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Non-covalent Thrombin Inhibitors Featuring P_3-Heterocycles with P_1-Bicyclic Arginine Surrogates†

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Abstract—Novel, potent, and highly selective classes of thrombin inhibitors were identified, which resulted from judicious combination of P_4-aromatics and P_2-P_3-heterocyclic dipeptide surrogates with weakly basic (calcd pK_a ~non-basic—8.6) bicyclic P_1-arginine mimics. The design, synthesis, and biological activity of achiral, non-covalent, orally bioavailable inhibitors **NC1–NC44** featuring P_1-indazoles, benzimidazoles, indoles, benzotriazoles, and aminobenzisoxazoles is disclosed.

Thrombosis, or excessive blood clotting, is a significant factor in cardiovascular and related diseases, accounting for nearly half of US and European deaths annually. Although thrombus formation occurs normally in blood vessels to repair minor internal injuries, major vessel injury or other pathophysiological conditions can cause the thrombus to become very large. Acute myocardial infarction (MI, heart attack), unstable angina (serious chest pain preceding MI), ischemic stroke, deep vein thrombosis (DVT), pulmonary embolism and disseminated intravascular coagulation (DIC) all result from such aberrant thrombosis.[1]

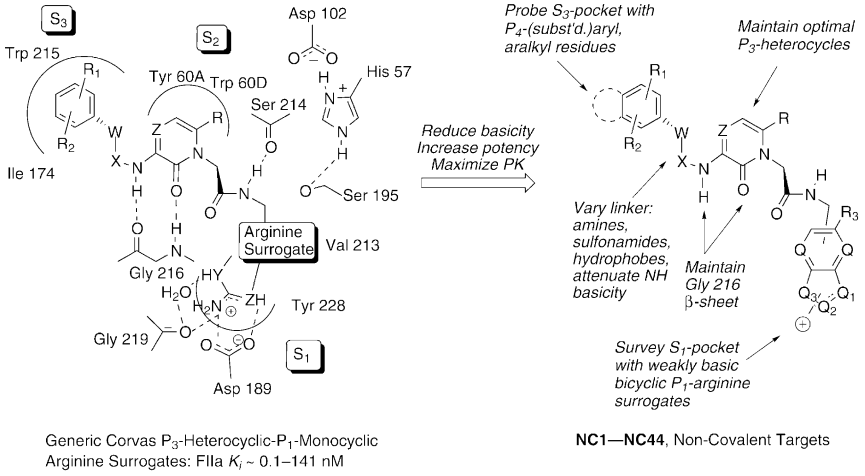

Figure 1. Design of non-covalent thrombin inhibitors **NC1–NC44** featuring weakly basic bicyclic P_1-arginine mimics.

†J.E.S. dedicates this paper with great admiration and respect to his industrial mentor, Dr. James E. Powell, Ph.D., Shell Development BSRC and DuPont Agricultural Products, on the occasion of his 60th birthday.

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Thrombin (fIIa), a multifunctional serine protease with trypsin-like specificity, plays a central role in thrombosis and hemostasis by regulating the blood coagulation cascade and platelet activation. Serving as the terminal enzyme of the cascade, thrombin cleaves the zymogen fibrinogen to fibrin, which ultimately combines with platelets and other components to form a blood clot.[2] Limited efficacy and side effects of established antithrombotics including heparin, warfarin, and aspirin have provided an impetus for the development of alternate drug classes.[3] Thrombin, along with the other procoagulants factor Xa (fXa) and prothrombinase (PTase), are deemed attractive targets for therapeutic intervention and continue to attract enormous attention in the pharmaceutical industry.[4]

