



# MEET NIKEM RESEARCH AT .....



**Friday 9 April 2010**

**Session: Outsourcing Strategies**

**09:30 - Driving for Success in Lead Optimisation**

***Rod Porter, Business Development Director, NiKem Research***

The importance of the close integration of NiKem Research's extensive range of ADME/toxicology and PK protocols with our highly experienced medicinal chemists in driving successful lead optimization programmes will be discussed.



**28<sup>th</sup> Camerino-Cyprus-  
Noordwijkerhout Symposium**

**Camerino, May 16-20, 2010**

**Trekking through Receptor Chemistry**



**Tuesday (morning), May 18th, 2010**

**11:30-12:00 - S. Ronzoni (NiKem Research, I)**

**Novel potent and selective ORL-1 antagonists with efficacy in animal models of Parkinson's disease and neuropathic pain**

**NOVEL POTENT AND SELECTIVE ORL-1 ANTAGONISTS WITH EFFICACY  
IN ANIMAL MODELS OF PARKINSON'S DISEASE AND NEUROPATHIC PAIN**

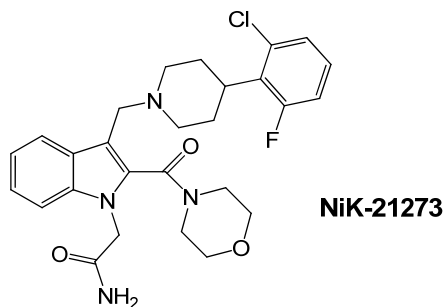
Silvano Ronzoni

*NiKem Research srl, Via Zambelletti 25, 20021 Baranzate (MI), Italy.*

Since the discovery of the NOP/ORL-1 receptor in 1994, many research groups have devoted a considerable effort in the identification of selective non-peptide ligands to better elucidate the biological role of this receptor. ORL-1 antagonists in particular have been suggested to be useful, amongst other therapeutic applications, in neuropathic pain, depression, Parkinson's disease, food intake inhibition and learning and memory.

During the course of our previous studies we identified a novel ORL-1 antagonist, SB-612111/NiK-10001 (1) which, notwithstanding a promising preliminary profile (2), was endowed with significant liabilities (mainly high hERG binding affinity and low oral bioavailability) which hampered its potential as a preclinical candidate. A medicinal chemistry programme focused at the identification of novel chemical scaffolds was therefore undertaken, resulting in the discovery of indole derivatives possessing high affinity and selectivity for the ORL-1 receptor.

Detailed structure-activity relationship studies aimed at the optimisation of this new chemical class, with particular focus on early ADME parameters, will be presented. This investigation led to the identification of NiK-21273, a potent and selective ORL-1 antagonist with efficacy in animal models of neuropathic pain and Parkinson's disease. These results confirmed that ORL-1 antagonists could be of clinical relevance for the treatment of intractable neuropathic pain and for the symptomatic therapy of parkinsonism.



(1) Zaratin, P.F. et al. *J.Pharmacol. Exp. Ther.* **2004**, 308, 454-461.

(2) Rizzi, A. et al. *J.Pharmacol. Exp. Ther.* **2007**, 321, 968-974.



## EFMC-ISMC 2010 - 21st International Symposium on Medicinal Chemistry Brussels, Belgium, September 5-9, 2010

### SYNTHESIS AND PRECLINICAL PROFILING OF NOVEL HYDROXAMIC ACIDS AS HISTONE DEACETYLASE INHIBITORS

**Stefania Gagliardi<sup>‡</sup>, Andrea Colombo<sup>‡</sup>, Florian Thaler<sup>∞†</sup>, Antonello Mai<sup>‡</sup>, Raffaella Amici<sup>∞†</sup>, Chiara Bigogno<sup>‡</sup>, Roberto Boggio<sup>||</sup>, Anna Cappa<sup>∞||</sup>, Simone Carrara<sup>‡</sup>, Tiziana Cataudella<sup>||</sup>, Fulvia Fusar<sup>||</sup>, Eleonora Gianti<sup>∞†</sup>, Maurizio Moroni<sup>‡</sup>, Davide Munari<sup>||</sup>, Gilles Pain<sup>†</sup>, Nickolas Regalia<sup>‡</sup>, Luca Sartori<sup>∞||</sup>, Stefania Vultaggio<sup>||</sup>, Giulio Dondio<sup>‡</sup>, Saverio Minucci<sup>⊥∅</sup>, Ciro Mercurio<sup>||</sup>, Mario Varasi<sup>∞||</sup>**

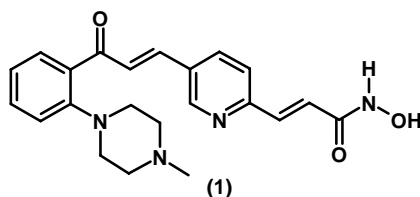
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The histone deacetylases (HDACs) are able to regulate gene expression and histone deacetylase inhibitors (HDACi) emerged as a new class of agents in the treatment of cancer as well as other human disorders such as neurodegenerative diseases.<sup>1</sup> SAHA (Suberoylanilide hydroxamic acid), one of the early HDAC inhibitor discovered by Breslow and colleagues,<sup>2</sup> was approved by FDA in October 2006 for the treatment of patients with cutaneous T-cell Lymphoma (CTCL).<sup>3</sup> Dozens of other inhibitors with different structures are now in various stages of clinical trial.

