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Identification of potent and reversible cruzipain inhibitors for the treatment of Chagas disease

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ABSTRACT

Identification of potent and reversible cruzipain inhibitors for the treatment of Chagas disease is described. The identified inhibitors bearing an amino nitrile warhead in P1 exhibit low nanomolar in vitro potency against cruzipain. Further SAR in P2 portion led to the identification of compounds, such as **26**, that have a unique selectivity profile against other cysteine proteases and offering new opportunities for safer treatment of Chagas disease.

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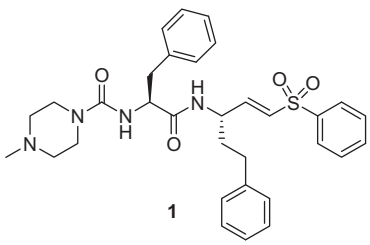
Chagas disease is a parasitic trypanozomial infection endemic to South America where an estimated 10–12 million individuals are afflicted and over 15,000 deaths are incurred each year.[1] The causative agent, *Trypanosoma cruzi*, is transmitted by an insect vector, blood transfusions, maternal vertical transmission and, rarely, oral infection. There are currently no FDA-approved drugs for the treatment of Chagas infections and development of effective therapies is currently a major focus of organizations such as the Drugs for Neglected Diseases initiative (DNDi) and OneWorld Health (iOWH).[1–3]

While numerous approaches for the treatment of Chagas are under consideration, one family of enzymes, cysteine proteases of the cruzipain family, is considered to be a promising target.[4–6] The cruzipain enzymes have been identified as critical to the viability of the parasite,[7] and the ability of the trypanosomal form to invade tissues[8,9] and evade the host immune response.[10,11] For these reasons, cruzipain inhibitors have been proposed as potential therapies for *T. cruzi* infection. Elegant studies with the irreversible inhibitor K-777 (**1**, Fig. 1) have demonstrated the anti-parasitic activity of a cruzipain inhibitor.[12] K-777 has also shown efficacy in pre-clinical models of Chagas disease.[13,14] Other efforts toward identifying irreversible covalent inhibitors have also been reported by several research groups in recent years.[15–18] These inhibitors contain electrophilic groups (e.g., vinyl sulfonate, tetrafluorophenoxymethyl ketone, epoxy ketone) that serve as a 'warhead' that binds to cruzipain through a covalent bond to the active site cysteine. Unfortunately, irreversible inhibitors may also inhibit host protease enzymes and possibly cause unacceptable side effects due to off-target activity and/or autoimmune and inflammatory phenomena associated with protein covalent binding.[7,19] Thus, the identification of reversible and specific inhibitors of cruzipain would be of particular interest for the development of a safer therapeutic agent for Chagas disease. However, only rare examples of reversible covalent inhibitors of cruzipain have been reported[19] and specificity, if reported at all, is poor.

Cruzipain is a cysteine protease enzyme, closely related to the cathepsin family of enzymes.[20] Recently, the discovery of reversible cathepsin K (Cat K) inhibitors has been reported. Odanacatib (**2**) (Fig. 2), a cathepsin K inhibitor having an amino nitrile warhead,[21] is currently in Phase III clinical studies.[22] This nitrile



1

Figure 1. Structure of K-777 (**1**).

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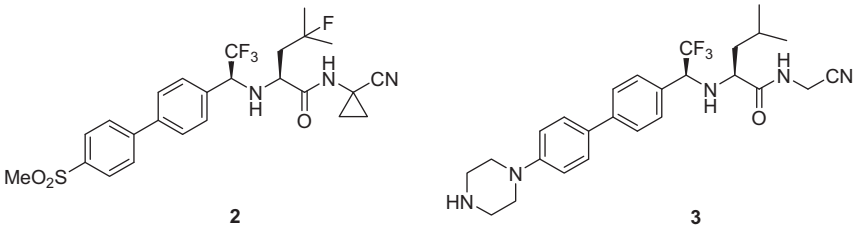


Figure 2. Structure of odonacatib (**2**) and of basic cruzipain inhibitor **3**.

