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Design, Synthesis and Biological Activity of Novel Non-Amidine Factor Xa Inhibitors. Part 1: P_1 Structure–Activity Relationships of the Substituted 1-(2-Naphthyl)-1*H*-pyrazole-5-carboxylamides

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Abstract—Based on DuPont Pharmaceuticals' monobenzamidine lead structure SN429, we have designed the biphenyl 1-(2-naphthyl)-1*H*-pyrazole-5-carboxylamides as a novel series of non-basic factor Xa inhibitors. We have discovered that the displacement of the benzamidine moiety with substituted 2-naphthyl structures not only results in highly potent factor Xa inhibitors, but also significantly increases their enzyme specificity and oral bioavailability.

Cardiovascular diseases involving acute occlusive thrombosis are a leading cause of death worldwide. Warfarin, the only orally bioavailable anticoagulant on the market for thrombotic disorders, suffers from a number of shortcomings which limit its application. With a narrow therapeutic index, it requires careful and costly monitoring of clotting time to achieve desired efficacy and dose titration to minimize excessive bleeding and potential drug–drug interactions.[1] Blood coagulation factor Xa (fXa), a trypsin-like serine protease that plays a pivotal role in the blood coagulation cascade, has emerged as a very attractive target for the design of new therapeutic agents with potential for the treatment of arterial and venous thrombosis.[2] At the final convergence point of both the intrinsic and extrinsic coagulation pathways, fXa forms a prothrombinase complex with factor Va, Ca^{2+}, and phospholipid.[3] FXa is the active enzyme in the prothrombinase complex, converting inactive prothrombin to active thrombin. This activation is a highly amplified process.[4] One molecule of fXa in the prothrombinase complex can generate 138 molecules of thrombin per minute. Therefore, inhibition of fXa not only interrupts both the coagulation pathways of thrombin production, but also is theoretically more therapeutically effective to prevent thrombosis than direct thrombin inhibition. As suggested by preclinical studies, fXa inhibitors have less potential for increasing the risk of abnormal bleeding and have a wider therapeutic index. Representing an important advance in the management of both arterial and venous thrombosis, fXa inhibitors have been actively pursued as new orally active antithrombotic agents. A promising fXa inhibitor must demonstrate adequate potency, enzyme specificity, oral bioavailability, duration of action (half-life, $t_{1/2}$) and efficacy. It should be highly specific for fXa versus thrombin, trypsin and other enzymes such as tissue plasminogen activator (t-PA), activated protein C (aPC), plasmin and kallikrein.

In early 1999, the recurring structural feature of many fXa inhibitors was the presence of a benzamidine moiety. One of the most potent P_1 monobenzamidine fXa inhibitors was DuPont's SN429.[5] Although it indicated a very low in vitro human fXa K_i at 0.013 nM, SN429 was not a promising orally active antithrombotic agent due to its poor oral bioavailability (4% in dog) and short half-life (0.82 h in dog). The amidino group is likely a major contributor to the low oral bioavailability due to its high basicity. In order to improve oral bioavailability, clearly the amidino group should be replaced with less basic moieties. It should be pointed out that SN429 also inhibits thrombin (K_i 300 nM), trypsin (K_i 16 nM) and kallikrein.

