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(1-Benzimidazolonyl)alanine (Bia): preliminary investigations into a potential tryptophan mimetic†

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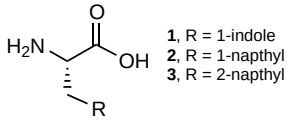
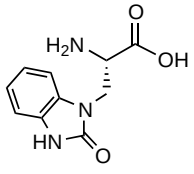
Abstract—The new amino acid (1-benzimidazolonyl)alanine (Bia) was synthesized as a potential biomimetic replacement of tryptophan. The replacement of tryptophan with this amino acid in a truncated analog of p53 showed nearly identical binding to HDM2 as the same length of the natural peptide. Similar peptides containing 1- and 2-naphthylalanine in lieu of Trp showed weaker binding than either of the other peptides. The synthesis of Bia and the binding of the p53 analogs to HDM2 are reported.

Tryptophan (**1**) has been implicated as the key residue for strong and selective molecular interactions in a number of important proteins.[1] However, the reactivity of the indole group[2] makes Trp difficult to use in high-throughput organic synthesis, particularly in the presence of electrophiles. Hence, mimetics of this amino acid may be important to many drug discovery programs.



1, R = 1-indole
2, R = 1-napthyl
3, R = 2-napthyl



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The few acid-stable protecting groups for the indole nitrogen of Trp are, by necessity, not easily removed on resin.[3,4] Consequently, these protecting groups can be difficult to incorporate into high throughput synthetic strategies. A number of unnatural amino acids, e.g. L-1-(**2**) and L-2-naphthylalanine (**3**) (1-Nal and 2-Nal, respectively), have been used to mimic the aromatic π-system of tryptophan while possessing different hydrogen bonding patterns and reactivity.[5] While the Nal derivatives can be considered isosteric models of **1**, their lack of a hydrogen-bond donor may limit their general utility as Trp mimetics. The general utility of other Trp mimetics, such as isotryptophan,[5a] may be limited by their synthetic difficulty. Therefore, we have initiated a program to identify mimetics of tryptophan that retain the biological activity of the natural amino acid, while avoiding some of the chemical incompatibility associated with the indole group.

Based on the gross structural similarities, we envisioned that benzimidazolonylalanine (**4**) might adequately mimic both the steric and stereoelectronic properties of **1**.[6] Compound **4** was also expected to be stable towards electrophilic centers that may be present during synthesis of target molecules.

Commercially available *N*-α-Cbz-L-diaminopropionic acid (**5**)[7] was chosen as the starting point for the synthesis of the Alloc-protected version of **4** (Scheme 1). The acid was esterified without protecting the free amine using standard Fischer esterification conditions.[8] The crude amino ester was then reacted with 2-fluoro-1-nitrobenzene in the presence of TEA in refluxing MeCN to give **6** in good yield.[9] The reduction of the nitro group of **6** with H_2–Pd/C was accomplished with the concomitant removal of the Cbz group. The resulting crude mixture was carried forward without purifica-

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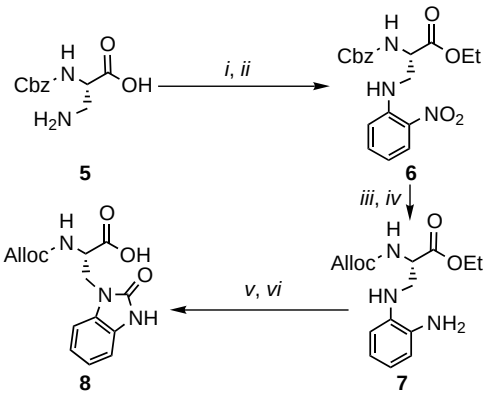

Scheme 1. *Reagents and conditions*: (i) EtOH, MsOH, reflux, (81%); (ii) 2-fluoro-1-nitrobenzene, TEA, MeCN, reflux (87%); (iii) H_2, Pd/C, THF, MeOH; (iv) Alloc-Cl, NHS, TEA, THF (85%, steps (iii) and (iv)); (v) $COCl_2$, pyr, DCM, 0°C (70%); (vi) NaOH, MeOH, H_2O, HCl (93%).

tion. A solution of the crude triamine in THF was added to a premixed solution of allyl chloroformate, TEA and *N*-hydroxysuccinimide in THF, thereby giving compound **7** in good yield.[10]

