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PLATELET GLYCOPROTEIN IIb-IIIa RECEPTOR (GPIIb-IIIa) ANTAGONISTS DERIVED FROM AMIDINOINDOLES.

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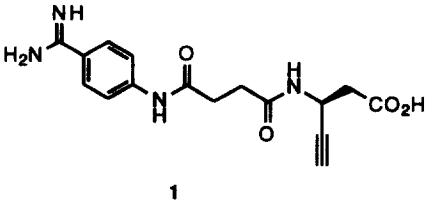
Abstract: A series of substituted amidinoindoles have been prepared as mimics of the RGD sequence and were studied as antagonists of the platelet glycoprotein IIb-IIIa receptor (GPIIb-IIIa). The agents were potent and selective antagonists of GPIIb-IIIa. Compared to their acyclic counterparts, the amidinoindole series bound with 10- to 20-fold greater affinity, indicating the advantages of added conformational restriction and/or hydrophobicity in the basic region of RGD mimics.

Platelet activation and subsequent aggregation are primary contributors to a number of vaso-occlusive disorders including unstable angina, myocardial infarction, and reocclusion following thrombolytic therapy or angioplasty.[1] While a number of physiological agonists will stimulate platelets, aggregation proceeds through a final obligatory step, namely the binding of fibrinogen to the platelet glycoprotein IIb-IIIa receptor (GPIIb-IIIa).[2] A major determinant of the GPIIb-IIIa/fibrinogen interaction is receptor recognition of the tripeptide sequence Arg-Gly-Asp (RGD), which occurs four times in the primary sequence of fibrinogen.[3] Accordingly, peptides and peptidomimetics that contain or mimic the RGD sequence act as antagonists of fibrinogen binding to activated platelets and effectively block platelet aggregation, irrespective of the agonist.[4]

A number of flexible, linear RGD mimics have been designed based on the use of benzamidine as a basic substitute for arginine, including for example agents **1**[5] and **2**[6]. During our investigation into RGD mimetics, we



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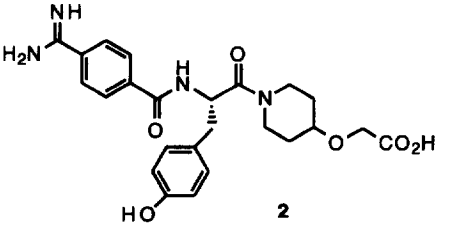

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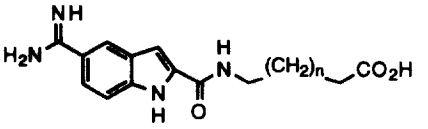
were interested in exploring the use of amidinoindoles, since they had been previously employed as probes for the specificity pocket of arginine endopeptidases.[7] Indole amidines offer additional degrees of conformational restriction compared to benzamidine and might offer further insight into the impact of introducing conformational definition into the basic component of otherwise flexible RGD mimics. Initially we wanted to determine the optimal spatial relationship between the guanidyl and carboxyl binding sites in receptor bound RGD sequences and studied a series of agents in which acidic tails of varying length were appended to the 2-position of 5-amidinoindole (**3-6**; **Table 1**). In both a purified receptor binding assay[8] and a platelet aggregation assay,[9]

amidino acid **5** was at least a full order of magnitude more potent than any of the other derivatives, indicating that an eight atom tether between the amidinoindole nuclues and the carboxylate tail provides the closest approximation of the critical distance between the basic guanidine and the acidic carboxylate of receptor bound RGD.

Table 1. Antagonism of the Platelet GPIIb-IIIa by 2,5-Disubstituted Amidinoindole Acids **3 - 6**.



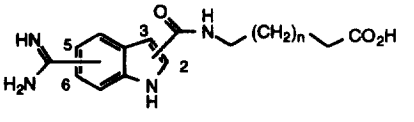
3: n = 2
4: n = 3
5: n = 4
6: n = 5

Compound #	Ligand Binding ELISA IC_{50} (μM)[8]	Platelet Aggregation PRP IC_{50} (μM)[9]
3	48 ± 8.0	> 100
4	30 ± 4.0	> 100
5	0.70 ± 0.04	2.7 ± 0.05
6	9.8 ± 1.9	> 100

The strict spatial requirements governing this series is evidenced by the fact that shortening or lengthening the acidic sidechain by the distance of only a single carbon-carbon bond (1.4 Å) causes at least a 13-fold decrease in binding affinity. Even with the freely flexible acidic sidechain, agent **6** cannot readily undergo minor conformational alterations in order to mimic the optimal distance that is approximated by agent **5**. The lower activity of specific compounds when tested in PRP versus the ELISA assay could be due to a variety of factors including protein binding and/or the high fibrinogen concentrations present in the PRP assay.

