TRANSLATIONAL RESEARCH SOLUTIONS

IMPROVING REPRODUCIBILITY: BEST PRACTICES FOR SMALL MOLECULES

INTRODUCTION

Small Molecules are used as modulators for cellular processes, as substrates for and readouts of cellular activity, and as part of screening libraries to identify promising compounds. Though antibodies, cell lines, and cell culture conditions often come under scrutiny as key contributors to confounding variation between experiments, proper use and storage of small molecules are also important to ensure experiments can be reproduced.

Many of the small molecules used in bioassays and screening libraries are shipped with certificates of analysis verifying their purity, but factors such as structure are often overlooked. In one instance, the work of several research groups was invalidated when it was discovered that the wrong isomer of bosutinib, a pharmacologically active molecule was distributed. While HPLC analysis indicated high purity, the molecule was not subjected to quality control tests specific enough to distinguish the isomers.[1,2] Furthermore, lack of attention to physical and chemical properties, such as melting temperature and light sensitivity, can cause a change in the phase or the chemical composition of the molecule that may affect its stability, as well as the results of downstream assays.

Vendors typically test small molecules to confirm structure and validate purity. However, differences in stereochemistry can go unnoticed for years, as was the case with the aforementioned bosutinib, and significantly alter the outcome of an assay. Confirming the identify of a small molecule is an everyday practice for many chemists and material scientists, but can be a challenge for researchers without access to instruments to perform nuclear magnetic resonance (NMR) spectroscopy, for example.

SMALL MOLECULES

Small molecules are used as modulators for cellular processes, as substrates for and readouts of cellular activity, and as part of screening libraries to identify promising compounds. Though antibodies, cell lines, and cell culture conditions often come under scrutiny as key contributors to confounding variation between experiments, proper use and storage of small molecules are also important to ensure experiments can be reproduced.

Vendors typically test small molecules to confirm molecular structure and validate for purity. However, differences in stereochemistry can go unnoticed for years, as was the case with the aforementioned bosutinib, and significantly alter the outcome of an assay. Confirming the identity of a small molecule is an everyday practice for many chemists and material scientists, but can be a challenge for researchers without access to instruments to perform nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry, for example.

Quality vendors will supply information and data proving they have performed these analyses (See **Box 1: Four Factors to Consider When Selecting a Small Molecule Vendor** and **Box 2: Validation Techniques for Small Molecules**). The most important step a researcher can take to avoid problems with small molecules, aside from performing these tests in their own labs, is to educate themselves about the molecule's characteristics, including its structure, concentration, and method of purification.

Proper handling and storage practices (See **Box 3: Storage and Usage Tips for Small Molecules**) are also crucial to ensure optimum performance of small molecules, whether solid or dissolved in solution. Sigma-Aldrich recommends that researchers be mindful of the three S's: solvent, solubility, and stability.

Solvent — The solvent used to dissolve a small molecule may be toxic to cultured cells. The solvent could also confound colorimetric and fluorescence assay results, for example by changing the absorbance maximum of a molecule or quenching fluorescence, respectively.

Solubility — When a molecule "crashes" out of solution, its concentration is no longer accurately known. Read reagent bottles, resources such as the CRC Handbook of Chemistry and Physics, or peer-reviewed papers to determine the solubility of the molecule. The solvent will also play a role in a molecule's solubility. The old adage of "like dissolves like" is important here.

Stability — If a molecule is light or air-sensitive, any exposure can lead to decomposition or alteration of the functional groups of a molecule. Labeling information and/or the Material Safety Data Sheet (MSDS) should specify instructions for handling and storing the molecule. Expiration dates are often provided on reagents found in assay kits.

BOX 1: Four Questions to Consider When Selecting a Small Molecule Vendor

Asking a few simple questions of your vendor before purchasing a small molecule or reagent kit can help ensure quality. A reputable vendor will adequately address all of the questions below.

1. Does the vendor supply a CofA showing the analysis performed on the molecule?
2. Does the vendor supply references for the synthesis of the molecule?
3. If the molecule is used in a colorimetric or fluorescence, does the vendor supply the appropriate spectra?
4. Is technical support available and will they answer specific questions about purification and analysis?

BOX 2: Validation Techniques for Small Molecules

Synthetic and analytical chemists use the methods below to purify and identify the small molecules used as reagents or components in chemical libraries. While it is not practical for most life science researchers to use the methods below to validate their reagents, it is important to be familiar with those that may be listed on the product specification sheet and CofA.

Molecule purification/separation

Liquid chromatography (LC) and gas chromatography (GC) are used to separate molecules in solution. GC is used specifically on volatile molecules.

Molecular weight determination

Mass Spectrometry (MS) is a technique to measure the mass-to-charge ratio of a molecule, which is then used to determine its molecular weight. MS to determine molecular weight often follows LC or GC to separate molecules of interest.

Structure

There are two types of techniques commonly used to gain structural information about a molecule.

Nuclear Magnetic Resonance (NMR) spectroscopy measures the behavior of atomic nuclei in the presence of electromagnetic radiation, which is used to determine structure and location of atoms in a molecule. The two most common types of NMR are 1H NMR ("proton NMR"), which is used to determine the location and stereochemistry of hydrogen atoms, and ^{13}C NMR ("carbon NMR"), which is used to determine the location, substitution of, and stereochemistry around carbon atoms.

Elemental analysis is used to determine the chemical formula of a molecule or the specific percentage of a certain element in a molecule. It is often used in conjunction with other techniques such as NMR and chromatography.

BOX 3: Tips for Storing and Using Small Molecules

Proper storage and handling of small molecules reagents will maintain the integrity and stability of the molecule, as well as reduce the incidence of contamination and mislabeling.

Storage

- Write the open date on the outside of the package. Record lot number, product number, and molecular structure in a lab notebook when a new product is opened. Without this information, doubts or mistakes at a later cannot be addressed.
- Keep an eye out for and adhere to printed expiration dates on reagents and reagent kits.
- Label all prepared solutions with chemical name, concentration, solvent, and date.

Usage

- Each researcher should maintain a stock of reagents, if possible, to reduce contamination.
- Vortex or mix reagents before use.
- Do not pipette directly from or stick a spatula into reagent containers.
- If research has an intended clinical application, GMP reagents/materials may be required. GMP materials are manufactured under high quality and regulated standards.

References

1. Halford, B. Bosutinib Buyer Beware. *Chemical & Engineering News* (2013). <http://cen.acs.org/articles/90/web/2012/05/Bosutinib-Buyer-Beware.html%3E.
2. Levinson, N. M. & Boxer, S. G. Structural and spectroscopic analysis of the kinase inhibitor bosutinib and an isomer of bosutinib binding to the Abl tyrosine kinase domain. *PloS one* **7**, e29828, doi:10.1371/journal.pone.0029828 (2012).

sh8827
1075