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Sulfonylated Aminothiazoles as New Small Molecule Inhibitors of Protein Phosphatases

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Abstract—Based on a previously identified lead structure, **SC-ααδ9**, we have developed a versatile new chemical scaffold that can be readily modified to generate libraries of both Tyr and dual specificity phosphatase inhibitors with reduced molecular weight and lipophilicity. The most potent analogue identified to date, aminothiazole **8z**, inhibits the dual specificity phosphatase Cdc25B with a K_i of 4.6 ± 0.4 μM and a Hill coefficient of 2.

The control of biological pathways exerted by reversible phosphorylation of enzymes and receptors extends from cell division, cell growth, and cellular transport to apoptosis and muscle contraction.[1] As part of a program to develop phosphatase inhibitors as mechanistic probes in cell biology and as potential antiproliferative agents,[2–4] we have used combinatorial chemistry strategies to develop pharmacophores **1** and **2** as inhibitors of Ser/Thr protein phosphatases (PP1 and PP2A), Tyr protein phosphatases (PTP1B), and, especially, Ser/Thr/Tyr dual specificity phosphatases (Cdc25 and VHR).[5] While the Tyr protein phosphatases and dual specificity phosphatases share a common $HC(X)_5R$ catalytic site and mechanism of catalysis, they have unique biological roles. Thus, PTP1B is believed to regulate intracellular insulin signaling[6a] while VHR functions to dephosphorylate the mitogen activated protein kinase substrates Erk1 and Erk2.[6b] Cdc25A and its isoforms B and C play a crucial role in the regulation of the cell cycle.[7] Each Cdc25 isoform is involved in a distinct cell cycle event: Cdc25A expressed early in the G1 phase controls entry into S phase and appears to be a crucial player in the DNA damage checkpoint mechanism.[1a,8] Cdc25B is expressed in G1 and G2 and is essential for preinitiation of G2/M transition and possibly S phase progression.[9] Cdc25C activates the mitotic kinase Cdk1 at the G2/M checkpoint.[10] Overexpression of Cdc25 has been implicated in cancerogenesis,[7] and given the importance of cell cycle checkpoints in maintaining genetic integrity and immune function, the identification of phosphatase-selective inhibitors should be potentially useful for the development of novel chemotherapeutic agents.

Our prior lead structure, the combinatorial library-derived **SC-ααδ9**, displayed low-micromolar activity against Cdc25B and sub-micromolar inhibition of PTP1B as well as moderate cancer cell-specific in vitro toxicity,[3] but in our SAR studies with scaffolds **1** and **2**, we were unable to reduce the lipophilicity and molecular weight of this compound without ablating enzyme inhibitory activity.[4] An intriguing aspect of the structure–activity profile of **SC-ααδ9**, however, was the pronounced influence of substituents at the oxazole moiety on the biological effects of this compound.[2,3] Accordingly, we decided to study a new heterocyclic library developed around the oxazole segment of **SC-ααδ9**.

Aminothiazoles have almost ubiquitous presence in pharmaceutical test samples and have been used as building blocks in dopamine and fibrinogen (GpIIb-IIIa) receptor antagonists,[11,12] DNA gyrase inhibitors,[13] antibiotics[14] and inhibitors of kynurenine 3-hydroxylase cyclin dependent kinases,[15,16] among others. *N*-Sulfonamide derivatives of aminothiazoles offer many possibilities for substituent modifications without being subjected to the ready metabolism of amide derivatives. α,β-Unsaturated sulfonamide derivatives retain considerable electrophilic character,[17] and since DSPases contain an active-site cysteine residue,[18] we investigated sulfonamides of type **8** as a new class of potentially irreversible Cdc25 inhibitors. The preparation of the aminothiazole component was readily accomplished by

