NANOBRET™ TARGET ENGAGEMENT PLATFORM FOR HIGH THROUGHPUT SCREENING OF NLRP3 INFLAMMASOME INHIBITORS

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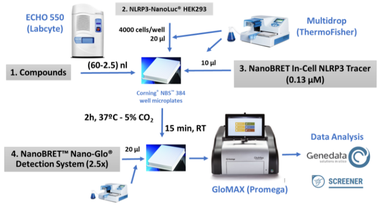
EU-OPENSCREEN is a not-for-profit European Research Infrastructure Consortium (ERIC) for chemical biology and **early drug discovery**.

EU-OS-DRIVE will further help the EU-OS-ERIC to deliver its added-value via constant re-use of generated data and tools by users across the globe and to support the competitiveness of European life science industries.

WorkPackage7-EU-OS-DRIVE_Industry engagement: co-developments of novel screening technologies between industry technology providers and specialized screening partner sites are established. One of these co-developments has been performed with the collaboration of **Fundacion MEDINA** and **Promega**.

NanoBRET Platform: Miniaturization & Automation

Fundación MEDINA is a non-profit research organization focused on the discovery of novel bioactive compounds. **MEDINA** is one of the eight high-capacity screening centers of the EU-OPENSCREEN-ERIC [3]. Leveraging its industrial-derived experience, high qualified research team and cutting-edge technology platforms, MEDINA is today a reference in drug discovery [4-6].

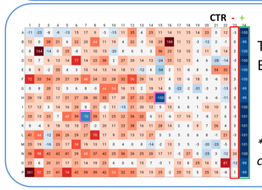


Target And Technology To Implement NLRP3 Inhibitors HTS Platform

NLRP3 inflammasome is a critical component of the **innate immune system** that mediates caspase-1 activation and the secretion of proinflammatory cytokines such as IL-1β in response to microbial infection and cellular damage. NRLP3 has a potential **therapeutic interest** for **COVID-19** and **inflammatory diseases** [1,2].

The **NanoBRET™** Target Engagement Assays (**Promega**) measure the apparent affinity of test compounds by competitive displacement of the NanoBRET™ tracer, reversibly bound to a NanoLuc® luciferase fusion expressed **in live cells**. The system uses **NanoLuc®** as a BRET (Bioluminescence Resonance Energy Transfer) energy donor and a target protein labeled with the NanoBRET™ fluorophore.

Screening



Testing the **2,500 Bioactive** compounds from the **ECBL** (European Chemical Biology Library) ***Pilot library*** at 10 µM

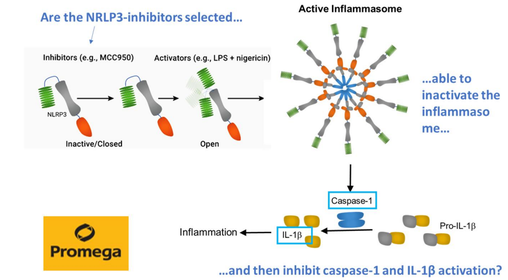
Z'factor=0.78
Hits > 40% activity → **28 actives ***

** One of the most potent compounds from Pilot library was MCC950, which is the positive control used in the assay, **confirming the accuracy of the screening** method*

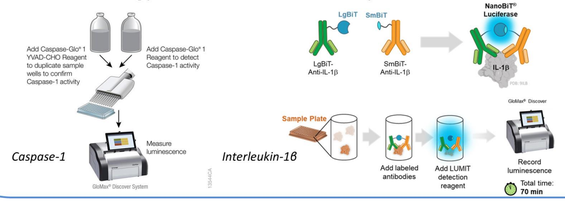
Confirmation

- ❑ Dose-response curves were tested, and **27 compounds** were confirmed in **MEDINA, 17** of them showed IC_{50}<3 µM and **13** were selected to continue to the next step.
- ❑ A second laboratory **(Promega US) confirmed NLRP3 inhibition in 10** of the 13 compounds selected

