

Imide-Substituted 4-Benzyl-2H-phthalazin-1-ones: Potent Inhibitors of Poly(ADP-ribose) Polymerase-1 (PARP-1)



MAYBRIDGE

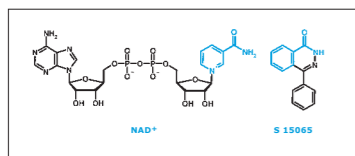
Frank Kerrigan*, Xiaoling Cockcroft*, Krystyna J. Dillon*, Lesley Dixon*, Gill D. Douglas*, Penny J. Eversley*, Janet Hoare*, Niall M.B. Martin*, Roger F. Newton*, Jane M. Paul*, Graeme C.M. Smith*, Julia Vile* and Rick C. White*,
a. Maybridge plc, Trevillet, Tintagel, Cornwall, PL34 0HW, UK b. ChemOvation Ltd., Foundry Lane, Horsham, West Sussex, RH13 5PX, UK c. KuDOS Pharmaceuticals Ltd., 327 Cambridge Science Park, Milton Road, Cambridge, CB4 0GZ

Introduction

Poly(ADP-ribose) Polymerase-1 (PARP-1), the most abundant member of a family of Poly(ADP-ribosylating) enzymes¹, is a nuclear protein implicated in a range of cellular processes including DNA repair, cell replication and differentiation, necrosis, particularly in response to oxidative injury, and promotion of pro-inflammatory signal transduction processes². Inhibition of the activity of PARP-1 therefore has wide-ranging therapeutic potential, from enhancing the efficacy of cytotoxic agents during cancer chemotherapy³ to the reduction of neuronal damage following cerebral ischaemia⁴.

PARP-1 binds to, and is activated by single and double strand breaks in DNA. Utilising nicotinamide adenine dinucleotide (NAD⁺), the activated enzyme synthesises ADP-ribose homopolymers onto a range of protein acceptors (including PARP-1 itself)². It has been postulated that formation of these negatively charged polymers causes electrostatic repulsion of modified PARP-1 and other targets from the DNA, thereby facilitating recruitment of the base excision repair complex⁵. Most classical PARP inhibitors mimic the nicotinamide moiety of NAD⁺ and, therefore, interfere with binding of the substrate to the active site of the enzyme.

Starting from the phthalazinone nicotinamide mimic **S 15065**, identified by screening the Maybridge Compound Collection, and overcoming poor ADME-PK characteristics of early compounds, we have developed a novel series of imide-substituted 4-benzyl-2H-phthalazin-1-ones, which are potent PARP-1 inhibitors in both isolated enzyme and cell-based assays. A member of the series has subsequently been shown to enhance the efficacy of a cytotoxic agent in a mouse xenograft model.



Early SAR Development of S 15065 (Table 1)

Variations on the fused or pendant benzene rings of **S 15065** gave modest enhancements in activity, reaching a plateau of IC₅₀ around 200nM for several diamines such as **1**. However, homologation to the benzyl compound **2** significantly enhanced activity and substitution on the benzylic ring indicated excellent scope for SAR optimisation. In particular, carbonyl-containing substituents at the 3-position gave potent PARP-1 inhibitors such as **3** and **4**.⁶

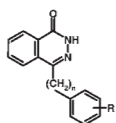
Focused analogue libraries were designed around both compounds, using simple chain variation and more complex Maybridge Reactive Intermediates in parallel synthesis methodologies. This poster will focus on amides based on **4**, but similar potency enhancements were achieved with esters based on **3**.

Chain extension to the propionamide (**MRU 687**) significantly increased activity (IC₅₀ 20nM). Further chain extension (**5**) or branching (**6**) tended to reduce potency, although constraining the branch into a cyclopropane ring (**7**) reversed this trend. Several compounds with more complex amide substituents (e.g. **8**) were also potent PARP-1 inhibitors.

