Supporting Information

for

New unsymmetrical bisacridine derivatives non-covalently attached to quaternary quantum dots improve cancer therapy *via* enhancing cytotoxicity towards cancer cells and protecting normal cells

Joanna Pilch,[a] Edyta Matysiak-Brynda,[b] Agata Kowalczyk,[b] Piotr Bujak,[c] Zofia Mazerska,[a] Anna M. Nowicka,[b,*] Ewa Augustin[a*]

[a] Faculty of Chemistry, Gdańsk University of Technology, Narutowicza Str. 11/12, 80-233 Gdańsk, Poland

[b] Faculty of Chemistry, University of Warsaw, Pasteura Str. 1, PL-02-093 Warsaw, Poland

[c] Faculty of Chemistry, Warsaw University of Technology, Noakowskiego Str. 3, 00-664 Warsaw, Poland.

*Corresponding authors: anowicka@chem.uw.edu.pl (A. Nowicka), ewa.augustin@pg.edu.pl (E. Augustin)

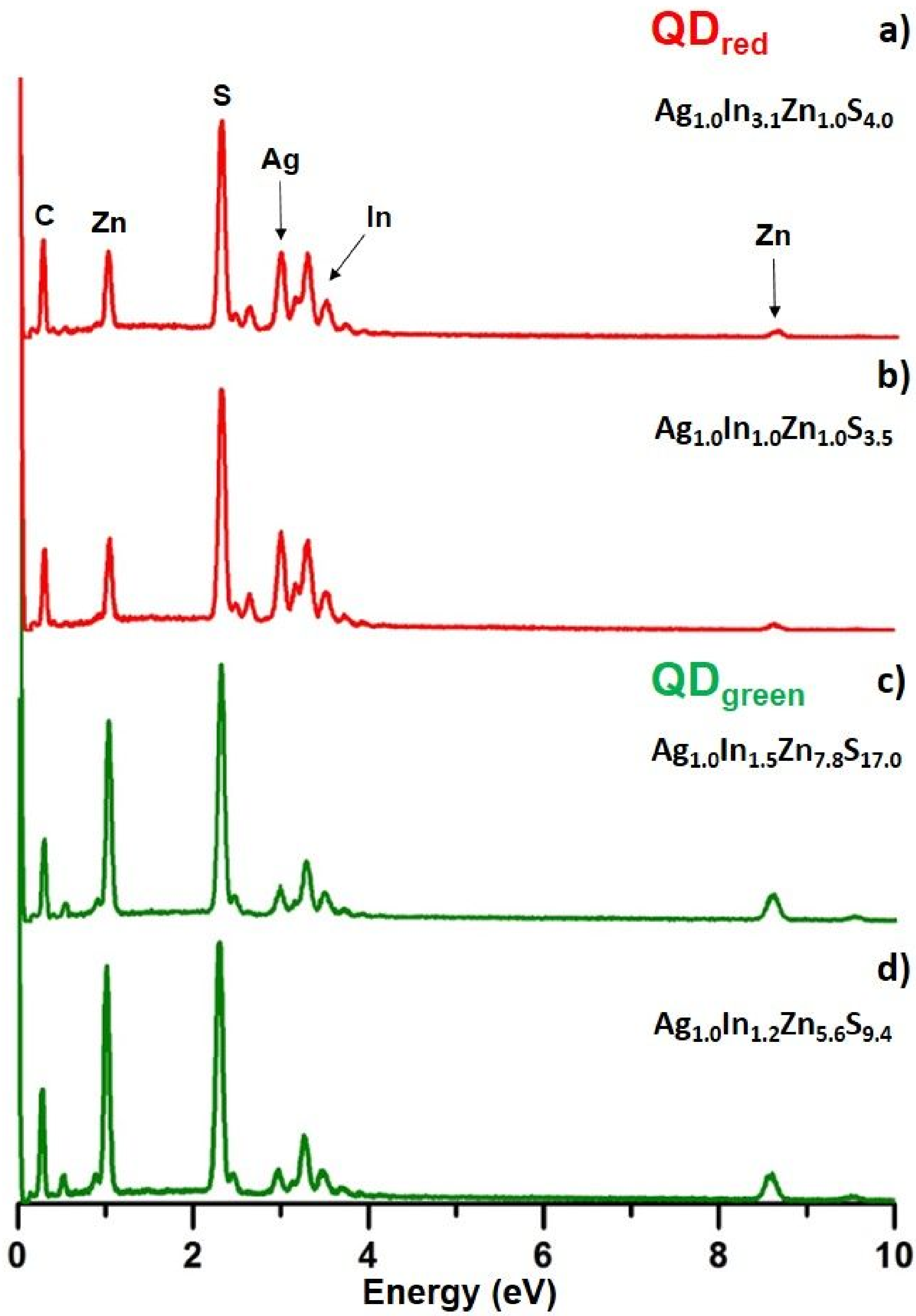

Figure S1. EDS spectra of QD_{red} and QD_{green}.

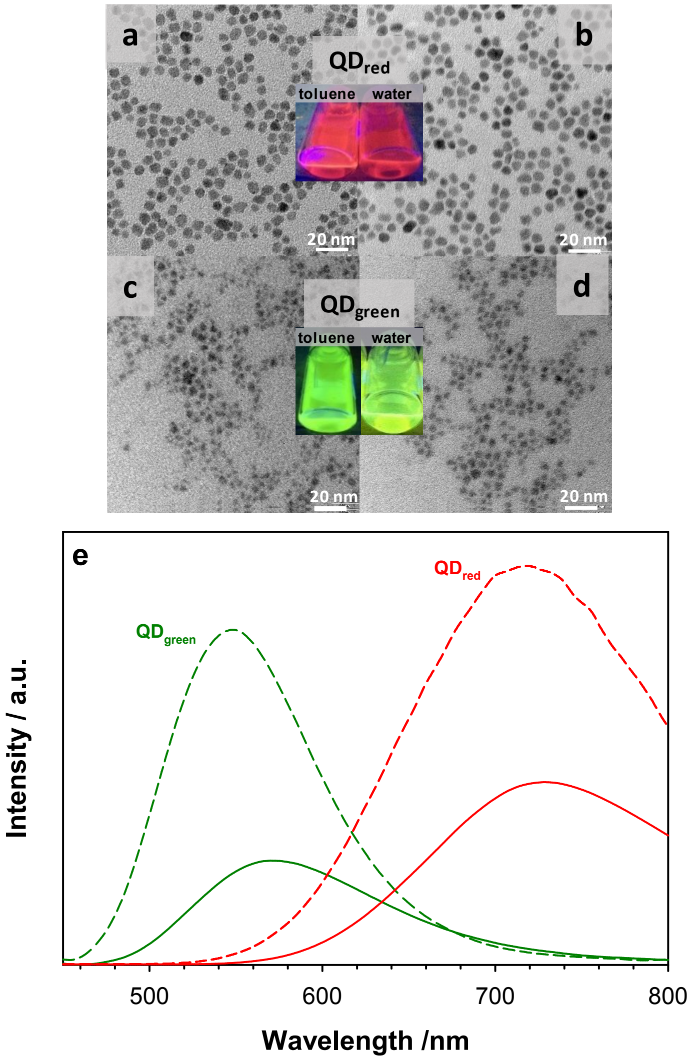

Figure S2. TEM images of Ag-In-Zn-S nanocrystals (QD_{red} and QD_{green}) before (a, c) and after (b, d) ligands exchange for MUA. (e) Comparison of Ag-In-Zn-S (QD_{red} and QD_{green}) nanocrystals photoluminescence spectra for dispersions in toluene (dashed lines) and water (solid lines).

