Peptide cell-display for selection of inhibitors against human glutathione transferase P1-1 (hGSTP1-1) allozymes

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ABSTRACT. We developed a combinatorial strategy aiming at designing peptide inhibitors against the hGSTP1-1 isoenzyme involved in MDR. We developed a combinatorial strategy aiming at designing peptide inhibitors against the hGSTP1-1 isoenzyme. We employed a combinatorial peptide library featuring engineered E. coli cells harboring a plasmid able to express a fusion protein containing random 12peptides. After five selection rounds, clones were screened for hGSTP1-1 binding (dot blot hybridization) and those with the strongest signal were selected and sequenced. Sequence alignments showed a core binding sequence which, along with selected peptide fragments, were synthesized using the solid phase methodology . The synthetic peptides were studied for their inhibition potency against three human GSTP1-1 allozymes, A, B and C

INTRODUCTION.

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a large family of isoenzymes catalysing the conjugation of the tripeptide glutathione (GSH) to a variety of hydrophobic endogenous and exogenous compounds, rendering them hydrophilicity and facilitating their eventual secretion from the cell. They catalyse the conjugation of glutathione (GSH) to a variety of hydrophobic endogenous and exogenous substrates, rendering them hydrophilicity and facilitating their metabolic processing and eventual secretion from the cell [1]. Based on the same detoxification mechanisms, cancer cells may acquire resistance against certain chemotherapeutic drugs by overexpressing GST isoenzymes, mainly of the α, π and μ classes [2,3]. Therefore, there is an increasing interest for new GST synthetic inhibitors, as a strategy to eventually overcoming MDR attributed to GST overexpression[5-8]. We report on the design, synthesis and enzymological evaluation of peptides as inhibitors for hGSTP1-1 allozymes A,B,C.
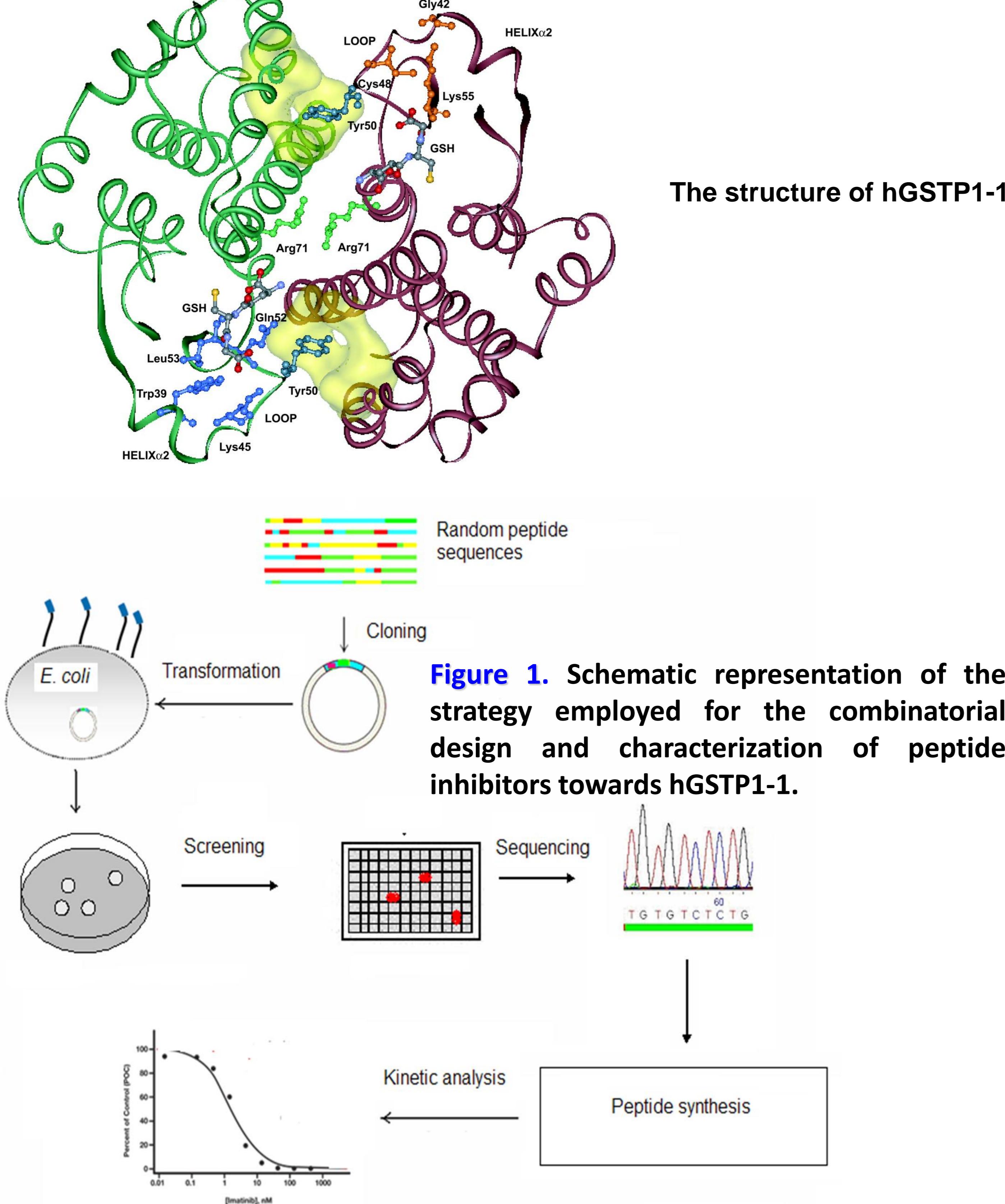

The structure of hGSTP1-1

Figure 1. Schematic representation of the strategy employed for the combinatorial design and characterization of peptide inhibitors towards hGSTP1-1.

