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Adamantane and Nipecotic Acid Derivatives as Novel β-Turn Mimics

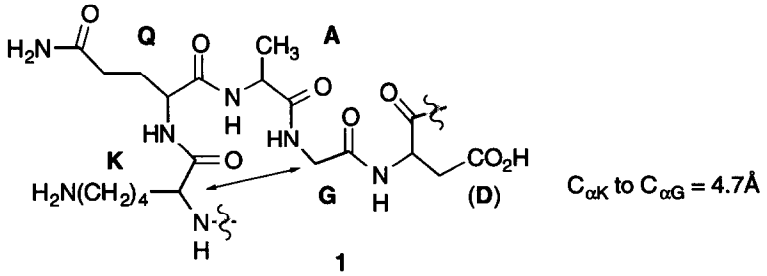
William J. Hoekstra, Jeffery B. Press, Mary Pat Bonner,
Patricia Andrade-Gordon and Patricia M. Keane
The R. W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania 19477

Kathleen A. Durkin and Dennis C. Liotta
Emory University, Atlanta, Georgia 30322

Kevin H. Mayo
Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Abstract: 1,3-Adamantanediamine and nipecotic acid were used as scaffolds in designing β-turn peptide mimetic derivatives **3** and **5**, respectively, targeted as fibrinogen-GPIIB/IIIA antagonists. The design focused on the 406-409 KQAG region of the γ-chain of fibrinogen which appears as a β-turn in solution NMR structures of Fg-γ385-411.

As part of our program in cardiovascular agents, we became interested in novel fibrinogen receptor antagonists which might have utility as platelet aggregation inhibitors. While several laboratories are studying RGD mimetics designed from segments of fibrinogen's α-chain, we are unaware of any examples of γ-chain mimics under investigation.[1] This is especially surprising since the RGD sequence is present in a large number of blood adhesive proteins (hence RGD mimetics are potentially unselective), while the γ-chain is unique to fibrinogen and thus offers the possibility of improved selectivity.[2] Since the 385-411 sequence of the γ-chain (derived from BrCN degradation of fibrinogen) has good inhibition of fibrinogen binding to immobilized GPIIb/IIIa (IC_{50} = 3.4 μM) as well as platelet antiaggregatory activity, we undertook a research program investigating structural mimics of this region. A thorough NMR investigation of γ385–411 at pH 3.5 and 6.0 in 20% TFE/water solution gave rise to families of solution structures with secondary structural features in the 400-411 binding domain. Several families of these structures show a tight turn region encompassing KQAG (406–409) without the commonly observed *i* to *i+3* hydrogen bond. The average Cα–Cα distances between the *i* and *i*+3 residues in these turn regions ranged between 4.2 and 4.7 Å, suggesting a type II β-turn (**1**).[3,4] In this Letter, we describe one aspect of our research project that addressed the design and synthesis of mimics of this KQAG β-turn region.[5]

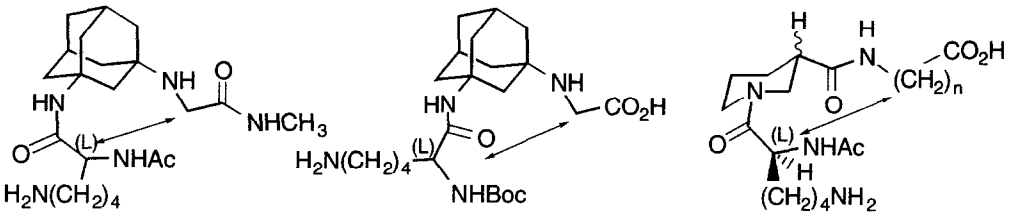


1

KQAG(D) region of γ-chain of fibrinogen [406-409 (410)]

One of our design goals was to utilize commercially available compounds with requisite functionality as scaffolding so that additional side chains could be conveniently introduced as necessary. The 3-aminoadamantane unit of model compound **2** was initially proposed as a "QA" dipeptide replacement. In addition to the commercial availability of precursor 1,3-adamantanediamine, preliminary calculations using SYBYL predicted the $C_{\alpha K}$ to $C_{\alpha G}$ distance to be in the range of 4.5–4.9Å (observed in several conformer populations during a systematic search), agreeing well with the experimentally determined 4.7Å and close to that of a Type II β-turn. Furthermore, it was determined from the γ400-411 peptide structure-activity relationship that amino acid replacement of K^{406} and D^{410} greatly reduced the biological activity of γ400-411.[2,6] As an additional design requirement, we decided to incorporate the K residue as well as the side chain of the flanking D residue into our mimetics. The Q and A corner residues of the peptide turn may function as turn-inducing residues which enforce a conformational constraint securing the spatial orientation of the binding-critical K and D side chains. Presumably, this conformational constraint would be provided by the synthetic scaffold.

