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Preparation, characterization and in vivo conversion of new water-soluble sulfenamide prodrugs of carbamazepine

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Abstract—Improved synthetic methods are reported for the preparation of sulfenamide derivatives of carbamazepine (CBZ) for evaluation as prodrugs. These sulfenamide prodrugs were designed to rapidly release CBZ in vivo by cleavage of the sulfenamide bond by chemical reaction with glutathione and other sulfhydryl compounds. Physicochemical characterization and in vivo conversion of a new prodrug of CBZ was evaluated to further establish the proof of concept of the sulfenamide prodrug approach.

Carbamazepine (CBZ, **1**) is effective in the treatment of partial and generalized tonic–clonic epileptic seizures,[1] and is less sedating and causes less cognitive impairment than other anticonvulsants such as phenytoin, phenobarbital, and primidone.[2] Unfortunately, CBZ's relatively poor aqueous solubility (120 μg mL^{-1}) hampers parenteral administration,[3] and despite the need, there is no commercially available injectable formulation. The poor solubility of CBZ can be addressed to some extent by use of cosolvents and/or surface active agents,[4] but the effectiveness of these approaches is limited and these additives are undesirable for intravenous (iv) formulations due to the risk of severe allergic reactions and formulation related toxicities.[5] A water-soluble prodrug of CBZ that could be administered intravenously in a safe and effective formulation would be of considerable medical benefit.

There is a significant void in the literature for prodrug strategies available for weakly acidic NH-acid drugs such as amides, carbamates, and ureas.[6] A new prodrug approach involving the preparation and evaluation of sulfenamide prodrugs of a variety of NH-acids has recently been reported by Guarino et al.[6,7] The general route for the synthesis of these derivatives involved the reaction of the appropriate disulfide with sulfuryl chloride to generate a sulfenyl chloride intermediate, which was then further reacted in situ with the NH-acid to form the sulfenamide derivative. One of the sulfenamide prodrugs synthesized and evaluated was **3**, an *O*-ethyl cysteinyl prodrug of CBZ. While the sulfenyl chloride synthetic route was considered adequate for most of the amine, amide and imide drug, and model compounds studied, the yields for this approach with the urea drug example CBZ were very poor (overall yield 1–2%). It was apparent that improved synthetic methods would be required to further the study of sulfenamide derivatives of CBZ for evaluation as prodrugs.

In this communication, we report improved synthetic methods for the preparation of sulfenamide derivatives of CBZ and physicochemical characterization, and in vivo evaluation of a new water-soluble sulfenamide prodrug. *N*-cysteamine-CBZ (**4**) was designed as a water-soluble prodrug of CBZ to regenerate CBZ in vivo by cleavage of the sulfenamide bond by chemical reaction with glutathione and other endogenous sulfhydryl compounds. A successful in vivo result, supporting the proof of concept of the sulfenamide prodrug strategy, could further the application of this approach to improve the physicochemical properties and/or delivery characteristics of other NH-acidic drugs, including ureas.

Thiophthalimides are well-known sulfur transfer agents,[8] and were utilized as such here to attach various promoieties to the secondary nitrogen of CBZ. The

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reaction of *tert*-butyllithium with CBZ followed by *N*-(phenylthio)-phthalimide produce the sulfenamide *N*-(phenylthio)-CBZ (**2**) in good yield (76%, Scheme 1).[9] Although not further studied here, compound **2** provides a sulfenyl urea model with a UV-active promoiety to further investigate the stability and degradation reactions for this class of compounds.

