

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

Excerpt 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* anti-tumor effect of cinchona-chalcone hybrids with 1,4- or 1,5-disubstituted 1,2,3-triazole linker**

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Introduction

Combining different compounds with antitumor activity can enhance the effect of the hybrid compounds on tumor cells.

Quinine derivatives have several advantageous biological effects: it has been known for a long time as antimalarial agent.¹ Direct antitumor activity of these molecules was also described^{2,3} and they can be applied in combination therapy as well.⁴

Former studies proved the cytotoxic effect of cinchona hybrids on several tumor cell lines like PANC-1 human pancreatic carcinoma, COLO-205 human colon adenocarcinoma, A2058 human melanoma and EBC-1 lung carcinoma.^{5,6} Ferrocene–cinchona hybrids with triazolyl-chalcone linkers showed a marked anti-tumor effect on HT-29 human colon carcinoma and HepG2 human hepatocellular carcinoma⁶ and MCF-7 human breast cancer, SH-SY5Y human neuroblastoma and HL-60 human leukemia cell lines³ and they enhanced the production of reactive oxygen species, inhibited autophagy and increased paclitaxel sensitivity in MDR tumor cell lines as well.⁷

Chalcones are also a well-known group of molecules inducing antitumor activity. Antiproliferative effect elicited by isoliquiritigenin (4,2,4'-trihydroxychalcone) was reported on HeLa and MCF-7 human breast cancer cell lines, whereas other chalcones caused apoptosis *via* inhibition of p53 suppressor protein on tumor cells.⁸ In our research group, the cytotoxicity of 1,3-diphenylchalcone ($IC_{50} = 10.19 \mu\text{M}$) and 1-(*p*-methoxyphenyl)-3-phenyl chalcone ($IC_{50} = 12.43 \mu\text{M}$) was described on HL-60 human leukemia cell line.⁹ In the present study we investigated the *in vitro* cytostatic effect of novel cinchona-chalcone hybrids with 1,4- or 1,5-disubstituted 1,2,3-triazole linker (Figure 1).

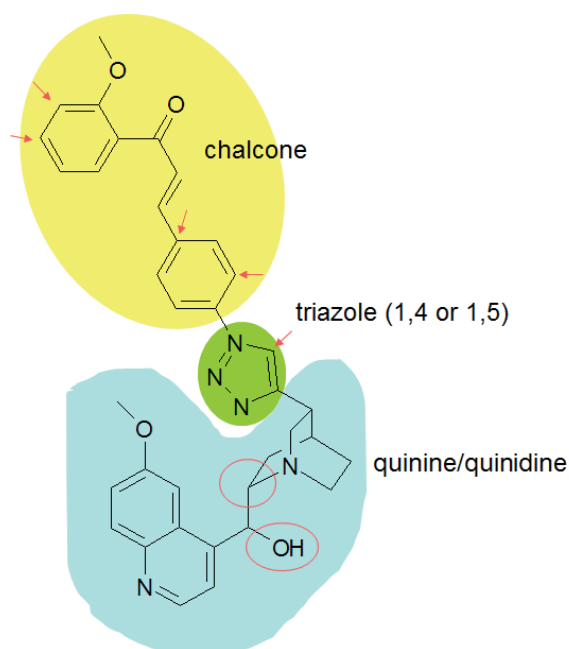


Figure 1. Structure of cinchona-chalcone hybrid molecules with a 1,2,3-triazole linker

Results

Synthesis of the compounds was performed by copper(I)- and ruthenium(II)-catalyzed click reactions according to Károlyi *et al.*³, in some cases modified by Jernei *et al.*⁵

In vitro cytostatic activity of the compounds were tested by MTT assay¹⁰ on tumor cell lines of different origin, including A2058 human melanoma, A431 human epithelial skin carcinoma, HT-29 human colon carcinoma, MDA-MB-231 and MCF-7 human breast carcinoma and HepG2 human hepatocellular carcinoma cell lines. Cells were treated with the compounds at 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). Treatment was taken place overnight, then compounds were removed, cells were washed and cultured for further 72 hours. The cytostatic effect was calculated with the following formula:

