Articles

Metabolism-Directed Optimization of 3-Aminopyrazinone Acetamide Thrombin Inhibitors. Development of an Orally Bioavailable Series Containing P1 and P3 Pyridines

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Recent efforts in the field of thrombin inhibitor research have focused on the identification of compounds with good oral bioavailability and pharmacokinetics. In this manuscript we describe a metabolism-based approach to the optimization of the 3-(2-phenethylamino)-6-methylpyrazinone acetamide template (e.g., **1**) which resulted in the modification of each of the three principal components (i.e., P1, P2, P3) comprising this series. As a result of these studies, several potent thrombin inhibitors (e.g., **20**, **24**, **25**) were identified which exhibit high levels of oral bioavailability and long plasma half-lives.

Introduction

Disorders resulting from the formation of an occlusive vascular thrombus due to improper maintenance of the blood coagulation system represent a major cause of mortality worldwide. Among the more prominent conditions that can result from excessive coagulation are deep vein thrombosis (DVT), pulmonary embolism (PE), and thromboembolic stroke.[1] The occurrence of DVT is most commonly associated with, but not limited to, vascularly invasive and incapacitating procedures such as knee or hip replacement surgery. DVT can frequently lead to other serious, life−threatening states such as PE, which is often difficult to detect. The severity of this condition is evidenced by the fact that 5−15% of U.S. hospital deaths are the result of PE, of which 70% were unsuspected antemortem.[2] Atrial fibrillation, a prevalent condition in the elderly, imparts a high risk of cardiogenic thromboembolism (due to blood stasis rather than vascular damage) and, consequently, ischemic stroke in the absence of anticoagulant therapy.[1]

Current therapies for the treatment of venous thrombosis and the prevention of cardiogenic thromboembolism consist of low molecular weight heparin (LMWH) and warfarin, in an acute and chronic setting, respectively. The limitations associated with each of these therapies have been well documented: the requirement for parenteral administration of LMWH limits its chronic utility, while the delayed onset of action, potential for drug−drug interactions, and need for continual monitoring of blood coagulation parameters render warfarin a challenging drug to safely administer.

Recent approaches to anticoagulant therapy have been directed toward identifying direct small molecule inhibitors of specific coagulation cascade enzymes[3] which possess the pharmacokinetic properties required for once daily oral administration. Assuming predictable pharmacokinetics, the direct antithrombotic action of these inhibitors should render routine monitoring of coagulation levels unnecessary. A prominent target to emerge from this effort is the trypsin-like serine protease thrombin (Factor IIa). Thrombin[4] occupies a central role in hemostasis, its primary procoagulant actions consisting of the activation of platelets and cleavage of fibrinogen to fibrin. Fibrin subsequently polymerizes and is cross-linked through the action of Factor XIIIa to comprise, along with platelets, the primary components of a vascular thrombus. Corroboration for this antithrombotic strategy is provided by the fact that argatroban, an intravenous thrombin inhibitor, has been approved in the U.S. for patients susceptible to heparin-induced thrombocytopenia.

Early thrombin inhibitor development focused on the modification of peptide structures based upon the sequences of known native substrates.[5] Although this approach yielded potent inhibitors indispensable in

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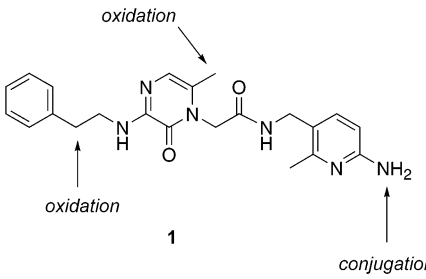
proof-of-principle experiments, a lack of selectivity (versus homologous proteases) and/or oral bioavailability plagued this early class of compounds. Guided by the wealth of enzyme structural information, impressive advances have been made in the development of peptidomimetic and nonpeptide thrombin inhibitors;[6] however, many of these retain a highly basic amidine that mimics the binding of the P1 Arg of natural peptide substrates to Asp-189 at the base of the S1 pocket. Although this salt-bridge interaction leads to a substantial amount of binding energy,[7] the incorporation of these extremely basic moieties is generally associated with low levels of oral bioavailability and high plasma clearance.[8] While a prodrug approach has been successfully employed to produce a benzamidine analogue with acceptable bioavailability (ximelagatran),[9] the majority of recent efforts in the field of thrombin inhibitor development have focused on establishing compounds that incorporate less basic P1 binding elements. These studies have begun to produce orally bioavailable thrombin inhibitors, and, presumably, clinical evaluations are in progress to determine if these candidates exhibit the pharmacokinetic and safety profile required to displace current anticoagulant therapies.

Recent reports from our laboratories have detailed the development of the aminopyrazinone acetamide thrombin−inhibitor template, which resulted in the identification of an efficacious, orally bioavailable compound **1** containing a moderately basic 2-aminopyridine P1.[10] Further study of **1** revealed three principal sites of



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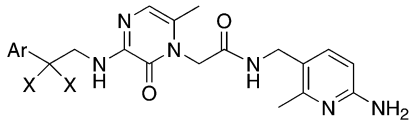
metabolism: oxidation of the P3 and P2 benzylic positions and Phase II conjugation of the P1 amino group.[11] Assuming that metabolism is the major route of clearance, elimination of these structural areas of metabolism could in turn lead to the generation of compounds with improved pharmacokinetics. In this manuscript we describe the metabolism-directed optimization process which resulted in the production of a series of potent, selective, and orally bioavailable compounds containing weakly basic pyridine P1 and P3 moieties; ultimately, metabolic considerations dictated the modification of each of the three principal components (i.e., P1, P2, P3) comprising this series.

Results and Discussion

A study to examine the effect of P3 benzylic substitution on thrombin inhibitory potency was initiated with the fundamental goal of sterically blocking metabolism at this site (Table 1). The inhibition constants (K_i) versus thrombin (IIa) and trypsin, and the concentration needed to double the activated partial thromboplastin time (2xaPTT) in human plasma were determined for each compound.[12] Simple mono- or dimethylation of the benzylic carbon exhibited minimal effect on thrombin inhibition (**2**, **3**: K_i = 1.1 nM). Introduction of a *gem*-difluoro group, however, led to an unexpected potency enhancement in both the enzyme inhibition and 2xaPTT clotting assay (**4**, K_i = 0.10 nM, 2xaPTT = 0.29 μM). Replacement of the P3 phenyl ring with a pyridine ring was well tolerated for each of the pyridine isomers: the 2-pyridyl substitution (**5**, K_i = 0.27 nM) improved potency while the 3- (**6**, K_i = 0.54 nM) and 4-pyridyl (**7**, K_i = 1.6 nM) isomers retained similar binding activity relative to phenyl. The tolerance of the S3 pocket to this change would prove to be a valuable finding, as it presents the option of integrating a phenyl or pyridyl P3 moiety to modulate the physicochemical properties of the inhibitors (vide infra). Incorporation of both the 2-pyridyl and *gem*-difluoro modifications afforded **8**, a potent thrombin inhibitor with excellent in vitro anticoagulant activity (K_i = 0.042 nM, 2xaPTT = 0.20 μM). As a result of the excellent potency and lack of obvious metabolic soft-spots the 2,2-difluoro-2-arylethylamino group emerged as the optimal P3.

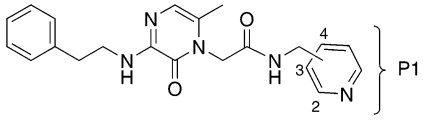
Table 1. Effect of P3 Benzylic Substitution on Thrombin (IIa) and Trypsin (tryp) Inhibition (K_i) and In Vitro Anticoagulant Potency (2xaPTT)



			K_i (nM)		
compd	Ar	X	IIa	tryp	2xaPTT (μM)
1	Ph	H, H	0.8	1800	0.41
2	Ph	H, Me	1.1	1600	0.61
3	Ph	Me, Me	1.1	800	0.50
4	Ph	F, F	0.10	855	0.29
5	2-pyr	H, H	0.27	3600	0.31
6	3-pyr	H, H	0.54	3800	0.28
7	4-pyr	H, H	1.6	4600	0.45
8	2-pyr	F, F	0.042	800	0.20

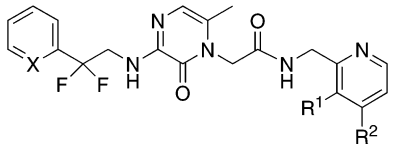
Due to the aforementioned metabolic issues, replacement of the P1 aminopyridine group was next addressed. At the outset this was projected to be a difficult task as the aminopyridine P1 conferred not only excellent potency but also high levels of oral bioavailability to this series of inhibitors.[10] As anticipated, excision of the amino group from **1** to give the 2-methyl-3-pyridyl analogue **9** resulted in a substantial loss (350-fold) in potency due to elimination of the interaction with Asp-189 (K_i = 280 nM, Table 2). Furthermore, removal of the methyl group from **9** led to an additional 4-fold deterioration in thrombin binding (**10**, K_i = 1200 nM).

Table 2. Effect of P1 Pyridine Isomers on Thrombin (IIa) and Trypsin (tryp) Inhibition (K_i)



		K_i	
compd	P1	IIa (nM)	tryp (μM)
9	2-Me-3-pyr	280	>100
10	3-pyr	1200	898
11	2-pyr	390	>1000
12	4-pyr	17000	251

Table 3. Effect of P1 Pyridine Substitution on Thrombin (IIa) and Trypsin (tryp) Inhibition (K_i), in Vitro Anticoagulant Potency (2xaPTT) and Dog Pharmacokinetic Parameters



				K_i				
compd	X	R^1	R^2	IIa (nM)	tryp (μM)	2xaPTT (μM)	C_{max} (μM)[a]	$t_{1/2}$ (h)[b]
13	N	H	H	15	>1000	2.2		
14	CH	H	Cl	1.3	47	0.89		
15	CH	H	Me	4.8	270	1.7		
16	N	H	Me	2.8	431	0.59	4.12	2.4
17	CH	F	Me	1.2	40	0.72	1.83	0.7
18	N	F	Me	1.0	118	0.48	1.66[c]	1.0
19	CH	F	H	6.8	501	1.26	3.73	2.0
20	N	F	H	4.2	252	0.67	5.64	3.5

[a] Dose = 1 mg/kg unless otherwise noted. [b] po half-life. [c] Dose = 0.7 mg/kg.

Of the simple pyridine isomers, the 2-pyridyl analogue **11** displays the highest inherent binding affinity (K_i = 390 nM) and as a result this P1 was chosen for further optimization.

Although **11** represents a 500-fold loss in thrombin inhibition from the parent compound **1**, the potency enhancements established in P3 could potentially compensate for this deficit. Combination of the optimized 2,2-difluoro-2-pyridylethylamino P3 and the 2-pyridyl P1 structural modifications exhibited an additive benefit (**13**: K_i = 15 nM, 2xaPTT = 2.2 μM; Table 3); however, the functional potency was still not within the targeted range (2xaPTT < 1 μM).[13] In a previous study it had been established that simple *meta* substitution of P1 aryl groups can access a lipophilic recess in the S1 pocket and lead to a large improvement in thrombin inhibitory potency.[14] Indeed, incorporation of this structural arrangement into the P1 pyridine in the form of a 4-chloropyridine afforded a compound with improved potency (**14**, K_i = 1.25 nM, 2xaPTT = 0.89 μM) and excellent trypsin selectivity. Cognizant of the inherent liability associated with reactive 4-halopyridines, an isosteric 4-methylpyridine was prepared. This modification resulted in a modest loss in potency in both the enzyme and coagulation (2xaPTT) assays (**15**, K_i = 4.8 nM, 2xaPTT = 1.7 μM). The functional activity could be regained by implementing the previously established strategy of incorporating the more basic P3 pyridine (**16**, K_i = 2.8 nM, 2xaPTT = 0.59 μM). This 3-fold improvement in the 2xaPTT (i.e., in vitro clot inhibition) is likely due to the differences in inhibitor physicochemical properties of **16** vs **15** (i.e., reduced lipophilicity).[15]

As the primary objective of the current study was the identification of thrombin inhibitors with superior pharmacokinetics, compounds meeting our established potency criteria were directly subjected to dog pharmacokinetic experiments (Table 3). Upon oral dosing of **16** (1 mg/kg), a 4.12 μM maximum plasma concentration (C_{max}) and a 2.4 h plasma half-life ($t_{1/2}$) were achieved (F = 68%). Additionally, **16** demonstrated complete efficacy in the rat $FeCl_3$ arterial thrombosis model upon iv dosing (0/6 occlusions, final plasma concentration = 736 ± 31 nM).[16] The in vitro and in vivo performance of this compound established that the P1 aminopyridine could be replaced with a less basic, simple pyridine and still deliver potent, efficacious, selective, and orally bioavailable thrombin inhibitors.

With **16** serving as a template, efforts were directed toward improving the $t_{1/2}$ while maintaining potency and efficacy (Table 3). In the absence of any information regarding the mode of clearance, it was postulated that oxidative metabolism was occurring at the P1 pyridine ring nitrogen or 4-methyl group. Introduction of a 3-fluoro group onto the P1 pyridine ring could, by reducing the pyridine basicity, diminish the potential for N-oxidation.[17] Additionally, this modification could have a simultaneous effect on the putative metabolism of the benzylic methyl group.[18] In practice this alteration produced an improvement in potency (**17**, K_i = 1.2 nM; **18**, K_i = 1.0 nM); although the good C_{max} was retained, the half-life was not increased and the in vivo efficacy was compromised (**18**, 3/6 occlusions, final plasma concentration = 606 ± 43 nM). The increased potency observed upon incorporation of the 3-fluoro substituent permitted removal of the benzylic methyl group (**19**, K_i = 6.8 nM; **20**, K_i = 4.2 nM). In combination with the pyridyl P3, this maneuver not only resulted in a restoration of efficacy (**20**, 1/6 occlusions, 1 reflow; final plasma concentration = 799 ± 72 nM), but also excellent pharmacokinetics. Upon dosing of **20** (1 mpk) to dogs, a C_{max} of 5.64 μM and a substantial improvement of the $t_{1/2}$ to 3.5 h were observed.

