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Smac-Derived Aza-Peptide As an Aminopeptidase-Resistant XIAP BIR3 Antagonist

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Abstract: The peptidic nature of anti-IAPs N-terminus Smac-derived peptides precludes their utilization as potential therapeutic anticancer agents. Recent advances in the development of novel Smac-derived peptidomimetics and non-peptidic molecules with improved anti-IAPs activity and resistance to proteolytic cleavage have been reported and led to a number of candidates that are currently in clinical trials including LCL-161, SM-406/AT-406, GDC-0512/GDC-0917, and birinapant. As an attempt to improve the proteolytic stability of Smac peptides, we developed the Aza-peptide Aza-Ala-Val-Pro-Phe-Tyr-NH₂ (1). Unlike unmodified peptide Ala-Val-Pro-Phe-Tyr-NH₂ (1), analogue (2) exhibited resistance towards proteolytic cleavage by two aminopeptidases; LAP and DPP-IV, while retaining its IAP inhibitory activity. This was due to the altered planar geometry of the P1 residue side chain. Our findings showed that using aza-isosteres of bioactive peptide sequences imbue the residue with imperviousness to proteolysis; underscoring a potential approach for developing a new generation of Smac-derived Aza-peptidomimetics.

Keywords: Aminopeptidases, Apoptosis, Aza-peptides, Caspase 9, IAPs, Smac.

INTRODUCTION

Inhibition of the anti-apoptotic IAPs proteins by short peptides derived from the N-terminal sequence of the pro-apoptotic mitochondrial protein Smac is an emerging strategy for re-engaging apoptosis in resistant cancer cells over-expressing IAPs. Although Smac-derived peptides have been demonstrated to augment the activity of anticancer drugs both in vitro and in vivo [1, 2], their entirely peptidic nature almost certainly precludes their utilization as anticancer therapeutic agents. This is because of the innate and generally impaired bioavailability of peptide ligands mainly due to their instability towards proteolytic cleavage. There have been long standing efforts in developing protease-resistant Smac-derived peptidomimetics (monovalent and bivalent) and non-peptidic analogues by ourselves [3] and many other groups [4-9] that led in some cases to IAP inhibitors that reached clinical trials [10] such as LCL-161 [11,12], SM-406/AT-406 [13,14], GDC-0512/GDC-0917 [15,16], and birinapant [17,18], and thus are drug-like compounds with suitable efficacy, bioavailability and tolerability.

Consequently, we have been interested in enhancing the proteolytic resistance of the N-terminal Smac-derived pentapeptide analogue Ala-Val-Pro-Phe-Tyr-NH₂ (3). With regards to the N-terminus Smac sequence, many studies have shown the crucial role of the P1 Ala residue for its IAP inhibitory activity. According to the reported NMR structure (PDB: 1G3F) [19], P1 Ala is involved in electrostatic interaction with the proximal Glu314 of XIAP BIR3 through the N-terminal amino group (Fig. 1A, PDB: 1G3F [19]). Also, the Ala α-methyl group snugly fits into the hydrophobic pocket formed by W310, interacting with the indole ring of the pocket (Fig. 1B, PDB: 1G3F [19]). Thus, protecting the crucial P1 Ala residue against hydrolysis by aminopeptidases has to be achieved without interference with these key interactions.

Since the acylation of the amino function of the P1 Ala residue of Smac abolished the binding affinity of the Smac peptide to XIAP BIR3 [19], as did N,N-dimethylation [4], such structural modifications cannot be applied for hindering the possible exposure of the N-terminal segment of the peptide to proteolytic cleavage. It is worth mentioning here that only the N-terminally mono-methylated (N-methyl) Smac-derived peptides have preserved IAP inhibitory activity [4], and similar modification is thus currently widely used as an aminopeptidase resistant residue at P1 in the Smac-derived peptidomimetic and in non-peptidic clinical candidates [11-18].

Based on the above discussed SAR, we have investigated the replacement of the N-terminal alanine of peptide (1), with aza-alanine (AzAAla) residue. Many studies demonstrated that the incorporation of aza-amino acids into susceptible peptide cleavage sites in peptides renders them less likely to be hydrolysed and thus enhancing the peptide sequence metabolic stability, biological action and elongating the duration of action [21]. We had anticipated that...
and were purified by RP-HPLC as previously described [3].

**METHODS**

**Peptide Synthesis (MW-SPPS)**

Peptides were synthesized using MW-SPPS on Rink Amide MBHA resin (substitution 0.65 mmol/g, 100-200 mesh) using the general protocol for the microwave assisted solid phase synthesis [MW-SPPS] [3].

