THE DEVELOPMENT OF MULTIFUNCTIONAL FLUORESCENT PROBES OF RHODAMINE-PYRIMIDINE TYPE

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Introduction

One of the most important organelle studied with respect to the cell viability is mitochondrion. The fluorescent visualization represents a convenient method for study of processes in the mitochondria.[1] Although several fluorescent systems for mitochondria visualization were developed there are still some gaps that remain unsolved.[2,3] This report is focused on the development of fluorescent systems suitable for staining the mitochondria, using cheap and highly accessible Rhodamine B. The method employs the solid-phase chemistry with application of combinatorial approach which allows the high throughput synthesis for systematic study of fluorescent properties of prepared derivatives. The studied fluorescent systems are formed by central core, unit for control of permeability or solubility and a fluorescent dye bound via appropriate linker (Figure 1).

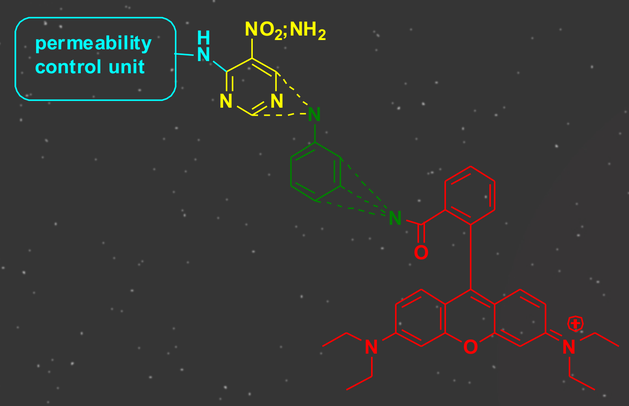
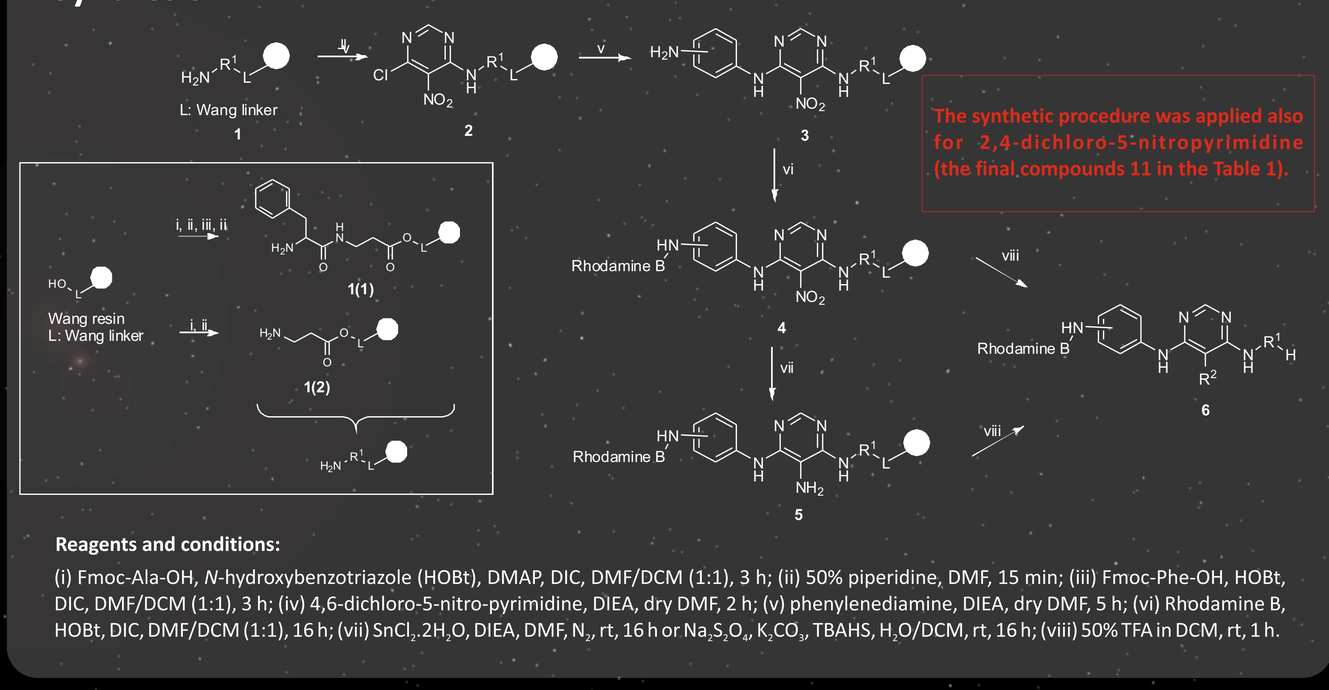


