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A chemical genetics approach to investigate accelerated ageing in Werner syndrome

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wales
college of medicine
cymru



Werner Syndrome

- Rare, autosomal recessive genetic disease
- Premature ageing of a subset of tissues
 - Premature development of osteoporosis, bilateral cataracts, type II diabetes, thymic atrophy, dysadipocytokinemia, soft tissue calcification, ulceration, and an overall aged appearance
 - Elevated risk of atherosclerosis and predominantly mesenchymal cancers
 - **Affected tissues are mainly those containing cells that are division-competent during life**



George Martin and German Werner individual (age 36)



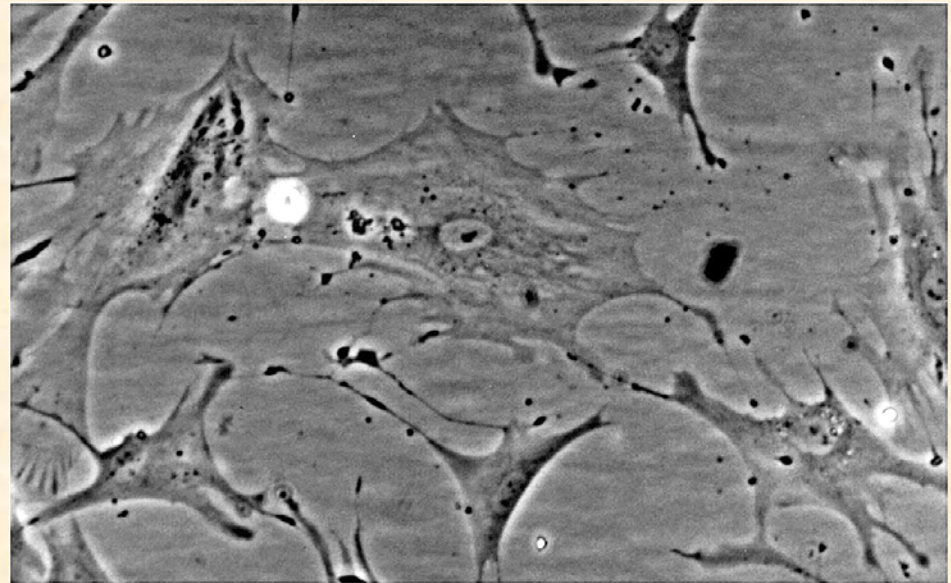
American Werner syndrome individual (age ~50)

What causes Werner Syndrome?

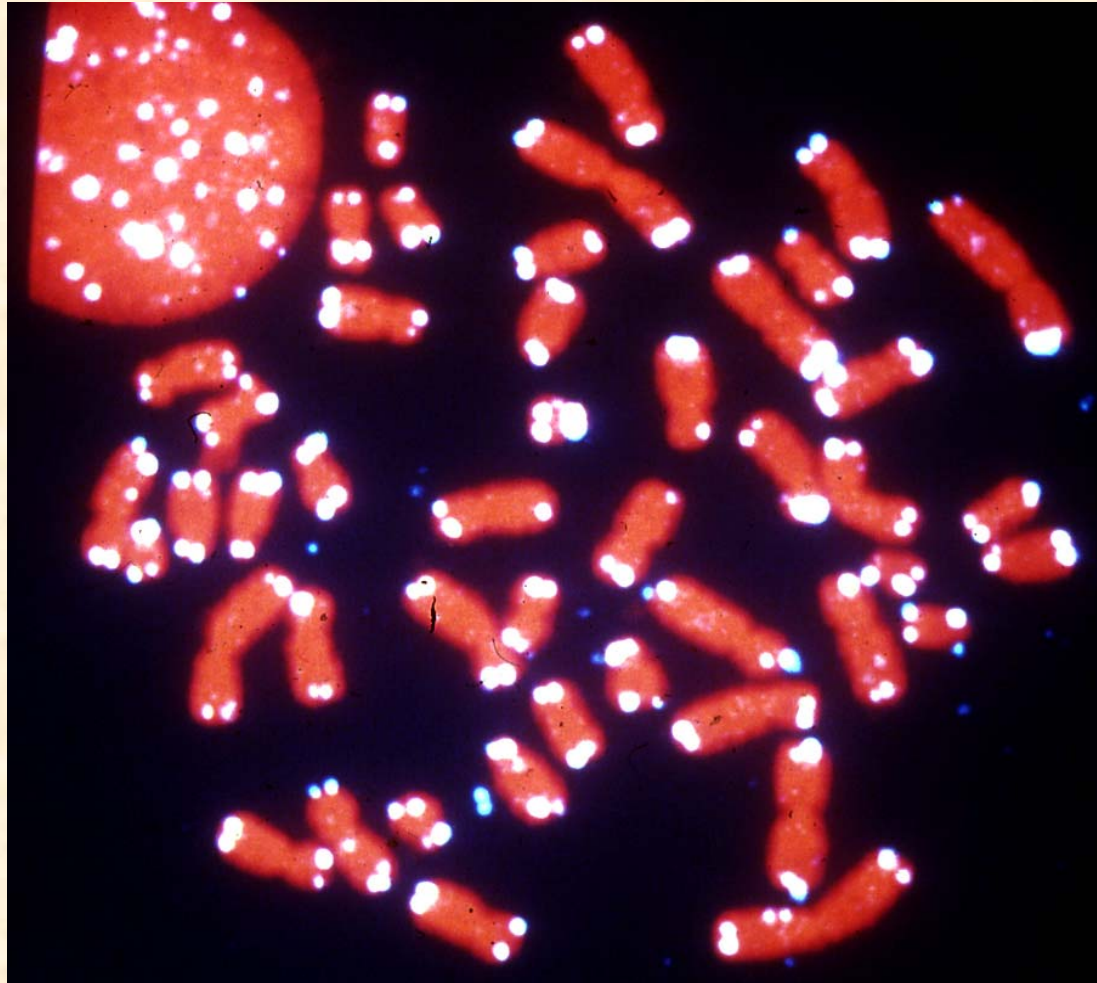
- *WS is caused by mutation in a recQ helicase (wrn)*
 - Lack of WRNp results in stalling of DNA replication, and problems with DNA repair
- *Shortened replicative lifespan of cells in culture*
 - The evidence is consistent with the premature ageing of WS patients being caused by accelerated replicative senescence

Replicative senescence *in vitro*

- Finite lifespan of normal human cells in culture
- **Senescence**
 - is viable cell cycle arrest
 - may act as a barrier to tumour formation
- **Senescent cells**
 - remain metabolically active
 - display altered patterns of gene expression, including secreted proteins



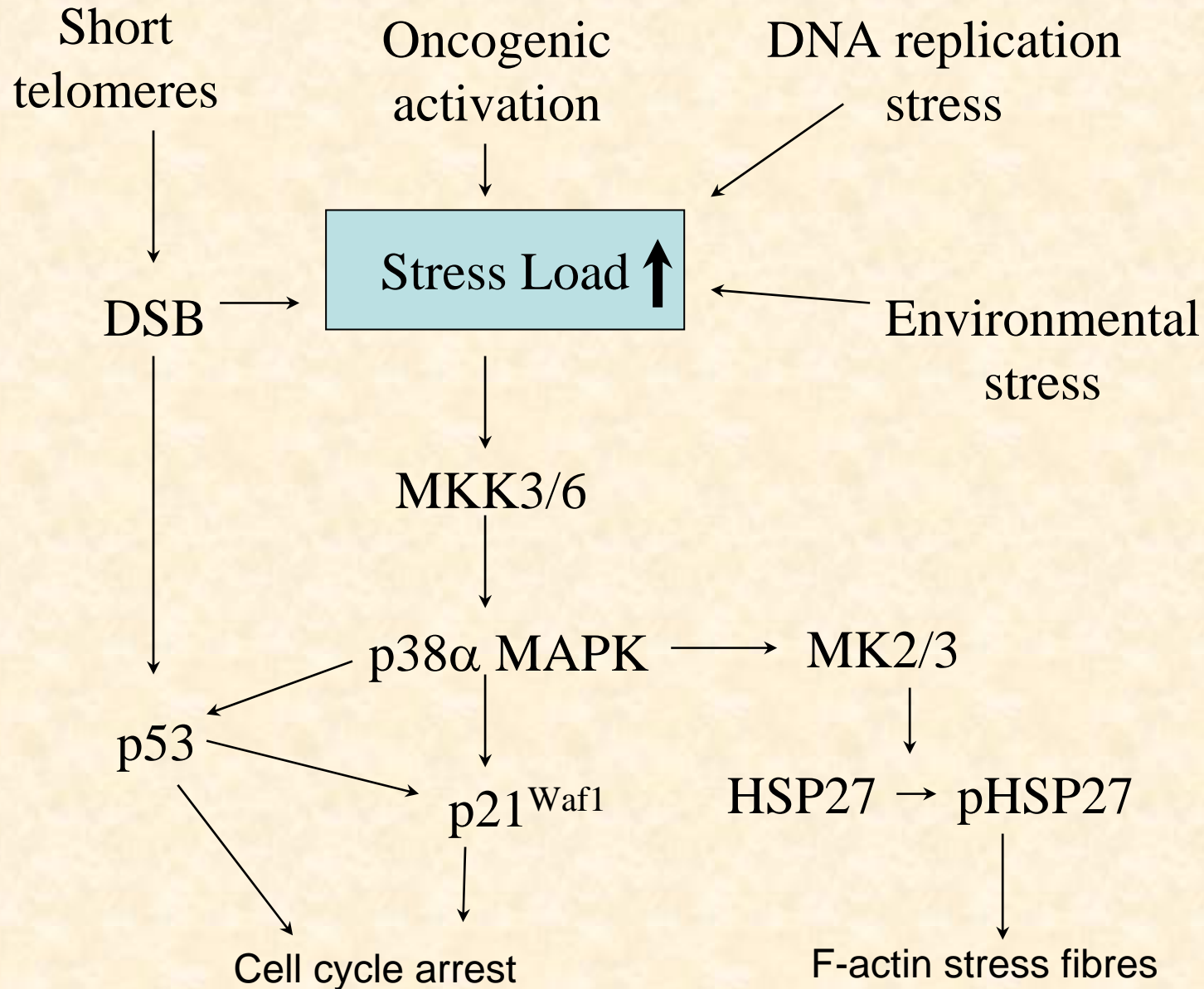
Telomere dependent senescence (TDS)



Telomere-independent senescence (TIS)

- TIS can be induced by extrinsic stimuli
 - e. g. oxidative stress, DNA damage
- Intrinsic signals can also induce TIS
 - e. g. activated oncogenes (Ras), genome instability

Growth arrest pathways in senescence



Senescence is a potential
ageing mechanism

Physiological cell loss

(wear-and-tear, wounding, haemodynamic stress)

Normal

Replacement cell division

Progressive accumulation of senescent cells

**Altered cellular
microenvironment**

**Reduced division
capacity**

Aged tissue



Physiological cell loss

(wear-and-tear, wounding, haemodynamic stress)



Replacement cell division



Progressive accumulation of senescent cells



Altered cellular
microenvironment



Reduced division
capacity



Accelerated Ageing

WS

Why do Werner Syndrome cells show premature replicative senescence?

