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Poster presentation



# Distinct molecular requirements for activation or stabilization of soluble guanylyl cyclase upon haem oxidation-induced degradation

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## Background

In endothelial dysfunction, signalling by nitric oxide (NO) is impaired because of the oxidation and subsequent loss of the soluble guanylyl cyclase (sGC) haem [1]. The sGC activator 4-[((4-carboxybutyl){2-[(4-phenethylbenzyl)oxy]phenethyl}amino)methyl [benzoic]acid (BAY 58-2667) is a haem-mimetic able to bind with high affinity to GC when the native haem (the NO binding site) is removed and it also protects sGC from ubiquitin-triggered degradation [2-4]. Here we investigate whether this protection is a unique feature of BAY 58-2667 or a general characteristic of haem-site ligands such as the haem-independent sGC activator 5-chloro-2-(5-chlorothiophene-2-sulphonylamino-*N*-(4-(morpholine-4-sulphonyl)-phenyl)-benzamide sodium salt (HMR 1766), the haem-mimetic Zn-protoporphyrin IX (Zn-PPIX) or the haem-dependent sGC stimulator 5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-4-ylamine (BAY 41-2272).

## Experimental approach

The sGC inhibitor 1H-(1,2,4)-oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) was used to induce oxidation-induced degradation of sGC. Activity and protein levels of sGC were measured in a Chinese hamster ovary cell line as well as in primary porcine endothelial cells. Cells expressing mutant sGC were used to elucidate the molecular mechanism underlying the effects observed.

## Results

Oxidation-induced sGC degradation was prevented by BAY 58-2667 and Zn-PPIX in both cell types. In contrast, the structurally unrelated sGC activator, HMR 1766, and the sGC stimulator, BAY 41-2272, did not protect. Similarly, the constitutively haem-free sGC mutant $\beta_1$H105F was stabilized by BAY 58-2667 and Zn-PPIX.

## Conclusion

The ability of BAY 58-2667 not only to activate but also to stabilize oxidized/haem-free sGC represents a unique example of bimodal target interaction and distinguishes this structural class from non-stabilizing sGC activators and sGC stimulators such as HMR 1766 and BAY 41-2272 respectively.

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