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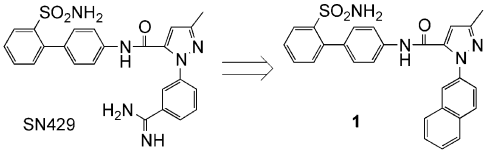
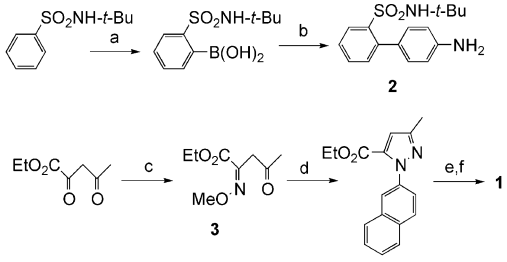
Our approach toward non-benzamidine fXa inhibitors was to substitute the benzamidine moiety with a less basic or totally neutral replacement. We envisioned the neutral 2-nathphyl group as a novel replacement for the P_1 benzamidine in the fXa S_1 pocket. SN429 presented to us a useful template to exercise this hypothesis. For the proof of principal, compound **1** was designed. The aromaticity and planarity of the 2-naphthyl group matched the predicted shape of the S_1 pocket very well. While a reduction in fXa affinity was predicted relative to SN429 due to the elimination of an important hydrogen bond interaction of the benzamidine with fXa Asp[189] carboxylate residue, the potentially improved oral bioavailability and pharmacokinetic profile were enticing. Compound **1**'s enzyme specificity profile should be better than that of SN429 by eliminating the additional hydrogen bond interaction of the P_1 benzamidine with the trypsin Ser[190] hydroxyl group. The corresponding fXa Ala[190] residue contributed to the hydrophobic character of the S_1 pocket, which should favorably affect binding of the P_1 naphthyl moiety.[6] The 2-naphthyl ring system also presented multiple potential sites for substitution and derivatization in order to increase the fXa affinity.

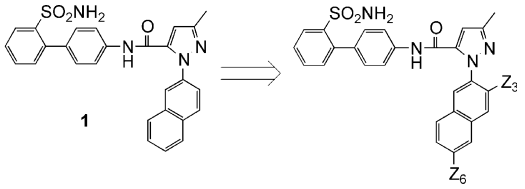


Compound **1** was synthesized according to the route shown in Scheme 1. Biphenylamine **2** was prepared in 70 to 85% yield by Suzuki coupling.[5] The condensation of **3** with the 2-naphthylhydrazine afforded the desired pyrazole product in excellent regioselectivity. Weinreb method[7] was employed to build the amide connection. The fXa IC_{50} value of compound **1** is 104 nM. Though much less potent than SN429, compound **1** exhibited significantly improved enzyme specificity and pharmacokinetic profile. Its IC_{50} values against thrombin, trypsin, t-PA, aPC, plasmin, and kallikrein are all higher than an 11 μM threshold. By orally dosing Sprague–Dawley rats, it displayed 24.5% oral bioavailability and 2.4 h half-life.

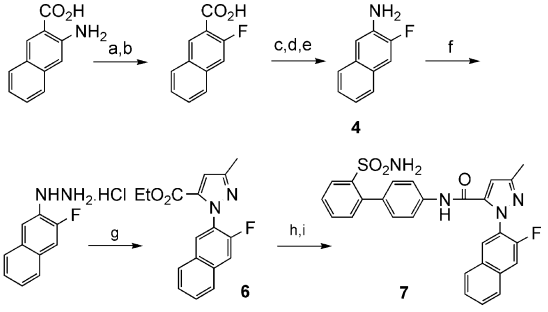


Scheme 1. (a) (1) BuLi (1.6 M in hexane, 2 equiv), THF, 0 °C; (2) $(i\text{PrO})_3B$ (1.4 equiv), 10 °C, 1 h; (3) HCl (1 N), 0 °C, overnight; extraction; (b) 4-bromoaniline (1 equiv), $Pd(Ph_3P)_4$ (0.1 equiv), Cs_2CO_3 (3 equiv), *n*-BuOH/H_2O/PhMe (1:2:4), 80 °C, 5 h; (c) $H_2NOMe{\cdot}HCl$ (1.1 equiv), EtOH, reflux, 2 h; (d) 2-naphthylhydrazine.HCl (1 equiv), THF/HOAc (1:2), reflux, 3 h; (e) **2** (1 equiv), $AlMe_3$ (2 M in hexane, 5 equiv), DCM, overnight; (f) TFA.

