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Islet Amyloid Polypeptide (IAPP)

- type-II diabetes

novel oligoamide helix mimetics

- backbone H-bonding
- side-chain lipophilicity
- in vitro and in vivo activity

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Amphiphilic oligoamide α -helix peptidomimetics inhibit islet amyloid polypeptide aggregation[†]

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ABSTRACT

The abnormal deposition of proteins as insoluble plaques is associated with many diseases, including Alzheimer's, Parkinson's and type II diabetes. There is an unmet need for synthetic agents that are able to mediate particular steps in the pathway between soluble proteins in their native unfolded state and their insoluble β -sheet rich aggregates. We have previously reported classes of α -helix mimetic that agonise or antagonise islet amyloid polypeptide aggregation, depending on the presence of a lipid bilayer. Here we investigate a novel mixed benzamide and pyridylamide scaffold that gives improved activity and investigate the role of side-chain polarity, backbone rigidity and curvature in inhibiting lipid-catalyzed fibrillization.

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The deposition of protein aggregates (amyloidosis) is a characteristic of the pathology of many disease classes including Alzheimer's, Parkinson's, prion diseases and type II diabetes.^{1–3} Typically, misfolding of the native protein leads to extended and highly stable β -sheet oligomers, which undergo further aggregation to ultimately form proteolysis-resistant fibrils. However, the precise oligomeric intermediates involved in amyloidosis, and the mechanism by which they aggregate remains an unsolved problem. In the case of type II diabetes, the protein that undergoes misfolding is the intrinsically disordered 37-residue hormonal peptide islet amyloid polypeptide (IAPP). We have previously described the role played by membrane-bound aggregates of α -helical intermediate conformers *en-route* to forming β -oligomers from the intrinsically disordered native structure of IAPP (Figure 1A).⁴ This provides an opportunity to retard amyloidosis by stabilizing or disrupting these α -helical intermediates, thus preventing progression of the disease pathology.

The oligobenzamide and oligopyridylamide classes of α -helix mimic provide a scaffold from which the phenol ether substituents accurately reproduce the spatial and angular projection of the side-chains at residues *i*, *i*+3/4 and *i*+7 of a natural α -helix (Figure 1B).^{5–12} The transient α -helical domain of IAPP is known to contain multiple cationic side-chains, hence we have utilized benzamide and pyridylamide homo-oligomers with complementary polyanionic carboxylate side-chains for the

Figure 1. (A) α -helical IAPP conformers – putative intermediates in amyloidosis and helix mimetic as potential therapeutic agents. Colours indicate complementary side-chains; (B) study design – investigating the role of backbone H-bonding and lipophilic side-chain structure for inhibition of fibrillization. Colours indicate charged (blue) and hydrophobic (grey) side-chains.

[†] This paper is dedicated to the memory of Harry H. Wasserman (1920–2013).

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inhibition of IAPP aggregation.^{13–16} However, the use of exclusively anionic side-chains is an over-simplification: the relevant cationic residues of IAPP (e.g. Arg11 and His18) occupy the (*i*) and (*i*+7) positions, with the intercalated (*i*+3/4) residues either lipophilic (Phe15) or neutral and polar (Asn14).¹⁵ Recently we described the incorporation of a lipophilic side-chain in the central (*i*+3/4) position, leading to increased potency in the oligopyridylamide series.¹⁷ Here we outline the synthesis of a series of oligobenzamides and mixed benzamide-pyridylamide trimeric helix mimetics possessing amphiphilic side-chain substituents (Figure 1B). We seek to understand the role played by the backbone pyridine and lipophilic side-chain in modulating the inhibition of amyloidosis.

Nitroaryl ester **1** was used as the starting point for the synthesis of the trimeric oligoamide helix mimetics (Scheme 1). Ester hydrolysis under basic conditions afforded carboxylic acid **2**, while reduction with hydrogen and palladium on carbon furnished the corresponding aniline **3**,¹⁸ which was coupled with a number of aryl ethers¹⁹ using Mukaiyama's reagent,²⁰ affording benzamide dimers **4–6** in modest yields. Hydrogenation of the aromatic nitro groups with palladium on carbon generated the required anilines **7–9** in yields of 76–99%. The protected trimeric benzamide- and pyridylamide-helix mimetics **11–15** were obtained by treatment of anilines **7–9** with benzoic acid **2** or picolinic acid **10**, employing Mukaiyama's reagent to effect the coupling (Scheme 2). Yields were generally good (55–85% for **12**, **13** and **15**), although **11** and **14** was obtained in a more modest 32% and 52% yield respectively. It is likely that this reduced reactivity is due to the increased steric bulk of the isopropyl ether adjacent to the aniline inhibiting its participation in the coupling reaction. Global deprotection using trifluoroacetic acid revealed terminal carboxylic acid side-chains, and thus furnished a focused library of amphiphilic helix mimetics **16–20**.

Scheme 1. Two-residue mimic synthesis. *Reagents and conditions:* (i) LiOH, THF/H₂O, RT, 2 h; (ii) H₂, Pd/C, CH₂Cl₂/EtOAc, RT, 16 h; (iii) Mukaiyama's reagent, Et₃N, CH₂Cl₂, reflux, 2 d.

