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A Four Component Coupling Strategy for the Synthesis of D-Phenylglycinamide-derived Non-Covalent Factor Xa Inhibitors

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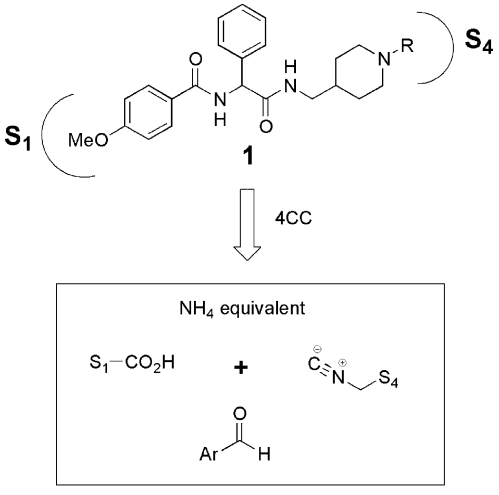
Abstract—A novel isonitrile derivative was synthesized and used in an Ugi four component coupling reaction to explore aryl group substitution effects on inhibition of the coagulation cascade serine protease factor Xa.

Discovery of new agents for the treatment and moderation of thrombotic disorders is an area of intensive research within the pharmaceutical and biotechnology communities.[1] Presently, thrombosis related complications are the leading cause of death in the industrial world.[2] Current therapies suffer from an unfavorable margin of safety which requires the blood of patients to be frequently monitored to prevent excessive pharmacology, namely life-threatening bleeding.[3] It is well understood that thrombus formation is dependant upon activation of the coagulation cascade which ends in the thrombin mediated conversion of soluble fibrinogen to insoluble fibrin. Once formed, fibrin then combines with activated platelets to form the thrombus. The serine protease enzyme factor Xa is central to both the intrinsic and extrinsic coagulation pathways and represents a promising target for the regulation of thrombin generation and thus control of clot formation.[4]

Our team recently discovered a series of non-covalent inhibitors of factor Xa derived from D-phenyl glycinamide and we wished to explore the effect of substitution on the central phenyl ring. In order to accomplish this quickly, we desired a synthetic route that would not require the individual synthesis of each newly desired central amino acid. The Ugi four component coupling (4CC) reaction represented an intriguing approach to our scaffold and offered the potential advantage of enabling us to draw upon the commercial availability of a wide variety of aryl aldehydes as the requisite starting materials (Scheme 1).



Scheme 1. General scheme for the 4CC approach to phenyl glycinamide derived factor Xa inhibitors.

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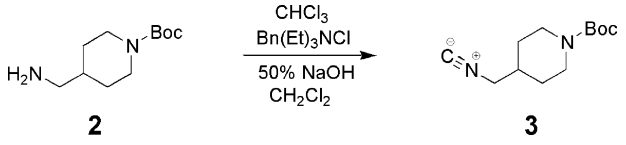
Our initial efforts focused on two interconvertible isonitriles that are reported in the literature.[5] After some experimentation, we found that development of a new isonitrile that already contained much of our desired S4 functionality, offered us the most flexibility with respect to tolerance of the starting aryl aldehyde component. Isonitrile formation from *N*-*tert*-butoxycarbonyl-4-aminomethyl-piperidene **2** was straightforward and could be performed on large scale.[6,7] Isonitrile **3** was isolated as a white solid which could be stored for prolonged periods in the freezer without significant decomposition (Scheme 2).[8]

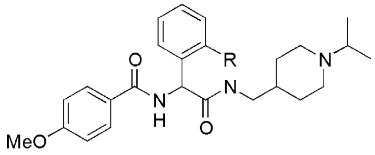


Scheme 2. Preparation of isonitrile **3**.

With multigram quantites of isonitrile **3** in hand, the 4CC route proved to be adequate for the investigation of a variety of aryl substituents. Although not individually optimized, purified yields for the Ugi reaction with aryl aldehydes, isonitrile **3**, *p*-methoxybenzoic acid, and 2,4-dimethoxybenzyl amine ranged from 15–78% providing compounds of type **4**. Subsequent removal of the Boc and 2,4-DMB protecting groups with trifluoroacetic acid provided, after basic workup, products **5** which were generally taken on as crude material through a final reductive amination with acetone to afford the final products **6** in acceptable yields (Scheme 3).[9,10]

The effects of *ortho*-substitution on the central phenyl ring of **1** are shown in Table 1.[11] A variety of *ortho*-substituted aryl aldehydes served as diversity elements for the Ugi reaction and the 4CC products were carried on to the corresponding inhibitors of type **7** (Table 1). Many of the surveyed *ortho*-substituents provide an increase in binding affinity for factor Xa.[12] Electron-withdrawing groups such as Cl, and Br provide apparent association constants (Kass) of 5.1 and 4.6 million L/mol, respectively. Interestingly, electron-donating groups such as methyl and methoxy boost binding affinity as well. The results in Table 1 also illuminate factor

