Epigenetic Screening Libraries

Epigenetic regulation of gene expression implies acetylation, methylation, phosphorylation, ubiquitination and other modifications of chromatin structure that alter DNA transcription mechanisms. These modifications are known as epigenetic marks, and the most important of them are chromatin remodeling and DNA methylation. There are separate groups of proteins, known as “writers”, “readers” and “erasers” of such marks.

Epigenetic “writers” catalyze the addition of chemical substituents onto either histone tails or DNA. These marks are not necessarily permanent modifications; they can be removed by “erasers”. In particular, the bromodomain-containing family of proteins recognizes,
or ”reads” modified lysine residues within histone proteins. These mechanisms together regulate gene expression and can contribute to or trigger the development of a number of disorders.

As a result of its research in the above field Life Chemicals presents its three new Epigenetic Libraries designed with both ligand-based and structure-based approaches:

- Epigenetic Focused Library - 2D Fingerprint Similarity Search (**650** compounds);
- Epigenetic Targeted Library - Docking Screening (**7,000** compounds);
- SIRT Targeted Library (**750** compounds).

The Libraries contain drug-like compounds carefully selected by computational chemistry and virtual screening techniques.

Epigenetic Focused Library - 2D Fingerprint Similarity Search

2D Fingerprint Similarity search allowed us to select **650** compounds making up the Library. This approach means that referent molecules (active compounds found in literature) and molecules of interest are represented as a set of small fragments encoded in a bit strings (“fingerprints”). Both sets of “fingerprints” are compared to estimate their degree of similarity. When designing this library, reference sets of known epigenetic modulator compounds were obtained from the ChEMBL database. The upper IC_{50} value threshold for all reference compounds was 1.1 μM against each target. Similarity search was performed with SYBYL-X software (SELECTOR, GALAHAD, Surflex-Sim).

Epigenetic Targeted Library - Docking Screening

The Library contains **7,000** compounds picked out by virtual screening against the following targets:

DNA Methyltransferase:

- DNMT1
- DNMT3A
- DNMT3B
- Lysine-Specific Histone Demethylase 1

Histone Deacetylase:

- HDAC I
- HDAC II

Histone Acetyltransferase:

- P300

Histone-Lysine N-methyltransferase:

- DOTL1
- Histone H3 Lysine 4 Specific Methyltransferase (KMT4)

Protein Arginine Methyltransferase:

- PRMT1
- PRMT3
- PRMT4/CARM1
- PRMT5

DNA Methyltransferase

In the course of creating this library, our primary interest was focused on DNA methyltransferases: DNMT1, DNMT3A, and DNMT3B, the proteins that are promising epigenetic targets in cancer therapy.

An additional sub-library for Lysine-Specific Histone Demethylase 1 was developed as well to expand and complete our DNA Methyltransferase library.

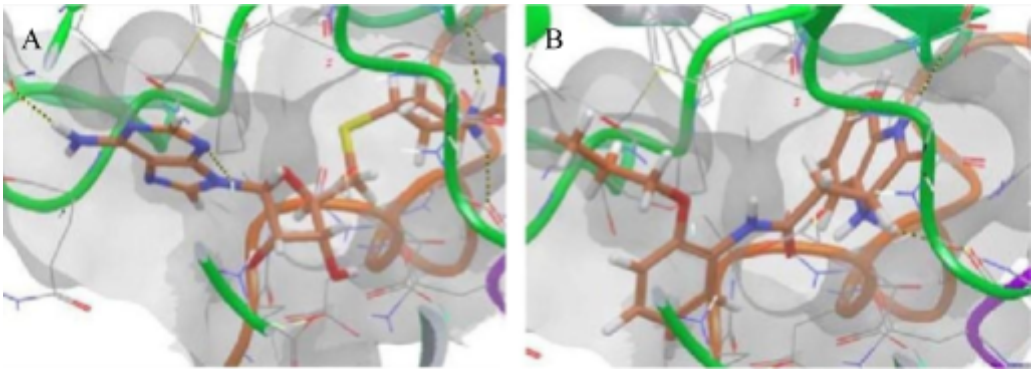

Figure 1. A. DNMT1 in a complex with S-adenosyl-homocysteine. **B**. Binding mode of compound F0016-0153 in the active site of DNMT1.