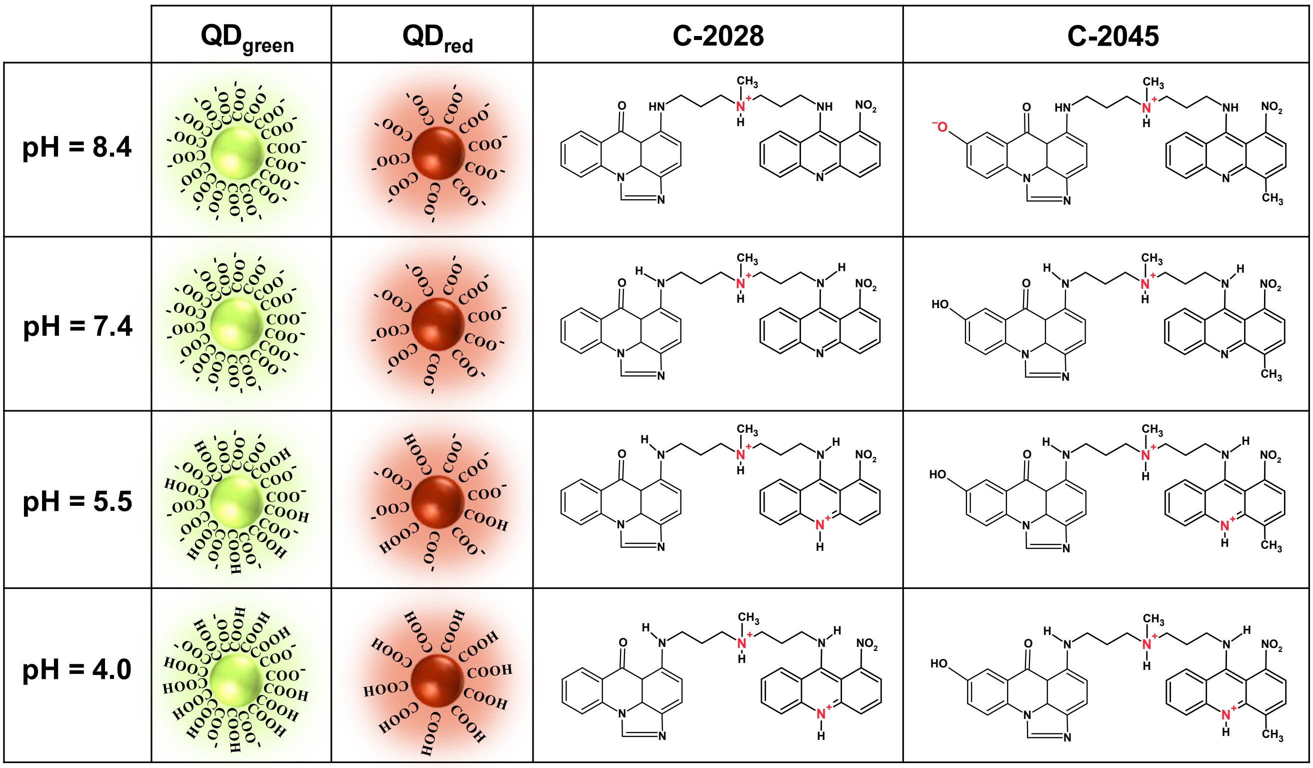

Figure S3. Forms of QD nanocrystals and UAs compounds at different values of pH.

Table S1. Assignments of bands in FTIR spectra based on ref.[1,2]

Origin	Wavenumber (cm^{-1})	Assignment
	QDs	
O-H	1410	carboxylate (carboxylic acid salt)
C=O	1555-1560	
	UAs compounds	
C-H	871-957	aromatic out-of-plane
C-H	1050-1260	aromatic in-plane
C-N	1090-1270	secondary and tertiary amine
O-H	1292	phenol
N=O	1330	nitro group
C-N	1381-1384	aliphatic chain
C-H	1447	from $–CH_2–$ in aliphatic chain
N=O	1493	nitro group
C=N	1522-1534	in aromatic ring
C=C		
N-H	1587-1601	secondary amine
C=C	1627-1668	in aromatic ring
C=O		carbonyl group

Table S2. Hydrodynamic diameter values determined from DLS measurements and zeta potential values for UAs compounds, QD nanocrystals and QD-UAs nanoconjugates obtained in 0.02 M PBS buffer at various pH.

