Key Organics

Chemistry | Innovation | Quality

Your Partner for Fragment-Based Drug Discovery (FBDD)

SUMMARY

Use of a fragment-based lead generation strategy in a discovery program is now a well-established option for modern biotech and pharmaceutical companies. Multiple drugs have received regulatory approval which had their origins from a fragment-based approach, and there are many more compounds in industry drug pipelines at various stages of pre-clinical and clinical development.

Key Organics has a long history of providing support to teams working on Fragment-Based Drug Design (FBDD) programs, ranging from provision of the original fragment libraries used in screening, through the fragment growth and lead optimisation process, all the way to scale-up and beyond.

This technical note provides a short history of fragment-based drug design and is followed by an overview of the main stages and key value inflection points for a discovery program using the approach. Finally, a variety of case studies from the literature are included to illustrate the main points and to highlight the bright future for this discovery paradigm.

INTRODUCTION

Multiple lead generation techniques are employed in modern small molecule drug discovery programs. Broadly speaking, these fall into two categories. The first is centred on screening of compound libraries – be they focused, 'random' or virtual. Such techniques include HTS (High Throughput Screening), MTS (Medium Throughput Screening), DEL (DNA-Encoded Libraries), natural products, VS (Virtual Screening), and others. The second is built upon a more tightly defined knowledge-based approach, wherein the lead generation strategy is focused on (for example) knowledge of the endogenous ligand for a particular biological pathway of interest or utilises a 'patent busting' (sometimes called scaffold hopping) approach.

Fragment-based approaches fall squarely into the first category and rely on the ability of a screening assay to be able to measure relatively low affinity ligand – protein interactions, and to use these 'weak binders' as start points to evolve and build onto during the optimisation process. An important aspect is that fragments are much smaller and simpler than conventional (small molecule) hits, leads or drugs, but will have at least one functional group or motif which can act as a pharmacophore to interact with a target biomolecule (e.g. a protein). A 2003 definition from Congreve et al. described fragments as conforming to a 'Rule of 3', with a useful update 10 years later by the same team. This stated that fragments had a molecular weight <300 Daltons and a calculated LogP of ≤3. The rules also suggested that the number of hydrogen bond donors was ≤3 and the number of hydrogen bond acceptors as ≤3 (thereby conceptually aligning with Lipinski's rules for small molecule drugs). Examples of fragments from a commercially available library are illustrated in *Figure 1*.

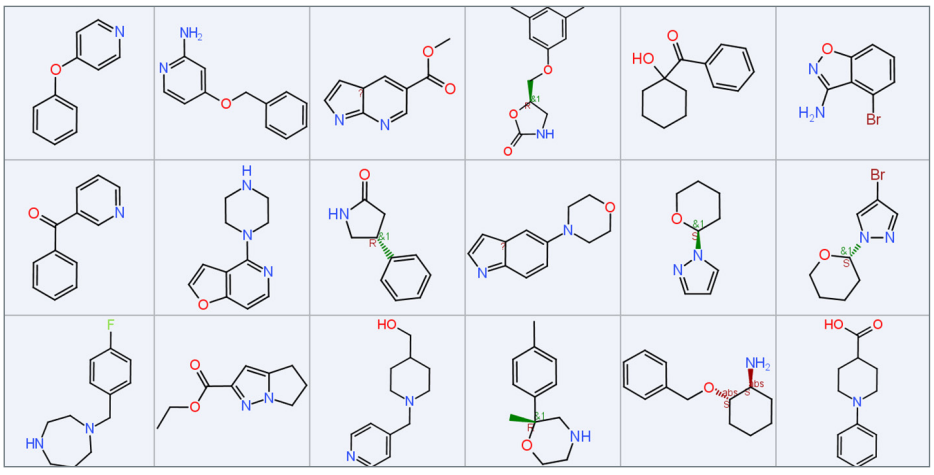

Figure 1: Examples from Key Organics' 2nd generation Premium Fragment Library (*visualised in DataWarrior*)

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Although fragment-based approaches had their origins in much earlier work it was not until pioneers including Astex, Vernalis and others began to champion it that the approach began to gather wider momentum in the early 2000's. Although there is limited published data on the success of FBDD compared to other strategies within industry, peer reviewed literature provides useful guidance. Publications metrics suggests that use of FBDD in industry and academia has since gone from strength to strength (*Figure 2*).

