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Small Molecule Analogues of the Immunomodulatory Protein, ES-62: Anti-inflammatory Compounds and Mechanism of Action

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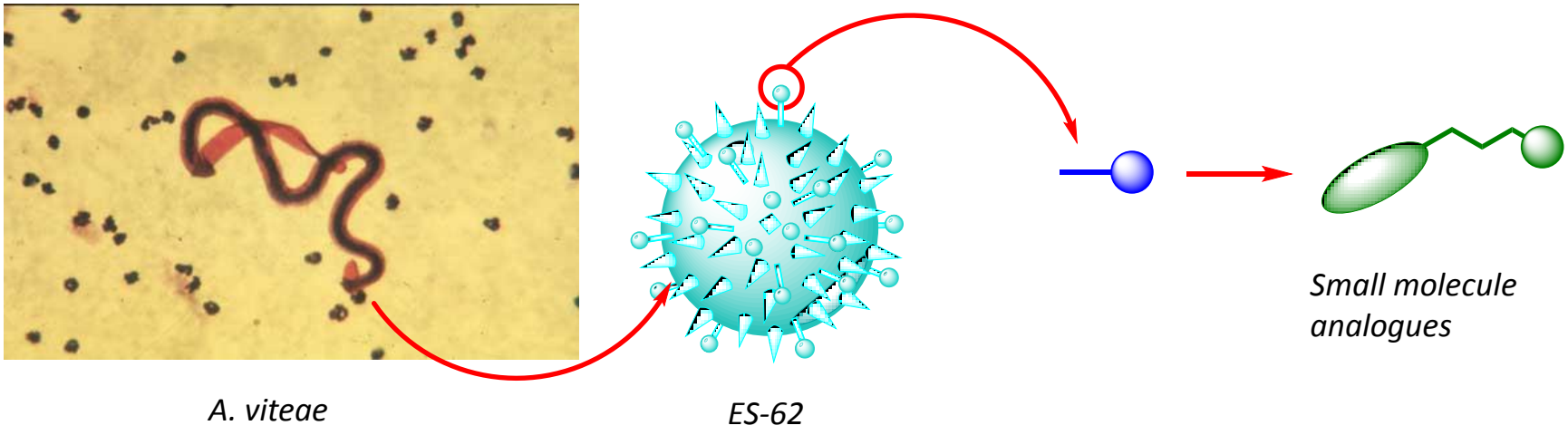
Small Molecule Analogues of the Immunomodulatory Protein, ES-62: Anti-inflammatory Compounds and Mechanism of Action

Graphical Abstract



ES-62: An immunomodulatory protein secreted by the filarial nematode, *Acanthocheilonema viteae*. Activity is dependent on surface-expressed covalently-bound phosphorylcholine, the 'warhead'.

Abstract: ES-62 is a protein secreted by the parasitic filarial nematode, *Acanthocheilonema viteae*, and is a highly effective immunomodulator. It interferes with the pro-inflammatory responses of a number of immune system cells. ES-62 has protective effects in a number of mouse models of inflammatory disease, e.g. collagen-induced arthritis, ovalbumin-induced airway-hyper-responsiveness, oxazolone-induced skin hypersensitivity, the MRL/Lpr model of systemic lupus erythematosus, and the *Gld*.ApoE$^{-/-}$ model of accelerated atherosclerosis in lupus. The biological activity of ES-62 depends upon post-translational attachment of phosphorylcholine (PC) to an *N*–type glycan. To obtain therapeutic effects not accessible to a protein, a library of small molecule analogues (SMAs) based upon the active PC but containing chemically stable substitutes for the phosphate ester were prepared. Phosphonates, sulfones and sulfonamides were all investigated of which the sulfones had the most favourable immunomodulatory properties. Sulfone-containing SMAs have since been found to be effective in treating mouse models of inflammatory diseases noted above. Here potential mechanisms of action, including receptor binding and various downstream signalling modifications are considered. Blocking essential interactions of the scaffolding protein MyD-88 with its partners is found to reduce the effect of TLR4 activation which in turn leads to a reduction in the release of pro-inflammatory cytokines from the targeted cells. In another context, downregulation of inflammasome activity suggesting a covalent engagement of an SMA with Keap regulatory protein has emerged as a possibility.

Keywords: immunomodulators, inflammation, sulfones

ES-62 – an immunomodulatory protein from a parasitic worm

This study describes some of the properties of small molecule drugs based upon the structure and properties of ES-62, a tetrameric glycoprotein secreted by the parasitic worm, *Acanthacheilonema viteae.* ES-62 interacts directly with cells of the immune system to inhibit cell signalling pathways. It does this by making atypical use of the receptor TLR4 and thereby causing degradation of certain signalling scaffold molecules such as MyD88 and enzymes such as PKCs.

The activity of ES-62 is dependent on phosphorylcholine (PC) moieties covalently attached to an *N*-type glycan that is post-translationally added to the protein. The net effect of ES-62 is the induction of an anti-inflammatory immunological phenotype, for example, the inhibition of pro-inflammatory cytokine production in antigen-presenting cells (APCs) and mast cells.

Based upon this property, several studies have shown that ES-62 is protective in certain mouse models of inflammatory diseases. In autoimmune disease, the following have been successful:

Collagen-induced arthritis (CIA)

MRL/Lpr model of systemic lupus erythematosus

Gld.ApoE$^{-/-}$ model of accelerated atherosclerosis in lupus

In allergic disease, the following have been successful:

Acute airway hypersensitivity

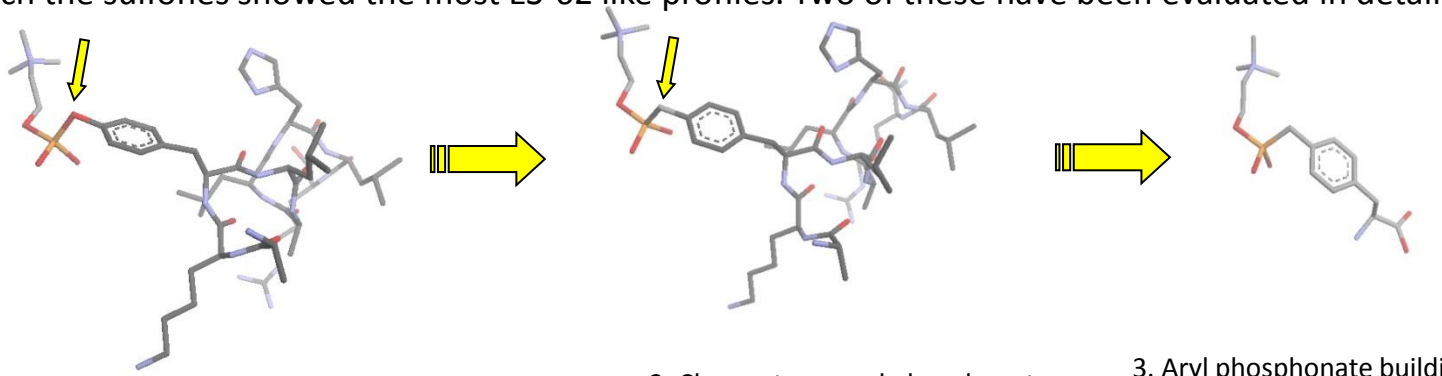
Chronic airway hypersensitivity

Oxazolone-induced skin hypersensitivity

Reference: Pineda, M.A. et al. **2014**, *Mol. Biochem. Parasitol. 194,* 1-8.