The performance in the aforementioned assays prompted further in vivo characterization of **20** (Table 4). This inhibitor exhibited good to moderate bioavailability in dog (F = 87%, n = 2), rat (F = 55%, n = 4) and rhesus monkey (F = 12%, n = 4), although the half-life decreased in the latter two species.

Table 4. Pharmacokinetics Parameters of **20** in Dog, Rat, and Rhesus Monkey

species	po dose (mg/kg)	po C_{max} (μM)	iv $t_{1/2}$ (h)	F (%)
dog	1	5.64	3.5	87
rat	10	2.16 ± 0.32	0.57 ± 0.07	55 ± 14
rhesus	5	1.10 ± 0.86	0.99 ± 0.34	12 ± 10

All of the P1 2-pyridyl containing thrombin inhibitors listed in Table 3 possessed no significant activity versus trypsin (>40 μM) or any other proteases assayed: t-PA, plasmin, factor Xa, plasma kallikrein, activated protein C, urokinase, and chymotrypsin.

Determination of the X-ray crystal structure of **20** complexed to thrombin revealed the same general binding motif as observed with **1**:[10] the fluoropyridine occupies the S1 specificity pocket, the methyl pyrazinone fills the insertion loop, and the P3 aryl group binds in the distal hydrophobic pocket (Figure 1). The antiparallel β-sheet hydrogen bonding motif between the aminopyrazinone and Gly-216 is maintained (d = 2.75 Å, 2.84 Å); similarly the Ser-214 H-bond to the inhibitor amide is conserved (d = 3.48 Å). The edge-to-face $\sigma-\pi$ interaction in S3 between the P3 aryl group and the π-rich Trp-215 is further reinforced by the incorporation of the electron-deficient P3 pyridine, providing greater overall binding affinity versus its phenyl counterpart. In agreement with this analysis is the improved potency incurred upon incorporation of the *gem*-difluoro group which inductively reinforces this $\sigma-\pi$ interaction.[19] In

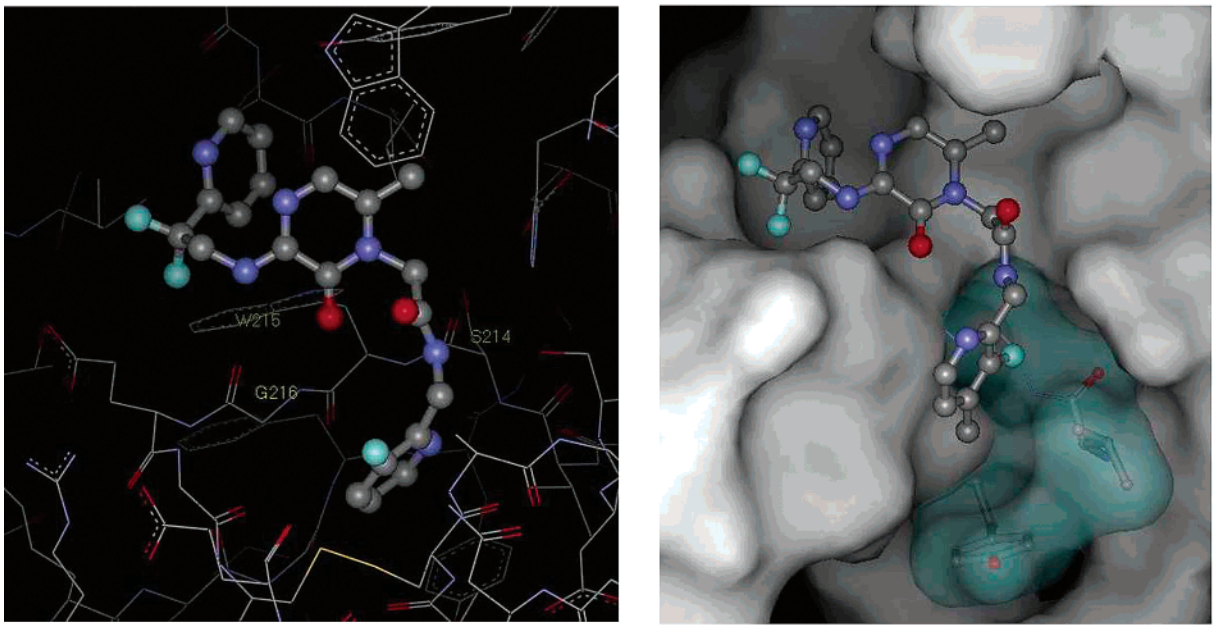


Figure 1. X-ray crystal structures of **20** and **18** bound in the thrombin active site. The solvent accessible surface is depicted for **18** (the lipophilic surface created by the sidechains of Tyr-228 and Val-213 is highlighted in blue).

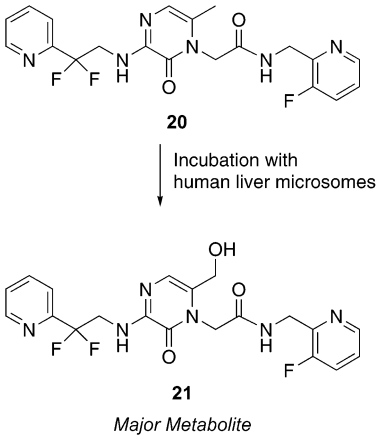
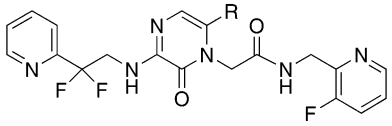


Figure 2. Primary metabolism of **20** upon incubation with human liver microsomes.

S1 the fluoropyridine does not interact with Asp-189: the ring nitrogen projects toward the enzyme backbone and the fluorine is solvent exposed and appears not to be involved in any specific interaction. The structure of **18** bound to thrombin revealed that incorporation of the 4-Me group induces a 180° rotation of the P1 fluoropyridine ring. This conformational change is driven by the lipophilic binding interactions between the 4-Me and 3-F groups and the surface created by the side chains of Tyr-228 and Val-213 (Figure 1, blue surface), consistent with the overall potency of **18** versus **20**.

Although **20** represents an inhibitor which displayed good bioavailability across three species, the plasma half-life could be improved. Toward this end, a study to identify the primary sites of metabolism of **20** revealed that the 6-hydroxymethylpyrazinone **21** was the major metabolite upon incubation with human liver microsomes (Figure 2).[20] In view of these data, metabolically resistant replacements for the 6-methyl group were investigated (Table 5). Elimination of the 6-methyl group resulted in a significant loss of potency (**22**, K_i = 140 nM), as did incorporation of a trifluoromethyl group (**23**, K_i = 44 nM).[21] Substitution of the methyl with a

Table 5. Effect of P2 Pyrazinone 6-Substitution on Thrombin (IIa) and Trypsin (tryp) Inhibition (K_i), in Vitro Anticoagulant Potency (2xaPTT) and Dog Pharmacokinetic Parameters



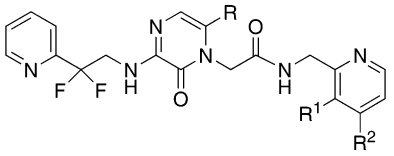
		K_i				
compd	R	IIa (nM)	tryp (μM)	2xaPTT (μM)	C_{max} (μM)[a]	$t_{1/2}$ (h)[b]
20	Me	4.2	252	0.67	5.64	3.5
22	H	140	>1000			
23	CF_3	44	136			
24	Cl	5.2	202	0.78	1.38	6.6
25	CN	2.3	213	0.41	1.17	9.7

[a] Dose = 1 mg/kg unless otherwise noted. [b] po half-life.

chloro group afforded a compound (**24**, K_i = 5.2 nM) that was nearly equipotent to the parent, whereas incorporation of a nitrile led to a more potent inhibitor (**25**, K_i = 2.3 nM). More importantly, and as anticipated from the metabolism study, both the chloro ($t_{1/2}$ = 6.6 h) and cyano ($t_{1/2}$ = 9.7 h) modifications imparted significantly improved half-lives versus **20**. While chloropyrazinone **24** maintained good in vivo efficacy (2/6 occlusions, final plasma concentration = 245 ± 9 nM), the cyanopyrazinone analogue **25** demonstrated compromised performance in the rat thrombosis assay (5/6 occlusions, final plasma concentration = 460 ± 46 nM).

Having established the 6-Cl and 6-CN substitutions as acceptable replacements for the 6-Me group, a reinvestigation into the P1 structure−activity relationship was undertaken (Table 6); particular attention was focused on the impact of these modifications on plasma half-life. Substitution of the 3-fluoro with a chlorine degraded potency (**26**, K_i = 7.9 nM, 2xaPTT = 1.41 μM) and, significantly, afforded lower plasma levels after oral administration (C_{max} = 0.062 μM). As in the 6-methylpyrazinone series, incorporation of either a 4-methyl or 3-fluoro-4-methylpyridine P1 yielded potent

Table 6. Effect of P1 Pyridine Substitution on Thrombin (IIa) and Trypsin (tryp) Inhibition (K_i), in Vitro Anticoagulant Potency (2xaPTT) and Dog Pharmacokinetic Parameters



				K_i				
compd	R	R^1	R^2	IIa (nM)	tryp (μM)	2xaPTT (μM)	C_{max} (μM)[a]	$t_{1/2}$ (h)[b]
24	Cl	F	H	5.2	202	0.78	1.38	6.6
26	Cl	Cl	H	7.9	567	1.41	0.062	5.7
27	Cl	H	Me	5.3	429	0.91	2.32	2.1
28	Cl	F	Me	0.90	80	0.48	0.67	3.2
29	CN	F	Me	0.40	61	0.31	1.70	2.4
30	Cl	OMe	H	15	706	2.19	0.90	2.3
31	Cl	H	OMe	3.6	665	0.71	0.53[c]	2.0

[a] Dose = 1 mg/kg unless otherwise noted. [b] po half-life. [c] Dose = 0.5 mg/kg.

Table 7. Pharmacokinetics Parameters of **24** in Dog, Rat, Rhesus, and African Green Monkey

species	dose (mg/kg)	po C_{max} (μM)	iv $t_{1/2}$ (h)	F (%)
dog	0.65	1.16	4.48	66
rat	10	1.06 ± 0.49	0.42 ± 0.23	23
rhesus	5	0.310	0.73 ± 0.21	5.2
African green	5	1.71 ± 1.75	1.6 ± 0.3	66 ± 54

analogues (**27**, K_i = 5.3 nM; **28**, K_i = 0.9 nM); however, neither modification enhanced the pharmacokinetic duration. The 6-cyanopyrazinone analogue of **28** was very potent (**29**, K_i = 0.4 nM), but displayed an inferior half-life ($t_{1/2}$ = 2.4 h) versus the parent **25**. Similarly, the 3- and 4-methoxypyridines **30** and **31** conferred no pharmacokinetic advantage. Thus, the same S1 and S3 binding elements that proved to be optimal in terms of potency[22] and pharmacokinetics in the 6-methyl series were also superior in the 6-chloro- and 6-cyanopyrazinone series.

As in the 6-methylpyrazinone series all compounds in Table 6 exhibit minimal (>61 μM) activity versus trypsin and against other proteases assayed: t-PA, plasmin, factor Xa, plasma kallikrein, activated protein C, urokinase, and chymotrypsin.

Due to its good C_{max}, long $t_{1/2}$ in dog and performance in the rat $FeCl_3$ efficacy assay, **24** was examined in further detail (Table 7). This compound exhibits good oral bioavailability in dog (66%, n = 2), rat (23%, n = 4) and African green monkey (66%, n = 4).[23] Furthermore, **24** demonstrates good stability to human liver microsome and hepatocyte preparations and, based on in vitro metabolic scaling studies, is predicted to be a low-clearance compound in man.[24]

Chemistry. The general strategy employed for the synthesis of these 3-aminopyrazinone acetamide thrombin inhibitors followed that established previously.[10,25] The modular nature of this series allows the independent preparation of three individual subunits: a P3 2-arylethylamine **A**, an ethyl 3-halopyrazin(1*H*)-2-one-1-acetate **B**, and a P1 benzylamine **C** (Figure 3). In practice, installation of the P3 amine **A** onto the pyrazinone **B** was followed by amide bond formation with the P1 amine **C** to deliver the final products.

Synthesis of 2,2-difluoro-2-(2-pyridyl)ethylamine **35**

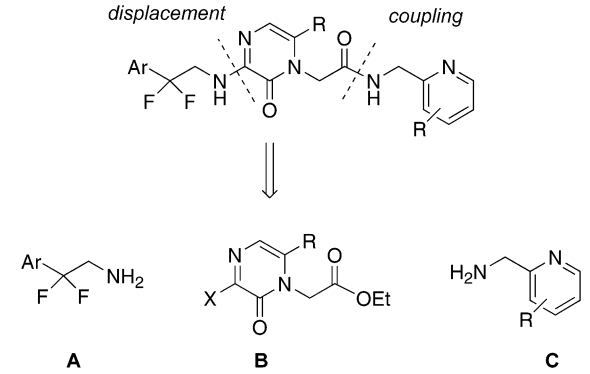
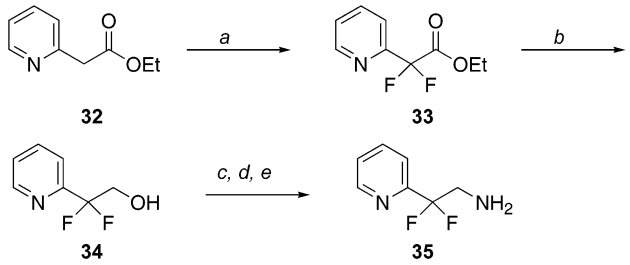


Figure 3. Synthetic approach to pyrazinone thrombin inhibitors.

