HERIOT WATT UNIVERSITY

Isoform-Selective EPAC Activators: Therapeutic Opportunities for Cardiopulmonary Inflammation

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27/4/23

LEADERS IN IDEAS AND SOLUTIONS

EPACs: Key cAMP Sensors

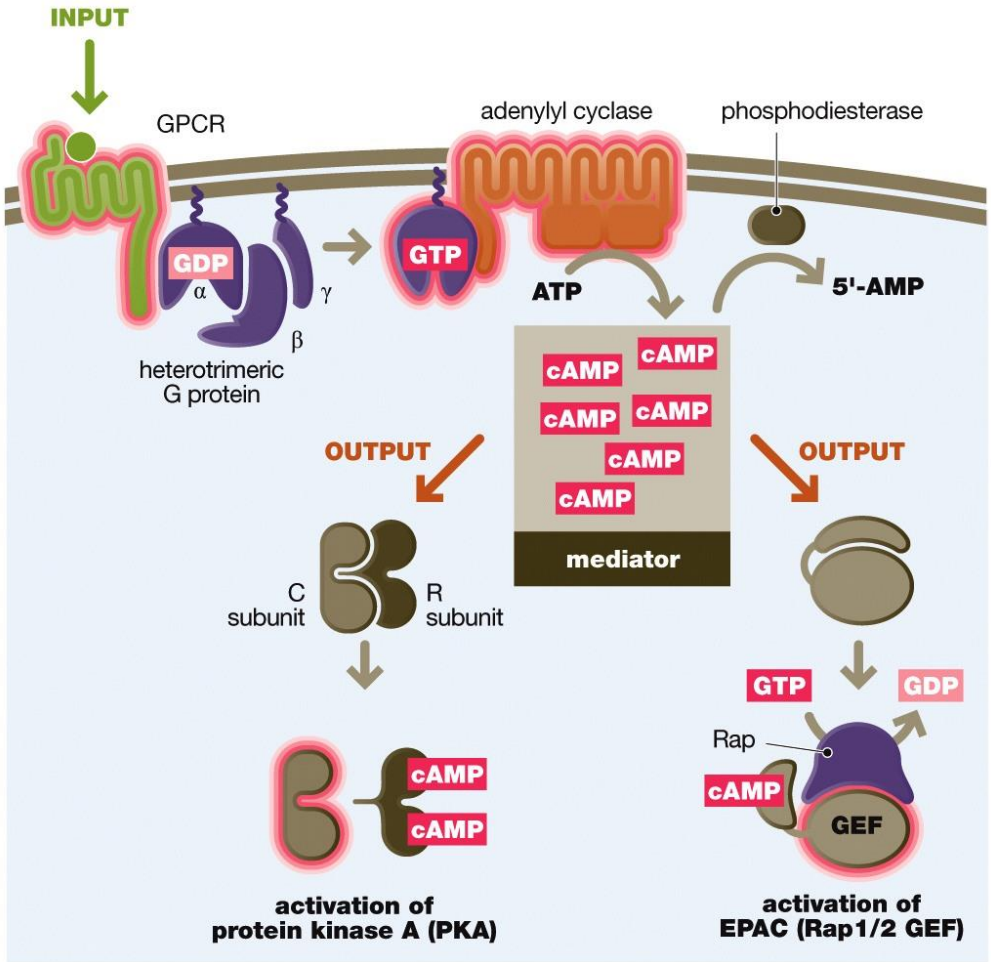
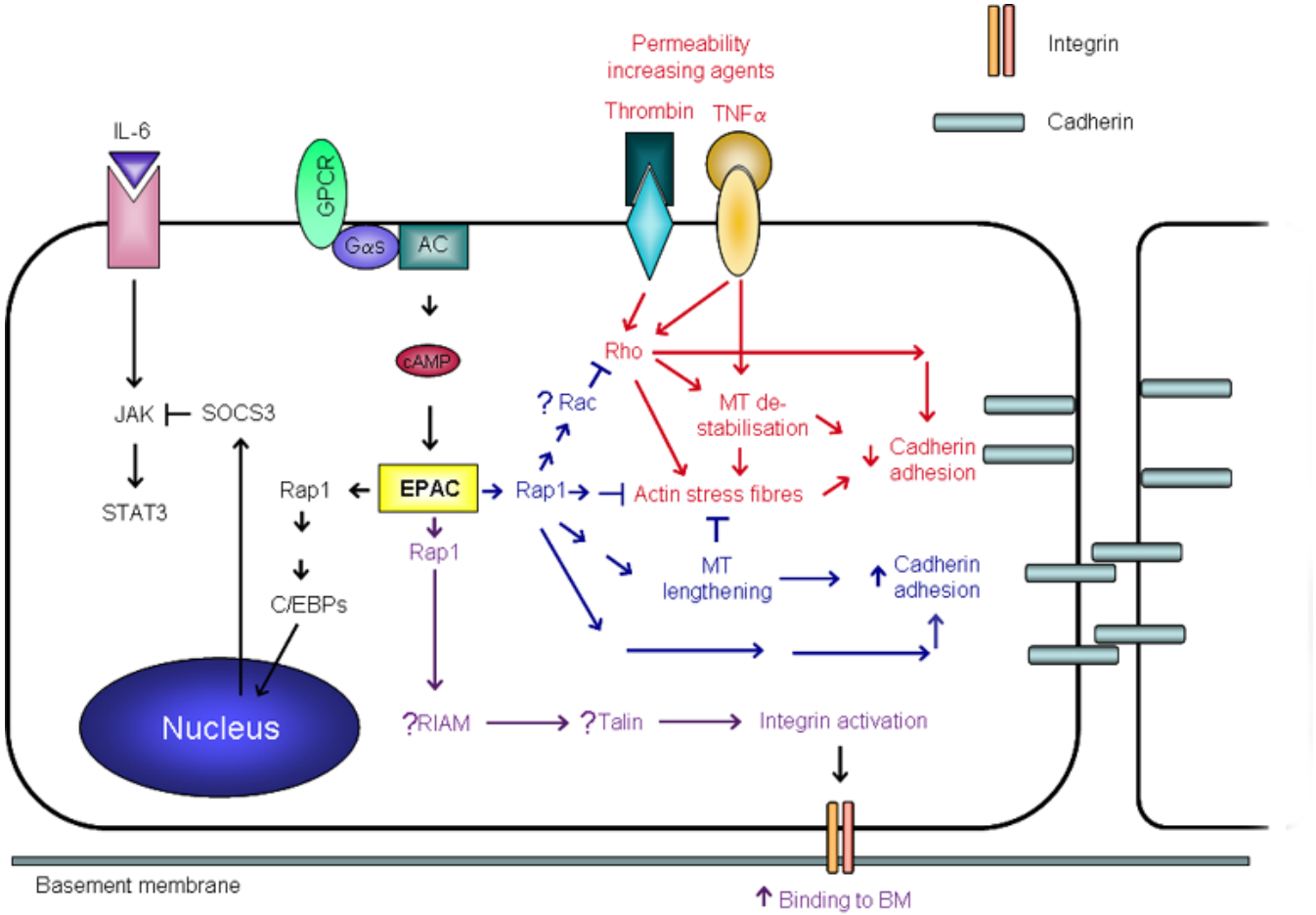


Figure 6.5 Cell Signaling (© Garland Science 2015)

- Agonism of Gs-coupled GPCRs results in synthesis of cAMP
- Well know: cAMP activates PKA
- cAMP also activates EPAC1 & 2, which are GEFs for Rap 1 & Rap2
- cAMP also interacts with cyclic nucleotide-gated ion channels, Popeye domain-containing proteins.

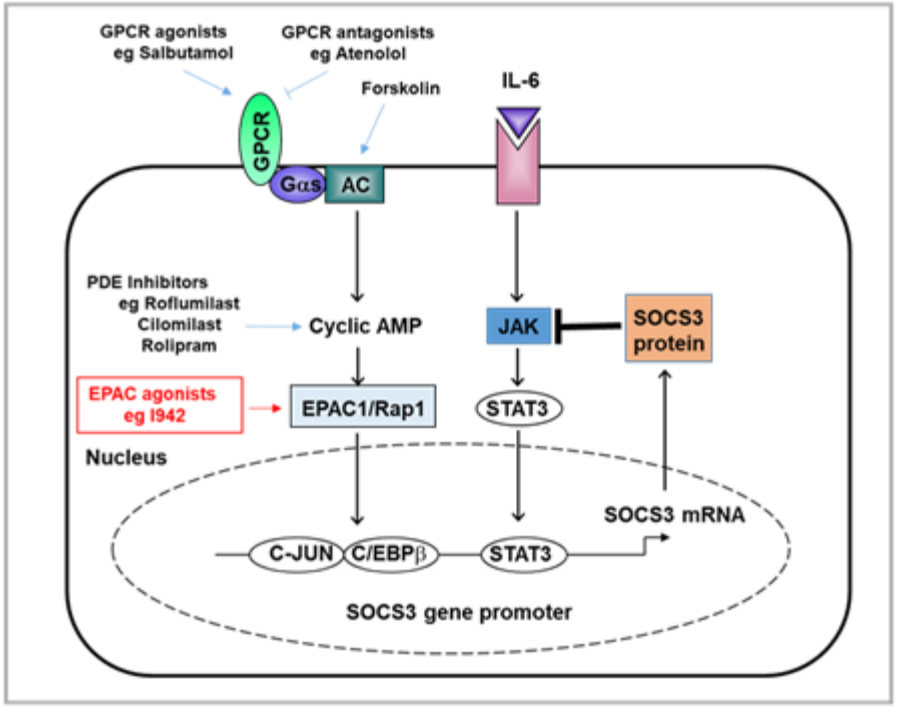
G. Barker *et al.*, *J. Cardiovasc. Dev. Dis.*, 2017, **4**, 22
U. Luchowska-Stańska *et al.*, *Biochem. Soc. Trans.*, 2019, **47,** 1415

Multiple Anti-Inflammatory Responses



Y. F. Chen *et al.*, *Respir. Res.*, 2019, **20**, 285
Y. Jiang *et al.*, *J. Inflamm. Res.*, 2019, **12,** 153
A. E. Dunne *et al.*, *Am. J. Respir. Cell Mol. Biol.*, 2019, **60**, 445
A. Oldenburger *et al.*, *FASEB J.*, 2017, **28**, 4617
R. K. Kopperud *et al.*, *Acta Physiol.*, 2017, **219**,. 441

