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Synthesis of Squaramides with Anti-tumor Activity

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OrganoCatálisis Asimétrica
Asymmetric OrganoCatalysis

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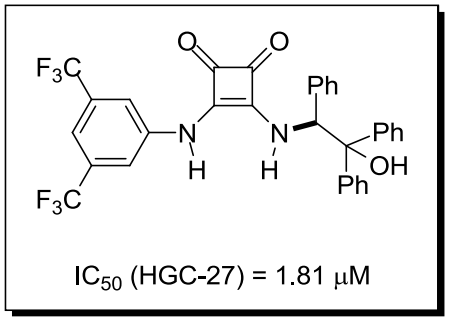
Synthesis of Squaramides with Anti-tumor Activity

Graphical Abstract

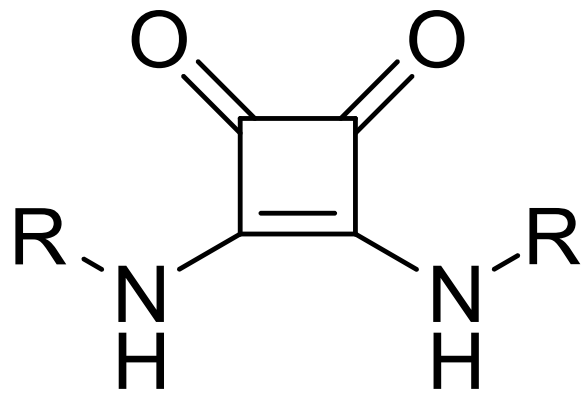


specificity against HGC-27 cells

Abstract:
In this study, the cytotoxic effects of different squaramides were tested against diverse cancer cells, such as HGC-27, HeLa, T98 and U87 cells, and non-cancer cells, such as EK293, MDCK and Vero cells. We found a disubstituted squaramide that showed an IC_{50} of 1.81 µM against HGC-27 cells, which is considerably lower than the IC_{50} observed in the rest of the cell lines.
Furthermore, the mechanism of action of this compound was evaluated. The results indicate that the decrease in cell viability produced by the squaramide is probably caused by G_0/G_1 cell cycle arrest and caspase-mediated apoptosis. Additionally, the cell death produced by this compound is accompanied by autophagy induction having a protective effect and no signs of cathepsin-mediated cell death or necroptosis have been observed.
The creation of compounds that trigger a specific cell death subroutine is preferred since it might avoid potential side-effects and nonspecific cytotoxic effects. Therefore, this squaramide and its derivatives could be promising molecules for the treatment of gastric carcinoma.

Keywords: squaramide; cancer; HGC-27; anti-tumor

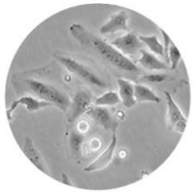
Introduction

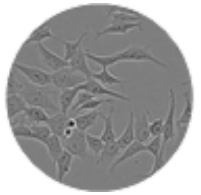


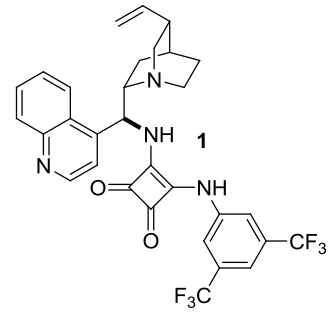
Squaramides
in Medicinal Chemistry

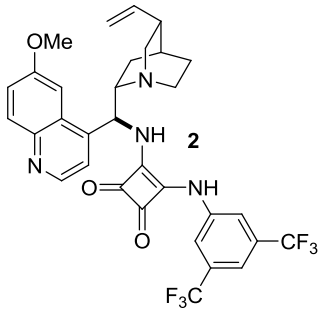
- Linkers for biomolecules
- Phosphate isosteres
- Antibiotics
- In this study… **Anti-tumor activity**

