Bioorganic & Medicinal Chemistry Letters 21 (2011) 172–175

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

MDCK cell permeability characteristics of a sulfenamide prodrug: Strategic implications in considering sulfenamide prodrugs for oral delivery of NH-acids

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ARTICLE INFO

Article history:
Received 17 October 2010
Accepted 5 November 2010
Available online 11 November 2010

Keywords:
Sulfenamide prodrug
Oral delivery
Prodrug Strategy
Supersaturation
NH-acid

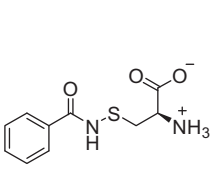
ABSTRACT

The objective of this Letter is both to report the permeability results of a linezolid-based sulfenamide prodrug in an MDCK cell model (enterocyte surrogate system) and to discuss the strategic implications of these results for considering sulfenamide prodrugs to enhance the oral delivery of weakly acidic NH-acids (e.g., amides, ureas, etc.). The two main findings from this study are that the sulfenamide prodrug does not appear to survive intracellular transport due to conversion to linezolid and that there appears to be an apically-oriented surface conversion pathway that can additionally serve to convert the sulfenamide prodrug to linezolid upon approach of the apical membrane. It is hoped that these findings, along with the discussion of the strategic implications, will facilitate a greater awareness of the potential strengths and weaknesses inherent in the sulfenamide prodrug approach for enhancing the oral delivery of weakly acidic NH-acid drugs.

Given that the sulfenamide prodrug approach for weakly acidic NH-acids (amides, ureas, etc.) was first reported only recently,[1,2] there is very limited general guidance available for the application of this technology for oral delivery enhancement relative to the more established prodrug approaches for NH-acids.[3] The objective of this Letter is both to report findings on the MDCK (Madin-Darby canine kidney) cell permeability characteristics of a linezolid-based sulfenamide prodrug and to provide a broader discussion of the strategic implications these results offer when considering the sulfenamide prodrug approach to enhance the oral delivery of weakly acidic NH-acid drugs. While a recent report[4] has investigated the extension of the sulfenamide prodrug approach to a highly basic amine drug (metformin), the objective of this Letter is to provide guidance towards the use of sulfenamides as prodrugs to enhance the oral delivery of *weakly acidic NH-acid* drugs.

Sulfenamides[5] are identified by a single N–S bond involving a bivalent sulfur atom where the bond's polarization makes the sulfur subject to nucleophilic attack. Sulfenamides are known to react rapidly and quantitatively with thiols to form mixed disulfides and a NH compound, and have been reported in the synthetic literature as effective sulfenylating agents.[6] It has been recently shown[1,2] that an optimally designed sulfenamide of a weakly acidic amide compound (e.g., **1**) can possess good hydrolytic stability (projected $t_{1/2}$ of 6.3 yr at 25 °C and pH 6), while maintaining a rapid reactivity at neutral pH with endogenous thiols (e.g., glutathione, cysteine, free thiol-containing proteins, etc.), which is the proposed conversion mechanism in vivo for sulfenamide prodrugs.[1,2,7,8] Regarding IV delivery, the promise of sulfenamides as prodrugs has been previously demonstrated both through dog whole blood spiking experiments of linezolid-based prodrugs (including **3**)[1,2] and a rat IV PK study of a carbamazepine-based sulfenamide prodrug,[9] demonstrating that sulfenamides of weakly acidic NH-acids 'instantaneously' and quantitatively release the NH-acid drug once delivered into the blood stream.

With oral delivery being the preferred route for many therapies, having a similar level of understanding for how



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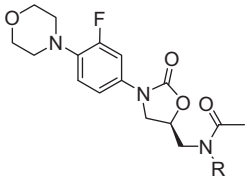

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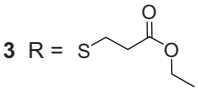

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sulfenamides of NH-acids might behave following oral dosing would be critical to making a rational judgment of whether the sulfenamide approach can be expected to overcome the existing oral delivery barrier for an amide-type drug. As part of this Letter, we

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doi:10.1016/j.bmcl.2010.11.043

share findings from a set of in vitro permeability studies evaluating the potential of a linezolid-based sulfenamide prodrug, **3**, to modulate the delivery of linezolid, **2**, across a MDCK cell monolayer (enterocyte surrogate).[10] The synthesis and structural characterization of **3** has been described previously.[1,2] We believe that these MDCK cell permeability findings carry important strategic implications (that will be discussed) and help elucidate the potential strengths and/or limitations of the sulfenamide prodrug approach for enhancing the oral delivery of weakly acidic NH-acid drugs.

