

# SYNTHESIS OF GLUTATHIONE ANALOGUES AND SCREENING AS SUBSTRATES & INHIBITORS FOR HUMAN GLUTATHIONE TRANSFERASE P1-1

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## INTRODUCTION

**The role of GSTs in medicine:** A major detoxification mechanism of the cell involves the glutathione transferase (GST)-catalyzed formation of glutathione (GSH) conjugates with various xenobiotics [1]. Based on the same mechanism, GST overexpression may lead to multidrug resistant phenotypes [2]. Therefore, several compounds with inhibitory potencies against GSTs have been developed as potential tools fortackling GST-attributed MDR [3-8]. Several individual compounds and prodrugs have been proposed as GST-inhibiting substances. In addition, GSH analogues have been considered as specific GST inhibitors, with particular attention been directed towards the synthesis of GSH analogues stable against  $\gamma$ -glutamyltranspeptidase ( $\gamma$ GT) and peptidases, as GST inhibitors [9-11].

**Aim of the present work:** we aimed to identify GSH-analogues that discriminate between the expression products of the polymorphic human GSTP1 gene locus involved in MDR. These proteins, designated as hGSTP1\*A (Ile<sup>104</sup>/Ala<sup>113</sup>), hGSTP1\*B (Val<sup>104</sup>/Ala<sup>113</sup>) and hGSTP1\*C (Val<sup>104</sup>/Val<sup>113</sup>), are expressed differentially in normal and cancer cells, also metabolizing differentially electrophilic substrates, including chemotherapeutic agents [12].

**What we did:** We synthesized 25 GSH analogues, using solid phase technology, and studied them with the human GSTP1 gene products. In particular, we identified GSH analogues displaying selective reactivity with the hGSTP1 allozymes. These analogues may prove useful tools for the selective probing of the hGSTP1 activities as well as for the design of novel conjugates bearing a GSH-mimetic moiety linked to an hGST-inhibiting heterocyclic one.

## MATERIALS AND METHODS

### Peptide Synthesis

25 new peptides analogues of GSH (Table 1) were synthesized manually—following the Fmoc/tBu strategy [13]. H-Gly-2-2-chlorotrityl resin (CTC) (0.5 mmol/g capacity) or H-Ala-2CTC resin (0.5 mmol/g capacity) was used as a solid support for the analogues possessing Gly or Ala at position 3 while, CTC resin (1.2 mmol/g capacity) was used for the synthesis of all the other peptides.

Purification of crude peptides was achieved by semipreparative HPLC on Supelcosil C18 Analytical HPLC equipped with a Waters C18 column at a 1 mL/min flow rate with the same solvent system as in the preparative HPLC—produced single peaks with at least 98% of the total peak integrals (integrated with Empower software). Electron Spray Ionization-Mass Spectrometry was in agreement with the expected mass. The structures and some physicochemical properties of the GSH analogues are shown in Table 1.

**Screening the GSH analogues as substrates for hGSTP1-1.** The enzyme assay was performed by adding the ingredients in the following order (1 mL assay volume): potassium phosphate buffer (100 mM, pH 6.5), an appropriate quantity of GSH analogue (in water or DMSO), equal to the  $K_m^{(GSH)}$  value for the particular allozyme (see Table 2) and purified enzyme ( $\approx 0.06 \Delta A_{340}/\text{min}$ ). The mixture was incubated at 25 °C for 1 min, prior to adding 1  $\mu\text{mol}$  of CDNB (in ethanol). The observed rate was used to calculate the fractional activity (%), taking as 100% initial activity value the rate observed after replacing the GSH analogue by an equal concentration of GSH.

**Screening the GSH analogues as inhibitors for hGSTP1-1.** The enzyme assay was performed by adding the ingredients in the following order (1 mL assay volume): potassium phosphate buffer (100 mM, pH 6.5), 1  $\mu\text{mol}$  of CDNB (in ethanol) and 25 nmol of GSH analogue and purified enzyme ( $\approx 0.06 \Delta A_{340}/\text{min}$ ). The mixture was incubated at 25 °C for 1 min, prior to adding an appropriate quantity of GSH (in water), equal to the  $K_m^{(GSH)}$  value for the particular allozyme (see Table 2). The observed rate was used to calculate the remaining activity (%), taking as 100% initial activity value the rate observed after replacing the GSH analogue by an equal volume of buffer or DMSO.

