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Acyl sulfonamide anti-proliferatives. Part 2: Activity of heterocyclic sulfonamide derivatives

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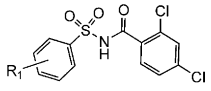
Abstract—The anti-proliferative activity of acylated heterocyclic sulfonamides is described in Vascular Endothelial Growth Factor-dependent Human Umbilical Vascular Endothelial Cells (VEGF-HUVEC) and in HCT116 tumor cells in a soft agar diffusion assay.

We have recently reported on the in vitro activity of acylsulfonamide anti-proliferative agents **1**, demonstrating a correlation between cytoxicity as determined in Vascular Endothelial Growth Factor-dependent Human Umbilical Vascular Endothelial Cells (VEGF-HUVEC) and anti-tumor activity as measured in a soft-agar disk diffusion (SADD) assay versus HCT116 colon carcinoma cells.[1,2] The mechanism of action of the compounds remains under investigation, but the series shows efficacy in vivo in the tumor xenograft model, HCT 116, with no correlation to known drugs in the NCI COMPARE analysis. In these studies, 3,4-disubstituted benzenesulfonamides were found to be highly active, and it was therefore of interest to determine the activity of a variety of heterocyclic sulfonamide derivatives (Table 1).

We found that fused bicyclic heterocyclic derivatives that mimic the 3,4-disubstitution pattern of benzene–sulfonamide are comparably active to **1c** (Table 2). Thus, the attachment of the sulfonamide to the aromatic six-membered ring at position 5 or 6 (e.g., **2b**,**c**, **3b**, **4a**) is preferred approximately twofold over to attachment of the sulfonamide to the 2-position of the five-membered ring (**2a** and **3a**). The least active compounds are those substituted by the sulfonamide at the 4- or 7- site in these 5/6 fused ring systems (**2d**,**e**, **3c**, **4b**). This finding was consistent with the SAR of parent series **1**. Placement of the sulfonyl in the *ortho* position resulted in the least active members of that family, and the 4- and 7-substitution of **2**, **3**, and **4** is a pseudo-*ortho* position for these heterocycles.

Fused heterocycles containing a saturated ring are also active, and oxygen-containing analogs attached through the aromatic ring have IC_{50} values <0.5 μM (Table 3;

Table 1. VEGF-HUVEC inhibition by previously reported compounds



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Compound	R_1	Ave. VEGF-HUVEC IC_{50}[a] (μM)	HCT116, SADD zone of inhibition[b]	Dose (μg, disk)
1a	(H)	0.53	700	115
1b	4-Cl	0.17	660	200
1c	3,4-diCl	0.46	560	200

[a] Values are means of at least three experiments.
[b] Zone of inhibition units (200 units = 6.5 mm).

Keywords: Cytotoxic; Oncolytic.
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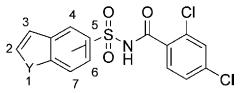
Table 2. VEGF-HUVEC inhibition by 5/6 fused heterocyclic sulfonamide analogs[a]

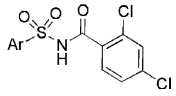


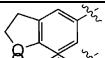
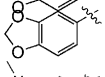
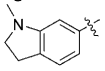
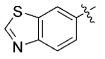
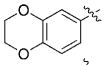
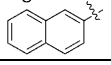
2; X = S
3; X= O
4; X=NH

Compound	Y	Point of sulfonamide attachment	VEGF-HUVEC IC_{50} (μM)
2a	S	2-	0.96
2b	S	6-	0.64
2c	S	5-	0.62
2d	S	4-	3.30
2e	S	7-	4.16
3a	O	2-	0.97
3b	O	6-	0.59
3c	O	7-	5.21
4a	NH	6-	1.87
4b	NH	7-	3.04

[a] Values are means of at least three experiments.

Table 3. VEGF-HUVEC inhibition by fused heterocyclic sulfonamide analogs[a]



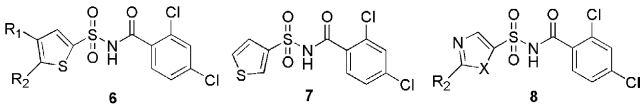
Compound	Ar	VEGF-HUVEC IC_{50} (μM)
5a		0.43
5b		0.48
5c		1.54
5d		0.89
5e		0.40
5f		0.80

[a] Values are means of at least three experiments.

