The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Molecular design of fluorescence probes for cell bio-imaging from a new "Store Operated Calcium Entry/Orai1" inhibitor of pancreatic cancer: the *delikine* DAD3.473

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

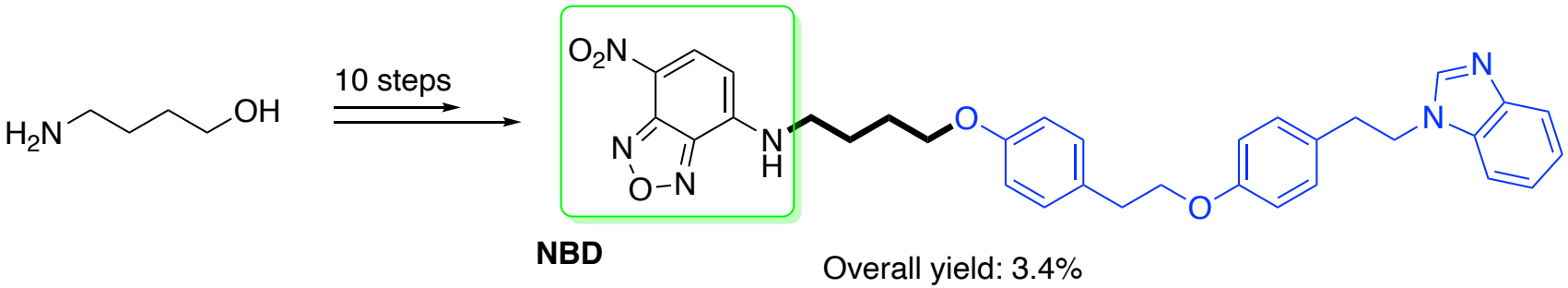
Amandine Asséké [1,2,*], Lou-Anna Voli [1], Camile Déliko Dago [2], Pascale Even-Hernandez [1], Valérie Marchi [1], Janat Mamyrbékova [2], Yves-Alain Békro [2], Olivier Mignen [3], Rémy Le Guével [4], Thierry Charlier [4] and Jean-Pierre Bazureau [1]

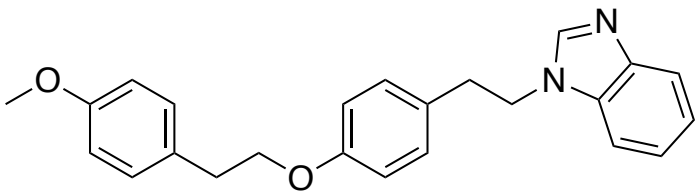
[1] Institut des Sciences Chimiques de Rennes ISCR UMR CNRS 6226, Bât. 10A, Campus de Beaulieu, CS 74205, 263 Avenue du Général Leclerc, 35042 Rennes Cédex, France
[2] Laboratoire de Chimie Bio-Organique & de Substances Naturelles LCBOSN, Université Nangui-Abrogoua, Voie Express d'Abobo-Adjamé, 02 BP 801, Abidjan 02, Côte d'Ivoire.
[3] Lymphocytes B et Auto-Immunité LBAI, Inserm U1227, CHU Morvan, Université de Bretagne Occidentale, 22 Avenue Camille Desmoulins, 29200 Brest Cedex, France.
[4] ImPACcell platform, SFR Biosit, Inserm U018, Université de Rennes 1', Campus de Villejean, Bât. 08, 2 Avenue du Prof. Léon Bernard, CS 34317, 35043 Rennes Cedex, France.

* Corresponding author: amandine.asseke@univ-rennes1.fr

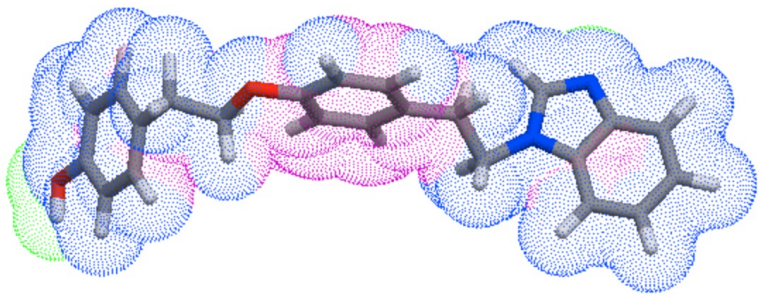
Molecular design of fluorescence probes for cell bio-imaging from a new “Store Operated Calcium Entry/Orai1” inhibitor of pancreatic cancer: the *delikine* DAD3.473

