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ARTICLE

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Design and Synthesis of Biphenyl and Biphenyl Ether Inhibitors of Sulfatases

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Abstract

Inhibitors of sulfatase-2 are putative anticancer agents, but the discovery of potent small molecules targeting this enzyme has proved challenging. Based on molecular modelling, two series of sulfatase-2 inhibitors have been developed with biphenyl and biphenyl ether scaffolds judiciously substituted with sulfamate, carboxylate and other polar groups (e.g. amino). Inhibition of aryl sulfatase A and B was also determined. The biphenyl ether derivatives were less selective for sulfatase-2 over aryl sulfatase B than the biphenyl series. All biphenyl ether derivatives inhibited aryl sulfatase A, whereas only amino derivatives inhibited aryl sulfatase B significantly. In the biphenyl series few derivatives exhibited activity against aryl sulfatase B. The trichloroethylsulfamate group was identified as a new pharmacophore enabling potent inhibition of all of the sulfatases studied.

Introduction

Sulfatase-1 (Sulf-1) and sulfatase-2 (Sulf-2) catalyse the hydrolysis of the 6-*O*-sulfate group attached to glucosamine residues in heparan sulfate proteoglycans (HSPGs).¹ The HSPG substrates consist of a protein core linked to heparan sulfate polysaccharide chains derived from repeating disaccharide units comprising a hexuronic acid (glucuronic acid or iduronic acid) and glucosamine. Removal of the 6-*O*-sulfate affects the mobilisation of heparan bound growth factors and cytokines,² including fibroblast growth factor (FGF) and wingless-related integration site (wnt).³ The role of FGF in cell proliferation, invasion, migration and angiogenesis,⁴ and of wnt in cell growth and proliferation,⁵ implicate the sulfatases as potential targets for therapeutic intervention in certain diseases including pulmonary fibrosis⁶ and prostate,⁷ colorectal,⁸ ovarian, and breast cancers.⁹ Very few small molecule inhibitors of Sulf-1 and Sulf-2 have been reported.^{10, 11} On re-synthesis, the purported

activity against Sulf-2 of a group of monosaccharide sulfamate-based inhibitors could not be replicated.¹² The nitron 'OKN-007' is a suboptimal Sulf-2 inhibitor tool compound, having several proposed potential chemical and biological mechanisms of action in addition to Sulf-2 inhibition, including suppression of NO production, S-nitrosylation of critical proteins and inhibition of NF- κ B activation.¹³ The difficulty in identifying small molecule inhibitors of Sulf-2 has led to the investigation of indirect methods to affect Sulf-2 through inhibition of the proteosomal machinery.¹⁴ However, in this paper we report new saccharide mimics that inhibit Sulf-2 directly. We have also identified the trichloroethylsulfamate group as a new pharmacophore for Sulf-2 inhibition. Among the compounds described are the first relatively potent small molecule inhibitors of Sulf-2 that will aid further biological studies and assist elucidation of the role of Sulf-2 in cancer.

De Novo Inhibitor Design

There is no crystal structure for Sulf-2 and a lack of either small molecule inhibitors or biological assay suitable for a high-throughput screening campaign. Given these constraints, the structure of heparin sulfate proteoglycans, which are the endogenous ligands of Sulf-2, was used as a template for the design of potential Sulf-2 inhibitors. With no solved structures of a HSPG, the principal structural motifs of HSPG reported to be substrates for Sulf-2 are the highly sulfated regions, which closely resemble heparin in structure.¹⁵ However, structures of heparin in solution have been solved by NMR (pdb code 1hpn).¹⁶ These were compared with structures of heparin bound to protein partners such as the NK1 growth factor (pdb code 1gmn), 3-*O*-sulfotransferase (pdb code 1t8u) and heparin lyase (pdb code 3ina). The bound form was found to resemble the solution α -helical form, with interactions with protein binding partners being dominated by contacts between the

sulfate and carboxylate functionalities on the surface of the heparin helix. Negatively charged sulfate and carboxylate groups cover most of the accessible surface of the heparin polymer, and have been shown to regulate the binding of heparin to the enzyme 3-*O*-sulfotransferase.^{16, 17} X-ray crystal structures of FGF-1 and FGF-2 in complex with heparin oligosaccharides also revealed the importance of the sulfate and carboxylate groups for binding.¹⁸ These studies demonstrated that interactions of the charged sulfate and carboxylate groups on the heparin oligosaccharide with basic amino acids on protein binding partners are the main contributors to tight and specific binding. Mimicking these ionic interactions was a key consideration for *de novo* design of potential non-saccharide based Sulf-2 inhibitors.

