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2-(2-Chloro-6-fluorophenyl)acetamides as potent thrombin inhibitors

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Abstract—2-(2-Chloro-6-fluorophenyl)acetamides having 2,2-difluoro-2-aryl/heteroaryl-ethylamine P3 and oxyguanidine P1 substituents are potent thrombin inhibitors (K_i = 0.9–33.9 nM). 2-(5-Chloro-pyridin-2-yl)-2,2-difluoroethylamine was the best P3 substituent, yielding the most potent inhibitor (K_i = 0.7 nM). Replacing the P3 heteroaryl group with a phenyl ring or replacing the difluoro substitution with dimethyl or cyclopropyl groups in the linker reduced the affinity for thrombin significantly. The aminopyridine P1s also provided an increase in potency.

The discovery of small molecule inhibitors of thrombin, a key serine protease in the coagulation cascade, continues to be an important goal for anti-thrombotic therapy.[1] Thrombin, a trypsin-like peptidase, mediates the cleavage of fibrinogen to fibrin and the activation of platelets, leading to the formation of blood clots.[2] Inhibition of thrombin would provide effective treatment for venous or arterial thromboembolism, e.g., prevention of postoperative deep venous thrombosis (DVT) and pulmonary embolism (PE), prevention of stroke during atrial fibrillation, and treatment of acute coronary syndrome (ACS).[3] The inhibition of thrombin has been clinically validated by the availability of Argatroban and the recombinant hirudins, used to treat heparin induced thrombocytopenia.

Pyridinones have been shown to be suitable P2 scaffolds,[4] providing the appropriately spaced hydrogen bonding partners to interact with the backbone Gly216, a key binding interaction in the thrombin active site (Fig. 1). Herein, we describe a series of 2-(2-chloro-6-fluorophenyl)acetamides capable of maintaining the Gly216 hydrogen bonding interaction, and exploration of the P3 group to provide potent thrombin inhibitors.

The synthesis of 2-(2-chloro-6-fluorophenyl)acetamides was achieved using the chemistry depicted in Scheme 1 (compounds **2**–**4** and **9**–**11**).[5]

First, diethyl malonate was deprotonated with NaH and reacted with 1,2,3-trifluoro-4-nitro-benzene (**I**). Subsequent decarboxylation using LiCl[6] gave the ester **II**. Then, the aromatic nucleophilic substitution (S_NAr) was accomplished by the displacement of the aryl fluoride (**II**) with the requisite aryl/heteroaryl amine.[7] Following protection of the aniline group as a trifluoroacetate, conversion of the nitro group to chloride **IV** was accomplished by reduction to the corresponding aniline and a Sandmeyer reaction. Deprotection of the aniline and ester, followed by coupling to the *O*-guanidine segment, completed the synthesis to render target compounds (**V**).

Alternatively, the acetamides were synthesized using the chemistry shown in Scheme 2 (compounds **1**, **8**, and **14**–**16**). 1,2,3-Trifluoro-4-nitro-benzene (**I**) was treated with diethyl malonate sodium salt, followed by acetic acid/hydrochloric acid mediated decarboxylation and saponification to yield acid **VI**. The acid was reduced using sodium borohydride. Then, the aniline group was introduced via S_NAr of *t*-Bu-amine and **VII** followed by deprotection of the *tert*-butyl group. The alcohol (**VIII**) was protected as the acetate and the P3-unit was introduced using the requisite acid chloride, giving compound **X**. The 2-Cl substituent was similarly converted from its nitro precursor via reduction to the

