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Towards more drug-like proteomimetics: two-faced, synthetic α -helix mimetics based on a purine scaffold†

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Mimicry of two faces of an α -helix might yield more potent and more selective inhibitors of aberrant, helix-mediated protein–protein interactions (PPI). Herein, we demonstrate that a 2,6,9-tri-substituted purine is capable of disrupting the Mcl-1-Bak-BH3 PPI through effective mimicry of key residues on opposing faces of the Bak-BH3 α -helix.

Protein–protein interactions (PPIs) participate in pivotal roles in cell signaling pathways, and their dysregulations can lead to a wide range of pathologies, including cancer, cardiovascular and neurodegenerative diseases.^{1–4} In addition, PPIs featuring exogenous proteins are involved in the transmission of infectious diseases, such as HIV.⁵ Given the potential health benefits, it is no surprise that the disruption of PPIs by synthetic agents has rapidly become a major goal in contemporary medicinal chemistry.^{6–9} The intensity with which these biological interfaces are being interrogated is likely fuelled by the various successful approaches reported over the last decade, quashing the pessimism that this is too formidable a goal. In particular, there have been exciting advances in the realm of helix-mediated PPIs; the most notable examples of which are ABT-737¹⁰ and the Nutlins,¹¹ which are low-molecular-weight, nanomolar inhibitors of the oncoproteins Bcl-x_L and HDM2, respectively.

One strategy to disrupt helix-mediated PPIs is through synthetic α -helix mimetics.^{12–15} Traditionally, helix mimetics reproduce key functionality located on only one face of an α -helix, even though many α -helices that engage in PPIs utilize multiple faces to accomplish recognition of their target pro-

teins.¹⁶ Most typically, the *i*, *i* + 3/4 and *i* + 7 hydrophobic side chains are emulated.^{12–15} A potential caveat with this strategy is that the resulting molecules are rather hydrophobic, particularly the pioneering terphenyls, with *clogP* values often around 5 or higher, which might result in poor solubilities and off-target effects.¹⁷ Also, the molecular weights (MW) of these compounds are commonly greater than 500. Thus, in at least two ways, some of the original helix mimetics contravene Lipinski's rules. The mimicry of multiple faces of an α -helix is expected to afford more potent agents that can better discriminate between protein surfaces through a richer recognition profile and may, therefore, provide a source of selectivity. Accordingly, towards the discovery of novel and potent Mcl-1 inhibitors, we recently described amphipathic α -helix mimetics based on a 1,2-diphenylacetylene scaffold.¹⁸ Other groups have also developed two-faced, synthetic α -helix mimetics,^{19–22} although all of these designs suffer from lengthy syntheses.^{18–22} As part of our group's continued interest in disrupting helix-mediated PPIs with small-molecule α -helix mimetics, we sought to discover more drug-like (MW < 500, *clogP* < 5) agents with therapeutic utility in which mimicry of two faces is once more accomplished, and in which the target molecules may be more readily accessed. At the same time, it is acknowledged that mimicry of just one face of an α -helix might afford inhibitors that exhibit polypharmacologies,^{23,24} hitting multiple targets as a more effective strategy to kill cancer cells, for example. In this latter scenario, a convenient means of installing a solubilizing group would be of utility in a location that has limited interference with the PPI, such as the opposing face to that emulated at the protein–protein interface. This would complement work by Rebek, Hamilton and Wilson who have described helix mimetics with a “wet” edge.^{25–27}

Lim and colleagues have described single-faced helix mimetics based on a rigid pyrrolopyrimidine scaffold wherein side chains are projected from the 2, 6 and 7 positions.²⁸ Their molecules proved potent disrupters of the MDM2–p53 PPI, indicating that a flexible scaffold is not crucial for effective helix mimicry. We hypothesized that a related 2,6,9-tri-substi-

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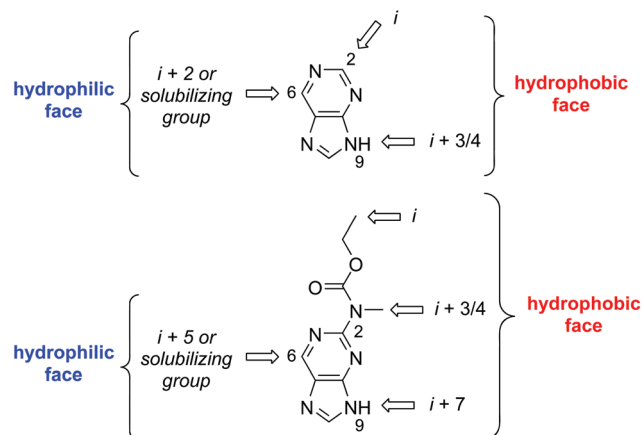