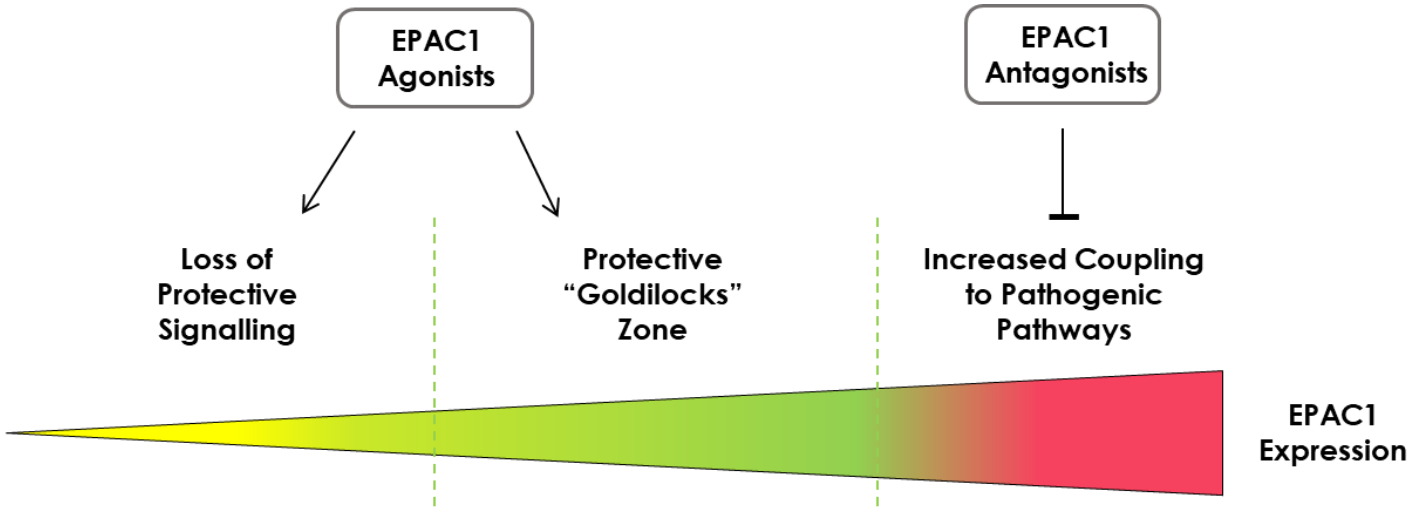
SOCS3 Anti-Inflammatory Responses



- EPAC1 activation promotes expression of the SOCS3 "saviour gene" in vascular endothelial cells
- SOCS3 normally activated by active IL-6 receptor, acts as break on inflammation
- Overriding SOCS3 can cause "cytokine storm"

H. D. Woolson *et al.*, *Cell. Signal.*, 2009, **21**, 1706
G. Borland *et al.*, *J. Biol. Chem.*, 2009, **284,** 17391
E. Parnell *et al.*, *Biochem. Soc. Trans.*, 2012, **40**, 1
J. Wiejak *et al.*, *FEBS Lett.*, 2014, 588
T. M. Palmer *et al.*, *J. Cell Signal.*, 2015, **1**, 1

EPAC1: Therapeutic Opportunities



Pathological Consequences:

Asthma
Pulmonary Arterial Hypertension
COPD
Tissue Fibrosis
Neutrophil Dysfunction
Kidney Disease

Protective Actions of EPAC1:

↓ Fibrotic Gene Expression
↓ Immune Cell Activation & Migration
↓ Cytokine Production & Signalling
↓ Thrombosis
↑ Endothelial Barrier Function
↑ Epithelial Barrier Function
Salt/Water Balance
↑ Vascular Tone
= Energy Homeostasis

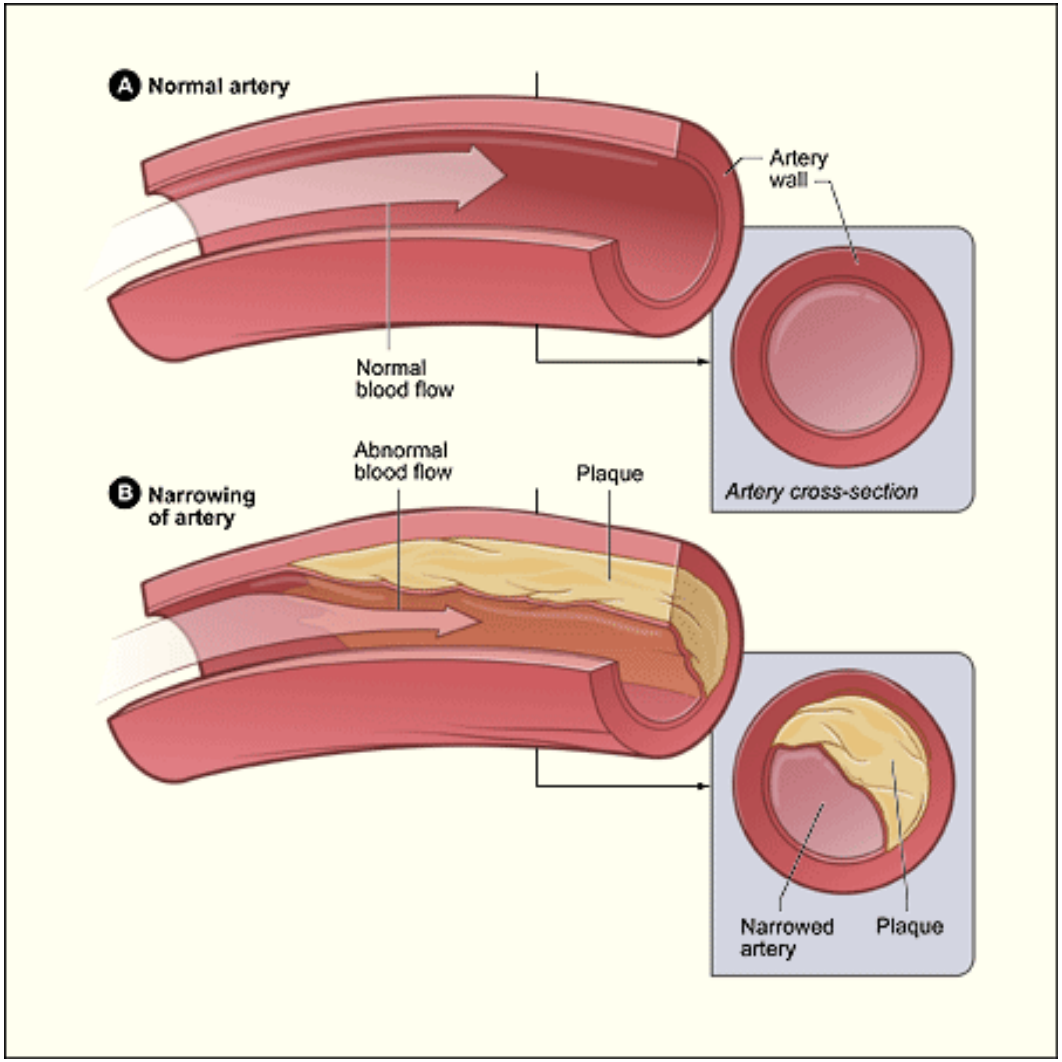
Pathological Consequences:

Alzheimer's Disease
Retinal neurodegeneration
Heart Failure
Cancer (Gastric, Breast & Pancreas)
Neointimal Hyperplasia
Late Stage Atherosclerosis

G. Barker *et al.*, *J. Cardiovasc. Dev. Dis.*, 2017, **4**, 22
U. Luchowska-Stańska *et al.*, *Biochem. Soc. Trans.*, 2019, **47,** 1415

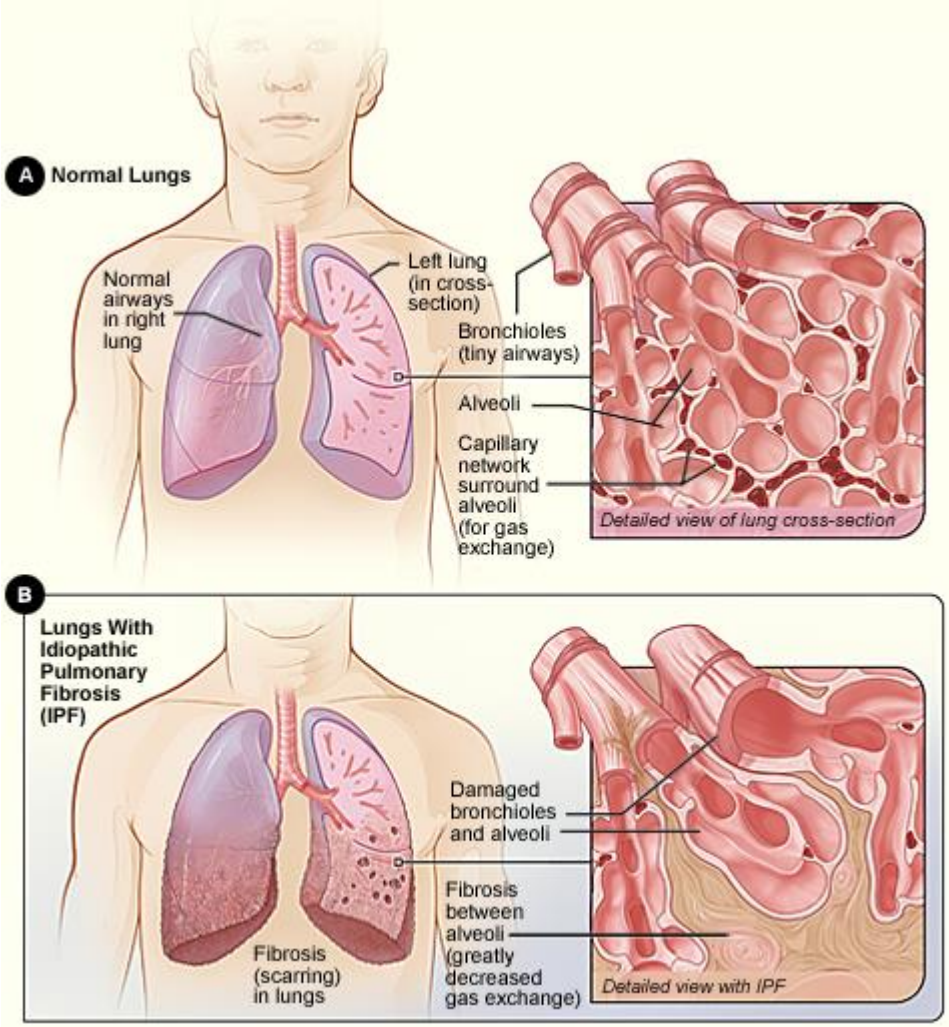
EPAC in CV Inflammation

- Atherosclerosis: the underlying cause of most cardiovascular diseases
- Irreversible
- 100% of the population over 65 y/o affected by atherosclerosis
- Over a quarter of all UK deaths are from cardiovascular diseases*
- Leading cause of death in the world*



The British Heart Foundation, https://www.bhf.org.uk/what-we-do/who-we-are, (accessed October 2021).

