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DESIGN, SYNTHESIS, AND BIOLOGICAL ACTIVITY OF NOVEL PURINE AND BICYCLIC PYRIMIDINE FACTOR Xa INHIBITORS

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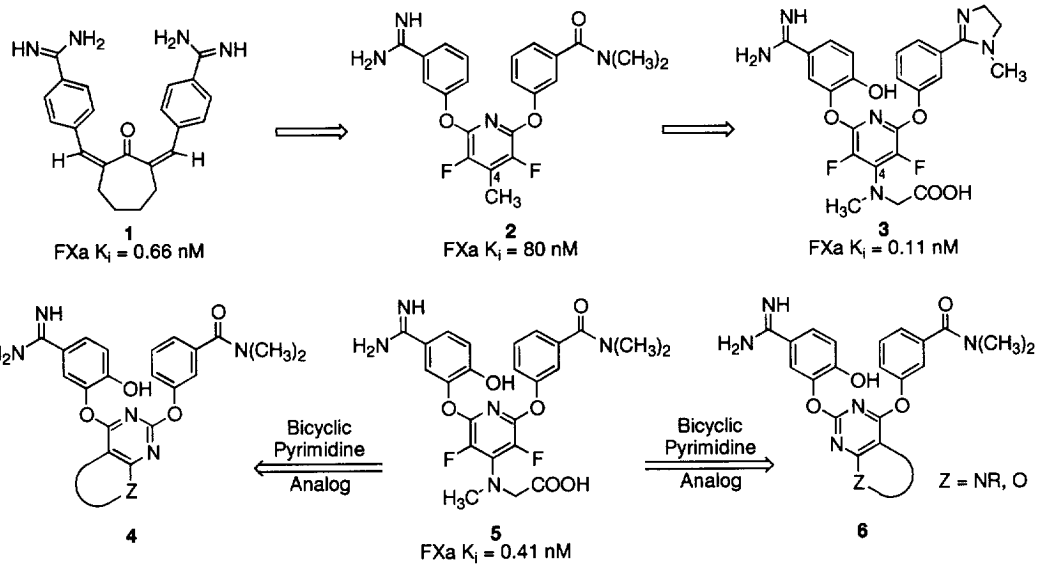
Abstract: The synthesis of amidinoaryloxy 9-benzyl-8-methyl-9*H*-purine, 7,8-dihydropteridine-6(5*H*)-one and 5,7-dihydropyrimido[4,5-*b*][1,4]oxazine-6-one inhibitors of Factor Xa is described. These compounds show nanomolar potency against FXa and maintain high selectivity over thrombin and trypsin.

Introduction

The prevention of blood coagulation is of primary importance in a number of pathological situations. Factor Xa (FXa) is a serine protease strategically situated at the intersection of the intrinsic and extrinsic arms of the blood coagulation pathway. FXa activates prothrombin to generate thrombin, which plays a critical role in thrombosis by not only converting fibrinogen to fibrin for clot formation, but also by strongly inducing platelet aggregation.[1] Since direct thrombin inhibitors have shown a tendency to undesirably prolong bleeding, the development of FXa inhibitors has emerged as a primary focus for the treatment and prevention of thrombotic disorders.



1 FXa K_i = 0.66 nM

2 FXa K_i = 80 nM

3 FXa K_i = 0.11 nM

4

5 FXa K_i = 0.41 nM

6

We have described the discovery and characterization of (Z,Z)-2,7-bis-(4-amidinobenzylidene)-cycloheptan-1-one (**1**) as a potent and selective inhibitor of FXa,[2] as well as its evolution, via **2** and **5**, into **3**, a highly potent, selective and orally active FXa inhibitor.[2b,3] In an effort to obtain higher potency and better pharmacological properties we designed a series of regioisomeric bicyclic pyrimidine analogs **4** and **6** as surrogates for the 4-nitrogen substituted pyridine core of **5**. This led to the discovery of novel pteridines **16a** and **16b**, pyrimidooxazine **17**, and purines **22a** and **22b** FXa inhibitors, whose synthesis and activity are described in this paper.[4]