The reaction of diamine **7** with carbonyldiimidazole and pyridine according to literature procedures gave incomplete and inconsistent yields;[11] whereas, the reaction of **7** with phosgene or triphosgene and pyridine in dry THF gave consistently high yields of the desired benzimidazolonyl compound.[12] Saponification of the ethyl ester with NaOH cleanly gave the desired compound **8**. Using this procedure, Alloc-Bia-OH was isolated in 39% overall yield.[13]

It was important to demonstrate that the structural similarities between **1** and **4** were sufficient for the latter to be biologically relevant. We felt that the ideal way to study the potential biological activity of **4** was to substitute tryptophan for this residue into a molecule of known activity, either a peptide or small molecule, and then measure the activity of the modified compound. It was assumed that the differences in the binding of the native and modified compound would be indicative of the degree to which **4** mimicked the properties of **1**.

The binding of p53,[14] a protein involved in cell cycle regulation, to HDM2, a down regulatory protein, was chosen as the model system for this study. Previous studies have shown that tryptophan was essential to the biological activity of p53.[15] Structurally, the p53 Trp residue was shown to have strong van der Waals and hydrogen bonding interactions with HDM2.[15c] The fact that the p53 Trp residue has been implicated in both hydrophobic and hydrogen bonding interactions with HDM2 makes the study of this system highly attractive. Other reasons for choosing this system were that truncated analogs of p53 were shown to retain HDM2 binding activity,[16] and the native peptide plus analogs containing **4**, as well as other amino acids could be conveniently synthesized.

H-Glu-Thr-Phe-Ser-Asp-Leu-**Xaa**-Lys-Leu-Leu-OH

9, Xaa = Trp
10, Xaa = 1-Nal
11, Xaa = 2-Nal
12, Xaa = Bia
13, Xaa = Ala

A truncated analog of p53 (**9**) was synthesized, along with other analogs having the tryptophan residue substituted with mimetics 1-Nal (**10**), 2-Nal (**11**) and Bia (**12**). The Ala containing analog **13** was also prepared as a negative control. These decapeptides were built-up stepwise on a solid support using appropriately protected amino acids. The peptides were obtained after TFA cleavage followed by purification by preparative HPLC.[17]

The binding of decapeptides **9**–**13** to recombinant HDM2 (residues 1–188) was then studied using a fluorescence polarization (FP) assay.[18–20] Changes in the polarization of the fluorescence correlate to the degree to which the peptide displaced the fluorescence probe (Fig. 1). From this data the peptides IC_{50} against HDM2 was found to be 0.864, 20.3, 7.21, 4.24 and $\gg$100 μM for **9**–**13**, respectively.[21]

The dramatic loss of activity observed when substituting Trp with alanine was not fully anticipated. Peptide **13** was expected to form a helical structure, like that of **9**, since alanine is known to form a helical peptide conformation.[16a] Hence, the secondary structure of peptide **13** may not have been responsible for the observed loss in its binding activity. This result was extremely important because it validated the hypothesis that the activity of the peptide was in part based on the degree to which aa-7 acts as a mimic of tryptophan.

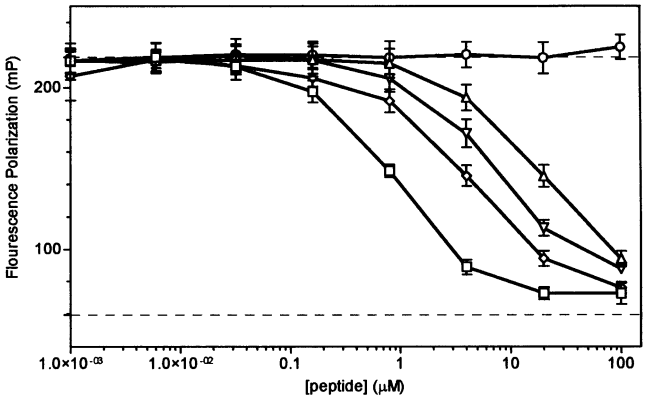

Figure 1. Fluorescence polarization of probe versus peptide binding to HDM2 as a function of [peptide]. Lower and upper dashed lines represent the binding probe (2 nM) alone and in the presence of HDM2 (5 μg/mL), respectively. Probe and HDM2 levels were held constant while [**9**] (-□-), [**10**] (-△-), [**11**] (-▽-), [**12**] (-◇-) and [**13**] (-○-) were varied.