Having identified the critical tether length, the regioisomeric derivatives **7-10** were prepared in order to determine the optimal orientation between the basic amidine and the acidic sidechain (**Table 2**). While the

Table 2. Antagonism of the Platelet GPIIb-IIIa by Regioisomeric Amidinoindole Acids **7 - 10**.



7: 2,6-disubstituted, n = 4
8: 3,6-disubstituted, n = 4
9: 3,5-disubstituted, n = 4
10: 3,5-disubstituted, n = 5

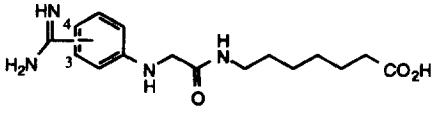
Compound #	Ligand Binding ELISA IC_{50} (μM)[8]	Platelet Aggregation PRP IC_{50} (μM)[9]
7	0.15 ± 0.02	1.3 ± 0.4
8	0.10 ± 0.01	0.65 ± 0.19
9	> 100	> 100
10	> 100	> 100
RGDS	14.5 ± 3.0	> 100
Mpr-RGDWR-Pen-NH_2	0.12 ± 0.02	0.28 ± 0.08

interatomic distances between indole atom pairs C-2/C-5 (**5**), C-2/C-6 (**7**), and C-3/C-6 (**8**) are all similar (4.5 Å, 4.5 Å, and 4.2 Å respectively), the interatomic distance between C-3 and C-5 (**9**) is only 3.7 Å.[10] In an attempt to compensate for the 0.8 Å discrepancy between the sites of attachment of the amidine and acidic tail in agents **5** and **9**, the one carbon homologue **10** was also prepared and evaluated. For comparative purposes, the acyclic peptide RGDS and a potent cyclic RGD-containing peptide Mpr-RGDWR-Pen-NH_2[11] were also evaluated.

In both assays, the 2,6- and 3,6-disubstituted regioisomers (**7** and **8** respectively) displayed greater activity than the parent compound **5**, while neither of the 3,5-disubstituted analogs (**9** and **10**) displayed detectable activity in either assay. The lack of detectable activity of compounds **9** and **10** compared to derivatives **3**, **5**, and **6** indicates that either the 'cup-like' shape imposed by the 3,5-disubstitution pattern is not recognized by the receptor or agents **9** and **10** cannot mimic the critical distance between the guanidyl and carboxyl binding sites within the GPIIb-IIIa receptor. The distance between the sites of substitution and their geometrical relationship to one another are nearly identical in regioisomers **5**, **7**, and **8**. However derivatives **7** and **8** bind with a 5-fold higher affinity than does agent **5**, suggesting the presence of a preferred spatial orientation between the amidine and the hydrogen bond donating, indole N-H. In the ELISA, both amidino acids **7** and **8** were 100-fold more potent than the freely flexible RGDS sequence, and bound equally well as the conformationally-restricted peptide Mpr-RGDWR-Pen-NH_2. Derivatives **7** and **8** were significantly less active than Mpr-RGDWR-Pen-NH_2 in the platelet aggregation assay.

It has been recently documented that conformational definition in the central region of RGD mimics enhances their binding affinity to the GPIIb-IIIa receptor.[12] To assess whether the additional conformational restriction offered by the indole nucleus compared to a simple disubstituted benzene enhances the binding affinity of this series, the acyclic benzamidine derivatives **11** and **12** were prepared and their activities compared to their indoleamidine counterparts, **5** and **7** respectively.

Table 3. Comparison of Amidinoindoles **5** and **7** with Their Acyclic Counterparts **11** and **12**.