a: Hydrodynamic diameter (nm)				
C-2028*		2.1 ± 0.4		
C-2045*		2.2 ± 0.3		
QD_{red}		9.1 ± 0.8		
QD_{green}		9.4 ± 0.7		
	pH = 4.0	***pH = 5.5***	***pH = 7.4***	***pH = 8.4***
QD_{red}-C-2028	9.2 ± 0.5	10.2 ± 0.9	10.8 ± 0.8	10.9 ± 0.9
QD_{red}-C-2045	9.4 ± 0.3	11.9 ± 0.5	12.6 ± 0.9	12.4 ± 0.6
QD_{green}-C-2028	9.9 ± 0.6	10.1 ± 0.8	11.9 ± 0.7	11.8 ± 0.8
QD_{green}-C-2045	11.4 ± 0.7	11.7 ± 0.9	12.5 ± 0.4	12.7 ± 0.6

b: Zeta potential values				
	pH = 4.0	***pH = 5.5***	***pH = 7.4***	***pH = 8.4***
C-2028	-15.3 ± 3.4	-14.3 ± 2.5	-19.3 ± 1.2	-18.1 ± 1.5
C-2045	-20.4 ± 1.6	-17.7 ± 1.7	-13.6 ± 2.2	-20.5 ± 1.1
QD_{red}	-13.6 ± 1.5	-34.2 ± 2.9	-34.1 ± 2.5	-34.8 ± 2.8
QD_{green}	-19.2 ± 1.8	-28.9 ± 1.8	-38.5 ± 1.9	-37.5 ± 2.5
QD_{red}-C-2028	-5.5 ± 1.1	-31.1 ± 1.9	-33.4 ± 2.6	-30.2 ± 2.5
QD_{red}-C-2045	-7.5 ± 1.3	-30.1 ± 1.5	-43.2 ± 2.5	-36.7 ± 1.5
QD_{green}-C-2028	-18.5 ± 2.5	-30.3 ± 1.8	-39.0 ± 1.5	-37.5 ± 2.7
QD_{green}-C-2045	-24.0 ± 2.0	-26.8 ± 1.3	-40.1 ± 2.5	-36.6 ± 2.2

*the diameters of UAs compounds were determined with the help of the ChemSketch program where it was assumed that the molecules are circular

Below we present the representative picture and the tables with the statistic for the *in vivo* test. Table S3. presents the data for Figure 9. There are the mean tumor volume and the standard deviations of these values.

Table S3.

Day	Glucose		Irinotecan		C-2028		C-2045		QD_{red}		QD_{red}-C-2028		QD_{red}-C-2045	
	mean	*SD*	mean	*SD*	mean	*SD*	mean	*SD*	mean	*SD*	mean	*SD*	mean	*SD*
1	117.5	*12.6*	116.7	*14.1*	114.4	*13.6*	116.4	*22.3*	117.7	*14.8*	117.5	*17.0*	115.0	*20.4*
4	218.2	*29.0*	163.8	*27.1*	173.0	*15.6*	200.3	*27.0*	206.7	*32.0*	215.0	*37.8*	236.1	*50.1*
6	291.1	*41.3*	221.3	*38.5*	265.7	*30.6*	309.6	*35.0*	324.2	*48.8*	371.2	*57.3*	331.2	*61.1*
8	448.8	*57.0*	280.1	*48.7*	389.1	*38.9*	427.7	*42.0*	466.5	*52.6*	572.4	*85.2*	529.3	*99.0*
10	756.5	*111.1*	352.1	*54.6*	542.0	*54.9*	615.7	*50.1*	718.7	*93.4*	829.3	*111.8*	861.1	*169.4*
12	1010.5	*112.4*	434.5	*62.7*	667.7	*81.8*	739.0	*52.3*	881.0	*115.5*	862.1	*117.6*	1015.2	*176.1*
14	1341.9	*138.0*	508.5	*71.4*	810.9	*100.3*	984.4	*92.3*	1156.2	*163.2*	1136.1	*153.5*	1306.0	*213.7*
17	1523.6	*163.4*	431.1	*60.9*	887.4	*136.9*	1088.5	*114.2*	1424.8	*248.9*	1181.6	*139.5*	1577.9	*234.6*

The Figure below presents the same results in the form of a bar graph.