*MDCK permeability results for **2** and **3***. Apical-to-basolateral (A–B) and basolateral-to-apical (B–A) transport studies[2,11] were conducted for both **2** and **3** (separately) to determine whether **3** could modulate the delivery of **2** across the MDCK cell monolayer. The data from these experiments are displayed in Figure 1, where they are organized by direction (A–B vs B–A) and compartment (donor vs receiver); the circle symbols (open and closed) are data from the transport studies of **3**, while the triangle symbols are data from the separate transport studies for **2**. For the transport studies of **2**, only receiver compartment concentrations (Fig. 1B and D) were measured with time to obtain a permeability value (P_{app}; Eq. 1),[12] which was found to be very similar for both directions ($P_{app,A-B}$ = 90(±4) nm/s; $P_{app,B-A}$ = 106(±2) nm/s), suggesting that the predominant transport mechanism for **2** is passive diffusion with no efflux implications. For the transport studies of **3**, both receiver and donor concentrations of **3** and **2** were monitored with time. Unlike for **2**, the permeability of **3** could not be directly determined due to conversion of **3** to **2** during the transport studies.

$$P_{app} = \frac{\left(\frac{dC_r}{dt}\right)(V_r)}{(C_d)_0(A)} \quad (1)$$

Two main findings from the transport studies of **3** are listed below, on which further discussion will be based:

1. For both A–B and B–A directions, the presence of **3** (the prodrug) was never detected on the *receiver* side.
2. The disappearance of **3** from the donor side is significantly faster in the A–B study compared to the B–A study. Also, in terms of concentration, the appearance of **2** in both the donor and receiver compartments is highly asymmetrical in the A–B study, while essentially symmetrical in the B–A study.

Discussion of MDCK results. The inability to detect **3** on the receiver side of both A–B and B–A studies clearly demonstrates that **3** is unable to survive passage across the cell in either direction under the conditions employed.[2,11] Furthermore, the loss of **3** is accompanied by the appearance of **2**, suggesting that the degradation of **3** is following the intended pathway to release **2** (i.e., cleavage of sulfenamide bond). The symmetrical versus asymmetrical appearances of **2** in the donor and receiver compartments for the B–A and A–B studies, respectively, suggests that in the A–B study, there is