Graphical Abstract





delikine DAD3.473

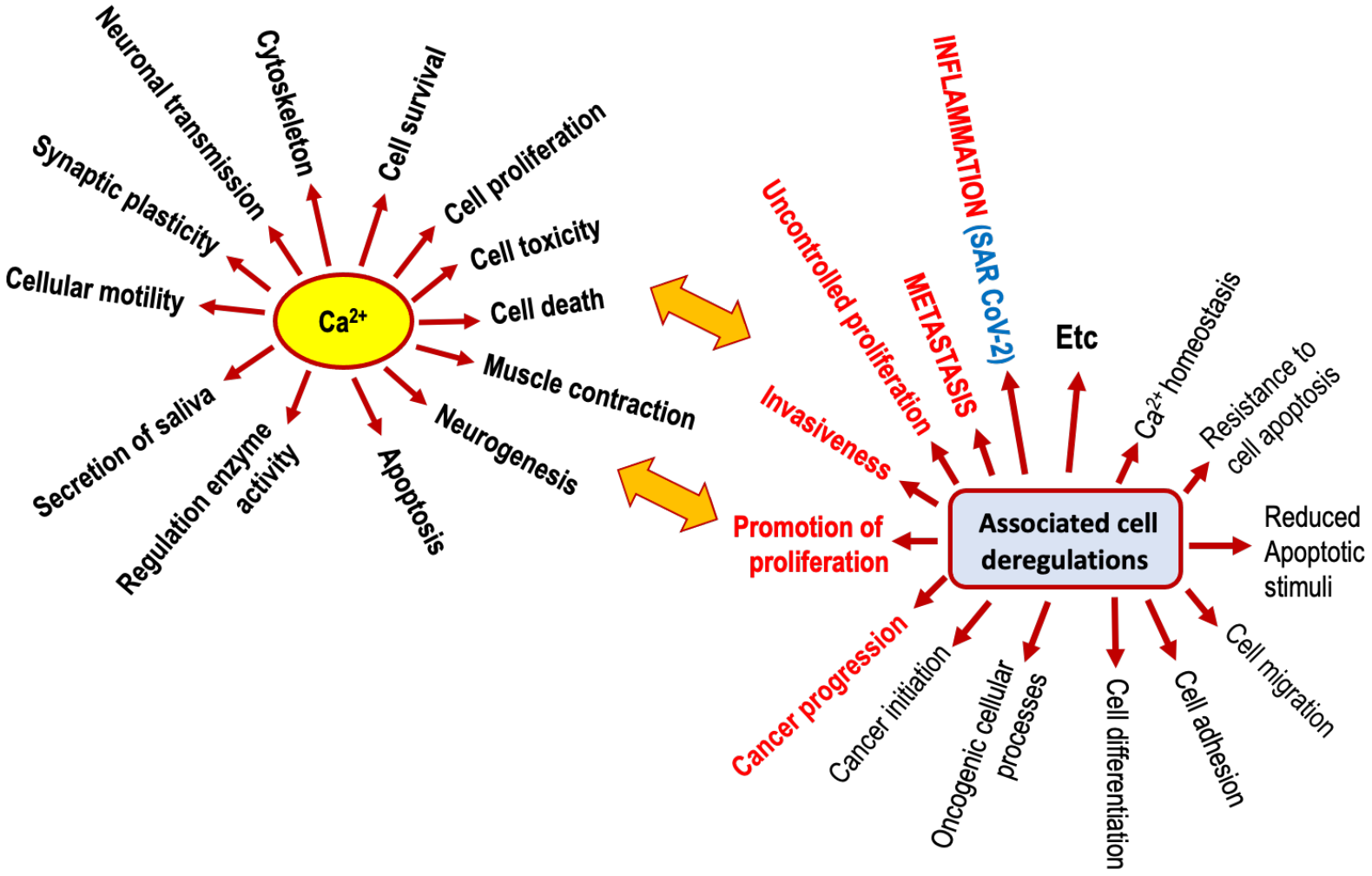


Abstract:
Calcium channel Orai1 is currently considered as an emerging and relevant target in cancer due to its indirect contribution, particularly in cell migration/invasion and metastatic spread. Therefore, the calcium channel Orai1 represents a promising therapeutic lead and accessibility to a selective inhibitor is a challenge for preventive treatment of metastases. During a **S**tructure **A**ctivity **R**elationship study around SKF-96365 (a molecule used as reference for measuring calcium influx since 1991), *delikine* molecules were identified as a new family of selective inhibitors targeting SOCE calcium influx controlled by the membrane protein Orai1. Thus, the *delikine* DAD3.4733 was identified and patented in 2020 as an active "hit" on pancreatic carcinoma cells PANC1 and on breast carcinoma cells MDA-H321 during the RSA study. To date, the mechanism of action of industrial SOCE/Orai1 inhibitors (GSK-96365 and CM4620) in clinical studies, is not or is poorly controlled. In this context and to try to build proof of concept around this new SOCE/Orai1 inhibitor, we decided to develop fluorescence probes derived from the *delikine* DAD3.473. To achieve this objective, we opted for the use of a short linker (3 or 4 carbon atoms) so as not to disturb the intrinsic character of this SOCE inhibitor, and to graft it onto the West, East or North of this inhibitor. The terminal part of the linker comprises the fluorophore NBD (7-nitro-1,2,3-benzoxadiazole). For this 8th ECMC presentation, the results of the multi-step syntheses of these fluorescence probes will be presented as well as the cell bio-imaging works.

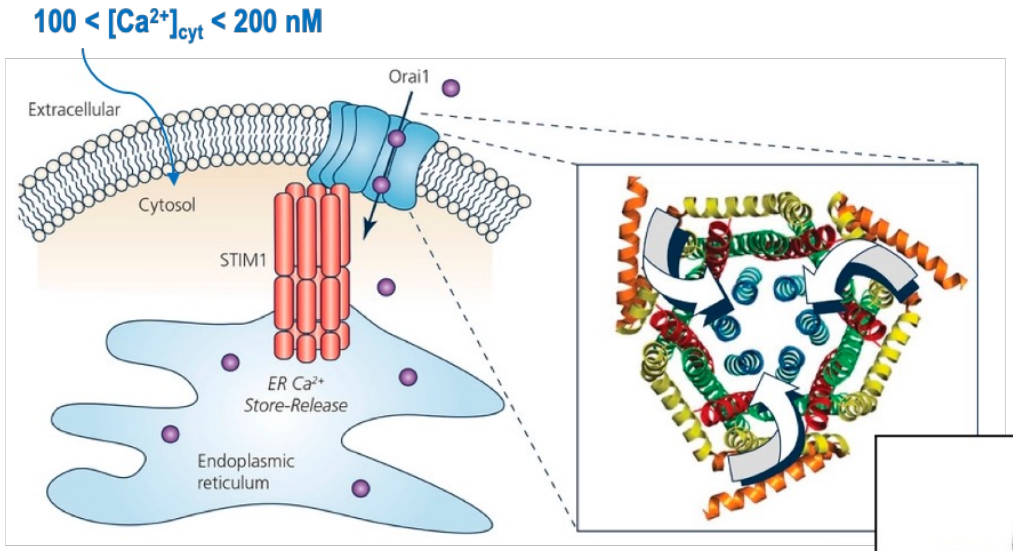
Keywords: calcium channel; store-operated calcium entry (SOCE); Orai1 protein; pancreatic cancer; Orai1 inhibitor; *delikine*; fluorescence probe; NBD.

Introduction

Importance of Ca^{2+} signaling in cell & associated cell deregulations *via* SOCE



''Store Operated Calcium Entry'' (SOCE) in CRAC ''Calcium Channel'': Membrane protein Orai1 & transmembrane protein STIM1 (ER)

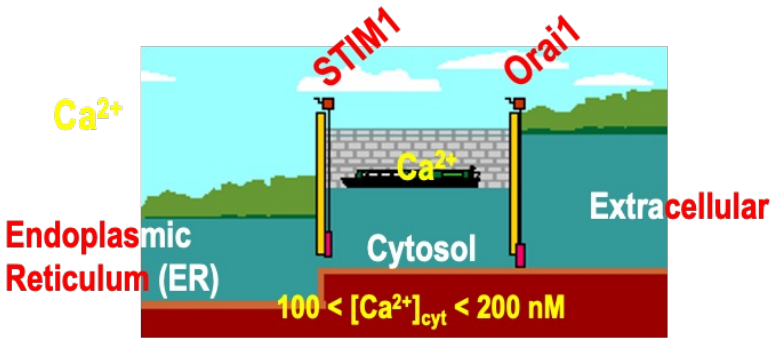


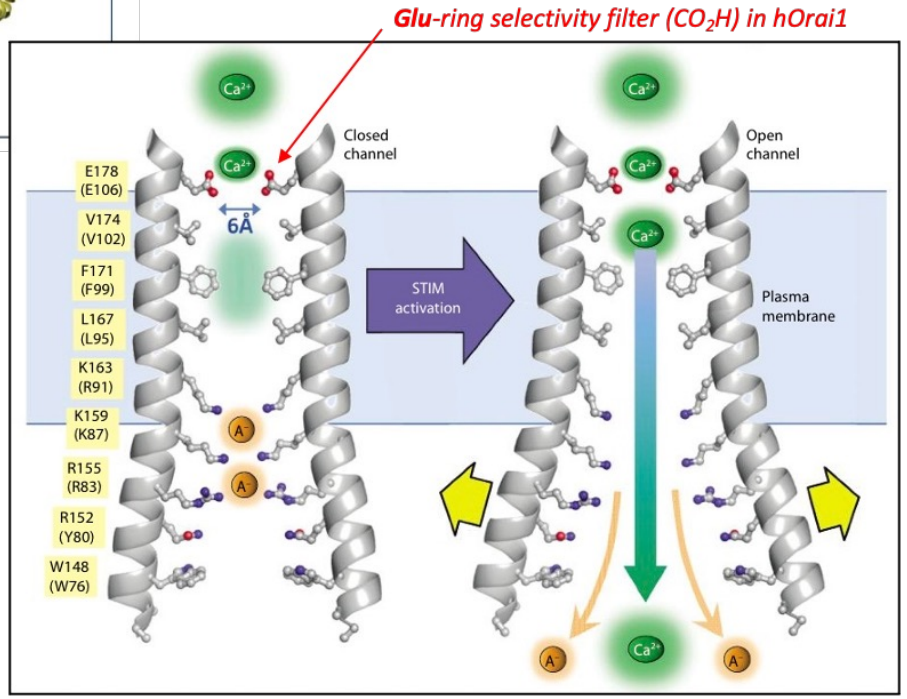
CRAC: **C**alcium **R**elease-**A**ctivated **C**hannel

