6th International Electronic Conference on Medicinal Chemistry
1-30 November 2020
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Norhierridin B, a New Hierridin B-Based Hydroquinone with Improved Antitumor Activity

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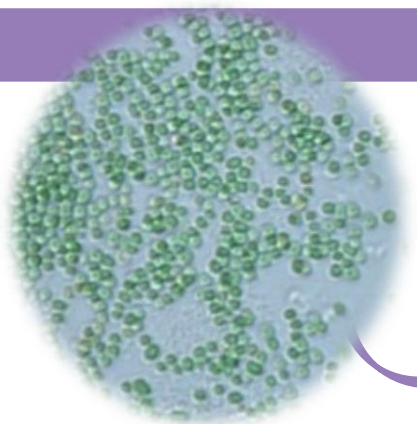
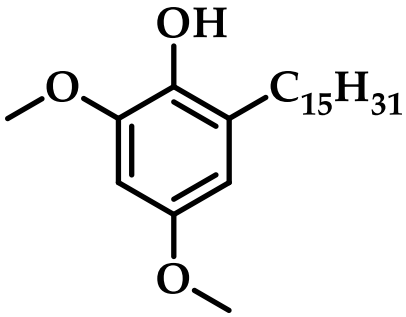
Norhierridin B, a New Hierridin B-Based Hydroquinone with Improved Antitumor Activity

Natural Product



Cyanobium sp. LEGE 06113

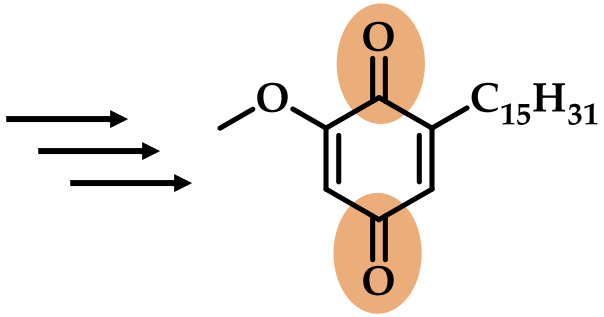


Hierridin B

GI_{50} > 50 µM

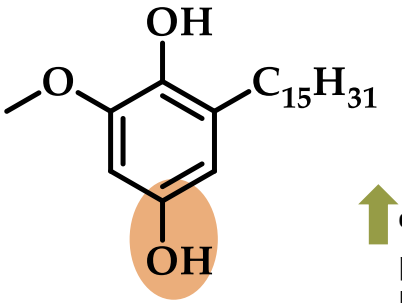
In MDA-MB-231, SKBR3, MDA-MB-468, A375, Huh-7, HCT116 cell lines

Synthetic Analogues



1,4-benzoquinone

GI_{50} = 2.95-29.6 µM



Norhierridin B

GI_{50} = 0.61-3.2 µM

↑ % of dead cells
p21 protein
Bax

↓ Bcl-2

Abstract:

Quinones/hydroquinones constitute a family of metabolites, widespread in nature, with a wide range of biological activities, including cytotoxicity to cancer cells [1]. Recently, **hierridin B, a methylated hydroquinone derivative with a C15 aliphatic chain isolated from the marine picocyanobacterium Cyanobium sp. LEGE06,113 [2], was identified as moderate inhibitor of the growth of colon adenocarcinoma HT-29 cell line, with a GI_{50} of 100.2 µM** [2]. In order to obtain new structurally-related quinone/hydroquinone with improved antiproliferative activity in cancer cell lines, the demethylated hierridin B derivative, norhierridin, as well as structurally-related quinone, were synthesized and evaluated for their growth inhibitory effect in a panel of human tumor cell lines. **Norhierridin B showed a great improvement of the antitumor activity when compared to hierridin and its structurally-related quinone with a potent growth inhibitory effect on all cancer cell lines, being the growth inhibitory effect on MDA-MB-231 cells associated with cell cycle arrest and apoptosis**. Norhierridin B interfered with several p53 transcriptional targets, increasing p21, Bax, and MDM2, while decreasing Bcl-2 protein levels, which suggested the potential activation of a p53 pathway. **Particularly, norhierridin B displayed a prominent growth inhibitory activity against TNBC cells, which are characterized by high therapeutic resistance**.

