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SOLID-PHASE PARALLEL SYNTHESIS APPLIED TO LEAD OPTIMIZATION: DISCOVERY OF POTENT ANALOGUES OF THE GPIIb/IIIa ANTAGONIST RWJ-50042

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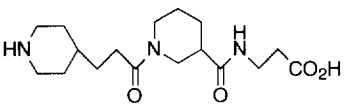
Abstract: A study of β-turn peptide mimetics, related to the C-terminal γ-chain of fibrinogen and containing a nipecotic acid scaffold, led to RWJ-50042 (**1**), an interesting fibrinogen receptor (GPIIb/IIIa) antagonist. To enhance potency, we employed solid-phase parallel synthesis for the preparation of over 200 analogues in a protocol of optimization cycles. This strategy produced several promising nipecotamide analogues, such as **25**, which is 35 times more potent than **1** in vitro.

Combinatorial chemistry methods have unleashed a powerful strategy for diversity-based discovery of new leads with desired chemical, biological, and physical properties.[1] Resin-based parallel synthesis of discrete chemical mini-libraries is a focused adaptation of combinatorial chemistry that provides an attractive tool for optimizing biological activity.[2] Through the solid-phase parallel synthesis of analogues of RWJ-50042 (**1**), our prototypical fibrinogen (Fg) receptor (GPIIb/IIIa) antagonist,[3] we have achieved a marked compression of the timeline for moving from a drug lead to a development candidate, vis-à-vis traditional methods.

Nipecotamide **1** is an orally active antagonist of the platelet Fg receptor (binding IC_{50} = 6 nM), which exhibits inhibition of collagen-induced platelet aggregation (ex vivo) after oral administration of 10 mg/kg to dogs.[4] This series was discovered by using the solution structure of the C-terminal γ-chain of Fg for drug design.[3,5,6] The substituted nipecotic acid unit modeled the β-turn structure contained within the KQAGD sequence of the γ-chain (residues 406-410). Although **1** displays high in vitro potency, its in vivo potency is deficient, requiring the exploration of new analogues. Given the highly competitive environment surrounding this area of antithrombotic therapy,[7] we decided to pursue a synthetic protocol that would expedite the study of analogues: namely, solid-supported, parallel organic synthesis.

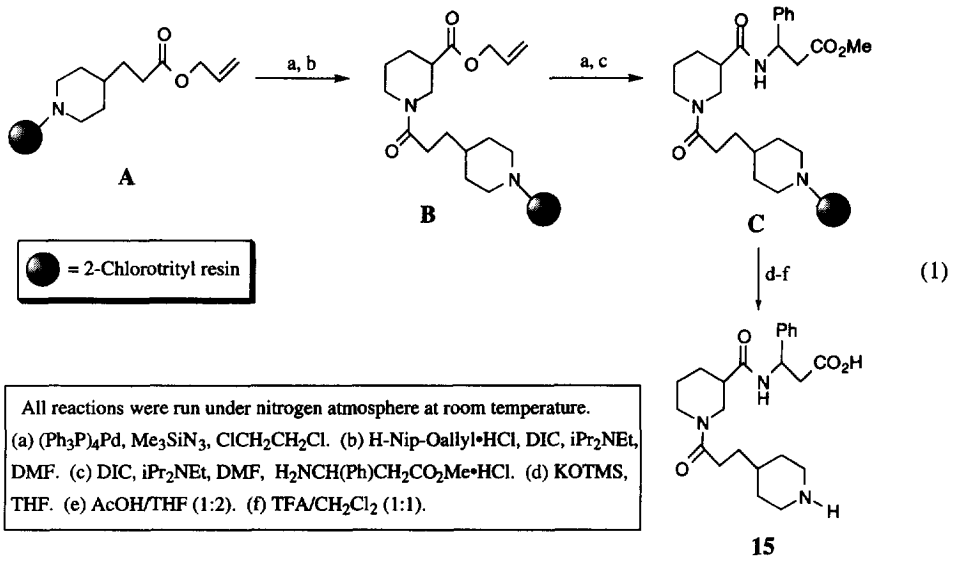


RWJ-50042 (**1**; racemic)
prototype GPIIb/IIIa antagonist

Synthesis of Analogues

A solid-phase parallel synthesis protocol was established to prepare analogues of **1** in a rapid fashion. Given that the lead molecule consists of two amide bonds/three structural components, variation of each element represents a practical, systematic strategy for potency improvement (Table 1). Since variants of the β-amino acid

