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Design and synthesis of an optimized positional scanning library of peptoids: identification of novel multidrug resistance reversal agents

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Abstract—Herein is reported the optimized solid-phase synthesis of a library of 5,120 trimeric *N*-alkylglycines (peptoids) using the positional scanning format and the submonomer strategy. Diversity at the N-terminal position was generated from 20 commercially available primary amines, whereas 16 primary amines were employed for the middle and C-terminal positions of the trimers. Formation of undesirable side-products observed in a previous library synthesis (Humet, M. et al. *J. Comb. Chem*. **2003**, *5*, 597–605) was averted by restricting the use of primary amines functionalized with tertiary amino groups to the third amination step. Screening of the new library for the identification of chemosensitizers yielded two peptoids, compounds **1** and **2**, with potent in vitro activity as multidrug resistance (MDR) reversal agents. The structures of the lead peptoids are consistent with a pharmacophore model generated from the interaction of various known inhibitors with the MDR-implicated transmembrane glycoprotein P-gp.

1. Introduction

Oligomers of *N*-alkylglycines, also known as peptoids, are a family of non-natural molecules with a wide range of biological activities, thus making them attractive candidates for drug discovery. These compounds generally exhibit greater proteolytic stability and bioavailability than their respective peptide analogues, a consequence of their side-chains being bound to nitrogen atoms as opposed to α-carbons.[1] From a synthetic perspective, the modular nature of peptoids makes them amenable to combinatorial strategies. In this context, the pioneering work of Zuckermann and co-workers led to the development of two solid-phase based complementary approaches for the preparation of *N*-alkylglycine oligomers. The first strategy involved the condensation of activated *N*-Fmoc-protected *N*-alkylglycines, which requires pre-preparation of a set of protected *N*-substituted glycine monomers.[2] The second approach comprised the systematic assembly of two readily available submonomers, a α-haloacetyl moiety and a primary amine.[2–4] The latter technique is advantageous since the α-haloacetyl monomer is common to all backbone elongation processes, while the broad commercial availability of primary amines facilitates structural diversification.

Peptoid from libraries constructed by the split-and-mix format have been identified as ligands for membrane receptors,[1,5,6] and have exhibited specific protein-binding activity.[7] Furthermore, the inherent conformational flexibility of these oligomers has led to their use in the disruption of protein–protein, protein–nucleic acid and/or protein/membrane interactions.[8–10] Split-and-mix derived peptoid libraries have been also used for the identification of new antibacterial compounds.[11,12]

The modular composition of peptoids raised the question of whether libraries of these oligomers could be synthesized and screened in a positional scanning format.[13–15] It was envisaged that a single peptoid library in this format could be used to identify hit compounds for multiple biological targets. In this context, the synthesis of the first library of *N*-alkylglycine trimers constructed under the positional scanning format was reported by our group.[16] This library, which contained over 10,000

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compounds, provided $TRPV_1$ channel[17] and NMDA receptor open-channel[18] blockers when screened on a subset of membrane receptors. The library was also screened in a whole-cell based assay, leading to the discovery of peptoids with in vivo neuroprotectant activity.[19] More recently, compounds exhibiting antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria were also identified.[16]

While the results obtained from the screening of this library were satisfactory, syntheses of many of its constituent peptoids were prone to low yields. This can be attributed to side-reactions that occurred when primary amines functionalized with tertiary amino moieties were used at the medial or C-terminal position of the trimer. Moreover, the complexity of the resulting crude reaction mixtures complicated purification of the desired peptoid.[16] These observations indicated that the peptoid library was not rigorously validated, raising doubts over the presence, at least in predictable concentrations, of the anticipated compounds. Although this risk is inherent to working with libraries of controlled mixtures, reassessment of the methodology used for construction of the library was required. A novel library was sought, which preserved the positional scanning format and use of tertiary-amino functionalized primary amines of the earlier library, but which contained all of the expected products in comparable concentrations. A recent paper from the group of Zuckermann substantiates the value of this type of approach for the solid-phase synthesis of peptoids using amines containing heterocyclic nitrogen atoms.[20]

Herein are described the results of the synthesis of an optimized library of *N*-alkylglycine trimers. The new library, containing over 5000 peptoids, was characterized by the use of primary amines exclusively in the third amination step to circumvent possible side-reactions. Subsequent screening of the library yielded two novel chemosensitizers with efficacies comparable to that of the widely recognized MDR reversal agent verapamil. Finally, preliminary modelling studies incorporating the structure of these peptoid hits into a pharmacophore model are also presented.