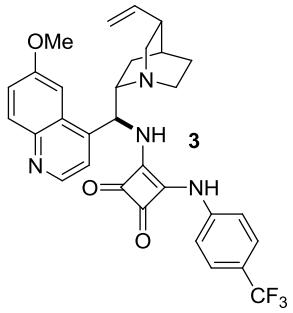
Results and discussion

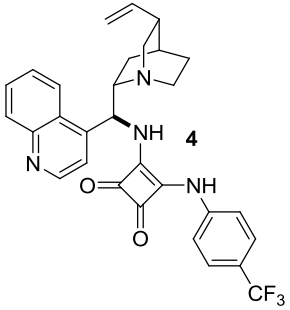


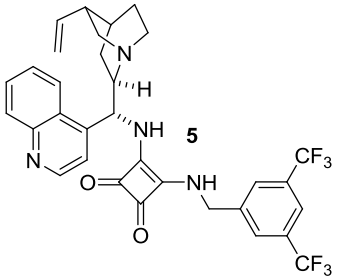


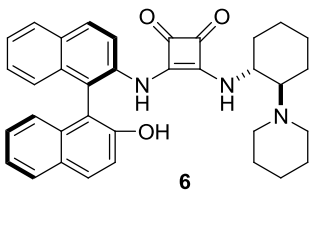


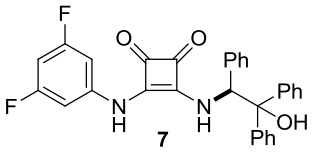


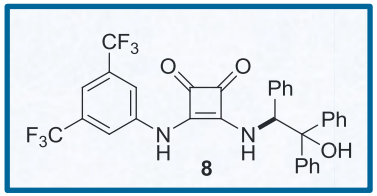


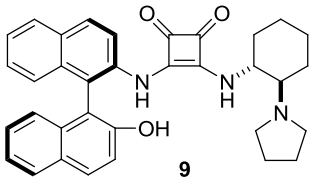








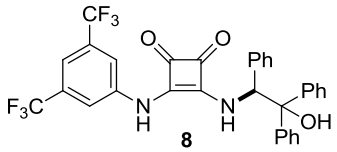




Squaramide	Mean IC_{50} (95% CI) in μM[a]	
	HeLa cells	HGC-27 cells
1	11.3 (10.3-12.2)	8.1 (7.1-9.2)
2	15.2 (13.2-17.4)	8.2 (7.7-8.8)
3	10.8 (9.5-12.3)	4.5 (3.8-5.4)
4	>20	12.8 (11.8-13.7)
5	12.1 (11.4-13.9)	3.0 (2.3-5.0)
6	>20	11.1 (10.5-11.7)
7	>20	3.4 (2.9-4.0)
8	34.6 (28.0-42.9)	1.8 (1.5-2.2)
9	>20	10.8 (10.3-11.2)
Cisplatin	--	20.4 (19.8-22.2)
Doxorubicin	--	15.82 (9.52-26.2)

[a] Measured by a MTT or the SRB assay (Doxorubicin). IC_{50}values are indicated as the average SD of three individual experiments.

Results and discussion



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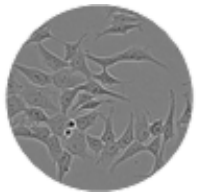
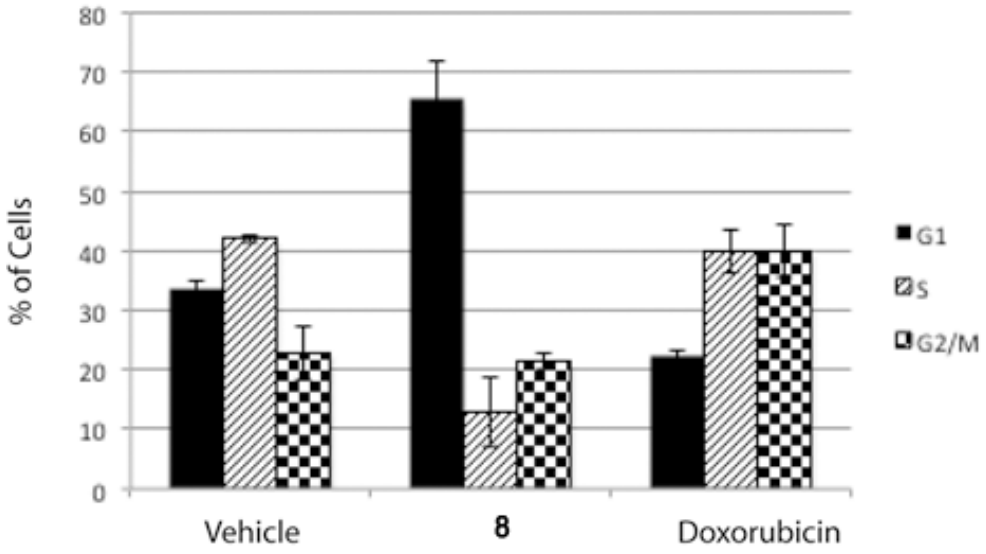
Squaramide	Mean IC_{50} (95% CI) in μM[a]						
	HeLa cells	HGC-27 cells	T98 cells	U87 cells	HEK293 cells	MDCK cells	Vero cells
8	34.6 (28.0-42.9)	1.8 (1.5-2.2) 0.66[b] (0.57-0.76)	7.2 (6.1-8.3)	60.3 (41.5-87.4)	9.0 (7.1-11.5)	70.2 (50.6-97.4)	33.4 (28.0-39.9)