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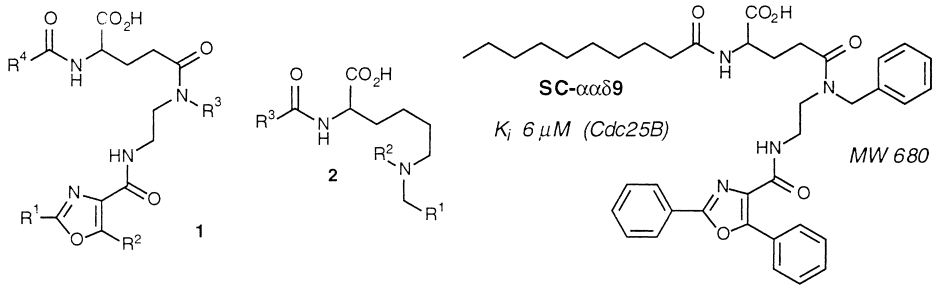
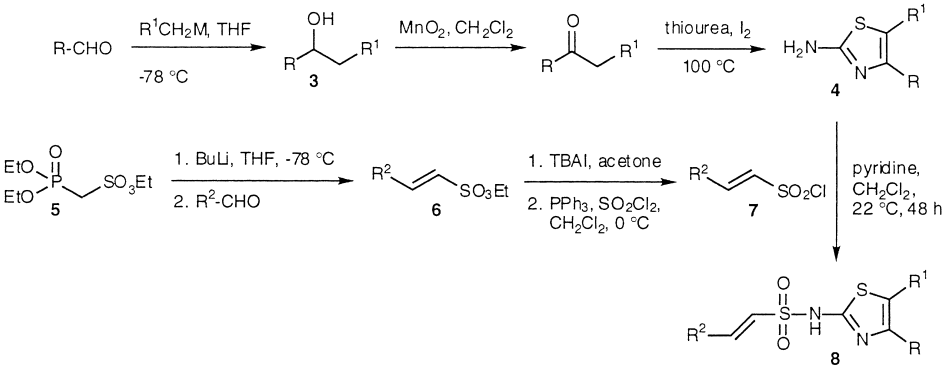


a Hantzsch synthesis (Scheme 1).[19] According to the method of King and Hlavacek,[20] aldehydes were converted to the α-methylene ketones that were not commercially available and exposed to a mixture of thiourea and iodine at 100 °C. α,β-Unsaturated sulfonylchlorides were obtained according to the protocols of Gennari and Roush.[17,21] Sulfonates **6** were isolated exclusively as *trans*-isomers after condensations of commercially available aldehydes with phosphonate **5** and were converted to the desired sulfonylchlorides **7** by cleavage of the ethyl group and treatment with triphenylphosphine and sulfuryl chloride.[22] A small library of *N*-sulfonated aminothiazoles was synthesized by treatment of **4** with **7** in the presence of pyridine as an acid scavenger.[23] Yields for the coupling reaction varied from 10% to 73% depending on the reactivity of the sulfonyl chlorides. In accordance with the SAR derived from the oxazole segment of **SC-ααδ9**, most of the substituents were aliphatic or aromatic groups.

Sulfonylated aminothiazoles **8** were evaluated for their ability to inhibit the in vitro enzyme activity of full length, recombinant, human Cdc25B, VHR, and PTP1B by our previously described methods[3] using *O*-methylfluorescein monophosphate as a substrate. Many aminothiazoles were effective in the low micromolar range, although none had submicromolar median inhibitory concentration (IC_{50}) values. As illustrated in Table 1, 15 of the 35 newly synthesized compounds had IC_{50} values for Cdc25B < 50 μM and five had IC_{50} values ≤ 25 μM, indicating the rich potential of this minimal pharmacophore. Among the best inhibitors, namely **8w**, **8y**, **8dd**, **8e**, **8ii**, and **8z**, except for **8e** all were substituted with halogenated aromatics in R and R^2. Because the parent SC-ααδ9 pharmacophore favored diphenyl oxazoles,[3] it was interesting that all of the most reactive compounds except **8e** had *n*-propyl moieties in R^1. A more detailed analysis of the modification on these domains was informative. For example, some variation in the aromatic halogens was tolerated on the R domain for Cdc25B inhibition but steric limitations existed. Thus, the 4-Cl phenyl containing thiazole **8dd** and the 4-CF_3 phenyl containing **8ii** had IC_{50} values of 21 and 25 μM, respectively, while the biphenyl containing **8cc** had an IC_{50} value of 40 μM. The 4-CF_3 phenyl containing **8y** ($IC_{50} = 20$ μM) and 4-chlorophenyl containing **8aa** ($IC_{50} = 34$ μM) were more potent Cdc25B inhibitors compared to the biphenyl containing **8t** ($IC_{50} = 46$ μM). Similarly, both **8w** and **8z** showed low micromolar IC_{50} values while **8ee** showed an IC_{50} for Cdc25B of >100 μM. The R^1 position had a considerable role in defining the potency of the sulfonylated aminothiazoles with phenyl substitutions being more potent than the ethyl or *n*-decyl substitutions. For example, **8d** had an IC_{50} of 26 μM while both **8o** and **8r** were essentially inactive at 100 μM. Similarly, **8a** and **8e** were more active than their congeners **8n** or **8q**. The R^2 domain had considerable flexibility in accepting substitutions. Generally, an aromatic residue containing an electron-withdrawing group performed well. Thus, **8cc** was superior to congeners lacking the difluorophenyl substitution, such as **8x**, **8u**, or **8v**, but was equivalent to **8bb**. Kinetic analyses of **8z** were most compatible with competitive inhibition of Cdc25B with a K_i of 4.6 ± 0.4 μM and a Hill coefficient of 2. For **8e** a K_i of 36 ± 6 μM was found when the best fit model, a competitive *S*-parabolic model, was used, which is consistent with two molecules of the inhibitor binding with the enzyme. It is interesting that the crystal structure of the Cdc25B catalytic domain revealed two independent potential binding sites for substrates.[24] Our



