Synthesis and preliminary evaluation of model compounds targeting the NLRP3 inflammasome pathways

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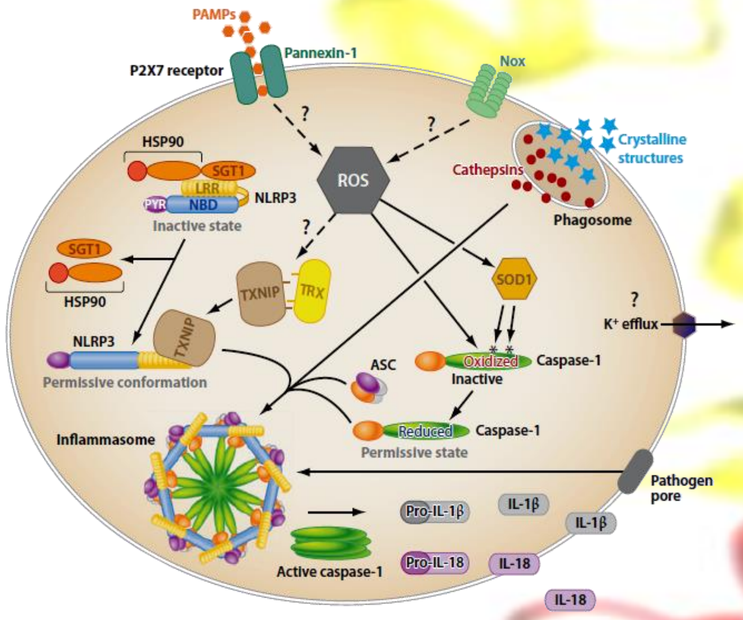
Introduction

Inflammasomes have recently emerged as key mediators of inflammation and immunity. Four inflammasome complexes have been described to date. The most intensely studied is the NLRP3 inflammasome formed by the nucleotide-binding domain leucine-rich repeat family member NLRP3 and the adapter protein ASC, its assembling is triggered by a diverse series of endogenous and exogenous stimuli.[1] (figure 1).



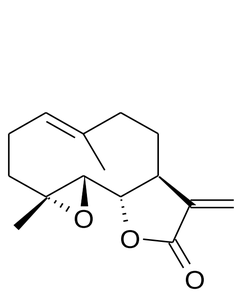
NLRP3 assembly leads to caspase-1 activation which causes the maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18 .

Fig. 1. Model of inflammasome activation.
Coordination of a manifold series of signals culminates in the activation of the inflammasome.

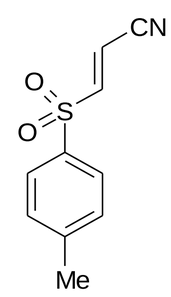
A series of autosomal and dominant or *de novo* mutations of nlrp3 lead to auto-inflammatory syndromes known as CAPS (cryopyrin–associated periodic syndromes) which are characterized by high levels of IL-1β release and associated sterile systemic inflammation. The onset of chronic inflammation has also been linked to a wide range of metabolic disorders, such as type 2 diabetes, atherosclerosis, and Alzheimer's disease.[2]

Design of inhibitors of NLRP3-related pathways

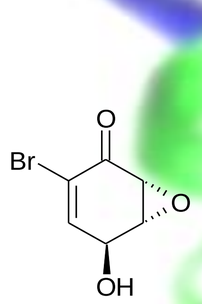
Few small molecules inhibitors of NLRP3 inflammasome-related effects have been described, among them **parthenolide**, **Bay 11-7082** and **bromoxone** are the most studied.



parthenolide



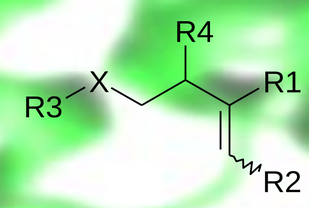
Bay 11-7082



bromoxone

These molecules share the ability to behave as **Michael acceptors**. Although Michael acceptors are traditionally shunned in modern drug discovery, many biologically relevant and druggable pathways are targeted by thiol-reactive compounds.[3]

To explore the use of **Michael acceptors** as pharmaco-chemical tools modulating inflammatory pathways we designed some model compounds (figure 2).

