Building a Diverse and Experimentally-Curated Fluorine Fragment Library

Key Organics
Chemistry | Innovation | Quality

Andrew Lowerson,[1] Louis Vaillancourt,[2] Yann Ayotte,[3] Sacha Larda,[2] Simon Woo,[2,3]
Michael Serrano-Wu,[4] and Steven R. LaPlante[2,3]

1. Key Organics Limited, Camelford, Cornwall, United Kingdom
2. NMX Research and Solutions Inc., Laval, Quebec, Canada
3. INRS Centre Armand-Frappier Santé Biotechnologie, Laval, Quebec, Canada
4. 3 Point Bio, Cambridge, Massachusetts, USA

NMX Research and Solutions Inc.

Introduction

A BIONET Fluorine Fragment Library has been constructed employing Rule of Three and industry standard substructure filtering including PAINS analysis. All fragments in the Fluorine Fragment Library have been analyzed by ^{1}H NMR for structure verification, purity, solubility, and lack of aggregation. Spectra are available to customers for Chemical Shift Encoding (CSE), thus allowing custom pools to be built with significant time and cost savings.

Build Strategy

RULE OF 3 COMPLIANT
- MW ≤ 300
- clogP ≤ 3
- HBA ≤ 4
- HBD ≤ 2
- Rot Bonds ≤ 3

REMOVE THE UNDERSIRABLES
- PAINS
- FAFDrugs4
- Lilly Med Chem Rules
- Med Chem Curation

QC BY NMR
- Structure Verification
- Purity
- Solubility
- Aggregation Status

SUITABILITY FOR FRAGMENT SCREENING
- Pooling
- Fragment Screening
- Hit Identification
- Singleton Confirmation

Remove the Undesirables

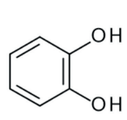
As part of our Fragment selection process, industry-standard substructure filtering - including PAINS filtering - was implemented and as a result the BIONET 2nd Generation Fluorine Fragment Library does not include substructures identified as promiscuous or reactive by empirically determined rejection rules. A fragment was rejected if it failed any one of 3 rejection rules: PAINS,[1] FAFDrugs4[2] and Lilly MedChem Rules.[3]

Focus on Pan Assay Interference Compounds (PAINS) substructure filtering – a deciding factor in the quality of a fragment library.

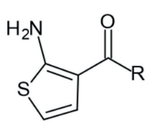
PAINS are compounds that frequently show up as screening hits, but that act through non-specific mechanisms such as covalent attachment to proteins or generation of hydrogen peroxide. The problem with PAINS is that they may show convincing biochemical and even cell based activity, but mechanistically be useless for further advancement to drugs or even chemical probes. PAINS remain common in many vendors' Fragment Libraries. PAINS compounds have been identified and substructure filters constructed that recognise these compounds.[1]

Examples of PAINS

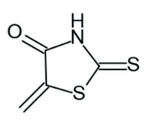
Catechols



2-Amino-3-carbonyl thiophenes



Rhodanines

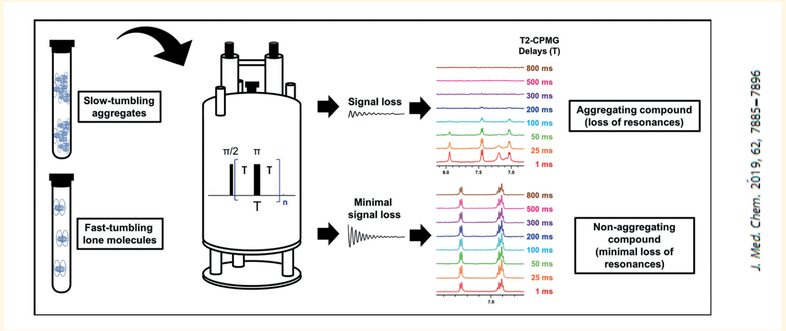


Aggregation Filtering

Key Organics Fluorine Fragment Library excludes fragments likely to form aggregates.

The spin–spin relaxation Carr–Purcell–Meiboom–Gill NMR (CPMG) experiment has been employed to detect and remove aggregate species from Key Organics BIONET Premium and Fluorine Fragment libraries.[4]

Small molecules can self-assemble in aqueous solution into a wide range of nanoentity types and sizes (dimers, n-mers, micelles, colloids, etc.), each having their own unique properties. This has important consequences in the context of drug discovery including issues related to nonspecific binding, off-target effects, and false positives and negatives. The T2-CPMG NMR experiment is sensitive to molecular tumbling rates and can expose larger aggregate species that have slower rotational correlations in solution. The strategy easily distinguishes lone-tumbling molecules versus nanoentities of various sizes. The technique is also highly sensitive to chemical exchange between single molecule and aggregate states and can therefore be used as a reporter when direct measurement of aggregates is not possible.



QC by NMR

^{19}F and ^{1}H NMR Curation for Fragment Prioritisation and Library Characterisation

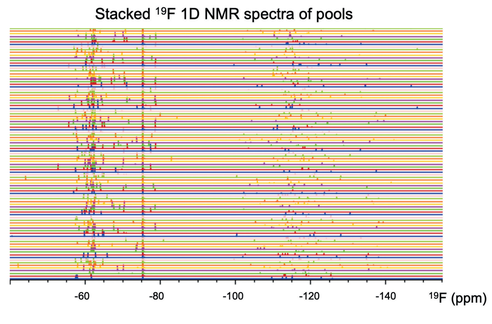
^{19}F and ^{1}H NMR were employed to select compounds with appropriate solution behavior amenable for rigorous biophysical analysis in physiologically relevant aqueous solution conditions. Each singleton sample consisted of nominal 300 µM compound in buffer (50 mM sodium phosphate pH 7.4, 100 mM NaCl). ^{1}H NMR spectra were acquired on a 600 MHz spectrometer equipped with a helium cryoprobe that significantly increased signal-to-noise. Simple 1D ^{19}F and ^{1}H NMR spectra were acquired along with a series of 1D ^{1}H T2-CPMG spectra, which were used to detect compounds showing potential aggregation in aqueous solution. Compounds with solubility at 100 µM and higher were prioritized given that most fragment screens and assays require high concentrations.