Keywords: antiproliferative activity, hydroquinones, quinones

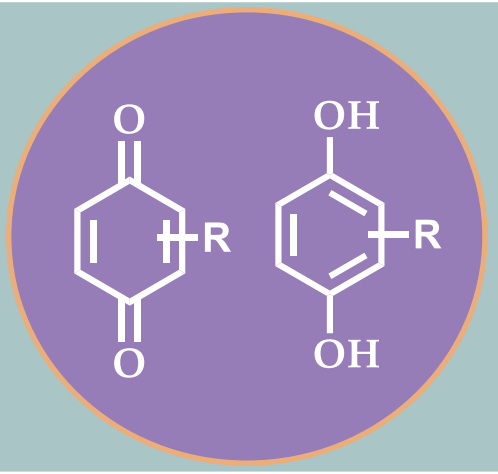
[1] Sunassee, N. and Davies-Coleman, T. Natural product reports, 2012. **29**(5): p. 513-535. [2] Leão, P. et al. PLoS One, 2013. **8**(7): p. e69562.

Introduction: Quinones and Hydroquinones

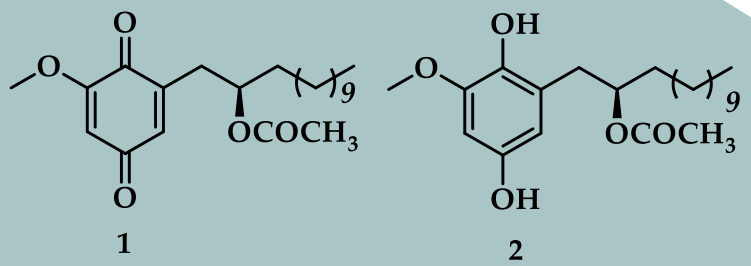
Quinones/ hydroquinones,

- Family of **metabolites** widespread in Nature
- wide range of **biological activities**

→ *in vitro* **growth inhibitory** effect against several human cancer cell lines



Natural quinones/hydroquinones with *in vitro* growth inhibitory effect against cancer cell lines:



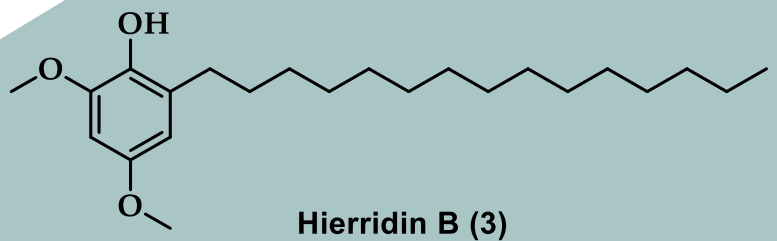
1 2

GI_{50} = 2.54- 11.32 µM

GI_{50} = 2.21- 9.42 µM

isolated from *Ardisia virens*

Chang, S. et al., *Phytochemistry.* **2009,** *70*, (17-18), 2064-2071.