Table 1. Kinetic constants for hGSTP1 allozymes A, B & C.

Allozyme	Substrate	V_{max} ($\mu mol \cdot min^{-1} \cdot mL^{-1}$)	k_{cat} (min^{-1})	K_m (mM)	k_{cat}/K_m ($min^{-1} \cdot mM^{-1}$)
hGSTP1A	GSH	0.028	1052	0.128	8237
	CDNB	0.069	2991	1.320	2265
hGSTP1B	GSH	0.017	458	0.149	3078
	CDNB	0.032	892	1.163	767
hGSTP1C	GSH	0.014	206	0.127	1615
	CDNB	0.023	457	1.069	427

RESULTS/CONCLUSIONS.

We developed a combinatorial strategy aiming at designing peptide inhibitors against the hGSTP1-1 isoenzyme (Figure 1). We employed a combinatorial peptide library featuring engineered *E. coli* cells harboring a plasmid able to express a fusion protein containing random 12peptides which were inserted into the active loop of thioredoxin, which itself was inserted into the dispensable region of the flagellin gene (Figure 2) engineered to express a fusion protein containing random dodecapeptides that were inserted into the active loop of thioredoxin. When the fusion protein becomes an integral part of the flagellar filaments on the bacterial cell surface, the dodecapeptides become available to interact with target proteins. After 5 continuous selection rounds, different clones were screened for hGSTP1-1 binding by dot blot (Figure 3) and the clones exhibiting the strongest signal were selected and their sequence determined by nucleotide sequencing. Sequence alignments showed a core binding sequence (*TH10*: PATAISLGGG) which, along with selected peptide fragments (*TH5N*: PATAI, *TH5C*: SLGGG, *TH4*: AISL), were synthesized using the solid phase methodology and Fmoc/tBu chemistry on 2-chlorotrityl chloride solid support.