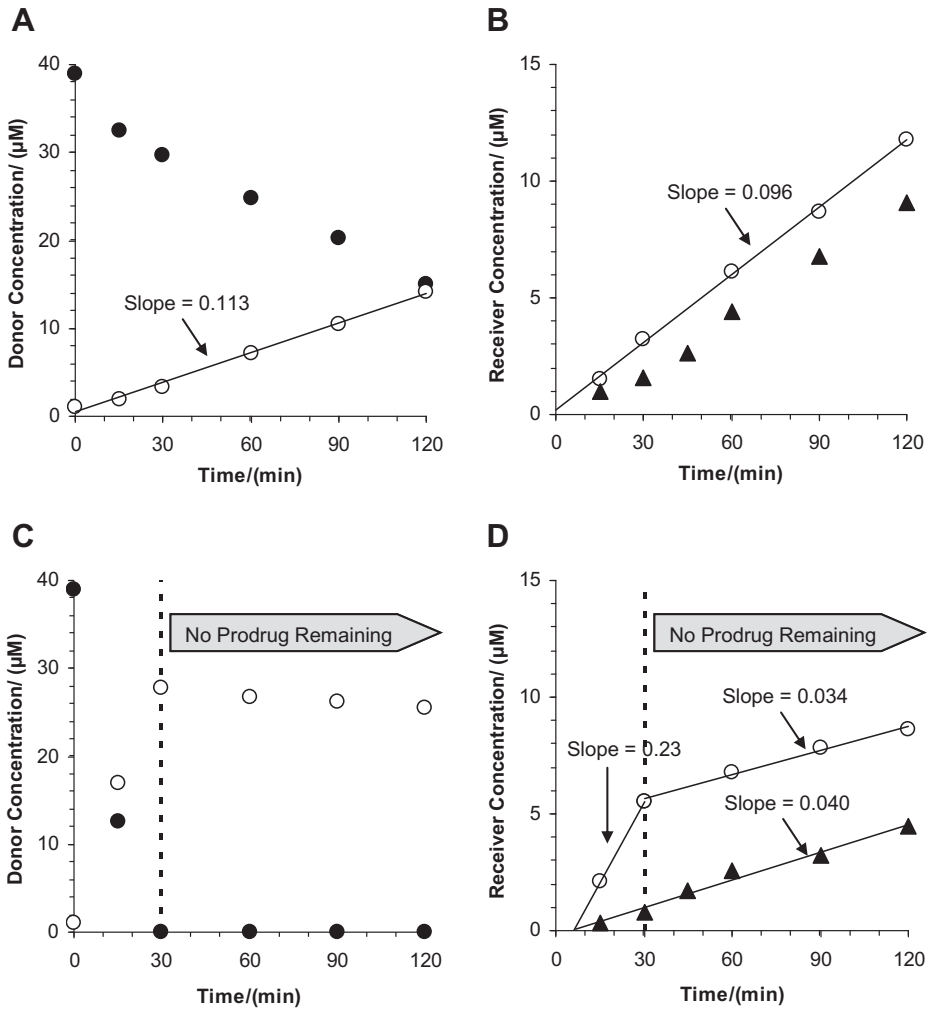
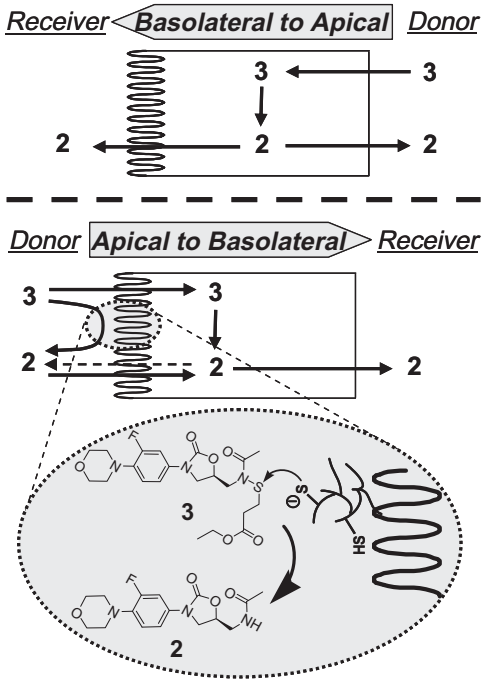


Figure 1. (A) Donor compartment concentrations of **3** (●) and **2** (○) from B–A experiment for **3**. (B) Overlay of receiver compartment concentrations of **2** for B–A experiments for both **3** (○) and **2** (▲). (C) Donor compartment concentrations of **3** (●) and **2** (○) from A–B experiment for **3**. (D) Overlay of receiver compartment concentrations of **2** for A–B experiments for both **3** (○) and **2** (▲).

likely an apical surface-based conversion for **3** that possibly involves reaction with a free thiol-containing protein[8] (discussed later in more detail).

To bring the above points together, Scheme 1 illustrates the proposed pathways involved for both the A–B and B–A transport studies of **3**. Starting with the simpler B–A transport behavior of **3** (Scheme 1; top), it is proposed that **3** crosses the basolateral membrane of the MDCK cell intact, but then degrades in a rapid fashion to form **2** intracellularly; because **2** is only formed intracellularly, and because the previous studies for **2** supported that its transport occurs predominantly by passive diffusion, there is a driving force for diffusion of **2** into both the donor and receiver compartments, which is consistent with the observed essentially symmetrical concentration-time profiles of **2** in both compartments (receiver and donor appearance slopes of 0.096 and 0.113 μM/min, respectively; Fig 1A and B open circles). While Figure 1B shows that **3** is unable to significantly enhance the delivery of **2** to the receiver compartment in the B–A direction, this is mainly due to the observed back-flux of **2** into the donor compartment (Fig 1A). Using the proposed B–A scheme (Scheme 1; top), the appearance of **2** in both the donor and receiver compartments can serve as a 'marker' representing the minimum amount of prodrug that must have crossed the basolateral membrane intact, and therefore conducting a mole-based accounting of **2** in both compartments from this B–A transport study of **3**, and comparing that total number to the total moles of **2** that appear in the receiver compartment from the separate B–A transport study of **2**, per se, should give a comparison of 'permeability' across this first basolateral membrane. This molar accounting approach is illustrated in Figure 2 and a simple ratio of the slopes suggests that **3** has at least a ~4-fold enhanced ability to cross the basolateral membrane relative to **2**; however, since **3** can not survive the intracellular transit, it loses part of its ability to deliver **2** to the receiver compartment due to back-flux of **2** into the donor compartment.

