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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1740–1743

Design and modular parallel synthesis of a MCR derived α-helix mimetic protein–protein interaction inhibitor scaffold

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Received 24 October 2005; revised 30 November 2005; accepted 30 November 2005
Available online 19 January 2006

Abstract—A terphenyl α-helix mimetic scaffold recognized to be capable of disrupting protein–protein interactions was structurally morphed into an easily amenable and versatile multicomponent reaction (MCR) backbone. The design, modular in-parallel library synthesis, initial cell based biological data, and preliminary in vitro screening for the disruption of the Bcl-w/Bak protein–protein interaction by representatives of the MCR derived scaffold are presented.

Protein–protein interactions (PPI) play an important role in the regulation of a number of critical biological functions. The formations of antibody–antigen, hormone-receptor or certain enzyme-inhibitor complexes represent a few examples of such PPI. The nature of these protein–protein interface interactions is complex. They are usually formed by discontinuous protein-sequence and protein-surface elements and the contact area is normally rather large. Attempts to classify the different types of protein–protein interfaces have resulted in a rather larger number of families.[1] Nevertheless, PPI constitute an important target class for novel drug discovery projects, and a growing number of small molecule inhibitors of these interactions continue to be identified.[2]

The major secondary structure elements in key apoptosis regulating PPI (e.g., Bcl-2 family proteins, p53/HDM2) are α-helices.[3] Since the disruption of these PPI could constitute an effective and selective means for the treatment of cancer, we have been interested in the design and discovery of α-helix mimetic PPI inhibitors as potentially novel, apoptosis promoting cancer chemotherapeutic agents, utilizing a chemical genomics type approach.[4]

In contrast to protein secondary structure elements such as β-sheets or β-turns, small molecule α-helix mimetics have rarely been described in the literature (Fig. 1). 1,1,6-Trisubstituted indanes suggested to operate as mimetics of the $i-1$, i, and $i+1$ sidechain residues of an α-helix have been shown to be NK1 binders with micromolar affinity.[5] 2,6,3′,5′-Tetrasubstituted biphenyls have been proposed to mimic the side chains of the i, $i+1$, $i+3$, and $i+4$ residues of an α-helix[6] and recently, Hamilton and co-workers have reported on 3,2′,2″-trisubstituted terphenyls as mimetics of the side chains at the i, $i+3$, and $i+7$ positions of an α-helix, capable of disrupting PPI.[7]

Our efforts into the design of novel α-helix mimetics that disrupt PPI and induce cellular apoptosis were prompted by Hamilton's terphenyl α-helix mimetic concept. One major disadvantage inherent in the terphenyl scaffold is the rather lengthy, multi-step synthetic approach needed for its assembly.[8] Hence, as a starting point, we investigated alternate α-helix mimetic scaffolds that would be accessible by a multicomponent reaction (MCR), ideally in a single reaction step and in a modular fashion. Molecular modeling[9] derived structural considerations led us to the selection of an appropriately

Keywords: Protein interaction antagonist; Cancer; Bcl family; Apoptosis; Multicomponent reaction; Isocyanide.
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0960-894X/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2005.11.102

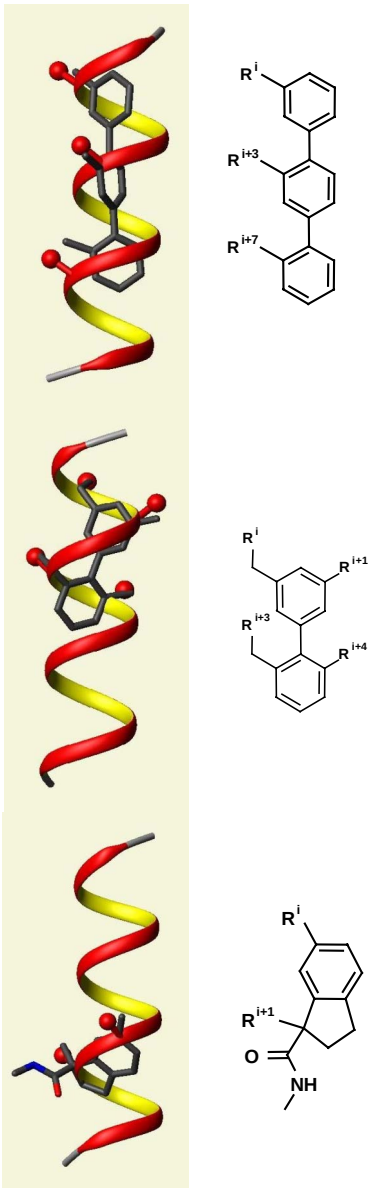


Figure 1. Rationally designed α-helix mimetic scaffolds, superimposed on an ideal α-helix. The relative location in the sequence of the α-helix residues addressed by the small molecules has been indexed.