EPAC in Pulmonary Fibrosis

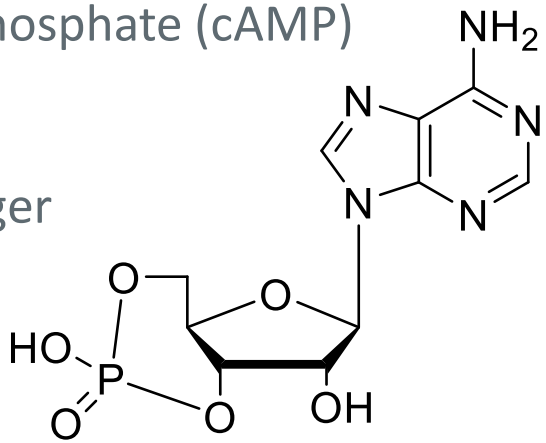


- 1/2000 people in UK suffer from PF.
- 5-year survival rate: 20-40%
- Irreversible
- Current treatments suffer from severe side effects
- Patients require very high flow rate supplemental oxygen

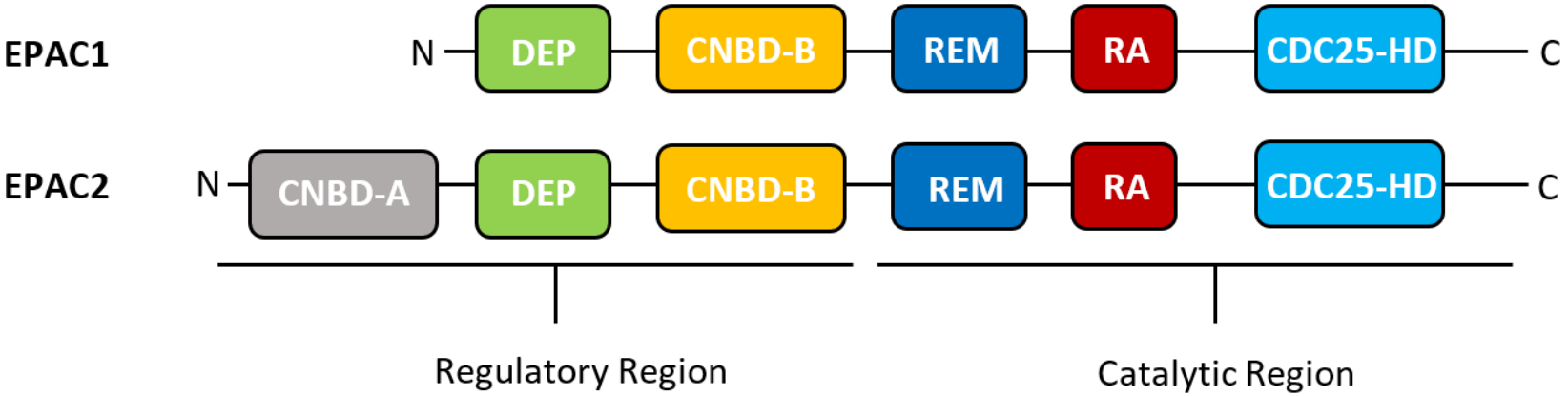
National Heart, Lung & Blood Institute, https://www.nhlbi.nih.gov/health/idiopathic-pulmonary-fibrosis/causes, (accessed April 2023).

EPAC Signalling

- Exchange Proteins activated by cyclic adenosine monophosphate (cAMP)
- Two main EPAC isoforms, EPAC1 and EPAC2
- Natural ligand cAMP, is an important secondary messenger
- However, cAMP also activates other sensors (e.g. PKA)

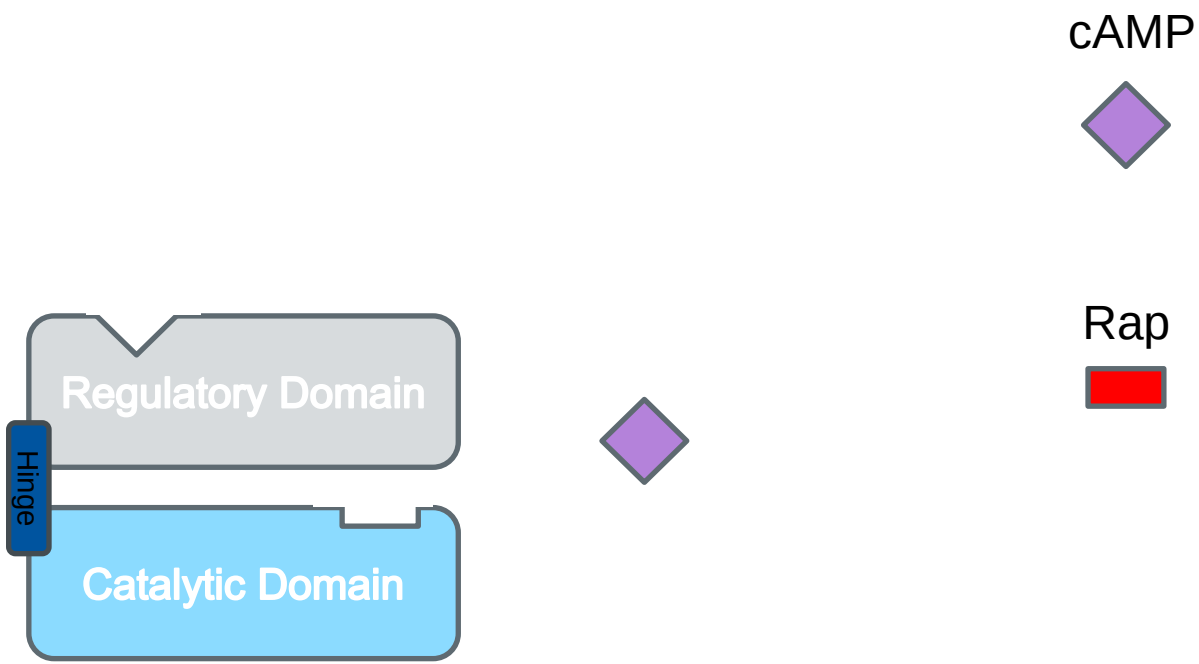


cAMP



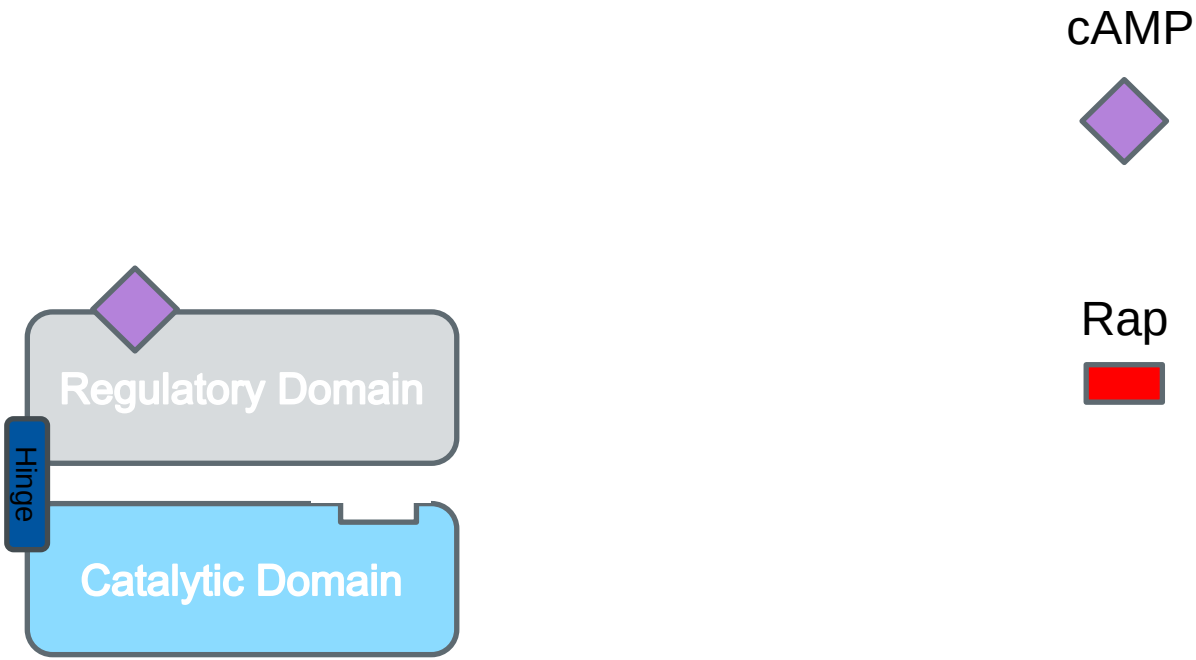
G. Barker, E. Parnell, B. Van Basten, H. Buist and S. J. Yarwood, *J. Cardiovasc. Dev. Dis.*, 2017, **4**, 22