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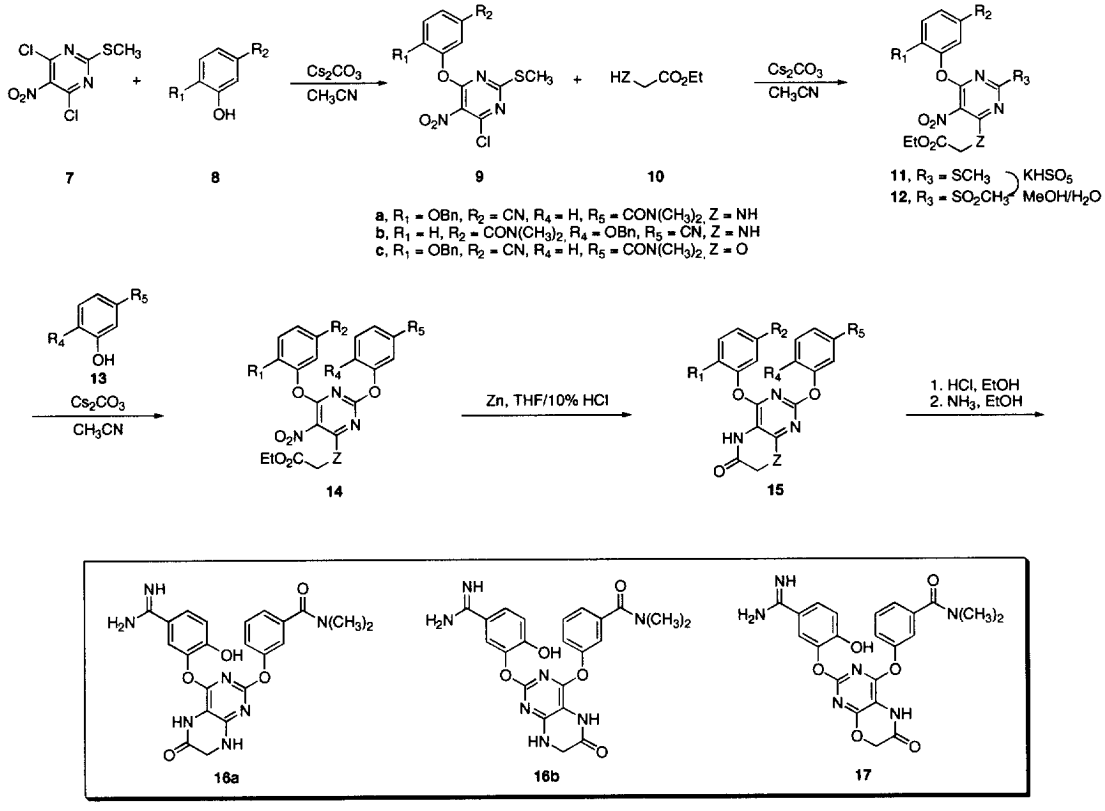
Synthesis

We chose 4,6-dichloro-5-nitro-2-methylthiopyrimidine (**7**) as the starting material for the construction of the analogs (Schemes 1 and 2). This starting material contains the readily oxidized 2-thiomethyl moiety as a latent leaving group that allows the first two nucleophilic additions to occur regioselectively at the 4-chloro and 6-chloro positions. The bicyclic pyrimidine compounds are synthesized from the common pyrimidine intermediate **7** via the sequential addition of (a) phenol **8**, (b) benzylamine or glycine analog **10** as the 4-substituent required for cyclization to the appropriate bicyclic analog, and (c) second phenol component **13**. The first nucleophilic attack on 2-methylthio-4,6-dichloro-5-nitropyrimidine displaces the chlorine at the 4-position; subsequent nucleophilic attack occurs at the 6-position.

