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**COVALENT
LIBRARIES**

2024

Mass Spectrometry Screening

Save on time and costs by running your MS-based Screening directly at Enamine

Enamine Covalent Collection is constantly being updated and enhanced with at least 10,000 newly synthesized compounds yearly. Comprising 120K compounds and representing 26 types of covalent warheads, this Collection is currently the world's largest source of reliable covalent binders.

Together with our collaborators and recognized experts in the field, we have designed **Covalent Libraries** aiming at meeting most of the early-stage research demands and efficient initiation of drug discovery projects. The diversity and utility of our libraries address all the practical aspects of hit finding.

All libraries are available in customized pre-plated formats for the most convenient and fast access. Using our libraries for hit finding our clients receive multiple benefits including on-site MS-screening, Hit confirmation, and follow-up support:

- Resupply of hits from dry powders and the same batch hit confirmation with additional QC check and HPLC repurification upon request, 90%+ purity used as standard.
- Resynthesis of hit compounds and enumeration of follow-up libraries. Library synthesis within just 4 weeks and with 80%+ success rate.
- MS-based screening, Kinetic, and Intrinsic reactivity measurements. Binding site localization for Cys-containing proteins.

If you choose to run your screening campaign at Enamine you will benefit in time and cost. Using our screening service, you will only pay an access fee instead of buying a full library. *No delivery and formatting delays!*

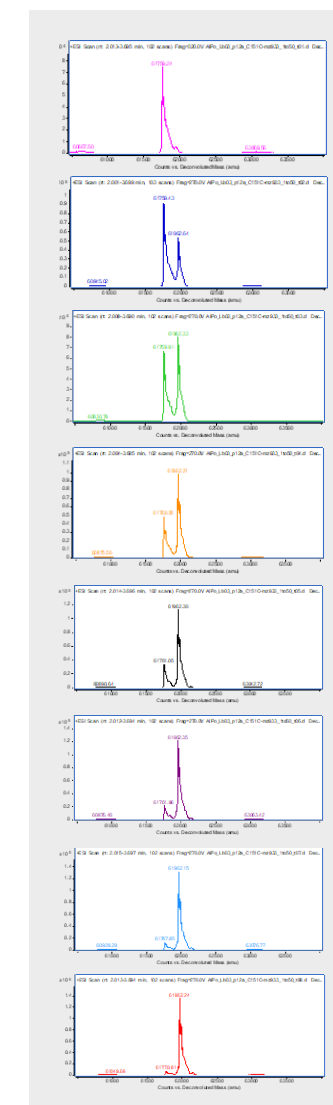
Mass spectrometry (MS) has become an important tool for high-throughput screening, owing to its ability to directly probe interactions between target proteins and ligands from the libraries of drug-like molecules. Screening by mass spectrometry, both covalent and non-covalent, includes several approaches with different areas of application. To address the growing demand of our drug discovery partners, MS-based Screening group has been established at Bienta in 2019.

Being a part of Enamine, the largest manufacturer of screening compounds for drug discovery, Bienta has gained experience in covalent screening by MS. Among the variety of the covalent probes, Cysteine-specific binders are currently in the highest demand. Our MS-based screening group is working with a broad choice of Cysteine-reactive covalent fragments and screening compounds libraries, as well as cus-tom compound selections from specific Cysteine-targeted warhead classes, such as acrylamides, chloroacetamides, vinyl sulfone compounds, and others. The libraries are being constantly updated, to ensure a novel and diverse chemical space for the quests for new hits.

Our team is equipped with two Agilent Q-TOF LC-MS systems dedicated for MS screening projects. A typical covalent screening project consists of 2 stages: (1) *method development and validation* and (2) the high-throughput screening *per se*, including hit confirmation and a counter-screen. We mostly work with the libraries in 384-well plate format.

For the data analysis, our software team developed an in-house data parser which goes through MS peak lists from individual wells, searching for protein-ligand conjugate peaks, and calculates % of adduct formation. To satisfy all customers' requests and answer additional questions that may arise during the screening, our MS-based Screening group can perform a variety of hit follow-up services, including di-verse orthogonal screens, intrinsic reactivity, kinetic studies, and ligand binding localization studies.

The MS-based Screening group works in close collaboration with the Recombinant Protein Expression group. Target proteins can be produced on a preparative scale in *E.coli*, baculovirus, or mammalian systems.