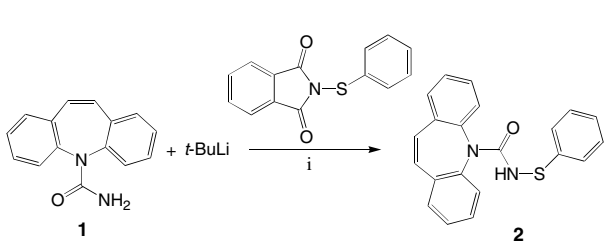
The improvement in synthetic yield of **3** over the sulfenyl chloride method is shown by the reaction of *tert*-butyllithium with CBZ followed by *N*-(Boc-Cys-*O*Et)-phthalimide, which yielded *N*-(Cys-*O*Et)-CBZ (**3**) with improved overall yield (55%, Scheme 2) compared to the overall yield using the synthetic method reported by Guarino et al.[7]

N-(Cys-*O*Et)-CBZ (**3**) is a water-soluble CBZ derivative that likely acts as a prodrug and the stability of **3** was recently characterized in detail.[7] Cysteamine was selected as the promoiety for this in vivo study to avoid any potential complication caused by hydrolysis of the ethyl ester functionality of **3**. The aqueous chemical stability of **4** was also found to be superior to that of **3**. The essential cysteamine derivative of phthalimide, *N*-(Boc-cysteamine)-phthalimide, was prepared by reaction of phthalimide with Boc-cysteamine in the presence of bromine and pyridine (96%, Scheme 3).

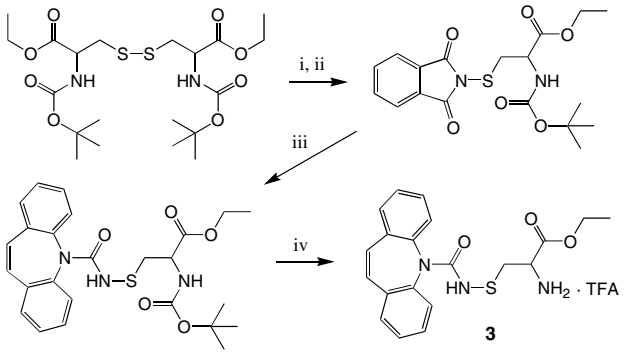
N-(Boc-cysteamine)-phthalimide was then reacted with lithium CBZ to yield N-(Boc-cysteamine)-CBZ, followed by reaction with TFA to yield **4** as a white powder in good overall yield (66%, Scheme 4).

The white powder **4** dissolved rapidly in pure water with apparent solubility in excess of 100 mg mL^{-1} (final pH value of 2.6). The chemical stability of **4** was determined in aqueous solution at various pH values. Figure 1 shows the pH-rate profile for the aqueous degradation of **4** at 70 °C.

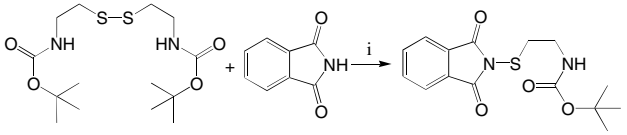
Eq. 1 expresses the observed rate constant (k_{obs}) for the loss of **4** in terms of rate and equilibrium constants as a function of hydrogen ion concentration, or pH where $[H^+] = 10^{-pH}$ and $K_w = 1.51 \times 10^{-13}$ at 70 °C.[10] The rates of hydrolytic reactions of the ionized and neutral forms of **4** include acid catalyzed hydrolysis (k_H), water hydrolysis (k_o), and base catalyzed hydrolysis (k_{OH}) of the protonated form and base catalyzed hydrolysis of the neutral form (k'_{OH}).



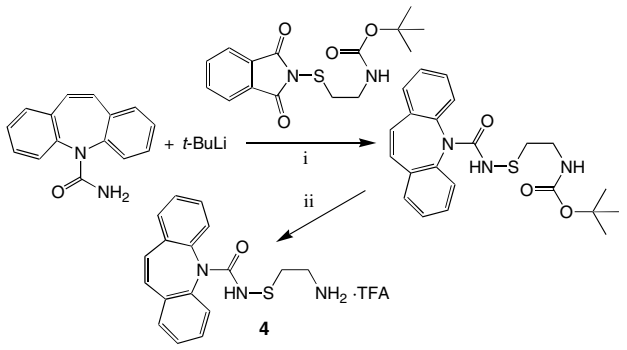
Scheme 1. Synthesis of *N*-(phenylthio)-CBZ (**2**). Reagents and condition: (i) −78 °C, THF, Ar.



