3rd International Electronic Conference on Medicinal Chemistry

1-30 November 2017

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Synthesis of Luminescent Squaramide Monoesters: Cytotoxicity and Cell Imaging Studies in HeLa Cells

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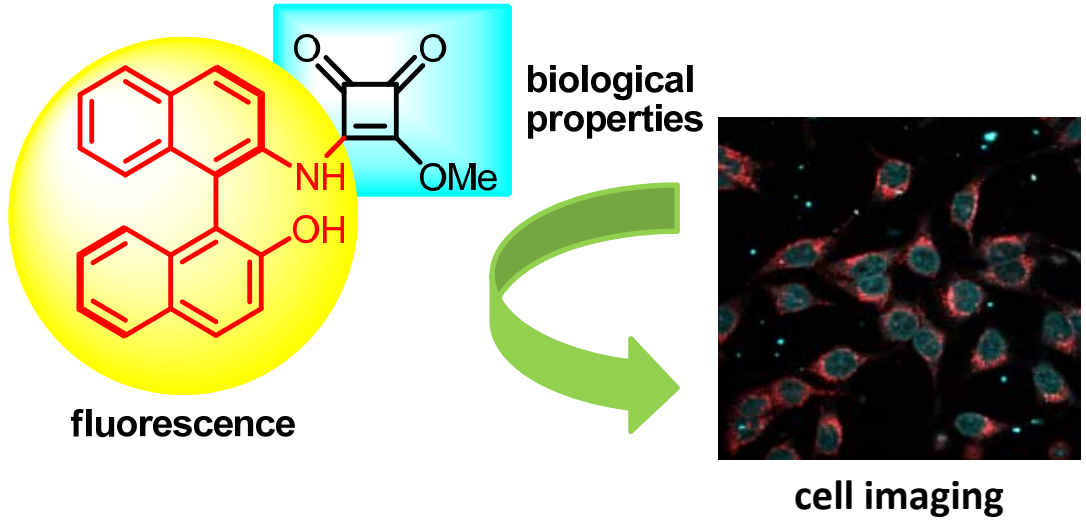
Graphical Abstract



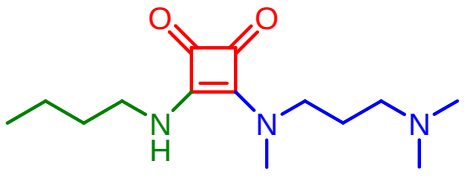
Herrera, Gimeno and co-workers, *RSC Adv.* **2016**, *6*, 14171-14177.

Abstract:
Novel luminescent squaramide monoesters functionalized with different fluorophore groups have been synthesized and explored in cell imaging for the first time.
Cytotoxicity studies performed in HeLa cervical cancer cells revealed high activity for some of these novel structures, highlighting the importance of the fluorescent fragment in the efficiency of these promising anticancer agents.
In addition, fluorescence cell microscopy disclosed the different biodistribution behavior depending on the fluorescent moiety, and the possibility of nuclear localization of chiral non planar squaramide monoesters.

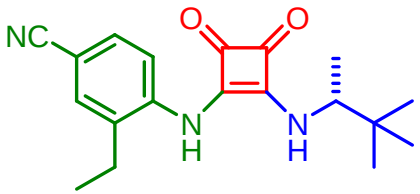
Keywords: squaramide, luminescence, cancer, HeLa

Introduction

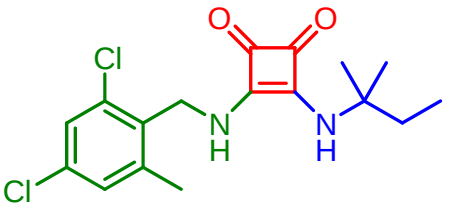
Squaramide motif has gained increased interest in medicinal chemistry being considered as valuable candidates for drug design.



anti-Chagasic drug



WAY-133537
smooth muscle relaxant



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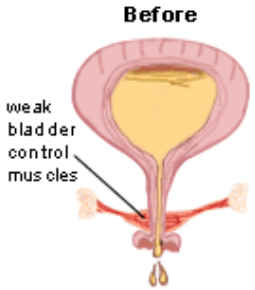
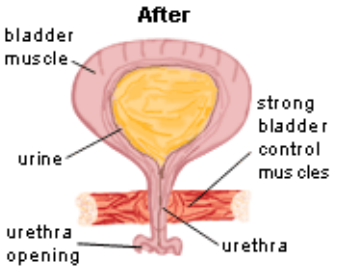
smooth muscle relaxant