Origin of SMAs – a peptide with ES-62-like activity

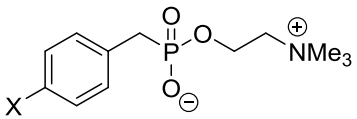
Being a protein and available only in very small quantity, ES-62 is not itself suitable for use as a drug, despite its remarkable properties. Small molecule analogues (SMAs) of ES-62 were therefore devised based upon the structure of a PC-bearing peptide that had some of the properties of ES-62. PC attached to tyrosine provided the basis for the design of the SMAs as shown below. Several structural types were built from this design of which the sulfones showed the most ES-62 like profiles. Two of these have been evaluated in detail extensively.

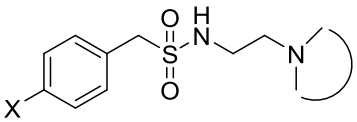


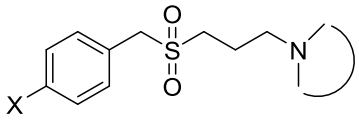
1. Peptide with some ES-62 like activity as template. Contains an aryl phosphate - relatively unstable

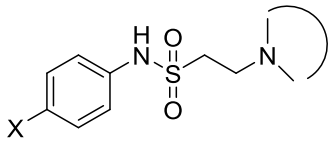
2. Change to an aryl phosphonate - relatively stable

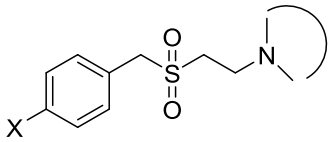
3. Aryl phosphonate building block – leads to several derivatives





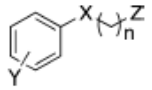






Results and discussion

Table 1. Modulatory Effect of the Library of Small Molecule Analogues on TLR-Dependent Cytokine Production[a]



(A) phosphonates and phosphates

SMA	X	Y	*n*	Z	LPS IL-12	LPS IL-6	BLP IL-12	BLP IL-6	CPG IL-12	CPG IL-6
5a	$CH_2PO_3^-$	4-Br	2	NMe_3^+	↓	↑	↓			
5b	$CH_2PO_3^-$	4-Br	2	NMe_2		↑				↑
5c	$CH_2PO_3^-$	4-Me	2	NMe_2	↑					
5d	$CH_2PO_3^-$	4-NO_2	2	NMe_3^+	↑	↑				
5e	$CH_2PO_3^-$	4-Me	2	NMe_2	↑		↑			
6	OPO_3^-	4-BOCNH	3	NMe_2		↑				↑

(B) sulfones

SMA	X	Y	*n*	Z	LPS IL-12	LPS IL-6	BLP IL-12	BLP IL-6	CpG IL-12	CpG IL-6
11a	CH_2SO_2	4-Br	2	Me_2N	↓	↓	↓		↓	↓
11b	CH_2SO_2	4-Me	2	Me_2N	↓	↓				
11c	CH_2SO_2	4-Me	2	pyrrol	↓	↓	↓	↓		
11d	CH_2SO_2	4-Br	2	pyrrol	↓					
11e	CH_2SO_2	3-F	2	NMe_2	↑	↑				
11f	CH_2SO_2	3-F	2	diam						
11g	CH_2SO_2	3-F	2	morph			↑			
11h	CH_2SO_2	3-F	2	pyrrol		↑	↓			
11i	CH_2SO_2	4-F	2	NMe_2			↓	↓		
11j	CH_2SO_2	4-F	2	morph		↓	↑	↑		
11k	CH_2SO_2	4-F	2	pyrrol	↑		↓			
11l	CH_2SO_2	4-F	2	diam			↓			
11m	CH_2SO_2	3-F	2	*S*-2-Me-but						
11n	CH_2SO_2	3-F	2	*R*-2-Me-but			↓	↓		
11o	CH_2SO_2	4-Br	2	*S*-2-Me-but			↓			
11p	CH_2SO_2	4-Br	2	*R*-2-Me-but						
12a	CH_2SO_2	4-Br	2	Me_3N^+						
12b	CH_2SO_2	4-Me	2	Me_3N^+	↓		↓		↓	↓

Pro-inflammatory compounds

Anti-inflammatory compounds

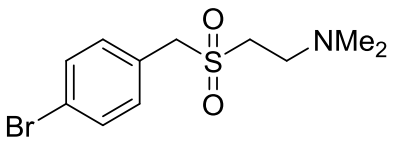
Mixed behaviour

Levels of cytokine output were measured by ELISA. Arrows indicate respectively up or downregulated levels.
J. Med. Chem. **2013**, *56*, 9982-10002. **11a** and **12b** showed the most ES-62 like profiles.

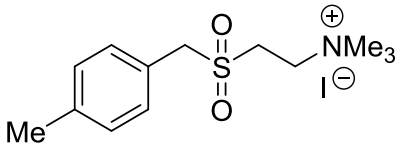
ES-62/SMA *in vivo* disease model activity summary

ES-62 SMAs are effective in

- Collagen-induced arthritis (CIA)
- MRL/Lpr model of systemic lupus erythematosus
- Acute airway hypersensitivity
- Chronic airway hypersensitivity
- Oxazolone-induced skin hypersensitivity



S3 or 11b



S5 or 12b

Both curative and prophylactic activity has been found typically at doses of ~50 μg/kg in mice.

ES-62 SMAs are not effective in

- NOD mouse model of type 1 diabetes
- EAE model of multiple sclerosis
- DSS models of inflammatory bowel disease

References to some biological properties of ES-62 SMAs

1. Designing Anti-inflammatory Drugs from Parasitic Worms: A Synthetic Small Molecule Analogue of the Acanthocheilonema viteae Product ES-62 Prevents Development of Collagen-Induced Arthritis L. Al-Riyami et al., *J. Med. Chem.* **2013**, *56*, 9982-10002.
2. Small molecule analogues of the immunomodulatory parasitic helminth product ES-62 have antiallergy properties. J. Rzepecka, et al., *Int. J. Parasitol*, **2014**, *44*, 669-674.
3. Prophylactic and therapeutic treatment with a synthetic analogue of a parasitic worm product prevents experimental arthritis and inhibits IL-1β production via NRF2-mediated counter-regulation of the inflammasome, J. Rzepecka et al., *J. Autoimmunity*. **2015**, *60*, 59-73.
4. The parasitic worm-derived immunomodulator, ES-62 and its drug-like small molecule analogues exhibit therapeutic potential in a model of chronic asthma J. C. Coltherd, et al., *Scientific Reports* **2015**, *5*, 19224.
5. Protective effect of small molecule analogues of the *Acanthocheilonema viteae* secreted product ES-62 on oxazolone-induced ear inflammation. L. Al-Riyaami et al., *Experimental Parasitology,* **2015**, doi:10.1016/j.exppara.2015.03.025.
6. Drug-like analogues of the parasitic worm-derived immunomodulator ES-62 are therapeutic in the MRL/Lpr model of systemic lupus erythematosus. D. Rodgers, et al., *Lupus* **2015**, *24*, 1437–1442.
7. Testing Small Molecule Analogues of *Acanthocheilonema viteae* immunomodulator ES-62 against clinically relevant allergens L. Janicova et al., *Parasite Immunology,* **2016**, DOI: 10.1111/pim.12322
8. Dendritic cells provide a therapeutic target for synthetic small molecule analogues of the parasitic worm product, ES-62 F. E. Lumb, et al.,*Scientific Reports*, **2017**, 17, 1704

Properties of S3 and other SMAs

The medicinal chemical parameters associated with **S3**/**11a** suggest no problems for developability.