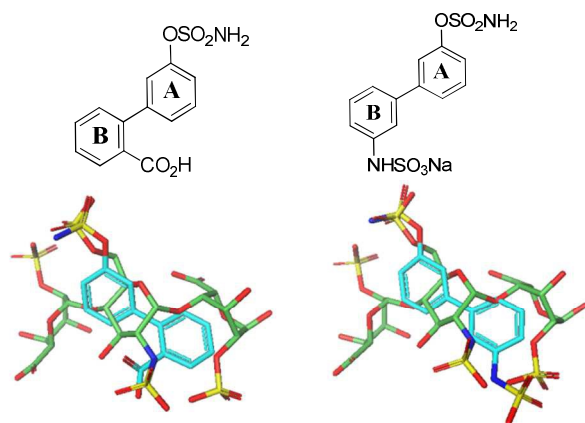


Figure 1: Representative overlays of biphenyl targets (cyan) with a heparin-derived trisaccharide template (green) created using the proprietary structural visualisation program MoViT (Pfizer).

Potential sulfamate inhibitors were designed with the aim of identifying hit compounds with improved Sulf-2 inhibition and physicochemical properties compared to the monosaccharides.⁶ The arylsulfamate group has been shown to be a privileged structure for the inhibition of sulfatases,¹⁹ although much of this work was focused on steroid sulfatase (STS). Potential Sulf-2 inhibitors were designed initially by overlaying a primary phenylsulfamate (A-ring) with the 6-*O* sulfate of glucosamine of a representative trisaccharide taken from the reducing end of the heparin solution structure (1hpn). Vectors where the phenyl ring could be substituted to allow incorporation of a second polar group that could overlay a surface polar group on the heparin helix were explored. Compounds from both biphenyl (Figure 1) and biphenyl ether sulfamate (Figure 2) series were energy minimised and found to enable such a superposition of polar functionality. A primary sulfamate group at the 3-position of the (A-ring) in the biphenyl series enabled substituents on the second phenyl ring to overlay the *N*-sulfate of glucosamine or the *O*-sulfate of iduronic acid in the template structure. In addition to these rationally designed derivatives, the biphenyl core was used to probe additional polar and ionic interactions through incorporation of polar functional groups at the *ortho*, *meta* and *para* positions of the B-ring. In the biphenyl ether sulfamate scaffold the oxygen linker allows different conformations with alternative positioning of groups on the B-ring relative to the A-ring.

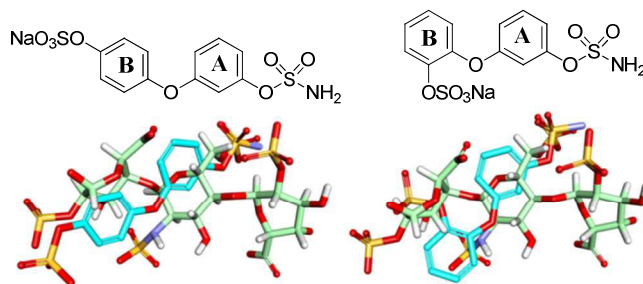
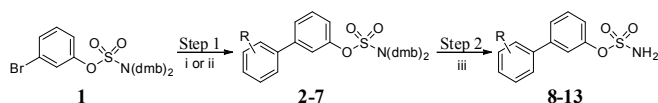


Figure 2: Representative overlays of biphenyl ethers (cyan) with a heparin-derived trisaccharide template (green)

Synthesis of Inhibitors

A number of highly efficient synthetic routes were implemented that should have wide applicability for sulfamate synthesis. Key to the synthesis of the desired templates was our previously reported sulfamate protecting group methodology using the *N*-2,4-dimethoxybenzyl (dmb) group,²⁰ which was applied to the synthesis of libraries of biphenyl- and biaryl ether-sulfamates. Microwave-assisted Suzuki-Miyaura cross-coupling of **1** in the presence of potassium carbonate and Pd(PPh₃)₄ afforded **2** in 75% yield (Method i, Scheme 1 and Table 1). Alternative Suzuki conditions (Method ii, Scheme 1 and Table 1) were required for the preparation of **3-7**. All deprotections proceeded at room temperature in dilute TFA, resulting in high yields of the desired primary sulfamates **8-13**.