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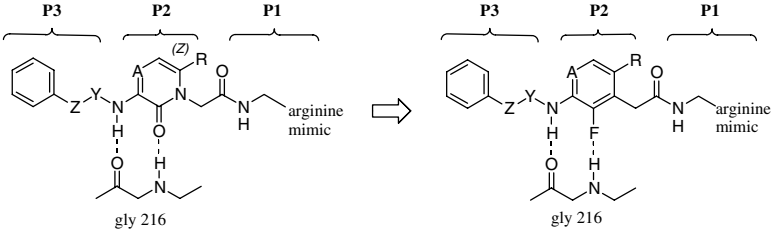
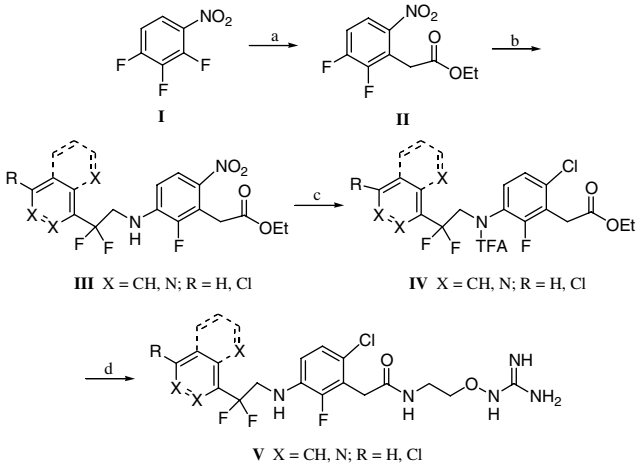
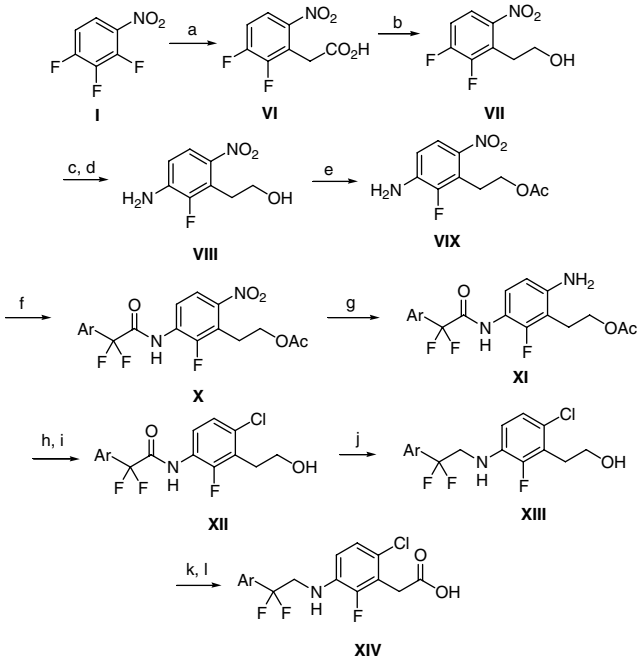


Figure 1. Replacement of pyridinone scaffold with aryl fluoride.



Scheme 1. Reagents and conditions: (a) i—NaH (2 equiv), diethyl malonate (2 equiv), THF, 0 °C–rt, 3 h; ii—LiCl, H_2O, DMSO, 150 °C, 2 h, 78%; (b) $ArCF_2CH_2NH_2$ (0.95 equiv), DIEA (1.2 equiv), DMSO, 81–85%; (c) i—TFAA (5 equiv), DIEA (1.2 equiv), DCM, 99%; ii—Zn (11 equiv), AcOH (22 equiv), DCM, 99%; iii—*tert*-butyl nitrite (1.5 equiv), $CuCl_2$ (1.2 equiv), CH_3CN, 48–52%; (d) i—NaOH (2.5 equiv), MeOH; ii—$H_2NCH_2CH_2ONHC(=NBoc)NHBoc{\cdot}HCl$ (2 equiv), BOP (6 equiv), DIEA (6 equiv), DMF, 83%; iii—TFA, DCM, 82–85%.



Scheme 2. (a) i—$NaCH(CO_2Et)_2$; ii—concd HOAc, 4 N HCl, reflux, 14 h, 58%; (b) $NaBH_4$, BF_3, 99%; (c) *t*-$BuNH_2$, toluene, reflux, 16 h, 62%; (d) i—concd HCl, EtOH, reflux, 2 h; ii—$NaHCO_3$, 93%; (e) AcCl, THF, 0 °C, DIEA, 69%; (f) $ArCF_2COCl$, DIEA, DCM, 92%; (g) Pd/C, H_2, EtOH, 92%; (h) i—K_2CO_3 (aq), MeOH, ii—HCl, 92%; (i) i—HOAc, concd HCl, $NaNO_2$; ii—CuCl, concd HCl, 48%; (j) BH_3, THF, reflux, 20 h, 81%; (k) SO_3·pyridine, DMSO, DIEA, 99%; (l) i—$NaClO_2$, H_2O, DMSO, NaH_2PO_4; ii—HCl aq, 51%.