2. Results and discussion

2.1. Design and synthesis of the new library of *N*-alkylglycines

The submonomer synthetic strategy was used for the synthesis of the peptoid library.[3,4] In accordance with results obtained in our former library, and in order to optimize chemical diversity, 16 commercially available primary amines were selected for the C-terminal and medial positions of the *N*-alkylglycine trimers. None of these amines had additional amino groups that could promote the undesired side-reactions observed in the first library. The same amines, as well as four more that contained an additional tertiary amino group, were chosen for the N-terminal position (Fig. 1). According to the positional scanning format, the library was divided into three sublibraries: OXX (256 compounds per mixture), XOX and XXO (320 compounds per mixture), in which O represents a defined diversity position and X refers to an iso-reactive mixture of all amines, for a total of 52 controlled mixtures representing 5120 compounds. As shown in Figure 1, all primary amines with the exception of $\mathbf{a_1}$, $\mathbf{a_2}$ and $\mathbf{a_8}$ had an aminoethyl residue to exhibit similar reactivities towards the haloacetyl moiety. In addition, choice of reagents reflected chemical diversity in terms of hydrophobic and lipophilic substituents, aromatic residues, polar chains and hydrogen bond donors, and also took into account the most bioactive structures from the first library. As a result, some previously used amines were replaced with the fluorinated amines $\mathbf{a_{15}}$ and $\mathbf{a_{16}}$.

The 52 controlled mixtures were synthesized on solid phase via the sequence shown in Scheme 1. The solid support was confined in sealed tea bags.[21] Thus, after

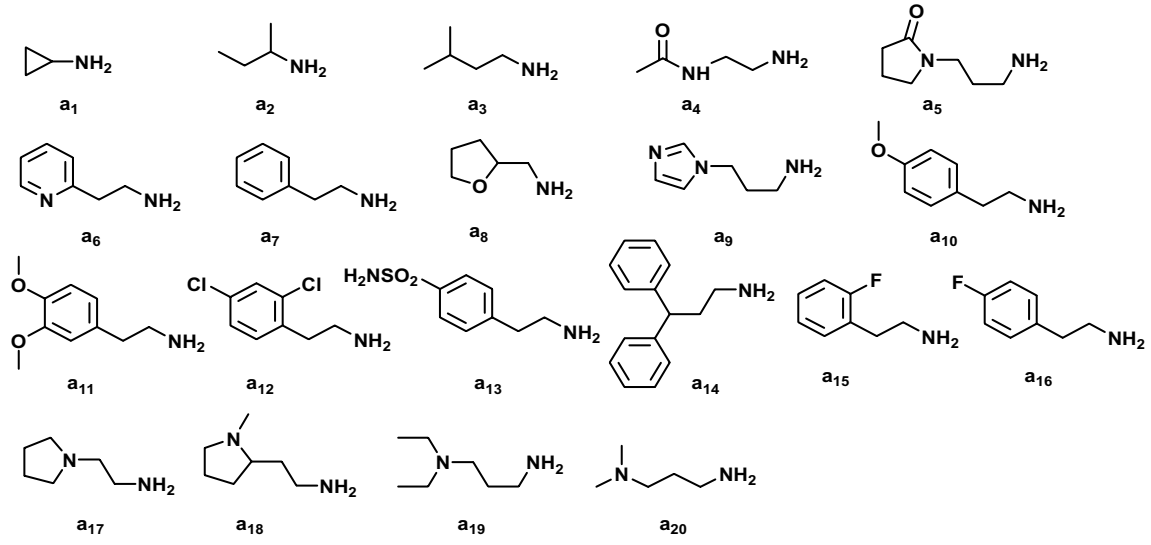
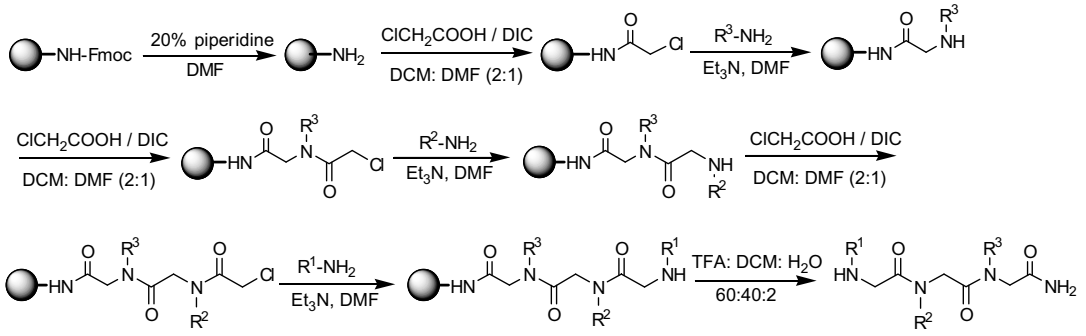