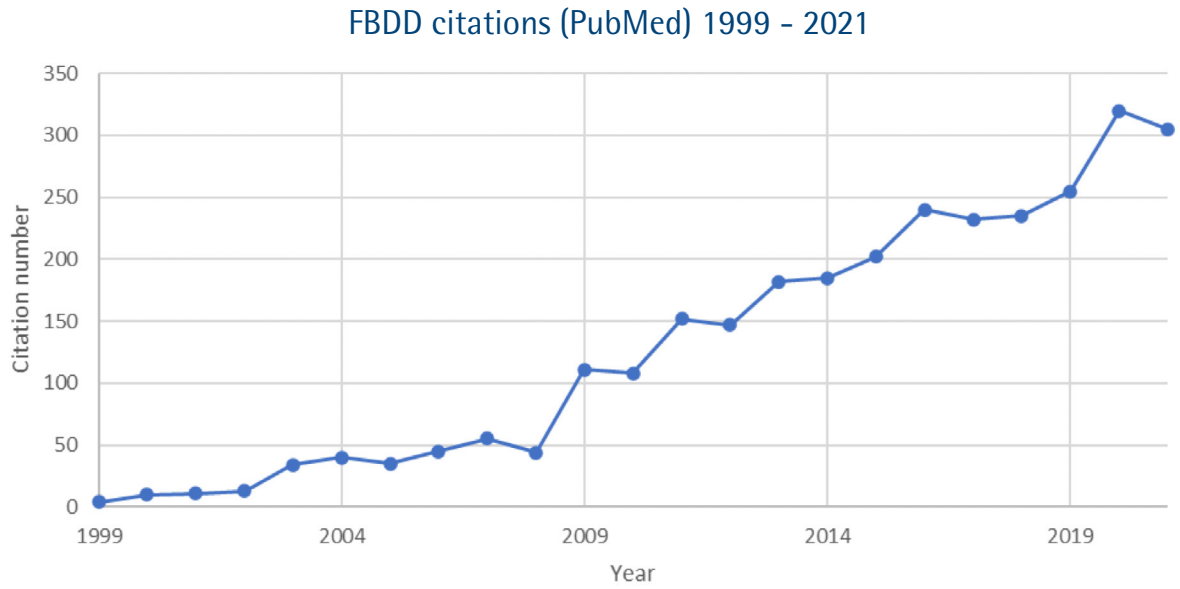


Figure 2: PubMed citations (to 2021), search term 'fragment-based drug discovery'

The potential for FBDD took a little while to be recognised by industry, with the idea that a low molecular weight / 'small' starting point having binding affinity in the millimolar range could be successfully and effectively developed into a drug with high affinity and selectivity attracting scepticism, since at the time most organisation were focused on high throughput screening campaigns for hit generation. As work progressed in the field, more was learned and understood about how best to approach FBDD, and successful case studies began to emerge. It became clear that this was an approach with considerable potential, and a drug discovery force to be reckoned with. Given that many organisations were focusing their programs on chemical feedstock with origins mainly in HTS campaigns, it also became apparent that one's perspective of hit generation needed to shift from being viewed solely through the lens of high potency in a primary assay.

GENERAL PRINCIPLES

The fundamental concept behind screening low molecular weight fragments is that a compound is more likely to bind to biomolecules such as proteins the smaller it is, due to there being a higher probability of there being an appropriate protein – ligand 'match', and this probability decreases significantly as a compound grows in size / complexity. The general concept which runs alongside that is that it is more efficient to start from a small, relatively weakly binding molecule and successively build onto it during the optimisation process, than to start with a larger molecule and try and 'cut back' compound bulk, or otherwise tweak the existing molecular real estate in order to improve the overall profile. In other words, it is preferable to start with a small fragment and undertake carefully thought out, structurally enabled optimisation in a forward sense, since this will result in a lead compound which is more ligand efficient and can have other drug-like properties designed in at an early stage.

