The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)
01–30 November 2023 | Online

Marine-inspired compounds with anti-inflammatory properties and potential anti-pruritic activity

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

Márcia S. Martins [1,2], Solida Long [3], Madalena M. M. Pinto [1,2], Isabel F. Almeida [4,5], Maria T. Cruz [6,7], Emília Sousa [1,2*]

[1] Natural Products and Medicinal Chemistry, CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal;
[2] Laboratory of Organic and Pharmaceutical Chemistry, FFUP-Faculty of Pharmacy, University of Porto, Portugal
[3] Department of Bioengineering, RUPP - Royal University of Phnom Penh, Cambodia,
[4] Laboratory of Pharmaceutical Technology, Department of Drug Sciences, UCIBIO-Applied Molecular Biosciences Unit, MedTech, FFUP-Faculty of Pharmacy, University of Porto, Portugal,
[5] Associate Laboratory i4HB-Institute for Health and Bioeconomy, FFUP-Faculty of Pharmacy, University of Porto, Portugal,
[6] CNC-Center for Neuroscience and Cell Biology, Coimbra, Portugal;
[7] FFUC-Faculty of Pharmacy, University of Coimbra, Portugal.
***** Corresponding author: esousa@ff.up.pt

Marine-inspired compounds with anti-inflammatory properties and potential anti-pruritic activity

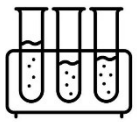
Substance P antagonists

Marine-inspired compounds

***In silico* studies**
Docking studies
Pharmacokinetic studies

***In vitro* studies**
Cytotoxicity
Anti-inflammatory activity evaluation

Pruritic inflammatory diseases



***In chimico* studies**
Nitric Oxide scavenging potential

Synthesis
Two different pathways

Abstract: Chronic pruritus has been associated with the neurokinin 1 receptor (NK1R) and its agonist substance P (SP). With the recent disclosure of the receptor's crystallographic structure, the design of new NK1R antagonists was facilitated. In the marine environment, several SP antagonists were isolated inspiring the synthesis of novel compounds. Since pruritus and inflammation often go together, developing compounds with antipruritic and anti-inflammatory activities is a promising strategy. Therefore, we aim for the structure-based drug design of new SP antagonists based on marine natural products (MNP) to obtain innovative compounds for topical treatment of pruritus-associated inflammatory skin diseases and to evaluate their activity *in silico* and *in vitro*.

In silico, eighteen marine-inspired compounds were found to bind to NK1R with better or equal docking scores than the natural MNP and demonstrated positive pharmacokinetic properties for skin permeation. *In vitro*, no relevant cytotoxicity and a 50% reduction in the release of the pro-inflammatory mediator nitric oxide (NO) was detected. The significant decrease in inducible nitric oxide synthase (iNOS) protein levels and NO release, together with the absence of NO-scavenging potential, suggests the blockage of pro-inflammatory signaling pathways upstream of iNOS synthesis. A structure-activity relationship was established, and two new compounds have already been synthesized by two different pathways and structurally characterized.

Marine-inspired products are promising sources of anti-inflammatory compounds and NK1R antagonists for the treatment of skin conditions characterized by pruritus and inflammation.

Keywords: Inflammation; marine-inspired compounds; neurokinin 1 receptor; pruritus, substance P antagonist.

Pruritus

Innate response capable of protecting the skin against irritants

> Six weeks

Chronic Pruritus (CP)

"unpleasant sensation that elicits the desire or reflex to scratch"

Samuel Hafenreffer, 1660

CP can be associated:

- Systemic diseases
- Neurological diseases
- Psychiatric disorders
- Dermatological conditions
  - **Common skin diseases**
    - Atopic dermatitis
    - Psoriasis
  - **Rare skin diseases**
    - Prurigo nodularis
    - Epidermolysis bullosa