- Have shown that WS cells do not show more rapid telomeric losses therefore mechanism is not through TDS (Baird et al, 2004, Hum Mol Genet **13**, 1515)

Why do Werner Syndrome cells show premature replicative senescence?

- **Postulate that:**
 - The shortened lifespan of WS cells results from an additional process of telomere-independent senescence
 - This is superimposed upon normal telomere-driven senescence
 - These two mechanisms together define WS cell lifespan

So what might be causing
TIS?

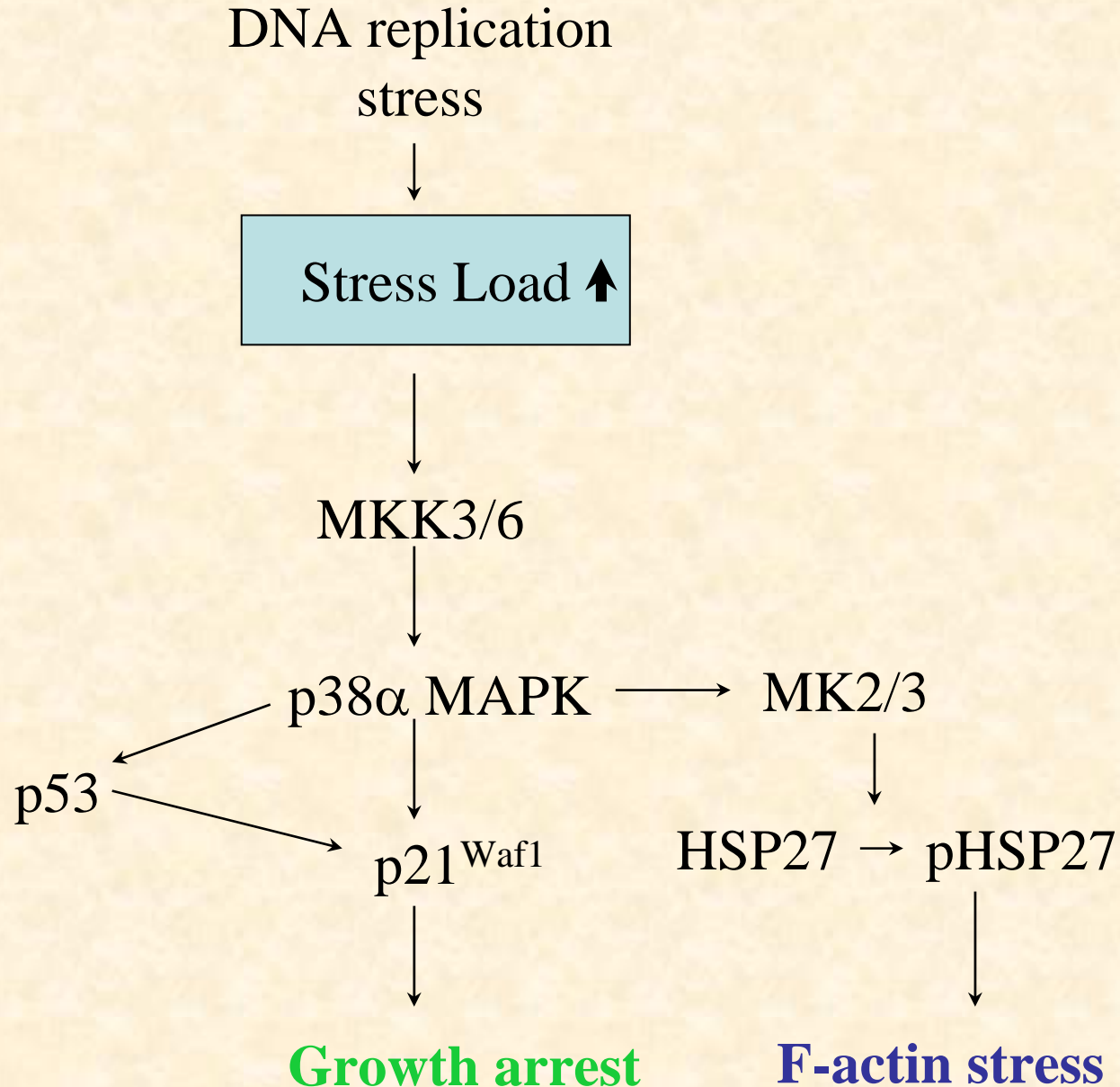
Stress and WS cells

- WS cells have genome instability
- DNA replication frequently stalls
- DNA repair defects

Any of above could provide trigger for activation of stress pathways

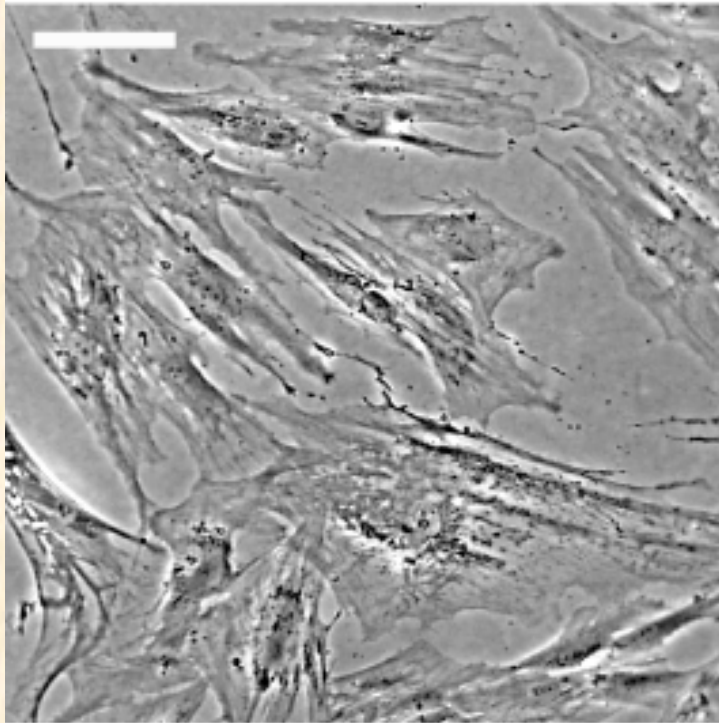
Such stress is transduced by the p38 MAP kinase pathway

Stress pathways in WS cells

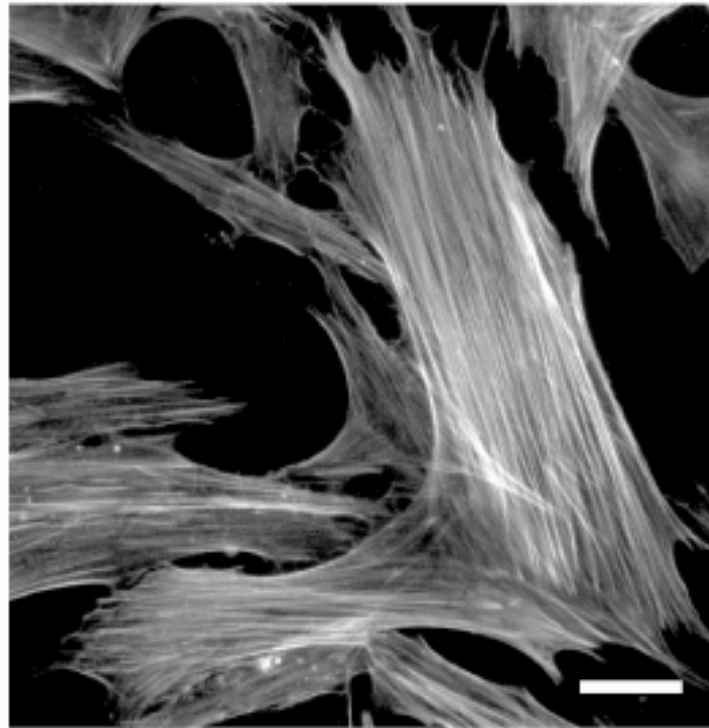


Evidence for Stressed cells in WS

young AG05229 cells



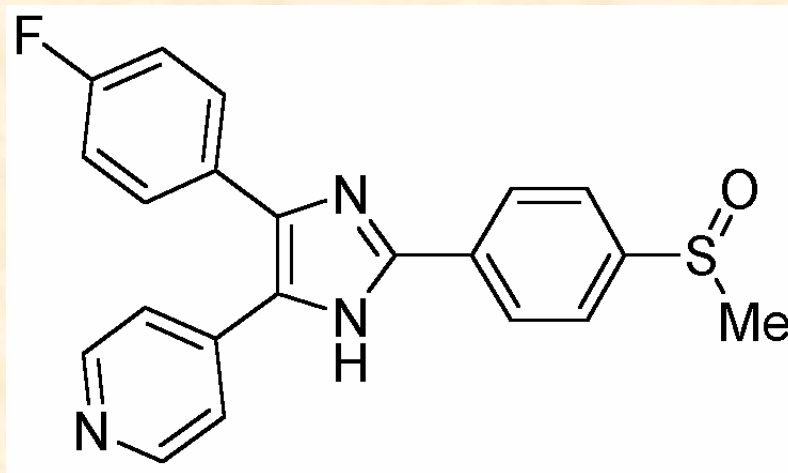
phase contrast x10;