Since a significant part of the optimisation process for hits arising from HTS or similar is improving compound properties distinct from the intended primary pharmacology, the appeal of fragments, with their in-built favourable physicochemical properties is doubly appealing.

As with other approaches to lead generation, it was shown relatively early on that FBDD would be able to most rapidly demonstrate its value in particular types of project and against certain target classes. Enzymes, and particularly those where structural data was available were very much top of the list, since this allowed for an iterative, structurally enabled optimisation process, though as X-ray crystallographic data became available for other target classes (e.g. receptors such as GPCRs), or other approaches became possible the opportunities for fragment based approaches became more numerous.

This important aspect of a fragment-based approach being informed by structural data for the target protein, and protein-ligand complexes is used as an integral part of the design-make-test-analyse (DMTA) cycle for compound optimisation. Thus, being able to access these data quickly and easily improved the efficiency of FBDD programs. Originally these structural data were provided from X-ray structures (and hence organisations with a deep expertise in this field quickly led the field), but more recently it has become possible to use other techniques such as NMR based structures, providing opportunities to a wider range of organisations. For example, Key Organics has a long standing commercial relationship with NMX Research and Solutions Inc. to facilitate these types of projects.

Optimisation using fragment approaches typically falls into one of two design strategies, both appending carefully positioned molecular equity onto the original fragment hit in a very atom-efficient manner. The most commonly used is the so-called 'fragment expansion' (or 'fragment growing') technique wherein new groups are added to the original fragment hit. An interesting alternative, termed 'fragment linking', instead connects together multiple low affinity binding fragments to form a single compound of higher affinity than the originals. More recently modification of low affinity fragments to high affinity fragments ('fragment to fragment optimisation') have also been successfully employed (see case studies). Targeted degradation strategies including PROTACs, RIBOTACs and molecular glues could also be argued to have their origins aligned with fragment-based approaches.

THE FBDD PROCESS

The operational workflow for a fragment-based discovery program (*Figure 3*) is broadly similar to that used with a conventional small molecule screening approach such as HTS.