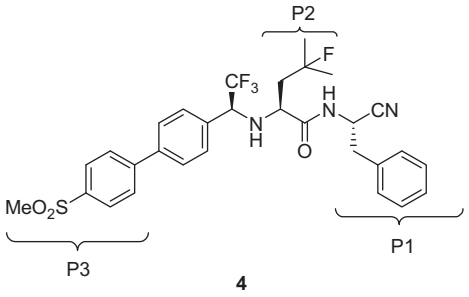
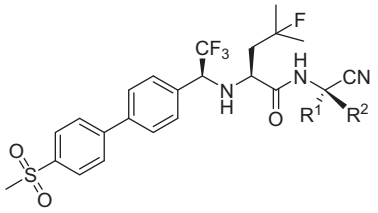


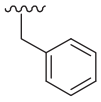
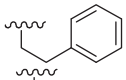
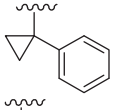
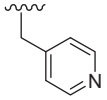
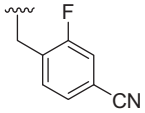
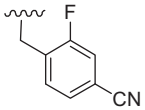
Figure 3. Structure of non-basic cruzipain inhibitor **4**.

moiety forms a reversible thioimidate[21] with the active site cysteine of cathepsin K. Given the structural similarities between cruzipain and the cathepsins, a drug discovery effort was mounted to identify an amino nitrile-containing cruzipain inhibitor.

Focused screening of our cysteine protease inhibitors collection identified compound **3**[23] (Fig. 2) as a potent cruzipain inhibitor with an IC_{50} of 1.8 nM. In vitro studies with *T. cruzi* and a preliminary in vivo proof-of-concept study in mice demonstrated that this basic cruzipain inhibitor significantly reduces the blood parasitemia in mice infected with the Brazilian strain of *T. cruzi* without any evidence of toxicity.[24] However the basic nature of **3** is likely associated with lysosomotropism in which the accumulation of the compound in lysosomes could lead to unexpected activity

Table 1
P1 SAR: IC_{50} values for **4**–**11**



Compds	R^1	R^2	Cruz. IC_{50}[a] (nM)	Cat L IC_{50}[a] (nM)	Cat B IC_{50}[a] (nM)	Cat F IC_{50}[a] (nM)
4		H	8	966	122	67
5	H	H	183	3819	312	547
6		H	130	2628	668	654
7		H	510	>10,000	397	3242
8		H	>10,000	>10,000	>10,000	>10,000
9		H	6	219	79	12
10		H	1	49	9	4
11	H		17	711	260	76

[a] IC_{50} values represent an average of at least three titrations. Standard deviations for these assays were typically within 35% of the IC_{50} values. See Refs. 28,29 for assay conditions.

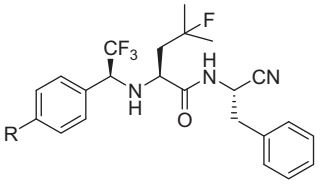
against human cathepsins such as Cat B, L, and F.[25] This prompted us to develop a non-basic inhibitor of cruzipain for safer treatment of Chagas disease. Fortunately, among the compounds tested in our screening campaign we also identified the non-basic inhibitor **4** (Fig. 3) as a potent inhibitor of cruzipain. However, the potency, selectivity profile against human cathepsins and pharmacokinetic profile in mice ($T_{1/2}$ <30 min) of **4** were sub-optimal and optimization of these parameters became the main objective of the SAR studies conducted with this new series of compounds.

