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β-Carbonyl Substituted Glutathione Conjugates as Inhibitors of *O. Volvulus* GST2

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Abstract—A series of β-carbonyl substituted glutathione conjugates were prepared and evaluated as inhibitors of OvGST2. Their specificity for the parasite derived protein was assessed through comparison with their inhibition of human πGST. Inhibition of OvGST2 has been demonstrated at low micromolar concentrations for these conjugates and selectivity for OvGST2 over human π-GST of greater than 10-fold has been achieved.

Introduction

The filarial nematode parasite *Onchocerca volvulus* causes onchocerciasis; a major cause of preventable blindness and severe dermatitis in Africa. Glutathione *S*-transferases (GSTs) are the linchpin of parasitic helminth defence mechanisms, providing their major phase II detoxification system and accounting for up to 4% of the total soluble protein. Two *O. volvulus* GSTs have been reported to date, OvGST1 and OvGST2. We have previously reported the isolation of purified active recombinant OvGST2.[1] GSTs detoxify electrophilic compounds,[2] including many anthelmintics and cytotoxins arising from the effector mechanism of the immune response,[3] through catalysis of their conjugation to glutathione.

Unlike mammalian GSTs, parasitic nematode GSTs are not well characterised. Biochemical, immunological and primary amino acid sequence analysis fails to place OvGST2 into any of the five characterised mammalian species independent soluble GST families (α, μ, π, θ and σ). Homology models indicate critical structural differences at the active site between host and parasite enzymes. In particular, the parasitic enzymes, whilst strongly topologically related to the mammalian π-GST family, have a very open hydrophobic binding cleft.[4] This is in contrast to that found in the mammalian enzymes, which have a more constricted feature. By contract, the glutathione binding site is closely related to that found in the mammalian enzymes: Notably the Tyr 115 residue is almost identically placed in both the mammalian and parasite enzymes. This residue is a potential target for binding of a number of π-GST inhibitors, all bearing a carbonyl moiety β to the point of where glutathione conjugation occurs.[5,6]

Given the significant divergence from host GSTs, we propose that selective inhibition of parasite derived GST enzymes is a realisable chemotherapeutic target. This should ultimately tip the molecular balance in favour of the host during the characteristically chronic nematode infection and may be an important adjunct in effective immuno- and chemotherapy.[3] Selective inhibitors of nematode GST will also provide new tools to study host–parasite interactions.

It is our hypothesis that suitable derivatisation of β-keto substituted glutathione *S*-conjugates will engender specificity along with established abolition of target enzyme activity. As part of our ongoing investigations into the utility of GSTs as a target for antiparasitic agents, we have synthesised a series of β-carbonyl substituted glutathione conjugates as inhibitors of OvGST2.

Chemistry

Enones were synthesized by the base catalysed Aldol condensation and the α,β -unsaturated esters were synthesized

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by acid catalysed esterification of maleic acid. The glutathione conjugates were prepared through a base catalyzed Michael-type addition. Cyclohex-2-enone and *S*-(dicarboxyethyl)glutathione (**8**) were obtained commercially. Unexpectedly, the reaction between glutathione and dibutyl maleate yielded the monobutyl succinate glutathione conjugate (**11**) (R′ = H in Figure 1b). In all other cases, the diester conjugate was formed. Conditions and reagents are shown in Figure 1.

Inhibition of OvGST2 was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as the second substrate in the standard assay:[7] Reduced glutathione (1 mM), inhibitor (0–1 mM) and purified OvGST2 (10 μg/mL) were combined in potassium phosphate buffer (pH 6.5, 100 mM) and equilibrated at room temperature for 5 min. The reaction was initiated by addition of 1-chloro-2,4-dinitrobenzene in ethanol (1 mM). The increase in absorbance at 340 nm due to production of *S*-(2,5-dintrophenyl)glutathione was determined over 5 min at room temperature. I_{50} was determined as the concentration of inhibitor leading to 50% decrease in the rate of *S*-(2,5-dintrophenyl)glutathione production. The inhibition of OvGST2 and human π GST are presented as IC_{50} values in Table 1.

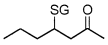
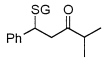
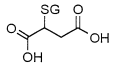
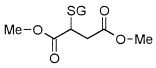
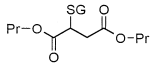
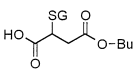
Results and Discussion

It is likely that the GST most closely related to OvGST2 in a human host would be in the π-GST class.[4] For this reason inhibition data is shown for both OvGST2 and human π-GST, giving a measure of selectivity of the inhibitor for the target parasite enzyme (Table 1).