The observed A–B transport behavior of **3** (Fig. 1C and D) appears to be significantly more complex relative to that observed in the B–A study. While in the B–A study, **3** was detectable for



Scheme 1. Proposed transport and reconversion processes for B–A study of **3** (top) and A–B study of **3** (bottom) in a MDCK cell model with magnified view of proposed apical conversion pathway involving free thiol-containing surface protein.

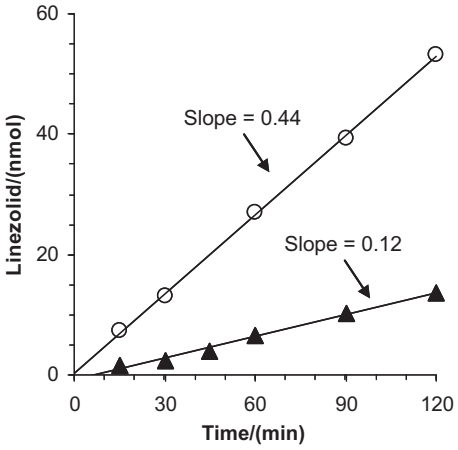


Figure 2. Comparison of receiver compartment appearance of **2** (▲) from B–A transport experiment for **2** with the sum of donor and receiver appearance of **2** (○) from B–A transport experiment for **3**.

the entire 120 min and the concentration-time profiles of **2** were symmetrical in both donor and receiver compartments, at just the 30 min time point of the A–B study, **3** is no longer detectable in the donor compartment and **2** has appeared in a very *asymmetrical* fashion with the donor concentration being nearly 6-times that of the receiver compartment. To account for this behavior, an additional conversion reaction is proposed to occur at the apical surface that directly releases **2** into the donor compartment (Scheme 1; bottom). While the exact identity of this surface reaction has not been determined, one hypothesis is a reaction with a free-thiol-containing protein extending from the apical surface of the cell.[8] This surface degradation pathway is proposed to occur in parallel to the permeation pathway such that only a fraction of **3** in the donor compartment is effectively available for permeation into the cell. It is important to note that apical conversion of **3** via leached chemicals from the cells is highly unlikely based on a control experiment where **3** was shown to be stable in transport media that had been previously incubated in contact with the apical side of the MDCK cell.

Using this proposed A–B transport model, it is striking that despite an apparently large portion of **3** being subject to the surface conversion, the delivery rate of **2** to the receiver compartment during this first 30 min is nearly 6-fold greater than the rate obtained in the separate A–B transport study of **2**, per se, where actually 100% of the molecules of **2** were available to permeate (Fig. 1D). Beyond the 30 min time point, **3** is no longer detectable in the donor compartment and the subsequent delivery of **2** to the receiver compartment appears to occur solely by passive diffusion following the existing concentration gradient of **2**, which is consistent with both the fall in donor concentration of **2** (Fig. 1C) and the observation that the rate of receiver compartment appearance of **2** closely resembles the rate observed in the separate transport study of **2**, per se (Fig. 1D). So the biphasic concentration-time profile for **2** in the receiver compartment of the A–B study of **3** is proposed to be due to a significantly enhanced ability of **3** to deliver **2** to the receiver compartment combined with a short lifetime of **3** in the donor compartment (less than 30 min) due to an apical surface conversion. These results suggest that **3** has in *intrinsic* ability to cross the apical membrane that is at least 6-fold greater than **2**, per se, and possibly much greater, given that the proposed surface conversion should effectively eliminate a fraction of **3** from even getting the chance to permeate.

