Tagging the Untaggable: A Difluoroalkyl-Sulfinate Ketone-Based Reagent for Direct C–H Functionalization of Bioactive Heteroarenes

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ABSTRACT: We have developed a new difluoroalkyl ketal sulfinate salt reagent suitable for direct derivatization of heteroarene C–H bonds. The reagent is capable of introducing a ketone functional group on heteroarene bioactive compounds via a one-pot reaction. Remarkably, in three examples the ketone analog and its parent drug had almost identical cytotoxicity. In a representative example, the ketone analog was bioconjugated with a delivery vehicle via an acid-labile semicarbazone linkage and with a photolabile protecting group to produce the corresponding prodrug. Controlled release of the drug–ketone analog was demonstrated in vitro for both systems. This study provides a general approach to obtain taggable ketone analogs directly from bioactive heteroarene compounds with limited options for conjugation. We anticipate that this sodium ketal-sulfinate reagent will be useful for derivatization of other heteroarene-based drugs to obtain ketone-taggable analogs with retained efficacy.

Bioconjugation has become an essential tool in chemical biology for attaining controlled release of a small molecule with medicinal activity.1−5 It is typically implemented through chemoselective modification of native functional groups present on the target molecule.1 For example, amines can be chemoselectively derivatized through amide linkages, azides, or acetylenes through the “click” reaction,4,5 carboxylic groups through ester bonds,6 disulfide bridges through a thiol coupling,7 and carbonyl groups through the oxime ligation.8 Although many medicinal agents contain traditional “taggable” functional groups, some compounds present the challenge of not having any apparent chemical handles. As a further layer of complexity, some molecules require an orthogonal handle for bioconjugation. We have recently introduced a new method to modify unactivated C–H bonds based on the controlled decomposition of alkane sulfinate reagents to reactive radicals in situ via a simple, cheap oxidative process.9−13 This strategy has opened a new gateway for derivatization of medicinal molecules by a one-step synthetic route carried out in aqueous mixtures, open to the air with operationally simple thermal initiation conditions. The chemical functionalization of the inert C–H bond of small molecules with a fluoroalkyl substituent bearing a “clickable” functional group was recently demonstrated using an azido-alkyl handle.13 However, bioconjugation of such molecules with targeting carriers produces an irreversible nonlabile linkage (triazole), which lacks the ability for site-selective release of the bioactive entity.

The introduction of a ketone onto a heteroarene would allow for the conjugation of bioactive molecules via an acid-labile hydrazone linkage or through a photolabile ketal, for controlled-release applications. However, functionalization of a bioactive molecule to produce an analogue with a taggable functional group will only be effective if the obtained analogue retains the efficacy of the native compound. The ketone is a functional group bearing a lower potential for interacting with other functionalities, in comparison to the amine, hydroxyl, or thiol groups. Thus, we anticipate that in many instances the ketone would be less susceptible to interfere with binding the natural target of the native bioactive compound. Here we report the synthesis and evaluation of a new difluoroalkyl-sulfinate ketone-protected reagent suitable for direct C–H bond functionalization of heteroarens that have either limited or no tagging option.

Sodium difluoroalkyl ketal sulfinate 1 is a ketone-protected form of our designed reagent. Such a reagent is capable of reacting with a heteroarene C–H bond under oxidative acidic conditions to produce the corresponding ketone derivative 1 (Figure 1). Deprotection of the ketal should occur in situ under the hydrolytic acidic conditions of the reaction to afford the ketone functional group. The heteroarene–ketone analog can...
be readily masked through an acid-labile semicarbazone linkage\(^{14-16}\) (II) or a photolabile ketal protecting group\(^{16,17}\) (III).

The synthesis of ketal sulfinate 1 was achieved in an analogous manner to that recently described for other sulfinate salts (Figure 2).\(^3,13\) Thus, alkylation of pyridine derivative 1a by ketal 1b generated sulfone 1c. The latter was reacted with sodium mercaptoethanol to afford sulfinate salt 1. This synthesis could be easily performed on a multigram scale to produce sulfinate 1 with good yields. Heteroarene difluoroalkylation by sulfinate salt 1 to produce the corresponding ketone derivative was initially established on caffeine as a test substrate. Caffeine successfully reacted with sulfinate 1 through direct functionalization of its C–H bond to afford ketone derivative 1d in 87% yield.

Next, we sought to evaluate the functionalization-capability of sulfinate salt 1 on four selected known heteroarene antineoplastic drugs camptothecin (CPT), temozolomide (TMZ), bosutinib, and methotrexate (MTX). CPT (2) is a topoisomerase inhibitor with limited options for bioconjugation through its tertiary hydroxyl group. TMZ (3) is a cytotoxic alkylating agent, which is considered an untaggable compound due to the absence of a suitable functional group. Likewise, bosutinib (4), an approved tyrosine kinase inhibitor based drug, has no functional group available for conjugation. MTX (5), an antifolate-based chemotherapeutic drug, is a heteroarene with limited bioconjugation options (Table 1).

