Chemical Genomics Core Facility

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MELVIN AND BREN SIMON CANCER CENTER
INDIANA UNIVERSITY

Abstract

The Chemical Genomics Core Facility (CGCF) facilitates the identification of chemical tools to study biological pathways and the discovery of lead compounds for the development of novel therapeutics. The CGCF strives to provide excellence and innovation in high throughput screening and medicinal chemistry. As the first shared resource facility of its kind to be established in an academic setting in Indiana, the CGCF has a proven record of providing screening and medicinal chemistry expertise and service to Indiana University Melvin and Bren Simon Cancer Center (IUSCC) members and researchers across Indiana and beyond. The CGCF provides IUSCC investigators with cutting edge technologies and cost-effective access to large-scale, high-throughput screening of structurally diverse, drug-like small molecules, hit confirmation and resupply, and hit to lead optimization chemistry to furnish research tools or to develop new experimental small molecule therapeutics.

This shared resource has been designed to be highly flexible in order to meet the needs of multiple users employing a range of different types of assays. The facility staff works closely with each investigator through all stages of the drug discovery process, providing an opportunity for cancer center members to gain experience and training in high throughput screening and medicinal chemistry at the facility. The CGCF became fully operational in July 2006 and has been a shared resource for IUSCC since 2007. The number of investigators utilizing the CGCF increases every year, from 10 in FY 2007 to 48 in FY2012, a 480% increase in user base. The majority of CGCF users are Cancer Center investigators. Of the 48 CGCF users in FY2013, 28 of them are peer-review funded (58%) IUSCC members and 20 of them (42%) are non-IUSCC members. As a shared resource of IUSCC, the CGCF has assisted Cancer Center members in their research by providing quality services to enable numerous high throughput screening and medicinal chemistry projects aimed at identifying small molecule probes and potentially new therapeutics against novel cancer targets.

Services Provided

•Consultation for assay development
•Assistance in assay implementation and validation
•Assistance in carrying out high-throughput screening of chemical libraries
•Access to compound libraries pre-plated, available for use in a 96- or 384-well format
•Training in the use of facility-maintained instrumentation
•Assistance with data analysis and compound selection
•Lead validation
•Design and synthesis of focused library for lead optimization
•Chemical synthesis of literature-cited compounds

Facilities and Location

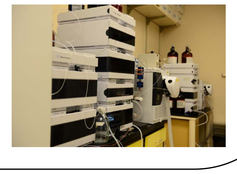
Medical Sciences Building
635 Barnhill Drive, MS 1005

Personnel

Zhong-Yin Zhang, Ph.D.-Director
Lan Chen, Ph.D.-HTS Director
Lily Wu, Facility Manager
Sheng Zhang, Ph.D.-Research Associate
Andrea Gunawan, Research Analyst

Key Instrumentation

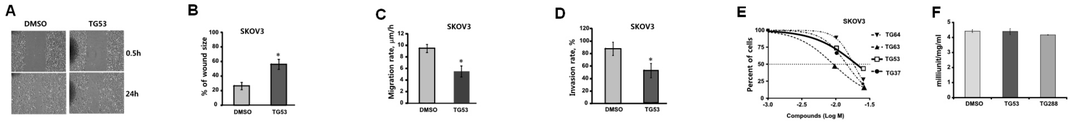
- Freedom EVO Workstations (one using disposable pipette tips, the other sing fixed tip block and active wash station; both can use a 384-well format pin tool)
- Multidrop 384 Lipuid Dispensers (one with robotic stackers and the other placed in biosafety hood for cell culture dispensing)
- Precision Microplate Sample Processor
- EnVision 2102 Mltilabel Plate Reader (with AlphaScreen ® and LANCE ® (hTRF) upgrades, bukilt-in stackers)
- Ultra 384 Multilabel Plate Reader
- SpectraMax Plus 384 Spectrometer
- VICTOR Light Luminescence Counter (with dual injectors and stackers)
- BenchCel Microplate Handling System
- VCode bar code printing and applying station
- PlateLoc and ALPS Plate Sealers
- iTC200 Isothermal Titration Calorimeter
- Chemical hoods, LC-MS and HPLC's
- Peptide/organic synthesizer and microwave reactor
- NMR