The IC_{50} results suggest that **4** more closely mimics tryptophan than either of the naphthyl derivatives (**2** or **3**); albeit, the differences between the peptides containing **4** and **2** are quite small. This result may be due to two reasons. The 6,5-ring system of **4** may fit the HDM2 binding cavity somewhat better than the 6,6-systems of **2** and **3**, although its not clear how the oxo-group in **4** will be tolerated in general. Alternatively, the N–H proton of the benzimidazolonyl moiety in **4** may be interacting with a hydrogen bond acceptor in the HDM2 binding pocket more strongly than possible for the comparable aryl C–H proton of **2** or **3**. A priori it is easy to suggest that the greater HDM2 binding by **12** compared to **10** and **11** is due to a combination of these two arguments; although, the extent to which the individual factors affect the ligand–protein binding have yet to be determined.

Molecular modeling of decapeptide **14** to HDM2 suggested that the substitution of Bia for Trp leads to little disruption of the protein binding interactions. Of particular interest, the oxo group of the benzimidazolonyl moiety appeared to point towards the solvent and away from the HDM2 backbone. The previously described hydrogen bonds and van der Waals contacts are expected between the benzimidazolonyl moiety of **12** and HDM2 were evident.[15c] Moreover, additional van der Waals contacts are expected between the benzimidazolonyl moiety of **12** and Leu-57, Phe-86, Phe-91 Met-102 and Ile-103.

The results of the decapeptide–HDM2 binding studies clearly suggest that in some cases **4** may be an outstanding mimic for **1**. However, the complexity of protein–substrate interactions preclude any overarching conclusions regarding the suitability of **4** as a general Trp mimetic. Nevertheless, it may be argued that the greater activity of peptide **12** compared to **10** and **11** may in part be due to the ability of compound **4** to mimic both the electrostatic and hydrogen bonding properties of **1**. If a combination of these properties are in fact correct, Bia may prove to be a highly versatile mimic for **1** compared to those amino acids that only act as steric or hydrogen bond mimics. Clearly, further studies into this class of benzimidazolonyl amino acids and their potential in biologically active compounds are warranted.

Acknowledgements

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6. (2*S*)-2-Amino-3-[1-(1,3-dihydro-2*H*-benzimidazol-2-one)]-propionic acid (**4**) abbreviated as (1-benzimidazolonyl)-alanine or Bia.
7. α-Z-DAP is conveniently synthesized from Z-Asn by the method of Zhang, L.-H.; Kauffman, G. S.; Pesti, J. A.; Yin, J. *J. Org. Chem.* **1997**, *62*, 6918–6920.
8. 2-Cbz-L-2,3-diamino-1-propionic acid (10 g, 44 mmol) was suspended in absolute ethanol (200 mL) to which methane sulfonic acid (3.4 mL, 53 mmol) was added. The resulting solution was heated to reflux and stirred for 14 h. The reaction mixture was then carefully neutralized with saturated aq. $NaHCO_3$ then dried in vacuo giving a viscous semi-solid. The residue was partitioned between ethyl acetate and saturated $NaHCO_3$. The organic solution was separated and washed with additional sat. $NaHCO_3$ then brine. The organic solution was dried over $MgSO_4$, filtered and concentrated in vacuo giving the desired product as viscous oil which turned solid upon standing at −20°C. The crude product was used without further purification (9.1 g, 81% yield). Mp = 58–58.5°C. Proton, carbon and IR spectra were consistent with the desired compound. Anal. calcd for $C_{13}H_{18}N_2O_4 \cdot 0.2H_2O$: C, 57.85; H, 6.87; N, 10.38. Found: C, 57.80; H, 6.55; N, 10.31%.
9. α-Z-Dap-OEt (5.0 g, 20 mmol) was dissolved in MeCN (150 mL) to which 2-fluoro-1-nitrobenzene (6.2 mL, 60 mmol) and TEA (8.2 mL, 60 mmol) were added. The resulting solution was heated to reflux and stirred under N_2 for 48 h. The solvent and excess reagents were then removed in vacuo. The dark viscous residue was dis-

