Bioorganic & Medicinal Chemistry Letters 20 (2010) 966–970

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel bis-arylsulfonamides and aryl sulfonimides as inactivators of plasminogen activator inhibitor-1 (PAI-1)

Nadine C. El-Ayache [a], Shih-Hon Li [b], Mark Warnock [b], Daniel A. Lawrence [b], Cory D. Emal [a,*]

[a] Department of Chemistry, Eastern Michigan University, 225 Mark Jefferson, Ypsilanti, MI 48197, United States
[b] Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan Medical School, 7301 MSRB III, 1150 W. Medical Center Dr., Ann Arbor MI 48109-0644, United States

ARTICLE INFO

Article history:
Received 15 October 2009
Revised 9 December 2009
Accepted 14 December 2009
Available online 21 December 2009

Keywords:
PAI-1 inhibitors
Bis-arylsulfonamide
Aryl sulfonimide

ABSTRACT

Inactivators of plasminogen activator inhibitor-1 (PAI-1) have been identified as possible treatments for a range of conditions, including atherosclerosis, venous thrombosis, and obesity. We describe the synthesis and inhibitory activity of a novel series of compounds based on bis-arylsulfonamide and aryl sulfonimide motifs that show potent and specific activity towards PAI-1. Inhibitors containing short linking units between the sulfonyl moieties and a 3,4-dihydroxy aryl substitution pattern showed the most potent inhibitory activity, and retained high specificity for PAI-1 over the structurally-related serpin anti-thrombin III (ATIII).

Plasminogen activator inhibitor-1 (PAI-1) is member of the serpin superfamily of protease inhibitors and is the primary inhibitor of urokinase type plasminogen activator (uPA) and tissue type plasminogen activator (tPA).[1] At normal physiologic levels PAI-1 plays a significant role in multiple processes, including fibrinolysis, angiogenesis, cell migration, and wound healing.[2–6] Pathologic levels of PAI-1 have been connected with obesity and metabolic syndrome, tumor metastasis, and vascular diseases such as venous thrombosis and atherosclerosis, making it an attractive pharmacological target.[7–13] However, PAI-1 has proven to be a challenging target for drug design as it is present in multiple conformational forms and has a flexible reactive center loop. Despite the challenges inherent in the development of small-molecule PAI-1 inactivators, several have been reported recently, although each has drawbacks that have impeded further progress in their development.[14–22] The most studied of these inhibitors, tiplaxtinin,[3,19,23–26] has a reported IC_{50} in the low micromolar range although PAI-1 inhibitors with IC_{50} values as low as 0.2 μM have been reported.[14,20]

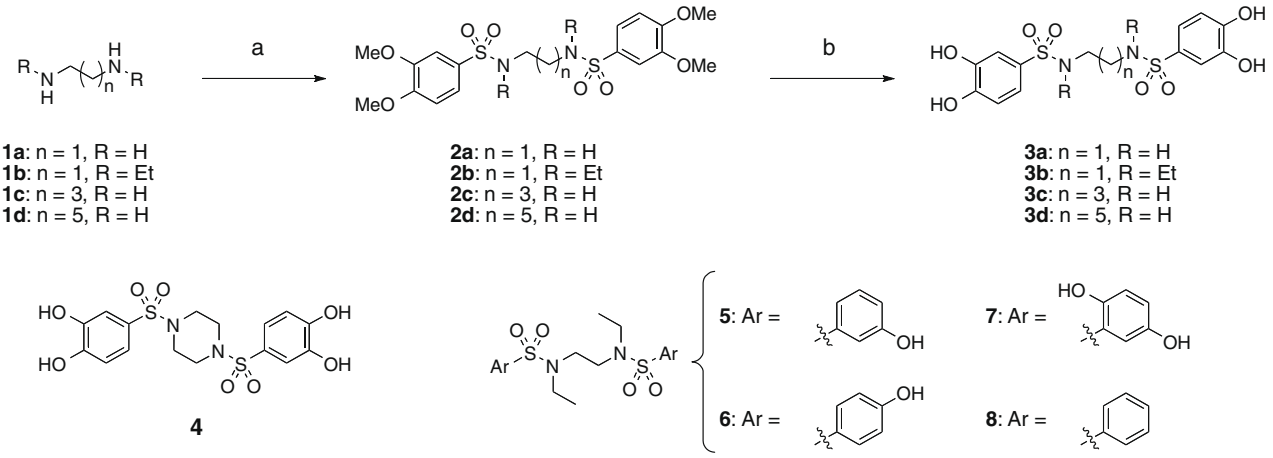
Here we report the development of a novel class of PAI-1 inhibitors based on an aryl sulfonamide or aryl sulfonimide motif that shows up to 30-fold more potent inhibitory activity toward PAI-1 than that of tiplaxtinin. Based on insights gained from a screen for anti-PAI-1 activity of a library of structurally diverse compounds,[27] we developed a set of compounds designed to probe structural requirements for PAI-1 inactivation. Our general design strategy was to synthesize compounds containing two polyphenolic moieties separated by linking units of various length and composition. Sulfonamides were chosen as a basis for the linking unit due to the moiety's widespread use in pharmaceutical design and the flexible synthetic access to structural diversity it would provide. As we were also interested in the level of specificity for the inhibition of PAI-1, we assayed the set of compounds for activity against anti-thrombin III (ATIII), a structurally similar mammalian serpin.

