allen JE, Krigsfeld G, Mayes PA, Patel L, Dicker DT, Patel AS, Dolloff NG, Messaris E, Scata KA, Wang W, Zhou JY. Wu GS. El-Deiry WS. *Sci Transl Med* **5:**171ra17 (2013)
2. Ishida C T, Zhang Y, Bianchetti E, Shu C, Nguyen TTT, Kleiner G, Sanchez-Quintero MJ, Quinzii CM, Westhoff MA, Karpel-Massler G, Prabhu VV, Allen JE, Siegelin MD. *Clin Cancer Res* **24:** 5392-5406 (2018)
3. Cheng L, Liu YY, Lu PH, Peng Y, Yuan Q, Gu XS, Jin Y, Chen MB, Bai XM. *Oncotarget* **8:** 28385-28394 (2017)
4. Lev A, Lulla AR, Wagner J, Ralff MD, Kiehl JB, Zhou Y, Benes CH, Prabhu VV, Oster W, Astsaturov I, Dicker DT, El-Deiry WS. *Oncotarget* **8:** 81776-81793 (2017)
5. Vashisht Gopal YN, Jayaraju D, Kondapi AK. *Arch Biochem Biophys* **376:** 229-235 (2000)
6. Zsoldos-Mády V, Csámpai A, Szabó R, Mészáros-Alapi E, Pásztor J, Hudecz F, Sohár P. *ChemMedChem* **1:** 1119-1125 (2006)
7. Miklán Zs, Szabó R, Zsoldos-Mády V, Reményi J, Bánóczi Z, Hudecz F. *Biopolymers* **88:** 108-114 (2007)
8. Pigeon P, Wang Y, Top S, Najlaoui F, Garcia Alvarez MC, Bignon J, McGlinchey MJ, Jaouen G. *J Med Chem* **60:** 8358-8368 (2017)
9. Farzaneh S, Zeinalzadeh E, Daraei B, Shahhosseini S, Zarghi A. *Anticancer Agents Med Chem* **18:** 295-301. (2018)
10. Bárány P, Oláh-Szabó R, Kovács I, Czuczi T, Szabó CL, Takács A, Lajkó E, Láng O, Kőhidai L, Schlosser G, Bősze Sz, Mező G, Hucecz F, Csámpai A. *Molecules* **23:** 2248 (2018)
11. Mosmann T. *J Immunol Methods* **65:** 55-63 (1983)
12. Patent Application No. 1292889-3119/MOI (2019)