The four heteroarene drugs were successfully functionalized by sulfinate salt 1 to afford the corresponding ketone analogs in moderate to good yields. For CPT, bosutinib, and MTX, sulfinate salt 1 was able to selectively functionalize the heteroarenes at the most electron-deficient C–H bond.

As mentioned above, this functionalization will be useful only if the taggable ketone-derivative maintains the biological activity of the native drug. Therefore, in order to find out whether the new derivatives retain their efficacy, the in vitro cytotoxicity of the ketone analogs was evaluated in comparison to that of the parent drugs. Figure 3 shows representative cell-growth inhibition plots for each drug and its ketone analog. The applied cell lines and the calculated IC\(_{50}\) values are presented in Table 2.

Remarkably, for three of the four drugs, tumor cell-growth inhibition assays showed almost identical cytotoxicity (IC\(_{50}\) values) for the ketone-derivatives and their native drugs. Functionization of CPT, TMZ, and bosutinib using sulfinate salt 1 resulted in ketone analogs that retained their cytotoxic activity. However, the ketone analog of MTX lost its cytotoxicity by 3 orders of magnitude. The assays were repeated with several human tumor cell lines and the obtained results were similar (see Supporting Information). These results indicate that it is possible for certain biologically relevant...
heteroarenes to maintain their original activity, after C–H functionalization by sulfinate salt 1, at an appropriate position.

To determine the usefulness of these drug analogs harboring a ketone for controlled release and bioconjugation, we selected CPT-ketone 2a for further evaluation. As presented in Figure 1, a ketone functional group can be masked either through an acid-labile semicarbazone linkage or with a photolabile ketal protecting group. CPT-ketone 2a was masked with a photolabile protecting group to produce ketal derivative 6 (Figure 4). This derivative can be considered the prodrug form of CPT-ketone 2a. Irradiation of prodrug 6 with UV (λ = 360 nm) light over 2 h under physiological conditions resulted in the release of CPT-ketone 2a (Figure 4A). No release was observed without irradiation. The cytotoxicity of prodrug 6 was then evaluated in a standard cell-growth inhibition assay before and after irradiation with UV light (Figure 4B). As expected, derivative 6 before irradiation exhibited typical prodrug behavior, with an IC_{50} value of 350 nM. Prodrug 6 after irradiation has shown significantly higher cytotoxicity with an IC_{50} value of 10 nM, similarly to that obtained for CPT-ketone 2a (IC_{50} = 5 nM). These results demonstrate how the newly installed ketone of the heteroarene analogs can be used to obtain prodrugs with a photolabile controlled-release pathway. In this example, the prodrug was activated with UV light; however, there are analogous protecting groups, which can be removed through a visible or near-infrared light. To the best of our knowledge, this is the first demonstration of a prodrug photoactivation, which is based on unmasking of a ketone group.

Next, we sought to evaluate the acid-labile hydrazone linkage, for controlled-release and bioconjugation between CPT-ketone 2a and a targeting vehicle. Hydrolysis via an acid-labile linkage is a useful controlled-release mechanism for cell-penetrating vehicles such as folic acid conjugate (FA) conjugate (Figure 5). Thus, CPT-ketone 2a was reacted with a semicarbazide derivative of folic acid (7a), via a semicarbazone linkage, to generate CPT–folic acid conjugate 7 with an acid-labile controlled-release mechanism (see Supporting Information for synthesis). A

<table>
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<tr>
<th>Heteroarene Drug</th>
<th>Heteroarene-Ketone Derivative</th>
<th>Isolated Yield</th>
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<tr>
<td>Camptothecin (CPT)</td>
<td><img src="image" alt="Camptothecin" /></td>
<td><img src="image" alt="Camptothecin-Ketone" /></td>
</tr>
<tr>
<td>Temozolomide (TMZ)</td>
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</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td><img src="image" alt="Methotrexate" /></td>
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</tr>
<tr>
<td>Bosulbinib</td>
<td><img src="image" alt="Bosulbinib" /></td>
<td><img src="image" alt="Bosulbinib-Ketone" /></td>
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polyethylene glycol (PEG) linker derivative was used as a spacer to connect between the folic acid and CPT-ketone moieties.

To validate the release of CPT-ketone from folic acid conjugate 7, we monitored the stability of the semicarbazone linkage under various pHs (physiological conditions—pH 7.4; early stage endosomes, pH 6.5 and 6.0; and late-stage endosomes, pH 4.8). The kinetic plots of CPT-ketone release are presented in Figure 6. The semicarbazone-based conjugate exhibited excellent selective hydrolysis under acidic and physiological conditions. Fast release kinetics of CPT-ketone were observed under acidic pHs ($t_{1/2} < 1$ h), while no release at all was observed over 24 h at physiological pH. Importantly, at relatively mild acid conditions (early stage endosomes, pH 6.5), release of CPT-ketone still effectively took place ($t_{1/2} < 5$ h).