Compounds from a reference set collected from the literature were docked in the prepared S-adenosyl-l-homocysteine binding sites. Docking (Fig. 1, 2) was carried out with Glide software (Schrödinger), which provides an efficient and flexible prediction of protein-ligand complexes based on constraints in the electrostatic grid maps and hydrophobic regions. Finally, the compounds have been selected by score values according to the results of the docking validation experiment that employed the reference set of compounds.

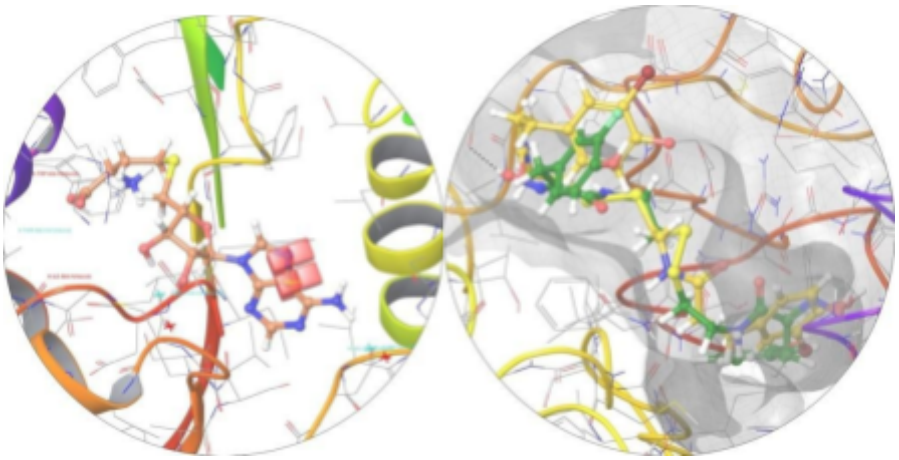

Figure 2. Structures of DNMT3A (left) and DNMT3B (right), the surface of the binding pocket is shown.

HATs, PRMTs and KMTs

Docking in these series of proteins was carried out with Glide (Fig. 3) after analysis of co-crystallized ligand binding modes. Information about the binding modes was obtained from published data and available crystal structures. The analysis included relaxation of protein-ligand complexes with molecular dynamics simulation and protein conformations clustering.

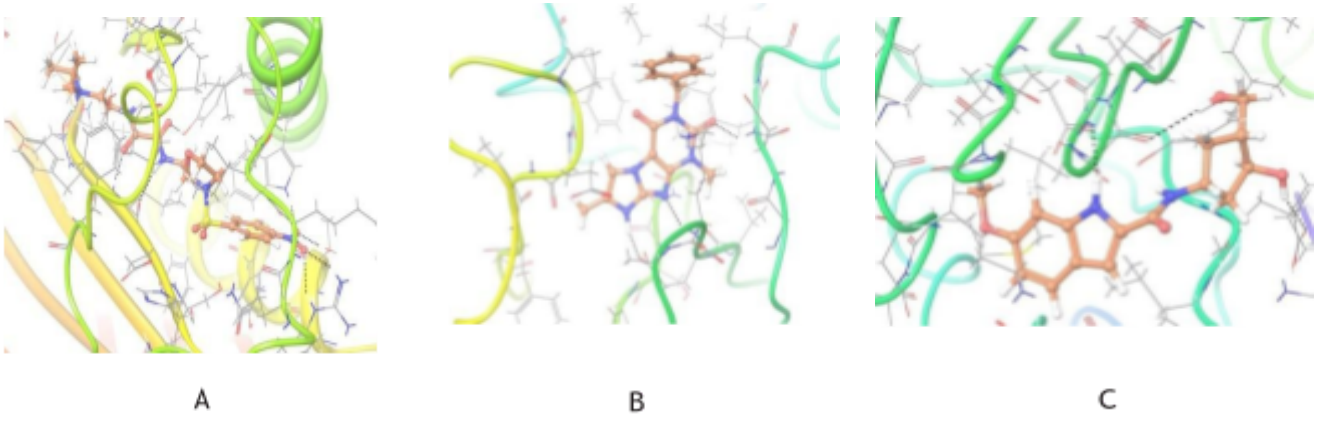

Figure 3. A. Compound F1843-0098 in the binding site of p300 (HAT). **B**. Compound F3234-0953, which occupies the binding site of DOTL11 (KMT). **C**. A complex of PRMT5 and compound F6127-0221.