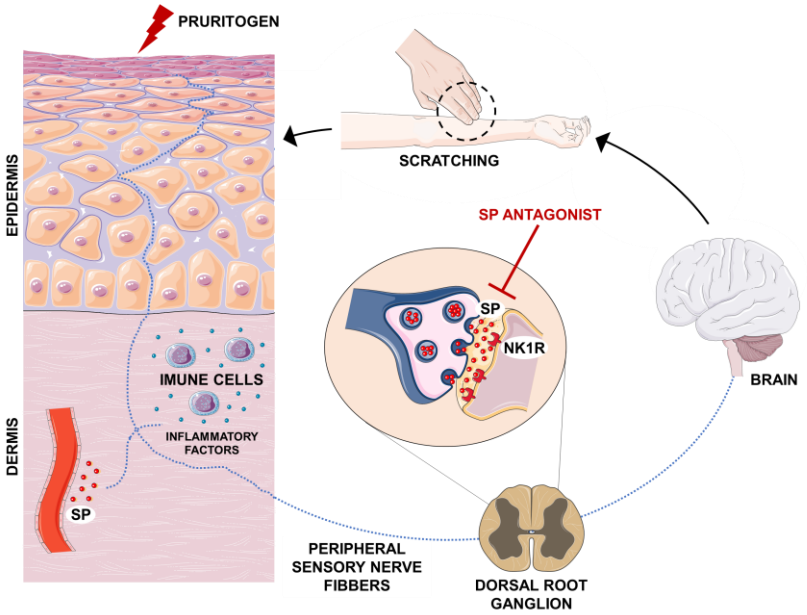
Most bothersome symptom

⇩ **Quality of life**

Ikoma, A., *et al.*, *Nature Reviews Neuroscience* **2006,** *7* (7), 535-547
Dong, X., *et al.*, *Neuron* **2018,** *98* (3), 482-494
Richard, M. A., *et al.*, Journal of the European Academy of Dermatology and Venereology **2022**, 36 (*7*), 1088-1096

Substance P → **Agonist** → **Neurokinin 1 receptor**

Nausea
Cancer progression
Pruritus



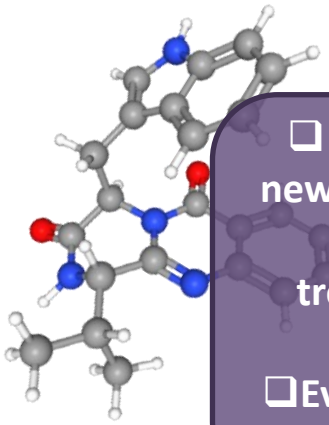
Pruritus is normally associated with **inflammation**