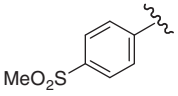
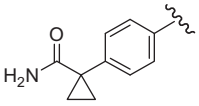
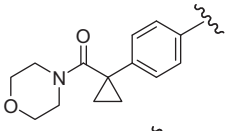
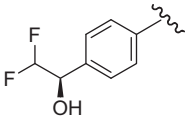
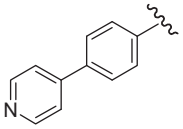
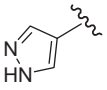
The scaffold of this series was divided into three portions that we identified as P1, P2, and P3 (Fig. 3). SAR effort around the P1 portion highlighted the importance of this substituent for potency against cruzipain (Table 1). Presence of the phenylalanine-like motif in P1, was clearly important. Indeed, the absence of P1 group or its replacement by a group smaller than phenylalanine, as in **5** and **6**, resulted in a >10-fold loss of potency against cruzipain. Homologation of the phenylalanine moiety in compound **7** was also found to be detrimental for potency, and attempts to further functionalize the phenylanine's methylene (**8**) were also unsuccessful. Interestingly, heterocycles were found to be tolerated in P1, but did not contribute to additional potency or selectivity as observed with compound **9**. Further exploration of the substitution pattern on the phenylalanine moiety led to the discovery of the 4-cyano-2-fluoro-phenylalanine **10** which exhibited an eightfold increase in potency over the unsubstituted phenylalanine **4**. In addition, the synthesis of the *R* isomer (**11**) demonstrated the importance of having the *S* stereochemistry in P1 for potency.

Given the minimal effect that P1 modifications had on the selectivity of cruzipain inhibitors over human cathepsins, we evaluated the SAR in P3 to explore the potential to improve the selectivity profile of our compounds. The ease of the chemistry in this region allowed the evaluation of a wide range of moieties in P3. The most potent analogs were found to share a common *para*-substituted biphenyl motif in P3. As shown in Table 2, most of the P3 groups introduced were well tolerated (**12–16**). However, none of these changes contributed to a significant gain of potency or selectivity compared to the 4-methanesulfonyl biphenyl moiety starting point. Nonetheless, the variety of functional groups being accepted in P3 could be very useful to modulate the physicochemical properties and the metabolic stability of inhibitors.

At this point, we knew very little about the SAR around the P2 portion of the series. However, previous studies in the Cat K program had indicated that this region was instrumental in achieving selectivity amongst the cathepsins.[21] Indeed, this synthetically more demanding portion represented our last option to improve the selectivity of this series. Replacement of the fluoroleucine in **4** by a simple leucine (**17**) resulted in a 16-fold increase in potency (Table 3). However, inhibitor **17** was found to be almost equipotent on Cat F and was metabolically unstable due to methine oxidation of the leucine.[21] The cyclopropylalanine analog **18** and phenylalanine analog **20** were found to be equipotent to **4**, but did not provide additional selectivity. To improve the metabolic stability of leucine analog **17**, the dichloro alanine analog **19** was prepared. This halogenated derivative exhibited sub-nanomolar potency

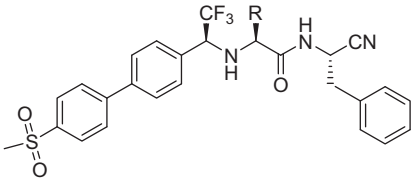
Table 2
P3 SAR: IC_{50} values for **12–16**

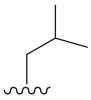
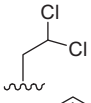
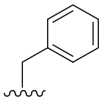


Compds	R	Cruz. IC_{50}[a] (nM)	Cat L IC_{50}[a] (nM)	Cat B IC_{50}[a] (nM)	Cat F IC_{50}[a] (nM)
4		8	966	122	67
12		3	426	27	37
13		3	1084	64	22
14		8	1086	48	64
15		8	436	54	51
16		33	379	59	24

[a] IC_{50} values represent an average of at least three titrations. Standard deviations for these assays were typically within 35% of the IC_{50} values. See Refs. 28,29 for assay conditions.