CRAC ''Calcium Channel'': membrane protein Orai1 / transmembrane protein STIM1 (ER)

STIM1: **S**ensor **S**tromal **I**nteraction **M**olecule

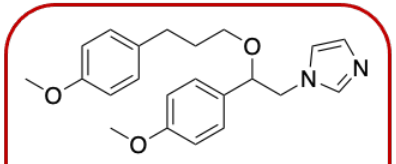
SOCE: *to replace Ca^{2+} lost from ER with Ca^{2+} that enters the cytoplasm through Plasma Membrane (PM) Channels (Orai1)*



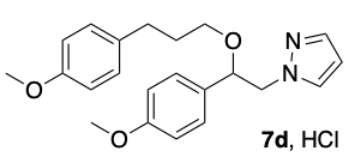


B. S. Rothberg, Y. Wang, D. L. Gill, Orai channel pore properties and gating by STIM: Implications from the Orai crystal structure. *Sci. Signal.* **2012**, *6*, pe9

''Store-Operated Calcium Entry'' SOCE inhibitors cited in literature (patent, articles)

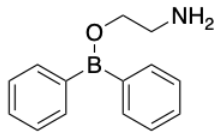


(R,S) **SKF 96365** (*)
Smith Kline French UK
(1991) ''the first''

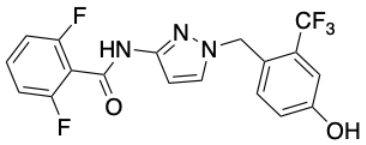


7d, HCl

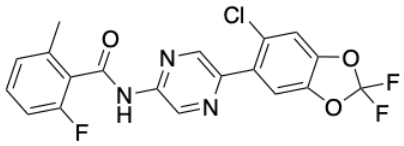
(R) & *(S)* **7d** (**)
Dago et al. **2018**



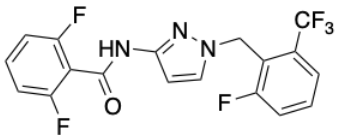
2-APB
J. Biochem **1997**



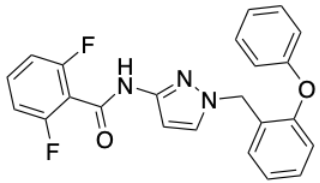
GSK-7975A
Glaxo, Smith & Kline Ltd, US
Phase IIa (2015), acute pancreatitis



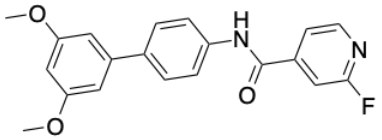
CM-4620
Calcimedica Inc. US
Phase IIa (2015), acute pancreatitis
Phase IIIa (2019), acute pancreatitis



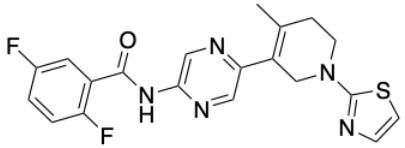
GSK-5498A
Glaxo, Smith & Kline Ltd, US



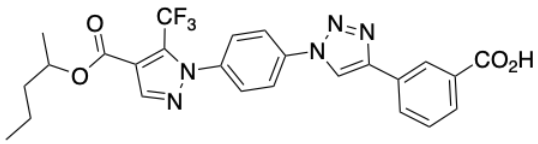
GSK-5503A
Glaxo, Smith & Kline Ltd, US



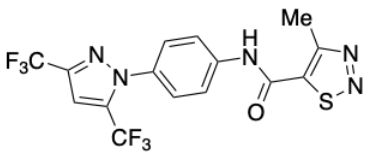
Syntha 66
Synta Pharmaceuticals Corp, US



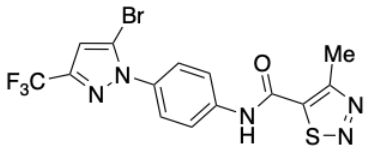
RO2959
Hoffmann-La-Roche Inc., US



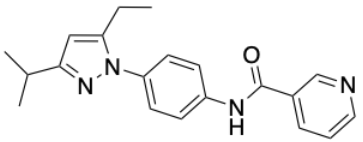
CIC-37
ChemICare S.r.l., Ital



RP4010 (YM-58483)
Rhizen Pharmaceuticals SA, CH
Phase I/IIb, non-Hodgkin's lymphoma



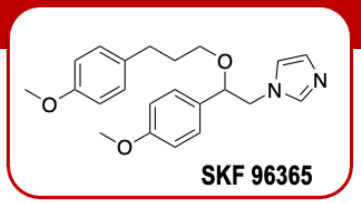
YM-09
ex Yamanouchi Pharm., JP
Astella Pharma Inc. , JP



BI-14
Boehringer Ingelheim
Pharmaceuticsl Inc., US

(*) C.D. Dago, Y-A Békro, O. Mignen, C. Brigaudeau, J-P Bazureau *Molbank* **2016**, *2016*, M909
(**) C.D. Dago, P. Le Maux, T. Roisnel, Y-A. Békro, C. Brigaudeau, O.Mignen, J-P. Bazureau, *Int. J. Mol. Sci.* **2018**, *19(3)*, 856

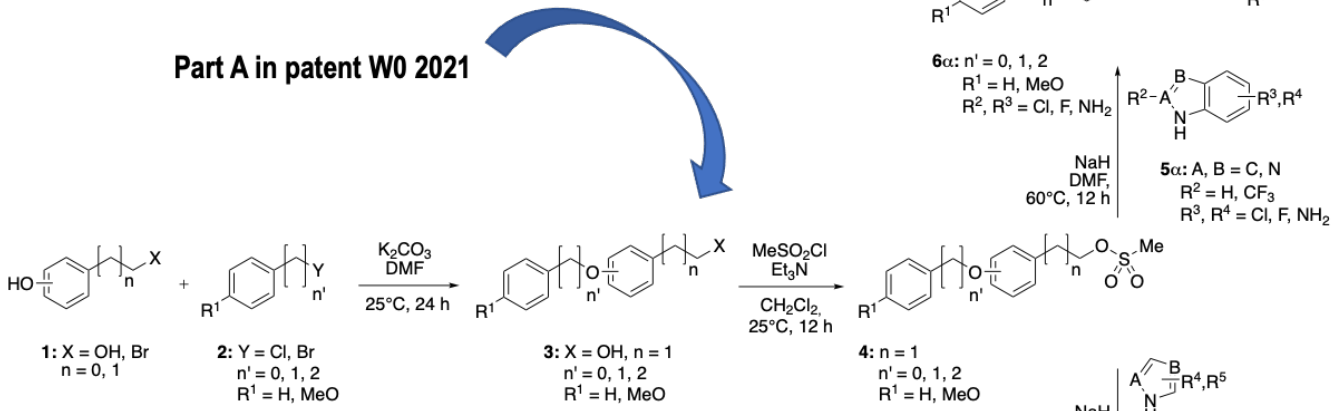
''Store-Operated Calcium Entry'' RelationShip Activity (RSA) study around SKF-96365: *Delikine* as new family of SOCE/Orai1 inhibitors

