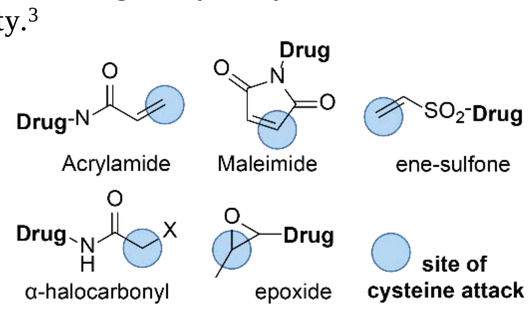
Heteroaromatic sulfones as warheads for Targeted Covalent Inhibitors (TCIs)

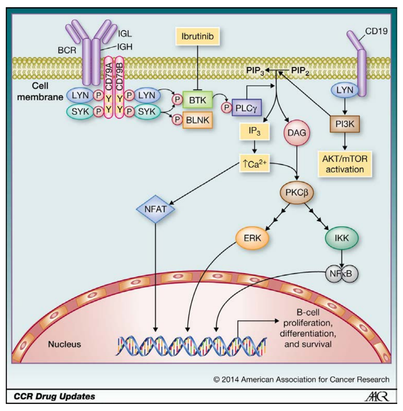
Ruxandra Moraru *[a], Beatriz Valle-Argos [b], Lara Buermann [b], Annabel Minton [b], Graham Packham [b], Matthias Baud†[a]

[a] School of Chemistry, Faculty of Engineering and Physical Sciences, University of Southampton
[b] School of Cancer Sciences, Faculty of Medicine, University of Southampton

1. Introduction

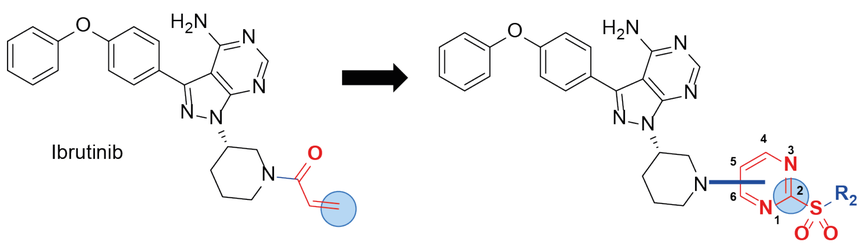
Targeted covalent inhibitors (TCIs) are a subclass of covalent inhibitors focused on targeting poorly conserved amino acids.[1] TCIs consist of a scaffold which binds non-covalently to the enzyme pocket to arrange the warhead in the best orientation to form a bond with the nucleophile. The model system we use is Bruton's Tyrosine Kinase (BTK) which is a highly researched protein with Cys481 being the most explored nucleophile.[2] Ibrutinib is a covalent BTK inhibitor which binds to Cys481 and is used to treat lymphoma and leukaemia. Acrylamide warheads are the most popular warheads for covalent inhibitors although they carry several disadvantages such as retro Michael addition and low tunability.[3]





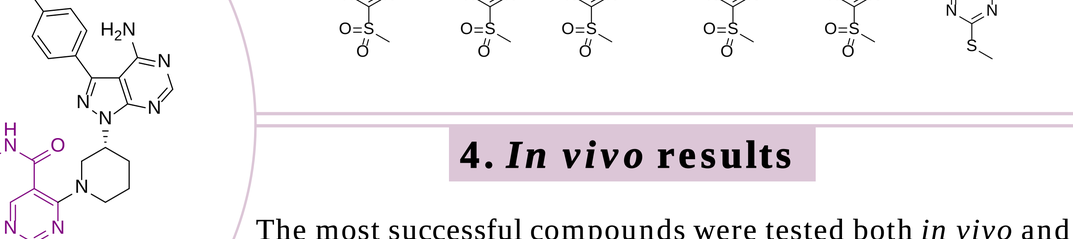
Our aim is to overcome these limitations by introducing a new set of warheads based on the 2-Sulfonylpyrimidine (2-SP) functional group.

The pyrimidine moiety is commonly found in natural products and drug discovery. By using 2-SPs we move away from Michael addition to S_NAr as well as giving the opportunity for tunability by using all the positions on the pyrimidine ring.