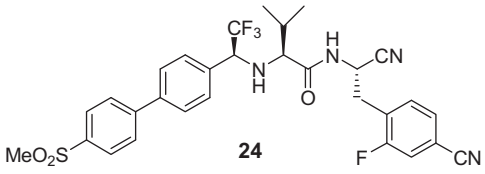
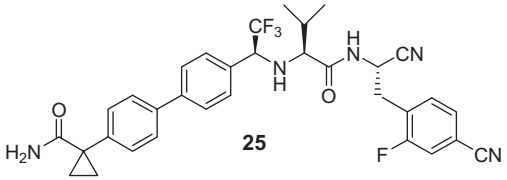
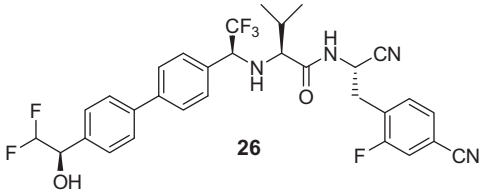
Table 3
P2 SAR: IC_{50} values for **17–23**



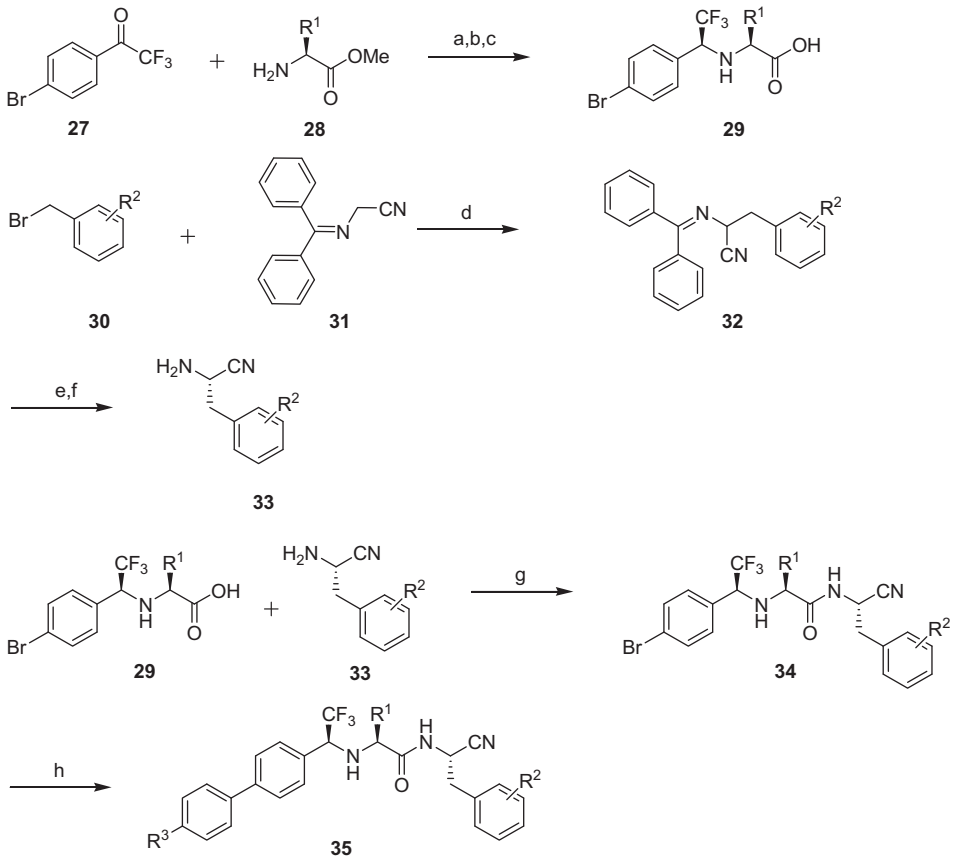
Compds	R	Cruz. IC_{50}[a] (nM)	Cat L IC_{50}[a] (nM)	Cat B IC_{50}[a] (nM)	Cat F IC_{50}[a] (nM)
4		8	966	122	67
17		0.2	117	163	0.3
18		16	5246	231	147
19		0.1	42	383	4
20		7	458	577	769
21		2	631	433	174
22		7	751	68	80
23		380	>10,000	>10,000	>10,000

[a] IC_{50} values represent an average of at least three titrations. Standard deviations for these assays were typically within 35% of the IC_{50} values. See Refs. 28,29 for assay conditions.

