Dual Inhibition of Phosphodiesterase 4 and Matrix Metalloproteinases by an (Arylsulfonyl)hydroxamic Acid Template

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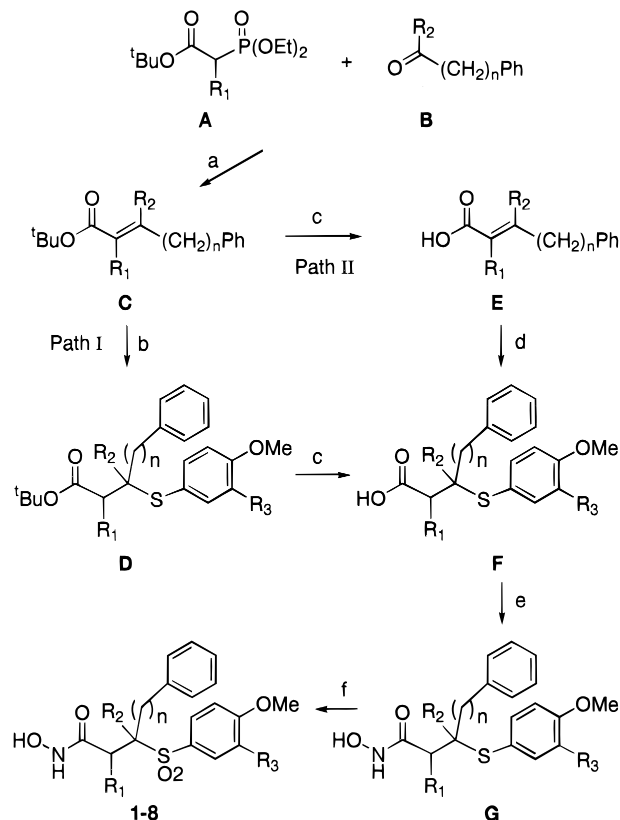
Introduction. Two biological targets of considerable interest in the field of inflammatory disease are phosphodiesterase type 4 (PDE4)[1] and selected members of the matrix metalloproteinase (MMP) family, such as collagenase, gelatinase, and stromelysin.[2] PDE4 is a cyclic AMP-specific phosphodiesterase and is the predominant isozyme of the inflammatory and immune cell types.[3] Inhibition of PDE4 in these cells elevates intracellular concentrations of c-AMP which produces a response that has been associated with a general suppression of proinflammatory cellular activity, including the regulation of the proinflammatory cytokine TNF-α.[4] The successful utilization of PDE4 inhibitors has been demonstrated in preclinical and clinical studies for disease states such as rheumatoid arthritis (RA),[5] multiple sclerosis (MS),[6] atopic dermatitis (AD),[7] and psoriasis.[8] These encouraging results employing PDE4 inhibitors have, in many respects, been paralleled by studies using MMP inhibitors.

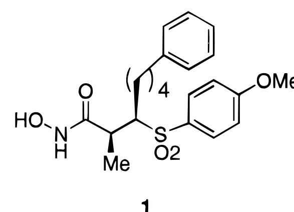
The MMPs are a family of zinc-dependent endopeptidases involved in the degradation of the proteins which comprise the extracellular matrix (ECM). The substrate specificity of the MMPs divides the group into roughly three subfamilies: collagenases, gelatinases, and stromelysins.[9] Remodeling of the ECM is important in maintaining connective tissue and basement membrane integrity for normal physiology; however, a number of pathological conditions involve the overactivation of this class of enzymes. Small-molecule inhibitors of the MMPs have been developed,[2] and in fact, a selection of these inhibitors shows positive results in the same disease models of RA,[10] MS,[11] AD,[12] and psoriasis[13] which have been used to examine the efficacy of PDE4 inhibitors.

Therefore, since it is possible to achieve a beneficial pharmacological effect by the modulation of two independent biological pathways (PDE4 and MMPs), this creates the intriguing possibility that dual inhibition of PDE4 and MMPs may offer a therapeutic advantage in disease states for which models show a positive response by monotherapy alone. *Indeed, in this Communication we wish to report the discovery of the first compounds, represented by the β-(arylsulfonyl)hydroxamic acid **1**, that simultaneously inhibit both PDE4 and MMPs 1, 2, and 3.*[14]

Scheme 1[a]



[a] Reagents: (a) NaH, THF, then **B**; (b) ArSH, cat. BuLi, THF; (c) TFA/CH_2Cl_2; (d) ArSH, cat. piperidine, Δ; (e) i. $(COCl)_2$, CH_2Cl_2, ii. $TMSONH_2$, CH_2Cl_2; (f) Oxone, MeOH/H_2O.