phalloidin x20

Blocking the p38 stress response

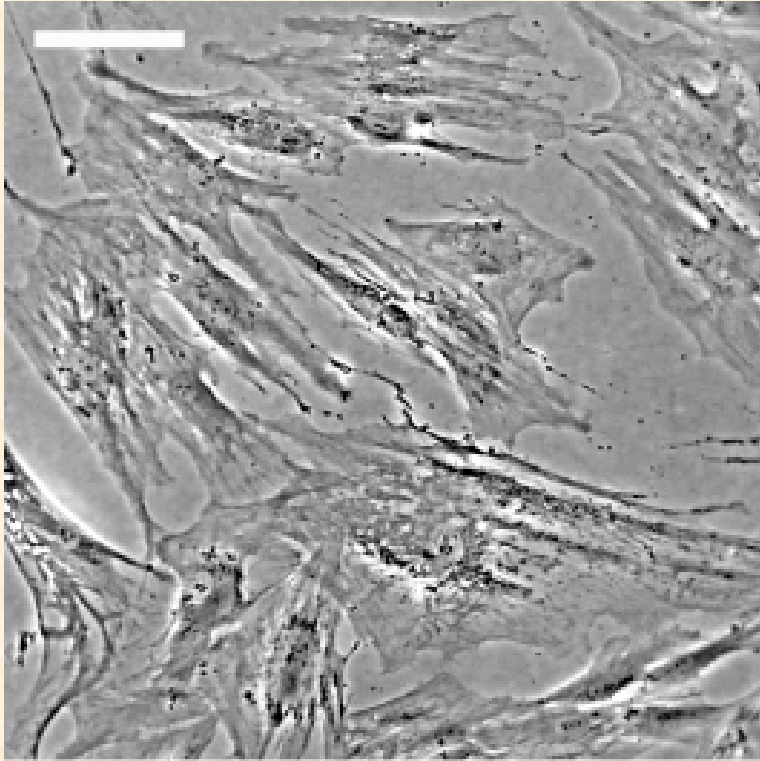
SB203580



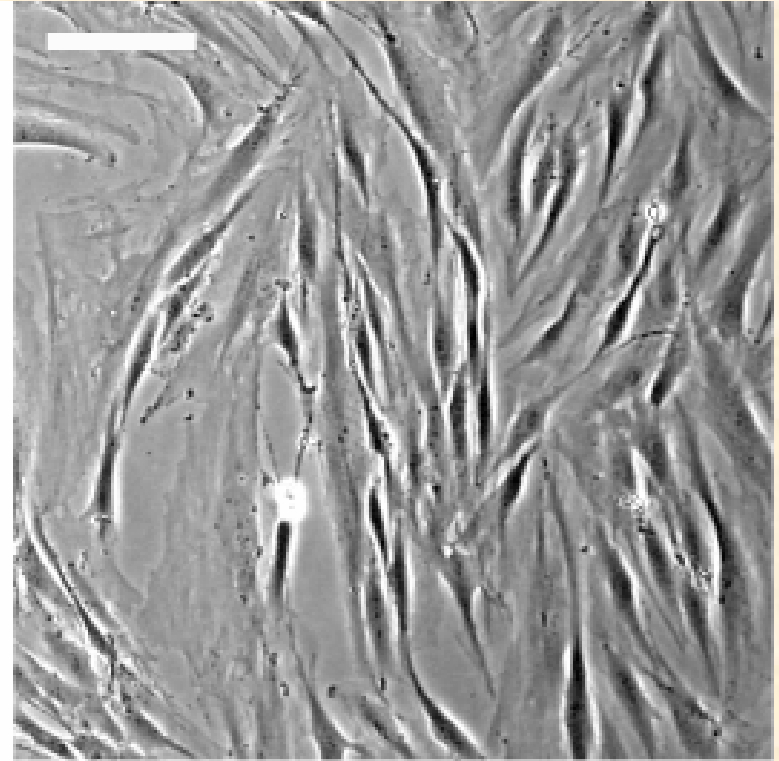
Inhibits the α and β forms of p38 MAPK (and some other targets)

