19-Substituted Geldanamycins: Potential Therapeutics in Neurodegenerative Disease

Conformational Switch, Protein Crystallography and Hsp90 Inhibition of Novel 19BQAs

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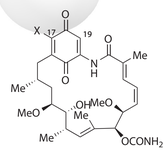
1 Hsp90 and Neurodegeneration

- Hsp90 is one of the most abundant proteins in eukaryotic cells.[1]
- Molecular chaperone responsible for the folding of nascent proteins.[1]
- Inhibition can disrupt many cancer-causing pathways.
- The modulation of protein folding also makes Hsp90 a relevant target for neurodegenerative conditions such as Alzheimer's and Parkinson's diseases.
- Inhibition of Hsp90 induces chaperones Hsp70 and Hsp27,which have been shown to be beneficial in Parkinsonian mouse models.

2 Geldanamycin

- Benzoquinone ansamycin (BQA) isolated from *Streptomyces hygroscopicus* var. *geldanus* in 1970.[2]
- Potent inhibitor of Hsp90, binding to ATP-site in the *N*-terminal domain.
- Binding to Hsp90 has been studied by X-ray crystallography and NMR.
- However, studies revealed significant hepatotoxicity.



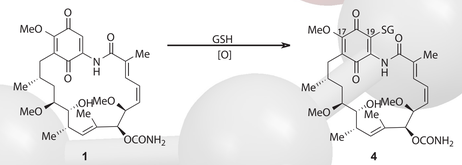
X = OMe **1**
X = $NHCH_2CH{=}CH_2$ **2**
X = $NHCH_2CH_2NH_2$ **3**

Figure 1: Geldanamycin **1** and its semi-synthetic derivatives 17-AAG **2** and 17-DMAG **3**.

- The 17-aminoquinone geldanamycin derivatives AAG **2** and DMAG **3** were synthesised to increase the stability and solubility.
- However, toxicity was still an underlying problem.

3 Hypothesis and Aims

- Toxicity issues are thought to arise from the reaction with glutathione at the 19-position of the quinone ansamycin.[3]
- We postulated that blocking the 19-position might suppress the conjugate addition of glutathione and thus ameliorate the toxicity (Scheme 1).



Scheme 1: Conjugate addition of glutathione with geldanamycin.

- Geldanamycin **1** is known to undergo a change of conformation upon binding to Hsp90.
- The unbound substrate prefers an extended conformation with a *trans*-amide. On binding, a 'C-clamp' conformation with a *cis*-amide is adopted (Figure 2).
- Substitution at the 19-position might force the unbound substrate to adopt the C-clamp conformation, potentially influencing the binding and potency.

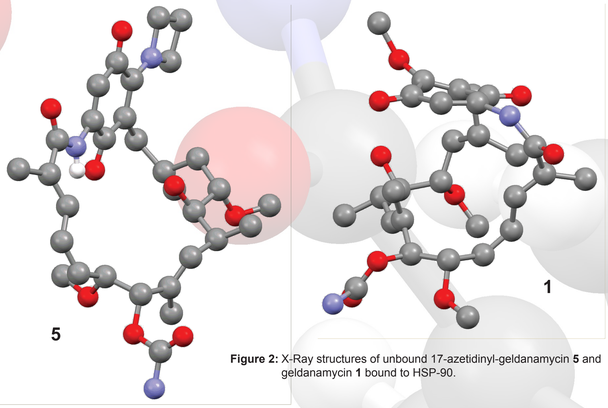
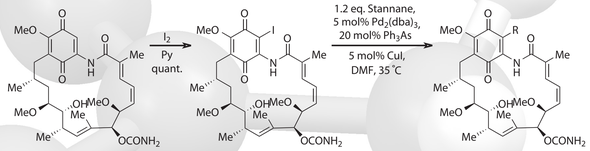


Figure 2: X-Ray structures of unbound 17-azetidinyl-geldanamycin **5** and geldanamycin **1** bound to HSP-90.

- We aimed to synthesise a range of 19-substituted geldanamycin analogues, in order to study the effect on their conformation, toxicity and Hsp90 inhibition.
- We also wanted to investigate their use as treatments for neurological diseases.

4 Synthesis

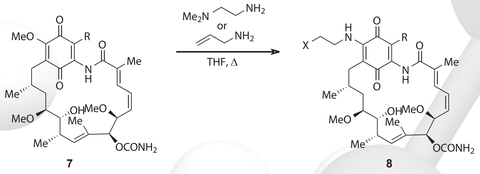
- We were attracted to a cross-coupling strategy, utilising readily available 19-iodogeldanamycin **6**.[4]
- Most conditions proved incompatible with the sensitive substrate.
- Specialised Stille conditions were successful, with compounds **7** obtained in moderate to excellent yield (Scheme 2).[5]



Scheme 2: Synthesis of 19-substituted BQAs.

5 Aminoquinone Analogues

- Since 17-AAG **2** and 17-DMAG **3** have the most clinical promise, we synthesised the 19-substituted amino derivatives (Scheme 3).



Scheme 3: Synthesis of 19-substituted aminoquinone analogues.

Entry	R	17-AAG Yield (%)	17-DMAG Yield (%)
1	Me	quant.	quant.
2	Ph	quant.	quant.
3	4-MeO-C_6H_4	quant.	quant.
4	4-F-C_6H_4	77	75
5	4-R_2N-C_6H_4	quant.	quant.
6	$CH{=}CH_2$	0	0
7	2-furanyl	96	92
8	2-thienyl	48	92
9	2-pyridyl	96	92

Table 1: Synthesis of 19-substituted aminoquinone analogues.