Data analyses involved combinations of manual and automation tools. The CMC Assist automation software (Bruker Spectrospin Inc.) had multiple practical uses. It allowed for an automatic readout of the fragment concentration that was experimentally derived from integrating the NMR resonances of each singleton sample and referencing to standardized samples using the ERETIC module. The CMC Assist module also allowed for verification of each singleton spectrum to determine if the spectral attributes were consistent with the proposed primary structure of the corresponding fragment. This exercise was also complemented by an automated analysis using Spectral DB software (Advanced Chemistry Development, Inc.).

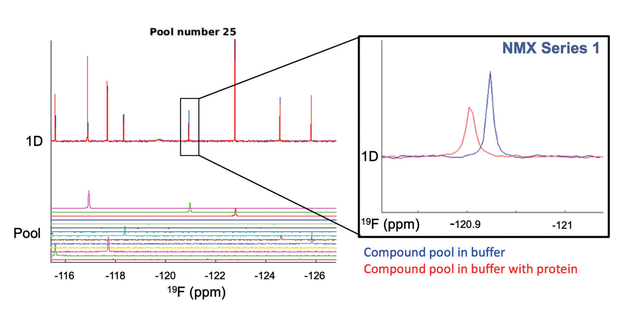
The compounds and respective spectra were then re-subjected to visual inspection by medicinal chemists experienced in the art of fragment-based drug discovery. Compounds that were inconsistent with the NMR spectra were removed, along with those that showed signs of insolubility, instability, or aggregation. Moreover, the NMR curation also allowed us to evaluate and characterize the final library. This experimental data served to verify structural integrity, purity, solubility, stability, aggregation status, and chemical shift positions. The final library consisted of 719 fragments that were soluble to at least 100 µM in PBS aqueous buffer.

Fragment Screening at NMX Research and Solutions Using Fluorinated Compound Library

Fragment Pools: Pooling of the fragments facilitates rapid screening of the library by ^{19}F NMR techniques. Factors taken into consideration for the pooling process included chemical compatibility of the fragments and adequate separation of the ^{19}F NMR signals. As shown in the figure below, ^{19}F NMR spectra of the pooled compounds were then collected to confirm compound integrity in the pools. Due to the wide chemical shift range of ^{19}F NMR, pools of up to 15 compounds could be routinely achieved with no spectral overlap.

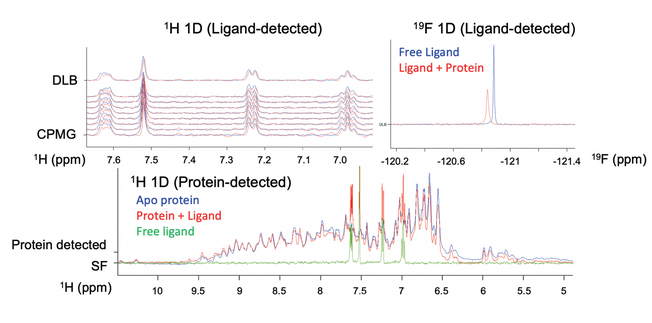


Fragment Screening: A subset of the ^{19}F library (31 pools) was screened against a known "undruggable" target that was of interest for our internal drug discovery program. Through a comparison of the ^{19}F NMR spectra of each pool in the absence (blue trace below) and presence (red trace below) of the target protein, differential line broadening of the ^{19}F NMR signal was used to detect binding of fragments. The identity of binders was easily determined through comparison of the chemical shift of the binder to those of the components in the pool.



Singleton Confirmation: The binding of hits identified in the fragment screen were confirmed with the individual compounds using a combination of ^{19}F and ^{1}H NMR techniques. Scores generated by proprietary software during this step were used to rank-order and prioritize the hits for follow-up studies.

One of the hits identified during this screen has formed the foundation of an ongoing drug discovery campaign, the results of which will be reported in due course.[5]



References

1. Jonathan B. Baell and Georgina A. Holloway. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. *Journal of Medicinal Chemistry* **2010**, 53, 2719-2740.
2. David Lagorce, Lina Bouslama, Jerome Becot, Maria A Miteva, and Bruno O Villoutreix. FAF-Drugs4: free ADME-tox filtering computations for chemical biology and early stages drug discovery. Bioinformatics 2017 Nov 15;33(22):3658-3660. BMC Bioinformatics 2008, 9:396.
3. Robert F. Bruns and Ian A. Watson. Rules for identifying potentially reactive or promiscuous compounds. *Journal of Medicinal Chemistry* **2012**, 55, 9763-9772.
4. Yann Ayotte, Victoria M. Marando, Louis Vaillancourt, Patricia Bouchard, Gregory Heffron, Paul W. Coote, Sacha T. Larda, and Steven R. LaPlante. Exposing Small-Molecule Nanoentities by a Nuclear Magnetic Resonance Relaxation Assay. *Journal of Medicinal Chemistry* **2019**, 62, 7885-7896.
5. Manuscript in preparation.

For more information, please contact us at:
www.keyorganics.net
www.nmxresearch.com