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

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

Results indicate that most of the cinchona-chalcone hybrids studied proved to be effective anti-tumor agents under the conditions described above. Overall, MCF-7 human breast cancer cell line proved to be the most sensitive to the cinchona-chalcone hybrids (IC_{50} values were $< 3 \mu\text{M}$, except for one compound), but in case of some hybrids, lower IC_{50}

values could be obtained on HT-29 human colon carcinoma and A2058 human melanoma cells. The most effective compounds were JT-226 with a quinine skeleton, 1,4-disubstituted triazole linker carrying a 3,4,5-trimethoxybenzoyl substituent in the chalcone moiety ($IC_{50} = 0.57 \pm 0.23 \mu\text{M}$ on HT-29 cells), and JT-227 with a quinidine skeleton, and also a 1,4-disubstituted triazole linker and a 3,4,5-trimethoxybenzoyl group in the chalcone moiety ($IC_{50} = 0.52 \pm 0.06 \mu\text{M}$ on A2058 cells (Table 1., Figure 1).

Table 1. *In vitro* cytostatic effect of cinchona-chalcone hybrids JT-181–JT-227 on HT-29, HepG2, A2058, MDA_MB_231 and MCF-7 tumor cell lines

code	HT-29		HepG2		A2058		MDA-MB-231		MCF-7	
	IC_{50} [μM]	\pm SD	IC_{50} [μM]	\pm SD	IC_{50} [μM]	\pm SD	IC_{50} [μM]	\pm SD	IC_{50} [μM]	\pm SD
JT-181	4.07	0.77	8.92	0.08	8.15	0.34	5.70	4.70	2.9	0.50
JT-182	3.28	0.48	8.03	0.38	2.51	0.43	6.80	5.10	3.1	0.60
JT-218	9.25	0.48	12.25	1.10	3.35	0.08	11.15	0.40	2.3	0.10
JT-219	8.99	1.08	34.18	0.00	3.36	0.26	6.05	2.10	3.1	0.60
JT-220	7.92	0.28	10.37	0.38	3.67	0.46	12.40	5.10	8.4	0.40
JT-221	3.66	0.72	9.34	0.07	2.31	0.01	6.60	4.50	3.1	0.20
JT-222	27.40	9.50	24.25	9.00	2.59	0.06	6.56	4.20	2.6	0.60
JT-223	8.14	0.56	6.88	3.86	1.49	0.33	9.15	2.10	2.8	0.30
JT-224	2.88	0.09	6.62	0.82	2.50	0.06	2.80	0.00	1.8	0.60
JT-225	10.86	0.99	n.d.	n.d.	7.27	0.90	2.60	0.10	1.4	0.40
JT-226	0.57	0.23	0.99	0.52	1.10	0.08	2.15	0.40	1.6	0
JT-227	1.32	0.04	2.98	0.70	0.52	0.06	5.70	4.70	2.9	0.50

