Compound decomposition: a new drug discovery tool?

'Chemical decomposition reactions might represent an unexplored and exciting research tool, potentially leading to the discovery of completely new molecular diversity.'

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The drug discovery process has been in constant evolution, from the empirical exploration of natural products, through both the systematic testing of synthetic compounds and 'rational' drug design, to the recent high-throughput screening (HTS) of chemical compounds. Combinatorial chemistry has now become fully integrated into the modern drug discovery process, allowing broader investigation of molecular diversity for drug research and development.

Interestingly, the introduction of combinatorial chemistry and associated HTS, aimed at speeding up the progress of research programs, has generated new issues related to an area often neglected, at least in the past, by many drug discovery organizations – compound management. Collections of large numbers of chemical compounds (obtained through traditional synthesis, combinatorial synthesis and purchasing) are usually best stored in vials and/or plates for years, and are regularly used for hit screening, hit validation, structure–activity relationships and many other steps in the drug discovery pathway.

Compound storage: stability and solubility issues hamper the drug discovery process

Compound stability during the storage process is a major concern for any HTS operation. The loss of compounds as a result of decomposition affects assay consistency, producing false positives and negatives, thereby increasing the time required for hit finding. Many pharmaceutical organizations store their screening compound collections as frozen dimethyl sulfoxide (DMSO) solutions, generally at 4°C in a low humidity atmosphere. It has generally been accepted that the integrity of a sample in DMSO depends on chemical stability and DMSO solubility, which both potentially depend on different chemical features. Today, solubility evaluation can be addressed using various techniques [1], many of which have been implemented by Chris Lipinski at Pfizer (http://www.pfizer.com/main.html). However, chemical stability remains largely unexplored, and the rest of this article will address this important issue more specifically.

Many factors such as humidity, temperature and exposure to oxygen are thought to play a role in the stability of a compound. Recently, two publications [2,3] reported on experimental data concerning the impact of these factors on compound stability during long-term storage: (i) Procter & Gamble Pharmaceuticals (http://www.pgpharma.com/index.shtml) reported on the stability of ~7200 diverse compounds stored as 20-mM anhydrous DMSO solutions under ambient conditions for one year [2]. Using electrospray ionization mass spectrometry, they showed that the probabilities of still observing the original compound were 92%, 83% and 52% after three-, six- and 12-month storage in DMSO, respectively. This study provided the first published results on the effect of room-temperature storage on the stability of compounds in DMSO. Although it is obvious that such results vary according to the chemical nature of the compounds involved in the study, it clearly highlights the potential importance of chemical decomposition in the field of HTS. (ii) Abbott (http://abbott.com/) reported the stability study of a set of 644 compounds stored in 10-mM DMSO, including an accelerated stability study at 40°C [3]. Quantitative liquid chromatography, UV spectroscopy and mass spectrometry were used to characterize decomposition of the samples stored under various conditions (i.e. DMSO +/– water, +/– oxygen). Some major findings indicated that:

- Most compounds stored in water-free DMSO were stable for 15 weeks at 40°C.
- The decomposition rate at 40°C was approximately two times that observed at room temperature
- Water caused more compound decomposition than oxygen, and the presence of water also induced compound precipitation.
- No significant compound loss occurred after 11 freeze–thaw cycles. By contrast, a study by Procter & Gamble Pharmaceuticals reported a 25% compound loss after 15 freeze–thaw cycles [4]. Interestingly, the sample losses

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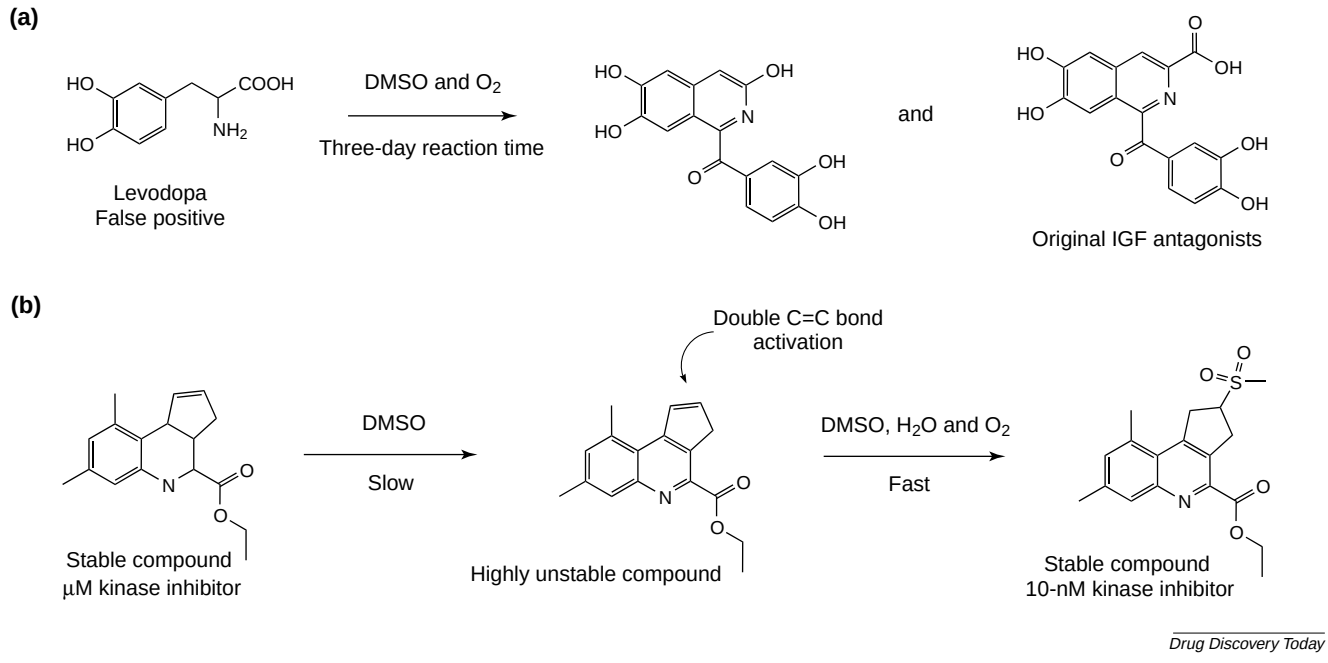