To increase fXa potency, we carried out a systematic structure–activity relationship (SAR) exploration of the substitution on the 2-naphthyl moiety. The proximity of the C-3 position to the available Ser[195] hydroxyl group suggested potential hydrogen bond interactions could be gained by appropriate substitutions. This interaction might be direct or mediated through a water molecule. A variety of groups with hydrogen bond donating and/or accepting ability were considered for Z_3, including halides, –CN, –OH, $–NH_2$, $–NO_2$, –OMe, $–SO_2Me$, $–SO_2NH_2$, $–CO_2H$, $–CO_2R$, $–CONH_2$, $–C(NH)NH_2$, $–C(NH)NR_2$, $–CH_2OH$ and $–CH_2NH_2$. The depth of the fXa S_1 pocket, generally defined by the location of Tyr[129], provided impetus for additional SAR exploration by substituting at position C-6. Favorable van der Waals and potential hydrogen bond interactions were considered in choosing halides, –OH and –OMe as Z_6 substituents.

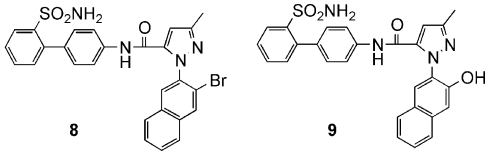


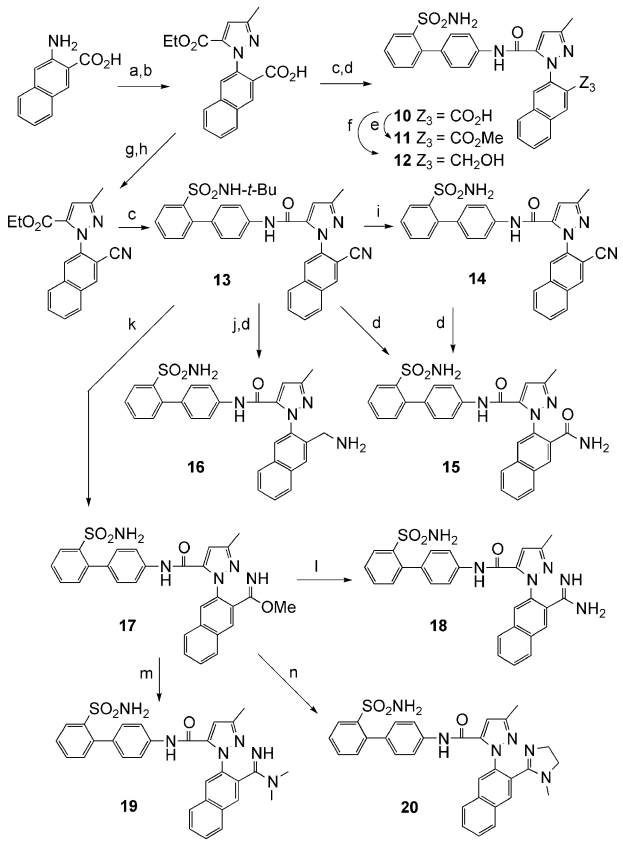
The 3-fluoronaphthyl target **7** was prepared according to Scheme 2. Schiemann reaction was used to convert the amine to fluoride in over 80% yield. Curtius rearrangement cleanly converted the carboxylic acid to amine. The hydrazine salt was produced by $SnCl_2$-reduction of the diazonium salt and was used without purification. Pyrazole **6** was isolated in >70% yield.



Scheme 2. (a) (1) $NaNO_2$ (1.2 equiv), concd HCl, −10 °C, 30 m; (2) HBF_4 (48% aq, 2 equiv), −10 °C, 1 h; filtration; (b) xylene, reflux, 4 h; (c) $(COCl)_2$ (3 equiv), DMF (cat.), DCM, 3 h; evaporation; (d) NaN_3 (2 equiv), dioxane, −5 °C, 2 h; evaporation; workup; (e) DMF/water (2:1), reflux, 4 h; (f) (1) $NaNO_2$ (1.2 equiv), concd HCl, −5 °C, 30 m; (2) $SnCl_2{\cdot}2H_2O$ (2.5 equiv), concd HCl, −5 °C, 1 h; filtration; (g) **3** (1.5 equiv), THF/HOAc (1:2), reflux, 2 h; (h) **2** (1 equiv), $AlMe_3$ (2 M in hexane, 5 equiv), DCM, overnight; (i) TFA.

The 3-bromo and 3-hydroxy targets (**8** and **9**) were prepared in a manner similar to that described in Scheme 2 using the corresponding commercial 2-naphthylamines.