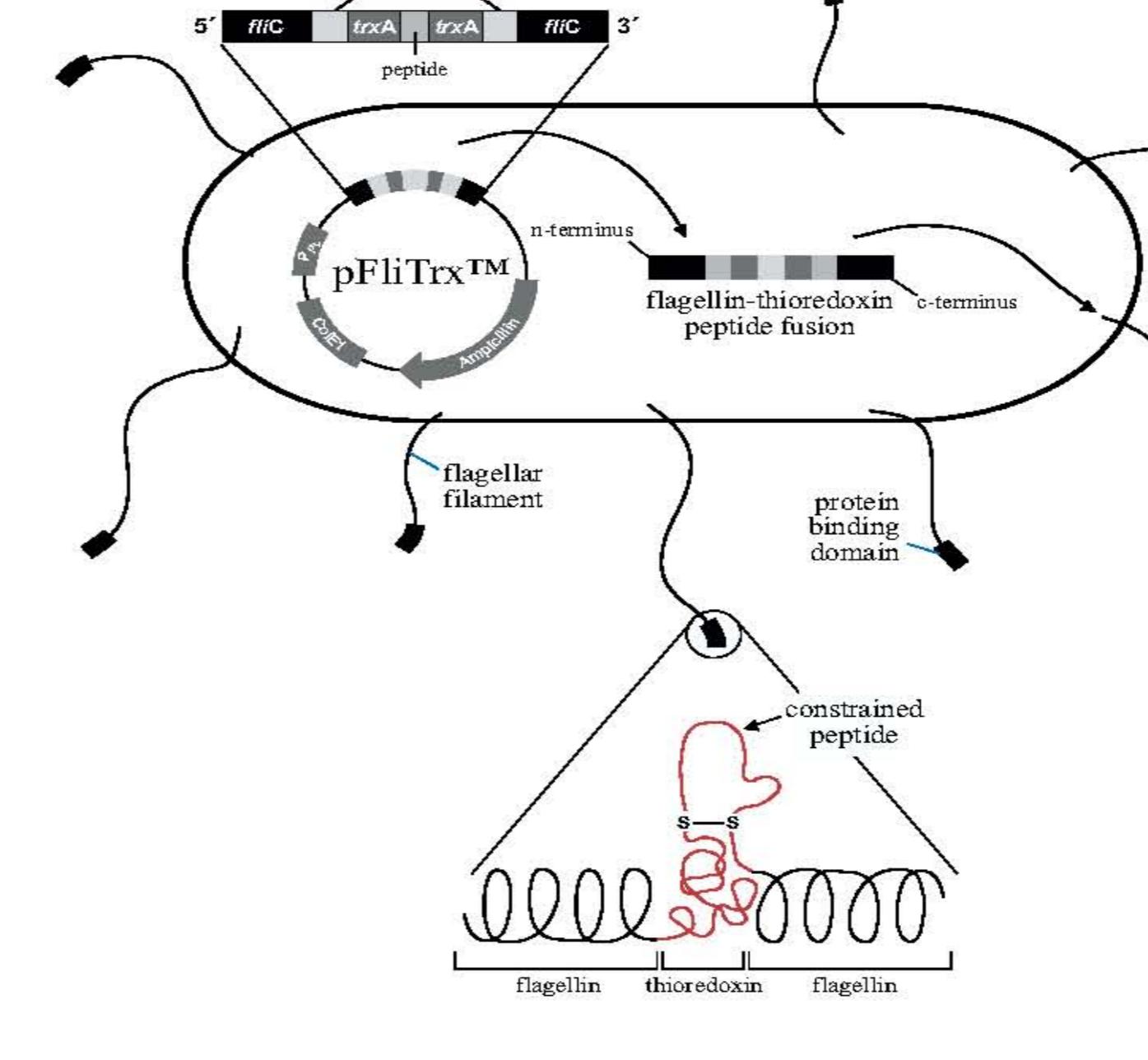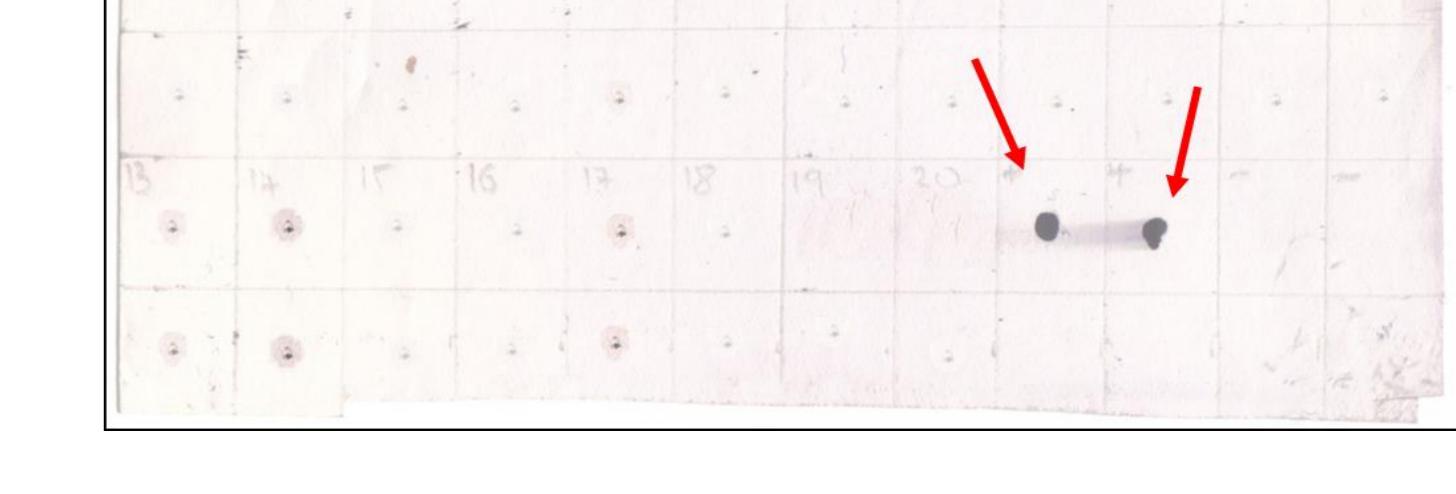**Figure 2.** The structure of the plasmid pFlitrx.

Figure 3. Dot blot analysis for the selection hGSTP1-1 binding peptides.

The purified allozymes were subjected to kinetic study and inhibition tests with the designed peptides *TH10, TH5N, TH5C & TH4*.

It appears that the mutations of the allozymes hGSTP1*A (Ile104/Ala113), hGSTP1*B (Val104/Ala113) & hGSTP1*C (Val104/Val113) have small influence on the *binding affinity* between substrate & enzyme (Table 1, K_m values), but result in significant changes of the *reaction rate* (Table 1, k_{cat} values) and, hence, the overall *catalytic efficiency* (Table 1, k_{cat}/K_m values). Furthermore, these mutations influence the inhibitory ability of the designed peptides. TH10 is the most effective inhibitor (Table 2), with the shorter counterparts showing varied inhibitory potency depending on the allozyme (Table 2).

Table 2. Inhibition of hGSTP1 allozymes by peptides designed on the basis of results from the *E. coli* displayed combinatorial library.

Peptide	Inhibition of hGSTP1-1 (%, compared in the absence of inhibitor)		
	A	B	C
TH10 :	32.2	58.0	25.4
TH5N :	29.2	46.6	14.3
TH5C :	34.2	42.2	18.7
TH4 :	15.0	30.6	16.8

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