Figure 1: The fluorescent system

Synthesis



Reagents and conditions:

(i) Fmoc-Ala-OH, *N*-hydroxybenzotriazole (HOBt), DMAP, DIC, DMF/DCM (1:1), 3 h; (ii) 50% piperidine, DMF, 15 min; (iii) Fmoc-Phe-OH, HOBt, DIC, DMF/DCM (1:1), 3 h; (iv) 4,6-dichloro-5-nitro-pyrimidine, DIEA, dry DMF, 2 h; (v) phenylenediamine, DIEA, dry DMF, 5 h; (vi) Rhodamine B, HOBt, DIC, DMF/DCM (1:1), 16 h; (vii) $SnCl_2.2H_2O$, DIEA, DMF, N_2, rt, 16 h or $Na_2S_2O_4$, K_2CO_3, TBAHS, H_2O/DCM, rt, 16 h; (viii) 50% TFA in DCM, rt, 1 h.

Table 1: Summary of the prepared compounds

Entry	R^1	R^2	R^3	Purity[a] [%]	Yield[b] [%]	λ_{ex} [nm]	λ_{em} [nm]	QY (DMSO)	penetration
6(1,1,1)	1(1)	NO_2	*p*-	82	61	556	580	0.2132	no
6(1,2,1)	1(1)	NH_2	*p*-	77	36	550	572	0.6912	no
6(1,1,2)	1(1)	NO_2	*m*-	82	86	566	591	0.0011	yes
6(1,2,2)	1(1)	NH_2	*m*-	65	30	393	459	0.0313	yes
6(1,1,3)	1(1)	NO_2	*o*-	85	83	557	581	0.2254	yes
6(1,2,3)	1(1)	NH_2	*o*-	67	70	386	463	0.0871	no
6(2,1,1)	1(2]	NO_2	*p*-	83	25	559	580	0.1809	yes
6(2,2,1)	1(2)	NH_2	*p*-	59	32	350	475	0.0067	yes
6(2,1,2)	1(2)	NO_2	*m*-	73	37	558	582	0.3845	yes
6(2,2,2)	1(2)	NH_2	*m*-	62	23	550	568	0.1238	yes
6(2,1,3)	1(2)	NO_2	*o*-	61	5	560	582	0.0373	yes
6(2,2,3)	1(2)	NH_2	*o*-	69	11	336	463	0.0052	yes
11(1,1,1)	1(1)	NO_2	*p*-	70	70	555	580	0.2712	yes
11(1,2,1)	1(1)	NH_2	*p*-	80	23	553	583	0.3019	no
11(1,1,2)	1(1)	NO_2	*m*-	89	92	560	586	0.1841	yes
11(1,2,2)	1(1)	NH_2	*m*-	56	14	557	582	0.2149	no
11(1,1,3)	1(1)	NO_2	*o*-	72	76	568	592	0.0528	yes
11(1,2,3)	1(1)	NH_2	*o*-	77	67	568	590	0.0284	yes
11(2,1,1)	1(2)	NO_2	*p*-	69	14	560	590	0.2562	yes
11(2,1,2)	1(2)	NO_2	*m*-	78	22	566	590	0.0995	yes
11(2,1,3)	1(2)	NO_2	*o*-	68	35	350	489	0.0064	no
11(2,2,1)	1(2)	NH_2	*p*-	55	4	568	590	0.2549	yes
11(2,2,2)	1(2)	NH_2	*m*-	51	7	373	439	0.0233	yes
11(2,2,3)	1(2)	NH_2	*o*-	72	15	562	586	0.3125	yes

[a]Purity of crude products estimated from LC traces. [b]Isolated yield after HPLC purification.

pH stability and photostability

The pH dependence study was performed for derivatives **6(1,1,2)**, **6(2,1,3)**, **(1,1,3)** and **11(2,1,3)**. The fluorescence emission measured under various pH is demonstrated in Figure 3.

