A High Throughput Technique for Rapid Measurement of Fragment Solubility

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Introduction

As the field of Fragment–Based Drug Discovery continues to mature there is an increasing need for access to fragments of the highest quality.[1] Whilst structural and pharmacophysical properties are still key criteria when selecting compounds to screen, the question of fragment solubility is an increasingly important factor for researchers and practitioners in the field to consider.

Regardless of the biophysical technique applied (SPR, XRD, NMR, ITC, HCA etc), full dissolution in the aqueous assay media is a prerequisite for successful in vitro testing as poor solubility can compromise the robustness of the screening data through aggregation and promiscuous inhibition.[2] Fragment hits with poor aqueous solubility are also more prone to lead to evolved analogues with inferior ADME properties such as plasma protein binding and poor systemic distribution. Selection of high quality, soluble fragments as start-points can reduce the likelihood of attrition when developing leads and further expedite the overall drug discovery process.

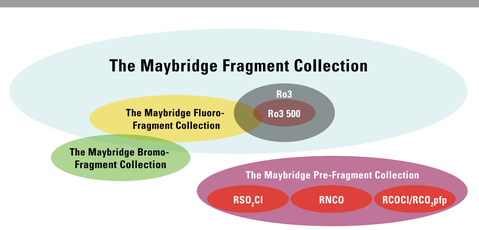
The Maybridge Ro3 Fragment library is acknowledged to provide high quality fragments that harness the diverse and pharmacophore rich nature of the Maybridge portfolio as well as incorporating the strict pharmacophysical attributes required in meeting the Rule of 3 criteria.[3]

Although a number of solubility prediction tools are available, most have been built around drug-like molecules which fit Lipinski's rules, the same calculations when applied to smaller fragment-like molecules are much less reliable. As part of the continued drive to enrich the data surrounding each fragment a study was carried out, in collaboration with STEM®, a sister company within Thermo Fisher Scientific, to experimentally assess the solubility of each member of the library. The Clarity solubility station with integrated Integrity data handling software allowed for 100-200 experiments to be run per day (depending on the protocol), providing data on compound solubility in both DMSO and aqueous phosphate buffer.

The Maybridge Fragment Range

The Maybridge Fragment range has been developed to provide not only the quality and structural diversity scientists have come to expect from Maybridge products, but also the flexibility to meet their needs. The choice of superior off the shelf collections and the ability to "cherry-pick" from our carefully selected fragment lists when creating bespoke libraries provides an essential blend of convenience and control for the design and execution of Fragment screening programmes.

FIGURE 1. The Maybridge Fragment Range



The Maybridge Ro3 (1000) and Ro3 500 libraries have been carefully engineered to provide a premium selection of high quality fragments which are available in range of convenient formats to support the broad arena of fragment screening research.[4] Both libraries benefit from a number of key features such as high purity (≥95%), Rule of Three (Ro3) compliance,[4] quantifiable diversity through the application of industry standard chemometrics.[5]

Both Ro3 libraries and their sister sets in the Maybridge Fragment Range tap into the pharmacophore-rich Maybridge portfolio which has been developed over the past 45 years and which spans the small molecule building block and screening compound arenas, similar to the fragment based drug discovery bridges the gap between ligands and hits.

Materials & Methods

Clarity Solubility Station[6]

Developed in collaboration with partners at Pfizer and the Illinois Institute of Technology, the Clarity solubility station with integrated Integrity software is a powerful tool for determining solubility and crystallisation profiles. Precise heating and data collection of up to 10 reactor cells in parallel provides rapid measurement of solubility under a range of conditions whilst each individual infra red transmission detector allows turbidity/solubility measurement to a standardised endpoint (threshold).

Solubility Protocol

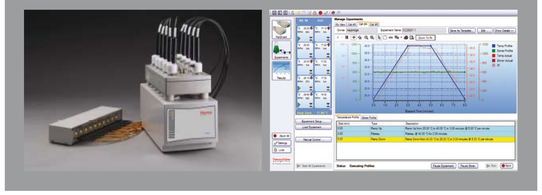
A solubilisation protocol was designed to be representative of the techniques used by fragment screening practitioners and returns a definitive "soluble" or "insoluble" result for each fragment at the following concentrations:

a) 200mM DMSO
b) 5mM aqueous buffer (containing 2.5% DMSO)
c) 1mM aqueous buffer (containing 0.5% DMSO)

The experimental conditions were engineered to incorporate a three stage warming/cooling cycle to maximise the chances for dissolution and mirror techniques often used for screening sample preparation.

- Warm from 25°C to 40°C over 3min
- Hold at 40°C for 2minutes
- Cool from 40°C to 25°C over 3min

FIGURE 2. Stem Clarity Solubility station and Integrity Software

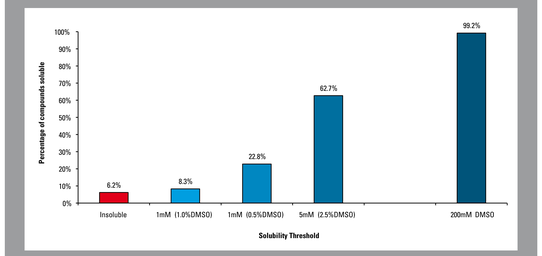


The choice of buffer and concentration is driven by developments in the field where practitioners opt for the convenience of DMSO stock for throughput purposes and increased solubility in aqueous systems. The aqueous buffer of 20mM phosphate buffer (adjusted to pH 7.5) is widely used in fragment sample preparation and screening as it is both NMR transparent and at the optimal concentration to provide pH control whilst not promoting unwanted salting effects.