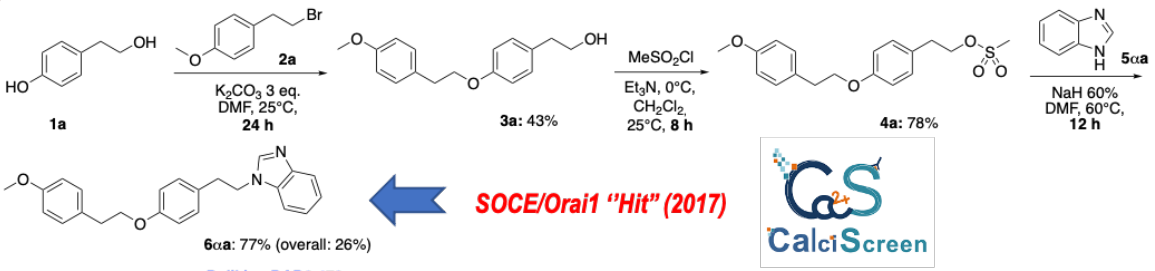


Réseau Canaux ioniques Cancéropôle

2012: Ion Channel Network

Part A in patent W0 2021





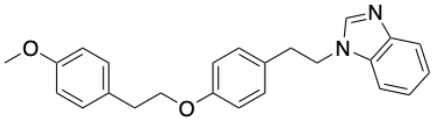
~ 100 *delikine* derivatives

LBAI U1227

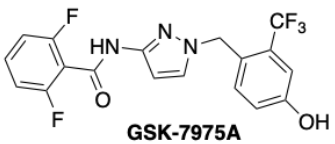
CalciScreen

J-P. Bazureau, C.D. Dago, L-A. Voli, Y-A. Békro, O. Mignen, C. Brigaudeau, Eur. Patent 2020/201775509.7, 19 Mai **2020**, patent WO 2021/233994 A1, 25 Nov. **2021**

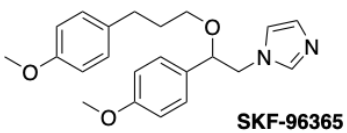
Delikine DAD3.473 as ''SOCE''/Orai1 inhibitor/modulator for pancreatic cancer
Results of biology



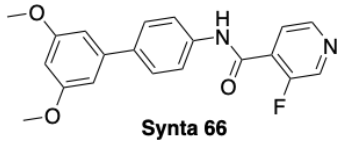
Delikine DAD3.473



GSK-7975A



SKF-96365

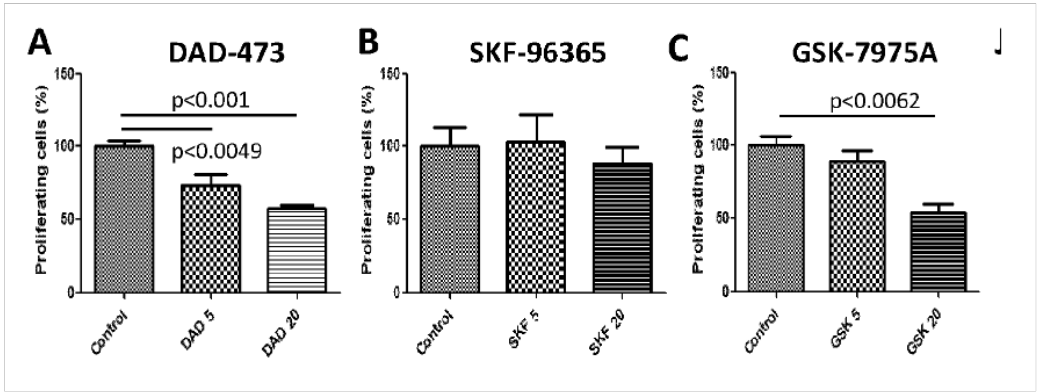


Synta 66

		SOCE IC_{50} (μM) in tumor cell lines		
Compound	Log P_{calc}	**HEK 293** (embryonic kidney cells)	**HEK 651 HS1** (solid tumor of kidney)	**PLP-B lymphocytes**
Delikine **DAD3.473**	5.78	3.1	20	3.1
GSK-7975A	3.83	2.0	44	2.3
SKF-96365	4.09	ND [a]	40	60
Syntha 66	3.55	ND [a]	ND [a]	8.8

[a] ND = Not Determined

Antiproliferative effect on PANC1 cells

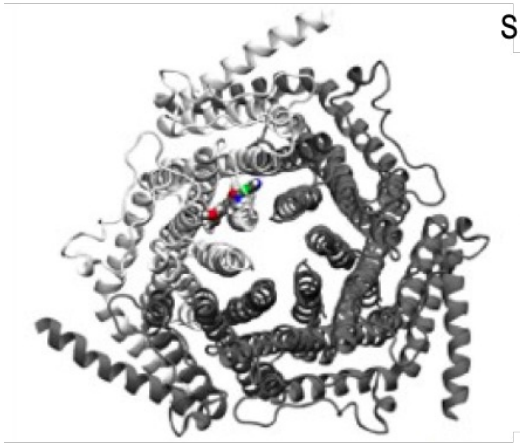


J-P. Bazureau, C.D. Dago, L-A. Voli, Y-A. Békro, O. Mignen, C. Brigaudeau, patent WO 2021/233994 A1, 25 Nov. **2021**

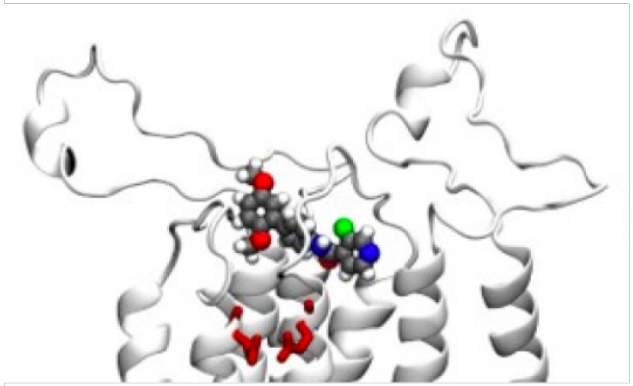
Interaction of *Delikine* DAD3.473 as ''SOCE'' inhibitor with Orai1 in CRAC channel ?
Molecular design of fluorescence probes for bio-imaging

- Inhibitory action of **Syntha 66** in Hs Orai1 pore using docking:

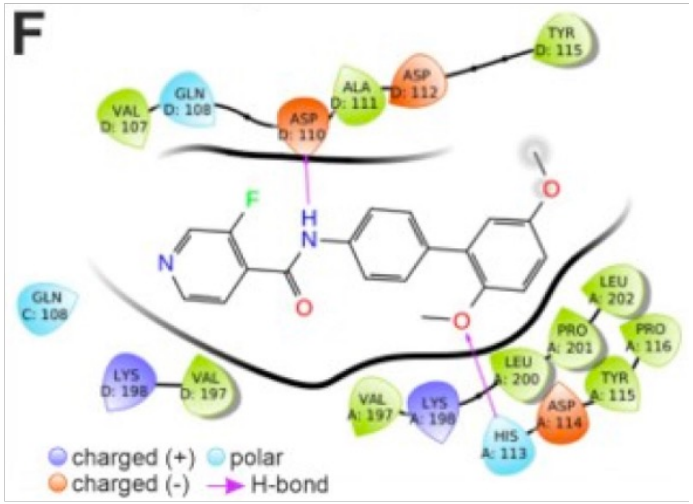
S. Patz, R. Schindl et al. *Cancers* **2020**, *12*, 2876