EPAC Signalling



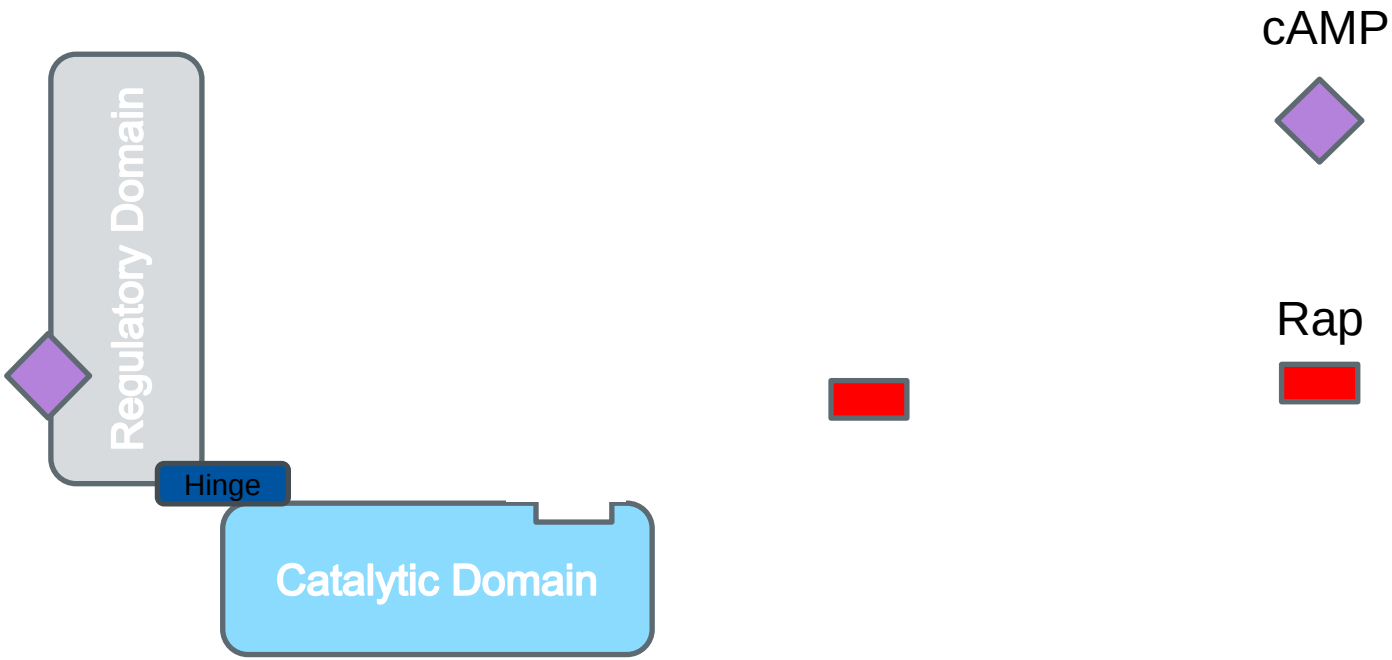
Inactive State

EPAC Signalling



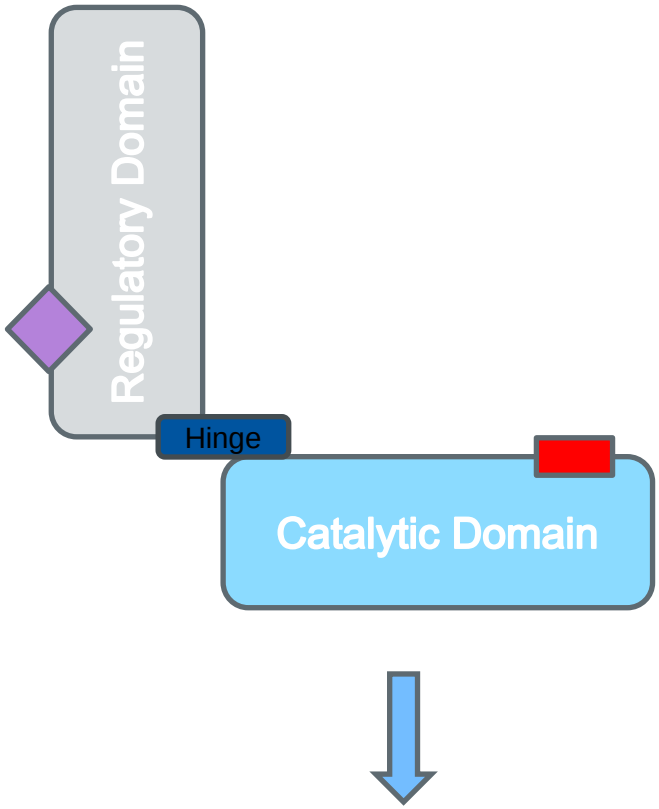
Active State

EPAC Signalling



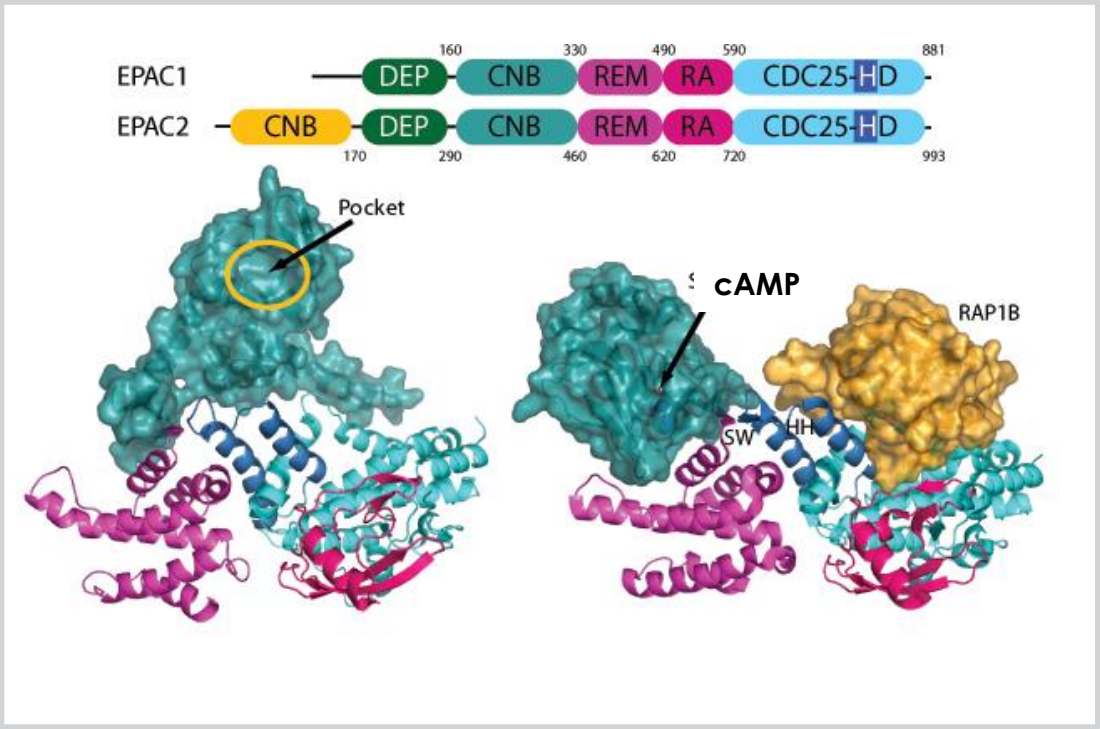
Active State

EPAC Signalling

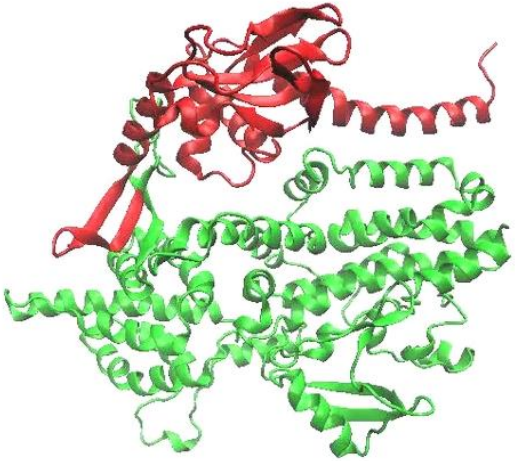


Further Downstream Signalling

EPAC Activation Confromations

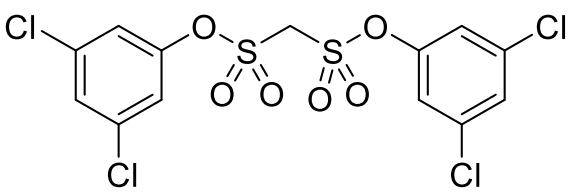


Conformational Changes in EPAC1

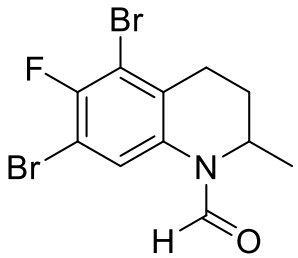


Previous EPAC Regulators

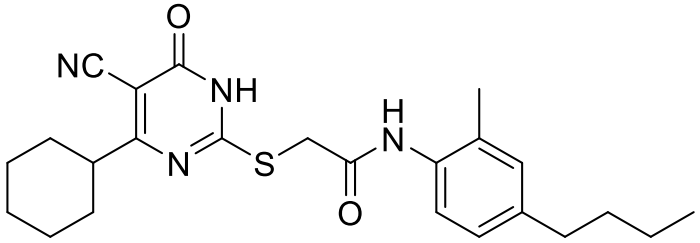
Antagonists



ESI-04

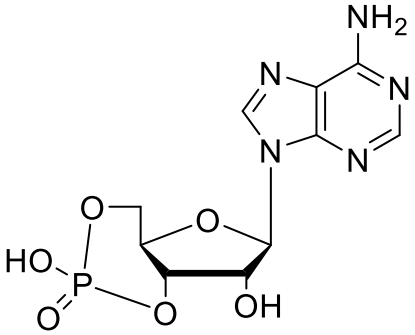


CE3F4

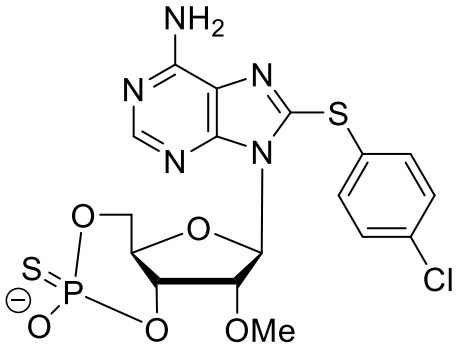


ESI-06

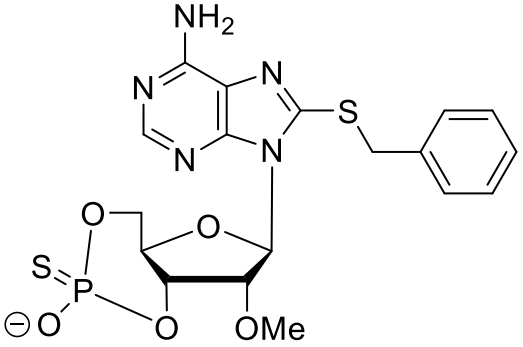
Agonists



cAMP



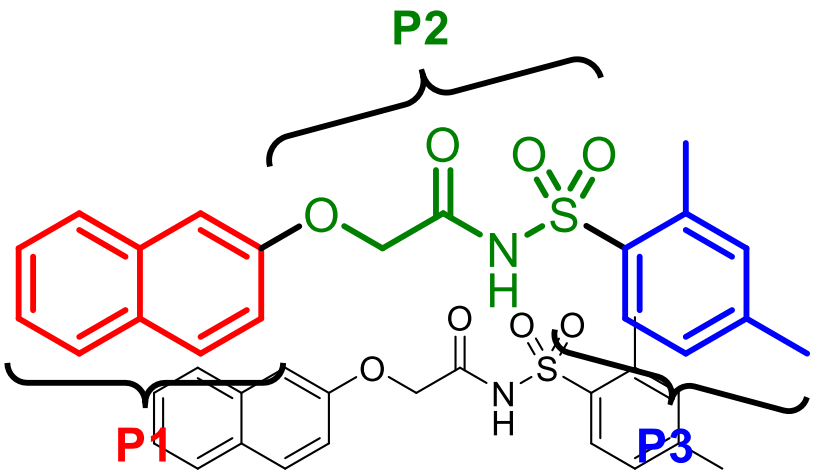
Sp-8-CPT-2'-O-Me-cAMPS
2 (007)