unit are readily available as esters, commercially or synthetically, an initial set was selected for target synthesis. Our procedure involved the synthesis of 12 resin-bound compounds with each contained in a magnetically-stirred, fritted-disk reaction vial.[2,8] The N-terminal pseudodipeptide portion was held constant in the matrix. Importantly, we chose a strategy of N-terminal attachment to the resin to allow coupling of the numerous β-amino esters late in the synthesis, thereby timing the 12-way resin division immediately before the second amide bond formation (Eq 1). A corresponding C-terminal attachment strategy would have incorporated less available N-protected β-amino acids and compelled resin splitting prior to the first amide bond-forming step.

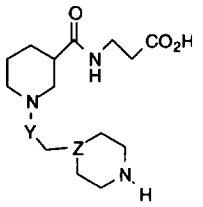


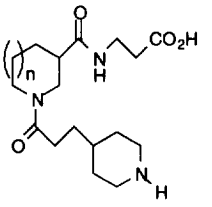
Isonipecotic acid, and the five- and seven-membered-ring congeners of nipecotic acid, were used to study scaffold size/conformation change for the "central ring." While 288 variants (6 x 4 x 12) were targeted for synthesis, in principle, a *concurrent refinement process* was implemented to select increasingly optimal sets of components for subsequent analogue synthesis/evaluation (see Discussion of Biological Activity). By using a focused compound mini-library, we discarded inferior components to avoid unnecessary synthesis and bioassay of presumed weakly active agents.

Resin-based preparation of **15**, a 3-phenyl analogue of RWJ-50042, typifies our strategy of convergent, high-throughput synthesis. N-Attachment of 2-chlorotrityl chloride resin to allyl 4-piperidinepropanoate yielded "N-protected" intermediate **A**. Allyl ester removal under mild Pd(0) conditions, followed by diisopropylcarbodiimide coupling to allyl piperidinepropanoate, gave pseudodipeptide **B**. Starting at the N-terminus allows one to accrue large quantities of resin-bound **B** in a common reaction vessel. After saponification of **B**, the resin was split for coupling to 12 readily available β-amino esters, leading to the final products. For modifications at the tertiary amide, analogous urethane and urea couplings at the *N*-nipecotyl position were performed, by using *p*-nitrophenylchloroformate[9] conditions and the appropriate resin-bound primary alcohol or amine (e.g., **2** or **3**). Solution-phase coupling of Cbz-4-piperidineethanesulfonyl chloride to ethyl nipecotate gave a sulfonamide

intermediate (e.g., **4**).[10] Intermediate **B** was saponified and coupled with methyl 3-phenyl-3-aminopropionate to render **C**. To convert methyl or ethyl β-amino esters to carboxylate targets, an organic solution-based saponification with $KOSiMe_3$/THF was applied to intermediates such as **C**. Effective resin swelling permitted complete ester cleavage, whereas aqueous-base conditions generally did not. The carboxylates were acidified with dilute HOAc and cleaved with CF_3CO_2H to give products, exemplified by 3-phenyl derivative **15** (70% yield and 90% purity, starting with 0.23 mmol resin/compound; 50-90% yields for solid-phase products in Table 1).

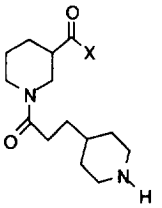
Table 1. Inhibition of human platelet aggregation and fibrinogen binding by analogues of **1**.