Marine Natural Products

Source of unique chemical structures with unexplored activities

High potential to furnish drug candidates

Known SP antagonists

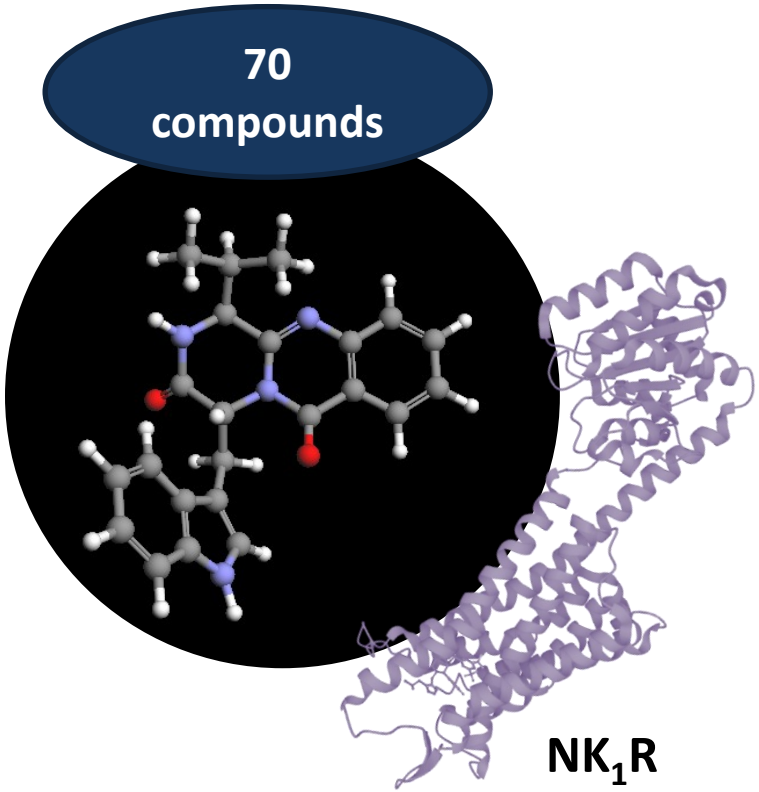


Main goals

- ❑ Structure-based drug design of new SP antagonists based on marine natural products for topical treatment of pruritus-associated inflammatory skin diseases
- ❑ Evaluation of their activity *in silico* and *in vitro*

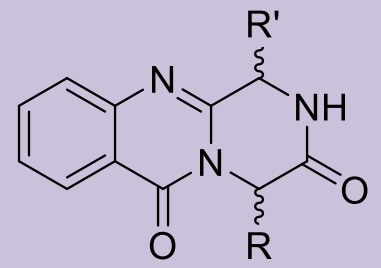
Wong L-S; Wu T; Lee C-H, *International Journal of Molecular Sciences.* **2017,** *18* (7), 1485.
Malve H. *Journal of Pharmacy and Bioallied Sciences.* **2016**, *8* (2), 83-91.

In silico studies



NK_1R
PDB: 6E59

Compound	Binding affinity (kcal/mol)
Aprepitant (control)	-11.2
10a	-11.8
10b	-11.7
36c	-11.0
10a_1	-10.8
10b_1	-10.7
Marine natural product SP antagonist	-10.1



General structure of the studied compounds

NK_1R-Neurokinin 1 receptor **SP**-Substance P

Pharmacokinetic studies - SwissADME software

Compound	MW	Consensus Log P	Log Kp (cm/s)
10a	540.61	4.78	-5.62
10a1	450.49	3.20	-6.36
10b	540.61	4.76	-5.62
10b1	450.49	3.15	-6.36
23c	609.50	5.76	-5.15
For skin permeation	**< 500**	**Log P < 5**	**-7 < Log Kp < -5**

