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TROPANE-BASED AMINO ACIDS FOR PEPTIDE STRUCTURE–FUNCTION STUDIES: INHIBITORS OF PLATELET AGGREGATION

Philip E Thompson,* David L Steer, Marie-Isabel Aguilar, and Milton T W Hearn

Centre for Bioprocess Technology, Department of Biochemistry and Molecular Biology, Monash University, Clayton 3168, Australia

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Abstract: Novel tropane (azabicycloheptane) and azabicyclohexane containing amino acids have been prepared and incorporated into analogues of reported inhibitors of platelet aggregation. The influence of these central constraints upon biological activity suggest their utility in peptide structure function studies.

Introduction

Novel, readily available amino acids that induce conformational constraint in peptides are important tools in peptide structure-function studies and peptidomimetic research.[1] The potential of these amino acids has been amplified in recent years by the emergence of the combinatorial library and parallel synthesis strategies whereby the synthesis of vast arrays of molecules incorporating novel amino acids can be readily achieved.[2]

An area of notable success in this field has been in the design of peptidomimetics of the Arg-Gly-Asp integrin receptor recognition sequence.[3] Present in an array of endogenous and natural product ligands with affinity for an array of receptors, the RGD sequence has been successfully modified by conformational constraint at each of the amino acid residues in the tripeptide sequence. In particular, inhibition of platelet aggregation by antagonism of the binding of fibrinogen to the platelet membrane receptor glycoprotein IIb/IIIa (GpIIb/IIIa) has been demonstrated and a number of these agents are in clinical trial.[4] An important requirement of these constrained peptidomimetics is that they act selectively and avoid interactions with other integrins which may result in severe side effects.[5] In this regard, a second class of inhibitors has been described based upon the fibrinogen γ-chain specific binding domain.[6]

Amino acid derivatives of the tropane alkaloid natural products[7] (Figure 1) have been synthesised and evaluated for potential application as peptidomimetics. These molecules contain the structural elements desirable in a peptidomimetic-amino and carbonyl groups held in an relatively rigid orientation by a cyclic structure. The structural diversity within this class of heterocycle allows for a variety of conformational families

* Current address: Thrombogenix Pty. Ltd., Department of Medicine, Monash University, Box Hill Hospital, Arnold St., Box Hill 3128 Australia. email: Phil.Thompson@boxhill.med.monash.edu.au.

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to be surveyed, while at the same time retaining a relatively simple structure, well precedented approaches to synthesis[8] and incorporation into peptides via solid-phase synthesis methods.

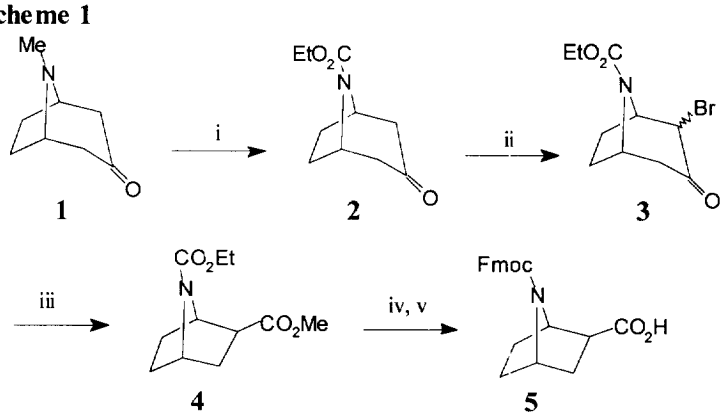
We have incorporated these amino acids into GPIIb/IIIa inhibitors including both γ-chain and RGD targets to establish if useful structure-function data could be gathered using a family of tropane template structures, and whether such a strategy could expedite the generation of pharmacophores for peptides and peptidomimetics.