4-[5-(Fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine

Effect of SB203580 on cell morphology

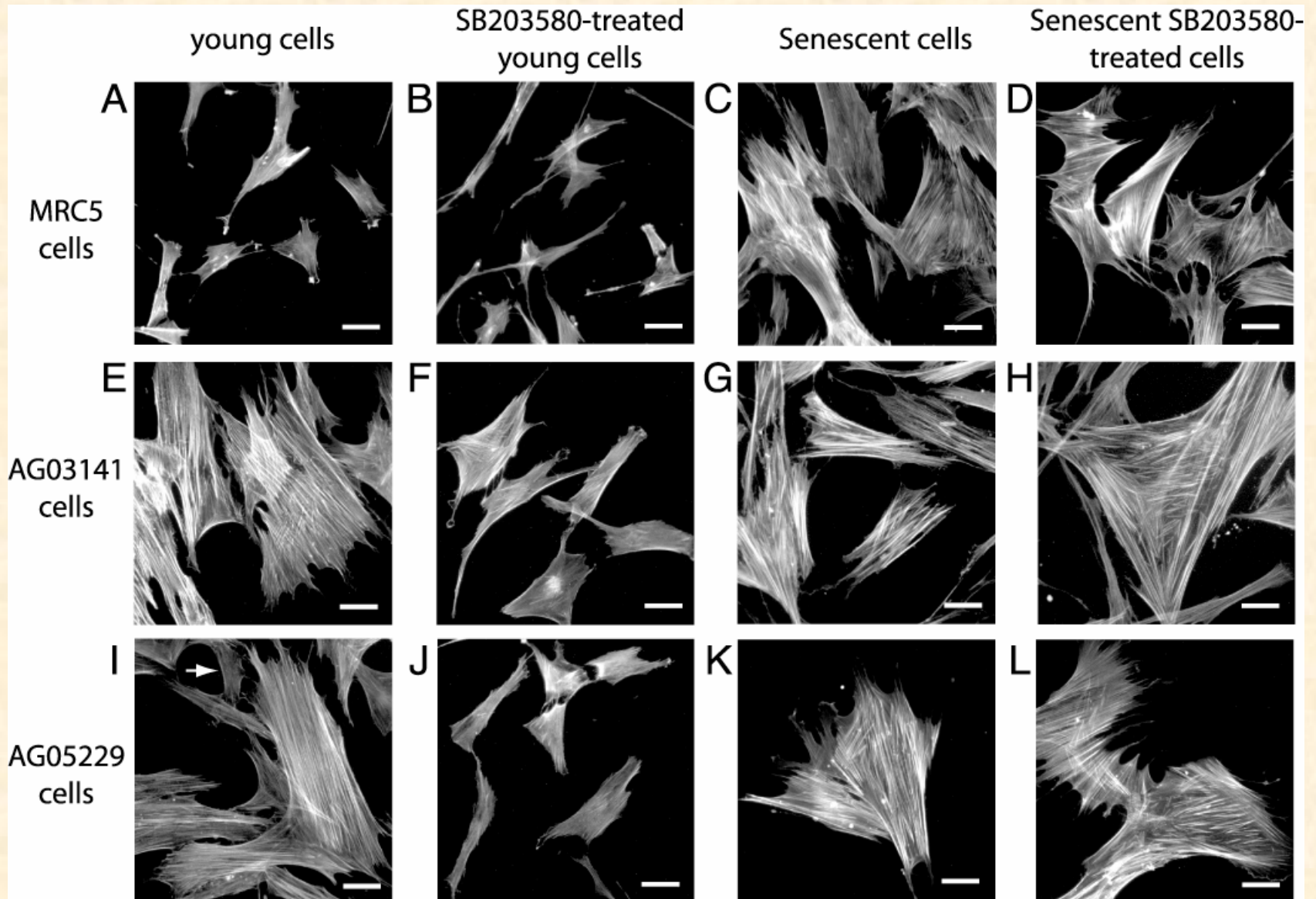


young control
AG05229 cells

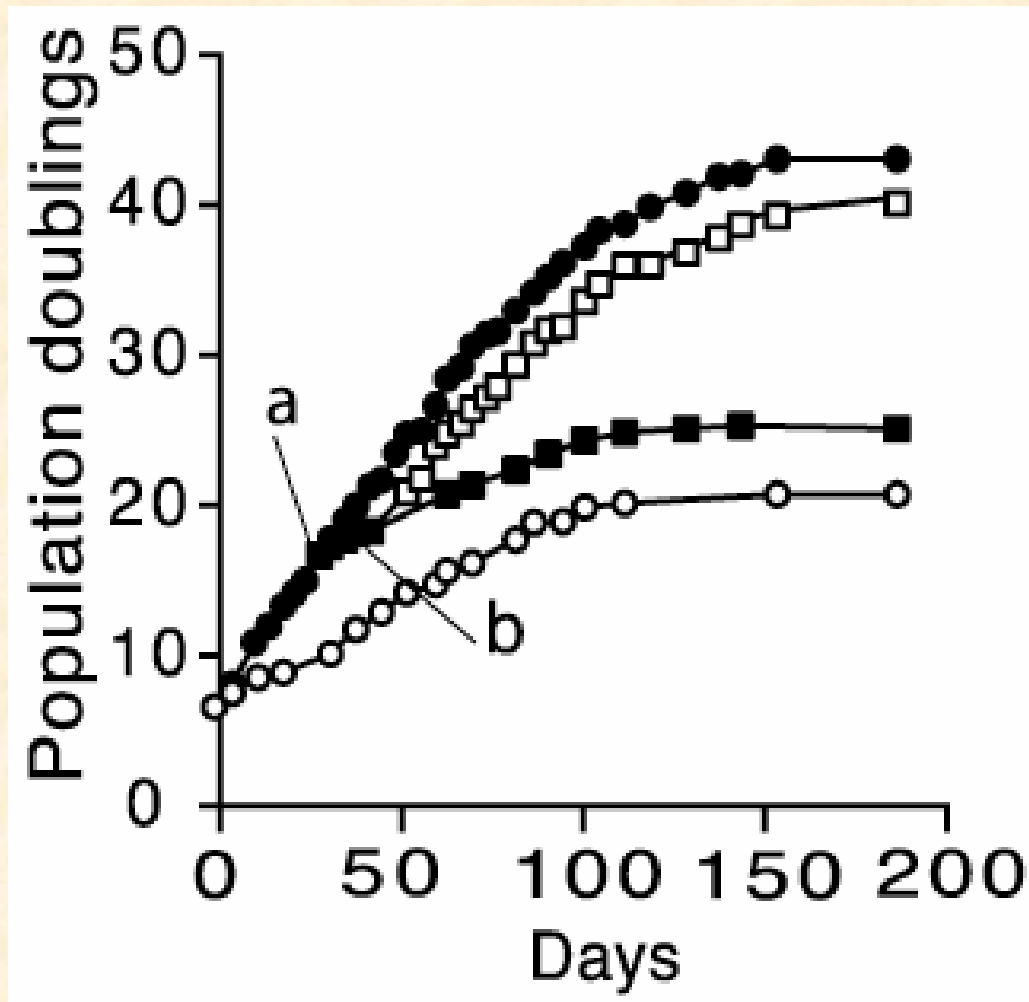


young SB203580 treated
AG05229 cells

Effect of SB203580 on stress fibres



Effect of SB203580 on WS cell growth



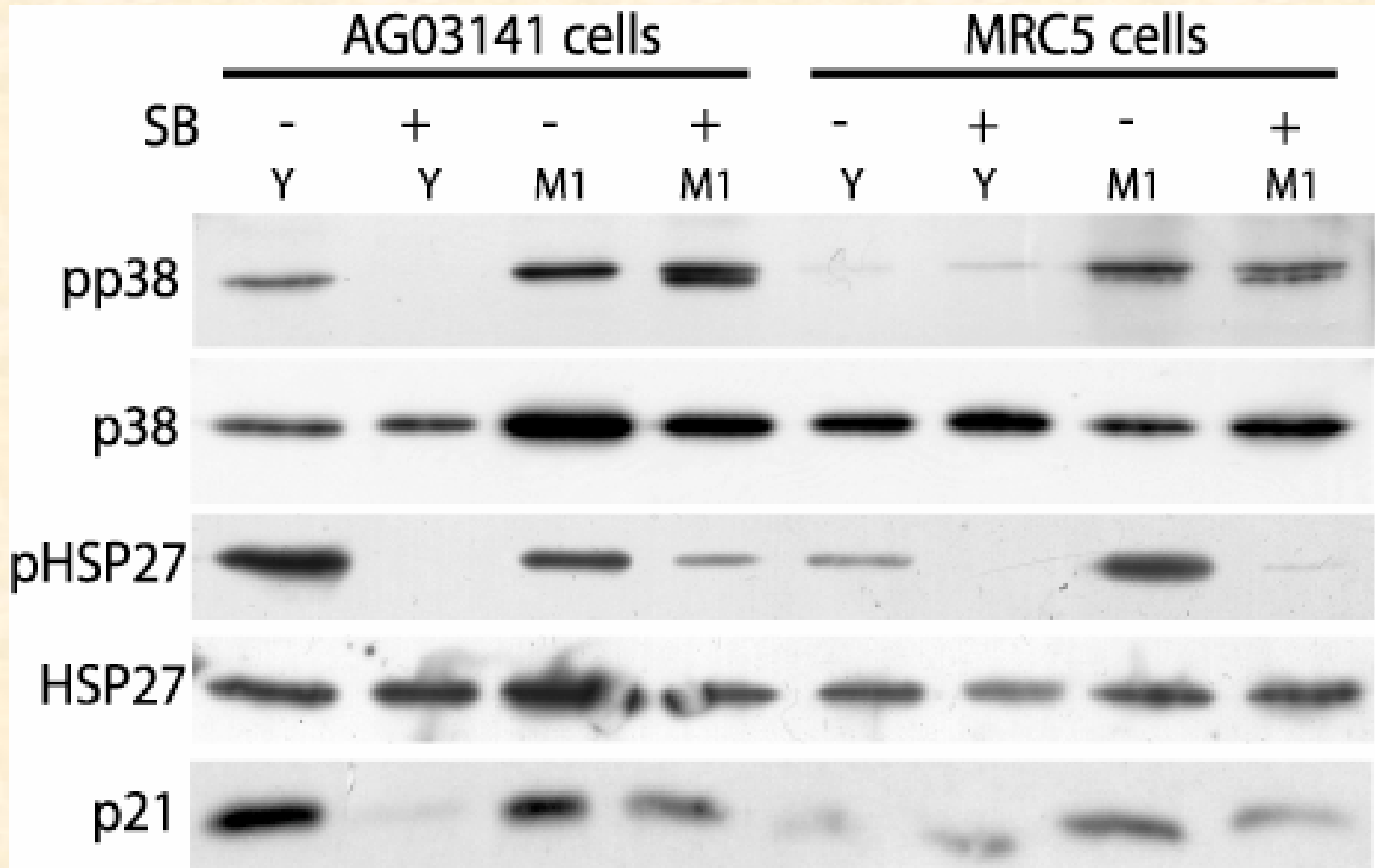
Open circles: control

Closed circles: SB-treated

Closed squares: SB-treated to point a, then drug removed

Open squares: SB-treated to point a, then drug removed; drug restored at point b

Immunoblot analysis of p38 activity



Summary

With regard to cell ageing, SB203580 makes WS cells behave like normal cells

Therapeutic opportunities for WS?

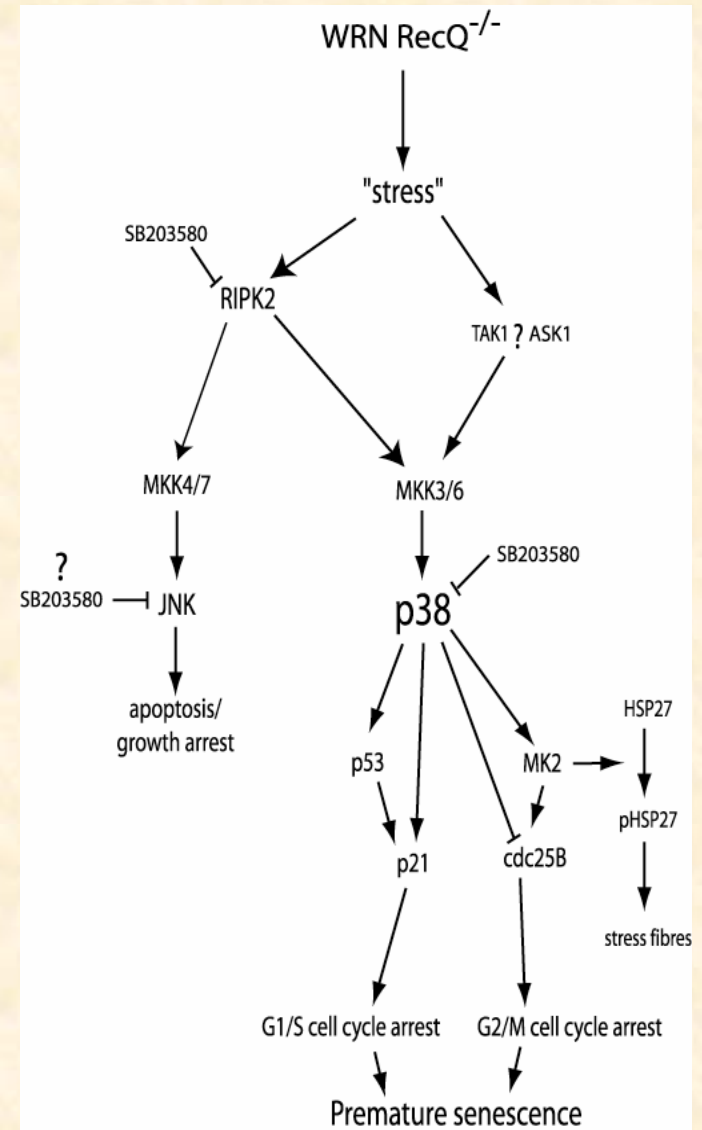
Use of p38 inhibitors to treat WS?

If accelerated replicative decline does underlie rapid ageing in WS then these inhibitors may help alleviate this and lead to longer life.

This may also be applicable to normal ageing?!?

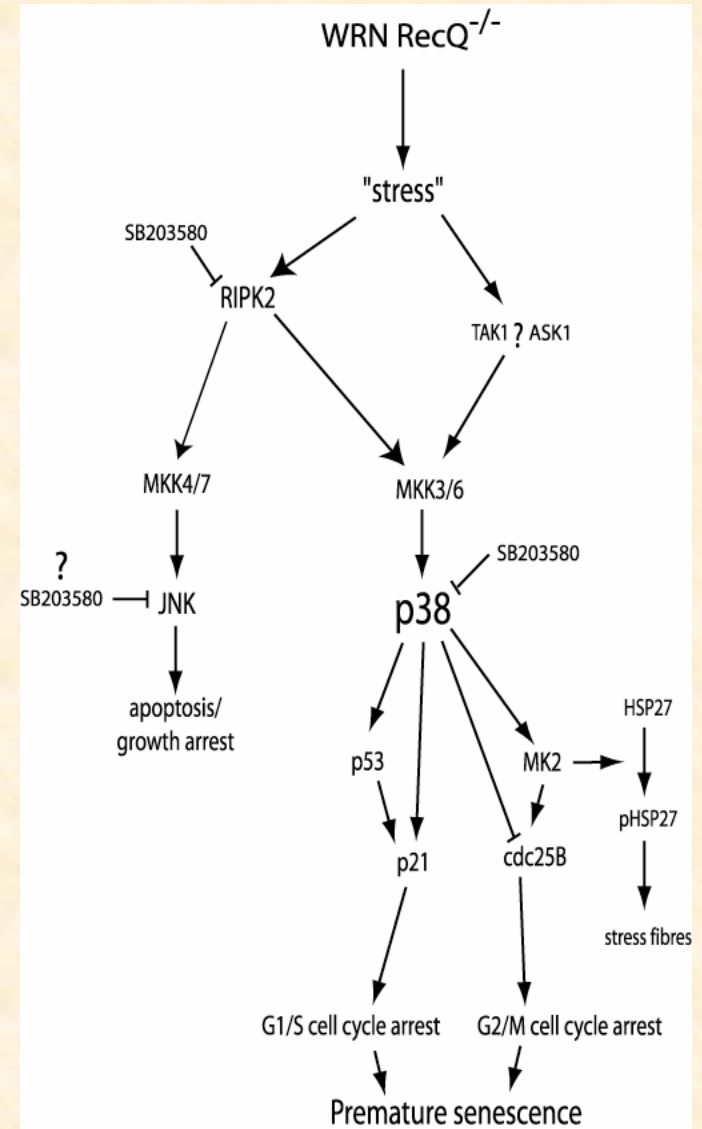
However!