Chemistry. The (arylsulfonyl)hydroxamates were prepared according to the pathways presented in Scheme 1. Condensation of a phosphonate (**A**) with an aldehyde or ketone (**B**)[15] provided the α,β-unsaturated *tert*-butyl ester (**C**). Conversion to the intermediate carboxylic acid (**F**) was accomplished by one of two paths. Lithium thiolate-catalyzed Michael addition[16] (path I) provided the thiol addition product (**D**) which was subsequently deprotected using TFA to yield the carboxylic acid (**F**). Alternatively, hydrolysis of the *tert*-butyl ester followed by thiol addition to the unsaturated acid (**E**) at elevated temperatures (path II) produced the intermediate β-(arylthio)carboxylic acid (**F**). Conversion to the hydroxamic acid (**G**) was accomplished by reaction of the acid chloride with excess *O*-(trimethylsilyl)hydroxylamine. Finally, sulfide oxidation with Oxone provided the target β-sulfonylhydroxamic acids **1**−**8** (Table 1).

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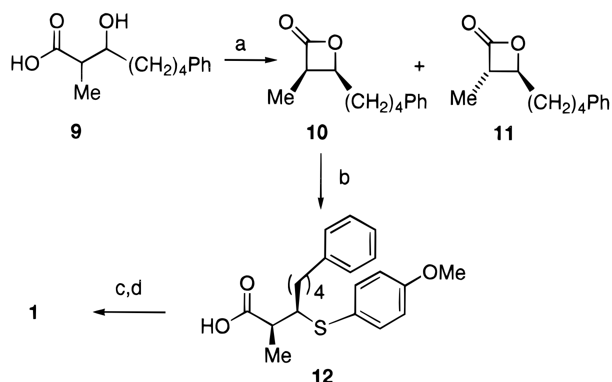
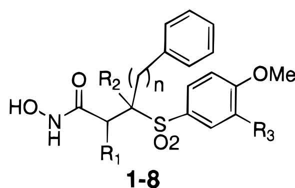
Scheme 2[a]



[a] Reagents: (a) i. $(PyS)_2$, PPh_3, $CHCl_3$, ii. $Hg(OMs)_2$, CH_3CN, Δ, (66%); (b) *p*-MeOPhSH, NaOH, iPrOH, (90%); (c) i. $(COCl)_2$, CH_2Cl_2, ii. $TMSONH_2$, CH_2Cl_2, (93%); (d) Oxone, MeOH/THF/H_2O, (95%).

Table 1. Structures and Enzymatic Activities for Compounds **1**−**8**



compd[a]	*n*	R_1	R_2	R_3	PDE4[b]	MMP-1[c]	MMP-2[c]	MMP-3[c]
2 (B)	1	H	H	H	9	1	0.02	0.3
3 (B)	2	H	H	H	9	4	0.03	0.2
4 (B)	3	H	H	H	3	2	0.05	0.6
5 (A)	4	H	H	H	0.5	5	0.02	0.7
6 (A)	4	H	H	OMe	0.001	>10	>10	>10
7 (B)	4	H	Me	H	9	2	0.05	1
8[d] (A)	4	Me	H	H	0.3	2	0.06	0.6
1 (C)	4	Me	H	H	0.03	2	0.01	0.5

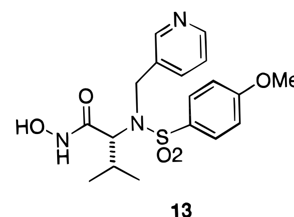
[a] Method of preparation in parenthesis: (A) path I, Scheme 1; (B) path II, Scheme 1; (C) Scheme 2. [b] IC_{50} (μM), guinea pig macrophage PDE4; see ref 20. [c] K_i (μM); MMP-1, fibroblast collagenase; MMP-2, gelatinase A; MMP-3, stromelysin-1; see ref 21. [d] Anti stereoisomer (2R^*,3R^*), de 80%.

In general, the yield of each step throughout the reaction sequence was very good (>80%), and the choice of pathways was inconsequential apart from two exceptions: Preparation of compound **7** (R_2 = Me) required the use of path II to obtain a satisfactory yield for the thiol addition step, and preparation of compounds **1** and **8** (R_1 = Me) produced different ratios of the two diastereomeric products depending upon the path chosen. For **1** and **8**, path II produced a poorly separable 50/50 mixture of syn and anti diastereomers following thiol addition to the (*E*)-isomer of unsaturated acid **E** (where R_1 = Me, R_2 = H, n = 4). In contrast, path I provided stereoselective (9:1) anti addition[16] to the (*E*)-isomer of *tert*-butyl ester **C** (R_1 = Me, R_2 = H, n = 4) which established the diastereomeric relationship of **8.**

For the preparation of isomer **1**, an independent synthesis was used as outlined in Scheme 2. Hydroxy acid **9**[17] was cyclized using a double activation procedure[18] to yield an equimolar mixture of β-lactones (**10**, **11**). The isomers were readily separated by flash column chromatography and identified by their distinctive coupling constants.[19] Ring opening of **10** with sodium 4-methoxyphenylthiolate in 2-propanol gave clean inversion to provide carboxylic acid **12**. Conversion of **12** to sulfonyl hydroxamic acid **1** proceeded without racemization using our standard two-step protocol (vide supra).