Bis-arylsulfonamides **3a–d** were prepared as shown in Scheme 1. Diamines (**1a–d**) were treated with 3,4-dimethoxybenzenesulfonyl chloride in the presence of triethylamine to form the bis-arylsulfonamides **2a–d**. Exposure of the bis-sulfonamides to boron tribromide in methylene chloride achieved deprotection of the aryl methoxy groups, providing the fully deprotected tetraphenols **3a–d**. Bis-sulfonamide **4** was similarly produced by this route, using piperazine as the initial diamine. Variously substituted bis-arylsulfonamides **5–7** were synthesized by analogous routes using the appropriate sulfonyl chlorides. Unsubstituted bis-benzenesulfonamide **8** was synthesized directly by the reaction of benzenesulfonyl chloride and *N,N′*-diethylethylenediamine in the presence of triethylamine.

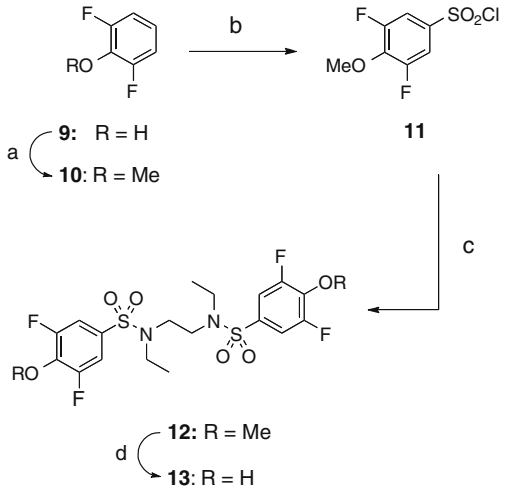
The synthesis of bis-3,5-difluoro-4-hydroxybenzenesulfonamide **13** proceeded as shown in Scheme 2. A solution of 2,6-difluorophenol in dimethylformamide was treated with iodomethane and anhydrous potassium carbonate to provide 2,6-difluoroanisole (**10**),[28] which was then exposed to chlorosulfonic acid[29] to provide the aryl sulfonyl chloride **11**. *N,N′*-Diethylethylenediamine was added dropwise to a solution of **11** in pyridine to give the

* Corresponding author. Tel.: +1 734 487 0305; fax: +1 734 487 1496.
E-mail address: cemal@emich.edu (C.D. Emal).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.051



Scheme 1. Preparation of bis-arylsulfonamides. Reagents and conditions: (a) 3,4-dimethoxybenzenesulfonyl chloride, triethylamine, ethyl acetate, 35–100%; (b) 1M BBr_3 in CH_2Cl_2, CH_2Cl_2, 0 °C to rt, 36–86%.



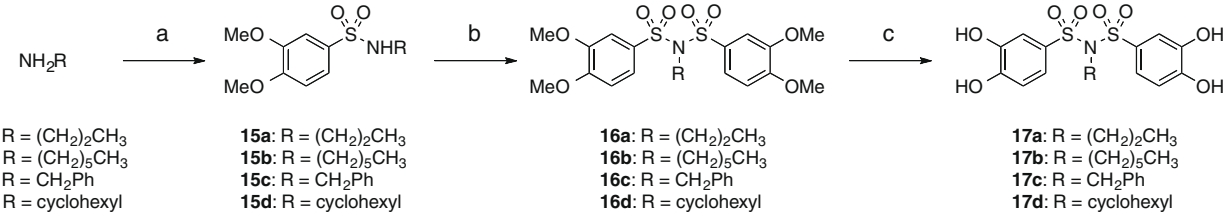
Scheme 2. Preparation of bis-3,5-difluoro-4-hydroxybenzenesulfonamide **13.** Reagents and conditions: (a) K_2CO_3, CH_3I, DMF, 50 °C, 65%; (b) chlorosulfonic acid, CH_2Cl_2, reflux, 2 h, 85%; (c) *N,N′*-diethylethylenediamine, Et_3N, EtOAc, 40%; (d) 1M BBr_3 in CH_2Cl_2, CH_2Cl_2, 0 °C to rt, 67%.