Figure 1. Primary amines used in the synthesis of the library of peptoids.



Scheme 1. Solid-phase synthesis of the library of peptoids.

release of the Fmoc group from the Rink amide resin, successive steps of acylation with chloroacetic acid, followed by the corresponding amination of the chloromethyl intermediate with the specific primary amine or mixture of amines, were conducted. The main difference with respect to the methodology employed in the previous peptoid library was the use of chloroacetic acid instead of chloroacetyl chloride for the acylation. Burkoth et al. reported the use of this acid instead of bromoacetic acid for an efficient synthesis of split-and-mix peptoid libraries containing heterocyclic nitrogens.[20] All reaction steps were carried out in duplicate to ensure maximum conversion. Peptoids were released from the resin with a 60:40:2 trifluoroacetic acid–dichloromethane–water mixture. The synthesis of a 'reporter peptoid', containing three different 2-arylsubstituted ethylamines to facilitate UV monitoring, was also incorporated into the library construction sequence. The MS analysis at different stages of the reporter synthesis indicated the presence of the expected peptoid fragments, and the RP-HPLC profile of the final product showed a major peak that accounted for the 70% of the total peak profile.

2.2. Screening of the library of *N*-alkylglycine trimers for the identification of $TRPV_1$ receptor channel antagonists

This first screening was planned as a validation test for the new peptoid library. Since the primary amines integrated in the peptoids identified as antagonists of the $TRPV_1$ receptor channel in the screening of the first peptoid library[17] were also present in this new library (e.g., $\mathbf{a_{12}}$, $\mathbf{a_{18}}$ and $\mathbf{a_{19}}$), it was expected that the new library would afford similar results. The deconvolution process selected the amines expected for each corresponding *N*-alkylglycine moiety (e.g., $\mathbf{a_{12}}$ at the inner and C-terminal positions, and $\mathbf{a_{18}}$ and $\mathbf{a_{19}}$ at the N-terminal position, results not shown).

2.3. Screening of the library of *N*-alkylglycine trimers for the identification of chemosensitizers

Multidrug resistance to anticancer agents remains a major cause of treatment failure in cancer chemotherapy. MDR describes the cross-resistance of tumour cell lines to several structurally unrelated chemotherapeutic agents after exposure to a single cytotoxic drug.[22] This phenomenon is often associated with overexpression of a 170-kd transmembrane glycoprotein (P-gp) encoded by the human MDR1 gene and which acts as a drug-efflux pump.[23,24] This protein is thus considered a prime therapeutic target in cancer chemotherapy.[23,25] Screening of the peptoid library was carried out using an in vitro assay based on accumulation of daunomycin in drug resistant, P-gp overexpressing murine cell lines. Defined mixtures from the library revealed the presence of multidrug resistance reversal agents. Quantification of these results for the entire library (Fig. 2) led to the selection of the most active mixtures by a twofold serial dilution

