Supplemental material

Targeting Canine *KIT* Promoter by Candidate DNA G-quadruplex Ligands

Authors:

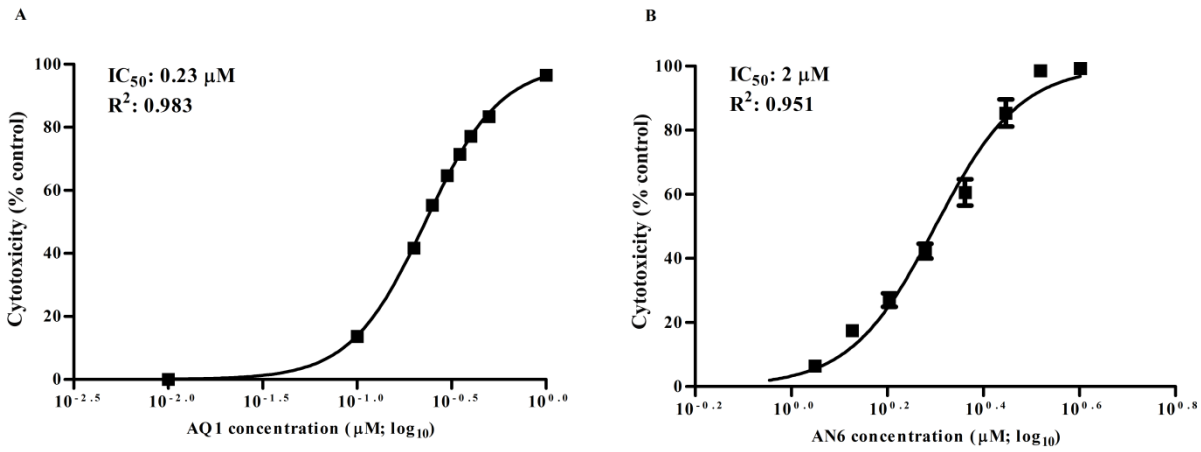
Eleonora Zorzan, Silvia Da Ros, Mery Giantin, Lara Zorro Shahidian, Giorgia Guerra, Manlio Palumbo, Claudia Sissi, and Mauro Dacasto

The Journal of Pharmacology and Experimental Therapeutics

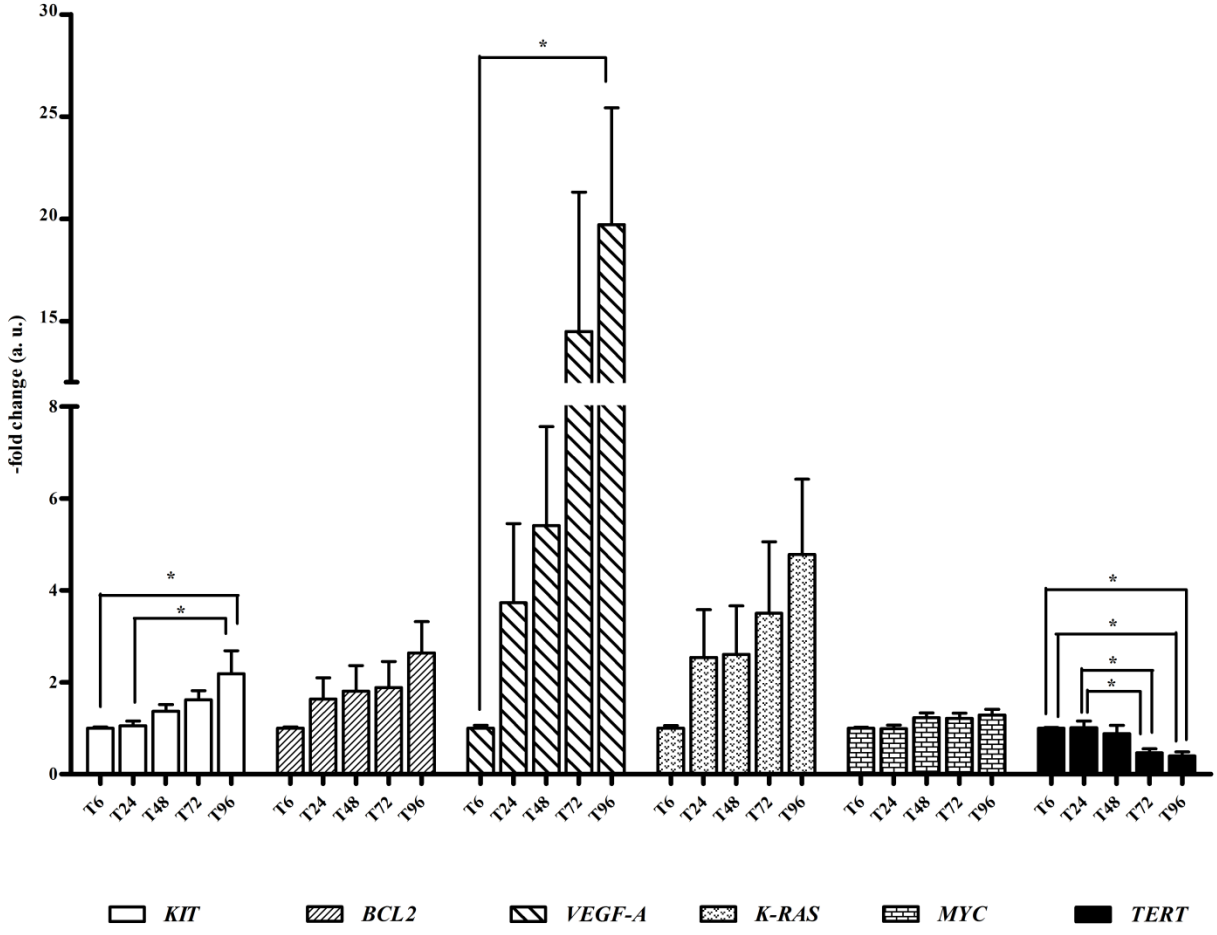
Supplementary Table 1. qPCR assay standard curve parameters obtained in C2 and NI-1 cell lines.