Sp-8-Bnt-2'-O-Me-cAMPS

U. Luchowska-Stańska, D. Morgan, S. J. Yarwood and G. Barker, *Biochem. Soc. Trans.*, 2019, **47**, 1415

EPAC1 Activators: PW Series



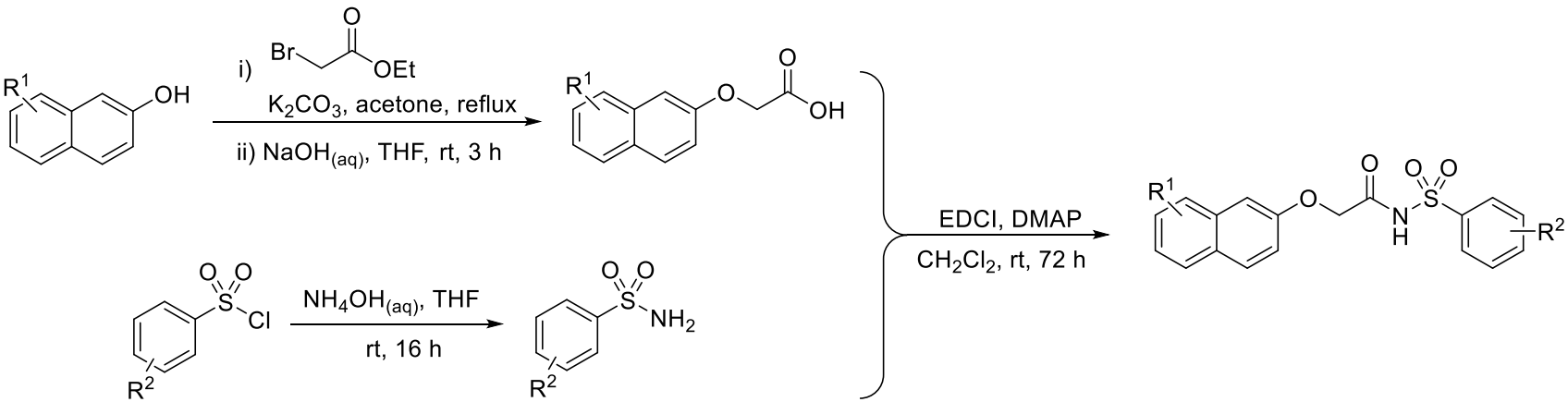
I942

EPAC1 pIC50 = 4.9

- Halogenated and methoxy substituted **naphthalene group (P1)** tolerated
- **Sulfonamide linker group (P2)** found to be vital for binding
- Six analogues with differing groups to ***m*-xylyl (P3)** showed improved binding than I942

P. Wang, U. Luchowska-Stańska, H. Chen, Z. Liu, J. Wiejak, P. Whelan, D. Morgan, E. Lochhead, G. Barker, H. Rehmann, S. J. Yarwood, and J. Zhou, *J. Med. Chem*. 2020, **63**, 5159−5184.

PW Series – Trivial Synthesis!



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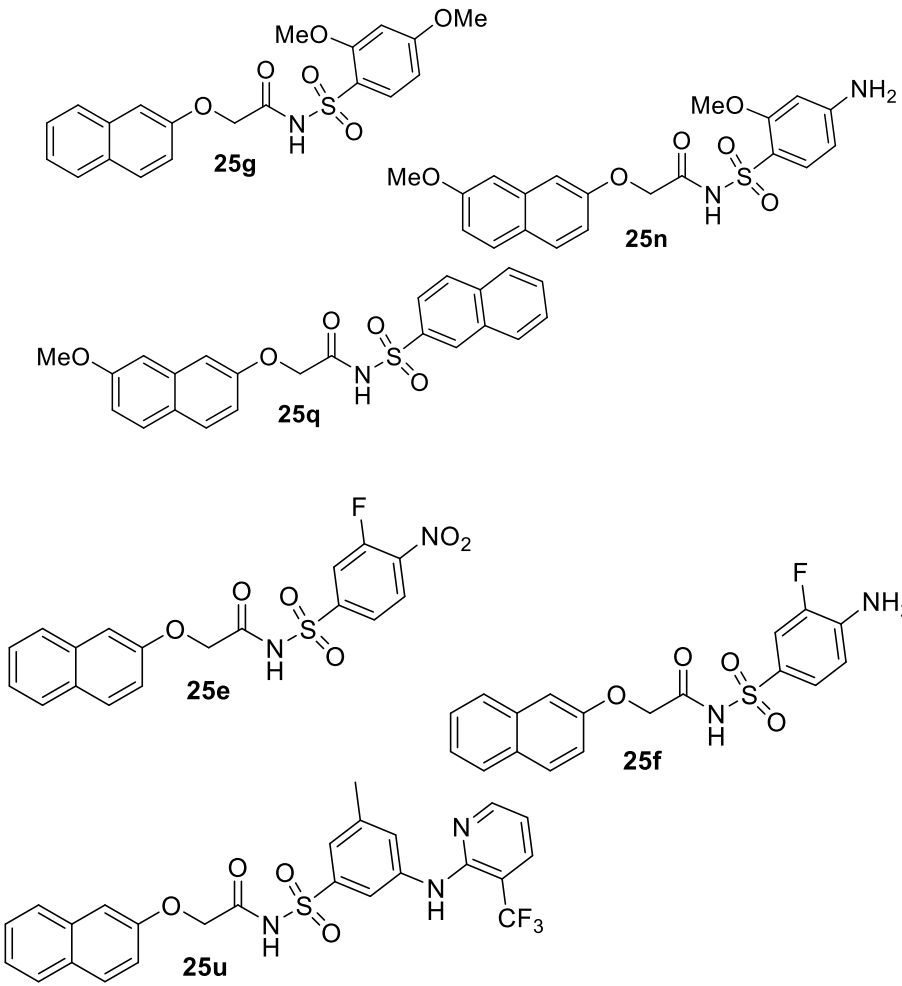

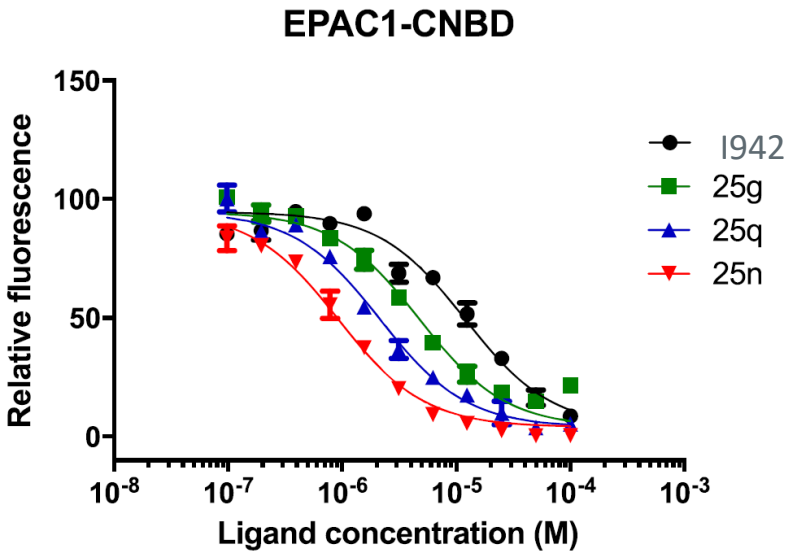

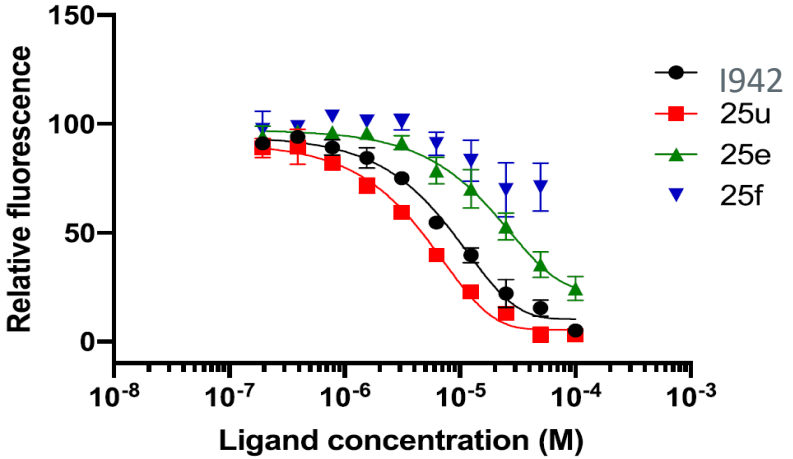

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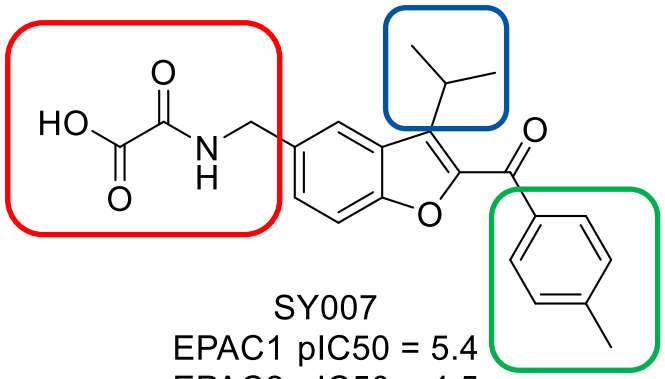
EPAC1 Activators: SY Series

3-substituent governs selectivity:
^{i}Pr optimal for EPAC1
Et optimal for EPAC2

Oxoacetic acid critical for binding; must be in benzofuran 5-position



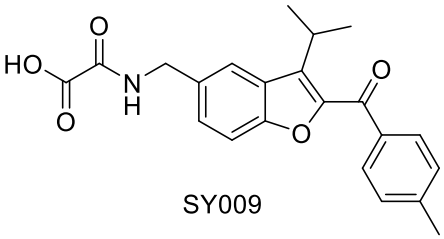
SY007
EPAC1 pIC50 = 5.4
EPAC2 pIC50 = 4.5

Saturated heterocycles tolerated; not fully explored

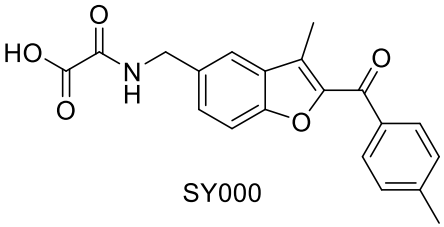
Also:
- No alternative to benzofuran core found (yet)
- Ketone 2-substituent required for EPAC1 selectivity

E. M. Beck, E. Parnell, A. Cowley, A. Porter, J. Gillespie, J. Robinson, L. Robinson, A. D. Pannifer, V. Hamon, P. Jones, A. Morrison, S. McElroy, M. Timmerman, H. Rutjes, P. Mahajan, J. Wiejak, U. Luchowska-Stańska, D. Morgan, G. Barker, H. Rehmann and S. J. Yarwood, *Cells*, 2019, **8**, 1425

