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Synthesis of Amino-ADT Provides Access to Hydrolytically Stable Amide-Coupled Hydrogen Sulfide Releasing Drug Targets

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Abstract

As additional physiological functions of hydrogen sulfide (H_2S) are discovered, developing practical methods for exogenous H_2S delivery is important. In particular, nonsteroidal anti-inflammatory drugs (NSAIDs) functionalized with H_2S -releasing anethole dithiolethione (**ADT-OH**) through ester bonds are being investigated for their combined anti-inflammatory and antioxidant potential. The chemical robustness of the connection between drug and H_2S -delivery components, however, is a key and controllable linkage in these compounds. Because esters are susceptible to hydrolysis, particularly under acidic conditions such as stomach acid in oral drug delivery applications, we report here a simple synthesis of amino-ADT (**ADT-NH₂**) and provide conditions for successful **ADT-NH₂** derivatization with the drugs naproxen and valproic acid. Using UV-vis spectroscopy and HPLC analysis, we demonstrate that amide-functionalized ADT derivatives are significantly more resistant to hydrolysis than ester-functionalized ADT derivatives.

Graphical abstract

Keywords

sulfur; dithiolethione; ADT-OH; hydrogen sulfide; H_2S donors

Hydrogen sulfide (H_2S) has joined carbon monoxide (CO) and nitric oxide (NO) as the third endogenously produced gaseous signaling molecule, or gasotransmitter.¹ Biosynthesized through enzymatic and nonenzymatic pathways, cellular H_2S regulates critical cardiovascular, immune, nervous, respiratory, and gastrointestinal system functions and is implicated in a number of diseases.² In addition to central nervous system diseases, such as

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Supporting Information

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Down syndrome,³ Alzheimer's,⁴ and Parkinson's disease,⁵ the role of H₂S in inflammation and angiogenesis has been of particular interest.^{6,7} Different studies have shown H₂S to exhibit either anti-inflammatory or pro-inflammatory effects depending on which model is being examined. For example, H₂S-mediated activation of K_{ATP} channels limits leukocyte adherence to vascular endothelium and reduces edema formation in rats, both anti-inflammatory effects.⁸ In models of septic shock, however, inflammatory responses are observed in conjunction with elevated levels of H₂S,⁹ and inhibition of enzymatic H₂S production during endotoxemia mitigates both inflammation and organ injury.¹⁰ Furthermore, enhanced levels of K_{ATP} activation have been linked with LPS-induced models of hypotension and hyporeactivity.¹¹ These observations suggest that the interactions of H₂S with K_{ATP} channels during inflammation may be circumstantially either beneficial or deleterious. In addition to inflammation, signaling actions of H₂S have significant effects on angiogenesis. H₂S promotes endothelial cell growth through upregulation of vascular endothelial growth factor (VEGF) expression,¹² and topical H₂S administration to burn wounds has been shown to stimulate wound healing on rats.¹³ More recently, H₂S treatment was shown to enhance angiogenesis after ischemic stroke through cooperation of astrocytes and endothelial cells.¹⁴ Taken together, these broad-reaching roles of H₂S highlight the pharmacological potential of exogenously administered H₂S.