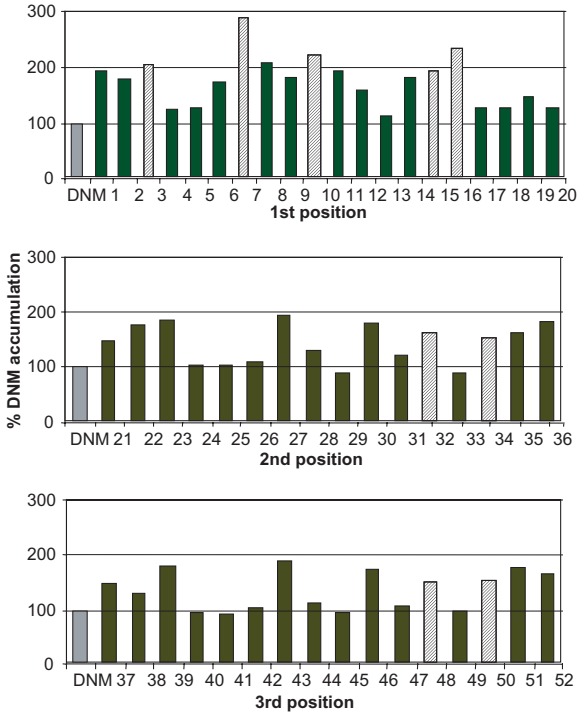


Figure 2. Screening of the library of *N*-alkylglycine trimers for multidrug resistance (MDR) reversal agents. The individual bars in the panels represent the relative percentage of intracellular daunomycin (DNM) accumulation for each peptoid mixture, with the *x*-axis representing the defined amine ('O' position), for the three separate positional sublibraries: OXX, XOX and XXO. The results at 0.1 mg/mL. Striped bars indicate the mixtures selected for the identification of individual peptoids. Mixtures were selected for deconvolution according to their activity and cytotoxicity. Values for DNM (3 mM) are also indicated for each bar panel.

assay. The corresponding deconvolution process selected amines $\mathbf{a_3}$, $\mathbf{a_7}$, $\mathbf{a_{10}}$, $\mathbf{a_{15}}$ and $\mathbf{a_{16}}$ for the N-terminal position sublibrary (OXX) and amines $\mathbf{a_{12}}$ and $\mathbf{a_{14}}$ for both the central position and the C-terminal position sublibraries (XOX and XXO, respectively) (Fig. 2). These amines were selected because of their relatively high activity and low cytotoxicity. When considered together, the screening data provided sufficient information for the identification of the 20 compounds in the library.[16]

2.4. Synthesis and biological activity of peptoids retrieved from the deconvolution

The 20 selected peptoids were independently synthesized and evaluated against the MDR phenotype (Fig. 3). The results led to the identification of 2 peptoids (**1** and **2**, Fig. 4) that caused a higher intracellular accumulation of daunomycin than verapamil at the same dose (5 μM). Cytotoxicity assays revealed that these peptoids decreased MDR resistance in leukaemia cells by $\geqslant$3-fold, presumably by blocking P-gp drug efflux activity. Full details regarding the in vitro and in vivo activity and toxicity studies of compounds **1** and **2** will be reported elsewhere.

2.5. Modelling studies

In addition to the biological results obtained, the potential of peptoids **1** and **2** for development as P-gp inhibitors was also studied computationally. A three-dimensional pharmacophore model based on structural features common to a diverse set of P-gp inhibitors and substrates was thus constructed using Catalyst® software. Several pharmacophore patterns have been reported for P-gp related drugs using computational approaches.[26–28] From those studies, which used a broader set of compounds, the presence of aromatic rings, two hydrophobic regions and at least one hydrogen bond acceptor were inferred as common structural features for eliciting the P-gp inhibitory activity.

