POSTER SESSION

Cytotoxics (including Antimetabolites, Anthracyclin, Alkylating agents, Aurora kinases, Polo-like kinase, Topos-isomerase inhibitors, Tubulin-binding compounds)

182 (PB062)

N-1,2,3-Triazole-Isatin derivatives in lymphoma cell lines

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Background: Molecular hybrid constructs are an interesting approach to merge individual pharmacophores with different mechanisms of action, potentially decreasing side effects. The 1,2,3-triazole unit is present in many bioactive compounds and it is characterized by its ability to be stable towards hydrolysis to increase the compounds lipophilicity. Hybrids containing this pharmacophore together with isatin and its analogues have shown a wide spectrum of potential therapeutic activities, also against cancer. Burke et al. have recently reported new *N*-1,2,3-triazole-isatin hybrids with *in vitro* anti-tumor activity in solid tumor cell lines (RSC Medicinal Chemistry 2022; EP3400938). Here, we present the *in vitro* anti-lymphoma activity and structure activity relationships (SAR) of 9 *N*-1,2,3-triazole-isatin hybrids.

Methods: Anti-proliferative activity assessed by 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide (MTT) assay at 72 h. Cell cycle assessed by FACS. IC_{50} values defined as the concentrations corresponding to 50% viability inhibition.

Results: Cell lines derived from diffuse large B-cell lymphoma (DLBCL) of the germinal center B-cell-like (GCB) type (DOHH-2, VAL) and of the activated B-cell-like (ABC) type (OCI-LY-10, SU-DHL-2) were exposed to increasing concentrations of 9 chemically modified oxindole derivatives (Table 1). While 6 compounds did not show any activity at concentrations <20 μM, compounds (1a-c) were active, with IC_{50}s lower in ABC- than in GCB-DLBCL. Specifically, compound (1c), which carries a methyl group in the 5-position of the aromatic ring of the isatin scaffold, was the most active. The chiral non-racemic *N*-1,2,3-triazole-oxindole derivatives (2) did not show activity. Compounds (1a) and (1c) (10 μM; OCI-LY-10; 48, 72 h) induced an accumulation of cells in the sub-G_0 phase, suggestive of the induction of cell death, slightly higher with (1c) than with (1a), in agreement with the IC_{50}s. In terms of SAR, compounds with the free carbonyl unit in the 3-position, i.e. (1a)-(1c) gave the best results against the ABC-DLBCL cell lines. Since both (1a) and (1c) were more active than (1b), the *N*-benzyl unit might also be important in determining anti-tumor activity.

Table 1. Chemically modified oxindole derivatives and their IC_{50} values obtained in DLBCL cell lines. Compound names according to Burke *et al*., RSC Medicinal Chemistry 2022. IC_{50} values in μM.

Compound	DOHH2	VAL	OCI-LY-10	SU-DHL-2
(1a)	>20	>20	10	0.75
(1b)	>20	>20	7	15
(1c)	10	>20	5	0.75
(*S*)-(2a)	>20	>20	>20	>20
(*R*)-(2b)	>20	>20	>20	19
(*R*)-(2d)	>20	>20	>20	>20
(*S*)-(2f)	>20	>20	>20	>20
(*S*)-(2i)	>20	>20	>20	>20
(*R*)-(2j)	>20	>20	>20	>20

Conclusions: *In vitro* anti-tumor activity in ABC-DLBCL models was observed for specific *N*-1,2,3-triazole-isatin hybrids, indicating that their structures represent the starting point to design compounds with stronger activity. *AJB and FB: co-senior authors.*

Conflict of interest:
Ownership: Patent EP3400938

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The antimetabolite KAT/3BP has in vitro and in vivo anti-lymphoma activity

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Background: Reprogramming of cellular metabolism is one of the hallmarks of cancer (Hanahan, 2022), thus representing an important therapeutic target. 3-bromopyruvate (3BP or KAT/3BP) is a small, highly reactive molecule formed by the bromination of pyruvate (Ko et al, 2012). The very high similarity of its structure with pyruvic acid and lactic acid is the basis of its mechanism of action as an anti-cancer agent. Indeed, 3BP enters cancer cells via monocarboxylic acid transporters and it can then inhibit glycolysis and oxidative phosphorylation process. KAT/3BP has received FDA Orphan Drug Designation for different solid tumors and is about to enter the early controlled clinical evaluation. Here, we present *in vitro* and *in vivo* assessments of KAT/3BP in lymphoma models.

Materials and Methods: Cell lines were exposed to increasing concentration of KAT/3BP by MTT assay for 72 h. Apoptosis and cell cycle were evaluated by FACS. BALB/c mice (A20 cells in the left flank; 5 mice/group) were treated (4 weeks on, 1 off) with: oral (PO) and intratumoral (IT) vehicles, PO KAT/3BP [2.5 (low), 10 mg/kg (high)], IT KAT/3BP [0.5 (low), 2 mM (high)], PO-low/IT-low, PO-high/IT-high.

Results: KAT/3BP showed dose-dependent anti-proliferative activity in cell lines derived from diffuse large B-cell lymphoma (DLBCL; n = 8) and mantle cell lymphoma (MCL; 4), with median IC50 s of 8 μM and 5.5 μM, respectively. The compound was also tested in marginal zone lymphoma (MZL; 2) cell lines and in their derivatives with acquired resistance to idelalisib (2), ibrutinib (1), and copanlisib (1). 3BP was equally active in parental and resistant cell lines. KAT/3BP (5 μM, 72 h) was able to induce strong apoptosis in cell lines (1 DLBCL, 1 MCL) already after 24 h of treatment.

An *in vivo* pilot experiment using the murine syngeneic model (A20 lymphoma cells, BALB/c mice) confirmed the *in vitro* observed anti-tumor activity. All mice in the control groups died after nearly 20 days. A tumor reduction compared to the control vehicle was observed in all the treatment groups based on slope values extrapolated by a linear regression model. In particular, PO-high KAT/3BP lead to complete tumor reduction in 3/5 mice (2 still alive at D92, 1 dead at D36). After D26, also 1 mouse in IT-low and 1 in PO-low/IT-low survived and showed reduced tumor mass. Peripheral and focal tumor necrosis was seen in the tumor in PO-high/IT-high, PO-high, and IT-high (1 each). Necrosis was more extensively observed at histology in IT-high and PO-high/IT-high groups.

Conclusions: KAT/3BP showed *in vitro* activity in MCL, DLBCL, and MZL, including models resistant to PI3 K/BTK inhibitors. *in vivo* activity was also seen in a syngeneic mouse model. KAT/3BP induced apoptosis *in vitro* and necrosis *in vivo*.

*CT, FS: Equally contributed

Conflict of interest:
Advisory Board: Gilead, AbbVie, Janssen, AstraZeneca, MSD, BMS/Celgene, Roche, Mei Pharma, Astra Zeneca,
Celltrion Healthcare, Incyte, Kite/Gilead
Corporate-sponsored Research: Celgene, Roche, Janssen, Acerta, ADC Therapeutics, Bayer AG, Cellestia, CTI Life Sciences, EMD Serono, Helsinn, ImmunoGen, Menarini Ricerche, NEOMED Therapeutics 1, Nordic Nanovector ASA, Oncology Therapeutic Development, PIQUR Therapeutics AG, Gilead, AbbVie, Janssen
Other Substantive Relationships: Work supported by Ko Discovery LLC.

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Transcriptome and computational analysis assess the anti-tubulin activity of [1,2]oxazole derivatives in lymphoma

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