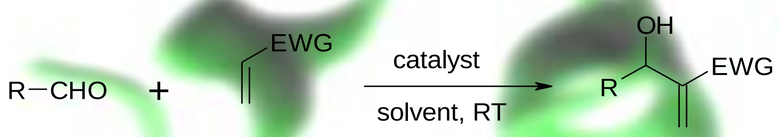


X = alkyl, cycloalkyl, aryl, substd aryl, heteroaryl

Fig. 2. General structure of designed Michael acceptors

Synthesis

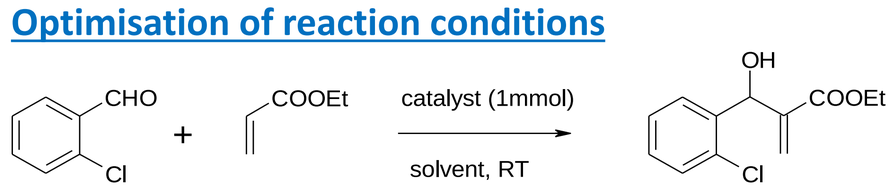
Designed compounds were synthesized via the Morita-Baylis-Hillman (MBH) reaction (*scheme 1*).



Scheme 1. The Morita-Baylis-Hillman reaction

- Commercially available starting materials
- Mild reaction conditions
- Flexible and multifunctional adducts
- Atom economic reaction

Optimisation of reaction conditions



1 mmol 3 mmol

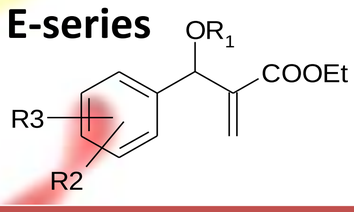
Solvent	catalyst	Time (d)	Yield [a]	Solvent	catalyst	Time (d)	Yield [a]
$CHCl_3$	Et_3N	7	-	CH_3CN/H_2O	Et_3N	7	-
$CHCl_3$	DMAP	19	54 %	CH_3CN/H_2O	DMAP	5	42 %
$CHCl_3$	DBU	12	25 %	CH_3CN/H_2O	DBU	20	trace
$CHCl_3$	Im	7	-	CH_3CN/H_2O	Im	20	< 2%
$CHCl_3$	DABCO	7	-	CH_3CN/H_2O	DABCO	7	67 %
$CHCl_3$	PPh_3	7	c	CH_3CN/H_2O	PPh_3	4	c
$CHCl_3$	TMEDA	7	-	CH_3CN/H_2O	TMEDA	20	trace
				Dioxane/H_2O	DABCO	20	-
				THF/MeOH/H_2O	DABCO	4	44 %
				CH_3CN/H_2O	DABCO	7	65 % [b]
				CH_3CN/H_2O	DABCO	2 [d]	40 % [b]

Tab. 1 a) Isolated yields; b) reaction run on 10 mmol scale; c) complex mixture ; d) run at 80 °C Solvents: dioxane/H_2O (1/1); CH_3CN /H_2O (9/1); THF/MeOH/H_2O (1/1/2)

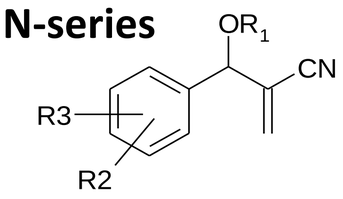
- ✓ **Use of polar protic medium is preferred**
- ✓ **Use of DABCO or DMAP gives best results**
- ✓ **Long reaction times are required**
- ✓ **Scale-up can be effectively obtained**

The synthesis of **E- N- X-** series of compounds was obtained through reaction of ethylacrylate or acrylonitrile with different aldehydes.

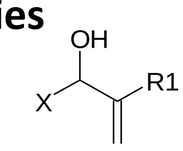
E-series



N-series



X-series



	E compounds			Reaction conditions				N compounds				Reaction conditions		
	R1	R2	R3	solv	catalyst	yield		R1	R2	R3	solv	catalyst	yield
1E	-H	-H	-H	B	DABCO	45%	1N	-H	-H	-H	D	DABCO	69 %
2E	-H	2-NO_2	-H	A	DABCO	60 %	2N	-H	2-NO_2	-H	A	DABCO	44 %
3E	-H	2-F	-H	A	DABCO	66 %	4N	-H	2-Cl	-H	D	DABCO	quant
4E	-H	2-Cl	-H	B	DABCO	67 %	5N	-H	2-Br	-H	D	DABCO	quant
5E	-H	2-Br	-H	B	DABCO	quant	7N	-H	2-Cl	4-Cl	D	DABCO	37 %
6E	-H	4-Cl	-H	B	DMAP	38 %	8N	-H	2-Cl	6-Cl	D	DABCO	21 %
7E	-H	2-Cl	4-Cl	B	DMAP	58 %	X compounds				Reaction conditions		
8E	-H	2-Cl	6-Cl	B	DABCO	36 %		X		R1	Solv	catalyst	yield
9E	-H	3-Cl	5-Cl	B	DABCO	40 %	13E	2-piridyl		-COOEt	A	DABCO	48 %
10E	-$(CH_2)_2$COOEt	2-Cl	-H	B	DMAP	8 %	13N	2-piridyl		-CN	A	DABCO	70 %
11E	-$(CH_2)_2$COOEt	2-Cl	4-Cl	B	DMAP	16 %	14N	2-naftalenyl		-CN	D	DABCO	72 %
12E	-$(CH_2)_2$COOEt	2-Cl	6-Cl	B	DMAP	11 %	15E	cyclohexyl		-COOEt	C	DABCO	33 %
							16E	methyl		-COOEt	B	DABCO	21 %