The ability of folate-conjugate 7 to bind to FR receptors was evaluated in a $[^3H]$-folic acid competition assay with high FR KB cells (HiFR-KB). CPT-PEG derivative 7b, which lacks the folic acid moiety, was used as a control (Figure 7A). The obtained measurements showed 82% and 14% competition of $[^3H]$-folic acid by conjugate 7 and control 7b, respectively, when compared to free folic acid (896.5%). Cytotoxicity evaluation of CPT-folic acid conjugate 7 on HiFR-KB cells revealed relative IC$_{50}$ values for CPT-ketone 2a and its folic acid conjugate 7. CPT-PEG derivative 7b showed a 15-fold higher IC$_{50}$ value compared to conjugate 7. These results support FR-mediated uptake of the folic acid-CPT conjugate followed by intracellular CPT-ketone controlled-release through the acid-labile semicarbazone linkage.

The sodium difluoroalkyl ketone 1 has recently become commercially available from Sigma-Aldrich (product no. 792446). The reagent was demonstrated to be useful for direct derivatization of bioactive heteroarenes. The obtained analogs are equipped with a new ketone functional group, suitable for bioconjugation. Such chemistry can open a new door for obtaining drug analogs that had either a limited or no practical option for covalent controlled-release. The approach was demonstrated to be effective for three chemotherapeutic drugs, where the ketone analog fairly maintained the biological activity of the native drug. In one option, the ketone functional group of the analogs could be masked in form of a photoremovable ketal to produce a corresponding prodrug. In another option, the ketone was used for bioconjugation via an acid-labile hydrazone linkage with an appropriate targeting carrier. If a more stable linkage is required, one can use the oxime ligation.

<table>
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<tr>
<th>drug and ketone analog</th>
<th>IC$_{50}$ (nM)</th>
<th>cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT (2)</td>
<td>7</td>
<td>U87</td>
</tr>
<tr>
<td>CPT-Ketone (2a)</td>
<td>10</td>
<td>U87</td>
</tr>
<tr>
<td>TMZ (3)</td>
<td>36</td>
<td>U251</td>
</tr>
<tr>
<td>TMZ-Ketone (3a)</td>
<td>35</td>
<td>U251</td>
</tr>
<tr>
<td>Bosutinib (4)</td>
<td>17</td>
<td>SF295</td>
</tr>
<tr>
<td>Bosutinib-Ketone (4a)</td>
<td>17</td>
<td>SF295</td>
</tr>
<tr>
<td>MTX (5)</td>
<td>28</td>
<td>SF295</td>
</tr>
<tr>
<td>MTX-Ketone (5a)</td>
<td>&gt;1000</td>
<td>SF295</td>
</tr>
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In summary, we have developed a new difluoroalkyl ketal sulfinate salt reagent suitable for direct derivatization of heteroarene C–H bonds. The reagent is capable of introducing a ketone functional group on heteroarene bioactive compounds via one-pot reaction. In three out of four examples, the ketone analog and its parent drug had almost identical cytotoxicity. The ketone analog of CPT was conjugated with a delivery vehicle via an acid-labile semicarbazone linkage and with a photolabile protecting group to produce a corresponding prodrug. Controlled-release of the CPT-ketone analog was demonstrated in vitro for both systems. This study provides a general approach to obtain taggable ketone-analogs directly from heteroarene bioactive compounds. We anticipate that our sodium ketal-sulfinate reagent will be useful for derivatization of other heteroarene-based drugs, affording ketone-taggable analogs.

Figure 4. (A) Release of CPT-ketone 2a with (blue) and without (orange) irradiation of prodrug 6 (150 μM in PBS 7.4:DMSO, 95:5 v/v). (B) Cell growth inhibition assay with human primary glioblastoma cell line U-87; prodrug 6 before (yellow) and after (gray) irradiation, CPT-ketone 2a (blue).

Figure 5. Release of CPT-ketone 2a from a folic acid conjugate via acid-labile controlled-release mechanism of a semicarbazone linkage.
Figure 6. Hydrolysis rate of CPT-ketone-semicarbazone-PEG-FA 7 (150 μM) as a function of time after incubation in buffer at pH 4.8 (blue), pH 6.0 (orange), pH 6.5 (yellow), and pH 7.4 (green) at 37 °C.

Figure 7. (A) [3H]FA competitive binding assays using HiFR-KB cells. (B) Cytotoxicity assays using HiFR-KB cells.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.6b00382.

General information, synthesis procedures, and spectra (PDF)

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Notes
The authors declare no competing financial interest.
REFERENCES