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Solid-Phase Synthesis of an Arylsulfone Hydroxamate Library

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Abstract—Synthesis of an arylsulfone hydroxamate lead optimization library is presented. Biological activity of representative examples is given to demonstrate the value of this approach for lead optimization.

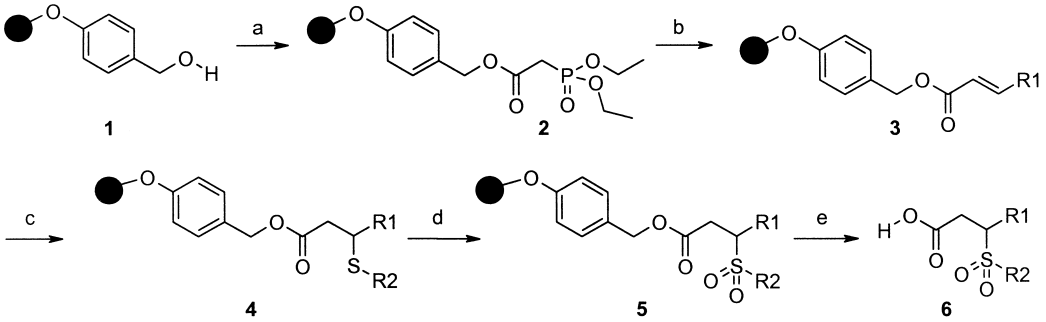
Introduction

The solid-phase synthesis and biological activity of an arylsulfone hydroxamate library is described. The goal of this work was to develop a solid-phase route to the arylsulfone hydroxamate chemical series[1] to facilitate optimization of this lead series. The preliminary SAR on a β-arylsulfone hydroxamate scaffold capable of inhibiting either MMPs or PDE4 has recently been disclosed. These targets are of considerable interest as anti-inflammatory agents.[1,2] As part of this study a general synthetic route for chemical libraries of carboxylic and hydroxamic acids was designed and carried to fruition. In this report we wish to disclose this solid-phase synthesis and the resulting SAR required for potent, selective MMP or PDE4 inhibitors.

Chemistry

A five-step solution route to the arylsulfone inhibitors (compound **8**; Scheme 2), had been developed prior to starting this project.[1] However, this route involved chromatographic purification after each synthetic step.

A robust solid-phase route would facilitate purification and thus make the entire process more efficient. A solid-phase synthesis of the carboxylic acid sulfone was developed (Scheme 1).[3] This synthesis was flexible enough to allow for the isolation and screening of all the carboxylic acid intermediates. A resin bound hydroxylamine was employed to transform the carboxylic acid into the hydroxamic acid[4] (Scheme 2). Typically, the reaction products displayed >80% purity as determined by



Scheme 1. Reagents. (a) Phosphonoacetic acid (3 equiv), 2,6-dichlorobenzoylchloride (3.2 equiv), anhydrous pyridine (6.4 equiv), DMF, 25 °C, 8 h; (b) Lithium bis(trimethylsilyl)amide (5 equiv), THF, 0 °C to 25 °C, 60 min then filter under argon, add R_1CHO (4 equiv), 60% cyclohexane in THF 25 °C 24–48 h; (c) HSR_2 (5 equiv), THF, nBuLi (0.1 equiv), 25 °C, 12 h; (d) mCPBA (5 equiv), dioxane, 25 °C, 12h; (e) 30% TFA in CH_2Cl_2 (excess), 25 °C, 1 h.

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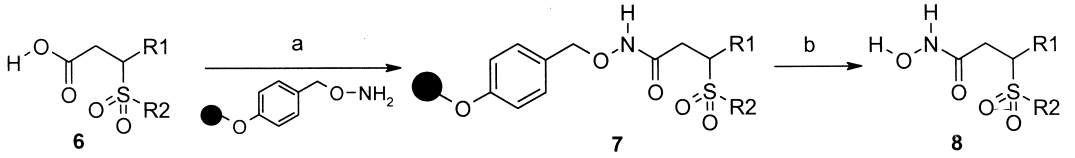
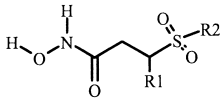
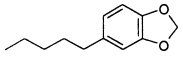
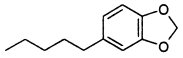
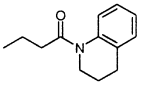
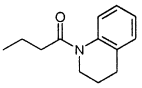
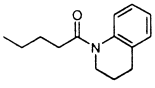
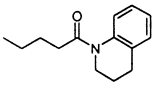


Scheme 2. Reagents. (a) EDC (3 equiv), DMF, Hydroxylamine resin, DMF, 25 °C 4 h; (b) 30% TFA in CH_2Cl_2 (excess), 25 °C, 1 h.

