

187
187

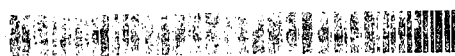
Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 03-107405-00007-0000

Fecha de inscripción: 05/12/2007
Tipo de registro: NUEVAS CREACIONES
Clase de registro: FASE NACIONAL II
Número de folios: 45

Re: Expediente No. 03-107.408

Solicitud de patente titulada: "DERIVADOS DE PIRROLIDINA COMO INHIBIDORES DEL FACTOR Xa".

Solicitante: GLAXO GROUP LIMITED

Nuestra Ref.: P2003/000166

RESPUESTA AL OFICIO No. 15927 NOTIFICADO POR FIJACIÓN EN LISTA No. 445,
DE FECHA 07 DE DICIEMBRE DE 2007
(SEGUNDO EXAMEN DE FONDO)

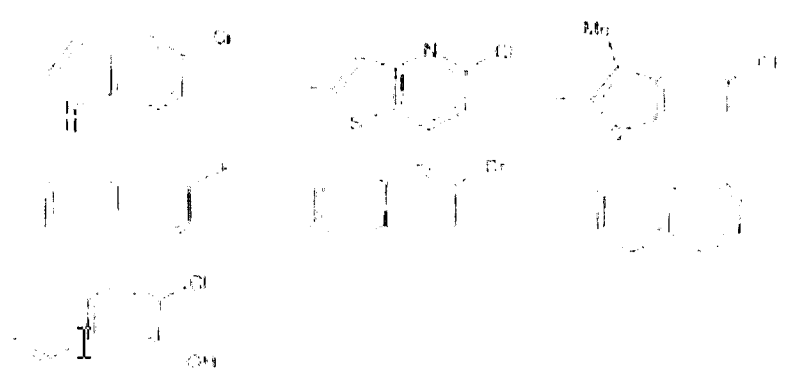
CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 45 de la Decisión 486:

1. En primer lugar, con el ánimo de brindar mayor claridad a la materia reivindicada, presento un **Capítulo Reivindicatorio Modificado** que reemplaza en su totalidad el Capítulo Reivindicatorio adjuntado en respuesta al primer examen de fondo. Para tal efecto, adjunto un recibo de pago que cubre la tasa de modificación correspondiente.

En la Reivindicación 1, el sustituyente A ha sido restringido a los grupos:



188
188



Lo anterior encuentra sustento en la página 7, líneas 18 en adelante, de la Descripción. Con esta modificación la nueva Reivindicación 1 abarca todos los compuestos, con excepción de los Ejemplos 60, 65, 66, 67, 68, 80, 81 y 87. Así, el Capítulo Reivindicatorio Modificado se dirige únicamente a la protección de los compuestos más activos, como lo discutiré más adelante.

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486 de la CAN, ya que éstas de ninguna manera implican una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial, por el contrario, el Capítulo Reivindicatorio Modificado es una franca limitación de lo originalmente reclamado.

2. a- El Examinador considera que los documentos WO01/19795 (D1) y US 5.489.583 (D2) afectan el nivel inventivo de la presente solicitud:

El Examinador insiste en que el documento D1 describe compuestos inhibidores del factor Xa, que al igual que los de la presente solicitud, tienen un núcleo básico de pirrolidin-2-ona sustituido en la posición 3 del anillo con una sulfonamida. Adicionalmente, el Examinador menciona que D2 revela compuestos de pirrolidina, que actúan como inhibidores de trombina que, al igual que D1, son útiles para tratar enfermedades vasculares.

El Examinador concluye que el arte previo enseña que los compuestos que presentan un núcleo de pirrolidin 2-ona, sustituido con una sulfonamida en la posición 3, son activos sobre enzimas y factores de coagulación.

El Examinador considera adicionalmente que en la presente invención no existe evidencia que al incluir un grupo morfolina o un $-CF_3$, es posible proveer agentes con una con una actividad mejorada en comparación del arte previo más cercano. Así mismo, establece que aún cuando la

189
189
189

presente invención provee datos $IC_{50} < 0.1 \mu M$, estos resultados no han sido comparados con compuestos similares del estado de la técnica y por lo tanto no es posible establecer que los mismos presentan una mayor efectividad o cualquier otro efecto inesperado respecto de los compuestos ya conocidos, igualmente útiles en el tratamiento de enfermedades vasculares.

b- i) Llamo respetuosamente la atención del Examinador al hecho que, aun cuando los compuestos de la presente invención comparten el mismo núcleo estructural con los compuestos de D1, correspondiente a 3-amino sustituido-1-(2-oxoetil)pirrolidin-2-onas, D1 no brinda sugerencia alguna respecto a la conveniencia de incluir un grupo morfolina en lugar del grupo 2-oxoetil; esto es, el reemplazo del grupo etoxiguanidina de D1 por un grupo morfolina. El único documento que generaría tal motivación para hacer este reemplazo se encuentra precisamente en la solicitud ventilada en este expediente, documento que obviamente no puede citar el Examinador.

Y por el contrario, de seguir las enseñanzas de D1, cualquier persona medianamente versada en la materia, estaría motivada únicamente a perseguir la obtención de derivados N-sustituidos-(etoxiguanidina)-2-(3-amino sustituido-2-oxopirrolidin-1-il)acetamidas, sin considerar un grupo morfolino.

ii) En cuanto a D2, la presente invención no considera la inclusión de un grupo 4-metilbenzamidina sustituyendo la posición 1 del grupo 2-oxoetil. Dicha posición en la presente invención está sustituida con R^2 y R^3 , donde dichos radicales pueden ser hidrógeno, C_{1-3} -alquil o $-CF_3$ (solamente uno de ellos).

Adicionalmente, los compuestos de D2 también difieren de los de la presente invención en el sustituyente A, puesto que los compuestos aquí reivindicados se relacionan con grupos arilo y heteroarilo específicos, mientras que los compuestos de D2 en dicha posición (Ar) comprenden un grupo $-NR^1R^2$ o un grupo $-OR^3$. Así las cosas, el experto en la materia, basado únicamente en las indicaciones estructurales de de D2, estaría predispuesto a incluir un grupo voluminoso sustituyendo la posición 1 del grupo -2-oxoetil, como el metilbenzamidina allí ilustrado, pero no estaría motivado a remover dicho grupo voluminoso y a incluir hidrógenos, C_{1-3} -alquil o $-CF_3$, como en los compuestos de la presente invención.

iii) Así mismo, debo resaltar el hecho que una persona versada en la materia enfrentada al problema de diseñar fármacos inhibidores del factor Xa no tendría en cuenta las enseñanzas de D2, por cuanto los compuestos allí divulgados están dirigidos a una enzima

190
190
9

completamente diferente (trombina), cuyo dominio de unión dista estructuralmente de aquel presente en el factor Xa y por lo tanto, las moléculas que se unen a ellos son diferentes tanto electrónica como espacialmente.

En ese sentido, me permito subrayar que la evaluación de nivel inventivo en el análisis de patentes debe realizarse sobre la base de qué hubiera sido obvio para una persona versada en el arte. Claramente, la búsqueda del estado de la técnica de anterioridades, basada únicamente en la estructura molecular, sin considerar también los diferentes mecanismos de acción, no es representativa de la práctica realizada en el ámbito de la investigación farmacéutica. La persona versada en la técnica con la tarea de desarrollar fármacos con actividad inhibidora del factor Xa, evidentemente enfocaría su investigación en el estudio estructural de agentes en el estado de la técnica con ese mecanismo de acción particular. Por esta razón, la persona versada en la técnica jamás consideraría relevante enseñanzas previas de cómo diseñar agentes con un mecanismo de acción diferente, como ocurre en el presente caso respecto de la materia revelada en D2, que enseña compuestos con una actividad de inhibición de trombina.

iv) En relación a lo anterior, llamo respetuosamente la atención del Examinador al artículo escrito por Chan et.al, adjunto con la presente comunicación (ANEXO 1), en el cual se establece claramente que los compuestos activos sobre el Factor Xa no son necesariamente activos sobre trombina. De hecho algunos de los compuestos de la presente invención son selectivos para el factor Xa y no para trombina. En la página 1551 de dicho artículo se menciona que “con respecto a las demás proteasas en el proceso de coagulación, se han identificado análogos con alta afinidad por trombina pero actividades insignificantes frente a la proteína C activada, al factor/ TF VIIA y al factor XIIa”. Con lo anterior se confirma que una persona versada en el arte con la tarea de diseñar inhibidores del Factor Xa no consideraría D2 como punto de partida, puesto que éste solamente brinda enseñanzas respecto de de trombina.

Con el ánimo de brindar mayor sustento al nivel inventivo de la presente invención, me permito presentar datos adicionales de la actividad biológica de los compuestos reclamados en la nueva Reivindicación 1 (ANEXO 2), los cuales fueron evaluados en el ensayo cromogénico y demostraron valores de IC₅₀ menores a 15µm. De esta forma, el alcance de la reclamación actual se limita únicamente a los compuestos más activos frente al factor Xa, cuya actividad ventajosa no es predecible a partir de la divulgación de D1 y/o D2.

191
191

3. Finalmente, deseo poner bajo su conocimiento que en relación con la presente solicitud además de los privilegios otorgados en Europa (EP 1395553B1) y Estados Unidos (US 7.186.717B1), para el concepto inventivo involucrado en la presente solicitud han sido concedidas patentes en Australia (AU2002311451), China (ZL02815400.2), Chipre (CY1103408), Hong Kong (1063471), México (237917), Mónaco (1395553), Nueva Zelanda (530004), Filipinas (1-2003-501261), Singapur (10/220) y Sur África (2003/9367).

Una vez más, aunque dichas concesiones en otros países no son vinculantes en el sentido que obliguen a la Superintendencia a tomar una decisión en el mismo sentido, éstas evidentemente sí sugieren claramente el cumplimiento de los requisitos de patentabilidad de la materia reivindicada, ya que los criterios de evaluación y estudio aplicados para determinar la novedad y nivel inventivo resultan equivalentes a los que la Superintendencia debe emplear.

4. En conclusión, considero que las objeciones presentadas por el Examinador han sido resueltas y por consiguiente solicito respetuosamente que se proceda a otorgar el privilegio de patente a la presente invención. En el caso que el Examinador considere que aún existen reivindicaciones no patentables, solicito respetuosamente emitir un examen de fondo adicional que brinde la oportunidad al solicitante de hacer las aclaraciones correspondientes.

Atentamente,



CARLOS R. OLARTE

C.C. No. 79.782.747 de Bogotá

T.P. 74.295

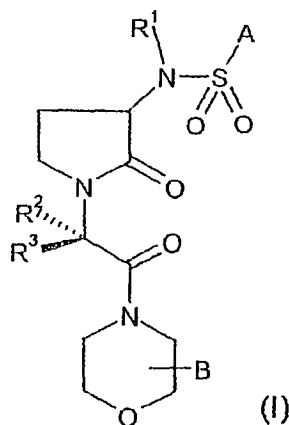
Anexos:

- Capítulo Reivindicatorio Modificado.
- Recibo de pago correspondiente a la tasa de modificación.
- ANEXO 1: Chuen Chan et. al. Factor Xa inhibitors: S1 binding interactions of a series of N-{(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamides. J. Med. Chem. 2007, 50, 1546-1557.
- ANEXO 2: Datos de actividad biológica de los compuestos reclamados en la nueva Reivindicación 1.

192
190

CAPITULO REIVINDICATORIO MODIFICADO

1. La presente invención proporciona compuestos de la fórmula (I):



5 en la que:

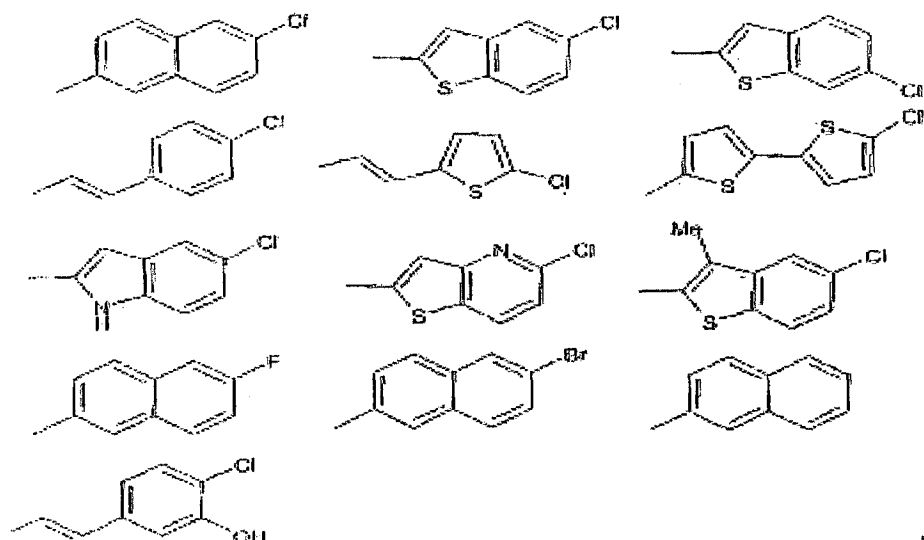
R^1 representa hidrógeno, -alquilo C_{1-6} , -alqueno C_{3-6} , -(alquil C_{2-3}) NR^bR^c , -(alquil C_{2-3}) $NHCO R^b$, fenilo o un grupo heterocíclico aromático de 5 o 6 miembros, estando el fenilo o el grupo heterocíclico aromático de 5 o 6 miembros opcionalmente sustituidos con halógeno, o R^1 representa un grupo X-W, en el que X representa -alqueno C_{1-3} y
10 W representa -CN, -CO₂H, -CONR^bR^c, -COalquilo C_{1-6} , -CO₂alquilo C_{1-6} , fenilo o un grupo heterocíclico aromático o no aromático de 5 o 6 miembros que contiene al menos un heteroátomo seleccionado entre O, N o S, estando el fenilo o el grupo heterocíclico aromático opcionalmente sustituidos con uno o más sustituyentes seleccionados entre: -alquilo C_{1-3} , -alcoxi C_{1-3} , -(alquil C_{1-3})OH, halógeno, -CN, -CF₃, -NH₂, -CO₂H y
15 -OH;

R^2 y R^3 representan independientemente hidrógeno, -alquilo C_{1-3} o -CF₃ con la condición de que uno de R^2 y R^3 es -alquilo C_{1-3} o -CF₃ y el otro es hidrógeno;

R^b y R^c representan independientemente hidrógeno o -alquilo C_{1-3} ;

A representa un grupo seleccionado entre:

193
2193



Z representa uno o dos sustituyentes opcionales seleccionados independientemente entre halógeno y OH,

W representa un sustituyente opcional -alquilo C₁₋₃,

5 alq representa alquileo C₂₋₃ o alquenileno C₂₋₃,

T representa un heteroátomo seleccionado entre O, N o S;

B representa uno o más sustituyentes opcionales sobre los átomos de carbono del anillo seleccionados entre:

- (i) uno o más sustituyentes seleccionados entre -CF₃, -F, -CO₂H, -alquilo C₁₋₆, -(alquil C₁₋₆)OH, -(alquil C₁₋₃)NR^bR^c, -(alquil C₀₋₃)CONR^bR^c y -(alquil C₀₋₃)CO₂alquilo C₁₋₃, -CONH(alquil C₂₋₃)OH, -CH₂NH(alquil C₂₋₃)OH, -CH₂Oalquilo C₁₋₃ y -CH₂SO₂alquilo C₁₋₃;
- 10 (ii) un grupo -Y-R^e,

Y representa -alquileo C₁₋₃, -CO-, -(alquil C₁₋₃)NH-, -(alquil C₁₋₃)NHCO-, -(alquil C₁₋₃)NHSO₂-, -CH₂NHSO₂CH₂- o un enlace directo,

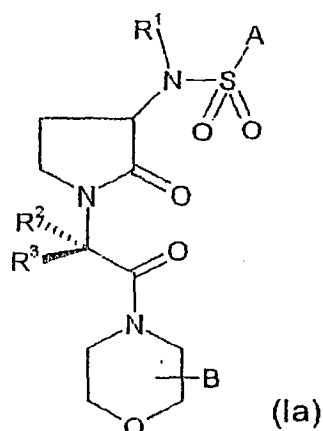
- 15 R^e representa fenilo, un cicloalquilo de 5 o 6 miembros o un heterociclo de 5 o 6 miembros que contiene al menos un heteroátomo seleccionado entre O, N o S, cada uno de los cuales está opcionalmente sustituido con uno o más sustituyentes seleccionados entre: -alquilo C₁₋₃, -alcoxi C₁₋₃, -(alquil C₁₋₃)OH, halógeno, -CN, -CF₃, -NH₂, -CO₂H y -OH; o

- 20 (iii) un segundo anillo R^f que está condensado con el anillo heterocíclico; en el que R^f representa fenilo, un grupo cicloalquilo de 5 o 6 miembros o un grupo heterocíclico aromático de 5 o 6 miembros que contiene al menos un heteroátomo seleccionado entre O, N o S, y el grupo bicíclico condensado está opcionalmente sustituido con uno o más sustituyentes seleccionados entre: -alquilo C₁₋₃, -alcoxi C₁₋₃, -(alquil C₁₋₃)OH, halógeno, -CN, -CF₃, -NH₂, -CO₂H y -OH;
- 25

y sus derivados farmacéuticamente aceptables.

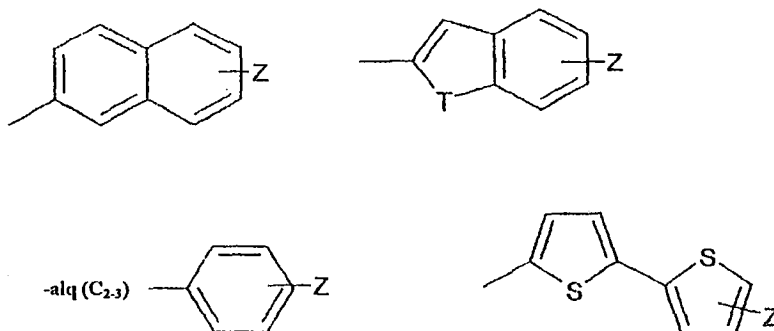
2. Un compuesto según la reivindicación 1, que tiene la fórmula (Ia):

17/194
20



en la que:

- R^1 representa hidrógeno, -alquilo C_{1-6} , -alquenilo C_{2-6} o un grupo X-W, en el que X representa -alquileo C_{1-3} y W representa -CN, $-CO_2H$, $-CONR^bR^c$, $-COalquilo C_{1-6}$, $-CO_2alquilo C_{1-6}$, fenilo o un grupo heterocíclico aromático de 5 o 6 miembros que contiene al menos un heteroátomo seleccionado entre O, N o S, estando el fenilo o el grupo heterocíclico aromático opcionalmente sustituidos con uno o más sustituyentes seleccionados entre: -alquilo C_{1-3} , -alcoxi C_{1-3} , $-(alquil C_{1-3})OH$, halógeno, -CN, $-CF_3$, $-NH_2$, $-CO_2H$ y $-OH$;
- R^2 y R^3 representan independientemente hidrógeno, -alquilo C_{1-3} o $-CF_3$ con la condición de que cuando uno de R^2 y R^3 es -alquilo C_{1-3} o $-CF_3$, el otro es hidrógeno;
- R^b y R^c representan independientemente hidrógeno o -alquilo C_{1-3} ;
- A representa un grupo seleccionado entre:



- Z representa un sustituyente halógeno opcional,
alq representa alquileo o alquenileno,
T representa un heteroátomo seleccionado entre S o N;
B representa uno o más sustituyentes opcionales sobre los átomos de carbono del anillo seleccionados entre:
- (i) uno o más sustituyentes seleccionados entre $-CF_3$, $-F$, $=O$, $-CO_2H$, -alquilo C_{1-6} , $-(alquil C_{1-6})OH$, $-(alquil C_{1-3})NR^bR^c$, $-(alquil C_{0-3})CONR^bR^c$ y $-(alquil C_{0-3})CO_2alquilo C_{1-3}$;
- (ii) un grupo $-Y-R^e$,

195
195
CN

Y representa -alquileo C_{1-3} -, -CO-, -(alquil C_{1-3})NH-, -(alquil C_{1-3})NHCO-, -(alquil C_{1-3})NHSO₂-, -CH₂NHSO₂CH₂- o un enlace directo,

R^e representa fenilo, un cicloalquilo de 5 o 6 miembros o un heterociclo de 5 o 6 miembros que contiene al menos un heteroátomo seleccionado entre O, N o S, cada uno de los cuales está opcionalmente sustituido con uno o más sustituyentes seleccionados entre: -alquilo C_{1-3} -, -alcoxi C_{1-3} -, -(alquil C_{1-3})OH, halógeno, -CN, -CF₃, -NH₂, -CO₂H y -OH; o

(iii) un segundo anillo R^f que está condensado con el anillo heterocíclico, en el que R^f representa fenilo, un grupo cicloalquilo de 5 o 6 miembros o un grupo heterocíclico aromático de 5 o 6 miembros que contiene al menos un heteroátomo seleccionado entre O, N o S, y el grupo bicíclico condensado está opcionalmente sustituido con uno o más sustituyentes seleccionados entre: -alquilo C_{1-3} -, -alcoxi C_{1-3} -, -(alquil C_{1-3})OH, halógeno, -CN, -CF₃, -NH₂, -CO₂H y -OH;

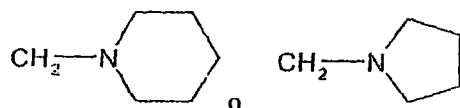
y sus sales y solvatos farmacéuticamente aceptables.