Scheme 1[a]

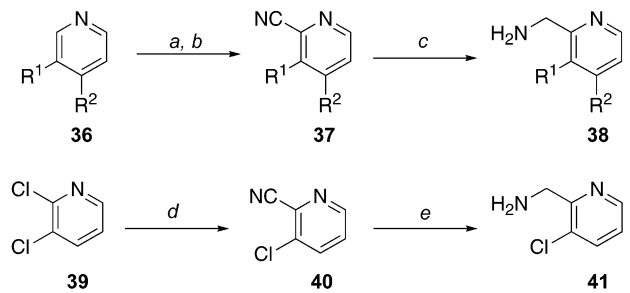


[a] (a) KHMDS, $MnBr_2$, −78 °C, *N*-fluorobenzenesulfonimide, THF; (b) $NaBH_4$, EtOH, 0 °C; (c) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, −78 °C, CH_2Cl_2; (d) NaN_3, DMF, 60 °C; (e) 1 atm H_2, 10% Pd/C, EtOAc.

commenced with electrophilic *gem*-difluorination of the potassium enolate of ethyl 2-pyridyl acetate **32** with *N*-fluorobenzenesulfonimide (Scheme 1).[26] The use of manganese(II) bromide was critical to the success of this transformation, as a significant amount of the monofluorinated product was obtained in its absence. Elaboration to the primary amine **35** was most effectively accomplished through a four-step sequence. Sodium borohydride reduction was followed by triflation of the resultant primary alcohol **34** employing 2,6-di-*tert*-butyl-4-methylpyridine as the base. Displacement with azide and subsequent hydrogenolysis afforded the amine **35** as a low-melting solid.

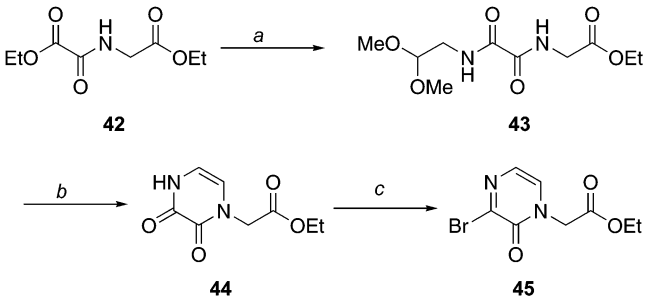
The substituted P1 2-aminomethylpyridines were produced via a three-step sequence (Scheme 2). Pyridine N-oxidation (MCPBA or H_2O_2 with $MeReO_3$ catalysis[27]) was followed by TMSCN-promoted cyanation,[28] to produce the 2-cyanopyridines **37** with good regioselectivity. Hydrogenation under acidic conditions afforded the

Scheme 2[a]



[a] (a) MCPBA, CH_2Cl_2 or H_2O_2, cat. $MeReO_3$, CH_2Cl_2/H_2O; (b) TMSCN, Et_3N, CH_3CN, reflux; (c) 10% Pd/C, 35 psi H_2, HCl, EtOH; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 95 °C; (e) Raney Ni, 1 atm H_2, EtOH, NH_3.

Scheme 3[a]



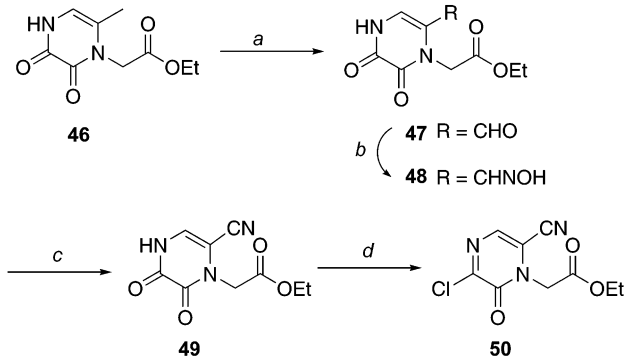
[a] (a) Aminoacetaldehyde dimethylacetal, ^{i}PrOH; (b) AcOH, HCl, reflux; (c) $POBr_3$, DCE, reflux.

primary amines **38** as their hydrochloride salts. The 3-chloropyridine **41** was prepared by a different route: selective Pd-mediated cyanation of 2,3-dichloropyridine **39**, followed by Raney-Ni-catalyzed reduction, delivered the primary amine **41**.

A modification of the Cheeseman pyrazinedione synthesis was employed for P2 subunit production (Scheme 3).[29,25] Addition of aminoacetaldehyde dimethylacetal to oxamate **42**[25] afforded the cyclization precursor **43**. Refluxing oxamide **43** in acetic acid, with a catalytic amount of HCl, affected cyclization to the crude pyrazinedione **44**. Crystallization of this black tar from hot ethanol produced **44** as a tan solid; notably, this sequence was conducted on a multigram scale without chromatography. Bromination with $POBr_3$ produced bromopyrazinone **45**, which was used in subsequent reactions without purification. The scaleability of this sequence would prove valuable, as substrate **45** would additionally serve as the precursor for the 6-chloropyazinone thrombin inhibitors (vide infra, Scheme 5).

The 6-methylpyrazinone intermediate **46**[25] was utilized for the preparation of the 6-cyanopyrazinone **50** (Scheme 4). Oxidation to the aldehyde **47** was effected with selenium dioxide, and subsequent condensation with hydroxylamine produced the oxime **48**. Direct conversion to 3-chloro-6-cyanopyrazinone **50** via a one-pot dehydration/chlorination sequence with $POCl_3$ proved unreliable; alternatively, the two-step PPh_3−CCl_4-mediated dehydration and $POCl_3$ chlorination afforded the P2 6-cyanopyrazinone **50** in good yield.

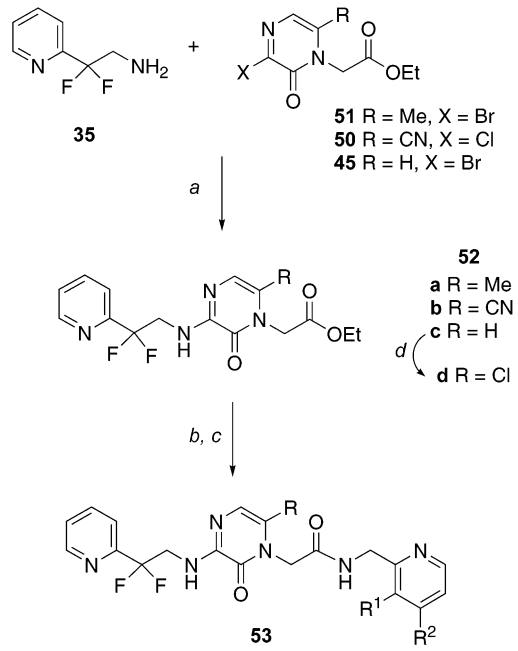
Scheme 4[a]



[a] (a) SeO_2, dioxane, reflux; (b) NH_2OH·HCl, pyridine, EtOH, reflux; (c) PPh_3, DCE, CCl_4, reflux; (d) $POCl_3$, NH_4Cl, reflux.

Addition of the P3 2,2-difluoro-2-pyridylethylamine **35** to the 3-bromopyrazinones **45** and **51** (Scheme 5) required elevated temperature and extended reaction

Scheme 5[a]



[a] (a) $PhCH_3$, EtOH, sealed tube, 60−125 °C; (b) KOH, MeOH; (c) 2-aminomethylpyridine, EDC, HOAT, Et_3N, DMF; (d) NCS, DCE, 80 °C.

times; conversely, reaction of the activated 3-chloro-6-cyanopyrazinone **50** was complete within 1 h at 60 °C. Chlorination of **52c** with NCS in DCE occurred with complete regioselectivity to afford the 6-chloropyrazinone **52d**. Stoichiometry was crucial, as any excess chlorinating agent produced the inseparable 5,6-dichloropyrazinone. Hydrolysis of ethyl esters **52a**−**d** afforded the corresponding acids, which underwent EDC-mediated amide coupling with the P1 amines to afford the final products **53**.

Conclusion

Metabolism-directed optimization of the 3-aminopyrazinone acetamide template (e.g., **1**) has been achieved through modification of each of the three principal components (i.e., P1, P2, P3) comprising this series. Consequently, several potent thrombin inhibitors containing weakly basic P1 and P3 pyridines (e.g., **20**, **24**, **25**) were identified which demonstrate good oral bioavailability and plasma half-lives. It remains to be determined if clinical candidates emerging from this structural class of inhibitors will possess the essential safety and pharmacokinetic profile to supplant heparin and warfarin anticoagulant therapy.

Experimental Section

Unless otherwise noted, all nonaqueous reactions were carried out under a N_2 atmosphere with commercial grade reagents and solvents. Melting points were determined in open capillary tubes in a Thomas-Hoover apparatus and are uncorrected. The ^{1}H NMR were recorded on a Varian Unity Inova 400 MHz spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane. Flash column chromatography was performed using EM silica gel 60 (230−400 mesh). Reverse phase preparative HPLC were performed using a Waters Prep LC 2000 and a Waters C_{18} PrepPak 500 column. Analytical HPLC was performed using an Agilent Zorbax SD-C8 4.6 × 75 mm 3.5 μm column with a 4 min linear gradient from 95:5 to 1:99 0.1% H_3PO_4:CH_3CN at a flow rate of 3 mL/min (system

A) or a Waters Xterra RP_{18} 4.6 × 50 mm 3.5 μm column with a 4 min linear gradient from 95:5 to 5:95 0.1% H_3PO_4:CH_3CN at a flow rate of 4 mL/min (system B) with UV detection at 215 and 254 nm. Procedures for protease inhibition and 2xaPTT assays as well as animal pharmacokinetic studies have been previously described.[10]

Ethyl 2,2-Difluoro-2-pyridyl Acetate (33). To a −78 °C solution of 5.99 g (30.0 mmol) of potassium bis(trimethylsilyl)-amide in 90 mL of THF was added 1.52 mL (10.0 mmol) of ethyl 2-pyridyl acetate via syringe. The off-white slurry was stirred for 45 min, then 4.30 g (20.0 mmol) of $MnBr_2$ was added in one portion. The tan slurry was stirred for 30 min, then 8.90 g (28.2 mmol) of *N*-fluorobenzenesulfonimide was added in one portion. After a further 30 min at −78 °C, the reaction was allowed to warm to room-temperature overnight. The thick slurry was poured into saturated aqueous $NaHCO_3$ (200 mL) and CH_2Cl_2 (300 mL), the layers were separated, and the aqueous layer extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were washed and dried over $MgSO_4$, and the solvent was evaporated. The residue was flash chromatographed using 20 to 50% EtOAc−hexanes to give 1.19 g of the title compound: ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H), 4.35−4.4 (m, 2H), 7.4−7.45 (m, 1H), 7.75 (d, 1H), 7.95 (d, 1H), 8.45 (d, 1H).

2,2-Difluoro-2-(2-pyridyl)ethanol (34). To a 0 °C stirred solution of 3.97 g (19.7 mmol) of ethyl 2,2-difluoro-2-pyridyl acetate **33** in 40 mL of methanol was added 757 mg (20 mmol) of sodium borohydride in several portions. After stirring for 0.5 h, the cold bath was removed and stirring continued another 0.5 h. The reaction was quenched with 2 M HCl, and the solvents removed under reduced pressure. The residue was partitioned between ether and 10% Na_2CO_3, the aqueous layer was extracted with several portions of ether, and the combined organic layers were washed with brine and dried over $MgSO_4$. Concentration gave 2.97 g of a yellow oil that was flash chromatographed using 99:1 to 97:3 CH_2Cl_2−CH_3OH to give 1.02 g of the title compound as solid: ^{1}H NMR ($CDCl_3$) δ 8.61 (d, 1H, 4.5 Hz), 7.88 (td, 1H, 8.0, 1.7 Hz), 7.73 (d, 1H, 7.8 Hz), 7.4−7.45 (m, 1H), 4.25 (td, 2H, 12.5, 7.1 Hz), 3.46 (t, 1H, 6.6 Hz).

2,2-Difluoro-2-(2-pyridyl)ethyl Trifluoromethanesulfonate. To a stirred solution of 5 g (31.4 mmol) of 2,2-difluoro-2-(2-pyridyl)ethanol **34** and 9.69 g (47.2 mmol) of 2,6-di-*tert*-butyl-4-methylpyridine in 110 mL of methylene chloride at −78 °C was added 7.93 mL (47.2 mmol) of triflic anhydride dropwise. After 1 h, the reaction was diluted with 100 mL of pentane and filtered. The filtrate was concentrated and treated again with pentane and filtered. Concentration of the filtrate gave 10.5 g of the title compound as a brown oil, contaminated with 2,6-di-*tert*-butyl-4-methylpyridine: ^{1}H NMR ($CDCl_3$) δ 8.66 (d, 1H, 4.9 Hz), 7.89 (td, 1H, 7.7, 1.7 Hz), 7.76 (d, 1H, 7.9 Hz), 7.45−7.49 (m, 1H), 5.12 (t, 2 H, 11.9 Hz).

2,2-Difluoro-2-(2-pyridyl)ethyl Azide. A solution of 105 mg (0.31 mmol) of 2,2-difluoro-2-(2-pyridyl)ethyl trifluoromethanesulfonate and 43 mg (0.66 mmol) of sodium azide in 1.0 mL of DMF was heated at 60 °C under Ar. After 1.5 h, the mixture was cooled to room temperature, diluted with water, and extracted with two portions of ether. The combined organic layers were washed twice with water, brine, and dried over $MgSO_4$. The solvents were removed at reduced pressure and a bath temperature of 20 °C to give 50 mg of a brown oil: ^{1}H NMR ($CDCl_3$) δ 8.68 (d, 1H, 4.2 Hz), 7.86 (td, 1H, 7.8, 1.5 Hz), 7.72 (d, 1H, 7.8 Hz), 7.40−7.45 (m, 1H), 4.03 (t, 2 H, 13.2 Hz).

