Peptide Coupling Challenges on Route to Aza-Pipecolyl Smac Mimetic

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Introduction

Azopeptides are reactive peptide analogs bearing a diazo dicarbonyl moiety as a dehydro *N*-(acyl)amino acid surrogate [1]. Azopeptides have been employed in the synthesis of peptide analogs bearing semicarbazides as amino amide surrogates, so called azapeptides [2]. For example, the Diels-Alder reaction of a diazine residue with cyclopentadiene provided aza-methano-pipecolyl peptide **5** (Scheme 1) [1]. In the context of research towards anticancer drugs, we perceive the aza-methano-pipecolyl structure as a constrained valine analog for insertion into a mimic of the *N*-terminal AVPI binding motif of the second mitochondria-derived activator of caspases (Smac). Coupling to the aza-methano-pipecolyl residue proved however challenging during efforts to prepare Smac mimetic **4**.

Results and Discussion

The AVPI peptide amide **1** has served as a lead for the development of Smac mimetics that bind the inhibitor of apoptosis protein-3 and induce apoptosis (Figure 1) [3-6]. Considering the turn conformation adopted by this peptide on receptor binding, constrained analogs such as indolizidinone amino acids have been used to rigidify the Val-Pro dipeptide to enhance potency (e.g., **2**) [7-9]. Although such bicyclic amino acid analogs represent an important class of Smac mimetics, their multiple step synthesis has restricted analog development. Employing aza-amino acyl proline analogs as indolizidinone amino acid surrogates, azapeptide smac mimetics (e.g., **3**) have been developed that induce cell death by a caspase-9 mediated apoptotic pathway [6]. In the interest to further restrict the conformation of the aza-amino acid residue, aza-methano-pipecolate was pursued as a rigid valine analog in smac mimetic **4**.

Fig. 1. AVPI-NH₂ and peptide mimetic activators of caspase-9.

Hydrogenation aza-Diels-Alder adduct **5** removed the Cbz group and reduced the olefin to provide bicyclic semicarbazide **6** (Scheme 1). The acylation of semicarbazide **6** with Boc-Ala proved particularly challenging presumably because of the combination of steric hindrance and electronic deactivation. A variety of coupling condition was examined to achieve acylation, albeit with minimal success. For example, various coupling conditions failed to afford the Boc protected aza-tripeptide amide **7**: TBTU/HOBT [10], EDCI/HOBT [11], and DIC/AtOH [12]. Moreover, attempts were unsuccessful using the mixed anhydride generated from Boc-Ala, *iso*-butyl chloroformate and *N*-methylmorpholine [13,14]. On the other hand, PyBOP and di-*iso*-propyl ethyl amine (DIEA) proved effective in the reaction of semicarbazide **6** with Boc-Ala, and gave tripeptide amide **7** in 58% yield [15]. Removal of the Boc group with 25% TFA in dichloromethane, salt exchange using 1N HCl for 30 min and freeze-drying afforded hydrochloride salt **4** (HRMS m/z calculated for $\text{C}_{27}\text{H}_{34}\text{N}_5\text{O}_3$ $[\text{M}+\text{H}]^+$ 476.2656; found 476.2663).

Scheme 1. Synthesis of aza-methano-pipecolyl Smac mimetic 4.

In conclusion, we have disclosed the incorporation of a novel constrained valine into an azapeptide Smac mimetic analog. Application of PyBOP surmounted difficulties in coupling to the bulky and electron deficient aza-methano-pipecolyl residue. Smac mimetic analog **4** will be examined for bioactivity to probe further structure-activity relationships at the AVPI binding site towards the development of improved therapy for treating cancer.

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References

- Chingle, R., Lubell, W.D. *Submitted*.
- Proulx, C., Sabatino, D., Hopewell, R., Spiegel, J., García Ramos, Y., Lubell, W.D. *Future Med. Chem.* **3**, 1139-1164 (2011), <http://dx.doi.org/10.1021/jm300557t>
- Kerr, J.F., Wyllie, A.H., Currie, A.R. *Br. J. Cancer* **26**, 239-257 (1972), <http://dx.doi.org/10.1038/bjc.1972.33>
- Fadeel, B., Orrenius, S. *J. Intern. Med.* **258**, 479-517 (2005), <http://dx.doi.org/10.1111/j.1365-2796.2005.01570.x>
- Deveraux, Q.L., Reed, J.C. *Genes Dev.* **13**, 239-252 (1999), <http://dx.doi.org/10.1101/gad.13.3.239>
- Bourguet, C.B., Boulay, P.-L., Claing, A., Lubell, W.D. *Bioorg. Med. Chem. Lett.* **24**, 3361-3365 (2014), <http://dx.doi.org/10.1016/j.bmcl.2014.05.095>
- Sun, H., Nikolovska-Coleska, Z., Yang, C.-Y., Xu, L., Tomita, Y., Krajewski, K., Roller, P.P., Wang, S. *J. Med. Chem.* **47**, 4147-4150 (2004), <http://dx.doi.org/10.1021/jm0499108>
- Sun, H., Nikolovska-Coleska, Z., Yang, C.-Y., Xu, L., Liu, M., Tomita, Y., Pan, H., Yoshioka, Y., Krajewski, K., Roller, P.P. *J. Am. Chem. Soc.* **126**, 16686-16687 (2004), <http://dx.doi.org/10.1021/ja047438>
- Khashper, A., Lubell, W.D. *Org. Biomol. Chem.* **12**, 5052-5070 (2014), <http://dx.doi.org/10.1039/C4OB00777H>
- Bourguet, C.B., Sabatino, D., Lubell, W.D. *Peptide Science* **90**, 824-831 (2008), <http://dx.doi.org/10.1002/bip.21103>
- Chakraborty, T.K., Ghosh, A., Sankar, A.R., Kunwar, A.C. *Tetrahedron Lett.* **43**, 5551-5554 (2002), [http://dx.doi.org/10.1016/S0040-4039\(02\)01141-3](http://dx.doi.org/10.1016/S0040-4039(02)01141-3)
- Hemmerlin, C., Cung, M.T., Boussard, G. *Tetrahedron Lett.* **42**, 5009-5012 (2001), [http://dx.doi.org/10.1016/S0040-4039\(01\)00917-0](http://dx.doi.org/10.1016/S0040-4039(01)00917-0)
- Anderson, G.W., Zimmerman, J.E., Callahan, F.M. *J. Am. Chem. Soc.* **89**, 5012-5017 (1967), <http://dx.doi.org/10.1021/ja00995a032>
- Zaitsev, A.B., Adolfsson, H. *Org. Lett.* **8**, 5129-5132 (2006), <http://dx.doi.org/10.1021/ol062227q>
- Coste, J., Le-Nguyen, D., Castro, B. *Tetrahedron Lett.* **31**, 205-208 (1990), [http://dx.doi.org/10.1016/S0040-4039\(00\)94371-5](http://dx.doi.org/10.1016/S0040-4039(00)94371-5)