Non-Peptide Glycoprotein IIb/IIIa Antagonists. 11. Design and *in Vivo* Evaluation of 3,4-Dihydro-1(1*H*)-isoquinolinone-Based Antagonists and Ethyl Ester Prodrugs

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The structure−activity relationship of a series of orally active glycoprotein IIb/IIIa antagonists containing a nitrogen heterocycle grafted onto a 3,4-dihydro-1(1*H*)-isoquinolinone core is described. These compounds are structurally novel analogs of the progenitor compound **1** (L-734,217, [[3(*R*)-[2-(piperidin-4-yl)ethyl]-2-oxopiperidinyl]acetyl]-3(*R*)-methyl-*β*-alanine) in which the lactam chiral center has been removed. The 4-piperazinyl- and 4-piperidinyl-substituted 3,4-dihydro-1(1*H*)-isoquinolinones were found to be optimal for *in vitro* potency. In addition, substitution at the 3-position of the *β*-amino acid enhanced potency with the 3-pyridyl and 3-ethynyl analogs being the most potent prepared. Attempts to improve the *in vivo* profile of these compounds focused on modification of the physical properties. Ester prodrugs were prepared to increase the lipophilicity and remove the zwitterionic nature of the antagonists. The prodrug approach, coupled with the arylpiperazine terminus (pK_a = ∼9.0), afforded moderately basic and relatively nonpolar compounds. The acid *N*-[[7-(piperazin-1-yl)-3,4-dihydro-1(1*H*)-oxoisoquinolin-2-yl]acetyl]-3(*S*)-ethynyl-*β*-alanine, **6d** (L-767,679), is a potent fibrinogen receptor antagonist able to inhibit the ADP-induced aggregation of human gel-filtered platelets with an IC_{50} of 12 nM. Although **6d** is orally active based on the results of an *ex vivo* dog assay at 0.3 mg/kg, the ethyl ester prodrug of this compound, **19** (L-767,685), is better absorbed at this dose than **6d**. Upon oral dosing, the ester **19** is converted to **6d** *in vivo* in dog with an estimated oral systemic availability of >17% (0−8 h, $AUC_{\mathbf{19}po}/AUC_{\mathbf{6d}iv}$). In addition, studies in monkey at an oral dose of 1 mg/kg show that **19** affects the complete inhibition of the *ex vivo* platelet aggregation in response to ADP between 2 and 8 h postdose with the level of inhibition remaining at 40% at 12 h postdose. This level of activity was superior to that observed for **6d** and **1** at the same dose. Using *ex vivo* ADP-induced aggregation data from rhesus monkey (n = 2, 0−8 h using the $AUC_{\mathbf{19}po}/AUC_{\mathbf{6d}iv}$), the estimated systemic oral availability of **6d** when dosed as **19** is 32%.

Introduction

The hemostatic system is designed to maintain fluid blood flow under normal physiological conditions while being capable of reacting rapidly to form a clot to seal sites of vascular damage. When vascular injury occurs, subendothelial adhesion proteins such as collagen and von Willebrand factor are exposed leading to subsequent platelet adherence and activation. Activated platelets secrete agonists such as ADP, serotonin, and thromboxane A2 which promote further activation of platelets. The symmetrical protein fibrinogen bridges platelets to form the hemostatic plug.[1] This process may also occur when an atherosclerotic plaque is ruptured, and the resulting thrombus can lead to heart attacks, strokes, and angina.[2] Thus, a method to inhibit the formation of a thrombus would have wide clinical significance.[3]

The binding of fibrinogen to platelet glycoprotein (GP) IIb/IIIa is the final obligatory step in the platelet aggregation cascade and is independent of the numerous activation mechanisms. For this reason, it has been identified as an attractive target for the development of novel antithrombotics.[4] It has been shown that a recognition sequence present in fibrinogen involves the subunit arginine-glycine-aspartic acid (RGD). Thus small molecule mimetics of RGD (which are capable of antagonizing the GP IIb/IIIa receptor) should prevent the fibrinogen-mediated cross-linking of platelets. Indeed, a GP IIb/IIIa specific antibody[5] has been shown to be clinically effective in this regard,[6] and currently a number of RGD mimetics are also undergoing clinical evaluation.[7−16]

Recently, we have described the design and synthesis of novel, low molecular weight, and orally active fibrinogen receptor antagonists.[10] From this work, the lactam **1** (L-734,217) was selected for further evaluation in clinical trials. Utilizing this compound as a starting point, we have continued to focus on the preparation of structurally simple antagonists which display good oral bioavailability. In this paper, we will present the structure−activity relationships (SAR) and *in vivo* evaluation of a novel series of antagonists employing an achiral bicyclic core. From this study, the 3,4-dihydro-1(1*H*)-isoquinolinone (benzolactam) acid **6d**, as the ethyl ester prodrug **19**, was found to be orally active in both dog and monkey at doses lower than that required for **1** to produce a similar response.

Chemistry

The chemistry for the construction of the benzolactam core and the subsequent elaboration to the target compounds is shown in Scheme 1. Alternative hetero-

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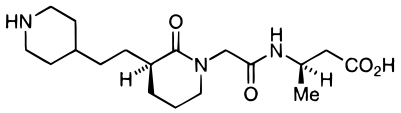
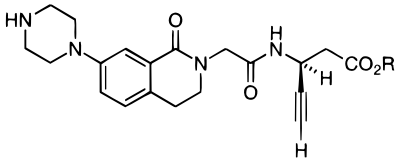


1 (L-734,217)



6d R = H (L-767,679)
19 R = Et (L-767,685)

cyclic core structures were synthesized according to the routes given in Schemes 2−5. For all the antagonists, standard methodologies were used for the preparation of substituted β-alanines.[17]

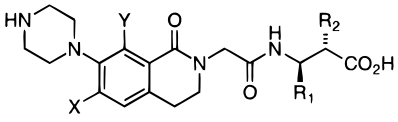
The synthesis of the key intermediate benzolactam **3a** commences with **2a** and employs previously described chemistry.[18] The piperazine was then installed using bis(chloroethyl)amine hydrochloride in *n*-butanol/H_2O with 1 N NaOH, the product was protected as the Boc derivative (52% for two steps), and subsequent alkylation with ethyl bromoacetate yielded **4a** in 85% yield. In a similar fashion, **4b** was prepared from **2b**. Chlorination of **4a** using *N*-chlorosuccinimide in THF yielded a 3:1 mixture of the 8-chloro (**5b**) to 6-chloro (**5a**) products which were separable by silica gel chromatography and identified by NMR. Finally, hydrolysis of either **4** or **5** followed by a peptide coupling with the appropriate β-alanine derivative,[19] deprotection, and HPLC purification then gave **6a**−**k** (see Table 1).

Compound **8a**, containing a morpholine terminus, was derived from **3a** by condensation with bis(bromoethyl) ether in DMF followed by alkylation with *tert*-butyl bromoacetate. Alternatively, transformation of the amine of **3a** to the iodide, alkylation with ethyl bromoacetate, and then a palladium-catalyzed conversion of iodine to tributyltin yielded the aryltin intermediate **7**. The remaining compounds (**8b**−**d**) were obtained via cross-coupling reactions of **7** with either 4-iodoimidazole[20] (to give **8b**) or 4-bromopyridine (to give **8c**). Catalytic reduction of **8c** and protection yielded the piperidine analog **8d**. Standard chemical transformations, as before, allowed the conversion of **8a**−**d** to **9a**−**d**, and the compounds prepared in this manner are listed in Table 2.

A series of analogs with variations in the bicyclic heterocyclic nucleus were also synthesized, and the key steps are outlined as follows. Scheme 2 shows the preparation of the dehydrobenzolactam analog **11a** via **10**. Dehydrogenation of the benzolactam **4a** was achieved using 10% Pd on carbon in cyclohexene/ethanol to give **10** in 95% yield.