**Methods**

**General Protocol for the Microwave Assisted Solid Phase Peptide Synthesis (MW-SPPS)**

Peptides were synthesized using MW-SPPS on Rink Amide MBHA resin (substitution 0.65 mmol/g, 100-200 mesh) and were purified by RP-HPLC as previously described [3].

**Figure 2.** Configuration of L-Ala and AzaAla residues.

modified peptide AzaAla-Val-Pro-Tyr-NH₂ (2) could still make the important electrostatic contacts within the XIAP BIR3 binding pocket, by virtue of the retained and unmodified primary amino function of the AzaAla residue, whilst exhibiting enhanced stability towards aminopeptidases (exemplified mainly by LAP). All this is due to the altered geometry at P1, where the side chain residue of the SP² hybridised aza-amino acid is considered to adopt a planar conformation when compared to the tetrahedral conformation of the natural SP³ hybridised L-amino acid (Fig. 2). This letter thus reports on the synthesis of the Smac-derived Aza-peptide (2), testing its stability towards LAP compared to peptide (1), and an examination of its ability to re-engage caspase-9 activation for apoptosis in cell lysates obtained from MDA-MB-231 breast cancer cells.

**Figure 1.** Smac/DIABLO-derived N-terminal sequence AVPI (shown in the XIAP BIR3 binding pocket, generated from PDB: 1G3F [19]) reconstituted and the shape of the binding cavity is shown with the surface-like representation coloured based on the location of the negative and positive charge residues. Reprinted from Bioorganic and Medicinal Chemistry 2012, 20, Elsawy M. A., Martin L., Elshammas A. C.-G. Wallace, The solid-phase synthesis of Smac/ DIABLO-derived peptides using a ‘Safety-Catch’ resin: Identification of potent XIAP BIR3 antagonists, 5004-5011, Copyright 2013, with permission from Elsevier [3].

**Figure 2.** Configuration of L-Ala and AzaAla residues.

**L-Configuration  Planar geometry**

To a stirred solution of 1-(tert-butyloxycarbonyl)-2-(9H-fluoren-9-methoxycarbonylamino)-1-methylhydrazine (4) To a stirred solution of 1-(tert-butyloxycarbonyl)-2-(9H-fluoren-9-methoxycarbonylamino)-1-methylhydrazine (4) in THF:H₂O (70:70 ml, 3 in a 500 ml two neck round bottom flask was added solid sodium bicarbonate (9.3 g, 110.7 mmol) followed by a solution of Fmoc-C₅H (20.3 g, 60.76 mmol) in THF (140 ml). The latter was added dropwise using a dropping funnel over 30 minutes. The reaction mixture was stirred overnight at room temperature, after which the THF was separated and the aqueous phase was washed with diethyl ether (3 x 25 ml). The combined organic extracts were washed with H₂O, separated, dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to afford yellow viscous oil (18 g) (4) that was directly used in the next step without purification.

1-(tert-Butoxy carbonyl)-2-(9H-fluoren-9-methoxycarbonylamino)-1-methylhydrazine (5)

TFA (50 ml) was added to the yellow oil (4) obtained above- and the reaction mixture was stirred, at room temperature, overnight. The reaction mixture was then concentrated under vacuum in a well-ventilated fume hood to give reddish viscous oil. The residual oil was dissolved in H₂O/ethyl acetate mixture (50:30 ml), stirred at room temperature and sodium bicarbonate solid was added till no effervescence was observed (pH 9). A white precipitate was formed, which was filtered, washed with ethyl acetate and dried under vacuum to afford 12.56 g (84.6%) of 2-(9H-Fluoren-9-methoxycarbonylamino)-1-methylhydrazine (5) as a white powder of melting point 145-150 °C.

**Figure 1.** Smac/DIABLO-derived N-terminal sequence AVPI (dark grey with Ala in black) in the XIAP BIR3 binding pocket (light grey) with only the residues directly interacting with the ligand are visualized; generated from PDB: 1G3F [19].
ried using RP-HPLC (check under general protocols for the MW-SPSS) to obtain AzaAla-Val-Pro-Phe-Tyr-NH2 (2) (8% yield and 98% purity after semi-preparative RP-HPLC purification). ESI-Mass m/z: 618.6 [M+Na]+.