5a,**b**,**e**). The least active within this set is the naphthyl derivative **5f**, but the observation of decreased activity when the distal ring is the six-membered aromatic is consistent with the activity of the 2-benzofuranyl and 2-benzothienyl analogs **2a** and **3a**, respectively. The benzodioxinyl analog **5e** was more active than **5f**, but the saturated heterocycle **5e** may allow for greater flexibility in fitting into the target active site.

Monoheterocyclic sulfonamides were also highly active in the primary assay (Table 4). The thiophene analogs **6a**–**m**, whether substituted at the 4- or 5-position of the thiophene, showed similar potency to the substituted phenyl analogs **1**. Optimal cases were the thiophenes with small substituents, such as halo or alkyl examples **6a**–**j**, with IC_{50} values <1 μM. The activity of thiophenes

Table 4. VEGF-HUVEC inhibition by five-membered ring heterocyclic sulfonamide analogs[a]



6 **7** **8**

Compound	R_1	R_2	X	VEGF-HUVEC IC_{50} (μM)	c Log *P*
6a	H	H	—	0.54	3.23
6b	H	Br	—	0.25	4.19
6c	Br	H	—	0.60	4.19
6d	Br	Br	—	0.22	4.89
6e	Cl	Br	—	0.76	4.80
6f	H	Cl	—	0.36	4.04
6g	Cl	H	—	0.39	4.04
6h	H	Me	—	0.60	3.73
6i	Me	H		0.70	3.73
6j	H	Et	—	0.31	4.26
6k	H	MeO	—	0.68	3.31
6l	H	MeO_2C	—	1.39	3.51
6m	H	NC	—	3.16	3.10
7	—	—	—	1.27	3.23
8a	—	Cl	S	1.09	3.30
8b	—	OMe	S	0.54	3.37
8c	—	H	S	2.24	2.58
8d	—	Me	S	1.18	3.08
8e	—	Et	S	0.60	3.60
8f	—	Et	NH	>20	2.88

[a] Values are means of at least three experiments.

6a–**m** appears to be independent of the substituents' electronic effects, but more closely correlated to their lipophilicity as predicted by c log *P*,[3] with a value of >4.0 tending to prevail in the most potent thiophene analogs. A loss of potency relative to **6a** is observed if the thiophene itself is attached as a 3-sulfonamido derivative (e.g., **7**), but in the absence of a molecular target, the reasons for this deviation are not clear.

Addition of nitrogen within the five-membered ring resulted in decreased activity. Thiazoles **8a**–**e** were two- to fivefold less potent than the corresponding thiophenes, with one exception (**8b**), and imidazole analog **8f** was inactive under the assay conditions. More generally, inclusion of nitrogen in the ring systems in Tables 2–4 results in a decreased activity relative to the oxygen and sulfur analogs with sulfonamide attached at the same position (e.g., **4a** vs **2b** and **3b**). The indole, thiazole, and imidazole analogs presumably are more hydrophilic than the thiophene series because of the ring nitrogen, and may not penetrate the HUVEC cell membrane as efficiently as the thiophenes.[1] An alternative explanation may be that the decrease in activity of the nitrogen heterocycles reflects an unfavorable interaction with the receptor.

Representative potent compounds from each series were additionally assayed against HCT116 tumor cells in a SADD assay that provided a qualitative measure of the compounds oncolytic, cytotoxic activity. We earlier observed that approx. 80% of compounds with IC_{50} values <1 μM in the VEGF-HUVEC assay were also active in HCT116, with lesser effect on normal fibroblasts and

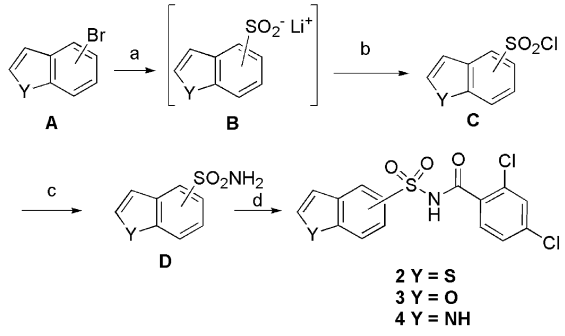
Table 5. SADD activity of compounds with VEGF-HUVEC $IC_{50} < 1$ μM[a]

Compound	HCT116	L1210	Normal fibroblasts	Dose (μg/disk)
2a	500	0–100	0–370	210
5a	680	0	0–400	210
5b	610	0	0–400	200
5c	630	0–60	0–330	210
5e	580	0–250	0–400	200
5f	470–530	200–270	0–270	210
6a	490	0	0–350	190
6b	600	0–180	0–390	190

[a] SADD Zone of Inhibition reported, zone of inhibition units: 200 units = 6.5 mm. A zero zone means no inhibition.