This strategy allows the synthesis of either of the two 2,6-diaryloxy regioisomers **14a** and **14b** or **20a** and **20b** by switching the addition sequence of the phenol components. Oxidation of the methylthio group to the methanesulfonyl group[5] allows the final addition to occur readily at the 2-position.[6] This plan was well suited to our synthesis of tri-substituted pyrimidines required for the generation of the bicyclic final products, since each of the three components could be added stepwise to construct tri-substituted pyrimidine intermediates **14** or **20**.

Scheme 1



7 8 9 10 11, R_3 = SCH_3 12, R_3 = SO_2CH_3

a, R_1 = OBn, R_2 = CN, R_4 = H, R_5 = $CON(CH_3)_2$, Z = NH
b, R_1 = H, R_2 = $CON(CH_3)_2$, R_4 = OBn, R_5 = CN, Z = NH
c, R_1 = OBn, R_2 = CN, R_4 = H, R_5 = $CON(CH_3)_2$, Z = O

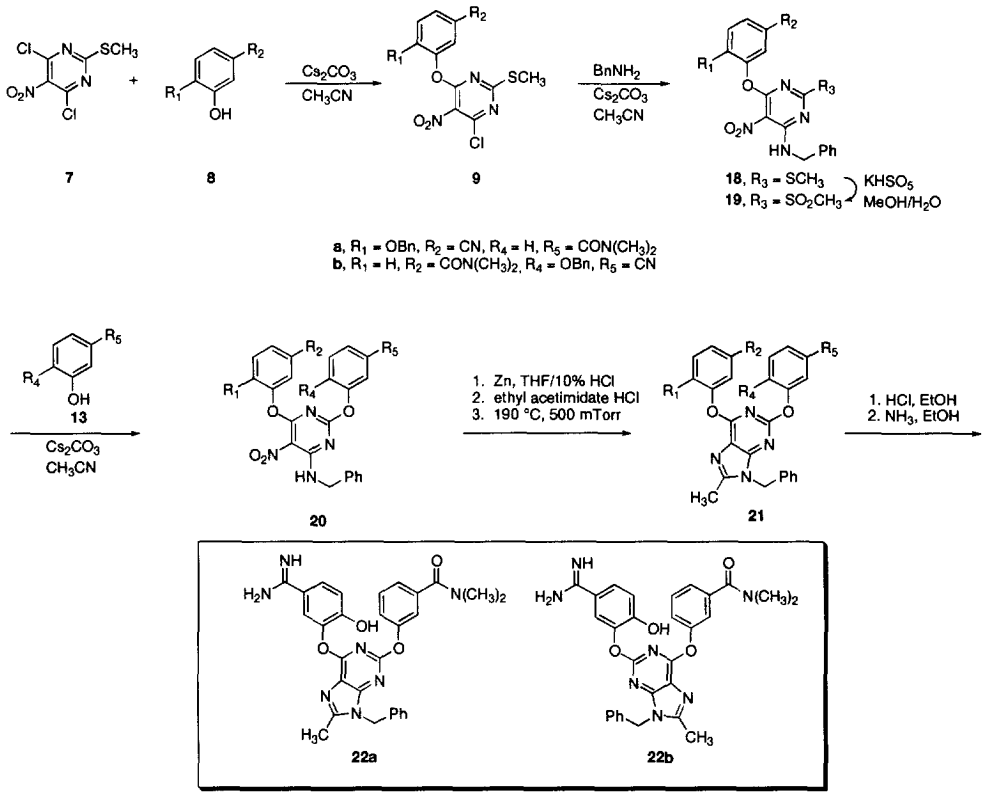
13 14 15

16a 16b 17

The syntheses of 2,6-diaryloxy-7,8-dihydropteridin-6(5*H*)-ones **16a, 16b** and dihydro-pyrimido[4,5-*b*][1,4]oxazine-6-one **17** proceed as follows (Scheme 1). Addition of the cesium salt of phenol **8a** or **8b** to chloropyrimidine **7** in acetonitrile at 0 °C gives **9a** (56%) or **9b** (83%). Glycine ethyl ester **10a** is added to