Coupling of 2-(9H-Fluoren-9-methoxycarbonylamo)-1-methylhydrazine (5) to Val-Pro-Phe-Tyr-Resin (7) (Scheme 1, Routes B and C)

For 0.25 mM scale microwave-assisted synthesis of AzaAla-Val-Pro-Phe-Tyr-NH2 (2), 2-(9H-Fluoren-9-methoxycarbonylamino)-1-methylhydrazine (5) (11.5 mmol, 402 mg, 6 equivalent) and carbonyldimidazole (1.5 mmol, 243 mg, 6 equivalent) or p-nitrophenyl chloroformate (1.5 mmol, 302 mg, 6 equivalent) were dissolved in DMF (6 mL). DIEA was used as activator base, where 2-Fmoc-1-methylhydrazine (5) (DIEA/carbonyldimidazole or p-nitrophenyl chloroformate (1/2/1) for one coupling cycle. The coupling reagents were transferred to the microwave reaction vessel for coupling (5) to the resin bound peptide H2N-Val-Pro-Phe-Tyr-Resin (7). The reaction was repeated for 7 cycles (coupling completion was monitored by Kaiser Test and ESI-MS), each coupling cycle was performed for 10 minutes, at 22 Watts, at a temperature of 75 °C.

Aminopeptidase Susceptibility Assays

In 1.5 mL microcentrifuge tube, 940 µL of assay buffer (DPP-IV assay buffer: 25 mM Tris, 140 mM NaCl and 10 mM KCl at pH 8.5) and 10 µL of peptide DMSO solution (0.5 mg/mL EDTA-Na 2 and 2 µg/mL Aprotinin), and was subjected to the Kaiser Test and ESI-MS, in order to assess the N-terminus amino group of resin tethered peptides is required for completion of the reaction.

Caspase-9 Activity Fluorimetric-Assay

MDA-MB-231 cell lysate was prepared by solubilising cells pellet (~5 x 10^5 cells) in 1 mL ice cold lysis buffer (50 mM KCl, 5 mM EGTA, 2 mM MgCl2, 1 mM DTT, 0.2% (w/v) CHAPS and 50 mM HEPES; pH 7.4), supplemented with a protease inhibitor cocktail (50 µg/mL Antipain, 2HCl, 40 µg/mL Bestatin, 6 µg/mL Chymostatin, 7 µg/mL Pepstatin, 50 µg/mL Phosphoramidon, 1 mg/mL Pefabloc SC, 0.5 mg/mL EDTA-Na2 and 2 µg/mL Aprotinin), and was incubated on ice for 15 min. The samples were injected onto an analytical RP-HPLC to check the peptide degradation/stability (using the same analytical RP-HPLC protocol described under general protocols for the MW-SPSS).

Caspase-9 Activity Fluorimetric-Assay

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RESULTS AND DISCUSSION

Synthesis of AzaAla-Val-Pro-Phe-Tyr-NH2 (2)

The Micro-wave-assisted solid phase synthesis (MW-SPSS) of the target peptide included the coupling of 2-(9H-Fluoren-9-methoxycarbonylamino)-1-methylhydrazine (5) to the tetrapeptide sequence Val-Pro-Phe-Tyr-tethered to the resin (7) (Scheme 1), but first the synthesis of (5) was required. Compound (5) was synthesized from 1-tert-butoxycarbonyl-1-methylhydrazine (3), using the previously reported synthesis protocol by Busnel et al. [22] and others [23-25]. The synthesis comprises two steps, an initial Fmoc-protection of the primary amino function of the hydrazine, followed by Boc removal of the secondary amine functionality (Scheme 1). The introduction of the Fmoc-group was accomplished using Fmoc-OSu, in the presence of sodium bicarbonate, to obtain (4), which was then treated with TFA in order to effect the removal of the Boc-group. The final product, N-Fmoc-methylhydrazine (8), was obtained in high yield (85%, based on the starting amount of 4).

The synthesis of the target Aza-peptide (2) was achieved as shown in Route A (Scheme 1) through the activation of (5) by treatment with triphosgene in the presence of N-3-chlorosulfonyl-2-(9H-Fluoren-9-methoxycarbonylamino)-1-methylhydrazine (6) was obtained in high yield (75% based on the amount of starting material 5) and was used directly to acylate the resin-bound H2N-Val-Pro-Phe-Tyr-sequence (7) using microwave-assisted coupling, employing DIEA as activator base (Scheme 1, Route A). Samples of resin were subjected to the Kaiser Test and ESI-MS, in order to assess the extent of coupling (6). It was found that seven repeat coupling cycles (10 minutes/cycle, 22 Watts, 75 °C) were required for completion of the reaction.

