Discovery of Carbohydrate Sulfotransferase Inhibitors from a Kinase-Directed Library**

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Carbohydrate sulfotransferases have recently emerged as an important and relatively unexplored class of therapeutic targets. For example, the seminal discovery that sulfated sialyl LewisX mediates the adhesion of leukocytes to inflamed endothelium established carbohydrate sulfotransferases as potential targets for anti-inflammatory therapy. Ongoing genome sequencing projects have uncovered numerous carbohydrate sulfotransferase genes in the past few years. It is now apparent that carbohydrate sulfotransferases comprise a large family of enzymes with overlapping tissue distribution and substrate specificities. To deconvolute the precise role of each sulfotransferase gene product and elucidate its contribution to normal and pathological processes, cell-permeable and highly specific small-molecule antagonists need to be identified. Surprisingly, no inhibitor of a carbohydrate sulfotransferase has been reported to date. At present, the limited structural and mechanistic information about this class of enzymes impedes rational approaches to inhibitor design. To identify lead inhibitors of carbohydrate sulfotransferases, we therefore adopted a strategy that involved the screening of small-molecule libraries.

To narrow our search, we focused on the similarities between the substrates utilized by sulfotransferases and kinases, a widely studied family of enzymes for which diverse and potent inhibitors are available. Carbohydrate sulfotransferases catalyze the transfer of a sulfonate group from the universal sulfate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a hydroxy (or amino) group of the acceptor oligosaccharide (Scheme 1).

![Scheme 1. Reactions catalyzed by carbohydrate sulfotransferases and kinases.](image)

Kinases catalyze a similar anionic group transfer reaction using adenosine 5′-triphosphate (ATP) as a phosphoryl donor. Thus, both enzyme classes recognize adenosine-based substrates, PAPS and ATP. Furthermore, the hydrophobic adenine binding pockets of the recently crystallized estrogen sulfotransferase and heparin N-sulfotransferase are similar to those of several kinases. On the basis of these parallels, we chose to screen a panel of previously reported kinase-
directed inhibitors that are competitive with ATP for cross-reactivity with carbohydrate sulfotransferases. Here we report the discovery of the first carbohydrate sulfotransferase inhibitors from a kinase-directed library.

We selected the well-characterized GlcNAc-6-sulfotransferase NodH from *Rhizobium meliloti* as an initial enzymatic target.[7] NodH is involved in the biosynthesis of the sulfated nodulation factor 1 (Scheme 2) which induces root nodulation in the symbiotic host plant alfalfa.[8] NodH can also utilize the simple disaccharide chitobiose (2) as a substrate.[9]

We developed a medium-throughput radiolabel transfer assay similar to that of Bourdineaud et al.[10] that involves thin-layer chromatography separation of the product, 35S-labeled 6-sulfo-chitobiose from excess substrate, and 35S-labeled PAPS. Using this assay we measured a $K_M$ value for PAPS of 4.3 (±0.3) $\mu$M and a $K_I$ value for the product 3',5'-ADP of 1.36 (±0.08) $\mu$M (data not shown).

Our initial inhibitor screen included 139 compounds comprising selected structures from purine libraries (3 and 4),[4b, 11] as well as commercially available protein kinase inhibitors (16 tyrphostins (5) and four representatives from other kinase-inhibitor families) (Figure 1). One set of purines (3) possessed conserved N9-methyl and N2-carboxamide groups, but varied in structure at N6. The second set of purines (4) contained diverse substituents at all three positions. These libraries were originally directed toward cyclin-dependent kinases (CDKs).[4b, 11]

Enzyme assays in the presence of the test compounds (200 $\mu$M) identified several purine analogues and one tyrphostin analogue (compound 10, Figure 2) as lead inhibitors;
As the library above comprises known kinase inhibitors, a major concern is the possible cross-reactivity of the identified “hits” with kinases. We tested the compounds for inhibitory activity against two serine/threonine kinases, p38/MAP kinase, and cyclin-dependent kinase 2 (CDK2), the original target of the library we had screened. Against the former, the compounds displayed IC\textsubscript{50} values greater than 60 \mu M. However, not surprisingly, they were more active against CDK2, with IC\textsubscript{50} values in the range of 15 to 40 \mu M. Our next goal is to explore structural modifications that enhance potency against the sulfotransferases while simultaneously reducing kinase inhibitory activity.