Enantiomeric Pairs Library

960 compounds

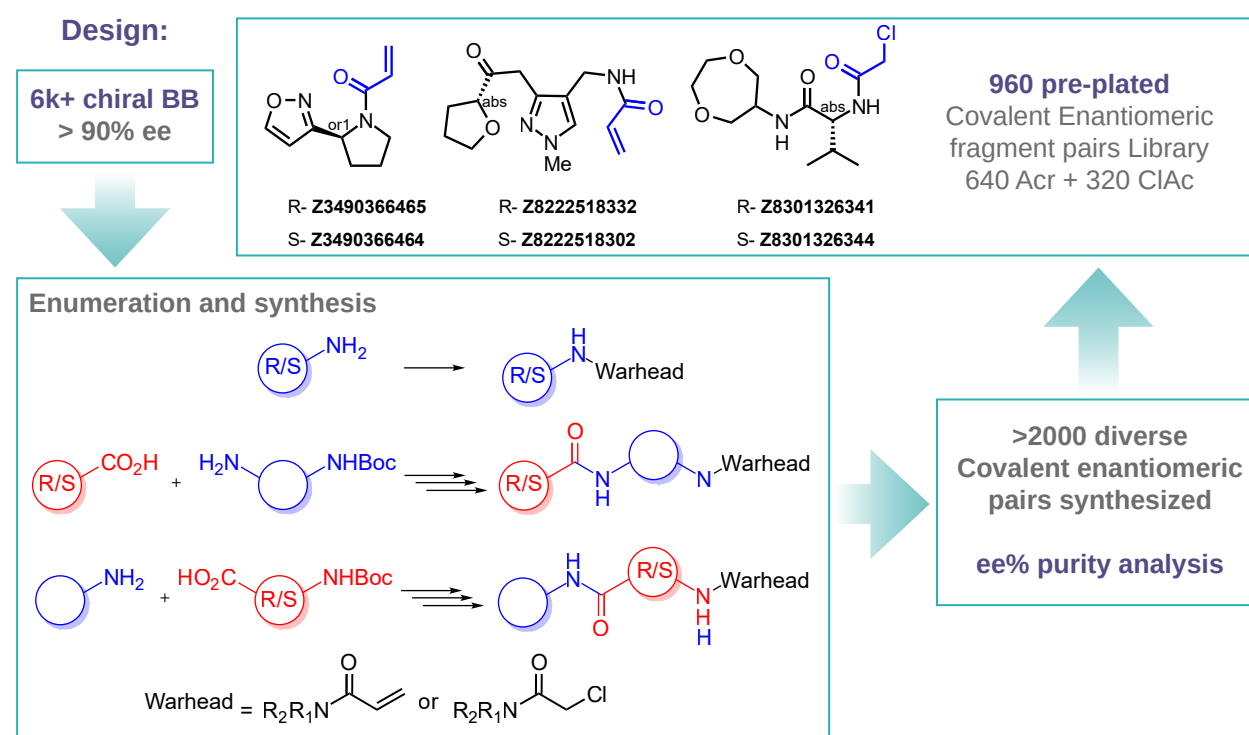
Enantiomeric pairs of covalent electrophilic fragments

Covalent chemical probes have become a valuable tool in drug discovery within the last few years. Modern technologies and mass spectrometric imaging open a new horizon for proteome-wide screening and proteomics discoveries. The impressive number of successful applications brought aspiration to the discovery and synthesis of new covalent probes. Previously developed by Cravatt research group mapping of the ligandable proteome using fully functionalized enantiomeric probe pairs makes this field especially attractive for further investigations. To support further research of the stereoselective interaction of proteins with chiral covalent small-molecules we designed and specially synthesized two sets of the most robust covalent binders – acrylamides and chloroacetamides.

More than 6000 chiral building blocks with enantiomeric excess (ee) purity of 90%+ were analyzed and triaged for further synthesis of covalent binder pairs. The resulting set of over 2000 of the most diverse novel covalent modifiers has been synthesized. The enantiomeric purity of the corresponding enantiomeric pairs was analyzed by chiral chromatography. Compounds that passed rigorous QC and selection based on the diversity and Ro3 criteria were assembled into Covalent Enantiomeric Pairs Library.