Calculated values for S3/11a and (ranges) for compounds studied

tPSA: 37 (37 – 98); cLogP: 1.84 (1.1 – 4.7); pK_a: 7.8 (5.6 – 7.8); MW: 306 (240 – 380)

Safety indications

No liabilities were observed at 10 μM for **S3**/**11a** in the *Cerep Safetyscreen 44*
S3/**11a** has no effect on cell viability, as determined using 7-AAD staining, at ~15 μM.
hERG inhibition > 25 μM

Pharmacokinetic parameters for S3/11a (Cyprotex)

Mouse hepatocyte stability CL_{int} 269 (mL/min/10^6cells)
$t_{1/2}$ (mouse) 5.2 min
Plasma stability (mouse) ~ 90% @ 120 min.
Protein binding (mouse) 55.4% unbound

Metabolic properties – CYP inhibition

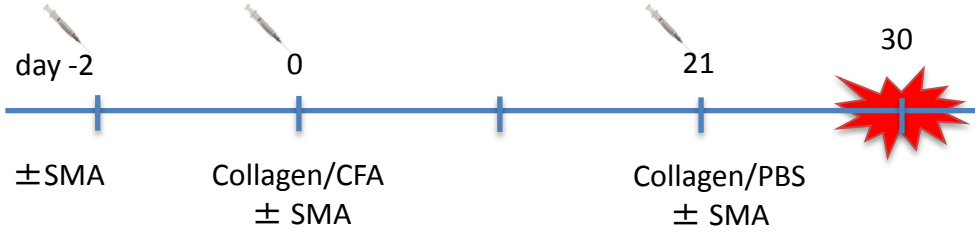
CYP3A4 >25 μM; CYP2D6 >25 μM; CYP2C13 10 μM; CYP2C3 >25 μM; CYP1A >25 μM

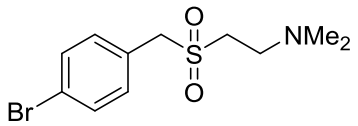
Comments

These properties are all encouraging although the half-life is short. Bearing in mind the effectiveness in *in vivo* models, this appears not to be a problem, a fact that is consistent with the possible actions of SMAs as triggers stimulating the rebalancing of the immune system. With such a mode of action, they would not require continuous target site occupation as is found with most drugs. Nevertheless, optimisation is still necessary.

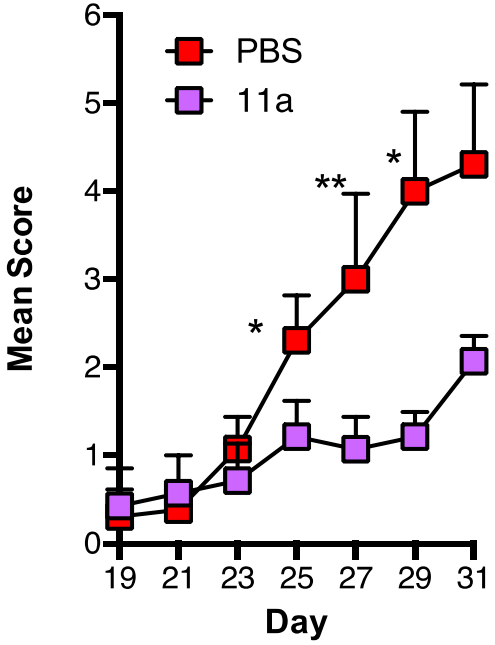
ES-62-based SMAs suppress CIA

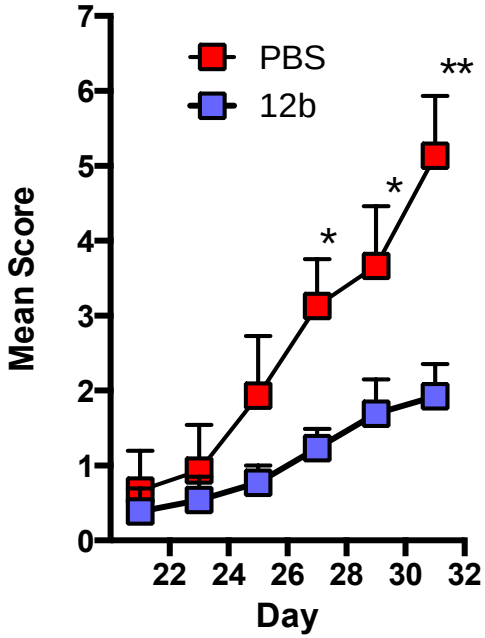
In this model mice are sensitised and challenged with collagen and the effect of the SMAs on arthritis development in paws measured.