Evolution of earlier covalent P_2-P_3-heterocyclic thrombin[5] and fXa[6] inhibitor classes coupled with a desire to overcome the limitations imposed by the P_1-argininal functionality, whose high basicity negatively impacted oral bioavailability (OBA) and pharmacokinetic (PK) profiles while imposing chiral lability and scale-up issues, led us to pursue novel classes of non-covalent thrombin inhibitors. Along these lines, we recently described potent, selective P_3-(benzo)lactams[7a] and P_3-heterocycles[7b] featuring monocyclic P_1-arginine mimics of varying basicity (calcd pK_a ~non-basic—14.1). Achiral inhibitor candidates with optimal P_4-(substituted)aromatic groups and weakly basic monocyclic P_1-moieties emerged that displayed moderate to good PK profiles when dosed in dogs po at 1 mg/kg.[7b]

Concurrent SAR development, modeling of prototypes (Fig. 1), and perusal of the reference inhibitors **L374,087**[8] and **L375,378**[9] (Table 1, both feature the P_1-2-amino-6-methyl-5-pyridylmethyl group, **AmMePyr**, calcd pK_a ~7.2) led us to design a new generation of achiral thrombin inhibitors **NC1–NC44**.[10] These targets exploit a range of weakly basic bicyclic P_1-hetero-aromatic arginine mimetics, which in turn were expected to positively impact OBA and PK profiles. As such however, they were predicted a priori to be less potent inhibitors in vitro than conventional P_1-benzylamidines and this potential issue necessitated the elucidation of alternate active-site interactions so as to restore the requisite potency levels.[7,10,11]

As summarized in Figure 1, our design strategy included: (a) Packing the thrombin S_3 specificity pocket with optimally substituted P_4-aromatics (increased potency, selectivity, metabolic stability), (b) Linking P_4-aromatics from the P_3-heterocyclic NH function via tethers composed of 2–3 tetrahedral atoms, (c) Maintaining intrinsic potency from P_3-pyridones and pyridazinones via β-sheet H-bond with Gly216 plus other interactions at the 60′s loop of S_2, and (d) Surveying the S_1 binding pocket with diverse bicyclic P_1-heteroaromatic arginine surrogates[12] of low basicity (~neutral or calcd pK_a ~3.6–8.6).[13] Ideally, such P_1-surrogates would preserve important H-bonds and/or salt bridges with Asp189 and glean additional hydrophobic interactions at S_1.

The bicyclic heteroaromatic P_1-arginine mimics investigated are collected in Figure 2 and include 3-substituted indazoles, 3-aminobenzisoxazoles, benzimidazoles, indoles, benzotriazoles, and 2-aminobenzothiazoles. They are identified SAR in Table 1 by the indicated acronyms. The calculated pK_a values are included.[13] Several potent, selective, and orally bioavailable thrombin inhibitors resulted from this exercise.

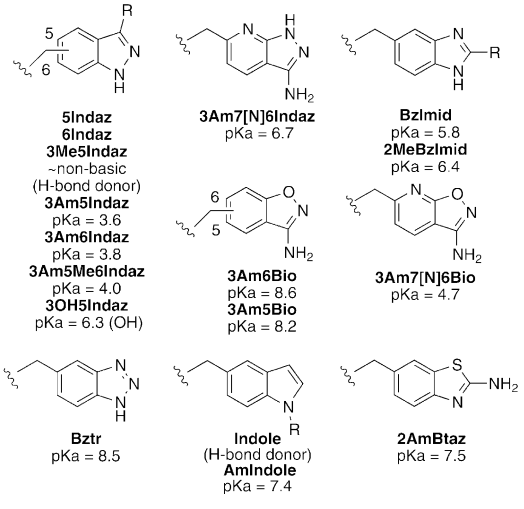
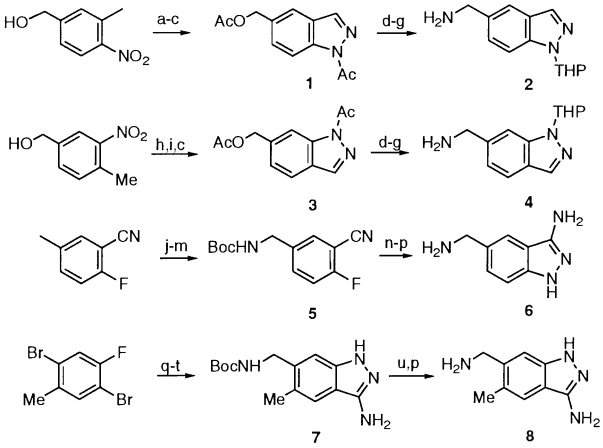


Figure 2. Acronyms and calculated pK_a values for representative bicyclic P_1-arginine surrogates.