Results and Discussion. The β-(arylsulfonyl)-hydroxamic acid template provides potent inhibitors of either PDE4[20] or the MMPs[21] and also delivers inhibitors with potent activity against both classes of enzymes. A preliminary outline of the SAR and the dual activity that has been revealed is presented in Table 1.

The β-(arylsulfonyl)hydroxamic acids **1**−**8** have some structural similarity to an arylsulfonamide class of MMP inhibitors represented by the clinical candidate CGS 27023A (**13**).[22] Thus, beginning with compound **2**, which contains a benzyl substituent at the β-position and has some analogy to **13**, we observed potent MMP



inhibition but only weak activity against PDE4. Modification at the β-position revealed that as the chain length was increased beyond phenethyl (**3**) to phenpropyl (**4**) and phenbutyl (**5**), inhibition of PDE4 was increased with little change in the MMP profile. This chain elongation demonstrated for the first time that the (arylsulfonyl)hydroxamic acid template could be used to produce compounds with potent inhibitory activity against MMPs and submicromolar potency against PDE4.

The next stage of our work revealed that when a 3′-alkoxy group was added (such as 3′-OMe, compound **6**) to produce the classic 3′,4′-dialkoxyphenyl pharmacophore present in a number of PDE4 inhibitors,[1] a dramatic improvement in PDE4 potency was obtained. This finding is in accordance with a report that certain (dialkoxyaryl)hydroxamic acids are potent PDE4 inhibitors.[23] The dialkoxy substitution pattern, however, resulted in a dramatic loss of MMP activity, thereby revealing an apparent pharmacophore "switch" between the mono- and dimethoxyphenyl analogues of this template.[24] Throughout our work with this template, the suppressive effect of dialkoxy substitution upon MMP inhibitory activities was so dominant that the monoalkoxy pharmacophore was required to maintain potency against the MMPs.

During further exploration of the SAR at the β-position (within the *p*-alkoxy series), it became apparent that the incorporation of a second alkyl group (e.g., **7**) produced much weaker PDE4 inhibition. In contrast, our initial investigation of substitution at the α-position has been more fruitful, as exemplified by the α-methyl derivatives **8** and **1**. Examination of diastereomer **8** revealed little difference in overall activity when compared to the unsubstituted analogue **5**, whereas isomer **1** resulted in a 15-fold improvement in activity against PDE4 (IC_{50} = 0.03 μM) while still maintaining a favorable MMP profile, particularly for MMP-2 (K_i = 0.01 μM). While the origin of this potency increase against PDE4 is unknown, it is speculated to be the result of either a positive hydrophobic interaction within

the enzyme active site or a more favorable conformational bias imparted by the substitution. The ability to introduce this substitution at the α-position not only provides an increase in potency with **1** but also affords the possibility of increasing the metabolic stability of the hydroxamic acid. Indeed, the stabilizing benefit of substitution α to hydroxamic acids has been observed for a number of MMP inhibitors, including several compounds in the sulfonamide series (i.e., **13**), which has been attributed to a decrease in the hydrolysis and enzymatic metabolism (via reduction and glucuronidation) of the hydroxamate.[22]

Several features of the SAR within our β-(arylsulfonyl)hydroxamic acid series suggest that this template binds in the active site of the MMPs with an orientation similar to that of the sulfonamides.[25] As related to our series, the key elements of this proposed binding model are the chelation of the hydroxamic acid to the active-site zinc and the placement of the aryl sulfone in the well-defined S1′ hydrophobic pocket. The β-substituent occupies the shallow S2′ domain and makes limited contact with the enzyme. This model, therefore, places a strong dependence upon the aryl sulfone moiety for binding and limits the effect of the β-substituent. Indeed, the SAR observed throughout our work with this template (represented by compounds **2**−**6**) reflects this aryl sulfone dependence in determining the inhibitory activities and MMP selectivity profile of the series. Although there is not the same structural data available for PDE4, the inhibitors of this β-(arylsulfonyl)hydroxamic acid series fit a previously proposed active-site model[23] and, likewise, reveal a strong dependence upon the hydroxamic acid for activity.[26]

In conclusion, we have described the discovery and initial activity profile of a novel β-(arylsulfonyl)hydroxamic acid-based structural template. This template can not only provide potent and selective inhibitors of PDE4 or certain MMPs but, as described herein, also reveal compounds with potent *dual* activity (such as **1**) depending upon the appropriate scaffold elaboration. The ability to simultaneously inhibit both PDE4 and MMPs with a single compound may offer a new pathway for treating pathologies such as RA, MS, AD, and psoriasis in which both enzyme families are implicated. Our research in this area will be reported in due course.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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