MRU 687 was also active at 1µM in a cell-based assay of PARP-1 inhibition and was considered suitable for further progression. However, early ADME-PK studies indicated that this compound exhibited poor metabolic stability both *in vitro* (mouse hepatic microsomes: t_{1/2} = 5.5 min; Cl_i = 12.8 mL/min/g liver) and *in vivo* (mouse plasma t_{1/2} = 13.6 min after 20 mg/kg *i.v.*). The ensuing programme therefore focussed on enhancing metabolic stability.

Table 1: Early SAR development of S 15065

Compound	n	R	PARP-1 IC ₅₀ (µM)
S 15065	0	H	1.2
1	0	3-NH ₂ /4-NMe ₂	0.24
2	1	H	0.77
3	1	3-OCOMe	0.036
4	1	3-NHCOMe	0.09
MRU 687	1	3-NHCOEt	0.02
5	1	3-NHCOPr ⁿ	0.09
6	1	3-NHCOPr ⁿ	0.37
7	1	3-NHCO-	0.055
8	1	3-NHCO-	0.010



The Search for Metabolic Stability (Scheme 1)

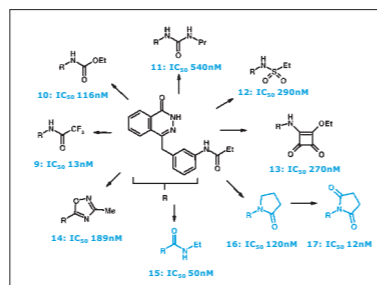
The exocyclic amide was considered the most likely point of metabolic instability, so several manoeuvres were undertaken to stabilise this group. Fluoro-substitution α to the carbonyl retained potency (**9**) but reduced stability (mouse hepatic microsomes: Cl_i >50 mL/min/g liver). Several bioisosteric amide replacements were tried, such as carbamate (**10**), urea (**11**), sulfonamide (**12**), "squaramide" (**13**) and 1,2,4-oxadiazole (**14**), but all reduced PARP-1 inhibitory activity.

Reversal of the amide topology (**15**) retained activity and also considerably increased metabolic stability *in vitro* (mouse hepatic microsomes: Cl_i = 0.49 mL/min/g liver). This compound was used as the lead for synthesis of several analogue libraries, resulting in many potent PARP-1 inhibitors. This work will form the subject of future publications.

Our final strategem for stabilising **MRU 687** was to constrain the amide into a lactam ring (**16**). This achieved a moderate increase in stability (mouse hepatic microsomes: Cl_i = 4.94 mL/min/g liver), but a reduction in potency. However, when a second carbonyl was added to the ring, giving the imide **17**, there was a ten-fold increase in potency. In addition, **17** appeared much more stable to metabolism, being unchanged after incubation with mouse hepatic microsomes.

This initial imide lead exhibited only moderate activity in a cell-based assay, so further SAR optimisation was required.⁷

Scheme 1: Bioisosteric replacements for the amide moiety



Optimisation of the Imide Series (Table 2)

By this stage of the project, we were seeking IC₅₀ <20nM in an isolated enzyme assay, and PF₅₀ >2 in a cell-based assay. The PF₅₀ value is the Potentiation Factor, calculated as the ratio of concentration of the alkylating agent, methyl methanesulfonate, at which 50% of HeLa B cell growth survives with and without added test compound at a concentration of 200nM.

Insertion of a double bond (**18**) into the imide ring retained activity in the isolated enzyme assay, but abolished activity in the cell-based assay. Fusion with a benzene ring (**19**) reduced PARP-1 inhibitory activity overall.

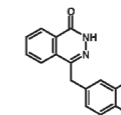
Substitution *ortho* to the imide moiety with chloro (**20**) or methoxy (**21**) groups did not increase activity, but there was a surprising enhancement of potency in both enzyme and cell-based assays when a fluoro substituent was introduced (**MRU 868**). In fact, for this compound activity in the cellular assay is maintained below 10nM. **MRU 868** exhibited good metabolic stability *in vitro* and was therefore chosen for more extensive evaluation (see below).