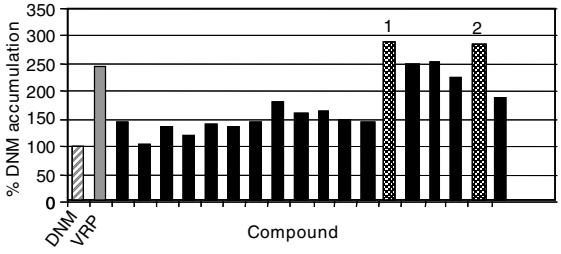


Figure 3. Relative percentage of intracellular daunomycin (DNM) accumulation of individual peptoids at 5 μM in the P-glycoprotein overexpressing, murine leukaemic resistant cell line L1210R. Values for DNM (3 μM) and verapamil (VRP, 5 μM) are also given.

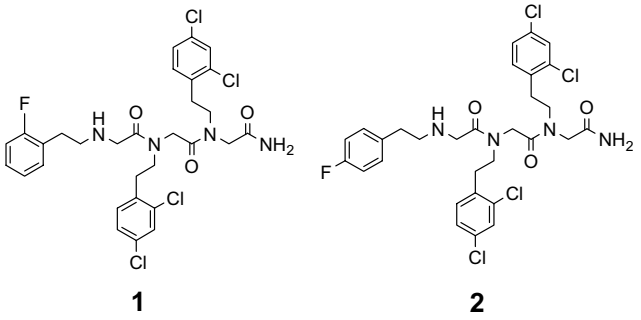


Figure 4. Structures of the peptoid hits identified in the present study.

The training set selected for the generation of our pharmacophore model was composed of (*R*)-verapamil, rhodamine 123, vinblastine, colchicine and calcein AM. All of these compounds bind to P-gp at a similar site; in addition, they contain hydrogen bond acceptors, hydrogen bond donors, hydrophobic regions and aromatic rings. The model generated agrees with previously reported models; it contains two hydrophobic groups (aromatic or aliphatic chains), one aromatic ring and two hydrogen bond acceptors. However, in agreement with work by Ekins et al.,[28] only one of these hydrogen bond acceptors plays an essential role for the compounds analyzed (Fig. 5). Once the pharmacophore model was built, peptoids **1** and **2** were tested to determine if they could interact with P-gp. A favourable alignment was revealed for some of the conformations of both compounds, although one of the hydrogen bond acceptors was not aligned in any case (Fig. 5).

In conclusion, a positional scanning library of 5120 *N*-alkylglycine trimers has been synthesized on solid phase,

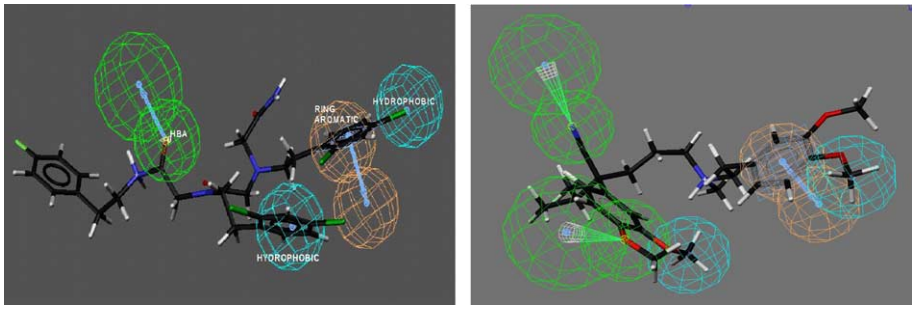


Figure 5. (Left) Peptoid **2** fit into the pharmacophore model generated by Catalyst. The pharmacophore is composed of two hydrophobic regions (cyan), one aromatic ring (orange) and two hydrogen bond acceptors (green). One of the hydrogen bond acceptors, which did not interact with peptoid **2,** has been removed for clarity. (Right) Verapamil fit into the same pharmacophore for comparison purposes.

employing a set of 20 commercially available primary amines as a source of chemical diversity. Screening of the library yielded two potent chemosensitizers, compounds **1** and **2**. The structures of these peptoids are consistent with a pharmacophore model generated from the interaction of various known inhibitors with the MDR-implicated transmembrane glycoprotein P-gp.