For the tetrahydroisoquinoline analog **11b** (Scheme 3), the direct reduction of various advanced benzolactam intermediates was unsuccessful, so the tetrahydroisoquinoline core was constructed first. Thus, the trifluoroacetamide **12** was reacted with paraformaldehyde in HOAc and H_2SO_4 to give (after hydrolysis of the trifluoroacetamide) a 75% yield of the desired tetrahydroisoquinoline.[21] This was alkylated with ethyl bromoacetate in Et_3N/CH_2Cl_2 and the nitro group reduced catalytically to produce **13** in near-quantitative yield.

Table 1

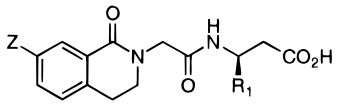


6

compd	X	Y	R_1	R_2	inhibition of aggregation[a] IC_{50} (nM)
1					32
6a	H	H	H	H	140
6b	H	H	Me	H	30
6c	H	H	$Ph(CH_2)_2$	H	16
6d	H	H	C≡CH	H	12
6e	H	H	3-pyridyl	H	13
6f	H	H	3-pyridyl *N*-oxide	H	18
6g	H	H	H	NH_2	630
6h	H	H	H	$NHSO_2Ph$	140
6i	F	H	C≡CH	H	15
6j	Cl	H	C≡CH	H	19
6k	H	Cl	C≡CH	H	35

[a] *In vitro* inhibition of human gel-filtered platelets using ADP agonist. See the Experimental Section for details.

Table 2



9

cpd	Z	R_1	Inhibition of aggregation[a] IC_{50} (nM)
9a		3-pyridyl	>300,000
9b		3-pyridyl	320
9c		C≡CH	160
9d		C≡CH	6
6d		C≡CH	12

[a] *In vitro* inhibition of human gel-filtered platelets using ADP agonist. See the Experimental Section for details.

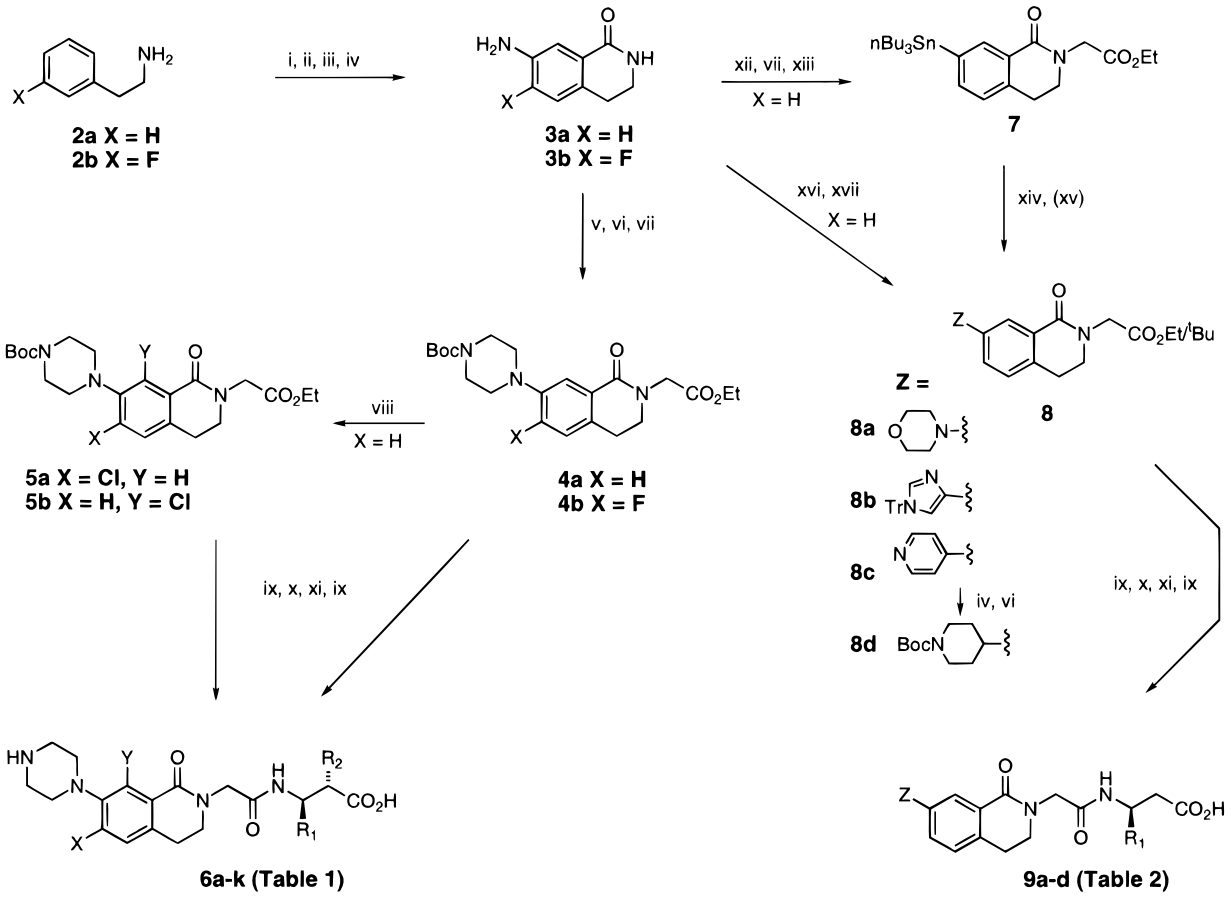
The preparation of the quinazolinone **11c** commences with chloronitrobenzoic acid **14** (Scheme 4) which was fused with piperazine hydrochloride to install the piperazine group. Protection with Boc anhydride and coupling with ethylglycine afforded **15** which was reduced and ring closed to yield the core quinazolinone **16**.

Finally, Scheme 5 outlines the preparation of the quinazolinedione **11d**. In this case, the quinazolinedione ring was constructed by first coupling **17** with ethylglycine and then cyclization with carbonyldiimidazole. Reduction of the nitro group then yielded **18** which was elaborated (piperazine formation and peptide coupling) as before to give the target compound.

Results and Discussion

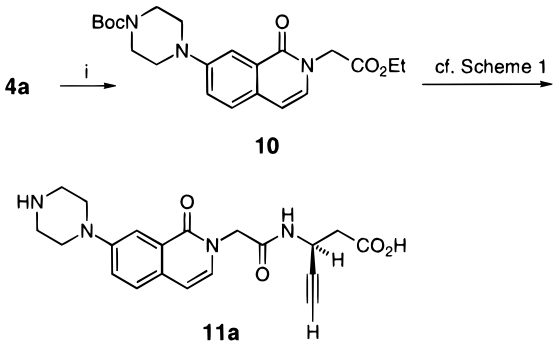
The compounds described were evaluated in a platelet aggregation assay to assess the ability to inhibit ADP-induced aggregation of human gel-filtered platelets (GFPs). The results are given as IC_{50}s (concentration required for 50% inhibition) and shown in Tables 1−3.

Scheme 1[a]



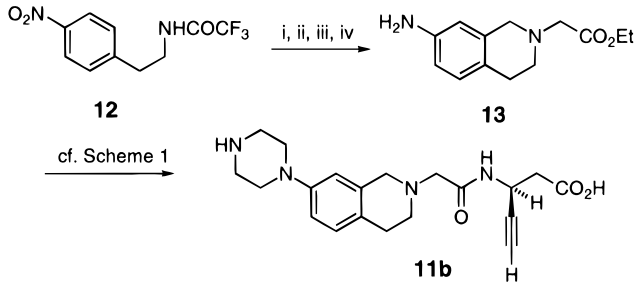
[a] Reagents: (i) EtOCOCl, Et_3N, THF; (ii) PPA, 150 °C; (iii) KNO_3, H_2SO_4; (iv) 10% Pd−C, H_2, MeOH; (v) $HN(CH_2CH_2Cl)_2$·HCl, *n*-BuOH, 110 °C; (vi) $(Boc)_2O$, K_2CO_3, THF, H_2O; (vii) NaHMDS, THF, $BrCH_2CO_2Et$; (viii) NCS, THF; (ix) 1 N LiOH, MeOH; (x) β-alanine ester, EDC, HOBT, Et_3N, DMF; (xi) HCl(g), EtOAc; (xii) $NaNO_2$, H_2O, 3 N HCl, KI; (xiii) $(n\text{-}Bu_3Sn)_2$, $(PPh_3)_4Pd$, 1,4-dioxane, 80 °C; (xiv) haloheteroaryl, $(PPh_3)_4Pd$, DMF, 120 °C; (xv) trityl chloride, Et_3N, DMF; (xvi) $O(CH_2CH_2Br)_2$, DIPEA, DMF, 100 °C; (xvii) NaHMDS, THF, $BrCH_2CO_2tBu$;

Scheme 2[a]



[a] Reagents: (i) 10% Pd−C, EtOH, cyclohexane, 75 °C.

