SciBX
Science-Business eXchange

Closer to class IIa HDAC inhibitors

By Tracey Baas, Senior Editor

GlaxoSmithKline plc and its **Tempero Pharmaceuticals Inc.** spinout have identified selective, first-in-class compounds that bind and inhibit the catalytic domain of class IIa histone deacetylases,[1] a not-so-well-understood subset of the target family that has been genetically linked to several diseases.

Both companies initially plan to study the trifluoromethyloxadiazole (TFMO) molecules in autoimmune disease.

There are four distinct classes of histone deacetylases (HDACs), which are classified according to primary structure: class I (HDAC1, HDAC2, HDAC3 and HDAC8); class II, which is broken down into class IIa (HDAC4, HDAC5, HDAC7 and HDAC9) and class IIb (HDAC6 and HDAC10); class III (sirtuin 1 (SIRT1)–SIRT7); and class IV (HDAC11).

> **"Taking this forward by using genomewide acetylproteomics might be most interesting and relevant here. The compounds in the present study are powerful tools for broad scientific exploration, comprising a major advance."**
>
> ***—James Bradner, Dana-Farber Cancer Institute***

Little is known about the specific cellular and biological functions of each class outside of class I. The difficulty in teasing out that information is exacerbated by the fact that few class-specific HDAC inhibitors exist.

At least 23 companies have HDAC inhibitors in clinical trials, mostly for cancer, and the majority are nonselective. For example, the two marketed HDAC inhibitors, **Merck & Co. Inc.**'s Zolinza vorinostat and **Celgene Corp.**'s Istodax romidepsin, inhibit at least four HDAC isoforms. Both are approved for cutaneous T cell lymphomas (CTCLs).

Development of selective class IIa HDACs has been particularly challenging,[2,3] in part because the targets have the least mechanistic overlap with other HDAC classes. Indeed, histones are not the substrates of class IIa HDACs—their endogenous enzymatic substrates have not been identified, and their deacetylase activity is almost nonexistent.

Instead, the class IIa HDAC catalytic domain is thought to serve more as an acetyllysine reader, which imprints or erases an epigenetic mark without actually cleaving the post-translational modification, and the noncatalytic domain is thought to take part in multiprotein complexes that can act as transcriptional repressors.

Typically these multiprotein complexes include a class I HDAC to provide deacetylase activity.

Despite the opacity surrounding the function of class IIa HDACs, there have been genetic associations with alopecia, Huntington's disease (HD), glucose homeostasis, muscular dystrophies, autoimmunity and ischemic stroke. Whether the genetic associations are causative or correlative has not been determined, and the dearth of class IIa HDAC inhibitors for use as investigational probes has kept the enzymes poorly validated as targets of human disease.

The Tempero-GSK team started with recombinant HDAC9 and a synthetic trifluoroacetyllysine analog as a substrate and screened about 2 million compounds from GSK's diversity collection. Of the 93 hits with an IC_{50} value less than 10 μM, 43 contained the chemical moiety TFMO. Moreover, three of the four most potent hits contained TFMO.

To determine selectivity, the researchers tested the top hit, dubbed TMP269, in HDAC1–11 inhibition assays. In both recombinant enzyme and whole-cell inhibition assays, the small molecule showed preferential inhibition of only class IIa enzymes. The compound did not lead to cytotoxicity in healthy cells, a common side effect of nonselective HDAC inhibitors, at concentrations up to 10 μM.

The team then worked backward to determine how the TFMO-containing molecules achieved preferential inhibition of class IIa HDACs. The first step was getting a crystal structure of TMP269 bound to HDAC7, which showed that the compound bound directly to the catalytic active site zinc and took on a U-shaped conformation.

Most HDAC inhibitor metal-binding groups, such as hydroxamates, show strong metal-chelating properties. Based on other reported heterocycle–metal ion interactions, the authors were surprised that the oxygen of the TFMO—not the nitrogen—was closest to the zinc and, together with one of the fluorine atoms, established the metal binding.

Because the catalytic pocket of class IIa HDACs is larger than those of class I and IIb enzymes, the researchers hypothesized that the compound was excluded from binding the zinc of other HDACs because the other classes could not accommodate the large size of TFMO as a metal-binding group.

Indeed, when TFMO was replaced with the smaller metal-chelating hydroxamate, the compound series lost their class IIa HDAC selectivity.

Together, these results indicate that the U-shape conformation and the bulky but weak-binding TFMO group provided class IIa selectivity.

In stimulated human peripheral blood mononuclear cells, one of the lead hits, TMP195, led to gene expression changes in a subset of monocytes but not in T cells or B cells. These gene expression profiles are the first clear identification of transcriptional readouts that show endogenous class IIa HDACs use a catalytic or acetyllysine reader function to modulate monocyte responses to nonspecific stimulation.

Results were published in *Nature Chemical Biology*.

"Having these types of compounds available may help to unveil novel biology that has not been able to be investigated before," said Christian Steinkühler, scientific director of the **Immaculate's Dermatology Institute** research hospital and treatment center and

cofounder and CEO of oncology company **Exiris**.

He was project leader of the Merck team that originally identified trifluoroacetyllysine as the first synthetic *in vitro* substrate of class IIa HDACs.