**Table 1.** Yield of synthesis and physicochemical properties of GSH analogues

	Peptide Code	Sequence	Retention time; $t_R$ (min)*	Yield (%)	
	GSH	$\gamma$ -Glu-Cys-Gly	2.36	-	
Group A	I	pGlu-Cys-Gly	2.86	84	
	II	Tic-Cys-Gly	4.72	72	
	III	Sar-Cys-Gly	1.48	85	
	IV	Hyp-Cys-Gly	1.52	82	
	V	Ac- $\gamma$ -Glu-Cys-Gly	3.11	92	
	VI	NMeGlu-Cys-Gly	1.55	81	
	VII	Pro-Cys-Gly	1.45	93	
Group B	VIII	$\gamma$ -Glu-Met-Gly	3.55	89	
	IX	$\gamma$ -Glu-Thi-Gly	4.91	87	
	X	$\gamma$ -Glu-Cys(tButhio)-Gly	7.35	93	
	XI	$\gamma$ -Glu-Cys(Acm)-Gly	1.52	86	
	XII	$\gamma$ -Glu-NMeCys-Gly	1.47	89	
	Group C	XIII	$\gamma$ -Glu-Cys-Nva	4.75	91
XIV		$\gamma$ -Glu-Cys-Nle	6.07	88	
XV		$\gamma$ -Glu-Cys-Sar	3.03	91	
XVI		$\gamma$ -Glu-Cys-Aib	3.80	82	
XVII		$\gamma$ -Glu-Cys-a-t-butyl-Gly	5.27	91	
Group D		XVIII	pGlu-Cys(Trt)-Gly	9.75	68
		XIX	Sar-Cys(Trt)-Gly	7.94	71
	XX	Tic-Cys-Ala	5.20	88	
	XXI	Hyp-Cys-Ala	2.11	87	
	XXII	Gly-Cys-Hyp	2.12	79	
	XXIII	Tic-Cys-Aib	5.77	86	
	XXIV	Tic-Cys(Acm)-Gly	4.51	92	
	XXV	Tic-Cys-Sar	4.94	93	

