

DISCOVERY OF 2-MERCAPTONICOTINOYL GLYCINE

A NEW POTENT BRIGHTENING AGENT FOR HYPERPIGMENTATION MANAGEMENT, EXHIBITING A LOW ENVIRONMENTAL IMPACT

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1 INTRODUCTION

Research of brightening agents is an important but difficult task due to multi-parameter considerations. An innovative methodology is described, based notably on high-throughput screening test (HTS), *in silico* method predicting physico-chemical properties and environmental profile, and highly physiologic pigmented biological models, which led to the discovery of 2-mercaptionicotinoyl glycine (2-MNG). Their capacity to inhibit melanin production via topical application on 3D pigmented reconstructed epidermis was superior to 4-*n*-butylresorcinol, a tyrosinase inhibitor chosen as a reference on the market.

2 MATERIALS & METHODS

HTS melanin production assay

Raw materials to be evaluated were solubilized in dimethyl sulfoxide (DMSO) at 30 mM and diluted in culture medium in a 2-fold serial dilution manner to obtain 10 doses starting from 200 μ M. Normal human melanocytes (NHM) were cultivated as described by Duval *et al.*, (2001) in 384-well plates with 80 μ l of M154 (thermo Fisher) culture medium per well. The raw materials were added after 24h incubation. Cells were incubated at 37°C for 72 hours. Optical density was measured at 340 nm reflecting melanin content and data were normalized in comparison with untreated cells. Dose-response curves were generated and IC50 was determined (50% inhibition of melanin signal).

Environmental profile assessment

Aquatic ecotoxicity and biodegradability were determined from data calculated using softwares recognized by the public authorities. Evaluation used EPI Suite™ v4.11 and CATALOGIC v5.12.1 software. Kow logs calculated with other softwares, especially ClogP, were also taken into account when available.

Determination of water footprint index (WFI) is described in the article « Eco-design of cosmetic formulae: methodology and application » (L'Haridon *et al.*, 2018). Lower is WFI less is the environmental impact.

<https://www.epa.gov/tsc-screening-tools/epi-suite-estimation-program-interface>

In silico predictions

An internal digital tool was used to select the most desirable mercaptionicotinoyl amide structures to be synthesized, taking into consideration physico-chemical parameters, environmental, absorption, distribution, metabolism, excretion and toxicity parameters.

Pigmented reconstructed epidermis (PRE) assay

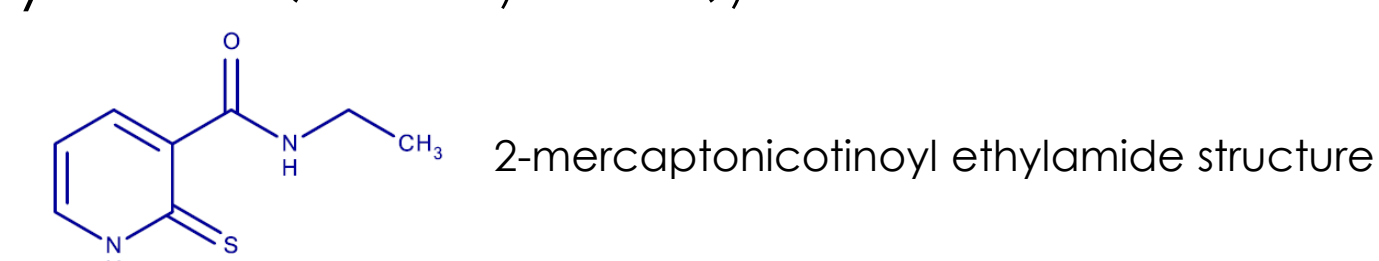
The assay was designed following the description detailed by Qiu *et al.*, 2016. Briefly human pigmented epidermis were reconstructed according to the technique described by Duval *et al.*, (2001). They were daily treated via topical application with DMSO 1/1000 alone, 4-*n*-butylresorcinol (4-*n*-BR) at 100 μ M, or 2-MNG at 100 and 300 μ M over 9 days. Melanin was quantified by image analysis on Fontana masson stained skin sections. The area occupied by melanin was quantified using Histolab image analysis software (Microvision, Evry, France). Mean comparison versus vehicle was then performed using a Mann-Whitney U test ($p < 0.05$).