Chemistry

We have previously described the synthesis of two Fmoc-protected tropane amino acids, (±)-Fmoc-Ntc[2α] and (±)-Fmoc-Nec (Figure 1) via synthetic routes developed for structural studies of cocaine.[8] We describe here the synthesis and application of a third novel amino acid, (±)-7-Fmoc-7-azabicyclo[2.2.1]hexane-2*exo*-carboxylic acid [(±)-Fmoc-Abh, **5**] derived from a reported precursor to epibatidine.[9] The expectation that work related to important natural products would provide the platform for our own syntheses has been an important element of the overall design strategy.

Epibatidine, a potent nicotinic agonist, has been the focus of much attention and a number of syntheses have utilised a carboxyl-substituted azabicyclohexane structure intermediate.[10] Xu et al.[9] have reported a very rapid entry into a key protected precursor. In short, dealkylation of tropinone (**1**) and protection as the ethyl carbamate, was followed by bromination. The bromo adduct (**3**) underwent Favorskii rearrangement to yield the *exo* configuration amino acid protected as the carbamate ester (**4**). Deprotection via TMSI hydrolysis and N-Fmoc protection yielded our target Fmoc amino acid (**5**)[11] (Scheme 1).

Scheme 1

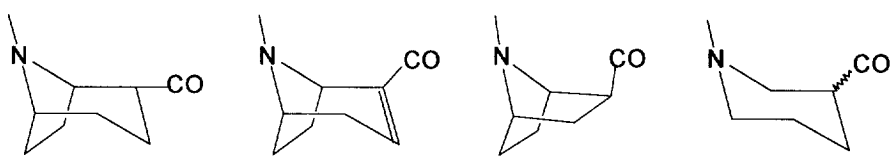


Reagents and Conditions: i $ClCO_2Et$ (2 eq), K_2CO_3 (cat), reflux, 3 h; ii $CuBr_2$ (2 eq), $CHCl_3$, EtOAc, reflux, 1 h; iii NaOMe (3 eq), DME, rt, 0.5 h; iv TMSI, $CHCl_3$, reflux 13 h; v Fmoc-Cl, dioxan, Na_2CO_3 (10% aq.), rt, 15 h then RP-HPLC.

These amino acids (Figure 1) were then incorporated into standard Fmoc-based solid-phase peptide synthesis protocols. Three groups of peptides were prepared, (A) Ac-Lys-Xxx-β-Ala, (B) Ac-D-Lys-Xxx-β-Ala related to inhibitors described by Hoekstra et al.,[12] and (C) Arg-Xxx-Asp-Ser, the GpIIb/IIIa antagonist derived from the endogenous motif of fibrinogen. The peptides were synthesised using standard Fmoc-based solid-phase synthesis on Wang Resin[13] and generally utilised the novel amino acids in reduced loading to preserve material. Nonetheless couplings proceeded quite smoothly under these conditions. Peptides were cleaved from the resin using 95% TFA for groups (A) and (B) and Reagent K[14] for group (C). Crude peptides were purified by RP-HPLC using a acetonitrile/water gradient buffered with 0.1% TFA. Purity was characterised by analytical RP-HPLC and structures were confirmed by MS and amino acid analysis.

Platelet Aggregation Assays

Compounds **1–9** were assayed for their ability to inhibit the aggregation of washed human platelets by thrombin.[15] Of particular interest was the direct comparison of the tropane substituted peptide with its "native" analogue. To washed platelets (8.3×10^7 platelets/mL, 360 μL) in Tyrodes buffer was added $CaCl_2$ (100 mM, 4 μL) and test compound (40 μL) and the mixture incubated at 37 °C for 2 min. Thrombin (50 U/mL, 1 μL) was added and the extent of aggregation after 4 min measured in a 4 channel aggregometer (Kyoto Daiichi, Japan) with stirring at 950 rpm. The determination of IC_{50} values (summarised in Table 1) represent duplicate experiments upon a single platelet preparation.