L1210 leukemia cells.[1] The heterocyclic sulfonamide derivatives possessed a similar profile as shown in Table 5, indicating the heterocyclic analogs are likely to be active at the same receptor.

The primary synthetic focus was the preparation of the heterocyclic sulfonamides, and a variety of methods were employed. In the preparation of 4-, 5-, 6-, and 7-sulfonamido 5/6 fused heterocycles, the key precursors were the brominated analogs **A**,[4] which were subjected to strong base to effect lithium–halogen exchange. The bromoindole precursors for products **4a** and **4b** required no protection, but were first deprotonated by KH at 0 °C, then reacted with an excess of *t*-BuLi at −78 °C to effect the halogen/metal exchange. In all cases, the lithiated intermediate was treated with SO_2 gas, followed by oxidation in situ of the lithium sulfinate intermediates **B** by *N*-chlorosuccinimide (NCS). Stirring the resultant sulfonyl chlorides with ammonium hydroxide provided the desired sulfonamides **D** (Scheme 1).[5] Preparation of 2-sulfonamido-benzothiophene and 2-sulfonamido-benzofuran was accomplished respectively by deprotonation of benzothiophene and benzofuran with *n*-BuLi, followed by treatment with SO_2, NCS, and NH_4OH as above.[6] The saturated, oxygenated bicyclic heterocyclic sulfonamides giving rise to products **5a**,**b**, and **5e** were prepared by literature methods.[7] The sulfonamides were reacted with 2,4-dichlorobenzoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and *N*,*N*-dimethylaminopyridine (DMAP) to provide the acylsulfonamide products.



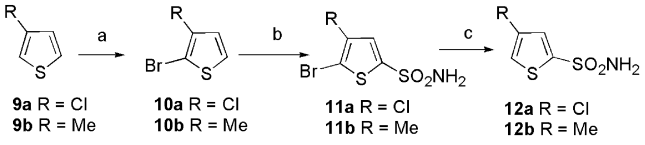
Scheme 1. Reagents and conditions: (a) (i) base, THF, −78 °C; (ii) SO_2, −78 °C to rt; (b) NCS; (c) NH_4OH; (d) 2,4-dichlorobenzoic acid, EDC, DMAP, CH_2Cl_2, rt.

Preparation of the 4-substituted thiophene-2-sulfonamides presented the greatest challenge, as deprotonation/sulfonylation of **9a** or **9b** would have yielded a 2,3-disubstitution pattern. Thus, a bromine blocking group was first introduced at the 2-position (Scheme 2). Sulfonylation by PCl_5 and chlorosulfonic acid followed by treatment in concentrated ammonium hydroxide gave the trisubstituted sulfonamides **11a** and **11b**. Removal of the blocking group was accomplished by treatment of the bromothiophenes with zinc dust to yield **12a** and **12b**.

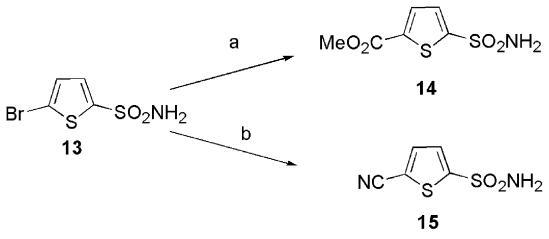
Thiophene derivatives were also prepared from commercially available 5-bromothiophene-2-sulfonamide **13** (Scheme 3). Palladium-catalyzed methoxy-carbonylation provided ester **14**,[8] and palladium-catalyzed cyanation of the bromide resulted in nitrile **15**.[9]

Thiazolyl sulfonamides **18** were prepared by deprotonation/sulfonylation, followed by oxidation of the lithium sulfinate salt by one of two methods (Scheme 4). Alkyl and halo substituted thiazole sulfinate derivatives were treated with *N*-chlorosuccinimide followed by aqueous ammonium hydroxide.[6,10] Alternatively, the lithium sulfinate of an alkoxy or unsubstituted thiazole was aminated by treatment with hydroxylamine-*O*-sulfonic acid to provide the sulfonamides.