Deprotection of benzamides **16–18** was generally achieved in slightly higher yield (80–91%) than the pyridylamides **19** and **20** (65% and 72% respectively).

Scheme 2. Mimetic **16–20** synthesis. *Reagents and conditions:* (i) Mukaiyama's reagent, Et₃N, CH₂Cl₂, reflux, 2 d (ii) TFA, CH₂Cl₂, RT, 3–5 h

Figure 2. (A) Typical curve for IAPP fibrillization assay; (B) inhibition of IAPP fibrillization by helix mimetics **16-20**. Assay: IAPP (10 μ M), mimetic (100 μ M), DOPG:DOPC, 1:1 (630 μ M) and ThT (5 μ M), buffer (pH 7.4); (C) CTB viability assay of mimetics **16-19**; 10 μ M, 1:1 compound:*h*IAPP, viability measured at 72 h; (D) dose-dependence of CTB assay as above for **16** and **19**; (E) intramolecular hydrogen bonding stabilizes the mimic in the desired conformation, leading to greater efficacy relative to its benzamide congener. Lipophilicity and steric factors in the ether substituent are also vital in ensuring biological activity.

We have previously described the crucial role played by lipid bilayers in catalyzing the deposition of amyloid, and demonstrated that while appropriate helix mimics lead to reduced fibrillization in the presence of lipids, the same compounds lead to *increased* fibrillization in their absence.^{4,13,21} In this study we chose to investigate the effects of the targeted library of mimics **16-20** purely in the presence of a lipid bilayer, to assess their *in vitro* inhibition of amyloid formation (Figure 2A). Compounds **16-20** were added to a solution of *h*IAPP, a DOPG:DOPC phospholipid bilayer formed *in situ* and thioflavin-T (ThT).¹³ ThT undergoes selective fluorescence enhancement in the presence of amyloid, and therefore the fluorescence intensity is used as an indirect measure of the degree of fibrillization. The effectiveness of **16-20** can therefore be described in terms of the time required for 50% conversion of *h*IAPP to amyloid (t_{50}) expressed relative to the same value obtained in the absence of the inhibitor ($t_{50,control}$). All compounds tested displayed a measurable inhibitory effect. The mimics containing a pyridylamide (**19** and **20**) caused a significantly longer delay before the onset of fibrillization than the oligobenzamides (**16-18**), suggesting that the structural preorganization caused by the formation of an intramolecular hydrogen bond in the pyridylamides is advantageous in terms of their inhibitory effect. For example, benzamide **16** gave the weakest inhibition of the compounds tested ($t_{50}/t_{50,control} = 1.3$), while the corresponding pyridylamide gave the strongest ($t_{50}/t_{50,control} = 2.4$). There was also significant variation in the inhibition of IAPP aggregation between compounds of the same class. Within the benzamide series isobutyl **17** gave the strongest inhibition and isopropyl **16** the weakest. However, within the pyridylamide series the same substituents exhibited the opposite trend, with isopropyl pyridylamide **19** giving the strongest inhibition and isobutyl **18** the weakest.

The *in cellulo* activity of helix mimetics **16-19** was also measured in a CellTiter-Blue® (CTB) assay (Figure 2C). Cell survival was determined by fluorescent readout after 72 h in pancreatic INS-1 cells exposed to *h*IAPP in the presence and absence of helix mimetics, and thus the ability of compounds to protect cells from amyloid-induced cell apoptosis was determined. All compounds tested conferred some protection, resulting in increased cell viability relative to the control. Pyridylamide **19** gave the greatest protection with a cell viability of 79% (vs. 59% for control) after 72 h, and in good agreement with the ThT assay, where this was also the most effective. The CTB assay was also employed to examine dose-dependence in cell viability. Helix mimetics **16** and **19** were selected to allow

direct comparison of the effect of the bifurcated hydrogen bond (Figure 2D), with both compounds exhibiting the expected dose-response curve. The superiority of **19** over **16** was observed across the complete range of concentrations.

Pyridylamide **19** gave the most impressive results in both assays, giving strong evidence for the importance of the N-H...N hydrogen bond in pre-organising the mimetic into a functional conformation, thus reducing the entropic penalty for binding to helical IAPP intermediates (Figure 2E). We and others have previously noted that this intramolecular hydrogen bond leads to increased curvature in the mimetic backbone,^{18,22-24} in this case potentially improving its binding interaction by providing a better shape match with the surface of IAPP helices. The role of the alkyl ether substituent is less clear: while both isopropyl compounds **16** and **19** gave the greatest increases in cell viability, benzamide **16** gave only weak inhibition of fibrillization *in vitro*. It is likely that the significance of substitution at this position is due to changes in: (i) the binding interaction with IAPP due to hydrophobic interactions and sterically-driven changes to ground-state conformation; and (ii) the partitioning of the mimetics between the membrane and bulk solution due to hydrophobicity. We have previously shown that alkyl substituents in a related system undergo structurally-consequential hydrophobic effects in the solid state.¹⁹

In summary, we have synthesized a focussed library of oligobenzamide and pyridylamide helix mimetics. These were tested against both *in vitro* fibrillization and cell viability assays. Pyridylamides generally performed better, and this is postulated to be due to the increased molecular rigidity of the mimics due to the formation of a structural hydrogen bond, which reduces the entropic penalty for binding IAPP helices. Lipophilic substituents were introduced at the central ether linkage and their precise structure was found to have a significant influence on efficacy. It is anticipated that these results will inform future development of small-molecule peptidomimetic inhibitors of IAPP aggregation, and provide a tool for the treatment of amyloid diseases.