Key feature

- Stereoselective interaction of target with enantiomeric covalent binder provides evidence of ligand-protein interaction
- Information about “correct” stereochemistry of hit on early stage
- Most common and well-validated warheads



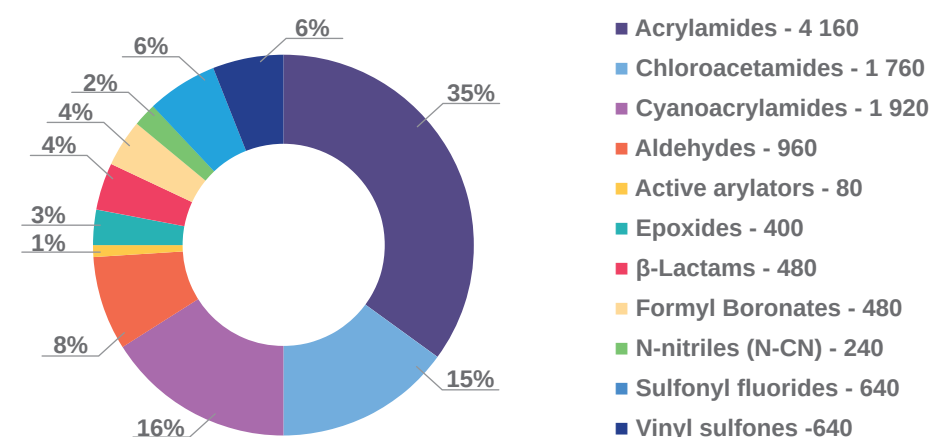
Enantiomeric Pairs Fragment Library is plated at 100 mM concentration and is available for fast supply in different formats. The Library consists of two sublibraries, which can be acquired separately.

Covalent Screening Library

11 760 pre-plated compounds

Diverse Ro5-compliant molecules with the most attractive warheads

Covalent probes play an essential role in the discovery of new technologies, investigation of new pro-teins, and assessment of their druggability. We have been working for years on the synthesis of covalent compounds and the development of new covalent warheads to make them accessible to a wide R&D community. Enamine focused on the elaboration of parallel synthesis approaches to synthesize a series of new valuable covalent compounds with balanced reactivity. All our efforts over the years resulted in the production of the largest commercially available Collection of Covalent Compounds. The most interesting and popular covalent classes were assembled into the diverse Screening Library, aimed to represent Enamine's Covalent Collection.



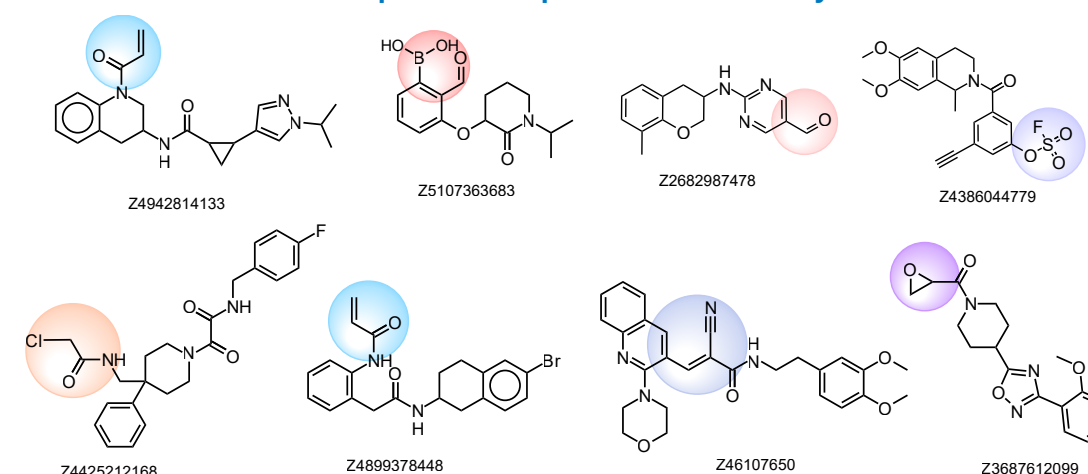
Key Features

- The most diverse core structures within each covalent class
- Only attractive pharmacophores, no simple reagents, or small fragments
- Plated at 10 mM concentration in DMSO
- Each class of covalent binders can be acquired separately

The library is available in different pre-plated formats, with compound pooling available as a formatting option. Each covalent class is plated in separate plates.

Screen the Library directly at Enamine and save on turn-around time and cost!