Scheme 2. Improved synthesis of **3**. Reagents: (i) SO_2Cl_2, THF; (ii) potassium phthalimide, THF; (iii) deprotonated CBZ (with *t*-BuLi), THF; (iv) TFA, CH_2Cl_2.



Scheme 3. Synthesis *N*-(Boc-cysteamine)-phthalimide. Reagents: (i) Br_2, pyridine, CH_3CN.



Scheme 4. Preparation of **4**. Reagents and condition: (i) −78 °C, THF, Ar; (ii) TFA, CH_2Cl_2.

$$k_{obs} = \frac{k_H[H^+]^2 + k_o[H^+] + k_{OH}K_w + \frac{k'_{OH}K_aK_w}{[H^+]}}{[H^+] + K_a} \quad (1)$$

The results for k_{obs} from the accelerated stability studies of **4** conducted at pH 4 were 9.39×10^{-6} s^{-1} for 70 °C, 1.67×10^{-6} s^{-1} for 60 °C, and 3.96×10^{-7} s^{-1} for 50 °C. The Eyring behavior was assumed to be linear and the values obtained were: $\Delta H^{\ddagger} = 143$ kJ mol^{-1} (34.2 kcal mol^{-1}) and $\Delta S^{\ddagger} = 74$ J mol^{-1} K^{-1} ($R^2 = 0.9949$). The $\Delta H^{\ddagger}$ value appears to be relatively high compared to the activation energies reported for the hydrolysis of many other drug compounds.[11] The predicted hydrolysis rate (k_{obs}) for the aqueous degradation of **4** extrapolated to 25 °C at pH 4.0 is 3.8×10^{-9} s^{-1}, which corresponds to a half-life ($t_{1/2}$) of 5.7 years and a time for 10% degradation (t_{90}) of 320 days. Extrapolation to 4 °C predicts a k_{obs} value of 4.4×10^{-11} s^{-1} and a t_{90} value of 75 years indicating that a refrigerated ready-to-use injectable formulation of **4** with a shelf-life of >2 years may be possible. While the time for 10% degradation (t_{90}) appears to be more than adequate

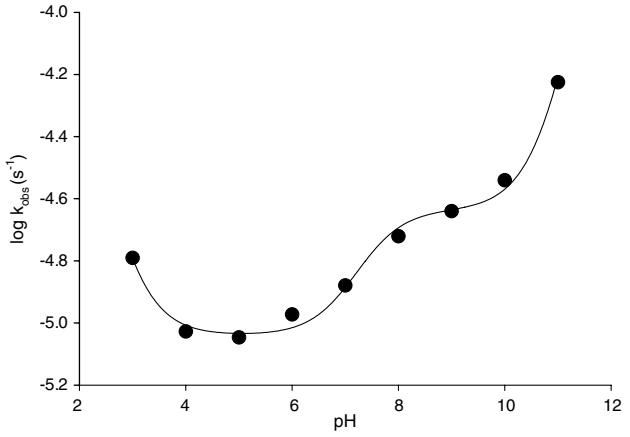


Figure 1. pH-rate profile for the aqueous degradation of **4** at 70 °C. The solid line represents the fit of the data to Eq. 1. The values for the rate constants obtained from the fit of the pH-rate profile were: $k_H = 7.06 \times 10^{-3}\ s^{-1}M^{-1}$, $k_o = 9.04 \times 10^{-6}\ s^{-1}$, $k_{OH} = 5.92\ s^{-1}\ M^{-1}$, and $k'_{OH} = 1.01 \times 10^{-10}\ s^{-1}\ M^{-1}$, and the value obtained for the pK_a of **4** was 7.4.