Fig. 1 Substitution of the 2, 6 and 9 positions of the purine scaffold putatively permits mimicry of two faces of an α -helix. Generic examples illustrated are amphipathic helix mimetics, but other two-faced helix mimetics may be prepared similarly.

tuted purine scaffold would permit mimicry of the i and $i + 3/4$ side chains on one face of the helix, and the $i + 2$ side chain (or simply a solubilizing group) on the opposing face, as depicted in Fig. 1A. Mimicry of opposing faces of one turn of an α -helix in this way might afford potent and selective inhibitors that exhibit lower-molecular-weights than the terphenyls and their counterparts. Additionally, the purine scaffold is more hydrophilic than the terphenyl scaffold, which would serve to lower the $\log P$ of the helix mimetic. Finally, elaboration of functionality introduced at the 2-position, such as a carbamate or a sulfonamide, might allow suitable emulation of both the $i + 3/4$ and the $i + 7$ side chains from the same position (Fig. 1B).

To test our hypothesis, we designed molecules to inhibit the anti-apoptotic Bcl-2 protein Mcl-1, which, along with its relative Bcl-x_L, is overexpressed in a range of cancers, including pancreatic cancer and acute myeloid leukaemia.^{29,30} Mcl-1 seizes the BH3 α -helical domains of pro-apoptotic Bcl-2 family members, including Bak and Bim, through a hydrophobic crevice on its surface.²⁹ Under normal conditions, the levels of anti- and pro-apoptotic Bcl-2 proteins are tightly regulated to maintain a healthy population of cells but in many cancers, the high levels of Mcl-1 and other anti-apoptotic proteins results in the neutralization of the pro-apoptotic proteins and, thus, tumorigenesis.²⁹ Akin to the successful inhibition of Bcl-x_L and HDM2,^{11,12,31,32} it has been proposed that low-molecular-weight ligands that can mimic these BH3 α -helices should compete with Mcl-1 binding, freeing up the pro-apoptotic proteins to initiate the intrinsic apoptosis pathway, and, thereby, destroy the cancer cell.^{33,34}

Alanine scanning mutagenesis has revealed that the key side chains of the Bak-BH3 α -helix that engage the anti-apoptotic Bcl-2 proteins include Val74, Leu78 (i), Ile81 ($i + 3$), Ile85 ($i + 7$), on one face, which bind the p1, p2, p3 and p4 pockets, respectively, and Asp83 ($i + 5$) on the other face, which binds Arg139 of Bcl-x_L and Arg263 of Mcl-1.^{29,35} Since potent inhibitors have been fashioned by mimicking only three of the four hydrophobic residues,^{12–15} we designed amphipathic α -helix mimetics that reproduced the functionality of only Leu78, Ile81 and Ile85 on the hydrophobic face and Asp83 on the opposing face; these residues are highlighted in Fig. 2A. In Fig. 2B, the corresponding purine-based synthetic α -helix mimetic of the helix in Fig. 2A is given. Fig. 2C illustrates the Bak-BH3 α -helix overlaid with an MM2 energy-minimized conformation of the helix mimetic in Fig. 2B; very good mimicry of the key side chains is observed. Fig. 2D describes our simplified, two-faced α -helix mimetic design, wherein R¹