Examples of compounds in the library



Covalent Fragment Library

8 480 covalent fragments

Diverse covalent warheads with balanced reactivity

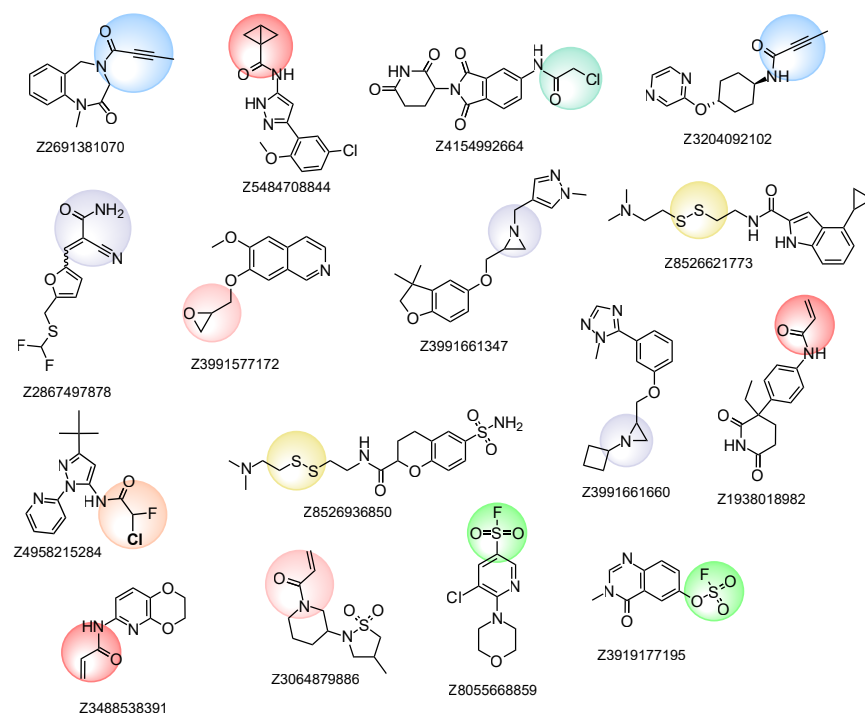
Over the years, many important practical details about the synthesis and storage of new covalent compounds have been found. The acquired experience and knowledge on stability and selectivity were applied in the design of our new edition of Covalent Fragment Library.

The renewed library has been designed to represent Enamine's latest scaffolds in combination with the most interesting covalent anchors. All compounds in the library meet the stringent Ro3 criteria with core molecules that do not exceed MW of 280, ClogP of 2.8, and rotatable bonds less than three.

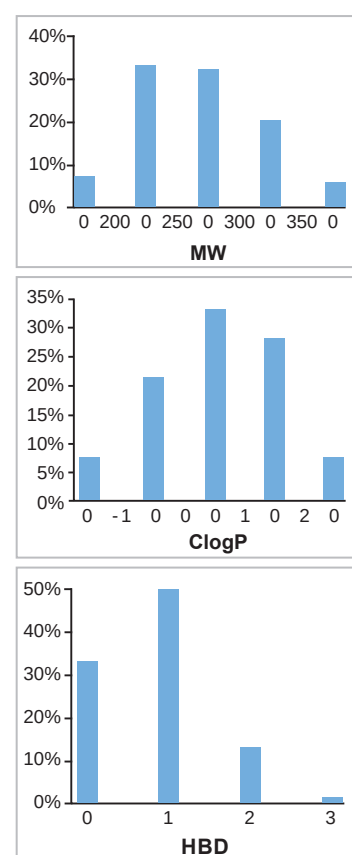
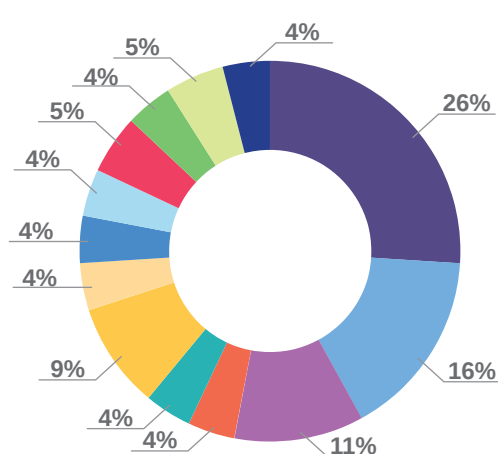
All compounds pass rigorous QC before formatting and are regularly monitored by LCMS analysis and freeze-thaw cycles to ensure the high quality of our library.

Covalent Fragment Library is plated at 100 mM concentration and is available for fast supply in multiple formats. The Library consists of 13 distinct sublibraries, which can be acquired separately.