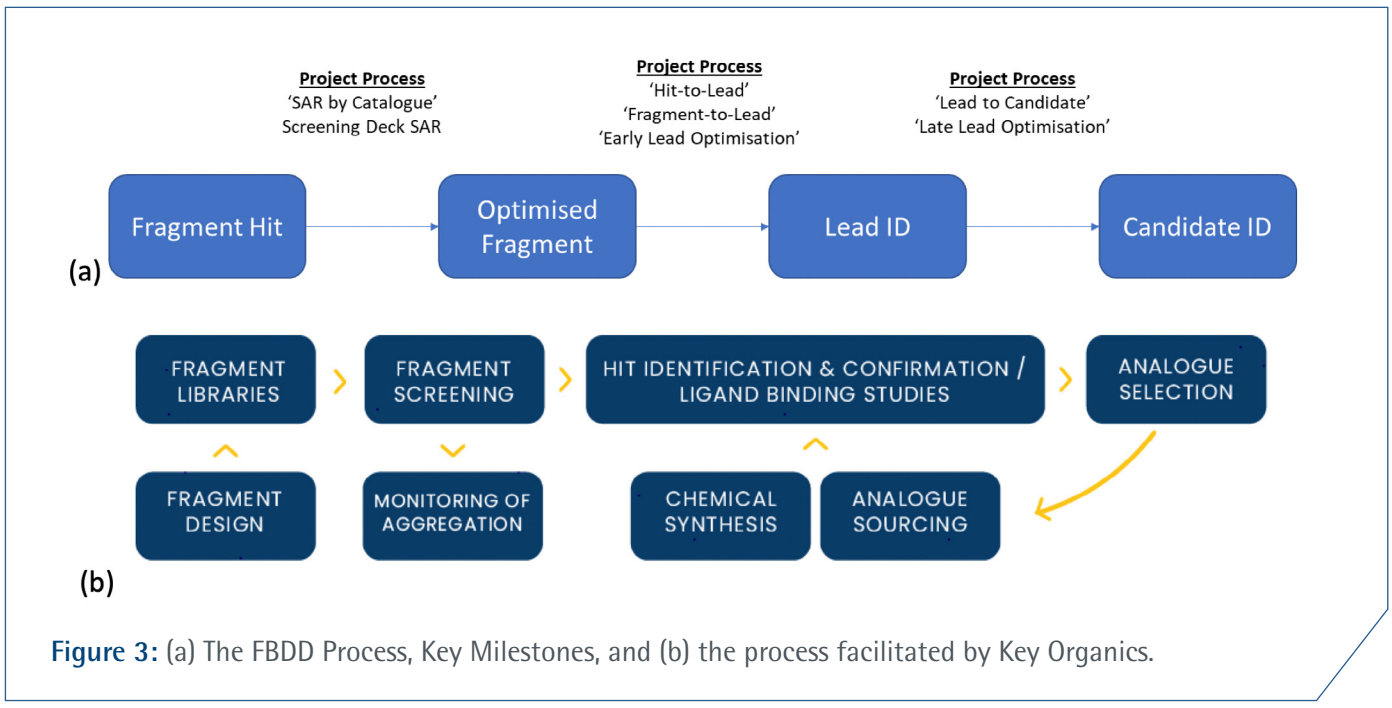


Figure 3: (a) The FBDD Process, Key Milestones, and (b) the process facilitated by Key Organics.

However, there are several subtle but important differences between fragment programs and those using HTS; these are highlighted in the subsequent paragraphs.

TARGET SELECTION / SCREENING

The same priorities exist for FBDD programs as for any other in terms of target selection. Typically, this will mean there is a clear link for the specific target protein / biomolecule with the disease for which a drug is sought. This, along with validation data sufficient to provide confidence in druggability, and overall commercial aspects which when combined provide a compelling business case for an organisation to invest the significant capital that will be required to push a drug through to market approval.

On a technical level, since FBDD is a heavily structurally enabled technique, successful progression of the program is significantly enhanced by having crystallographic data. Interestingly however, fragment approaches have recently been applied to projects based on phenotypic screening approaches.

Given that several of the assay technologies which are employed in early-stage screening for FBDD require significant amounts of protein, these aspects all play an important part in target selection.

HIT IDENTIFICATION: FRAGMENT LIBRARY SIZE & CONTENT

As with other screening approaches, the composition and quality of the compound screening set used for FBDD defines pretty much everything about what follows. One initial point to note is that the screening deck is much smaller for FBDD, typically of the order of 1,000 to 10,000 members, whereas for HTS this can reach into the millions (and beyond for DEL enabled programs).

Since fragments are conventional ligands for biomolecules such as proteins in the same way as members of HTS libraries, the similar principles apply in that the compound needs to have one or more functional groups which will interact with the target and form a favourable binding interaction. Much analysis has taken place on the optimal size, design and composition of fragment screening libraries. Screening techniques used in FBDD to measure protein-ligand interactions and identify potential 'hits' are much more sensitive for FBDD, with typical affinities found for hits being in the mM – µM range. Alongside this it has been shown by Hann and others that for small molecule fragments (MW < 150 Da) it is possible to cover a much broader range of chemical space with a very much smaller compound library; thus one can maximise the possibility of identifying multiple diverse viable start points for a program with a relatively modest starting number of compounds. Recent analysis has also shown that assembly of a diverse fragment library which will maximise coverage of chemical space requires careful thought and consideration.

Physicochemical properties of the fragments are important, since compound screening can take place at relatively high concentration (up to mM), with good solubility profiles being needed, since problems with assay interference including aggregation or precipitation could confound assay readouts.