Future

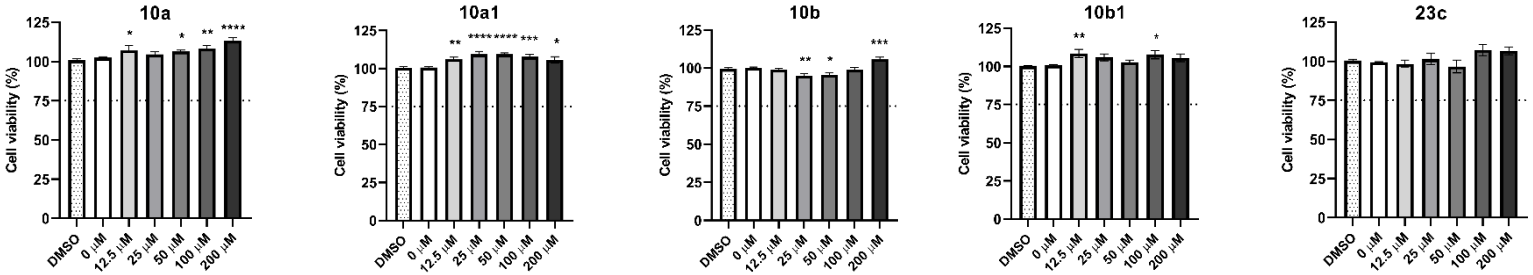
Molecular modifications

↓ Molecular weight
Log P

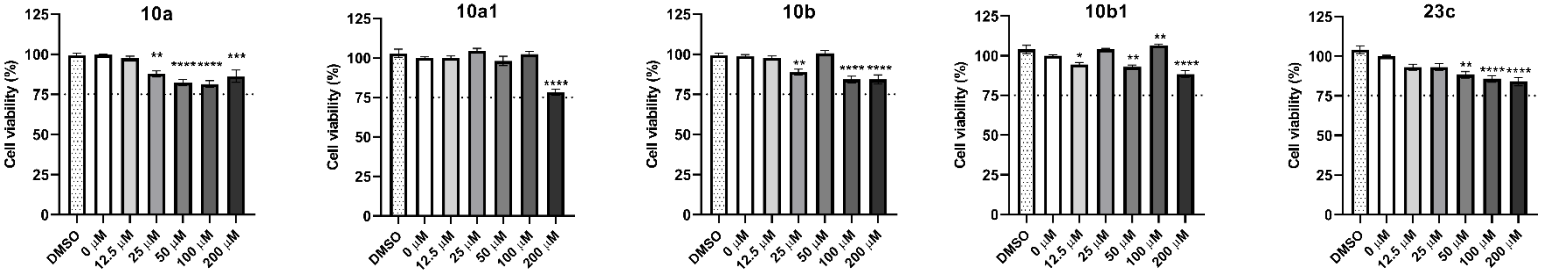
MW- molecular weight; **Log P-** oil/water partition coefficient; **Log Kp-** skin permeability

In vitro studies - Cytotoxicity

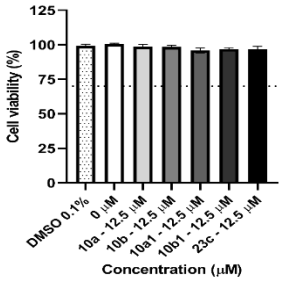
Raw 264.7



HaCaT



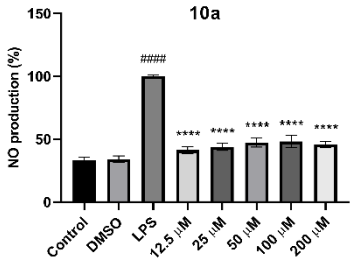
3T3

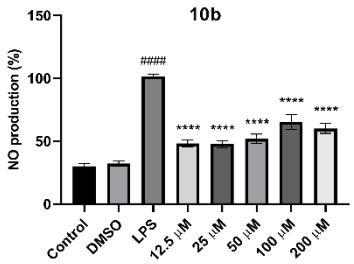


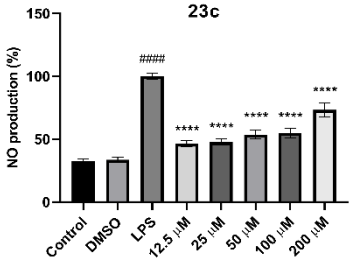
Cell viability was higher than 75 %

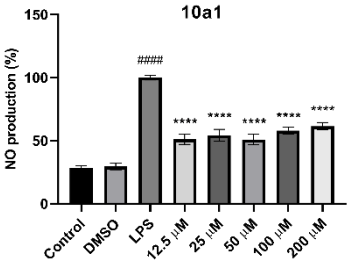
Effect of the compounds (12.5 – 200 µM) on cell viability on macrophages (Raw 264.7), keratinocytes (HaCaT), and fibroblasts (3T3) evaluated by the resazurin reduction 24 h after exposure. Each value represents the mean ± SEM from at least 3 experiments, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$, compared to control, as determined by one-way ANOVA, followed by Dunnett's multiple comparisons test.

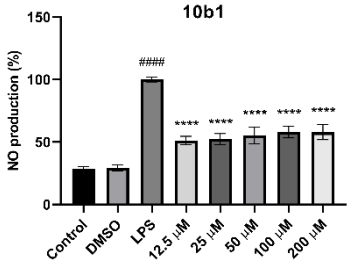
In vitro studies - Anti-inflammatory activity evaluation