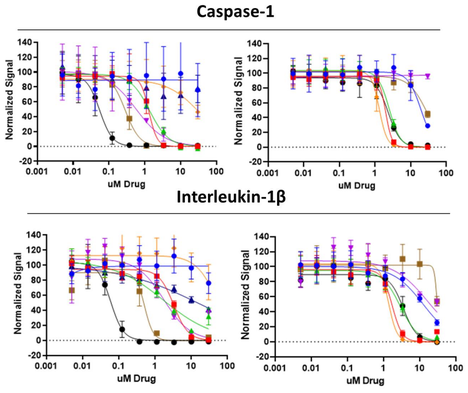
Functional Results Validation (Promega US Lab)



✓Caspase-Glo 1 Inflammasome Assay
✓Lumit™ Technology for measurement of IL-1β Release



Dose-response curves_IC_{50} calculation



Caspase-1 and IL-1β assays were performed in human monocytic THP-1 cell line treated with lipopolysaccharide from E. coli (LPS) and nigericin for selected compounds

Summary table of Results

Compound #	IC50 (µM) NLRP3-NanoBRET (MEDINA)	IC50 (µM) NLRP3-NanoBRET (Promega)	NLRP3 confirmation	IC50 (µM) Caspase-1 (Promega)	IC50 (µM) IL-1β (Promega)	Functional correlation
#1	<0.8	0.3	**yes**	19.40	12.30	low
#2	**0.9**	**0.8**	**yes**	**1.98**	**1.51**	**good**
#3	**1**	**0.8**	**yes**	**2.49**	**2.88**	**good**
#4	**1.3**	**1.2**	**yes**	**0.06**	**0.06**	**good**
#5	**1.8**	**0.9**	**yes**	**0.29**	**0.46**	**good**
#6	1.9	0.7	**yes**	>30uM	15.00	low
#7	**2**	**1.0**	**yes**	**0.66**	**1.31**	**good**
#8	2.2	-	no	none	16.52	no
#9	**2.3**	**1.6**	**yes**	**1.37**	**1.32**	**good**
#10	2.9	32.4	no	34.33	10.22	low
#11	**2.9**	**0.7**	**yes**	**1.35**	**1.79**	**good**
#12	**3**	**0.8**	**yes**	**2.30**	**3.60**	**good**
#13	2.8	11.6	no	28.07	35.12	low

#Compound_4 was previously reported as inhibitor of NRLP3 with interesting MoA (ubiquitination)
#Compound_5 was previously reported as modulator of the systemic inflammation

References

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[4]Nat Rev Chem. 2021;5(10):726-749. doi: 10.1038/s41570-021-00313-1
[5] Drug Discov Today. 2021 Jun;26(6):1369-1381. doi: 10.1016/j.drudis.2021.02.024
[6] Curr Opin Microbiol. 2019 Oct;51:81-87. doi: 10.1016/j.mib.2019.10.012

Conclusions

✓ The **NanoBRET_NLRP3** assay was implemented at the **MEDINA facility** using the **ECHO acoustic pipetting** system and its suitability to be used in HTS campaigns was verified.
✓ 2,500 compounds of Bioactive library of **ECBL** were tested (Z´factor > 0.5); 27 out of 28 compounds confirmed the activity in the confirmation assay (DR curves); 17 compounds showed activity with IC_{50} < 3 µM; 13 were selected for functional assays.
✓ 10 out of 13 were confirmed in second lab (**Promega US**) and 8 **showed functional correlation in a human cell model** by inhibiting downstream signaling: inhibiting caspase-1 and IL-1β activity.
✓ Could these inhibitors be effective in the progression of **COVID-19** or **in inflammatory diseases**? Promising results are shown in this proof of concept.

- EU-OPENSCREEN ERIC has received funding from European Union's Horizon 2020 research and innovation program under grant agreement no. 823893 (EU-OPENSCREEN-DRIVE)
- Ayudas a infraestructuras y equipamientos de I+D+i para entidades de carácter privado convocada, en régimen de concurrencia competitiva, en el ámbito del Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020) y de la Estrategia de Innovación de Andalucía (RIS3 Andalucía). IEPR-0031
- Spanish Ministry of Science and Innovation under grant agreement INP-2011-0016-PCT-010000-ACT7– 2011
- Ministerio de Ciencia e Innovación, Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica y el Fondo Europeo de Desarrollo Regional (FEDER).PCT_300000-2009-0016; PCT-010000-2010-3; INP-2011-0016-PCT-010000-ACT7