Results

A series of validation analyses were carried out in order to attune the arbitrary transmission scale to a visual "pass". The value of 78 ATU (Arbitrary Transmission Units) was found to be the optimal threshold value for the study, above which the sample was soluble.

FIGURE 3. Maybridge Ro3 1000Solubility Profile

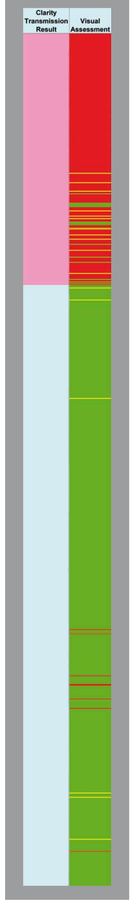


The initial phase of the study, where solubility in DMSO at 200mM was assessed, confirmed that >99% of the 1000 compounds tested were experimentally soluble. The insoluble compounds were either salts which subsequently dissolved in aqueous buffer and three compounds which had decomposed and the compound was immediately removed from the library.

The 200mM DMSO solutions were used as stock for aqueous dilution to either 5mM or 1mM. The concentration of DMSO ranged from 2.5% to 0.5% depending on dilution. It was found that 94% of the 1000 Ro3 compounds were experimentally soluble at ≥1mM with the majority soluble at ≥5mM (62%) (see figure 3). Only 26 compounds required heat (40°C) for dissolution.

The study found 62 compounds which were insoluble under the protocol conditions. These compounds were replaced with structurally similar, Ro3 compliant alternatives from the Maybridge Fragment Collection, which have had their aqueous solubility experimentally assured.

FIGURE 4. Clarity Vs Visual Data (5mM)



Protocol Validation and Data Analysis

Validation of the Clarity Endpoint Data

As part of the study a visual check was made for each sample in order to assess the accuracy of the Clarity solubility station and the validity of the results obtained by applying a fixed transmission endpoint.

When viewed graphically (figure 4, where red/pink are insoluble and green/light green are soluble) the two sets of data for solubility assessment at 5mM show good correlation (96.9%).

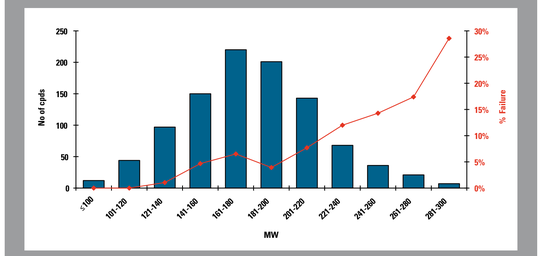
Closer visual inspection of those outlying samples where a negative visual result / positive transmission result was obtained, showed that macro-aggregation or "oiling" were the cause. The reasons are less clear when a positive visual result/negative transmission result was obtained and further investigation is needed for these cases.

The Effect of Molecular Weight on Solubility

The results show that molecular weight is directly proportional to the percentage of the 62 insoluble compounds at a given molecular weight. Figure 5 shows the molecular weight distribution of the Maybridge Ro3 library along with the percentage of compounds in each molecular weight category which were shown to be insoluble at ≥ 1mM.

As you would expect the graph shows an upward trend for insolubility as the size of the fragment molecule increases. This data lends more credence to the view that "smaller is better" in terms of fragments for screening and has clear implications for fragment library designers

FIGURE 5. Maybridge Ro3 1000 MW Distribution / Percentage Insoluble (for a given MW)

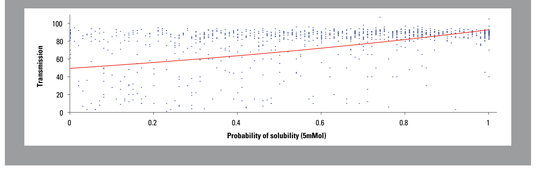


Experimental Vs Calculated data

A study was recently carried out in collaboration with Dr Igor Tetko of VCCLab and the University of Neuherberg to investigate the accuracy of in silico solubility prediction for small fragment-like molecules, using the Ro3 library as a model set.

The ALogPS algorithm derived aqueous solubility data was used to predict the probability of a molecule to be insoluble at a pre-defined solubility threshold (5mmol/l).[7, 8, 9] Figure 6 illustrates the correlation between experimental and calculated data and shows a clear trend towards higher transmission values (solubility) as the probability for dissolution increases.

FIGURE 6. Measured IR Transmission Vs ALogPS Predicted Probability of Solubility at 5mMol

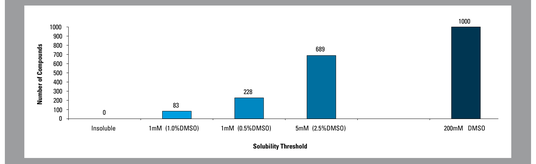


Conclusions

- Compounds in the Maybridge Ro3 Fragment Library are experimentally assured to be soluble at >1mM in aqueous media at biological pH.
- Compounds in the Maybridge Ro3 Fragment Library have a high solubility in DMSO solution (>200mM).

The solubility profile of the recently upgraded Maybridge Ro3 Library is shown in Figure 7.

FIGURE 7. Maybridge Ro3 1000 - 2009 Upgrade Solubility Profile



- The Stem Clarity Solubility Station allows rapid generation of solubility data to a standardised endpoint and removes the subjective aspect of a purely visual assessment.
- The Clarity transmission data has been vaildated against the observed data and has been shown to be 97% accurate.
- The results show a trend for greater solubility at lower molecular weight.
- Predicted solubility data, generated using the ALogPS calculator, show a positive correlation in terms of probability of solubility against actual solubility.

The outcome of this work is a valuable upgrade of the popular Maybridge Ro3 available Fragment Library and provide experimental aqueous solubility assurances for the entire 1000 compounds.

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