METHANETHIOSULFONATE DERIVATIVES AS LIGANDS OF STAT3-SH2 DOMAIN

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Introduction

Inflammatory conditions in selected organs increase the risk of cancer. Compounds of the inflammatory tumor microenvironment include leukocytes, cytokines, complement components, and are orchestrated by transcription factors, such as **STAT3** (Signal Transducer and Activator of Transcription 3) and **NF-kB**. Two main approaches have been explored to inhibit STAT3 signalling:

- ***indirect*,** inhibiting the upstream tyrosine kinases that are responsible for STAT3 activation or blocking factors such as JAK, Src, Bcr-Abl, FLT3 and EGFR that are involved in the activation of STAT3 signalling, inducing tumour-cell apoptosis but it is poor selective.
- ***direct*,** by interaction of small molecules with the protein. In this selective approach the starting point is the crystallographic structure of **STAT3-SH2** domain.

Objectives

S-methylmethanethiosulfonate has been shown to inhibit colon tumor incidence when administered to rats during the post-initiation phase of carcinogenesis [**1**]. Recently, a new methanethiosulfonate derivative of valproic acid (**ACS33**) was reported by some of us to show good *in vitro* antiproliferative activity and to inhibit *in vivo* the growth of PC3 in subcutaneous xenograft mice models [**2**].

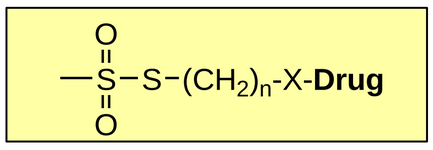


Fig.1: Structure of the studied thiosulfonate hybrids.

Since the influence of methanethiosulfonates on STAT3 activity has not been yet studied, we decided to synthesize a set of thiosulfonate-drug hybrids (**Fig.1**) and to submit them and their parent compounds to the **AlphaScreen-based assay**, to investigate their **ability to inhibit the binding of STAT3-SH2 domain** to its phosphopeptidic ligand [**3**] Moreover, in order to check the selectivity of our molecules, we also decided to test their ability to bind SH2 domain of STAT1, which exhibits a high degree of sequence homology to STAT3.

Method

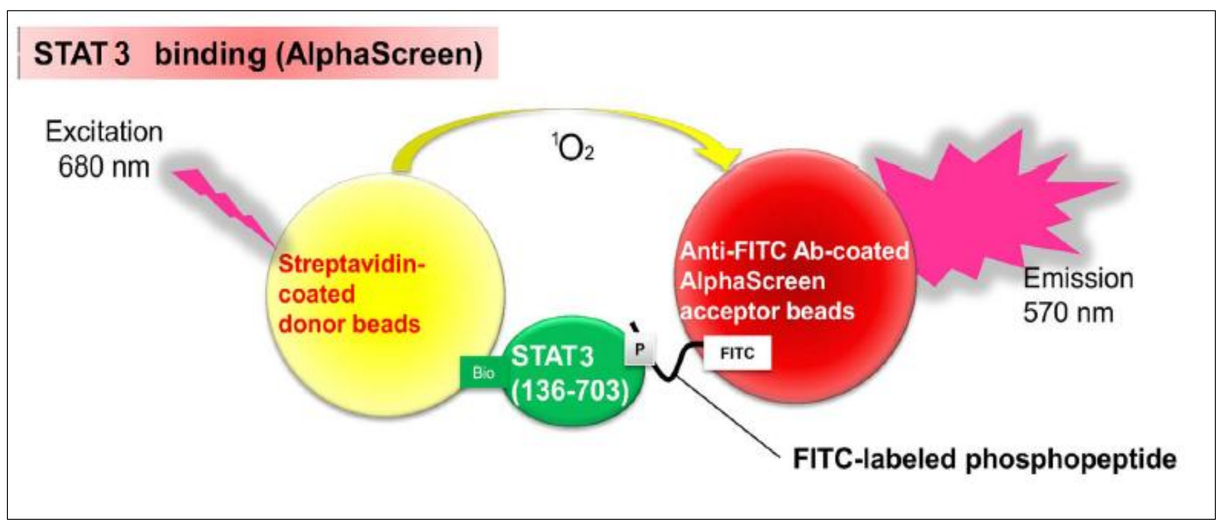


Fig.2: Schematic diagram of the multiplexed Alpha assay for STAT3 [**3**].

AlphaScreen technology is capable of analyzing protein-protein or protein-peptide interactions and it is an useful method to detect the interactions between the SH2 domain and the pTyr-containing peptide. This test is bead-based non-radioactive assay system for detecting biomolecular interactions in a microlitre plate format. In details sandwich antibody complexes are captured by AlphaScreen **donor** and **acceptor beads** (**Fig.2**), bringing them into close proximity: the excitation of the donor bead provokes the relase of a singlet oxygen molecules that triggers a cascade of energy transfer in the acceptor bead, resulting in a fluorescent signal between 520 and 620 nm.

Results and Conclusions

Most of the synthesized thiosulfonate-hybrids are able to strongly and selectively bind STAT3-SH2 domain (Table 1), whereas the parent drugs were completely devoid of this ability (Table 2).
Studies are ongoing to better define the profile of our new methanethiosulfonate derivatives as potential dual STAT3/NF-kB inhibitors.