trisubstituted imidazole backbone (Fig. 2), which could be easily obtained utilizing the synthetic approach of van Leusen et al.[10]

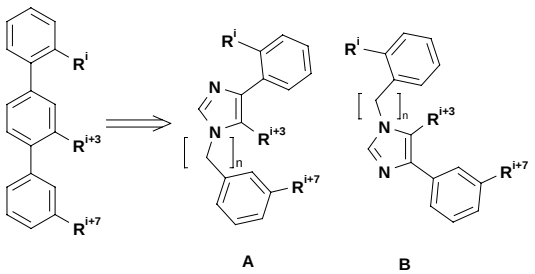


Figure 2. Structural morphing of the terphenyl scaffold into a van Leusen imidazole backbone ($n = 0$–2).

Phenyl-substituted tosylmethyl isocyanides (TosMICs) constitute one of the versatile starting materials in our synthesis of a MCR derived imidazole based α-helix mimetic. They are easy to synthesize or are commercially available.[11] During the course of this work, we prepared several substituted phenyl TosMICs by reacting the corresponding substituted benzaldehyde, formamide, and *para*-methyl phenyl sulfinic acid in a MCR (α-aminoalkylation) followed by dehydration (Fig. 3).[12] The chosen substituents were selected to effectively mimic one of the side-chain residues of the amino acids constituting a part of the triad *i*, *i* + 3, and *i* + 7.

For the synthesis of the desired trisubstituted imidazoles, we initially investigated the use of anilines (R^3 = substituted phenyl) as the amine component in the van Leusen synthesis (Fig. 4).

Under a variety of experimental conditions and for a small set of aldehydes investigated, *meta*-substituted anilines failed to react with *ortho*-substituted phenyl TosMICs to provide the desired imidazole products **A** (Fig. 2). Alternatively, when *meta*-substituted phenyl TosMICs were employed in the same reaction, the corresponding imidazoles were obtained, albeit in moderate yields only. Significantly, key inversely substituted imidazoles **B** could be generated starting from *ortho*-substituted anilines and *meta*-substituted phenyl TosMICs, although in low yields.

Due to the difficulty encountered with suitably reacting *ortho*-substituted phenyl TosMICs with anilines, we next turned our attention to the use of appropriately substituted benzylamines (R^3 = substituted phenylmethyl) as the amine component in the van Leusen imidazole synthesis (Fig. 4). Gratifyingly, we observed that benzylamines typically reacted much more robustly than the corresponding anilines in the van Leusen reaction. A

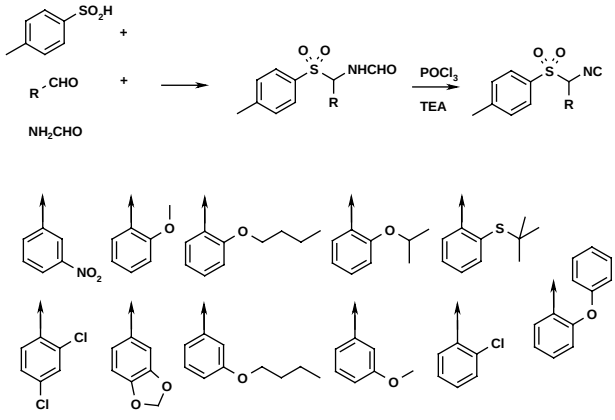


Figure 3. Preparation of phenyl-substituted TosMICs.

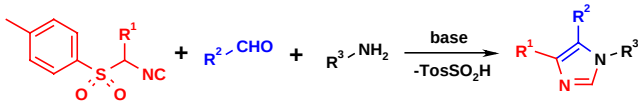


Figure 4. The van Leusen MCR synthesis of trisubstituted imidazoles.