Scheme 1: Synthesis of biphenyl sulfamates 8-13.

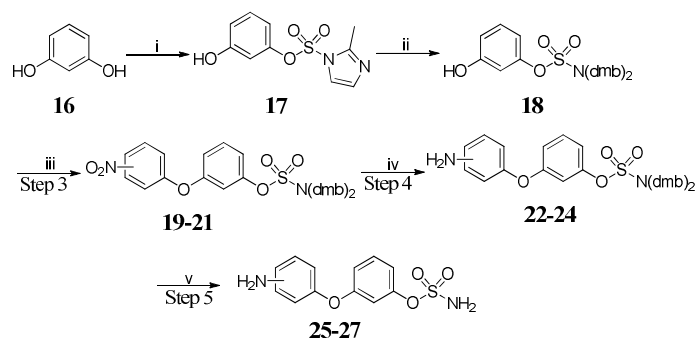


Reagents and conditions: (i) K₂CO₃, RC₆H₄B(OH)₂, Pd(PPh₃)₄, MeCN, 120 °C, μ W, 20 min; (ii) RC₆H₄B(pin), 2 M aq. Na₂CO₃, Pd(dppf)Cl₂, dioxane, 80 °C, μ W, 20 min; (iii) 10% TFA/DCM, RT, 2 h.

Table 1: Summary of yields for synthesis of biphenyl sulfamates.

R	Step 1		Step 2
	Method	Yield	Yield
2-CO ₂ Me	i	2 75%	8 88%
3-CO ₂ H	ii	3 79%	9 82%
4-CO ₂ H	ii	4 77%	10 90%
2-NH ₂	ii	5 82%	11 83%
3-NH ₂	ii	6 77%	12 82%
4-NH ₂	ii	7 72%	13 82%

The preparation of amino-substituted biaryl ether-sulfamates **25-27** started from resorcinol **16** (Scheme 2 and Table 2). The use of high dilution (0.08 M) and low temperature prevented side-reactions during the methylation/imidazolium displacement step. S_NAr reaction of fluoronitrobenzenes with **18** gave biphenyl ether intermediates **19-21**, which were reduced *via* palladium-catalysed flow hydrogenation. Deprotection of **22-24** gave high yields of the desired primary sulfamates **25-27**.

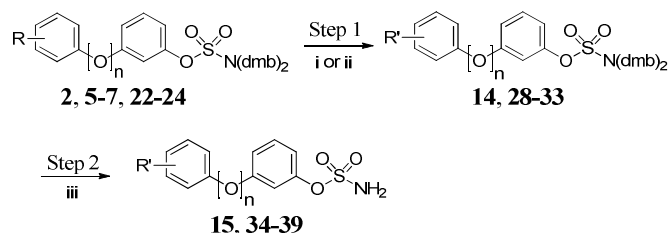
Scheme 2: Synthesis of biaryl ether-sulfamates **25-27**.

Reagents and conditions: (i) 1,1'-sulfonylbis(2-methyl-1H-imidazole), Cs₂CO₃, MeCN, 120 °C, μW, 15 min, 80%; (ii) a) Me₃O·BF₄, DCM:THF (8:1), 0 °C to RT, 9 h; b) bis(2,4-dimethoxybenzyl)amine, MeCN, 42 °C, 24 h, 60%; (iii) fluoronitrobenzene, K₂CO₃, DMF, 150 °C, μW, 20 min; (iv) H₂, 10% Pd/C, MeOH:THF (3:1), RT, 24 h; (v) 10% TFA/DCM, RT, 2 h.

Table 2: Summary of yields for synthesis of biaryl ether-sulfamates.

Position	Step 3	Step 4	Step 5
2'-	19 80%	22 85%	25 90%
3'-	20 75%	23 87%	26 82%
4'-	21 85%	24 86%	27 65%

Methyl ester **2** was hydrolysed under basic conditions and the resulting carboxylic acid **14** was deprotected to give **15** (Scheme 3 and Table 3). Acetylation of aniline derivatives **5-7** and **22-24** afforded acetamidobiphenyls **28-33**, which were deprotected to give **34-39**.