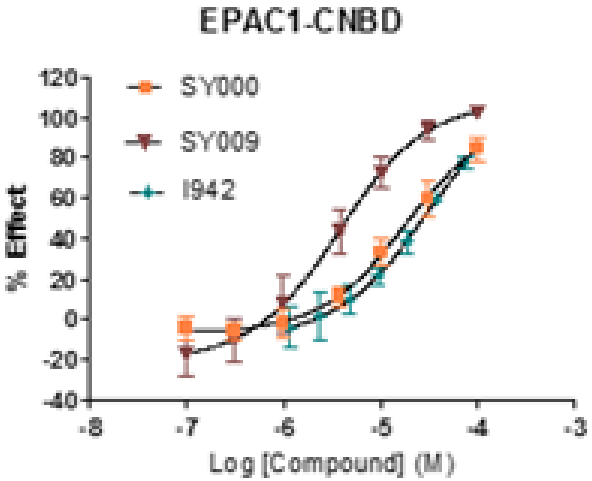
EPAC1 Activators: SY Series



SY009



SY000



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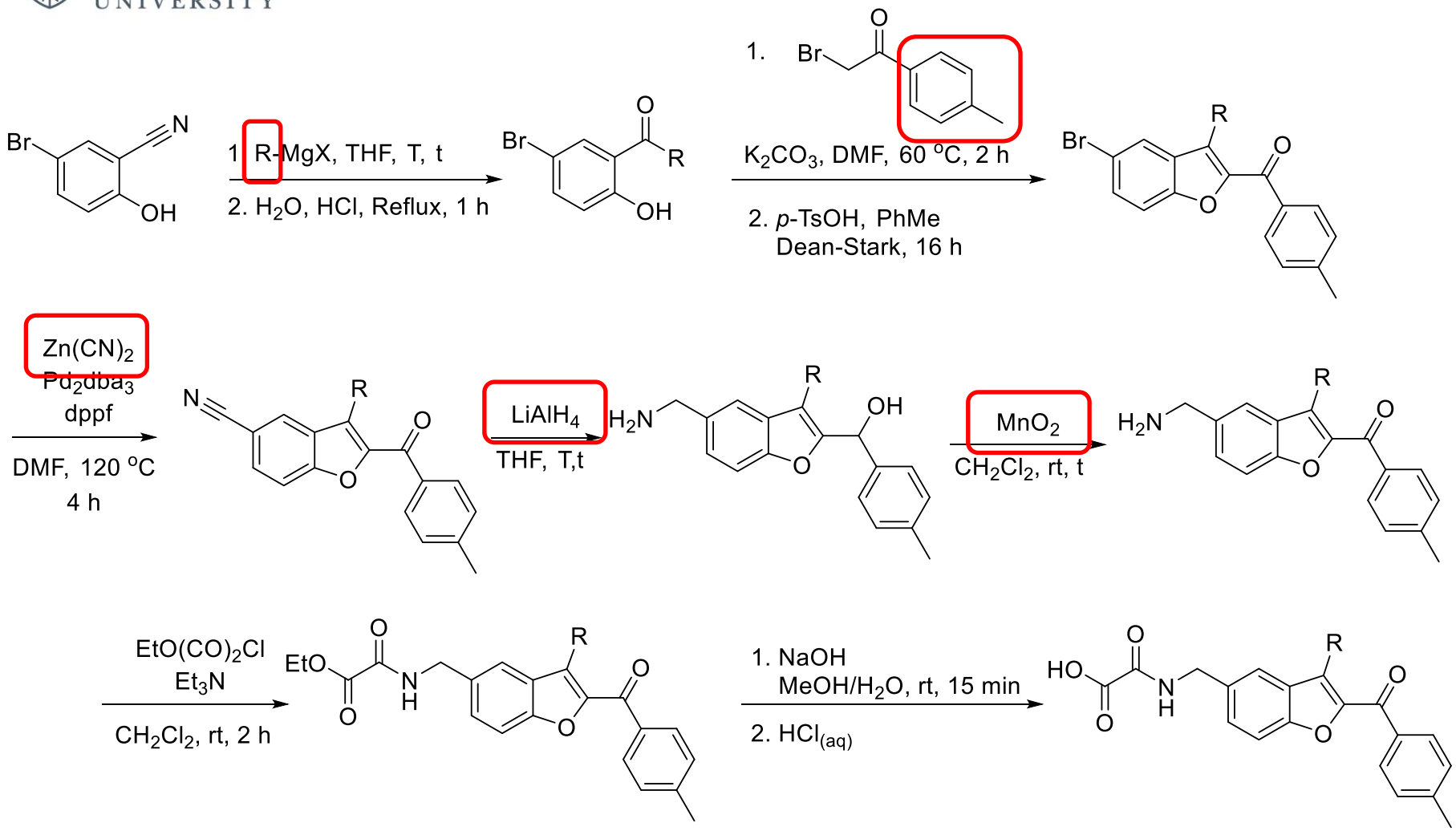
SY Series – Synthesis



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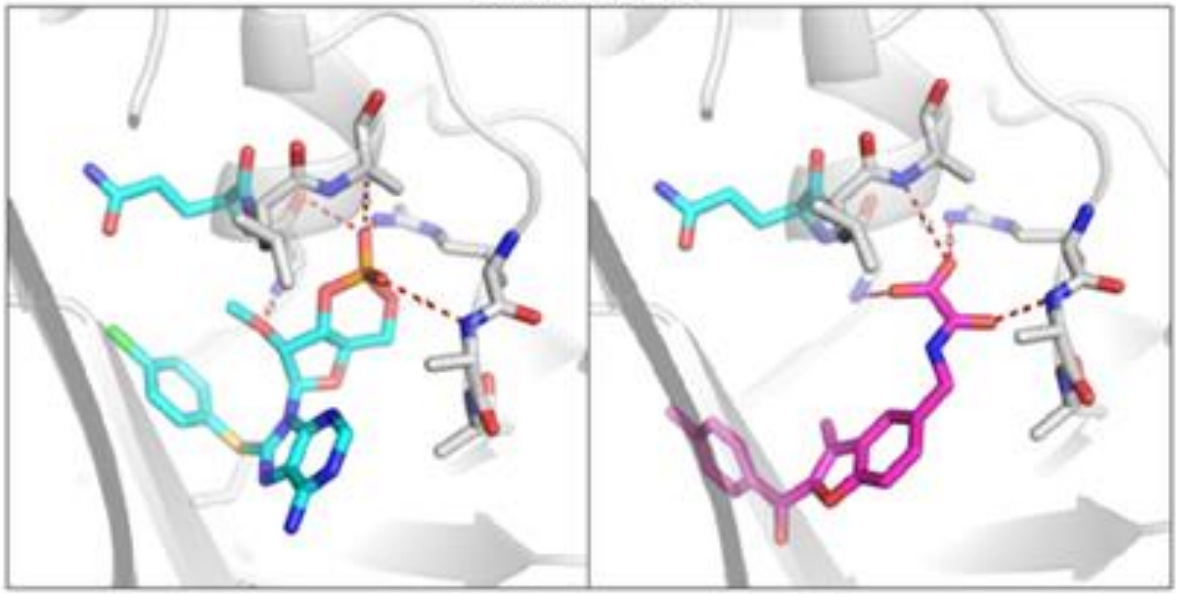
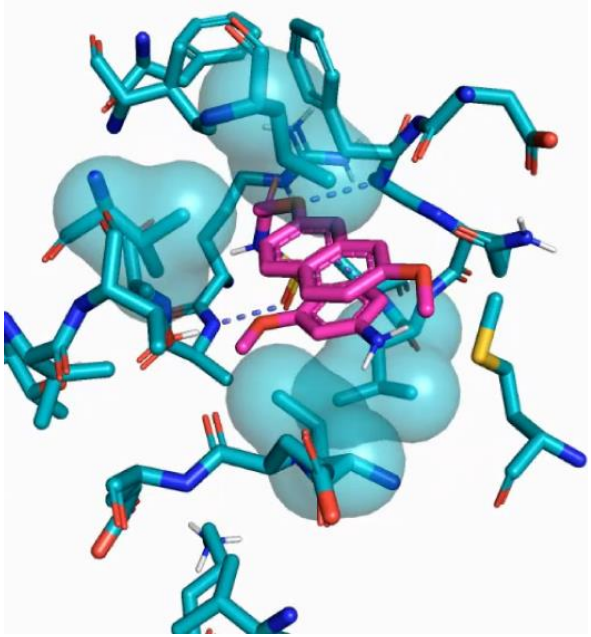
EPAC1 Activators: Docking Models