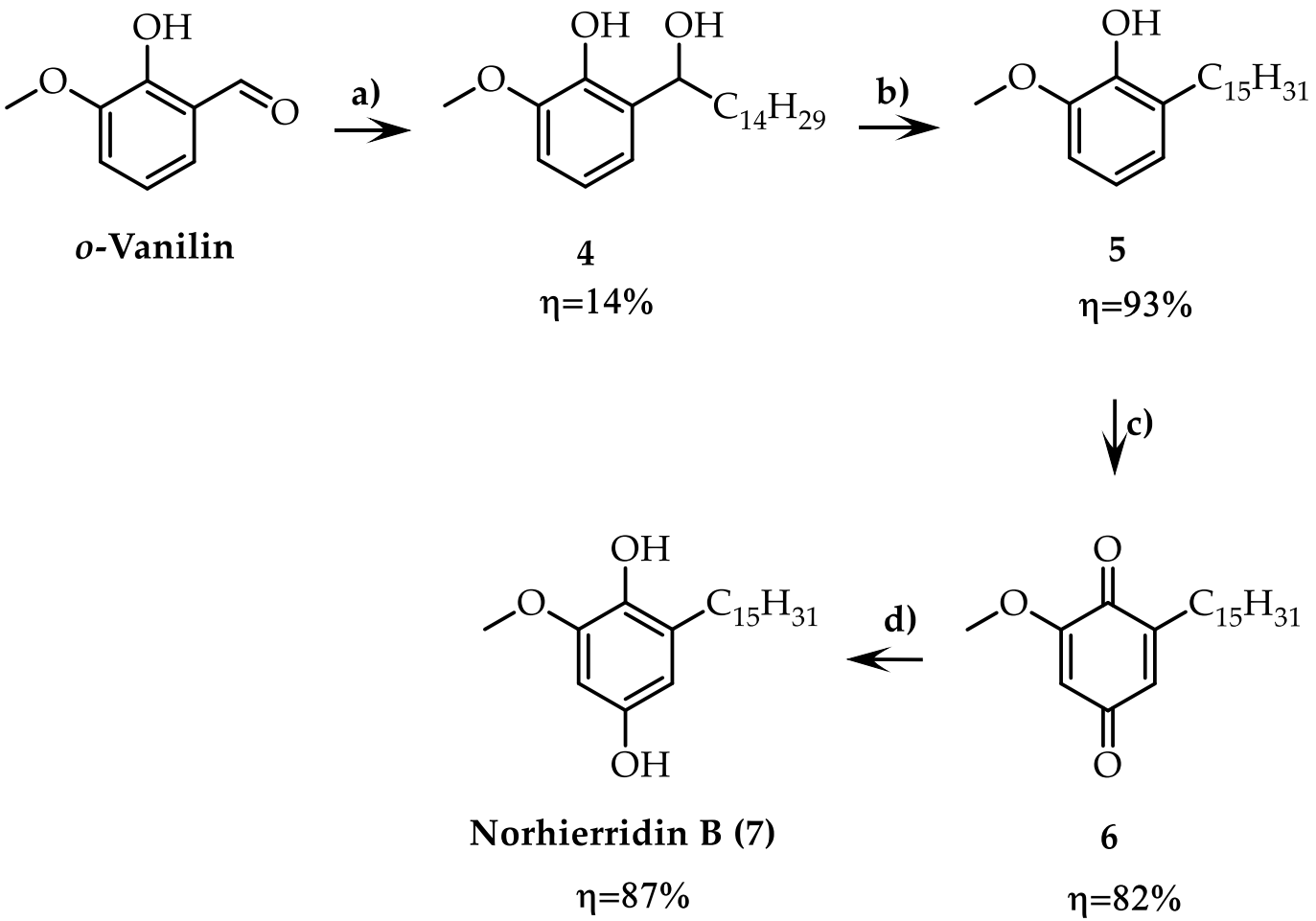
Hierridin B (3)

GI_{50} = 100.2 µM (HT-29 cell line)

isolated from *Cyanobium* sp. LEGE 06113, by CIIMAR research group

Leão, P. et al., *PLoS One.* **2013,** *8*, (7), e69562.