6 Conformational Studies

- NMR spectroscopy and molecular modelling of the nOe data supported a conformation with a *cis*-amide (Figures 3 and 4) .
- This was confirmed with an X-ray structure (Figure 5).[5]

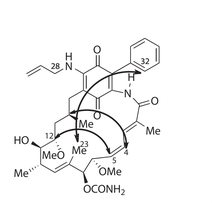


Figure 3: Key nOe interactions.

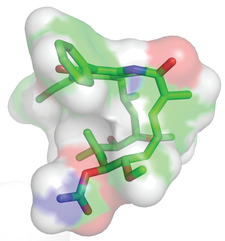


Figure 4: Molecular model from NMR data.

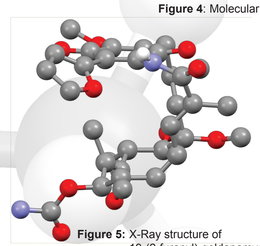
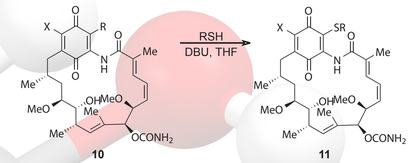


Figure 5: X-Ray structure of 19-(2-furanyl)-geldanamycin.

7 Reaction at C-19

- No reaction was observed with thiol nucleophiles at the 19-position (Scheme 4).[5]



Scheme 4: Reaction at C-19.

R = H, 21-62%
R ≠ H, **no reaction**

8 Hsp90-Bound X-ray

- 19BQAs bind in the same way to the *N*-terminal domain of Hsp90 as the parent quinones, with a *cis*-amide, C-clamp conformation.[5]
- However, steric constraints force a positional change of the quinone, altering some of the H-bonding interactions.

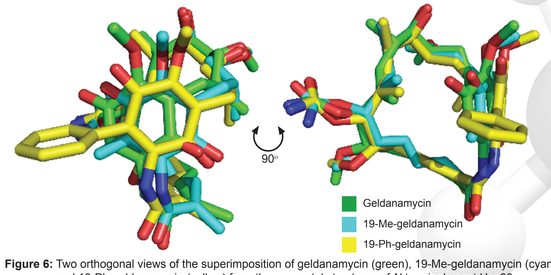


Figure 6: Two orthogonal views of the superimposition of geldanamycin (green), 19-Me-geldanamycin (cyan) and 19-Ph-geldanamycin (yellow) from the co-crystal structures of *N*-terminal yeast Hsp90.

9 Cellular Toxicity

- 19BQAs (particularly 19MeBQAs) exhibited significantly lower toxicity to dopaminergic SH-SY5Y cells than the parent compounds (Figure 7).[6]

Compound	IC_{50}	Compound	IC_{50}	Compound	IC_{50}
GA	133 nM	17DMAG	9.4 μM	17AAG	16.2 μM
19Ph-GA	>10 μM	19Ph-DMAG	>20 μM	19Ph-AAG	>20 μM
19Me-GA	>10 μM	19Me-DMAG	>20 μM	19Me-AAG	>20 μM

Figure 7: IC_{50} values for parent BQAs and 19BQAs. SH-SY5Y cells were exposed to different concentrations of GA, 17-AAG, and 17-DMAG series for 4h and cell viability was determined by MTT assay. The value represents a mean ± stdev (n=3). IC_{50} (the dose leads to 50% cell death) of 19BQAs and their parent quinones.

10 Application to Neurodegeneration

- Treatment of SH-SY5Y dopaminergic cells with 19Me-GA resulted in potent Hsp70 and Hsp27 induction, with minimal toxicity (Figure 8).[6]
- Consequently, 19BQAs have great potential for use as neuro-therapeutic agents.

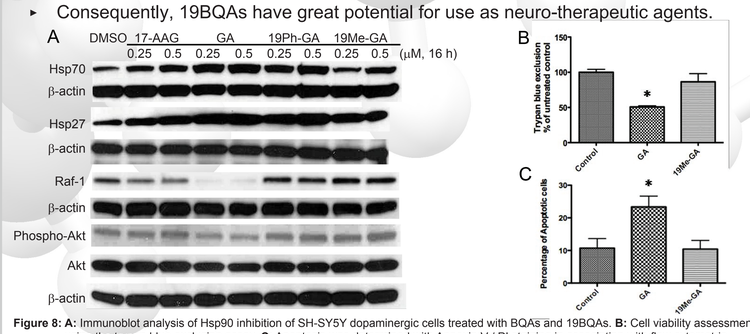


Figure 8: A: Immunoblot analysis of Hsp90 inhibition of SH-SY5Y dopaminergic cells treated with BQAs and 19BQAs. **B:** Cell viability assessment using the trypan blue exclusion assay. **C:** Apoptosis was determined with Annexin V / PI staining in association with flow cytometric analysis. Data is presented as the percentage of DMSO treated cells (*p < 0.05; ANOVA with Dunnet's multiple comparison test).

11 Conclusions

- Designed and synthesised a series of semi-synthetic 19-substituted geldanamycin analogues.
- 19BQAs adopt a C-clamp conformation and 19-substitution suppresses conjugation with nucleophiles.
- 19BQAs exert little cellular toxicity, yet induce upregulation of Hsps.
- Great potential for use as therapeutic agents for neuro-diseases.
- The application of 19BQAs against cancer cells and the effects of 19BQA Hsp90 inhibition on interactions with co-chaperones is discussed in a companion poster (Ross *et. al.*).

Acknowledgements

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