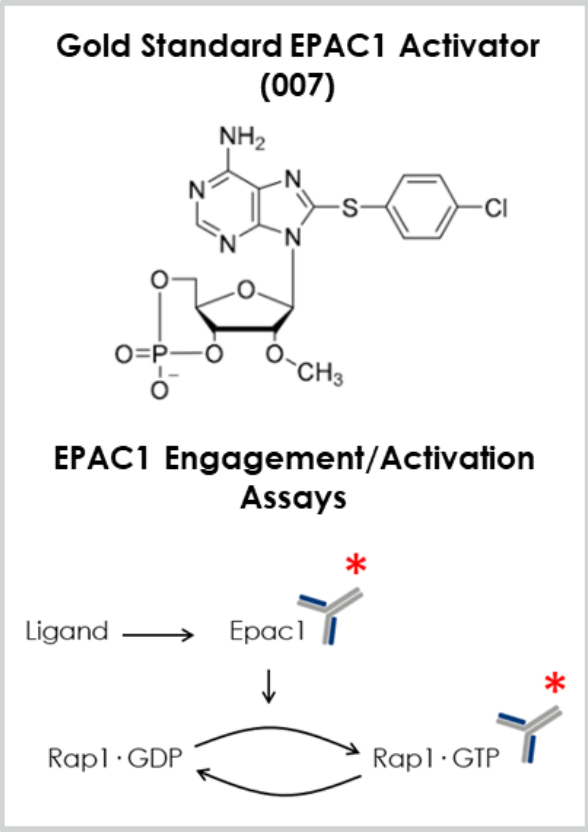
D-007 SY000

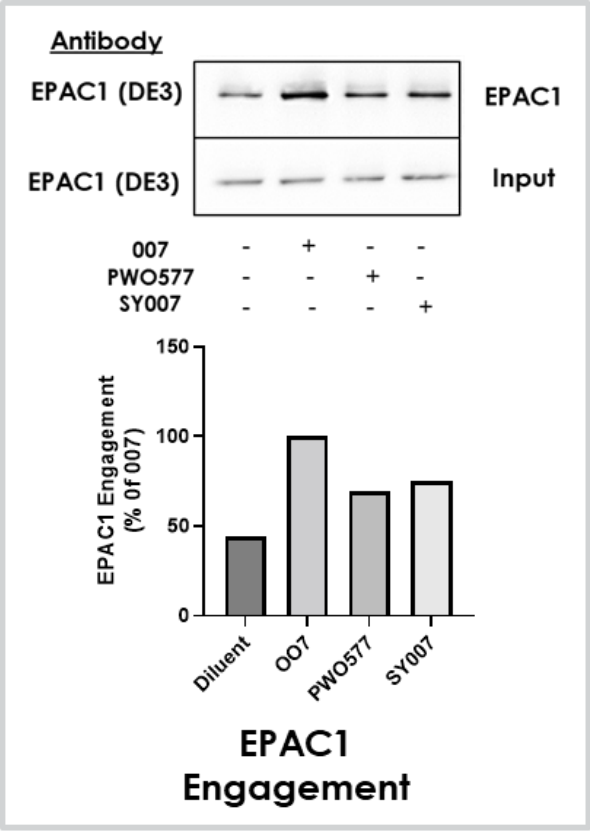


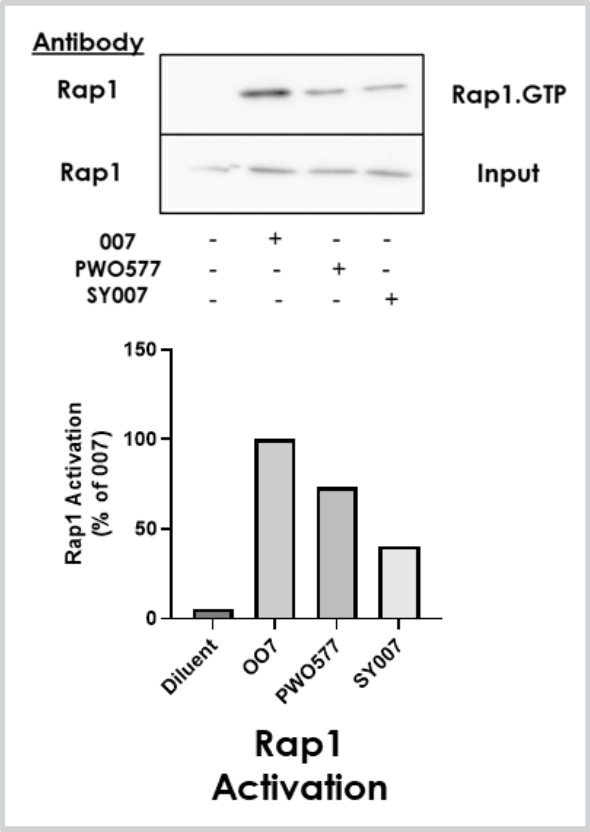
PW0577

E. M. Beck, E. Parnell, A. Cowley, A. Porter, J. Gillespie, J. Robinson, L. Robinson, A. D. Pannifer, V. Hamon, P. Jones, A. Morrison, S. McElroy, M. Timmerman, H. Rutjes, P. Mahajan, J. Wiejak, U. Luchowska-Stańska, D. Morgan, G. Barker, H. Rehmann and S. J. Yarwood, *Cells*, 2019, **8**, 1425

EPAC1 Activators: Rap Activation







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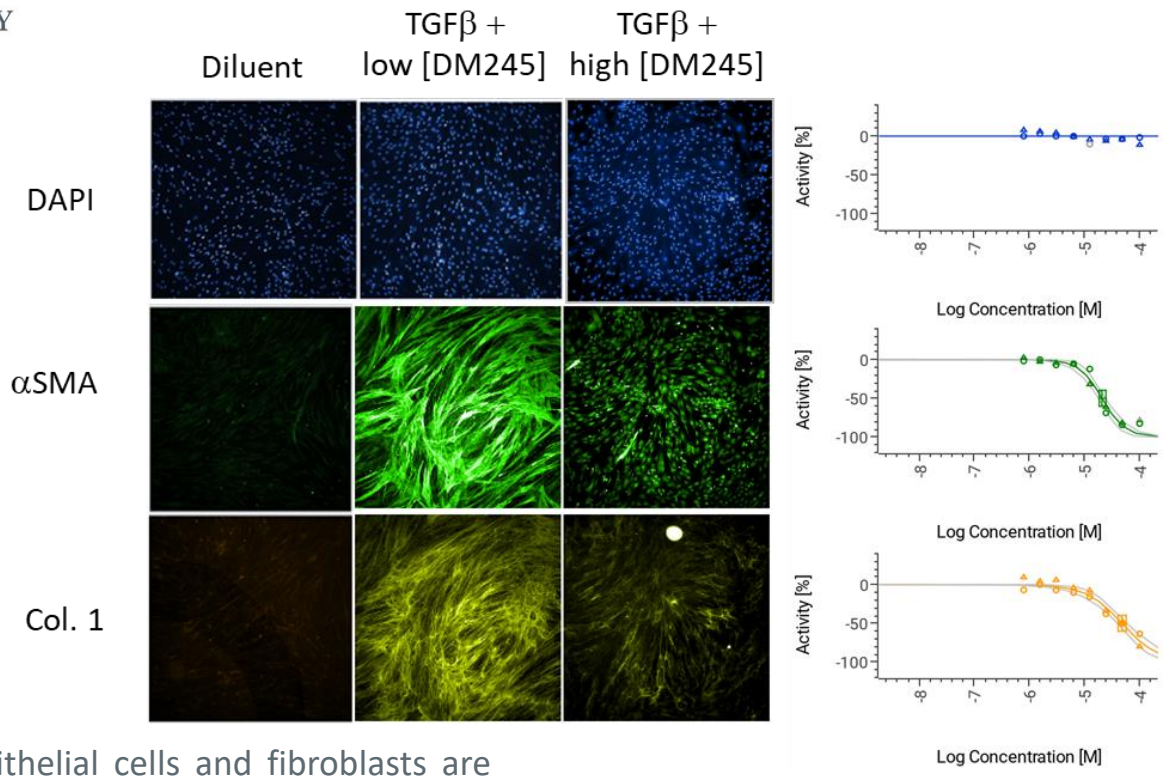
EPAC1 Activators: Fibrosis Models



- In IPF, healthy epithelial cells and fibroblasts are replaced by secretory cells (myoepithelial cells) that produce extracellular matrix (ECM) components, including collagen 1, leading to production of scar tissue and eventual loss of lung function.

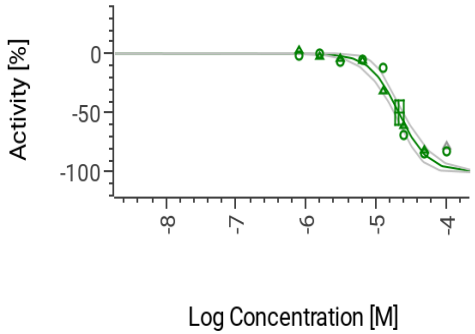
- EPAC1 inhibits the conversion to myoepithelial cells, by inhibiting the actions of fibrotic growth factors (eg TGFβ)

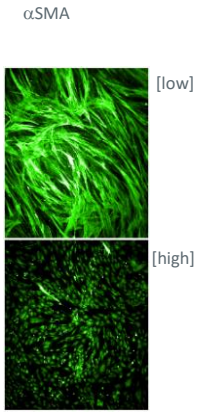
- In the diseased state TGFβ reduces the expression of EPAC1

- To counteract this we have created activators of EPAC1(eg DM245) to reduce the action of TGFβ in promoting myofibroblast production

EPAC1 Activators *vs.* Nintedanib

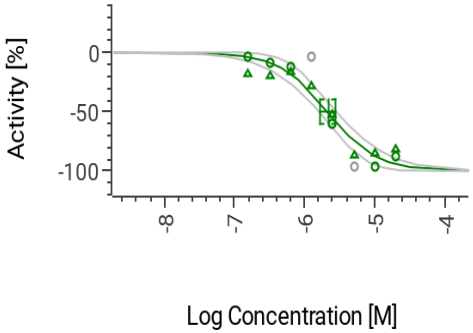
DM245

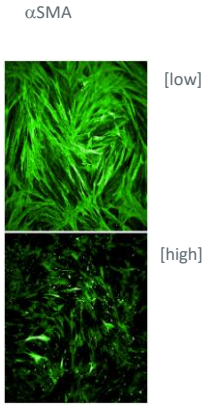




26.1 µM

Nintedanib

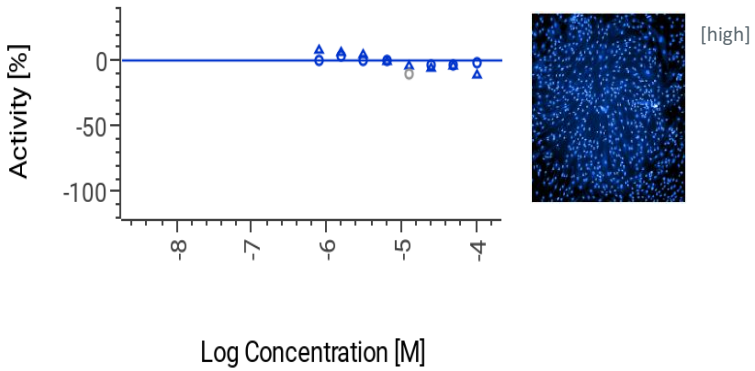




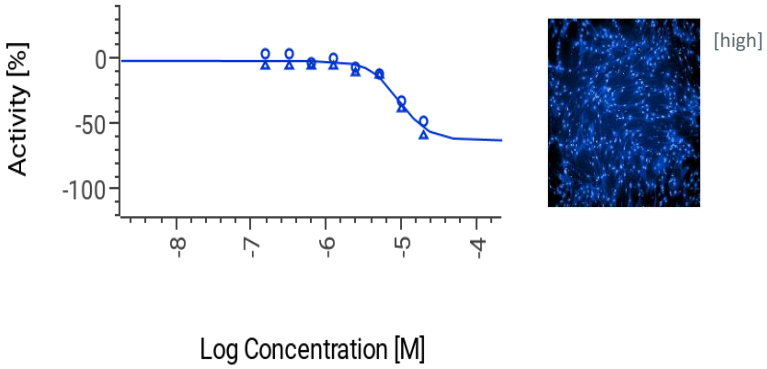
1.64 µM

EPAC1 Activators *vs.* Nintedanib

DM245



Nintedanib



- Our compounds have no measurable cytotoxicity up to 100 µM!