(±)-Ntc2α (±)-Nec (±)-Abh (±)-Nip

Figure 1. Amino acid structures and abbreviations

In the case of the Group A fibrinogen γ-chain mimics, Ac-L-Lys-(±)-Nip-β-Ala (**6**) proved more potent than the corresponding tropane Ac-Lys-(±)-Ntc$^{2\alpha}$-β-Ala (**7**), which was unable to fully inhibit aggregation even at concentrations of 0.5 mM and was nearly an order of magnitude less potent than the parent molecule. Compounds **8** and **9** showed negligible inhibitory activity. Substitution of D-Lys (Group B) yielded improved activity across the group. The stereoisomeric ligand Ac-D-Lys-(±)-Ntc$^{2\alpha}$-β-Ala (**11**) showed markedly improved inhibitory characteristics, equipotent to (**1**) but twofold less potent than the corresponding Ac-D-Lys-Nip-β-Ala (**10**). The Nec containing ligand (**12**) showed moderate inhibitory activity, however the Abh containing ligand (**13**) again failed to significantly inhibit aggregation.

In the case of Arg-Gly-Asp-Ser analogues (Group C), more success has been obtained in terms of retaining bioactivity. Substitution of (±)-Ntc$^{2\alpha}$ for the glycine residue resulted in a ligand (**15**) of increased potency relative to RGDS (**14**). Replacement with (±)-Nec (compound **16**) and (±)-Abh (compound **17**) resulted in a significant loss of inhibitory activity.

Table 1. Inhibiton of thrombin-induced platelet aggregation by tropane derivatives.

	Sequence	IC_{50} (μM)
6	Ac-Lys-(±)-Nip-β-Ala	60
7	Ac-Lys-(±)-Ntc$^{2\alpha}$-β-Ala	370
8	Ac-Lys-(±)-Nec-β-Ala	>500
9	Ac-Lys-(±)-Abh-β-Ala	>500
10	Ac-D-Lys-(±)-Nip-β-Ala	20
11	Ac-D-Lys-(±)-Ntc$^{2\alpha}$-β-Ala	30
12	Ac-D-Lys-(±)-Nec-β-Ala	160
13	Ac-D-Lys-(±)-Abh-β-Ala	>500
14	Arg-Gly-Asp-Ser	50
15	Arg-(±)-Ntc$^{2\alpha}$Asp-Ser	23
16	Arg-(±)-Nec-Asp-Ser	270
17	Arg-(±)-Abh-Asp-Ser	402

As tools for drug discovery, nonribosomal amino acids are an important bridge between peptide leads and nonpeptide drugs. The incorporation of increased structural definition while retaining the simplicity and reproducibility of standard solid phase peptide synthesis techniques may significantly expedite the gathering of structure-function data. While many examples of such amino acids have appeared in recent literature,[16] no single family of amino acids adequately surveys conformational properties of lead peptides. One thrust of recent reports has focussed upon the potential of beta amino acids. Klein et al. examined the use of cyclic and acyclic β-alanine based residues as glycine replacements in RGDV mimics.[17] From 13 analogues containing either N-alkyl or cyclic β-alanine residues, 10 contained increased potency as inhibitors of platelet aggregation from 1 to 100 times more potent, correlative to affinity for integrin GPIIb/IIIa. Also impressive was the stereoselectivity exhibited by pyrrolidin-3-carboxamido isomers. The nipecotamide derivatives of Hoekstra *et al* also show stereochemical selectivity.[18]

In this study, the potential for tropane-based amino acids to achieve similar selectivities has been examined. The results show the subtlety underpinning the selectivity of these peptides. In the γ-chain analogues, the differences between the nipecotamides (**6** and **10**) and the corresponding (±)-tropane-2α-carboxamides (**7** and **11**) were not anticipated to be great. In the event however the tropane derivatives showed reduced activity both in the L-Lys series and the more potent D-Lys series. The smaller azabicyclohexane peptides and unsaturate norecgonidine derivatives all showed reduced potency, suggesting low energy conformations not overlapping the parent nipecotamide.