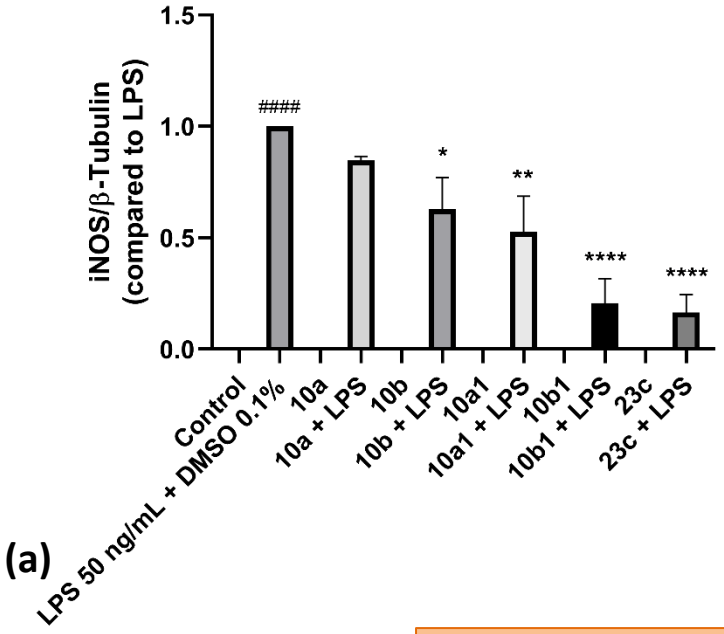




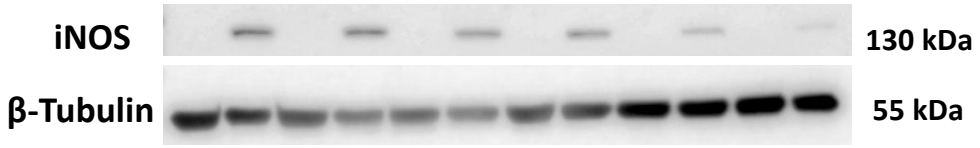
Reduction in NO production by approximately 50 %

Effect of the compounds on nitric oxide (NO) production induced by liposaccharide (LPS) in macrophages (RAW 264.7) after 24 h of exposure. Nitrite concentration was determined from a sodium nitrite standard curve, and the results are expressed as a percentagem of NO production by cells treated with LPS. Each value represents the mean ± SEM from at least 3 experiments #### $p < 0.0001$, compared to control; **** $p < 0.0001$ compared to LPS, as determined by one-way ANOVA, followed by Dunnett's multiple comparisons test.

In vitro studies - iNOS protein levels evaluation



(a)



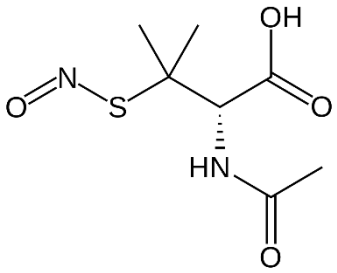
(b)

Significant decrease on iNOS protein levels

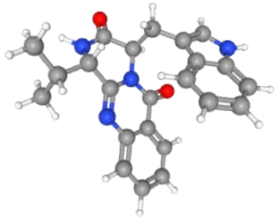
Inhibitory effect of compounds on inducible nitric oxide synthase (iNOS). (a) Quantification of iNOS protein levels. (b) Representative Western blots of iNOS. Cells were maintained in culture medium (control), or incubated with liposaccharide (LPS), or incubated with compounds (12.5 μM) alone or simultaneously with LPS, for 24 h. Results are expressed as percentage of protein levels relative to LPS. Each value represents the mean ± SEM from at least 3 experiments, #### $p < 0.0001$, compared to control; * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ compared to LPS, as determined by one-way ANOVA, followed by Dunnett's multiple comparisons test.