Scheme 1. Indazole intermediates-1. Reagents and conditions: (a) H_2, Pd/C, EtOH, 10 psi, 1.5 h, 99%; (b) Ac_2O, KOAc, $CHCl_3$, rt to reflux, 2 h; (c) *i*-amyl nitrite, 18-C-6, reflux, 28 h, Ac_2O, rt, 12 h, 90–95% for 2 steps; (d) HBr, rt, 25 h, 84–92%; (e) DHP, THF, reflux, 2 h, 72–80%; (f) NaN_3, DMF, 90 °C, 30 min, 83–99%; (g) $LiAlH_4$, THF, 0 °C, 1 h, 92–97%; (h) H_2, Pd/C, EtOAc, 10 psi, 48 h, 51%; (i) Ac_2O, KOAc, $CHCl_3$, rt to reflux, 2 h, 92%; (j) NBS, AIBN, CCl_4, reflux, 59%; (k) NaN_3, DMF, rt, ~quant; (l) Ph_3P, THF, H_2O, 0 °C to rt, 85%; (m) Boc_2O, CH_2Cl_2, 83%; (n) NH_2NH_2, *n*-BuOH, reflux, 52%; (o) 2 M HCl, dioxane, rt, 3 h, ~quant; (p) AG1-X8 (OH^-) resin, MeOH, rt, 96%-quant; (q) CuCN, DMF, reflux, 6 h, 76%; (r) NH_2NH_2, EtOH, reflux, 17 h, 91%; (s) BH_3·THF, 0 °C to rt, 15 h; (t) Boc_2O, $NaHCO_3$, THF, MeOH, rt, 60% for two steps; (u) 2 M HCl, MeOH, dioxane, rt, 4 h, ~quant.

Table 1. In vitro and in vivo activity of P_3-heterocyclic-P_1-bicyclic arginine surrogate thrombin inhibitors **NC1–NC44**