New Science – Examples of the projects supported by the CGCF

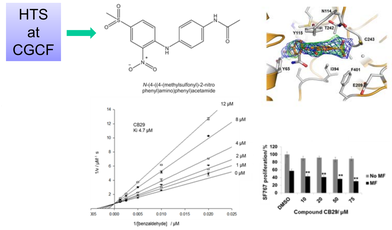
Small Molecule Inhibitors Target the Tissue Transglutaminase and Fibronectin Interaction
PI: Daniela Matei, MD (EDT Program)

Tissue transglutaminase (TG2) mediates protein crosslinking through generation of ε-(γ-glutamyl)lysine isopeptide bonds and promotes cell adhesion through interaction with fibronectin (FN) and integrins. Cell adhesion to the peritoneal matrix regulated by TG2 facilitates ovarian cancer dissemination. Therefore, disruption of the TG2-FN complex by small molecules may inhibit cell adhesion and metastasis. The Core has helped Dr. Matei's group developing a novel high throughput screening (HTS) assay based on AlphaScreen™ technology to measure the formation of a complex between His-TG2 and a biotinylated FN fragment, and a screen of 10,000 compounds was carried out at the CGCF to discover small molecules that inhibit this protein-protein interaction. Several hits were identified and validated in ELISA and other cell based assays measuring cell adhesion, migration, invasion, and proliferation. The top candidate (TG53) was found to inhibit ovarian cancer cell adhesion to FN, cell migration and invasion (see figures below) and could be further developed as a potential inhibitor for ovarian cancer dissemination. Further lead optimization is planned at the core facility.



Development of Selective Inhibitors for Human Aldehyde Dehydrogenase 3A1 to Enhance Cyclophosphamide Cytotoxicity in Cancer Therapy
PI: Thomas D. Hurley, PhD (EDT Program)

Aldehyde dehydrogenase 3A1 (ALDH3A1) plays an important role in many cellular oxidative processes, including cancer chemo-resistance by metabolizing activated forms of oxazaphosphorine drugs such as cyclophosphamide (CP) and its analogues such as mafosfamide (MF), ifosfamide (IFM), 4-hydroperoxy-cyclophosphamide (4-HPCP). Compounds that can selectively target ALDH3A1 may permit delineation of its roles in these processes and could restore chemo-sensitivity in cancer cells that express this isoenzyme. Dr. Hurley's group has carried out high throughput screens at the CGCF using a kinetic assay measuring the enzymatic activity of ALDH. A small molecule (CB29) was identified that specifically inhibits ALDH3A1. This compound and its derivatives were found to be able to enhance the anti-proliferative effects of mafosfamide in ALDH3A1 expressing lung cancer cells and brain cancer cells. It is promising that CB29 and its derivatives can become noval agents that increases chemosensitivity toward cyclophosphamide in ALDH3A1 expressing tumors and perhaps permit therapies to proceed with reduced marrow toxicity.

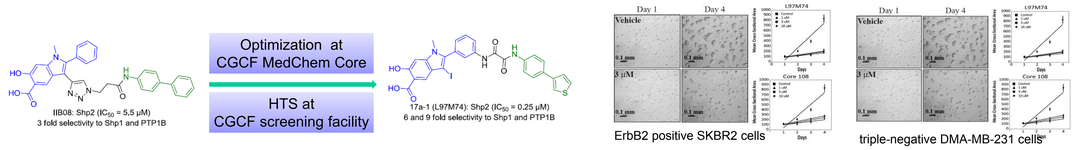


Screening for Selective Retinoblastoma Cell Cytotoxins
PI: Time Corson, PhD (EDT Program)

