Tuning covalent reactivity: A Chemist's toolbox

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Revival of covalent inihibitors in drug discovery

- Covalent drugs have proved to be successful therapies for various indications, with nearly 30% of drugs on the market acting via a covalent mechanism of action[1),2)]. However, largely owing to safety concerns, covalent inhibitors are often shunned by medicinal chemists and toxicologists alike. While the potential risks of covalent inhibition are known, the sustained duration of inhibition offers several advantages:
  (a) Improved biochemical efficiency
  (b) Lower, less frequent dosing reducing the burden on the patient
  (c) Dissociation of pharmacokinetics from pharmacodynamics
  In addition, success stories have been reported where previously considered as "difficult" or even "undruggable" proteins have been targeted by covalent inhibitors[3)].

  Among several reviews published recently highlighting the increased interest[4)-6)], Martin H. Johansson's paper focuses on reversible Michael additions[7)] and describes two major strategies to develop safe and efficient covalently acting drugs:

- Targeted Covalent Inhibition (TCI) of *less reactive electrophilic functional groups,* such as irreversible kinase inhibitors (e.g. EGFR inhibitors) are a classical example for this optimisation strategy (Figure 1).

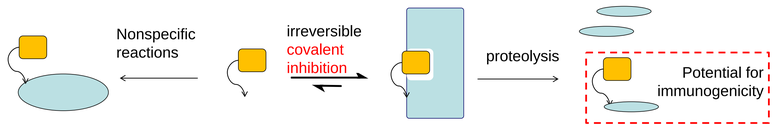


Figure 1: Representation of the irreversible inhibition of a protein using a reactive ligand (adapted from Ref. 5)

- Reversible Covalent inhibition of *more reactive electrophilic groups* e.g. aldehydes found in protease inhibitors, boronic acids (e.g. bortezomib), nitriles and Michael acceptors (Figure 2).

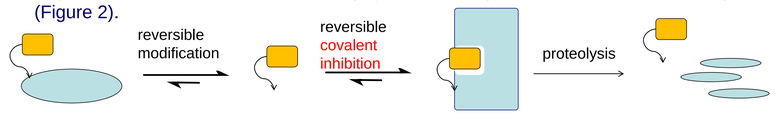


Figure 2: Representation of the reversible covalent inhibition of a protein by a reactive ligand (adapted from Ref. 5)

As interest in covalent inhibitors continues to grow, the tools to evaluate and characterise a covalent inhibitors will evolve[8)]. Herein we would like to present *in silico* and experimental methods which we evaluated and applied to the optimisation of reversible covalent Usp9x inhibitors.

Michael Acceptors as Reversible Covalent Usp9x Inhibitors

- A series of α–cyano acrylamides were previously reported as micromolar inhibitors of the deubiquitinase Usp9x[9)] and lead compounds WP1130 and VM030 served as starting point for the optimisation programme (Figure 3).

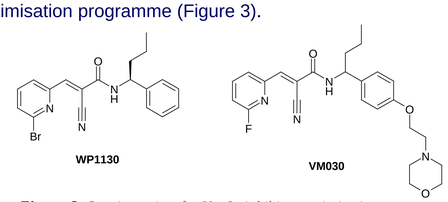
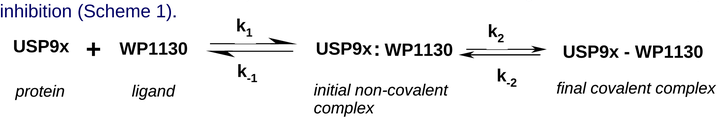


Figure 3: Starting points for Usp9x inhibitor optimisation programme

- The medicinal chemistry strategy consisted of a parallel approach to optimise the non-covalent (data not presented) as well as the covalent binding contribution to Usp9x inhibition (Scheme 1).



Scheme 1: Description of the general mechanism of action of a covalent inhibitor

In silico assessment of covalent reactivity

- The 1,4 Michael reaction is the addition of a nucleophile to an α,β-unsaturated carbonyl compound at the β carbon position. This type of reaction is dominated by orbital, rather than electrostatic, considerations. Not surprising, the corresponding E_{HOMO} (ε_H) and E_{LUMO} (ε_L) values have been used as descriptors to explain chemical reactivity together with other related properties such as the electrophilicity index.