3. Un compuesto según la reivindicación 1, en el que R^1 representa hidrógeno, -alquilo C_{1-6} -, -alqueno C_{3-6} -, -(alquil C_{2-3})NR^bR^e-, -(alquil C_{2-3})NHCOR^b-, fenilo o un heterociclo aromático de 5 o 6 miembros, o R^1 representa un grupo X-W, en el que X representa -alquileo C_{1-3} - y W representa -CN, -CO₂H, -CONR^bR^e-, -COalquilo C_{1-6} -, -CO₂alquilo C_{1-6} -, o un grupo heterocíclico aromático o no aromático de 5 o 6 miembros que contiene al menos un heteroátomo seleccionado entre O, N o S.

4. Un compuesto según una cualquiera de las reivindicaciones 1-3, en el que R^2 representa -alquilo C_{1-3} o hidrógeno.

5. Un compuesto según una cualquiera de las reivindicaciones 1-4, en el que R^3 representa -alquilo C_{1-3} o hidrógeno.

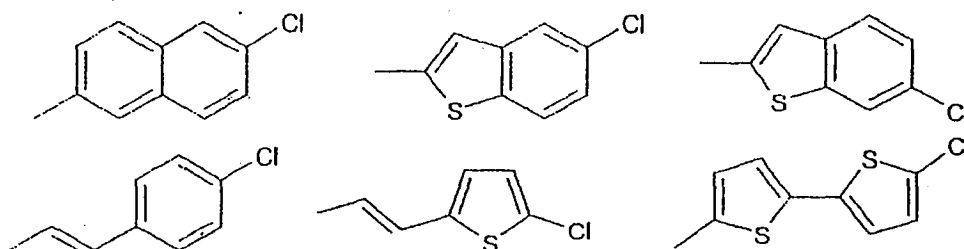
6. Un compuesto según una cualquiera de las reivindicaciones 1-5, en el que B representa hidrógeno o un sustituyente seleccionado entre -alquilo C_{1-6} -, -CONHCH₃-, -CONHCH₂CH(OH)CH₃-, -CH₂NH(CH₃)₂-, -CH₂OCH₃-, -CH₂SO₂CH₃-, -CH₂NHCH₂CH(OH)CH₃-,



7. Un compuesto según una cualquiera de las reivindicaciones 1-6, en el que B representa hidrógeno.

8. Un compuesto según una cualquiera de las reivindicaciones 1-7, en el que A representa un grupo seleccionado entre

196
196
80



9. Un compuesto según la reivindicación 1, seleccionado entre:

- (E)-2-(4-clorofenil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-3-il)-
etenosulfonamida,
- 5 6-cloro-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-3-il)naftalen-2-
sulfonamida,
5'-cloro-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-3-il)-2,2'-
bitiofen-5-sulfonamida,
N2-(((E)-2-(4-clorofenil)etenil)sulfonil)-N2-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-
10 2-oxopirrolidin-3-il)glicinamida,
N2-((5'-cloro-2,2'-bitien-5-il)sulfonil)-N2-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-
oxopirrolidin-3-il)glicinamida,
5'-cloro-N-(cianometil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-
3-il)-2,2'-bitiofen-5-sulfonamida,
- 15 N-((5'-cloro-2,2'-bitien-5-il)sulfonil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-
oxopirrolidin-3-il)glicinato de metilo,
5'-cloro-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-3-il)-N-(2--
oxobutil)-2,2'-bitiofen-5-sulfonamida,
N-((5'-cloro-2,2'-bitien-5-il)sulfonil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-
20 oxopirrolidin-3-il)glicina,
(E)-2-(4-clorofenil)-N-(cianometil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-
oxopirrolidin-3-il)etenosulfonamida,
(E)-2-(4-clorofenil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-3-il)-
N-(2-oxobutil)etenosulfonamida,
- 25 N-(((E)-2-(4-clorofenil)etenil)sulfonil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-
oxopirrolidin-3-il)glicinato de metilo,
6-cloro-N-(3-furilmetil)-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-
3-il)naftalen-2-sulfonamida,
formiato de 6-cloro-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-3-il)-
30 N-(piridin-3-ilmetil)naftalen-2-sulfonamida,
6-cloro-N-etil-N-((3S)-1-((1S)-1-metil-2-morfolin-4-il-2-oxoetil)-2-oxopirrolidin-3-
il)naftalen-2-sulfonamida,

198
197
V

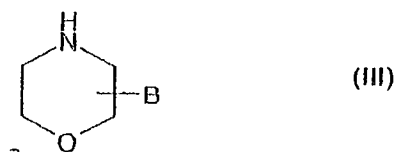
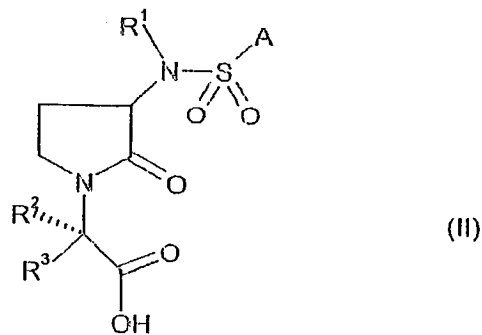
- 6-cloro-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il)-N-(2-oxobut-
til)naftalen-2-sulfonamida,
N-2-[(6-cloro-2-naftil)sulfonyl]-N-2-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-
oxopirrolidin-3-il)glicinamida,
- 5 6-cloro-N-(2-furilmetil)-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-
3-il)naftalen-2-sulfonamida,
6-cloro-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il)-N-(1,3--
tiazol-2-ilmetil)naftalen-2-sulfonamida,
N2-[(6-cloro-2-naftil)sulfonyl]-N2-((3S)-1-[(1S)-2-[(2R,6S)-2,6-dimetilmorfolin-4-il]-1-
10 metil-2-oxoetil]-2-oxopirrolidin-3-il)glicinamida,
6-cloro-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il)-N-[(2-metil-
1,3-tiazol-4-il)metil]naftalen-2-sulfonamida,
formiato de 6-cloro-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il)-
N-(piridin-2-ilmetil)naftalen-2-sulfonamida,
- 15 formiato de 6-cloro-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il)-
N-(piridin-4-ilmetil)naftalen-2-sulfonamida,
6-cloro-N-(cianometil)-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-
il)naftalen-2-sulfonamida,
6-cloro-N-metil-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3--
20 il)naftalen-2-sulfonamida,
6-cloro-N-(3,3-dimetil-2-oxobutil)-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-
oxopirrolidin-3-il)naftalen-2-sulfonamida,
N-alil-6-cloro-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3--
il)naftalen-2-sulfonamida,
- 25 N-[(6-cloro-2-naftil)sulfonyl]-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-
oxopirrolidin-3-il)glicinato de metilo,
N-[(6-cloro-2-naftil)sulfonyl]-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-
oxopirrolidin-3-il)glicinato de *tert*-butilo,
N-[(6-cloro-2-naftil)sulfonyl]-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2--
30 oxopirrolidin-3-il)glicina,
(E)-2-(5-clorotien-2-il)-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-
il)etenosulfonamida,
6-cloro-N-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il)-N-(2-
oxobutil)-1-benzotiofen-2-sulfonamida,
- 35 N2-[(6-cloro-1-benzotien-2-il)sulfonyl]-N2-((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-
2-oxopirrolidin-3-il)glicinamida,

198
~~198~~
12

- N2-[(6-cloro-2-naftil)sulfonyl]-N2-((3S)-1-[(1S)-2-[(2R,6S)-2,6-dimetilmorfolin-4-il]-1-metil-2-oxoetil]-2-oxopirrolidin-3-il)glicinamida,
formiato de 6-cloro-N-[(3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il]-N-(2-morfolin-4-iletel)naftalen-2-sulfonamida,
5 formiato de 6-cloro-N-[(3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il]-N-(2-pirrolidin-1-iletel)naftalen-2-sulfonamida,
formiato de 6-cloro-N-[2-(dimetilamino)etil]-N-[(3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il]naftalen-2-sulfonamida, y
N-[2-[(6-cloro-2-naftil)sulfonyl]((3S)-1-[(1S)-1-metil-2-morfolin-4-il-2-oxoetil]-2-oxopirrolidin-3-il)amino)etil]acetamida.

10. Una composición farmacéutica que comprende un compuesto según una cualquiera de las reivindicaciones 1-9, junto con un vehículo y/o excipiente farmacéutico.

11. Un procedimiento para preparar un compuesto de la fórmula (I) que comprende:
15 (a) hacer reaccionar un compuesto de la fórmula (II) con un compuesto de la fórmula (III):

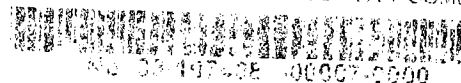


SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 16,727
FECHA : FEBRERO 26 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



NO. 08-107408-000007-0000
SE. SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
CALLE 14 N. 14-100, BOGOTÁ, D.C.
ART. 444 PARR. 2º, 3º Y 4º DEL CODIGO DE COMERCIO

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
OLARTE RAISBECK	CONSIGNACION	BANCO DE BOGOTA	062754387	1483223	26/02/2008	4,404,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
12		MARCAS Y LEMAS COMERCIALES	103,000.00
		TOTAL :	\$ 103,000.00

SON: CIENTO TRES MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

Factor Xa Inhibitors: S1 Binding Interactions of a Series of *N*-{[(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamides†

Chuen Chan,*‡ Alan D. Borthwick,‡ David Brown,§ Cynthia L. Burns-Kurtis,|| Matthew Campbell,‡ Laiq Chaudry,‡ Chun-wa Chung,‡ Máire A. Convery,‡ J. Nicole Hamblin,‡ Lisa Johnstone,‡ Henry A. Kelly,‡ Savvas Kleanthous,‡ Angela Patikis,‡ Champa Patel,‡ Anthony J. Pateman,‡ Stefan Senger,‡ Gita P. Shah,‡ John R. Toomey,|| Nigel S. Watson,‡ Helen E. Weston,‡ Caroline Whitworth,‡ Robert J. Young,‡ and Ping Zhou*

CVU UK Medicinal Chemistry Department, Screening and Compound Profiling UK, Assay Development UK, Computational and Structural Chemistry, CVU UK DMPK Department, GlaxoSmithKline Research and Development, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, United Kingdom, and Vascular Inflammatory Diseases, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, Pennsylvania 19406

Received July 24, 2006

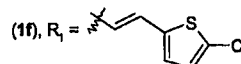
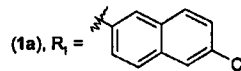
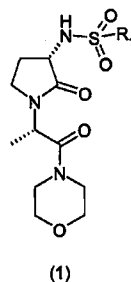
Factor Xa inhibitory activities for a series of *N*-{[(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamides with different *P*1 groups are described. These data provide insight into binding interactions within the S1 primary specificity pocket; rationales are presented for the derived SAR on the basis of electronic interactions through crystal structures of fXa–ligand complexes and molecular modeling studies. A good correlation between in vitro anticoagulant activities with lipophilicity and the extent of human serum albumin binding is observed within this series of potent fXa inhibitors. Pharmacokinetic profiles in rat and dog, together with selectivity over other trypsin-like serine proteases, identified 1f as a candidate for further evaluation.

Introduction

Thrombosis is a multifactorial disorder initiated by intravascular blood clotting leading to thrombus formation in an artery or vein. The resultant thrombi can be obstructive or dislodged into smaller blood vessels, depriving vital organs of oxygen (ischemia), which can be catastrophic. Manifestation of these life threatening conditions includes myocardial infarction, pulmonary embolism, and stroke. Drugs that inhibit the blood clotting process can prevent these events, and the development of antithrombotics is a major focus of pharmaceutical research.¹ A number of antithrombotics such as aspirin, heparin, low molecular weight heparins,² and warfarin³ are used clinically, but these drugs suffer from limited efficacy, low oral bioavailability, or a narrow therapeutic window. Indeed, administration of warfarin, a vitamin K epoxide reductase inhibitor, requires individual treatment with continuous dose adjustment and laboratory monitoring because of its interactions with other drugs and dietary vitamin K. Furthermore, warfarin has a slow onset of action.

A key driver of thrombus formation is the dysregulation of blood coagulation, which consists of the interconnected intrinsic and extrinsic pathways⁴ that converge onto the formation of activated factor X (fXa). When bound to factor Va, calcium, phospholipid, and prothrombin (prothrombinase complex), fXa converts prothrombin into thrombin which in turn proteolytically converts fibrinogen into fibrin. Ultimately, fibrin chains are cross-linked into an insoluble matrix (the blood clot). Thus, thrombin and fXa are attractive targets⁵ for pharmacological

intervention. Indeed, fondaparinux,⁶ a selective indirect fXa inhibitor suitable for subcutaneous administration, has been approved for the prevention of venous thromboembolism in patients undergoing major orthopaedic (hip or knee replacement) surgery. Furthermore, direct thrombin and fXa inhibitors have also shown encouraging results for the prevention of thromboembolic events in clinical studies.^{7a–d} Ximelagatran, the first registered, orally active thrombin inhibitor, was approved in Europe for the prevention of thrombosis in patients undergoing hip or knee replacement, but was withdrawn from the market due to concerns over toxicity in the liver.^{7e} Currently a number of orally active thrombin [e.g., MCC-977, dabigatran etexilate]⁸ and fXa [e.g., rivaroxaban, LY-517717, apixaban, and DU-176b]^{9a–b} inhibitors are being investigated in clinical trials for similar conditions. In addition, DX-9065a is currently in clinical trials for parenteral indications.^{9h–i} As part of our program to develop efficacious and orally bioavailable anticoagulants, we have identified highly selective and potent thrombin¹⁰ and fXa inhibitors with good anticoagulant activities and promising profiles for chronic oral administration. Factor Xa inhibitors containing the 3-aminopyrrolidin-2-one scaffold and highly basic benzamidine *P*1 substituents have been disclosed previously.^{11a} Our own studies^{11b} with this scaffold have led to a nonbasic series of novel and orally active fXa inhibitors incorporating 6-chloro-*N*-[(3*S*)-1-substituted-2-oxo-3-pyrrolidinyl]-2-naphthalenesulfonamides and identified 1a as our lead compound. The



† PDB ID codes for 1a, 1b, 1d, 1e, and 1x complexed with factor Xa are 2cjl, 2j95, 2j38, 2j34, and 2j94, respectively.

* To whom correspondence should be addressed. Tel.: (+44) 1438 768445. Fax: (+44) 1438 764869. E-mail: chuen.8.chan@gsk.com.

‡ CVU UK Medicinal Chemistry Department.

§ Screening and Compound Profiling UK.

|| Vascular Inflammatory Diseases.

‡ Assay Development UK.

‡ Computational and Structural Chemistry.

© CVU UK DMPK Department.

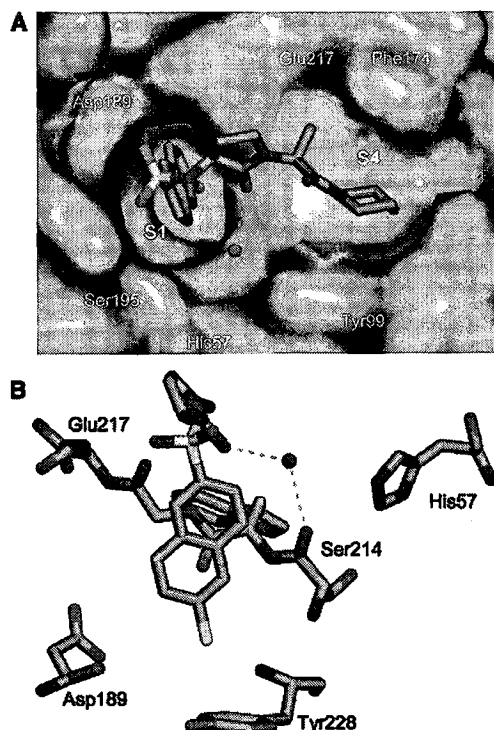


Figure 1. Crystal structure of **1a** bound to fXa showing a water-mediated hydrogen bonding interaction of pyrrolidin-2-one carbonyl to Ser214 carbonyl group. (A) Solvent accessible surface of fXa binding site depicting the 6-chloronaphthyl group binding into the S1 pocket and the alanyl morpholinamide into the S4 pocket while the methyl group binds into a subpocket within S4. (B) Alternative view without the solvent accessible surface showing the bound conformation of 6-chloronaphth-2-ylsulfonamido group in the S1 pocket and the key amino acid residues.

crystal structure of **1a** bound to fXa (Figure 1) clearly showed that the 6-chloronaphth-2-yl group bound in the S1 primary specificity pocket and the morpholino amide occupies the aromatic box (Tyr99, Try215, and Phe174) of the S4 pocket. The pendent methyl group of the alanyl motif binds to a small subpocket formed by Phe174 and the carbon atoms of the side-chain of Glu217. Herein, we extend our study and describe a systematic investigation into the effects of sulfonamido R_1 substituents on fXa inhibitory activities and anticoagulant activities within the N -{[(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]}sulfonamide series. Our understanding of the tolerance of sulfonamido groups within the S1 pocket through X-ray crystallography and molecular modeling studies is compared and contrasted with those reported in the literature for alternative series of fXa inhibitors. Correlation of anticoagulant activities with lipophilicity and the extent of human serum albumin (HSA) binding are highlighted together with pharmacokinetic data that led to the identification of **1f** as a candidate for further evaluation.

Chemistry

The key intermediate amine **2** was readily prepared from N -CBZ-L-methionine (Scheme 1) by coupling with L-alanine *t*-butyl ester under standard conditions using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N -hydroxybenzotriazole (HOBt) in the presence of triethylamine to give as an intermediate the protected dipeptide **3**. Activation of the methylthioether moiety of **3** with iodomethane, followed

by base-induced cyclization¹² with the hydroxide form of Dowex ion-exchange resin, established the pyrrolidin-2-one system, which was deprotected with TFA in DCM to provide the carboxylic acid **4**. Amide coupling with morpholine using O -(benzotriazol-1-yl)- N,N,N' -tetramethyluronium tetrafluoroborate (TBTU) and i -Pr₂NEt, followed by deprotection under palladium-catalyzed hydrogenolysis, thereby yielded the key amine intermediate **2**.