Figure 1. Decomposition and lead finding. Decomposition reactions such as oxidation and aromatization have led to the discovery of **(a)** insulin-like growth factor (IGF) antagonists and **(b)** kinase inhibitors. Abbreviation: DMSO, dimethyl sulfoxide.

under the experimental conditions used in this study could have been caused by precipitation rather than molecular degradation.

Currently, several companies are running similar studies to define the best possible storage conditions. For example, seven pharmaceutical companies; AstraZeneca (http://www.astrazeneca.com/), Bristol-Myers Squibb (http://www.bms.com/landing/data/), Eli Lilly (http://www.lilly.com/), Hoffmann-La Roche (http://www.roche.com/home.html), GlaxoSmithKline (http://www.gsk.com/index.htm), Merck (http://www.merck.com/) and UCB (http://www.ucb-group.com/) have teamed up with the Dutch company Specs (http://www.specs.net/), to initiate the COMDECOM (compound decomposition) project for monitoring the stability of tens of thousands of compounds over three years. Another study by Chembridge Corporation (http://chembridgeresearch.com/index.html) encompassed the HPLC and ^{1}H-NMR analysis of 1000 compounds stored as dry powder, dry film and in DMSO solution for five years (T.R. Webb, pers. commun.). The ultimate target of such studies is obviously to set up a predictive model correlating chemical structures with stability. One of the first systems trying to predict chemical stability, STABEX™, has been published by Karancsi *et al.* [5] from ComGenex (http://www.comgenex.com/cgi-bin/index.php). Thermal decomposition was the main factor taken into account to set up this kind of predictive model.

Another model has been designed to identify particular structural classes that are prone to degradation via known mechanisms such as intramolecular cyclization, retro-Michael decomposition or retro-Mannich hydrolysis, as well as unknown mechanisms. This model has been disclosed recently by researchers from ChemDiv (http://www.chemdiv.com/), who explored >210 molecular and topological descriptors (e.g. molecular weight, dipole moment, topological branching and conformational energy) that potentially affect compound degradability (N. Savchuk, pers. commun.). Based on such descriptors, the team developed a neural network model able to classify molecules into potentially stable and unstable chemical families, thus allowing the set-up of recommendations on handling and storing different compound classes. Only extensive testing of such models will help define their accuracy.

Chemical decomposition: merely induction of false positives?

Chemical decomposition during prolonged storage has certainly generated many false positives and negatives within numerous HTS campaigns, inducing frustration among the researchers ready to embark on hit explosion and optimization. However, this should not prevent us from taking advantage of the decomposition phenomenon to explore new ways of obtaining potential lead compounds, original scaffolds and even original chemical reactions one would

never have thought of. One recently published example perfectly illustrates this idea [6]. A group at Neurocrine Biosciences (http://www.neurocrine.com/home.html) identified what was supposed to be an initial hit, levodopa, in an HTS dedicated to the discovery of insulin-like growth factor (IGF)-binding inhibitors (Figure 1). Interestingly, this result could not be confirmed using a freshly prepared sample of this compound, whereas the 'aged' solution did show some activity after storage for three days in DMSO exposed to air. After separation and identification of the degradation products present in the sample, they identified the chemical pathway leading to these new hits (Figure 1). Furthermore, a relevant structure–activity relationship based on the isoquinoline 'degradation scaffold' was obtained, yielding nM IGF-binding inhibitors. Another example within the field of kinase inhibitors also nicely illustrates this rather original lead-finding approach (N. Savchuk, pers. commun.) (Figure 1). Many other examples of what can sometimes be considered a serendipitous approach might be found in many of the research laboratories performing drug discovery activities.

Such examples might encourage researchers to use the decomposition phenomenon as a new tool in the search for hits. Mimicking decomposition, or the accelerated decomposition (using heat, microwave heating, or enzymes as catalysts) of diverse sources of molecules such as small organic molecules, or even natural products, could lead to new scaffolds, new types of chemistries and new lead compounds. Obviously, this type of activity would need adequate chemical and analytical facilities and resources to identify and characterize the decomposition reactions clearly. Pfizer, for example, have performed degradation studies by exploring the effects of pH, oxidation, heat, humidity and photostability on chemical integrity [7]. With CambridgeSoft Corporation (http://www.camsoft.com/), the Degradation Resource Group at Pfizer set up a Windows-based program to manage all information related to drug degradation [7]. This database could be used to accumulate experimental data for the design of new chemical entities synthesized according to degradation reactions. Because most of these reactions occur very slowly and in very low yields, all of the organic chemist's expertise would be required to find fast and high-yielding synthetic pathways to make this approach attractive for drug research purposes.

It is generally accepted that the serendipitous discovery of unforeseen activity remains a major source of new lead compounds. Using compound decomposition as a tool for drug research could be another way to favor serendipity by simply looking more carefully at some of the chemical reactions involved in molecular degradation.

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