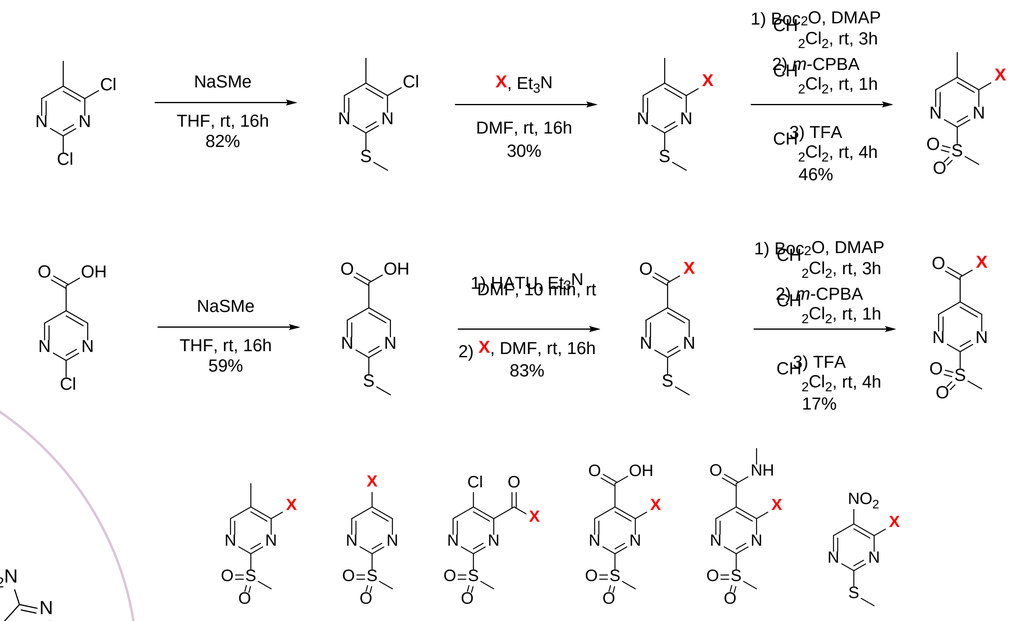


2. Design & Synthesis

Three types of linkages were envisioned in the design stage which can connect the warhead and the scaffold.

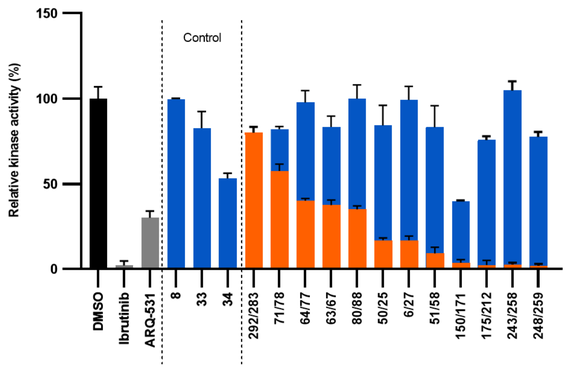


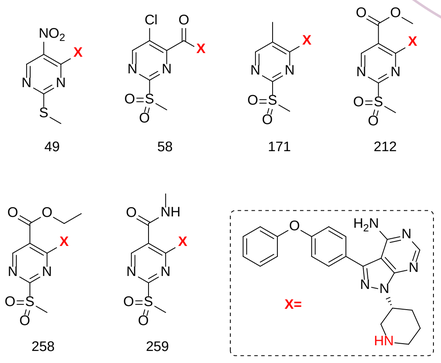
The *N*-arylation and *N*-acylation compounds are synthesised typically through a 5 step synthesis using mild conditions and resulting in good final yields. A general synthetic method is shown below for the 2 types of linkages.



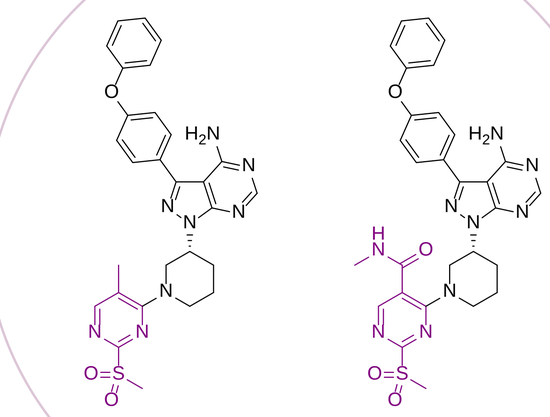
3. *In vitro* results

A total of 36 compounds were synthesised and tested *in vitro* with BTK using an ADP-Glo kinase assay kit. BTK assay results at 100 nM concentration. ARQ-531 is a non covalent inhibitor of BTK. The activity of sulfones is highlighted in orange compared to their thioether precursors in blue.





Selectivity (bottom left and middle) profiles showed how using a 2-SP as a warhead increased the selectivity with 171 and 259 showing the most promising selectivity increase. Plasma stability (bottom right) shows how 171 exceeded the stability of parent compound ibrutinib and 259 still holding above average stability after 3 h at 37 ˚C.