4 CONCLUSIONS

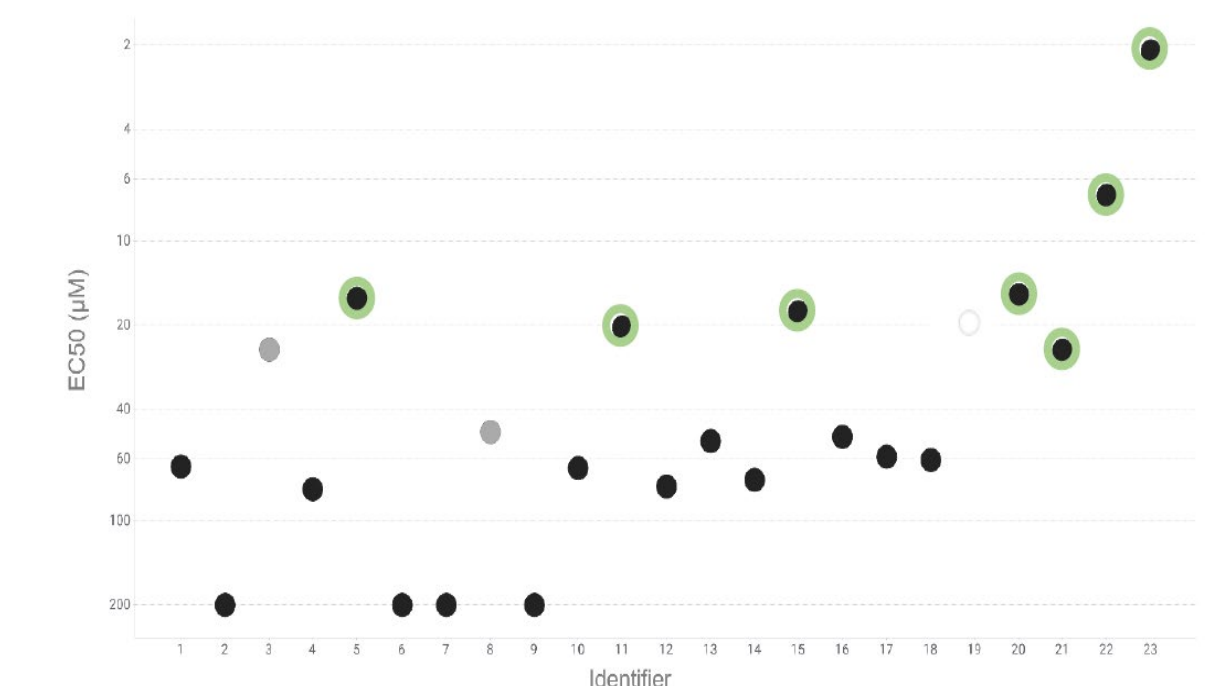
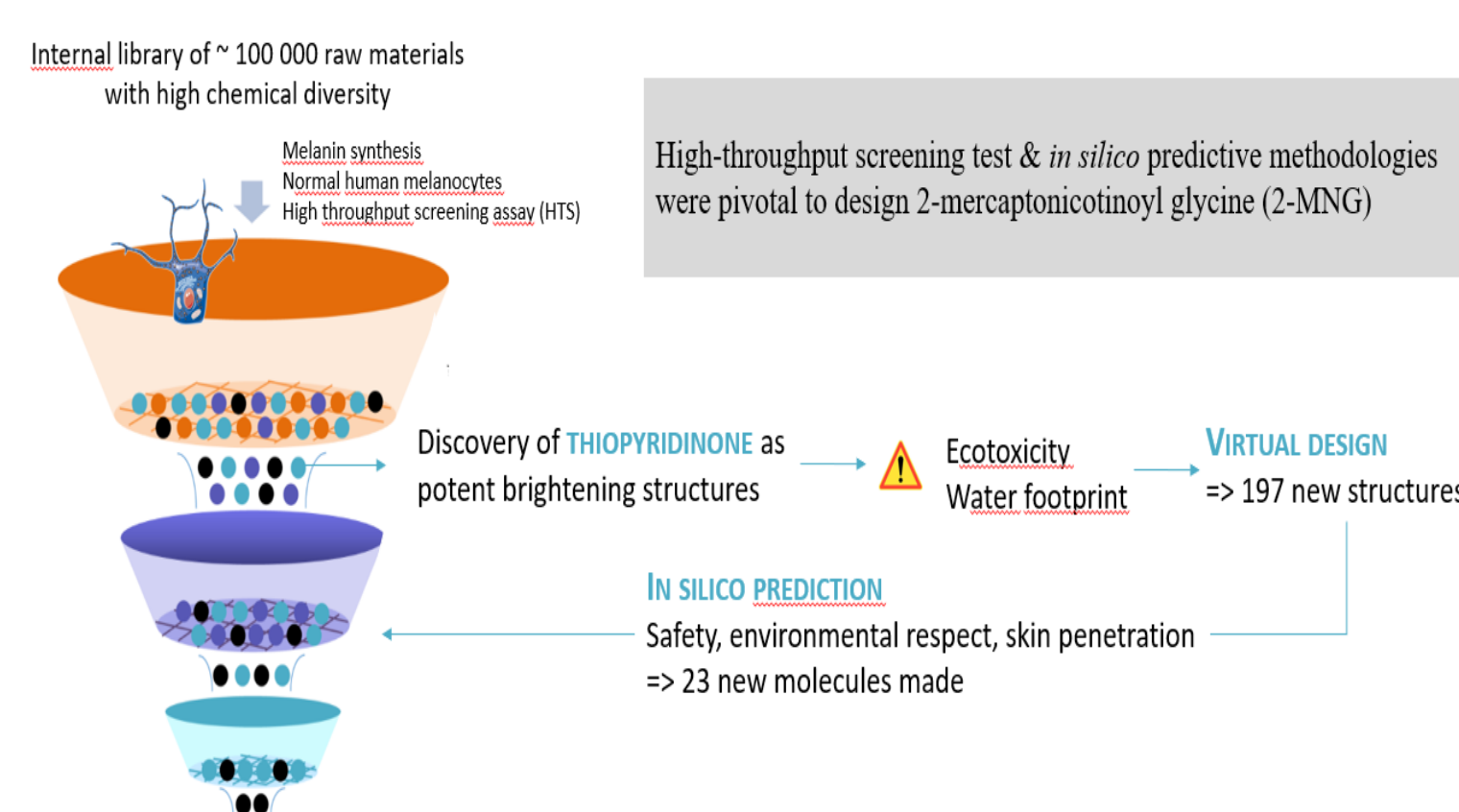
Research on new brightening agents considering efficacy, safety but also environmental impact is of great importance to significantly improve the profile of existing actives on the market and avoid unwanted side-effects. Here, the discovery of an innovative technology for hyperpigmentation management based on a thiopyridinone backbone is described. Thanks to high-throughput screening biological assay, we were able to identify after blind screening of over 100 000 chemical structures, the new thiopyridinone family of potent lightening agents for skin tone management. Using *in silico* prediction tools of physicochemical data, environmental profile, absorption, distribution, metabolism, excretion and toxicity parameters, we discovered 2-MNG, displaying the best compromise between efficacy, skin penetration and water footprint-profile.