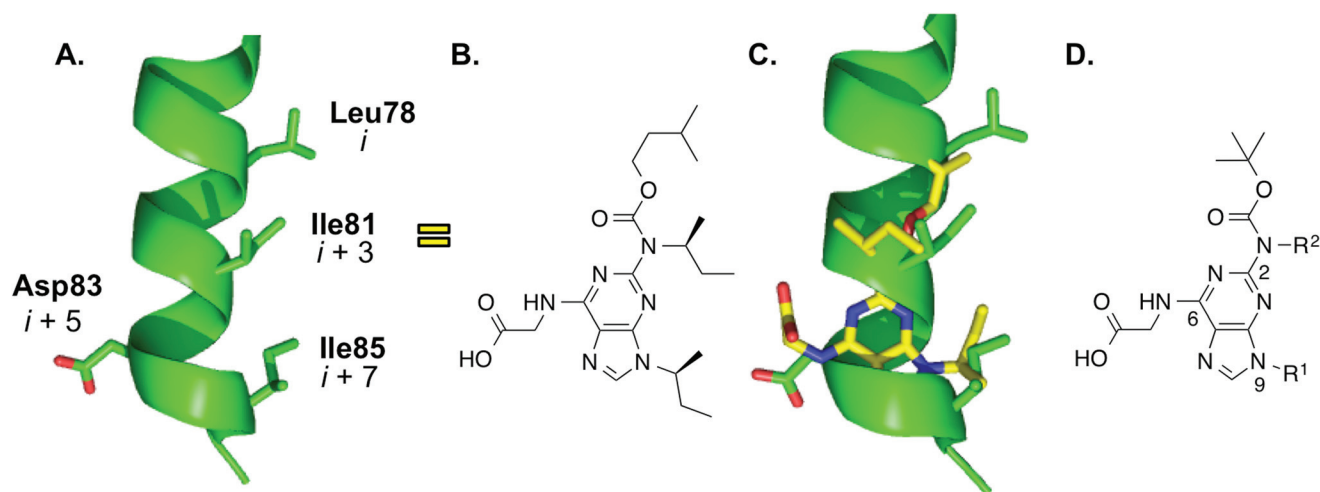
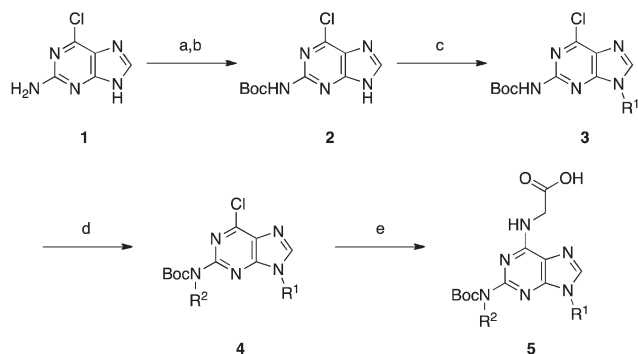


Fig. 2 A. Key residues of the Bak-BH3 α -helix. B. A purine-based α -helix mimetic designed to imitate Leu78, Ile81, Ile85 and Asp83. C. Superimposition of an MM2 energy-minimized conformation of the molecule in B. on the Bak-BH3 α -helix. D. Simplified Bak-BH3 α -helix mimetic where R¹ and R² are proposed to imitate the $i + 7$ and $i + 3$ side chains, respectively, whilst the Asp83 mimetic is fixed as CH₂COOH, and the Leu78 mimetic constrained as *tert*-butyl.

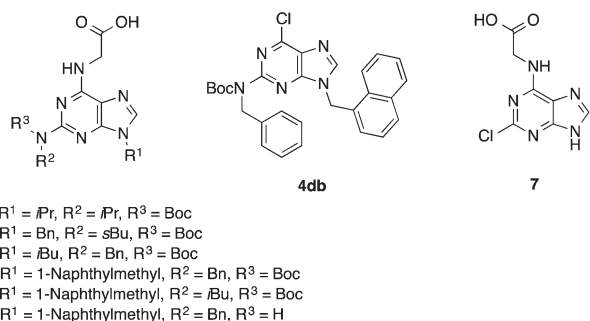


Scheme 1 (a) Boc_2O , cat. DMAP, DMSO, 0 °C to RT; (b) NaH, THF, 0 °C to RT; (c) R^1OH , DIAD, PPh_3 , THF, RT; (d) R^2OH , DIAD, PPh_3 , THF, 35 °C; (e) glycine, K_2CO_3 , iPrOH, reflux.

and R^2 are intended to mimic the $i + 7$ and $i + 3$ side chains, respectively, and, to expedite compound synthesis, we fixed the Asp83 mimetic ($i + 5$) as CH_2COOH , and the carbamate group as a Boc group. This last constraint was anticipated to result in sub-optimal mimicry of Leu78 (i) but would greatly facilitate the synthetic chemistry.

We have previously shown that N^2 -Boc-2-amino-6-chloropurine (2) can be regioselectively alkylated at the N9 position under standard Mitsunobu conditions.³⁶ NB: In the preparation of 2, NaH as a dispersion in mineral oil gave capricious results, whereas dry NaH consistently drove complete Boc transfer from the N^9 to the N^2 position. Therefore, as shown in Scheme 1, to ensure projection of the R^1 (the $i + 7$ side chain of the Bak-BH3 α -helix) and CH_2COOH ($i + 5$) groups from opposite faces of the helix mimetic, 2 was condensed with alcohols R^1OH in the presence of DIAD and PPh_3 . The resulting N^9 -alkylated products 3 were subjected to a second round of Mitsunobu chemistry using modified conditions (2.5 equiv. of R^2OH , PPh_3 and DIAD, and gentle warming) to introduce the R^2 group ($i + 3$ mimetic). Finally, an $\text{S}_{\text{N}}\text{Ar}$ reaction with glycine installed the $i + 5$ mimetic on the opposite face of the scaffold, providing the target molecules 5 in five simple steps.