solved in ethyl acetate and washed with saturated aq. $NaHCO_3$ (2×) then brine. The resulting organic solution was then dried over $MgSO_4$, filtered and concentrated in vacuo giving an orange-red oil. The crude product was purified by column chromatography (silica gel, 1% MeOH/DCM), giving **6** as a yellow solid (6.7 g, 87% yield). Mp = 78–79°C (dec.). Proton, carbon and IR spectra were consistent with the desired compound. Anal. calcd for $C_{19}H_{21}N_3O_5$: C, 58.91; H, 5.46; N, 10.85. Found: C, 58.95; H, 5.32; N, 10.84%.

10. Compound **6** (1.5 g, 3.9 mmol) was dissolved in 50% THF/CH_3OH (50 mL) to which moist 5% Pd/C (0.50 g) was added. The reaction vessel was sealed, evacuated then back-filled with H_2 at ambient pressure, and the resulting mixture was stirred for 3 h. The resulting mixture was filtered through a pad of Celite giving a reddish solution. The solvent was removed in vacuo giving the crude triamine as a viscous red oil. The crude residue was dissolved in THF (10 mL), then added to a mixture of TEA (2.2 mL, 16 mmol), NHS (0.91 g, 7.9 mmol) and allyl chloroformate (0.56 mL, 4.3 mmol) that had been stirred in THF for 1 h. The resulting mixture was stirred for 4 h then diluted with EtOAc and washed with saturated aq. $NaHCO_3$ (2×) and brine. The resulting organic solution was dried over $MgSO_4$, filtered, and concentrated in vacuo giving a yellow oily residue. The residue was dissolved in 33% EtOAc/hexanes then filtered through a pad of silica-gel giving an amber oil. This residue was crystallized from ether giving **7** as an off-white solid (1.0 g, 85% yield). Mp = 75–75.4°C. Proton, carbon and IR spectra were consistent with the desired compound. Anal. calcd for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67. Found: C, 58.57; H, 6.86; N, 13.55%.
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12. Diamine **7** (3.6 g, 12 mmol) was dissolved in dry THF (30 mL) followed by the addition of pyridine (7.5 mL, 93 mmol). The resulting solution was cooled to 0°C and a 20% solution of phosgene (6.1 mL, 12 mmol) in toluene was slowly added by syringe. The resulting mixture was stirred for 1 h. The reaction was then diluted with EtOAc and washed with 1N HCl (2×) then brine. The resulting organic solution was dried over $MgSO_4$, filtered and concentrated in vacuo giving a tan solid. The crude product was crystallized from Et_2O giving the desired product as an off-white solid (2.7 g, 70% yield). Mp = 135–136°C. Proton, carbon and IR spectra were consistent with the desired compound. Anal. calcd for $C_{16}H_{19}N_3O_5$: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.44; H, 5.57; N, 12.55%.
13. The methyl ester (2.4 g, 7.2 mmol) was suspended in 50% aqueous MeOH (100 mL), to which NaOH (0.64 g, 16 mmol) was added. The resulting solution was stirred for 3 h at room temperature. The reaction mixture was dried in vacuo giving a viscous residue. The crude residue was partitioned between water and 50% EtOAc/Hex. The aqueous phase was separated and acidified with 6N HCl. The immediately formed white precipitate was collected by filtration and washed with excess H_2O and Et_2O then dried in vacuo, giving **8** as a white solid (2.0 g, 93% yield). Mp = 237.7–238°C (dec.). $[\alpha]_D^{27} = -3.5$ (*c* 0.25, 25% AcOH/CH_3OH). Proton, carbon and IR spectra were consistent with the desired compound. Anal. calcd for $C_{14}H_{15}N_3O_5 \cdot 0.1H_2O$: C, 54.76; H, 4.99; N, 13.68. Found: C, 55.04; H, 5.23; N, 13.31%.
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17. Standard Fmoc-Xaa-Wang solid-phase peptide coupling procedures were used for the synthesis of **9**–**13**. All reactions were carried out on an 80 μM scale in 10 mL polyethylene syringes fitted with a fritted polyethylene disk. The peptides were cleaved from the resin using a 15:4:1 solution of TFA/DCM/H_2O (8 mL). All peptides were purified by preparative HPLC following standard procedures. Peptide **9** was isolated after purification in 29% yield (31.1 mg). Anal. calcd for $C_{68}H_{93}F_9N_{12}O_{23} \cdot 4H_2O$: C, 47.59; H, 6.11; N, 10.09. Found: C, 47.72; H, 5.78; N, 10.05%. MS (ES) calcd for $C_{60}H_{92}N_{12}O_{17}^{2+}$: *m*/*z*, 626.7. Found: *m*/*z*, 626.8 (100%). Peptide **10** was isolated after purification in 38% yield (42.3 mg). Anal. calcd for $C_{68}H_{94}N_{11}O_{23}F_9 \cdot 2H_2O$: C, 49.78; H, 6.02; N, 9.39. Found: C, 49.62; H, 5.76; N, 9.53%. MS (ES) calcd for $C_{62}H_{93}N_{11}O_{17}^{2+}$: *m*/*z*, 632.2. Found: *m*/*z*, 632.3 (100%). Peptide **11** was isolated after purification in 35% yield (38.5 mg). Anal. calcd for $C_{68}H_{94}F_9N_{11}O_{23} \cdot 2H_2O$: C, 49.78; H, 6.02; N, 9.39. Found: C, 49.77; H, 5.91; N, 9.59%. MS (ES) calcd for $C_{62}H_{93}N_{11}O_{17}^{2+}$: *m*/*z*, 632.2. Found: *m*/*z*, 632.2 (100%). Peptide **12** was isolated after purification in 25% yield (27.6 mg). Anal. calcd for $C_{65}H_{92}F_9N_{13}O_{24} \cdot 2H_2O$: C, 47.42; H, 5.88; N, 11.05. Found: C, 47.15; H, 5.68; N, 11.31%. MS (ES) calcd for $C_{59}H_{91}N_{13}O_{18}^{2+}$: *m*/*z*, 635.2. Found: *m*/*z*, 635.3 (100%). Peptide **13** was isolated after purification in 23% yield (23.2 mg). Anal. calcd for $C_{58}H_{88}F_9N_{11}O_{23} \cdot 0.5H_2O$: C, 46.84; H, 6.03; N, 10.36. Found: C, 46.50; H, 6.11; N, 10.65%. MS (ES) calcd for $C_{52}H_{87}N_{11}O_{17}^{2+}$: *m*/*z*, 569.2. Found: *m*/*z*, 569.2 (100%).
18. Recombinant HDM2 was expressed in *E. coli* utilizing the GST gene fusion vector pGEX-4T-3 (Amersham Pharmacia). A DNA fragment coding for amino acids 1–188 of HDM2 was generated by PCR amplification of human colon cDNA (Clontec). This DNA fragment was ligated into a pGEX-4T-3 vector modified by addition of a DNA sequence coding for six histidine residues. The resulting recombinant plasmid was transfected into *E.*