## RESULTS

**Table 2.** Screening of GSH-analogues as substrates and inhibitors for hGSTP1-1 allozymes.

peptide code	hGSTP1-A $K_m^{GSH} = 0.3 \pm 0.02$ mM		hGSTP1-B $K_m^{GSH} = 0.074 \pm 0.008$ mM		hGSTP1-C $K_m^{GSH} = 0.15 \pm 0.01$ mM	
	Inhibitor; RA (%) <sup>b</sup> (Assays: [CDNB] = 1 mM, [GSH] = 0.3 mM, [analogue] = 25 $\mu$ M)	Substrate; FA (%) <sup>b</sup> (Assays: [CDNB] = 1 mM, [GSH] = 0.074 mM, [analogue] = 0.3 mM)	Inhibitor; RA (%) (Assays: [CDNB] = 1 mM, [GSH] = 0.074 mM, [analogue] = 25 $\mu$ M)	Substrate; FA (%) (Assays: [CDNB] = 1 mM, [GSH] = 0.074 mM)	Inhibitor; RA (%) (Assays: [CDNB] = 1 mM, [GSH] = 0.15 mM, [analogue] = 25 $\mu$ M)	Substrate; FA (%) (Assays: [CDNB] = 1 mM, [GSH] = 0.15 mM, [analogue] = 0.15 mM)
Group A						
I	NA <sup>c</sup>	11.5 $\pm$ 10.4	79.2 $\pm$ 4.8	0	80.0 $\pm$ 3.6	0
II	NA	41.3 $\pm$ 2.9	83.1 $\pm$ 1.5	0	78.0 $\pm$ 1.9	0
III	NA	15.8 $\pm$ 3.8	83.5 $\pm$ 1.6	0	NA	7.0 $\pm$ 14.0
IV	NA	39.5 $\pm$ 3.8	89.9 $\pm$ 3.2	0	88.0 $\pm$ 1.5	0
V	102.3 $\pm$ 2.8	0	85.3 $\pm$ 1.2	0	NA	10.1 $\pm$ 0.7
VI	NA	3.9 $\pm$ 4.7	81.0 $\pm$ 1.4	0	72.3 $\pm$ 3.5	0
VII	NA	21.6 $\pm$ 4.0	82.6 $\pm$ 3.0	0	NA	10.4 $\pm$ 5.0
Group B						
VIII	79.4 $\pm$ 1.1	NA	70.1 $\pm$ 0.8	NA	70.7 $\pm$ 2.6	NA
IX	67.4 $\pm$ 4.2	NA	92.5 $\pm$ 3.9	NA	67.4 $\pm$ 3.6	NA
X	85.6 $\pm$ 0.4	NA	79.5 $\pm$ 3.7	NA	81.0 $\pm$ 1.2	NA
XI	85.2 $\pm$ 1.7	NA	64.0 $\pm$ 2.9	NA	59.4 $\pm$ 2.7	NA
XII	100.0	0	100.0	0	100.0	0
Group C						
XIII	NA	16.0 $\pm$ 2.7	80.1 $\pm$ 4.8	0	85.2 $\pm$ 0.1	0
XIV	NA	26.4 $\pm$ 2.7	80.9 $\pm$ 4.1	0	NA	11.6 $\pm$ 5.4
XV	NA	17.0 $\pm$ 4.3	NA	60.8 $\pm$ 3.3	NA	67.7 $\pm$ 1.2
XVI	100.0	0	82.7 $\pm$ 1.9	0	NA	15.8 $\pm$ 3.5
XVII	100.0	0	80.4 $\pm$ 5.0	0	79.1 $\pm$ 3.7	0
Group D						
XVIII	82.3 $\pm$ 0.2	NA	95.8 $\pm$ 2.1	NA	99.1 $\pm$ 2.3	NA
XIX	100.0	NA	102.3 $\pm$ 5.0	NA	98.2 $\pm$ 4.4	NA
XX	100.0	0	100.0 $\pm$ 1.1	0	92.1 $\pm$ 2.6	0
XXI	98.7 $\pm$ 2.0	0	81.3 $\pm$ 1.0	0	76.6 $\pm$ 3.5	0
XXII	87.4 $\pm$ 0.2	0	81.6 $\pm$ 5.0	0	65.9 $\pm$ 3.1	0
XXIII	80.1 $\pm$ 4.8	0	75.2 $\pm$ 5.0	0	84.2 $\pm$ 2.1	0
XXIV	68.5 $\pm$ 4.5	0	84.2 $\pm$ 5.0	0	68.6 $\pm$ 2.0	0
XXV	53.3 $\pm$ 0.0	0	72.0 $\pm$ 2.2	0	74.6 $\pm$ 3.6	0

**Table 3.** Selectivity of GSH analogues (as substrates) against the hGSTP1-1 allozymes (% values are relative to GSH as substrate)

GSH analogue	hGSTP1-1 allozyme		
	A	B	C
II Tic-Cys-Gly	+ (41 %)	-	-
III Sar-Cys-Gly	+ (16 %)	-	+ (7 %)
IV Hyp-Cys-Gly	+ (40 %)	-	-
V Ac- $\gamma$ -Glu-Cys-Gly	-	-	+ (10 %)
XIII $\gamma$ -Glu-Cys-Nva	+ (16 %)	-	-
XIV $\gamma$ -Glu-Cys-Nle	+ (26 %)	-	+ (12 %)
XVI $\gamma$ -Glu-Cys-Aib	-	-	+ (16 %)

## CONCLUSIONS

### Selection of GSH analogues with human GSTP1-1 activities

Table 3 summarizes GSH analogues able to discriminate between the three hGSTP1-1 allozymes, by acting as selective substrates (data taken from Table 2). Analogues II, IV and XIII are substrates only with allozyme A. Analogues V and XVI are substrates only with allozyme C. Analogues III and XIV are weak substrates with both allozymes A and C. Therefore, it appears that certain GSH analogues, from groups A and C (Table 1), act selectively as substrates for either A or C allozymes or both of them, however, no analogue showed selectivity exclusively with allozyme B. This is the first report on this mode of behavior for GSH analogues. The engagement of molecular modeling and dynamic approaches may rationalize these encouraging findings. Nevertheless, the identified GSH analogues of Table 3 may prove useful for the selective probing of the hGSTP1 allozyme activities and the design of novel conjugate inhibitors against hGSTP1s.

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