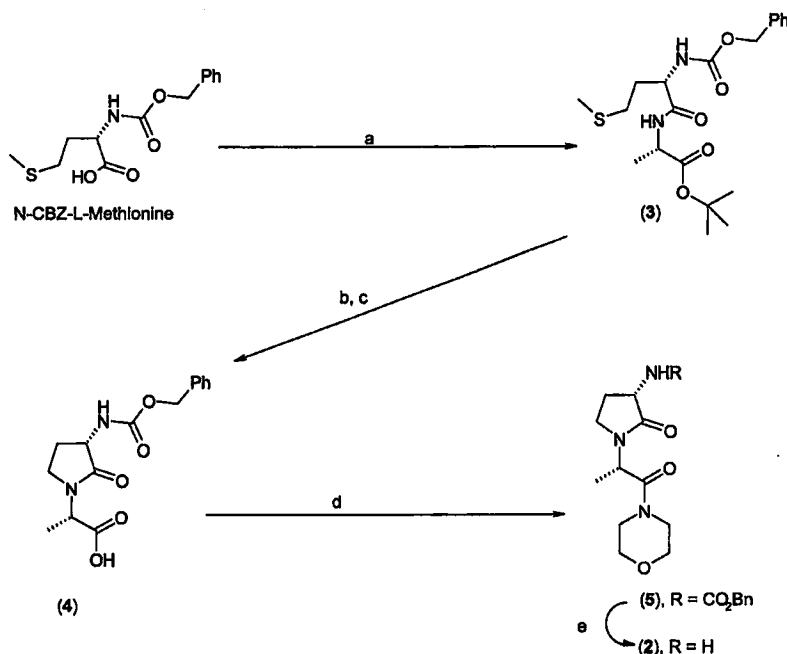
The secondary sulfonamides **1a–1h**, **1j–1p**, and **1s–1y** were prepared by sulfonylation of the (3*S*)-aminopyrrolidin-2-one alanylamide **2** with appropriate sulfonyl chlorides, R_1 SO₂Cl, **6**, or suitably protected derivatives thereof under standard conditions (see Method A in General Procedures). R_1 s are defined in Table 1. When Boc was used as a protecting group, its removal was effected with TFA in DCM (for **1s** and **1t**). With the exception of **6h**, **6n**, and **6u–6y**, all other sulfonyl chlorides were either available commercially (**6m**) or prepared according to literature procedures (**6a–6g**,^{13,14} **6j–6l**,^{14,15} **6o**,¹⁶ **6p**,¹⁶ N-Boc of **6s**,¹⁷ and N-Boc of **6t**¹⁷). Sulfonyl chlorides **6h**, **6n**, and **6u–6w** were synthesized from the corresponding chloroheteroaromatics by direct lithiation with n -BuLi at -78 °C, followed by sulfinylation with SO₂ and chlorinative oxidation with NCS (Scheme 2). In the case of **6y**, lithiation was carried out by metal–halogen exchange of 5-bromo-2-(5-chloro-2-thienyl)pyridine¹⁸ with t -BuLi, followed by sulfinylation and chlorinative oxidation as above to provide the requisite sulfonyl chloride. The chloro-bis(heteroaryls) were prepared by palladium-catalyzed coupling reactions. Thus, 5-(5-chloro-2-thienyl)-1,3-thiazole, 2-(5-chloro-2-thienyl)-1,3-thiazole, and 5-chloro-2-(2-thienyl)-1,3-thiazole were prepared by Suzuki coupling of either 2- or 5-bromothiazole^{19a} with 5-chlorothiophene-2-boronic acid or 2-bromo-5-chlorothiazole^{19b} with thiophene-2-boronic acid. 2-(5-Chloro-2-thienyl)furan was synthesized via Stille coupling of the tri- n -butyl-2-(furan)stannane with 2-bromo-5-chlorothiophene. 3-(5-Chloro-2-thienyl)-1*H*-1,2,4-triazole-5-sulfonyl chloride **6x** was prepared by the reaction of thiourea with 5-chloro-2-thiophenecarbohydrazide²⁰ at 160 °C in the absence of solvent, followed by careful oxidation of the resultant mercaptotriazolyl derivative with chlorine in AcOH (Scheme 3). The sulfonyl chlorides described above were generally used for sulfonylation with **2** without purification.

Due to complications in the synthesis of **6q**,²¹ an alternative and indirect synthesis for **1q** and **1r** was employed. Thus, 5-chlorination of the appropriate chloro-1,3-benzothiazole-2(3*H*)-thione²² using NCS, followed by sulfonylation with **2** and oxidation with KMnO₄, provided the 5- and 6-chloro-1,3-benzothiazole-2-sulfonamido derivatives **1q** and **1r** (Scheme 4).

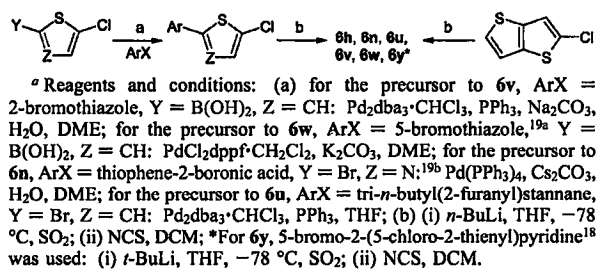
Compound **1i** was synthesized via the sulfonylation of **2** with 4-iodophenylsulfonyl chloride to give **7**. Sonagashira coupling with trimethylsilylacetylene in the presence of Et₃N and catalytic amounts of CuI and PdCl₂(PPh₃)₂ in DMF at room temperature, followed by the removal of the trimethylsilyl group with TBAF yielded the acetylenic compound **8**. Hydroborative chlorination²³ with t -hexylborane and cupric chloride afforded the required **1i** (Scheme 5).

Results and Discussion

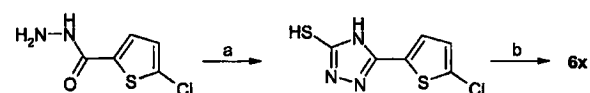
The influence of sulfonamido R_1 substituents on fXa inhibitory activities has been investigated within a series of N -{[(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]}sulfonamides (Table 1). 6-Chloronaphthyl, 5'-chloro-2,2'-bithienyl, 4-chlorostyryl, 5- and 6-chlorobenzothienyl, and 5-chlorothiophenylethynyl analogues **1a–1f** show good to excellent fXa inhibitory activities (K_i = 4–47 nM). Related findings for

Scheme 1^a

^a Reagents and conditions: (a) L-alanine *t*-butyl ester, HOBT, Et₃N, EDC; (b) MeI, acetone, then Dowex (HO⁻ form) ion-exchange resin; (c) TFA, DCM; (d) morpholine, *i*-Pr₂NEt, TBTU; (e) 10% Pd-C, H₂, EtOH.

Scheme 2^a

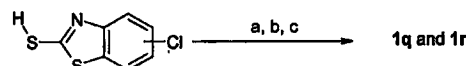
^a Reagents and conditions: (a) for the precursor to 6v, ArX = 2-bromothiazole, Y = B(OH)₂, Z = CH; Pd₂dba₃·CHCl₃, PPh₃, Na₂CO₃, H₂O, DME; for the precursor to 6w, ArX = 5-bromothiazole,^{19a} Y = B(OH)₂, Z = CH; PdCl₂dppf·CH₂Cl₂, K₂CO₃, DME; for the precursor to 6u, ArX = thiophene-2-boronic acid, Y = Br, Z = N;^{19b} Pd(PPh₃)₄, Cs₂CO₃, H₂O, DME; for the precursor to 6n, ArX = tri-*n*-butyl(2-furyl)stannane, Y = Br, Z = CH; Pd₂dba₃·CHCl₃, PPh₃, THF; (b) (i) *n*-BuLi, THF, -78 °C, SO₂; (ii) NCS, DCM; *For 6y, 5-bromo-2-(5-chloro-2-thienyl)pyridine¹⁸ was used: (i) *t*-BuLi, THF, -78 °C, SO₂; (ii) NCS, DCM.

Scheme 3^a

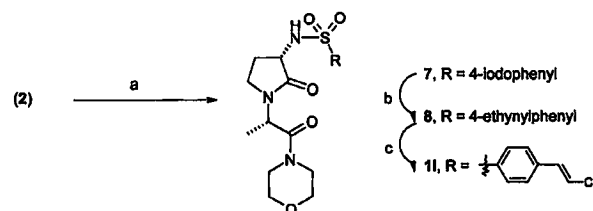
^a Reagents and conditions: (a) thiourea, 160 °C; (b) Cl₂, AcOH, 10 °C.

these and other chloroheteroaryl P1s have been reported^{13c,24,25} with other structural classes of fXa inhibitors. Basic P1 groups such as 3- and 4-substituted benzamides in first generation fXa inhibitors²⁶ have been reported to interact with Asp189 in the S1 pocket. In contrast, 1a–1f are nonbasic and, at the outset of our program, before X-ray crystal structures of our compounds bound to fXa were available, their binding orientation was uncertain. Evidence that chloroaromatic groups could bind in the S1 pocket was obtained by elegant ¹⁹F NMR studies of a 6-fluoronaphthyl containing inhibitor bound to fXa with and without Gd-EDTA^a complex as well as competitive displacement studies with a 6-chloronaphthyl containing fXa inhibitor.²⁷ Confirmation of a similar binding mode in the present series was established by X-ray crystal structures of these compounds

^a Abbreviations: Gd-EDTA, gadolinium–ethylenediaminetetraacetic acid complexes; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; Mes, 2-morpholinoethanesulfonic acid; PEG, polyethylene glycol; SD, standard deviation.

Scheme 4^a

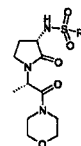
^a Reagents and conditions: (a) NCS, DCM; (b) pyridine, 2, MeCN; (c) KMnO₄.

Scheme 5^a

^a Reagents and conditions: (a) 4-iodophenylsulfonyl chloride, pyridine, MeCN; (b) (i) trimethylsilylacetylene, CuI, PdCl₂(PPh₃)₂, Et₃N, DMF; (ii) *n*-Bu₄NF, THF; (c) *t*-hexylborane, CuCl₂.²³

complexed with fXa (Figures 1–3), which showed clearly that the chloroaromatics in 1a, 1b, 1d, and 1e bound within the S1 pocket. The morpholino group occupied the S4 pocket with the alanyl methyl group bound to a small hydrophobic pocket near to S4. In contrast to a related series²⁸ in which 3*R* stereochemistry is preferred and the pyrrolidin-2-one C=O is hydrogen-bonded to Gly219,²⁸ the corresponding carbonyl in our series is indirectly hydrogen-bonded to the carbonyl of Ser214 via a water-mediated interaction. These interactions are similar to that reported for 1a (Figure 1) in our previous communication.^{11b} In contrast to the flat and rigid chlorobenzothienyl and chloronaphthyl P1s present in 1d, 1e, and 1a, respectively, the two thienyl rings in 1b are out of coplanarity (S–C–C–S torsion ca. 159°), which is in good agreement with that reported for the lowest energy conformer of bithiophene.²⁹ Our crystal structures show the chlorine atom is 3.7–4.2 Å from the centroid

Table 1. Affinities of 1a–1y in the Fluorogenic Factor Xa Assay



Compound	R ₁	K _i /nM	SD ^a
1a		6	2
1b		4	2
1c		11	5
1d		47	5
1e		15	3
1f		4	1
1g		109	9
1h		165	35
1i		314	119
1j		163	52
1k		2898	1404
1l		3670	1068
1m		> 15800	-
1n		3225	575
1o		285	14
1p		782	208
1q		4160	1710
1r		112	7
1s		90	20
1t		170	10
1u		1200	80
1v		5	4
1w		2	1
1x		534	80
1y		24	4

^a SD values are derived from at least n ≥ 2.

of the phenolic ring of Tyr228 in the S1 pocket. This interaction has been reported in the literature as hydrophobic for other series of fXa inhibitors.²⁴ However, we believe there is an electrostatic component to this interaction based on electrostatic potential maps for these chloroaromatics, which showed a slight positive charge at the center of the Cl atom.³⁰ Indeed, support for this

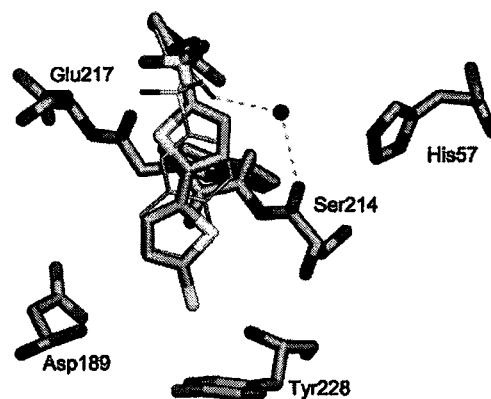


Figure 2. Crystal structure of 1b bound to fXa showing the conformation of bithienyl P1 (thick gray stick) with the 6-chloronaphth-2-yl P1 of 1a (thin gray stick) as comparison.

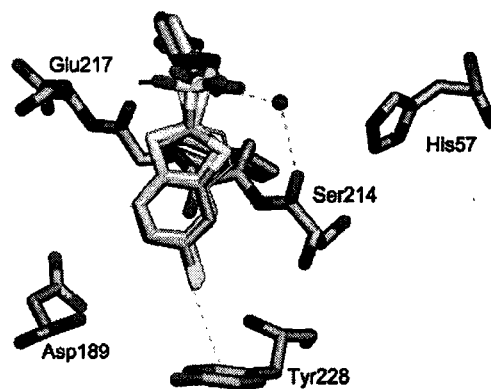


Figure 3. Crystal structures of 1d (thick wheat stick) and 1e (thick gray stick) bound to fXa showing the bound conformations of chlorobenzothienyl P1s. 6-Chloronaphth-2-yl P1 of 1a is shown (thin gray stick) as reference.

hypothesis arose by replacing the Cl atom with the isosteric methyl group, which resulted in over 10-fold loss in activity [1g vs 1a]. Furthermore, polarization of the aromatic proton facing Asp189 by the adjacent chlorine atom could provide an additional interaction (nonclassical hydrogen bonding),³¹ which could augment the binding affinity of chloroaromatics. However, this latter interaction is unlikely to be present in 1h and 1i, the thieno[3,2-b]thiophene analogue and the reversed analogue of 1c, respectively. In the bioactive conformation of 1h, the sulfur atom in the chlorothieno moiety is expected to occupy the equivalent volume of space that faces Asp189 and, therefore, cannot attain the nonclassical hydrogen bonding interaction mentioned above. When bound to fXa, the proton attached to the same carbon as the Cl atom in the chlorovinyl moiety of 1i is unlikely to align directly with the carboxylate of Asp189 and is also over 4 Å away to contribute any significant binding affinity. These considerations could provide an explanation for the moderate potency of 1h and 1i.

Replacement of the aromatic carbon atom by nitrogen in the chloroaryl ring is generally detrimental to their fXa inhibitory activities (1j–1n), which may be explained following close analysis of X-ray crystal structures of 1b, 1d, and 1e (Figures 2 and 3) bound to fXa. Analogous to 1a, the chloroaromatics in 1b, 1d, and 1e are bound to the S1 pocket and display a similar vector for the Cl–Tyr228 interaction. Assuming all chloroheteroaryl analogues maintain this highly favorable Cl–Tyr228 interaction, it is possible to predict the position of the

Table 2. Anticoagulant Activities and Human Serum Albumin Binding of Potent Factor Xa Inhibitors

compd	K _i (nM)	1.5XPT (μM)	SD	n	logD @ pH 7.4	HSA binding %
1a	6	5.4	0.46	3	1.79	79
1b	4	10.4	0.08	2	2.03	95
1c	11	2.7	0.5	2	1.49	63
1d	47	13.1	0.34	2	2.07	85
1e	15	7.5	0.9	5	1.85	88
1f	4	1.2	0.04	3	1.47	59
1v	5	24.7	1.4	2	2.18	93
1w	2	49.2	4	2	2.1	95
1y	24	27.2	1.3	2	2.24	89

Table 3. Selectivity Profiles of 1a–1f against Other Trypsin-Like Serine Proteases^a

compd	Thr ^b	TF/ fVIIa ^b	fXIIa ^b	aPC ^b	Plas ^b	Tryp ^b	Kall ^b
1a	37	>4500	>4500	>1000	>1000	>1000	>300
1b	298	>12 000	>12 000	>25 000	>7000	>2000	>1500
1c	19	>3500	>3500	>1000	>700	>700	>1300
1d	10 ^c	>350 ^{c,d}	nd ^e	>350 ^{c,d}	>750 ^c	>500 ^c	nd ^e
1e	22	nd ^e	nd ^e	>3000 ^c	>1500 ^c	>1000 ^c	nd ^e
1f	90	>30 000	>20 000	>4000	>20 000	>20 000	>1500

^a Unless otherwise stated, fluorogenic assays were performed on these compounds. ^b Ratio of K_i values (nM) to fXa. TF/fVIIa = tissue factor – factor VIIa; aPC = activated protein C; Plas = plasmin; Tryp = trypsin; and Kall = kallikrein. ^c These ratios were derived from chromogenic assays. ^d These ratios are based on IC₅₀ values. ^e nd = not determined.

nitrogen in the chloroheteroaromatic rings of compounds 1j–1n. The lone pair of electrons on the nitrogen atom in the ring of 1k–1n is placed in a highly electron-rich environment formed by the backbone carbonyl of Gly219 and the carboxylate of Asp189. Such a highly unfavorable interaction is reflected in their poor intrinsic activities. In contrast, 1j is moderately potent (K_i = 163 nM) and is predicted to bind with the nitrogen facing the other side of the S1 pocket, which is likely less electron rich and, therefore, a more favorable environment.

Modifications to the aromatic ring distal to the chlorine atom (i.e., directly attached to SO₂ of the sulfonamido group) are shown in 1o–1y. In the 5,6-fused aromatic systems, 1o–1t are moderate to weakly active. Interestingly, within each isomeric pair (5- and 6-chloro isomers) of benzofurans and benzothiazoles, one member (1p, 1q) is significantly less active than the other (1o, 1r). However, both chloroindolyl analogues (1s and 1t) have similarly moderate activities. Ab initio calculations for the conformational preference of the 5-membered ring heteroaryl-2-sulfonamides, as model systems for 1o–1t, showed³² that one of the S=O bonds prefers to be coplanar with the C–H, planar N–H, or S in these aromatic systems. When this conformational preference is translated into the 5,6-fused chlorobenzoheteroaryl systems and the important Cl–Tyr228 interaction is maintained, there are significant energy penalties for the bioactive conformations of 1p and 1q. Hence, 5-benzofuryl (1p) and 6-chlorobenzothiazolyl (1q) analogues are less potent than their corresponding isomers 1o and 1r. The similar activities of 5- and 6-chloroindolyl isomers (1s and 1t) could also be explained by the little difference in energy between the conformers of the sulfonamido S=O that eclipse the C–H or N–H of the pyrrole ring in 1H-pyrrole-2-sulfonamide.

The thienylsulfonamido heteroaromatic ring in the chlorobithienyl P1 analogue 1b is positioned further away from the bottom of the S1 pocket than the ring distal to the chlorine atom in the benzothienyl analogues (vide supra: 1b in Figure 2 vs 1d and 1e in Figure 3). Therefore, this thienyl ring is likely to be further away from potential unfavorable interactions in the lower half of the S1 binding pocket (cf. rationales for 1k–1n

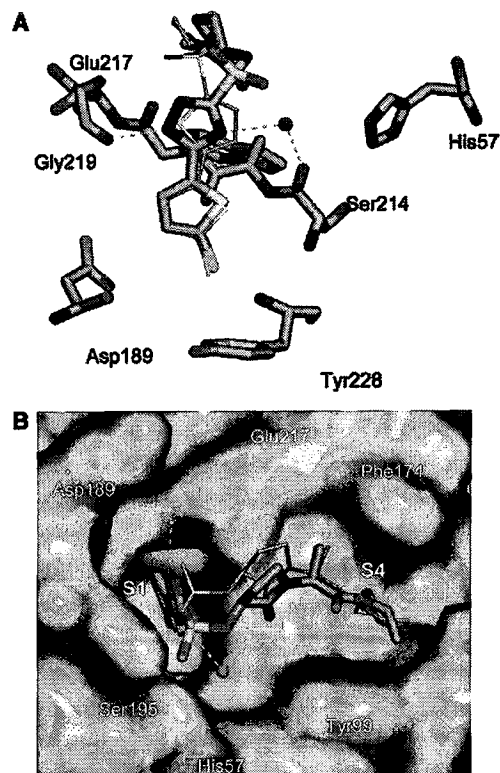


Figure 4. Crystal structure of 1x (thick stick) in fXa binding site. Compound 1b (thin stick) is shown for comparison. (A) P1 orientation of 1x showing the perturbed conformation of the triazolyl moiety relative to the bithienyl P1 in 1b. (B) S4 orientation in solvent accessible surface showing major changes in the bound conformation of the 3-sulfonamidopyrrolidin-2-one moiety in 1x relative to that in 1b.