bis-sulfonamide **12**, which was demethylated using BBr_3 to provide fully deprotected bis-phenol **13**.

Difficulties were encountered in forming aryl sulfonimides **17a–d** directly from primary amines **14a–d**; instead we installed each sulfonyl group in a stepwise fashion (Scheme 3). 3,4-Dimethoxybenzenesulfonyl chloride was exposed to a solution of the appropriate primary amine **14** and triethylamine in ethyl acetate to provide the respective sulfonamide **15**. Sulfonamide **15** was treated with sodium hydride and 3,4-dimethoxybenzenesulfonyl chloride to provide the aryl sulfonimide **16**, which was then deprotected with BBr_3 as before to provide the PAI-1 inhibitors **17a–d**.

Compounds **2b**, **3a–d**, **4–8**, **13**, and **17–d** were assayed in vitro against PAI-1 and anti-thrombin III (ATIII), a related mammalian serpin (Table 1).[30] Initially we varied the length of the linking unit in order to determine the distance between our sulfonamide units that would lead to maximal potency against PAI-1. We found that bis-sulfonamides containing longer linker chains (**3c** and **3d**) were less active than **3a**, an otherwise equivalent compound containing a single ethylene linking unit (IC_{50} = 518 μM, Table 1).[31] Replacement of the acidic hydrogens within the sulfonamide units of **3a** with ethyl groups resulted in an increase in inhibitor potency of about 55-fold (**3b**, IC_{50} = 9.32 μM) as compared to the non-alkylated version. Significantly, the anti-PAI-1 activity of **3b** equaled that of tiplaxtinin (IC_{50} = 9.7 μM).[19] Inclusion of the linking unit within a more conformationally-constrained 1,4-piperazinyl ring, as in **4**, resulted in a compound that lacked significant inhibitory activity towards PAI-1, indicating that the relative orientation of the aromatic rings that leads to the greatest degree of bioactivity are not available to this molecule.

We then investigated the importance of the substitution pattern of the aromatic ring. Given the high potency conferred by the *N,N′*-ethylethylenediamine linking unit of **3b**, we maintained that structural motif while varying the substitution pattern of the aromatic rings. Hydroxyl groups at both the 3- and 4-positions are critical for maximal activity as the compound containing this substitution, **3b**, was far more inactivating towards PAI-1 (IC_{50} = 9.32 μM) than those containing a single hydroxyl group at either the 3-position (**5**, IC_{50} = 343 μM) or 4-position (**6**, IC_{50} > 300 μM). Flanking the 4-OH moiety with fluorine atoms at the 3- and 5-positions, as in **13**, restored some of the activity lost with the absence of the 3-OH group (IC_{50} = 128 μM), but still resulted in an inhibitor roughly 15-fold less potent than the 3,4-dihydroxy version. Sulfonamide **7**,



Scheme 3. Preparation of aryl sulfonimides. Reagents and conditions: (a) 3,4-dimethoxybenzenesulfonyl chloride, Et_3N, EtOAc, 63-100%; (b) NaH (60% dispersion in oil), 3,4-dimethoxybenzenesulfonyl chloride, DMF, 25–64%; (c) 1M BBr_3 in CH_2Cl_2, CH_2Cl_2, 0 °C to rt, 48–65%.