Quality of the compounds is important for any library, but particularly so for fragment libraries. Since the binding affinities being measured are relatively weak, it is important to confirm that members of the screening deck are both pure and free from contaminants such as heavy metals. Failure to do so can have serious consequences for the output of the screen.

Finally, the class of biomolecule being targeted can play a part in the fragment library composition.

Despite initial misgivings, in recent years, drugs which form covalent, and essentially irreversible interactions with their protein targets have become more prolific. In parallel, interest in the screening of fragments which are capable of forming covalent interactions has become more popular (initially in the chemical biology field), and this is being applied to drug discovery targets.

Considerable evolution of fragment space has taken place in both commercially available and in-house collections, and it is now possible to access and purchase commercial fragment libraries which comprise traditional 'reversible' low-affinity compounds, covalent binders, or a mixture of both.

Key Organics offers a range of fragment libraries, including a Premium set, and collections based on Fluorine, Bromine and Boronic Acids. Recently a Covalent set has also been added.

The BIONET Premium Library has been constructed in collaboration with the Center for the Development of Therapeutics, Broad Institute and NMX Research and Solutions Inc. Key features & benefits of this collection include:

- 1166 Compounds with experimentally assured aqueous solubility in PBS buffer.
- Excludes fragments deemed to aggregate as determined by ^{1}H NMR spectra
- ^{1}H NMR pdf and raw data files provided for all compounds purchased, chemical shifts provided for all compounds in an excel file.
- Strictly meet Astex "Rule-of-3" including TPSA ≤ 60Å^{2} and number of atoms ≤ 16
- Includes 445 fragments found in approved drugs and >1100 sharing cores found in drugs
- Filtered to exclude promiscuous and reactive substructures
- The BIONET Premium Fragment library is now available in milligram or micromolar quantities. Cherry picking is available.

- ✓ Rule of 3 compliant: MW ≤300, CLogP≤3, number of HBA/HBD ≤3, PSA 60 and number of rotatable bonds ≤3
- ✓ Heavy atom count (HAC) ≤16
- ✓ Does not include substructures identified as promiscuous or reactive by empirically determined rejection rules
- ✓ Inclusion of diverse scaffolds that are present in bioactive compounds and that have 3-dimensionality
- ✓ Clustering and Diversity analysis
- ✓ Passes chemist visual inspection
- ✓ Solubility in PBS buffer and signs of aggregation determined by ^{1}H NMR spectra

FRAGMENT TO LEAD: THE OPTIMISATION PROCESS

Having identified and confirmed active fragment 'hits' from a library screen, the optimisation process can start in earnest. A common next step is to undertake an 'SAR by Catalogue' (SARbC) approach. In this process, fragments which have shown encouraging levels of binding to the target are used as inputs for a search of commercially available analogues which also sit within the fragment space, and can generate secondary SAR rapidly. A successful outcome from the SARbC process would be the identification of new fragments which offer improvements to the original hit(s). This could be due to them having improved binding, to be more readily optimisable (for example, easier compound vectors / points of attachment to work with), or to have preferable intellectual property positions.

Alongside this, where structural data for the bound fragment exists, and depending on the specific program, compound design, following analysis of the protein-ligand structure, it may be viable to identify suitable vectors to 'grow' the fragment using relatively simple chemistry, and to build in additional binding interactions.

LEAD TO CANDIDATE:

As with all discovery programs, successful optimisation is not just about discovery of compounds which have potency and selectivity for the target, these need to have appropriate physicochemical, DMPK and IP properties. Since fragment-based approaches are heavily structurally enabled and knowledge driven, adding new group, or modifying existing new ones so that following late lead optimisation, the overall compound meets an organisations criterion for advanced lead, and ultimately development candidate is quicker, more facile, and has a higher potential for success.

Recent examples are shown in *Figure 4* which followed either a 'fragment expansion' / 'fragment growing' or a 'fragment linking' approach.