**Experimental section**

Purine analogues were prepared as previously described.\textsuperscript{[14]} All other kinase inhibitors were purchased from Calbiochem (La Jolla, CA). Preparation of \textsuperscript{[\textit{35}S]}PAPS: Carrier-free \textsuperscript{[\textit{35}S]}PAPS was synthesized in vitro using a procedure similar to that described by Long and co-workers.\textsuperscript{[15]} Carrier-free \textsuperscript{[\textit{35}S]}Na\textsubscript{2}SO\textsubscript{4} (0.2 \textit{mCi}) was incubated for 24 h with 0.12 \textit{U} ATP sulfurylase (Sigma), 8.3 \textit{U} inorganic pyrophosphatase (Sigma), and 210 mg APS Kinase in buffer A (50 \textit{mM} Tris-HCl pH 8.0, 30 \textit{mM} KCl, 5 \textit{mM} MgCl\textsubscript{2}, 1 \textit{mM} EDTA, 1 \textit{mM} DTT, 10\% glycerol) containing 30 \textit{mM} ATP. The incorporation of \textit{35}S into \textsuperscript{[\textit{35}S]}PAPS was quantified by TLC on PEI-cellulose plates (eluting with 0.9 \textit{mL} LiCl) followed by phosphorimaging analysis (Model 445 SI, Molecular Dynamics). The reaction yield typically exceeded 90\% based on \textsuperscript{[\textit{35}S]}Na\textsubscript{2}SO\textsubscript{4}.

NodH sulfotransferase assay: Inhibitors were screened at 200 \mu M with 1 \mu L NodH (Calbiochem) 2.4 \mu M \textsuperscript{[\textit{35}S]}PAPS (1.0 \textit{mCi}) and 5 \textit{mM} \textit{N,N'-}diacetylchitobiose (Calbiochem) in buffer A. After preincubation of the enzyme and inhibitors for 20 min at room temperature, the enzymatic reaction was initiated by addition of \textsuperscript{[\textit{35}S]}PAPS and after 6 min at room temperature, terminated by dilution with MeOH (120 \mu L). A 25-\mu L aliquot of each reaction was spotted on Whatman LKTLC plates and eluted with 6:3:2 BuOH:EtOH:HB\textsubscript{2}O. The plates were dried and analyzed by phosphorimaging. For IC\textsubscript{50} determinations, serial dilutions of the inhibitors were prepared and tested in the presence of 2.4 \mu M \textsuperscript{[\textit{35}S]}PAPS (1.0 \textit{mCi}) and 5 \textit{mM} \textit{N,N'-}diacetylchitobiose in buffer A (100 \mu M total volume). Aliquots (5 \mu L) were removed and diluted with MeOH (30 \mu L) after 3, 6, 9, and 12 min. Reaction rates were determined by calculating the incorporation of radioactive sulfate into \textit{N,N'-}diacetylchitobiose-6-S\textsubscript{32}O\textsubscript{4} over time.

Supporting information contains a full list of compounds from libraries 1 to 5 as well as kinetic data for \textit{Km} and \textit{K}\textsubscript{i} measurements (6 pages).