Table 1
Inhibitory activity of bis-arylsulfonamides and aryl sulfonimides against PAI-1 and ATIII

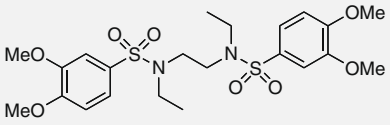
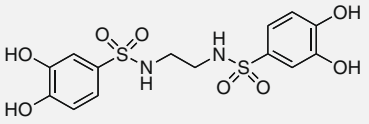
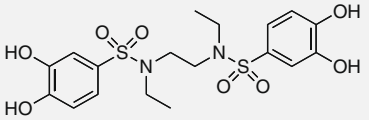
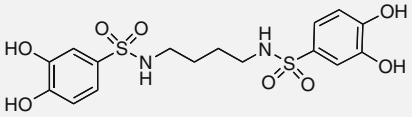
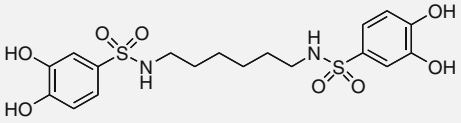
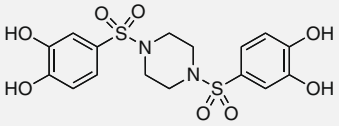
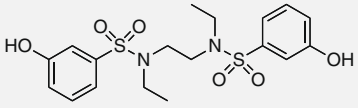
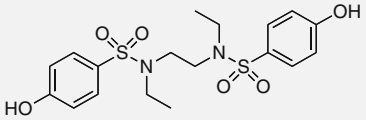
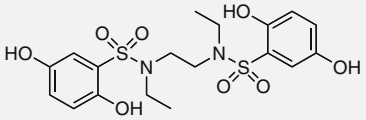
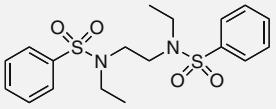
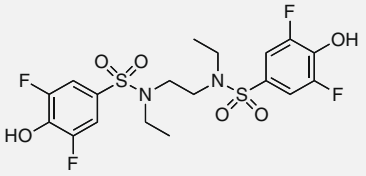
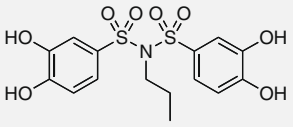
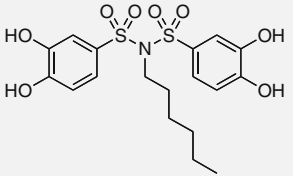
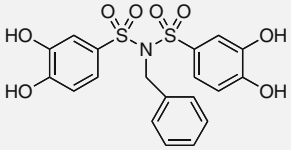
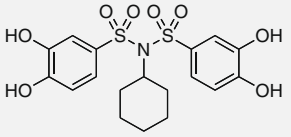
Compd	Structure	**PAI-1** Inhibition IC_{50}[a] (μM)	ATIII Inhibition IC_{50}[a] (μM)
2b		>300	>300
3a		518 (±64)	>3000[b]
3b		9.32 (±0.36)	>1000[b]
3c		>3000	>3000
3d		1384 (±112)	>3000
4		>300[c]	>300
5		343 (±14)	>1000
6		>300	>300
7		>300[c]	>300
8		>300	>300
13		128 (±18)	>3000
17a		2.67 (±0.27)	>1000
17b		0.284 (±0.016)	>300[b]

Table 1 (*continued*)

Compd	Structure	**PAI-1** Inhibition IC_{50}[a] (μM)	ATIII Inhibition IC_{50}[a] (μM)
17c		4.82 (±0.32)	>300
17d		0.594 (±0.086)	>300

[a] Each value is the average of at least three determinations; standard error is given in parentheses.
[b] <30% of ATIII was inhibited at the highest compound concentration used.
[c] <30% of PAI-1 was inhibited at the highest compound concentration used.

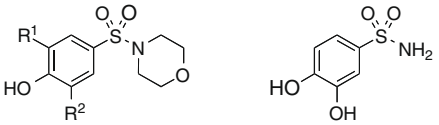
containing a hydroxyl group at each of the 2- and 5-positions, lacked significant potency towards PAI-1, supporting the importance of the 3,4-substitution pattern. Additionally, compounds that lacked hydroxyl groups entirely, such as **2b** and **8**, displayed no inhibitory activity against PAI-1 at the tested range of concentrations (>300 μM).

In order to confirm that two aryl sulfonyl groups are indeed required for anti-PAI-1 activity, three compounds containing a single such group (**18–20**, Fig. 1) were synthesized by routes analogous to those described in Scheme 1. These compounds showed a complete lack of activity towards PAI-1 at the range of concentrations tested (>3000 μM), confirming that both arylsulfonamide groups are required for inhibitory activity.