Scheme 3: Functionalisation/deprotection of protected biphenyl sulfamates.

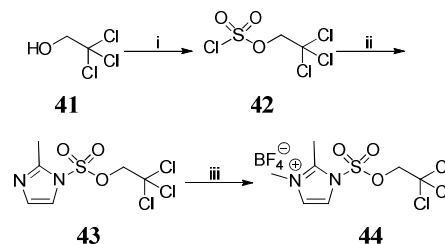
Reagents and conditions: (i) LiOH, H₂O/THF, 60 °C, 24 h, 80%; (ii) Ac₂O, NEt₃, DCM, RT, 24 h; (iii) 10% TFA/DCM, RT, 2 h.

Table 3: Summary of yields for functionalisation/deprotection of protected biphenyl sulfamates.

R	R'	n	Step 1		Step 2
			Method	Yield	Yield
2-CO ₂ Me	2-CO ₂ H	0	i	14 80%	15 89%
2-NH ₂	2-NHAc	0	ii	28 87%	34 86%
3-NH ₂	3-NHAc	0	ii	29 89%	35 91%
4-NH ₂	4-NHAc	0	ii	30 88%	36 84%
2-NH ₂	2-NHAc	1	ii	31 87%	37 85%
3-NH ₂	3-NHAc	1	ii	32 88%	38 94%
4-NH ₂	4-NHAc	1	ii	33 86%	39 92%

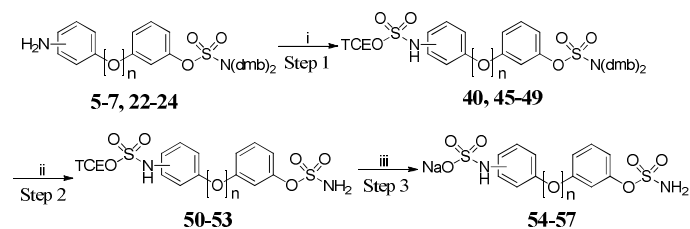
Attempted preparation of the 2'-sulfamic acid derivative by reaction of aniline **5** with sulfur trioxide-pyridine complex was

unsuccessful. Taylor *et al* reported the 2,2,2-trichloroethyl (TCE) group as an effective protecting group for arylsulfate esters²¹ and this methodology was adapted to the synthesis of protected sulfamic acids. TCE chlorosulfate **42** was obtained by reacting sulfur chloride with one equivalent of 2,2,2-trichloroethanol **41** (Scheme 4). Reaction of **42** with 2-methylimidazole gave 2-methylimidazole-1-sulfonate **43**, which was methylated with Meerwein's salt to give **44**.

Scheme 4: Synthesis of 2,3-dimethyl-1-((2,2,2-trichloroethoxy)sulfonyl)-1H-imidazol-3-ium tetrafluoroborate **44**.

Reagents and conditions: (i) SO₂Cl₂, pyridine, Et₂O, -78 °C, 4 h, 83%; (ii) 2-methylimidazole, THF, 0 °C to RT, 16 h, 95 %; (iii) Me₃O·BF₄, DCM, 0 °C to RT, 20 h, 86%.

Reacting the appropriate anilines with 3 molar equivalents of **44** under microwave irradiation in acetonitrile at 120 °C for 20 min afforded the *meta*- (**45** and **48**) and *para*- (**46** and **49**) derivatives, but poor conversion was observed with *ortho*-anilines **5** and **22** (Scheme 5 and Table 4). The trichloroethyl group was stable under the acidic conditions used for sulfamate deprotection. Cleavage of the trichloroethyl group was achieved using zinc in a mixture of methanol and acetate buffer with no desulfamoylation. The resulting sulfamic acids were converted to sodium salts using ion exchange chromatography, affording targets **54-57** in high yields.

Scheme 5: Synthesis of amino-sulfate derivatives **54-57**.

Reagents and conditions: (i) **44**, MeCN, 120 °C, μW, 20 min; (ii) 10% TFA/DCM, RT, 2 h; (iii) a) Zn powder, MeOH, acetate buffer pH 4.65, 60 °C, 2 h; b) Dowex 50W8X2 Na⁺ form, H₂O.