Table 4
Best P1, P2, and P3 matches: IC_{50} values for **24–26**

Compds	Cruz. IC_{50}[a] (nM)	Cat L IC_{50}[a] (nM)	Cat B IC_{50}[a] (nM)	Cat F IC_{50}[a] (nM)	Cat S IC_{50}[a] (nM)	Cat K IC_{50}[a] (nM)	Cat V IC_{50}[a] (nM)
 **24**	0.2	121	143	23	169	0.9	28
 **25**	0.2	98	41	16	408	3	79
 **26**	0.4	1060	87	56	322	2	69

[a] IC_{50} values represent an average of at least three titrations. Standard deviations for these assays were typically within 35% of the IC_{50} values. See Refs. 28,29 for assay conditions.



Scheme 1. General synthesis of compounds. Reagents and conditions: (a) K_2CO_3, MeOH, 50 °C; (b) $Zn(BH_4)_2$, MeCN, −40 °C; (c) 1 N HCl; (d) NaH, DMF, 0 °C; (e) AcOH, H_2O, THF, 40 °C; (f) HPLC chiral resolution; (g) HATU, 2,6-lutidine, DMF, 0 °C; (h) $ArB(OH)_2$, $Pd(dppf)Cl_2$, Na_2CO_3, DMF, 90 °C

against cruzipain (**17**), but exhibited poor selectivity against Cat F. This unnatural amino acid also represented a synthetic challenge, especially for large scale synthesis. Fortunately, the replacement of the fluoroleucine in P2 by a valine afforded the potent and selective compound **21**. With a potency of 2 nM and an encouraging selectivity profile of >80-fold against human cathepsins L, B, and F, the identification of **21** confirmed the potential of P2 SAR to modulate the selectivity against human cathepsins. However, the SAR around the valine in P2 was found to be very tight: replacing the isopropyl with a cyclopropyl (**22**) or a *t*-butyl group (**23**) resulted in compounds significantly less potent and/or less selective than **21**.

Due to its unique potency and selectivity profile, valine was judged the optimal P2 substituent and the compounds combining the best P1 and P3 portions were prepared. From this exercise, three compounds (**24–26**, Table 4) were found to show an excellent profile with sub-nanomolar potency on cruzipain and selectivity >80-fold against Cat L, B, F, S, and V. The low selectivity of these compounds against Cat K was not judged to be a significant development issue, as a selective Cat K inhibitor in clinical trials has shown an excellent safety profile.[22] The pharmacokinetic profile of compounds **24–26** was evaluated and **26** was determined to have the best profile with moderate bioavailability in rats and dogs ($F = 20\%$ and 30%, respectively) and good half-lives in both species (5.2 and 4.6 h, respectively). In addition, the difluoromethylcarbinol moiety enclosed within the P3 portion of compound **26** has demonstrated its potential to deliver compounds with suitable profile for drug development.[26] The overall profile of **26** is superior to any other known inhibitor of cruzipain and represents a great opportunity to evaluate a selective and reversible cruzipain inhibitor for the treatment of Chagas disease.

The general synthetic route to prepare compounds is outlined in Scheme 1. The P2 portion was first prepared through a stereoselective reductive amination[27] of the amino ester (**28**) with 1-(4-bromophenyl)-2,2,2-trifluoroethanone (**27**) to yield acid **29** with diastereomeric ratio >10:1 in favor of the desired diastereomer. The P1 aminonitrile was prepared by the alkylation of (diphenylmethylidene)amino acetonitrile with the substituted benzylic bromide (**30**). Hydrolysis of the resulting imine **32** yielded the racemic amino nitrile which was then resolved by chiral HPLC to yield the desired pure *S* isomer (**33**). Both P1 and P2 portions were then coupled to afford the bromophenyl intermediate **34**. The use of a Suzuki reaction with an appropriate aryl boronic acid allowed introduction of the P3 group to provide the final compounds.