Interested in exploring the inhibitory activity of bisarylsulfonyl compounds with even shorter linking units, we synthesized a set of N-substituted aryl sulfonimides, **17a–d**. The sulfonimides showed improved potency towards PAI-1, performing 2–30-fold better in vitro compared to the best of the bis-sulfonamides (cf. Table 1). Inhibitors containing *n*-hexyl (**17b**, IC_{50} = 0.284 μM) and cyclohexyl (**17d**, IC_{50} = 0.594 μM) substituents displayed somewhat better activity against PAI-1 compared to *n*-propyl (**17a**, IC_{50} = 2.67 μM) and benzyl (**17c**, IC_{50} = 4.82 μM) moieties. In addition, the most potent of the sulfonimides (**17b** and **17d**) proved to be 15–34-fold more potent than tiplaxtinin (IC_{50} = 9.7 μM).

Specificity for the target protein over structurally-related proteins is an important consideration in the drug development process, so to investigate the specificity of these new classes of PAI-1 inhibitors we tested **2b**, **3a–d**, **4-8**, **13**, **17a–d,** and **18–20** against anti-thrombin III (ATIII), a mammalian serpin that is closely related to PAI-1. None of the compounds displayed significant activity against ATIII, although three compounds (**3a**, **3b**, and **17b**) showed <30% inhibition of ATIII at the highest concentration of inhibitor. This lack of inhibitory activity towards ATIII indicates that an eventual drug candidate from these classes of inhibitors may display a high level of specificity for PAI-1 over related mammalian serpins.

In conclusion, we have described the design of a series of novel bis-arylsulfonamides and aryl sulfonimides that display highly potent and selective inhibitory activity towards PAI-1. We have determined the minimal structural requirements for inhibition of PAI-1 by these classes of compounds, as shorter linking units and 3,4-dihydroxy substitution of flanking aromatic rings are essential for maximal inhibitor potency. We will report further work based on this molecular scaffold in the near future.



18: R^1 = H, R^2 = OH
19: R^1 = F, R^2 = F

20

Figure 1. Tested compounds containing a single sulfonamide unit.

Acknowledgments

The authors wish to thank Robert Cody for his assistance with analysis by Direct Analysis in Real Time (DART) mass spectroscopy. This work was supported by NIH Grants HL55374, HL54710, and HL089407 to DAL.

Supplementary data

Supplementary data (containing synthetic details, spectroscopic characterization of novel compounds, and assay conditions) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.051.

References and notes

1. Yepes, M.; Loskutoff, D. J.; Lawrence, D. A. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*; Coleman, R. W., Marder, V. J., Clowes, A. W., George, J. N., Goldhaber, S. Z., Eds.; Lippincott Williams & Wilkins: Baltimore, 2006; pp 365–380.
2. McMahon, G. A.; Petitclerc, E.; Stefansson, S.; Smith, E.; Wong, M. K.; Westrick, R. J.; Ginsburg, D.; Brooks, P. C.; Lawrence, D. A. *J. Biol. Chem.* **2001**, *276*, 33964.
3. Leik, C. E.; Su, E. J.; Nambi, P.; Crandall, D. L.; Lawrence, D. A. *J. Thromb. Haemost.* **2006**, *4*, 2710.
4. Maquerlot, F.; Galiacy, S.; Malo, M.; Guignabert, C.; Lawrence, D. A.; d'Ortho, M. P.; Barlovatz-Meimon, G. *Am. J. Pathol.* **2006**, *169*, 1624.
5. Stefansson, S.; Lawrence, D. A. *Nature* **1996**, *383*, 441.
6. Cao, C.; Lawrence, D. A.; Li, Y.; Von Arnim, C. A.; Herz, J.; Su, E. J.; Makarova, A.; Hyman, B. T.; Strickland, D. K.; Zhang, L. *EMBO J.* **2006**, *25*, 1860.
7. Andreasen, P. A. *Curr. Drug Targets* **2007**, *8*, 1030.
8. Huang, Y.; Border, W. A.; Yu, L.; Zhang, J.; Lawrence, D. A.; Noble, N. A. *J. Am. Soc. Nephrol.* **2008**, *19*, 329.
9. Pinsky, D. J.; Liao, H.; Lawson, C. A.; Yan, S. F.; Chen, J.; Carmeliet, P.; Loskutoff, D. J.; Stern, D. M. *J. Clin. Invest.* **1998**, *102*, 919.
10. Schafer, K.; Fujisawa, K.; Konstantinides, S.; Loskutoff, D. J. *FASEB J.* **2001**, *15*, 1840.
11. Shah, C.; Yang, G.; Lee, I.; Bielawski, J.; Hannun, Y. A.; Samad, F. *J. Biol. Chem.* **2008**, *283*, 13538.
12. Lijnen, H. R. *Thromb. Res.* **2009**, *123*, S46.
13. Sobel, B. E.; Taatjes, D. J.; Schneider, D. J. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 1979.
14. Folkes, A.; Roe, M. B.; Sohal, S.; Golec, J.; Faint, R.; Brooks, T.; Charlton, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2589.
15. Gils, A.; Stassen, J. M.; Nar, H.; Kley, J. T.; Wienen, W.; Ries, U. J.; Declerck, P. J. *Thromb. Haemost.* **2002**, *88*, 137.
16. Einholm, A. P.; Pedersen, K. E.; Wind, T.; Kulig, P.; Overgaard, M. T.; Jensen, J. K.; Bodker, J. S.; Christensen, A.; Charlton, P.; Andreasen, P. A. *Biochem. J.* **2003**, *373*, 723.

