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# COMBINATORIAL DESIGN, SELECTION AND SYNTHESIS OF PEPTIDE INHIBITORS AGAINST HUMAN GLUTATHIONE TRANSFERASE P1-1

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**ABSTRACT.** Certain glutathione S-transferase (GST) isoenzymes detoxify the cell from xenobiotics, thus becoming inhibition targets when overexpressed in various tumours leading to MDR. We developed a combinatorial strategy aiming at designing peptide inhibitors against the hGSTP1-1 isoenzyme involved in MDR. 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. After five selection rounds, clones were screened for hGSTP1-1 binding 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 and Fmoc/tBu chemistry on 2-chlorotrityl chloride solid support. The four peptides were studied for their inhibition potency against hGSTP1-1 allozymes A, B and C.

**INTRODUCTION.** Glutathione S-transferases (GSTs, EC 2.5.1.18) are a family of isoenzymes that differ in their tissue-specificity expression and distribution. 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]. Cancer cells may acquire resistance by overexpressing GST activities, hampering the effectiveness of certain chemotherapeutic drugs [2,3]. Several synthetic compounds exhibiting inhibition potency against GSTs have been proposed as strategies to 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.

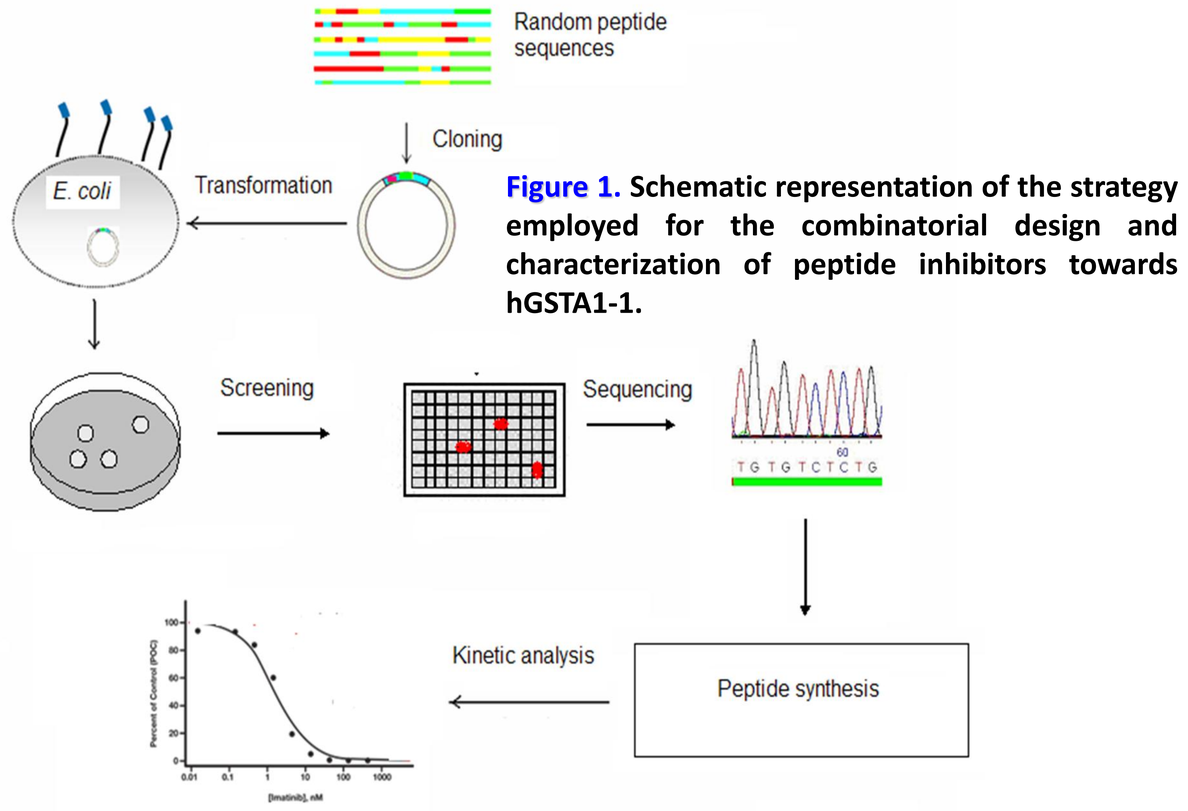



**Figure 1.** Schematic representation of the strategy employed for the combinatorial design and characterization of peptide inhibitors towards hGSTA1-1.