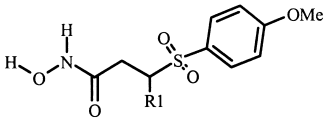
Table 1. Structure–activity relationships for PDE4 activity[a]



Compound	R_1	R_2	PDE4 IC_{50} nM[b]
9	-$(CH_2)_4$-Ph	-C_6H_3-4-OMe-3-OcC_5H_9	6.9
10[d]	-$(CH_2)_4$-Ph	-C_6H_3-4-OMe-3-OMe	1.3
11		-C_6H_3-4-OMe-3-OcC_5H_9	11
12		-C_6H_3-4-OMe-3-OMe	6.4
13[c,d]	-$(CH_2)_3$-Ph	-C_6H_3-4-OMe-3-OMe	14
14	-$(CH_2)_2$-Ph	-C_6H_3-4-OMe-3-OcC_5H_9	31
15	-$(CH_2)_2$-Ph	-C_6H_3-4-OMe-3-OMe	3030
16[c]	-Ph	-C_6H_3-4-OMe-3-OMe	580
17	-C_6H_4-4-OBn	-C_6H_3-4-OMe-3-OcC_5H_9	21
18	-C_6H_4-3-OBn	-C_6H_3-4-OMe-3-OcC_5H_9	89
19	-C_6H_4-2-OBn	-C_6H_3-4-OMe-3-OcC_5H_9	1500
20	-$(CH_2)_2$-C_6H_4-4-OBn	-C_6H_3-4-OMe-3-OcC_5H_9	63
21	-$(CH_2)_2$-C_6H_4-4-OBn	-C_6H_3-4-OMe-3-OMe	64
22	-$(CH_2)_2$-C_6H_4-4-OPh	-C_6H_3-4-OMe-3-OcC_5H_9	32
23	-C_6H_4-3-OPh	-C_6H_3-4-OMe-3-OcC_5H_9	260
24	-C_6H_4-3-O-C_6H_4-4-OMe	-C_6H_3-4-OMe-3-OcC_5H_9	49
25	-$(CH_2)_5CH_3$	-C_6H_3-4-OMe-3-OcC_5H_9	9.5
26	-$(CH_2)_2CH_3$	-C_6H_3-4-OMe-3-OcC_5H_9	55
27	-$(CH_2)_2CH_3$	-C_6H_3-4-OMe-3-OMe	5250
28	-$CH_2CH(CH_3)_2$	-C_6H_3-4-OMe-3-OcC_5H_9	102
29	-$CH_2CH(CH_3)_2$	-C_6H_3-4-OMe-3-OMe	>10,000
30		-C_6H_3-4-OMe-3-OcC_5H_9	16
31		-C_6H_3-4-OMe-3-OMe	564
32		-C_6H_3-4-OMe-3-OcC_5H_9	36
33		-C_6H_3-4-OMe-3-OMe	295
34	-$(CH_2)_3$-Phthalimide	-C_6H_3-4-OMe-3-OcC_5H_9	32
35	-$(CH_2)_3$-$CON(CH_3)$Ph	-C_6H_3-4-OMe-3-OcC_5H_9	118
36	-$(CH_2)_2$-$CON(CH_3)$Ph	-C_6H_3-4-OMe-3-OcC_5H_9	50
37	-$(CH_2)_2$-$OCON(CH_3)$Ph	-C_6H_3-4-OMe-3-OcC_5H_9	140
38	-$(CH_2)_2$-$N(CH_3)$COPh	-C_6H_3-4-OMe-3-OcC_5H_9	1040
39	-CH_2-$N(CH_3)$COPh	-C_6H_3-4-OMe-3-OcC_5H_9	974
40	-$(CH_2)_2$-$N(CH_3)CO_2$Bn	-C_6H_3-4-OMe-3-OcC_5H_9	460

[a]MMP IC_{50}s are all >10,000 nM, see ref 7.
[b]See ref 6.
[c]See ref 8; Compound **13** inhibits TNF α IC_{50} = 9 nM; Compound **16** inhibits TNF α IC_{50} = 450 nM.
[d]Purified analogues.