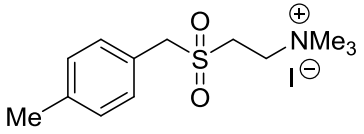




S3 or 11b

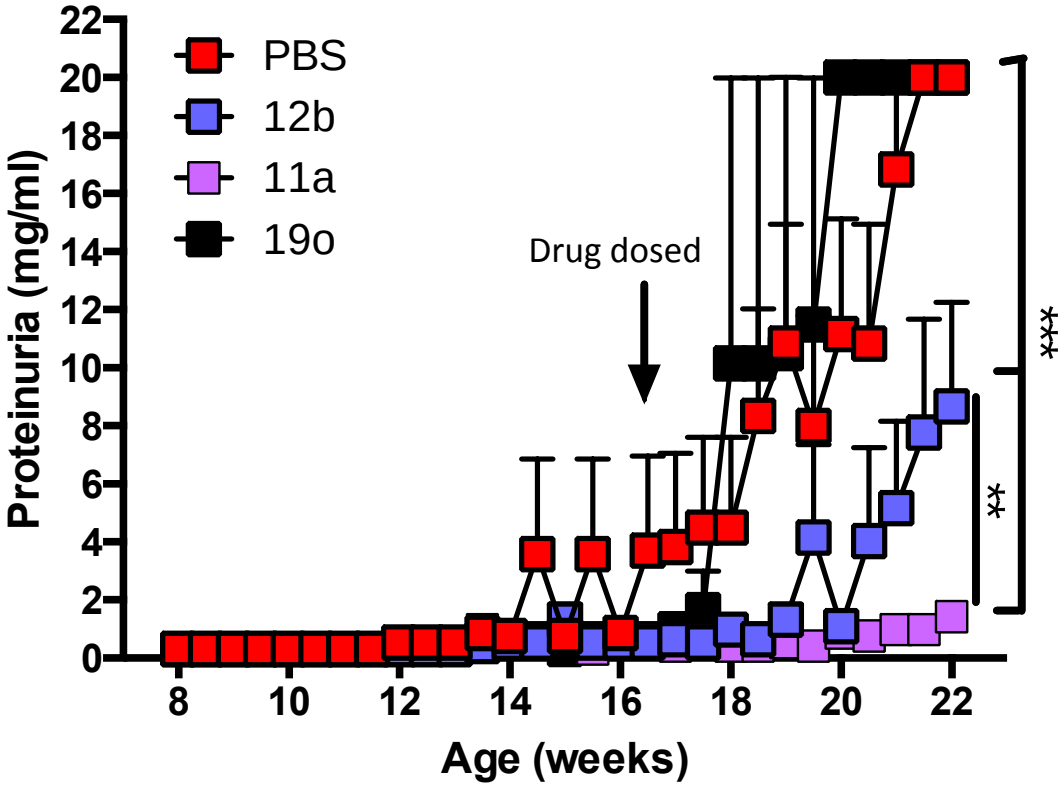


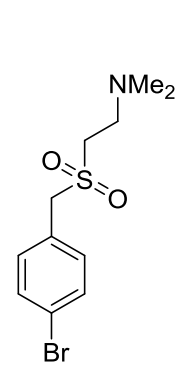




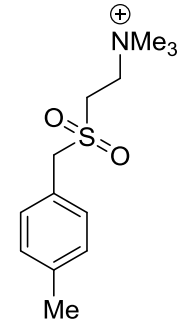
S5 or 12b

11a and 12b suppress lupus pathology after disease onset in MRL/*lpr* mouse

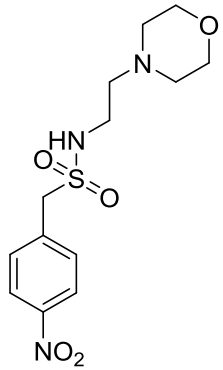




11a = S3



12b = S5



19o

Convincing evidence that the sulfone class of SMA (**11a** and **12b**) is active but the sulfonamide class (**19o**) is inactive in an *in vivo* model of lupus erythematosus. **11a** virtually completely suppresses the pathology of disease as measured by proteinuria (> 1 mg/ml) onset at a dose of 1 μg. Renal MyD88 was also substantially reduced for both **11a** and **12b** treatment. These results are also relevant to the molecular mechanism of action discussed below.

Some possibilities for mechanism of action

Cumulative biological evidence shows that SMAs **11a** and **12b** produce similar responses *in vitro* and *in vivo* to those produced by ES-62. Since ES-62 seems to operate in several ways the same is probable for the SMAs. The following can be considered:

1. Direct binding to a TLR?

The natural ligands for TLRs (lipoproteins and nucleotides, for example) are very different in chemical character from the SMAs. At first sight this possibility seems unlikely.

2. *Interference with TLR signal transduction in the TLR protein cluster?*

TLRs, like all cell surface receptors are coupled with intracellular proteins in a functional cluster that leads to the change in conformation of the intracellular protein mediators. Protein-protein interactions are crucial to these effects. Whilst few small molecule drugs have been successfully designed to interfere with protein-protein interactions, the many interfaces in the TLR cluster suggest that this possibility myst be considered.

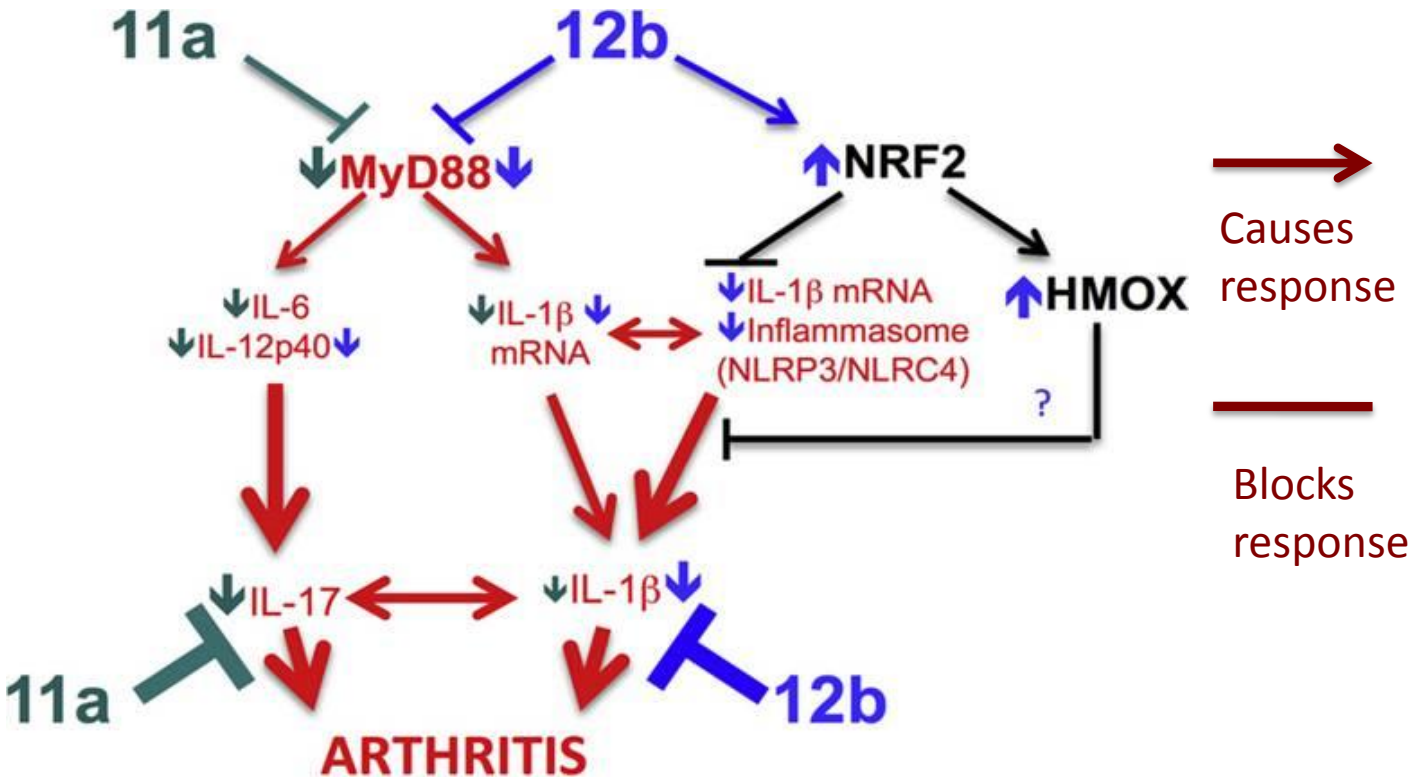
3. Interference with a different downstream event?

Protein clusters, typically of kinases, can be found downstream in the cascade initiated by TLR4 action. They too could be targets for SMAs by the same arguments as for 2 above.

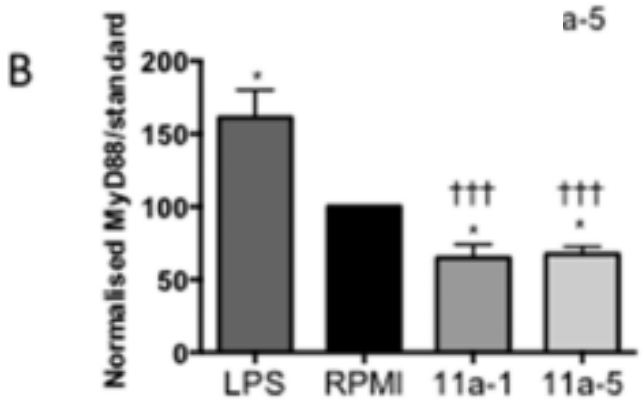
The following slides provide experimental evidence for two mechanisms: MyD88 binding inhibition and modulation of the inflammasome by perturbing NrF2/Keap interactions.