Top view of **Syntha 66** in Orai1 pore

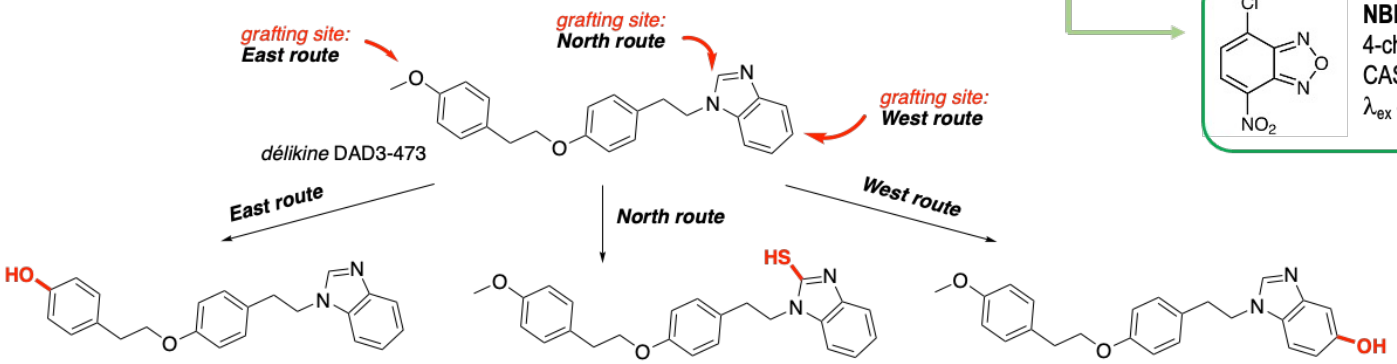


Side view of **Syntha 66** in Orai1 pore



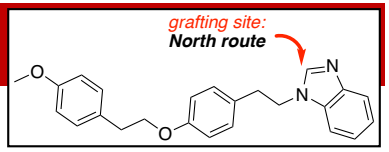
Two H-bonds of **Syntha 66** with amino-acids of Orai1 pore at: Asp110 and His113

- *Fluorescence probes*: grafting site on *delikine* **DAD3.473** for ''short linker-fluorophore''

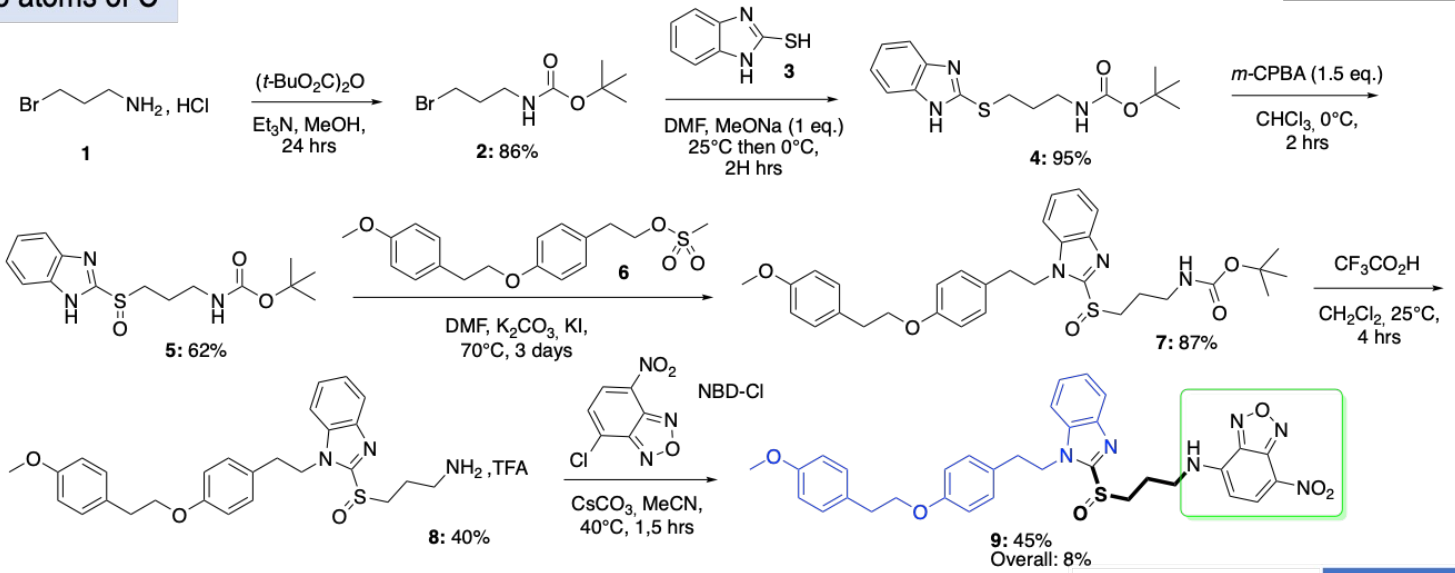


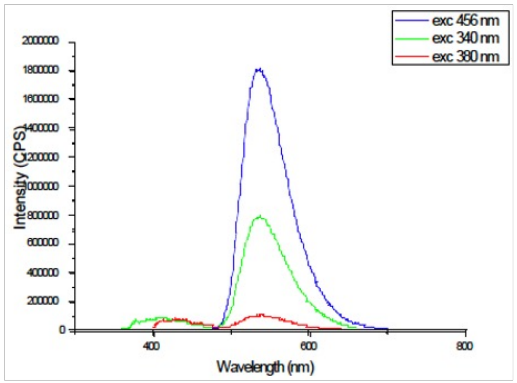
NBD-Cl:
4-chloro-7-nitro-2,1,3-benzoxadiazole
CAS 10199-89-0
λ_{ex} 475 nm, λ_{em} 550 nm

Fluorescence probes derived from *delikine* DAD3.473 for bio-imaging: North route



Linker: 3 atoms of C





Fluorescence emission spectrum of **9** in DMSO at different excitation wavelengths