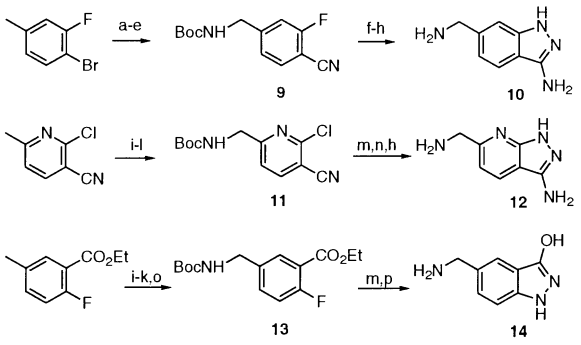
Compd	P_4	P_3	P_1	K_i FIIa (nM)[a]	Dog PK dosed po @ 1 mg/kg		
					AUC (μg*min/mL)	C_{max} (μg/mL)	$T_{1/2}$ (min)
Reference standards							
L374,087	$BnSO_2$	Pdn(6-Me)	AmMePyr	0.7	160±28	0.79±0.12	107±7
L375,378	3PhEtAm	Pdn[4aza](6Me)	AmMePyr	1.0	275±17	0.90±0.05	199±11
New targets							
NC1	$BnSO_2$	Pdn(6Me)	5Indaz	8.7	30.9±13.7	0.41±0.16	36±11
NC2	5-(2,3-Dihydro-benzofuran)-SO_2	Pdn(6Me)	6Indaz	1318			
NC3	5-(2,3-Dihydro-benzofuran)-SO_2	Pdn(6Me)	5Indaz	3.9			
NC4	PhEtAm	Pdn[4aza](6Me)	6Indaz	1587	132±26	0.96±0.09	25±10
NC5	PhEtAm	Pdn[4aza](6Me)	5Indaz	18.4			
NC6	5-(2,3-Dihydro-benzofuran)Et	Pdn[4aza](6Me)	5Indaz	2.9			
NC7	(3MeO)PhEtAm	Pdn[4aza](6Me)	5Indaz	10.4			
NC8	Ph((*R*)-2Me)EtAm	Pdn[4aza](6Me)	5Indaz	5.7			
NC9	Pyr-2EtAm	Pdn[4aza](6Me)	5Indaz	3.1			
NC10	(3-OMe)$PhSO_2$	Pdn	3Am5Indaz	325.4	237±43	0.93±0.11	274±86
NC11	(3-OMe)$PhSO_2$	Pdn	3Am6Indaz	3571	62.6±13.3	0.35±0.05	111±7
NC12	$BnSO_2$	Pdn(6Me)	3Am5Indaz	72.9			
NC13	$BnSO_2$	Pdn(6Me)	3Am6Indaz	223.8	78.7±6.12	0.58±0.31	84±24
NC14	$BnSO_2$	Pdn(6Me)	3Am7[N]6Indaz	1587			
NC15	$BnSO_2$	Pdn(6Me)	3Am5Me6Indaz	271.4			
NC16	5-(2,3-Dihydro-benzofuran)-SO_2	Pdn(6Me)	3Am5Indaz	28.7	17.9±9.7	0.23±0.09	72±29
NC17	PhEtAm	Pdn[4aza](6Me)	3Am5Indaz	89.2	291±106	1.03±0.20	166±35
NC18	(4MeO)Ph(2,2-cycloPr)EtAm	Pdn[4aza](6Me)	3Am5Indaz	9.0	196±70	1.37±0.32	89±10
NC19	(4F)Ph(2,2-cycloPr)EtAm	Pdn[4aza](6Me)	3Am5Indaz	15.4	177±79	0.88±0.23	124±25
NC20	Ph(2,2-diF)EtAm	Pdn[4aza](6Me)	3Am5Indaz	4.1	356±94	0.96±0.01	218±46
NC21	$BnSO_2$	Pdn(6Me)	3Am5Bio	14,300	37.8±5.42	0.24±0.04	132±25
NC22	$BnSO_2$	Pdn(6Me)	3Am6Bio	74.3	221±46	1.6±0.3	93±15
NC23	$BnSO_2$	Pdn(6Me)	3Am7[N]6Bio	203.2	14.2±3.5	0.28±0.07	36±17
NC24	(4Br)$BnSO_2$	Pdn(6Me)	3Am6Bio	22.2	328±54	1.52±0.12	109±14
NC25	PhEtAm	Pdn[4aza](6Me)	3Am6Bio	82.2	237±39	1.03±0.11	151±21
NC26	Ph_2EtAm	Pdn[4aza](6Me)	3Am6Bio	31.0	132±26	0.30±0.02	250±57
NC27	(4F)PhEtAm	Pdn[4aza](6Me)	3Am6Bio	17.6	281±58	0.64±0.06	261±41
NC28	(4F)Ph(2,2-cycloPr)EtAm	Pdn[4aza](6Me)	3Am6Bio	15.5	189±55	0.85±0.19	130±15
NC29	Ph(2,2-diF)EtAm	Pdn[4aza](6Me)	3Am6Bio	2.5	362±84	1.20±0.18	197±22
NC30	Pyr-2-(2,2-cycloPr)EtAm	Pdn[4aza](6Me)	3Am6Bio	25.9	134±13	0.77±0.08	100±6
NC31	Ph(2,2-cycloBu)-EtAm	Pdn[4aza](6Me)	3Am6Bio	10.2	129±41	0.62±0.16	117±15
NC32	(4Cl)$BnSO_2$	Pdn(6Me)	BzImid	6.5	12.1±6.1	0.17±0.08	69±60
NC23	PhEtAm	Pdn[4aza](6Me)	BzImid	16.7	98±13	0.39±0.01	106±15
NC34	(4MeO)PhEtAm	Pdn[4aza](6Me)	BzImid	5.0	27.5±1.5	0.19±0.01	91±18
NC35	Ph[2(*S*)Me]EtAm	Pdn[4aza](6Me)	BzImid	9.3	60.4±15.9	0.48±0.15	69±9
NC36	PhEtAm	Pdn[4aza](6Me)	2MeBzImid	>3968			
NC37	PhEtAm	Pdn[4aza](6Et)	Indole	23.7	31.4±8.7	0.31±0.08	84±17
NC38	Indane-5EtAm	Pdn[4aza](6Me)	Indole	29.8			
NC39	PhEtAm	Pdn[4aza](6Me)	Amindole	12,400			
NC40	PhEtAm	Pdn[4aza](6Me)	3Me5Indaz	444.0			
NC41	PhEtAm	Pdn[4aza](6Me)	3OH5Indaz	33.0	1.1±0.8	0.03±0.01	ND
NC42	Ph(2,2-cycloBu)EtAm	Pdn[4aza](6Me)	3OH5Indaz	10.0			
NC43	PhEtAm	Pdn[4aza](6Me)	2AmBtaz	>3968			
NC44	PhEtAm	Pdn[4aza](6Me)	Bztr	223.8			