Examples of compounds in the Library



- Acrylamides - 2 240
- Chloroacetamides - 1 360
- Sulfonyl fluorides - 960
- Bicyclobutan amides - 320
- Boronics - 400
- Cyanacrylamides - 800
- 2-Chloropropionamides - 320
- Chlorofluoroacetamides - 320
- Disulfides - 320
- Vinyl Sulfones - 400
- Epoxides - 320
- Butynamides - 400
- Aziridines - 320



Lysine focused Library

1 600 compounds

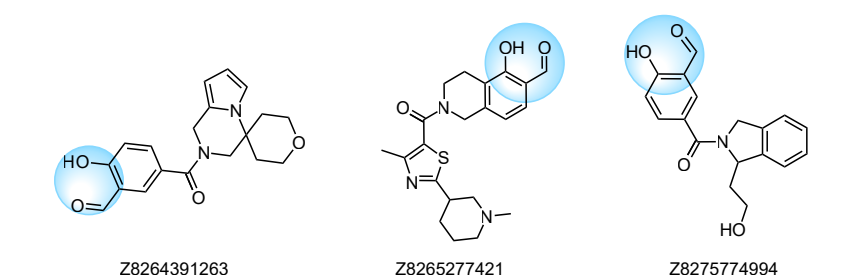
A unique set of Lys-specific binders

Until recently, covalent inhibitors targeting lysine side chain attracted less attention compared to those modifying Cysteine or Serine residues. Meanwhile, Lysine residue is found both at the surface and in active sites of many enzymes (e. g. some kinases, viral polymerases and integrases, aldolases, DOPA decarboxylase, P-glycoprotein), as well as in the "hot spots" of protein-protein interactions. Moreover, Lys residue is often involved as a key player in many essential signaling and metabolic processes. An example can be protein ubiquitination which mainly occurs through lysine residues on substrate proteins or itself. Thus, the selective targeting of Lysine is becoming increasingly attractive for the development of next-generation drugs.

To address the increased interest in Lysine-specific covalent binders, we have synthesized several libraries bearing warheads described as selective toward Lysine. Our renewed library contains only specially synthesized compounds with Lys-specific covalent warheads. The following classes were used for the library construction:

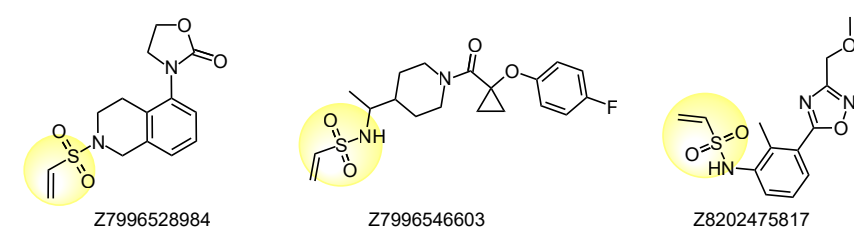
Salicylic aldehydes

- Lys-specific, reversible
- o-hydroxy group stabilizes covalent adducts
- Low reactivity
- 480 compounds



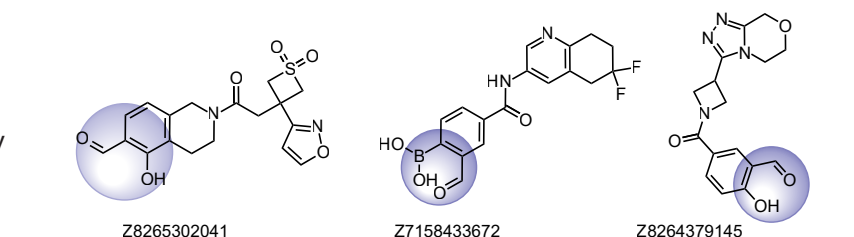
Vinyl sulfonamides

- Irreversible binders
- Showed some selectivity toward Lys over Cys
- Highly reactive
- 480 compounds



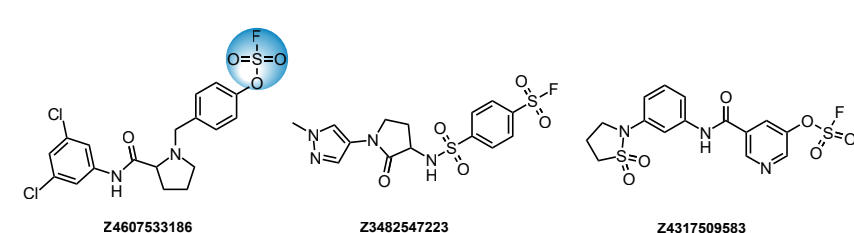
Formyl Boronics

- Reversible and Lys-specific
- Boronic residue in α -position dramatically enhances the stability of resulting adducts
- 320 compounds



Sulfonyl Fluorides

- Irreversible binders for Lys
- Moderate reactivity
- 320 compounds



Lysine focused Library is plated at 20 mM concentration in DMSO and is available for fast supply in multiple formats. Compounds can be pooled within the same class based on the largest possible difference in MW.