chloropyrimidine **9a** or **9b** in the presence of cesium chloride in acetonitrile at 0 °C for 2 h to afford **11a** (87%) or **11b** (95%). Alternatively, the sodium salt of ethyl glycolate **10c** is added to **9c** to afford **11c** (48%). Thiomethyl pyrimidine **11a**, **11b**, or **11c** is oxidized to the methanesulfonyl moiety **12a** (45%), **12b** (92%), or **12c** (40%) with potassium metabisulfite ($KHSO_5$, Oxone®) in methanol/water. The second phenol **13a** or **13b** is added in the presence of cesium carbonate in acetonitrile at 0 °C to afford diaryloxypyrimidine **14a** (18%), **14b**, (46%) or **14c** (30%). Reduction of the nitro group using zinc metal (THF/10% HCl, 70 °C, 30 min) gives reductive cyclization products **15a** (95%), **15b** (90%), or **15c** (90%). Nitrile intermediate **15a**, **15b**, or **15c** is converted to the ethyl acetimidate with simultaneous removal of the phenolic benzyl group by treatment with HCl in ethanol. Further treatment with ammonia provides the final amidines **16a**, **16b**, or **17**.[7]

Scheme 2



7 8 9 **18**, $R_3 = SCH_3$; **19**, $R_3 = SO_2CH_3$ 13 20 21 **22a** **22b**

The syntheses of 2,6-diaryloxy-9-benzyl-8-methyl-9*H*-purines **22a** and **22b** proceed as follows (Scheme 2). Monoaryloxy pyrimidine **9a** or **9b** is formed by addition of the cesium salt of phenol **8a** or **8b** in acetonitrile at 0 °C, as above. The addition of benzylamine in the presence of cesium carbonate in acetonitrile at 75 °C for 4 h gives 6-substituted pyrimidine **18a** (65%) or **18b** (48%). The methylthio group of **18a** or **18b** is oxidized to the methanesulfonyl moiety **19a** (95%) or **19b** (95%) by treatment with potassium metabisulfite ($KHSO_5$, Oxone®) in methanol-water, as above. The appropriate phenol **13a** or **13b** is added in the presence of cesium carbonate

in acetonitrile at 0 °C to afford 2,6-diaryloxy pyrimidine **20a** (52%) or **20b** (34%). Reduction of the nitro group as above (Zn, THF/10% HCl, 70 °C, 30 min) gives the diamine intermediate. Addition of ethyl acetimidate hydrochloride followed by heating under vacuum (190 °C, 500 mTorr) affords the cyclized purine **21a** (50%) or **21b** (75%). The nitrile intermediate **21a** or **21b** is converted to the final amidines **22a** (62%) or **22b** (72%), as above.[8]

Results and Discussion

In vitro screening results were determined for the bicyclic pyrimidine compounds that were synthesized. The compounds were tested in vitro for activity against human FXa, and selectivity against human thrombin and bovine trypsin (Table 1).[9] The most potent compound, **22a**, has a FXa K_i = 0.42 nM. This regioisomer has a *syn* relationship between the purine ring and the benzamidine group and is over twenty times more potent than the isomer that has a *syn* relationship between the dimethyl benzamide group and the purine ring (**22b** FXa K_i = 10 nM). Purine **22a** also shows high selectivity (FXa K_i / IIa K_i >1600) over human thrombin.