[a]Inhibition constants (K_i) of compounds **NC1–44** were derived from the corresponding IC_{50} values necessary to inhibit human thrombin (FIIa) cleavage of the chromogenic substrate described in ref 11a by 50%. Reported values for each compound are from one to three IC_{50} determinations that confirmed the initial range values.

The classic Huisgen protocol[14] was employed to construct indazole derivatives **2**, **4**, **6**, and **8**, proceeding via the intermediates **1**, **3**, **5**, and **7**, respectively (Scheme 1).[15] Approaches to 3-aminoindazole **10**[16] (regioisomer of **6**, via **9**), 3-amino-7-azaindazole **12** (via **11**)[17] and 3-hydroxyindazole **14** (via **13**)[16b] are summarized in Scheme 2. Key intermediates **15** and **17** were efficiently prepared by modification of the acetohydroxamic acid method (Scheme 3).[18] Two complimentary, scalable routes were established for construction of the 3-aminobenzisoxazole derivative **15** that proceeded through the intermediacy of either **9** or **16**. 3-Amino-7-aza-benzisoxazole **17** was obtained in seven steps from 2-chloro-3-cyano-6-methylpyridine via intermediate **11**.

Using variations of the above methods, benzimidazole-5-carboxylic acid precursors were elaborated via intermediates **18** and **20** to the corresponding protected amines **19** and **21** (Scheme 4). Indoles **22** and **23**, benzotriazole **24**, and 2-aminobenzothiazole **25** were secured employing analogous methods.

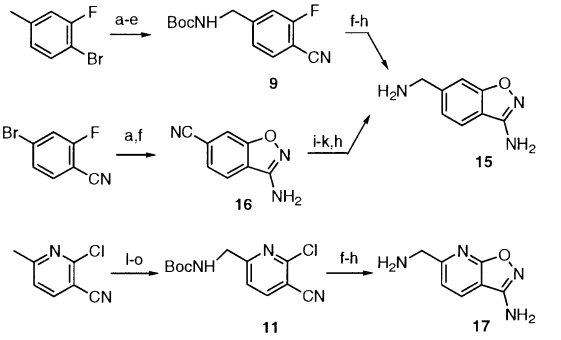
Scheme 5 outlines the final coupling and elaboration reactions between P_3-heterocyclic-P_2-acetic acid intermediates **26**–**28**[5,7–9] with the various P_1-arginine precursors. Coupling reactions generally employed EDC, HOBt (or HOAt) with Et_3N (or NMM) at ambient temperature in dry acetonitrile, THF, or DMF solvents or mixtures thereof. Optional final deprotections with