In the RGDS series, the retention of potency shows an overlap between the conformers of (±)-Ntc$^{2\alpha}$ and Gly residues. Again the (±)-Abh- and (±)-Nec-containing compounds (**16** and **17**) showed markedly reduced activity. Interestingly comparable monocyclic substitutions in the series prepared by Klein et al. all resulted in increased bioactivity.[17] It may be argued that the replacement of arginine by the rigid 4-guanidinocinnamic acid in that series has rendered the glycine conformation "sub-optimal", which is probably also the case with other reported RGD peptidomimetics. Our intention in the present investigation was to examine the tropane as a first pass test of structure function using the native inhibitor sequence.

It should also be noted that these studies have not used optically pure templates and so activity is not attributable to either or both isomers in each experiment. Resolution of these isomers will provide another layer of structure activity information and such studies are currently underway. Moreover other congeners of these tropanes: (±)-Nec$^{2\beta}$and the *endo* isomer of (±)-Fmoc-Abh, have been prepared and will be incorporated into further studies.

The results described here represent the first application of a tropane amino acid "construction kit" analogous to the sugar amino acid kit described by Graf von Roedern et al.[1] The tropane nucleus with its capacity for substitution, configurational diversity and relative synthetic accessibility offers significant potential in the generation of structurally defined pseudopeptide libraries.

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References and Notes

1. Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156.
2. Pavia, M. P.; Sawyer, T. K.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 387.
3. Ojima, I.; Chakravarty, S.; Dong, Q. *Bioorg. Med. Chem.* **1995**, *3*, 337.

4. Lefkovits, J.; Plow, E. F.; Topol, E. J. *New England J. Med.* **1995**, *332*, 1553.
5. Craig, W. S.; Cheng, S.; Mullen, D. G.; Blevitt, J.; Pierschbacher, M. D. *Biopolymers* **1995**, *37*, 157.
6. Kloczewiak, M.; Timmons, S.; Lukas, T. J.; Hawiger, J. *Biochemistry* **1984**, *23*, 1767.
7. Fodor, G.; Dharanipragada, R. *Nat. Prod. Rep.* **1993**, *10*, 199.
8. Thompson, P. E.; Hearn, M. T. W. *Tetrahedron Lett.* **1997**, *38*, 2907.
9. Xu, R.; Chu, G.; Bai, D. *Tetrahedron Lett.* **1996**, *37*, 1463.
10. (a) Hernández, A.; Marcos, M.; Rapoport, H. *J. Org. Chem.* **1995**, *60*, 2683. (b) Zhang, C.; Trudell, M. L. *J. Org. Chem.* **1996**, *61*, 7189.
11. Selected data for **5**: IS-MS: 364 (M + H), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.60 (dd (J = 4.9 Hz, 8.9 Hz), 1H, C2*H endo*).
12. Hoekstra, W. J.; Beavers, M. P.; Andrade-Gordon, P.; Evangelisto, M. F.; Keane, P. M.; Press, J. B.; Tomko, K. A.; Fan, F.; Kloczewiak, M.; Mayo, K. H.; Durkin, K. A.; Liotta, D. C. *J. Med. Chem.* **1995**, *38*, 1582.
13. Fields, G. B.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161.
14. King, D. S.; Fields, C. G., Fields, G. B. *Int. J. Peptide Protein Res.* **1990**, *36*, 255.
15. Schoenwalder, S. M.; Jackson, S. P.; Yuan, Y.; Teasdale, M. S.; Salem, H. H.; Mitchell, C. A. *J. Biol. Chem.* **1994**, *269*, 32479.
16. For example: Combs, A. P.; Kapoor, T. M.; Feng, S.; Chen, J. C.; Daudé-Snow, L. F.; Schreiber, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 287.
17. Klein, S. I.; Czejak, M.; Molino, B. F.; Chu, V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1773.
18. Hoekstra, W. J.; Maryanoff, B. E.; Andrade-Gordon, P.; Cohen, J. H.; Costanzo, M. J.; Damiano, B. P.; Haertlein, B. J.; Harris, B. D.; Kauffman, J. A.; Keane, P. M.; McComsey, D. F.; Villani Jr., F. J.; Yabut, S. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2371.