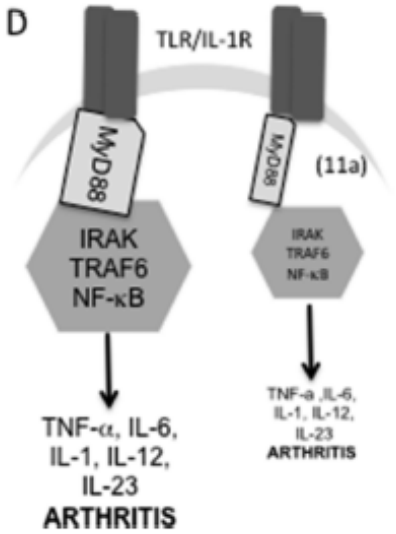
The case of arthritis

Biological evidence from the accumulated studies is consistent with the flow chart below. Both SMAs degrade MyD88 to inhibit inflammatory signaling with resultant, but somewhat different, effects on downstream cytokine production. **11a** tends to target IL-17 and **12b**, IL-1β: inhibiting production of either cytokine, reduces arthritis.



Molecular mechanism? MyD88 levels are lowered by 11a





Bone marrow macrophages (BmMs) treated with medium (RPMI), **11a** at 1 and 5 µg/mL (11a-1 or 11a-5), or LPS (100 ng/mL) for 20 h were analyzed by Western blotting for expression of MyD88 and loading control GAPDH. Levels of expression were determined by densitometry using Image-J software and expressed as the ratio of MyD88:GAPDH and normalized to the RPMI value.

(B) Quantitative Western Blot data from six analyses revealed that while LPS significantly increased MyD88 expression (*$p < 0.05$), both 11a-1 and 11a-5 reduced it (*$p < 0.05$) relative to the RPMI control. In addition, the level of MyD88 in cells treated with either 11a-1 or 11a-5 was significantly different (†††$p < 0.001$) to that in those exposed to LPS.

(D) Simple schematic of model of action of **11a**. **11a** downregulates MyD88 expression and hence induces a partial uncoupling of TLR/IL-1R from NF-κB activation and consequent pro-inflammatory cytokine production that both initiates pathogenic IL-17-mediated inflammation and perpetuates chronic vascular permeability, inflammation, and pathology in the joints. Thus **11a**-mediated downregulation of MyD88 impacting at one or more of these sites provides a molecular mechanism for the protection afforded in CIA; see below for more detailed information.

MyD88 has been identified as a potential drug target

Published examples:

Loiarro, M., *et al.*. Targeting the Toll-like receptor/interleukin 1 receptor pathway in human diseases: rational design of MyD88 inhibitors. *Clinical lymphoma, myeloma & leukemia* **13**, 222-226, doi:10.1016/j.clml.2013.02.003 (2013).

Loiarro, M. *et al.* Mutational analysis identifies residues crucial for homodimerization of myeloid differentiation factor 88 (MyD88) and for its function in immune cells. *Journal of biological chemistry* **288**, 30210-30222, doi:10.1074/jbc.M113.490946 (2013).

Kissner, T. L. *et al.* Therapeutic inhibition of pro-inflammatory signaling and toxicity to staphylococcal enterotoxin B by a synthetic dimeric BB-loop mimetic of MyD88. *PLoS ONE* **7**, e40773, doi:10.1371/journal.pone.0040773 (2012).

Alam, S. *et al.* Structure-Based Design and Synthesis of a Small Molecule that Exhibits Anti-inflammatory Activity by Inhibition of MyD88-mediated Signaling to Bacterial Toxin Exposure. *Chemical biology & drug design* **86**, 200-209, doi:10.1111/cbdd.12477 (2015).

Kissner, T. L. *et al.* A small molecule that mimics the BB-loop in the Toll interleukin-1 (IL-1) receptor domain of MyD88 attenuates staphylococcal enterotoxin B-induced pro-inflammatory cytokine production and toxicity in mice. *The Journal of biological chemistry* **286**, 31385-31396, doi:10.1074/jbc.M110.204982 (2011).

Li, J., Wang, X., Zhang, F. & Yin, H. Toll-like receptors as therapeutic targets for autoimmune connective tissue diseases. *Pharmacology & therapeutics* **138**, 441-451, doi:10.1016/j.pharmthera.2013.03.003 (2013).

Olson, M. A. *et al.* Discovery of small molecule inhibitors of MyD88-dependent signaling pathways using a computational screen. *Scientific reports* 5, 14246, doi:10.1038/srep14246 (2015).

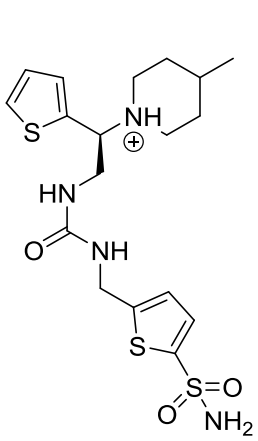
The last citation provided the basis for a collaborative evaluation of the possibility that MyD88 itself might be one of the molecular targets for ES-62.

Computational screening provides a way in

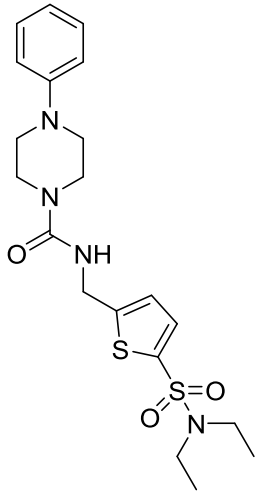
Two of us (Olson and Sheikh) and their colleagues have investigated small molecule inhibitors ("T-series" inhibitors) using virtual screening of a compound library containing 5 million members via binding to a protein-protein dimeric docking model of the TIR-domain of MyD88. From a first generation hit compound (T5910047, see next slide), others were identified from the PubMed database with more drug like properties and the most active compound (T6167923) evaluated in a range of immunological and biochemical assays relevant to MyD88 function, which indicated that it exhibited its anti-inflammatory actions via inhibition of MyD88 homodimerisation. The similarity of these T-series compounds in terms of immunological profile (reducing the release of pro-inflammatory cytokines from stimulated cells) with that of the SMAs of ES-62 together with a number of common structural features with the SMA library suggested that it was possible that the active SMAs, **11a** and **12b,** by binding to the same or nearby sites on the MyD88 surface, might also disrupt its dimerisation. The 'active' SMAs, **11a** and **12b**, and the 'inactive' SMA **19o** were therefore examined initially as ligands for MyD88 *in silico*.

A (next but one slide) shows the docking of molecule T5910047 in two different binding poses and the overall top-ranked scores from Vina Δ*G* and the computed *PC-Score*. The T5910047 score was used as a benchmark for assessing the three ES-62 SMA compounds. The two binding poses of T5910047 illustrated in **A** are nearly indistinguishable in terms of scoring and are given by Vina Δ*G* = -6.4 kcal/mol and *PC-Score* = -9.2 kcal/mol. For the three SMAs, docking successfully sampled favourable binding modes on the MyD88 model, although unlike T5910047 and T6167923, docking populated the three binding sites (**B**). There were some similarities observed at functional group level between the SMAs and T-series compounds. Figure **D**, for example, shows the docking of **11a** in a binding pose where the sulfone functional group is recognized by the same binding pocket (site-1) as T5910047. The *PC-Score* = -8.9 kcal/mol and is slightly less favorable than that for T5910047 (-9.2 kcal/mol). Of the three SMAs, **12b** scored most favourably in *PC-Score* (-10.4 kcal/mol), even performing better than T5910047 and T6167923.