Gene	C2			NI-1		
	Slope	**Efficiency (%)**	**Dynamic range (Ct)**	**Slope**	**Efficiency (%)**	**Dynamic range (Ct)**
ATP5β	-3.26	102.7	19.17-30.39	-3.39	97.4	17.87-29.09
BCL2	-3.42	95.9	26.36-36.46	-3.49	93.6	25.62-37.20
CCZ1	-3.34	99.2	25.30-33.35	-3.37	98.2	22.14-33.10
CGI-119	-3.43	95.7	23.23-31.64	-3.30	101	22.55-34.05
GOLGA1	-3.28	101.8	25.60-36.74	-3.23	104	25.36-36.18
KIT	-3.32	99.9	17.67-28.85	-3.34	99	18.05-29.05
KRAS	-3.33	99.7	20.97-32.11	-3.33	99.7	22.35-32.22
MYC	-3.34	99.4	22.40-33.06	-3.28	101.7	21.82-31.17
VEGFA	-3.29	101.4	22.04-31.05	-3.27	102.2	22.90-34.18
TERT	-3.21	105.1	27.12-37.92	-3.28	101.7	27.99-36.02

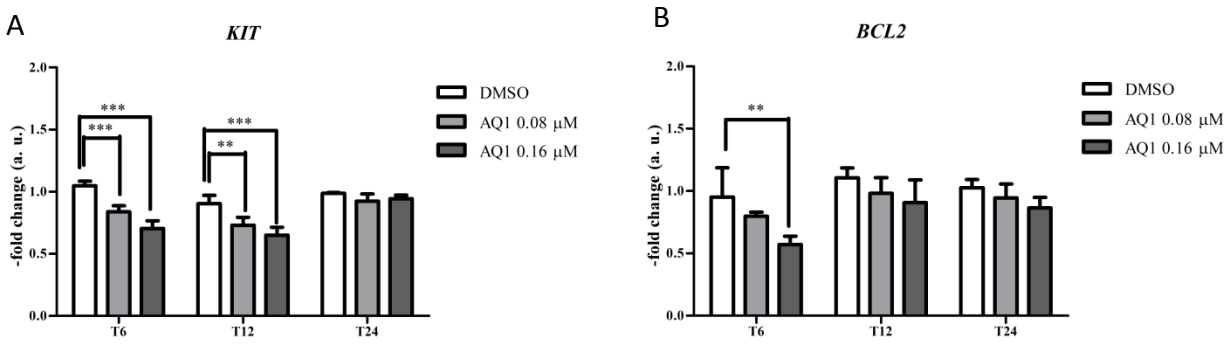
ATP5β, ATP synthase, H^+ transporting, mitochondrial F1 complex, beta polypeptide; *BCL2*, B-cell leukemia/lymphoma 2; *CGI-119*, transmembrane BAX inhibitor motif containing 4; *CCZ1*, vacuolar protein trafficking and biogenesis associated homolog; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; *GOLGA1*, Golgin A1; ICG, internal control gene; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *MYC*, v-myc avian myelocytomatosis viral oncogene homolog; qPCR, quantitative Real-time RT-PCR; *TERT*, telomerase reverse transcriptase; *VEGFA*, vascular endothelial growth factor A.