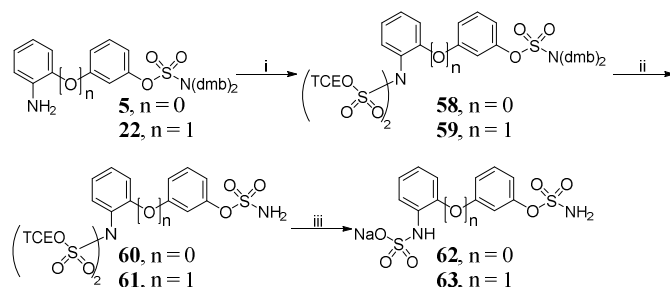
Table 4: Summary of yields for the synthesis of amino-sulfate derivatives.

Position	n	Step 1	Step 2	Step 3
2'-	0	40 0%	-	-
3'-	0	45 75%	50 86%	54 84%
4'-	0	46 82%	51 91%	55 80%
2'-	1	47 15%	-	-
3'-	1	48 78%	52 86%	56 75%
4'-	1	49 90%	53 89%	57 70%

Protection of 2-aminobiphenyls **5** and **22** with TCE chlorosulfate **44** produced bis-*N,N*-sulfated aminobiphenyls **58** and **59**, respectively (Scheme 6), which were deprotected to afford **60**

and **61**. A one-pot deprotection/mono-sulfate hydrolysis of **60** and **61** led to the isolation of **62** and **63**.

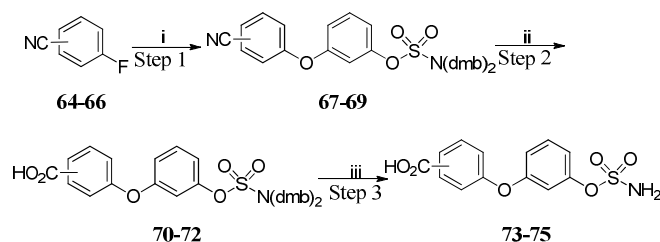
Scheme 6: Synthesis of amino-sulfate derivatives **62**, **63**.



Reagents and conditions: (i) **44**, NEt_3 , DMAP, THF, 0 °C to RT, 24 h, $n = 0$ 70%, $n = 1$ 55%; (ii) 10% TFA/DCM, RT, 2 h, $n = 0$ 90%, $n = 1$ 86%; (iii) Zn powder, MeOH, acetate buffer pH 4.65, AcOH, RT, 24 h; Dowex 50W8X2 Na^+ form, H_2O , $n = 0$ 50%, $n = 1$ 33%.

$\text{S}_{\text{N}}\text{Ar}$ reactions with fluorobenzonitriles **64-66** gave cyanobiphenyl ethers **67-69** which were hydrolysed to the corresponding benzoic acids **70-72** (Scheme 7 and Table 5). Acidic deprotection of the sulfamate moiety afforded targets **73-75**. Thus, a set of 13 substituted biphenyl and 12 biphenyl ether sulfamates were prepared. Compounds **76-82** (Table 6) bearing a single substituent on the aromatic core were also prepared by analogous routes (see Supplementary data).

Scheme 7: Synthesis of benzoic acid derivatives **73-75**.



Reagents and conditions: (i) **18**, K_2CO_3 , DMF, 150 °C, μW , 20 min; (ii) 2 M aq. NaOH, dioxane, 130 °C, μW , 2 h; (iii) 10% TFA/DCM, RT, 2 h.

Table 5: Summary of yields for synthesis of benzoic acid derivatives.

Position	Step 1	Step 2	Step 3
2'-	67 81%	70 45%	73 90%
3'-	68 61%	71 80%	74 94%
4'-	69 72%	72 65%	75 92%

Table 6: Analogues lacking a primary sulfamate group

Cmpd			
	R	X	R'
76	Ph	O	NH_2
77	Ph	O	NMe_2
78	OPh	O	NH_2
79	Ph	NH	OCH_2CCl_3
80	OPh	NH	OCH_2CCl_3