Boeglin and Lubell have previously shown that coupling of various Fmoc-aza-amino acids (including (6) to the N-terminus amino group of resin tethered peptides is only complete after 6 h reaction in DCM with DIEA as activator base using conventional SPPS, and in some cases the coupling has to be repeated to ensure completion [24]. Employing microwave-assisted coupling, we managed to significantly reduce the coupling reaction time to 70 minutes (7 cycles x 10 minutes/cycle). Unfortunately, even with this repetitive coupling protocol, (2) could only be obtained as a low purity (~25%) crude product, as estimated by analytical
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Scheme 1. On-resin microwave-assisted coupling of 2-(9H-Fluoren-9-methoxycarbonylamino)-1-methylhydrazine (5) for the synthesis of AzaAla-Val-Pro-Phe-Tyr-NH₂ (2); triphosgene activation (Route A), p-nitrophenyl chloroformate activation (Route B) and carbonyldiimida-
zole activation (Route C).

RP-HPLC (data not shown). This could be due to the infeasibility of purifying the highly active and unstable intermediate (8), which necessitates using it as a crude product for coupling to the resin bound peptide (7). However, the crude Aza-peptide (2) was purified successfully, by semi-preparative RP-HPLC, to give a final product with a yield of only 8%, but of 98% purity (Fig. 3).

Triphosgene is not compatible for use within a micro-
wave-assisted synthesizer, due to it being highly reactive and
with potential for interaction with the amide groups of the
microwave compatible solvents DMF and NMP. Therefore
in attempts to improve the yield and purity of the product
and to automate the synthesis, additional carbonylating
agents were tried for coupling N-Fmoc-methylhydrazine (5)
Figure 3. Panel (A) is ESI-MS data for purified AzaAla-Val-Pro-Phe-Tyr-NH₂ (2) (calcd. m/z: 595, found m/z: 618 [M+Na]+, 100% relative abundance) produced by triphosgene-mediated carbonylation of N-Fmoc-methylhydrazine (5). Panel (B) is the analytical RP-HPLC spectrum for purified aza-peptide (2) of retention time 35 min (peak identified by ESI-MS).

to the N-terminal amino group of the resin-tethered peptide (7), under microwave conditions. We tried both para-nitrophenyl chloroformate (Scheme 1, Route B) and carbonyldiimidazole (Scheme 1, Route C) mediated carbonylation and coupling using the conditions described in the experimental section. Disappointingly, we did not obtain any of the target peptide, even after 7-repeat coupling cycles in case of para-nitrophenyl chloroformate (data not shown) and incomplete coupling was observed when carbonyldiimidazole was used as activator, as indicated by ESI-MS analysis (Fig. 4). Similar observations have been reported by Han et al., in the attempted incorporation of two aza-amino acids into target sequences when using para-nitrophenyl chloroformate and carbonyldiimidazole [26]. This could be due to a low inherent reactivity of the respective aryl carbamates and N-carbonyl imidazoles, or the poor accessibility of these derivatives to the amino functionality of the tethered peptide within the resin matrix, as reported by André et al. [27]. Ultimately, the AzaAla-Val-Pro-Phe-Tyr-NH₂ (2) synthesis was only successful through the triphosgene-mediated carbonylation approach (Scheme 1, route A).

Aminopeptidase Susceptibility

Compound (2) was then tested for its stability against two aminopeptidases; namely LAP and DPP-IV, and in comparison to the wild-type Smac-derived native sequence Ala-Val-Pro-Phe-Tyr-NH₂ (1) and the N-methyl alanil analogue N-Me-Ala-Val-Pro-Phe-Tyr-NH₂ (9).
Figure 4. ESI-MS trace for crude AzaAla-Val-Pro-Phe-Tyr-NH$_2$ (2) produced by carbonyldiimidazole-mediated carbonylation of N-Fmoc-methylhydrazine (5) showing peaks of unreacted Val-Pro-Phe-Tyr-NH$_2$ even after 7-repeated coupling cycles, indicating incomplete coupling.

Figure 5. RP-HPLC profiles of (a), Smac-derived pentapeptide analogue (1) incubated with LAP, for 30 min, at 37°C (b), AzaAla-analogue (2) and (c), N-Me-analogue (9) incubated with LAP, for 6 h, at 37°C.

