7-Halogenated indoles as potential DYRK1A/B degraders

Maren Flaßhoff [1,2], **Gerrit Wilms** [3], **Samuele Bordi** [4], **Benedikt Wanner** [4], **Walter Becker** [3], **Conrad Kunick** [1,2]

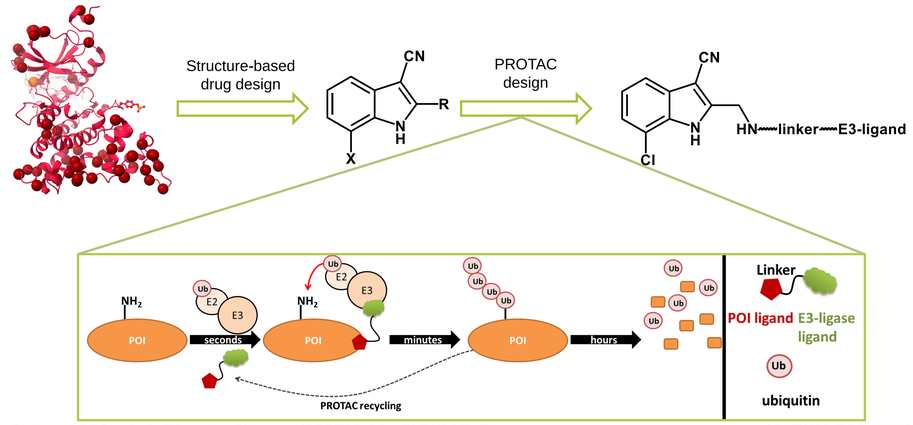
[1] TU Braunschweig, Institute of Medicinal and Pharmaceutical Chemistry, Beethovenstraße 55, 38106 Braunschweig, Germany [2] TU Braunschweig, PVZ - Center of Pharmaceutical Engineering, Franz-Liszt-Straße 35A, 38106 Braunschweig, Germany [3] RWTH Aachen University, Institute of Pharmacology and Toxicology, Wendlingweg 2, 52074 Aachen, Germany [4] Synple Chem AG, Kemptpark 18, 8310 Kemptthal, Switzerland

m.flasshoff@tu-braunschweig.de | Phone +49 (0) 531 391-8450

Funded by DFG Deutsche Forschungsgemeinschaft German Research Foundation

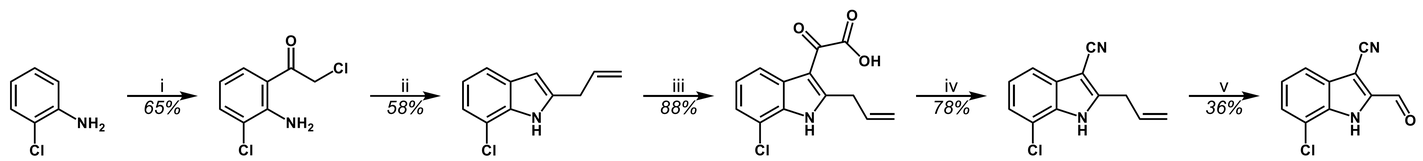
Targeting DYRK1A/B is a promising and challenging goal

The closely related protein kinases DYRK1A (dual-specificity tyrosine phosphorylation-regulated kinase 1A) and DYRK1B are involved in several types of cancer. Despite the fact that they have partly the same substrates, they have different functions. For example, DYRK1B acts only as a tumor promotor, whereas DYRK1A has additional tumor suppressor properties.



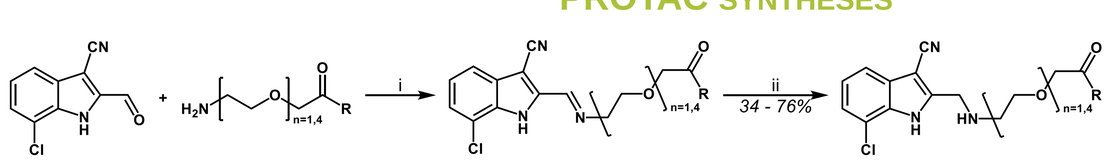
Design of PROteolysis-TArgeting Chimeras (PROTACs) for DYRK1A/B. The dark red spheres in the crystal structure of DYRK1A (PDB: 4YLK) point out the different amino acids between DYRK1A and DYRK1B. The orange sphere highlights the only different amino acid in the ATP binding pocket. POI: protein of interest, here DYRK1A/B.

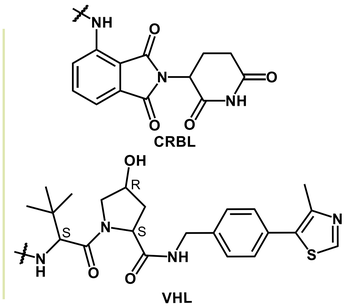
Indole syntheses



Reaction conditions: i) 1. 2-Chloroacetonitrile, $AlCl_3$, BCl_3, DCM, 0 °C, 0.5 h, reflux, 6 – 12 h; 2. HCl_{aq}, reflux, 30 min. ii) Allylmagnesium bromide, toluene, 0 °C, 0.5 h, rt, 1.5 – 24 h. iii) Oxalyl dichloride, Et_2O, rt, 5.5 – 7.5 h. iv) Hydroxylammonium chloride, sodium acetate, EtOH:H_2O (2:1), reflux, 11.5 – 12 h. v) Osmium tetroxide, sodium periodate, THF:H_2O (2:1), rt, 22 h.