This enhancement of potency by fluoro-substitution appeared quite general for this series, and the remaining compounds in **Table 2** retain this substitution.

Addition of a methyl group α to the carbonyl (**22**) increases activity in cells, possibly due to enhanced cell penetration as a result of increased lipophilicity. Longer alkyl chains retain activity (not shown), but *geminal*-dialkylation (**23**) produces a slight decrease in potency in both assays. Larger aromatic substituents also retain high activity (**24**). A high potency inhibitor (**25**) was also obtained by fusion of a cyclopropane ring. Ring expansion of the imide to a piperidinedione (**26**) and inclusion of a further heteroatom to give a piperazinedione (**27**) also retain good activity in both assays.

Table 2: Imide SAR development

Compound	X	R	PARP-1 IC ₅₀ (nM)	PF ₅₀ at 200nM
17	H		12	1.74
18	H		13	0.94
19	H		180	-
20	Cl		19.2	1.51
21	OMe		33.8	1.5
MRU 868	F		5.0	5.62
22	F		3.8	18.2
23	F		9.8	2.96
24	F		6.8	5.38
25	F		5.0	14.4
26	F		4.1	6.6
27	F		9.5	3.1



Further Evaluation of MRU 868

The synthetic route used for scale-up of **MRU 868** is shown in **Scheme 2**. In unfluorinated analogues, the nitrobenzylidene phthalide **28** can be converted directly into the aminobenzyl phthalazinone **29** by heating with an excess of hydrazine. For **MRU 868**, displacement of the fluorine atom is facile, so stepwise reduction of the nitro group followed by ring formation with hydrazine is required. Formation of the imide ring initially proved difficult by standard methodologies, but this two-step process involving reaction with a cyclic anhydride to give the amidoacid **30**, followed by cyclodehydration with a peptide coupling reagent proved generally applicable.

A proposed binding mode for **MRU 868**, together with selectivity and early metabolic stability data for **MRU 868** are shown in **Table 3**.

The physiological roles of other members of the PARP "family" are not fully delineated, although it is known that Tankyrase is involved in promotion of telomere elongation, but selectivity for PARP is considered desirable. **MRU 868** exhibits selectivity for PARP-1 and PARP-2 over other family members.

Compared with **MRU 687**, **MRU 868** exhibits good metabolic stability (mouse microsome t_{1/2} = 5.5 min and 78 min respectively). The increase in stability was not quite so marked *in vivo*. After *i.p.* dosage, plasma t_{1/2} was 66 min, compared with 11.6 min for **MRU 687**. However, plasma levels remain above 200nM beyond 1 hour and, in fact, tumour PK studies indicated that **MRU 868** concentrates in tumour tissue and levels above 1µM are maintained beyond 2 hours.

These stability levels were considered sufficient to progress **MRU 868** to a key efficacy study in a mouse xenograft model.

Scheme 2: Synthesis of MRU 868

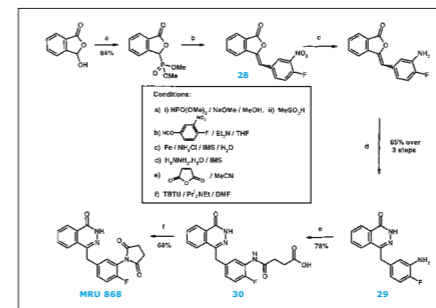
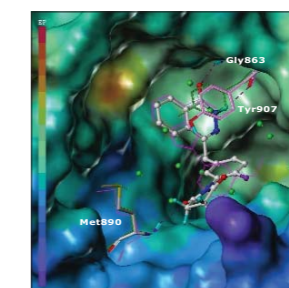