In the present investigation, we describe the synthesis, the biological evaluation and preclinical profiling of compounds derived from the expansion of N-hydroxy-arylacrylamide scaffold:

- N-Hydroxy-3-phenyl-2-propenamide and N-hydroxy-3-(pyridin-2-yl)-2-propenamide as core structures containing a phenyloxopropenyl moiety, either unsubstituted or substituted by a 4-methylpiperazin-1-yl or 4-methylpiperazin-1-ylmethyl group;
- N-Hydroxyacrylamide-propenylamido derivatives.

The compounds were evaluated for their ability to inhibit nuclear extract HDACs as well as for their *in vitro* antiproliferative activity. Moreover, their metabolic stability in human and mouse microsomes and solubility was studied. Selected compounds were further characterized by *in vivo* pharmacokinetic experiments. The compounds showed a remarkable stability *in vivo*, also compared to hydroxamic acid HDAC inhibitors already entered in clinical trials. One representative compound (**1**) was submitted to an *in vivo* efficacy experiment and showed a significant antitumor activity in a human colon carcinoma xenograft model.



#### References

- <sup>1</sup>Sleiman, S.F.; Basso, M.; Manishi, L.; Kozikowski, A.P.; Donhoe, M.E.; Langley, B.; Ratan, R.R. *Expert Opin Investig Drug* **2009**, *18*, 573-574.
- <sup>2</sup>Breslow, R.; Marks, P. A.; Jursic, B.. WO 93/07148, April 15, 1993.
- <sup>3</sup>(a) Grant, S.; Easley, C.; Kirkpatrick, P. *Nature Rev. Drug Discovery* **2007**, *6*, 21-22; (b) Butler, L. M.; Agus, D. B.; Scher, H. I.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T. *Cancer Research* **2000**, *60*, 5165-5170.

**XX National Meeting on Medicinal Chemistry**  
**September 12-16<sup>th</sup>, 2010**  
**Abano Terme (Padova), Italy**

**INDENO-PYRAZINE-DICARBONITRILE DERIVATIVES AS INHIBITORS OF DEUBIQUITINATING ENZYMES**

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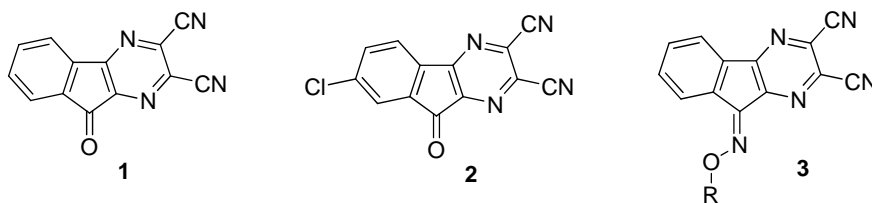
**Abstract**

Deubiquitinating enzymes (DUBs), mainly belonging to cysteine protease family, are enzymes able to mediate the removal and processing of ubiquitin, a highly-conserved protein expressed in eukaryotes cells, whose prominent function is to address proteins to proteasomal degradation. Among the subclasses of DUBs, Ubiquitin-Specific Protease (USP) class is the most represented. Due to their protease activity and their involvement in several human pathologies, USPs are emerging as potential biological targets for pharmacological interference in the ubiquitin regulatory machinery. In particular, USP7 and USP8 showed functional connections with essential viral proteins and oncogenic pathways, thus indicating that targeting these enzymes with small-molecule inhibitors may be useful for the treatment of cancer and viral diseases.

High-throughput screening of a wide collection of chemically diverse compounds identified polycyclic dicyanopyrazine structures as potentially active compounds towards USPs. In particular, compound **1** showed inhibition of deubiquitinating activity with IC<sub>50</sub> in the micromolar range. Starting from the chemical structure of **1** and with the aim of increasing potency and selectivity, three different variation points were identified: introduction of substituents on the phenyl ring, transformation of cyano group(s) and functionalization of the ketone moiety.

Functionalization of phenyl ring of **1** led to the identification of compound **2** able to inhibit reversibly USP7 and USP8 deubiquitinating activity with an IC<sub>50</sub> in the submicromolar range.

Compounds **3**, coming from the derivatization of carbonyl moiety of **1** with O-alkyl hydroxylamines, were totally inactive on USP7 but showed activity in the submicromolar range on USP8. This selectivity was confirmed by assessing the inhibitory effect of these compounds against an extended panel of cysteine proteases. Compounds **3** were further evaluated for their efficacy in cancer cells and they were found to affect the viability of HCT116 colon and PC-3 prostate cancer cell lines with IC<sub>50</sub> values from 0.5 to 1.5 μM.



**References**

COLLAND, F. *et al.*, 2009. Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. *Molecular Cancer Therapeutic*, 8, 2286-2295.

COLOMBO, M. *et al.* Synthesis and biological evaluation of analogues of 9-oxo-9H-indeno[1,2-b]pyrazine-2,3-dicarbonitrile as potential inhibitors of deubiquitinating enzymes. *ChemMedChem*, in press.