coli host BL21 (DE3)pLysS (Promega). The full-length recombinant protein contained amino acids 1–188 of HDM2 flanked by an amino terminal GST fusion and a carboxyl terminal six histidine fusion. Purification was performed by affinity chromatography using Glutathione Sepharose 4B (Amersham Pharmacia).

19. p53/HDM2 binding was measured using a fluorescence polarization assay. FITC-Aha-ETFSDLWKLL was used as a HDM2 binding probe. Inhibitory peptides were resuspended in DMSO at a concentration of 10 mM and stored at −20°C until needed. Binding reactions were carried out in 96-well polypropylene plates (Costar #3365), each well containing 200 μL of 100 mM Tris, 150 mM NaCl, 0.1% Tween 20, 10 mM MgCl, 10 mM dithiothreitol, 2 nM FITC probe and 5 μg/mL GST-HDM2 with and without inhibitory peptides. Reactions were incubated at on a rotary plate shaker for 30 min at 25°C. Following incubation, 50 μL aliquots of each reaction were added to triplicate wells of an opaque black 384 well plate (Nalge Nunc #26455). Plates were read on a LJL Analyst fluorescence detector using an excitation wavelength of 484 nM and an emission wavelength of 530 nM.
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21. No binding of peptide **13** to HDM2 was detected up to [**13**] = 100 μM.