Table 4. Pharmacokinetic Parameters of 1f in Male Rats and Dogs Following Intravenous and Oral Administration at Nominal Doses of 1 mg/kg i.v. and 2.5 mg/kg p.o.

species	t _{1/2} ^a (h)	Clp ^b (mL/min/kg)	V _{ss} ^c (L/kg)	F ^d (%)
Spague Dawley rats	0.7 ± 0.1	8.0 ± 1.7	0.29 ± 0.02	75 ± 23
Beagle dogs	1.2 ± 0.3	4.6 ± 1.7	0.42 ± 0.03	53 ± 9

^a t_{1/2} = half-life of the test compound expressed in hours. ^b Clp = plasma clearance of the test compound expressed mL/min/kg. ^c V_{ss} = steady-state volume of distribution of test compound expressed as L/kg. ^d F = oral bioavailability of test compound expressed as percentage.

above), and replacement of aromatic carbons by nitrogen are expected to be better tolerated. Furthermore, the rotatable bond between the two thienyl rings in 1b can be twisted to alleviate any possible unfavorable interactions. Indeed both the thiazolyl analogues (1v and 1w) retain potent fXa inhibitory activities (K_i = 5 and 2 nM, respectively) in contrast to the benzothiazolyl analogues (1q and 1r, K_i = 4160 and 112 nM, respectively). Even replacing the thiazolyl ring by a 6-membered pyridyl ring (1y) retains good potency (K_i = 24 nM). However, both the furyl (1u) and triazolyl (1x) analogues are significantly less potent. The weak fXa inhibitory activity of 1u may be rationalized by the preferred conformation of the P1 sulfonamido group in which one of the S=O bonds eclipses the CH of the furan ring (vide supra) while maintaining the highly favorable Cl–Tyr228 interaction.³² This would position the furyl oxygen atom on the same side as the sulfur atom of the chlorothienyl unit and could change to a less favorable bioactive conformation of 1u relative to 1b. A comparison of the crystal structures of

Table 5. Crystallographic and Refinement Data

compound	1a ^a	1b ^a	1d ^a	1e ^a	1x ^b
RCSB code	2cji	2j95	2j38	2j34	2j94
resolution	2.1	2	2.1	2	2.1
cell dims	56.968	56.914	56.794	56.867	56.739
	72.633	72.731	72.731	73.147	72.563
	79.784	79.901	79.895	80.125	78.924
resolution	2.1	2	2.1	2	2.1
(high shell)	(2.1–2.18)	(2.0–2.07)	(2.1–2.18)	(2.0–2.07)	(2.1–2.21)
Rmerge	0.077	0.050	0.068	0.070	0.109
(high shell)	(0.531)	(0.381)	(0.288)	(0.486)	(0.431)
unique reflection	19 989	22 752	18 209	21 497	18 402
completeness	99	99.5	94.1	94.2	94
multiplicity	4.0	4.0	3.8	3.8	3.8
Rfactor	0.192	0.193	0.211	0.178	0.181
Rfree	0.242	0.242	0.302	0.220	0.234
rms bond	0.016	0.017	0.016	0.017	0.015
rms angle	1.52	1.571	1.697	1.513	1.588
total no. atoms	2397	2443	2485	2462	2441
avg B	31.259	36.95	24.28	24.837	34.27
avg B for catalytic domain	31.128	32.338	24.018	24.57	33.778
avg B for inhibitor	37.337	40.723	31.682	31.588	44.206
avg B for solvent	32.524	36.117	26.585	28.143	39.735

^a The X-ray intensity data was collected at the Synchrotron Radiation Source (Daresbury, UK) on station 9.6. ^b The X-ray intensity data was collected at the European Synchrotron Radiation Facility (Grenoble, France) on beamline ID29.

the triazolyl analogue **1x** with **1b** (Figure 4) in fXa revealed such a distortion, which could account for the weaker intrinsic activity of **1x**. While the S atom of the thiophene ring proximal to the sulfonamido group in **1b** is orientated toward Gly219, the N atom at the 1 position of the 1,3,4-triazolyl group in **1x** appears to be indirectly hydrogen-bonded to Ser214 via a water molecule. This distortion is amplified in the binding of the pyrrolidin-2-one and the morpholinoalanylamide in which the previously observed water-mediated interaction of the pyrrolidin-2-one C=O with Ser214 is now absent in **1x**.

The anticoagulant activities of compounds with fXa K_i values ≤ 47 nM (**1a–1f**, **1v**, **1w**, and **1y**) together with their logD values, determined at pH 7.4, and binding to immobilized human serum albumin (HSA) in a high throughput HPLC assay³³ are shown in Table 2. These compounds showed a broad range of anticoagulant activities (1.5xPT 1.2–49.2 μ M), which did not correlate with their intrinsic fXa affinities. Indeed the fall off in plasma-based activities relative to their fXa inhibitory activities ranged from 2 to 4 orders of magnitude. The biggest fall off in 1.5xPT values was observed with the bithienyl and related bi(heteroaromatic) P1s (**1b**, **1v** and **1w**). In particular, there is over 20 000-fold difference between fXa affinity and anticoagulant activity for **1w**. These more lipophilic analogues (logD 2.03–2.18 at pH 7.4) showed high protein binding (>90% bound to HSA), which could account for their weaker than expected anticoagulant activities (1.5xPT 10.4–49.2 μ M). Indeed there is a good correlation of the degree of HSA binding with lipophilicity (logD at pH 7.4) for this series of compounds. Thus, good anticoagulant activities (1.5xPT 1.2–7.5 μ M) were observed for compounds (**1a**, **1c**, **1e**, and **1f**) that are more hydrophilic (logD 1.47–1.85 at pH 7.4) and showed <90% HSA binding, while having similar potent fXa inhibitory activities (K_i = 5–16 nM).

Selected compounds (**1a–1f**) with good anticoagulant activities were evaluated against a panel of other trypsin-like serine proteases (Table 3). These compounds have excellent selectivity over trypsin, kallikrein, and the fibrinolytic enzyme plasmin. Against other proteases in the coagulation process, thrombin activities were weaker with good selectivities seen for **1a**, **1b**, and **1f**, while no significant activities against activated protein C, TF/factor VIIa, and factor XIIIa were detected.

The pharmacokinetic profiles of compounds with good anticoagulant activities were initially ranked using either one to two animals or a cassette dosing regiment with four animals. Compounds **1a–1f** showed promising pharmacokinetic properties (plasma clearance 3–18 mL/min/kg, volumes of distribution 0.17–0.6 L/kg, half-lives 0.57–1.5 h) and high oral bioavailabilities (69–100%) in rat. Definitive studies ($n = 3$) in both rat and dog were then carried out with **1f**, which showed encouraging pharmacokinetic profiles with high oral bioavailabilities (Table 4). Based on a combination of attractive in vitro and pharmacokinetic profiles, **1f** was selected as a candidate for further evaluation. Its antithrombotic profile and further pharmacological evaluation will be reported separately.³⁴

Conclusions

Systematic modifications to a series of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}-sulfonamides have resulted in a number of highly potent fXa inhibitors (**1a–1f**, **1s**, **1v**, **1w**, and **1y**). X-ray crystallography of selected ligands in fXa and molecular modeling studies has provided rationales for the derived SAR. The importance of both electrostatic and steric interactions of the chlorine atom to the phenolic centroid of Tyr228 has been highlighted. Modifications to the chloroaromatic ring through the replacement of C–H by nitrogen atom are generally detrimental due to the presence of polar groups (e.g., carboxylate of Asp189 and carbonyl backbone of Gly219) which form one side of the S1 pocket. However, there is a greater scope to modify the aromatic ring attached to the sulfonamido group, which is also influenced by the torsional preference of the NSO₂–heteroaryl bond. Relative to their fXa affinities, good anticoagulant activities have been identified for the more hydrophilic analogues that show <90% binding to HSA. Against a panel of other trypsin-like serine proteases, these compounds showed excellent selectivities against trypsin, kallikrein, and the fibrinolytic enzyme, plasmin. With respect to other proteases in the coagulation process, analogues with good selectivities against thrombin and no significant activities against activated protein C, TF/factor VIIa, and factor XIIIa have been identified. Together with the good pharmacokinetic profile in rats and dogs, **1f** was selected as a candidate for further evaluation.

Experimental Section

General Procedures. All general purpose solvents used were Fisons analytical reagents. Anhydrous solvents (Sureseal) were purchased from Aldrich and Fluka. All other reagents were usually obtained from Aldrich, Fluka, or Lancaster. All reactions involving organometallic reagents were carried out under nitrogen atmosphere. Reactions were monitored either by LC-MS or TLC using Merck 60 F₂₅₄ silica gel glass backed plates (5 × 10 cm), eluted with suitable solvent mixtures and visualized by UV light, followed by heating with aqueous phosphomolybdic acid. LC-MS were run on a Hewlett-Packard 1050 coupled with a Micromass Platform Series II or Waters ZQ [using electrospray (ES) positive or negative ionization modes] equipped with a Supelcosil ABZ+PLUS column (3 μm, 3.3 cm × 4.6 mm ID). Standard conditions were eluent systems A (H₂O, 0.1% formic acid, 10 mmol ammonium acetate) and B (95% MeCN and 0.05% formic acid in H₂O): gradient 100% A 0.7 min, 100% A–100% B 4.2 min, 100% B 1.1 min, 100–0% B 0.2 min; flow rate = 3 mL/min. Purifications were performed on silica SPE (solid-phase extraction) eluted under suction or using an OPTIX-10 system, Biotage chromatography using prepacked Kieselgel columns, preparative hplc or mass-directed autoprep. SPE refers to the use of cartridges sold by International Sorbent Technology, Ltd. OPTIX-10 refers to the ISCO OPTIX 10 automated chromatography system. This uses pumps to give gradient control of solvents and variable wavelength UV detection and trigger for fraction collection (λ = 254 nm, unless specified). Biotage chromatography refers to purification carried out using equipment sold by Dyax Corporation (either the Flash 40i or Flash 150i) and cartridges prepacked with KPSil. Preparative HPLC refers to methods where the material was purified by high performance liquid chromatography on a Supelcosil ABZ+ PLUS column (5 μm, 10 cm × 21.2 mm i.d.) with 0.1% HCO₂H in water (A) and MeCN (0.5% HCO₂H; B) utilizing the generic gradient elution conditions expressed as "x to y" gradient with a gradient system as follows: 0–1.45 min x %B, 1.45–20 min x to y %B, 20–24 min y to 95% B, 24–30 min 95% B, 32–34 min 95 to x %B at a flow rate of 8 mL/min. The Gilson 233 fraction collector was triggered by UV (254 nm unless specified). Mass directed autoprep refers to methods where the material was purified by high performance liquid chromatography on a HPLCABZ+ 5 μm column (5 cm × 10 mm i.d.) with 0.1% HCO₂H in water and 95% MeCN, 5% water (0.5% HCO₂H) utilizing the following gradient elution conditions: 0–1.0 min 5% B, 1.0–8.0 min 5 to 30% B, 8.0–8.9 min 30% B, 8.9–9.0 min 30–95% B, 9.0–9.9 min 95% B, 9.9–10 min 95–0% B at a flow rate of 8 mL/min (System 2). The Gilson 202-fraction collector was triggered by a Micromass ZMD Mass Spectrometer on detecting the mass of interest. Where appropriate, test compounds were isolated from their aqueous solutions, if necessary, in the presence of 1,4-dioxan, by freeze-drying using Edwards Freeze Dryer Modulyo. GC-MS was performed on a HP5973MSD with CTC Combipal injector, using 1/100 split injection and chemical ionization with ammonia or methane as collision gas, equipped with HP5 5% phenyl methyl siloxane column (30 m × 0.25 mm × 0.25 mm). Standard GC condition was 80–320 °C at 50 °C/min over 10 min using helium as carrier gas at 1.5 mL/min. Mass spectra were run by an electrospray Hewlett-Packard 5989B instrument. All NMR spectra were run on a Bruker DPX 400 MHz instrument in an appropriate deuterated solvent using tetramethylsilane or residual hydrogenated solvent as standard. Elemental microanalyses were determined by Butterworth Laboratories Limited, Teddington, Middlesex, U.K. High-resolution mass spectra were obtained as positive ion accurate mass centroided data using a Micromass Q-ToF 2 hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface, over a mass range of 80–1200 Da, with a scan time of 0.95 s, and an interscan delay of 0.07 s, using reserpine as the external mass calibrant ([M + H]⁺ = 609.2812 Da).

***t*-Butyl *N*-[(Benzyloxy)carbonyl]-L-methionyl-L-alaninate 3.** *N*-(benzyloxycarbonyl)-L-methionine (10 g, 35.3 mmol) was dissolved in DMF (200 mL) and EDC (1.2 equiv, 8.13 g, 42.4 mmol)

was added followed by HOBt (1.2 equiv, 5.72 g, 42.33 mmol) and triethylamine (4 equiv, 19.7 mL, 141.34 mmol). The mixture was stirred for 1 h, *L*-alanine *t*-butyl ester (7.7 g, 53 mmol) was then added, and stirring was continued for 18 h. The mixture was concentrated under reduced pressure and partitioned between diethyl ether and water. The separated organic phase was washed with 1 M hydrochloric acid, saturated aqueous sodium bicarbonate solution, and brine, dried (MgSO₄), and concentrated under reduced pressure to give **3** (11.9 g, 82%) as an orange oil, which crystallized on standing. ¹H NMR (CDCl₃): δ 1.37 (d, 3H, *J* = 7 Hz), 1.46 (s, 9H), 1.92–2.16 (m, 2H), 2.11 (s, 3H), 2.59 (br t, 2H, *J* = 7 Hz), 4.33–4.48 (m, 2H), 5.11 (s, 2H), 5.55 (br d, 1H, *J* = 7.5 Hz), 6.62 (br d, 1H, *J* = 6.5 Hz), 7.28–7.40 (m, 5H).

(2*S*)-2-[(3*S*)-3-[(Benzyloxy)carbonyl]amino]-2-oxopyrrolidin-1-yl) Propanoic Acid 4. A solution of **3** (11.9 g, 29 mmol) in acetone (75 mL) was treated with methyl iodide (10 equiv, 18 mL, 0.29 mol) and stirred at room temperature for 72 h. The reaction mixture was then concentrated under reduced pressure to give an orange solid that was dissolved in MeCN (200 mL). Dowex resin (hydroxide form, 19.42 g) was added, and the mixture stirred for 18 h at room temperature. The mixture was filtered and the resin was washed with EtOAc. The filtrate was concentrated under reduced pressure to afford a yellow oil that was purified by Biotage chromatography (eluting with cyclohexane/EtOAc 3:2) to give the *t*-butyl (2*S*)-2-[(3*S*)-3-[(benzyloxy)carbonyl]amino]-2-oxopyrrolidin-1-yl)propanoate (2.92 g, 28%) as a colorless oil. ¹H NMR (CDCl₃): δ 1.39 (d, 3H, *J* = 7.5 Hz), 1.45 (s, 9H), 1.86–1.97 (m, 1H), 2.64–2.76 (m, 1H), 3.27–3.36 (m, 1H), 3.48 (t, 1H, *J* = 9 Hz), 4.25–4.36 (m, 1H), 4.72 (q, H, *J* = 7 Hz), 5.13 (s, 2H), 5.36 (br s, 1H), 7.28–7.38 (m, 5H).

t-Butyl (2*S*)-2-[(3*S*)-3-[(benzyloxy)carbonyl]amino]-2-oxopyrrolidin-1-yl)propanoate (0.5 g, 1.38 mmol) was dissolved in DCM (7 mL), and TFA (4.7 mL) was added. The mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure to give **4** (0.423 g, 99.9%) as a colorless oil, which after azeotroping with toluene, crystallized to a white solid. ¹H NMR (MeOH-*d*₄): δ 1.43 (d, 3H, *J* = 7.3 Hz), 1.88–2.02 (m, 1H), 2.37–2.50 (m, 1H), 3.34–3.52 (m, 1H), 4.43 (dd, 1H, *J* = 10.5 and 9 Hz), 4.67 (q, 1H, *J* = 7.3 Hz), 5.09 (s, 2H), 7.25–7.50 (m, 5H). LC-MS: *t*_R 2.43 min; *m/z* (ES) 307 (MH⁺), 305 (M – H)[–].

Benzyl (3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-ylcarbamate 5. Compound **4** (84.5 g, 0.276 mol) was dissolved in DMF (2 L) and TBTU (1.8 equiv, 161 g, 0.5 mol) was added, followed by *N,N*-diisopropylethylamine (2.4 equiv, 92 mL, 0.66 mol) and morpholine (1.9 equiv, 46 mL, 0.527 mol). The mixture was stirred under nitrogen for 2.5 h, and saturated aqueous ammonium chloride was added. The mixture was stirred for 15 min then partitioned between water and EtOAc. The separated organic phase was washed with aqueous lithium chloride (10% w/v), followed by saturated sodium bicarbonate and brine. The organic layer was dried (over Na₂SO₄) and concentrated under reduced pressure to give **5** (65 g, 63%) as a yellow solid. ¹H NMR (MeOH-*d*₄): δ 1.28 (d, 3H, *J* = 7.1 Hz), 1.95–2.08 (m, 1H), 2.34–2.44 (m, 1H), 3.31–3.55 (m, 4H), 3.56–3.72 (m, 6H), 4.23 (t, 1H, *J* = 9.5 Hz), 5.03–5.12 (m, 3H), 5.09 (s, 2H), 7.25–7.39 (m, 5H). LC-MS: *t*_R 2.39 min; *m/z* (ES) 376 (MH⁺), 374 (M – H)[–].

(3*S*)-3-Amino-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-pyrrolidin-2-one 2. A mixture of **5** (20 g, 53.3 mmol), 10% palladium on carbon (10% w/w, 2 g), and ethanol (1.3 L) was stirred under an atmospheric pressure of hydrogen for 16 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure to give **2** (12.3 g, 96%) as a pale white oil. ¹H NMR (CDCl₃): δ 1.31 (d, 3H, *J* = 7 Hz), 1.64–1.76 (m, 3H), 2.38–2.49 (m, 1H), 3.30 (td, 1H, *J* = 9.5 and 6.5 Hz), 3.39 (td, 1H, *J* = 9.5 and 1.7 Hz), 3.47–3.73 (m, 9H), 5.07 (q, 2H, *J* = 7 Hz).

General Procedures. Method A: Sulfonylation of 2 with Sulfonyl Chlorides 6. To a solution of **2** (typically 50–100 mg) in anhydrous MeCN were added the appropriate sulfonyl chloride **6** (1.05–1.2 equiv) in MeCN or DCM and a mild base (2.2 equiv) such as pyridine or *N,N*-diisopropylethylamine, and the mixture

was stirred at room temperature overnight (typically 18 h). Saturated ammonium chloride solution was added, and the resultant mixture was stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure, and the residue was partitioned between chloroform and a 1:1 mixture of hydrochloric acid (2 M) and water. The organic layer was washed with a 1:1 mixture of saturated aqueous sodium bicarbonate and water and brine. The organic layer was separated, dried (MgSO₄), and concentrated under reduced pressure to give the corresponding sulfonamide, which may have required purification by SPE and/or (mass-directed) autoprep before being freeze-dried in an aqueous dioxan solution.