Table 1. Effect of *ortho* substitution on binding to factor Xa



7a-r

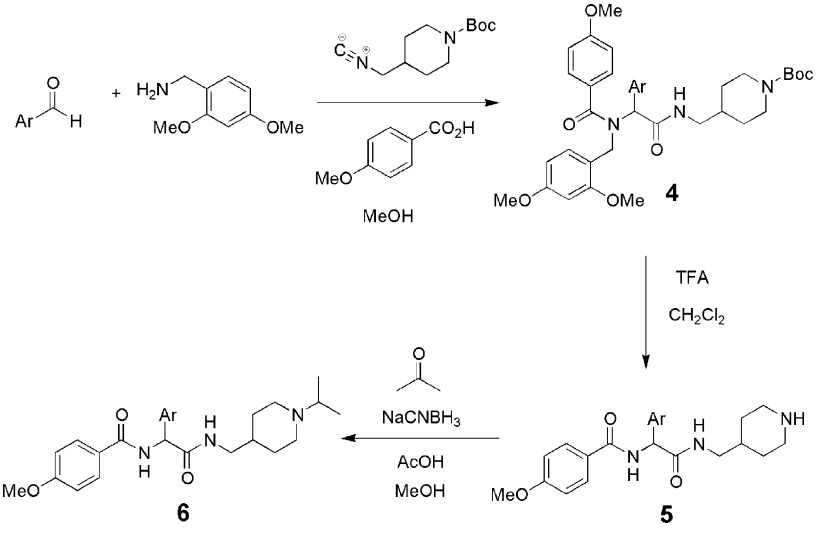
Compd	R	K_{ass} (10^6 L/mol)[a]	% Yield of 4CC Reaction
7a	H	0.71	—
7b	CH_3	2.5	42
7c	CF_3	3.1	32
7d	F	2.0	72
7e	Cl	5.1	61
7f	Br	4.6	50
7g	I	4.1	65
7h	OH	1.3	51[b]
7i	OMe	1.9	60
7j	OEt	2.8	66
7k	OBn	2.6	51
7l	OCF_3	4.1	56
7m	OPh	1.3	69
7n	NO_2	4.2	56
7o	NH_2	0.75	—[c]
7p	NMe_2	1.1	—[d]
7q	SMe	2.9	74
7r	S-*t*Bu	1.7	78

[a] K_{ass} is the apparent association constant reported in units of 10^6 L/mol and is approximately equal to $1/K_i$. K_{ass} values were obtained by the method of Smith et al.[14] and are the result of a single experiment (performed in triplicate at each of four to eight inhibitor concentrations).[15]

[b] Two step yield obtained from deprotection (H_2, Pd/C, quantitative yield) of 2-benzyloxybenzaldehyde **4CC** adduct **4k**.

[c] Compound **7o** was obtained in quantitative yield from **7n** (H_2, Pd/C).

[d] Compound **7p** was obtained in 31% yield from **7o** ($(CH_2O)_n$, $NaCNBH_3$, HOAc).



Scheme 3. The Ugi 4CC route to glycinamide factor Xa inhibitors.

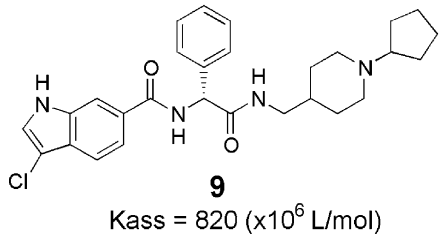
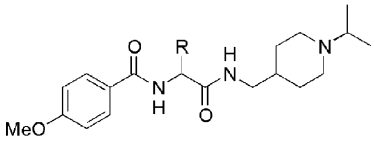


Figure 1. A factor Xa inhibitor with a 3-chloro-6-indolyl S1 unit.

Table 2. Effect of heteroaromatic substitution on binding to factor Xa



Compd	R	K_{ass}, (10^6 L/mol)	%Yield of 4CC Reaction
7a	C_6H_5	0.71	—
8a	2-Furan	0.36	17
8b	3-Furan	0.20	15
8c	2-Thiophene	1.1	15
8d	3-Thiophene	0.88	32
8e	2-Imidazole	0.21	30
8f	4-Quinoline	1.3	21
8g	3-Quinoline	0.05	25

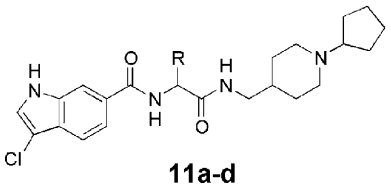
Xa's ability to accommodate increasing steric bulk at this position on inhibitors of type **7**.[13]

Heterocyclic and bicyclic replacements for the central aryl ring are shown in Table 2. Heterocycles such as 2-thiophene and 2-furan are tolerated, but provide no additional boost in binding affinity toward factor Xa.