11: 3-Amidino
12: 4-Amidino

Compound #	Ligand Binding ELISA IC_{50} (μM)[8]	Platelet Aggregation PRP IC_{50} (μM)[9]
5	0.70 ± 0.04	2.7 ± 0.05
12	23 ± 2.6	67 ± 2.4
7	0.15 ± 0.02	1.3 ± 0.4
11	64 ± 7.5	> 100

Amidinoindoles **5** and **7** bind to GPIIb-IIIa with at least twenty-fold greater affinity than their acyclic counterparts **11** and **12**. A number of plausible explanations might explain the greater activity of the indole series. One, the one-half log unit difference in clog P values between indole and N-methylaniline (2.14 versus 1.66 respectively),[13] might lead to additional hydrophobic interactions. Second, the indole nucleus might provide

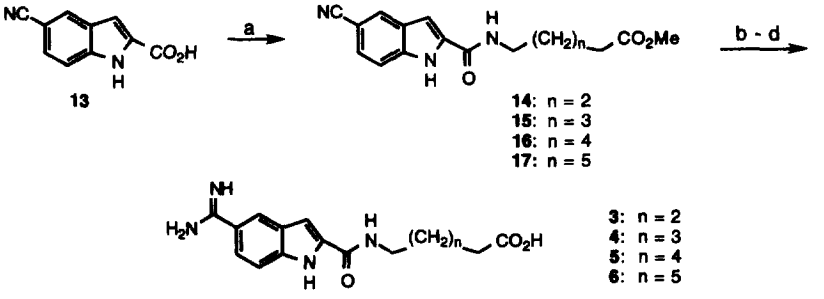
additional π-stacking within that region of the GPIIb-IIIa receptor. Finally, the greater conformational definition of the indole nucleus could force the amidine, hydrogen-bond donating NH, and acidic sidechain into a more favorable geometrical relationship. It is unclear at this point whether the enhanced activity of the amidinoindoles results from one or more of the above reasons.

The RGD motif is also recognized by other integrins such as the vitronectin receptor ($\alpha_v\beta_3$) and therefore mimics of the RGD sequence can serve as antagonists at sites other than GPIIb-IIIa. As a representative from this series, amidinoindole **8** was studied and was found to bind selectively to the GPIIb-IIIa receptor, failing to compete with vitronectin for binding to the vitronectin ($\alpha_v\beta_3$) receptor even at concentrations up to 100 μM.[14]

In summary, we have employed amidinoindoles as arginine surrogates in the design of novel, non-peptidal RGD mimics which selectively antagonize the GPIIb-IIIa receptor. It has been shown for the first time that restricting conformation in the basic region of an RGD mimic can greatly enhance the binding affinity of the agent. A few of the agents within this series bind with affinities that rival those of cyclic RGD-containing peptides. The attractiveness of this series lies in their structural simplicity and the opportunities that are presented for future development of the structure-activity relationships. Ongoing studies will define the structure-activity relationships surrounding this series of novel GPIIb-IIIa receptor antagonists.

CHEMISTRY:

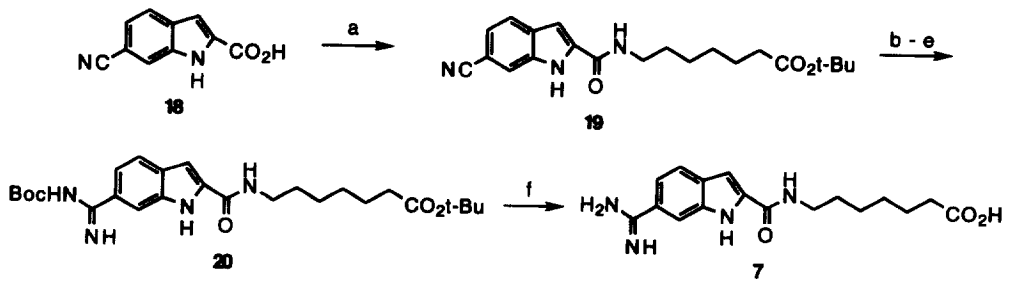
The target amidino acids **3 - 6** were derived from 5-cyanoindole-2-carboxylate (**13**; Scheme 1) which was readily prepared using the Reissert indole synthesis.[15,16] Condensation of acid **13** with the appropriate aliphatic amino esters afforded the amidino ester precursors **14-17** which were readily converted to the corresponding amidines using the Pinner reaction.[17] Basic hydrolysis of the esters afforded agents **3-6** which were purified by recrystallization or reverse phase chromatography.[18]