Method B: Preparation of Sulfonyl Chlorides 6 from Parent or Bromo-chloro(hetero)aromatics. *n*-Butyl lithium (1.6 M in hexanes, 1.1 equiv) was added to a cooled (−78 °C) solution of the appropriate chloro(hetero)aromatics (typically 100 mg) in anhydrous THF (typically 5–10 mL) over 15 min. The reaction was stirred for 20 min. Sulfur dioxide gas was then condensed into the reaction for 10 min. The reaction was allowed to warm to room temperature, stirred for 1 h, and then evaporated in vacuo. The resultant residue was stirred at room temperature (typically for 2–18 h) with *N*-chlorosuccinimide (1.23 equiv) in anhydrous DCM (typically 6–10 mL). The solution was filtered, and the filtrate was concentrated under reduced pressure to give the corresponding sulfonyl chloride as a crude product, which was used in Method A without further purification.

(*E*)-2-(5-Chlorothiophen-2-yl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}ethanesulfonamide 1f. Compound 1f (19.3 g, 70% yield) as a white solid was synthesized from 2 (14.9 g, 61.8 mmol) and (*E*)-2-(5-chloro-2-thienyl)ethanesulfonyl chloride^{13c} (16.5 g, 67.9 mmol) using Method A under General Procedures. ¹H NMR (CDCl₃): δ 1.35 (d, 3H, *J* = 7 Hz), 1.99–2.12 (m, 1H), 2.59–2.68 (m, 1H), 3.31–3.40 (m, 1H), 3.47–3.74 (m, 9H), 3.96 (ddd, 1H, *J* = 11, 8 and 4 Hz), 5.02 (br d, 1H, *J* = 3 Hz), 5.07 (q, 1H, *J* = 7 Hz), 6.55 (d, 1H, *J* = 15 Hz), 6.91 (d, 1H, *J* = 4 Hz), 7.09 (d, 1H, *J* = 4 Hz), 7.49 (d, 1H, *J* = 15 Hz). LC-MS: *t*_R 2.70 min; *m/z* (ES) 448/450 MH⁺, 446/448 (M – H)[−]. Anal. (C₁₇H₂₂ClN₃O₅S₂): C, H, N.

4-Iodo-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}benzenesulfonamide 7. Compound 7 (3.217 g, 71%) was synthesized from 2 (2.163 g, 8.96 mmol) and 4-iodophenylsulfonyl chloride (1.1 equiv, 2.983 g, 9.86 mmol) using Method A under General Procedures. ¹H NMR (CDCl₃): δ 1.29 (d, 3H, *J* = 7 Hz), 1.91–2.05 (m, 1H), 2.45–2.65 (m, 1H), 3.29 (td, 1H, *J* = 10 and 6 Hz), 3.42–3.74 (m, 9H), 3.82 (dd, 1H, *J* = 10, and 8 Hz), 5.03 (q, 1H, *J* = 7 Hz), 6.27 (br s, 1H), 7.64 (d, 2H, *J* = 8.6 Hz), 7.87 (d, 2H, *J* = 8.6 Hz). LC-MS: *t*_R 2.60 min; *m/z* (ES) 508 MH⁺, 506 (M – H)[−].

4-Ethynyl-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}benzenesulfonamide 8. A mixture of 7 (547 mg, 1.08 mmol), CuI (3 mol%, 6.2 mg, 0.032 mmol), dichlorobis(triphenylphosphine)palladium(II) (3 mol%, 22.7 mg, 0.032 mmol), trimethylsilylacetylene (3 equiv, 0.458 mL, 3.24 mmol), and triethylamine (2 mL) in DMF (2 mL) was stirred at room temperature for 22 h. The reaction mixture was evaporated in vacuo to give a dark brown residue that was purified using SPE (20 g SiO₂, 50 to 70% EtOAc–cyclohexane, EtOAc then 5% MeOH–EtOAc) to give *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-4-[(trimethylsilyl)ethynyl]benzenesulfonamide as a brown foam (514 mg, 99.9%). ¹H NMR (CDCl₃): δ 0.26 (s, 9H), 1.30 (d, 3H, *J* = 7 Hz), 1.92–2.05 (m, 1H), 2.49–2.59 (m, 1H), 3.29 (td, 1H, *J* = 10 and 6 Hz), 3.43–3.77 (m, 10H), 5.01 (q, 1H, *J* = 7 Hz), 5.56 (bd, 1H, *J* = 3 Hz), 7.59 (d, 2H, *J* = 8.6 Hz), 7.84 (d, 2H, *J* = 8.6 Hz). LC-MS: *t*_R 3.20 min; *m/z* (ES) 478 MH⁺, 476 (M – H)[−].

To a solution of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-4-[(trimethylsilyl)ethynyl]benzenesulfonamide (391 mg, 0.82 mmol) in dry THF (4 mL) at −78 °C, tetra *n*-butylammonium fluoride (1 M in THF, 0.819 mL, 0.82 mmol) was added. The mixture was stirred at −78 °C for 1 h and then at room temperature for another hour and evaporated in vacuo. The resultant residue was dissolved in EtOAc (80 mL) and

washed with water (3 × 30 mL), dried (MgSO₄), and filtered. Removal of solvent gave a light brown residue that was purified using SPE (10 g SiO₂, EtOAc) to give 8 (276.5 mg, 83%) as a white solid. ¹H NMR (CDCl₃): δ 1.30 (d, 3H, *J* = 7 Hz), 1.95–2.08 (m, 1H), 2.54–2.63 (m, 1H), 3.27 (s, 1H), 3.31 (td, 1H, *J* = 10 and 6 Hz), 3.43–3.77 (m, 10H), 5.01 (q, 1H, *J* = 7 Hz), 5.27 (br s, 1H), 7.63 (d, 2H, *J* = 8.6 Hz), 7.87 (d, 2H, *J* = 8.6 Hz). LC-MS: *t*_R 2.46 min; *m/z* (ES) 406 MH⁺, 404 (M – H)[−].

4-[(*E*)-2-Chloroethenyl]-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}benzenesulfonamide 1i. To a slurry of 8 (132.7 mg, 0.327 mmol) in anhydrous THF (4 mL) at 0 °C under nitrogen, *t*-hexylborane (prepared from 0.69 mL of each of THF solution of 1 M borane and 2 M 2-methyl-2-butene according to standard procedure) was added and stirred for 4 h. Copper(II) chloride (2.4 equiv, 105.6 mg, 0.785 mmol) followed by 1,3-dimethyl-2-imidazolidinone (0.5 mL) and water (0.014 mL, 0.785 mmol) were added.²³ The mixture was stirred at 0 °C to room temperature for 18 h and quenched with water (5 mL). Extraction with EtOAc (3 ×) gave a yellow oil that was purified using autopreparative reverse phase HPLC to give 1i (49.6 mg, 34%) as a creamy white solid. ¹H NMR (CDCl₃): δ 1.30 (d, 3H, *J* = 7 Hz), 1.96–2.08 (m, 1H), 2.53–2.64 (m, 1H), 3.26–3.36 (m, 1H), 3.43–3.75 (m, 9H), 3.74 (dd, 1H, *J* = 10.5 and 8 Hz), 5.01 (q, 1H, *J* = 7 Hz), 5.15–5.60 (br s, 1H), 6.84 (ABq, 2H, *J* = 13.6 Hz), 7.44 (d, 1H, *J* = 8 Hz), 7.86 (d, 1H, *J* = 8 Hz). LC-MS: *t*_R 2.70 min, 98% purity; *m/z* (ES) 442/444 MH⁺, 440/442 (M – H)[−]. HRMS calcd for C₁₉H₂₃N₃O₅ClS *m/z* (MH⁺), 442.1203; found, 442.1213.

5-(5-Chloro-1,3-thiazol-2-yl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-2-thiophenesulfonamide 1n. To a mixture of thiophene-2-boronic acid (1.1 equiv, 0.71 g, 5.54 mmol), 2-bromo-5-chlorothiazole^{19b} (1 g, 5.04 mmol), and Cs₂CO₃ (2.6 equiv, 4.27 g, 13.1 mmol) under nitrogen, degassed water (12 mL) was added and stirred for 10 min. DME (12 mL) followed by Pd(PPh₃)₄ (5 mol%, 291 mg, 0.25 mmol) were then added. The mixture was heated at 70 °C overnight. The crude reaction mixture was extracted with Et₂O (3 × 90 mL), dried, and evaporated to give a dark brown oil (1.152 g). SPE (20 g SiO₂, cyclohexane, 3 to 10% EtOAc–cyclohexane) followed by further purification on OPTIX 10 (2 × 10 g SiO₂, 5% EtOAc–cyclohexane) gave the 5-chloro-2-(2-thienyl)-1,3-thiazole as a yellow solid (527 mg, 51.9%). ¹H NMR (CDCl₃): δ 7.07 (dd, 1H, *J* = 5 and 4 Hz), 7.40–7.44 (m, 2H), 7.55 (s, 1H). LC-MS: *t*_R 3.37 min; *m/z* (ES) 202/204 MH⁺.

Using Method A under General Procedures, 1n (3.3 mg) was synthesized from 2 (101.6 mg, 0.42 mmol) and 5-(5-chloro-1,3-thiazol-2-yl)-2-thiophenesulfonyl chloride 6n [139.5 mg; prepared from 5-chloro-2-(2-thienyl)-1,3-thiazole (104 mg, 0.52 mmol) using Method B under General Procedures and used without further purification]. ¹H NMR (CDCl₃): δ 1.33 (d, 3H, *J* = 7 Hz), 2.04–2.16 (m, 1H), 2.62–2.73 (m, 1H), 3.32–3.40 (m, 1H), 3.45–3.73 (m, 9H), 3.92 (br t, 1H, *J* = 9 Hz), 5.03 (q, 1H, *J* = 7 Hz), 5.48 (br s, 1H), 7.37 (d, 1H, *J* = 4 Hz), 7.61 (d, 1H, *J* = 4 Hz), 7.63 (s, 1H). LC-MS: *t*_R 2.83 min, 95% purity; *m/z* (ES) 505/507 MH⁺, 503/505 (M – H)[−]. HRMS calcd for C₁₈H₂₁ClN₃O₅S₂ *m/z* (MH⁺), 505.0441; found, 505.0431.

6-Chloro-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}-1,3-benzothiazole-2-sulfonamide 1q. *N*-Chlorosuccinimide (0.37 g, 2.77 mmol) was added to 6-chloro-1,3-benzothiazole-2(3*H*)-thione²² (0.5 g, 2.48 mmol) in DCM (15 mL) under nitrogen, and stirred at room temperature for 3 h. A solution of 2 (0.569 g, 2.36 mmol) and triethylamine (1.04 mL, 7.49 mmol) in anhydrous DCM (5 mL) were added, and the resulting mixture was stirred at room temperature under nitrogen for 2 h. The solution was filtered, and the filtrate was diluted with DCM. The organic solution was washed with water and brine, dried (MgSO₄), and concentrated under reduced pressure. The residue was purified by SPE (silica, eluting with cyclohexane–EtOAc 1:1, increasing polarity to EtOAc–MeOH 19:1) to give (3*S*)-3-[[6-chloro-1,3-benzothiazol-2-yl]thio]amino]-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]pyrrolidin-2-one (300 mg, 29%) as a white

solid. ^1H NMR (CDCl_3): δ 1.34 (d, 3H, J = 6.6 Hz), 2.06–2.20 (m, 1H), 2.40–2.51 (m, 1H), 3.30–3.44 (m, 2H), 3.47–3.78 (m, 8H), 4.03 (ddd, 1H, J = 12, 8 and 3.5 Hz), 4.35 (d, 1H, J = 3.5 Hz), 5.10 (q, 1H, J = 6.6 Hz), 7.37 (dd, 1H, J = 9 and 2 Hz), 7.72 (d, 1H, J = 9 Hz), 7.77 (d, 1H, J = 2 Hz). LC-MS: t_R 2.82 min; m/z (ES) 441/443 MH^+ , 439/441 ($\text{M} - \text{H}$) $^-$.

(3S)-3-[[[6-Chloro-1,3-benzothiazol-2-yl]thio]amino]-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]pyrrolidin-2-one (0.1 g, 0.227 mmol) was stirred at room temperature in anhydrous acetone (3 mL) and 5% aqueous potassium permanganate (1.35 mL) for 3 h, after which additional acetone (3 mL) and 5% aqueous potassium permanganate (1.35 mL) were added. The reaction mixture was stirred for a further 18 h and filtered through Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by mass directed preparative HPLC to give **1q** (6.2 mg, 6%) as a white solid. ^1H NMR (CDCl_3): δ 1.32 (d, 3H, J = 7 Hz), 2.08–2.21 (m, 1H), 2.70–2.81 (m, 1H), 3.37 (td, 1H, J = 10.5 and 6 Hz), 3.43–3.74 (m, 9H), 4.31 (dd, 1H, J = 10.8 and 8 Hz), 5.01 (q, 1H, J = 7 Hz), 5.86–6.03 (br s, 1H), 7.68 (dd, 1H, J = 8.8 and 2 Hz), 7.97 (d, 1H, J = 2 Hz), 8.10 (d, 1H, J = 8.8 Hz). LC-MS: t_R 2.98 min, 98% purity; m/z (ES) 473 MH^+ . HRMS calcd for $\text{C}_{18}\text{H}_{21}\text{ClN}_4\text{O}_5\text{S}_2$ m/z (MH^+), 473.0719; found, 473.0719.

5-Chloro-*N*-{[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]-1*H*-indole-2-sulfonamide 1s. *t*-Butyl 5-chloro-2-[[[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]amino]sulfonyl]-1*H*-indole-1-carboxylate (13 mg, 25%) as a colorless glass was synthesized from **2** (44 mg, 0.18 mmol) and 1,1-dimethylethyl 5-chloro-2-(chlorosulfonyl)-1*H*-indole-1-carboxylate¹⁷ (33 mg, 0.094 mmol) using Method A under General Procedures. ^1H NMR (CDCl_3): δ 1.27 (d, 3H, J = 7 Hz), 1.75 (s, 9H), 2.06–2.18 (m, 1H), 2.64–2.73 (m, 1H), 3.32 (td, 1H, J = 10 and 6.5 Hz), 3.41–3.73 (m, 9H), 3.90 (ddd, 1H, J = 11, 8, and 3 Hz), 5.00 (q, 1H, J = 7 Hz), 6.75 (d, 1H, J = 3 Hz), 7.42 (dd, 1H, J = 8 and 2 Hz), 7.42 (s, 1H), 7.62 (d, 1H, J = 2 Hz), 7.95 (d, 1H, J = 8 Hz). LC-MS: t_R 3.41 min; m/z (ES) 555/557 MH^+ , 553/555 ($\text{M} - \text{H}$) $^-$.

t-Butyl 5-chloro-2-[[[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]amino]sulfonyl]-1*H*-indole-1-carboxylate (11 mg, 0.02 mmol) was dissolved in 1:1 TFA/DCM (0.5 mL) and allowed to stand at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was partitioned between saturated aqueous sodium bicarbonate and DCM. The separated organic phase was dried (MgSO_4) and concentrated under a stream of nitrogen to give **1s** (8.2 mg, 91%) as a white solid. ^1H NMR (CDCl_3): δ 1.36 (d, 3H, J = 7 Hz), 2.05–2.17 (m, 1H), 2.58–2.68 (m, 1H), 3.37 (td, 1H, J = 10 and 6.5 Hz), 3.39–3.70 (m, 9H), 4.17–4.28 (m, 1H), 5.03 (q, 1H, J = 7 Hz), 5.69 (d, 1H, J = 6.5 Hz), 7.06 (d, 1H, J = 2 Hz), 7.21–7.30 (m, 2H), 7.63 (d, 1H, J = 1 Hz), 10.9 (s, 1H). LC-MS: t_R 2.95 min, 96% purity; m/z (ES) 455/457 MH^+ , 453/455 ($\text{M} - \text{H}$) $^-$. HRMS calcd for $\text{C}_{19}\text{H}_{23}\text{ClN}_4\text{O}_5\text{S}$ m/z (MH^+), 455.1156; found, 455.1158.

5-(5-Chloro-2-thienyl)-*N*-{[(3S)-1-[(1S)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl]-2-furansulfonamide 1u. A mixture of 2-bromo-5-chlorothiophene (0.307 mL, 2.8 mmol), tri-*n*-butyl(2-furanyl)stannane (0.882 mL, 2.8 mmol), tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct ($\text{Pd}_2\text{-dba}_3\cdot\text{CHCl}_3$; 2.5 mol%, 72.5 mg, 0.07 mmol), and PPh_3 (10 mol%, 73.4 mg, 0.28 mmol) in THF (15 mL) was heated under nitrogen at 75 °C for 21.5 h. KF (10 g) and water (5 mL) were added to the cooled reaction mixture and stirred at room temperature for 7 h. Aqueous NaOH (2 M, 5 mL) was then added. The resultant mixture was evaporated in vacuo to remove most THF, diluted with water (20 mL), extracted with ether (3 $\times$ 30 mL), and dried (MgSO_4). Removal of solvent gave an oily brown solid (1.31 g) that was purified by SPE (20 g SiO_2 , cyclohexane) to give 2-(5-chloro-2-thienyl)furan (299 mg, 58%) yellow liquid. ^1H NMR (CDCl_3): δ 6.43–6.48 (m, 2H), 6.86 (t, 1H, J = 3.5 Hz), 7.01 (t, 1H, J = 3.5 Hz), 7.40 (s, 1H). GC-MS: t_R 7.07 min; m/z (APCI for $\text{C}_8\text{H}_8\text{ClO}_2\text{S}$) 184/186 M^+ .

Using Method A under General Procedures, **1u** (37 mg, 19% based on **2**) as a light brown foam was synthesized from **2** (97.4 mg, 0.4 mmol) and 5-(5-chloro-2-thienyl)-2-furansulfonyl chloride **6u** [226 mg; prepared from 2-(5-chloro-2-thienyl)furan (167.5 mg, 0.91 mmol) using Method B under General Procedures and used without further purification]. ^1H NMR (CDCl_3): δ 1.33 (d, 3H, J = 7 Hz), 1.99–2.13 (m, 1H), 2.59–2.68 (m, 1H), 3.35 (td, 1H, J = 10 and 6 Hz), 3.42–3.74 (m, 9H), 3.96 (dd, 1H, J = 10.6 and 8.1 Hz), 5.04 (q, 1H, J = 7 Hz), 5.63 (br s, 1H), 6.50 (d, 1H, J = 3.5 Hz), 6.91 (d, 1H, J = 4 Hz), 7.12 (d, 1H, J = 3.5 Hz), 7.18 (d, 1H, J = 4). LC-MS: t_R 2.93 min; m/z (ES) 488/490 MH^+ , 486/488 ($\text{M} - \text{H}$) $^-$. Anal. ($\text{C}_{19}\text{H}_{22}\text{ClN}_4\text{O}_5\text{S}_2\cdot 0.55\text{CH}_2\text{O}_2$): C, H, N.

2-(5-Chloro-2-thienyl)-*N*-{[(3S)-1-[(1S)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl]-1,3-thiazole-5-sulfonamide 1v. To a mixture of 2-bromothiazole (0.55 mL, 6.1 mmol) and 5-chlorothiophene-2-boronic acid (991 mg, 6.1 mmol) in DME (10 mL) under nitrogen, a solution of sodium carbonate (1.68 g, 15.86 mmol) in water (10 mL), bubbled with nitrogen for 10 min, followed by $\text{Pd}_2\text{dba}_3\cdot\text{CHCl}_3$ (2.5 mol%, 109 mg, 0.105 mmol) and a solution of PPh_3 (10 mol%, 110.5 mg, 0.421 mmol) in DME (10 mL) were added. The mixture was heated at 80 °C under nitrogen for 16 h and evaporated in vacuo. The resultant aqueous mixture was extracted with DCM (3 $\times$ 20 mL), dried (MgSO_4), and filtered. Removal of solvent gave a brown oil that was purified by SPE (20 g SiO_2 , 3–5% EtOAc-cyclohexane) to give the 2-(5-chloro-2-thienyl)-1,3-thiazole (374 mg, 30%) as a light brown oil. ^1H NMR (CDCl_3): δ 6.91 (d, 1H, J = 4 Hz), 7.26 (d, 1H, J = 3 Hz), 7.28 (d, 1H, J = 4 Hz), 7.76 (d, 1H, J = 3 Hz). LC-MS: t_R 3.26 min; m/z (ES) 202/204 MH^+ .