Therapeutic potential

Based on the gene-transcription profiles, Tempero and GSK think their data point toward a role for the class IIa HDACs in macrophage differentiation.

"We first selected to work with immune cells because published work with Hdac9-deficient mice revealed that they were protected in a model of T cell–driven colitis. With these new tool compounds in hand, we are interested to understand their therapeutic potential in autoimmune disease," said Michael Nolan, principal scientist of biology at Tempero and coleader of the Tempero and GSK team.

"Our far-reaching goals are to further our understanding of class IIa HDACs as therapeutic targets and also to elucidate their molecular activity," added Nolan. "Our current data demonstrate that occupying the acetyllysine binding site in class IIa HDACs has distinct and measurable consequences in cell biology, providing evidence that the catalytic domains have an endogenous function."

Timothy McKinsey, associate professor and associate division head for translational research at the **University of Colorado Denver School of Medicine**, wanted to see more details on what happens when class IIa HDACs are blocked. "Even with the compounds in hand, determining an appropriate readout is challenging for measuring the function and inhibition of class IIa HDAC enzymes," he said. "For other classes, people can rely on monitoring histone acetylation, but not for class IIa enzymes, which lack histone deacetylase activity. These novel compounds should enable discovery of class IIa HDAC substrates."

"Indeed, taking this forward by using genomewide acetylproteomics might be most interesting and relevant here," said James Bradner. "The compounds in the present study are powerful tools for broad scientific exploration, comprising a major advance."

Bradner is an investigator in the Department of Medical Oncology at **Dana-Farber Cancer Institute** and an assistant professor in the Department of Medicine at **Harvard Medical School**. He also is a founder of epigenetics companies **Acetylon Pharmaceuticals Inc.** and **Tensha Therapeutics Inc.**

Steinkühler cautioned that "although a class IIa–specific HDAC inhibitor is available, each individual target—HDAC4, HDAC5, HDAC7 and HDAC9—will likely have its own contribution to biology. Molecular tool optimization should not yet be over."

Class act

GSK and Tempero are not the only companies pursuing class IIa HDAC inhibitors.

Merck identified a selective HDAC4 inhibitor in 2008, which contained a 2-trifluoroacetylthiophene oxadiazole moiety,[4] but the program was discontinued.

In January, **Novartis AG** filed for a U.S. patent covering TFMO derivatives and their use in treating neurodegeneration, muscle atrophy or metabolic syndrome by way of HDAC4 inhibition.

Meanwhile, **Active Biotech AB** reported last year that its tasquinimod is an allosteric inhibitor of HDAC4.[5] The compound is in Phase III testing to treat prostate cancer. The intended target of the oral quinoline-3-carboxamide derivative is S100 calcium binding protein A9 (S100A9; calgranulin B; MRP14).

In November 2012, the company showed that the compound interacts with the noncatalytic binding zinc to lock HDAC4 into a conformation that prevents the multiprotein complex formation of HDAC4, nuclear receptor corepressor 1 (NCOR1) and HDAC3. Blocking this complex inhibits deacetylation of histones and transcription factors such as hypoxia-inducible factor 1α (HIF1A; HIF1α). The company said this regulation contributes to tasquinimod's antiangiogenic and anticancer activity.

The patent status of the molecules described in the *Nature Chemical Biology* paper by Tempero and GSK is undisclosed.

"Even with the compounds in hand, determining an appropriate readout is challenging for measuring the function and inhibition of class IIa HDAC enzymes. For other classes, people can rely on monitoring histone acetylation, but not for class IIa enzymes, which lack histone deacetylase activity. These novel compounds should enable discovery of class IIa HDAC substrates."
—Timothy McKinsey, University of Colorado Denver School of Medicine

Baas, T. *SciBX* 6(13); doi:10.1038/scibx.2013.301
Published online April 4, 2013

REFERENCES

1. Lobera, M. *et al. Nat. Chem. Biol.*; published online March 24, 2013; doi:10.1038/nchembio.1223
   Contact: Michael A. Nolan, Tempero Pharmaceuticals Inc., Cambridge, Mass.
   e-mail: mnolan@temperopharma.com
2. Lahm, A. *et al. Proc. Natl. Acad. Sci. USA* **104**, 17335–17340 (2007)
3. Bradner, J.E. *et al. Nat. Chem. Biol.* **6**, 238–243 (2010)
4. Muraglia, E. *et al. Bioorg. Med. Chem. Lett.* **18**, 6083–6087 (2008)
5. Isaacs, J.T. *et al. Cancer Res.* **73**, 1386–1399 (2013)

COMPANIES AND INSTITUTIONS MENTIONED

Acetylon Pharmaceuticals Inc., Boston, Mass.
Active Biotech AB (SSE:ACTI), Lund, Sweden
Celgene Corp. (NASDAQ:CELG), Summit, N.J.
Dana-Farber Cancer Institute, Boston, Mass.
Exiris, Rome, Italy
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Harvard Medical School, Boston, Mass.
Immaculate's Dermatology Institute, Rome, Italy
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Tempero Pharmaceuticals Inc., Cambridge, Mass.
Tensha Therapeutics Inc., Cambridge, Mass.
University of Colorado Denver School of Medicine, Denver, Colo.