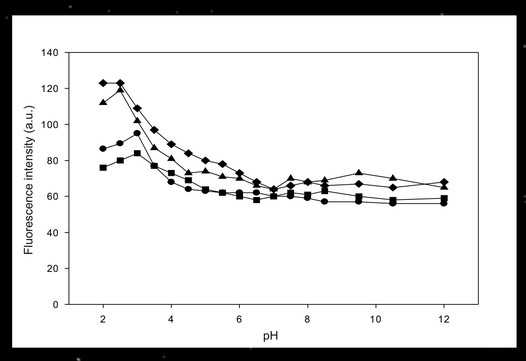


Figure 3: Dependence of fluorescence intensity on pH for compounds **6(1,1,2)** ▲, **6(1,1,3)** ♦, **6(2,1,3)** ● and **11(2,1,3)** ■.

The photostability of selected compounds was tested. During one hour there was no decreasing of intensite in all cases (Figure 4).

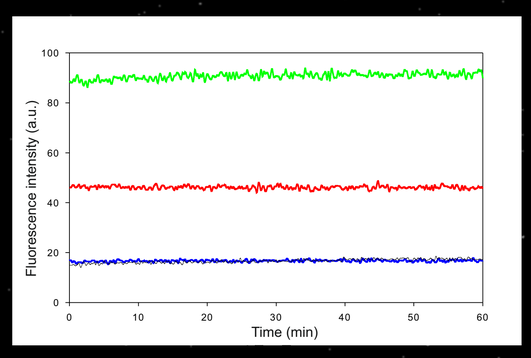


Figure 4: Intensity of fluorescence of derivatives **6(1,1,2)**, **6(1,1,3)**, **6(2,1,3)** and **11(2,1,3)** during one hour measurement. **6(1,1,2)**, black; **6(1,1,3)**, blue; **6(2,1,3)**, green; **11(2,1,3)**, red.

The basic fluorescent properties

All compounds were tested for their basic fluorescent properties in DMSO. The excitation and emission spectra of prepared compounds mostly showed similar shape and maxima as Rhodamine B. The various substitutions of central group as well as different linker affect significantly the fluorescence intensity. Interestingly some derivatives do not exhibited rhodamine excitation maximum (around 560 nm), but only one maximum between 336-393 nm (Table 1). The emission intensity of this compounds at rhodamine excitation was much more higher when the measurement was done in water (Figure 2).

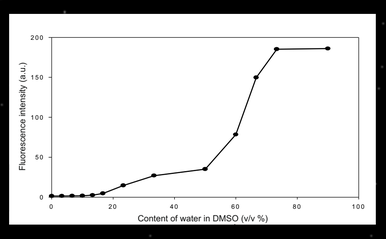


Figure 2: Fluorescence intensity of derivative **6(1,2,3)** according to water/DMSO ratio. Excitation wavelength is 560 nm.

Table 2: Fluorescence properties of studied compounds.

	λ_{ex}/ λ_{em} (QY)	
Compound	**DMSO**	**Water**
6(1,1,2)	393/459 (0,03)	0,0076
6(1,1,3)	386/463 (0,0871)	0,2997
6(2,2,2)	350/475 (0,0067)	0,1862
6(2,1,3)	336/463 (0,0052)	0,1024
11(2,1,3)	350/489 (0,0064)	0,1928
11(2,2,3)	373/439 (0,0233)	0,3603

Penetration the cell membrane

The prepared compounds were studied for the ability to penetrate to the living cells (U-2-OS human osteosarcoma). From the 24 studied compounds 18 of them were able to penetrate into the cell interior (Table 1). According to the fluorescent microscopy observation all of them were selectively accumulated in mitochondria (Figure 5).

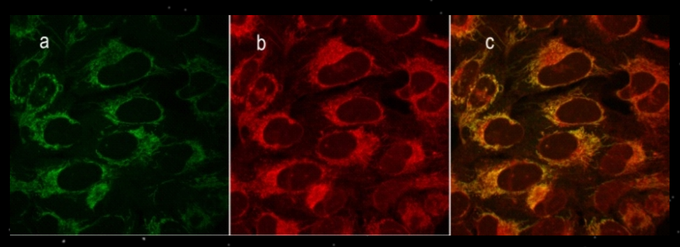


Figure 5: Fluorescent image of the compound with ability to penetrate to U-2-OS cell: a) NADH autofluorescence at 355 nm visualizing live mitochondria; b) cells after 15 min. treatment with **11(2,2,3)** excited at 561 nm; c) overlay of (a) and (b)

References:

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This work was supported by the National Program of sustainability (project LO1304) and Biotrend (CZ.1.07/2.2.00/28.0184).

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