- In fact, this global molecular electrophilicity introduced by Parr in 1999 (see below)[10), 11)] is one of the most widely applied theoretical scale for reactivity having been extensively validated against experimental data:

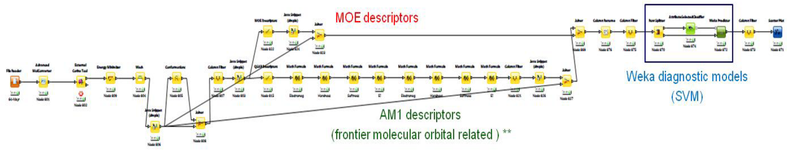
  Electrophilicity Index (EI or ω): $\omega = -\mu^2/2\eta$ (1)

  where electronegativity (μ) is described as $\mu \approx -(\varepsilon_H + \varepsilon_L)/2$ (2)

  and chemical hardness (η) calculated as $\eta \approx (\varepsilon_L - \varepsilon_H)/2$ (3)

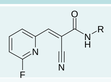
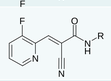
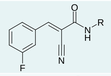
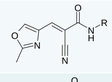
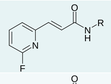
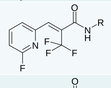
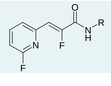
- On the experimental side, Herbert Mayr and colleagues[12)] have explained diverse types of reactions by quantifying the electrophile's and nucleophile's strengths and a comprehensive web-based resource is now available[13)].

- In addition, we implemented a KNIME®-based prediction (see below) of Mayr Electrophilicity Scale (MES) via a QSAR approach using QM descriptors.



- A good correlation was observed between EI and Usp9x potency, which allowed *in silico* optimisation and prioritisation of future analogues for synthesises (Table 1). Outliers in the trend could be explained by changes in the non-covalent binding contribution (e.g. steric clashes).

Table 1: *In-silico* descriptors, ^{1}H NMR shift of β-hydrogen and Usp9x inhibition (R = constant)

Structure	AM1_LUMO	Hardness	EI	δ^1H ($CDCl_3$, 400 MHz) ppm	Usp9x IC_{50} [μM]
	-1.27	8.60	1.81	8.20	13
	-1.34	8.50	1.83	8.57	11
	-0.99	8.20	1.58	8.26	>20
	-0.96	8.58	1.60	8.09	>40
	-0.86	8.31	1.51	7.5 (d, J 15 Hz)	>40
	-1.14	8.94	1.76	7.08	>40
	-0.89	8.71	1.58	6.68	>40

NMR approaches to assess electrophilicity of α,β-unsaturated carbonyl systems

- Several NMR experimental set-ups have been developed to not only assess the reversibility of the Michael addition, but also to determine the relative electrophilicity of the β-carbon[14), 15)].

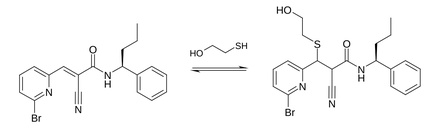
- A general correlation between δH(β-hydrogen) NMR-shift and Usp9x potency was observed.

- Prioritisation of compounds for synthesis was performed based on prediction by commercial NMR software package (MestReNova Chemist 8.1).

In vitro reactivity studies

- Reaction rates have been measured using β-mercaptoethanol (BME) as the model thiol functional group by adaptation of published conditions[8)] (Scheme 2).

- The reaction progress was measured with a UV/VIS spectrophotometer (Fig. 4), as LC-MS/HPLC was unsuitable due to sample dilution causing reaction reversal.



Scheme 2: Model reaction of WP1130 with BME to assess *in vitro* reactivity

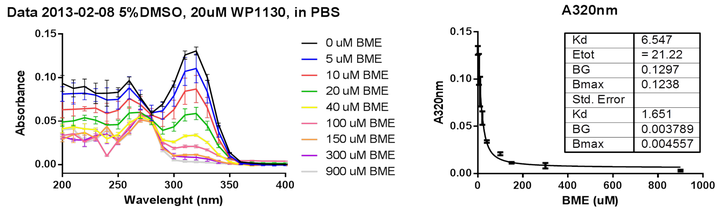


Figure 4: UV/VIS Absorption spectrum of model reaction with increasing BME concentrations (M. Young, University of Michigan)

Summary

Emerging interest to harness the power of covalent inhibitors and the potential of making "undruggable" biological targets "druggable", has led us to strategically establish experimental methods to evaluate covalent binders with the aim to support and drive future medicinal chemistry optimisation strategies. We exemplified the application of – for chemists readily accessible – methods to assess, rank and predict the reactivity of Michael acceptors.

References

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