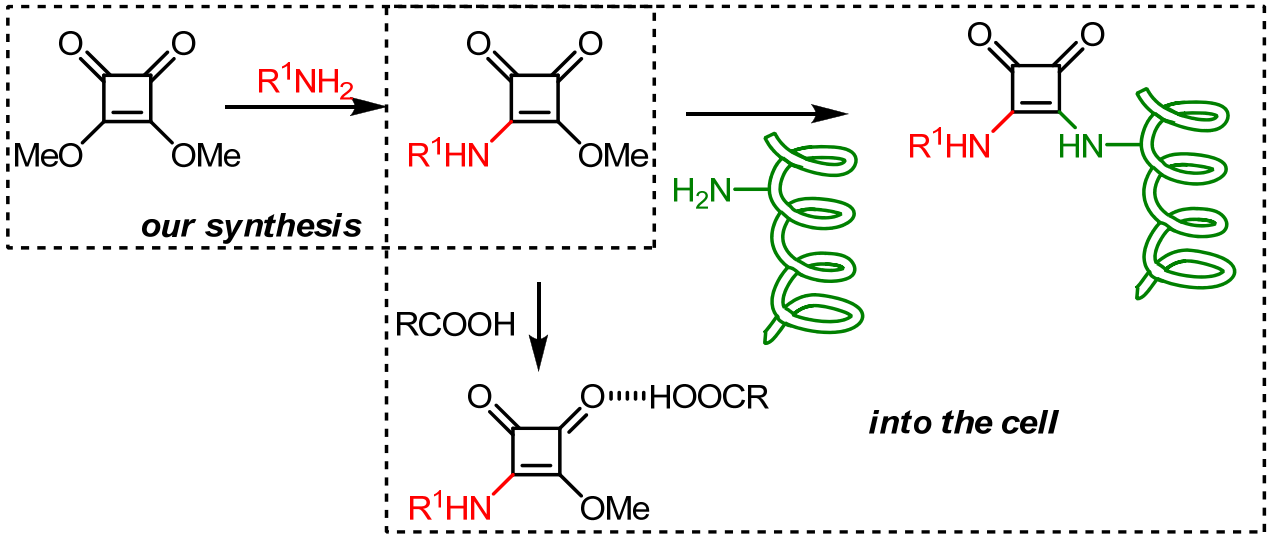




Herrera and co-workers. *RSC Adv.* **2015**, *5*, 33450-33462.

In contrast, the study of **squaramide monoesters as cytotoxic agents or for cell imaging has been overlooked in the literature so far**, and no examples as potential anticancer agents have been previously reported.

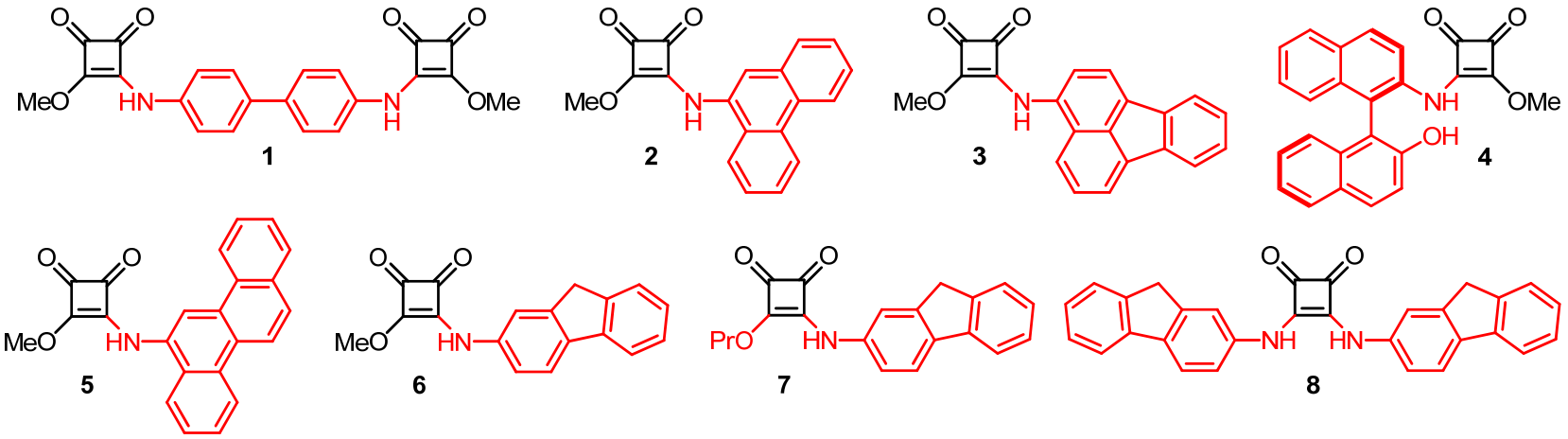
Hypothesis of work



Therefore, we hypothesized that: 1) squaramide monoesters could form plausible covalent bonds with internal amine groups belonging to biological molecules in the cells, or 2) could be anchored by acidic organelles; to help in both cases in the internalization into the cells.