Cmpd	Y	Z	Pl. Aggr.[a] IC_{50} (μM)	Bndg.[b] IC_{50} (nM)
1	$COCH_2$	CH	0.7 ± 0.05	6 ± 4
2[c]	CO_2	CH	5 ± 1	20 ± 28
3[c]	CONH	CH	7 ± 1	20 ± 0.5
4	SO_2CH_2	CH	20 ± 4	20 ± 5
5[c,e]	$COCH_2$	CH	2 ± 0.2	6 ± 0.1
6	$COCH_2$	N	>10	2 ± 0.8

Cmpd	n	Pl. Aggr.[a] IC_{50} (μM)	Bndg.[b] IC_{50} (nM)
1	1	0.7 ± 0.05	6 ± 4
7[c]	0	7 ± 1	7 ± 3
8[c]	2	8 ± 3	10 ± 3
9[c,d]	1	≥10	>100
10[f]	1	0.3 ± 0.08	6 ± 2
11[g]	1	3 ± 0.3	4 ± 2

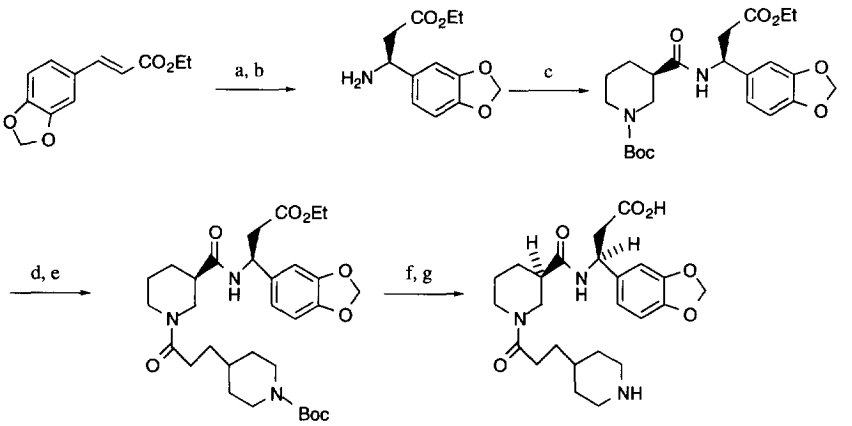


Cmpd	X	Pl. Aggr.[a] IC_{50} (μM)	Bndg.[b] IC_{50} (nM)
1	β-Ala	0.7 ± 0.05	6 ± 4
12	N-Me-β-Ala	28 ± 2	200 ± 0
13	3-Me-β-Ala	2 ± 0.4	3 ± 2
14	3-*i*-Bu-β-Ala	4 ± 0.7	2 ± 0.5
15	3-Ph-β-Ala	4 ± 0.4	3 ± 1
16	L-Asp-OMe	1 ± 0.2	3 ± 2

Cmpd	X	Pl. Aggr.[a] IC_{50} (μM)	Bndg.[b] IC_{50} (nM)
17	2-Me-β-Ala	≥10	>100
18	2-OH-β-Ala	0.8 ± 0.2	5 ± 2
19	4-oxo-nipecotic acid	65[h]	9 ± 4
20	3-NH-*c*-$C_6H_{10}CO_2H$	>50	>50,000
21	$NH(CH_2)_2SO_3H$	11 ± 1	200 ± 70
22	$NH(CH_2)_2$-5-tetrazole	40 ± 10	600 ± 500

a. Thrombin-induced human gel-filtered platelet aggregation (n = 3).[3] b. Inhibition of biotinylated Fg binding to immobilized GPIIb/IIIa (n = 2).[3] c. Twelve examples were prepared with racemic β-amino acids typified by compounds **23-27**. d. Isonipecotic acid derivative. e. *N*-methylpiperidine. f. 3-(*R*) nipecotic acid. g. 3-(*S*) nipecotic acid. h. n = 1.

