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A differentiating and apoptotic therapy for acute myeloid leukaemia using potent *human dihydroorotate dehydrogenase* inhibitors

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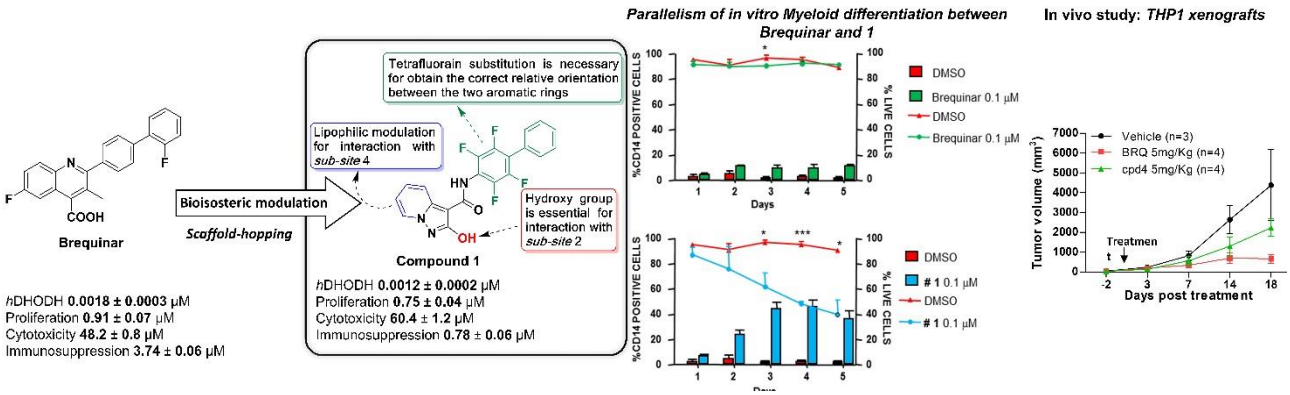
Why my work is important. In acute myeloid leukaemia (AML), blasts lose their ability to differentiate into mature cells and undergo apoptosis. Accordingly, a proapoptotic and differentiating therapy (arsenic and all trans retinoic acid, ATRA) has dramatically improved survival in acute promyelocytic leukaemia; however, such combination therapy is not available for other AML subtypes. While, in 2016, inhibition of dihydroorotate dehydrogenase (DHODH), a key enzyme of the pyrimidine biosynthesis, was found to induce differentiation in several AML models. In fact, brequinar (BRQ) was utilized in vivo studies.[1]

What the specific objectives of my work were/are? We are optimising *h*DHODH inhibitors to improve potency and drug-like proprieties. Moreover, we would like to evaluate how different parameters such as, pK_a, LogD$^{7.4}$ of different carboxylic acid bioisosteres can influence *in vitro* and *in vivo* studies. The main objective is to identify the best inhibitor suitable for use in *in vivo* studies on AML animal model.

A brief explanation of the methods I have used. Bio(iso)steric replacement is a widely used approach in medicinal chemistry to improve the bioavailability, selectivity, potency and other properties of a lead compound. Since 2006, the authors have investigated hydroxylated heterocyclic systems, in order to create a sophisticate tool able to bioisosterically mimic the carboxylic and other acidic functions. Optimized chemical strategies for the synthesis of hydroxylated pentatomic heterocycles (substituted triazoles, pyrazoles, 1,2,5-oxadiazole, thiadiazole), as well as hydroxylated ring fused systems (pyrazolo[1,5‑a]pyridine and benzoisoxazole) will be discussed, and each system analysed in terms of acidity and lipophilicity. The use of these systems in the modulation of acidic lead brequinar, led to a library of potent *h*DHODH inhibitors.[2]

A succinct statement of the results and my conclusions. In this work we will present a new generation of *h*DHODH inhibitors able to reach the enzymatic BRQ inhibition potency levels. Our data showed that cpd **1**, the best of two series, was found able to restore the myeloid differentiation in leukaemia cell lines (U937 and THP1) at concentrations one digit lower than those achieved in experiments with BRQ. Moreover, we characterized cpd **1** with *in vitro* and *in vivo* experiments, showing that it had a significant pro-apoptotic effect in several AML cell lines, which was at least partially independent from the differentiating effect. Furthermore cpd 1 had a significant pro-apoptotic effect on several AML cell lines, but not on non-AML cell lines. Finally, our preliminary results from *in vivo* experiments showed that i) cpd **1** wasn't toxic on Balb/c mice after 5 weeks of intraperitoneal administration; ii) the half-life was limited to 4-6 hours and iii) cpd **1** had a good antileukemic activity (approximately 50% reduction of the tumour volume compared with control, after 18 days of treatment in THP1-xenograft models obtained from NSG mice). Theoretical design, modeling, synthesis, SAR, X-ray crystallographic data, biological assays, Drug-Like proprieties, pharmacokinetic studies and *in vivo* evaluations on AML models will be here presented and discussed.



(1) Sykes, D. B.; et al., *Cell* **2016**, 167, 171-186.e15.

(2) Sainas, S.; et al., *J. Med. Chem.* **2018**, 61 (14), 6034–6055.