Results and discussion

Squaramide monoesters synthesized and tested in this study

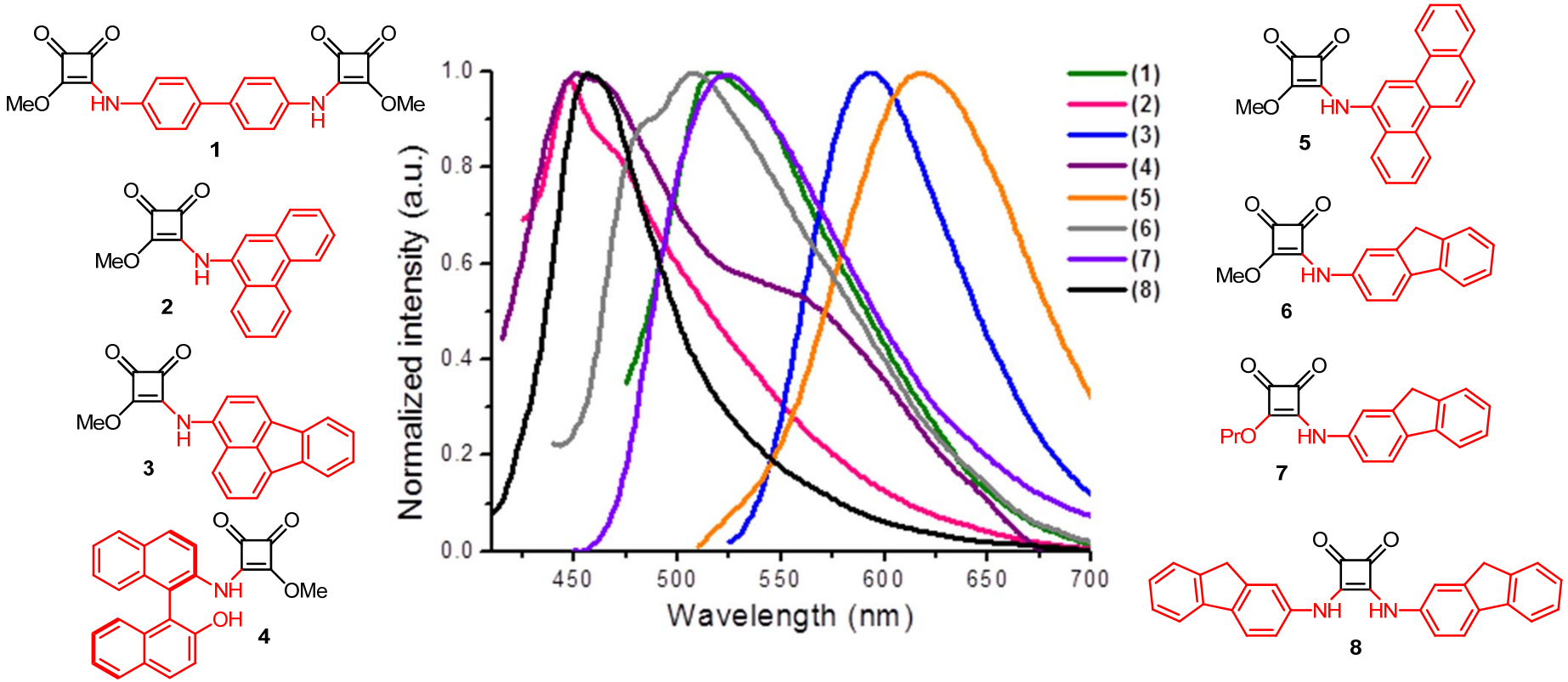


Photophysical properties

UV-visible absorption, emission and excitation spectra of compounds **1**-**8** were recorded in a DMSO solution at 298 K and the most significant data are collected below.