Scheme 3[a]

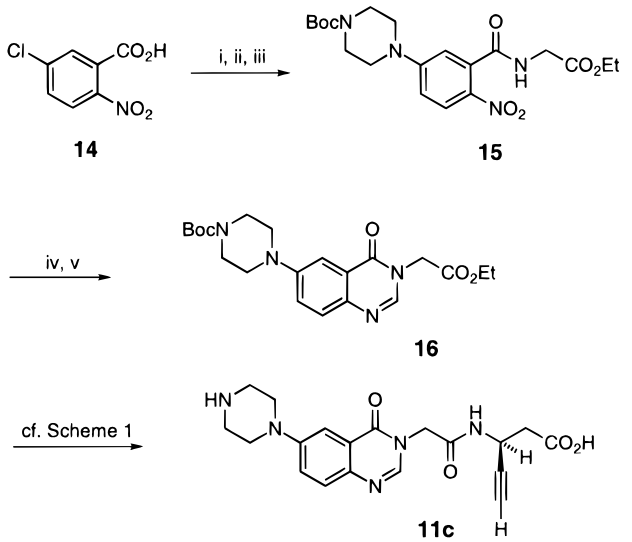


[a] Reagents: (i) HOAc, H_2SO_4, $(CH_2O)_x$; (ii) 1 N LiOH, MeOH, THF; (iii) $BrCH_2CO_2Et$, Et_3N, CH_2Cl_2; (iv) 10% Pd−C, H_2, EtOH.

Our initial attempt to constrain the piperidine side chain of **1** by incorporation of a benzene ring resulted in the achiral analog **6a** which had an IC_{50} of 140 nM. Installation of a 3(*R*)-methyl group (**6b**), to provide the conformational bias present in **1**, improved the potency further to 30 nM. That **6b** and **1** were equipotent was a gratifying result as this achieved our initial goal of simplifying the molecule by removing a chiral center. In addition, this modification yielded a compound in which the basicity of the N-terminus is considerably reduced compared to that of **1**. The pK_a of the piperidine is ~11, while the aryl piperazine is about 2 log units lower at ~9.[22] It was hoped that this reduced basicity could be harnessed to boost the oral performance of these compounds (vide infra).

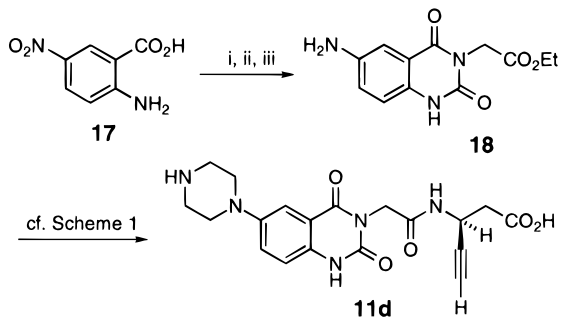
Several other 3-substituted β-alanine analogs were prepared for testing since substitution at this site has been shown to significantly enhance potency. Thus, incorporation of a phenethyl moiety afforded **6c** (16 nM) which is about 2-fold more potent than **6b**. The β-acetylene derivative **6d** and the β-3-pyridyl derivative **6e** were found to be equipotent in the platelet aggregation assay at 12 and 13 nM, respectively. The excellent potency-enhancing effect achieved with these two substituents was originally discovered by workers at Monsanto-Searle[11,19c] culminating in the discovery of xemilofiban which is a prodrug in phase III clinical trials as an oral agent.[11] In fact, **6d**,**e** represent the most

Scheme 4[a]



[a] Reagents: (i) piperazine, 110 °C, neat; (ii) 1 N NaOH, $(Boc)_2O$, 1,4-dioxane; (iii) $H_2NCH_2CO_2Et$·HCl, EDC, HOBT, NMM, DMF; (iv) 10% Pd−C, H_2, EtOH; (v) $HC(OEt)_3$, reflux.

Scheme 5[a]



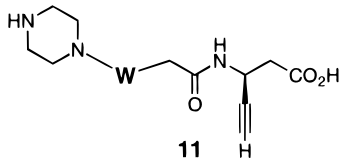
[a] Reagents: (i) $H_2NCH_2CO_2Et$·HCl, EDC, HOBT, NMM, DMF; (ii) CDI, THF, reflux; (iii) 10% Pd−C, H_2, EtOH.

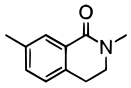
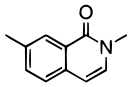
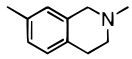
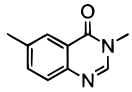
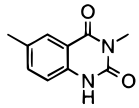
potent compounds prepared in this series to date. Interestingly, the pyridine *N*-oxide **6f** is also very potent (IC_{50} = 18 nM) indicating that the receptor will tolerate both lipophilic (e.g., phenethyl) and very polar substituents at this position.

In previous publications, we reported on a novel series of fibrinogen receptor antagonists which employ a sulfonamide α to the C-terminus acid.[9a,23] The sulfonamide appears to bind noncovalently to the GP IIb/IIIa receptor at an exosite and confers, in certain cases, a considerable enhancement in potency. It was, therefore, of interest to see if this effect could be utilized in the benzolactam series. Accordingly, the sulfonamide **6h** and the corresponding amine **6g** were prepared. As can be seen by comparison of the unsubstituted analog **6a** with the amine **6g** (140 versus 630 nM), the presence of an α-amino group has a detrimental effect on potency. Addition of the sulfonamide functionality to give **6h** gives an antagonist of modest potency (IC_{50} = 140 nM). Unfortunately, this is considerably less potent than the 3-substituted *β*-alanine analogs described herein, and clearly the sulfonamide group is not a general solution to the problem of potency enhancement for fibrinogen receptor antagonists.

Substitution of the aromatic ring with F or Cl was undertaken to deactivate this electron rich system and to probe the steric effects between the heteroatom and the piperazine ring. These compounds were all made

Table 3



cpd	W	Inhibition of aggregation[a] IC_{50} (nM)
6d		12
11a		81
11b		320
11c		330
11d		27

[a] *In vitro* inhibition of human gel-filtered platelets using ADP agonist. See the Experimental Section for details.

using the 3-alkynyl-*β*-alanine C-terminus. The 6-F (**6i**) and 6-Cl (**6j**) derivatives are equipotent to the parent compound **6d**. In contrast, the 8-Cl analog **6k** is 3 times less potent than **6d** (IC_{50} = 35 compared to 12 nM) at inhibiting the aggregation of GFPs. Presumably, the presence of the chlorine at C-8 causes the piperazine to adopt a less favorable conformation or interferes (sterically or electronically) with an interaction between the lactam amide and the receptor.

Examination of a series of heterocycles at the N-terminus (Table 2) shows clearly the requirement for a basic nitrogen to obtain very potent inhibitors. The morpholine analog **9a**, wherein the oxygen atom replaces the piperazine nitrogen, is inactive at 300 μM. Moderately basic heterocycles such as imidazole (**9b**) and pyridine (**9c**) have modest potency in the platelet aggregation assay, whereas the highly basic piperidine **9d** is roughly equipotent to that of the corresponding piperazine **6d**.