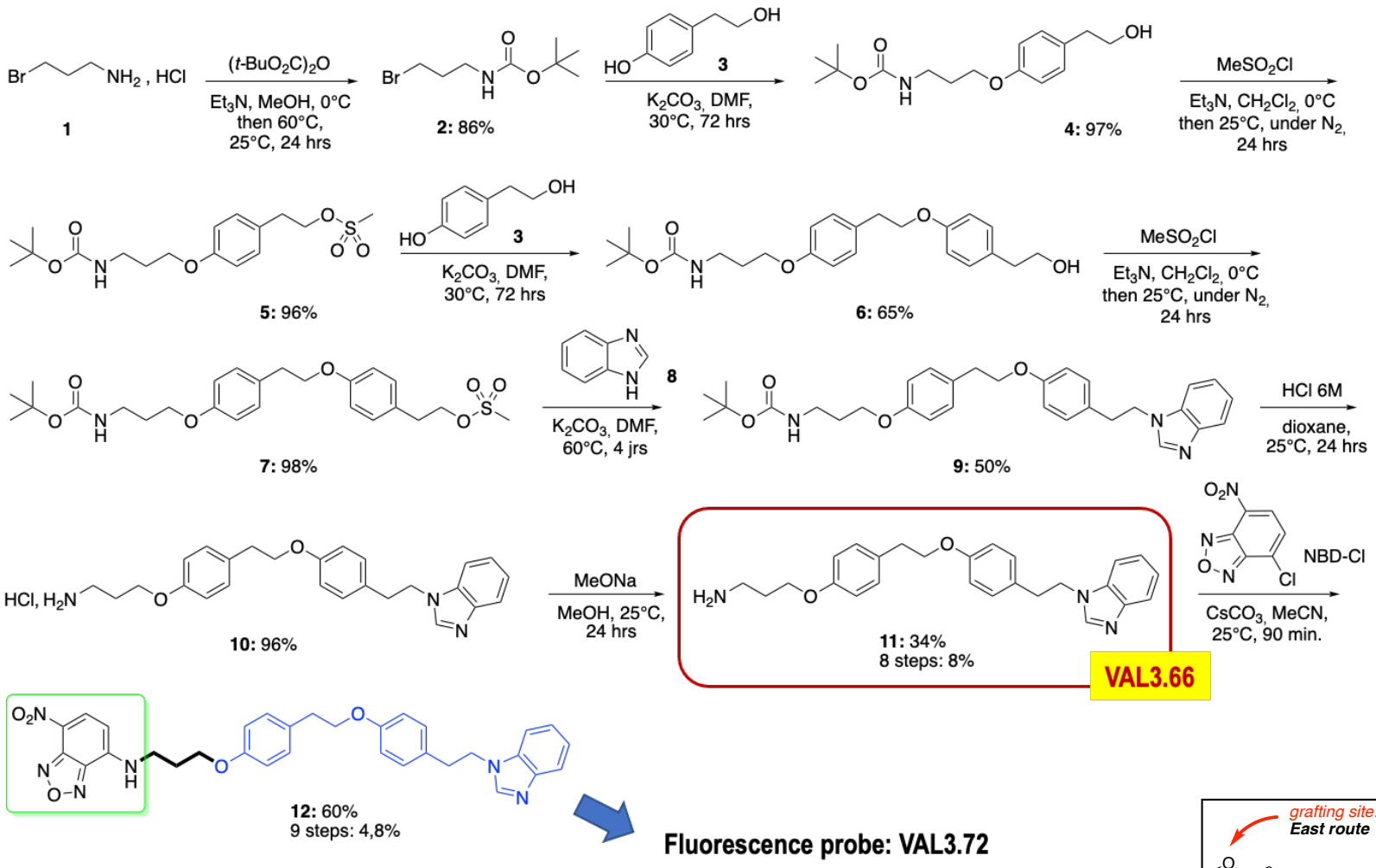
Concentration range of **9** under UV light at 254 nm

λ_{max} = 538 nm in DMSO
ε_9 = 2.23 .10^4 mol^{-1}.l.cm^{-1}
ε_{NBDCl} = 2.2 .10^4 mol^{-1}.l.cm^{-1}

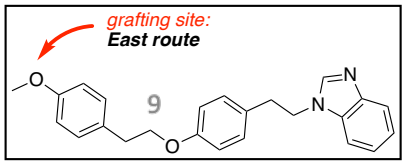
	SOCE IC_{50} (µM)
Compound	**PLP-B lymphocytes**
Delikine **DAD3.473**	3.1
8 (without TFA)	> 10
9	> 25

Voli Lou-Anna, PhD thesis, Janu. 29, **2020**
L-A Voli, C.D. Dago et al., **2022**, unpublished

Fluorescence probes derived from *delikine* DAD3.473 for bio-imaging: East route
Part 1: linker with 3 atoms of C

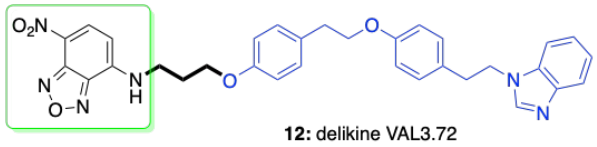


L-A Voli, C.D. Dago et al., **2022**, unpublished results



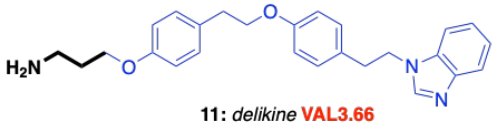
Fluorescence probes derived from *delikine* DAD3.473 for bio-imaging: East route - Part 1

Results of photophysics and biology

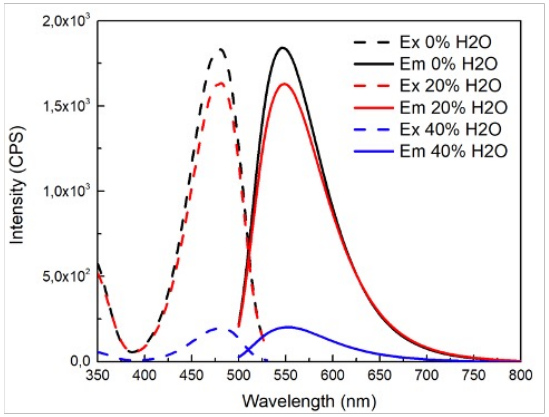


12: delikine VAL3.72

λ_{max} (ex) VAL3.72 = 479 nm in DMSO
λ_{max} (em) VAL3.72 = 550 nm in DMSO



11: *delikine* VAL3.66

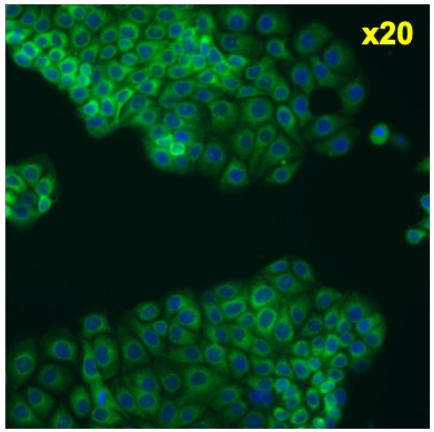


Compound	SOCE IC_{50} (µM) PLP-B lymphocytes
Delikine **DAD3.473**	3.1
12 (VAL3.72)	4.2
11 (VAL3.66)	**2.5**

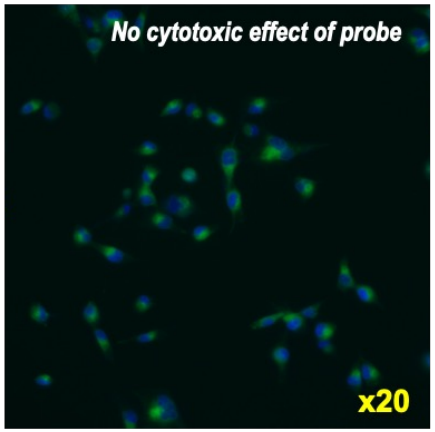
Results of bio-imaging / cell labelling (after 48 h) in various tumoral cells with *delikine* VAL3.72

ImPACcell

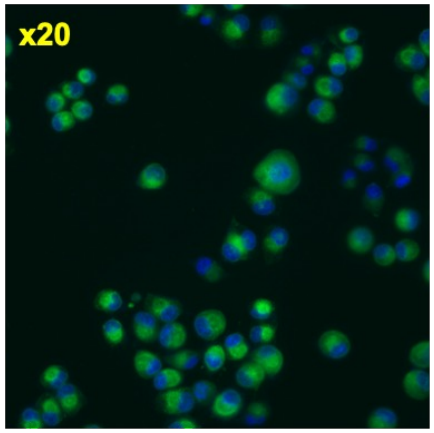
Fluo Zeiss microscope



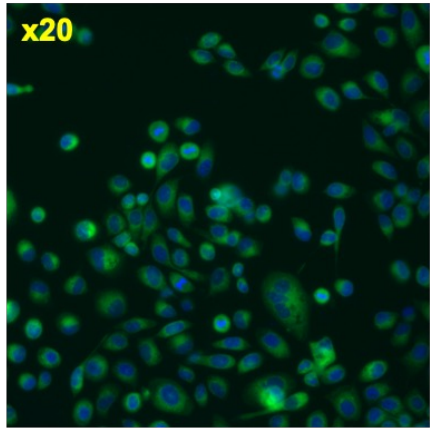
MCF7, 3 µM
(sein)