[a] Measured by MTT. IC_{50}values are indicated as the average SD of three individual experiments. [b] IC_{50} measured after 48 h of treatment.

specificity against HGC-27 cells



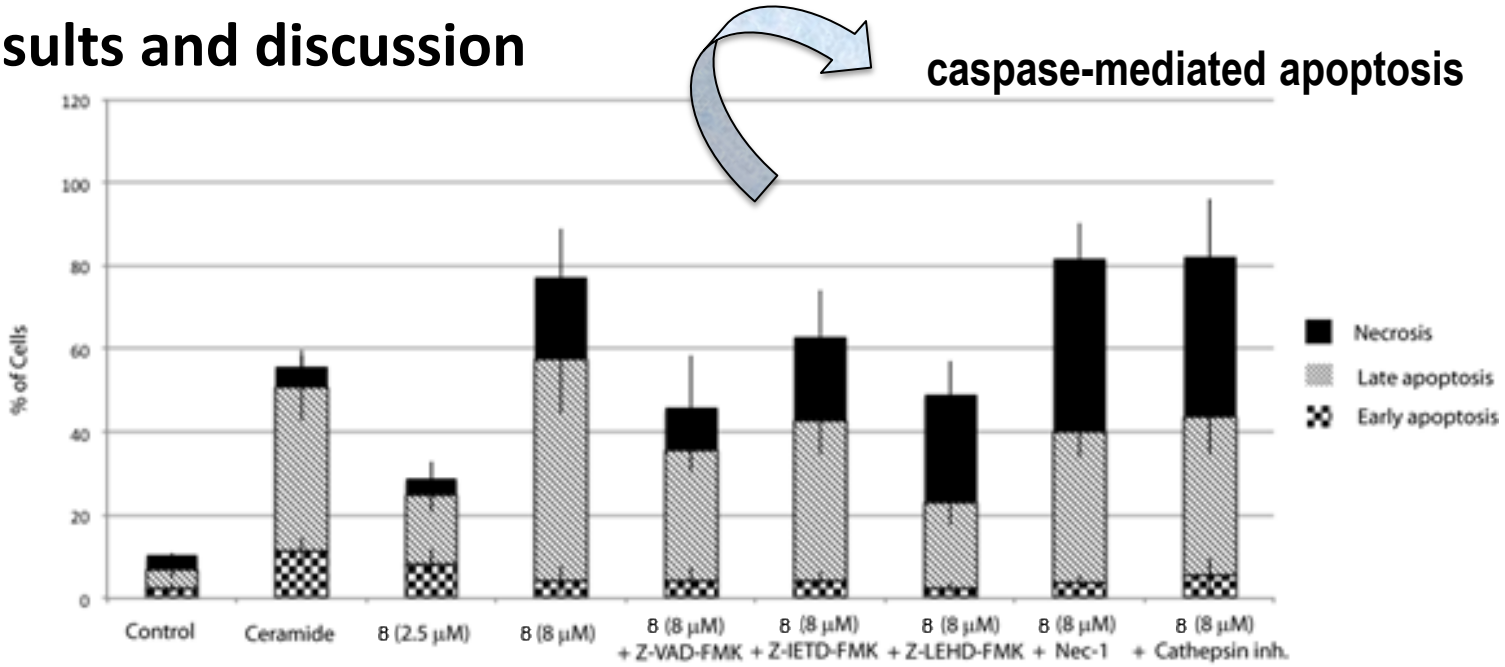
Results and discussion



Cell cycle distribution of HGC-27 cells with and without treatment of compound **8** (5 μM in DMSO) for 24 h. Doxorubicin (500 nM in DMSO) was included as a positive control.