Results and Discussion: Synthesis of quinones and hydroquinones with structure related with Hierridin B (3)



o-Vanilin

4
η=14%

5
η=93%

Norhierridin B (7)
η=87%

6
η=82%

Scheme 1. Synthesis of norhierridin B (**6**). **a)** $C_{14}H_{29}Br$, Mg, Et_2O, 35 °C, 2 h; **b)** HCOOH, Pd/C, EtOH/H_2O, 80 °C, 4 h; **c)** O_2, salcomine, DMF, r. t., 24 h; **d)** $Na_2S_2O_4$, $CHCl_3$/H_2O, r. t., 10 min

Results and Discussion: Biological evaluation

Tumor cell growth inhibitory activity evaluation in Triple Negative Breast Cancer (TNBC) cell lines

Table 1. Effect of compounds **3**-**7** on the growth of human cancer cell lines.

	GI_{50} (μM)				
Cell line/Compound	**3**	**4**	**5**	**6**	**7**
MDA-MB-231	>50	9.65±0.15	28.8±3.3	5.83±1.10	**0.61±0.07**
SKBR3	>50	14.0±0.0	>50	4.35±0.15	**0.77±0.06**
MDA-MB-468	>50	7.85±2.15	31.00±7.00	6.65±0.90	**0.68±0.13**
A375	>50	16.0±1.7	32.9±4.2	20.6±1.9	**2.0±0.4**
Huh-7	>50	27.5±0.5	>50	2.95±0.15	**0.61±0.03**
HCT116	>50	26.5±0.5	26.0±3.2	29.6±0.5	**3.2±0.6**

Norhierridin B (**7**) showed to be the most effective against all cancer cell lines, exhibiting much lower GI_{50} values than its methyl derivative hierridin B (**3**).