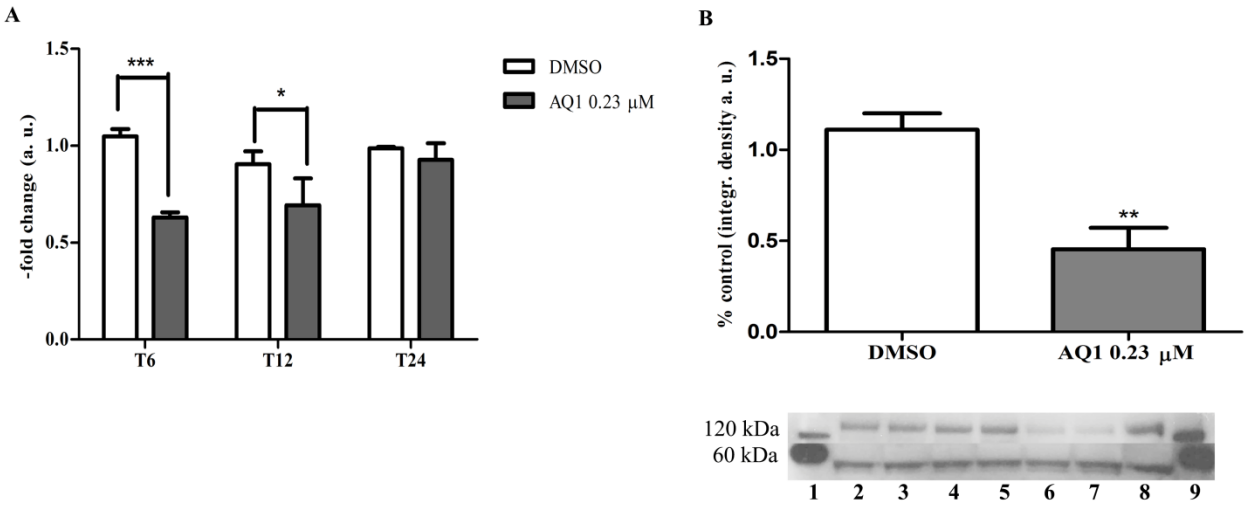
Supplementary Figure 1. Cytotoxicity (dose-response curves) of AQ1 and AN6 in canine NI-1 MCT cell line. NI-1 cells were exposed to AQ1 (A) and AN6 (B) and their cytotoxicity was measured using the Alamar blue assay. Cytotoxicity was calculated as [100-(T/control mean*100)]. Data are expressed as mean values ± standard deviation (S.D.) of three independent experiments (each concentration performed in sestuplicate) performed in different passages.



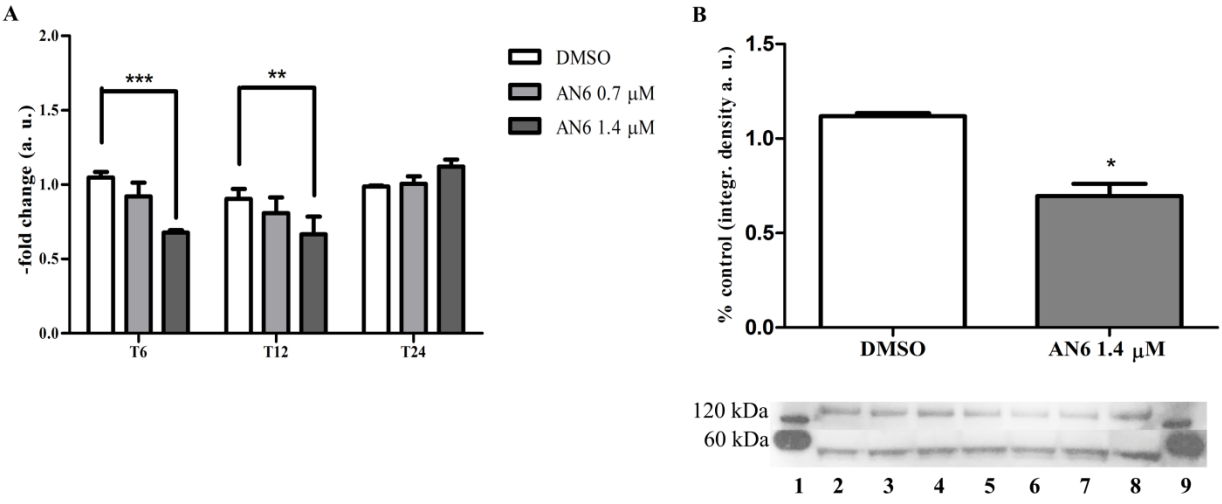
Supplementary Figure 2. Effects of culturing time (6, 24, 48, 72, 96 hours) on the expression of genes containing putative G4 structures in their promoter in the canine NI-1 MCT cell line. Total RNA was isolated from NI-1 cells and *KIT*, *BCL2*, *VEGFA*, *KRAS*, *MYC* and *TERT* mRNA levels were measured using qPCR. Data (arithmetic means± S.D.) are expressed as n-fold change (a.u.) normalized to the RQ mean value of cells stopped at T_6, to whom an arbitrary value of 1 was assigned. Experiments were performed in triplicate and, for each experiment, three biological replicates were included. The one-way ANOVA was used to measure statistical differences between different times culture. *: $P<0.05$.