Using Method A under General Procedures, **1v** (66 mg, 32%) was synthesized from **2** (98 mg, 0.41 mmol) and 2-(5-chloro-2-thienyl)-1,3-thiazole-5-sulfonyl chloride **6v** [165 mg, prepared from 2-(5-chloro-2-thienyl)-1,3-thiazole (133.2 mg, 0.66 mmol) using Method B under General Procedures and used without further purification]. ^1H NMR ($\text{MeOH}-d_4$): δ 1.27 (d, 3H, J = 7 Hz), 1.80–1.93 (m, 1H), 2.39–2.49 (m, 1H), 3.33–3.41 (m, 2H), 3.43–3.70 (m, 8H), 4.26 (dd, 1H, J = 10.4 and 8.6 Hz), 4.99 (q, 1H, J = 7 Hz), 7.08 (d, 1H, J = 4 Hz), 7.66 (d, 1H, J = 4 Hz), 8.16 (s, 1H). LC-MS: t_R 2.96 min; m/z (ES) 505/507 MH^+ , 503/505 ($\text{M} - \text{H}$) $^-$. Anal. ($\text{C}_{18}\text{H}_{21}\text{ClN}_4\text{O}_5\text{S}_3\cdot 0.6\text{H}_2\text{O}$): C, H, N.

5-(5-Chloro-2-thienyl)-*N*-{[(3S)-1-[(1S)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl]-1,3-thiazole-5-sulfonamide 1w. To a mixture of 5-chlorothiophene-2-boronic acid (1.041 g, 6.41 mmol) and 5-bromothiazole^{19a} (92% in ether, 1.143 g, 6.41 mmol) in DME (30 mL), bubbled with N_2 for 10 min before use, a solution of sodium carbonate (2.6 eq., 1.77 g, 16.67 mmol) in water (15 mL), bubbled with N_2 for 20 min before use followed by tetrakis(triphenylphosphine)palladium(0) (10 mol%, 741 mg, 0.64 mmol) were added. The mixture was heated at 70–80 °C under nitrogen for 5.5 h. The cooled reaction mixture was evaporated in vacuo, extracted with ether (4 $\times$ 30 mL), dried (MgSO_4), and filtered. Removal of solvent gave a brown oil (1.7 g). Purification on a SPE (20 g SiO_2) and eluted with cyclohexane followed by 5–10% EtOAc-cyclohexane gave the 5-(5-chloro-2-thienyl)-1,3-thiazole as a yellow solid (708 mg, 51%). ^1H NMR (CDCl_3): δ 6.89 (d, 1H, J = 3.8 Hz), 6.99 (d, 1H, J = 3.8 Hz), 7.90 (s, 1H), 8.71 (s, 1H). LC-MS: t_R 3.24 min; m/z (ES) 202/204 MH^+ .

Using Method A under General Procedures, **1w** (140 mg, 66.5%) was synthesized from **2** (100.6 mg, 0.42 mmol) and 5-(5-chloro-2-thienyl)-1,3-thiazole-2-sulfonyl chloride **6w** [175 mg; prepared from 5-(5-chloro-2-thienyl)-1,3-thiazole (103 mg, 0.51 mmol) using Method B under General Procedures and used without further purification]. ^1H NMR (CDCl_3): δ 1.33 (d, 3H, J = 7 Hz), 2.06–2.19 (m, 1H), 2.65–2.74 (m, 1H), 3.37 (td, 1H, J = 10 and 6 Hz), 3.45–3.74 (m, 9H), 4.27 (dd, 1H, J = 10.8 and 8 Hz), 5.04 (q, 1H, J = 7 Hz), 6.20 (br s, 1H), 6.93 (d, 1H, J = 4 Hz), 7.07 (d, 1H, J = 4 Hz), 7.89 (s, 1H). LC-MS: t_R 2.97 min; m/z (ES) 505/507 MH^+ , 503/505 ($\text{M} - \text{H}$) $^-$. Anal. ($\text{C}_{18}\text{H}_{21}\text{ClN}_4\text{O}_5\text{S}_3\cdot 0.5\text{H}_2\text{O}$): C, H, N. For N: calcd, 10.90%; found, 10.34%.

3-(5-Chloro-2-thienyl)-1*H*-1,2,4-triazole-5-sulfonyl Chloride 6x. A mixture of 5-chloro-2-thiophenecarboxylic acid hydrazide

(197 mg, 1.12 mmol) and thiourea (90.3 mg, 1.12 mmol) was heated at 160 °C²⁰ under nitrogen without solvent for 6.5 h. The cooled mixture was stirred with aqueous NaOH (5%, 20 mL) for 4 h and filtered to remove any insolubles. The basic solution was acidified with acetic acid (1.65 mL) to pH 5. The resultant precipitate was collected and dried in vacuo at 50 °C overnight to give the 5-(5-chloro-2-thienyl)-1,2-dihydro-3H-1,2,4-triazole-3-thione as a creamy white solid (198 mg, 82%). ¹H NMR (DMSO-*d*₆): δ 7.69 (d, 1H, *J* = 3.8 Hz), 7.85 (d, 1H, *J* = 3.8 Hz). LC-MS: *t*_R 3.04 min; *m/z* (ES) 218/220 MH⁺, 216/218 (M - H)⁻.

To a solution of 5-(5-chloro-2-thienyl)-1,2-dihydro-3H-1,2,4-triazole-3-thione (45.5 mg, 0.21 mmol) in acetic acid (2 mL) at about 10 °C (bath temperature), a solution of chlorine in acetic acid (0.11 g/mL, prepared by bubbling chlorine gas into 10 mL of acetic acid in ice-water bath for 15 min, 3.3 equiv, 0.444 mL, 0.63 mmol) was added. The mixture was allowed to warm to room temperature slowly, stirred for 3 h, and evaporated to dryness to afford 3-(5-chloro-2-thienyl)-1H-1,2,4-triazole-5-sulfonyl chloride **6x**, which was used without purification. An amount of this crude product was treated with excess methylamine in THF. LC-MS confirmed the formation of the corresponding *N*-methyl sulfonamide [*t*_R 2.68 min; *m/z* (ES) 279/281 MH⁺, 277/279 (M - H)⁻].

3-(5-Chloro-2-thienyl)-*N*-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl]-1H-1,2,4-triazole-5-sulfonamide **1x.** Compound **1x** (23 mg, 23% based on **2**), as a white solid, was synthesized from **2** (50 mg, 0.21 mmol) and 3-(5-chloro-2-thienyl)-1H-1,2,4-triazole-5-sulfonyl chloride **6x** (crude, obtained as above) using Method A under General Procedures. ¹H NMR (CDCl₃): δ 1.39 (d, 3H, *J* = 7 Hz), 2.13–2.26 (m, 1H), 2.67–2.76 (m, 1H), 3.39–3.74 (m, 1H), 4.58 (dd, 1H, *J* = 10.3 and 8.6 Hz), 5.03 (q, 1H, *J* = 7 Hz), 6.87 (d, 1H, *J* = 4 Hz), 7.42 (d, 1H, *J* = 4 Hz). LC-MS: *t*_R 2.55 min; *m/z* (ES) 489/491 MH⁺, 487/489 (M - H)⁻. Anal. (C₁₇H₂₁ClN₆O₅S₂·1.2H₂O): C, H, N.

FXa Crystal Structures with Bound Ligands. Preparation of the Crystals. Purified des-Gla fXaβ was purchased from Enzyme Research Laboratories (South Bend, IN). The protein was further purified on a weak cation-exchange resin (carboxymethyl (CM) sepharose, Amersham Biosciences, Uppsala, Sweden) equilibrated in a solution containing 25 mM sodium acetate (pH 5.0) from which protein was eluted with a NaCl gradient. Protein was then buffer exchanged into a solution containing 5 mM Mes^a-NaOH (pH 6.0), 5 mM CaCl₂, and up to 1 μM crystallization tool compound RPR128515³⁵ prior to concentration to approximately 5 mg/mL for crystallization experiments in a Vivaspin ultracentrifugation unit (Vivascience, Hanover, Germany). Crystallization was carried out using the hanging drop vapor diffusion method in 2 μL drops containing a 1:1 mixture of protein and well solution. Well solution contained 16–20% PEG^a 6 K, 50 mM Mes^a-NaOH (pH 5.7–6.0), 5 mM CaCl₂, and 50 mM NaCl. Streak seeding followed by macroseeding, using the derived small crystals, routinely gave needle-shaped crystals (size 200 × 50 × 50 μm) suitable for synchrotron X-ray data collection.

X-ray Data Collection and Processing. Compound was prepared as a 100 mM stock in DMSO for soaking experiments. Crystals were soaked in 20 μL well solution containing 1–5 mM compound and 5–10% DMSO for up to 1 week. Prior to freezing at 100 K, the crystals were briefly transferred to well solution with 20% glycerol included. The X-ray intensity data was collected at either the Synchrotron Radiation Source (Daresbury, UK) on station 9.6 or the European Synchrotron Radiation Facility (Grenoble, France) on beamlines ID29 or ID14 eh4. Data processing and scaling was carried out using DENZO and SCALEPACK,³⁶ except for **1x**, which was processed using MOSFLM.³⁷ All crystals have *P*2₁2₁2₁ symmetry and similar cell dimension, *a* ≈ 56.8 Å, *b* ≈ 72.3 Å, and *c* ≈ 79.5 Å.

Structure Solution and Crystallographic Refinement. The initial structure was solved by molecular replacement using the coordinates of 1ezq.³⁵ a fXa complex structure from the RCSB Protein Data Bank with ligand removed as the search model. Subsequent structures were solved using difference Fourier. Model building, including ligand fitting, was carried out in QUANTA2000

(Accelrys). All other data manipulation was carried out using programs from the CCP4 suite,³⁸ including crystallographic refinement using REFMAC.³⁹ All nonglycine residues fall within the energetically favorable regions of the Ramachandran plot. All of the atoms of the inhibitors and residues in the vicinity of the active site lie in well-defined electron density. The majority of the rest of the protein is in well-determined electron density, except for the EGF1 domain, which was not visible. The statistics of the crystallographic refinement are shown in Table 5. Atomic coordinates have been deposited with the RCSB Protein Data Bank (2cjj for **1a**, 2j95 for **1b**, 2j38 for **1d**, 2j34 for **1e**, and 2j94 for **1x**).

In Vitro Assay for Inhibition of Factor Xa. The ability of test compounds to inhibit human fXa in vitro was determined in a fluorescence assay, using rhodamine 110, bis-(Boc-L-glycylglycyl-L-arginine amide) as the fluorogenic substrate. Test compounds were diluted from a 10 mM DMSO solution to the final assay concentration range of 166 μM to 0.09 nM. Assay was performed at room temperature using buffer consisting of 50 mM HEPES^a, 150 mM NaCl, and 5 mM CaCl₂ at pH 7.4 containing human factor Xa (0.2 nM final concentration). Test compound and enzyme were preincubated for 15 min prior to addition of the substrate (0.01 mM final concentration). Incubation was for 3 h, after which a LJL Analyst (now marketed by Molecular Devices Corporation) was used to monitor the fluorescence at 485/535 nm wavelength. Analysis of the derived data using Activity Base, a curve fitting software from IDBS, gave the pIC₅₀ and pK_i values from which K_i values were derived.

Prothrombin Time (PT) Assay.⁴⁰ Blood from two female and two male donors, supplied by Biological Specialty Corporation (Colmar, PA), was collected into 3.8% sodium citrate vacutainers and mixed gently. Plasma was generated by centrifugation of the citrated blood samples at 1200 *g* for 20 min at room temperature and stored at -20 °C until use. Prior to assay, plasma from four different human subjects (two male and two female) was thawed and pooled.

PT assays were performed at 37 °C using the Dade Behring BCS Coagulation System (*n* = 2 trials). An amount equal to 50 μL of plasma containing test compound at concentrations ranging from 0.03 to 100 μM (made from a 100 μM plasma stock containing 1% DMSO and prepared by the coagulation analyzer) was combined with 100 μL of thromboplastin C Plus (Dade Behring, Newark, DE). Absorbance at 405 nm was then monitored and time (sec) to clot formation at each concentration of test compound was determined and compared to time to clot formation of control (human plasma in the absence of test compound) and reported as the fold-increase in PT.

The concentration of test compound, which yield 1.5-fold extension in PT in the human plasma, was estimated using a mathematical model.⁴¹ The extrapolated 1.5× concentration was validated by spiking the plasma with this exact concentration of test compound, and the resulting PT was compared to that of control plasma. The 1.5× concentration was considered accurate if they caused the plasma to clot 1.5-fold slower than control plasma.

Pharmacokinetic Studies. The formulation used for both i.v. and p.o. dosing to rat and dog was a 5:95% (v/v) mixture of DMSO and 50:50 PEG^a-200/sterile water. For all animal studies, serial blood samples were collected into heparinised containers at various time-points and blood centrifuged to yield plasma.

Acknowledgment. The authors would like to thank the Physicochemical Characterisation group of Analytical Chemistry, GSK Stevenage, for carrying out human serum albumin binding and log D measurements for compounds highlighted in Table 2 and Mark E. Burgert (Principal Statistician at CVU CEDD, Upper Merion) for the design of a mathematical model for the analysis of the effect of concentrations of test compounds on the coagulation parameters.

Supporting Information Available: Electrostatic potential maps of selected *N*-methyl P1 sulfonamide and additional experi-