81	Ph	O	OCH_2CCl_3
82	Ph	NH	OCH_2CF_3
83	H	NH	OCH_2CCl_3

Biological Evaluation

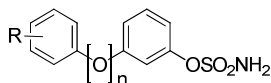
Compounds were assayed for their ability to inhibit the Sulf-2-catalysed desulfation of 4-methylumbelliferyl sulfate (4-MUS) to the fluorescent 4-methylumbelliferone (MU). To determine their sulfatase selectivity, inhibition of aryl sulfatases A (ARSA) and B (ARSB) was also assessed. To date no suitable benchmark inhibitors of these sulfatase have been reported. In the biphenyl series (Table 7A), only the trichloroethylsulfamates (**50** and **51**) exhibited high Sulf-2 inhibition giving almost complete inhibition of sulfatase activity at 1 mM concentration. The position and nature of the substituent on the B ring had a pronounced effect on the sulfatase inhibition. In the majority of cases the presence of a substituent on the B-ring was detrimental to Sulf-2 inhibition when compared with unsubstituted biphenyl sulfamate **76**. Aminosulfates **54** and **62** and carboxylic acid **15** retained potency. Surprisingly, the synthetic trichloroethylsulfamate intermediates **50** and **51** exhibited potent inhibition. Compounds **8**, **10**, **15**, **50** and **51** were also relatively potent ARSA inhibitors. The tertiary dimethylsulfamate **77** exhibited no Sulf-2 inhibition (0% inh @ 1 mM). Similar SAR was observed with the 3-phenoxyphenyl template (Table 7B) with the trichloroethylsulfamates **52** and **53** again proving to be superior inhibitors against all sulfatases tested. Aminosulfates **56** and **63** were the best Sulf-2 inhibitors lacking a trichloroethylsulfamate group. In summary, the design strategy of introducing polar groups to mimic polar groups of the endogenous substrate did not give the anticipated improvement in Sulf-2 inhibition, whereas the presence of a relatively large, lipophilic trichloroethylsulfamate group on the B-ring in both templates provided superior inhibitors.

To understand the origin of sulfatase inhibition by compounds **50-53**, compounds **79** and **80**, lacking a primary sulfamate, were prepared and found to also be potent Sulf-2 inhibitors (Table 8), indicating that the primary sulfamate group is not essential for sulfatase inhibition with these templates. Compound **79** exhibited some selectivity over ARSA and ARSB, whereas compound **80** was a good inhibitor for all of the sulfatases. Compound **83**, the simple phenyl trichloroethylsulfamate derivative did not retain Sulf-2 inhibitory activity, suggesting that the trichloroethylsulfamate group is not sufficient *per se* to impart sulfatase inhibition and is not conferring pan-sulfatase activity through a non-specific mechanism of action. Compound **83** did, however, possess inhibitory activity against both ARSA and ARSB. Trichloroethylsulfate **81** and trifluoroethylsulfamate **82** exhibited no Sulf-2 inhibition, but retained activity against ARSA and ARSB. Thus, it appears that the trichloroethylsulfamate group is a new motif for Sulf-2 inhibition, and that, in combination with lipophilic groups, inhibitors of Sulf-2 lacking a primary sulfamate moiety can be developed.

Sulf-2 IC_{50} values were determined for the most potent inhibitors (Table 9). Compound **50** had the highest Sulf-2 inhibitory activity with an IC_{50} value of 167 μM . The ARSA and ARSB inhibitory activity of all compounds tested proved to be higher than their Sulf-2 inhibitory activity, providing the first reported inhibitors of ARSA and ARSB with $\text{IC}_{50} < 1$ mM. Following screening at 1 mM, ARSA inhibition by **80** was evaluated over the concentration range 0.025-1 mM. Concentration-dependent inhibition was not observed and hence an IC_{50} value could not be determined. Aryl sulfamate-based inhibitors of steroid sulfatase have demonstrated irreversible inhibition profiles.¹⁵ Further studies will be required to determine if the aryl sulfamates such as **50**, and

trichloroethylsulfamates such as **80** behave as irreversible sulfatase inhibitors.

Table 7: Sulfatase inhibition data for biphenylsulfamates. Data are means of two or more determinations. n.d. = not determined.