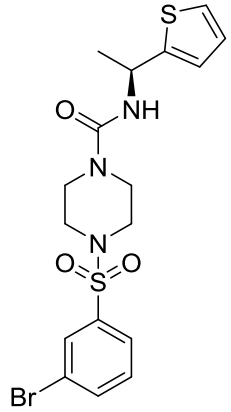
Sulfones and sulfonamide look superficially similar



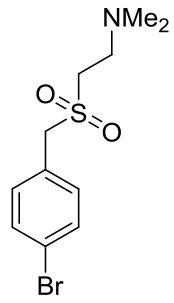
T5910047



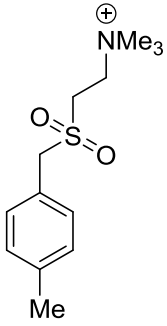
T5996207



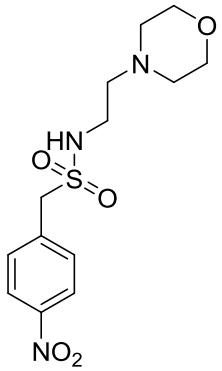
T6167923



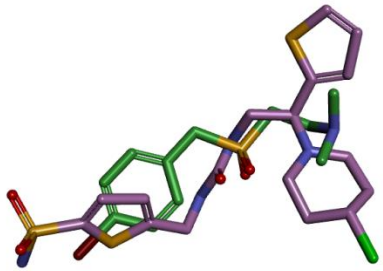
11a = S3



12b = S5

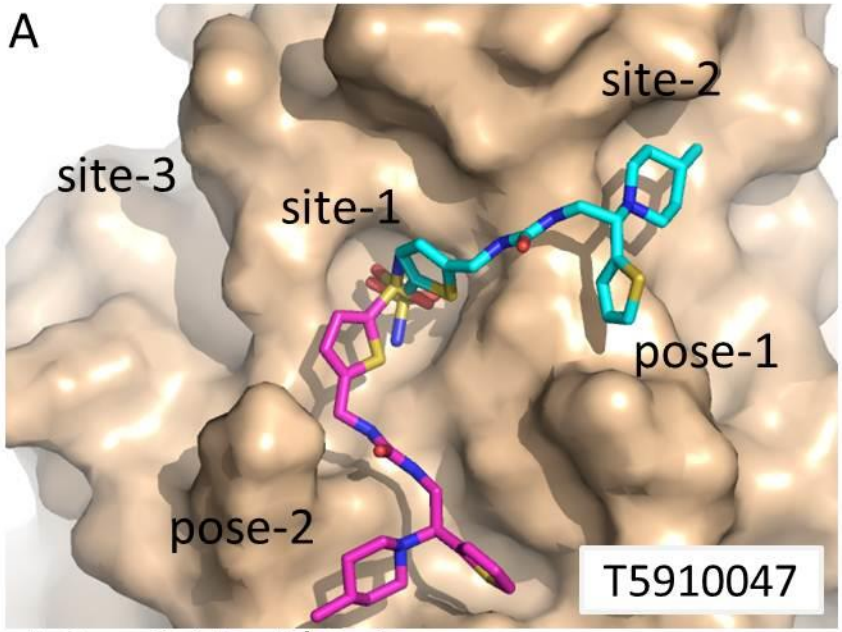


19o

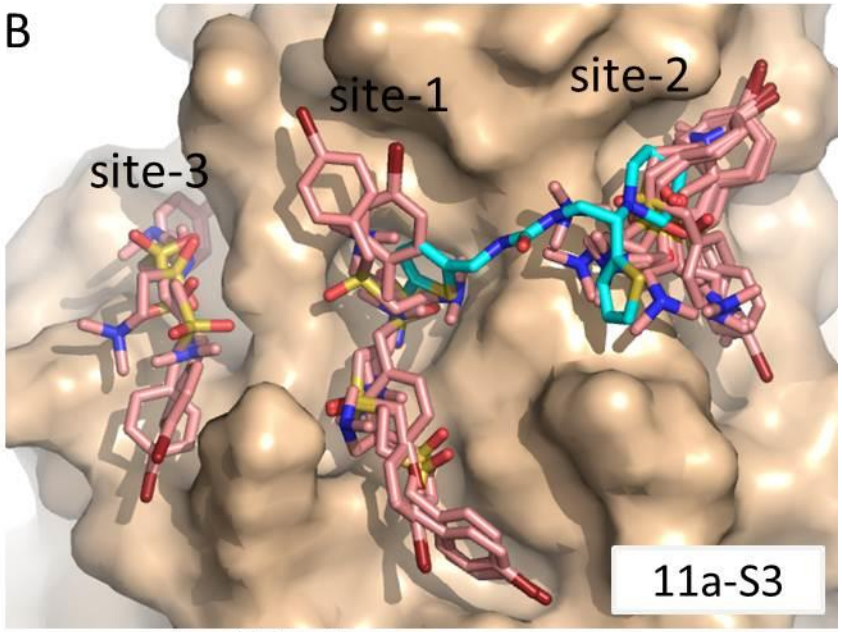


Overlay of **T5910047** (magenta backbone) and **11a** (green backbone) shows that the sulfone and sulfonamide probably have different roles in protein binding and that the situation is more complex than at first sight.

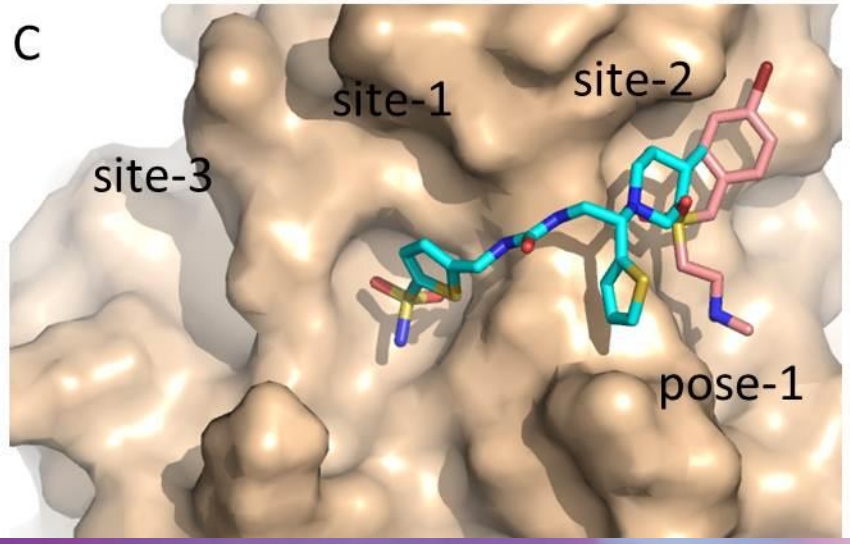
Docking with a MyD88 surface provides further information (next slide) and is consistent with the possibility that the SMAs act at least in part by inhibiting dimerisation of MyD88.