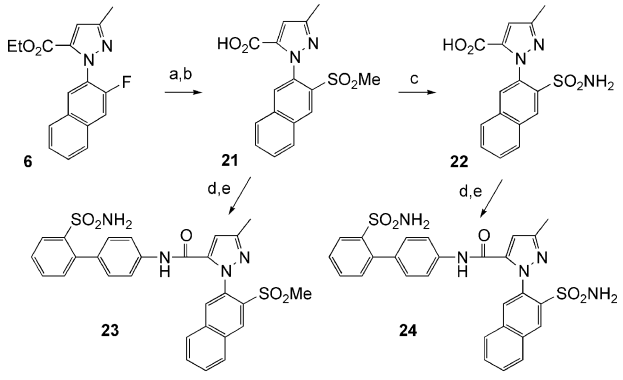
Scheme 3. (a) (1) $NaNO_2$ (1.2 equiv), concd HCl, −5 °C, 30 m; (2) $SnCl_2{\cdot}2H_2O$ (3 equiv), concd HCl, −5 °C, 1 h; filtration; (b) **3** (1.5 equiv), THF/HOAc (1:2), reflux, overnight; (c) **2** (1 equiv), $AlMe_3$ (2 M in hexane, 5 equiv), DCM, overnight; (d) TFA; (e) $TMSCH_2N_2$ (2 M in hexane, 2 equiv), THF, 2 h; (f) BH_3 (1 M in THF, 4 equiv), THF, 0 °C, 1 h; (g) NH_3 (g), BOP (1.2 equiv), DMF, 10 m; (h) $(Cl_3CO)_2CO$ (1.2 equiv), Et_3N (2 equiv), DCM, 1 h; (i) BF_3OEt_2 (30 equiv), DCM, 2 days; (j) $NaBH_4$ (8 equiv), $CoCl_2$ (2 equiv), DMF, overnight; (k) HCl (g), MeOH, 0 °C to rt, overnight; (l) NH_4OAc (5 equiv), MeOH, reflux, 1 h; (m) $HNMe_2$ (2M in MeOH, 5 equiv), MeOH, reflux, 1 h; (n) $MeNHCH_2CH_2NH_2$ (5 equiv), MeOH, reflux, 1 h.

Compounds derived from Z_3 carboxylic acid and nitrile were synthesized according to the routes shown in Scheme 3. Direct methylation and reduction of carboxylic acid **10** yielded **11** and **12**, respectively. To accomplish the 3-cyano substitution, the 3-carboxylic acid was first converted to the corresponding amide, which was then dehydrated using triphosgene. The 3-cyano function in **13** however was labile in strong acid. Removal of the *t*-butyl by TFA afforded the corresponding amide **15** along with **14**, even at room temperature and in lower concentration. Clean deprotection of **13** was achieved using $BF_3{\cdot}OEt_2$. The 3-aminomethyl target **16** was prepared via reduction of nitrile **13**. Amidines **18**, **19**, and **20** were prepared via the methyl imidate **17**, freshly produced by saturating a methanolic solution of nitrile **13** with dry HCl gas.

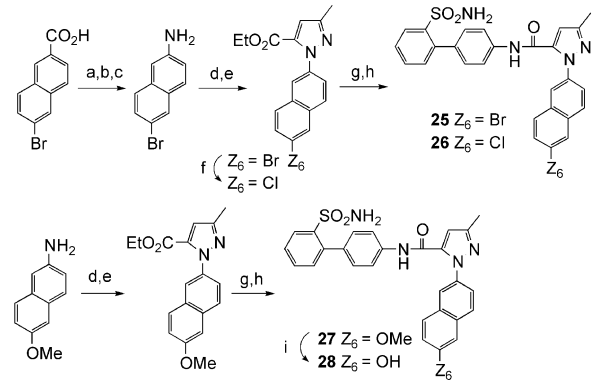
The 3-methylsulfonyl and 3-aminosulfonyl targets (**23** and **24**) were prepared from 3-fluoro intermediate **6** according to Scheme 4. A one-pot reaction employing Huang's method[8] implemented the conversion of methylsulfone **21** to sulfonamide **22** in high yield. The couplings of the carboxylic acids and biphenylamine **2** were promoted by $POCl_3$.