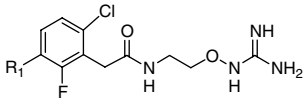
aniline, deprotection of the acetate group and a Sandmeyer reaction using CuCl (compound **XII**). The acetamide was reduced using a borane reduction, giving the desired P3 segment. Oxidation then provided the necessary carboxylic acid (**XIV**), ready for attachment to the P1 moiety.

Compounds **1**–**18** were evaluated for the inhibition of thrombin using a standard chromogenic assay[8] (Tables 1 and 2). In summary, the SAR directed us to electron-deficient mono- and bicyclic 2,2-difluoroethylamine moieties as the most effective P3s. For example, switching from a phenyl group (**1**, K_i = 47.1 nM) to a 2-pyridyl group (**9**, K_i = 8.6 nM) increases the potency more than fivefold. This is due to better edge to face σ–π interactions of the electron-deficient aryl groups with the backbone π-rich Trp215 in the P3 region.[9] This effect is also seen in compounds **3** and **4**. The *p*-Cl group in compound **11** increases the binding affinity for thrombin almost fivefold compared with compound **9**. Biaryl rings with an electron withdrawing substituent are also sufficient to achieve nM potency, such as compound **8**.

Several H-bond interactions were revealed in the X-ray crystal structure of compound **1** in human thrombin (Fig. 2). As expected, the aniline-NH contributes to a hydrogen bonding interaction with the thrombin backbone (Gly216, 3.05 Å).[9] The amide NH also makes important hydrogen bonding interactions with the backbone protein (Ser214, 3.01 Å; Ser195 3.11 Å), and the *O*-guanidine moiety, an arginine mimic, effectively anchors the ligand with multiple (4) hydrogen bonding interactions (Gly219, 2.90 Å; Asp189, 2.69 Å, 3.19 Å; Ala190, 3.25 Å).

An interesting insight from the crystal structure was the observed distance between the P2 fluorine and the backbone nitrogen of Gly216 (F–N distance 3.17 Å). This equates to a F–H distance of 2.14 Å, as N–H bond lengths are 1.03 Å.[10] While organofluorine may only rarely be a H-bond acceptor,[11] F–H distances of $\leqslant$2.35 Å are regarded as strong evidence that such an H-bond exists.[11,12] It should be noted, however, that while the CF–HN hydrogen bond is undoubtedly

Table 1. Effect of R1 on potency of 2-fluoro-4-chloro-3-phenyl acetamide *O*-guanidines



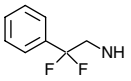
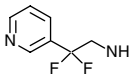
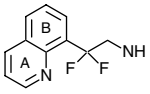
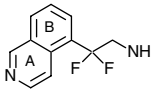
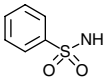
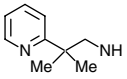
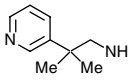
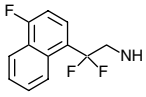
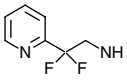
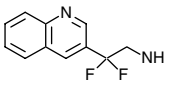
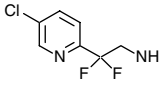
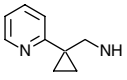
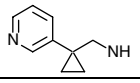
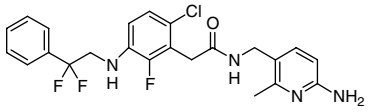
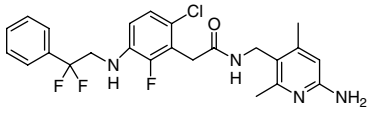
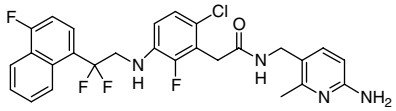
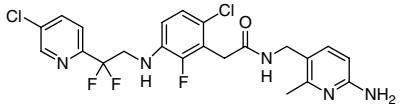
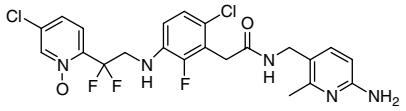
Compound	R1	K_i (nM)
1		47.1
2		33.9
3		2.7
4		4.7
5		1281
6		58.0
7		128
8		3.1
9		8.6
10		169
11		1.8
12		57.0
13		142