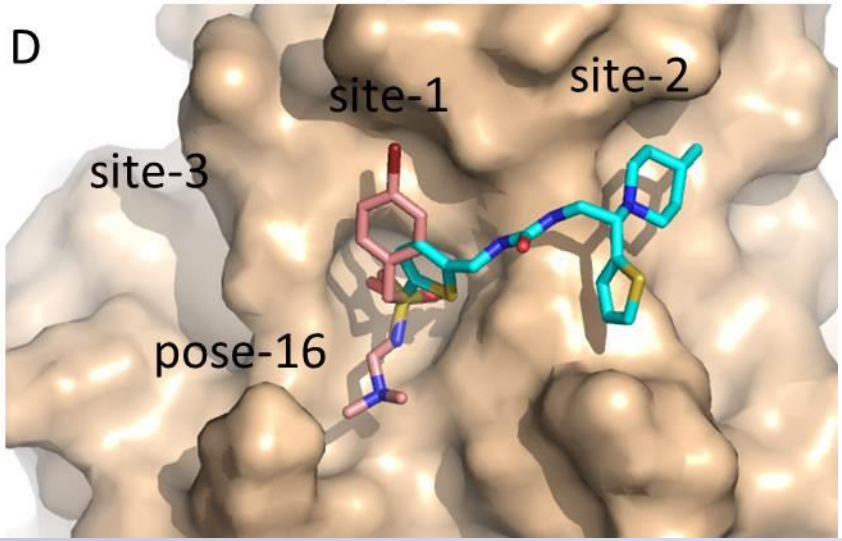


ΔG = -6.4 kcal/mol

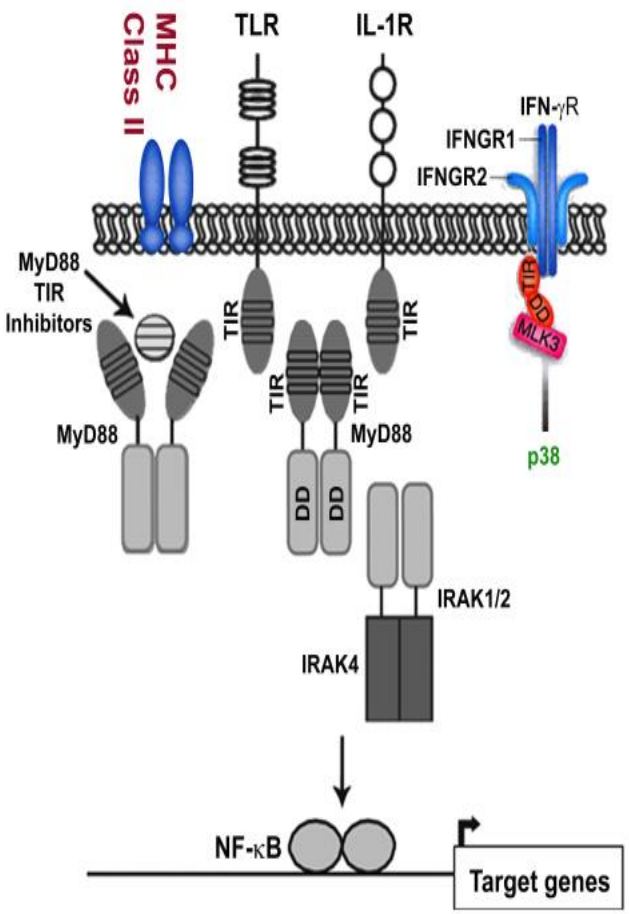


top-ranked binding poses





Consequences of MyD88 interference



A proposed model of small molecule inhibitor binding to TIR domain showing how it disrupts MyD88-mediated pro-inflammatory signaling.

Such inhibitors block the surface of the TIR domain preventing dimerisation to form the functional complex that then activates the next proteins in the cascade, IRAKs. The signal is then no longer passed further via IKKs (not shown) to reduce the activation of NFκB and the transcription of the pro-inflammatory genes that respond to NFκB. In the SMA series, **11a** and **12b** bind to an appropriate patch on the surface but **19o**, whilst evidently binding to a part of the surface not associated with dimerisation, causes no downstream effects. Inhibited MyD88 is degraded in the proteasome leading to its reduced levels as observed.

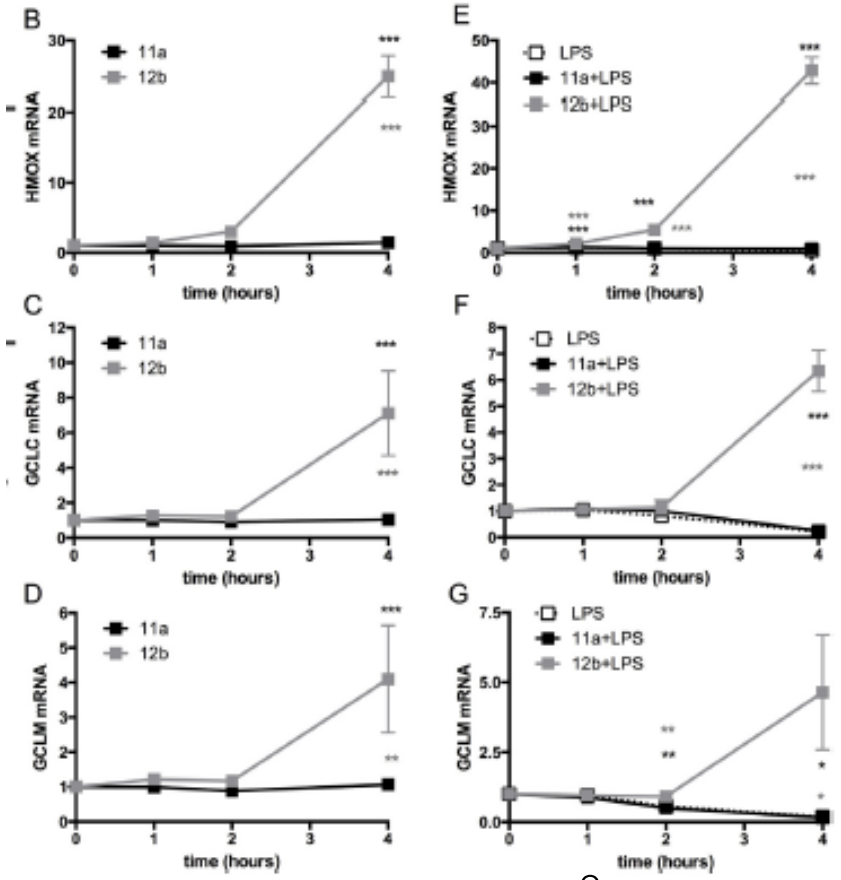
Regulation of inflammation – 11a compared with 12b

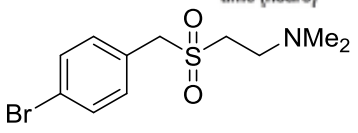
Many small molecules stimulate inflammation by disturbing the balance of expression of anti-oxidant supporting genes by disrupting the binding of the transcription factor, NRF2, to its normal partner, KEAP. (A Small-Molecule Inducer of the Antioxidant Response Element W. Hur, et al., *Chemistry and Biology* 2010, *17*, 537–547.)