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Facile and Clean Oxidation of Alcohols in Water Using Hypervalent Iodine(III) Reagents**

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Oxidation of alcohols to carbonyl compounds is a pivotal reaction in organic chemistry and numerous methods using a variety of reagents and conditions have been developed. Recent demand for eco-conscious chemical processes has encouraged the development of several clean and practical oxidation reactions,[5] and awaits further development of high-yielding, clean, safe, and economical methods for the oxidation of alcohols. Hypervalent iodine reagents have been used extensively in organic syntheses as a result of their low toxicity, ready availability, and easy handling.[3] For example, the highly utilized pentavalent iodine reagents such as Dess–Martin periodinane (DMP)[3] and o-iodoxybenzoic acid (IBX)[4] oxidize alcohols mildly and efficiently to carbonyl compounds in high yields in organic solvents such as CH₂Cl₂, DMSO, and acetone. However, despite their utility, iodine(Ⅴ) reagents are potentially explosive, cannot be stocked, and the generated iodine(Ⅲ) species are usually not utilized (only iodine(Ⅴ) species can be used for the oxidation of alcohols). Therefore, a facile and efficient use of the readily available and relatively stable iodine(Ⅲ) reagents in place of iodine(Ⅴ) reagents has been long desired. In contrast to oxidations with iodine(Ⅴ)-based reagents, only a few examples of the oxidation of alcohols using iodine(Ⅲ) reagents have been described.[5] Existing methods are limited to organic solvents and usually involve activation of Ru or Yb catalysts[8,9] or of 2,2,6,6-tetramethyl-1-piperidinylxoyl (TEMPO)[5] by an iodine(Ⅲ) co-oxidant. As an exception, readily oxidizable benzyl alcohol derivatives do not require catalysts.[6,13a] We have been studying the activation of hypervalent iodine reagents using several additives and solvents,[7] and report herein an efficient oxidation of alcohols using iodosobenzene (PhI–O) with an inexpensive inorganic salt, KBr, in water under neutral conditions. This method was extended to an environmentally benign polymer-supported (diaetoxyiodo)benzene (PSDIB) oxidation procedure.

Various aqueous oxidation reactions have been developed since economic and environmental concerns encourage the use of water as a reaction medium.[5] Very recently we have achieved a novel catalytic activation of PhI–O using a cationic surfactant, cetyltrimethylammonium bromide (CTAB), under neutral conditions. Its low solubility in water and in most organic solvents normally limits the reactivity of PhI–O, however, our micellar and reversed micellar systems have expanded the choice of solvents (from nonpolar solvents to water) for hypervalent iodine oxidation.[9] In this study we first examined the oxidation of alcohols using PhI–O-CTAB in water, by the CTAB-catalyzed (20 mol %) oxidation of 2-octanol (1 e) and 1-phenylethanol (2 e) in water to give 2-octanone (2 e) in 67 % yield. Thus, we re-examined the activation of PhI–O in this reaction with a variety of additives including alkali metal salts. The addition of bromide salts such as NaBr, KBr, and LiBr was found to activate PhI–O remarkably to give 2 e in good yields (94 % yield (KBr)), while salts other than bromide (NaX: X = F, Cl, I, HSO₄, BF₄, HCO₃, ClO₄, OAc, NO₃) did not catalyze the reaction effectively.[10] We therefore chose KBr, the most economical alkali metal bromide, for further studies. The oxidation of activated alcohols such as benzylic or allylic alcohols proceeds quantitatively just by activating PhI–O using several additives and solvents,[7] and report herein an efficient oxidation of alcohols using iodosobenzene (PhI–O) with an inexpensive inorganic salt, KBr, in water under neutral conditions. This PhI–O KBr system is applicable to various oxidations of activated alcohols such as benzylic or allylic alcohols proceeds quantitatively just by activating PhI–O with a small amount of water (10 equiv.), and without addition of KBr (Table I, entries 1–3). With benzylic alcohol (1 a), the reaction rate is enhanced remarkably by adding a catalytic amount of KBr. In contrast, a catalytic amount of KBr is indispensable for the oxidation of saturated primary and secondary alcohols. This PhI–O KBr system is applicable to the oxidation of a variety of primary and secondary alcohols bearing functional groups such as ether, ester, sulfonamide, and azido groups (see Table 1). Oxidation of primary alcohol 1 k only yields carboxylic acid 2 k (52 % yield) and none of the corresponding aldehyde when using 1.1 equivalents of PhI–O.

** COMMUNICATIONS


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