Replacement of the benzolactam core with alternative bicyclic heterocycles (as shown in Table 3) was also investigated. Dehydrogenation of the lactam ring afforded **11a** which has an IC_{50} = 81 nM and is approximately 4-fold less potent than the parent compound **6d**. The detrimental effect of removing the carbonyl group of **6d** is demonstrated with the amine **11b**. In this case, **11b** is significantly less potent (IC_{50} = 320 nM) than **6d**, and this may reflect the altered conformation of the side chain or, perhaps, the fact that a basic site in the center of the molecule gives rise to unfavorable ionic interactions. Interestingly, the quinazolinone **11c** is also a relatively poor fibrinogen receptor antagonist, whereas the close analog quinazolinedione **11d** is some 10 times more potent (IC_{50}s of 330 and 27 nM, respectively). Calculations show that

there is little difference in the electronic character of the lactam carbonyl present in compounds **6d** and **11a**−**d**; therefore, the observed differences in relative potencies may reflect subtle differences in the conformations of the side chains. Nevertheless, the results of the SAR studies presented show that the benzolactam core in conjunction with a 3-substituted β-alanine and a piperazine or piperidine N-terminus yields compounds that are superior in potency to the lactam **1**. In addition, the asymmetric center present in the lactam of **1** has been removed and the N-terminus side chain constrained by appending an aromatic ring. This has the additional effect of reducing the basicity of the N-heterocycle, especially in the case of the arylpiperazine which is approximately 2−3 log units lower in pK_a[22] than the piperidine of lactam **1**.

The oral profile of the more potent inhibitors described herein (deemed as those with an IC_{50} < 30 nM, i.e., **6b**−**e**,**i**,**j**, **9d**, and **11d**) was assessed in dogs by measuring the inhibition of the extent of *ex vivo* ADP-induced aggregation of platelet rich plasma as a function of time. Compounds were screened with an oral dose of either 0.3, 0.5, or 1 mg/kg (n = 2−5), and selected compounds were also evaluated as the corresponding ethyl ester prodrug. The results are displayed in Table 4. All of the parent acids showed good peak activity (maximum inhibition >80%), with the exception of **6c** and **11d** which are essentially inactive at this dose. The time to reach peak inhibition of aggregation (T_{max}) was also fairly constant, with all compounds achieving a T_{max} between 1 and 3 h. Unfortunately, the duration of activity was less than desired as, in all cases, the inhibitory effects returned to base line within 6−8 h.

We next examined the effect of ester prodrugs of several of these compounds to see if this would lead to increased absorption and a prolonged duration of action. Indeed, there are several fibrinogen receptor antagonist prodrugs that show enhanced absorption and/or duration which are currently under clinical investigation.[11,13,14a,15] By masking the carboxylate functionality as an ester, the zwitterionic character is removed and the polarity of the molecule is therefore attenuated. Both of these factors may aid absorption. In addition, for those compounds containing an arylpiperazine moiety, it was postulated that an ester prodrug may be better absorbed due to the relatively low basicity of the piperazine.

Of the original compounds investigated, the ethyl esters of **6d**,**e**,**i**, **9d**, and **11d** were prepared. In all cases, the esters prepared had an IC_{50} of >1 μM in the platelet aggregation assay. In addition, the log P[24] of the parent acids were approximately 2−3 orders of magnitude lower than that of the corresponding ethyl ester. For example, the acid **6d**, with a log P of less than −3.3, is considerably more polar than the ethyl ester prodrug **19**, log P of −0.85. To examine the effect of the ester prodrug *in vivo*, the prodrugs were dosed orally in dogs, and the results are included in Table 4. As with the quinazolinedione acid **11d**, the ethyl ester **23** showed no *ex vivo* activity despite the relatively high dose of 1 mg/kg. Of the arylpiperazine-containing compounds (**6d**,**e**,**i**), the β-pyridyl analog **20** (ester of **6e**) was disappointing as the activity was less than that observed for the free acid when given at the same dose. In contrast, both the β-acetylene derivatives **19** and **21** (esters of **6d**,**i**, respectively) showed excellent activity. In particular, dogs dosed at 0.5 mg/kg with **19** were completely inhibited from 2.5 h until the end of the 8 h experimental period. The 6-F analog **21** also showed complete inhibition at 8 h using the same dose, but in this case, the time to peak activity was longer (7 h). Interestingly, **22**, the ethyl ester of piperidine analog **9d**, showed poor activity when dosed orally at 0.3 mg/kg. The contrast in behavior between the ethyl esters **19** and **22** perhaps reflects a difference in the absorption of the two compounds. This may be related to the increased polarity and basicity of the arylpiperidine of **22** (log P = −1.54 in comparison to the arylpiperazine of **19**, log P = −0.85) which diminishes the ability of **22** to cross the intestinal wall.

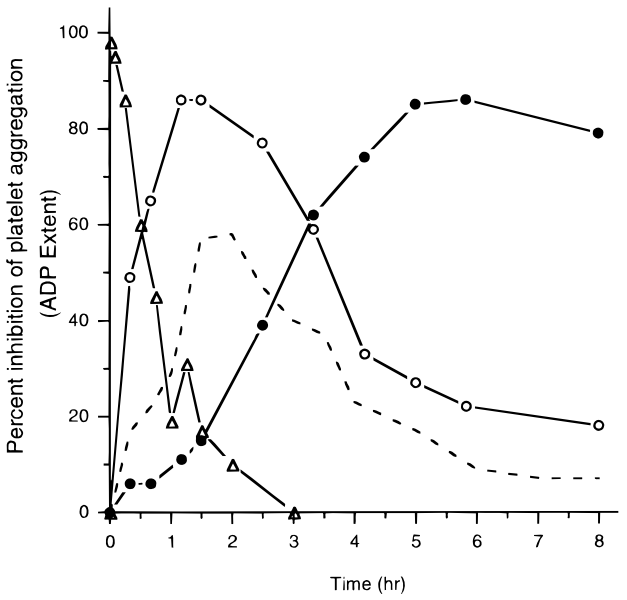


Figure 1. Effect of drug on *ex vivo* platelet aggregation response to ADP agonist expressed as percent inhibition over time (h) in conscious dogs. Drug was administered as follows: 0.3 mg/kg po (n = 5) for (− −) **1**, (○) **6d**, and (●) **19**; 0.01 mg/kg iv bolus (n = 3) for (△) **6d**.

Table 4. Effect of Compound on Inhibition of the Extent of *ex Vivo* ADP-Induced Aggregation in Canine Platelet Rich Plasma

free acid				ethyl ester prodrug			
compd	po dose (mg/kg)	max inhibition (%)	T_{max} (h)	compd	po dose (mg/kg)	max inhibition (%)	T_{max} (h)
6b	0.5	100	1				
6c	0.5	20	0.5				
6d	0.3	80	1.5−3	**19**	0.5	100	2.5−8
6e	0.5	100	1−4	**20**	0.5	75	2.5
6i	0.5	95	1−3	**21**	0.3	100	7−8
6j	0.5	80	1−1.5				
9d	0.3	100	1−4	**22**	0.3	40	4
11d	0.5	0		**23**	1.0	0	

Due to the excellent activity of **6d** when dosed orally as the ethyl ester prodrug **19** at 0.5 mg/kg, we decided to evaluate this compound in depth and demonstrate that it has potential for b.i.d. dosing in two species (dog and monkey) at a lower dose than that required for **1**. Figure 1 shows the effects in dog on *ex vivo* platelet aggregation in response to ADP for **1**, **6d**, and **19**. When **6d** was administered orally at 0.3 mg/kg (n = 5), the inhibition of ADP-stimulated platelet aggregation peaked at 85% from 1 to 1.5 h and fell rapidly back to base line levels by 8 h. However, when **19** was administered orally at the same dose (n = 5), the degree of inhibition