In conclusion, a new and unique series of potent, selective, and reversible cruzipain inhibitors has been identified. Compound **26** has a good selectivity profile (>100-fold) against human cathepsins L, B, S, and F and its pharmacokinetic profile is suitable for further evaluation in the search for a safe and effective treatment of Chagas disease.

References and notes

1. Nature Outlook, June 24th, 2010.
2. Ribeiro, I.; Sevcsik, A.-M.; Alves, F.; Diap, G.; Don, R.; Harhay, M. O.; Chang, S.; Pecoul, B. *PLoS Neglected Trop. Dis.* **2009**, *3*, e484.
3. Cavalli, A.; Bolognesi, M. L. *J. Med. Chem.* **2009**, *52*, 7339.
4. Urbina, J. A. *Curr. Pharm. Des.* **2002**, *8*, 287.
5. Cazzulo, J. J.; Stoka, V.; Turk, V. *Curr. Pharm. Des.* **2001**, *7*, 1143.
6. Cazzulo, J. J. *Curr. Top. Med. Chem.* **2002**, *2*, 1261.
7. McKerrow, J. H.; Engel, J. C.; Caffrey, C. R. *Bioorg. Med. Chem.* **1999**, *7*, 639.
8. Meirelles, M. N. L.; Juliano, L.; Carmona, E.; Silva, S. G.; Costa, E. M.; Murtha, A. C. M.; Scharfstein, J. *Mol. Biochem. Parasitol.* **1992**, *52*, 175.
9. Aparicio, I. M.; Scharfstein, J.; Lima, P. C. A. *Infect. Immun.* **2004**, *72*, 5892.