The preparation of 6-bromo, 6-chloro, 6-methoxy and 6-hydroxy targets (**25**, **26**, **27**, and **28**) is shown in Scheme 5. Boron tribromide was used to unmask the 6-hydroxy.

The fXa inhibition data for the substituted 2-naphthyl inhibitors are shown in Table 1. For C-3 substitution, fluoro (**7**), cyano (**14**), amide (**15**), amidine (**18**), aminomethyl (**16**), methylsulfone (**23**) and sulfonamide (**24**) increase fXa potency 3- to 15-fold; conversely, hydroxy (**9**), hydoxymethyl (**12**), carboxylic acid (**10**), carboxylate (**11**), and substituted amidines (**19** and **20**) decrease fXa potency. For C-6 substitution, chloro (**26**) and bromo (**25**) increase fXa potency 6- to 10-fold; methoxy (**27**) and hydroxy (**28**) decrease fXa potency. It should be noted that none of the compounds display an IC_{50} value lower than 11 μM against thrombin, trypsin, *t*-PA,

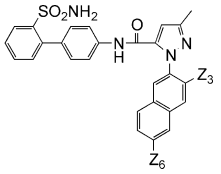


Scheme 4. (a) NaSMe (4 equiv), DMSO, 100 °C, 1 h; (b) MCPBA (2.5 equiv), DCM, 1 h; (c) (1) LiHMDS (1 M in hexane, 4 equiv), THF, 0 °C, 30 m; (2) Bu_3B (5 equiv), rt, 2 h; then 90 °C, 2 h; cool to rt; (3) NaOAc (10 equiv), water, H_2NOSO_3H (8 equiv), 18 h; removal of THF; concd HCl (till pH 1); extraction; (d) **2** (1 equiv), $POCl_3$ (3 equiv), pyr, 0 °C, 1 h; (e) TFA.

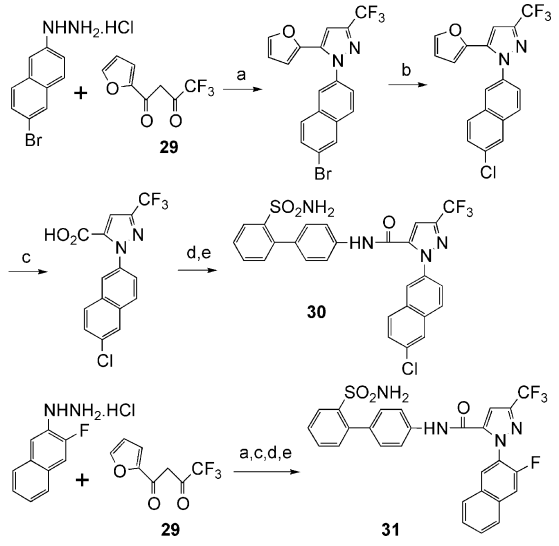


Scheme 5. (a) $(COCl)_2$ (3 equiv), DMF (cat), DCM, 3 h; evaporation; (b) NaN_3 (2 equiv), dioxane, −5 °C, 2 h; evaporation; workup; (c) DMF/water (2:1), reflux, 4 h; (d) (1) $NaNO_2$ (1.2 equiv), concd HCl, −5 °C, 30 m; (2) $SnCl_2{\cdot}2H_2O$ (3 equiv), concd HCl, −5 °C, 1 h; filtration; (e) **3** (1.5 equiv), THF/HOAc (1:2), reflux, 4 h; (f) CuCl (10 equiv), CuI (1 equiv), DMF, reflux, 1 h; (g) **2** (1 equiv), $AlMe_3$ (2 M in hexane, 5 equiv), DCM, overnight; (h) TFA; (i) BBr_3 (1 M in DCM, 1.5 equiv), DCM, overnight.