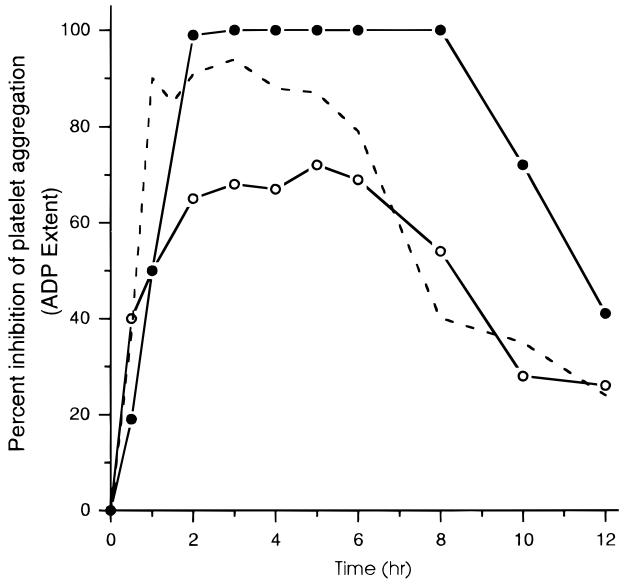


Figure 2. Effect of drug on *ex vivo* platelet aggregation response to ADP agonist expressed as percent inhibition over time (h) in conscious rhesus monkeys. Drug was administered as follows: 1.0 mg/kg po for (− −) **1** ($n = 2$), (○) **6d** ($n = 3$), and (●) **19** ($n = 2$).

was equal to or greater than 75% from 4 to 8 h. In contrast, **1** did not perform as well reaching a maximum inhibition of 55% at 2 h with the effect lost at 6 h. For an estimate of the systemic oral availability of **19**, the acid **6d** was given at 0.01 mg/kg iv ($n = 3$). Maximum inhibition was observed at the first time point (100% inhibition at 5 min), and by 2 h the level of inhibition had returned to base line. From the AUC data, the systemic oral availability (0−8 h) of **6d** is 13% and, when dosed orally as the ethyl ester prodrug **19**, is increased to at least 17%. This is a grossly underestimated value since, at the end of the study at 8 h, the level of inhibition for **19** is still 80%. In fact, in a separate experiment in which two dogs were dosed orally with **19** (same dose, 0.3 mg/kg) and monitored over 12 h, the profile was similar to that shown in Figure 1 and the level of inhibition at 10 and12 h was 64% and 51%, respectively. Thus it appears that **19** is suitable for b.i.d. dosing in dog.

A similar study was performed in rhesus monkey comparing **1**, **6d**, and **19** at an oral dose of 1 mg/kg during a 12 h protocol. The effects on *ex vivo* platelet aggregation in response to ADP are shown in Figure 2. At this dose, **6d** achieved a maximum inhibition of 65−70% between 2 and 6 h falling below 30% inhibition at 10 h. In contrast, the prodrug **19** showed full inhibition of aggregation between 2 and 8 h, declining to 40% inhibition by 12 h after dosing. This result with a single dose suggests that **19** may be suitable for b.i.d. dosing in primates. The lactam **1** did not perform as well as **19**, since the peak activity of **1** occurred between 1 and 5 h and the level of inhibition dropped below 40% at 8 h. In a separate 8 h study (data not shown) in rhesus monkey ($n = 2$, cross-over using 0.25 mg/kg iv **6d** and 1.25 mg/kg po **19**), the systemic oral availability (using the $AUC_{\mathbf{19}po}/AUC_{\mathbf{6d}iv}$ of the inhibition of ADP-stimulated platelet aggregation) was estimated to be 32%. Thus, the ethyl ester **19** is well absorbed and converted *in vivo* to the acid **6d** in both dog and rhesus monkey.

In conclusion, we have discovered a novel series of orally active fibrinogen receptor antagonists containing a piperazine grafted onto a benzolactam core. These compounds are structural analogs of the progenitor compound lactam **1** which embody only one chiral center. Substitution at the 3-position of the β-amino acid enhanced potency with the 3-pyridyl (**6e**) and 3-ethynyl (**6d**) congeners being the most potent prepared. Attempts to improve the *in vivo* profile of these compounds focused on modification of the physical properties. Ester prodrugs were prepared to increase the lipophilicity and remove the zwitterionic nature of the antagonists. This, coupled with the arylpiperazine terminus ($pK_a = \sim 9.0$), afforded moderately basic and relatively nonpolar compounds. Thus, although the acid **6d** is orally active in the inhibition of *ex vivo* aggregation in the dog and monkey, the ethyl ester prodrug of this compound (**19**) appears better absorbed in both species and is converted to **6d** *in vivo*. The estimated oral systemic availability of **6d** (when dosed as **19**) is >17% in dog and 32% in rhesus monkey. In addition, in both dog and rhesus monkey the ester **19** appears suitable for b.i.d. administration, and it has been demonstrated to be more efficacious in these species than **1** at the same dose.

Experimental Section

***In Vitro* Inhibition of Gel-Filtered Platelets Assay.** Human blood was collected into 0.1 vol of acid citrate/dextrose, and platelets were isolated by differential centrifugation followed by gel filtration on Sepharose 2B in divalent ion-free Tyrode's buffer (pH 7.4) containing 2% bovine serum albumin.

Platelet aggregation was measured at 37 °C in a Chronolog aggregometer. The reaction mixture contained gel-filtered human platelets (2×10^8/mL), fibrinogen (100 μg/mL), Ca^{2+} (1 mM), and the compound to be tested. The aggregation was initiated by adding 10 μM ADP 1 min after the other components were added. The reaction was then allowed to proceed for at least 2 min. The extent of inhibition of aggregation is expressed as the percentage of the rate of aggregation observed in the absence of inhibitor. The IC_{50} is the dose of a particular compound inhibiting aggregation by 50% relative to a control lacking the compound.

Experimental Protocol for *in Vivo* Studies. Conscious, purpose-bred mongrel dogs of either sex (8−12 kg) were administered test compounds as an intravenous bolus (vehicle = saline) or orally by gastric lavage (vehicle = water). During these studies the dogs rested comfortably in nylon slings. At specified time points, 5 mL blood samples were drawn from either saphenous or cephalic veins (0.38% sodium citrate, final concentration) for the measurement of *ex vivo* platelet aggregation responses to ADP and for whole blood platelet counts.[25] Blood samples were obtained before compound administration (base line) and at 1, 5, 15, 30, 45, 60, 75, 90, 120, and 180 min after dosing for the iv studies and at 20, 40, 70, 90, 150, 200, 250, 300, 350, and 480 min after dosing for the oral studies in dogs. Compounds were also administered orally by nasogastric lavage (vehicle = water) to conscious rhesus monkeys (5−7 kg) which were placed in primate restraint chairs. Blood samples (3 mL) were obtained before compound administration (base line) and at 30, 60, 120, 180, 240, 300, 360, 480, 600, and 720 min after dosing.