Compound	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	λ_{exc}/nm	λ_{em}/nm	ϕ
1	292 (22500) sh, 353 (54000)	450	515	0,2
2	262 (40200), 274 (37100), 319 (16900), 383 (3400)	414	474	27,9
3	259 (25100), 337 (14800), 483 (14000)	514	591	45,7
4	283 (27800), 318 (21500), 337 (18500) sh, 378 (7020) sh	375	490	0,7
5	266 (76200), 348 (18000), 416 (3300)	490	616	0,6
6	334 (32300), 394 (5400) sh	440	508	0,5
7	280 (14900) sh, 332 (30000), 434 (11000)	457	525	2,3
8	280 (32600), 374 (88900), 430 (7600)	434	525	7,3

Luminescence spectra of all species **1-8** showed a broad emission band between 475 and 615 nm which was tentatively attributed to IL transition processes among the fluorophores and the squaramide.



Biological properties

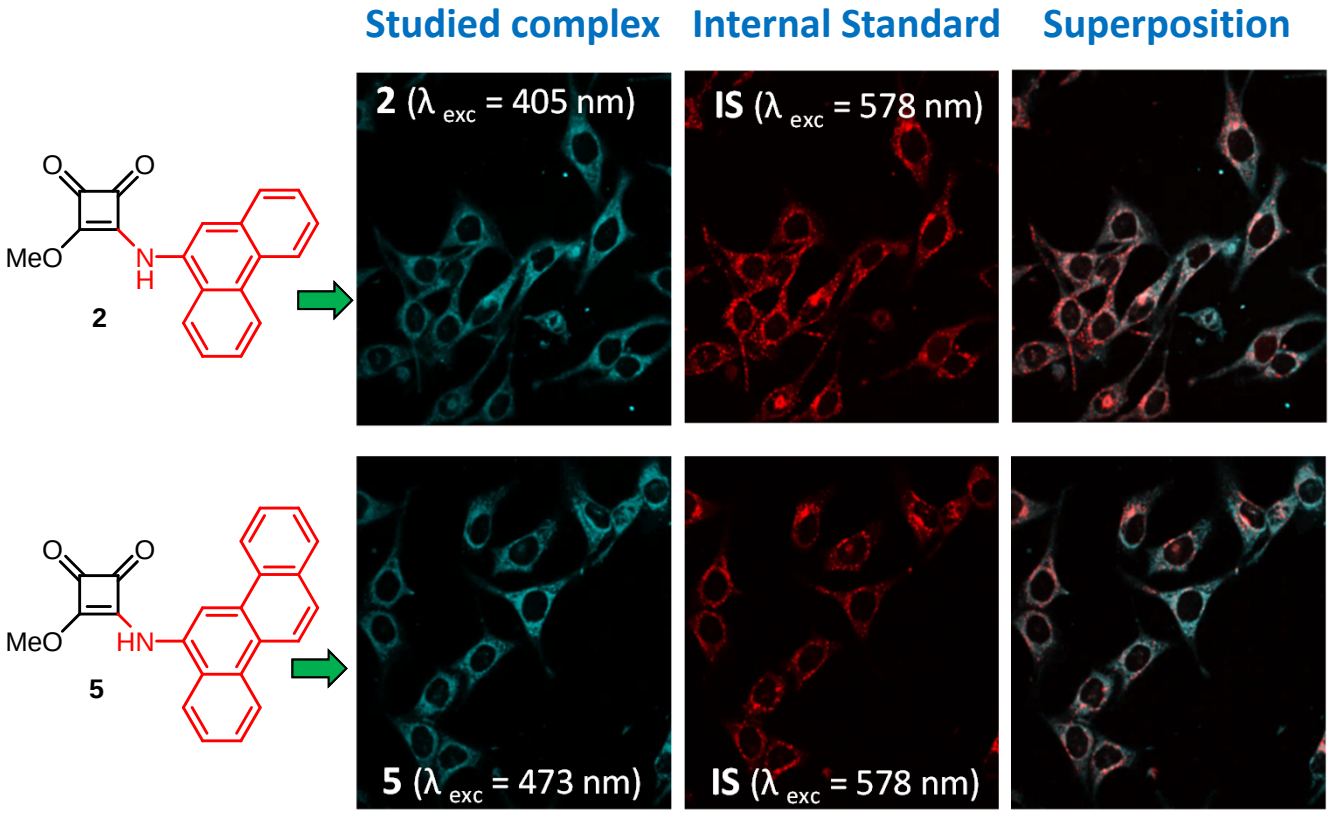
In view of the excellent emission properties of compounds **1**-**8**, a series of experiments were undertaken to test their cytotoxic activity and viability as specific **cell imaging agents** in *human HeLa cervical cancer cells*.