Table 1. Activity of Bicyclic Pyrimidine Inhibitors against FXa, FIIa and Trypsin

Compound	Inhibition human FXa (K_i, nM)	Inhibition human thrombin (K_i, nM)	Inhibition bovine trypsin (K_i, nM)
1	0.66	430	30
2	80	> 5000	2700
3	0.11	2000	280
5	0.41	4500	1100
16a	19	1000	320
16b	81	> 5000	2200
17	106	> 5000	> 5000
22a	0.42	710	150
22b	10	1300	760

Pteridines **16a** (FXa K_i = 19 nM), **16b** (FXa K_i = 81 nM), and **17** (FXa K_i = 106 nM) show lower potency than purines **22**. The relationship of the activity between the regioisomers **22a** and **22b** parallels that of **16a** and **16b**. Pyrimidine **16a**, the regioisomer that has a *syn* relationship between the pteridine ring and the benzamidine group, is over 4 times more potent than the isomer that has a *syn* relationship between the dimethyl benzamide group and the pteridine ring (e.g., **16b** FXa K_i = 81 nM).

Molecular modeling studies of the FXa enzyme–inhibitor complex provide a rationale for the 23-fold greater activity of regioisomer **22a** over **22b**.[10] The crystal structures of 4-substituted analogs of **2** and **5** bound to Trypsin show that the benzamidine binds to the S1 pocket and that the dimethyl benzamide binds to the S4 pocket.[11] The binding of related benzamidine inhibitors is similar in both Trypsin[12] and FXa,[13] thus we anticipate that **22a** and **22b** will bind in FXa as they do in Trypsin. Both purines **22a** and **22b** exist in either of two low energy conformations in which the 9-benzyl group may lie to either side of purine ring plane (Figure 1 and 2). While the more active regioisomer **22a** shows no deleterious interactions with any residue of the enzyme backbone (Figure 1), **22b** shows close contacts between the benzyl ring and the active site in both low energy conformations (Figure 2). One conformation of **22b** shows a destabilizing interaction with Gln193

(carbon–nitrogen distance = 2.3 Å), while the other shows a destabilizing interaction with Gly216 (carbon–carbon distance = 3.5 Å) (Figure 2). In addition, a potential favorable interaction exists for the more active purine **22a**. Both conformations of **22a** allow a possible positive hydrogen bonding interaction between the purine 7-nitrogen position and the amide hydrogen of Gln193 (Figure 1).

In conclusion, purines **22** and pteridines **16** and **17** show nanomolar potency against FXa and maintain high selectivity over other serine proteases in the blood coagulation cascade. The most potent compound **22a** (FXa K_i = 0.42 nM) is the regioisomer that has a *syn* relationship between the purine ring and the benzamidine.

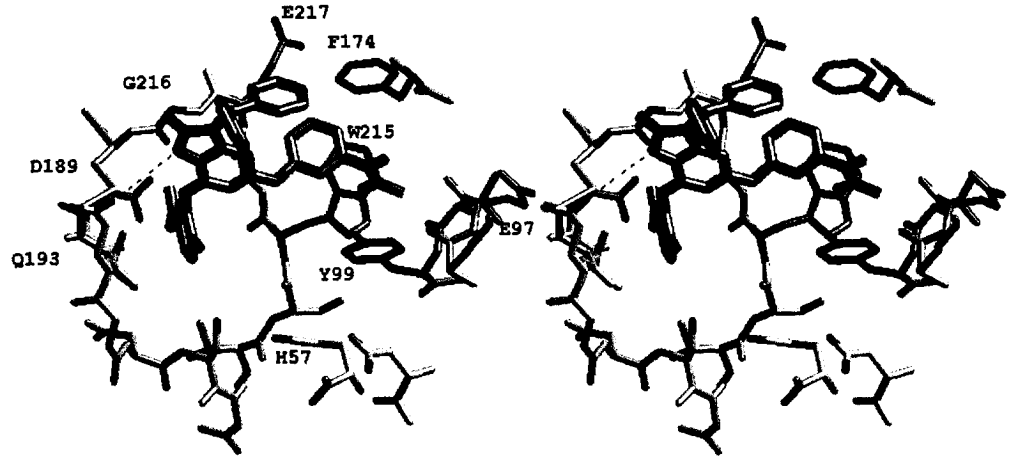


Figure 1. **22a** Modeled in Active Site of FXa. Both conformations superimposed with the benzyl group in pink.