- SB203580 not suitable for *in vivo* use
- SB203580 inhibits several kinases other than p38
- Thus need to clarify things



However!

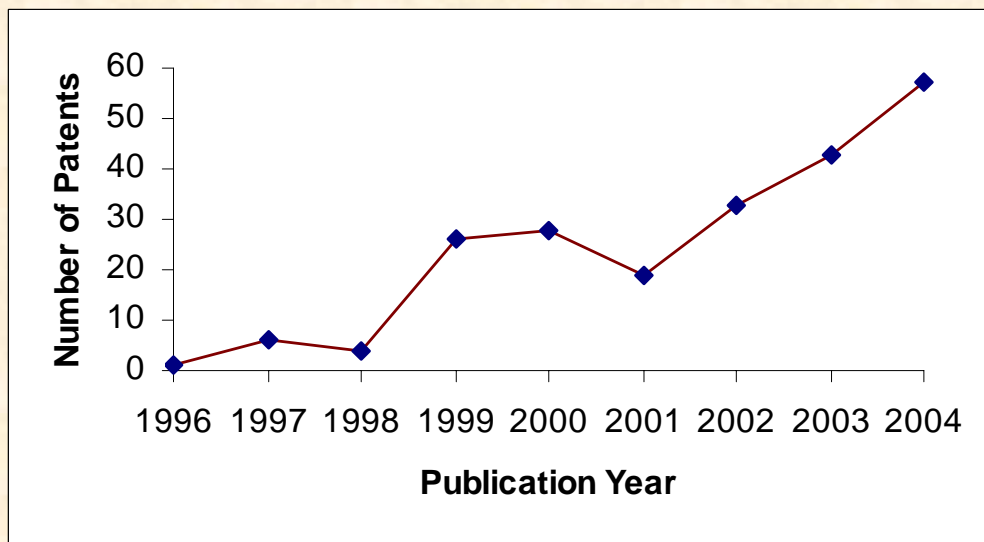
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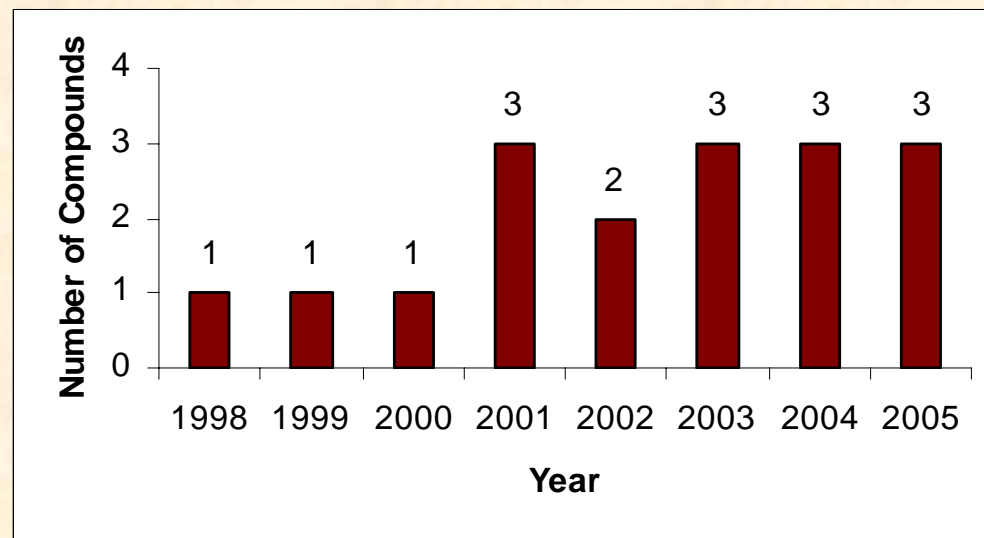
Enter Mark

Clinical Inhibitors of p38 MAPK

- Applications of p38 kinase inhibitors by number of patents



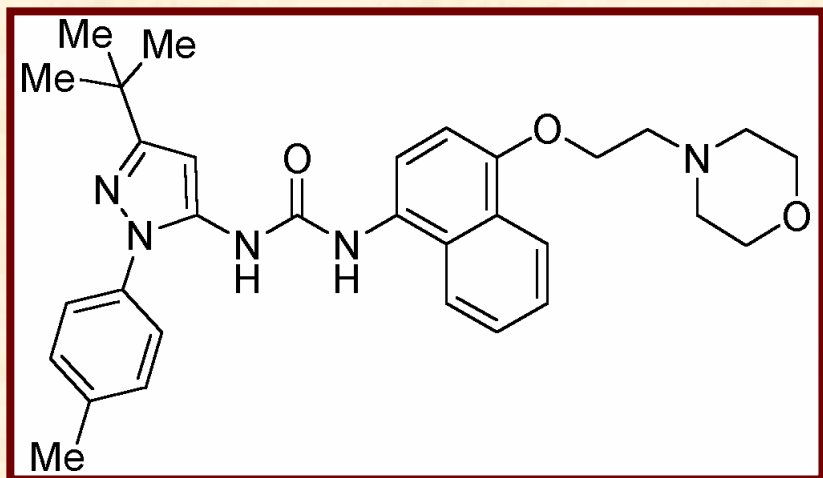
- P38 kinase inhibitors in clinical development for treatment of inflammatory diseases



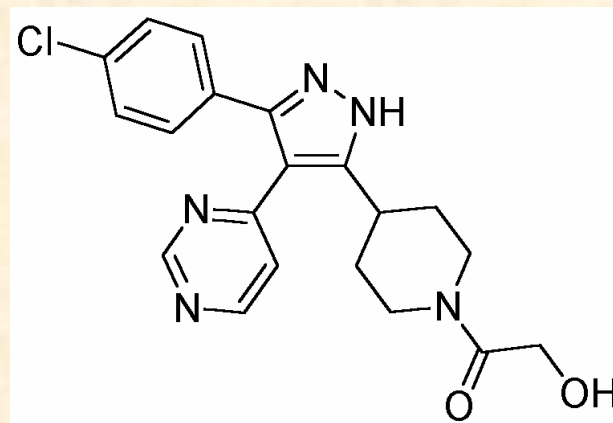
SCIBS: chemistry biology interface

- *BBSRC Selective Chemical Intervention into Biological Systems (SCIBS) grant*
 - A collaboration between MEDICINE and CHEMISTRY
- **Objective**
 - Design and test new chemical inhibitors of the p38^{MAPK} signalling pathway using WS cells as model test system.
- **Goals**
 - Design of new tools for probing the pathway
 - Determine the roles played by the various members of the pathway in the accelerated senescence seen in WS cells
 - Move to pre-clinical trials with mouse WS model

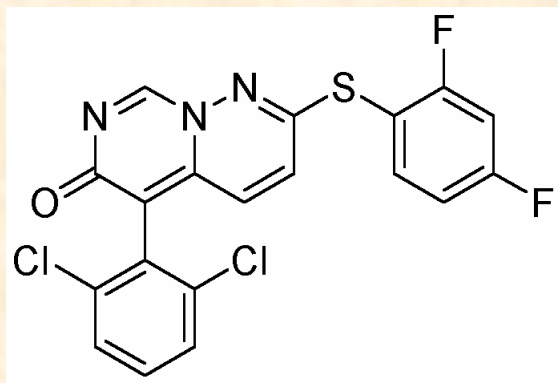
Structurally Diverse Clinical Candidates



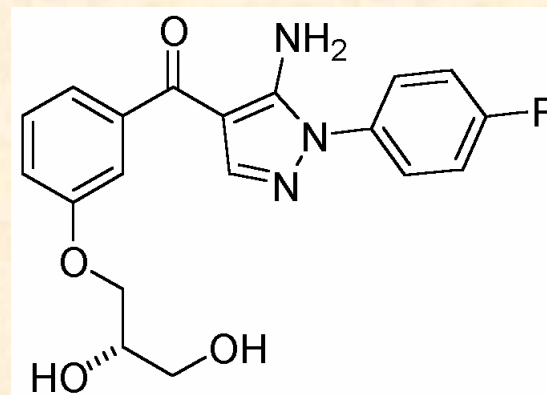
BIRB 796 (*Boehringer Ingelheim*)
P38 α : K_d 76 pM, IC_{50} 63 nM



SD-06 (*Pfizer*)
P38 α : IC_{50} 80 nM (p38 β 26000 nM)