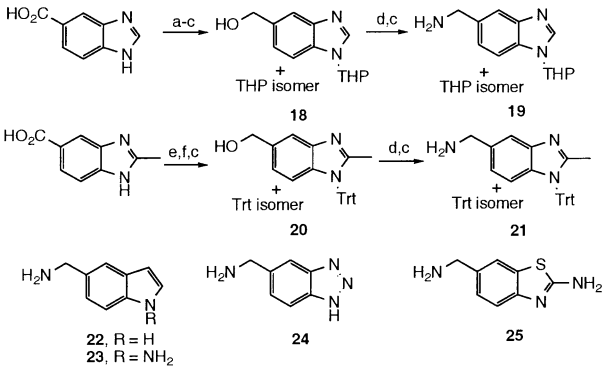
Scheme 2. Indazole intermediates-2. Reagents and conditions: (a) CuCN, DMF, reflux, 5 h, 94%; (b) NBS, AIBN, CCl_4, reflux, 4.5 h, 54%; (c) phthalimide, Cs_2CO_3, DMF, rt, 30 min, 91%; (d) NH_2NH_2, *n*-BuOH, reflux, 5 min; (e) Boc_2O, CH_2Cl_2, rt, 1 h, 99% for 2 steps; (f) NH_2NH_2, *n*-BuOH, reflux, 22 h, 64%; (g) 2 M HCl, dioxane; (h) AG1-X8 resin (OH^-), 94%-quant for 2 steps; (i) NBS, $(PhCO_2)_2$, CCl_4, reflux, 6–18 h, 35–65%; (j) NaN_3, DMF, rt, 15 h, 79%; (k) Ph_3P, THF, H_2O, 0 °C to rt, 15–18 h, 52–98%; (l) Boc_2O, THF, rt, 15 h, 42%, (m) NH_2NH_2, *n*-BuOH, reflux, 4–48 h, ~70%-quant; (n) 2 M HCl, dioxane, ~quant; (o) Boc_2O, $NaHCO_3$, H_2O, dioxane, rt, 18 h, 65%; (p) 4 M HCl, dioxane, MeOH, rt, 2 h, ~50–70% overall.

HCl were followed by RP-HPLC purification to deliver the final targets **NC1–44**, generally in satisfactory overall yields.

The in vitro biological activity of our targets **NC1–NC44** along with the standards **L374,087**[8] and **L375,378**[9] is summarized in Table 1 (note P_3 ring: Pdn = pyridone, Pdn(6Me) = 6-methylpyridone and Pdn[4aza](6Me) = 6-methylpyrazinone). In vivo PK data from oral cassette dosing studies in fed dogs at 1 mg/kg is included for most compounds. Moderate to excellent levels of thrombin inhibition were observed for several analogues, with K_i's = 2.5–33 nM for the best candidates. All new inhibitors were selective against the ubiquitous digestive enzyme trypsin, the thrombolytic enzymes plasmin, tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), as well as



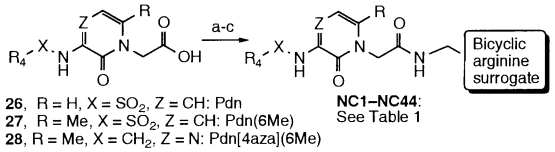
Scheme 3. P_1-Aminobenzisoxazole derivatives. Reagents and conditions: (a) CuCN, DMF, reflux, 5–18 h, 90–94%; (b) NBS, AIBN, CCl_4, reflux, 4.5 h, 54%; (c) phthalimide, Cs_2CO_3, DMF, rt, 30 min, 91%; (d) NH_2NH_2, *n*-BuOH, reflux, 5 min; (e) Boc_2O, CH_2Cl_2, rt, 1 h, 99% for 2 steps; (f) $CH_3CONHOH$, KO-*t*-Bu, DMF, rt, 15 h; 70 °C, 24 h, 55–75%; (g) 2 M HCl, dioxane; (h) AG1-X8 resin (OH^-), 94%-quant for two steps; (i) BH_3·THF, THF, rt, 15 h; (j) Boc_2O, NaOH, THF-MeOH, rt, 18 h; (k) 4 M HCl, dioxane, MeOH, rt, 3 h, 78% overall; (l) NBS, $(PhCO_2)_2$, CCl_4, reflux, 6 h, 35%; (m) NaN_3, DMF, rt, 15 h, 79%; (n) Ph_3P, THF, H_2O, 0 °C to rt, 15 h, 52%; (o) Boc_2O, THF, rt, 15 h, 42%.