Although a variety of H₂S-delivery methods exist, different results are often observed depending upon which H₂S donor is used,⁶ and developing new types of synthetic H₂S-donating compounds is an active area of investigation.¹⁵ By contrast to the more commonly utilized inorganic sulfide sources such as Na₂S and NaHS, which deliver an immediate burst of H₂S into an experimental system, small-molecule donors are designed to more closely resemble continuous, endogenous H₂S release. One of the most extensively studied H₂S donors is 5-(4-hydroxyphenyl)-3*H*-1,2-dithiol-3-thione (**ADT-OH**), which contains the H₂S-releasing dithiolethione moiety. This phenolic compound is easily functionalized via ester linkages (Scheme 1), and a number of ester-bound ADT derivatives of nonsteroidal anti-inflammatory (NSAIDs) and other drugs have been prepared and are being investigated for their synergistic anti-inflammatory and antioxidant potential. For example, an ADT-functionalized diclofenac derivative, S-diclofenac, not only exhibited greater anti-inflammatory effects than the parent diclofenac in LPS-injected rats, but also displayed reduced gastrointestinal toxicity.¹⁶ Other demonstrations of ester-functionalized ADT drugs include derivatives of naproxen,¹⁷ valproic acid,¹⁸ aspirin,¹⁹ sildenafil,²⁰ and mesalamine.²¹ One practical challenge associated with implementing these ADT derivatives in drug delivery, however, is the potential for hydrolysis of the ester bond linking the two drug components. A recent report demonstrated that hydrolysis of a polymeric ester-bound ADT in phosphate-buffered saline occurred with a half-life of approximately 30 minutes.²² Therefore, stomach acid would also likely catalyze hydrolysis and component fragmentation, which would be particularly problematic in oral drug delivery systems. One logical solution to this unwanted hydrolysis is to link ADT to the drugs through amide, rather than ester, bonds.²³

To better enable access to more robust ADT-functionalized NSAIDs, we report here a straightforward synthesis and purification of 5-(4-aminophenyl)-3*H*-1,2-dithiol-3-thione

(amino-ADT, **ADT-NH₂**) and compare the hydrolytic stability between amide- and ester-coupled ADT. We also provide conditions for coupling of **ADT-NH₂** with common drugs, including naproxen and valproic acid and demonstrate similar cytotoxicities for the ester- and amide-linked versions of these drug conjugates.

To access **ADT-NH₂**, direct conversion of the phenolic group in **ADT-OH** to an amine would be preferred. Our attempts using a reported one-pot S_NAr–Smiles rearrangement strategy (Scheme 2,A)²⁴ or palladium-catalyzed Buchwald–Hartwig amination of triflated ADT (Scheme 2,B) were unsuccessful. We reasoned that the presence of highly metalophilic sulfur atoms in the dithiolethione moiety, combined with the sulfophilicity of palladium, makes palladium-catalyzed cross-couplings with ADT problematic. Therefore, we reasoned that **ADT-NH₂** would need to be constructed from an aniline precursor and that any metal-mediated steps must be incorporated prior to installation of the dithiolethione moiety (Scheme 2,C).

Fortunately, organotrifluoroborate salts have emerged as excellent substrates for Suzuki–Miyaura reactions due to their improved air-stability and reduced cost.²⁵ For our purposes, they offer an attractive alternative to expensive vinyl boronic acid and boronate substrates.²⁶ Utilizing this strategy, we found that **ADT-NH₂** is accessible in two steps from commercially available starting materials with 56% overall yield (Scheme 3,A). Palladium-catalyzed coupling of 4-chloro-(*N*-Boc)aniline with potassium *trans*-1-propenyltrifluoroborate installs a propenyl dithiolethione precursor onto the protected aniline to form compound **1**.²⁷ Subsequent reaction with elemental sulfur at 180 °C both forms the dithiolethione and deprotects the aniline to give **ADT-NH₂**.^{28,29} Methylated **ADT-NMe₂** (Scheme 3,D) was synthesized with the same methodology using 4-bromo-*N,N*-dimethylaniline as starting material. **ADT-OH** and **ADT-OMe** were prepared using previously reported methods,¹⁶ and we report an updated purification method in the Supporting Information. Importantly, dithiolethione formation from reaction of elemental sulfur with an aryl propenyl group appears to be general, as similar reaction conditions may be used to form **ADT-NH₂**, **ADT-NMe₂**, and **ADT-OMe** from their respective propenyl starting materials.