Results and Discussion: Biological evaluation

Tumor cell growth inhibitory activity evaluation

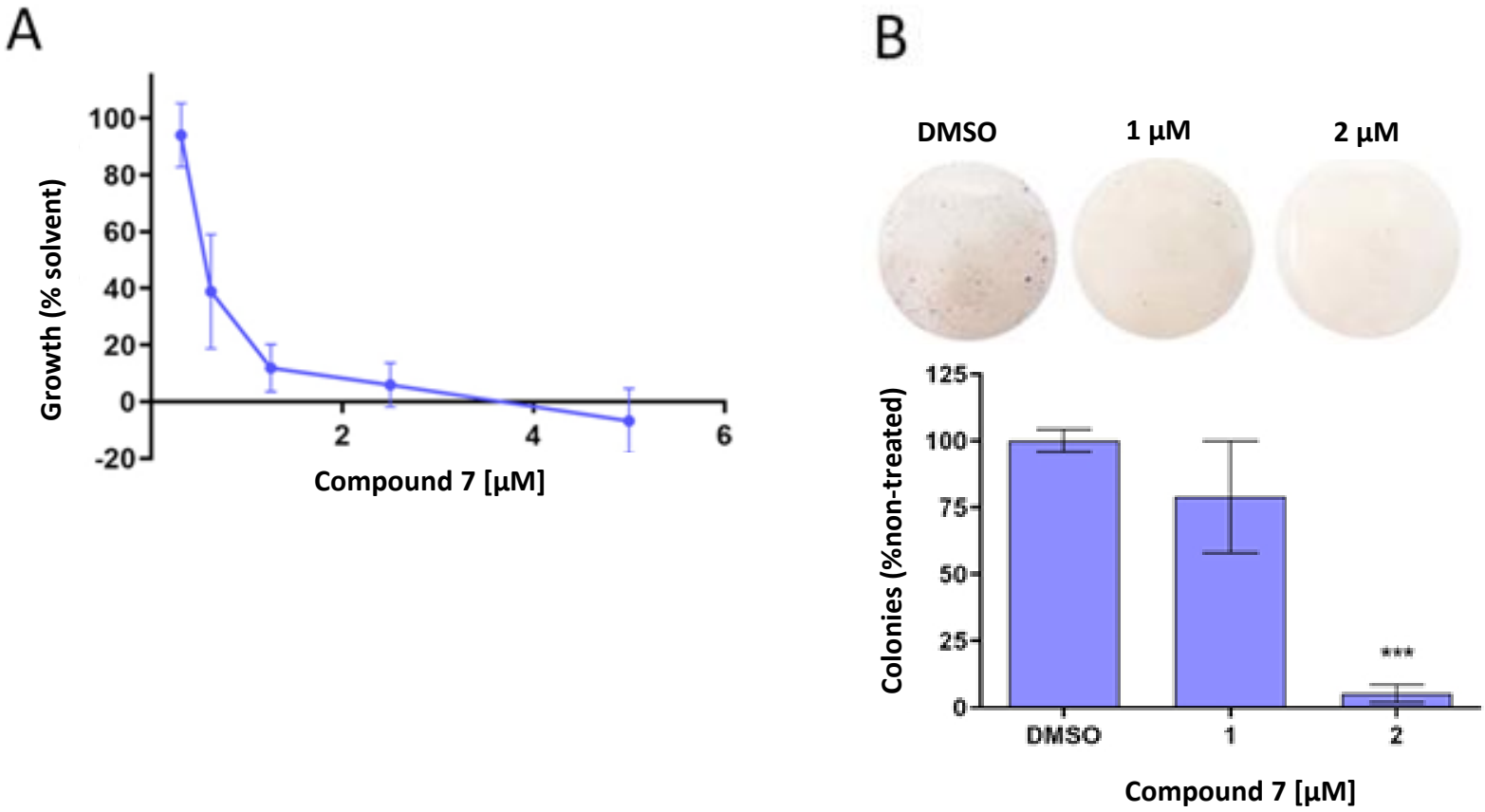


Figure 2: **Norhierridin B (7) inhibits the growth of breast adenocarcinoma MDA-MB-231 cells**. (**A**) Dose-response curve for the growth of MDA-MB-231 cells treated with compound **7** for 48 h, determined by SRB assay; data are mean ± SEM of four independent experiments; growth obtained with vehicle was set as 100%. (**B**) Colony formation assay for MDA-MB-231 cells treated with compound **7** for nine days; images correspond to a representative experiment of two; graph represents mean ± SEM of two independent experiments; values significantly different from DMSO: *$p < 0.05$, unpaired Student's *t*-test.