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- Stephen Yarwood
  scientific founder

AUDAX Therapeutics

- Chris Wardhaugh
  commercial founder

- Graeme Barker
  founder: chemistry

https://www.audaxtherapeutics.com/

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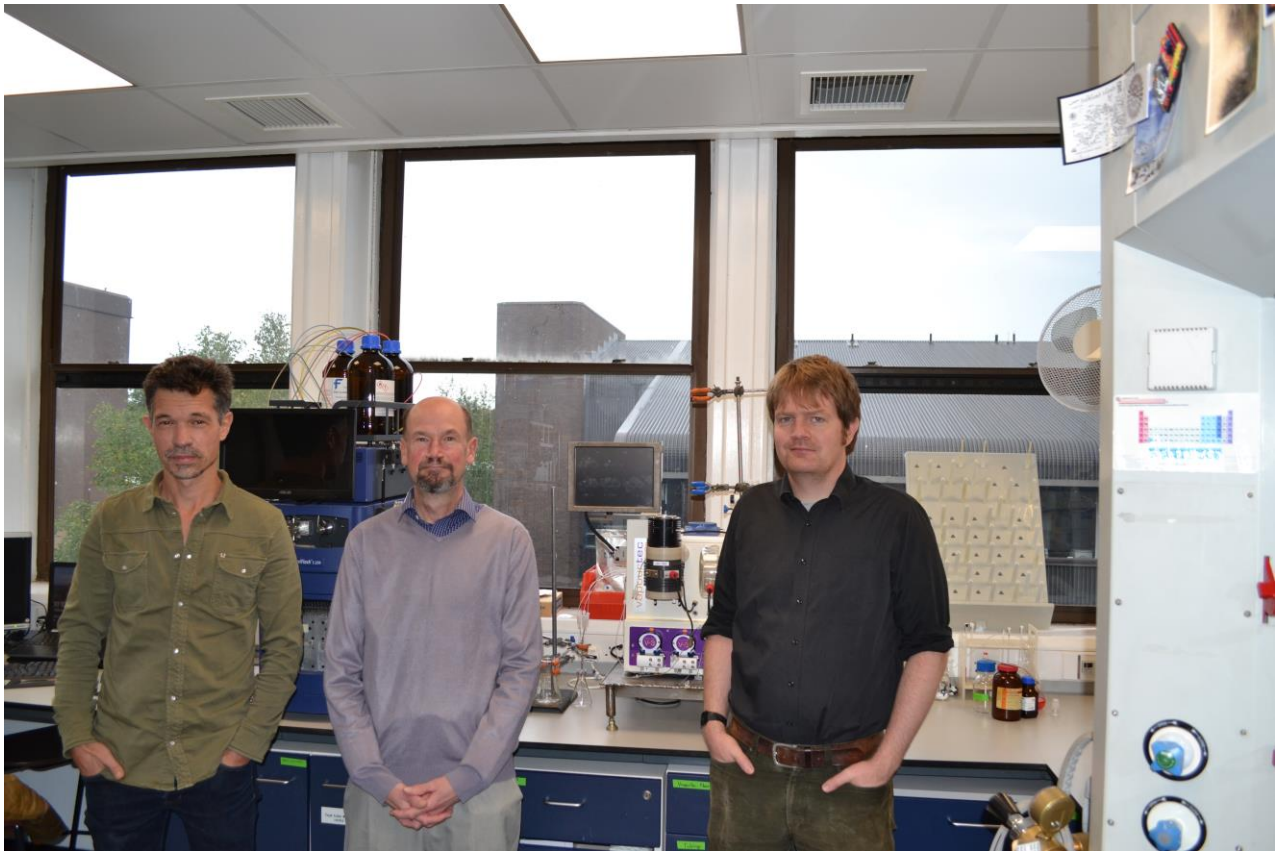
flow lab



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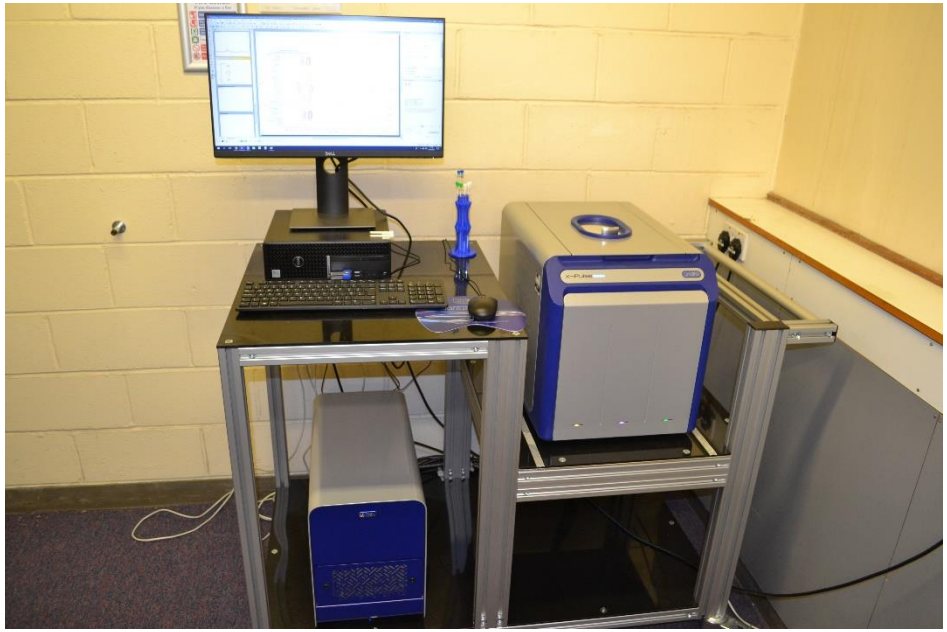

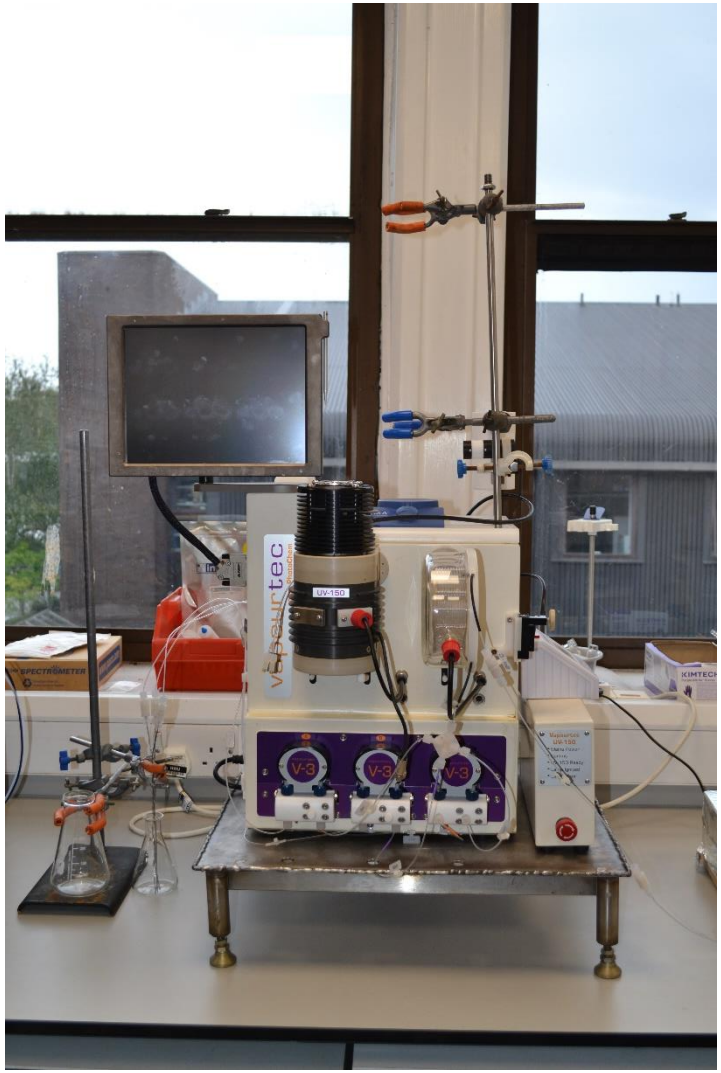

- Additional UV/Vis and HPLC monitoring
- Now with in-line flash column chromatography!
- Contact: Continuum@hw.ac.uk (facility), F.Vilela@hw.ac.uk (Director)
  Graeme.Barker@hw.ac.uk (Co-Director), D.Ellis@hw.ac.uk (Co-Director)

C. G. Thomson, C. Banks, M. Allen, G. Barker, C. R. Coxon, A.-L. Lee and F. Vilela, *J. Org. Chem.*, 2021, **86**, 14079

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Thanks!

UKRI Engineering and Physical Sciences Research Council

British Heart Foundation

THE CARNEGIE TRUST FOR THE UNIVERSITIES OF SCOTLAND

TENOVUS SCOTLAND SUPPORTING MEDICAL RESEARCH

ROYAL SOCIETY OF CHEMISTRY

Scottish Enterprise

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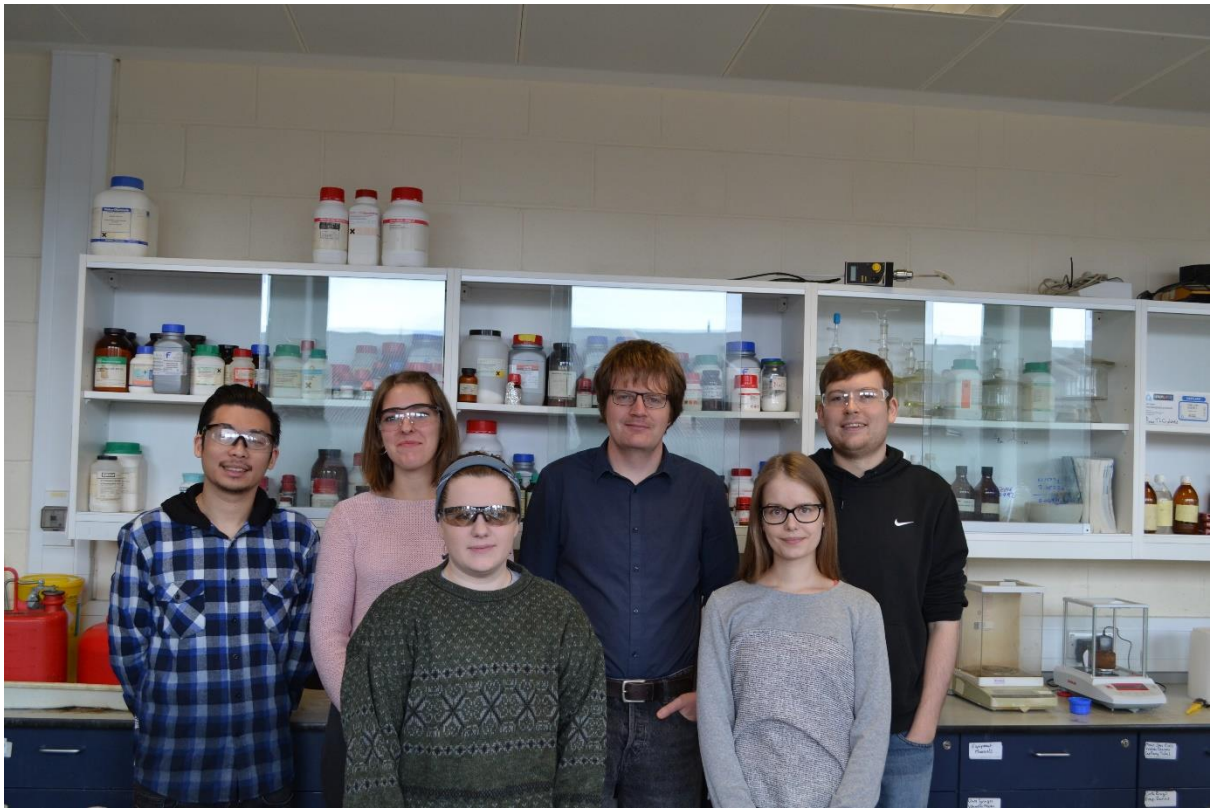
Thanks!



Hear about our metalation & flow chemistry!

RSC INTEREST GROUP
BIOLOGICAL AND MEDICINAL CHEMISTRY SECTOR

3rd Synthesis in Drug Discovery & Development
23rd – 24th May
Zoom

ROYAL SOCIETY OF CHEMISTRY | HETEROCYCLIC AND SYNTHESIS GROUP

25th Lakeland Symposium
7th – 11th May
Grasmere, Lake District