A High Throughput Technique for Rapid Measurement of Fragment Solubility


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Materials & Methods

Clarity Solubility Station

Developed in collaboration with partners at Pfizer and the Illinois Institute of Technology, the Clarity solubility station with integrated infrared software is a powerful tool for determining solubility and crystallization profiles. Precise heating and data collection of up to 10 reactor cells in parallel provides rapid measurement of solubility and the range of conditions whilst each individual tube and transmission detector allows turbidity/solubility measurement to a standardized 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:

- 200mM DMSO
- 5mM aqueous buffer (containing 2.5% DMSO)
- 1mM aqueous buffer (containing 0.5% DMSO)

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

- Warm from 25ºC to 40ºC over 3min
- Hold at 40ºC for 2min
- Cool from 40ºC to 21ºC over 3min

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 200mM DMSO was chosen to cover the complete solubility range and as it is both NMR transparent and at the optimal concentration to provide pH control while not promoting unwanted salting effects.

Results

A series of validation analyses were carried out in order to assess the arbitrary transmission scale to a real “pass.” The value of 78.0% (Xtreme Transmittance) was chosen to be the optimal threshold value for the study, which shows what the sample was soluble.

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 other salts which subsequently dissolved in aqueous buffer. Although the compound had decomposed and the compound was immediately removed from the library.

The 200mM DMSO solutions were used as stock for aqueous dilution to either 1mM or 5mM concentration of DMSO ranged from 2.5% to 0.5% depending on dilution. It was found that 98% of the 1000 Ro3 compounds were experimentally solubile at 200mM with the majority soluble at 1mM (94%) (see figure 3). Only 26 compounds required heat (40ºC) 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 assessed.

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 viability of the results obtained by applying a final transmission endpoint.

When viewed graphically (Figure 4), where redpink is insoluble and green/blue is soluble, the two sets of data for solubility assessment at 5mM show good correlation (96.9%).

Closer visual inspection of these output graphs where a negative visual result/positive transmission result was obtained revealed a potential equipment-related issue. As a result a re-run of these samples was performed for each of the cases. The reasons are less clear when a positive visual result/negative transmission 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 52 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 5mM.

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 fragment molecule size and has clear implications for fragment library designers.

Conclusion

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

The Stearn Clarity Solubility Station allows rapid generation of solubility data for a miniaturized endpoint and removes the subjective aspect of a purely visual read.

The Clarity transmission data has been validated against the observed data and has been shown to be 98% accurate.

The results show a trend for greater solubility at lower molecular weight.

Preclinical 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 Fragment Library and provides experimental aqueous solubility assurance for the entire 1000 compounds.

References

6. http://www.stemcorp.co.uk
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Experimental vs Calculated data

A study was recently carried out in collaboration with Dr Ilop Tetko of VCLlab and the University of Nottingham 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 as a pre-defined solubility threshold (insoluble). Figure 5 illustrates the correlation between experimental and calculated values and shows a clear trend towards higher transmission values (solvency) as the probability for dissolution increases.