Retinoblastoma is a pediatric ocular cancer, responsible for 1% of childhood cancer deaths and 5% of childhood blindness. Despite this disease burden, there has been little pharmaceutical company interest or published successes in development of novel chemotherapeutics specific for this blinding childhood cancer. Dr. Corson and his collaborators believe that first step toward targeted therapies for retinoblastoma is to find small molecules that have an effect on retinoblastoma cell lines without an effect on normal cells of the eye. Using a cell viability assay, screens of a library of known drugs and bioactives were performed against several retinoblastoma cell lines at the CGCF. Cell line-specific and common hits were identified and currently they were under further analysis and validation.

Hydroxyindole Carboxylic Acid Based Inhibitors for Oncogenic Src Homology-2 Domain Containing Protein Tyrosine Phosphatase-2 (Shp2)
PI: Zhong-Yin Zhang, PhD (EDT Program)

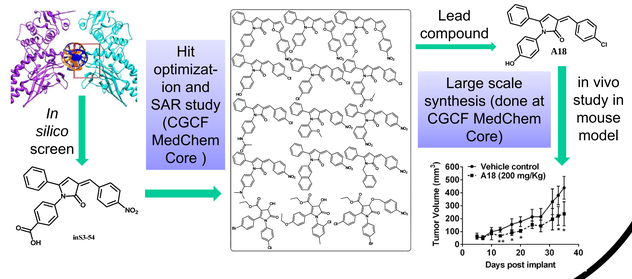
The Src homology 2 domain-containing protein tyrosine phosphatase 2 (Shp2) plays a positive role in various signaling transduction downstream of growth factor and cytokine receptors. Gain-of-function Shp2 mutations are known to cause the autosomal dominant Noonan Syndrome, a variety of leukemia and solid tumors. Potent and selective Shp2 inhibitors may provide new treatment for Noonan Syndrome, leukemia and cancers. Dr. Z.-Y. Zhang's lab has previously developed a Shp2 inhibitor, II-B08, which has efficacious cellular activity and favorable in vivo anti-leukemia ability; but the potency of this compound is at μM level, which is not adequate for preclinical investigation. Optimization of II-B08 was performed at the Medicinal Chemistry Core at the CGCF to improve the potency and selectivity. Four focused libraries were designed and synthesized to optimize different positions of the molecule. The library compounds were also screened at the Core. A compound, 17a-1 (L97M74), was identified with >20 fold higher inhibitory activity towards Shp2 than the parental II-B08 and better selectivity. This compound was found to inhibit the growth of a number of cancer cell lines, including breast cancer and thus represents a promising new lead for further development.



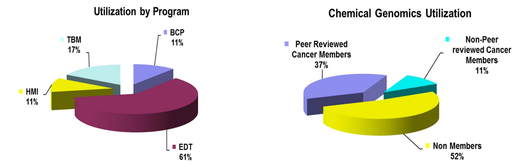
Small molecule compounds targeting DNA binding domain of STAT3 for inhibition of tumor growth and metastasis
PI: Jian-Ting Zhang, PhD (EDT Program)

Signal transducer and activator of transcription 3 (STAT3) is constitutively activated in malignant tumors, and its activation is associated with high histological grade and advanced cancer stage. Thus, inhibiting STAT3 promises an attractive strategy for treatment of advanced tumors with metastatic potential. Dr. J.-T. Zhang's lab has identified a STAT3 inhibitor, inS3-54, by targeting DNA-binding site of STAT3 using an in-silico screening approach. However, inS3-54 was found not to be appropriate for further studies because of low specificity for STAT3 and poor absorption in mice.