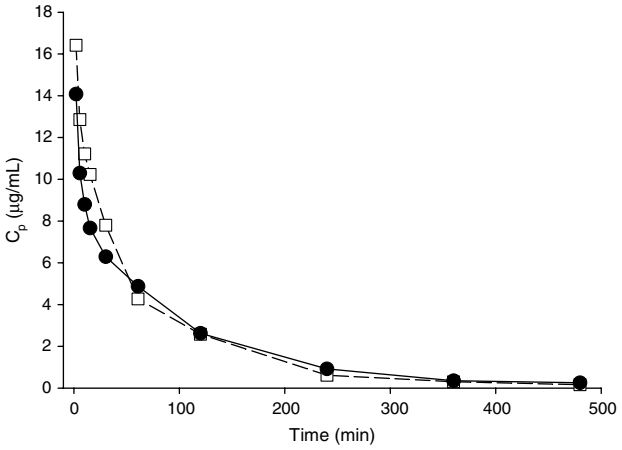


Figure 2. Plasma concentration of CBZ versus time profile following crossover iv administration of **4** (•) and CBZ control (□) in rat 1.

for this indication, it is likely that the precipitation of insoluble degradation products would be the limiting factor.

A 59 μM solution of **4** in 25 mM isotonic pH 7.4 phosphate buffer was found to rapidly and completely convert to CBZ in the presence of a 10-fold molar excess of L-cysteine at 25 °C. The conversion was nearly instantaneous as the immediate HPLC analysis of this sample showed no detectable **4**. Similarly, it was found that **4** rapidly and completely converted to CBZ in rat whole blood at ambient temperature.

A limited animal study was performed to establish the bioavailability of CBZ following iv administration of **4** in rats. A crossover design was chosen because of the large degree of interanimal variation seen in pharmacokinetics studies with CBZ in rats.[12] Rats were given an iv bolus dose of CBZ control (dissolved in 10% HP-β-CD) followed 48 h later by an equimolar iv dose of **4** in sterile saline or vice versa in a crossover design.[13] *N*-methylmaleimide was added to drawn blood samples to trap any available sulfhydryl compounds to limit the ex vivo conversion of **4** to CBZ to less than 1.8% during blood sample processing, refrigerated storage, and analysis. The iv bolus dose administration of **4** resulted in the immediate appearance of the peak plasma level of CBZ and no intact **4** was observed in any blood samples. The plasma concentration versus time profiles of CBZ from iv doses of **4** and CBZ control for rat 1 are shown in Figure 2. The area under the curve values for CBZ were 964 μg min mL^{-1} for CBZ from **4** and 959 μg min mL^{-1} for CBZ from control.

A second rat showed a similar plasma CBZ profile from administration of **4**, but only one data point was able to be collected for the CBZ control dose due to failure of the carotid artery from blood clot blockage. Values for the dose amounts of CBZ (D_{CBZ}) and **4** (D_4), AUC values for CBZ from **4** and control, and CBZ C_{max} values are listed in Table 1. Compound **4** was not detected in any blood or urine samples.

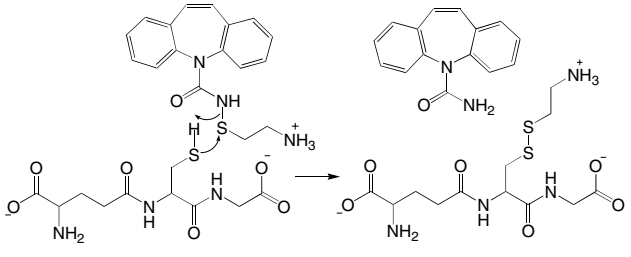
All rats dosed with CBZ and/or **4** did not seem to exhibit any pain or discomfort with the injections. Prior to dosing, rats appeared active, and within minutes following dosing the rats calmed down and went to sleep and were no longer active for several hours. The observed behavior was identical following doses of CBZ and **4**.

In summary, these results show that **4** acts as a prodrug of CBZ and the conversion of **4** to CBZ is rapid and quantitative in vitro and in vivo. This supports the hypothesis that sulfenamide prodrugs release parent NH-acidic drugs by reaction with glutathione and other endogenous sulfhydryl containing compounds (Scheme 5).