Supplementary Figure 3. Effect of AQ1 (0.08 µM and 0.16 µM) on *KIT* (A) and *BCL2* (B) mRNA levels in canine NI-1 MCT cell line. Gene expression profiles were measured by using qPCR, and data (arithmetic means ± S.D.) are expressed as n-fold change (a.u.) normalized to the RQ value of corresponding control cells (T_6, T_{12}, T_{24}) to whom an arbitrary value of 1 was assigned. Experiments were performed in triplicate and, for each experiment, three biological replicates were included. Two-way ANOVA and Bonferroni post-test were used to check for statistical differences between doses and time of treatment. **: $P<0.01$; ***: $P<0.001$.



Supplementary Fig. 4. Effect of AQ1 (0.23 µM) on *KIT* gene expression (A) and c-kit protein (B) in the canine NI-1 MCT cell line. (A) *KIT* mRNA levels were measured by qPCR, and data (arithmetic means ± S.D.) are expressed as n-fold change (a.u.) normalized to the RQ of control cells at each time (T_6, T_{12}, T_{24}), to whom an arbitrary value of 1 was assigned. Experiments were performed in triplicate and, for each experiment, three biological replicates were included. Two-way ANOVA and Bonferroni post-test were used to find out statistical differences between doses and time of treatment. (B) The effect of AQ1 on c-kit protein amount was measured by using immunoblotting, and data are expressed as n-fold change (a.u.) with respect to the untreated cells densitometry. Experiments were performed in triplicate and, for each experiment, three biological replicates were included. The Student *t*-test was used to check for statistical differences between cells treated with AQ1 and those treated with the vehicle only (DMSO). A representative immunoblot image is reported. Legend: 1, ladder; 2-3, control cells; 4-5, DMSO (vehicle); 6-7, cells exposed to AQ1 (24 hours); 8, TF1 control cells; 9, ladder. *,**,***: $P<0.05$; $P<0.01$; $P<0.001$.



Supplementary Figure 5. Effect of AN6 (0.7 µM and 1.4 µM) on *KIT* mRNA levels (A) and c-kit protein (B) in canine NI-1 MCT cell line. (A) Gene expression profiles were measured by using qPCR, and data (arithmetic means ± S.D.) are expressed as n-fold change (a.u.) normalized to the RQ value of corresponding control cells (T_6, T_{12}, T_{24}) to whom an arbitrary value of 1 was assigned. Experiments were performed in triplicate and, for each experiment, three biological replicates were included. Two-way ANOVA and Bonferroni post-test were used to check for statistical differences between doses and time of treatment. (B) The effect of AN6 on c-kit protein amount was measured by immunoblotting, and data are expressed as n-fold change (a.u.) with respect to the untreated cells densitometry. Experiments were performed in triplicate and, for each experiment, three biological replicates were included. The Student t-test was used to check for statistical differences between cells treated with AN6 and those treated with the vehicle only (DMSO). A representative immunoblot image is shown. Legend: 1, ladder; 2-3, control cells; 4-5, DMSO (vehicle); 6-7, cells exposed to AQ1 (24 hours); 8, TF1 control cells; 9, ladder. *,**,***: $P<0.05$; $P<0.01$; $P<0.001$.