After synthesis of a few adamantane derivatives, we were gratified to find that adamantane **3**[7] afforded some activity in our biochemical assays (44% inhibition of fibrinogen binding @ 50 μM, 40% inhibition of platelet aggregation @ 20 μM).[8] Prior to undertaking an extensive synthesis program, **3** was examined rigorously using modelling techniques which included molecular dynamics simulations (heating to 900°K over 50 picoseconds). A $C_{\alpha K}$ to $C_{\alpha G}$ distance of 7.2–7.7Å, considerably longer than our target distance of 4.7Å, was calculated for a number of conformational families. This elongated distance may arise from side chain flexibility and may be the cause of the relatively low potency of **3**.



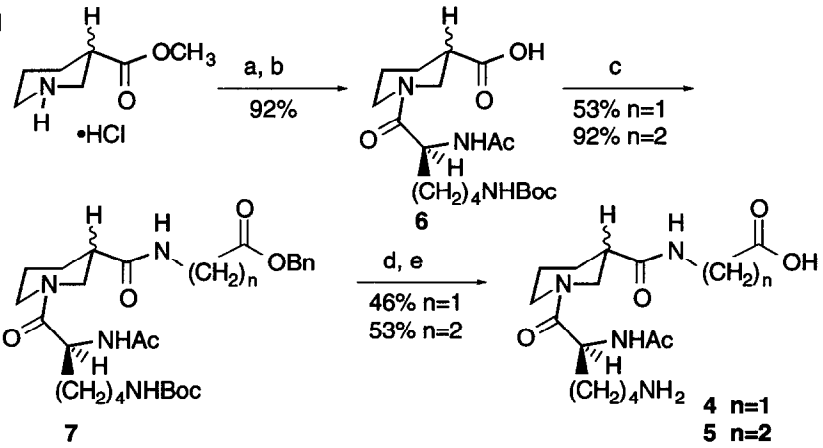
2, avg. $C_{\alpha K}$ to $C_{\alpha G}$ = 4.9Å **3**, avg. $C_{\alpha K}$ to $C_{\alpha G}$ = 7.7Å **4**, n = 1 **5**, n = 2

We next examined eight bis-functionalized, commercially available scaffolds using molecular dynamics simulations in an effort to find an improved fit of these potential mimetics to the NMR-derived KQAG structure. While maintaining the L-configuration for lysine, examination of 1,8-naphthalenediamine, isomannide, 5-aminophthalimide, 2-aminoperimidine, 9-amino-1-fluorenecarboxylic acid, tetrahydropyrimidone, pseudopelletierine and nipecotic acid derivatives led to the prediction that the 3-*S*-(+)-diastereomer of nipecotamide **4** had the best fit with a calculated $C_{\alpha K}$ to $C_{\alpha G}$ distance of 5.7Å and a very narrow conformer population distribution of 5.0-7.0 Å.

Compound **4** meets our design goals of creating a β-turn mimic from commercially available materials (Scheme 1), but produces only modest biological activity of 24% inhibition of fibrinogen binding at 50 μM. Assuming the β-turn of **4** in fact mimics the γ406-409 region as predicted by our computer models, the diminished activity of **4** is suggestive of the importance of precise positioning of the D^{410} side chain in the γ-chain of fibrinogen (*vide supra*). Extension of the C-3 side chain of nipecotamide **4** by one methylene unit (β-

alanine) provided compound **5** (**5** is a 1:1 mixture of diastereomers by NMR and HPLC), which showed dramatic improvement in biological activity (vis-á-vis **4**) with an IC_{50} of 0.074 μM in fibrinogen binding and 86% inhibition of platelet aggregation at 50 μM.[8] This effect may be due to an improved geometric alignment of the carboxy terminus of **5** with the native peptide.

Scheme 1



(a) Ac-L-Lys(Boc)-OH, BOP-Cl/NMM, CH_2Cl_2; (b) LiOH, aq. THF;
(c) $H_2N(CH_2)_nCO_2Bn$, EDC, NMM; (d) H_2/Pd-C, THF, aq. AcOH; (e) TFA or aq. HCl

The successful use of adamantane and, more importantly, nipecotic acid as β-turn scaffolds adds two new tools to the field of peptide mimics. Furthermore, we have demonstrated the utility of these mimetics for our specific therapeutic targets by facile modification of the region representing the D^{410} side chain of the fibrinogen γ-chain. Further modifications and more detailed biological evaluation of this nipecotamide series of GPIIb/IIIa antagonists are the subject of a future publication.[10]