**RESULTS/CONCLUSIONS.** Peptide design (Figure 1) was accomplished through an iterative binding cycle called panning using a bacterial surface peptide display library (FliTrx) with an estimated diversity of $1.77X10^8$ possible peptide combinations using hGSTP1-1 as the target. The bacterial peptide display library was comprised of *E. coli* cells harbouring a plasmid (pFlitrx, Figure 2) engineered to express a fusion protein containing random dodecapeptides that were inserted into the active loop of thioredoxin, which itself was inserted into a dispensable of flagellin, the major constituent of flagellar filaments. 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 (PATAISLGGG) which, along with selected peptide fragments (PATAI, SLGGG, AISL), were synthesized using the solid phase methodology and Fmoc/tBu chemistry on 2-chlorotrityl chloride solid support.

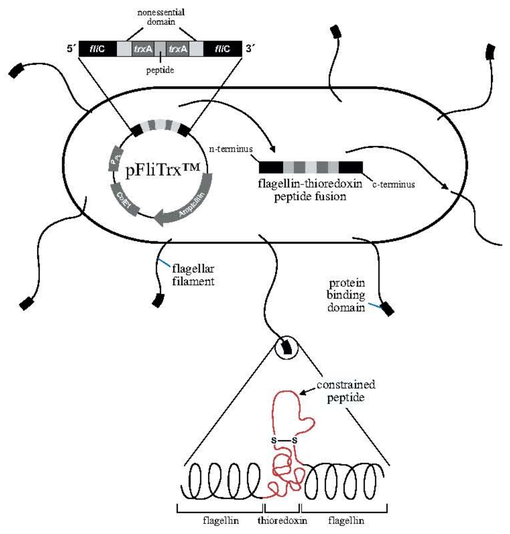
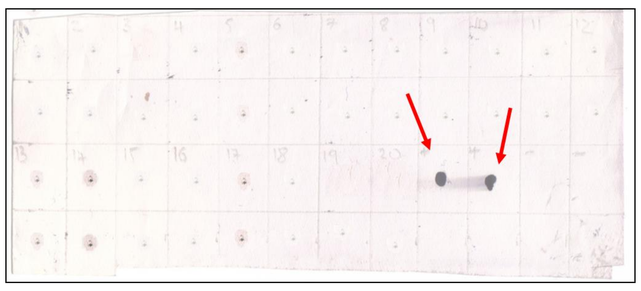



**Figure 2.** The structure of the plasmid pFlitrx.

**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 desiged peptides.

It appears that the mutations of the allozymes hGSTP1*A ($Ile^{104}/Ala^{113}$), hGSTP1*B ($Val^{104}/Ala^{113}$) & hGSTP1*C ($Val^{104}/Val^{113}$) have small influence on the *binding affinity* between substrate & enzyme (Table 1, $K_m$ values), but result in significant changes of the *catalytic reaction* (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.

Rationalization of the above results requires the engagement of molecular modeling and dynamic approaches.

**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 | **0.0284** ±0.0005 | **1051.85** ±18.52 | **0.1277** ±0.0065 | **8236.88** ±594.55 |
| | CDNB | **0.0688** ±0.0023 | **2991.30** ±100,00 | **1.3205** ±0.0844 | **2265.28** ±235.56 |
| hGSTP1B | GSH | **0.0165** ±0.0003 | **458.33** ±8.34 | **0.1489** ±0.0081 | **3078.11** ±236.31 |
| | CDNB | **0.0321** ±0.0005 | **891.67** ±13.89 | **1.1627** ±0.0488 | **766.90** ±46.06 |
| hGSTP1C | GSH | **0.0140** ±0.0003 | **205.88** ±4.41 | **0.1275** ±0.0071 | **1614.75** ±131.84 |
| | CDNB | **0.0233** ±0.0003 | **456.86** ±5.89 | **1.0692** ±0.0366 | **427.29** ±20.85 |

**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 :*** Pro-Ala-Thr-Ala-Ile-Ser-Leu-Gly-Gly-Gly | 32.2 | 58.0 | 25.4 |
| ***$TH5^N$ :*** Pro-Ala-Thr-Ala-Ile | 29.2 | 46.6 | 14.3 |
| ***$TH5^C$ :*** Ser-Leu-Gly-Gly-Gly | 34.2 | 42.2 | 18.7 |
| ***TH4 :*** Ala-Ile-Ser-Leu | 15.0 | 30.6 | 16.8 |

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