2,2-Difluoro-2-(2-pyridyl)ethylamine (35). A stirred solution of 1.8 g (9.7 mmol) of 2,2-difluoro-2-(2-pyridyl)ethyl azide and 500 mg of 10% Pd/C in 100 mL of ethyl acetate was hydrogenated under a balloon for 1 h. The catalyst was removed by filtration, and the solvents were removed at reduced pressure to give 1.2 g of the title compound as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.66 (d, 1H, 4.2 Hz), 7.82 (td, 1H, 7.7, 1.7 Hz), 7.68 (d, 1H, 8.1 Hz), 7.37−7.40 (m, 1H), 3.44 (t, 2 H, 14.3 Hz), 1.41 (br s, 2H).

***N*-(Ethyl carboxymethyl)-*N*′-(2,2-dimethoxyethyl)oxamide (43).** To a solution of oxamate **42**[25] (84.0 g, 414 mmol) in 2-propanol (500 mL) was added aminoacetaldehyde dimethyl acetal (45.7 g, 435 mmol) in one portion. After being stirred overnight at room temperature, the reaction mixture was concentrated to a thick orange oil. This thick slurry was diluted with 2-propanol (300 mL), and the solid was broken up with a spatula. Filtration afforded a solid which was further rinsed with an additional portion of 2-propanol. Removal of residual 2-propanol was accomplished via high vacuum to afford a light orange solid (89.8 g): ^{1}H NMR ($CDCl_3$) δ 7.82 (br s, 1H), 7.50 (br s, 1H), 4.41 (t, 1H, 5.3 Hz), 4.24 (q, 2H, 7.1 Hz), 4.09 (d, 2H, 5.9 Hz), 3.47 (dd, 2H, 5.3, 6.2 Hz), 3.40 (s, 6H), 1.30 (t, 3 H, 7.1 Hz).

Ethyl 3-Hydroxypyrazin-(1*H*)-2-one-1-acetate (44). A solution of the oxamide **43** (89.8 g, 343 mmol), acetic acid (400 mL), and concentrated HCl (2 mL) was heated to reflux. After 1 h the black reaction was concentrated to a thick oil (high vacuum employed to ensure complete removal of AcOH) which was diluted with EtOH (150 mL) and MeOH (150 mL). Scraping the thick black oil with a spatula induced precipitation of the product. The MeOH was removed via rotary evaporation, and the remaining slurry was filtered and rinsed with EtOH (200 mL) to deliver a tan solid. Recrystallization from refluxing EtOH (300 mL) afforded 33.0 g of an off-white powder: ^{1}H NMR (CD_3OD) δ 6.50 (d, 1H, 5.9 Hz), 6.36 (d, 1H, 5.9 Hz), 4.58 (s, 2H), 4.23 (q, 2H, 7.1 Hz), 1.28 (t, 3 H, 7.1 Hz). Further crude dione could be obtained upon concentration of the mother liquor.

Ethyl 3-Bromopyrazin-(1*H*)-2-one-1-acetate (45). A solution of the hydroxypyrazinone **44** (25.0 g, 126 mmol) and phosphorus oxybromide (37.9 g, 132 mmol) in 1,2-dichloroethane (250 mL) was heated to reflux. After 8 h the reaction mixture was treated with saturated aqueous Na_2CO_3 (250 mL) and stirred for 1 h. The mixture was diluted with water (100 mL) and dichloromethane (100 mL), the layers were separated, and the aqueous layer was backwashed with EtOAc (3 × 200 mL). The combined organic layers were dried ($MgSO_4$), and concentrated to give an oil which was stored on a high vacuum line overnight to afford 28.0 g of a brown solid: ^{1}H NMR ($CDCl_3$) δ 7.17 (d, 1H, 4.2 Hz), 7.07 (d, 1H, 4.2 Hz), 4.65 (s, 2H), 4.27 (q, 2H, 7.2 Hz), 1.31 (t, 3 H, 7.2 Hz).

Ethyl 6-Formyl-3-hydroxypyrazin-(1*H*)-2-one-1-acetate (47). A suspension of the hydroxypyrazinone **46**[25] (5.0 g, 23.6 mmol) and selenium(IV) oxide (2.62 g, 23.6 mmol) in 1,4-dioxane (100 mL) was heated to reflux for 24 h. The dark reaction mixture was cooled and filtered through a pad of Celite with MeOH. Concentration and flash chromatography with 3:97 to 10:90 MeOH:CH_2Cl_2 afforded 2.5 g of the title compound as an orange solid: ^{1}H NMR (CD_3OD) δ 9.11 (s, 1H), 7.39 (s, 1H), 5.12 (s, 2H), 4.22 (q, 2H, 7.1 Hz), 1.29 (t, 3 H, 7.1 Hz).

Ethyl 6-Formoximyl-3-hydroxypyrazin-(1*H*)-2-one-1-acetate (48). To a suspension of the formylpyrazinone **47** (5.43 g, 24.0 mmol) and hydroxylamine hydrochloride (1.67 g, 24.0 mmol) in ethanol (100 mL) was added pyridine (1.90 mL, 24.0 mmol). After 2 h at reflux, the reaction mixture was cooled and concentrated. This crude solid was recrystallized from ethanol (100 mL) to deliver 2.80 g of the title compound as a solid. An additional 1.90 g of product was obtained by concentration of the filtrate and trituration with water (50 mL): ^{1}H NMR (DMSO) δ 11.85 (d, 1H), 11.19 (s, 1H), 7.82 (s, 1H), 6.79 (d, 1H, 5.9 Hz), 5.05 (s, 2H), 4.12 (q, 2H, 7.1 Hz), 1.20 (t, 3 H, 7.1 Hz).

Ethyl 6-Cyano-3-hydroxypyrazin-(1*H*)-2-one-1-acetate (49). A slurry of the hydroxypyrazinone **48** (2.70 g, 11.2 mmol) and polymer-bound triphenylphosphine (1.55 mmol/g resin: 15.1 g, 23.5 mmol) in 1,2-dichloroethane (90 mL) and carbon tetrachloride (9 mL) was heated to reflux for 1.5 h. The reaction mixture was cooled and filtered and the resin rinsed with of 1:1 MeOH:CH_2Cl_2 (200 mL). Concentration of the filtrate yielded 2.70 g of the product as a tan solid: ^{1}H NMR ($CDCl_3$) δ 7.06 (s, 1H), 4.73 (s, 2H), 4.29 (q, 2H, 7.1 Hz), 1.33 (t, 3 H, 7.1 Hz).

Ethyl 3-Chloro-6-cyanopyrazin-(1*H*)-2-one-1-acetate (50). A suspension of the hydroxypyrazinone **49** (450 mg, 2.02 mmol) and ammonium chloride (237 mg, 4.44 mmol) in phosphorus oxychloride (2 mL) was heated at reflux for 1.5 h. The reaction mixture was cooled, and the volatiles were removed via rotary evaporation. The residue was quenched with water, and solid Na_2CO_3 was added until the mixture was basic. This aqueous mixture was extracted with dichloromethane (3×), and the combined organic layers were dried (Na_2SO_4) and concentrated to give 415 mg of the product as an amber oil: ^{1}H NMR ($CDCl_3$) δ 7.60 (s, 1H), 4.87 (s, 2H), 4.32 (q, 2H, 7.1 Hz), 1.31 (t, 3 H, 7.1 Hz).

Ethyl 3-(2,2-Difluoro-2-(2-pyridylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetate (52a). A solution of 7.13 g (45.1 mmol) of 2,2-difluoro-2-(2-pyridyl)ethylamine **35** and 12.4 g (45.1 mmol) of ethyl 3-bromo-6-methylpyrazin-(1*H*)-2-one-1-acetate **51**[25] in 15 mL of toluene and 15 mL of ethanol was heated to 125 °C in a sealed tube overnight. The reaction was concentrated, and the residue was diluted with ethyl acetate, washed with 15% $NaHCO_3$ and the aqueous layer backwashed with ethyl acetate (3×). The combined organic layers were dried over $MgSO_4$ and the solvents removed at reduced pressure to give an oil that was flash chromatographed using 50:50 hexanes−EtOAc to give 9.67 g of the title compound as a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.67 (d, 1H, 4.8 Hz), 7.80 (t, 1H, 7.9 Hz), 7.68 (d, 1H, 7.9 Hz), 7.36−7.39 (m, 1H), 6.71 (s, 1H), 6.31 (br t, 1H), 4.69 (s, 2H), 4.35 (td, 2H, 14.1, 6.6 Hz), 4.24 (q, 2H, 7.1 Hz), 2.11 (s, 3H), 1.29 (t, 3 H, 6.8 Hz).

3-(2,2-Difluoro-2-(2-pyridylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic Acid (52a acid). To a stirred solution of 9.67 g (27.5 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetate **52a** in 100 mL of methanol was added 8.58 g (153.0 mmol) of potassium hydroxide in 20 mL of water. After 1 h, the solution was concentrated at reduced pressure, and the residue was dissolved in 25 mL of water. This solution was neutralized (pH ∼ 7) using 1.3 M HCl and concentrated at reduced pressure to give 19.8 g of a yellow solid containing potassium chloride and the title compound: ^{1}H NMR (CD_3OD) δ 8.65 (d, 1H, 4.7 Hz), 7.95 (td, 1H, 7.9, 1.8 Hz), 7.72−7.74 (m, 1H), 7.50−7.54 (m, 1H), 6.64 (d, 1H, 1.09 Hz), 4.78 (s, 2H), 4.31 (t, 2H, 14.1 Hz), 2.16 (s, 3H).

Ethyl 3-(2,2-Difluoro-2-(2-pyridyl)ethylamino)-6-cyanopyrazin-(1*H*)-2-one-1-acetate (52b). A mixture of 0.300 g (1.9 mmol) of 2,2-difluoro-2-(2-pyridyl)ethylamine **35**, 0.35 mL (2.5 mmol) of triethylamine, and 0.42 g (1.75 mmol) of ethyl 3-chloro-6-cyanopyrazin-(1*H*)-2-one-1-acetate **50** in 3 mL of toluene and 0.5 mL of ethanol was heated to 60 °C for 1 h. The reaction was concentrated and the residue partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The aqueous phase was backwashed with CH_2Cl_2 (2×), dried, and concentrated. Flash chromatography using 25−50% EtOAc/hexanes afforded 0.38 g of the title compound as a tan powder: ^{1}H NMR ($CDCl_3$) δ 8.67 (d, 1H, 4.8 Hz), 7.86 (ddd, 1H, 1.6, 7.8, 7.8 Hz), 7.71 (dd, 1H, 0.9, 8.0 Hz), 7.43 (m, 2H), 7.35 (br t, 1H), 4.79 (s, 2H), 4.62 (dt, 2H, 6.5, 13.5 Hz), 4.29 (q, 2H, 7.1 Hz), 1.32 (t, 3 H, 7.1 Hz).

3-(2,2-Difluoro-2-(2-pyridyl)ethylamino)-6-cyanopyrazin-(1*H*)-2-one-1-acetic Acid (52b acid). To a stirred solution of 0.38 g (1.06 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridyl)ethylamino)-6-cyanopyrazin-(1*H*)-2-one-1-acetate **52b** in dimethoxyethane (10 mL) was added 1.6 mL of lithium hydroxide solution (1.0 M in water). After 1 h, the solution was neutralized using 1 M HCl. Concentration at reduced pressure (azeotrope with $PhCH_3$) afforded 0.400 g of an off-white solid containing lithium chloride and the title compound, which was used directly in the amide couplings.

Ethyl 3-(2,2-Difluoro-2-(2-pyridylethylamino)pyrazin-(1*H*)-2-one-1-acetate (52c). A solution of 4.80 g (30.4 mmol) of 2,2-difluoro-2-(2-pyridyl)ethylamine **35**, 4.24 mL (30.4 mmol) of triethylamine, and 7.93 g (30.4 mmol) of ethyl 3-bromopyrazin-(1*H*)-2-one-1-acetate **45** in 12 mL of toluene and 4 mL of ethanol was heated to 120 °C in a sealed tube overnight. The reaction was concentrated, the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$, and the aqueous layer was backwashed with CH_2Cl_2 (4×). The combined organic layers were dried over $MgSO_4$ and the solvents removed at reduced pressure to give an oil that was flash chromatographed using 60:40 to 40:60 hexanes−EtOAc to give 6.81 g of the title compound as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.67 (dd, 1H, 4.8, 0.7 Hz), 7.81 (ddd, 1H, 7.8, 7.8, 1.7 Hz), 7.69 (dd, 1H, 7.8, 1 Hz), 7.38 (dd, 1H, 5.1, 7.0 Hz), 6.86 (d, 1H, 4.8 Hz), 6.54 (br t, 1H, 5.9 Hz), 6.40 (d, 1H, 4.6 Hz), 4.54 (s, 2H), 4.38 (td, 2H, 14.0, 6.4 Hz), 4.24 (q, 2H, 7.1 Hz), 1.29 (t, 3 H, 7.1 Hz).

Ethyl 3-(2,2-Difluoro-2-(2-pyridylethylamino)-6-chloropyrazin-(1*H*)-2-one-1-acetate (52d). To a stirred solution of 6.81 g (20.1 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)pyrazin-(1*H*)-2-one-1-acetate **52c** in 100 mL of 1,2-dichloroethane was added 2.42 g (18.1 mmol) of *N*-chlorosuccinimide. An additional 242 mg (1.81 mmol) and 75 mg (0.56 mmol) of NCS were added to the reaction mixture after 1 and 1.5 h, respectively. After 2.5 h total, the solution was cooled to room temperature and partitioned between CH_2Cl_2 (150 mL) and saturated aqueous $NaHCO_3$ (200 mL). The layers were separated, and the aqueous phase was backwashed with CH_2Cl_2 (2 × 200 mL). The combined organic layers were dried over $MgSO_4$, and the solution concentrated to a volume of ∼10 mL. This liquid was directly loaded onto a SiO_2 column and eluted with 65:35 to 55:45 hexanes−EtOAc to give 6.50 g of the title compound as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.68 (d, 1H, 4.8, Hz), 7.83 (ddd, 1H, 7.7, 7.7, 1.6 Hz), 7.9 (dd, 1H, 7.9 Hz), 7.40 (dd, 1H, 4.9, 7.3 Hz), 6.96 (s, 1H), 6.49 (br t, 1H, 5.9 Hz), 4.89 (s, 2H), 4.38 (td, 2H, 13.9, 6.5 Hz), 4.26 (q, 2H, 7.1 Hz), 1.30 (t, 3 H, 7.1 Hz).