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References and notes

- 1 C. M. Dobson, *Nature*, **2003**, *426*, 884–890.
- 2 I. W. Hamley, *Angew. Chem. Int. Ed.*, **2007**, *46*, 8128–8147.
- 3 S.-Y. Ow and D. E. Dunstan, *Protein Sci.*, **2014**, *23*, 1315–1331.
- 4 J. D. Knight and A. D. Miranker, *J. Mol. Biol.*, **2004**, *341*, 1175–1187.
- 5 J. T. Ernst, J. Becerril, H. S. Park, H. Yin and A. D. Hamilton, *Angew. Chem. Int. Ed.*, **2003**, *42*, 535–539.
- 6 L. A. Estroff, C. D. Incarvito and A. D. Hamilton, *J. Am. Chem. Soc.*, **2004**, *126*, 2–3.
- 7 O. V. Kulikov, S. Thompson, H. Xu, C. D. Incarvito, R. T. W. Scott, I. Saraogi, L. Nevola and A. D. Hamilton, *Eur. J. Org. Chem.*, **2013**, *2013*, 3433–3445.
- 8 I. Saraogi and A. D. Hamilton, *Chem. Soc. Rev.*, **2009**, *38*, 1726–1743.
- 9 V. Azzarito, K. Long, N. S. Murphy and A. J. Wilson, *Nat. Chem.*, **2013**, *5*, 161–173.
- 10 M. K. P. Jayatunga, S. Thompson and A. D. Hamilton, *Bioorg. Med. Chem. Lett.*, **2014**, *24*, 717–724.
- 11 S. Marimganti, M. N. Cheemala and J. Ahn, *Org. Lett.*, **2009**, *11*, 4418–4421.
- 12 S. Thompson, R. Vallinayagam, M. J. Adler, R. T. W. Scott and A. D. Hamilton, *Tetrahedron*, **2012**, *68*, 4501–4505.
- 13 I. Saraogi, J. A. Hebda, J. Becerril, L. A. Estroff, A. D. Miranker and A. D. Hamilton, *Angew. Chem. Int. Ed.*, **2010**, *49*, 736–739.
- 14 S. Kumar and A. D. Miranker, *Chem. Commun.*, **2013**, *49*, 4749–4751.
- 15 J. A. Hebda, I. Saraogi, M. Magzoub, A. D. Hamilton and A. D. Miranker, *Chem. Biol.*, **2009**, *16*, 943–950.
- 16 S. Kumar, M. A. Brown, A. Nath and A. D. Miranker, *Chem. Biol.*, **2014**, *21*, 775–781.
- 17 S. Kumar, D. E. Schlamadinger, M. A. Brown, J. M. Dunn, B. Mercado, J. A. Hebda, I. Saraogi, E. Rhoades, A. D. Hamilton and A. Miranker, *Chem. Biol.*, **2015**, *Accepted manuscript*.
- 18 I. Saraogi, C. D. Incarvito and A. D. Hamilton, *Angew. Chem. Int. Ed.*, **2008**, *47*, 9691–9694.
- 19 O. V. Kulikov, C. Incarvito and A. D. Hamilton, *Tetrahedron Lett.*, **2011**, *52*, 3705–3709.
- 20 T. Mukaiyama, M. Usui, E. Shimada and K. Saigo, *Chem. Lett.*, **1975**, 1045–1048.
- 21 J. A. Hebda, M. Magzoub and A. D. Miranker, *Protein Sci.*, **2014**, *23*, 1341–1348.
- 22 V. Azzarito, P. Prabhakaran, A. I. Bartlett, N. S. Murphy, M. J. Hardie, C. A. Kilner, T. A. Edwards, S. L. Warriner and A. J. Wilson, *Org. Biomol. Chem.*, **2012**, *10*, 6469–72.
- 23 W. Q. Ong, H. Zhao, Z. Du, J. Z. Y. Yeh, C. Ren, L. Z. W. Tan, K. Zhang and H. Zeng, *Chem. Commun.*, **2011**, *47*, 6416–8.
- 24 J. L. Yap, X. Cao, K. Vanommeslaeghe, K.-Y. Jung, C. Peddaboina, P. T. Wilder, A. Nan, A. D. MacKerell, W. R. Smythe and S. Fletcher, *Org. Biomol. Chem.*, **2012**, *10*, 2928–33.