Table 1. Values for dose amounts of CBZ (D_{CBZ}) and **4** (D_4), AUC values for CBZ from **4** and control, and peak plasma CBZ concentration (C_{max})

	Rat 1	Rat 2
Rat mass (g)	187	227
D_{CBZ} (mg)	2.54	3.08
D_4 (mg)	4.54	5.50
CBZ $AUC_{control}$[a] (μg min^{-1} mL^{-1})	959	ND
CBZ AUC_4[a] (μg min^{-1} mL^{-1})	964	545
C_{max} (control) (μg mL^{-1})	16.4	17.4
C_{max} (from **4**) (μg mL^{-1})	14.1	16.3

[a] Values determined by trapezoidal method.



Scheme 5. Proposed conversion mechanism for reaction of **4** with glutathione to release to CBZ in vivo.

Glutathione activity is known to be high in red blood cells and tissues such as the liver and kidney of rats and humans.[14] The rapid and complete conversion of **4** to CBZ in rat whole blood is consistent with the proposed mechanism of conversion via reaction with glutathione.

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9. *N-(phenylthio)CBZ* (**2**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.76 (s, 1H), 6.97 (s, 2H), 7.16–7.27 (m, 2H), 7.28–7.34 (m, 3H), 7.34–7.44 (m, 4H), 7.45–7.51 (m, 2H), 7.52–7.57 (m, 2H).
   N-(OEt-Cys)-CBZ (**3**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (t, 3H), 2.22–2.36 (m, 1H), 3.03–3.14 (m, 1H), 4.00–4.08 (m, 1H), 4.16 (q, 2H), 6.97 (s, 2H), 7.09 (s, 1H), 7.31–7.38 (m, 2H), 7.39–7.45 (br m, 6H), 8.41 (br s, 3H).
   N-cysteamine-CBZ (**4**). ^{1}H NMR (400 MHz, DMSO-*d*6) δ 2.74 (t, 2H), 2.89 (q, 2H), 7.02 (s, 2H), 7.06 (s, 1H) 7.33–7.42 (m, 2H), 7.45–7.50 (m, 6H), 7.81 (br s, 3H). The addition of two drops of D_2O to the NMR sample resulted in the disappearance of the peaks at 7.06 and 7.81. HRMS (TOF ES$^+$) *m/z* calcd $C_{17}H_{18}N_3OS$ $(M+H)^+$ 312.1171, found 312.1155.
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13. *Animal studies*. Adult male Sprague–Dawley rats (200–227 g) were purchased from Harlan (Indianapolis, IN) with jugular and carotid artery pre-cannulated. Rats were attached to a swivel device allowing the rat free mobility and access to water during dosing and blood sampling experiments, but fasted for 12 h prior to dosing. A 13.58 mg kg^{-1} CBZ control dose solubilized with 10% HP-β-CD in sterile saline followed by an equivalent dose of **4** (24.30 mg kg^{-1}) in sterile saline (or vice versa) were administered intravenously in a crossover design following a 48-h washout period. Each dose was prepared in excess to provide the specified dose of **1** or **4** in a volume of 1.00 mL. Dosing syringes and needles were completely filled and the 1.00- mL dose was administered as indicated by the change in position of the syringe plug. Doses were given iv through the jugular vein cannula as a bolus dose over 1 min. Blood samples were collected manually. Blood (200 μL) was drawn from the carotid artery cannula at 2, 15, 30, 60, 120, 240, 360, and 480 min post-dose. Excess blood was returned and 200 μL of normal saline was infused into the jugular vein cannula following each blood sample to replace lost blood volume. A volume of heparinized saline (100 UI mL^{-1}) equivalent to the cannula volume was infused into the cannula to create a heparin lock after replacing the lost blood volume. Drawn blood samples were immediately added to a solution of 0.10 M *N*-methylmaleimide and 1.0 M acetic acid in methanol (1–10 parts blood) and vortexed. Processed samples were stored at 4 °C until analyzed.
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