Peptoids are simple molecules with a high degree of conformational mobility. Hence some of the conformations of compounds **1** and **2** fit into the model very efficiently, with energies close to the minimum conformer, while in other cases the interactions were inconsistent with the pharmacophore. The structural simplicity of peptoids makes these peptidomimetics amenable to structural manipulation, thus facilitating the optimization of lead molecules for drug-like properties. Peptoid libraries can be considered a valuable link between modern drug discovery technology and traditional lead development.[29,30] However, the high conformational mobility of these compounds can also generate selectivity problems due to unwanted interactions with non-target molecules. Synthesis of conformationally restricted analogues of **1** and **2** could amplify activity and selectivity in the peptoids by effectively 'freezing' their respective computationally predicted, most active conformers. Work addressed to this aim is currently underway in our laboratory.

3. Experimental

3.1. General

Solvents, amines and other reagents were purchased from commercial suppliers and used without further purification. HPLC analyses on an analytical scale were carried out with a Hewlett Packard Series 1100 (UV detector 1315A) modular system using a Kromasil 100 C8 (15×0.46 cm, 5 μm) column, with CH_3CN–H_2O mixtures containing 0.1% TFA as mobile phase, at a flow rate of 1 mL/min and detection at 220 nm. High resolution mass spectra (HRMS-FAB) were carried out at the Mass Spectrometry Service of the University of Córdoba (Spain).

3.2. Synthesis of the peptoid library

The synthesis of the library was carried out on a 1% cross-linked polystyrene resin bearing the Fmoc-protected Rink amide linker AM RAM (0.7 mmol/g, Rapp Polymer; Germany) using an IKA Labortechnik HS501 digital stirrer. An eight-step protocol similar to that previously described[16] was used. All treatments were carried out in duplicate at room temperature. Initial Fmoc deprotection was monitored using the TNBS test, while acylation and amination steps were checked with the chloranil test.

The 52 tea bags of the library, as well as three extra tea bags for reaction control, were filled with 0.4 g of Rink amide resin (load of 0.7 mmol/g, 0.28 mmol) and divided into three groups, one per sublibrary, as follows: $(1 \times 20) + (2 \times 16)$. Common reactions (deprotection, acylation and the introduction of a random diversity position) were carried out in tea bags placed into 1 L polypropylene bottles, and amination reactions at the defined positions were performed in 50 mL polypropylene bottles. *Deprotection*: a solution containing 378 mL or 306 mL of 20% piperidine in DMF was added, correspondingly, to each of the three 1 L bottles containing the tea bags and the resulting mixtures were stirred for 30 min. The resin was drained and washed with DMF (3×360 mL), *i*PrOH (3×360 mL) and CH_2Cl_2 (3×360 mL). *Acylation:* the resin was treated with a solution of chloroacetic acid (20 equiv/bag) and *N*,*N*′-diisopropylcarbodiimide (20 equiv/bag) in either 378 or 306 mL of 1:1 DMF/CH_2Cl_2, for 30 min. The resin was drained and washed with CH_2Cl_2 (3×360 mL), *i*PrOH (3×360 mL) and DMF (3×360 mL). *Amination conditions: (a) Defined positions*. The tea bags corresponding to the defined position, as well as the control bags, were separated and introduced individually into 30 mL bottles. The desired primary amine (20 equiv, 5.6 mmol) and triethylamine (0.78 mL, 20 equiv, 5.6 mmol) in DMF (17 mL), were added to the appropriate tea bag, and the mixture was stirred for 3 h. The tea bag was drained and washed with DMF (3×18 mL), *i*PrOH (3×18 mL) and CH_2Cl_2 (3×18 mL). *(b) Random position*. The other tea bags corresponding to the sublibraries with a random position were maintained together in 1 L bottles. An equimolar mixture of the 16 (or 20, depending upon the position) primary amines (20 equiv per amine, 1.25 or 1.0 mmol amine/bag), and triethylamine (12.5 or 15.5 mL, 20 equiv/bag) in 275 or 345 mL of DMF, were added to the bags and the mixture was stirred for 3 h. The tea bags were drained and washed with DMF (3×360 mL), *i*PrOH (3×360 mL) and CH_2Cl_2 (3×360 mL). *Cleavage*: the resin was treated with a mixture of 60:40:2 (v/v/v) TFA/DCM/H_2O for 30 min. The cleavage mixture was filtered, all filtrates were combined and the solvent was removed by evaporation under reduced pressure followed by lyophilization. The recovered average weight for all mixtures was approximately 70%. Finally, all 52 mixtures were redissolved in 10% DMSO at 10 mg/mL and stored at −20 °C until use.