17. Crandall, D. L.; Elokdah, H.; Di, L.; Hennan, J. K.; Gorlatova, N. V.; Lawrence, D. A. *J. Thromb. Haemost.* **2004**, *2*, 1422.
18. Liang, A.; Wu, F.; Tran, K.; Jones, S. W.; Deng, G.; Ye, B.; Zhao, Z.; Snider, R. M.; Dole, W. P.; Morser, J.; Wu, Q. *Thromb. Res.* **2005**, *115*, 341.
19. Gorlatova, N. V.; Cale, J. M.; Elokdah, H.; Li, D.; Fan, K.; Warnock, M.; Crandall, D. L.; Lawrence, D. A. *J. Biol. Chem.* **2007**, *282*, 9288.
20. Gardell, S. J.; Krueger, J. A.; Antrilli, T. A.; Elokdah, H.; Mayer, S.; Orcutt, S. J.; Crandall, D. L.; Vlasuk, G. P. *Mol. Pharmacol.* **2007**, *72*, 897.
21. Rupin, A.; Gaertner, R.; Mennecier, P.; Richard, I.; Benoist, A.; De Nanteuil, G.; Verbeuren, T. J. *Thromb. Res.* **2008**, *122*, 265.
22. Izuhara, Y.; Takahashi, S.; Nangaku, M.; Takizawa, S.; Ishida, H.; Kurokawa, K.; van Ypersele de Strihou, C.; Hirayama, N.; Miyata, T. *Arterioscler. Thromb. Vasc. Biol.* **2008**, *28*, 672.
23. Elokdah, H.; Abou-Gharbia, M.; Hennan, J. K.; McFarlane, G.; Mugford, C. P.; Krishnamurthy, G.; Crandall, D. L. *J. Med. Chem.* **2004**, *47*, 3491.
24. Hennan, J. K.; Elokdah, H.; Leal, M.; Ji, A.; Friedrichs, G. S.; Morgan, G. A.; Swillo, R. E.; Antrilli, T. M.; Hreha, A.; Crandall, D. L. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 710.
25. Crandall, D. L.; Quinet, E. M.; El Ayachi, S.; Hreha, A. L.; Leik, C. E.; Savio, D. A.; Juhan-Vague, I.; Alessi, M. C. *Arterioscler. Thromb. Vasc. Biol.* **2006**, *26*, 2209.
26. Baxi, S.; Crandall, D. L.; Meier, T. R.; Wrobleski, S.; Hawley, A.; Farris, D.; Elokdah, H.; Sigler, R.; Schaub, R. G.; Wakefield, T.; Myers, D. *Thromb. Haemost.* **2008**, *99*, 749.
27. Cale, J. M.; Li, S-H.; Warnock, M.; Su, E. J.; North, P. R.; Sanders, K. L.; Puscau, M. M.; Emal, C. D.; Lawrence, D. A. submitted for publication.
28. Zhang, L.; Zheng, J.; Hu, J. *J. Org. Chem.* **2006**, *71*, 9845.
29. Adapted from the procedure of: Sato, M.; Kawashima, Y.; Goto, J.; Yamane, Y.; Chiba, Y.; Jinno, S.; Satake, M.; Iwata, C. *Eur. J. Med. Chem.* **1995**, *30*, 403–414.
30. See Supplementary data for detailed assay conditions and the synthesis and spectroscopic characterization of tested compounds.
31. Attempts to synthesize analogous compounds containing a hydrazine-based linking unit were unsuccessful, possibly due to unintended side-reactions similar to those described by: Shyam, K.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1985**, *28*, 525.