12b causes an increase in NRF2 activity, which is associated with an anti-inflammatory response. A key question is how **12b** is able to promote NRF2 activation. Like **12b**, **11a** causes inhibition of NF-κB but it does not appear to activate NRF2. The major structural difference between **11a** and **12b** is that the latter is a quaternary ammonium salt as opposed to a tertiary amine, a difference that would be expected to have a substantial influence both on binding to receptors (through differences in hydrogen bonding ability and steric bulk) and on access to cells and cellular compartments (**12b** is permanently positively charged).
The 4-substituent on the benzene ring (bromo in **11a** and methyl in **12b**) is also significantly different, particularly in terms of size, and might also influence receptor binding. However the most plausible explanation for the difference in effect on NRF2 is that **12b** but not **11a** can be converted by β-elimination within the cell to a vinyl sulfone, a structure known to cause activation of NRF2 (see pdb 4czt and below).
The **12b**-derived vinyl sulfone, as an electrophile, could in theory interact with thiol groups on cysteine residues of the NRF2 repressor protein, Keap-1, which could cause a conformational change that would allow release of NRF2, and translocation to the nucleus to drive expression of target genes such as HMOX1 which, like NRF2 itself , has been shown to be protective against inflammatory arthritis. Evidence for such gene expression effects of **12b** is shown on the next slide and the mechanistic interpretation on the following slide.

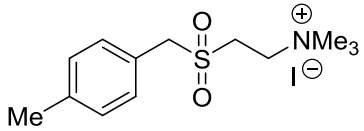
(Prophylactic and therapeutic treatment with a synthetic analogue of a parasitic worm product prevents experimental arthritis and inhibits IL-1β production via NRF2-mediated counter-regulation of the inflammasome, J. Rzepecka et al., *J. Autoimmunity*. **2015**, *60*, 59-73.)

12b upregulates mRNA levels of antioxidant genes





11b



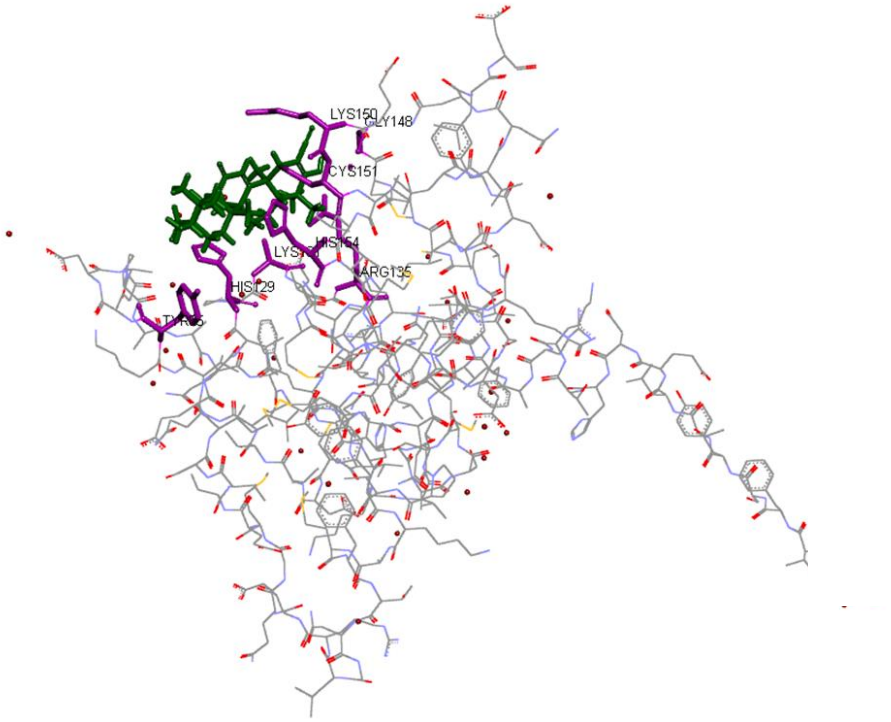
12b

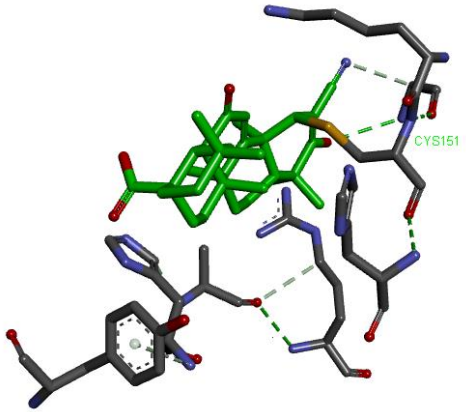
The effect of exposure of bmMs to SMAs-**11a** and -**12b** over 4 h (both at 5 mg/ml) on the steady state- and LPS-induced mRNA levels of HMOX1 (B & E); GCLC (C & F) and GCLM (D & G) as assessed by qRT-PCR where the levels of the gene of interest were normalized to the level of GAPDH and expressed as a fold change with respect to the medium control.

Data are presented as the means ± SEM of values pooled from 3 individual experiments. *p < 0.05; **p < 0.01 and ***p < 0.001. Black* represent significance between 12b (or 11a) and control whereas grey* represents significant differences between 12b- and 11a-treated cells.

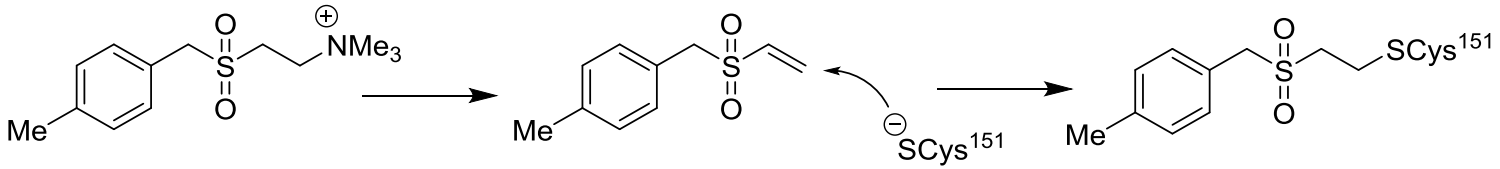
The contrast between the effects of **11a** and **12b** is striking.

BB region of Keap binding protein: another drug target





Keap binding protein with steroid drug showing C-S-C bond with Cys151 (pdb 4czt).
A similar event could occur with **12b** after protein catalysed elimination to give an intermediate vinyl sulfone.



Michael addition by C151 blocks Keap/NRF2 interaction?

Conclusions

SMAs constitute remarkable small molecule drug candidates that successfully replicate properties of a tetrameric protein, ES-62 both in *in vitro* evaluation and in mouse models of inflammatory disease.

The medicinal chemical profile of SMAs shows properties that are suitable for development as drugs but is also capable of further optimisation with a specific therapeutic indication in mind.

SMAs are effective at small doses both curatively and prophylactically, a fact that may be associated with an unusual mechanism of action.

Different SMAs replicate different features of the behaviour of ES-62 suggesting that there may be more than one mechanism of action for the SMAs.

Of possible molecular mechanisms of action, there is evidence to support immunoregulation by inhibition of MyD88 dimerisation and subsequent degradation of the adaptor. There is also evidence that interaction with the Keap regulator that controls the NRF2 response of the inflammasome is a target.

SMAs appear to be highly unusual but remarkably effective drug candidates owing their effects to modulation of cellular responses in contrast to the conventional on/off switch of drugs acting at single targets. This profile, although unconventional, may prove to be of great value in the treatment of inflammatory diseases.

Acknowledgments

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