3 RESULTS & DISCUSSION

Identification thiopyridinone chemical backbone for pigmentation management A screening of around 100,000 chemical (internal sample library) was performed in the HTS assay. This assessment led to the identification of thiopyridinone as a new family of potent lightening agents for skin tone management, represented by 2-mercaptionicotinoyl ethylamide (Marat, 2010), the most efficient candidate.

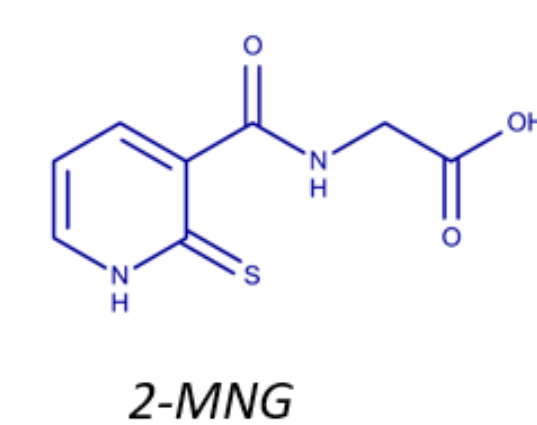


Eco-design of mercaptionicotinoyl amides with low environmental impact: A virtual library was built based on a rational design from 2-mercaptionicotinoyl ethylamide chemical structure. Digital assessment of structure-activity and structure-ecotoxicity relationships on 197 derivatives of this chemical family allowed us to identify 23 compounds most promising.



HTS evaluation of the 23 virtually optimized compounds. Compounds are plotted on the x-axis. Their related IC50 are plotted on the y-axis in descending order, the higher position defining the most efficient actives. Darker is the spot better is cell viability. Compounds fitting with both efficacy and viability expectations are surrounded in green.