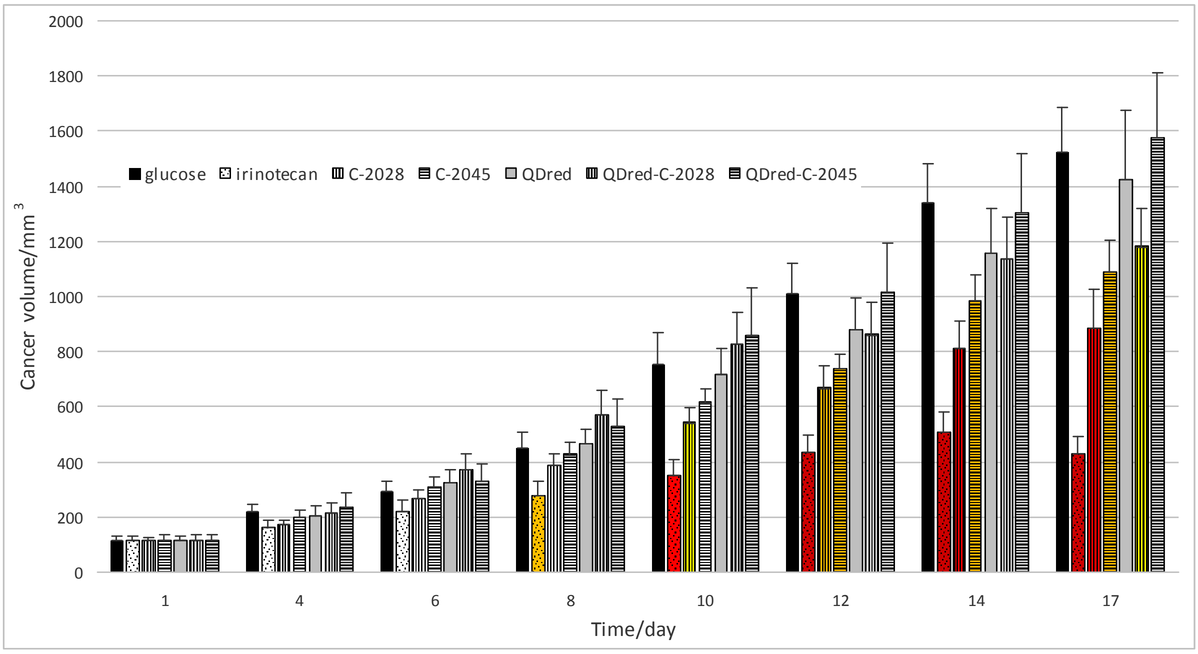

Figure S4. *In vivo* antitumor efficacy of C-2028 and C-2045 non-covalently attached to QD_{red}, QD_{red} and pure bisacridines in HCT116 xenografts in nude mice. Positive control: irinotecan.

Furthermore, we present in Table S4 the values of Student's t-statistics for the significance of tumor volume differences in relation to control for the selected days of experiments. Levels of significance are presented additionally in appropriate colors and with sign "+" or "*".

The same code of colors is used in Figure S4.

Table S4.

Day	Irinotecan		C-2028		C-2045		QD_{red}		QD_{red}-C-2028		QD_{red}-C-2045	
	t stat.	signif.	t stat.	signif.	t stat.	signif.	t stat.	signif.	t stat.	signif.	t stat.	signif.
1	0.040		0.158		0.044		0.010		0.000		0.106	
4	1.258		1.166		0.415		0.252		0.066		0.317	
6	1.135		0.437		0.309		0.496		1.120		0.543	
8	2.037	*	0.757		0.264		0.209		1.207		0.724	
10	2.751	**	1.458	+	0.966		0.235		0.430		0.518	
12	3.819	***	2.177	*	1.836	*	0.751		0.855		0.023	
14	4.539	***	2.746	**	1.878	*	0.833		0.945		0.142	
17	5.167	***	2.692	**	1.915	*	0.333		1.440	+	0.189	

p value	critical value	code
< 0.1	1.316	+
< 0.05	1.708	*
< 0.01	2.485	**
< 0.001	3.450	***

(1) Coates, J. *Interpretation of Infrared Spectra, a Practical Approach,* in: Meyers, R.,A.; (Ed.), *Encyclopedia of Analytical Chemistry*, John Wiley&Sons Ltd, Chichester, **2000**, pp.10815-10837.

(2) Thomas, G. J.; Kyogoku, Y. *Biological Science*, in: Brame, E. G.; Grasselli, J.G.; (Eds.), *Infrared and Raman Spectroscopy (part C)*, Marcel Dekker, New York, **1977**, pp. 717-861.