Cyclodextrin encapsulated pH sensitive dyes as fluorescent cellular probes: self-aggregation and in vitro assessments

Monica Sardaru 1,2, Oana Carp 1, Elena Laura Ursu 1, Anda-Mihaela Craciun 1, Corneliu Cojocaru 1, Mihaela Silion 1, Vladyslava Kovalska 3,4, Ionel Mangalagiu 2, Ramona Danac 2

and Alexandru Rotaru 1,*.

1 “Petru Poni” Institute of Macromolecular Chemistry, Romanian Academy, Grigore Ghica Voda Alley 41 A, 700487 Iasi, Romania
2 Alexandru Ioan Cuza University of Iasi, Chemistry Department, 14 Carol I, 700506 Iasi, Romania
3 Institute of Molecular Biology and Genetics, NASU, 150 Zabolotnogo St., 03143 Kyiv, Ukraine
4 Scientific Services Company Otava Ltd, 150 Zabolotnogo St., 03143 Kyiv, Ukraine

* Correspondence: rotaru.alexandru@icmpp.ro;

Content

1. ESI-MS spectra – Figures S1, S2, S3
2. TEM images – Figure S4
3. UV-Vis spectra – Figures S5, S6
4. Fluorescence spectra – Figure S7
5. Molecular docking models – Figures S8, S9
6. Compounds uptake into HeLa cells – Figures S10, S11
7. Compounds intracellular distribution and co-staining with LysoTracker Red – Figures S12, S13
8. Compounds intracellular distribution and co-staining with MitoTracker Red – Figures S14, S15
Figure S1. Examples of ESI-MS spectra of the 1a_CD at 1:5 molar ratio between indolizine 1a and CD. Peaks corresponding to: β-CD (MNa⁺ ion at m/z 1157), the formation of 1:1 inclusion complex (M⁺-Br + CD ion at m/z 1687) and 1:2 (M⁺-Br + 2CD ion at m/z 2822) have been identified.
Figure S2. Examples of ESI-MS spectra of the 1b_CD at 1:5 molar ratio between indolizine 1b and CD. Peaks corresponding to: β-CD (MNa⁺ ion at m/z 1157), the formation of 1:1 inclusion complex (M⁺-Br + CD ion at m/z 1733) and 1:2 (M⁺-Br + 2CD ion at m/z 2867) have been identified.
Figure S3. Examples of ESI-MS spectra of the 1c_CD at 1:5 molar ratio between indolizine 1c and CD. Peaks corresponding to: the formation of 1:1 inclusion complex (M^+ - Br + CD ion at m/z 1803) and 1:2 (M^+ - Br + 2CD ion at m/z 2939) have been identified.
Figure S4: Examples of TEM images for compounds 1a_CD (A); 1b_CD (B); 1c_CD (C).

Figure S5: UV-Vis spectra of compounds 1(a-c) at pH value of 1.0 (0.1 M HCl), 7.4 (1xTAE) and 13.0 (0.1 M NaOH).
Figure S6: UV-Vis spectra of compounds 1(a-c)_CD at pH value of 1.0 (0.1 M HCl), 7.4 (1xTAE) and 13.0 (0.1 M NaOH).
Figure S7: Fluorescence spectra at pH = 1.0 (0.1 M HCl), 7.4 (1xTAE) and 13.0 (0.1 M NaOH) for compounds: (A) compound 1b, (B) compound 1b_CD, (C) compound 1c and (D) compound 1c_CD.
Figure S8: Examples of molecular docking models of compound 1b in complex with β-CD showing the possibility of the 1:1 (A) and 1:2 (B) inclusion complexes formation.

Figure S9: Examples of molecular docking models of compound 1c in complex with β-CD showing the possibility of the 1:1 (A) and 1:2 (B) inclusion complexes formation.
Figure S10. Compound 1b_CD uptake into HeLa cells after 15 min (left) and 24 hours (right) incubation.

Figure S11. Compound 1c_CD uptake into HeLa cells after 15 min (left) and 24 hours (right) incubation.
**Figure S12.** Examples of images for intracellular distribution of compound 1b_CD after 24 hours (A) compared to LysoTracker Red (B) and corresponding overlay (C).
Figure S13. Examples of images for intracellular distribution of compound 1c_CD after 24 hours (A) compared to LysoTracker Red (B) and corresponding overlay (C).
Figure S14. Examples of images for intracellular distribution of compound 1b_CD after 24 hours (A) compared to MitoTracker Red (B) and corresponding overlay (C).
Figure S15. Examples of images for intracellular distribution of compound 1c_CD after 24 hours (A) compared to MitoTracker Red (B) and corresponding overlay (C).