3-(2,2-Difluoro-2-(2-pyridylethylamino)-6-chloropyrazin-(1*H*)-2-one-1-acetic Acid (52d acid). To a stirred solution of 7.27 g (19.5 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)-6-chloropyrazin-(1*H*)-2-one-1-acetate **52d** in 200 mL of methanol was added 39 mL (39.0 mmol) of 1 M aq potassium hydroxide. After 3 h the solution was neutralized (pH ∼ 7) using concentrated HCl, and concentrated at reduced pressure (azeotrope with $PhCH_3$) to give 9.30 g of a white solid containing potassium chloride and the title compound: ^{1}H NMR (CD_3OD) δ 8.64 (d, 1H, 4.8 Hz), 7.93 (ddd, 1H, 7.7, 7.7, 1.5 Hz), 7.70 (d, 1H, 8.0 Hz), 7.49 (dd, 1H, 5.2, 7.4 Hz), 6.80 (s, 1H), 4.67 (s, 2H), 4.27 (t, 2H, 13.9 Hz).

***R*/*S*-2-Amino-6-methylpyridyl-3-(2-methyl-2-(phenyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide Dihydrochloride (2).** Prepared according to the previous procedure for **1**:[10] mp > 200 C; ^{1}H NMR (CD_3OD) δ 8.86 (t, 1H, 5.6 Hz), 7.85 (d, 1H, 9.2 H), 7.21−7.34 (m, 5H), 6.83 (d, 1H, 9.2 Hz), 6.52 (d, 1H, 1.1 Hz), 4.74 (s, 2H), 4.33 (d, 2H, 5.5 Hz), 3.63 (m, 2H), 3.19 (m, 1H), 2.52 (s, 3H), 2.17 (d, 3H, 1.1 Hz), 1.37 (d, 3H, 7.0 Hz); HRMS (FAB) calcd $C_{23}H_{30}N_6O_2$ (M + 1) 421.2352, found 421.2355. Anal. ($C_{23}H_{29}N_6O_2$·2.75HCl) C, H, N. Calcd 53.04, 5.95, 16.14. Found 53.44, 5.56, 15.95.

2-Amino-6-methylpyridyl-3-(2,2-dimethyl-2-(phenyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (3). Prepared according to the previous procedure for **1**:[10] mp 179−181 C; ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 4H), 7.21 (tt, 1H, 7.1, 1.4 Hz), 7.13 (d, 1H, 8.2 Hz), 6.8 (t, 1H, 5.1 Hz), 6.71 (d, 1H, 0.9 Hz), 6.2 (d, 1H, 8.1), 5.64 (t, 1H, 5.9 Hz), 4.55 (s, 2H), 4.43 (s, 2H), 4.22 (d, 2H, 5.5 Hz), 3.56 (d, 2H, 6.0 Hz), 2.22 (s, 6H), 1.37 (s, 6H). Anal. ($C_{24}H_{30}N_6O_2$·0.05H_2O) C, H, N. Calcd 66.20, 6.97, 19.30. Found 66.24, 6.99, 18.93.

5-(2-Amino-6-methyl)-pyridylmethyl 3-(2,2-Difluoro-2-(phenyl)ethylamino)-6-methylpyrazin-2-one-1-acetamide (4). To a stirred solution of 350 mg (1.09 mmol) of 3-(2,2-difluoro-2-(phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid[30] and 228 mg (1.09 mmol) 2-amino-5-aminomethyl-6-methylpyridine dihydrochloride in 3 mL of DMF were added 209 mg (1.09 mmol) of EDC, 147 mg (1.09 mmol) of HOBT, and 2 mL of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ and the aqueous layer backwashed with CH_2Cl_2 (3×). The combined organic layers were dried over $MgSO_4$ and the solvents

removed at reduced pressure. This solid was purified by reverse phase HPLC and lyophilized to give 130 mg of the TFA salt of the title compound as a white solid: ^{1}H NMR (CD_3OD) δ 7.84 (d, 1H, 9.2 Hz), 7.57 (m, 2H), 7.46 (m, 3H), 6.82 (d, 1H, 9.0 Hz), 6.65 (d, 1H, 1.1 Hz), 4.72 (s, 2H), 4.31 (d, 2H, 4.0 Hz), 4.10 (t 2H, 14.5 Hz), 2.51 (s, 3H), 2.16 (d, 3H, 0.9 Hz); Anal. ($C_{22}H_{24}N_6O_2F_2$·2.15TFA·0.85H_2O) C, H, N. Calcd 44.94, 3.99, 11.96. Found 44.90, 3.96, 11.97; LRMS 443.2 $(MH)^+$.

5-(2-Amino-6-methyl)-pyridylmethyl 3-(2-(2-Pyridyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (5). To a stirred solution of 189 mg (0.46 mmol) of 3-(2-(2-pyridyl-ethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid (TFA salt) and 110 mg (0.46 mmol) of 2-*tert*-butoxycarbonylamino-5-aminomethyl-6-methylpyridine in 7 mL of DMF were added 107 mg (0.55 mmol) of EDC, 75 mg (0.55 mmol) of HOBT, and 161 μL (1.15 mmol) of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$, H_2O (4×), and brine. The organic layer was dried over $MgSO_4$ and the solvents removed at reduced pressure. This residue was triturated with EtOAc (with heating) to give 130 mg of the product as a white solid. Through a stirred solution of this material in EtOAc was bubbled HCl(g) for 10 min at −78 °C. The reaction was allowed to warm to room-temperature overnight. The solution was concentrated and the residue triturated with EtOAc/MeOH to give the title compound as a white solid (90 mg): ^{1}H NMR (CD_3OD) δ 8.78 (d, 1H, 4.9 Hz), 8.59 (dd, 1H, 7, 7 Hz), 8.12 (d, 1H, 7.9 Hz), 8.00 (dd, 1H, 6.7, 6.7 Hz), 7.87 (d, 1H, 9.0 Hz), 6.83 (d, 1H, 8.9 Hz), 6.70 (s, 1H), 4.78 (s, 2H), 4.33 (s, 2H), 3.98 (t, 2H, 6.7 Hz), 3.49 (t, 2H, 6.6 Hz), 2.54 (s, 3H), 2.23 (s, 3H); Anal. ($C_{21}H_{25}N_7O_2$.2.0 HCl·0.35H_2O) C, H, N. Calcd 48.21, 5.53, 18.74. Found 48.22, 5.88, 18.55; LRMS 408.2 $(MH)^+$.

5-(2-Amino-6-methyl)-pyridylmethyl 3-(2-(3-Pyridyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (6). To a stirred solution of 162 mg (0.28 mmol, 56% purity) of 3-(2-(3-pyridylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid (HCl salt) and 73 mg (0.31 mmol) of 2-*tert*-butoxycarbonylamino-5-aminomethyl-6-methylpyridine in 6 mL of DMF were added 66 mg (0.34 mmol) of EDC, 44 mg (0.34 mmol) of HOBT, and 97 μL (0.70 mmol) of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$, H_2O (4×), and brine. The organic layer was dried over $MgSO_4$, and the solvents were removed at reduced pressure and flash chromatographed with 0.5 to 5% MeOH:CH_2Cl_2 to give 81 mg of a white solid. Through a stirred solution of this material in EtOAc was bubbled HCl(g) for 10 min at −78 °C. The reaction was allowed to warm to room-temperature overnight. The solution was concentrated and the residue triturated with EtOAc/MeOH to give the title compound as a tan solid (76 mg): ^{1}H NMR (CD_3OD) δ 8.95 (s, 1H), 8.79 (d, 1H, 5.7 Hz), 8.66 (d, 1H, 8.1 Hz), 8.07 (dd, 1H, 5.9, 8.1 Hz), 7.86 (d, 1H, 9.2 Hz), 6.83 (d, 1H, 9.2 Hz), 6.66 (d, 1H, 0.9 Hz), 4.78 (s, 2H), 4.33 (s, 2H), 3.86 (t, 2H, 7.1 Hz), 3.28 (t, 2H, 7.1 Hz), 2.53 (s, 3H), 2.21 (s, 3H); Anal. ($C_{21}H_{25}N_7O_2$.3.0 HCl·1.55H_2O) C, H, N. Calcd 46.30, 5.75, 18.00. Found 45.96, 5.38, 18.06; LRMS 408.3 $(MH)^+$.

5-(2-Amino-6-methyl)-pyridylmethyl 3-(2-(4-Pyridyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (7). To a stirred solution of 503 mg (0.87 mmol, 50% purity) of 3-(2-(4-pyridylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid and 210 mg (0.87 mmol) 2-*tert*-butoxycarbonylamino-5-aminomethyl-6-methylpyridine in 8 mL of DMF were added 211 mg (1.04 mmol) of EDC, 211 mg (1.04 mmol) of HOBT, and 150 μL (1.04 mmol) of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$, H_2O (4×), and brine. The organic layer was dried over $MgSO_4$, and the solvents were removed at reduced pressure and flash chromatographed with 2 to 7% MeOH:CH_2Cl_2 to give 252 mg of the product as an off-white solid. Through a stirred solution of this material in EtOAc was bubbled HCl(g) for 10 min at −78 °C. The reaction was allowed to warm to room-temperature overnight. The solution was concentrated and the residue triturated with EtOAc/MeOH to give the title compound as a yellow solid (225 mg): ^{1}H NMR (CD_3OD) δ 8.80 (d, 2H, 6.6 Hz), 8.11 (d, 2H, 6.6 Hz), 7.86 (d, 1H, 9.0 Hz), 6.83 (d, 1H, 9.1 Hz), 6.66 (d, 1H, 1.1 Hz), 4.78 (s, 2H), 4.33 (d, 2H, 5.5 Hz), 3.91 (t, 2H, 7.2 Hz), 3.65 (t, 2H, 7.1 Hz), 2.53 (s, 3H), 2.21 (d, 3H. 0.7 Hz); Anal. ($C_{21}H_{25}N_7O_2$·3.0HCl·0.60H_2O) C, H, N. Calcd 47.80, 5.58, 18.58. Found 47.41, 5.81, 18.93; LRMS 408.4 $(MH)^+$.

5-(2-Amino-6-methyl)-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)ethylamino)-6-methylpyrazin-2-one-1-acetamide (8). To a stirred solution of 50 mg (0.15 mmol) of **52a acid** and 32 mg (0.15 mmol) 2-amino-5-aminomethyl-6-methylpyridine dihydrochloride in 1 mL of DMF were added 35 mg (0.18 mmol) of EDC, 2.0 mg (0.15 mmol) of HOAT, and 105 μL (0.75 mmol) of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was diluted with saturated aqueous $NaHCO_3$, filtered, and rinsed with H_2O. This solid was flash chromatographed using 2 to 15% MeOH:CH_2Cl_2 to give 30 mg of the title compound as a white solid: ^{1}H NMR (CD_3OD) δ 8.63 (d, 1H, 4.8 Hz), 7.92 (ddd, 1H, 1.6, 7.8, 7.8 Hz), 7.71 (dd, 1H, 1.0, 7.8 Hz), 7.49 (br t, 1H, 6.3 Hz), 7.34 (dd, 1H, 8.2 Hz), 6.62 (d, 1H, 1.1 Hz), 6.38 (d, 1H, 8.4 Hz), 4.68 (s, 2H), 4.26 (m, 4H), 2.33 (s, 3H), 2.17 (d, 3H, 0.9 Hz); LRMS 444.3 $(MH)^+$; t_R = 1.47 min (99% @ 215 and 98% @ 254 nm, system A) and t_R = 0.83 min (99% @ 215, system B); HRMS (ES) calcd $C_{21}H_{23}N_7O_2F_2$ (M + 1) 444.1954, found 444.1919.

3-(2-Methyl)-pyridylmethyl 3-(2-Phenylethylamino)-6-methylpyrazin-2-one-1-acetamide (9). To a stirred solution of 442 mg (1.54 mmol) of 3-(2-phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid[10] and 183 mg (1.50 mmol) of 3-aminomethyl-2-methylpyridine in 5 mL of DMF were added 288 mg (1.50 mmol) of EDC, 203 mg (1.50 mmol) of HOBT, and 329 mg (3.25 mmol) of NMM. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was partitioned between EtOAc and H_2O and the aqueous layer backwashed with EtOAc. The combined organic layers were dried over $MgSO_4$, and the solvents were removed at reduced pressure and flash chromatographed using EtOAc (100 mg): ^{1}H NMR (DMSO) δ 8.67 (br t, 1H), 8.32 (d, 1H, 4.6 Hz), 7.56 (d, 1H, 7.6 Hz), 7.28−7.15 (m, 6H), 6.84 (br t, 1H), 6.64 (s, 1H), 4.63 (s, 2H), 4.28 (d, 2H, 5.6 Hz), 3.46 (dt, 2H, 7.6, 7.6 Hz), 2.83 (t, 2H, 7.3 Hz), 2.49 (d, 3H, 1.1 Hz), 2.05 (s, 3H); Anal. ($C_{22}H_{25}N_5O_2$·0.30H_2O) C, H, N; Calcd 66.57, 6.50, 17.65. Found 66.25, 6.56, 18.03. LRMS 392.2 $(MH)^+$.