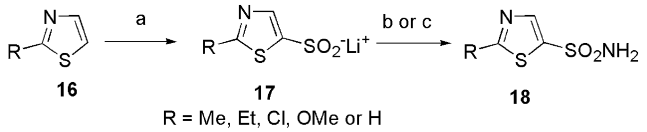
Finally, the imidazole analog was prepared from 2-ethylimidazole as shown in Scheme 5. This route required protection of N-1 in compound **19** by [2-(trimethylsilyl)-ethoxy]-methyl chloride (SEM chloride), and then proceeded as above, with a sequence of a deprotonation, SO_2 quench, NCS oxidation, and treatment with aqueous concentrated ammonium hydroxide, yielding sulfonamide **22**. Following the EDC coupling of **22** to



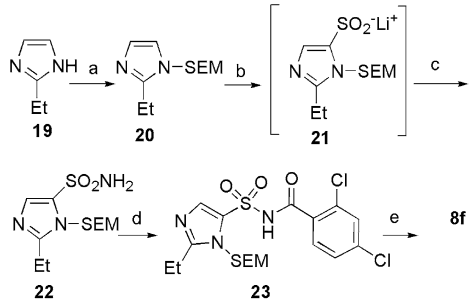
Scheme 2. Reagents and conditions: (a) NBS, $CHCl_3$, AcOH, rt; (b) (i) PCl_5, $ClSO_3H$; (ii) NH_4OH, acetone; (c) Zn dust, AcOH.



Scheme 3. Reagents and conditions: (a) CO, $Pd(OAc)_2$, $Ph_2P(CH_2)_3PPh_2$, Et_3N, DMF, MeOH; (b) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, μ-wave.



Scheme 4. Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 °C; (ii) SO_2, −78 °C to rt; (b) for R = Me, Et, or Cl: (i) NCS; (ii) NH_4OH; (c) for R = OMe or H: H_2NOSO_3H, NaOAc.



Scheme 5. Reagents and conditions: (a) SEMCl, NaH, THF, 0 °C; (b) (i) *n*-BuLi, THF, −78 °C, (ii) SO_2; (c) (i) NCS, (ii) NH_4OH; (d) 2,4-dichlorobenzoic acid, EDC, DMAP, CH_2Cl_2, rt; (e) HCl, EtOH, 50 °C.

2,4-dichlorobenzoic acid, removal of the SEM protecting group under acidic conditions provided **8f**.

Thus, several mono- and bicyclic heterocyclic sulfonamides were found to possess activity in VEGF-HUVEC and HCT116 SADD assay comparable to the parent biaryl series **1**. The preferred point of sulfonamide substitution for 5/6 fused bicyclic compounds is at the 5- or 6-position, and for thiophenes is at the 2-position. The in vivo activity of these analogs in tumor xenograft models is under investigation.

Acknowledgements

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5. A representative example for the conversion of a heterocyclic bromide to a sulfonic acid amide is as follows: To a solution of 4-bromobenzo[*b*]thiophene (1.43 g, 6.71 mmol) in anhydrous Et_2O (31 mL) at −78 °C was added *t*-BuLi (9.1 mL of 1.7 M in pentane, 15.4 mmol) dropwise. After stirring for 40 min at −78 °C, the reaction was warmed to 0 °C for 5 min then re-cooled to −78 °C. Sulfur dioxide was bubbled over the solution for 5 min then the reaction was warmed to room temperature overnight. *N*-Chlorosuccinimide (2.69 g, 20.1 mmol) was added to the reaction and the mixture was stirred for 1.5 h. The reaction was filtered under vacuum and the precipitate was washed with Et_2O. The filtrate was concentrated under vacuum to give the crude sulfonyl chloride (1.07 g), which was dissolved in acetone (50 mL) and was added to a stirred solution of concentrated NH_4OH (20 mL) in acetone (20 mL) at 0 °C and the reaction was stirred for 1 h. The reaction was partitioned between EtOAc and H_2O and the aqueous layer was separated and extracted with EtOAc (2 ×). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under vacuum to give the crude product. Flash chromatography on silica gel eluting with hexane–CH_2Cl_2 (4:1) afforded the benzo[*b*]thiophene-4-sulfonamide (281 mg, 20%).
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