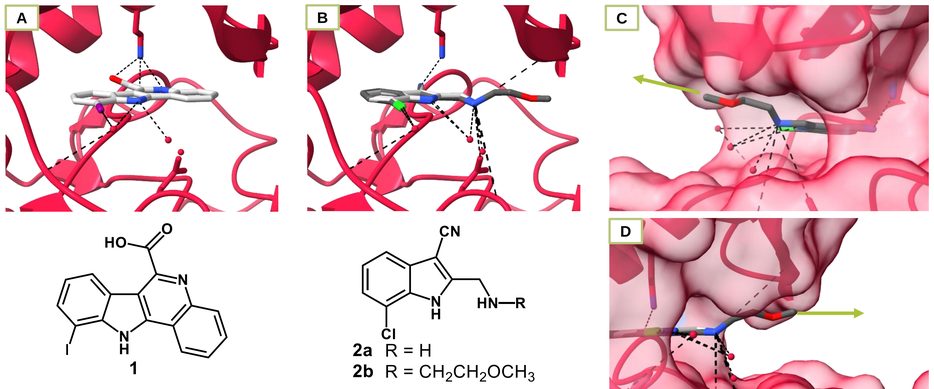
PROTAC syntheses





PROTAC syntheses were performed at Synple Chem AG using a modified workflow with SYNPLEs fully automated capsule based prototype. **Reaction conditions:** i) DCM:HFIP (3:1), Si-Et_3N, DCM, rt, 25 min; 40 °C, 2.5 – 3 h. ii) DCM, HFIP, Si-$CNBH_3$, SCX-2, rt, 4 h.

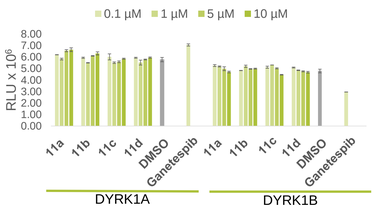
Molecular docking studies visualize the possibility to introduce a linker in 2-position of the indole



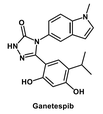
Prediction of the binding mode of 2a and 2b by molecular docking. A similar binding mode to **1** (cocrystal structure, PDB: 4YLJ, shown in A) was predicted for **2a/2b**, whose top ranked poses overlap well (shown in B). The green arrows in C/D illustrate the possibility to exit the binding pocket with a corresponding linker.

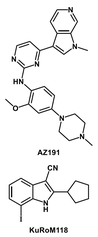
Docking: GOLD, v. 5.2.2[5]; Preparation: MOE, v.2018.01[6]; Visualization: ChimeraX1.3[7]

Direct linker attachment in 2-position results in loss of DYRK1A and DYRK1B binding

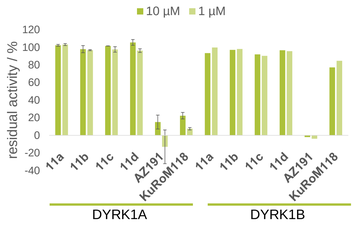


DYRK1A and DYRK1B degradation assay for 11a – 11d. In the absence of HSP90 (heat shock protein 90) DYRK1B aggregates whereas the solubility of DYRK1A is not affected. Therefore, ganetespib, a HSP90 inhibitor, functions as a positive control. Quantification of soluble protein was performed using HiBit Lytic detection systems.



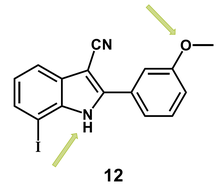


Evaluation of the DYRK1A and DYRK1B residual activity. AZ191 is a known DYRK1B inhibitor with about 5-fold selectivity over DYRK1A. KuRoM118 is a DYRK1A inhibitor identified in structure-based drug design mentioned above. Both act as positive controls in the KinaseGlo assay using recombinant GST-DYRK1A/B and DYRKtide as a substrate. For DYRK1B, only a one-point measurement was performed.



Modification of linker attachment is required to restore activity

In addition to **11a** – **11d**, other compounds with a flexible side chain directly linked to the 2-position of the indole also show no inhibitory activity at both DYRK1A and DYRK1B. Besides changing the linker attachment, the 7-chloro substituent will be replaced by an iodine.



12

Acknowledgements

MF and CK are grateful for funding through the German Research Foundation (DFG grant KU-1371/10-1).

References

[1] Boni, J. *et al.*, *Cancers*, **2020**, *12*, [2] Crews, C.M., *Chem Biol.*, **2010**, *17*, 551–555. [3] Huang, H.-T. *et al.*, *Cell Chem. Biol.*, **2018**, *25*, 88–99.e6.[4] Meine, R. *et al.*, *Molecules*, **2018**, *23*, 64. [5] Jones, G. *et al.*, *J. Mol. Biol.*, **1997**, *267*, 727 – 748. [6] Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2018**. [7] Pettersen, E. F. *et al.*, *Protein Sci.*, **2021**, *30*, 70 – 82.