Table 2. Replacement of *O*-guanidine P1 with aminopyridines

Compound	K_i (nM)
14 	10.3
15 	3.3
16 	2.8
17 	0.7
18 	0.9

contributing to the potency of the series, it is also possible that the P2 (and P3) fluorines are providing potency by increasing the strength of the aniline NH–CO hydrogen bond via increased aniline NH acidity. Indeed, the calculated pK_a[13] of the *gem*-difluoro aniline NH of **2** (−1.1) is 3 pK_a units lower than the fourfold less potent *gem*-dimethyl aniline NH of **7** (2.2), which is in line with the known effect of β-fluorine on amine pK_a.[14]

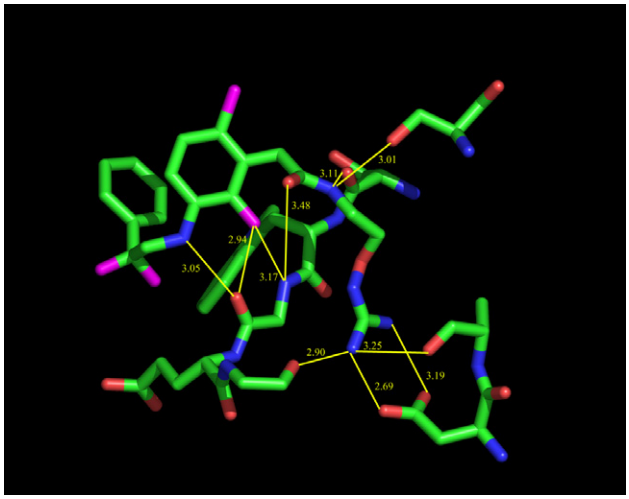


Figure 2. X-ray crystal structure of compound **1** in human thrombin (PDB ID: 2R2M).

Aminopyridines have been reported to be suitable arginine (P1) surrogates.[15] A brief survey revealed that replacement of the *O*-guanidine group in compound **1** with a methyl aminopyridine P1 (compound **14**) and a dimethyl aminopyridine (compound **15**) increased the potency 4- and 14-fold, respectively. The methyl aminopyridine also improved the potency of compound **11**, giving compound **17** with sub-nM potency (K_i = 0.7 nM). Interestingly, the corresponding *N*-oxide compound **18** has comparable potency to **17**.[9]

In conclusion, we have developed a series of non-peptidic, 2-(2-chloro-6-fluorophenyl)acetamides which have excellent potency against human thrombin. Proper selection of the P3 substituent was critical in attaining sub-nM potency. The *O*-guanidine moiety was a suitable arginine replacement. Replacement of the *O*-guanidines with aminopyridines further improved the thrombin potency.

