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Investigations into novel antagonists of the chemokine receptor CXCR4 to prevent the migration of cancerous cells

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Introduction: Tumor metastasis is the cause of 90% of deaths in cancer patients[1]. One mediator of this is the chemokine family and their cognate receptors such as CXCR4, the most commonly overexpressed chemokine receptor on tumor cells[2]. The development of therapeutic CXCR4 antagonists could slow down the process of metastasis. The aim of this study was to investigate whether three novel CXCR4 antagonists, Ac-Arg-Ala-[Cys-Arg-Phe-Phe-Cys]-COOH (AZ3-2), Ac-Arg-Ala-[DCys-Arg-Phe-Phe-Cys]-COOH (AZ6-2)[3] and AZ9-2 are more effective at inhibiting CXCR4 than the licensed CXCR4 antagonist AMD3100/Plerixafor.

Method: Chemotaxis: Two leukemic cell lines, Jurkat and THP-1, were harvested and incubated with either AZ3-2, AZ6-2, AZ9-2 or AMD3100. 25-50 x 10^4 cells were loaded onto a chemotaxis plate and stimulated with 1-5 nM CXCL12 for 5 hours. Migrating cells were counted using a haemocytometer. Calcium release assay: Jurkat, THP-1 and the breast cancer cell line MCF-7 cells were harvested and loaded with 1 µM of either AZ3-2, AZ6-2 or AMD3100 and 4 µM Fura-2AM at 37°C for 30 minutes. Cells were resuspended at a density of $2x10^6$ cells/mL and analysed using a BMG Labtech Flurostar OPTIMA fluorometer. MTS assays: Jurkat cells were seeded into 96 well plates at a density of $5x10^5$ cells/mL and incubated with a range of different concentrations of the test compounds for 72 hours and assessed using MTS reagent (Promega). Analysis was performed using one-way AVONA using GraphPad Prism.

Results: AZ3-2, AZ9-2 and AMD3100 (at 1000 nM and 100 nM) significantly blocked cellular migration in both THP-1 and Jurkat cell lines (n=3/4), while AZ6-2 caused significant reduction in cellular migration at much lower doses (0.01 nM to 1000 nM) (n=4, Fig. 1, Table 1). Additionally, calcium flux assays demonstrated that AZ6-2 and AMD3100 significantly inhibited calcium release in Jurkat and MCF-7 cells but AMD3100 does not block calcium release in THP-1 cells. These results are not due to the compounds exhibiting any cell toxicity, as determined by cell proliferation assays in Jurkat cells.

Table 1: EC50 values using two leukemic cell lines and four CXCR4 antagonists. Data given as mean± SEM

Peptide	Cell Line	
	Jurkat	THP-1
AZ3-2	42.47 nM ± 36.20	
AZ6-2	0.28 nM ± 0.18	0.56 nM ± 0.19
AZ9-2		19.19 nM ± 17.82
AMD3100	11.14 nM ± 6.86	59.47 nM ± 57.33

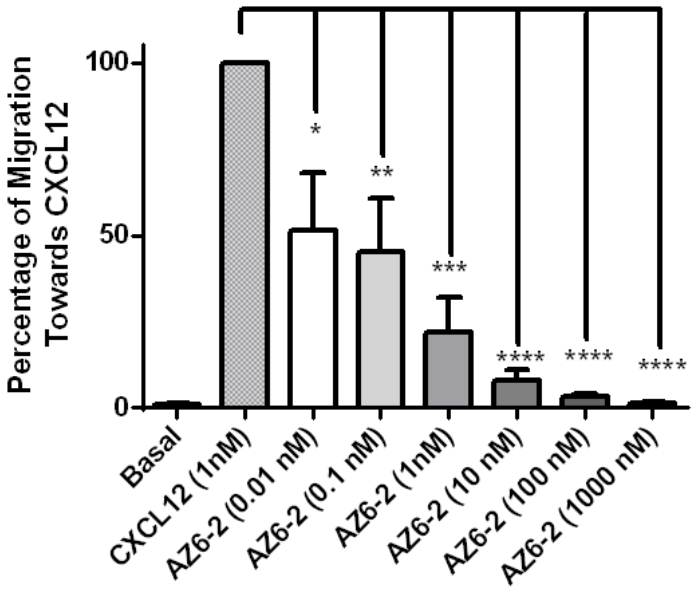

Fig. 1. Effect of AZ6-2 upon Jurkat cellular migration.

Conclusion: All four compounds caused a decrease in cellular migration with only the novel CXCR4 antagonist, AZ6-2 inhibiting calcium release across all cell lines.

References:

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2. Salazar N et al .(2013). Crit Rev Eukaryot Gene Expr **23**(1).
3. Di Maro S et al. (2016). J Med Chem **59**(18): 8369-8380.