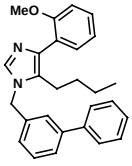
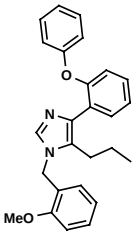
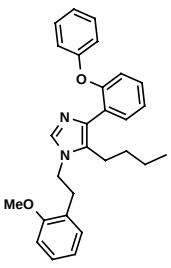
parallel solution phase synthesis of a focused library of about 60 trisubstituted imidazoles was conducted. Rapid parallel purification of the crude reaction products was accomplished via 'chromfiltration'[13] to afford the desired trisubstituted imidazole products in >80% purity.

Based on our primary interest in initially identifying novel compounds that inhibit cell proliferation and promote cellular apoptosis, the synthesized imidazole derivatives were initially profiled in phenotypic cell based assays and tested for apoptosis-inducing ability.

It is observed from Table 1 that representative trisubstituted imidazoles exhibited micromolar potency for cell growth inhibition[14] in a HL-60 cell line. The induction of apoptosis assessed in a DNA fragmentation assay in HL-60 cells[15] for two compounds listed in Table 1, **2** and **3** is shown in Figure 5. Both compounds were able to induce apoptosis in a dose-dependent manner consistent with their IC_{50} values in the cell proliferation assay.

To preliminarily delineate the mode of action of the synthesized trisubstituted imidazoles as apoptosis inducers, we screened them in a FP assay[17] established in-house for inhibition of Bcl family PPI. Significantly, the major secondary structure elements in the Bcl-2 subfamily of antiapoptotic proteins are α-helices, arranged in such a way that a large hydrophobic groove is formed on the protein surface. The BH3 domains of the proapoptotic proteins then bind as an amphipathic α-helix within this groove. Terphenyl based α-helix mimetics of a BH3 peptide epitope that are capable of inhibiting this PPI have been reported.[7]

Table 1. Cell proliferation data (HL-60) and in vitro FP data (Bcl-w)

Compound	Structure	GI_{50} (μM) HL-60	IC_{50} (μM) FP, Bcl-w
1		12.95 ± 0.11	26.85 ± 1.24
2		12.51 ± 2.68	13.99 ± 4.11
3		9.43 ± 1.3	8.09 ± 2.62

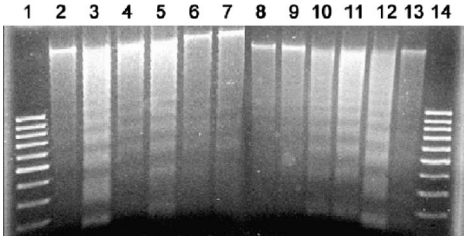


Figure 5. Apoptosis induced by representative trisubstituted imidazoles in HL-60 cells in a DNA fragmentation assay. Lanes 1, 14: 100 bp DNA markers; 2, 13: DMSO control; 3, 12: 1.25 μM camptothecin; 4, 5, 6, and 7: 100, 10, 1, and 0.1 μM **3**, respectively; 8, 9, 10, and 11: 0.1, 1, 10, and 100 μM **2**, respectively.

In our initial screen, three representative compounds (Table 1) were found to disrupt the interaction of Bcl-w with the Bak-BH3 peptide. Surprisingly, all three compounds were inactive against other Bcl family members such as Bcl-2 or Bcl-X_L (data not shown). Modeling studies on imidazole **2** demonstrated the ability of these compounds to mimic the critical *i*, *i* + 3, and *i* + 7 residues of an α-helix epitope of a proapoptotic Bcl family peptide (Fig. 6).

In summary, we have introduced the design of novel imidazole based α-helix mimetics that are conceptually derived from the Hamilton terphenyl compounds, but are synthetically amenable in one step utilizing a MCR. The described trisubstituted imidazoles comprise a novel class of lead molecules suitable for further optimization. Further studies are needed to fully delineate the biochemical mode of action of representatives of this lead series. The selection of a wider set of starting materials should lead to compounds with improved biological and pharmacokinetic activities.