Having developed a facile route to access **ADT-NH₂**, our next objective was to demonstrate effective functionalization of the amine. Acetamide (**ADT-NAc**) and acetate (**ADT-OAc**) **ADT** derivatives were synthesized as model compounds for hydrolysis study, both through reactions with acetyl chloride (Scheme 3,B). Ester- and amide-coupled ADT derivatives of naproxen and valproic acid (**ADT-ORox**, **ADT-OVal**, **ADT-NRox**, and **ADT-NVal**) were coupled using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·H·Cl) and catalytic 4-dimethylaminopyridine (DMAP) as coupling agents (Scheme 3,C). Both **ADT-NH₂** and **ADT-OH** are weak nucleophiles, making substitution reactions challenging. The dithiolethione group is inductively electron-withdrawing, as reflected in the increased acidity of **ADT-OH** compared with phenol (*pK_a* of 7.86 vs. 10.00), slightly more so than a nitrile group (*pK_a* of 4-cyanophenol is 7.95).³⁰ Resonance contributions from lone-pair electron delocalization produce partial zwitterionic character in these compounds, which further decreases their nucleophilicities (Scheme S1, Supporting Information). We found that **ADT-NH₂** is less reactive than **ADT-OH**, which suggests a higher degree of electron

delocalization. For example, esterification of ADT to form esters **ADT-ORox** and **ADT-OVal** proceed at room temperature, whereas 120 °C temperatures are required for the corresponding amide couplings of **ADT-NRox** and **ADT-NVal**. Nonetheless, **ADT-NH₂** is readily linked through amide bonds using these standard methodologies, which can likely be extended toward coupling with other carboxylic acid containing NSAIDs.

To compare the hydrolytic stability of ester- and amide-bound ADT derivatives in an acidic environment akin to the stomach, the hydrolysis of model compounds **ADT-OAc** and **ADT-NAc** in 0.10 M HCl was investigated using UV-vis spectroscopy and HPLC. After addition of **ADT-OAc** (20 μM) to a cuvette containing 0.10 M HCl, a decrease in absorption at 320 nm was observed with concomitant growth of a new peak at 358 nm, which is consistent with formation of **ADT-OH** after ester hydrolysis (Figure 1,A). By contrast, treatment of **ADT-NAc** (20 μM) resulted in only minimal hydrolysis to form **ADT-NH₂** ($\lambda_{\text{max}} = 311$ nm, Figure 1,B). Over the course of the 18 h experiment, 69% of the ester-bound ADT was hydrolyzed, whereas only 9% of the amide was hydrolyzed under identical conditions (Figure 1,C). **ADT-OH** and **ADT-NH₂** fragments were also identified as reaction byproducts using HPLC analysis. (Figure S1, Supporting Information). As expected, these results clearly demonstrate the enhanced chemical stability of amide linkages toward hydrolysis in ADT derivatives by contrast to ester linkages. As a preliminary demonstration of the biological activity of these amide-functionalized ADT-based donors, we also tested the cytotoxicity of the amide- and ester-linked ADT conjugates in HeLa cells using the MTT assay.³¹ As a whole, the amide and ester ADT derivatives generally displayed similar toxicity profiles (Figure S2, Supporting Information), suggesting that these compounds may find similar biological efficacies in future investigations.

In conclusion, amide-coupled ADT derivatives help provide a solution to challenges associated with undesired acid-catalyzed hydrolysis of ester-bound anethole dithiolethione NSAIDs in oral drug delivery applications. By utilizing advances in Suzuki–Miyaura cross-coupling using trifluoroborate salts, we successfully accessed the key intermediate **1** toward our target H₂S donor, **ADT-NH₂**. We hope that the proof of concept syntheses of **ADT-NVal** and **ADT-NRox** will assist future investigations toward unlocking the therapeutic potential of H₂S-releasing drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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27. **Synthesis of 1** In a glovebox, 4-chloro(N-Boc)aniline (350 mg, 1.54 mmol), potassium trans-1-propenyltrifluoroborate (273 mg, 1.85 mmol), Pd(OAc)₂ (35 mg, 0.16 mmol), RuPhos (144 mg, 0.308 mmol), and Cs₂CO₃ (1.5 g, 4.6 mmol) were added to an oven-dried three-neck round-bottom flask fitted with a reflux condenser. The sealed apparatus was removed from the glovebox, and a degassed solvent mixture of 4:1 THF–water (10 mL) was added via syringe; minimal solvent aids with full conversion and overall yield. The reaction mixture was stirred at 80 °C under N₂ for 14 h, after which the solvent was removed by rotary evaporation. The residue was dissolved in