Reagents: (a) DMAP, DIEA, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, amino ester; (b) EtOH, HCl; (c) NH_3, EtOH; (d) LiOH

Scheme 1

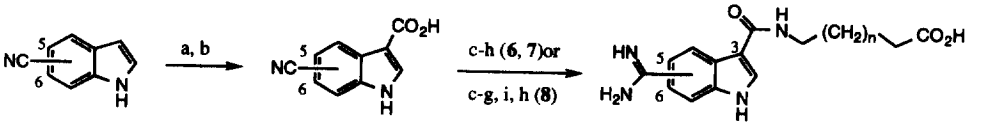
Regioisomer **5** was prepared from readily available 6-cyanoindole-2-carboxylate (**18**; Scheme 2)[19] by using chemistry similar to that employed in the preparation of agents **3 - 6**. In this case however, nitrile **19** was converted to the corresponding amidine by a known three step route[20] and was protected as the Boc derivative (**20**) to facilitate its purification. Simultaneous deprotection of both the amidine and the acid using triflouroacetic acid afforded the desired product **7** in analytically pure form.[18]



Reagents: (a) DMAP, DIEA, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, t-butyl 7-aminoheptanoate; (b) H_2S, pyr; (c) MeI; (d) NH_4OAc; (e) Boc_2O, K_2CO_3; (f) TFA, anisole

Scheme 2

Amidine **10** was derived from readily prepared 6-cyanoindole (**21**; Scheme 3)[21] while derivatives **11** and **12** were derived from commercially available 5-cyanoindole (**22**; Scheme 3).



21: 6-CN
22: 5-CN

23: 6-CN
24: 5-CN

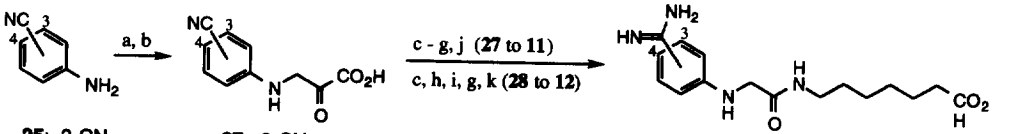
10: 6-amidino, n = 4
11: 5-amidino, n = 4
12: 5-amidino, n = 5

Reagents: (a) $POCl_3$, DMF; (b) $NaClO_2$, $NaH_2PO_4.H_2O$; (c) DMAP, DIEA, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride, t-butyl 7-aminoheptanoate (**6** and **7**) or methyl 8-aminocaprylate (**8**); (d) H_2S, pyr; (e) MeI; (f) NH_4OAc; (g) Boc_2O, K_2CO_3; (h) TFA, anisole; (i) NaOH, EtOH.

Scheme 3

Formylation of indoles **21** and **22**, and subsequent oxidation[22] afforded the nitrile acids **23** and **24**. Coupling the acids to the appropriate amino esters and conversion to the desired amidino acids was effected using previously described chemistry.[17, 20]

Acyclic amidines **13** and **14** were prepared in a straightforward manner from 3-amino and 4-aminobenzonitrile, respectively (**Scheme 4**). During the preparation of product **12**, conversion of nitrile **28** to



25: 3-CN
26: 4-CN

27: 3-CN
28: 4-CN

11: 3-Amidino
12: 4-Amidino

Reagents: (a) t-butyl bromoacetate, K_2CO_3; (b) TFA, anisole; (c) DMAP, DIEA, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride, t-butyl 7-aminoheptanoate; (d) H_2S, pyr; (e) MeI; (f) NH_4OAc; (g) Boc_2O, K_2CO_3; (h) EtOH, HCl; (i) NH_3, EtOH; (j) TFA, anisole; (k) NaOH, EtOH.

Scheme 4

the corresponding thioamide proceeded uneventfully with H_2S. However upon treatment of the intermediate thioamide with methyl iodide, starting nitrile **28** was the sole product isolated, necessitating the use of Pinner conditions[17] to effect the nitrile to amdine conversion.

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