Scheme 4. P_1-Benzimidazoles and other heteroaromatic derivatives. Reagents and conditions: (a) MeOH, H_2SO_4 reflux, 24 h, 64%; (b) dihydropyran, CSA (cat.), THF, reflux, 20 h, 99%; (c) $LiAlH_4$, THF, 0 °C, 30–40 min, 95–98%; (d) $(PhO)_2P(O)N_3$, DBU, THF, 0 °C to rt, 15 h, 62–72%; (e) EtOH, $SOCl_2$, reflux, 3 h, 93%; (f) TrtCl, Et_3N, CH_2Cl_2, rt, 90%.

activated protein C (PCa). In general, SAR trends indicated that in vitro potency decreased as a function of the P_1-arginine surrogate as follows: 5Indaz (**NC3**,**6**,**9**, ~non-basic, H-bond donor to Asp189 and/or water-bridge) ≥ BzImid (**NC32–35**, $pK_a = 5.8$) > Indole (**NC37**,**38**, ~non-basic, H-bond donor to Asp189 and/or water-bridge) > 3OH5Indaz (**NC41**,**42**, $pK_a = 6.3$, OH) > 3Am6Bio (**NC22**,**24**,**25–31**, $pK_a = 8.6$) ≥ 3Am5Indaz (**NC10**,**12**,**16**,**17–20**, $pK_a = 3.6$) > 3Am7[N]6Bio (**NC23**, $pK_a = 4.7$) > 3Am6Indaz (**NC13**, $pK_a = 3.8$) ~ Bztr (**NC44**, $pK_a = 8.5$) > 3Am5Me6Indaz (**NC15**, $pK_a = 4.0$) > 3Me5Indaz (**NC40**, ~non-basic) > 6Indaz (**NC2**,**4**, ~non-basic) > 3Am7[N]6Indaz (**NC14**, $pK_a = 6.7$) > 2AmBtaz (**NC43**, $pK_a = 7.5$) ~ 2MeBzImid (**NC36**, $pK_a = 6.4$) > Amindole (**NC39**, $pK_a = 7.4$) > 3Am5Bio (**NC21**, $pK_a = 8.2$). Potency enhancements resulting from incorporation of the P_4-arylsulfonamido- and P_4-arylalkyl-type moieties listed in Table 1 correlated well with prior work.[5,7–9] Although greater inhibitory potency usually results by increasing P_1-basicity (> p*K*a),[7] in this work retention of the intrinsically potent P_3-pyridone and P_3-pyrazinone residues allows for efficient accommodation of neutral or weakly basic bicyclic P_1-arginine surrogates. These heterocycles expressed good in vitro activity due to their favorable topography and ability to effectively participate in key H-bonding and hydrophobic interactions at the S_1 pocket. Further active-site interactions are discussed below.

Based on the in vivo parameters listed in Table 1 (oral dosing, AUC, C_{max}, $T_{1/2}$), relative oral bioavailability



Scheme 5. Coupling and elaboration to targets **NC1–NC44**. Reagents and conditions: (a) couple protected P_1-amine precursors **2**, **4**, **6**, **8**, **10**, **12**, **14**, **15**, **17**, **19**, and **21–25**: EDC, HOBt or HOAt, Et_3N or NMM, CH_3CN, THF, or DMF; 0 °C to rt, 12–15 h, 47%–quant; (b) optional deprotection of P_1-group: for **NC1–9**, **33–36**: HCl, dioxane, 0 °C to rt, (c) RP-HPLC, 40–90%.