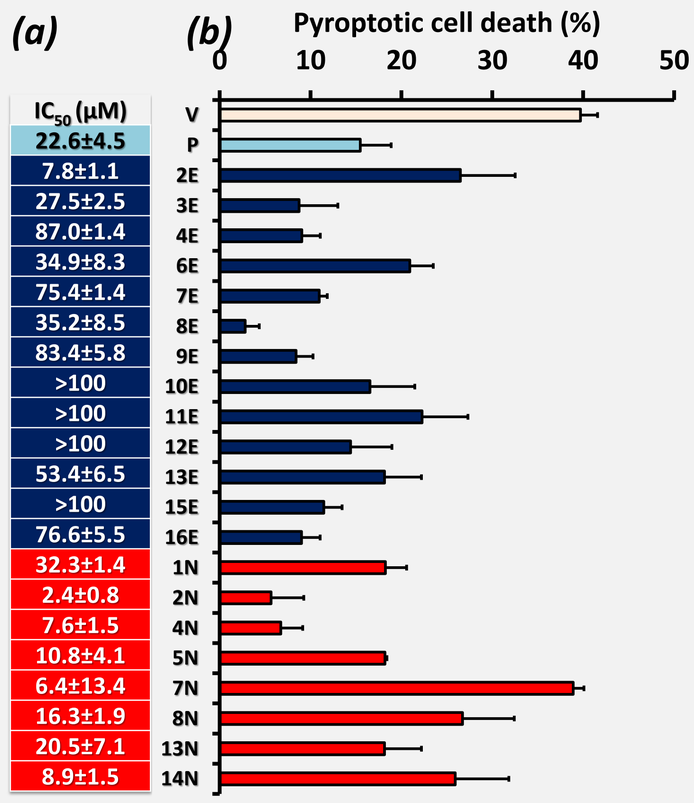
Tab. 2 A = dioxane / H_2O (1/1); B = CH_3CN / H_2O (9/1); C = formamide; D = THF / MeOH / H_2O (1/1/2)

Biological results

(a)

IC_{50} (μM)
22.6±4.5
7.8±1.1
27.5±2.5
87.0±1.4
34.9±8.3
75.4±1.4
35.2±8.5
83.4±5.8
>100
>100
>100
53.4±6.5
>100
76.6±5.5
32.3±1.4
2.4±0.8
7.6±1.5
10.8±4.1
6.4±13.4
16.3±1.9
20.5±7.1
8.9±1.5

(b)



(a) Cytotoxicity

Immortalized human tubular epithelial cells were exposed to increasing compound concentrations (0.1-100 μM). After 72 h cell viability was evaluated by MTT assay. Data are means ± S.E.M. of at least three independent experiments run in quadruplicate.

(b) Pyroptotic cell death

PMA-differentiated THP-1 cells were stimulated with LPS. Cells were treated with vehicle alone (V), parthenolide (P) or synthesized compounds (10 μM; 1 h), then pulsed with ATP in serum-free medium. The culture supernatants were collected and assayed for LDH activity. Data are means ± S.E.M. of at least three independent experiments run in triplicate.

- ➢ Preliminary data identify a number of compounds able to inhibit **pyroptotic cell death** which is recognized to be related to **NALP3 activation**.
- ➢ Compounds **4E**, **8E**, **9E**, **16E** showed a better profile with respect to parthenolide, used as reference compound.

References

1) D. De Nardo et al. *Trends Immunol.* **2011**, *32(8)*, 373-379. **2)** B. K. Davis et al. *Annu. Rev. Immunol.* **2011**, *29*, 707-735. **3)** S. Amslinger *ChemMedChem* **2010**, *5, 351-356.*