3-Pyridylmethyl 3-(2-Phenylethylamino)-6-methylpyrazin-2-one-1-acetamide (10). To a stirred solution of 143 mg (0.50 mmol) of 3-(2-phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid[10] and 56 mg (0.52 mmol) of 3-aminomethylpyridine in 10 mL of DMF were added 166 mg (0.87 mmol) of EDC, 94 mg (0.70 mmol) of HOBT, and 110 μL (1.0 mmol) of NMM. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was diluted with H_2O, filtered, and rinsed with saturated aqueous $NaHCO_3$ and H_2O to give 164 mg of the title compound as a solid: ^{1}H NMR ($CDCl_3$) δ 8.50 (m, 2H), 7.56 (d, 1H, 7.9 Hz), 7.32−7.20 (m, 6H), 6.89 (br t, 1H), 6.78 (s, 1H), 5.92 (br t, 1H, 5.8 Hz), 4.64 (s, 2H), 4.43 (d, 2H, 6.0 Hz), 3.65 (dt, 2H, 6.7, 6.7 Hz), 2.92 (t, 2H, 7.0 Hz), 2.26 (s, 3H); Anal. ($C_{21}H_{23}N_5O_2$·0.15H_2O) C, H, N; Calcd 66.34, 6.18, 18.42. Found 66.34, 6.34, 18.59.

2-Pyridylmethyl 3-(2-Phenylethylamino)-6-methylpyrazin-2-one-1-acetamide (11). Prepared according to procedure for **10** [using 3-(2-phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid[10] (143 mg, 0.50 mmol); 2-aminomethylpyridine (56 mg, 0.52 mmol); DMF (10 mL); EDC (166 mg, 0.87 mmol), HOBT (94 mg, 0.70 mmol); NMM (110 μL, 1.0 mmol)] to give 170 mg of the title compound as a solid: ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, 4.0 Hz), 7.64 (ddd, 1H, 1.8, 7.6, 7.6 Hz), 7.31−7.17 (m, 8H), 6.76 (s, 1H), 6.78 (s, 1H), 5.97 (br t, 1H), 4.71 (s, 2H), 4.55 (d, 2H, 5.1 Hz), 3.65 (dt, 2H, 6.7, 6.7 Hz), 2.93 (t, 2H, 7.1 Hz), 2.24 (s, 3H); Anal. ($C_{21}H_{23}N_5O_2$·0.20H_2O) C, H, N; Calcd 66.19, 6.19, 18.38. Found 66.11, 6.17, 18.43.

4-Pyridylmethyl 3-(2-Phenylethylamino)-6-methylpyrazin-2-one-1-acetamide (12). Prepared according to procedure for **10** [using 3-(2-phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid (143 mg, 0.50 mmol); 4-aminomethylpyridine (56 mg, 0.52 mmol); DMF (10 mL); EDC (166 mg, 0.87 mmol), HOBT (94 mg, 0.70 mmol); NMM (110 μL, 1.0 mmol)] to give 178 mg of the title compound as a solid: ^{1}H NMR (DMSO) δ 8.82 (br t, 1H, 6.0 Hz), 8.50 (m, 2H), 7.31−7.19 (m, 7H), 6.87 (br t, 1H, 5.9 Hz), 6.66 (s, 1H), 4.67 (s, 2H), 4.33 (d, 2H, 5.9 Hz), 3.48 (dt, 2H, 6.9, 6.9 Hz), 2.85 (t, 2H, 7.4 Hz), 2.08 (s, 3H); Anal. ($C_{21}H_{23}N_5O_2$·0.10H_2O) C, H, N; Calcd 66.50, 6.17, 18.47. Found 66.49, 6.17, 18.36.

2-Pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)ethylamino)-6-methylpyrazin-2-one-1-acetamide (13). To a stirred solution of 50 mg (0.15 mmol) of **52a acid** and 16 mg (0.15 mmol) 2-aminomethylpyridine in 1 mL of DMF was added 35 mg (0.18 mmol) of EDC, 2.0 mg (0.15 mmol) of HOAT and 105 μL (0.75 mmol) of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ and the aqueous layer backwashed with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvents removed at reduced pressure. This solid was rinsed with H_2O to give 40 mg of the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.67 (d, 1H, 4.2 Hz), 8.50 (d, 1H, 4.2 Hz), 7.80 (ddd, 1H, 1.5, 7.7, 7.7 Hz), 7.68 (d, 1H, 7.9 Hz), 7.64 (ddd, 1H, 1.7, 7.7, 7.7 Hz), 7.38 (br t, 1H, 6.0), 7.32 (br s, 1H), 7.2 (m, 2H), 6.75 (s, 1H), 6.33 (br t, 1H, 6.2 Hz), 4.70 (s, 2H), 4.55 (d, 2H, 5.1 Hz), 4.36 (td, 2H, 14.1, 6.6 Hz), 2.23 (s, 3H); LRMS 415.3 $(MH)^+$; t_R = 1.46 min (99% @ 215 and 254 nm, system A) and t_R = 0.71 min (95% @ 215 and 97% @254 nm, system B); HRMS (ES) calcd $C_{20}H_{20}N_6O_2$ F_2 (M + 1) 415.1689, found 415.1652.

4-Chloro-2-pyridylmethyl 3-(2,2-Difluoro-2-(phenyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (14). To a stirred solution of 100 mg (0.31 mmol) of 3-(2,2-difluoro-2-(phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid[30] and 48 mg (0.34 mmol) of 2-aminomethyl-4-chloropyridine in 5 mL of DMF were added 60 mg (0.31 mmol) of EDC, 42 mg (0.31 mmol) of HOBT, and 51 mg (0.50 mmol) of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was purified by reverse phase HPLC to give 53 mg of the TFA salt of the title compound: ^{1}H NMR (CD_3OD) δ 8.45 (d, 1H, 5.5 Hz), 7.60 (m, 2H), 7.53 (d, 1H, 1.8 Hz), 7.48 (m, 3H), 7.42 (dd, 1H, 2.1, 5.6 Hz), 6.64 (d, 1H, 1.1 Hz), 4.85 (s, 2H), 4.55 (s, 2H), 4.130 (t 2H, 14.5 Hz), 2.23 (d, 3H, 0.9 Hz); Anal. ($C_{21}H_{20}N_5O_2ClF_2$·2.30TFA·0.60H_2O) C, H, N. Calcd 42.65, 3.29, 9.72. Found 42.66, 3.58, 9.33; LRMS 448.2 $(MH)^+$.

4-Methyl-2-pyridylmethyl 3-(2,2-Difluoro-2-(phenyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (15). Prepared according to procedure for **14** [using 3-(2,2-difluoro-2-(phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid (68 mg, 0.21 mmol); (38 mg, 0.32 mmol) 2-aminomethyl-4-methylpyridine; DMF (5 mL); EDC (60 mg, 0.32 mmol); HOBT (43 mg, 0.32 mmol); triethylamine (202 mg, 2.0 mmol)] to give 22 mg of the TFA salt of the title compound as an off-white solid: ^{1}H NMR (CD_3OD) δ 8.58 (d, 1H, 5.7 Hz), 7.85 (s, 1H), 7.75 (d, 1H, 5.3 Hz), 7.56−7.47 (m, 5H), 6.67 (s, 1H), 4.83 (s, 2H), 4.73 (s, 2H), 4.12 (t 2H, 14.5 Hz), 2.65 (s, 3H), 2.20 (s, 3H); Anal. ($C_{22}H_{23}N_5O_2F_2$·2.05TFA·1.35MeOH) C, H, N. Calcd 46.80, 4.36, 9.94. Found 46.99, 4.01, 9.55; LRMS 428.2 $(MH)^+$.

4-Methyl-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (16). Prepared according to procedure for **14** [using **52a acid** (0.49 mmol); 2-aminomethyl-4-methylpyridine (61 mg, 0.50 mmol); DMF (4 mL); EDC (95 mg, 0.49 mmol); HOBT (67 mg, 0.49 mmol); NMM (202 mg, 2.0 mmol)] to give 158 mg of the TFA salt of the title compound as a yellow solid: ^{1}H NMR (CD_3OD) δ 8.67 (d, 1H, 4.6 Hz), 8.60 (d, 1H, 6.0 Hz), 7.99 (ddd, 1H, 1.5, 7.9, 7.9 Hz), 7.86 (s, 1H), 7.77 (app d, 2H, 7.5 Hz), 7.56 (dd, 1H, 4.9, 7.3 Hz), 6.69 (s, 1H), 4.87 (s, 2H), 4.74 (s, 2H), 4.39 (t, 2H, 14.4 Hz), 2.65 (s, 3H), 2.23 (s, 3H); Anal. ($C_{21}H_{22}N_6O_2F_2$·2.35TFA) C, H, N. Calcd 44.32, 3.52, 12.07. Found 44.20, 3.28, 12.37; LRMS 429.2 $(MH)^+$.

3-Fluoro-4-methyl-2-pyridylmethyl 3-(2,2-Difluoro-2-(phenyl)ethylamino)-6-methylpyrazin-2-one-1-acetamide (17). To a stirred solution of 76 mg (0.24 mmol) of 3-(2,2-difluoro-2-(phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid and 60 mg (0.28 mmol) of 2-aminomethyl-3-fluoro-4-methylpyridine dihydrochloride salt in 4 mL of DMF were added 46 mg (0.24 mmol) of EDC, 32 mg (0.24 mmol) of HOBT, and 276 mg (2.0 mmol) of NMM. After stirring for 1 d, the volatiles were removed at reduced pressure. The residue was flash chromatographed using 95:5 $CHCl_3$−MeOH to give the title compound as a white solid: ^{1}H ($CDCl_3$) δ 8.15 (d, 1H, 4.8 Hz), 7.54 (m, 2H), 7.42 (m, 4H), 7.07 (t, 1H, 6.0 HzHz), 6.70 (s, 1H), 6.16 (br t, 1H, 6.0 Hz), 4.73 (s, 2H), 4.60 (d, 2H, 4.8 Hz), 4.10 (td, 2H, 14.3, 6.4 Hz), 2.31 (s, 3H), 2.24 (s, 3H). Conversion to the dihydrochloride salt can be carried out by treating a EtOAc solution with 3 mL of 3.0 M HCl in EtOAc, followed by concentration: Anal. ($C_{22}H_{22}N_5O_2F_3$·2.40HCl) C, H, N. Calcd 49.58, 4.61, 13.14. Found 49.53, 5.01, 12.90; LRMS 446.3 $(MH)^+$.

3-Fluoro-4-methyl-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)ethylamino)-6-methylpyrazin-2-one-1-acetamide (18). To a stirred solution of 62 mg (0.17 mmol) of **52a acid** and 63 mg (0.30 mmol) of 2-aminomethyl-3-fluoro-4-methylpyridine dihydrochloride in 2 mL of DMF were added 50 mg (0.26 mmol) of EDC, 35 mg (0.26 mmol) of HOBT and 151 mg (1.5 mmol) of triethylamine. After stirring for 1 d, the volatiles were removed at reduced pressure. The resulting dark oil was diluted with ethyl acetate and washed with 5% $NaHCO_3$, and the aqueous layer was backwashed with EtOAc (3×). The combined organic layers were dried over $MgSO_4$ and the solvents removed at reduced pressure. This residue was flash chromatographed using 95:5 $CHCl_3$−MeOH to give 42 mg of the title compound as a white solid. Conversion to the hydrochloride salt can be carried out by treating a dioxane solution with three equiv of 4.0 M HCl in dioxane, followed by concentration: ^{1}H NMR (CD_3OD) δ 8.63 (d, 1H, 4.8 Hz), 8.17 (d, 1H, 5.0 Hz), 7.93 (dd, 1H, 7.7, 7.7 Hz), 7.70 (dd, 1H, 0.9, 7.9 Hz), 7.49 (dd, 1H, ∼6, 6 Hz), 7.24 (dd, 1H, 5.2, 5.2 Hz), 6.62 (s, 1H), 4.78 (s, 2H), 4.57 (s, 2H), 4.26 (t, 2H, 14.0 Hz), 2.38 (s, 3H), 2.33 (d, 3H, 0.9 Hz); LRMS 447.2 $(MH)^+$.

3-Fluoro-2-pyridylmethyl 3-(2,2-Difluoro-2-(phenyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (19). Prepared according to procedure for **18** [using 3-(2,2-difluoro-2-(phenylethylamino)-6-methylpyrazin-(1*H*)-2-one-1-acetic acid (80 mg, 0.24 mmol); 2-aminomethyl-3-fluoropyridine dihydrochloride salt (71 mg, 0.36 mmol); DMF (1 mL); EDC (46 mg, 0.24 mmol); HOBT (32 mg, 0.24 mmol); triethylamine (287 mg, 2.84 mmol)] to give 60 mg of the title compound as a white solid. Conversion to the hydrochloride salt can be carried out by treating a EtOAc solution with 3 mL of 3.0 M HCl in EtOAc, followed by concentration: ^{1}H NMR (CD_3OD) δ 8.56 (dd, 1H, 0.9, 5.3 Hz), 8.14 (dd, 1H, 9.0, 9.0 Hz), 7.80 (m, 1H), 7.62 (m, 2H), 7.54 (m, 3H), 6.64 (s, 1H), 4.89 (s, 2H), 4.75 (s, 2H), 4.19 (t 2H, 14.6 Hz), 2.25 (s, 3H); Anal. ($C_{21}H_{20}N_5O_2F_3$·2.05HCl) C, H, N. Calcd 49.83, 4.39, 13.84. Found 50.05 4.38, 13.47; HRMS (FAB) calcd $C_{21}H_{21}N_5O_2F_3$ (M + 1) 432.1642, found 432.1638; LRMS 432.2 $(MH)^+$.