mental data are available. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

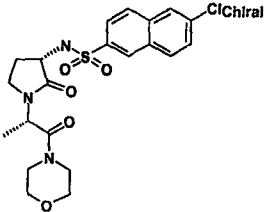
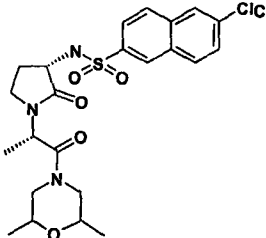
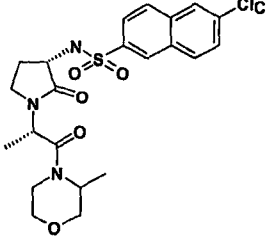
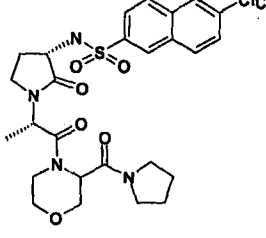
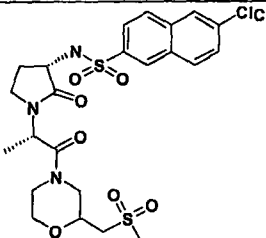
- (1) Fevig, J. M.; Wexler, R. R. Anticoagulants: Thrombin and Factor Xa Inhibitors. *Annu. Rep. Med. Chem.* 1999, 34, 81–100. Zhu, B.-Y.; Scarborough, R. M. Factor Xa Inhibitors: Recent Advances In Anticoagulant Agents. *Annu. Rep. Med. Chem.* 2000, 35, 83–102. Scarborough, R. M.; Pandey, A.; Zhang, X. Small Molecule Anticoagulant/Antithrombotic Agents. *Annu. Rep. Med. Chem.* 2005, 40, 85–101. Linkins, L.-A.; Weitz, J. I. New Anticoagulants. *Semin. Thromb. Hemostasis* 2003, 29 (6), 619–663.
- (2) Hirsh, J.; Warkentin, T. E.; Raschke, R.; Granger, C.; Ohman, E. M.; Dalen, J. E. Heparin and Low-Molecular-Weight Heparin: Mechanism of Action, Pharmacokinetics, Dosing Considerations, Monitoring, Efficacy, and Safety. *Chest* 1998, 114 (5), 489S–510S.
- (3) Hirsh, J.; Dalen, J. E.; Anderson, D. R.; Poller, L.; Bussey, H.; Ansell, J.; Deykin, D.; Brandt, J. T. Oral Anticoagulants: Mechanism of Action, Clinical Effectiveness, and Optimal Therapeutic Range. *Chest* 1998, 114 (5), 445S–469S.
- (4) Davie, E. W.; Fujikawa, K.; Kisiel, W. The Coagulation Cascade: Initiation, Maintenance, and Regulation. *Biochemistry* 1991, 30 (43), 10363–10370.
- (5) (a) Connolly, A. J.; Ishihara, H.; Kahn, M. L.; Farese, R. V., Jr.; Coughlin, S. R. Role of the Thrombin Receptor in Development and Evidence for a Second Receptor. *Nature* 1996, 381 (6582), 516–519. (b) Kaiser, B. Factor Xa—A Promising Target for Drug Development. *Cell. Mol. Life Sci.* 2002, 59 (2), 189–192. (c) Alexander, J. H.; Singh, K. P. Inhibition of Factor Xa: A Potential Target for the Development of New Anticoagulants. *Am. J. Cardiovasc. Drugs* 2005, 5 (5), 279–290. (d) Kubitz, D.; Haas, S. Novel Factor Xa Inhibitors for Prevention and Treatment of Thromboembolic Diseases. *Exp. Opin. Invest. Drugs* 2006, 15 (8), 843–855.
- (6) (a) Decousus, H.; Rivron-Guillot, K.; Girard, G.; Moulin, N. Clinical Efficacy and Safety of Fondaparinux in the Prevention and Treatment of Venous and Arterial Thrombosis. *Future Cardiol.* 2005, 1 (6), 743–758. (b) Bauersachs, R. M. Fondaparinux: An Update on New Study Results. *Eur. J. Clin. Invest.* 2005, 35 (Suppl. 1), 27–32. (c) Arixtra gets EU approval for VTE (venous thromboembolism) prophylaxis in major orthopaedic surgery. *Scrip*; 20th Nov 2003; No. 2860, p 27. (d) Expanded U.S. approval for Arixtra in hip fracture surgery. *Scrip*; 20th Jun 2003; No. 2748, p 22.
- (7) (a) Eriksson, B. Clinical Experience with Ximelagatran in Orthopedic Surgery. *Drugs* 2004, 64 (Suppl. 1), 27–35. (b) Boos, C. J.; Hinton, A.; Lip, G. Y. H. Ximelagatran: A Clinical Perspective. *Eur. J. Intern. Med.* 2005, 16 (4), 267–278. (c) Quan, M. L.; Lam, P. Y. S.; Han, Q.; Pinto, D. J. P.; He, M. Y.; Li, R.; Ellis, C. D.; Clark, C. G.; Teleha, C. A.; Sun, J.-H.; Alexander, R. S.; Bai, S.; Luetgen, J. M.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. Discovery of 1-(3'-Aminobenzisoxazol-5'-yl)-3-trifluoromethyl-N-[2-fluoro-4-[(2'-dimethylaminomethyl)imidazol-1-yl]phenyl]-1H-pyrazole-5-carboxamide Hydrochloride (Razaxaban), a Highly Potent, Selective, and Orally Bioavailable Factor Xa Inhibitor. *J. Med. Chem.* 2005, 48 (6), 1729–1744. (d) Eriksson, B. I.; Quinlan, D. J. Oral Anticoagulants in Development: Focus on Thromboprophylaxis in Patients Undergoing Orthopaedic Surgery. *Drugs* 2006, 66 (11), 1411–1429. (e) (i) Study backs controversial blood-thinning drug. *Reuters News*; 08 Feb 2005. (ii) More bad news for Exanta as France requests more data. *Scrip*; 20 Jan 2005.
- (8) *Dabigatran etexilate* (currently in phase III): (a) Sorbera, L. A.; Bozzo, J.; Castaner, J. Dabigatran/Dabigatran Etexilate: Prevention of DVT Prevention of Ischemic Stroke Thrombin Inhibitor. *Drugs Future* 2005, 30 (9), 877–885. (b) Stangier, J.; Eriksson, B. I.; Dahl, O. E.; Ahnfelt, L.; Nehmiz, G.; Staehle, H.; Rathgen, K.; Svaerd, R. Pharmacokinetic Profile of the Oral Direct Thrombin Inhibitor Dabigatran Etexilate in Healthy Volunteers and Patients Undergoing Total Hip Replacement. *J. Clin. Pharmacol.* 2005, 45 (5), 555–563. (c) Boehringer Ingelheim launches REVOLUTION program for dabigatran etexilate. *Prous*; 20th Jan 2006. MCC-977 (no structure or CAS number available, currently in phase II): (d) MCC-977 - Mitsubishi Pharma (JP): Phase II Clinical Trial. *Pharmaprojects*; 18th May 2006.
- (9) Rivaroxaban (BAY -59 - 7939; currently in phase III): (a) Eriksson, B. I.; Borris, L.; Dahl, O. E.; Haas, S.; Huisman, M. V.; Kakkar, A. K.; Misselwitz, F.; Kaelebo, P. Oral, Direct Factor Xa Inhibition with BAY-59-7939. For the Prevention of Venous Thromboembolism after Total Hip Replacement. *J. Thromb. Haemost.* 2006, 4 (1), 121–128. (b) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E. Discovery of the Novel Antithrombotic Agent 5-Chloro-N-((5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methylthiophene-2-carboxamide (BAY 59 - 7939): An Oral, Direct Factor Xa Inhibitor. *J. Med. Chem.* 2005, 48 (19), 5900–5908. LY-517717 (N-[(1R)-2-[4-(1-methyl-4-piperidinyl)-1-piperazinyl]-2-oxo-1-phenylethyl]-1H-indole-6-carboxamide, CAS number 313489-71-3, currently in Phase II): (c) LY-517717: A Novel Prophylactic for VTE following Major Orthopedic Surgery. *Prous*; 02 Jan 2006. (d) Saiah, E.; Soares, C. Small Molecule Coagulation Cascade Inhibitors in the Clinic. *Curr. Top. Med. Chem.* 2005, 5 (16), 1677–1695. DU-1766 (CAS number 912273-65-5, no structure available): (e) Daiichi Sankyo (JP): Phase II Clinical Trial. *Pharmaprojects*; 03 Apr 2006. (f) Cardiovasculars Take Centre Stage in Daiichi-Sankyo's R&D Plan. *Scrip*; 30 Mar 2006. Apixaban: (g) Apixaban Enters Phase II/III Trials as a Preventive Therapy for Venous and Pulmonary Thrombosis. *Prous*; 16 Sep 2005. DX-9065a ([S-(R*)]-7-(Aminoiminoethyl)- α -[4-[[1-(1-iminoethyl)-3-pyrrolidinyl]oxy]phenyl]-2-naphthalenepropanoic Acid, Monohydrochloride, Pentahydrate, CAS number 155204-81-2, currently in Phase II): (h) Kaiser, B. DX-9065a Daiichi. *Curr. Opin. Invest. Drugs* 2003, 4 (9), 1105–1112. (i) Daiichi Sankyo (JP): Phase II Clinical Trial. *Pharmaprojects*; Monday, 24 Jul 2006.
- (10) Watson, N. S.; Pass, M.; Patel, V. Preparation of 5-[2-(Pyridin-4-ylamino)ethoxy]benzamides as Thrombin Inhibitors. *PCT Int. Appl.* 1997, p 139, WO9722589A1; *Chem. Abstr.* 1997, 127, 121643.
- (11) (a) Ewing, W. R.; Becker, M. R.; Manetta, V. E.; Davis, R. S.; Pauls, H. W.; Mason, H.; Choi-Sledeski, Y. M.; Green, D.; Cha, D.; Spada, A. P.; Cheney, D. L.; Mason, J. S.; Maignan, S.; Guilloteau, J.-P.; Brown, K.; Colussi, D.; Bentley, R.; Bostwick, J.; Kasiewski, C. J.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. Design and Structure–Activity Relationships of Potent and Selective Inhibitors of Blood Coagulation Factor Xa. *J. Med. Chem.* 1999, 42, 3557–3571. (b) Watson, N. S.; Brown, D.; Campbell, M.; Chan, C.; Chaudry, L.; Convery, M. A.; Fenwick, R.; Hamblin, J. N.; Haslam, C.; Kelly, H. A.; King, N. P.; Kurtis, C. L.; Leach, A. R.; Manchee, G.; Mason, A. M.; Mitchell, C.; Patel, C.; Patel, V. K.; Senger, S.; Shah, G. P.; Weston, H. E.; Whitworth, C.; Young, R. J. Design and Synthesis of Orally Active Pyrrolidin-2-one-based Factor Xa Inhibitors. *Bioorg. Med. Chem. Lett.* 2006, 16, 3784–3788.
- (12) MacDonald, S. J. F.; Clarke, G. D. E.; Dowle, M. D.; Harrison, L. A.; Hodgson, S. T.; Inglis, G. G. A.; Johnson, M. R.; Shah, P.; Upton, R. J.; Walls, S. B. A Flexible, Practical, and Stereoselective Synthesis of Enantiomerically Pure *trans*-5-Oxohexahydropyrrolo[3,2-*b*]pyrroles (Pyrrolidine-*trans*-lactams), a New Class of Serine Protease Inhibitors, Using Acyliminium Methodology. *J. Org. Chem.* 1999, 64, 5166–5175.
- (13) (a) For 6a: Haginoya, N.; Kobayashi, S.; Komoriya, S.; Yoshino, T.; Suzuki, M.; Shimada, T.; Watanabe, K.; Hirokawa, Y.; Furugori, T.; Nagahara, T. Synthesis and Conformational Analysis of a Non-Aminine Factor Xa Inhibitor That Incorporates 5-Methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine as S4 Binding Element. *J. Med. Chem.* 2004, 47 (21), 5167–5182. (b) For 6c: Matier, W. L.; Comer, W. T.; Gomoll, A. W. Synthesis and Biological Properties of 5-Aryl-4H-1,2,4-thiadiazine 1,1-Dioxides. *J. Med. Chem.* 1974, 17 (5), 549–552. (c) For 6f: Choi-Sledeski, Y. M.; Kearney, R.; Poli, G.; Pauls, H.; Gardner, C.; Gong, Y.; Becker, M.; Davis, R.; Spada, A.; Liang, G.; Chu, V.; Brown, K.; Collussi, D.; Leadley, R., Jr.; Rebello, S.; Moxey, P.; Morgan, S.; Bentley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J.-P.; Mikol, V. Discovery of an Orally Efficacious Inhibitor of Coagulation Factor Xa which Incorporates a Neutral P1 Ligand. *J. Med. Chem.* 2003, 46 (5), 681–684. (d) For 6g: Ando, T.; Saito, Y.; Yamawaki, J.; Morisaki, H.; Sawada, M.; Yukawa, Y. 6-Methyl-2-naphthalenesulfonate (menasylate). New and Useful Leaving Group for Trifluoroacetylation. *J. Org. Chem.* 1974, 39 (16), 2465–2466.
- (14) For 6b, 6d, 6e, and 6j: Ewing, W. R.; Becker, M. R.; Choi-Sledeski, Y. M.; Pauls, H. W.; McGarry, D. G.; Davis, R. S.; Spada, A. Preparation of Heterocyclylsulfonylaminoxyrrrolidinylmethylbenzamides and Related Compounds as Factor Xa Inhibitors. 1998, p 36, U.S. Patent 5,731,315A; *Chem. Abstr.* 1998, 128, 243948.
- (15) (a) For 6k: Chan, C.; Hamblin, J. N.; Kelly, H. A.; King, N. P.; Mason, A. M.; Patel, V. K.; Senger, S.; Shah, G. P.; Watson, N. S.; Weston, H. E.; Whitworth, C.; Young, R. J. Preparation of 3-Sulfonylamino pyrrolidine-1-acetamides as Factor Xa Inhibitors. *PCT Int. Appl.* 2002, p 85, WO2002100830A; *Chem. Abstr.* 2002, 138, 39537. (b) For 6l: Choi-Sledeski, Y. M.; Pauls, H. W.; Barton, J. N.; Ewing, W. R.; Green, D. M.; Becker, M. R. Preparation of Sulfonic Acid or Sulfonylamino *N*-(Heteroalkyl)-azaheterocyclylamide Compounds as Inhibitors of Factor Xa. *PCT Int. Appl.* 1998, p 116, WO9825611A; *Chem. Abstr.* 1998, 129, 81744.
- (16) For 6o and 6p: Komoriya, S.; Haginoya, N.; Kobayashi, S.; Nagata, T.; Mochizuki, A.; Suzuki, M.; Yoshino, T.; Horino, H.; Nagahara, T.; Suzuki, M.; Isobe, Y.; Furugori, T. Design, Synthesis, and Biological Activity of Nonbasic Compounds as Factor Xa Inhibitors: SAR Study of S1 and Aryl Binding Sites. *Bioorg. Med. Chem.* 2005, 13 (12), 3927–3954.

- (17) For Boc-6s and Boc-6t: (a) Jia, Z. J.; Su, T.; Zuckett, J. F.; Wu, Y.; Goldman, E. A.; Li, W.; Zhang, P.; Clizbe, L. A.; Song, Y.; Bauer, S. M.; Huang, W.; Woolfrey, J.; Sinha, U.; Arfsten, A. E.; Hutchaleelaha, A.; Hollenbach, S. J.; Lambing, J. L.; Scarborough, R. M.; Zhu, B.-Y. *N,N*-Dialkylated 4-(4-Arylsulfonylpiperazine-1-carbonyl)-benzamides and 4-((4-Arylsulfonyl)-2-oxo-piperazin-1-ylmethyl)-benzamides as Potent Factor Xa Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14* (9), 2073–2078. (b) Ewing, W. R.; Becker, M. R.; Choi-Sledeski, Y. M.; Pauls, H. W.; He, W.; Condon, S. M.; Davis, R. S.; Hanney, B. A.; Spada, A. P.; Burns, C. J.; Jiang, J. Z.; Li, A.; Myers, M. R.; Lau, W. F.; Poli G.B. Substituted Piperazinone Derivatives and other Oxoazaheterocycli Compounds Useful as Factor Xa/IIa Inhibitors. PCT Int. Appl. 2001, p 460, WO2001007436A; *Chem. Abstr.* **2001**, *134*, 163059.
- (18) Yamamoto, T.; Zhou, Z.-H.; Kanbara, T.; Shimura, M.; Kizu, K.; Maruyama, T.; Nakamura, Y.; Fukuda, T.; Lee, B.-L.; Ooba, N.; Tomaru, S.; Kurihara, T.; Kaino, T.; Kubota, K.; Sasaki, S. π -Conjugated Donor–Acceptor Copolymers Constituted of π -Excessive and π -Deficient Arylene Units. Optical and Electrochemical Properties in Relation to CT Structure of the Polymer. *J. Am. Chem. Soc.* **1996**, *118*, 10389–10399.
- (19) (a) 5-Bromothiazole was prepared by monobromination of 2,5-dibromothiazole using a procedure in: Dondoni, A.; Mastellari, A. R.; Medici, A.; Negrini, E.; Pedrini, P. Synthesis of Stannylthiazoles and Mixed Stannylsilylthiazoles and their Use for a Convenient Preparation of Mono- and Bishalothiazoles. *Synthesis* **1986**, *9*, 757–760. (b) Urch, C. J.; Salmon, R.; Lewis, T.; Godfrey, C. R. A.; Clough, M. S. Preparation of 3-Aryl-3-cyano-8-azabicyclo[3.2.1]octanes as Insecticides and Acaricides. PCT Int. Appl. **1996**, p 77, WO9637494A1; *Chem. Abstr.* **1997**, *126*, 104021.
- (20) Saramet, I.; Banciu, M. D.; Draghici, C. Synthesis and Characterization of Some 5-Substituted-3-Mercapto-1,2,4-Triazoles. *Rev. Roum. Chim.* **1991**, *36*, 135–144.
- (21) (a) Attempts to prepare the sulfonyl chloride **6q** by oxidation of 6-chloro-1,3-benzothiazole-2(3H)-thione with chlorine in acetic acid followed by sulfonylation with **2** gave very little desired sulfonamide (**1q**). (b) Recently, Vedejs and Kongkittigam have shown that the stability of benzothiazole-2-sulfonyl chloride in solution could be extended with 2,6-di-*t*-butyl-4-methylphenol: Vedejs, E.; Kongkittigam, C. Solution-Phase Synthesis of a Hindered *N*-Methylated Tetrapeptide Using Bts-Protected Amino Acid Chlorides: Efficient Coupling and Methylation Steps Allow Purification by Extraction. *J. Org. Chem.* **2000**, *65*, 2309–2318.
- (22) (a) Pays, M.; Beljeun, M. Heterocyclic Hydrazines and Hydrazones. I. Synthesis of 2-Hydrazinobenzothiazoles, 2-Hydrazonaphtho[1,2-*d*] and -[2,1-*d*]thiazoles and 3-Methyl-2-benzothiazolinone and 3-Methyl-2-naphthothiazolinone Hydrazones. *Bull. Soc. Chim. Fr.* **1973**, *11* (Pt. 2), 3044–3051. (b) Baudin, J. B.; Hareau, G.; Julia, S. A.; Lorne, R.; Ruel, O. Stereochemistry of Direct Olefin Formation from Carbonyl Compounds and Lithiated Heterocyclic Sulfones. *Bull. Soc. Chim. Fr.* **1993**, *130*, 856–878.
- (23) Masuda, Y.; Hoshi, M.; Arase, A. Simple Stereospecific Syntheses of (*E*)-1-Chloro(or bromo)alk-1-enes from Alk-1-ynes via Hydroboration. *J. Chem. Soc., Perkin Trans. 1* **1992**, *21*, 2725–2726.
- (24) Maignan, S.; Guilleateau, J.-P.; Choi-Sledeski, Y. M.; Becker, M. R.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Miko, V. Molecular Structures of Human Factor Xa Complexed with Ketopiperazine Inhibitors: Preference for a Neutral Group in the S1 Pocket. *J. Med. Chem.* **2003**, *46*, 685–690.
- (25) Mederski, W. W. K. R.; Cezanne, B.; van Amsterdam, C.; Buhring, K. U.; Dorsch, D.; Gleitz, J.; Marz, J.; Tsaklakidis, C. Chlorothiophenecarboxamides as P1 Surrogates of Inhibitors of Blood Coagulation Factor Xa. *Bioorg. Med. Chem. Lett.* **2004**, *14* (23), 5817–5822.
- (26) (a) Gabriel, B.; Stubbs, M. T.; Bergner, A.; Hauptmann, J.; Bode, W.; Stuerzebecher, J.; Moroder, L. Design of Benzamidine-Type Inhibitors of Factor Xa. *J. Med. Chem.* **1998**, *41* (22), 4240–4250. (b) Katakura, S.; Nagahara, T.; Hara, T.; Iwamoto, M. A Novel Factor Xa Inhibitor: Structure–Activity Relationships and Selectivity between Factor Xa and Thrombin. *Biochem. Biophys. Res. Commun.* **1993**, *197*, 965–972. (c) Nar, H.; Bauer, M.; Schmid, A.; Stassen, J.-M.; Wienen, W.; Pripke, H. W. M.; Kauffmann, I. K.; Ries, U. J.; Haul, N. H. Structural Basis for Inhibition Promiscuity of Dual Specific Thrombin and Factor Xa Blood Coagulation Inhibitors. *Structure* **2001**, *9*, 29–37. (d) Fevig, J. M.; Pinto, D. J.; Han, Q.; Quan, M. L.; Pruitt, J. R.; Jacobson, I. C.; Galemno, R. A., Jr.; Wang, S.; Orwat, M. J.; Bostrom, L. L.; Knabb, R. M.; Wong, P. C.; Lam, P. Y. S.; Wexler, R. R. Synthesis and SAR of Benzamidine Factor Xa Inhibitors Containing a Vicinally-Substituted Heterocyclic Core. *Bioorg. Med. Chem. Lett.* **2001**, *11* (5), 641–645.
- (27) Chung, C.-W. Personal communication.
- (28) Levell, J. R.; Pauls, H. W.; Choi, Y.-M.; Ewing, W. R.; Becker, M. R.; McGarry, D. G.; Gardner, C. J.; Poli, G. B.; Czekaj, M.; Gong, Y.; Li, A.-W.; Davies, R.; Jiang, J. Z.; Chu, F.-H. V.; Maignan, S.; Mikol, V.; Liang, G.-Y. *Aminoquinazoline, Aminoquinoline and Aminoisoquinoline Substituted 3(R)- and 3(S)-Aminopyrrolidin-2-one Derived Factor Xa Inhibitors*; 226th ACS National Meeting, New York, United States, September 7–11, 2003, MEDI-077 (for example, RPR256580 in this poster).
- (29) Raos, G.; Famulari, A.; Marcon, V. Computational Reinvestigation of the Bithiophene Torsion Potential. *Chem. Phys. Lett.* **2003**, *379*, 364–372.
- (30) Details of ab initio calculations and selected electrostatic potential maps of *N*-methyl chloro(hetero)arylsulfonamides, truncated structures of **1**, are available in Supporting Information.
- (31) (a) Cappelli, A.; Giorgi, G.; Anzini, M.; Vomero, S.; Ristori, S.; Rossi, C.; Donati, A. Characterization of Persistent Intramolecular C–H...X(N,O) Bonds in Solid State and Solution. *Chem.—Eur. J.* **2004**, *10* (13), 3177–3183. (b) Manikandan, K.; Ramakumar, S. The Occurrence of C–H...O Hydrogen Bonds in α -Helices and Helix Termini in Globular Proteins. *Proteins: Struct., Funct., Bioinf.* **2004**, *56* (4), 768–781. (c) Pierce, A. C.; Sandretto, K. L.; Bemis, G. W. Kinase Inhibitors and the Case for C–H...O Hydrogen Bonds in Protein–Ligand Binding. *Proteins* **2002**, *49* (4), 567–576.
- (32) Senger, S.; Convery, M. A.; Chan, C.; Watson, N. S. Arylsulfonamides: A Study of the Relationship between Activity and Conformational Preferences for a Series of Factor Xa inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16* (22), 5731–5735.
- (33) Valko, K.; Nunhuck, S.; Bevan, C.; Abraham, M. H.; Reynolds, D. P. Fast Gradient HPLC Method to Determine Compounds Binding to Human Serum Albumin. Relationships with Octanol/Water and Immobilized Artificial Membrane Lipophilicity. *J. Pharm. Sci.* **2003**, *92* (11), 2236–2248.
- (34) Toomey, J. R.; et al. Manuscript in preparation, 2007.
- (35) RPR128515 is (α R)-3-(aminoiminomethyl)- α -[1(R)-1-[[3'-(aminomethyl)[1,1'-biphenyl]-4-yl]carbonyl]amino]ethyl]-benzenepropanoic acid, methyl ester and has a CAS number of 296761-71-2. Maignan, S.; Guilleateau, J.-P.; Pouzieux, S.; Choi-Sledeski, Y. M.; Becker, M. R.; Klein, S. I.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Mikol, V. Crystal Structures of Human Factor Xa Complexed with Potent Inhibitors. *J. Med. Chem.* **2000**, *43*, 3226–3232.
- (36) Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. *Methods Enzymol.* **1997**, *276*, 307–326.
- (37) Leslie, A. G. W. *Recent Changes to the MOSFLM Package for Processing Film and Image Plate Data*; Joint CCP4 and ESF-EAMCB Newsletter on Protein Crystallography; Daresbury Laboratory: Warrington, U.K., 1992; No. 26.
- (38) Collaborative Computational Project, Number 4. The CCP4 Suite: Programs for Protein Crystallography. *Acta Crystallogr.* **1994**, *D50*, 760–763.
- (39) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of Macromolecular Structures by the Maximum-Likelihood Method. *Acta Crystallogr.* **1997**, *D53*, 240–255.
- (40) Santoro, S. A.; Eby, C. S. Laboratory Evaluation of Hemostatic Disorders. In *Hematology—Basic Principles and Practice*; Benz, E. J., Cohen, H. J., Furie, B., Hoffman, R., McGlave, P., Shattil, S. J., Silberstein, L. E., Eds.; Churchill Livingstone: Philadelphia, 2000; pp 1841–1842.
- (41) The effect of various concentrations of test compound on the coagulation parameters was analyzed using a mathematical model which represents an alternative to an IC₅₀ curve in which there is no symmetric side for large values of *X*. This model is based on the modified Freundlich model with proportional weighting (larger values have less weight): $y = a + (b \times (X^c))$, where *y* is the fold-increase in time to clot formation, *a* is the *Y* offset (initial parameter estimate of 1), *b* is the rate scalar and orientation, *X* is the compound concentration, and *c* is the power factor.
- (42) These molecular graphics were generated using DeLano's PyMOL software available from <http://www.pymol.org>: DeLano, W. L. *PyMOL*, version 0.98; a molecular graphics system; DeLano Scientific LLC: Palo Alto, CA, 2002.