3.3. Synthesis of individual peptoids

Individual *N*-alkylglycine trimers were synthesized following the eight-step procedure described above with slight modifications (e.g., use of 5 equiv excess of reagents), and reactions were carried out in 10 mL polypropylene syringes. Compounds were purified from the crude reaction mixture by semi-preparative HPLC using a Kromasil C8 (25×2 cm, 5 m) column, CH_3CN–H_2O mixtures containing 0.1% TFA as mobile phases and a flow rate of at 5 mL/min. The NMR spectra of peptoids were recorded in a Varian Inova 500 apparatus (^1H NMR, 500 MHz; ^{13}C NMR, 125 MHz). The assignments of absorptions observed for the different peptoids were confirmed by gDQCOSY and gHSQC experiments. The occurrence of different conformations resulted in very complex spectra. For this reason, only

absorptions assigned to the major conformer are described.

[*N*-[2-(2′-Fluorophenyl)ethyl]glycyl]-[*N*-[2-(2′,4′-dichlorophenyl)ethyl]glycyl]-*N*-[2-(2′,4′-dichlorophenyl)ethyl]glycinamide (**1**) δ_H (500 MHz, CD_3OD; Me_4Si) 7.5 (1H), 7.4 (2H), 7.32 (4H), 7.2 (1H), 7.12 (2H), 4.2–3.8 ($3\times COCH_2N$), 3.60 (2H, m, NCH_2CH_2 (C_t)), 3.52 (2H, m, NCH_2CH_2), 3.23 (2H, m, $NHCH_2CH_2$ (N_t)), 3.12 (2H, NCH_2CH_2 (C_t)), 3.04 (2H, $NHCH_2CH_2$ (N_t)) and 2.92 (2H, NCH_2CH_2); δ_C (125 MHz, CD_3OD) 170.56 (CO), 169.73 (CO), 167.23, (CO), 161.59 (C_{Ar}), 135.97 ($2\times C_{Ar}$), 134.8 ($2\times C_{Ar}$), 134.2 ($2\times C_{Ar}$), 133.7 (CH_{Ar}), 133.37 (CH_{Ar}), 132.19 (C_{Ar}), 130.6 (CH_{Ar}), 130.5 (CH_{Ar}), 130.2 (CH_{Ar}), 128.9 (CH_{Ar}), 128.4 (CH_{Ar}), 125.8 (CH_{Ar}), 124.4 (C_{Ar}), 116.6 (CH_{Ar}), 50.6 ($COCH_2N$), 50.04 ($COCH_2N$), 49.38 (NCH_2CH_2 (C_t)), 49.02 (NCH_2CH_2), 48.79 ($COCH_2N$), 48.50 ($HNCH_2$-CH_2 (N_t)), 32.75 (NCH_2CH_2 (C_t)), 31.73 (NCH_2CH_2), 26.87 ($HNCH_2CH_2$ (N_t)); *m*/*z* (FAB) 545.0661 for ($M^+-C_7H_6F$. $C_{23}H_{25}Cl_4FN_4O_3$ requires 545.0681).