In vitro testing results on early products (**2-22**) indicated two important criteria for the selection of future analogues (Table 1). First, the N-terminal pseudodipeptide *N*-piperidinepropionylnipecotic acid unit of **1** was relatively optimal compared to other piperidine linkers and central rings (e.g., **1** vs. **2-6**; **1** vs. **7-9**). Second, substituted β-amino acids with a hydrophobic group in the β-position (**13-16**) represented a good site for potential potency improvement (although chirality issues had to be addressed). The ease of synthesis of β-amino esters furnished numerous subunits for attachment to solid-supported *N*-piperidinepropionylnipecotic acid.



(2)

25

(a) (*R*)-Me(Ph)CHNH$_2$, Me$_3$SiCl; BuLi. (b) H$_2$, Pd(OH)$_2$, EtOH. (c) Boc-(*R*)-nipecotic acid, HBTU, HOBT, NMM, MeCN (d) HCl, EtOAc. (e) Boc-piperidinepropanoic acid, HBTU, HOBT, NMM, MeCN. (f) LiOH, aq. THF. (g) HCl, CHCl$_3$.

Racemic 3-substituted β-amino esters were purchased or prepared by using a modified Knoevenagel procedure (RCHO/ammonium acetate/malonic acid).[11] Biological testing of racemic solid-phase targets revealed highly active Fg antagonists of interest for enantiospecific, solution-phase, scale-up synthesis. Enantiomerically enriched 3-aryl-3-amino esters were synthesized via Michael addition of (*R*)-1-phenylethylamine to arylacrylates (Eq 2).[12] Lithium acetylide addition to 4-benzoyloxy-2-azetidinone, followed by ring-opening and chiral chromatographic resolution of the resultant *O*-methylmandelamide, expedited preparation of acetylene intermediates.[13,14] Racemic 3-aryl-3-aminopropanoic acids were also resolved as their phenylacetamide derivatives by using penicillin amidase (3-quinoline and 2-thiophene entries).[15] All compounds gave satisfactory MS data; the 4-6 compounds per 12-compound array checked by ^{1}H NMR were also satisfactory, with purities of 85-95%. Compounds **5**, **6**, **10**, and **23-27** were fully characterized by ^{1}H NMR, MS, and elemental analysis.

A representative case involving asymmetric Michael addition[12] is illustrated for **25** (2). Addition of (*R*)-(+)-1-phenylethylamine to ethyl 3,4-methylenedioxybenzeneacrylate at -78°C proceeded with >90% diastereomeric excess. Hydrogenolytic removal of the α-methylbenzyl group was followed by coupling with Boc-(*R*)-(-)-nipecotic acid[16] by the agency of 2-(1H-benzotriazol-1-yl)tetramethyluronium hexafluorophosphate (HBTU) to afford a Boc-nipecotamide intermediate, which was N-terminally deprotected and coupled with Boc-piperidinepropanoic acid. Purification of **25** (as well as other species) was performed on the fully protected

coupling product by silica gel chromatography; **25** was isolated in 17% overall yield as an HCl salt [satisfactory microanalysis for C, H, N, Cl, and water (Karl Fischer analysis)].