References and Notes

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2. Kloczewiak, M.; Timmons, S.; Bednarek, M. A.; Sakon, M.; Hawiger, J. *Biochemistry* **1989**, *28*, 2915-2919.
3. Fan, F.; Kloczewiak, M.; Mayo, K. H., *Biochemistry*, submitted for publication. An observed β-turn in the QAGD sequence (407-410) of the shorter γ392-411 has been reported with 60% incidence at pH 5.2 only (M. Blumenstein *et al*, *Biochemistry* **1992**, *31*, 10692). We have made NMR determinations showing that the series of KQAG β-turns exists in the longer γ385-411 at the two disclosed pH's.
4. Because the NMR-derived solution structure of γ385-411 may not be indicative of its receptor binding conformation, transferred-NOE studies of the peptide "bound" to GPIIb/IIIa are currently underway. Preliminary experiments demonstrate that with high enough peptide to receptor ratios, γ385-411 is clearly interacting with its receptor, as indicated by the build-up of strong peptide/receptor NOEs. These studies should lead to structural families which are more representative of the bioactive conformation of the peptide.

5. For a recent review of β-turn mimetics, see Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039-3049.
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7. Compound **3**, a tan powder, was prepared from 3-amino-1-adamantane-*N*-glycine benzyl ester and N-Boc-L-Lys(Z)-OSu by standard solution phase peptide synthesis:[9] ^{1}H NMR (DMSO-d_6) δ 7.48 (br. s, 1H), 6.64 (d, J=7, 1H), 3.86 (m, 1H), 3.03 (br. s, 4H), 2.62 (m, 1H), 2.03 (m, 2 H), 1.99 (m, 1H), 1.7-1.9 (m, 10H), 1.62 (br. s, 2H), 1.4-1.6 (m, 7H), 1.37 (s, 9H), 1.26 (m, 3H); MS m/e 453 (MH^+); [a]$^{25}_D$ -26.25° (c 0.08, MeOH). Anal. calcd. for $C_{23}H_{40}N_4O_5 \cdot 2C_2H_4O_2 \cdot H_2O$: C, 54.90; H, 8.53; N, 9.48. Found: C, 54.67; H, 8.20; N, 9.73. 3-Amino-1-adamantane-N-glycine benzyl ester was prepared as follows: To a suspension of 1,3-adamantanediamine dihydrochloride (15.0 g, 0.063 mol) and DMF (300 mL) at RT was added NaH (5.64g, 0.19 mol, 80% mineral oil suspension). The mixture was warmed at 55°C for 2 h, cooled to RT, and treated with benzyl 2-bromoacetate (14.4g, 0.063 mol) dropwise over a 1 h period. The mixture was stirred for 18 h at RT, diluted with sat'd NH_4Cl (50 mL), sat'd $NaHCO_3$ (150 mL) and CH_2Cl_2 (200 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layers were washed with water (150 mL), filtered through $MgSO_4$, and evaporated to an oil. The oil was purified by flash chromatography (1%NH_4OH/10-40% iPrOH/CH_2Cl_2) to give the title compound (13.0 g, 65%) as a white powder: mp 172-174°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.05 (m, 3H), 7.40 (m, 5H), 5.15 (s, 2H), 3.37 (s, 2H), 2.18 (s, 2H), 1.2-1.9 (m, 12H); MS m/e 315 (MH^+).
8. *In vitro* biological methods: Solid Phase Purified Glycoprotein IIb/IIIa Binding Assay. RGD-affinity purified GPIIb/IIIa is immobilized in a TiterTek 96-well plate. Biotinylated fibrinogen (10 nM, final concentration) is added to wells containing test compounds and left at RT for 2-4 h. The solution is discarded and the plate washed extensively. Vecta Stain HRP-Biotin-Avidin reagent is added and incubated at RT for 15 min. The wells are allowed to develop for 3-5 min at RT after addition of a developing buffer. The absorbance is read at 490 nM. Peptide standard: RGDS, IC_{50} = 0.9 μM. Nonpeptide standard: Merck L-700462, IC_{50} = 0.001 μM.

   Platelet Aggregation. Blood from drug-free, normal donors is collected in tubes containing 0.13*M* sodium citrate. Platelet rich plasma (PRP) is collected by centrifugation. PRP is gel-filtered through Sepharose 2B, and platelet count is adjusted to $2x10^7$ platelets/ sample. Aggregation is monitored in a BIODATA aggregometer for 3 min after addition thrombin, 0.1unit/mL. Percentage platelet aggregation is calculated by increase in light transmission of compound-treated vs. control-treated platelet concentrate. Peptide standard: RGDS, IC_{50} = 30.0 μM.
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