Compound **1** shows optimal OvGST2 inhibition. Extension of the alkyl chain, such as in **2**, leads to slight increase in I_{50} however introduction of a branch, as found in **4** significantly increases $IC_{50.}$ Interestingly, replacement of tetrahedral branched feature in **4** with planar aryl group regains some of this lost activity. This may be due to removal of a steric constraint or acquisition of an aromatic–stacking interaction.

With the exception of the cyclic conjugate **3**, **5** shows the highest IC_{50} value of the enone derived series. By comparison with **1** this might be seen as the result of the extension of the chain on the side of the carbonyl away from the glutathione conjugation point, or as the result of chain shortening on the side of glutathione

Table 1. IC_{50} Inhibition data for selected glutathione conjugates

Compound		IC_{50} Data (μ M) [a]		
		OvGST2	πGST	Selectivity (πGST/ OvGST2)
1		4.1 ± 1.28	5.2 ± 3.0	1.3
2		8.4 ± 1.44	8.1 ± 2.6	1.3
3		220.0 ± 25.7	>250	>1.1
4		66.0 ± 8.49	>250	>3.8
5		90.0 ± 26.0	217 ± 9.6	2.1
6		41.0 ± 7.94	155 ± 13.2	3.8
7		31.9 ± 5.04	>250	>7.8
8		19.1 ± 0.8	>250	>13.9
9		>250	208 ± 29.3	< 0.6
10		143.3 ± 12.6	>250	>1.7
11		48.0 ± 9.7	40.3 ± 3.5	0.8

[a]Values shown as mean ± standard deviation ($n = 3$).

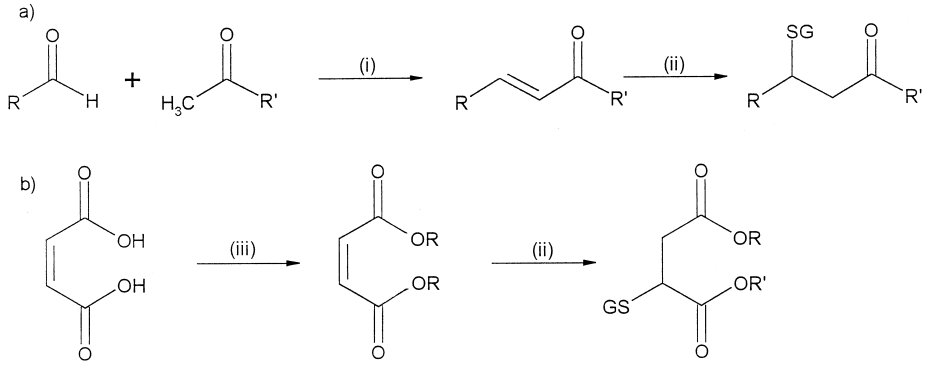


Figure 1. Reagents and conditions: (i) aldehyde (0.4 mmol), ketone (1.1 mmol), 0 °C, NaOH (10%, 10 cm^3), stir 2 h; (ii) glutathione (1.6 mmol), α,β - unsaturated carbonyl compound (6.4 mmol), methanol (2 cm^3) NaOH (2 M, 1cm^3), stir 25 °C, 24 h; (iii) Maleic acid (5.00 g), concd H_2SO_4 (1.5 mL), ROH (30 mL), reflux, 16 h.

conjugation. A comparison of **6** and **7** make the latter more likely.

The succinate conjugate, **8** can be considered as isosteric with **4** and **6**, however it is more active than either. This may suggest an additional electrostatic interaction is desirable. Indeed, dimethylation of the succinyl group whilst apparently not going beyond the steric boundaries set by **7** leads to a compound with negligible activity. The mono succinate ester conjugate **11** regains much of the activity lost by the succinate diesters. In summary, β-carbonyl substituted glutathione conjugates can demonstrate good OvGST2 inhibition. Extension of the chain beyond the carbonyl group is allowed, as is the introduction of an aryl group neighboring the glutathione conjugation point. It is likely that a hydrogen bond donating and/or accepting group in this position is especially favored.

Those conjugates with the requirements discussed above and possessing a point of branching next to the point of glutathione conjugation demonstrate the highest selectivity for OvGST2 over human πGST. This in accord with expectations of a more open active site in GSTs of parasitic origin.[4]

Conclusion

Inhibition of OvGST2 has been demonstrated at low micromolar concentrations for β-carbonyl glutathione conjugates. Whilst unsubstituted, straight-chain conjugates show the highest activity, substituted and branched conjugates show the greatest selectivity. Selectivity for OvGST2 over human π-GST of greater than 10-fold has been achieved. Further work is underway to enhance both activity and selectivity.

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