Serine focused Fragment Library

1 600 compounds

Special library of Ser-specific covalent binders

The Serine hydrolase (SH) enzyme family is one of the largest and most diverse protein classes, including proteases, lipases, esterases, thioesterases, amidases and peptidases. All these enzymes utilize a base-activated Serine residue to the cleavage of amide bonds in substrates via a covalent acyl-enzyme intermediate.

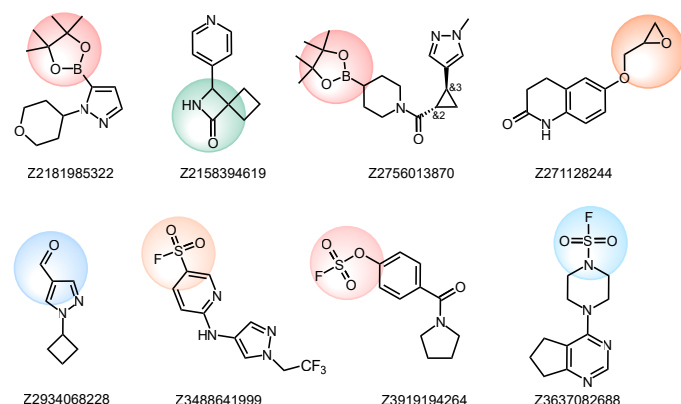
Various specific covalent-acting chemical probes are increasingly employed for proteome-wide target identification and imaging. These probes play a crucial role in discovering inhibitors with high specificity among related enzymes and enzyme isoforms. A significant number of known covalent drugs and natural products have proved the efficacy of this approach in drug discovery, especially for Serine hydrolases.

We designed our Serine-focused covalent library to cover as much core structure diversity as possible together with a Ser-specific covalent warhead. A number of compounds have been specially designed and synthesized for this library. The library has also been shaped with Ro3 criteria to meet all requirements of FBDD.

The following functional groups were used for the construction of Serine focused library:

- Sulfonyl fluorides, fluorosulfonates and sulfamoyl fluorides
- Epoxides
- β -Lactams
- Boronic acids and pinacolates
- Aldehydes and other

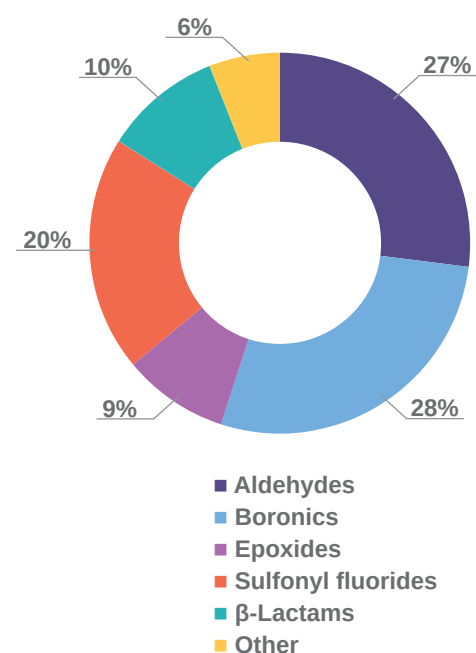
Examples of compounds in the library and composition by covalent warheads



Hits derived from this library can be quickly followed up with analogs from Enamine's stock of 120k Covalent Compounds or through synthesis of new compounds through REAL Database technology within just 3-4 weeks.

We provide **Hit Confirmation support** for all our libraries, including sample resupply from dry powders, QC check and HPLC repurification. All hits can be resynthesized in 2 weeks.

Serine focused covalent fragments are available in versatile pre-plated formats for the most convenient and fast delivery. All compounds passed QC (90%+ purity) before formatting, ensuring the high quality of the library.



Cysteine focused Library

3 200 compounds

Library of Cys-preferred electrophilic binders

We used a deep knowledge-based approach to design and synthesize our Cysteine focused library. Careful choice of covalent warheads based on their reactivity was performed by experienced chemists. The resulting set was refined with Rule of Three (Ro3) criteria applied to "core structures", yielding 3200 fragments capable of forming covalent bonds with cysteine residues.

