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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, and Cory D. Emal^{a,*}

^aDepartment of Chemistry, 225 Mark Jefferson, Eastern Michigan University, Ypsilanti, MI 48197, United States

^bDepartment 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

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).

Keywords

PAI-1 inhibitors; bis-arylsulfonamide; aryl sulfonimide

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 IC₅₀ in the low micromolar range although PAI-1 inhibitors with IC₅₀ values as low as 0.2 μM have been reported.^{14,20}

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

Supplementary material containing synthetic details, spectroscopic characterization of novel compounds, and assay conditions for this paper is available to all readers.

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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. Various 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 acid²⁹ to provide the aryl sulfonyl chloride **11**. *N,N'*-Diethylethylenediamine was added dropwise to a solution of **11** in pyridine to give the bis-sulfonamide **12**, which was demethylated using BBr₃ 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 BBr₃ 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 (IC₅₀ = 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**, IC₅₀ = 9.32 μM) as compared to the non-alkylated version. Significantly, the anti-PAI-1 activity of **3b** equaled that of tiplaxtinin (IC₅₀ = 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 ($IC_{50} = 9.32 \mu\text{M}$) than those containing a single hydroxyl group at either the 3-position (**5**, $IC_{50} = 343 \mu\text{M}$) or 4-position (**6**, $IC_{50} > 300 \mu\text{M}$). 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 ($IC_{50} = 128 \mu\text{M}$), but still resulted in an inhibitor roughly 15-fold less potent than the 3,4-dihydroxy version. Sulfonamide **7**, 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 ($>300 \mu\text{M}$).

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**, Figure 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 ($>3000 \mu\text{M}$), 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-fold to 30-fold better in vitro compared to the best of the bis-sulfonamides (cf. Table 1). Inhibitors containing *n*-hexyl (**17b**, $IC_{50} = 0.284 \mu\text{M}$) and cyclohexyl (**17d**, $IC_{50} = 0.594 \mu\text{M}$) substituents displayed somewhat better activity against PAI-1 compared to *n*-propyl (**17a**, $IC_{50} = 2.67 \mu\text{M}$) and benzyl (**17c**, $IC_{50} = 4.82 \mu\text{M}$) moieties. In addition, the most potent of the sulfonimides (**17b** and **17d**) proved to be 15- to 34-fold more potent than tiplaxtinin ($IC_{50} = 9.7 \mu\text{M}$).

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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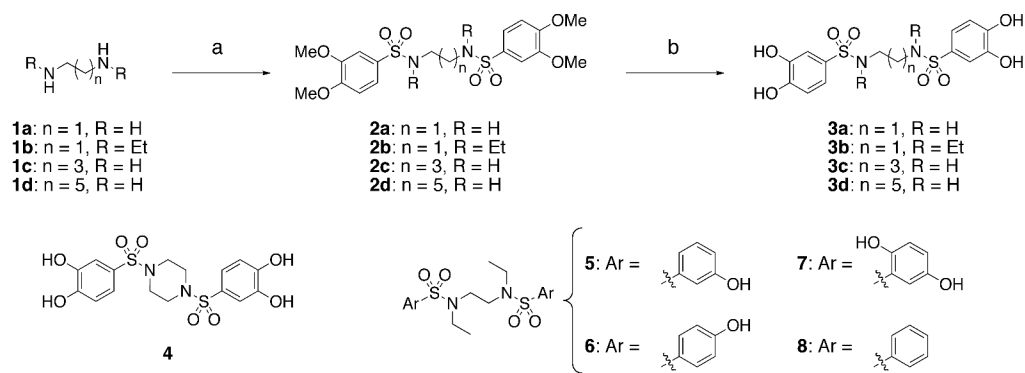
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30. See supplementary information for detailed assay conditions and the synthesis and spectroscopic characterization of tested compounds.
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Figure 1.
Tested compounds containing a single sulfonamide unit.

**Scheme 1.**

Preparation of bis-arylsulfonamides: (a) 3,4-dimethoxybenzenesulfonyl chloride, triethylamine, ethyl acetate, 35-100%; (b) 1M BBr₃ in CH₂Cl₂, CH₂Cl₂, 0 °C to rt, 36-86%.

**Scheme 2.**


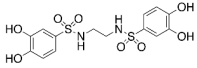

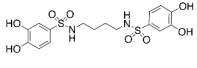
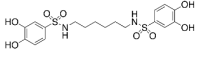

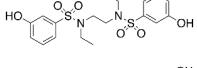
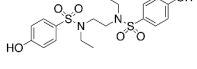



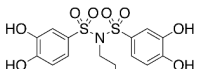


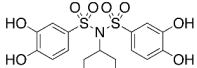
Preparation of bis-3,5-difluoro-4-hydroxybenzenesulfonamide **13**: (a) K_2CO_3 , CH_3I , DMF, 50 °C, 65%; (b) chlorosulfonic acid, CH_2Cl_2 , reflux, 2 hr, 85%; (c) *N,N'*-diethylethylenediamine, Et_3N , EtOAc, 40%; (d) 1M BBr_3 in CH_2Cl_2 , CH_2Cl_2 , 0 °C to rt, 67%.

**Scheme 3.**

Preparation of aryl sulfonimides: (a) 3,4-dimethoxybenzenesulfonyl chloride, Et₃N, EtOAc, 63-100%; (b) NaH (60% dispersion in oil), 3,4-dimethoxybenzenesulfonyl chloride, DMF, 25-64%; (c) 1M BBr₃ in CH₂Cl₂, CH₂Cl₂, 0 °C to rt, 48-65%.

Table 1

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

Cmpd	Structure	PAI-1 Inhibition IC ₅₀ , μM ^a	ATIII Inhibition IC ₅₀ , μM ^a
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
17c		4.82 (±0.32)	>300
17d		0.594 (±0.086)	>300

^a Each value is the average of at least 3 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.