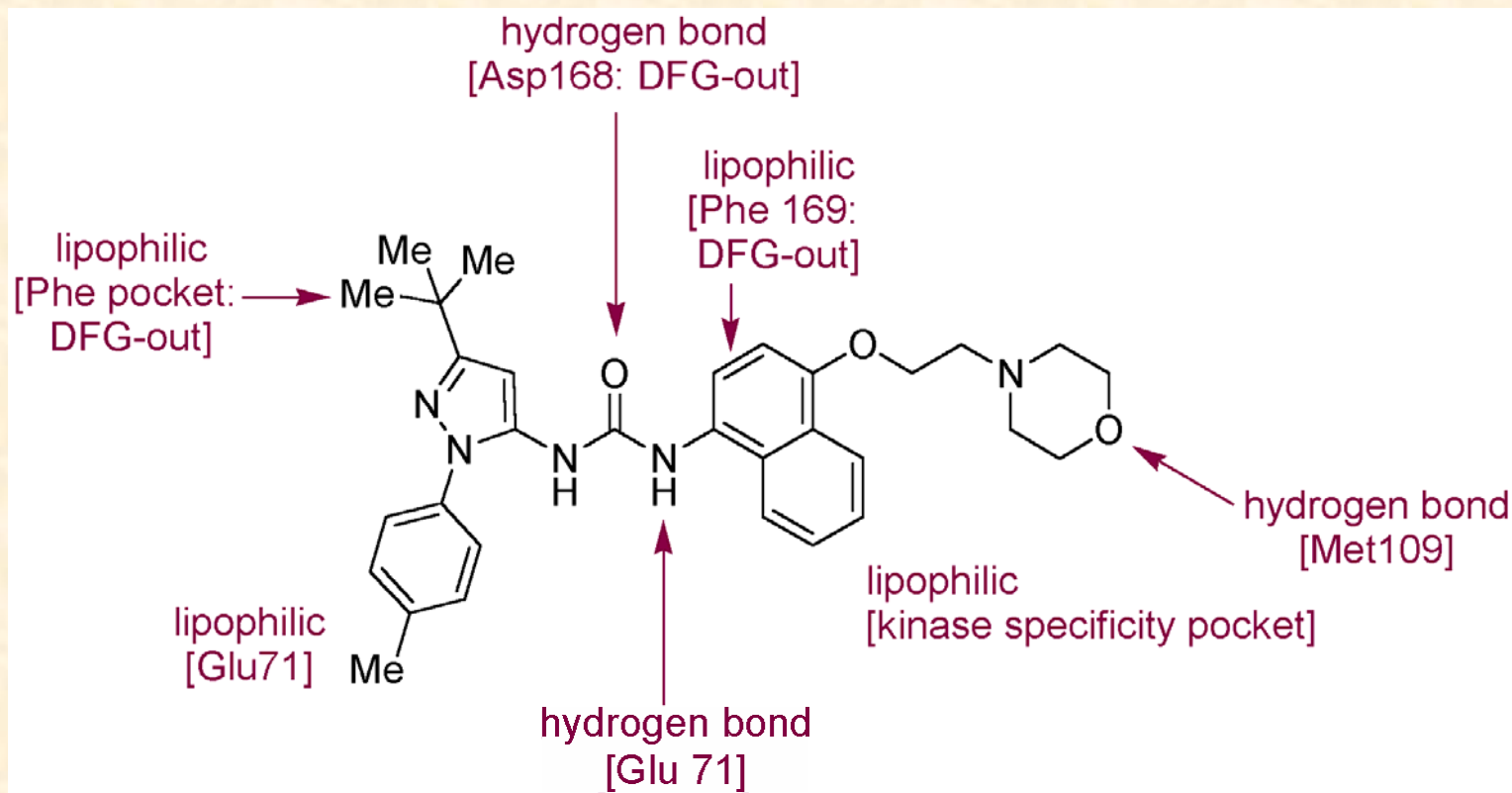


VX-745 (*Vertex*)
P38 α : IC_{50} 10 nM



RO3201195 (*Roche*)
P38 α : IC_{50} 700 nM (high selectivity)

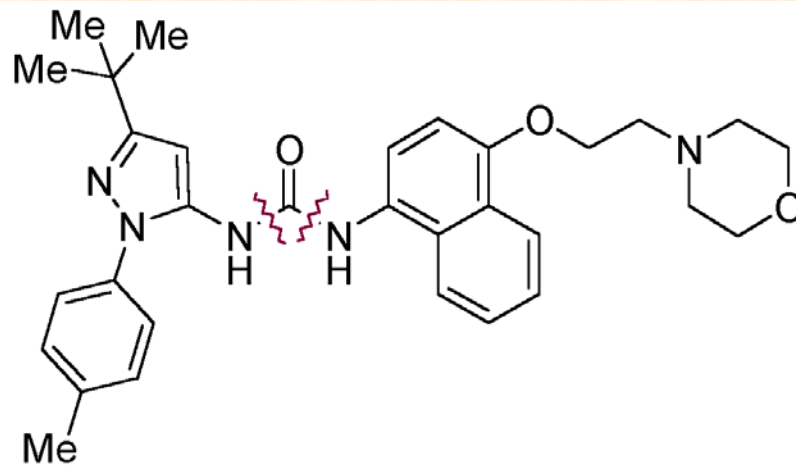
Key interactions of BIRB 796 with human p38 α



- In phase III Clinical Trials for the treatment of rheumatoid arthritis and other inflammatory diseases
- Formation of the allosteric binding site requires conformational change of the activation loop (DFG-out: Asp168-Phe169-Gly170)

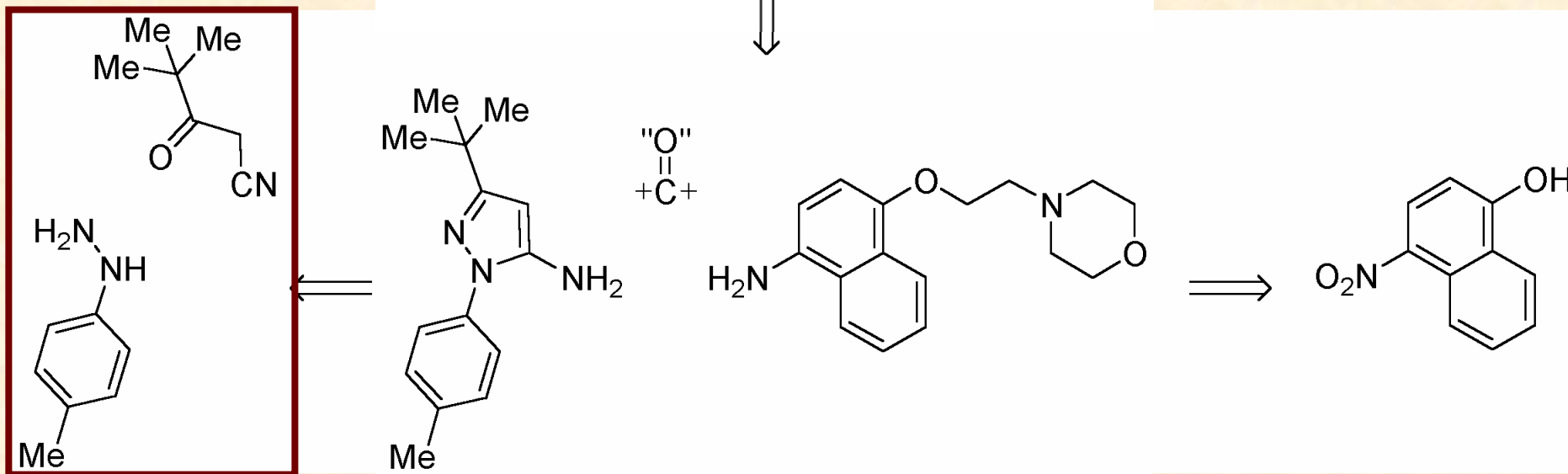
Disconnective Scheme for BIRB 796

BIRB 796



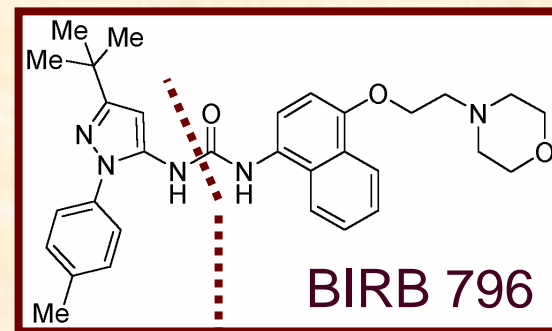
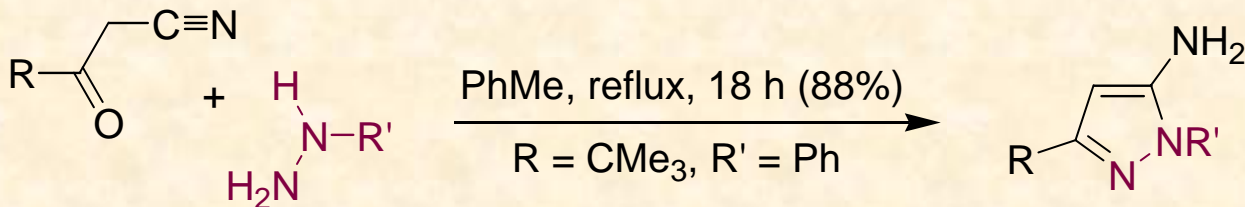
Synthetic needs:

- Convergent
- High purity
- Rapid
- Efficient



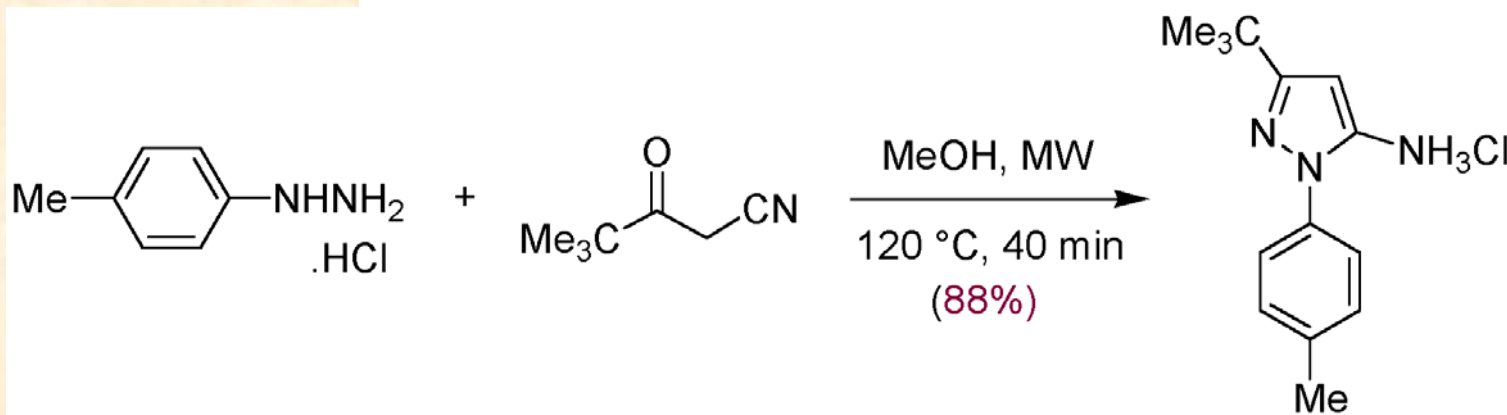
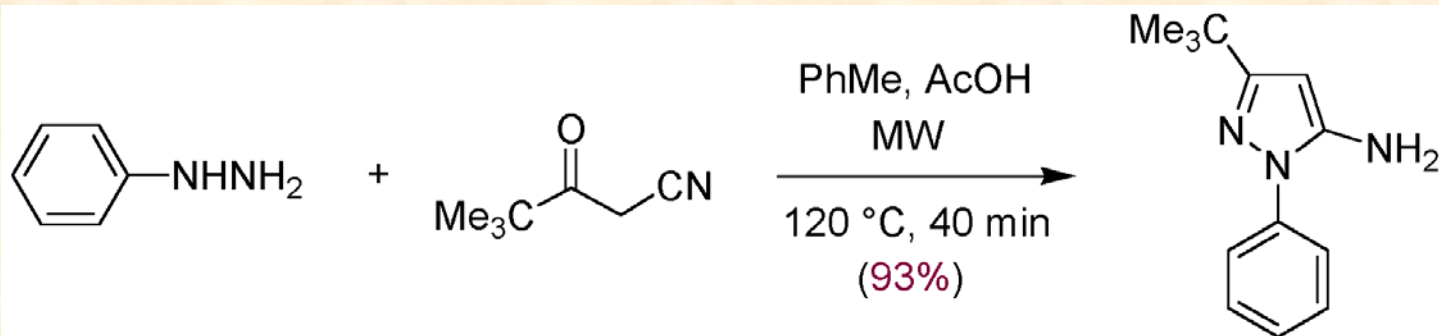
Synthesis of 2-aminopyrazoles

Traditional conductive heating:



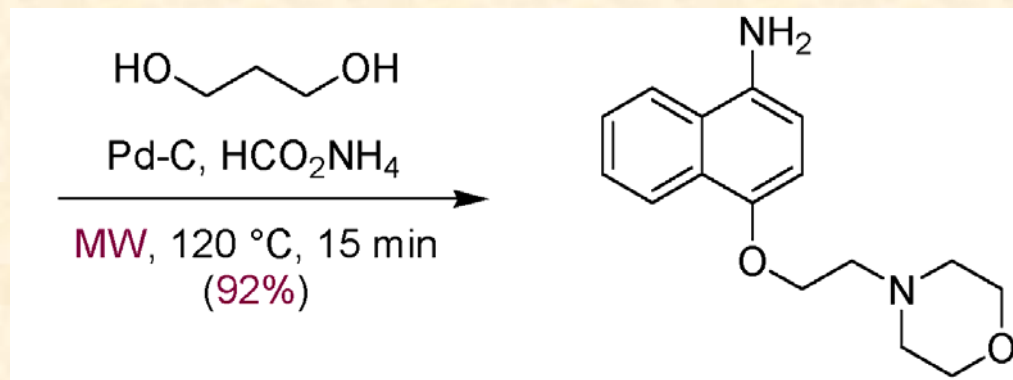
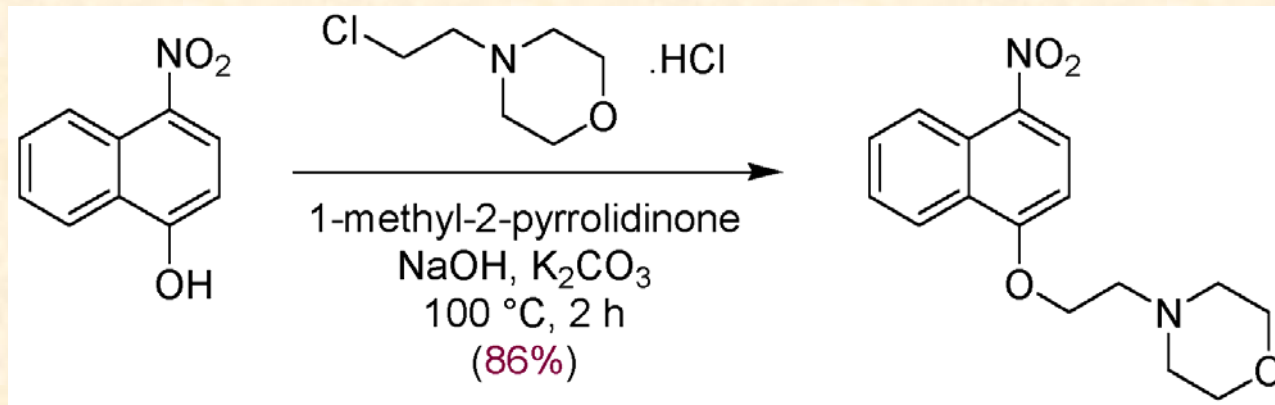
Aminopyrazole component

Microwave irradiation:

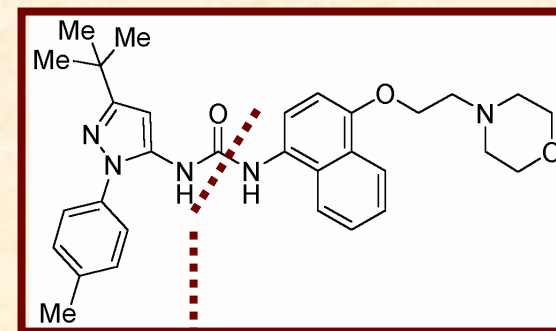


Amine or HCl salt

Synthesis of aminonaphthyl ether

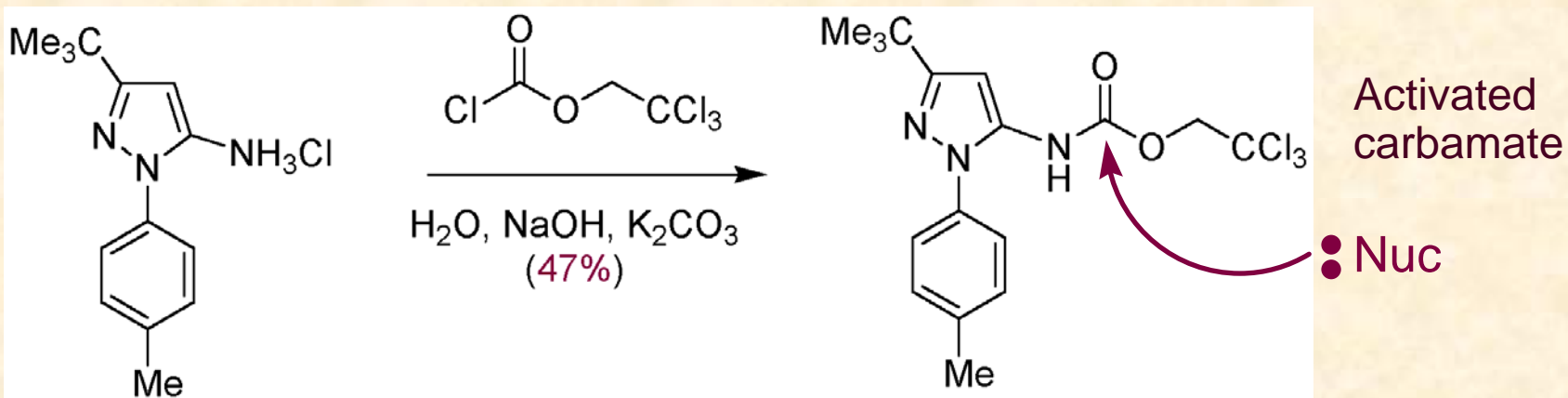
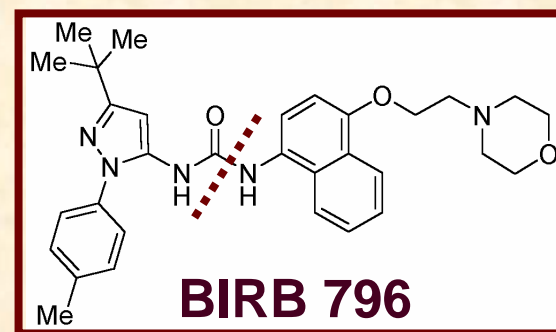
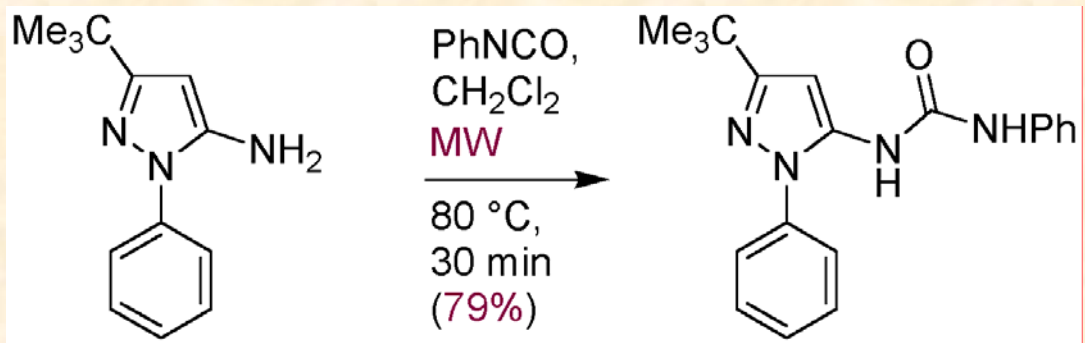