Table 2. Structure–activity relationships for MMP activity



Compound	R_1	TNF α IC_{50} nM[d]	PDE4 IC_{50} nM[a,b]	MMP-1, K_i (nM)[c]	MMP-2, K_i (nM)[c]	MMP-3, K_i (nM)[c]
41	-$(CH_2)_4$-Ph	>10,000	NA	5000	20	700
42	-$(CH_2)_3$-Ph	5000	NA	2580	44	1390
43	-CH_2-O-CH_2-Ph	>10,000	NA	>10,000	38	1430
44	-$(CH_2)_2$-Ph	>10,000	NA	2160	43	350
45	-$(CH_2)_2$-Cyclohexyl	>10,000	NA	400	36	590
46	-CH-(CH_3)Ph	>10,000	NA	1710	8	300
47[e]	-Ph	>10,000	4600	>10,000	270	3300
48	-C_6H_4-4-CO_2Me	>10,000	NA	1900	88	4420
49	-C_6H_4-4-OBn	1200	520	>10,000	>10,000	>10,000
50	-C_6H_4-4-OPh	730	280	>10,000	>10,000	>10,000
51	-C_6H_4-O-*iso*Propyl	>10,000	NA	>10,000	>10,000	>10,000
52	-C_6H_4-4-C_6H_4	4400	3600	>10,000	>10,000	>10,000
53	-cyclo-C_6H_{11}	>10,000	NA	1710	8	86
54	-cyclo-C_6H_{10}-3-CO_2Et	4600	NA	1010	0.8	60
55	-$(CH_2)_5CH_3$	1400	NA	9590	31	734
56	-CH(Et)$(CH_2)_3CH_3$	>10,000	NA	357	4	184
57	-$CH_2CH(CH_3)CH_2C(CH_3)_3$	8000	NA	1720	27	676
58	-$(CH_2)_3CH_3$	>10,000	NA	4170	30	404

[a]NA = data not available.
[b]See ref 6.
[c]See ref 7.
[d]See ref 8.
[e]Purified analogues.

HPLC/MS using UV220 detection. This purity was sufficient for biological evaluation and generation of structure–activity relationships. To confirm that the observed activity was derived from the expected compound, several analogues in the library were purified and re-screened.

Biological Results

Presented in Tables 1 and 2 are representative biological data to highlight this chemical library as a tool for generating SAR data rapidly. Carboxylic acid intermediates were screened but showed minimal activity at 10 μM concentration and therefore are not presented in the tables. The purpose of this study was to develop PDE4 SAR for the arylsulfonehydroxamate series. Screening against the various MMPs was performed mainly as a selectivity screen. Screening results indicate that a 3,4-dialkoxy-substituted moiety in R_2 such as 3,4-dimethoxyphenyl group provided inhibitors of PDE4 effective at nanomolar concentrations while suppressing activity against MMPs (Table 1). The use of the 3-*O*-cyclopentyl-4-*O*-methyl-benzene moiety for R_2 in some cases enhanced PDE4 activity in this series[5] (compounds **14**–**15**, **26**–**27**, **28**–**29**, **30**–**31** and **32**–**33**). A variety of R_1 groups were tolerated and reveal interesting SAR. The length of the alkyl sidechain is important for activity (compounds **25** and **26**). Importantly polar R_1 substituents were tolerated with only a 10 to 20 fold loss in activity (compounds **30** and **40**). Polar R_1 substituents may enhance the water solubility of the analogues, thus giving a handle to balance the pharmokinetic profile within the series.

Potent, selective MMP-2 inhibitors were found in the library. Due to the focus towards PDE4 inhibitors in this study only three R_2 groups were examined. Only the R_2 = 4-methoxy-phenyl analogues showed activity for the MMPs from this set of analogues. Variation in the R_1 position revealed interesting results. For example, alkyl groups were well tolerated, a cyclohexyl is better than a phenyl moiety (**47** vs **53**). Note the 10-fold increase in potency in the 3-carbethoxy-cyclohexyl analogue, (**53** versus **54**), resulting in a sub-nanomolar inhibitor against MMP-2 for this series.

Conclusion

In summary, a novel seven-step, solid-phase synthesis of potent MMP and PDE4 inhibitors has been accomplished. Both carboxylic and hydroxamic acids are available for screening from this synthetic route. This type of lead optimization library demonstrates the utility and the potential to accelerate the optimization process. Biological data could be generated directly from library compounds without any special purification. Selective compounds were shown to specifically inhibit several related enzymes and structure–activity relationships were easily extracted from the library.