The scope of the Ugi methodology was readily applicable to the preparation of derivatives containing the more potent 3-chloroindole S1 binding element found in compound **9** (Fig. 1).[16,17]

For example, the 4CC reaction of 3-chloroindole-6-carboxylic acid with 1-naphthaldehyde, 2,4-dimethoxybenzylamine, and isonitrile **3** provided **10a** in 53% isolated yield (Scheme 4).[18]

Table 3. 3-Chloroindolyl S1 factor Xa inhibitors



Compd	R	K_{ass} (10^6 L/mol)	2XPT (μM)[17]
11a	1-Naphthalene	250	3.8
11b	2-Naphthalene	79	4.1
11c	3-Quinoline	0.2	—
11d	2-Thiazole	380	0.79

Compounds **10a–d** were then converted in straightforward fashion under the same conditions shown in Scheme 3, replacing acetone with cyclopentanone to afford compounds **11a–d** (Table 3). It should be noted that despite the moderate yield (24%) of the 4CC reaction to form **10d**, we found the Ugi route to be superior to alternate approaches involving the synthesis of 2-thiazolyl glycine for multigram preparation of **11d**.[19]

In conclusion, a previously unknown isonitrile **3** was synthesized and used to develop an Ugi four component coupling reaction for the synthesis of reversible factor Xa inhibitors. It was discovered that *ortho*-substitution on the central phenyl ring with either electron withdrawing or electron donating groups provided a significant increase in binding affinity. The Ugi 4CC reaction allowed rapid access to a variety of substituents and heterocyclic replacements for the central aryl unit and enabled us to readily scale-up compounds for further evaluation.

Acknowledgements

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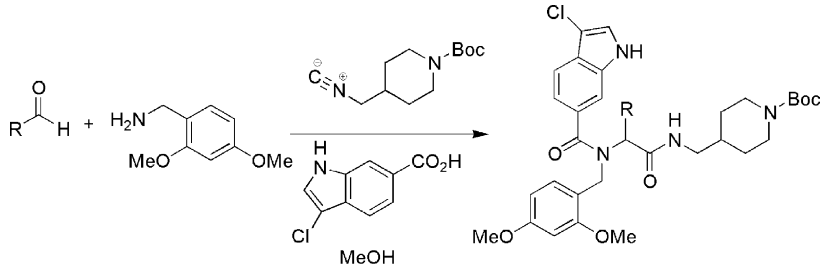

10a: R=1-naphthalene, 53%
10b: R=2-naphthalene, 35%
10c: R=3-quinoline, 29%
10d: R=2-thiazole, 24%

Scheme 4. Isolated yields for the Ugi 4CC reaction with 3-chloroindole-6-carboxylic acid.

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7. Preparation of isonitrile **3**: To a solution of **2** (77 g, 360 mmol) in 110 mL of CH_2Cl_2 at room temperature was added chloroform (40 mL, 500 mmol) and benzyl triethylammonium chloride (1.6 g, 7.2 mmol). 110 mL of a 50% (w/v) sodium hydroxide solution was added and the flask was fitted with a reflux condenser. The reaction spontaneously reached and maintained a gentle reflux for 90 min and the reaction was allowed to stir overnight at room temperature. The reaction was diluted with water and the product was extracted into CH_2Cl_2. The organic layer was dried over K_2CO_3, filtered, and concentrated under reduced pressure. The resultant oil was filtered through a SiO_2 plug (2:1 EtOAc:Hexane) to afford, after concentration 28 g (35%, 83% based on recovered starting material) of **3** as a white amorphous solid. The SiO_2 plug was then washed with 3:1 EtOAc:Isopropyl amine to provide 45g of recovered **2**.

3: Mp 58–59 °C; IR (neat) 2147, 1691, 1171 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) 1.16–1.26 (m, 2H), 1.44 (s, 9H), 1.72–1.84 (m, 3H), 2.67 (t, br, 2H, $J = 12.4$ Hz), 3.28 (d, 2H, $J = 6.0$), 4.13 (s, br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 28.8, 29.5, 36.3, 47.4, 47.5, 79.9, 154.7, and 157.1.

8. Stored under nitrogen at −20 °C in a sealed flask.

9. By the nature of its mechanism, the 4CC reaction as shown provides products in racemic form. Isolation of material enriched in the desired enantiomer requires subsequent separation by chiral HPLC. We have found that the majority of Xa binding affinity results from one enantiomer (unpublished results), and have tested the 4CC products as racemates for expedience.

10. All final products were purified by silica gel column chromatography and were judged to be of greater than 95% purity by ^1H NMR and HPLC analysis.