The current edition of the library has been validated in several MS-based screenings to remove promiscuous binders and overreactive covalent classes. *The library composition has been adjusted to optimize suitability for routine screening conditions, making it the most convenient tool for initial hit-finding campaigns.*

Hits derived from this library can be readily followed up with analogs from Enamine's stock or by synthesizing new compounds through REAL Database technology within 3-4 weeks.

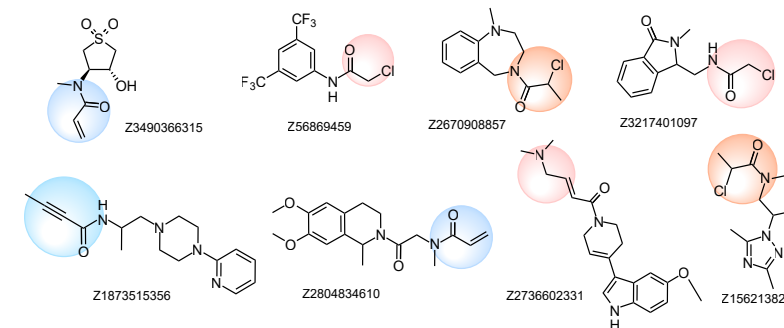
Key features

- Only well-validated covalent warheads that form easy-to-interpret adducts
- No overreactive, promiscuous binders
- Attractive scaffolds, derived from the latest core building blocks
- Plated at 100 mM concentration in DMSO

The following warheads were used for the library construction

- Acrylamides 1200
- Dimethylamine substituted acrylamides 480
- Chloroacetamides 800
- 2-Chloropropionamides 480
- Butynamides 240

Examples of the molecules in Cysteine focused Library



Typical library formats for fast delivery. Please request any custom formatting and our liquid handling team will be happy to provide the library in a format convenient for your project. Compounds pooling based on delta MW can be provided as a formatting option. Please request the list of expected shifts in MW for MS-based screenings.

Catalog ID	Plates	Amount	Format
CYS-3200-Y-10	10	10 μ L of 100 mM DMSO stock solutions	384-well eco-qualified microplates, 320 cmpds per plate
CYS-3200-X-25	40	25 μ L of 20 mM DMSO stock solutions	96-well microplates, Greiner 6501xx, 80 cmpds per plate
CYS-3200-X-50	40	50 μ L of 20 mM DMSO stock solutions	96-well plates, 2D-barcode Matrix microtubes

Electrophilic Covalent Probe Library

960 compounds

Library of electrophilic fragments with measured GSH reactivity and evaluated for promiscuity

Electrophilic Covalent Probe Library is aimed at the discovery of new inhibitors for cysteine-containing proteins. The library was developed by our collaborators at Weizmann Institute of Science (WIS), London lab and at Diamond Light Source, XChem group. All fragments were evaluated for thiol-reactivity and screened against 10 cysteine-containing proteins. The design process of this library is described in the paper published in *JACS*, 2019, 22, 8951. All fragments were evaluated for intrinsic reactivity with a newly developed GSH-based assay and screened against 10 cysteine-containing targets.

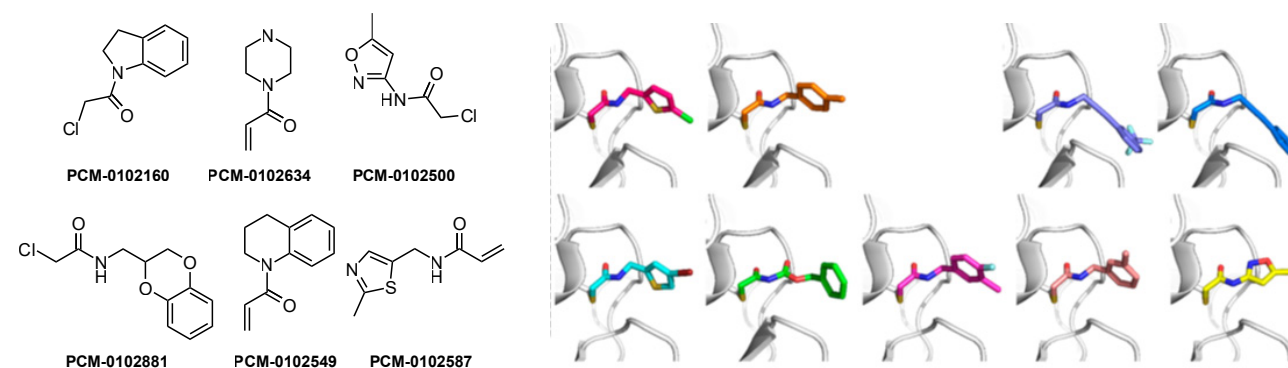
We refined a set of molecules initially proposed in the paper, by removing compounds found to be promiscuous binders in the screening of several proteins.