References and notes

1. (a) Hauptmann, J. *Eur. J. Clin. Pharmacol.* **2002**, *57*, 751; (b) Gustafssson, D.; Bylund, R.; Antonsson, T.; Nilsson, I.; Nystrom, J.-E.; Eriksson, U.; Bredberg, U.; Teger-Nilsson, A.-C. *Nat. Rev. Drug Discov.* **2004**, *3*, 649.
2. Colman, R. W.; Hirsch, J.; Marder, V. J.; Salzman, E. W., Eds.; *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 3rd ed.; J.B. Lippincott: Philadelphia, 1994.
3. Vlasuk, G. P. *Thromb. Haemostasis* **1993**, *70*, 212.
4. 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.-Wu; 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. *J. Med. Chem.* **1998**, *41*(23), 4466.
5. Compounds **6**, **7**, and **13** in Table 1 were prepared in three steps from (2-chloro-5-bromo-6-fluorophenyl)-acetic acid ethyl ester via Buchwald coupling (*J. Org. Chem.* **2000**, 65, 1144–1157) with the amine monomers followed by ester saponification and amide formation. The bromoarene was prepared from intermediate **II** in 34% overall yield via F → Br conversion (Sandmeyer reaction on the reduced azide) followed by nitro → Cl conversion (Sandmeyer reaction on the reduced nitro group). The amine monomers were prepared from treatment of the potassium salts of secondary nitriles with, e.g., fluoropyridines (*J. Am. Chem. Soc.* **2000**, 122, 712–713), followed by nitrile reduction with LAH.
6. Valpey, R. S.; Miller, D. J.; Estes, J. M.; Godleski, S. A. *J. Org. Chem.* **1982**, *47*(24), 4717.
7. (a) 3-Dimensional Pharmaceuticals Inc.; Pan, W., Lu, T., Markotan, T., Tomczuk, B. Aminopyridyl-, Aminoguanidinyl- and Alkoxyguanidinyl-Substituted Phenyl Acetamides as Protease Inhibitors, WO0228825; (b) 3-Dimensional Pharmaceuticals, Inc.; Kreutter, K., Lee, L., Lu, T., Mohan, V., Patel, S., Huang, H., Xu, G. Substituted Phenyl Acetamides and their Use as Protease Inhibitors, WO04091613.
8. Dissociation constants were determined at 37 °C in a 96-well format using a Molecular Devices plate reader. Varied concentrations of inhibitors in 10 μl dimethylsulfoxide were added to wells with 280 μl of assay buffer (pH 7.5) which contained 50 mM Hepes, 0.2 M NaCl, 1% dimethylsulfoxide, 0.05% *n*-octyl β-D-glucopyranoside, and substrate, and incubated for 15 min at 37 °C. Reactions were initiated by the addition of 10 μl of enzyme in assay buffer without dimethylsulfoxide and substrate, and the change in absorbance at 405 nm was monitored for 15 min. IC_{50}s were obtained from the inverse slope of plots of the ratio of initial velocity in the absence of inhibitor to initial velocity in the presence of inhibitor as a function of inhibitor concentration. All velocities were corrected for background substrate conversion (no enzyme addition). The dissociation constant (K_i) was calculated using the equation $K_i = IC_{50}/(1 + S/K_m)$ where S is the substrate concentration (Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol*. **1973**, *22*, 3099). K_m for the enzyme–substrate pair was determined from Hanes–Wolf plots using the same final buffer conditions as K_i determinations. Respective substrate, substrate concentration, and K_m values for human α-thrombin were: succinyl-Ala-Ala-Pro-Arg-*p*-nitroanilide, 100, and 320 μM. Within-run assay coefficient of variation (CV) was <10%; between-run CV was <20%.
9. Burgey, C. S.; Robinson, K. A.; Lyle, T. A.; Nantermet, F. G.; Selnick, H. G.; Isaacs, R. C. A. S.; Lewis, D.; Lucas, B. J.; Krueger, J. A.; Singh, R.; Miller-Stein, C.; White, R. B.; Wong, B.; Lyle, E. A.; Stranieri, M. T.; Cook, J. J.; McMasters, D. R.; Pellicore, J. M.; Pal, S.; Wallace, A. A.; Clayton, F. C.; Bohn, D.; Welsh, D. C.; Lynch, J. J.; Yan, Y.; Chen, Z.; Kuo, L.; Gardell, S. J.; Shafer, J. A.; Vacca, J. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1353.
10. Burley, S. K.; Petsko, G. A. *Adv. Protein Chem.* **1988**, *39*, 125.
11. Dunitz, J. D. *ChemBioChem* **2004**, *5*, 614.
12. Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613.
13. pK_a values calculated by Third Dimension Explorer?, a software package proprietary to Johnson & Johnson.
14. Grunewald, G. L.; Seim, M. R.; Lu, J.; Makboul, M.; Criscione, K. R. *J. Med. Chem.* **2006**, *49*, 2939.
15. Sanderson, P.; Stantan, M. G.; Dorsey, B. D.; Lyle, T. A.; McDonough, C.; Sanders, W. M.; Savage, K. L.; Naylor-Olsen, A. M.; Kruger, J. A.; Lewis, S. D.; Lucas, B. J.; Lynch, J. J.; Yan, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 795.