[*N*-[2-(4′-Fluorophenyl)ethyl]glycyl]-[*N*-[2-(2′,4′-dichlorophenyl)ethyl]glycyl]-*N*-[2-(2′,4′-dichlorophenyl)ethyl]glycinamide (**2**): δ_H (500 MHz, CD_3OD; Me_4Si) 7.5 (1H), 7.4 (2H), 7.34–7.2 (5H), 7.06 (2H), 4.2–3.8 ($3\times COCH_2N$), 3.6 (2H, NCH_2CH_2 (C_t)), 3.5 (2H, NCH_2CH_2), 3.17 (2H, m, $NHCH_2CH_2$ (N_t)), 3.10–2.97 (2H, NCH_2CH_2 (C_t)), 2.97 (2H, $NHCH_2CH_2$ (N_t)), 3–2.89 (2H, NCH_2CH_2); δ_C (125 MHz, CD_3OD) 172.4 (CO), 170.1 (CO), 167.7 (CO), 162.5 (C_{Ar}), 136.6 ($2\times C_{Ar}$), 135.9 (C_{Ar}), 134.8 ($2\times C_{Ar}$), 134.1 ($2\times C_{Ar}$), 133.8 (CH_{Ar}), 133.4 (CH_{Ar}), 131.6 ($2\times CH_{Ar}$), 130.5 (CH_{Ar}), 130.2 (CH_{Ar}), 128.9 (CH_{Ar}), 128.4 (CH_{Ar}), 116.6 ($2\times CH_{Ar}$), 50–48.8 ($3\times COCH_2N$ mixture of conformers), 49.85 ($HNCH_2CH_2$ (N_t)), 49.16 (NCH_2-CH_2), 49.08 (NCH_2CH_2 (C_t)), 32.71 (NCH_2CH_2 (C_t)), 32.34 ($HNCH_2CH_2$ (N_t)), 31.66 (NCH_2CH_2); *m*/*z* (FAB) 545.0654 for ($M^+-C_7H_6F$. $C_{23}H_{25}Cl_4FN_4O_3$ requires 545.0681).

3.4. Daunomycin accumulation assays

Daunomycin-resistant, P-glycoprotein (P-gp) overexpressing L1210R murine leukaemia cells were washed once with Hepes saline buffer (HBS), and the resulting pellet was resuspended in Hepes buffered saline (HBS) at 1×10^6 cells/mL per sample. Cells were then incubated with 3 μM daunomycin (control) and 5 μM verapamil (as a chemosensitizing effect reference) or the peptoid mixtures (0.1 mg/mL) for 1 h at 37 °C. After incubation, intracellular daunomycin accumulation was determined by flow cytometry using a previously described protocol.[31]

3.5. In vitro proliferation assays

In vitro proliferation assays compared the growth rate of L1210S and L1210R cells by both counting the number of viable cells after plating 2×10^4 cells/well on a P-24 flat-bottom plate for 5 days, as well as by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-2*H*-tetrazolium bromide (MTT, Sigma) assay after plating 2×10^4 cells in a 96-well plate for 48 h. MTT reagent was added to each well and incubated for 4 h at 37 °C. Thereafter, 100 μL/well of 0.1 N HCl–isopropyl alcohol were added, mixed thoroughly to dissolve the dark blue crystals, and plates were subsequently read on an ELISA reader at a wavelength of 570 nm.

3.6. Molecular modelling studies

Computational molecular modelling studies were performed using a Silicon Graphics Octane workstation. The 3D-structures of the different inhibitors of P-gp were constructed using Catalyst Version 4.7 (Molecular Simulations, San Diego, CA). The number of conformers generated using the 'best' feature of the program for each inhibitor was limited to a maximum of 250, with an energy range of 20 kcal/mol. Using the common-features hypothesis function (Hip-Hop) in Catalyst, 10 hypotheses were generated using the above conformers for each of the training set molecules. The eventual protonation of these inhibitors at physiological pH, as well as selected features such as hydrogen bond donors, hydrogen bond acceptors, hydrophobic regions and aromatic rings, were all taken into consideration. After the assessment of the 10 hypotheses, those exhibiting the lowest relative energy cost were selected. Compounds **1** and **2** were fitted into the selected hypothesis. The correct alignment was used with the best-fit procedure to assess how all conformers from both peptoids might orient within the hypothesis, minimizing the distance between the center of the hypothesis features and their mapping to atoms on the molecule.

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