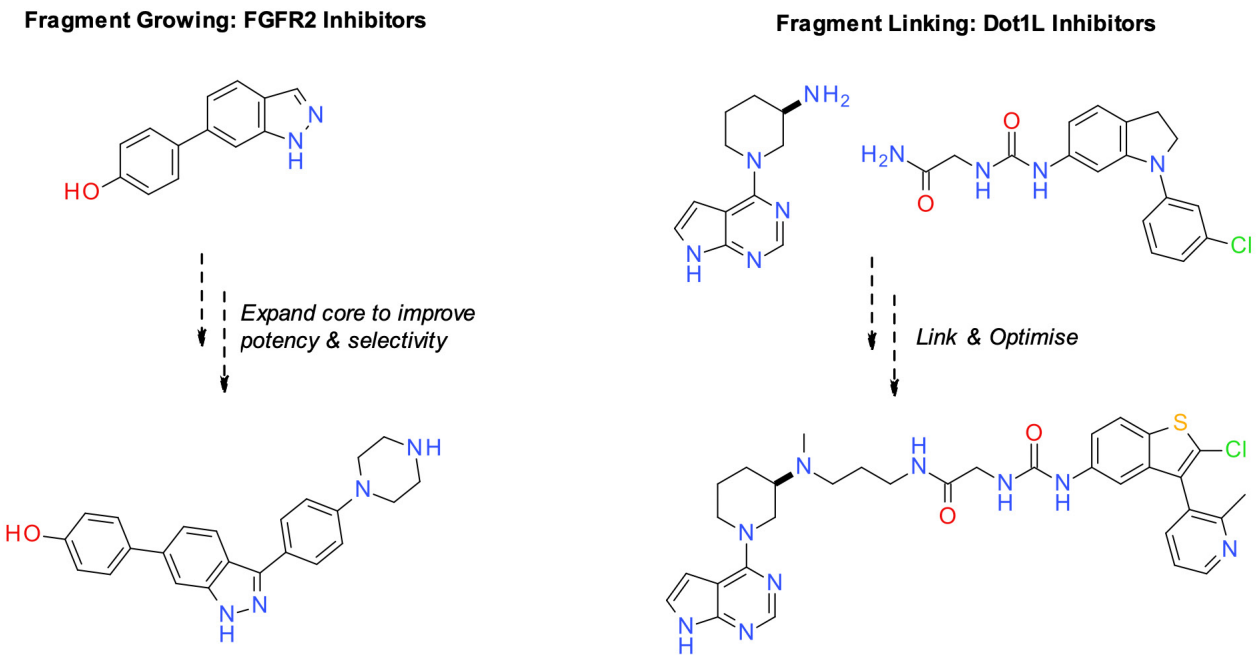


Figure 4: Late-stage lead optimisation using 'expansion' or 'linking' approaches

Candidate identification isn't the end of the story of course, and as the compound progresses into pre-clinical development and IND-enabling studies, ever increasing quantities of material are needed. Since the lead generation and optimisation approach used during FBDD could be viewed (albeit somewhat simplistically) as more modular from a synthetic perspective, one could speculate that the scale-up process may be more facile for a fragment-based program, as compared to a conventional HT- or MT-library screening derived lead, and an analysis of this would be informative and potentially add a further advantage to utilising the FBDD approach.

Key Organics has a long history and well-deserved reputation of applying their collective years of chemistry knowledge to projects from early stage discovery through to pre-clinical candidate stage. They occupy a unique position by being able to offer fragment and screening libraries for very early-stage projects. Key can follow this up by performing SAR by catalogue and custom synthesis of further analogues. Additionally, they offer FTE support for accelerating project progress – process development and scale up when the project approaches candidate selection phase. And finally, key Organics offers a deep well of experience in chemical procurement, which adds maximum value to all projects.

Case Studies

A number of excellent publications are available reviewing the fragment-to-lead work which has been published in the contemporary medicinal chemistry literature. Two articles of particular note were published in October 2016 (reviewing the literature in 2015) and in December 2021 (reviewing the literature in 2020).

Illustrative examples from the field having a particularly notable aspect are shown below.