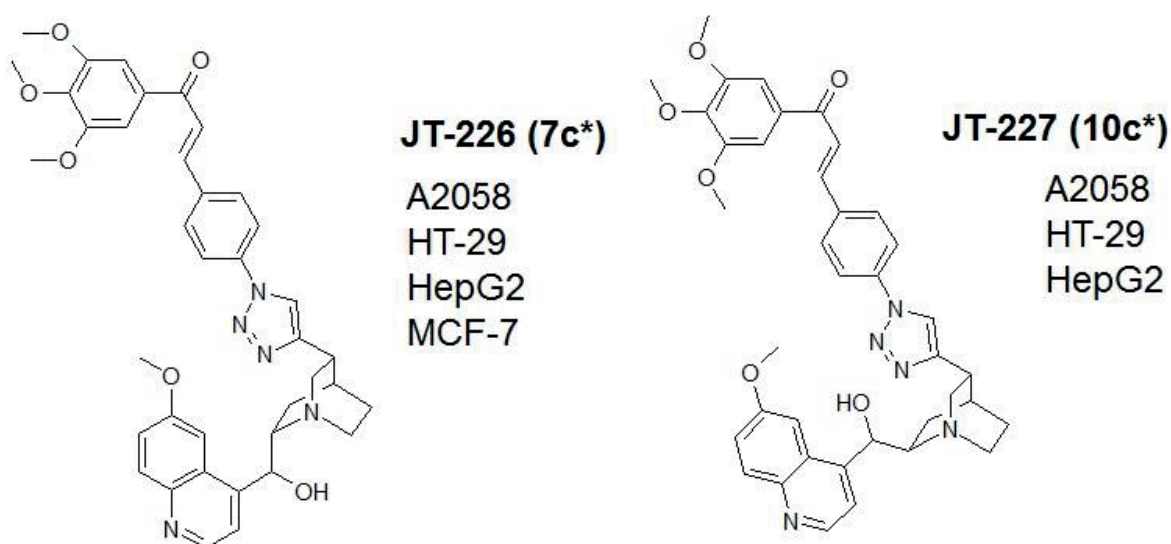


Figure 2. Structure of cinchona-chalcone hybrids elicited the most effective anti-tumor effect on HT-29 and A2058 cells (*numbering according to Jernei *et al.*⁵).

Based on these results, another set of compounds were tested on HT-29 and A2058 cells under the same conditions. We found two similarly effective compounds, with epiquinidine skeleton and 1,5-disubstituted triazole linker and 3,4,5-trimethoxybenzoyl group in the chalcone moiety (JT-382) or 3,5-dimethyl-4-hydroxybenzoyl derivative (JT-387) (Table 2)

Table 2. *In vitro* cytostatic effect of cinchona-chalcone hybrids JT-230–JT-387 on HT-29 and A2058 cell lines

code	HT-29		A2058	
	IC ₅₀ [μM]	± SD	IC ₅₀ [μM]	± SD
JT-230	10.27	2.34	9.86	0.49
JT-334/1	25.75	23.72	9.26	2.04
JT-334/2	9.71	0.17	10.04	1.51
JT-335/1	18.22	11.63	9.80	2.28
JT-335/2	>100	-	9.80	1.98
JT-354	25.24	18.37	23.11	15.36
JT-355	>100	-	52.72	10.51
JT-381	9.42	1.15	8.06	1.30
JT-382	4.48	2.59	2.78	0.06
JT-386	23.66	1.40	45.92	4.57
JT-387	3.54	0.11	4.40	0.16

Finally, a third group of hybrid compounds, four of them with quinuclidine skeleton, was also investigated on U87 human glioblastoma, A2058 human melanoma, A431 human skin carcinoma and HepG2 human hepatocarcinoma cell lines. Among these compounds, we found that JT-450 and JT-475 with 1,4-disubstituted triazole linker and 3,4,5-trimethoxybenzoyl substituent proved to be the most promising anti-tumor agents (IC₅₀ < 3 μM) on three of the four cell lines. The effect was cell dependent, A431 cells proved to be the most sensitive for these compounds (Table 3).

Table 3. *In vitro* cytostatic effect of cinchona-chalcone hybrids with quinuclidine skeleton on U87, A2058, A431 and HepG2 cell lines

code	U87		A2058		A431		HepG2	
	IC ₅₀ [μM]	±SD	IC ₅₀ [μM]	±SD	IC ₅₀ [μM]	±SD	IC ₅₀ [μM]	±SD
JT-446	11.51	-	9.19	-	11.00	-	14.11	-
JT-450	12.94	0.30	3.13	0.54	2.79	0.27	2.97	0.30
JT-471	9.56	-	7.26	-	4.41	-	8.54	-
JT-475	2.76	0.11	10.45	1.16	2.32	0.16	2.65	0.08
JT-521	14.74	0.18	3.14	0.45	5.33	0.35	11.78	0.65

In conclusion, we can state, that cinchona-chalcone hybrid compounds elicited an effective antitumor effect. The efficacy of these compounds was influenced by the skeleton as well as the position of the substituents on the linker, or the substituent in the chalcone moiety. Some hybrids showed outstanding effect with lower than micromolar IC₅₀ values; these molecules can be promising candidates for drug development for further clinical application.

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