MDA MBD-231, 3 µM
(adénocarcinome du sein)



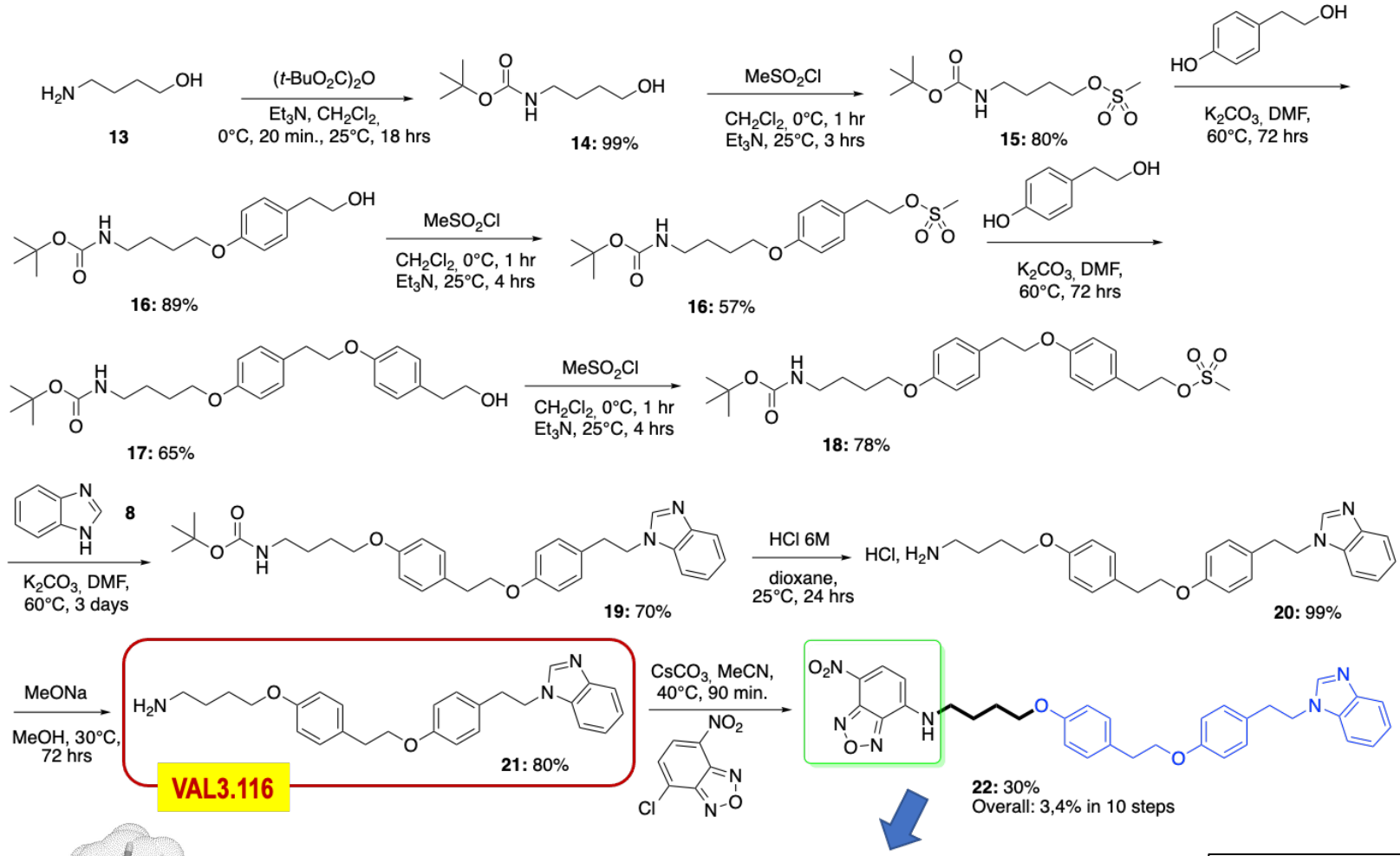
PANC1, 3 µM
(adénocarcinome du pancréas)



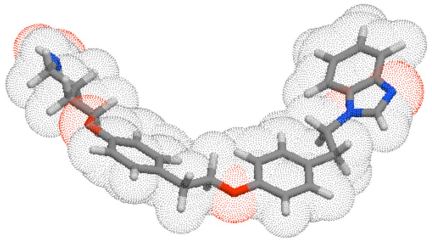
PC3, 3 µM
(prostate)

L-A Voli, C.D. Dago, R. Leguevel, T. Charlier et al., **2022**, unpublished results

Fluorescence probes derived from *delikine* DAD3.473 for bio-imaging: East route
Part 2: linker with 4 atoms of C

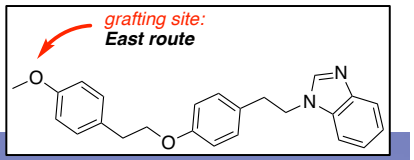


Fluorescence probe: VAL3.121

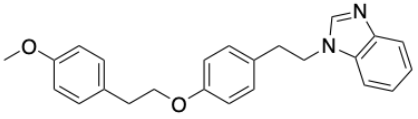


3D structure of VAL3.116

L-A Voli, C.D. Dago et al., **2022**, unpublished results



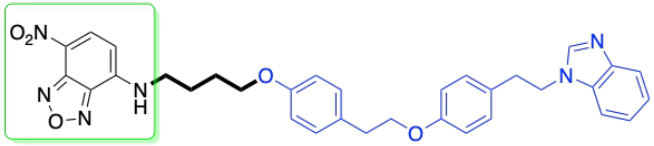
Fluorescence probes derived from *delikine* DAD3.473 for bio-imaging: East route - Part 2



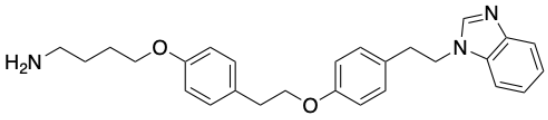
Delikine DAD3.473

Compound	SOCE IC_{50} (µM) PLP-B lymphocytes
Delikine **DAD3.473**	3.1
21 (VAL3.116)	**3.7**
22 (VAL3.121)	ND [a]