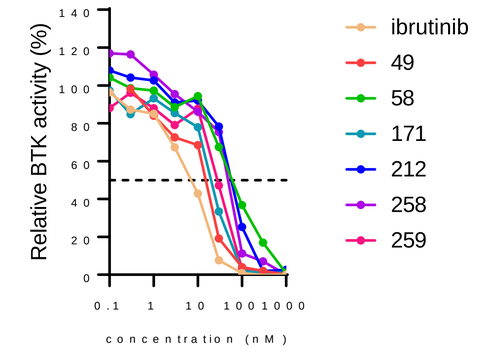
	WT *NanoBRET*	*WT* in vitro
Ib	1.1	9
171	8.2	24
212	38	57
258	26	35
259	0.6	28

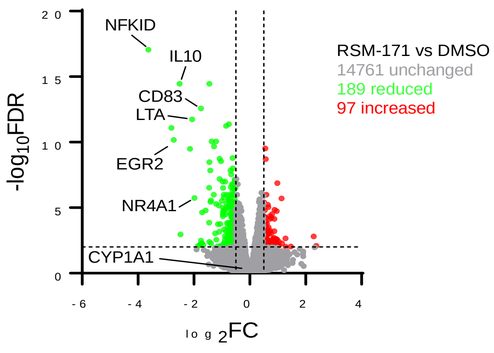
RNA-sequencing results in TMD8 cells with ibrutinib (bottom right), 171 (right). The most essential BTK transcripts reduced by the inhibition of these compounds are labelled in each experiment.

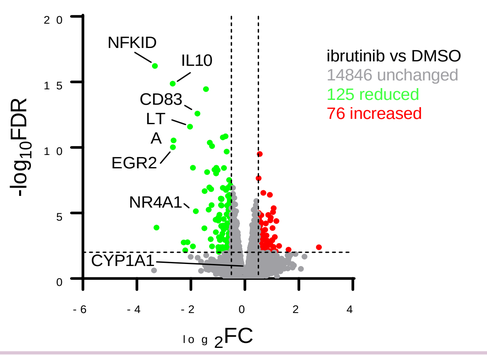
Cell apoptosis (below) of compounds 171 and 259 after exposure to TMD8 cells for 72h using annexin staining and flow cytometry.

4. *In vivo* results

The most successful compounds were tested both *in vivo* and *in vitro* for IC50s as well as cell Ca^{2+} inhibition. We looked further into the ability of these compounds to produce cell death over a 3 day period.

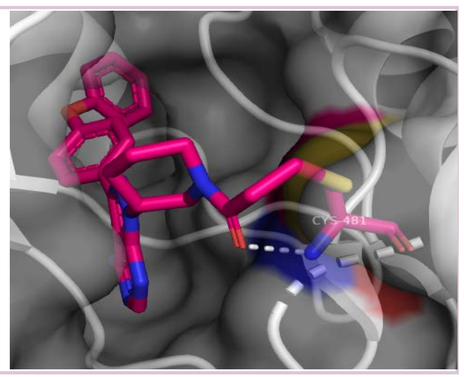






5. Conclusion & future work

Compounds 171 and 259 proved to have competitive activity across each of the different experiments shown above, providing evidence that this new class of warheads are superior to acrylamides both *in vitro* and *in vivo*. These are both *N*-arylated sulfonyl compounds which confirmed the initial hypothesis that sulfonyl compounds with an EWG attached on the pyrimidine ring makes a good leaving group. Whilst these compounds proved to be competitive with ibrutinib, the biggest question of this project is whether the selectivity across the kinome can be increased using new warheads, as it is one of the biggest challenges with this class of inhibitors. ScanTK experiments showed increased selectivity in compounds 171 and 259 with reduced EGFR inhibition which is a key kinase for common side effects. Future work includes protein mass spectrometry to prove the covalent modification at cysteine. It is known that the most common mutation of BTK is cysteine to serine, by using all the knowledge we have learnt we can focus on developing drug analogues which can bind covalently to serine.



References:
1. J. Singh, R. Petter et al, Nat. Rev. Drug Discov., **2011**, 10, 307-317 2. H. Wu, W. Wang et al, ACS Chem. Biol., **2014**, 9, 1086-1091 3. M. Gehringer and S. Laufer, J. Med. Chem., **2018**, 62, 5673-5724 4. A. Herrera and E. Jacobsen, Clinical Cancer Research, **2014**, 20, 5365-5371 5. Rohrbach S. et al, Angew. Chem. Int. Ed., **2019**, 58 (46), 16368-16388.

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Author contact details:
* r.moraru@soton.ac.uk
† m.baud@soton.ac.uk