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References and Notes

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2. Burns, C. J.; Groneberg, R. D.; Salvino, J.; McGeehan, G.; Condon, S. M.; Morris, R.; Morrissette, M.; Mathew, R.; Darnbrough, S.; Neuenschwander, K.; Scotese, A.; Djuric, S.; Ullrich, J.; Labaudiniere, R. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2848.
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5. The 3-*O*-cyclopentyl-4-*O*-methyl benzene moiety is a common pharmaaphcore for the rolipram class of PDE4 inhibitors. (a) Activity for RP73401 (compd **15j** in the reference) inhibits PDE4 with an $IC_{50}=1$ nM; Ashton, M.; Cook, D. C.; Fenton, G.; Karlsson, J.-A.; Palfreyman, M. N.; Raeburn, D.; Ratcliffe, A. J.; Souness, J. E.; Thurairatnam, S.; Vicker, N. *J. Med. Chem.* **1994**, *37*, 1696. (b) Kleinman, E. F.; Campbell, E.; Giordano, L. A.; Cohan, V. L.; Jenkinson, T. H.; Cheng, J. B.; Shirley, J. T.; Pettipher, E. R.; Salter, E. D.; Hibbs, T. A.; DiCapua, F. M.; Bordner, J. *J. Med. Chem.* **1998**, *41*, 266. (c) Newton, C.; Decicco, C. P. *J. Med. Chem.* **1999**, *42*, 2295. (d) Christensen, S.; Torphy, T. *Annu. Rep. Med. Chem.* **1994**, *29*, 185.
6. PDE4 inhibitory activity was measured as the mean of three determinations against guinea pig macrophage homogenate PDE4 according to the methods of Thompson, W. J.; Terasaki, W. L.; Epstein, P. M.; Strada, S. J. *Adv. Cyclic Nucl. Res.* **1979**, *10*, 69.
7. Inhibitory activity on recombinant MMP-1, MMP-2, and MMP-3 (biogenesis) was measured according to the methods of Knight, C. G.; Willenbrock, F.; Murphy, G. *FEBS Lett.* **1992**, *296*, 263.
8. All compounds shown to inhibit TNF-α release are inactive against TACE, and presumably inhibit TNF-α by PDE4 inhibition. *N*-(3,5-Dichloro-*N*-4-pyridyl-3-(cyclopentyloxy)-4-methoxybenzamide (RP73401) inhibits TNF-α release $IC_{50}=5$ nM and was used as a control. (a) In vitro inhibitory effects on TNF-α release by human monocytes. The effects of compounds on TNF-α production by human peripheral blood monocytes (PBMs) are examined as follows: blood is drawn from normal donors, mixed with dextran, and the erythrocytes allowed to sediment for 35 min at 37 °C. Leukocytes are fractionated by centrifugation through a discontinuous (18, 20, and 22%) metrizamide gradient. The mononuclear cell fraction comprising 30–40% PBMs is suspended in Hank's balanced salt solution and stored at 4 °C until use. Cells from the PBM-rich metrizamide fraction are spun down (200 g for 10 min at 20 °C), resuspended at 10^6 PBM s/mL of medium; RPMI 1640 containing 1% v/v FCS, 50 U/mL penicillin and 50 mg/mL streptomycin (Gibco, UK), then plated out in 96-well plates at 2×10^5 cells/well. The medium (200 μL) is changed to remove any non-adherent cells and the remaining, adherent PBMs left in the incubator overnight (18 h). One h prior to challenge, the medium is changed to that containing compound for test or drug vehicle. Control treatments and compounds for test are assayed in quadruplicate wells. Compounds are tested within the concentration range of 3×10^{-11} M to 3×10^{-6} M. Medium (50 μL) with or without 10 ng/mL LPS (*E. Coli*, 055 B5 from Sigma, UK) is then added. The incubation is then continued for a further 4 h. Cell supernatants are removed for storage at −20 °C. TNF-α levels in cell supernatants are quantified using a standard sandwich ELISA technique. ELISA plates (Costar, UK) are coated overnight at 4 °C with 3 mg/mL polyclonal goat anti-human TNF-α antibody (British Biotechnology, UK) in pH 9.9 bicarbonate buffer. Rabbit polyclonal anti-human TNF-α antiserum (Janssen Biochimicha, Belgium) at 1/500 dilution is used as the second antibody and polyclonal goat anti-rabbit IgG horseradish peroxidase (Calbiochem, USA) at 1/8000 dilution is used as the detection antibody. Color development is measured by absorbance at 450 nm using a Titek plate reader. TNF-α levels are calculated by interpolation from a standard curve using recombinant human TNF-α (British Biotechnology UK)(0.125-8 ng/mL). Data (log-concd vs log-resp) are fitted by linear regression ($P>0.99$) using a Multicalc (Wallac Pharmacia, UK) software program. Basal TNF-α levels are less than 100 pg/mL whilst LPS (lipopoly-saccharide) stimulation of the PBMs increases TNF-α levels to 3–10 ng/mL.