"FRAGMENT TO MARKETED DRUG"

Vemurafenib (*PLX4032, Figure 5*), is an orthosteric b-raf kinase inhibitor which was approved by the FDA in 2011. This was the first drug to reach the market having evolved using a fragment-based drug discovery strategy. The initial starting fragment, an anilino azaindole, was optimised to the candidate PLX4032 from early lead molecule PLX4720.

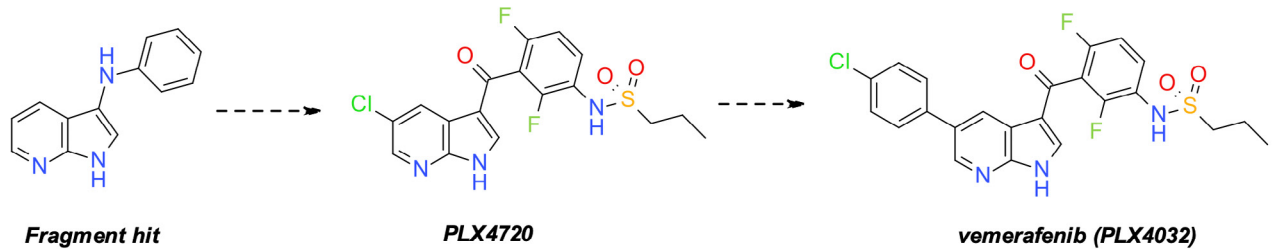


Figure 5: Evolution of Vemerafenib

Venetoclax (*ABT-199, Figure 6*) was the second FBDD originated drug, and one of the first examples of a protein-protein inhibitor class drug to reach the market. This gained regulatory approval by the FDA in 2016 for CLL. This compound functions by binding to the BH3 binding grove in Bcl-2, blocking binding interactions and resulting in programmed cell death. As well as evolving through an NMR based structurally enabled fragment-linking approach (via ABT-737, full details here), this drug is worthy of note for sitting well outside conventional small molecule / 'Lipinski'-type property space. It also contains relatively unusual structural features e.g. an aromatic nitro group and a cyclohexenyl motif.

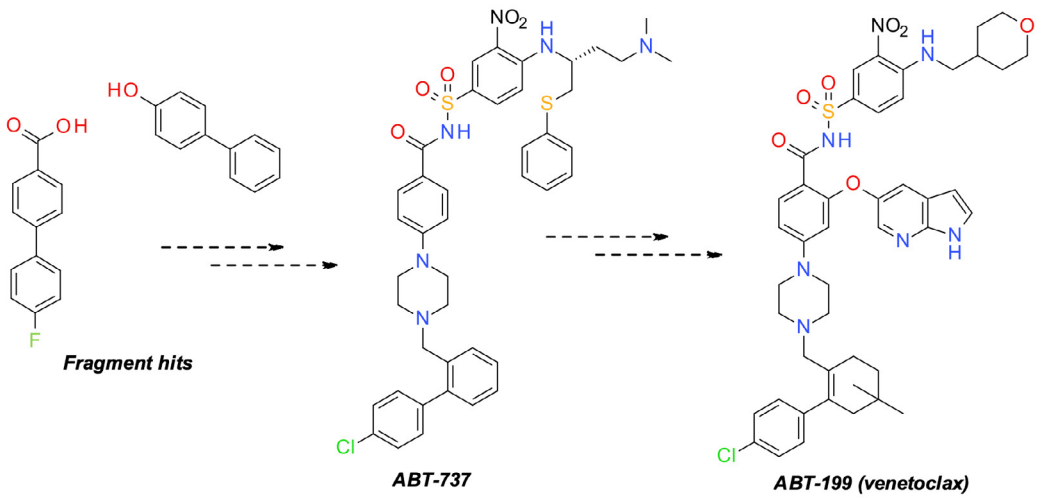


Figure 6: Evolution of Venetoclax

"COVALENT FRAGMENT TO CANDIDATE DRUG"

Sotorasib (*formerly AMG-510, Figure 7*) is an example of development of a late-stage clinical candidate against a very challenging target (KRAS) using a fragment-based approach. This compound is a covalent inhibitor, and started with the unusual disulfide 6H05, evolving through intermediate leads including ARS-1620, finally to the clinical candidate AMG-510.