EtOAc and filtered through Celite. The filtrate was washed with H₂O, brine, and dried with Na₂SO₄. The crude product was purified using column chromatography (20–50% hexane–EtOAc gradient) and further purified by recrystallization from hexanes to afford the pure product as a light brown solid (318 mg, 88% yield). ¹H NMR (500 MHz, DMSO): δ = 9.31 (s, 1 H), 7.38 (d, *J* = 8.5 Hz, 2 H), 7.24 (d, *J* = 8.6 Hz, 2 H), 6.31 (dd, *J* = 15.8, 1.43 Hz, 1 H), 6.11–6.18 (m, 1 H), 1.81 (dd, *J* = 6.56, 1.5 Hz, 3 H), 1.47 (s, 9 H) ppm. ¹³C{¹H} NMR (125 MHz, DMSO): δ = 152.67, 138.26, 131.34, 130.41, 125.94, 123.34, 118.08, 78.95, 28.11, 18.19 ppm. HRMS: *m/z* [M + Na]⁺ calcd for [NaC₁₄H₁₉NO₂]⁺: 256.1313; found: 256.1309.

28. **Synthesis of ADT-NH₂** Compound **1** (100 mg, 0.429 mmol) and sulfur (96 mg, 3.0 mmol) were added to an oven-dried pressure vessel and dissolved in DMA (6 mL) under N₂, and the reaction was stirred at 180 °C for 18 h. After stirring, the solvent was removed under vacuum while heated at 40 °C. The crude residue was diluted with H₂O, extracted into EtOAc. The combined organic fractions were washed with brine and dried with Na₂SO₄. The compound was purified using column chromatography (3% MeOH–CH₂Cl₂) to afford the pure product as a dark brown solid (66 mg, 68% yield). ¹H NMR (500 MHz, CD₂Cl₂): δ = 7.51 (d, *J* = 8.7 Hz, 2 H), 7.36 (s, 1 H), 6.71 (d, *J* = 8.7 Hz, 2 H), 4.27 (br, 2 H) ppm. ¹³C{¹H} NMR (125 MHz, CD₂Cl₂): δ = 215.05, 174.86, 151.60, 133.66, 129.25, 121.63, 115.29 ppm. HRMS: *m/z* [M + H]⁺ calcd for [C₉H₈NS₃]⁺: 225.9819; found: 225.9823
29. (a) We note that although the synthesis of **ADT-NH₂** is suggested in a patent,^{29b} the precursor to compound **1** in the patent application is not a known compound, and no preparation is presented. Wallace, J.; Cirino, G.; Caliendo, G.; Sparatore, A.; Santagada, V.; Fiorucci, S. US Patent. 2007/0197479 A1. 2007.
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Figure 1.

Hydrolysis of acyl ADT derivatives (20 μ M) in 0.10 M HCl at 37 $^{\circ}$ C. A) UV-vis timecourse of **ADT-OAc** (blue = initial, black = final) over 18 h overlaid with the absorbance spectrum of **ADT-OH** (red); B) time course of **ADT-NAc** (blue = initial, black = final) over 18 h overlaid with the absorbance spectrum of **ADT-NH₂** (red); C) calculated percent hydrolysis of **ADT-OAc** and **ADT-NAc**.

Scheme 1. Esterification of **ADT-OH** to form H₂S-releasing drugs

Scheme 2.
Potential synthetic routes to **ADT-NH₂**

Scheme 3.

Synthesis of ADT-containing compounds including (A) **ADT-NH₂**, (B) **ADT-OAc** and **ADT-NAc**, (C) ADT drug conjugates, and (D) **ADT-NMe₂** and **ADT-OMe**