(OBA) ranking of lead candidates decreased as a function of the P_1-arginine surrogate in the following order: 3Am6Bio (**NC22**, $pK_a = 8.6$) $\geq$ 3Am5Indaz (**NC10**, $pK_a = 3.6$) > 3Am6Indaz (**NC11**,**13**, $pK_a = 3.8$) > BzImid (**NC32**,**33**, $pK_a = 5.8$) > Indole (**NC37**, ~non-basic) $\geq$ 5Indaz (**NC1**, ~non-basic) > 3Am7[N]6Bio (**NC23**, $pK_a = 4.7$) > 3OH5Indaz (**NC41**, $pK_a = 6.3$, OH form). Although inhibitory potency was modest with our P_1-3-aminoindazole (3Am5Indaz, **NC10**) and 3-aminobenzisoxazole (3Am6Bio, **NC22**) prototypes, relative OBA profiles in fasted or fed dogs dosed at 1 mg/kg were quite intriguing. The impact of P_1-regioisomers on inhibitory potency and OBA was pronounced in both series; compare **NC10** versus **NC11** and **NC21** versus **NC22**. This empirical PK information, coupled with our prior SAR[5–7] and literature precedent,[4,8,9] led us to pursue a variety of novel second generation targets featuring tethered P_4-(substituted)aromatic groups judiciously combined with the optimal P_1-arginine surrogates identified above. This exercise resulted in the identification of the highly potent and selective inhibitors **NC17–20** and **NC24–31**, which demonstrated excellent PK properties in dogs comparable or superior to the reference compounds **L374,087** and **L375,378** (Table 1). Incorporation of other leading bicyclic heteroaromatic P_1-arginine surrogates (BzImid, Indole, 3Am7[N]6Bio, 3OH5Indaz) afforded potent and selective inhibitors in vitro, however they generally lacked useful in vivo efficacy.

Potency and selectivity in our series results from numerous key binding interactions at each of the S_1–S_3 specificity pockets in the thrombin active site (Fig. 1). As confirmed by an X-ray crystal structure of the **NC17**–fIIa complex,[10,11] inhibitor binding occurs in a normal substrate-like mode, with the rigid P_1-3-aminoindazole moiety participating in salt-bridge interactions with Asp189 and water-mediated hydrogen-bonding with Tyr228 at the S_1 specificity pocket. Related P_1-analogues may partake in additional hydrophobic interactions with Val213 at S_1. Other canonical β-sheet H-bonds and van der Waals interaction at S_2 and S_3 are conserved. Although many structural variations are represented in our 44 targets, it appears that overall drug potency and efficacy is governed by the appropriate balancing of several factors, including choice of P_4-hydrophobe, P_3-heterocycle and especially the P_1-arginine mimic. In general, P_1-arginine surrogates with pK_a's ~3.6–8.6 confer the best OBA in this series.

In conclusion, our investigations on novel thrombin inhibitor scaffolds incorporating P_3-heterocyclic dipeptide surrogates along with perusal of reference inhibitors led to the design, synthesis, and elucidation of potent, selective thrombin inhibitors **NC1–NC44** that feature a range of weakly basic bicyclic P_1-arginine mimics. After identifying the P_1-3-aminobenzisoxazole (3Am6Bio) and 3-aminoindazole (3Am5Indaz) pharmacophores from cassette oral dosing studies in dogs, we optimized potency/PK parameters by judicious combination of these arginine surrogates with several optimal P_4-(substituted)aromatic groups. Potent and selective inhibitor candidates **NC17–20** and **NC24–31** emerged that demonstrated excellent PK properties. Numerous favorable active-site interactions coupled with optimal physical properties are deemed as critical factors for conferring high potency, specificity, and useful PK properties in this class of inhibitors.

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