3-Fluoro-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-methylpyrazin-2-one-1-acetamide (20). Prepared according to procedure for **18** [using **52a acid** (200 mg, 0.60 mmol); 2-aminomethyl-3-fluoropyridine dihydrochloride salt (143 mg, 0.72 mmol); DMF (3 mL); EDC (119 mg, 0.62 mmol); HOBT (84 mg, 0.62 mmol); triethylamine (581 mg, 5.7 mmol)] to give 150 mg of the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.67 (dd, 1H, 0.7, 4.8 Hz), 8.31 (ddd, 1H, 1.3, 1.3, 4.6 Hz), 7.81 (ddd, 1H, 1.7, 7.7, 7.7 Hz), 7.69 (ddd, 1H, 0.9, 0.9, 8.1 Hz), 7.20 (br t, 1H), 7.37 (m, 2H), 7.23 (ddd, 1H, 8.6, 4.3, 4.3 Hz), 6.75 (d, 1H, 0.9 Hz), 6.34 (br t, 1H, 6.3 Hz), 4.73 (s, 2H), 4.63 (dd, 2H, 4.7, 1.6 Hz), 4.37 (td, 2H, 14.2, 6.5 Hz), 2.25 (d, 3H, 0.9 Hz). Conversion to the dihydrochloride salt can be carried out by treating a dioxane solution with 2 equiv of 4.0 M HCl in dioxane, followed by concentration: ^{1}H NMR (CD_3OD) δ 8.71 (br d, 1H, 4.6 Hz), 8.56 (dd, 1H, 0.9, 5.3 Hz), 8.15 (ddd, 1H, 0.9, 8.9, 8.9 Hz), 8.05 (ddd, 1H, 1.6, 7.8,

7.8 Hz), 7.83 (d, 1H, 8.1 Hz), 7.81 (m, 1H), 7.61 (dd, 1H, 5.1, 7.3 Hz), 6.68 (d, 1H, 0.9 Hz), 4.88 (s, 2H), 4.74 (d, 2H, 1.3 Hz), 4.44 (t, 2H, 14.4 Hz), 2.25 (d, 3H, 0.8 Hz); Anal. ($C_{20}H_{19}N_6O_2F_3$·2.50 HCl·0.70 MeOH) C, H, N. Calcd 45.53, 4.49, 15.39. Found 45.22, 4.36, 15.39; LRMS 433.4 $(MH)^+$.

3-Fluoro-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)pyrazin-2-one-1-acetamide (22). Prepared according to procedure for **18** [using **52c acid** (91 mg, 0.29 mmol); 2-aminomethyl-3-fluoropyridine dihydrochloride salt (70 mg, 0.35 mmol); DMF (1 mL); EDC (59 mg, 0.31 mmol); HOBT (42 mg, 0.31 mmol); triethylamine (363 mg, 3.6 mmol)] to give 40 mg of the title compound as a white solid. Conversion to the hydrochloride salt can be carried out by treating an EtOAc solution with 2.0 M HCl in EtOAc, followed by concentration. Recrystallization from hot 2-propanol gave 18 mg of pure material: ^{1}H NMR (CD_3OD) δ 8.71 (br d, 1H, 4.8 Hz), 8.58 (dd, 1H, 1.1, 5.5 Hz), 8.12 (dd, 1H, 8.6, 8.6 Hz), 8.05 (ddd, 1H, 1.7, 7.8, 7.8 Hz), 7.85 (m, 1H), 7.62 (dd, 1H, 4.9, 7.6 Hz), 7.10 (d, 1H, 5.7 Hz), 6.79 (d, 1H, 5.7 Hz), 4.79 (s, 2H), 4.75 (s, 2H), 4.47 (t, 2H, 14.4 Hz); Anal. ($C_{19}H_{17}N_6O_2F_3$·2.50 HCl·0.10 EtOAc) C, H, N. Calcd 45.07, 4.00, 16.26. Found 44.74, 4.21, 15.98; LRMS 419.1 $(MH)^+$.

3-Fluoro-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-trifluoromethylpyrazin-2-one-1-acetamide (23). To a stirred solution of 50 mg (remainder LiCl, 0.13 mmol) of 3-(2,2-difluoro-2-(2-pyridyl-*N*-oxide-ethylamino)-6-trifluoromethylpyrazin-(1*H*)-2-one-1-acetic acid[31] and 25 mg (0.13 mmol) 2-aminomethyl-3-fluoropyridine dihydrochloride in 1 mL of DMF were added 24 mg (0.13 mmol) of EDC, 14 mg (0.10 mmol) of HOAT, and 42 μL (0.3 mmol) of triethylamine. After stirring for 1 h, 25 mg of EDC was added and the reaction was allowed to stir overnight. The volatiles were removed at reduced pressure, the residue partitioned between CH_2Cl_2/saturated aqueous $NaHCO_3$ and the aqueous layer backwashed with CH_2Cl_2 (2×). The combined organic layers were dried over $MgSO_4$ and the solvents removed at reduced pressure. To a solution of this material in 5 mL of EtOH was added 10 mg 10% Pd/C and exposed to a balloon atmosphere of hydrogen. After 3 h, the reaction was filtered through Celite with EtOH and the filtrate concentrated. The residue was flash chromatographed using 1 to 10% MeOH:CH_2Cl_2 to give 35 mg of the title compound: ^{1}H NMR ($CDCl_3$) δ 8.67 (d, 1H, 4.8 Hz), 8.32 (d, 1H, 4.6 Hz), 7.84 (ddd, 1H, 1.4, 7.7, 7.7 Hz), 7.70 (d, 1H, 7.9 Hz), 7.4 (m, 3H), 7.26 (m, 1H), 7.19 (br t, 1H), 7.06 (br t, 1H, 6.1 Hz), 4.80 (s, 2H), 4.653 (d, 2H, 3.4 Hz), 4.46 (td, 2H, 13.7, 6.4 Hz); LRMS 487.3 $(MH)^+$; t_R = 2.34 min (97% @ 215 and 98% @ 254 nm, system A) and t_R = 1.90 min (95% @ 215 and 98% @ 254 nm, system B); HRMS (ES) calcd $C_{20}H_{16}N_6O_2F_6$ (M + 1) 482.1312, found 487.1280.

3-Fluoro-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-chloropyrazin-2-one-1-acetamide (24). To a stirred solution of 330 mg (0.536 mmol) of **52d acid** and 160 mg (0.805 mmol) of 2-aminomethyl-3-fluoropyridine dihydrochloride in 2 mL of DMF were added 128 mg (0.67 mmol) of EDC, 91 mg (0.67 mmol) of HOAT, and 0.30 mL (2.1 mmol) triethylamine. After stirring overnight, 80 mg of 2-aminomethyl-3-fluoropyridine dihydrochloride, 128 mg of EDC, 91 mg of HOAT, and 0.08 mL (2.1 mmol) triethylamine were added. The reaction was stirred for an additional 24 h, and the volatiles were removed en vacuo. The residue was diluted with saturated aqueous $NaHCO_3$ (10 mL) and water (15 mL) and filtered to afford a tan solid. This material was flash chromatographed using 1:99 to 5:95 MeOH−CH_2Cl_2 to give a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.67 (d, 1H, 4.6 Hz), 8.32 (d, 1H, 4.6 Hz), 7.81 (dd, 1H, 7.8, 6.4 Hz), 7.69 (d, 1H, 7.8 Hz), 7.41−7.37 (m, 2H), 7.27−7.22 (m, 2H), 6.97 (s, 1H), 6.49 (br t, 1H, 6.0 Hz), 4.93 (s, 2H), 4.66 (d, 2H, 4.0 Hz), 4.38 (td, 2H, 13.9, 6.5 Hz). Conversion to the hydrochloride salt can be carried out by diluting the product with ethyl acetate (2 mL) and treating with 5 mL of 1.5 M HCl in ethyl acetate, followed by filtration to afford a white powder (221 mg): ^{1}H NMR (CD_3OD) δ 8.72 (d, 1H, 4.6 Hz), 8.56 (dd, 1H, 0.9, 5.3 Hz), 8.16−8.11 (m, 2H), 7.87 (d, 1H, 8.1 Hz), 7.80 (ddd, 1H, 4.8, 4.8, 9.0 Hz), 7.68 (dd, 1H, 5.4, 7.2 Hz), 6.87 (s, 1H), 4.95 (s, 2H), 4.74 (d, 2H, 1.1 Hz), 4.35 (t, 2H, 13.8 Hz); Anal. ($C_{19}H_{16}N_6O_2ClF_3$·2.50HCl·0.45CH_2Cl_2) C, H, N. Calcd 40.12, 3.36, 14.44. Found 40.12, 3.16, 14.53.

3-Fluoro-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-cyanopyrazin-2-one-1-acetamide Hydrochloride (25). Prepared according to procedure for **24** [using **52b acid** (24 mg, 0.08 mmol); 2-aminomethyl-3-fluoropyridine dihydrochloride salt (24 mg, 0.12 mmol; 20 mg); DMF (1 mL); EDC (23 mg, 0.12 mmol; 25 mg); HOAT (16 mg, 0.12 mmol); triethylamine (56 μL; 50 μL)] to give a yellow solid (19 mg): ^{1}H NMR ($CDCl_3$) δ 8.66 (d, 1H, 4.8 Hz), 8.34 (dd, 1H, 1, 4.8 Hz), 7.84 (ddd, 1H, 1.5, 7.8, 7.8 Hz), 7.70 (dd, 1H, 1, 7.9 Hz), 7.44−7.39 (m, 3H), 7.33 (m, 2H), 7.26 (m, 2H), 4.83 (s, 2H), 4.68 (d, 2H, 4.4 Hz), 4.44 (dt, 2H, 6.5, 13.1 Hz); LRMS 444.5 $(MH)^+$; t_R = 2.02 min (98% @ 215 and 254 nm, system A) and t_R = 1.62 min (97% @ 215 and 96% @254 nm, system B); HRMS (ES) calcd $C_{20}H_{16}N_7O_2F_3$ (M + 1) 444.1390, found 444.1362.

3-Chloro-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-chloropyrazin-2-one-1-acetamide (26). To a stirred solution of 50 mg (0.19 mmol) **52d acid** and 62 mg (0.29 mmol) of 2-aminomethyl-3-chloropyridine dihydrochloride in 1 mL of DMF were added 56 mg (0.29 mmol) of EDC, 39 mg (0.29 mmol) of HOAT, and 0.50 mL Et_3N. After stirring overnight, the volatiles were removed in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$, filtered, and rinsed with water to afford a solid. Conversion to the HCl salt was accomplished by diluting the free base with MeOH (5 mL), treating with 2.5 M HCl (2 mL), and concentrating to a yellow solid (75 mg): ^{1}H NMR (CD_3OD) δ 8.77 (d, 1H, 5.0 Hz), 8.74 (d, 1H, 5.7 Hz), 8.57 (d, 1H, 8.3 Hz), 8.23 (dd, 1H, 7.8, 7.8 Hz), 7.94 (m, 2H), 7.78 (dd, 1H, 6.4, 6.4 Hz), 6.89 (d, 1H, 1.6 Hz), 5.0 (d, 2H, 1.1 Hz), 4.80 (s, 2H), 4.40 (t, 2H, 13.4 Hz); LRMS 468.8 $(MH)^+$.

4-Methyl-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-chloropyrazin-2-one-1-acetamide (27). Prepared according to procedure for **18** [using **52d** acid (75 mg, 0.21 mmol); 2-aminomethyl-4-methylpyridine dihydrochloride salt (49 mg, 0.25 mmol); DMF (1 mL); EDC (41 mg, 0.22 mmol); HOBT (30 mg, 0.22 mmol); triethylamine (213 mg, 2.1 mmol)] to give 40 mg of the title compound as a yellow solid. Conversion to the hydrochloride salt can be carried out by treating an EtOAc solution with 1.2 M HCl in EtOAc, followed by concentration. Recrystallization from hot 2-propanol gave 30 mg of pure material: ^{1}H NMR (CD_3OD) δ 8.66 (d, 1H, 4.9 Hz), 8.60 (d, 1H, 6.1 Hz), 7.98 (dd, 1H, 7.8, 7.8 Hz), 7.87 (br s, 1H), 7.81 (d, 1H, 6.0 Hz), 7.74 (dd, 1H, 0.9, 8.0 Hz), 7.55 (dd, 1H, 6.2, 6.2 Hz), 6.89 (d, 1H, 0.9 Hz), 4.98 (s, 2H), 4.72 (d, 2H, 3.5 Hz), 4.31 (t, 2H, 14.1 Hz), 2.67 (s, 3H); Anal. ($C_{20}H_{19}N_6O_2F_2Cl$·2.40HCl·2.10H_2O) C, H, N. Calcd 41.83, 4.49, 14.64. Found 42.23, 4.27, 14.25; LRMS 449.1 $(MH)^+$.

3-Fluoro-4-methyl-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)ethylamino)-6-chloropyrazin-2-one-1-acetamide (28). To a stirred solution of 80 mg (0.197 mmol, remainder KCl) of **52d acid** and 63 mg (0.296 mmol) of 2-aminomethyl-3-fluoro-4-methylpyridine dihydrochloride in 1 mL of DMF were added 77 mg (0.40 mmol) of EDC, 54 mg (0.40 mmol) of HOAT, and 0.13 mL (0.89 mmol) TEA. After stirring overnight, the volatiles were removed in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$, filtered, and rinsed with water to afford a brown solid. This material was flash chromatographed using 2−4% CH_3OH: CH_2Cl_2 to afford the title compound as a white solid. Conversion to the HCl salt was accomplished by diluting the free base with MeOH (5 mL), treating with 2.5 M HCl (2 mL), and concentrating to a solid (57.5 mg): ^{1}H NMR (CD_3OD) δ 8.82 (d, 1H, 4.1 Hz), 8.56 (d, 1H, 5.1 Hz), 8.33 (dd, 1H, 7.4, 7.4 Hz), 8.04 (d, 1H, 7.8 Hz), 8.00 (br t, 1H), 7.87 (br t, 1H, 5.6 Hz), 6.92 (s, 1H), 5.0 (s, 2H), 4.80 (s, 2H), 4.44 (t, 2H, 13.6 Hz), 2.621 (s, 3H).