Table 1: *In vitro* AlphaScreen assay results for the new synthesized compounds.

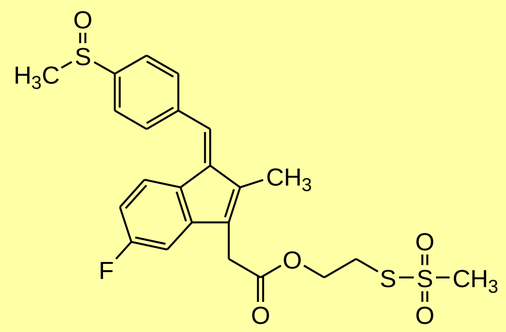
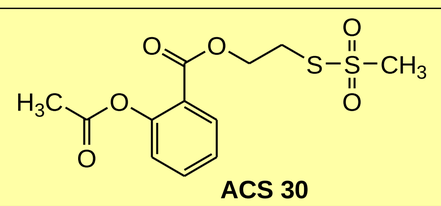
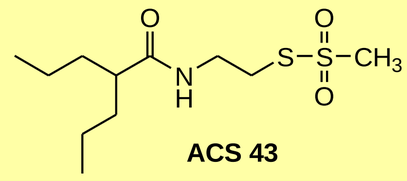
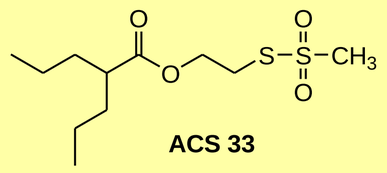
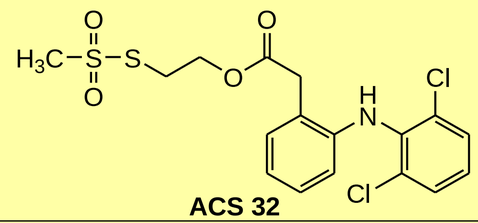
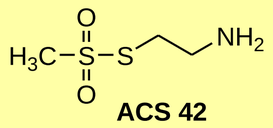
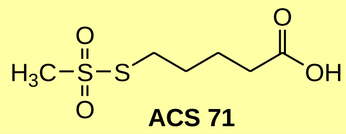
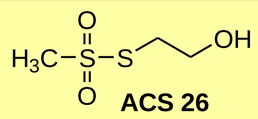
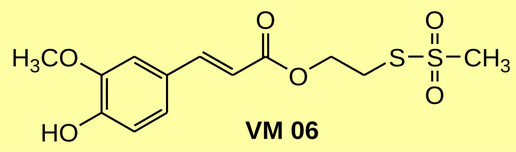
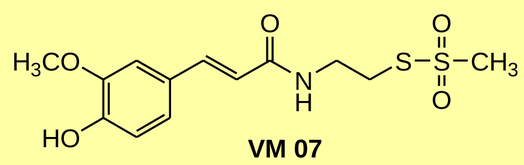
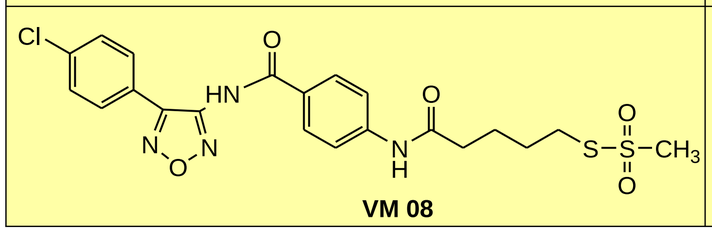
Compound	*STAT3* IC_{50} (μM) ±SD	*STAT1* IC_{50} (μM) ±SD
 ACS 27	0.5 ± 0.1	>3
 ACS 30	1.4 ± 0.1	3.6 ± 0.0
 ACS 43	1.4 ± 0.1	>3
 ACS 33	0.7 ± 0.1	>3
 ACS 32	0.5 ± 0.2	>3
 ACS 42	>3	>3
 ACS 71	4.7 ± 1.1	>3
 ACS 26	>30	>30
 VM 06	0.9 ± 0.0	7.0 ± 0.7
 VM 07	1.8 ± 0.5	>30
 VM 08	1.2 ± 0.1	13.4 ± 0.8

Table 2: *In vitro* AlphaScreen assay results for parent compounds, expressed as **inhibition %** .

Parent Compound (30 μM)	Inhibition % ± SD	
	STAT3	STAT1
Sulindac	4.2 ± 3.2	4.9 ± 2.0
Aspirin	3.8 ± 1.9	6.3 ± 1.7
Valproic Acid	0.2 ± 3.0	2.7 ± 3.0
Diclofenac sodium salt	4.5 ± 2.4	6.5 ± 2.1
Ferulic Acid	0.2 ± 8.2	n.t.

References

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3. Takakuma, K.; Ogo, N.; Uehara, Y.; Takahashi, S.; Miyoshi, N.; Asai, A. "Novel multiplexed assay for identifing SH2 domain antagonists of STAT family proteins" *PLOS ONE* **2013**, *8*, 1-11.

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