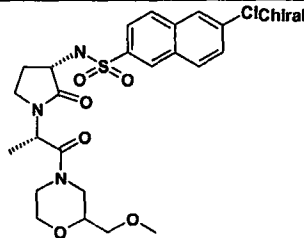
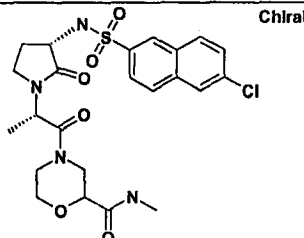
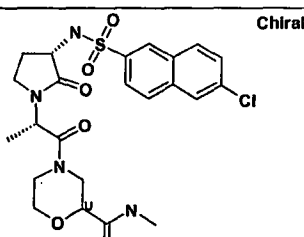
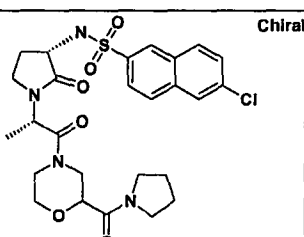
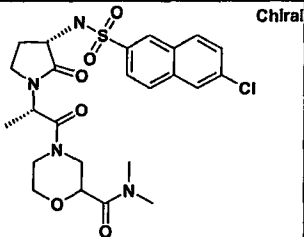
JM060870C

22
212
[Signature]

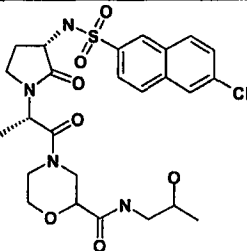
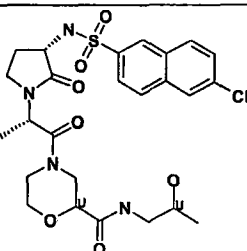
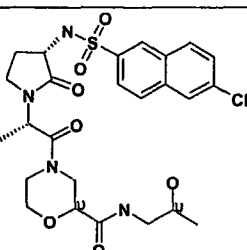
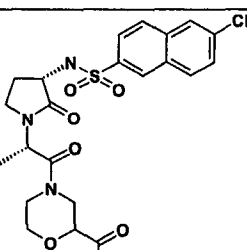
ENSAYOS DE ACTIVIDAD BIOLÓGICA

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
1	GW686675X	0.0436	3.13388046		6-Chloro-N-((3S)-1-((1S)-1-methyl-2-morpholin-4-yl)-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
2	GW697127X	0.413	0.24143056		6-Chloro-N-((3S)-1-((1S)-2-(2,0-dimethylmorpholin-4-yl)-1-methyl-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
3	GW699479X	0.143	0.01381342		6-Chloro-N-((3S)-1-((1S)-1-methyl-2-(3-methylmorpholin-4-yl)-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
4	GW725647X	2.415	62.2068748		6-Chloro-N-((3S)-1-((1S)-1-methyl-2-oxo-2-[3-(pyrrolidin-1-ylcarbonyl)morpholin-4-yl]ethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
5	GW725822X	0.911	0.439565		6-Chloro-N-[(3S)-1-((1S)-1-methyl-2-{2-[(methylsulfonyl)methyl]morpholin-4-yl}-2-oxoethyl)-2-oxopyrrolidin-3-yl]naphthalene-2-sulfonamide

213
213
213

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
6	GW725823X	0.851	0.17339658		6-Chloro-N-((3S)-1-((1S)-2-[2-(methoxymethyl)morpholin-4-yl]-1-methyl-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
7 & 8	GW767633X	0.415			4-[(2S)-2-((3S)-3-[(6-Chloro-2-naphthyl)sulfonyl]amino)-2-oxopyrrolidin-1-yl]propanoyl]-N-methylmorpholine-2-carboxamide [Isomer 1 and Isomer 2]
	GW767690X	2.287			
9	GW767634X	5.436	0.7953355		6-Chloro-N-((3S)-1-((1S)-1-methyl-2-oxo-2-[2-(pyrrolidin-1-ylcarbonyl)morpholin-4-yl]ethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
10	GW767635X	7.989	4.773642		4-[(2S)-2-((3S)-3-[(6-Chloro-2-naphthyl)sulfonyl]amino)-2-oxopyrrolidin-1-yl]propanoyl]-N,N-dimethylmorpholine-2-carboxamide

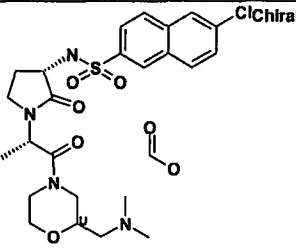
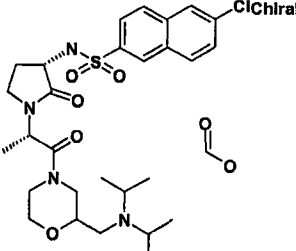
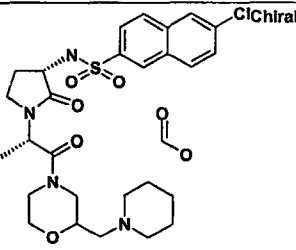
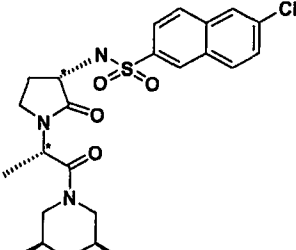
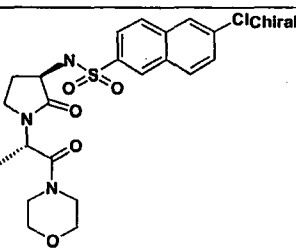
24/2/14
[Signature]

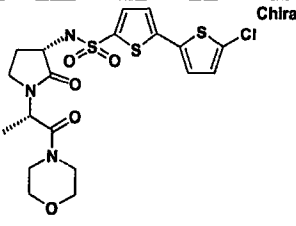
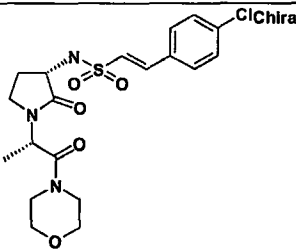
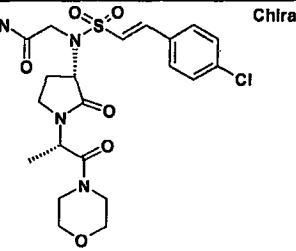
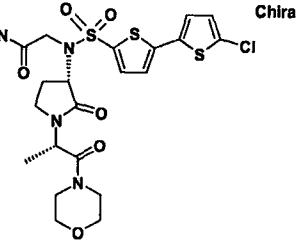
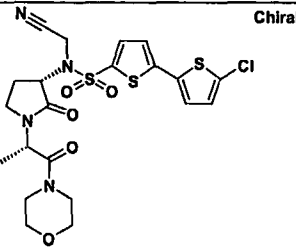
Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
11, 12 & 13	GW767693X	0.5	1.95053526		4-[(2S)-2-((3S)-3-[[[6-Chloro-2-naphthyl)sulfonyl]amino]-2-oxopyrrolidin-1-yl)propanoyl]-N-(2-hydroxypropyl)morpholine-2-carboxamide [Isomer 1, Isomer 2 and Isomer 3]
	GW767694X	0.618			
	GW767695X	2.195			
14	GW767729X	7.024	2.847883		4-[(2S)-2-((3S)-3-[[[6-Chloro-2-naphthyl)sulfonyl]amino]-2-oxopyrrolidin-1-yl)propanoyl]-N,N-diisopropylmorpholine-2-carboxamide

215
215
215

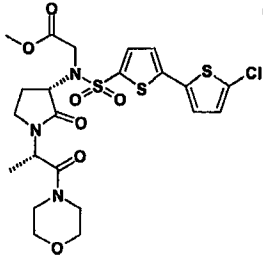
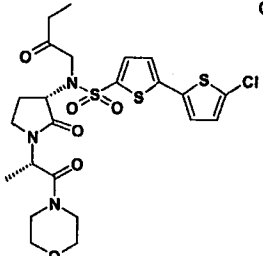
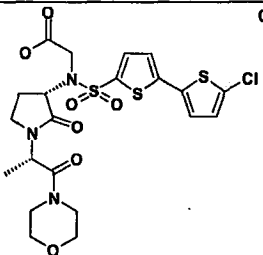
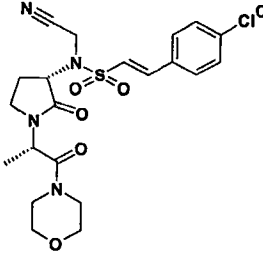
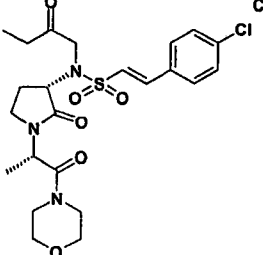
Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
15	GW767739X	3.404	58.1578596		6-Chloro-N-((3S)-1-((1S)-1-methyl-2-oxo-2-[2-(piperidin-1-ylcarbonyl)morpholin-4-yl]ethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
16	GW770608A	2.413	0.7900734		6-Chloro-N-[(3S)-1-((1S)-1-methyl-2-oxo-2-[(methylamino)methyl]morpholin-4-yl)-2-oxoethyl)-2-oxopyrrolidin-3-yl]naphthalene-2-sulfonamide formate
17	GW770609A	1.059	28.3800448		6-Chloro-N-((3S)-1-((1S)-1-methyl-2-oxo-2-[2-(pyrrolidin-1-ylmethyl)morpholin-4-yl]ethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide formate
18	GW770610A	0.638	0.3687208		6-Chloro-N-((3S)-1-((1S)-2-((2-hydroxypropyl)amino)methyl)morpholin-4-yl)-1-methyl-2-oxoethyl)-2-oxopyrrolidin-3-yl]naphthalene-2-sulfonamide formate
19 & 20	GW770611A	1.278			6-Chloro-N-[(3S)-1-((1S)-2-((dimethylamino)methyl)morpholin-4-yl)-1-methyl-2-oxoethyl)-2-oxopyrrolidin-3-yl]naphthalene-2-sulfonamide formate [Isomer 1 and Isomer 2]

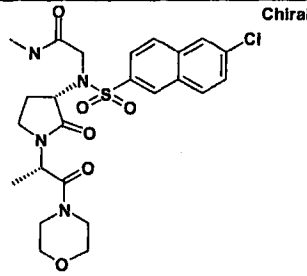
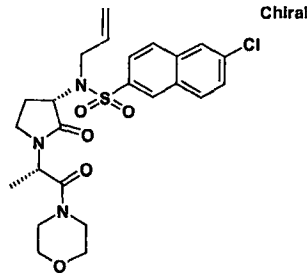
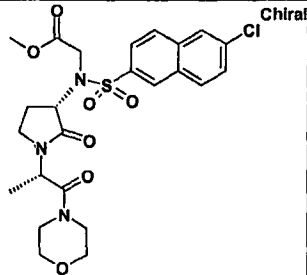
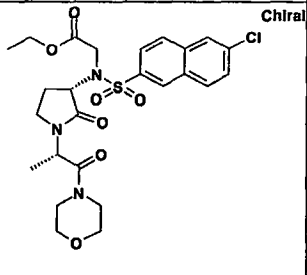
216
216
311

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
	GW770614A	2.197			
21	GW770612A	5.498	5.3327		6-Chloro-N-((3S)-1-((1S)-2-((diisopropylamino)methyl)morpholin-4-yl)-1-methyl-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide formate
22	GW770613A	0.802	5.9912896		6-Chloro-N-((3S)-1-((1S)-1-methyl-2-oxo-2-[2-(piperidin-1-ylmethyl)morpholin-4-yl]ethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide formate
23	GW797041X	0.114	0.1246405		6-Chloro-N-((3S)-1-((1S)-2-((2R,6S)-2,6-dimethylmorpholin-4-yl)-1-methyl-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide
24	GW781427X	0.371	0.0524807		6-Chloro-N-((3R)-1-((1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
25	GW702814X	0.023	0.00567716		5'-Chloro-N-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)-2,2'-bithiophene-5-sulfonamide
26	GW702821X	0.10	0.0182924		(E)-2-(4-Chlorophenyl)-N-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)ethanesulfonamide
27	GW779300X	0.054			N2-[(E)-2-(4-Chlorophenyl)ethenyl]sulfonyl-N2-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)glycinamide
28	GW779304X	0.014			N2-[(5'-Chloro-2,2'-bithien-5-yl)sulfonyl]-N2-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)glycinamide
29	GW702830X	0.028			5'-Chloro-N-(cyanomethyl)-N-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)-2,2'-bithiophene-5-sulfonamide

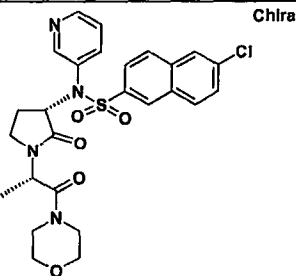
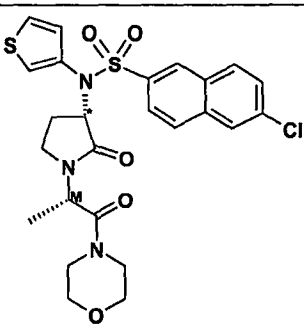
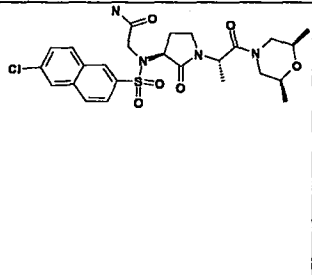
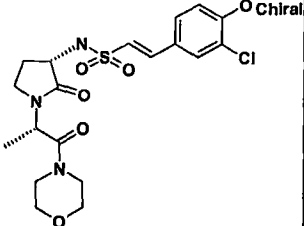
218
218
82

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
30	GW702834X	0.016			Methyl N-[(5'-chloro-2,2'-bithien-5-yl)sulfonyl]-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]glycinate
31	GW702836X	0.017			5'-Chloro-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]-N-(2-oxobutyl)-2,2'-bithiophene-5-sulfonamide
32	GW712430X	0.024	0.00388908		N-[(5'-Chloro-2,2'-bithien-5-yl)sulfonyl]-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]glycine
33	GW702839X	0.088			(E)-2-(4-Chlorophenyl)-N-(cyanomethyl)-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]ethenesulfonamide
34	GW702843X	0.06			(E)-2-(4-Chlorophenyl)-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]-N-(2-oxobutyl)ethenesulfonamide

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
53	GW727054X	Data not in Database			N2-[(6-Chloro-2-naphthyl)sulfonyl]-N1-methyl-N2-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]glycinamide
54	GW702549X	0.067			N-Allyl-6-chloro-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]naphthalene-2-sulfonamide
55	GW699471X	0.011			Methyl N-[(6-chloro-2-naphthyl)sulfonyl]-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]glycinate
56	GW727038X	0.358			Ethyl N-[(6-chloro-2-naphthyl)sulfonyl]-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]glycinate

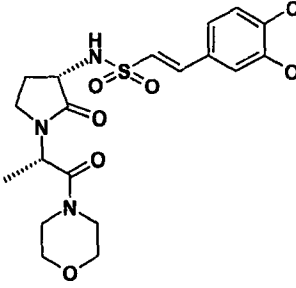
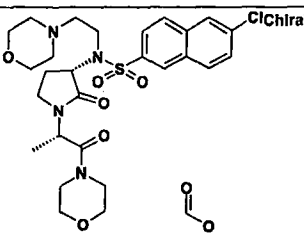
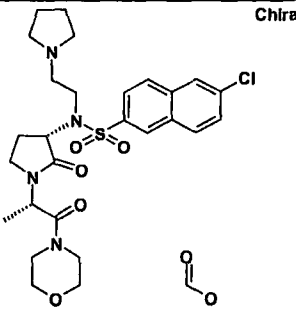
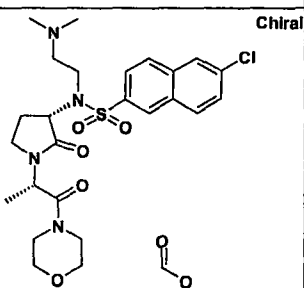
228
228

AR

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
77	GW791105X	0.238	0.141254		6-Chloro-N-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)-N-pyridin-3-ynaphthalene-2-sulfonamide
78	GW781538X	0.162			6-Chloro-N-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)-N-thien-3-ynaphthalene-2-sulfonamide
79	GL3234-28	0.038			N2-[(6-Chloro-2-naphthyl)sulfonyl]-N2-((3S)-1-[(1S)-2-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-1-methyl-2-oxoethyl]-2-oxopyrrolidin-3-yl)glycinamide
80	GW817109X	26.95			(E)-2-(3-Chloro-4-hydroxyphenyl)-N-((3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl)ethenesulfonamide

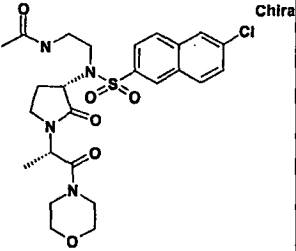
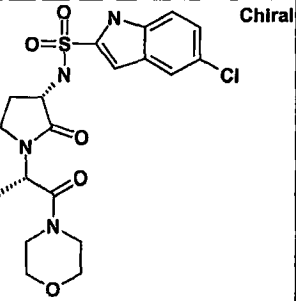
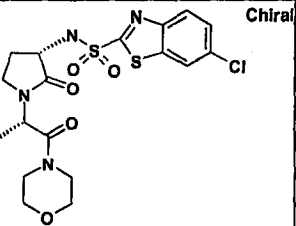
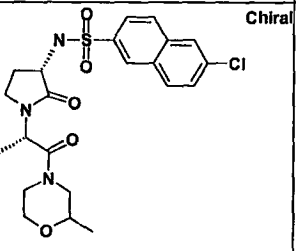
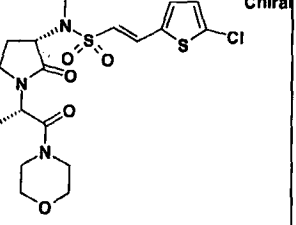
229
229

AB

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
81	GW823992X	1.94	8.793		(E)-2-(4-Chloro-3-hydroxyphenyl)-N-((3S)-1-((1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl)-2-oxopyrrolidin-3-yl)ethanesulfonamide
82	GW796927A	0.008			6-Chloro-N-((3S)-1-((1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl)-2-oxopyrrolidin-3-yl)-N-(2-morpholin-4-ylethyl)naphthalene-2-sulfonamide formate
83	GW803237A	0.012			6-Chloro-N-((3S)-1-((1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl)-2-oxopyrrolidin-3-yl)-N-(2-pyrrolidin-1-ylethyl)naphthalene-2-sulfonamide formate
84	GW803236A	0.024			6-Chloro-N-[2-(dimethylamino)ethyl]-N-((3S)-1-((1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl)-2-oxopyrrolidin-3-yl)naphthalene-2-sulfonamide formate

230
230

230

Example No.	Compound No.	Chromogenic IC50 MEAN uM	Flint IC50 MEAN uM	Structure	
85	GW803235X	0.057			N-[2-((6-Chloro-2-naphthyl)sulfonyl){(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}amino)ethyl]acetamide
86	GW805568X	0.237			5-Chloro-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]-1H-indole-2-sulfonamide
87	GW838607X R7815/80/2	25.053	6.74797		6-Chloro-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]-1,3-benzothiazole-2-sulfonamide
88	GW689803X	0.567	0.08305055		6-Chloro-N-[(3S)-1-[(1S)-1-methyl-2-(2-methylmorpholin-4-yl)-2-oxoethyl]-2-oxopyrrolidin-3-yl]naphthalene-2-sulfonamide
89	GW833146X	0.405	0.0446684		(E)-2-(5-Chlorothiophen-2-yl)-N-methyl-N-[(3S)-1-[(1S)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl]ethenesulfonamide