HDAC

The HDAC subset was designed for HDAC class I and HDAC class II proteins based on 7 existing crystal structures obtained from the Protein Data Bank. Docking score values of the reference set compounds correlated well with the corresponding activity values obtained experimentally. Glide docking mode assumed the implementation of hydrogen bond, volume, and hydrophobic interaction constraints to sort out unfavorable conformations of ligands before the screening.

UNITY modeling (SYBYL) was chosen as a screening tool. All protein sequences in both HDAC classes were aligned to evaluate the degree of amino acid homology of the HDAC active site, and one distinct UNITY model was built for each class. To address selectivity of the HDAC reference set a cross-docking procedure was applied to exclude cross-binders (about 20 compounds of high activity and 20 compounds of low activity were selected for each class).

To simulate HDAC flexibility tolerance features (donor/acceptor/volume) were adjusted (Fig. 4). The atom of Zn was transformed into a positively charged steric center with appropriate radii (gray sphere). Corresponding atoms of ASP93, GLY143, HIP134, HIP135, TYR298, TYR298 / ASP626, GLY678, HIS669, HIS670 were assigned as donors/acceptors (green and pink figures). The extended volume is colored yellow.

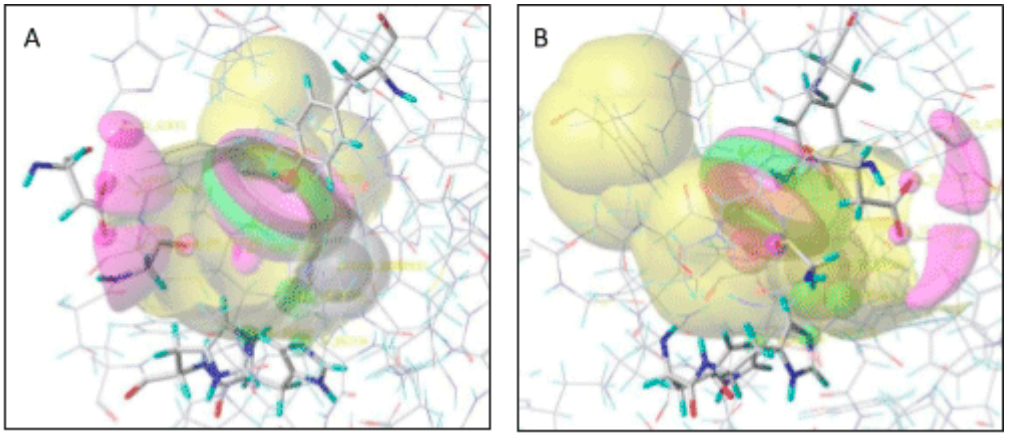

Figure 4. **A**. Front view of the Unity model of HDAC II. **B**. Right view of the Unity model of HDAC II.

SIRT Targeted Library

Sirtuins (SIRT) is a class of proteins that possess either mono-ADP-ribosyltransferase or deacylase activity, including the activity that is classified as an epigenetic factor. The SIRT Targeted Library includes potential sirtuin inhibitors and consists of **750** compounds. It was prepared by virtual screening of the Life Chemicals HTS Compound Collection applying Glide (Schrödinger) software.

The first docking model is based on SIRT 1, 2, 5 and 6 considering the similarity of their binding site (2 hydrogen bonds), and the second one is based on the structure of SIRT 3 (3 hydrogen bonds) (Fig. 5).