Measurement of *ex Vivo* Platelet Aggregation. Platelet rich plasma (PRP) was prepared by centrifugation of whole blood at 150*g* for 5 min, and the platelet concentration was adjusted to 2×10^8 platelets/mL with time-matched platelet poor plasma (PPP). PRP (300 μL, 2×10^8 platelets/mL) was incubated at 37 °C for 3 min prior to the addition of agonist. Platelet aggregation was measured by the change in light transmittance (PPP represents 100%) under stirring conditions (1100 rpm) at 37 °C in a Biodata platelet aggregation profiler,

Table 5. Analytical Data

compd	formula	analysis[a]
6a	$C_{18}H_{24}N_4O_4 \cdot 0.9H_2O \cdot 1.65TFA$	C, H, N
6b	$C_{19}H_{26}N_4O_4 \cdot 1.7H_2O \cdot 1.45TFA$	C, H, N
6c	$C_{26}H_{32}N_4O_4 \cdot 1.0H_2O \cdot 2.1TFA$	C, H, N
6d	$C_{20}H_{24}N_4O_4 \cdot 0.25H_2O \cdot 1.55TFA$	C, H, N
6e	$C_{23}H_{27}N_5O_4 \cdot 2.0H_2O \cdot 2.65TFA$	C, H, N
6f	$C_{23}H_{27}N_5O_5 \cdot 0.9H_2O \cdot 2.4TFA$	C, H, N
6g	$C_{18}H_{25}N_5O_4 \cdot 2.05H_2O \cdot 2.05TFA$	C, H, N
6h	$C_{24}H_{29}N_5O_6S \cdot 1.0H_2O \cdot 1.8TFA$	C, H, N
6i	$C_{20}H_{23}N_4O_4F \cdot 0.4H_2O \cdot 1.35TFA$	C, H, N.
6j	$C_{20}H_{23}N_4O_4Cl \cdot 1.25H_2O \cdot 1.75TFA$	C, H, N
6k	$C_{20}H_{23}N_4O_4Cl \cdot 0.15H_2O \cdot 1.45TFA$	C, H, N
9a	$C_{23}H_{27}N_4O_5$	*b*
9b	$C_{22}H_{21}N_5O_4 \cdot 1.2H_2O \cdot 1.65TFA$	C, H, N
9c	$C_{21}H_{19}N_3O_4 \cdot 0.25H_2O \cdot 1.2TFA$	C, H, N
9d	$C_{21}H_{25}N_3O_4 \cdot 0.8H_2O \cdot 1.35TFA$	C, H, N
11a	$C_{20}H_{22}N_4O_4 \cdot 0.6CH_3CN \cdot 2.0TFA$	C, H, N
11b	$C_{20}H_{26}N_4O_3 \cdot 3TFA$	C, H, N
11c	$C_{19}H_{21}N_5O_4 \cdot 0.5H_2O \cdot 2TFA$	C, H, N
11d	$C_{19}H_{21}N_5O_5 \cdot 1.45TFA$	C, H, N
19	$C_{22}H_{28}N_4O_4 \cdot 2.65HCl$	C, H, N

[a] All compounds (except **9a**) characterized by analysis, mass spectra, and ^{1}H NMR. [b] Exact mass and ^{1}H NMR obtained for this compound.

Model PAP-4. Aggregation in dog PRP was initiated by the addition of 10 μM ADP + 1 μM epinephrine; epinephrine is used to enhance the aggregation response of canine platelets to other agonists. Aggregation in monkey PRP was initiated by the addition of 20 μM ADP. The extent of aggregation was reported as the peak percent of aggregation achieved based on a maximum of 100% (standardized with PPP). The effect of compound treatment on the extent and rate of aggregation is expressed as the percent inhibition using the base line, pretreatment aggregation response as 100%.

Chemistry. Proton nuclear magnetic resonance spectra were obtained on a Varian 300 spectrometer, and proton chemical shifts are relative to tetramethylsilane (TMS) as internal standard. Elemental analyses and high-resolution mass spectra were provided by the Analytical Department and the Mass Spectroscopy Department of Merck, West Point, PA. Where elemental analyses are reported only by symbols of the elements, results are within 0.4% of the theoretical values. All reactions were carried out under an argon atmosphere, and all worked-up reaction solutions were dried using $MgSO_4$.

Final compounds were purified by reverse phase HPLC eluting with a gradient of acetonitrile/water containing 0.1% TFA. Excess acetonitrile was removed *in vacuo*, and the resulting solution was frozen and lyophilized to afford the TFA salt of the desired compound as an amorphous powder.

The physical data for the final compounds (prepared as described for compounds **6d**/**19** using the appropriate β-alanine derivatives and standard peptide reactions[17,19]) are give in Table 5.

Ethyl 7-(4-*N*-Boc-piperazin-1-yl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (4a). A solution of **3a**[18] (5.0 g, 30.8 mmol) and bis(2-chloroethyl)amine hydrochloride (6.3 g, 33.9 mmol) in *n*-butanol (250 mL) was stirred at 110 °C for 3 days. The precipitate was removed by filtration to provide the starting amine as the HCl salt (3.6 g). Evaporation of the n-butanol under reduced pressure afforded a dark oil which was purified by column chromatography (silica gel; EtOH/H_2O/NH_4OH, 10:0.5:0.5) to give 7-(piperazin-1-yl)-3,4-dihydro-1(1*H*)-isoquinolinone (2.22 g). ^{1}H NMR (CD_3OD): δ 2.86 (2H, t), 3.10 (4H, m), 3.22 (4H, m), 3.44 (2H, t), 7.18 (2H, m), 7.55 (1H, m).

This piperazine (2.22 g, 9.61 mmol), $(Boc)_2O$ (2.30 g, 10.6 mmol), and K_2CO_3 (1.33 g, 9.61 mmol) were stirred in THF (20 mL) and H_2O (20 mL) for 24 h. The solution was poured into water, extracted with EtOAc (3×), washed with water and then brine, dried ($MgSO_4$), and evaporated. Chromatography of the residue (EtOAc) gave a yellow solid which was swished with ether to afford 7-(4-*N*-Boc-piperazin-1-yl)-3,4-dihydro-1(1*H*)-isoquinolinone as a white solid (2.0 g, 63%). ^{1}H NMR ($CDCl_3$): δ 1.49 (9H, s), 2.92 (2H, t, J = 6.6 Hz), 3.19 (4H, m), 3.6 (6H, m), 6.15 (1H, br s), 7.02 (1H, dd, J = 8.3, 2.7 Hz), 7.13 (1H, d, J = 8.3 Hz), 7.62 (1H, d, J = 2.7 Hz).

A solution of this isoquinolinone (2.37 g, 7.16 mmol) in THF (30 mL) at −78 °C under argon was treated with NaHMDS (1 M solution in THF; 7.88 mL, 7.88 mmol), and the temperature was allowed to rise to −50 °C over 30 min. The solution was cooled back to −78 °C, and ethyl bromoacetate (1.19 mL, 10.7 mmol) was added in one lot. After stirring at ambient temperature for 3 h, the mixture was poured into EtOAc, extracted with 1 N HCl, washed with brine (twice), dried ($MgSO_4$), and evaporated to give an oil. Column chromatography (hexane/EtOAc, 1:1 and then 1:2) gave **4a** as a solid (3.0 g, quantitative). ^{1}H NMR ($CDCl_3$): δ 1.29 (3H, t, J = 7.1 Hz), 2.98 (2H, t, J = 6.6 Hz), 3.15 (4H, m), 3.6 (6H, m), 4.21 (2H, q, J = 7.1 Hz), 4.33 (2H, s), 7.0 (1H, dd, J = 8.3, 2.4 Hz), 7.10 (1H, d, J = 8.3 Hz), 7.64 (1H, d, J = 2.4 Hz).

Ethyl 6-Chloro-7-(4-*N*-Boc-piperazin-1-yl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (5a) and Ethyl 6-Chloro-7-(4-*N*-Boc-piperazin-1-yl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (5b). To a solution of **4a** (250 mg, 0.61 mmol) in THF (11 mL) was added NCS (114 mg, 0.85 mmol), and the reaction mixture was then stirred for 48 h. The solvent was removed and the residue chromatographed (silica gel; hexane/EtOAc, 3:2) to give the first eluted compound **5a** (30 mg). ^{1}H NMR ($CDCl_3$): δ 1.27 (3H, t), 1.46 (9H, s), 3.0 (6H, m), 3.5−3.7 (6H, m), 4.23 (2H, q), 4.33 (2H, s), 7.24 (1H, s), 7.75 (1H, s). This was followed by the slower eluting compound **5b** (100 mg). ^{1}H NMR ($CDCl_3$): δ 1.27 (3H, t), 1.50 (9H, s), 3.0 (6H, m), 3.6 (6H, m), 4.23 (2H, q), 4.35 (2H, s), 7.1 (2H, s).