To develop an effective and specific STAT3 inhibitor, the CGCF helped design and synthesize a focused library of analogues of inS3-54 for structure-activity relationship analysis. The best compound from this focused library (A18) was found to inhibit STAT3-dependent colony formation of hematopoietic progenitor cells, indicating a higher selectivity for STAT3 than the parental compound, inS3-54. In addition, A18 can reduce tumor growth in a mouse xenograft model of lung cancer with little effect on body weight. It was concluded that A18 as an inhibitor of STAT3 DNA-binding domain is feasible to serve as a lead compound for the development of anticancer therapeutics.



Utilization



Publication Resulted from CGCF Services in FY2013

1. Kimble-Hill, A.C., Parajuli, B., Chen, C.-H., Mochly-Rosen, D. and Hurley, T.D. "Development of selective inhibitors for Aldehyde Dehydrogenases based on substituted indole-2,3-diones", *J. Med. Chem.*, submitted (2013)
2. Parajuli, B., Fishel, M.L., and Hurley, T.D. "Selective ALDH3A1 inhibition by benzimidazole analogs increase mafosfamide sensitivity in cancer cells", *J. Med. Chem.*, submitted (2013)
3. Parajuli, B., Fishel, M.L. and Hurley, T.D. "Development of selective inhibitors for human aldehyde dehydrogenase 3A1 (ALDH3A1) for the enhancement of cyclophosphamide cytoxicity", *ChemBiochem.*, submitted to (2013)
4. Liu, D., Zhou, D., Mani, T., Knabe, W. E., Wang, F., Wang, B., Li, L., and Meroueh, S. O. "A New Class of Small Molecule Inhibitors of Protein Interac-tions of the Urokinase Receptor", *ACS. Med. Chem. Lett.*, Submitted (2013).
5. Kim, H.-S., Chen, Q., Kim, S.-K., Nickoloff, J.A., Hromas, R., Georgiadis, M.M., and Lee S.-H. "Functional evolution of a transposase into a human DNA repair protein." *Nucleic Acids Research,* Submitted (2013).
6. Yakubov, B., Chen, L., Belkin, A., Chelladurai, B., Zhang, Z.-Y., and D. Matei. "Small molecule inhibitors target the tissue transglutaminase and fibronectin interaction." *Molecular Therapeutics,* Submitted (2013).
7. Mani, T., Liu, D., Li, L., Zhou, D., Knabe, W. E., Wang, F., Oh, K., and Meroueh, S. O. "Probing Binding and Cellular Activity of Small Molecules Targeting the Urokinase Receptor", *ChemMedChem.* In Press (2013).
8. He, R., Zeng, L.-F., He, Y., Wu, L., Gunawan, A.M., and Zhang, Z.-Y. "Organocatalytic multicomponent reaction for the acquisition of a potent and selective inhibitor of mPTPB, a virulence factor of tuberculosis." *Chem. Commun.* 49, 2064-2066 (2013).
9. He, Y., Xu, J., Yu, Z.-H., Gunawan, A.M., Wu, L., Wang, L., and Zhang, Z.-Y. "Discovery and evaluation of novel inhibitors of mycobacterium protein tyrosine phosphatase B from the 6-hydroxy-benzofuran-5-carboxylic acid scaffold." *J. Med. Chem.* 56, 832-842 (2013).
10. Yu, Z.-H., Xu, J., Walls, C., Chen, L., Zhang, S., Zhang, R., Wu, L., Wang, L., Liu, S., and Zhang, Z.-Y. "Structural and mechanistic insights into LEOPARD syndrome associated SHP2 mutations." *J. Biol. Chem.* 288, 10472-10482 (2013).
11. Zeng, L.-F., Xu, J., He, Y., He, R., Wu, L., Gunawan, A. M., and Zhang, Z.-Y. "A facile hydroxyindole carboxylic acid-based focused library approach for potent and selective inhibitors of Mycobacterium protein tyrosine phosphatase B." *ChemMedChem.* 8, 904-908 (2013).
12. He, Y., Liu, S., Menon, A., Stanford, S., Oppong, E., Gunawan, A. M., Wu, L., Wu, D. J., Barrios, A. M., Bottini, N., Cato, A. C., and Zhang, Z.-Y. "A potent and selective small molecular inhibitor for the lymphoid-specific tyrosine phosphatase (LYP), a target associated with autoimmune diseases." *J. Med. Chem.* 56, 4990-5008 (2013).
13. Zhang, J., Luo, M., Marasco, D., Logdson, D., LaFavers, K.A., Chen, Q., Reed, A., Kelley, M.R., Gross, M.L. and M.M. Georgiadis." Inhibition of Apurinic/Apyrimidinic Endonuclease I's redox activity revisited." *Biochemistry. 52,* 2955–2966 (2013). PMID: 23597102.
14. Mani, T., Wang, F., Knabe, W.E., Sinn, A.L., Khanna, M., Jo, I., Sandusky, G.E., Sledge, G.W., Jones, D.R., Khanna, R., Pollok, K.E., and Meroueh, S.O. "Small-molecule inhibition of the uPAR·uPA interaction: Synthesis, biochemical, cellular, in vivo pharmacokinetics and efficacy studies in breast cancer metastasis." *Bioorg. Med. Chem.* 21, 2415-2155 (2013).
15. Li, M., Chen, X., Ye, Q.Z., Vogt, A., Yin, X.M., "A high-throughput FRET-based assay for determination of Atg4 activity." Autophagy. 8, 401-412, (2012).
16. Zhang, S., Chen, L., Lawrence, D. S. and Zhang, Z.-Y. "A combinatorial strategy for the acquisition of potent and specific protein tyrosine phosphatase inhibitors." *Methods in Molecular Biology,* 928, 53-65 (2012).
17. Wang, F., Knabe, E.W., Li, L., Jo, I., Mani, T., Roehm, H., Oh, K., Li, J., Khanna, M. and Meroueh, S.O. "Design, synthesis, biochemical studies, cellular characterization, and structure- based computational studies of small molecules targeting the urokinase receptor." *Bioorg. Med. Chem.* 20, 4760-4773 (2012).
18. Zhang, S., Liu, S., Tao, R., Wei, D., Chen, L., Shen, W., Yu, Z.-H., Wang, L., Jones, D. R., Dong, X. C. and Zhang, Z.-Y. "A highly selective and potent PTP-MEG2 inhibitor with therapeutic potential for type2 diabetes." *J. Am. Chem. Soc.* 134, 18116-18124 (2012).