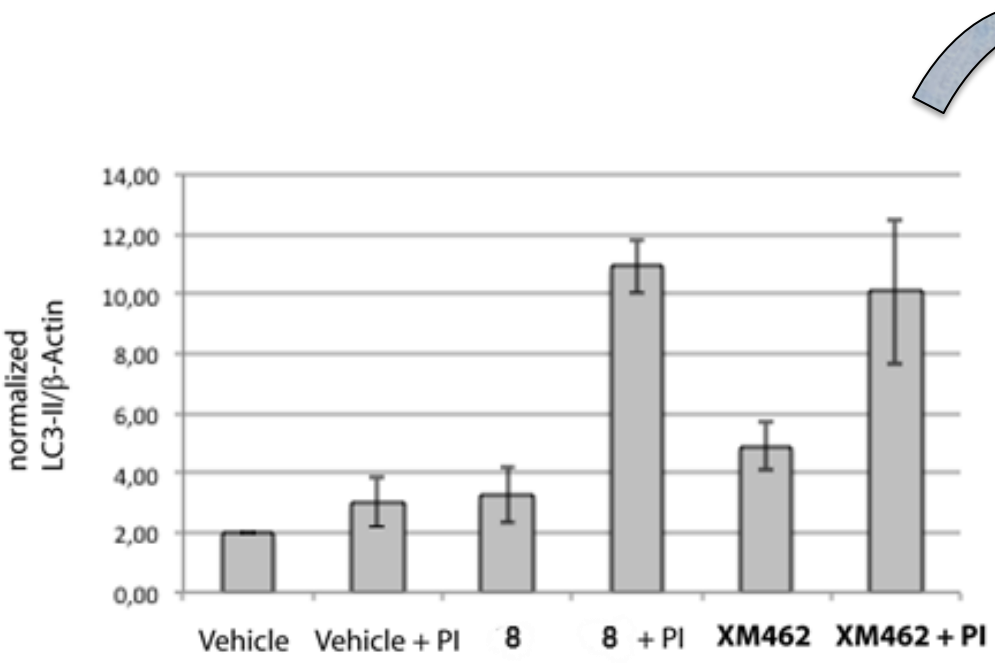
G_0/G_1 cell cycle arrest

Results and discussion



Apoptotic effect of squaramide **8** on HGC-27 cells. Cells were treated with **8** and different compounds: Z-VAD-FMK (20 μM), Z-IETD-FMK (20 μM), Z-LEHD-FMK (20 μM), Nec-1 (10 μM) or Cathepsin inhibitor III (10 μM). C8-Ceramide (20 μM) was used as a positive control. The figure shows the quantitative analysis of necrosis, early and late apoptosis.

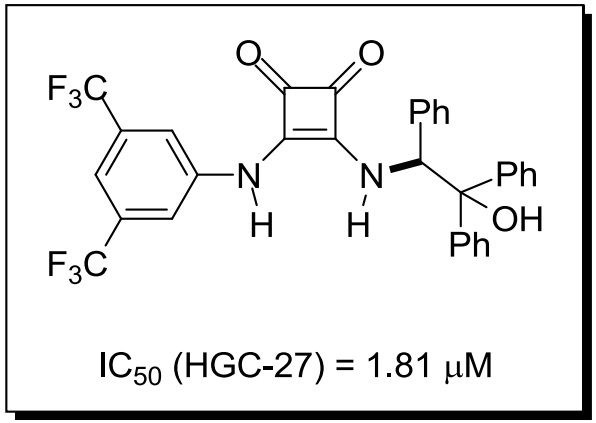
Results and discussion



autophagy induction

Quantified LC3-II levels respect to β-actin when HGC-27 cells were treated with **8** and different compounds: Cloroquine (CQ, 50 µM in EtOH) and 3-Methyladeninde (3-MA, 2 mM in DMSO). XM462 (10 µM), a known autophagy inducer in HGC-27 cells, was used as a positive control.

Conclusions



IC_{50} (HGC-27) = 1.81 μM

- Specificity against HGC-27 cells
- G_0/G_1 cell cycle arrest
- Caspase-mediated apoptosis
- Autophagy induction

Acknowledgments

Group E-104 Aragon Government

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