BIRB 796



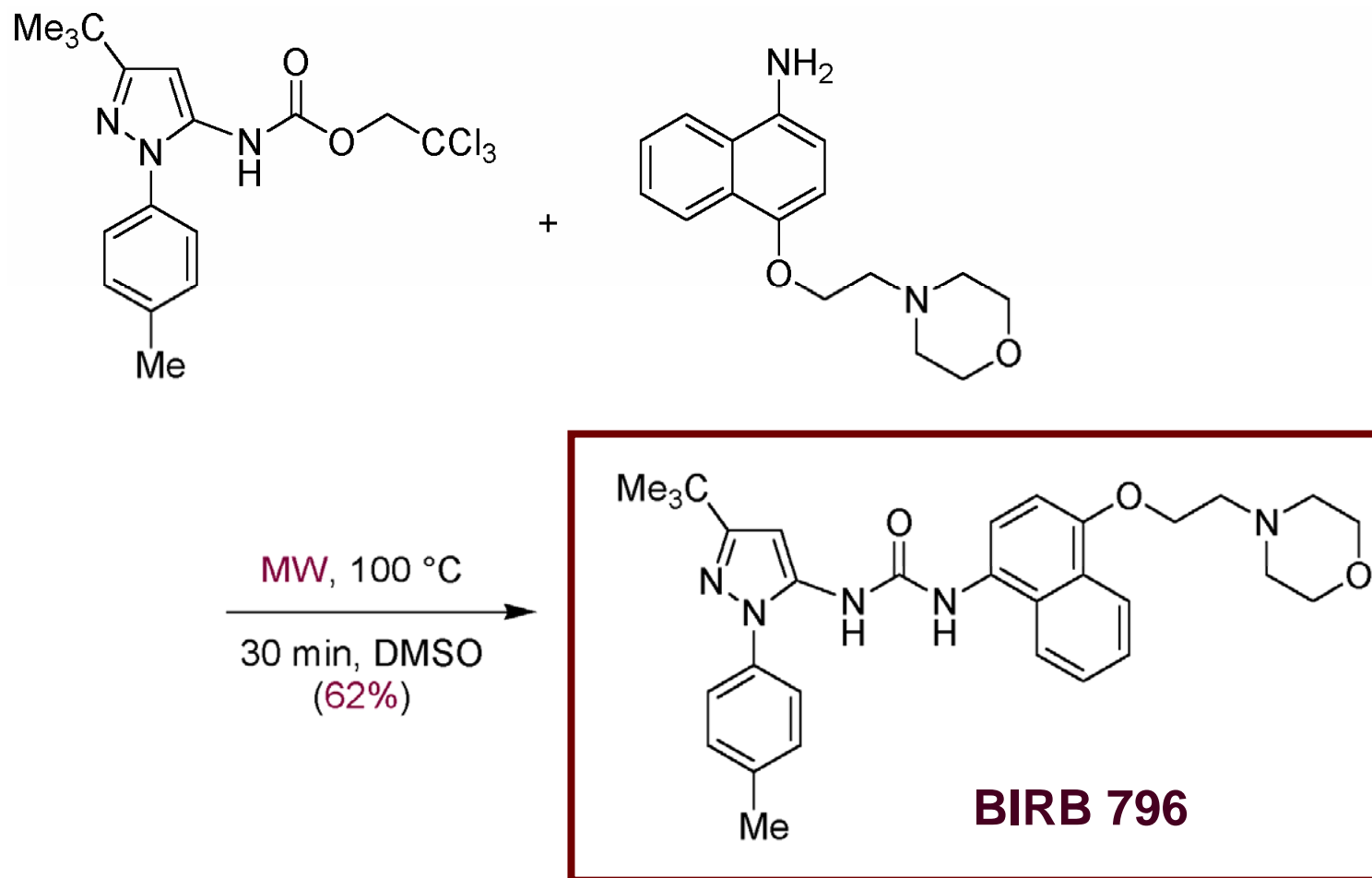
Aminonaphthol component

Synthesis of *N*-pyrazole ureas

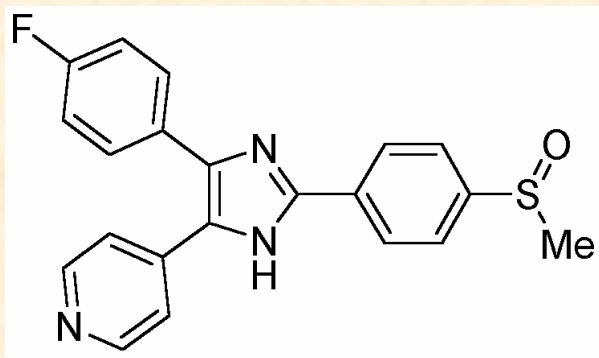
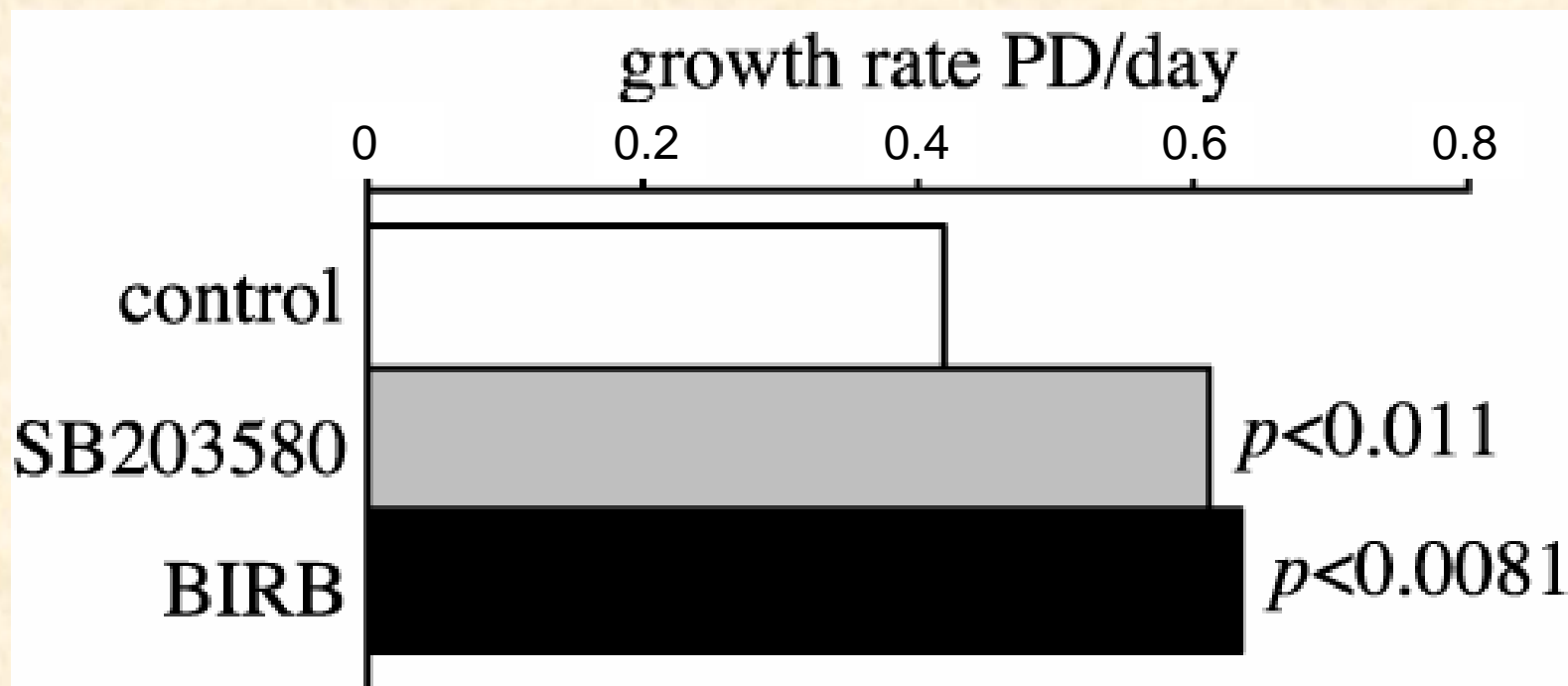


(other carbamates are under investigation)

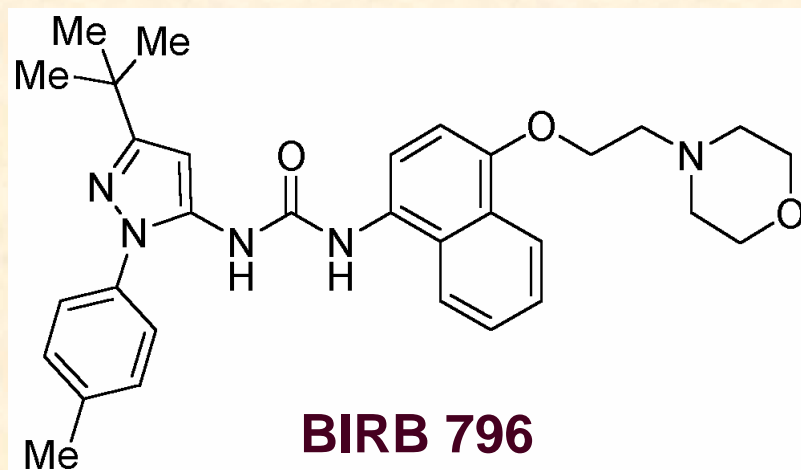
Synthesis of BIRB 796



Effect on growth rate of Werner Cells



SB203580



BIRB 796

Summary

With regard to cell ageing, SB203580 makes WS cells behave like normal cells

Other more selective inhibitors suitable for *in vivo* use can be prepared rapidly using microwave-assisted chemical synthesis

BIRB 796 may make WS cells behave like normal cells

Stress signals transduced by p38 α MAPK may be responsible for rapid ageing in WS cells

Pharmaceutical developments may offer clinical treatments for Werner's syndrome

Acknowledgements

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