Future Directions

The following goals reflect an important growth area that will ensure the CGCF continues to provide Cancer Center members state-of-the-art capabilities in chemical biology and drug discovery:

1)Enhanced capability in chemoinformatics: we plan to acquire the SYBYL-X suite form Tripos, which will enhance our ability to carry out molecular modeling from sequence through lead optimization. The package offers unique, competitive advantages in a number of key areas vital for successful discovery research, including 3D QSAR, ligand-based vitual screening, cheminformatics, and docking.
2)Implement high-content screening capabilities. A major challenge in early stage discovery of small molecule inhibitors is defining the efficacy in a cellular context. In addition, there is increasing demand for phenotypic assays to screen for compounds that selectively modulate cellular functions and signaling pathways. The acquisition of automated fluorescence-imaging platforms is the objective of CGCF expansion that we expect will be completed over the next project period. High-content screening would enable Cancer Center investigators to collect information-rich data on the effect of drugs on cell physiology and to discover novel small molecules through phenotypic screens.
3)A fast, HTS-compatible artificial membrane assay (PAMPA) will be developed to evaluate cell permeability of the lead compounds identified from HTS. This assay measures the ability of a compound to cross the cell membrane by passive diffusion, a mechanism by which the majority of the drugs utilize to enter the cell. This capability will further enhance CGCF's ability to help Cancer Center members to move lead molecules down the drug discovery pipeline.