Selection of 2-MNG as the most promising structure: Four structures among the 7 reaching efficacy and cytotoxicity requirements in HTS also had a WFI below 250, which was more favorable compared to on market benches: 4-*n*-BR WFI = 5813, kojic acid WFI= 316. 2-MNG (WFI=11) was selected for further investigation as displaying the best compromise between efficacy, safety and WFI parameters (Marat *et al.*, 2015)



Structure from single crystal of 2-MNG obtained by XRAY crystallography. CCDC deposition number: CCDC 2237379

Efficacy confirmation on PRE: Topical treatments of 2-MNG on PRE confirmed the potential of the molecule to inhibit melanin production significantly versus the vehicle and with a superior efficacy as 4-*n*-BR, chosen as cosmetic bench (fig 1). The treatment with 2-MNG did not impact melanocytes number or morphology (fig 2) not epidermis quality (fig 3)

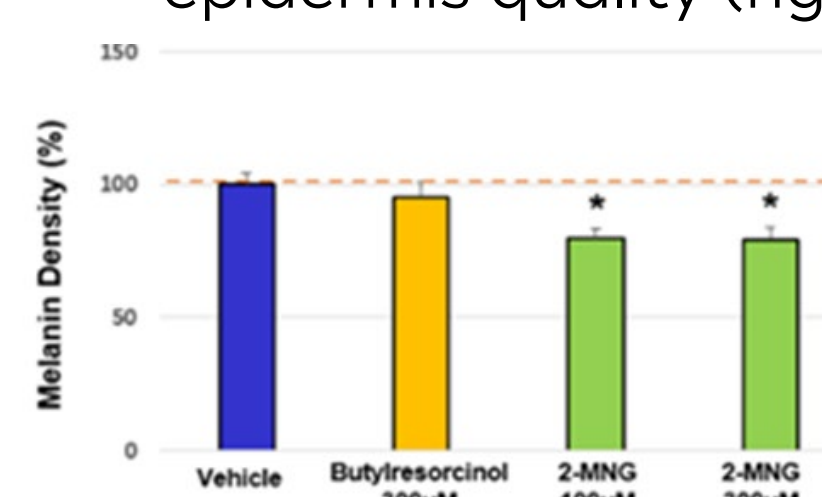


Fig 1 Melanin production chart bar

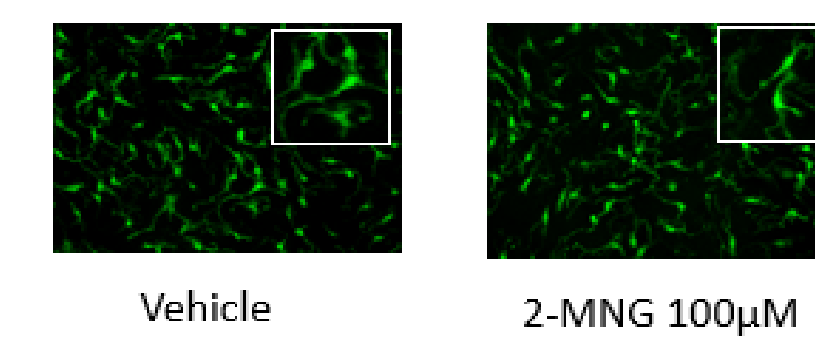


Fig 2 NHM in 3D reconstructed epidermis

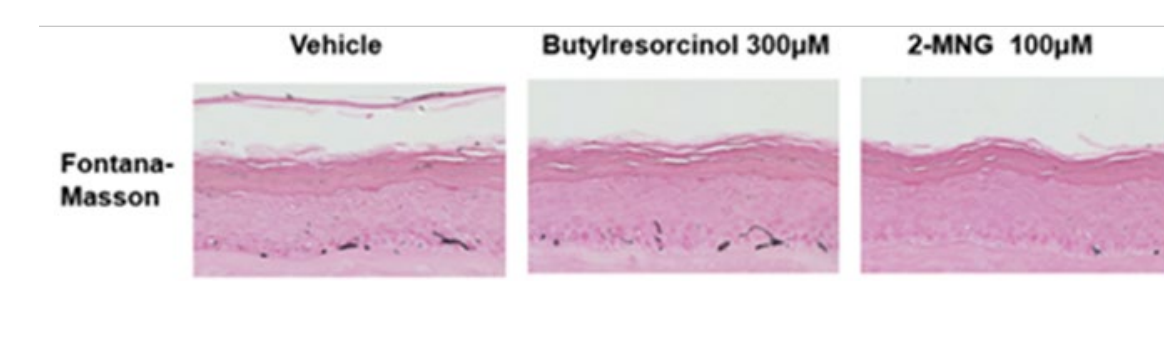


Fig 3 Pigmented reconstructed epidermis

REFERENCES

- 1- 2-MNG (2-mercaptionicotinoyl glycine) prevents uv-induced skin darkening and delayed tanning, R.deDormael, P.Sextius, N.Bourokba, E.Mainguene, R.Tachon, G.Kumar, H.Jouni, P.Bastien, S.Diridollou
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