Exploring dihydroBenzoImidazoTriazineDione (BITD) Core to Generate Selective ALDH1A1 Inhibitors: A Scaffold Repositioning Approach

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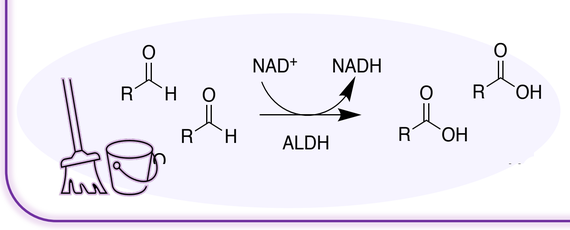
INTRODUCTION

Cancer is considered a grueling challenge for Public Health, being a leading cause of death worldwide. Multi-drug resistance and invasiveness along with the generation of Cancer Stem Cells (CSCs) seriously threaten the success of therapy and prognosis. In this context, Aldehyde Dehydrogenase (ALDH, EC: 1.2.1.3) is a family of detoxifying NAD-dependent enzymes involved in the conversion of reactive aldehydes into the corresponding carboxylic acids. Among them, the 1A1 isoform has recently garnered significant attention from the scientific community, since it was found overexpressed in several diseases, such as obesity and diabetes, and in some solid tumors. Moreover, ALDH1A1 has been identified as a CSC biomarker and is associated with chemoresistance mechanisms and aggressive cancer phenotypes. Inhibition of ALDH1A1 is a recognized successful strategy in the search for new anticancer agents with innovative mechanisms. [1,2]



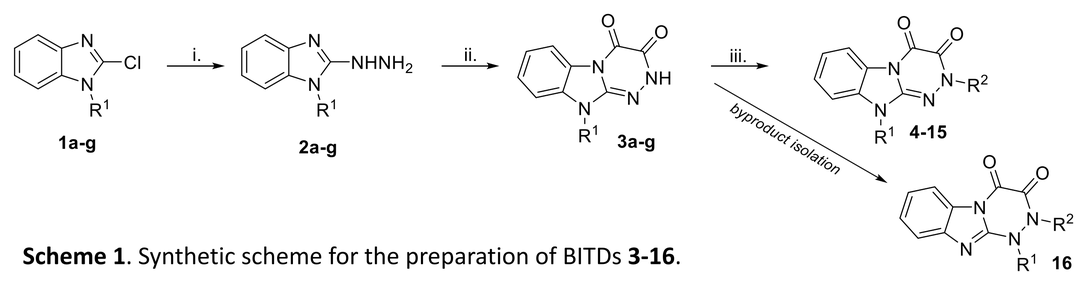
RATIONALE OF THE WORK

In the last years, derivatives of the natural **Isatin** (*e.g.*, ***N*-benzylisatin**, **KS99**, **KS111**) were reported to strongly inhibit ALDH enzymes.[3] Interestingly, the Isatin scaffold seems to share common chemical features with dihydro**B**enzo**I**midazo**T**riazine**D**ione (**BITD**), a core previously investigated by us as a perspective inhibitor of aldose reductase. [4]

Repositioning the BITD nucleus

Therefore, we repositioned the BITD nucleus by testing representative compounds from *in-house* libraries. Based on the retrieved knowledge of ALDH enzymes,[5,6] we designed new BITDs in order to improve affinity and isoform selectivity towards the targeted 1A1 enzyme by increasing lipophilicity and making the compounds more lipophilic and, thereby, more suitable for the ALDH1A1 catalytic site.

SYNTHESIS

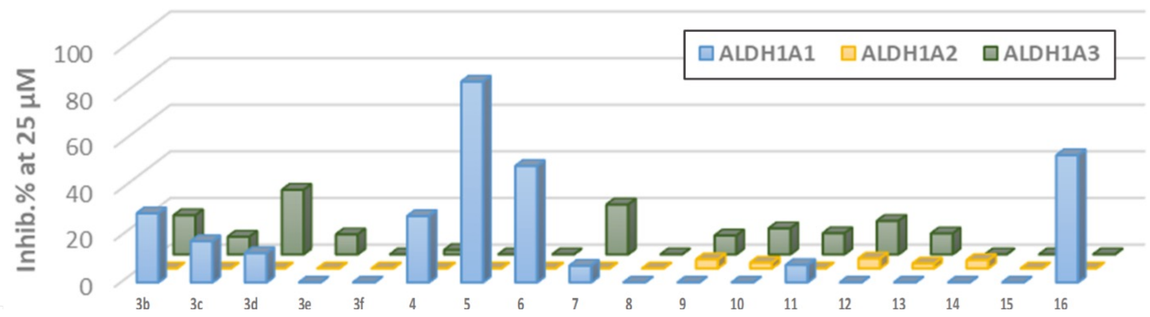


Scheme 1. Synthetic scheme for the preparation of BITDs **3-16**.

Reagents and conditions: i) $NH_2NH_2 \cdot H_2O$, MW, 120 °C, 15 min; ii) diethyl oxalate, MW, EtOH, 120 °C, 30 min; iii) appropriate halogen derivative, K_2CO_3, dry DMF, MW, 120 °C, 15 min.

A three-step synthetic pathway was performed by using a microwave-assisted protocol.

ENZYMATIC ASSAYS AND *IN SILICO* STUDIES



Inhibitory activity against human recombinant ALDH1As was evaluated at a compound concentration of 25 µM by spectrometric assay.[5] *In silico* studies were performed to rationalize the *in vitro* results and gain an understanding of the interactions network.

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HIGHLIGHTS

- The BITD core was repositioned
- *In house* BITDs were found inactive in inhibiting ALDH1A enzymes
- A new BITD derivatives library was developed
- Some compounds resulted to selectively inhibit ALDH1A1 isoenzyme

1) Marcato, P., et al., Cell Cycle, 2011. 10(9): p. 1378-84; 2) Li, B., et al., Eur J Med Chem, 2021. 209: p. 112940; 3) Dinavahi, S.S., et al., Eur J Med Chem, 2020. 187: p. 111962; 4) Da Settimo, F., et al., J Med Chem, 2001. 44(25): p. 4359-69; 5) Quattrini, L., et al., J Med Chem, 2020. 63(9): p. 4603-4616; 6)Quattrini, L., et al., ACS Med Chem Lett, 2020. 11(5): p. 963-970.