[a] ND = Not Determined



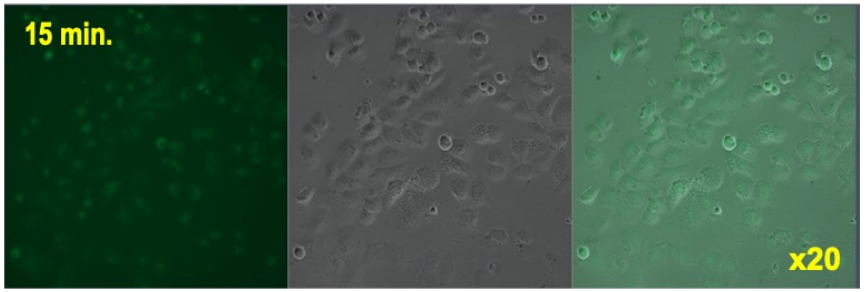
22: *delikine* VAL3.121

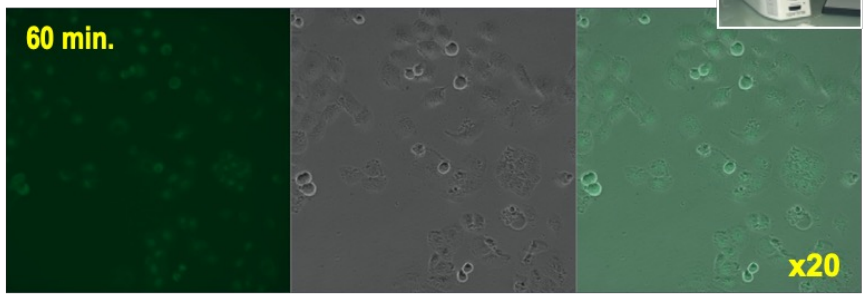


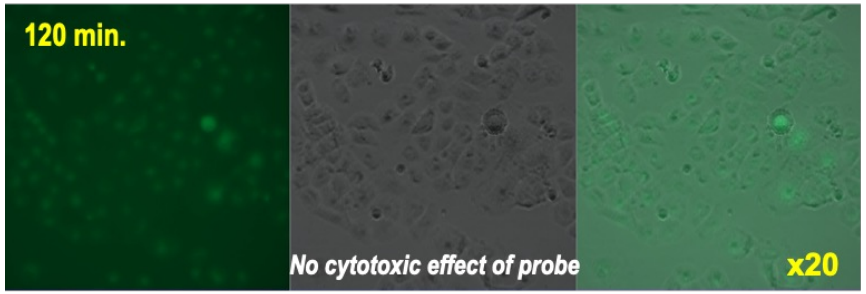
21: *delikine* VAL3.116

Fluo Zeiss microscope

Bio-imaging / kinetic cell labelling in PANC1 tumoral cells with *delikine* VAL3.121 (8 µM)







Conclusion:

- no penetration of fluorescence probe VAL3.121 in PANC cell,
- *delikine* VAL3.116 is better than DAD3.473 and
- this inhibitor works mainly in the outside part of the pore in Orai1

Near futur:

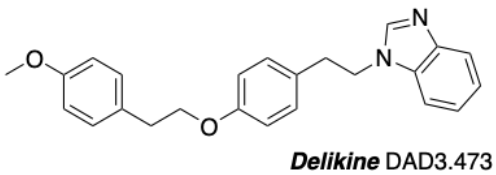
- To modifie the *East* 4-aminobutyl side chain in *delikine* DAD3.473

L-A Voli, C.D. Dago, R. Leguevel, T. Charlier, O.Mignen et al., **2022**, unpublished results

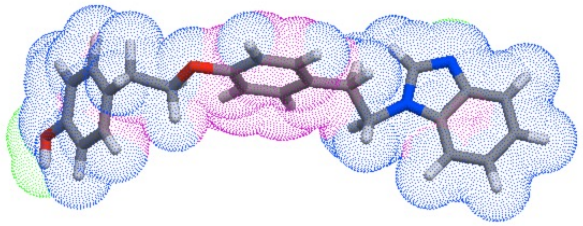
Summary of present work and post patent program on ‘’SOCE/Orai1” inhibitor

	IC_{50} (µM) SOCE		Antiprolif. effect	IC_{50} (µM) Cytotoxicity tumor cells	IC_{50} (µM) PK inhibition	Bio-imaging ‘’Proofs of concept”
HEK 293 (embryonic kindney cells)	HEK 651 HS1 (solid tumor cell)	PLP-B lymphocytes	PANC1	Huh7, Caco2, MDA-MB231, MDA-MB468, HCT116, PC3, MCF7	CDK5/p25, CDK9, Pim1, Haspin, GSK3β, CK1ε, DYRKs, CLKs	PANC1
3.1	20	3.1	Yes	> 25	> 25	In progress

Huh7: cellules tumorales foie **Caco2:** adénocarcinome colorectal **MDA-MB231:** cellule épithéliale cancer sein
MDA-MB468: cellule épithéliale cancer sein (métastase), patient > 51 ans **HCT116:** adénocarcinome colon (adulte mâle)
PC3: cancer prostate, métastase, grade 4 (adulte > 61 ans) **MCF7:** cancer sein (épanchement pleural)



Delikine DAD3.473



3D structure of *delikine* DAD3.473, issued from:
https://www.molinspiration.com/cgi-bin/galaxy

- **ACUTE TOXICITY of *Delikine* DAD3.473 (2022):** ‘’male & female” mice (nber: 12) up-and-down procedure OECD 425 guideline, iv 10 mg/kg/mice, vehicule DMSO

DAD3.473	$Sol._{max}$: 190 mg/ml	in DMSO 99.9%
	Solubility: 150 mg/ml	in DMSO 99.9%
Paracetamol	$Sol._{max}$: 1.24 mg/ml	in DMSO 99.9%

No toxicity of *delikine* DAD3.473 on mice

Conclusions

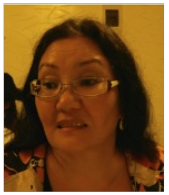
For this program on the *delikine* DAD3.473 as a SOCE/Orai1 inhibitor, we showed that it was possible to built a fuorescent probe bearing a short linker (3 or 4 atoms of C) and a NBD fluorophore on the positions East and North of this inhibitor. The grafting of the linker with a terminal primary amino function is not possible directly on this molecule and it was necessary to develop a linear synthetic strategy which induces to built the skeleton of the *delikine* DAD3.473 at the end of the synthetic sequence.

- ❑ Attaching the linker to the North position of the *delikine* was possible, but this position for this fluorescence probe disturbs the SOCE/Orai1 calcium regulation activity, so it was quickly abandoned.
- ❑ The East position proved to be more successful and we developed a linker with 3 and 4 carbon atoms bearing the NBD fluorophore on the *delikine* DAD3.473 backbone. These 2 fluorescence probes were evaluated on various cancer cell lines and made it possible to carry out bio-imaging on these cells. It should be noted that these fluorescence probes do not show any cytotoxic character and indirectly confirm that the *delikine* DAD3.473 does not show any toxic character on mice for a dose of 10 mg/Kg/mouse.
- ❑ On the basis of these results we plan in the near future to graft the *delikine* DAD3.473 with its linker in the East position on gold nanoparticles AuNPs in order to develop a therapeutic targeting strategy.

Actors of the *DELIKINE* program and AKNOWLEDGMENTS

Team CHEMISTRY

Pr Y-A Békro
UNA, Abidjan, Côte d'Ivoire



Pr J. Mamyrbékova
UNA, Abidjan, Côte d'Ivoire

Pr J-P Bazureau
CORINT / ISCR
Head MedChem

Dr C.D. Dago
PhD 2015 Dec
Post-Doc 2016-18

Dr L.A. Voli
PhD 2020 Jan
Post-Doc 2020-22

A. Asséké
PhD student
2020-23

Dr V. Marchi
MaCSE / ISCR
Fluo

Dr P. Even-H.
MaCSE / ISCR
Fluo

O. Bertrand
ENSCR 3rd year
Internship 2022

Team BIOLOGY

Dr O. Mignen
LBAI Inserm U1227
CHU Morvan Brest

Dr C. Brigaudeau
CalciScreen platform
CHU Morvan Brest

Dr R. Le Guével
ImPACcell platform
SFR Biosit UR1

Pr T. Charlier
ImPACcell platform
IRSET UR1

Dr S. Ruchaud
KISSf platform
Stat. Biol. Roscoff

Dr S. Bach
KISSf platform
Stat. Biol. Roscoff

Bourse Présidence République de la CI

Bourse d'Excellence MESR de la CI

Bourse Doctorale Fondation Benianh

Bourse Doctorale District d'Abidjan

Coop Contract 2019-2024

CD29 - AO 2013

CD35 - AO 2018

CD35 - AO 2021

AOi 2012 Ion Channel Network

BPO - AO 2020

Défis Scientif. AO 2020

PreMAT contract 2019-22