Strategic implications in considering sulfenamide prodrugs to enhance oral delivery. The following discussion is organized based on the two main observations made from this study: (1) the

sulfenamide prodrug does not survive transit across the MDCK cell due to intracellular conversion to the parent drug and (2) the sulfenamide prodrug is subject to an apical surface conversion pathway to also form the parent drug.

1. The intracellular conversion observed in the A–B and B–A MDCK transport studies suggest that a sulfenamide prodrug of an NH-acid is unlikely to survive passage across the enterocytes. This would obviously be a disadvantage if the goal is to deliver an intact prodrug into systemic circulation for purposes such as tissue targeting and/or by-passing hepatic first pass; but sulfenamides of NH-acids would likely be inappropriate for these purposes anyway given their known rapid conversion in whole blood.[1,2,9] In general, some potential disadvantages of conversion inside enterocytes is that a released drug with at least moderate permeability will likely be partially lost through back-flux to the GI tract while a released drug with poor permeability could reach high levels inside the enterocyte (note: a potential advantage if targeting the enterocyte). So if one is considering designing a lipophilic sulfenamide prodrug to increase permeability of a poorly permeable NH-acid parent drug, it is important to recognize that any amount of the sulfenamide prodrug that permeates into the enterocytes will likely convert intracellularly and the released parent drug could reach a high localized concentration if it can not permeate out.
2. The A–B MDCK results for **3** suggest that there is likely an apical surface conversion pathway available to sulfenamide prodrugs of NH-acids as they approach the enterocytes. This surface conversion can be a significant advantage or disadvantage depending on the goal for the prodrug. The advantage of an apical surface conversion for enhancing oral delivery is that it allows the prodrug to release the parent in proximity to the enterocytes without needing to possess the properties necessary to permeate into the enterocytes. This can work particularly well for solubilizing prodrugs of poorly soluble parent drugs since local supersaturated conditions can be achieved at the apical surface of the enterocytes which can aid in driving flux. In fact, many solubilizing prodrugs are too polar to significantly access the intracellular space of the enterocytes, so a surface-based conversion is ideal for these polar prodrugs. However, for surface conversion to successfully deliver drug to the systemic circulation, the parent drug must be able to cross the enterocytes following release. So it would appear that one promising application of the sulfenamide prodrug approach for oral delivery would be as a solubilizing approach for poorly soluble weakly acidic NH-acid drugs (amides, ureas, etc.) that possess sufficient permeability to cross the enterocytes once released at the apical surface. Solubilizing sulfenamide prodrugs can be made[1,2,9] by choosing ionizable sulfur-based promoieties such as, but certainly not limited to, the cysteine-based promoiety of **1**.

In conclusion, this Letter presented findings from permeability studies of a sulfenamide prodrug of the weakly acidic NH-acid drug linezolid in an MDCK cell surrogate model for the intestinal epithelium. The main findings were that the sulfenamide prodrug could not cross the MDCK cell intact and appeared to be subject to an additional surface degradation on the apical surface; however, despite these complexities, the sulfenamide prodrug was still able to deliver significantly higher levels of linezolid in the A–B direction across the MDCK cell monolayer. Furthermore, this surface-based conversion could be an advantage if designing solubilizing prodrugs of permeable NH-acid drugs where the goal is to avoid systemic exposure of the prodrug. With the sulfenamide approach being relatively new, it is hoped that these results and discussion of strategic implications will be useful to Drug Discovery efforts that are considering whether sulfenamides might be compatible with their drug delivery goals.

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11. MDCK-II WT cells were grown on Transwell® inserts. Transport experiments conducted at 37 °C. Initial donor concentration was ~40 μM in Hank's Balanced Salt Solution (HBSS) containing 0.5% DMSO (resulting from DMSO stock of compound). Receiver compartment contained only HBSS.
12. P_{app} is apparent permeability, dC_r/dt is slope of receiver compartment concentration versus time, V_r is receiver compartment volume (1.5 mL for B–A study; 2.5 mL for A–B study), $(C_d)_o$ is initial donor compartment concentration, and A is surface area of the permeable support (4.71 cm^2).