Accordingly, a small library of amphipathic α -helix mimetics based on 5 was rapidly developed (Scheme 2) and then each library member was analyzed for its ability to



Scheme 2 Purines prepared in this study.

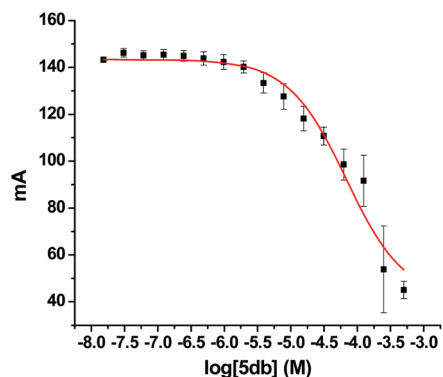


Fig. 3 FITC-BAK^{71–89} was competed off Mcl-1^{172–327} with 5db giving an estimated IC_{50} of $72.3 \pm 21.2 \mu\text{M}$. Data are an average of biological triplicates and errors are standard deviations. mA = milli-anisotropy. An absolute anisotropy of 40 mA indicates complete competition. For further details, please see the ESI.†

disrupt the Mcl-1-Bak-BH3 PPI using a fluorescence anisotropy competition assay with a fluorescein-labeled Bak-BH3 peptide GQVGRQLAIGDDINR, “FITC-Bak^{71–89}” (ESI†).³⁷ Unfortunately, only one compound, 5db, demonstrated appreciable activity with an estimated IC_{50} of $72.3 \mu\text{M}$ (Fig. 3). Notably, the construction of a sufficiently hydrophobic face was required since there is a seven-fold variation in the estimated IC_{50} values for 5db ($\text{R}^2 = \text{benzyl}$: $\text{IC}_{50} = 72.3 \mu\text{M}$) and 5dc ($\text{R}^2 = \text{isobutyl}$: $\text{IC}_{50} = 472 \mu\text{M}$), and no activity for 5aa ($\text{R}^1 = \text{R}^2 = \text{isopropyl}$) or compound 7. Furthermore, deletion of the Boc group (6db) abrogated binding. Also of particular significance, the carboxylic acid at the $i + 5$ position was required for binding to Mcl-1 (4db exhibited no inhibitory activity). Taken together, these data demonstrate that the appropriate functionalization of both faces of a purine scaffold furnished synthetic agents that are capable of recognition of Mcl-1. Although the MW of 5db is just over 500, its clogD at pH 7.4 (calculated using ACD Labs) is 2.56, which is considerably lower than those of analogous terphenyl-based helix mimetics (clogDs 7.22 (mono-acid), 5.27 (di-acid)). We anticipate that a structure-activity relationship (SAR) analysis of 5db will identify more potent Mcl-1 inhibitors that conform to Lipinski’s rules. More generally, these findings validate purine scaffolds as suitable frameworks to achieve two-faced, α -helix mimicry.

The inhibitory profile of 5db was investigated further by interrogating the HDM2-p53 and Bcl-x_L-Bak-BH3 PPIs using similar fluorescence anisotropy competition assays. In the latter case, the same Bak-BH3 peptide from the Mcl-1 experiments was utilized, whilst in the HDM2 experiments, a TAMRA-labeled p53 peptide SQETFSDLWLKPEN was employed (ESI†). Although no inhibition of HDM2 was observed, 5db weakly inhibited Bcl-x_L with an estimated IC_{50} of $201 \mu\text{M}$. Whilst the p53 helical peptide that binds HDM2 also presents a similar recognition pattern of amino acids (Phe19, Trp23, Leu26 on one face, and Asp21 on the other face),³⁸ the naphthyl group (a good mimetic of Trp) of 5db, is at a terminus of the helix mimetic and so is improperly posi-