3-Fluoro-4-methyl-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)ethylamino)-6-cyanopyrazin-2-one-1-acetamide Hydrochloride (29). Prepared according to procedure for **24** [using **52b acid** (60 mg, 0.16 mmol, remainder LiCl); 2-aminomethyl-3-fluoro-4-methylpyridine dihydrochloride (45

mg, 0.21 mmol; 45 mg); DMF (1 mL); EDC (40 mg, 0.21 mmol; 40 mg); HOAT (22 mg, 0.16 mmol); triethylamine (112 μL, 0.80 mmol; 60 μL)] to give a yellow foam (41 mg): ^{1}H NMR ($CDCl_3$) δ 8.67 (d, 1H, 4.8 Hz), 8.18 (d, 1H, 1, 4.9 Hz), 7.84 (ddd, 1H, 1.7, 7.7, 7.7 Hz), 7.70 (dd, 1H, 1.0, 8.0 Hz), 7.44 (s, 1H), 7.42 (m, 1H), 7.35 (br t, 1H), 7.32 (br t, 1H, 6.6 Hz), 7.10 (dd, 1H, 5.4, 5.4 Hz), 4.82 (s, 2H), 4.65 (d, 2H, 4.3 Hz), 4.45 (dt, 2H, 6.5, 13.7 Hz), 2.32 (d, 3H, 1.3 Hz); LRMS 458.1 $(MH)^+$.

3-Methoxy-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-chloropyrazin-2-one-1-acetamide (30). Prepared according to procedure for **24** [using **52d acid** (62 mg, 0.18 mmol); 2-aminomethyl-3-methoxypyridine dihydrochloride (52 mg, 0.27 mmol); DMF (1 mL); EDC (52 mg, 0.27 mmol); HOAT (37 mg, 0.27 mmol); NMM (200 μL)]. Conversion to the HCl salt was accomplished by diluting the free base with MeOH (5 mL), treating with 2.5 M HCl (2 mL), and concentrating to an off-white solid (45 mg): ^{1}H NMR (CD_3OD) δ 8.79 (d, 1H, 4.8 Hz), 8.33 (d, 1H, 5.5 Hz), 8.27 (m, 2H), 8.00 (m, 2H), 7.83 (dd, 1H, 5.5, 7.1 Hz), 6.91 (s, 1H), 4.99 (s 2H), 4.73 (s, 2H), 4.42 (t, 2H, 13.2 Hz), 4.13 (s, 3H); LRMS 465.1 $(MH)^+$.

4-Methoxy-2-pyridylmethyl 3-(2,2-Difluoro-2-(2-pyridyl)-ethylamino)-6-chloropyrazin-2-one-1-acetamide (31). Prepared according to procedure for **24** [using **52d acid** (86 mg, 0.25 mmol); 2-aminomethyl-4-methoxypyridine dihydrochloride (66 mg, 0.31 mmol); DMF (2 mL); EDC (80 mg, 0.26 mmol); HOAT (35 mg, 0.26 mmol); NMM (200 μL)]. Conversion to the HCl salt was accomplished by diluting the free base with MeOH (5 mL), treating with 2.5 M HCl (2 mL), and concentrating to a white solid (18 mg): ^{1}H NMR (CD_3OD) δ 8.74 (d, 1H, 5.3 Hz), 8.55 (d, 1H, 7.7 Hz), 8.17 (ddd, 1H, 3.7, 7.7, 7.7 Hz), 7.90 (d, 1H, 8.0 Hz), 7.72 (dd, 1H, 5.4, 7.4 Hz), 7.42 (m, 2H), 6.91 (s, 1H), 5.00 (s 2H), 4.70 (s, 2H), 4.37 (t, 2H, 13.7 Hz), 4.15 (s, 3H); LRMS 465.1 $(MH)^+$.

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Supporting Information Available: Procedures for the preparation of the P1 pyridines (**36**−**41**, Scheme 2) and the X-ray crystallography. This material is available via the Internet at http://pubs.acs.org.

References

(1) *Thrombosis in Cardiovascular Disorders*; Fuster, V., Verstraete, M., Eds.; W. B. Saunders Co.: Philadelphia, 1992.
(2) Stein, P. D.; Henry, J. W. Prevalence of Acute Pulmonary Embolism Among Patients in a General Hospital and at Autopsy. *Chest* **1995**, *108*, 978−981.
(3) Adang, A. E. P.; Rewinkel, J. B. M. A New Generation of Orally Active Antithrombotics: Comparing Strategies in the GPIIb/IIIa, Thrombin and Factor Xa Areas. *Drugs Future* **2000**, *25*, 369−383.
(4) Goldsack, N. R.; Chambers, R. C.; Dabbag, K.; Laurent, J. Molecules in Focus: Thrombin. *Int. J. Biochem. Cell Biol.* **1998**, *30*, 641−646.
(5) Rewinkel, J. B. M.; Adang, A. E. P. Strategies and Progress Toward the Ideal Thrombin Inhibitor. *Curr. Pharm. Design* **1999**, *5*, 1043−1075.
(6) Coburn, C. A. Small-molecule Direct Thrombin Inhibitors: 1997−2000. *Exp. Opin. Ther. Pat.* **2001**, *11*, 1−18. Sanderson, P. E. J. Chpater 8. Anticoagulants: Inhibitors of Thrombin and Factor Xa. *Annu. Reports Med. Chem.* **2001**, *36*, 79−88.
(7) For example *p*-aminobenzamidine has a K_i = 300 nM against thrombin: Hilpert, K.; Ackerman, J.; Banner, D. W.; Gast, A.; Gubernator, K.; Hadvary, P.; Labler, L.; Müller; K.; Schmid, G.; Tschopp, T. B.; Waterbeemd, H.v.d. Design and Synthesis of Potent and Highly Selective Thrombin Inhibitors. *J. Med. Chem.* **1994**, *37*, 3889−390
(8) These groups have also been implicated in provoking untoward cardiovascular effects (e.g. hypotension): Hauptmann, J.; Markwardt, F. Pharmacologic Aspects of the Development of Selective Synthetic Thrombin Inhibitors as Anticoagulants. *Semin. Thrombosis Hemostasis* **1992**, 200−217. Schumacher, W. A.; Balasubramanian, N.; St.Laurent, D. R.; Seiler, S. M. Effect of a Novel Thrombin Active-site Inhibitor on Arterial and Venous Thrombosis. *Eur. J. Pharmacol.* **1994**, *259*, 165−171.
(9) Hirsh, J. Synthetic Oral Thrombin Inhibitors: New Promises for Cardiovascular Therapy. Presented at the VII Congress International Society of Thrombosis and Haemostasis, July 6−12, 2001, Paris, France; Abstract SY111. Johansson, L. C.; Eriksson, L.; Frison, L.; Fager, G. A Comparison of the Pharmacokinetics of Ximelagatran in Young and Elderly Healthy Subjects. Presented at the VII Congress International Society of Thrombosis and Haemostasis, July 6−12, 2001, Paris, France; Abstract P782.
(10) Sanderson, P. E. J.; Lyle, T. A.; Cutrona, K. J.; Dyer, D. L.; Dorsey, B. D.; McDonough, C. M.; Naylor-Olsen, A. M.; Chen, I−W.; Chen, Z.; Cook, J. J.; Cooper, C. M.; Gardell, S. J.; Hare, T. R.; Krueger, J. A.; Lewis, S. D.; Lin, J. H.; Lucas, B. J.; Lyle, E. A.; Lynch, J. J.; Stranieri, M. T.; Vastag, K.; Yan, Y.; Shafer, J. A.; Vacca, J. P. Efficacious, Orally Bioavailable Thrombin Inhibitors Based on 3-Aminopyridinone or 3-Aminopyrazinone Acetamide Peptidomimetic Templates. *J. Med. Chem.* **1998**, *41*, 4466−4474.
(11) Sanderson, P. E.J; Stanton, M. G.; Balani, S. K. U.S. Patent No. 6,147,078, 2000. Riffel, K. A.; Hengchang, S.; Gu, X.; Yan, K.; Lo, M.-W. Simultaneous Determination of a Novel Thrombin Inhibitor and its Two Metabolites in Human Plasma by Liquid Chromatography/Tandem Mass Spectrometry. *J. Pharm. Biomed. Anal.* **2000**, *23*, 607−616.
(12) Lewis, S. D.; Ng, A. S.; Lyle, E. A.; Mellott, M. J.; Appelby, S. D.; Brady, S. F.; Stauffer, K. S.; Sisko, J. T.; Mao, S.-S.; Veber, D. F.; Nutt, R. F.; Lynch, J. J.; Cook, J. J.; Gardell, S. J.; Shafer, J. A. Inhibition of Thrombin by Peptides Containing Lysyl-α-Keto Carbonyl Derivatives. *Thromb. Haemost.* **1995**, *74*, 1107−1112.
(13) For a discussion of the general criteria for thrombin inhibitor development see: Tucker, T.J; Isaacs, R. C. A. The Development of Novel Noncovalent Thrombin Inhibitors. *Adv. Amino Acid Mimetics Peptidomimetics* **1999**, *2*, 53−87.
(14) Lumma, W. C.; Witherup, K. M.; Tucker, T. J.; Brady, S. F.; Sisko, J. T.; Naylor-Olsen, A. M.; Lewis, S. D.; Lucas, B. J.; Vacca, J. P. Design of Novel, Potent, Noncovalent Inhibitors of Thrombin with Nonbasic P-1 Substructures: Rapid Structure−Activity Studies by Solid-Phase Synthesis. *J. Med. Chem.* **1998**, *41*, 1011−1013.
(15) Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Baskin, E. P.; Woltmann, R.; Lynch, J. J.; Lyle, E. A.; Appleby, S. D.; Chen, I.-W.; Dancheck, K. B.; Vacca, J. P. Potent Noncovalent Thrombin Inhibitors That Utilize the Unique Amino Acid D-Dicyclohexylalanine in the P3 Position. Implications on Oral Bioavailability and Antithrombotic Efficacy. *J. Med. Chem.* **1997**, *40*, 1565−1569.
(16) Reported as number of carotid artery vesssels (n = 6) occluding after a 10 μg/kg/min 180 min infusion (arterial injury is initiated after 120 min, followed by a 60 min observation period). Reflow indicates that the vessel occluded then subsequently reopened. Kurz, M. D.; Main, B. W.; Sandusky, G. E. Rat Model of Arterial Thrombsosis Induced by Ferric Chloride. *Thromb. Res.* **1990**, *60*, 269−280. See also ref 12.
(17) Altuntas, T. G.; Gorrod, J. W. Factors Involved in the N-Oxidation of Isomeric Aromatic Diazines by Microsomal Preparations. *Drug Metab. Drug Interact.* **1995**, *12*, 117−130.
(18) Park, B. K.; Kitteringham, N. R. Effects of Fluorine Substitution on Drug Metabolism: Pharmacological and Toxicological Implications. *Drug Metab. Rev.* **1994**, *26*, 605−643.
(19) Hunter, C. A.; Sanders, J. K. M. The Nature of $\pi-\pi$ Interactions. *J. Am. Chem. Soc.* **1990**, *112*, 5525−5534.
(20) Singh, R.; Wong, B. Unpublished results.
(21) Isaacs, R. C. A.; Cutrona, K. J.; Newton, C. L.; Sanderson, P. E. J.; Solinsky, M. G.; Baskin, E. A.; Chen, I.-W.; Cooper, C. M.; Cook, J. J.; Gardell, S. J.; Lewis, S. D.; Lucas, B. J.; Lyle, E. A.; Lynch, J. J.; Naylor-Olsen, A. M.; Stranieri, M. T.; Vastag, K.; Vacca, J. P. C6 Modification of the Pyridinone Core of Thrombin Inhibitor L-374,087 as a Means of Enhancing Its Oral Absorption. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1719−1724.
(22) The X-ray structure of **24** bound to thrombin revealed that the 6-chloro group effectively occupies the lipophilic insertion loop and all other enzyme−inhibitor interactions are essentially the same as in the methylpyrazinone analogue **20** (see Supporting Information).
(23) The low oral bioavailability observed in rhesus monkey was determined to be due to extensive first-pass hepatic elimination.
(24) Wong, B. Unpublished results.
(25) Fleitz, F. J.; Lyle, T. A.; Zheng, N.; Armstrong, J. D., III; Volante, R. P. Kilogram Scale Synthesis of the Pyrazinone Acetic Acid Core of an Orally Efficacious Thrombin Inhibitor. *Synth. Commun.* **2000**, *30*, 3171−3180.
(26) Matsumura, Y.; Nakano, T.; Morisawa, Y. Preparation of Electron-withdrawing Group-containing α,α-difluoro Compounds. Patent No. JP 09110729 A2 970428, 1995.
(27) Copéret, C.; Adolfsson, H.; Khuong, T−A. V.; Yudin, A. K.; Sharpless, K. B. A Simple and Efficient Method for the Preparation of Pyridine *N*-Oxides. *J. Org. Chem.* **1998**, *63*, 1740−1741.

(28) Sakamoto, T.; Kaneda, S−I.; Nishimura, S.; Yamanaka, H. Site-Selectivity in the Cyanation of 3-Substituted Pyridine 1-Oxides with Trimethylsilanecarbonitrile. *Chem. Pharm. Bull.* **1985**, *33*, 565−571.
(29) Cheeseman, G. W. H.; Freestone, A. J.; Godwin, R. A.; Hough, T. L. Experiments on the Synthesis of Pyrazine Nucleosides. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1888−1891.
(30) Sanderson, P. E.J; Lyle, T. A.; Dorsey, B. D. WO 99/11267, 1999.
(31) Burgey, C. S.; Isaacs, R. C. A.; Dorsey, B. D.; Robinson, K. A.; Staas, D. D.; Sanderson, P. E. J.; Barrow, J. C. WO 01/38323, 2001.

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