In chimico studies - Nitric oxide scavenging potential evaluation

In culture medium



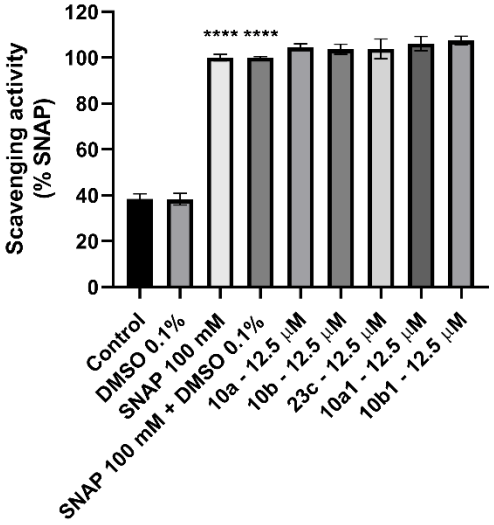
S-nitroso-*N*-acetyl-DL-penicillamine (SNAP)



Tested compounds

Griess assay

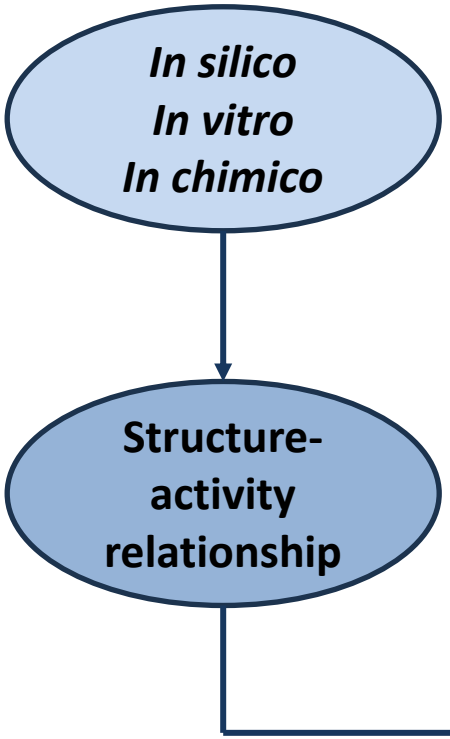
No Nitric Oxide scavenging potential



Nitric oxide (NO) scavenging potential of the tested compounds when incubated with SNAP (300 µM), a NO donor, for 3 h. Each value represents the mean ± SEM from at least 3 experiments, **** $p < 0.0001$, compared to control as determined by one-way ANOVA, followed by Dunnett's multiple comparisons test. No significant differences were observed between SNAP and compounds

Hit optimization

Performed studies



New Docking Studies on NK1R

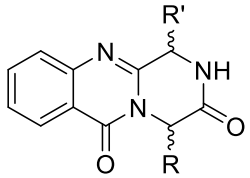
Ongoing synthesis	Binding affinity (kcal/mol)
MSM34	-11.9
MSM39	-11.2
MSM40	-10.8
MSM41	-11.0
MSM42	-10.5
MSM43	-11.3
MSM44	-11.6
MSM45	-11.9
MSM46	-11.4
MSM47	-11.4

Synthesis of new marine-inspired derivatives

Two different pathways

Structure characterization: Nuclear magnetic resonance spectroscopy

Yields: 17-23%



Conclusions

- **Cell viability higher than 75 %** was found for all the tested concentrations (12.5 – 200 µM) in **keratinocytes, macrophages and fibroblasts**
- **Reduction in NO production by approximately 50 %** for all the tested concentrations (12.5 – 200 µM)
- The **absence of NO scavenging activity** together with the **significant decrease in iNOS protein levels** suggest a **blockage of pro-inflammatory signaling pathways upstream of iNOS synthesis**
- **A high correlation between *in vitro* and *in silico*** results: Compounds with the **highest anti-inflammatory activity** presented the **highest binding affinity to NK1R**
- Establishment of a **structure-activity relationship** for **anti-inflammatory activity**
- **Hit optimization:** Ongoing

Acknowledgments

FCT–projects UIDP/04378/2020 and UIDB/04378/2020 UCIBIO, LA/P/0140/2020 i4HB, UIDB/04423/2020 and UIDP/04423/2020 (Group of Marine Natural Products and Medicinal Chemistry-CIIMAR), projects UIDB/04539/2020, UIDP/04539/2020, and LA/P/0058/2020 CIBB and project ref. NORTE-01-0247-FEDER-047239, co-financed by FEDER, NORTE2020, Portugal2020. M.S. Martins acknowledges her Ph.D. research grant 2021.05964.BD (FCT).