Table 3: MRU 868 Profiling

Binding mode of MRU 868 in PARP-1 Homology Model (Superimposed on Agouron Benzimidazole Inhibitor⁸ and conserved water of PARP X-ray structure)



Selectivity – IC₅₀

PARP-1	5nM
PARP-2	5nM
PARP-3	50nM
Tankyrase	1.5µM
VPARP	2µM

Metabolic stability

Mouse microsome t _{1/2}	78min
Mouse microsome Cl _i	0.034mL/min/g liver
Mouse plasma t _{1/2} (<i>i.v.</i>)	27min
Mouse plasma t _{1/2} (<i>i.p.</i>)	66min

Evaluation of MRU 868 in a Mouse Xenograft Model (Figure 1)

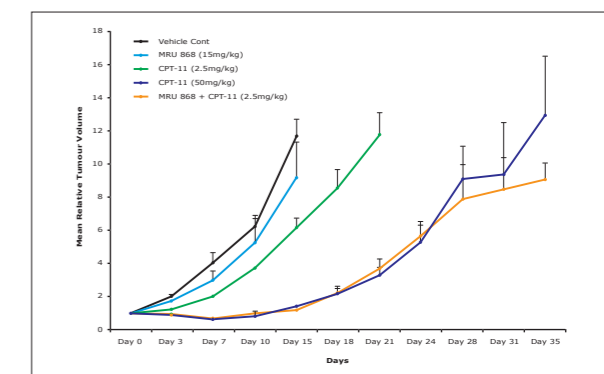
Nude mice were xenografted with human colon tumour cells LS174T. Twenty days later the mice were dosed *i.p.* with **MRU 868** (15mg/kg), followed 10 minutes later by the topoisomerase inhibitor, **Irinotecan (CPT-11)** *i.v.* (2.5mg/kg). Control groups of **MRU 868** (15mg/kg) and **CPT-11** (2.5mg/kg and 50mg/kg) were included.

Animals dosed with **CPT-11** exhibited significant dose-dependent inhibition of tumour growth.

Animals dosed with both **MRU 868** (15mg/kg) and **CPT-11** (2.5mg/kg) also exhibited highly significant inhibition of tumour growth over and above the **CPT-11** only group. The inhibitory effect of the combined dose was roughly equivalent to that obtained after treatment with high dose **CPT-11** (50mg/kg).

This clearly indicates that **MRU 868** acts as a potentiator of the effects of a cytotoxic agent in this animal model and therefore may have potential as an adjunct to cancer chemotherapy.

Figure 1: Efficacy of MRU 868 in a Mouse Xenograft Model



References

- Smith, S. The world according to PARP. *TIBS* **2001**, *26*, 174-179.
- D'Amours, D. et al. Poly(ADP-ribosylation) reactions in the regulation of nuclear functions. *Biochem. J.* **1999**, *342*, 249-268.
- Griffin, R.J. et al. The role of inhibitors of poly(ADP-ribose) polymerase as resistance-modifying agents in cancer therapy. *Biochimie* **1995**, *77*, 408-422.
- Plaschke, K. et al. The neuroprotective effect of cerebral poly(ADP-ribose) polymerase inhibition in a rat model of global ischemia. *Neurosci. Lett.* **2000**, *284*, 109-112.
- Dantzer, F. et al. Involvement of poly(ADP-ribose) polymerase in base excision repair. *Biochimie* **1999**, *81*, 69-75.
- International Patent Application WO 02/36576, KuDOS Pharmaceuticals Ltd & Maybridge plc.
- International Patent Application PCT/GB03/001817, KuDOS Pharmaceuticals Ltd & Maybridge plc.
- White, A.W. et al. Resistance-modifying agents. 9. Synthesis and biological properties of benzimidazole inhibitors of the DNA repair enzyme poly(ADP-ribose)polymerase. *J. Med. Chem.*, **2000**, *43*(22), 4084-4097.