Scheme 1.

Table 1. Structures of thiazoles **8** and IC_{50} values (μM ± SEM) for Cdc25B, VHR, and PTP1B[a]

Thiazole	R	R^1	R^2	Cdc25B	VHR	PTP1B
8a	Ph	Ph	(4-NO_2)Ph	54 ± 4	47 ± 3	58 ± 4
8b	Ph	Ph	(4-Me)Ph	>100	>100	>100
8c	Ph	Ph	2-Furyl	>100	>100	>100
8d	Ph	Ph	(2-Cl)Ph	26 ± 2	40 ± 2	38 ± 3
8e	Ph	Ph	2-Naphthyl	22 ± 2	30 ± 1	45 ± 2
8f	Ph	Ph	(3,4-diOMe)Ph	>100	>100	>100
8g	Ph	Ph	(4-CF_3)Ph	34 ± 3	25 ± 1	36 ± 1
8h	Ph	Ph	*n*-Nonyl	33 ± 1	24 ± 2	49 ± 2
8i	Ph	Ph	(3,4-diF)Ph	72 ± 12	33 ± 7	85 ± 4
8j	Ph	Ph	(4-$MeCO_2$)Ph	26 ± 4	20 ± 1	59 ± 20
8k	Ph	Ph	(4-*n*-Bu)Ph	58 ± 1	39 ± 3	>100
8l	Ph	Ph	(4-CN)Ph	76 ± 3	32 ± 1	58 ± 3
8m	Ph	Ph	(4-Ph)Ph	70 ± 20	34 ± 1	90 ± 7
8n	Ph	Et	(4-NO_2)Ph	88 ± 10	50 ± 2	83 ± 4
8o	Ph	Et	(2-Cl)Ph	>100	>100	>100
8p	Ph	Et	(4-$MeSO_2$)Ph	>100	>100	>100
8q	Ph	Et	2-Naphthyl	89 ± 11	32 ± 3	>100
8r	Ph	*n*-Decyl	(2-Cl)Ph	>100	>100	>100
8s	2-Naphthyl	*n*-Propyl	2-Naphthyl	69 ± 2	34 ± 5	98 ± 5
8t	(4-Ph)Ph	*n*-Propyl	(4-Me)Ph	46 ± 2	21 ± 1	58 ± 12
8u	(4-Ph)Ph	*n*-Propyl	(4-CF_3)Ph	92 ± 0	42 ± 1	>100
8v	(4-Ph)Ph	*n*-Propyl	2-Naphthyl	>100	57 ± 1	>100
8w	(4-CF_3)Ph	*n*-Propyl	(2-Cl)Ph	14 ± 4	25 ± 1	10 ± 5
8x	(4-Ph)Ph	*n*-Propyl	(4-*n*-Bu)Ph	80 ± 9	54 ± 12	>100
8y	(4-CF_3)Ph	*n*-Propyl	(4-Me)Ph	20 ± 4	31 ± 16	20 ± 3
8z	(4-Cl)Ph	*n*-Propyl	(2-Cl)Ph	26 ± 4	32 ± 9	24 ± 4
8aa	(4-Cl)Ph	*n*-Propyl	(4-Me)Ph	34 ± 8	48 ± 20	32 ± 6
8bb	(4-Cl)Ph	*n*-Propyl	(4-Ph)Ph	38 ± 8	27 ± 8	35 ± 5
8cc	(4-Ph)Ph	*n*-Propyl	(3,4-diF)Ph	40 ± 12	22 ± 6	77 ± 8
8dd	(4-Cl)Ph	*n*-Propyl	(3,4-diF)Ph	21 ± 4	20 ± 5	20 ± 8
8ee	(4-Ph)Ph	*n*-Propyl	(2-Cl)Ph	>100	82 ± 7	>100
8ff	(4-Cl)Ph	Me	(3,4-diF)Ph	33 ± 3	43 ± 1	51 ± 2
8gg	(4-Cl)Ph	H	(2-Cl)Ph	89 ± 3	>100	>100
8hh	(4-Cl)Ph	Me	(2-Cl)Ph	>100	>100	>100
8ii	(4-CF_3)Ph	*n*-Propyl	(3,4-diF)Ph	25 ± 1	31 ± 1	29 ± 2