Results and Discussion: Biological evaluation

Tumor cell growth inhibitory activity evaluation

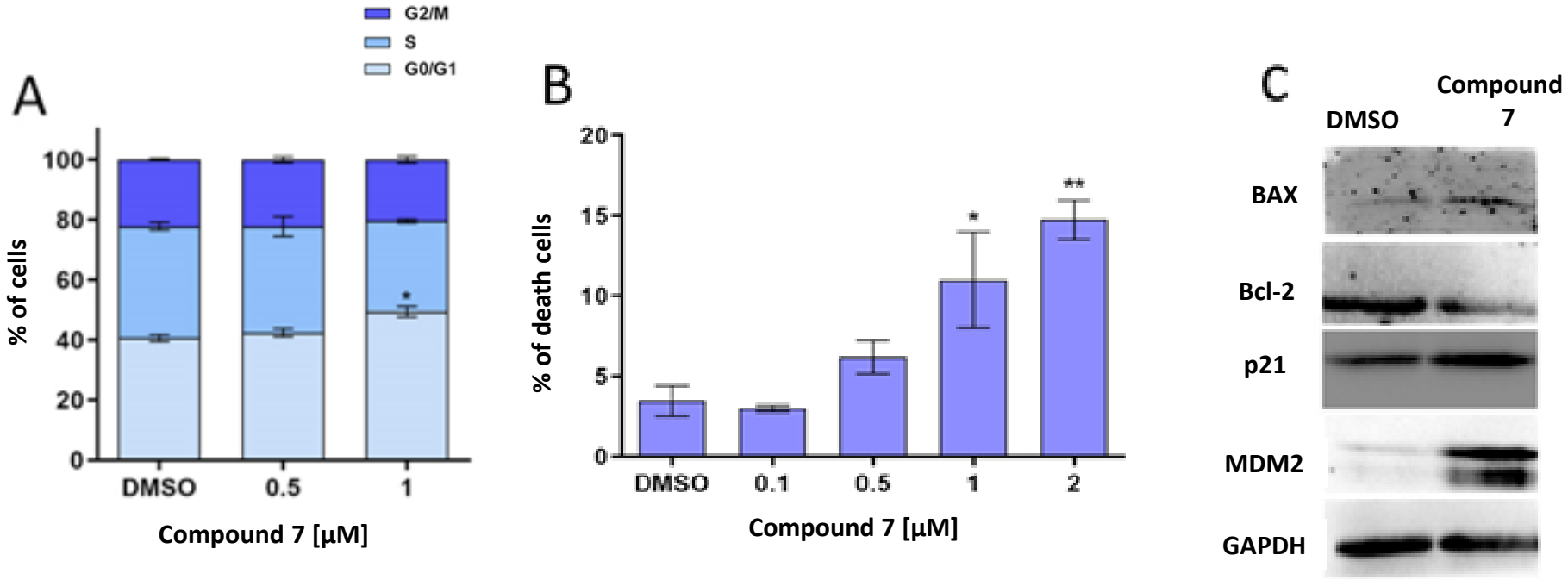
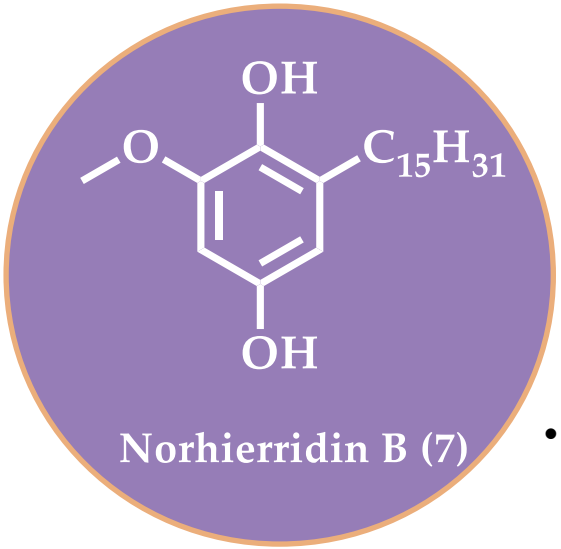


Figure 3: **Norhierridin B (7) induces cell cycle arrest and death and interferes with p53 transcriptional targets**. (**A**) Cell cycle progression was analyzed after 48 h treatment with compound **7** in MDA-MB-231 cells; data are mean ± SEM of three independent experiments; values significantly different from DMSO: *$p < 0.05$, unpaired Student's *t*-test. (**B**) Percentage of death cells determined by trypan blue assay for MDA-MB-231 cells treated with compound **7** for 48 h; data are mean ± SEM of three independent experiments; values significantly different from DMSO: *$p < 0.05$, **$p < 0.001$, unpaired Student's *t*-test. (**C**) Protein expression levels of p53 targets in MDA-MB-231 cells was analysed by western blot after 48 h treatment with 2 μM compound **7.** Immunoblots are representative of three independent experiments; GAPDH was used as loading control.

Conclusions

- …was **synthesized** for the **first time**



- …was the **most effective** in all cancer cell lines
- …exhibiting much lower GI_{50} values than its structural related quinone and hierridin B (**3**)
- …the effects were associated with **cell cycle arrest**, **increase** of **p21** and **Bax** and **decrease** of **Bcl-2** protein expression levels
- …could be an **effective cytotoxic agent against** TNBC cells, which deserve to be further explored in the future.

Acknowledgments

This research was partially supported by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry, CIIMAR), and also UIDB/50006/2020 through national funds provided by the FCT—Foundation for Science and Technology and European Regional Development Fund (ERDF), within the framework of the program PT2020 and the project PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736), co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF and by FCT through national funds. Joana Moreira and Joana Almeida acknowledge for their FCT grants (SFRH/BD/135852/2018 and 2020.05026.BD, respectively).

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