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12. For a depiction of the conformational constraint imparted by *ortho*-substitution in a computationally derived model of an *ortho*-substituted factor Xa inhibitor from this series docked into the factor Xa active site, see: Sheehan, S. M.; Masters, J. J.; Wiley, M. R.; Young, S. C.; Liebeschuetz, J. W.; Jones, S. D.; Murray, C. W.; Franciskovich, J. B.; Engel, D. B.; Weber, W. W.; Marimuthu, J.; Kyle, J. A.; Smallwood, J. K.; Foster, R. S.; Froelich, L. L.; Gifford-Moore, D. S.; Towner, R. D.; Snyder, D. W.; Chouinard, M. L.; Chastain, M. K.; Johnson, L. M.; Sipes, P. R.; Tluczek, J. P.; Craft, T. J.; Smith, G. F. *Abstracts of Papers, Part MEDI-230*, 225th National Meeting of the American Chemical Society, New Orleans, LA, March 23–27, 2003; American Chemical Society: Washington, DC, 2003.

13. While steric bulk is not problematic when located on one side of the phenyl ring, 2,6-substitution presents a substantial challenge to the enzyme. The 2,6-dichloro analogue of **7**, prepared from the Ugi reaction with 2,6-dichlorobenzaldehyde had an apparent K_{ass} of 0.11 million L/mol.

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17. (a) 2XPT is defined as the concentration of test compound required to double the time to clot formation in reconstituted human plasma, using the prothrombin time assay as described in: Wiley, M. R.; Weir, L. C.; Briggs, S.; Bryan, N. A.; Buben, J.; Campbell, C.; Chirgadze, N. Y.; Conrad, R. C.; Craft, T. J.; Ficorilli, J. V.; Franciskovich, J. B.; Froelich, L. L.; Gifford-Moore, S. S.; Goodson, T., Jr.; Herron, D. K.; Klimkowski, V. J.; Kurz, K. D.; Kyle, J. A.; Masters, J. J.; Ratz, A. M.; Milot, G.; Shuman, R. T.; Smith, T.; Smith, G. F.; Tebbe, A. L.; Tinsley, J. M.; Towner, R. D.; Wilson, A.; Yee, Y. K. *J. Med. Chem.* **2000**, *43*, 883.
(b) Inhibitor **9** has $>1000\times$ selectivity with respect to the following enzymes: Thrombin, Plasmin, Bovine Trypsin, Urokinase, aPC, Kallikrein, and tPA.

18. Representative Ugi procedure: To a solution of 1-naphthaldehyde (0.69 g, 4.4 mmol) in 2 mL of methanol was added 2,4-dimethoxybenzyl amine (0.74 mL, 4.4 mmol) and the reaction was allowed to stir at room temperature for 30 min. The reaction was diluted with 2 mL of methanol after which 3-chloroindole-6-carboxylic acid (0.67 g, 4.4 mmol) and isonitrile **3** (1.0 g, 4.4 mmol) were added. The reaction was then allowed to stir overnight at room temperature. The reaction was concentrated under reduced pressure and the resultant crude residue was subjected to flash silica gel chromatography to provide 1.7 g (53%) of **10a** as a white crystalline solid. Mp 150–151 °C; IR (neat) 1666, 1613, 1423 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 0.90–0.98 (m, 2H), 1.24–1.57 (m, 2H), 1.41 (s, 9H), 2.51 (s, br, 2H), 3.04 (s, br, 2H), 3.41 (s, 3H), 3.60 (s, 3H), 3.96 (s, br, 2H), 4.57 (dd, 2H, $J = 16$ and 45 Hz, 2H), 5.97

(s, br, 2H), 6.19 (t, 1H, $J=6.0$ Hz), 6.41 (s, br, 1H), 6.88 (d, 1H, $J=8.8$ Hz), 7.13 (d, 1H, $J=2.4$ Hz), 7.34–7.38 (m, 2H), 7.43–7.48 (m, 2H), 7.54 (d, 1H, $J=7.6$ Hz), 7.60–7.71 (m, 1H), 7.73–7.80 (m, 4H), 7.90 (s, br, 1H), and 9.78 (s, br, 1H); ^{13}CNMR (100 MHz, $CDCl_3$) 28.9, 30.0, 36.5, 45.5, 55.1, 55.5, 55.6, 79.7, 98.0, 103.7, 106.0, 111.9, 117.3, 118.1, 119.0, 123.2, 123.5, 125.2, 126.0, 126.6, 126.9, 127.8, 129.1, 129.5, 130.5, 132.4, 133.8, 134.6, 154.9, 157.5, 159.9, 170.5, and 174.5; HRMS(Acc ES+) calcd for $C_{41}H_{45}ClN_4O_6=725.3105$; Found = 725.3091.

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