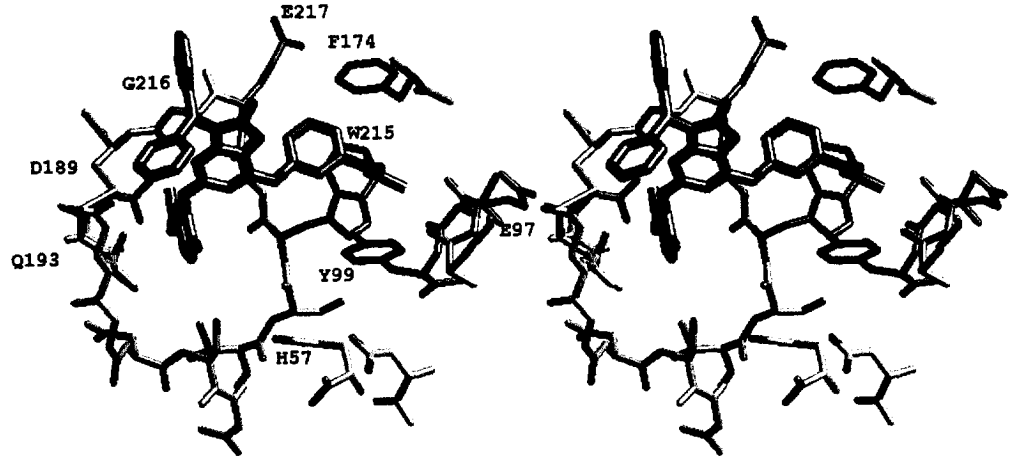


Figure 2. **22b** Modeled in Active Site of FXa. Both conformations superimposed with the benzyl group in pink.

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7. **16a** ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.30 (br s, 1), 9.10 (br s, 2), 8.80 (br s, 1), 8.00 (br s, 1), 7.70 (m, 2), 7.60 (dd, 1), 7.30–7.00 (m, 4), 4.00 (s, 2), 3.00 (s, 3), 2.80 (s, 3); **16b** (300 MHz, DMSO-d_6) δ 10.30 (br s, 1), 9.00 (br s, 2), 8.80 (br s, 2), 8.00 (br s, 1), 7.65 (m, 2), 7.60 (dd, 1), 7.30–7.20 (m, 4), 7.00 (d, 1), 4.00 (s, 2), 2.90 (s, 3), 3.00 (s, 3); **17** (300 MHz, DMSO-d_6) δ 9.10 (br s, 2), 8.80, br s, 2), 7.70 (m, 2), 7.50 (dd, 1), 7.40–7.20 (m, 4), 7.10 (d, 1), 4.90 (s, 2), 3.00 (s, 3), 2.90 (s, 3).
8. **22a** ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.10 (br s, 2), 8.80 (br s, 2), 7.80 (d, 1), 7.70 (d, 1), 7.50–7.10 (m, 10), 5.40 (s, 2), 3.00 (br s, 6), 2.70 (s, 3); **22b** (300 MHz, DMSO-d_6) δ 9.10 (br s, 2), 8.80 (br s, 2), 7.70 (d, 1), 7.65 (d, 1), 7.50 (dd, 1), 7.40–7.25 (m, 8), 7.10 (d, 1), 5.40 (s, 2), 3.00 (s, 3), 2.90 (s, 3), 2.70 (s, 3).
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10. The models were constructed using the program Cerius2 version 2.3 from Molecular Simulations, Inc. The models of the FXa binding of structures **22** were built based on the X-ray crystal structure of an analog of **5** bound to Trypsin. In constructing the models of **22a** and **22b**, the two phenoxy rings were held in the same relative position to each other as in the X-ray crystal structure bound to Trypsin.
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