10. Berasain, P.; Carmona, C.; Frangione, B.; Cazzulo, J. J.; Goni, F. *Mol. Biochem. Parasitol.* **2003**, *130*, 23.
11. Engel, J. C.; Doyle, P. S.; Palmer, J.; Hsieh, I.; Bainton, D. F.; McKerrow, J. H. *J. Cell Sci.* **1998**, *111*, 597.
12. Engel, J. C.; Doyle, P. S.; Hsieh, I.; McKerrow, J. H. *J. Exp. Med.* **1998**, *188*, 725.
13. Barr, S. C.; Warner, K. L.; Kornreic, B. G.; Piscitelli, J.; Wolfe, A.; Benet, L.; McKerrow, J. H. *Antimicrob. Agents Chemother.* **2005**, *49*, 5160.
14. McKerrow, J. H.; Doyle, P. S.; Engel, J. C.; Podust, L. M.; Robertson, S. A.; Ferreira, R.; Saxton, T.; Arkin, M.; Kerr, I. D.; Brinen, L. S.; Craik, C. S. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 263.
15. Moreira, D. R. M.; Leite, A. C. L.; Dos Santos, R. R.; Soares, M. B. P. *Curr. Drug Targets* **2009**, *10*, 212.
16. Choe, Y.; Brinen, L. S.; Price, M. S.; Engel, J. C.; Lange, M.; Grisostomi, C.; Weston, S. G.; Pallai, P. V.; Cheng, H.; Hardy, L. W.; Hartsough, D. S.; McMakin, M.; Tilton, R. F.; Baldino, C. M.; Craik, C. S. *Bioorg. Med. Chem.* **2005**, *13*, 2141.
17. Brak, K.; Doyle, P. S.; McKerrow, J. H.; Ellman, J. A. *JACS* **2008**, *130*, 6404.
18. Chen, Y. T.; Lira, R.; Hansell, E.; McKerrow, J. H.; Roush, W. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5860.
19. Mott, B. T.; Ferreira, R. S.; Simeonov, A.; Jadhav, A.; Ang, K K.-H.; Leister, W.; Shen, M.; Silveira, J. T.; Doyle, P. S.; Arkin, M. R.; McKerrow, J. H.; Inglese, J.; Austin, C. P.; Thomas, C. J.; Shoichet, B. K.; Maloney, D. J. *J. Med. Chem.* **2010**, *53*, 52.
20. Murta, A. C. M.; Persechini, P. M.; Padron, T. S.; Souza, W.; Guimaraes, J. A.; Scharfstein, J. *Mol. Biochem. Parasitol.* **1990**, *43*, 27.
21. (a) Gauthier, J. Y.; Chauret, N.; Cromlish, W.; Desmarais, S.; Duong, L. T.; Falgueyret, J. P.; Kimmel, D. B.; Lamontagne, S.; Léger, S.; LeRiche, T.; Li, C. S.; Massé, F.; McKay, D. J.; Nicoll-Griffith, D. A.; Oballa, R. M.; Palmer, J. T.; Percival, M. D.; Riendeau, D.; Robichaud, J.; Rodan, G. A.; Rodan, S. B.; Seto, C.; Thérien, M.; Truong, V. L.; Venuti, M. C.; Wesolowski, G.; Young, R. N.; Zamboni, R.; Black, W. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 923; (b) Oballa, R. M.; Truchon, J.-F.; Baily, C. I.; Chauret, N.; Day, S.; Crane, S.; Berthelette, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 998.
22. Stoch, S. A.; Zajic, S.; Stone, J.; Miller, D. L.; Van Dyck, K.; Gutierrez, M. J.; De Decker, M.; Liu, Q.; Liu, L.; Scott, B. B.; Panebianco, D.; Jin, B.; Duong, L. T.; Gottesdiener, K.; Wagner, J. A. *Clin. Pharm. Ther.* **2009**, *86*, 175.
23. Black, W. C.; Bayly, C. I.; Davis, D. E.; Desmarais, S.; Falgueyret, J.-P.; Léger, S.; Li, C. S.; Massé, F.; McKay, D. J.; Palmer, J. T.; Percival, M. D.; Robichaud, J.; Tsou, N.; Zamboni, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4741.
24. Ndao, M., manuscript in preparation.
25. Black, W. C.; Percival, M. D. *ChemBioChem* **2006**, *7*, 1525.
26. Isabel, I.; Bateman, K. P.; Chauret, N.; Cromlish, W.; Desmarais, S.; Duong, L. T.; Falgueyret, J. P.; Gauthier, J. Y.; Lamontagne, S.; Lau, C. K.; Léger, S.; LeRiche, T.; Lévesque, J.-F.; Li, C. S.; Massé, F.; McKay, D. J.; Mellon, C.; Nicoll-Griffith, D. A.; Oballa, R. M.; Percival, M. D.; Riendeau, D.; Robichaud, J.; Rodan, G. A.; Rodan, S. B.; Seto, C.; Thérien, M.; Truong, V. L.; Wesolowski, G.; Young, R. N.; Zamboni, R.; Black, W. C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 887.
27. Hughes, G.; Devine, P. N.; Naber, J. R.; O'Shea, P. D.; Foster, B. S.; McKay, D. J.; Volante, R. P. *Angew. Chem., Int. Ed.* **2007**, *46*, 1839.
28. Isabel, E.; Mellon, C.; Beaulieu, C. WO 2009/067797 A1, 2009.
29. Falgueyret, J. P.; Black, W. C.; Cromlish, W.; Desmarais, S.; Lamontagne, S.; Mellon, C.; Riendeau, D.; Rodan, S.; Tawa, P.; Wesolowski, G.; Bass, K. E.; Venkatraman, S.; Percival, M. D. *Anal. Biochem.* **2004**, *335*, 218.