[a]IC_{50} values were generally calculated with a minimum of five concentrations (0.1 to 100 μM) for the Cdc25B assays and three concentrations (1, 10, and 100 μM) for VHR and PTP1B assays.

analysis did not reveal any evidence for covalent interactions of aminothiazoles with Cdc25B.

Consistent with the conserved enzymology and signature motif of the active site of Cdc25, VHR, and PTP1B, almost all of the compounds that were inactive against Cdc25B were also inactive against VHR and PTP1B (Table 1). Nevertheless, exceptions were noted. Thus, while **8dd** and **8cc** exhibited difference in their inhibitory activity against Cdc25B and PTP1B, they were equally effective against VHR. Thiazoles **8i**, **8q**, **8u**, and **8v** retained anti-VHR activity even though they lacked significant inhibitory activity against Cdc25B or PTP1B. This may reflect differences in the variable amino acids juxtaposed to the catalytic cysteine and essential arginine in the catalytic site. As was previously noted,[3] the regions surrounding the active sites of Cdc25 and PTP1B are hydrophobic, which may explain the enhanced inhibition seen with aminothiazoles containing aromatic moieties.

In summary, we have identified a versatile new chemical scaffold that can be readily modified to generate libraries of both Tyr and dual specificity phosphatase inhibitors with reduced molecular weight and lipophilicity compared to our prior lead structure. Of particular interest is the lack of a requirement for an obvious surrogate phosphate, such as the carboxylate in **SC-ααδ9**. The parallel inhibitor profiles of most of the compounds in this limited library confirm the general similarity in the architecture of the active site of Cdc25, VHR, and PTP1B. The considerable activity differences among the members of the library, however, also reinforce the possibility that selective small molecule inhibitors should be attainable. Among the ca. 40 aminothiazoles of type **8** tested to date, we have already identified a compound (**8z**) with a K_i for Cdc25B below that of **SC-ααδ9**. Thus, we expect that the sulfonylated aminothiazole scaffold will continue to be useful in the pursuit of potent and selective phosphatase inhibitors.

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23. All new products were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. **A typical procedure:** 2-(2-Chlorophenyl)-ethenesulfonic acid ethyl ester (**6**, R^2 = 2-ClPh). To a solution of triethyl α-phosphonomethane sulfonate (**5**, 1.0 g, 3.8 mmol) in THF (50 mL) was added at −78 °C under N_2 *n*-BuLi (1.6 M in hexanes, 2.4 mL, 3.8 mmol). The reaction mixture was stirred for 15 min at −78 °C, then 2-chlorobenzaldehyde (0.39 mL, 3.5 mmol) was added dropwise as a solution in THF (5 mL). After the addition of aldehyde was complete, the reaction mixture was warmed up to 22 °C, stirred for 12 h, and quenched with satd aq NaCl. The organic layer was separated, the aqueous layer was extracted with Et_2O, the combined organic layers were dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The solid precipitated from the residue was recrystallized from CH_2Cl_2/hexane to give pure **6** (0.57 g, 66%) as a colorless powder. Alternatively, the crude mixture can be purified by chromatography on SiO_2 (hexanes:Et_2O, 6:1): mp 69–70 °C (hexanes); ^{1}H NMR ($CDCl_3$) δ 7.95 (d, *J* = 15.6 Hz, 1H), 7.57 (d, *J* = 6.9 Hz, 1H), 7.42–7.31 (m, 3H), 6.79 (d, *J* = 15.3 Hz, 1H), 4.27–4.20 (m, 2H), 1.38 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 140.4, 135.1, 132.4, 130.4, 130.3, 128.3, 127.5, 124.0, 67.4, 15.0; HRMS (EI) calcd for $C_{10}H_{11}ClO_3S$ 246.0117, found 246.0122.