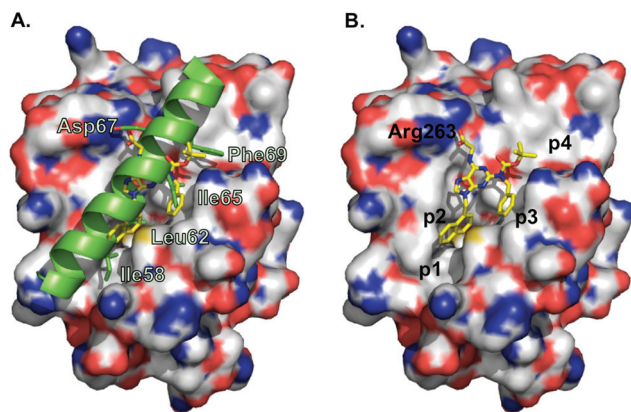


Fig. 4 GOLD docking of **5db** to Mcl-1 (PDB ID: 2PQK). Coloured by atom type: grey = carbon, red = oxygen; blue = nitrogen; yellow = sulfur. A. Superimposition of **5db** (yellow, coloured by atom type) with the Bim-BH3 α -helix (green, key side chains shown in stick format, labelled in green). B. Predicted binding mode of **5db** from A with BH3 α -helix removed: Arg263 and p1–p4 subpockets on Mcl-1 labelled in black.

tioned to emulate Trp23. Moreover, Asp21 does not play a functional role in binding HDM2. Rather, the carboxylic acid of Asp21 forms a hydrogen bond with the hydroxyl of Thr18 within the p53 peptide to ensure it folds into the correct helical conformation to permit recognition of HDM2.³⁸ Thus, appropriately-functionalized two-faced synthetic α -helix mimetics may, indeed, provide a source of selectivity over their single-faced counterparts.

To gain a better appreciation of the likely binding mode of **5db**, as well as a possible explanation to the approximate 3-fold selectivity for Mcl-1 over Bcl-x_L, we performed molecular docking simulations with GOLD. For this purpose, we used the crystal structure of Mcl-1 bound to the Bim-BH3 α -helix, which has a similar pattern of hydrophobic residues to Bak (Ile58, Leu62 (*i*), Ile65 (*i* + 3) and Phe69 (*i* + 7) on one face and Asp67 (*i* + 5) on the other (PDB ID: 2PQK)). For modelling experiments, the Bim-BH3 α -helix was first extracted to reveal the hydrophobic crevice binding site on the surface of Mcl-1. A high-scoring docking solution is given in Fig. 4A with **5db** shown in yellow, which is superimposed on the Bim-BH3 α -helix illustrated in green; key side chains of the helix are shown in stick format. It should be noted that the orientation of the molecule is anti-parallel to the original design. This finding may be due to the binding mode being driven by occupation of the p2 pocket by the large naphthylmethyl group, which, along with the salt bridge interaction with Arg263, is seen more clearly in Fig. 4B. The side chains of Leu62 (Leu78 in Bak), Ile65 (Ile81) and Asp67 (Asp83) overlapped well with the respective functionalities in **5db**. As expected, the *tert*-butyl of the Boc group inadequately simulated the spatial orientation of the side chain of Phe69 (Ile85 in Bak, originally intended to mimic Leu78), clearly providing a focal point for the design of second-generation compounds. Interestingly, the accommodation of the naphthylmethyl moiety in the p2

pocket may explain the observed two-fold selectivity for Mcl-1 over Bcl-x_L since occupation of this pocket appears to be a determinant in Mcl-1 specificity.³³ In closing, we would like to note that our work complements other research in the field of inhibiting helix-mediated PPIs. Particularly, occupation of the p2 pocket by the naphthylmethyl group of **5db** possibly explains the inverse selectivity of our purine-based helix mimetics for Mcl-1 over HDM2 for previously reported helix mimetics.³⁹ Also, a recently disclosed phenyl-piperazine-triazine-based helix mimetic demonstrates significantly greater selectivity for Mcl-1 over Bcl-x_L than that which we observed with our purines, although it is unclear if the groups mimicking the *i*, *i* + 3 and *i* + 7 side chains and/or the scaffold itself are responsible for this observation since only one compound was analysed for selectivity.⁴⁰

Conclusions

In conclusion, 2,6,9-trisubstituted purines were proposed and then validated as two-faced, α -helix mimetics, permitting reasonable mimicry of the *i*, *i* + 3 and *i* + 7 side chains on one face and *i* + 5 on the opposing face of the Bak-BH3 α -helix. Alternatively, functionalization at the *i* + 5 position may simply be applied to install a solubilizing group opposite the decorated face. Computational modelling indicated that the non-flexible *tert*-butyl group incorporated to facilitate the synthetic chemistry may have compromised helix mimicry somewhat; more flexible carbamates and sulfonamides at the N2 position are predicted to remedy this undesired outcome. In the course of this research, a novel inhibitor of Mcl-1 was discovered. Efforts in our laboratory are now underway to optimize this compound through an SAR campaign, a goal that will be directly facilitated by the short and simple synthesis.

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