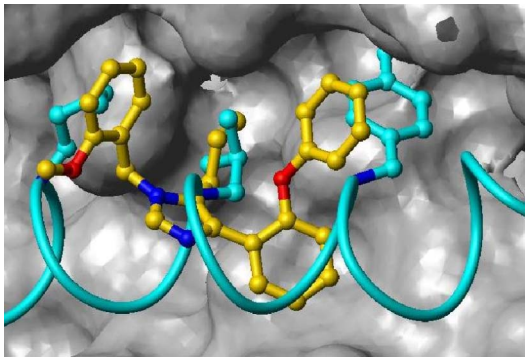


Figure 6. A designed van Leusen MCR derived trisubstituted imidazole α-helix mimetic modeled into the structure of the Bcl-X_L/Bad peptide complex (pdb-code 1G5J). Compound **2** (gold) is superimposed onto the α-helical Bad peptide epitope *i*, *i* + 3, and *i* + 7 residues. Thus, the phenyloxy substituent on the imidazole superimposes with the side chain of Tyr (*i* + 7), the *n*-propyl moiety with the side chain of Leu (*i* + 3), and the methoxyphenyl moiety with the side chain of Met (*i*).

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13. General procedure for the parallel, solution-phase multicomponent synthesis of 1,4,5-trisubstituted imidazoles; 5-butyl-1-(2-methoxybenzyl)-4-(2-phenoxy-phenyl)-1H-imidazole: A solution of 2-methoxy-benzylamine (13 μL, 0.10 mmol) and valeraldehyde (11 μL, 0.10 mmol) in MeOH (0.5 mL) was stirred at room temperature for 1 h. (2-Phenoxyphenyl) tosylmethyl isonitrile (36 mg, 0.10 mmol) and triethylamine (28 μL, 0.20 mmol) were now added and the resulting mixture was stirred and heated at 65 °C for 4 h. Solvent was evaporated under reduced pressure and the residue was redissolved in CH_2Cl_2 (0.5 mL). The crude product was loaded onto a short silica gel column (~1 g), packed in a disposable syringe (10 mL), that had been previously pre-eluted with CH_2Cl_2 (10 mL) containing MeOH (0.1%) and then sucked dry by applying suction from a water aspirator. The column was first eluted with 0.1% MeOH in CH_2Cl_2 (60 mL) to selectively remove reaction impurities. A subsequent wash with CH_2Cl_2 (20 mL) containing MeOH (1%) eluted the desired imidazole product (18 mg, 43%). MS (electrospray, + ions): *m/z* = 413 (M+H).
14. Cells were plated in 96-well plates in 50 μl of media at a density of 70,000 cells/well. Compounds were serially diluted into cell media as 2× stocks containing 1% DMSO and 50 μl of each dilution was added to the wells in triplicate. After 48 h of incubation with the compounds, cell survival was assessed by adding 10 μl WST-1 reagent (Roche Applied Science) in each well. After incubation for an additional 3 h, the plates were read in a plate reader (Spectramax 250 from Molecular Devices, Sunnyvale, CA) at 450 nm. The time 0-h for cells with only DMSO (final concentration 0.5%) was also assessed for each plate and was subtracted from the 48-h time points for both treated and untreated data points. The XLfit program (ID Business Solutions Inc., Parsippany, NJ) was used to obtain and calculate the GI50 values for each compound. Each experiment was done a minimum of three times.
15. Briefly 10^6 cells per well were plated into a 24-well plate. The cells were incubated with or without the compound in 0.5% DMSO for 24 h. After incubation, the cells were collected, the DNA was extracted and subjected to gel electrophoresis as described.[16] Camptothecin was used as a positive control in this assay.
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17. A primary screen for Bcl project is based on fluorescence polarization (FP) technology and is well described in the literature.[18] We have established assays for Bcl-2 (Santa Cruz Biotech), Bcl-X_L (R&D Systems), and Bcl-w (R&D Systems) using as the binding partner the 5-carboxyfluorescein-labeled 16-mer peptide tracer Flu-Bak-BH3 (sequence GQVGRQLAIIGDDINR is derived from the Bak BH3 domain). The assays were performed in a 384-well format, in 20 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA, 50 mM NaCl, and 0.05% Pluronic F-68. The final concentration of DMSO in all assays was 10%. The reaction was carried out in a 50 μL volume and the resulting polarization signal was measured at λ_{ex} = 485 nm/λ_{em} = 535 nm using an UltraReader (Tecan) after 2 h incubation of the reaction mixture at room temperature. Validation of the assays was performed using non-labeled Bak-BH3 peptide as a control inhibitor.
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