Ethyl 7-(Tributylstanyl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (7). To a solution of 7-iodo-3,4-dihydro-1(1*H*)-isoquinolinone (7.03 g, 25.7 mmol) in THF (125 mL) at −78 °C under argon was added NaHMDS (1 M in hexane; 28.3 mL, 28.3 mmol). The solution was warmed to −30 °C over 30 min and then cooled back to −78 °C and ethyl bromoacetate (4.27 mL, 38.6 mmol) added. After warming to room temperature, the mixture was stirred for 1 h and then partitioned between EtOAc and 10% citric acid solution. The organic extracts were washed with brine, dried, and evaporated to give an orange oil (11.6 g). Column chromatography (silica gel; hexane/EtOAc, 1:1) yielded ethyl 7-iodo-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate as a solid (9.8 g, quantitative).

The iodide (5 g, 13.9 mol), hexabutylditin (14 mL, 17.9 mmol), and tetrakis(triphenylphosphine)palladium(0) (805 mg, 0.7 mmol) were stirred in 1,4-dioxane (75 mL) at 80 °C for 16 h. After cooling, the solvent was removed and the residue purified on silica gel eluting with hexane/EtOAc (4:1) to give **7** as an oil (4.36 g, 60%). ^{1}H NMR ($CDCl_3$): δ 1.27 (3H, t), 1.03 (13H, m), 1.45 (17H, m), 3.05 (2H, t), 3.67 (2H, t), 4.22 (2H, q), 4.33 (2H, s), 7.14 (1H, d), 7.53 (1H, d), 8.19 (1H, s).

***tert*-Butyl 7-(4-*N*-Morpholinyl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (8a).** A solution of **3a** (391 mg, 2.41 mmol), diisopropylethylamine (1.26 mL, 7.2 mmol), and bis-(2-bromoethyl) ether (363 μL, 2.9 mmol) in DMF (15 mL) was heated at 100 °C for 16 h. The cooled solution was diluted with EtOAc, washed with saturated $NaHCO_3$ and then brine, dried, and concentrated. Chromatography of the residue (silica gel; 5% EtOH in EtOAc) afforded 7-(4-*N*-morpholinyl)-3,4-dihydro-1(1*H*)-isoquinolinone as a solid (402 mg, 72%).

This isoquinolinone was alkylated with *tert*-butyl bromoacetate using the conditions for **4a** to yield (after chromatography) **8a** as a solid (82%). ^{1}H NMR ($CDCl_3$): δ 1.48 (9H, s), 2.95 (2H, t), 3.18 (4H, m), 3.61 (2H, t), 3.86 (4H, m), 4.24 (2H, s), 7.00 (1H, dd), 7.10 (1H, d), 7.63 (1H, d).

Ethyl 7-(4-Pyridyl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (8c). A solution of **7** (4.36 g, 8.35 mol), 4-bromopyridine (1.79 g, 9.19 mmol), and tetrakis(triphenylphosphine)-palladium(0) (965 mg, 0.84 mmol) in DMF (40 mL) purged with argon was stirred at 120 °C for 16 h. The solution was poured into saturated $NaHCO_3$ and EtOAc, extracted with EtOAc, washed with brine, dried, and evaporated to give a brown oil. Chromatography (silica gel; EtOAc and then 5% MeOH in EtOAc) gave **8c** as a pale yellow oil (1.55 g, 60%). ^{1}H NMR (CD_3OD): δ 1.28 (3H, t), 3.18 (2H, t), 3.75 (2H, t), 4.21 (2H, q), 4.38 (2H, s), 7.48 (1H, d), 7.77 (2H, d), 7.92 (1H, d), 8.33 (1H, s), 8.60 (2H, d).

Ethyl 7-(4-*N*-Boc-piperidin-1-yl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (8d). The pyridine **8b** was hydrogenated in EtOH (100 mL) with HOAc (20 mL) using 10% Pd−C (200 mg added after each 24 h; 800 mg total) at 64 psi for 5 days. The mixture was filtered through Celite, the solvent removed and the crude product protected using $(Boc)_2O$, K_2CO_3 in THF/water (cf. preparation of **4a**). Chromatography (silica gel; hexane/EtOAc, 1:1) gave **8d** as a colorless oil (20% for two steps).

Ethyl 7-(1-Trityl-4-imidazolyl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetate (8b). This compound was prepared as for **8c** but using 4-iodoimidazole. The product was then protected using trityl chloride in Et_3N/DMF to yield **8b**. ^{1}H NMR ($CDCl_3$): δ 1.28 (3H, t), 3.05 (2H, t), 3.65 (2H, t), 4.31 (2H, s), 4.40 (2H, q), 7.19 (6H, m), 7.34 (9H, s), 7.47 (2H, m), 7.66 (1H, m), 8.05 (1H, d), 8.18 (1H, s).

***N*-[[7-(Piperazin-1-yl)-3,4-dihydro-1(1*H*)-oxoisoquinolin-2-yl]acetyl]-3(*S*)-ethynyl-β-alanine Ethyl Ester Hydrochloride (19).** The ethyl ester **4a** (2.9 g, 6.95 mmol), 1 N LiOH (20.9 mL, 20.9 mmol), and MeOH (50 mL) were stirred for 16 h at room temperature. The solvent was removed; the residue was taken up in EtOAc and washed with 1 N HCl. Repeated extraction of the aqueous phase followed by washing of the combined organic layers with brine (5 mL), drying ($MgSO_4$), and evaporation gave 7-(4-*N*-Boc-piperazin-1-yl)-3,4-dihydro-1(1*H*)-isoquinolinone-2-acetic acid as a solid (2.5 g, 92%). ^{1}H NMR (CD_3OD): δ 1.48 (9H, s), 2.99 (2H, t), 3.20 (4H, m), 3.62 (6H, m), 4.31 (2H, s), 7.22 (2H, s), 7.60 (1H, s).

To a solution of this acid (150 mg, 0.39 mmol), HOBT (63 mg, 0.46 mmol), and EDC (111 mg, 0.58 mmol) were added 3(*S*)-ethynyl-β-alanine ethyl ester hydrochloride (82 mg, 0.46 mmol) and Et_3N (80.7 mL, 0.58 mmol). The mixture was stirred at room temperature under argon for 16 h and then partitioned between EtOAc and 10% citric acid. The aqueous phase was extracted with EtOAc (×3), and the combined organic layers were then washed with saturated aqueous $NaHCO_3$ followed by brine. Removal of the dried ($MgSO_4$) solvent afforded a yellow oil which was purified by column chromatography (silica gel; hexane/EtOAc, 1:1) to give *N*-[[7-(4-*N*-Boc-piperazin-1-yl)-3,4-dihydro-1(1*H*)-oxoisoquinolin-2-yl]-acetyl]-3(*S*)-ethynyl-β-alanine ethyl ester as an oil.

HCl(g) was bubbled through a solution of the Boc-piperidine (176 mg, 0.37 mmol) in 50 mL of EtOAc for 5 min. The solvent was removed *in vacuo* to leave **19** as the HCl salt. ^{1}H NMR (CD_3OD): δ 1.25 (3H, t, J = 7.1 Hz), 2.75 (3H, m), 2.99 (2H, t, J = 6.4 Hz), 3.4 (8H, m), 3.64 (2H, t, J = 6.4 Hz), 4.14 (2H, q, J = 7.1 Hz), 4.24 (2H, s), 5.06 (1H, dd, J = 7.2, 2.4 Hz), 7.22 (2H, m), 7.55 (1H, d, J = 2.2 Hz).