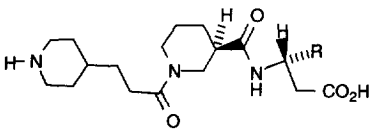
Discussion of Biological Activity

Compounds **1-27** represent more than 250 solid-phase synthesis targets that were tested against both Fg binding and platelet aggregation (Table 1). As precedented in this field,[7] antiplatelet data were correlated better with in vivo activity. Systematic changes at piperidine linker "Y" indicated that a tertiary amide at this site was preferred (e.g., **1**, platelet aggregation). While 12 β-amino acid variants of urethane (**2**) or urea (**3**) linkers were prepared on the resin, sulfonamide (**4**), *N*-methylpiperidine (**5**), and piperazine (**6**) examples represent singular samplings from solution-phase synthesis. Since the preferred central ring turned out to be nipecotic acid (n = 1; viz. **1**), about 180 variants were prepared while twelve each of examples **7-9** (Y = $COCH_2$) were isolated and tested. The initial 60 products established the N-terminal piperidinepropionyl-nipecotyl system as optimal for subsequent synthesis concurrent with early β-amino acid variation/improvement. The Fg binding assay of initial, diverse α-, β-, or N-substituted β-amino acid variants **12-22** indicated potential activity improvement for 3-alkyl (**13** and **14**), 3-aryl (**15**), or 2-oxy (**18**) substitutions. Aspartate methyl ester **16** was related to the "3-alkyl" case since its diacid derivative was inactive (IC_{50} > 25 μM). The subsequent synthesis and testing of 29 alkyl, alkenyl, and alkynyl 3-substituted compounds gave antagonists with up to four-fold potency improvement (e.g., **23** and **24**; Table 2) over the active enantiomer of RWJ-50042 (i.e., **10**). Large hydrophobic groups in this part of the molecule afforded the best activity; thus, numerous 3-aryl cases were subsequently prepared (120 compounds synthesized).

This parallel synthesis methodology rapidly yielded more than 15 analogues of **1** with potential for in-depth advanced study; they were targeted for enantiospecific scale-up synthesis and in vivo evaluation (some shown in Table 2). From an in vitro standpoint, some of the most promising compounds were the diastereomeric mixtures of the 3-(3,4-methylenedioxyphenyl) and 3-(3-quinoline) congeners. Indeed, enantiospecific synthesis of these Fg receptor antagonists afforded the most potent compounds of the series: the 3(*S*) enantiomers, **25** and **26**. 3-Aryl-β-aminopropionic acid analogues with the *R* absolute configuration exhibited ca. 100-fold weaker activity (data not shown). According to Fg binding data, **25** is 60 times more potent than **1**. In dog platelet-rich plasma, **10**, **24**, **25**, and xemlofiban[14] inhibited collagen-induced aggregation (n = 3) with IC_{50} values of 0.41, 0.45, 0.015, and 1.20 μM, respectively.[4] On oral administration to dogs, **25** inhibited ADP-induced platelet aggregation (ex vivo) with an ED_{50} value of 3 mg/kg and a duration of greater than 180 min.[4]

In conclusion, solid-phase parallel synthesis nicely facilitated lead optimization for the nipecotamide series of GPIIb/IIIa antagonists. First, it yielded several potent analogues of RWJ-50042 in a rapid fashion, whereas classical solution-phase chemistry would have required much more time and resources. Second, the number of improved antagonists obtained permitted the expeditious comparison of other critical in vivo properties, such as oral absorption, plasma half-life, and duration of action (to be published separately). Overall, the discovery-to-development cycle time was significantly compressed relative to traditional medicinal chemistry approaches.

Table 2. In vitro data for 3-substituted β-amino acid GPIIb/IIIa antagonists.



Cmpd	R	Pl. Aggr.[a] IC_{50} (μM)	Bndg.[b] IC_{50} (nM)
10	H	0.3 ± 0.08	6 ± 2
23	C≡C-Ph	0.08 ± 0.08	3 ± 0.1
24	C≡C-*t*-Bu	0.08 ± 0.02	21 ± 21
25	3,4-MD-Ph[c]	0.02 ± 0.01	0.5 ± 0.2
26	3-quinoline	0.02 ± 0.003	0.1 ± 0.03
27	2-thiophene	0.09 ± 0.02	0.1 ± 0.04
Xemlofiban (SC-54684)[d]		0.3 ± 0.1	2 ± 1

a. Same as for Table 1. b. Same as for Table 1. c. MD = methylenedioxy. d. Ref. 14.

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16. Although (*R*)-(-)-nipecotamides were identified early as being more potent than their epimeric counterpart,[3] racemic allyl nipecotate was employed in the high-throughput matrix synthesis. Boc-(*R*)-(-)-nipecotic acid was prepared in two steps from (*R*)-(-)-nipecotic acid ethyl ester (Chemi S.p.A.).

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