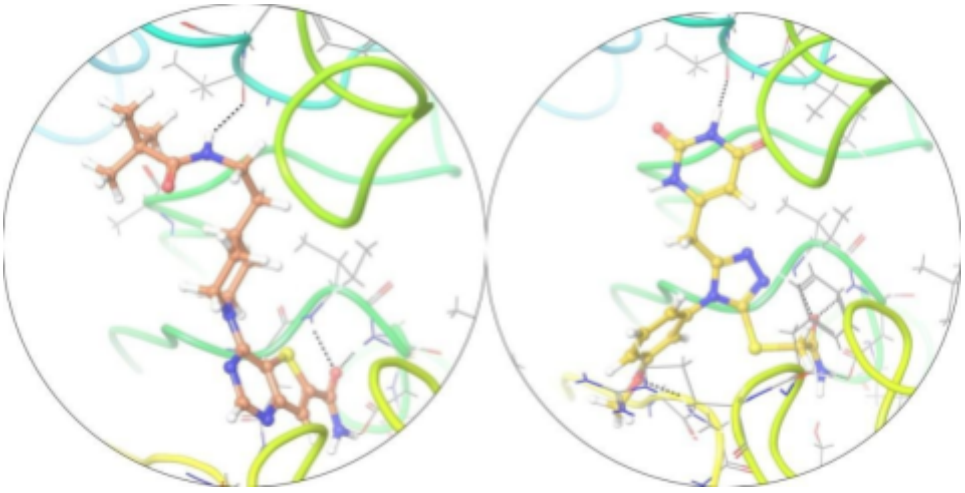

Figure 5. Test compound (on the left) and F0648-0360 (on the right) docked in SIRT3 model (4JT8)

References:

1. Chen T, Hevi S, Gay F, et al. Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. Nat Genet 2007; 39:391–6
2. Molecular modeling studies of the novel inhibitors of DNA methyltransferases SGI- 1027 and CBC12: implications for the mechanism of inhibition of DNMTs. Yoo J, Choi S, Medina-Franco JL. PLoS One. 2013 Apr 25;8(4): e62152
3. Novel and selective DNA methyltransferase inhibitors: Docking-based virtual screening and experimental evaluation. Dirk Kuck, Narender Singh, Frank Lyko, Jose L. Medina-Franco. Bioorg. Med. Chem. 18 (2010) 822–829
4. Virtual Screening and Biological Characterization of Novel Histone Arginine Methyltransferase PRMT1 Inhibitors. Ralf Heinke, Astrid Spannhoff, Rene Meier, Patrick Trojer, Ingo Bauer. ChemMedChem 2009,4,69–77
5. Studies on Hydroxamic Acid Histone DeacetylaseInhibitors(HDACI) by Molecular Docking and CoMFA. ZHANG Liang, XIANG Yu-Hong, Zhang Zhuo-Yong. Chemical Journal of Chinese Universities 2009, Vol. 30 Issue (11(Suppl.)): 52-57
6. Structure and function of histone acetyltransferases. R. Marmorstein. CMLS, Cell. Mol. Life Sci. 58 (2001) 693–703
7. Small molecule inhibitors that discriminate between protein arginine N-methyltransferases PRMT1 and CARM1. James Dowden, Richard A. Pike. Org. Biomol. Chem., 2011,9, 7814-7821
8. Power of inhibition activity screening and 3D molecular modeling approaches in HDAC 8 inhibitor design. Gamze BORA TATAR, TenzileDeniz TOKLUMAN, Kemal YELEKCI. Turk J Chem 35 (2011), 861 – 870
9. Identification of a better Homo sapiens Class II HDAC inhibitor through binding energy calculations and descriptor analysis. Usman Sumo Friend Tambunan and Evi Kristin Wulandari. BMC Bioinformatics 2010, 11(Suppl 7): S16
10. PLoS One. 2013;8(1):e51429. DOI: 10.1371/journal.pone.0051429. Epub 2013 Jan 28. Identification of inhibitor binding site in human sirtuin 2 using molecular docking and dynamics simulations. Sakkiah S1, Arooj M, Kumar MR, Eom SH, Lee KW
11. UNITY® - Tripos. http://www.tripos.com/tripos_resources/fileroot/pdfs/Unity_111408. pdf