Key features

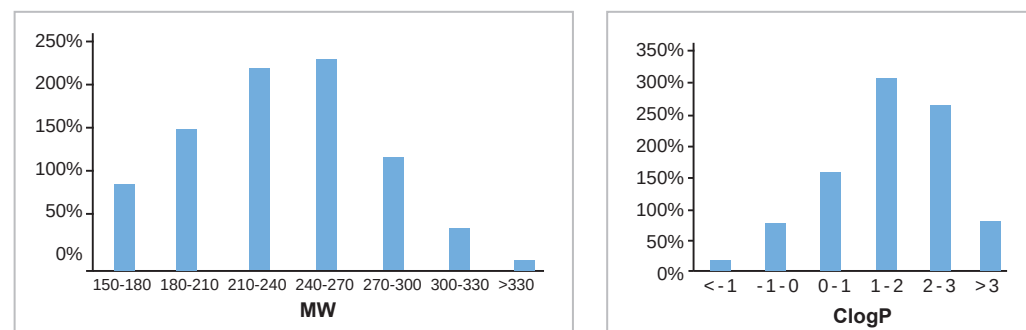
- Experimentally evaluated
- No overreactive and promiscuous covalent binders
- Initial SAR data based on the small clusters
- Ease of chemistry for fast follow-up

For the most convenient and fast delivery, the Electrophilic Covalent ProbeLibrary is available in versatile pre-plated formats and can be delivered *within a week only!* This library has also been evaluated for stability in DMSO and storage in solutions for at least two years.

Representative examples of cluster centroid molecules



Molecular Parameters of the Library

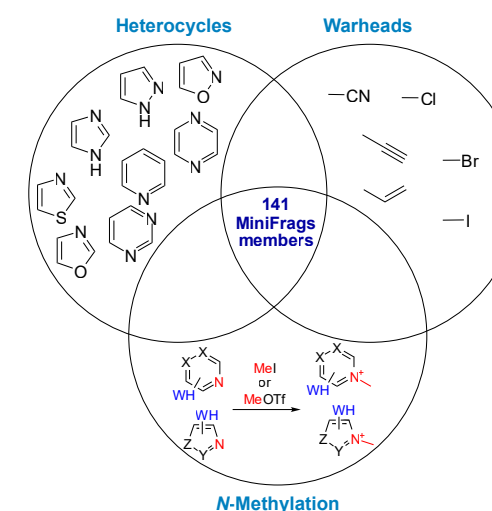


Covalent MiniFragments

141 compounds

Covalent heterocyclic fragment library for identification of Cryptic and Allosteric Pocket

This unique library of small heterocyclic electrophiles developed by the research group of Prof. György Keserű has shown to be effective in finding tiny new binding pockets for different protein targets (*J.Med.Chem.*, 2024, 67, 572; *MedChemComm* 2019, 10, 253; *Pharmaceuticals* 2022, 15,1484). The library combines the advantages of Astex's **MiniFragments** in exploring unprecedented binding sites with that of covalent binders in higher affinity and easier detection. It consists of six and five-membered heterocycles most abundant in approved drugs that are equipped with only one or two-atom covalent warheads. This makes it unique in contrast to those often used in covalent screening, much larger acrylamides and chloroacetamides which can significantly influence the binding mode of active molecules. Thus, applying the smallest possible covalent function helps to avoid promiscuity and keep the same recognition pattern of non-covalent scaffolds.



Key feature

- Most common nitrogen-containing heterocycles
- The smallest covalent warheads
- Experimentally characterized stability and intrinsic reactivity
- Evaluated on several targets, including KRAS^{G12C}

The CovalentMiniFragments library consists of 85 heterocyclic electrophiles and 56 N-methylated functional heterocycles. Both subsets are described in detail in scientific publications and are now available for your research.

Covalent MiniFragments library is a unique tool for searching for new binding pockets, elaboration of discovered hits, and growing vector identification. The **XChem facility** at Diamond LightSource UK is a strategic partner in pioneering applications of Covalent MiniFragments library.