***N*-[[7-(Piperazin-1-yl)-3,4-dihydro-1(1*H*)-oxoisoquinolin-2-yl]acetyl]-3(*S*)-ethynyl-β-alanine Trifluoroacetic Acid Salt (6d).** The ester **19** (70 mg, 0.16 mmol) was stirred in 10 mL of MeOH and 0.65 mL of 1 N LiOH for 16 h, and then 1 N HCl was added to give pH = 4. After removal of the solvent, the residue was purified by reverse phase HPLC (C_{18} column, H_2O/CH_3CN with 0.1% TFA as gradient) to give **6d** as the TFA salt. ^{1}H NMR (CD_3OD): δ 2.72 (3H, m), 2.99 (2H, t, J = 6.6 Hz), 3.4 (8H, m), 3.64 (2H, t, J = 6.6 Hz), 4.25 (2H, s), 5.05 (1H, m), 7.22 (2H, m), 7.55 (1H, d, J = 2.4 Hz), 8.62 (1H, d, J = 8.1 Hz).

Ethyl 7-(4-*N*-Boc-piperazin-1-yl)-1(1*H*)-isoquinolinone-2-acetate (10). A solution of **4a** (329 mg, 1 mmol) in EtOH (5 mL) and cyclohexene (5 mL) was treated with 300 mg of 10% Pd−C and heated to 75 °C for 48 h. The mixture was cooled and filtered through Celite (washing with copious amounts of EtOH) and the solvent removed to give **10** (311 mg, 95%) as a foam. ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, t), 1.47 (9H, s), 3.24 (4H, m), 3.6 (4H, m), 4.25 (2H, q), 4.70 (2H, s), 6.47 (2H, d), 6.88 (2H, d),7.33 (1H, dd), 7.43 (1H, d), 7.81 (1H, d).

Ethyl 7-Amino-1,2,3,4-tetrahydroisoquinoline-2-acetate (13). To a solution of **12** (10.5 g, 40.1 mmol) in 20 mL of HOAc was added a cooled, premixed solution of 120 mL of H_2SO_4 and 60 mL of HOAc followed by paraformaldehyde (1.44 g, 48.1 mmol). The mixture was stirred for 16 h, poured into ice, and extracted three times with EtOAc. After washing with water and then brine, the organic layer was dried and evaporated to give an oil (10.2 g). Chromatography on silica gel eluting with hexane/EtOAc (2:1) gave the tetrahydroisoquinoline (8.9 g, 81%).

This tetrahydroisoquinoline (750 mg, 2.7 mmol) was treated with 1 N LiOH (5.5 mL, 5.5 mmol), MeOH (8 mL), and THF (2 mL) for 1 h. The solution was poured into water, extracted with EtOAc, washed with brine, dried, and evaporated to give a solid (487 mg, 92%).

The tetrahydroisoquinoline (440 mg, 2.47 mmol), ethyl bromoacetate (0.29 mL, 2.6 mmol), and Et_3N (0.41 mL, 3.0 mmol) were stirred in CH_2Cl_2 (10 mL) for 16 h. After removal of the solvent, the residue was taken up in saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried, and evaporated. Chromatography (silica gel; EtOAc) yielded a yellow oil (627 mg, 96%).

To a solution of the nitrotetrahydroisoquinoline (627 mg, 2.38 mmol) in EtOH (30 mL) previously degassed with argon was added 10% Pd−C (60 mg), and then the mixture was placed under an atmosphere of H_2 (balloon) for 3 h. The mixture was filtered through Celite, and the solvent was removed to give **13** as an oil (526 mg, 95%). ^{1}H NMR ($CDCl_3$): δ 1.29 (3H, t), 2.73 (4H, m), 3.38 (2H, s), 3.72 (2H, s), 4.22 (2H, q), 6.35 (1H, d), 6.52 (1H, dd), 6.89 (1H, d).

***N*-[1-Nitro-4-(*N*-Boc-piperazin-1-yl)-2-benzoyl]glycine Ethyl Ester (15)**. Piperazine (4.65 g, 54 mmol) and 4-chloro-1-nitrobenzoic acid (**14**) (2.26 g, 11.2 mmol) were heated neat at 110 °C (oil bath temperature) for 2 h. After cooling, the residue was stirred in 10% aqueous $KHSO_4$ (pH ~ 4−5) for 15 min and the yellow-orange solid then collected by filtration (2.8 g).

This piperazine (2.8 g, 11 mmol) was dissolved in 1 N NaOH (50 mL) and 1,4-dioxane (100 mL) at 0 °C, and to this solution was added Boc-anhydride (4.0 g, 18 mmol). After stirring for 16 h, the mixture was concentrated *in vacuo*; the residue was taken up in H_2O (100 mL), filtered, and then acidified to pH ~ 3−4 with 10% aqueous $KHSO_4$. The precipitate was collected by filtration, dissolved in EtOAc, washed with 10% aqueous $KHSO_4$ and then brine, dried, and evaporated to yield a yellow solid (2.95 g).

To a solution of the acid (2.81 g, 8 mmol), glycine ethyl ester hydrochloride (1.12 g, 8 mmol), and HOBT (1.19 g, 8.8 mmol) in DMF were added NMM (1.76 mL, 11.6 mmol) and EDC (1.84 g, 9.6 mmol). After stirring for 16 h, the DMF was removed *in vacuo* and the residue partitioned between H_2O and EtOAc. The organic extracts were washed with 10% aqueous $KHSO_4$, brine, saturated $NaHCO_3$, and then brine, dried, and concentrated *in vacuo* to give a yellow solid. Column chromatography (silica gel; hexane/EtOAc, 2:3) afforded **15** as a yellow solid (3.1 g). ^{1}H NMR ($CDCl_3$): δ 1.25−1.35 (3H, t), 1.48 (9H, s), 3.34−3.5 (4H, m), 3.6 (4H, m), 4.19−4.32 (4H, m), 6.23−6.35 (1H, m), 6.75−6.86 (2H, m), 8.1 (1H, d).

Ethyl 6-(*N*-Boc-piperazin-1-yl)-4(3*H*)-quinazolinone-3-acetate (16). To a solution of **15** (3.0 g, 11.2 mmol) in EtOH (150 mL) previously degassed with argon was added 10% Pd−C (0.35 g), and then the mixture was placed under an atmosphere of H_2 (balloon) for 3 h. The mixture was filtered through Celite and the solvent removed to give a yellow-brown foam (2.8 g).

The aniline (1.4 g, 3.4 mmol) and triethyl orthoformate (15 mL) were heated at reflux for 16 h. After cooling, the excess solvent was removed *in vacuo* and the residue taken up in EtOAc, washed with H_2O, dried, and evaporated to give an oil. Chromatography on silica gel (hexane/EtOAc, 1:4) afforded **16** as a pale yellow solid (0.65 g). ^{1}H NMR ($CDCl_3$): δ 1.25−1.35 (3H, t), 1.49 (9H, s), 3.2−3.32 (4H, m), 3.54−3.66 (4H, m), 4.21−4.32 (2H, q), 4.69 (2H, s), 7.42 (1H, dd), 7.6−7.68 (2H, m), 7.85 (1H, s).

Ethyl 6-Amino-2,4(1*H*,3*H*)-quinazolinedione-3-acetate (18). 4-Amino-1-nitrobenzoic acid (**17**) (5.46 g, 30 mmol) was coupled with ethylglycine hydrochloride using the procedure described for **15**. After workup, a solid was obtained which was triturated with ether and filtered to give a yellow solid (6.62 g).

A solution of the aniline (1.34 g, 5 mmol) and CDI (0.97 g, 6 mmol) in THF (25 mL) was heated at reflux for 27 h. The

solvent was removed, and the residue was chromatographed (silica gel; hexane/EtOAc, 2:3) to yield the quinazolinedione (0.94 g) as a pale yellow solid.

The nitroquinazolinedione was reduced using H_2 and 10% Pd−C in EtOH. After filtration and removal of the solvent, the residue was chromatographed (silica gel; 2.5% MeOH in $CHCl_3$) to give **18** as a pale yellow solid (0.41 g). ^{1}H NMR (CD_3OD): δ 1.2−1.35 (3H, t), 4.14−4.3 (2H, q), 4.72 (2H, s), 6.99 (1H, d), 7.1 (1H, dd), 7.30 (1H, d).

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Supporting Information Available: NMR data for final compounds (2 pages). Ordering information is given on any current masthead page.

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