Table 1. 2-Naphthyl substitution on fXa potency



Compd	Z_3	Z_6	IC_{50} (nM)
1	H	H	104
7	F	H	9
8	Br	H	143
14	CN	H	6
9	OH	H	>11,000
12	CH_2OH	H	483
16	CH_2NH_2	H	38
10	CO_2H	H	376
11	CO_2Me	H	545
15	$CONH_2$	H	24
23	SO_2Me	H	45
24	SO_2NH_2	H	24
18	$C(NH)NH_2$	H	21
19	$C(NH)NMe_2$	H	1650
20	2-Imidazolinyl	H	7820
26	H	Cl	11
25	H	Br	18
27	H	OMe	3300
28	H	OH	3510

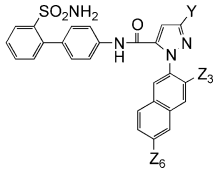


Scheme 6. (a) THF/HOAc (1:2), reflux, 4 h; (b) CuCl (10 equiv), CuI (1 equiv), DMF, reflux, 1 h; (c) $KMnO_4$ (5 equiv), acetone/water (2:1), 60 °C, 3 h; (d) **2** (1 equiv), $POCl_3$ (3 equiv), pyr, 0 °C, 1 h; (e) TFA.

aPC, plasmin and kallikrein. Compared to SN429, the enzyme specificity for fXa has been significantly improved by incorporating substituted 2-naphthyls as the P_1 moiety.

We also examined the 3-(trifluoromethyl)-1*H*-pyrazole nucleus since it has been known as a potency-enhancing replacement for 3-methyl-1*H*-pyrazole in fXa inhibitor design.[2] The 6-chloro and 3-fluoro targets (**30** and **31**) were prepared according to Scheme 6. Commercial diketone **29** was employed in the trifluoromethyl pyrazole formation. The furyl ring acted as a protected carboxylic acid, and was conveniently oxidized to the free acid using $KMnO_4$ in hot acetone and water.

Table 2. 3-Pyrazole substitution on fXa potency

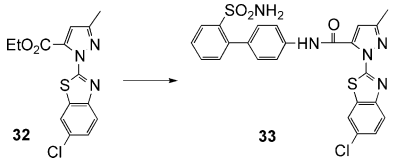


Compd	Y	Z_3	Z_6	IC_{50} (nM)
26	CH_3	H	Cl	11
30	CF_3	H	Cl	4
7	CH_3	F	H	9
31	CF_3	F	H	27

The fXa potency results for 3-methyl versus 3-trifluoromethyl substitution in the pyrazole nucleus are listed in Table 2. The trifluoromethyl group does not compromise the inhibitors' excellent enzyme specificity toward fXa.

The pharmacokinetic profile of this class of novel fXa inhibitors was explored by examining the representative analogue **7** (fXa K_i 5.7 nM). By orally dosing conscious Sprague–Dawley rats at a nominal dose of 4 mg/kg in 25% PEG-300, the oral bioavailability and half-life of compound **7** are 22% and 6.0 h, respectively. Intravenous administration to the conscious rats at a dose of 0.2 mg/kg in 50% PEG-300 demonstrated half-life, volume distribution and clearance values of 1.7 h, 2.6 L/kg and 17.7 mL/min/kg, respectively.

In order to compare 2-naphthyl with other neutral aromatic bicyclic structures as P_1 moieties in fXa inhibitor design, compound **33** was prepared from commercial ester **32** using the same chemistry as illustrated above. The lack of inhibiting activity against fXa potency (IC_{50} >11 μM) reinforced our focus on the 2-naphthyl P_1 fXa inhibitors.



In conclusion, we have designed a series of biphenyl 1-(2-naphthyl)-1*H*-pyrazole-5-carboxylamides as novel non-amidine fXa inhibitors possessing low nanomolar fXa potency, excellent enzyme specificity and respectable oral bioavailability and half-life. Several C-3 and C-6 2-naphthyl substitution patterns were discovered to increase fXa potency. Further SAR study and optimization of the biphenyl/biaryl P_4 and other novel P_4 structures, leading to subnanomolar fXa inhibitors, will be reported in future publications.

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