***Values of IC_{50} (μM) for species 1-8 in HeLa cells*.**

Comp.	**1**	**2**	**3**	**4**
IC_{50}	2.25±1.1	115.6±13.9	0.88±0.22	46.7±0.3
Comp.	**5**	**6**	**7**	**8**
IC_{50}	25.3±11.4	1.02±0.07	1.17±0.07	1.18±0.23

- On the basis of results, fluorescence confocal microscopy was only performed with selected species, ie. **2**, **4** and **5**.
- Species **1**, **3**, and **6**-**8** were discarded for this analysis because they displayed either high cytotoxicity, and/or poor solubility in the buffer media (features to avoid in the design of bioprobes for cell imaging application)

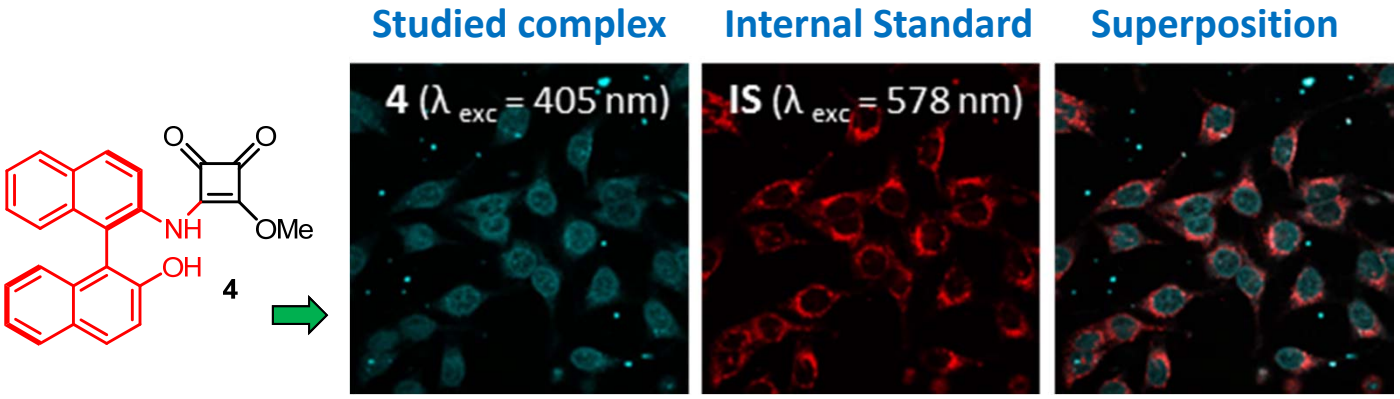
Fluorescence microscopy images of HeLa cells incubated with compounds **2** and **5**.



NOTE:
Blue: studied complex
Red: Internal standard
IS: Internal Standard: LisoTracker Red DND-99 (lisosomal dye)

- **Squaramide monoesters 2** and **5** seemed to have some accumulation in the lisosomes. Their pattern overlaps the pattern of the internal standard.

Fluorescence microscopy images of HeLa cells incubated with compounds **4**.



NOTE:
Blue: studied complex
Red: Internal standard
IS: Internal Standard: LisoTracker Red DND-99 (lisosomal dye)

Interestingly, compound **4** accumulated in the nuclear region. The differences in the internationalization could be due to:

- The acidic character between **4** *vs* **2** and **5** is different, or the cellular internalization mechanism did not occur via an endocytotic process.
- The different biodistribution could be also due to the chirality and non-planarity of the fluorophore fragment in **4**.
- In addition, the chirality of the probe may lead also to specific DNA binding and chiral recognition, a key feature for designing selective bioprobes.

Conclusions

- Novel luminescent squaramide monoesters have been explored as bioactive agents and in cell imaging for the first time.
- Cytotoxicity studies performed in HeLa cells revealed a high activity for some of these species, where the selection of the fluorescent fragment seems to be decisive for the efficiency of these promising anticancer agents in HeLa cells.
- Fluorophores containing the cyclopentadiene unit or with not fused phenyl rings are the most active.
- Fluorescence cell microscopy pointed out the different biodistribution behavior depending on the fluorescent moiety, and both lysosomal and nuclear localization has been observed, which highlights and proposes the possibility for chiral and non-planar bioprobes, as in the case of complex **4**, to exert a chiral recognition within the nucleus.
- Since the squaramide functionality provides a way to increase the transport ability of a receptor without significantly increasing the lipophilicity, it offers an ideal platform for designing future anion transporters.

Acknowledgments

Authors thank the Ministerio de Economía y Competitividad (MINECO/FEDER CTQ2013-48635-C2-1-P, CTQ2016-75816-C2-1-P and SAF2013-48626-C2-2-R), and DGA-FSE (E77 and E104) for financial support of this research.
J. V. A.-R. thanks to DGA for his pre-doctoral contract.