LAP is an aminopeptidase that can cleave N-terminal Ala residues from peptides [28]. As anticipated, the P1 Ala residue was completely hydrolysed from the native peptide sequence (1) in 30 minutes, to give the truncated hydrolysis product Val-Pro-Phe-Tyr-NH$_2$, as shown by RP-HPLC and ESI-MS (Fig. 5 (a)). Strikingly, the AzaAla-analogue (2) was completely resistant to hydrolysis after 6 h of exposure to LAP (Fig. 5 (b)), as was the case with the N-methyl alanyl protected analogue (9) (Fig. 5 (c)). These results suggest that designing Smac-derived analogues with a planar SP$^2$ hybridised Aza-alanyl modified N-terminal will improve the proteolytic resistance for those candidates, making them as resistant as the widely used N-methyl alanyl congeners.

Interestingly, the Smac-derived pentapeptide analogue (1), AzaAla-analogue (2) and N-Me-Ala-analogue (9) proved to be resistant to aminopeptidase DPP-IV after 6 h exposure to the enzyme, as observed by RP-HPLC and ESI-MS (data not shown). Although DPP-IV is an N-terminal dipeptidyl
peptidase that can cleave a dipeptide unit in ‘one bite’ from longer peptides with Val at the P2 position [29], it might have been expected that all the three Smac-derived peptides (1), (2) and (9) would have been subject to degradation by this peptidase (they all have Val at P2). However, the presence of Pro at P3 makes all of the N-terminal Smac-derived sequences intrinsically resistant to DPP-IV, since peptides with Pro at P3 are known to function as DPP-IV inhibitors [30].

**IAP Antagonistic Activity**

Subsequently, the antagonistic activity of AzaAla-analogue (2) against XIAP BIR3 was assessed by caspase-9 activity recovery assay. In this assay, the ability of the Smac-derived analogues to reactivate caspase-9 in the presence of its inhibitor rhXIAP BIR3 (500 nM) was determined by the increase in caspase-9 activity that they induced. Remarkably, the Aza-peptide analogue (2) retained the ability to antagonise the inhibitory effect of XIAP BIR3 on caspase-9 activation as effective as the wild-type (1) and the N-Methyl-Ala-sequence (9) (Fig. 6). In essence, (2) was able to restore 36.2 ± 4.9% of caspase-9 activity in the presence of 500 nM rhXIAP BIR3, at 1 μM compared to 43.1 ± 5.2% for the wild sequence (1) and 51.4 ± 7.1% for the N-Me-Ala-analogue (9), at the same concentration (mean ± SD, n=3) (Fig. 6). These results demonstrate that the planar conformation of the SP 2 hybridised AzaAla residue did not dramatically mis-locate the side-chain methyl group at P1 permitting the essential hydrophobic interaction with the indole ring of the W310 residue of XIAP BIR3 (Fig. 1[3]). Moreover, analogue (2) can still form the essential electrostatic interaction with the proximal Glu314 of XIAP BIR3, owing to the retained and unmodified N-terminal primary amino function of the AzaAla residue (Fig. 1[3]).

**CONCLUSION**

In the light of the above results, the modified peptide AzaAla-Val-Pro-Phe-Tyr-NH2 (2) was a successful bio-isostere for the N-terminal Smac-derived pentapeptide parent sequence Ala-Val-Pro-Phe-Tyr-NH2 (1). The Aza-peptide (2) is an equipotent analogue for (1) as a XIAP BIR3 inhibitor, whilst exhibiting enhanced stability towards aminopeptidases, exemplified mainly by LAP, owing to the planar geometry of the Aza-Ala residue at P1. Although the reported Aza-peptide (2) is not a therapeutic candidate per se, due to lack of cell permeability, it represents an important lead compound for the development of a new class of anti-cancer Smac-derived Aza-peptidomimetic therapeutic candidates. Our current studies focus on developing libraries of these novel candidates, where the N-terminal Ala residue at P1 is occupied with the Aza-Ala isostere instead of the widely used N-methyl substituted Ala, with P2, 3 and 4 residues modified as previously reported by ourselves [3] and others [4]. These structural modifications of the amin Smac-derived Aza-peptidomimetics are expected to improve the efficacy and proteolytic stability of these therapeutic candidates.

**LIST OF ABBREVIATIONS**

IAPs = Inhibitor of Apoptosis Proteins
Smac = Second mitochondria-derived activator of caspases
XIAP BIR3 = X-linked Inhibitor of Apoptosis Protein-Baculovirus IAP Repeat-3 domain
SAR = Structure Activity Relationship
LAP = Leucine Aminopeptidase
DPP-IV = Dipeptidyl Peptidase-IV

**CONFLICT OF INTEREST**

The authors declare there is no conflict of interest for this research work.

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**REFERENCES**


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Figure 6. Caspase-9 activation data for the N-terminal Smac-derived peptide library.