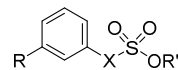
A: n = 0

Cpd	R	Sulf-2 % inh @ 1 mM	ARSA % inh @ 1 mM	ARSB % inh @ 1 mM
76	H	38	14	65
8	2'-CO ₂ Me	20	86	0
15	2'-CO ₂ H	31	98	0
9	3'-CO ₂ H	12	59	0
10	4'-CO ₂ H	22	85	0
11	2'-NH ₂	21	0	0
12	3'-NH ₂	20	0	0
13	4'-NH ₂	6	61	0
34	2'-NHAc	4	33	0
35	3'-NHAc	8	52	33
36	4'-NHAc	3	0	0
62	2'-NHSO ₃ Na	37	26	0
54	3'-NHSO ₃ Na	31	0	0
55	4'-NHSO ₃ Na	4	0	0
60	2'-N(SO ₃ CH ₂ CCl ₃) ₂	10	63	0
50	3'-NHSO ₃ CH ₂ CCl ₃	99	92	67
51	4'-NHSO ₃ CH ₂ CCl ₃	96	99	68

B: n = 1

Cpd	R	Sulf-2 % inh @ 1 mM	ARSA % inh @ 1 mM	ARSB % inh @ 1 mM
78	H	32	n.d.	n.d.
73	2'-CO ₂ H	10	44	0
74	3'-CO ₂ H	30	49	0
75	4'-CO ₂ H	23	33	0
25	2'-NH ₂	28	75	17
26	3'-NH ₂	31	75	0
27	4'-NH ₂	14	75	0
37	2'-NHAc	11	29	48
38	3'-NHAc	10	57	0
39	4'-NHAc	14	45	0
63	2'-NHSO ₃ Na	56	54	93
56	3'-NHSO ₃ Na	58	95	85
57	4'-NHSO ₃ Na	13	34	77
61	2'-N(SO ₃ CH ₂ CCl ₃) ₂	18	82	40
52	3'-NHSO ₃ CH ₂ CCl ₃	100	100	96
53	4'-NHSO ₃ CH ₂ CCl ₃	98	96	86

Table 8: Sulfatase inhibition data for analogues lacking a primary sulfamate group. Data are means of two or more determinations.



Cpd	R	X	R'	Sulf-2 % inh @ 1 mM	ARSA % inh @ 1 mM	ARSB % inh @ 1 mM
83	H	NH	CH ₂ CCl ₃	3	63	68
79	Ph	NH	CH ₂ CCl ₃	100	0	61
80	OPh	NH	CH ₂ CCl ₃	99	74	99
81	Ph	O	CH ₂ CCl ₃	1	70	85
82	Ph	NH	CH ₂ CF ₃	5	78	92

Table 9: Sulfatase IC₅₀ data for inhibitors **50-51**, **53**, **79-80**. IC₅₀ values were calculated using non-linear regression analysis for 7 different inhibitor concentrations each performed in duplicate to give the mean ± SEM.

Compound	Sulf-2 IC ₅₀ (μM)	ARSA IC ₅₀ (μM)	ARSB IC ₅₀ (μM)
50	167±5	55±11	130±6
51	566±28	91±4	101±8
53	390±11	108±2	116±10
79	254±3	No inhibition	30±11
80	298±3	nd	34±9

Conclusions

Two new series of sulfatase inhibitors have been developed based on biphenyl and biphenyl ether templates, with highly effective synthetic procedures providing access to a variety of sulfamates and aminosulfamates. The trichloroethylsulfamates **50-53**, **79** and **80** exhibited favourable Sulf-2 inhibition. The trichloroethylsulfamate group has therefore been identified as a new pharmacophore for sulfatase inhibition. Further studies will be required to determine the origin of sulfatase inhibition by aryl trichloroethylsulfamates. The most potent Sulf-2 inhibitors lacking a trichloroethylsulfamate group were biphenyl ether aminosulfamates **56** and **63**. The diphenyl ether derivatives also proved to be moderate to good inhibitors of ARSA, whereas significant inhibition of ARSB was only observed with substituted amino derivatives. This trend was not retained in the biphenyl series with few derivatives exhibiting activity against ARSB. However, the first inhibitors of ARSA and ARSB with IC₅₀ < 1 mM are reported. Additional SAR studies are underway to improve Sulf-2 potency and to delineate the factors affecting selectivity.

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Notes and references

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[†] Deceased 24 September 2014.

[‡] Electronic Supplementary Information (ESI) available for all experimental procedures: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

Author contributions: DCM, BTG and RJG designed the inhibitors; BTG, DCM and DRN wrote the manuscript; TR, AB (Bertoli) and AB (Brennan) performed the chemical synthesis supervised by CC, BTG, RJG and DCM; SFA and GSB performed the biological assays supervised by DRN and HLR.

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