2-(2-Chlorophenyl)-ethenesulfonyl chloride (**7**, R^2 = 2-ClPh). To a solution of **6** (0.44 g, 1.8 mmol) in acetone (50 mL) was added tetrabutylammonium iodide (0.99 g, 2.7 mmol). The reaction mixture was heated at reflux overnight, cooled to 22 °C, and concentrated under reduced pressure, and the residue was used without purification. To a solution of triphenylphosphine (0.94 g, 3.6 mmol) in CH_2Cl_2 (50 mL) was added at 0 °C under N_2 sulfuryl chloride (0.32 mL, 3.9 mmol) and after 15 min the crude ammonium salt obtained in the previous step as a solution in CH_2Cl_2 (10 mL). After stirring overnight, the solution was concentrated under reduced pressure and the crude residue was purified by chromatography on SiO_2 (Et_2O:hexanes, 1:4) to give sulfonyl chloride **7** (0.33 g, 78%) as a gray solid: mp 59–60 °C (hexanes); IR (neat) 3074, 1602, 1587, 1431, 1166 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.15 (d, *J* = 15.3 Hz, 1H), 7.63–7.27 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 141.3, 136.4, 133.6, 132.3, 131.0, 129.0, 128.9, 127.8; HRMS (EI) calcd for $C_8H_6Cl_2O_2S$ 235.9466, found 235.9473.

5-Propyl-4-(4-trifluoromethyl-phenyl)-thiazol-2-ylamine (**4**, R = 4-F_3CPh, R^1 = *n*-Pr). A mixture of thiourea (0.17 g, 2.2 mmol), I_2 (0.14 g, 1.1 mmol) and 1-(4-trifluoromethylphenyl)-pentan-1-one (0.25 g, 1.1 mmol) was stirred at 100 °C for 14 h. The cooled reaction mixture was washed onto a fritted filter with Et_2O and CH_2Cl_2. The filtrate was concentrated under

reduced pressure and the residue was dissolved in a minimum amount of boiling water, cooled, made basic with NH_4OH solution and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated to give an oil from which **4** (0.23 g, 74%) crystallized as a yellow solid: mp 47–49 °C (ethyl acetate); IR (neat) 3402, 3098, 2967, 1618, 1529, 1332, 1167 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.64 (s, 4H), 5.47 (bs, 2H), 2.73 (t, $J=7.7$ Hz, 2H), 1.70–1.61 (m, 2H), 0.96 (t, $J=7.4$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 165.4, 144.1, 138.8, 128.5, 125.1, 28.9, 25.2, 13.5; HRMS (EI) calcd for $C_{13}H_{13}F_3N_2S$ 286.0752, found 286.0752.

2-(2-Chlorophenyl)-ethenesulfonic acid [5-propyl-4-(4-trifluoromethylphenyl)-thiazol-2-yl]-amide (**8w**). A solution of **4** (0.079 g, 0.28 mmol) in pyridine (2 mL) was treated at 22 °C with a solution of sulfonyl chloride **7** (0.055 g, 0.23 mmol) in CH_2Cl_2 (1 mL). The reaction mixture was stirred at 22 °C for 2 days, and concentrated under reduced pressure. The crude residue was purified by chromatography on SiO_2 (CH_2Cl_2:Et_2O, 1:1) to give **8w** (72 mg, 64%) as a colorless solid: mp 162–163 °C (CH_2Cl_2); IR (neat) 3441, 2919, 1478, 1224, 1169, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.92 (d, $J=15.6$ Hz, 1H), 7.65–7.23 (m, 8H), 6.91 (d, $J=15.3$ Hz, 1H), 2.61 (t, $J=7.5$ Hz, 2H), 1.70–1.60 (m, 2H), 0.93 (t, $J=7.4$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.8, 135.8, 134.8, 132.7, 132.0, 131.3, 130.7, 130.1, 128.8, 128.1, 127.9, 127.2, 125.9, 124.1, 121.9, 28.5, 24.2, 13.6; HRMS (EI) calcd for $C_{21}H_{18}ClF_3N_2O_2S_2$ 486.0450, found 486.0460.

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