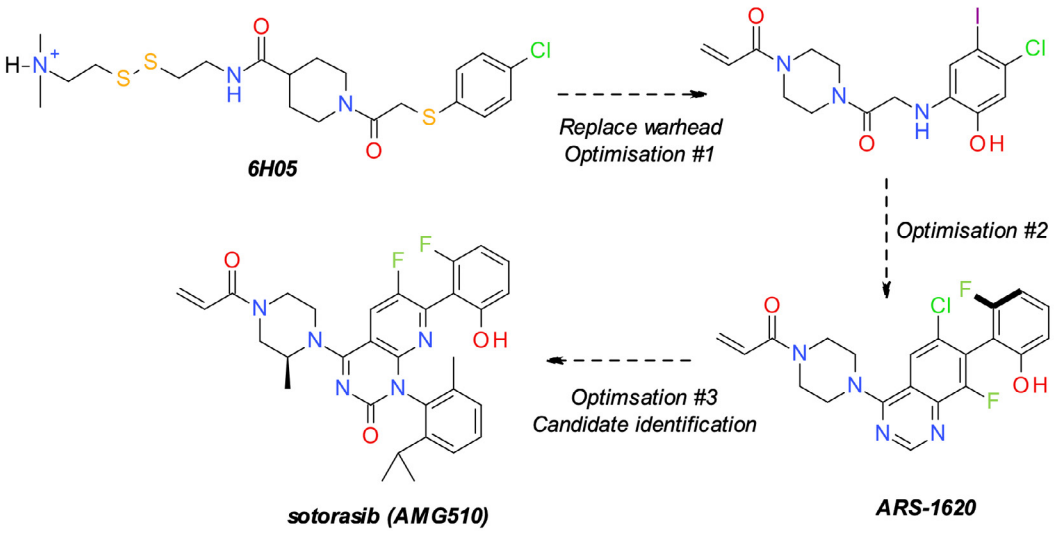
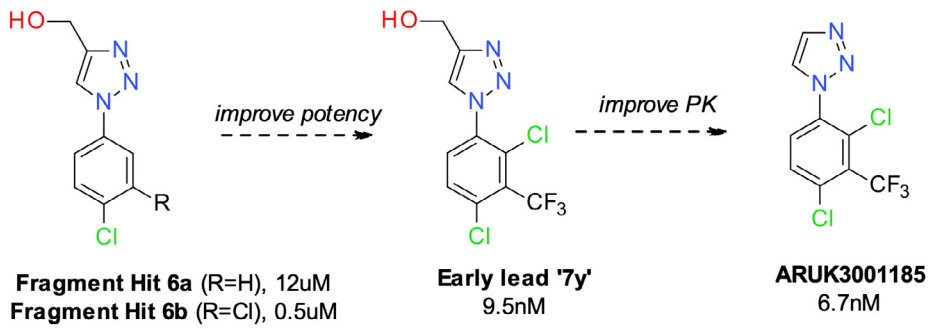


Figure 7: Evolution of Sotorasib

"FRAGMENT HIT TO FRAGMENT LEAD"

Fish and co-workers have recently published their lead identification work for inhibitors of notum, with support for the scale-up work on ARUK3001185 being provided by Key Organics. Interestingly, the final compound, ARUK3001185 itself has a fragment-like property profile.



Fragment Hit 6a (R=H), 12uM
Fragment Hit 6b (R=Cl), 0.5uM

Early lead '7y'
9.5nM

ARUK3001185
6.7nM

Figure 8: Evolution of ARUK3001185

OTHER UTILITIES FOR FRAGMENTS

With the emergence of new drug modalities such as targeted degradation and other bifunctional molecules, a range of other opportunities have opened up which may be facilitated by utilisation of fragment-sized molecules. A selection of illustrative opportunities are provided below.

- De novo PROTAC discovery
- E3 Ligase ligand discovery
- Molecular Glues
- Chemical Biology tools