Dipeptide Prodrugs of the Glutamate Modulator Riluzole

Jeffrey C. Pelletier,^{*,†,§} Suzie Chen,[‡] Haiyan Bian,[†] Raj Shah,[‡] Garry R. Smith,[†] Jay E. Wrobel,[†] and Allen B. Reitz^{*,†,§}[†]Fox Chase Chemical Diversity Center, Inc., 3805 Old Easton Road, Doylestown, Pennsylvania 18902, United States[‡]Department of Chemical Biology, Ernest Mario School of Pharmacy, 164 Frelinghuysen Road, Rutgers University, Piscataway, New Jersey 08854, United States[§]ALS Biopharma, LLC, 3805 Old Easton Road, Doylestown, Pennsylvania 18902, United States

S Supporting Information

ABSTRACT: We have previously reported a prodrug strategy based on the marketed drug riluzole (2-amino-6-trifluoromethoxybenzothiazole), associated with the benefits of lower patient to patient variability of exposure and potentially once daily oral dosing, as opposed to the large variance and twice daily dosing, which is currently observed with the parent drug. Riluzole is a glutamate modulator that is currently approved by the US FDA to treat amyotrophic lateral sclerosis (ALS). Riluzole also strongly suppresses the growth of melanoma cells that express the type 1 metabotropic glutamate receptor (GRM1, mGluR1). Riluzole is a substrate for the variably expressed liver isozyme CYP1A2, which has been shown to contribute to the variance in exposure of riluzole in humans upon oral administration. In addition, an elevated C_{max} following oral administration is a probable cause of increased liver enzyme levels in some patients. In order to mitigate these issues, a series of natural and unnatural dipeptide prodrugs of riluzole were prepared as products that bear lower first-pass hepatic clearance. The prodrugs were evaluated for their ability to produce riluzole in serum while remaining intact prior to absorption from the GI tract, characteristic of a type IIB prodrug. Here, we describe dipeptide conjugates of riluzole and report that the *t*-Bu-Gly-Sar-riluzole analog FC-3423 (**6**) is absorbed well and converts to riluzole in rats and mice in a regular and well-defined manner. FC-3423 strongly suppress tumor cell growth in mouse xenograft models of melanoma at a molar dose 10-fold less than that of riluzole itself.

KEYWORDS: Prodrugs, riluzole, glutamate modulator, peptide prodrugs, antitumor agents, pharmacokinetics

Nobel Prize winner James Black once said, “the best way to discover a new drug is to start with an old one.”¹ It is estimated that ~10% of the ~1500 FDA-approved drugs are prodrugs, and this number could be higher when taking into account the fact that the metabolites of many drugs also contribute to their therapeutic effect. We have described single amino acid prodrugs of the glutamatergic drug riluzole, which are stable in the gut and cleave in the blood following absorption to give riluzole itself (type IIB prodrug).² However, we found that such monoamino acid prodrugs undergo time-dependent spirocyclization of the α -amino group onto the 2-carbon of the benzothiazole bicyclic ring system of riluzole followed by further rearrangement,³ and they are not being considered further as drug candidates. We describe here dipeptide prodrug conjugates of riluzole, which overcome this chemical instability, and highlight FC-3423 in particular, as having promising pharmacokinetic properties as a prodrug of riluzole.

The natural physiological mechanisms of action of the glutamate system occur chiefly through the excitatory amino acid receptors NMDA, kainate, and AMPA, as well as the metabotropic glutamate receptors (mGluR1–5, GRM1–5). Glutamate in high concentrations leads to cell dysfunction and eventual death. Glutamatergic pathophysiology has been linked

to motor neuron disorders, psychiatric conditions, traumatic brain injury, and certain cancers such as melanoma, glioblastoma, colorectal cancer, and breast cancer.^{4–11} Previous cell based studies with the glutamate modulator riluzole (**1**)^{11–14} indicated significant growth inhibition of tumor cell lines selectively expressing the type 1 metabotropic glutamate receptor (GRM1) while having little effect on non-GRM1 phenotypes.¹⁵ Tumor growth inhibition was also shown in an *in vivo* xenograft model when using riluzole and confirmed by clinical studies involving patients that exhibit double refractory melanoma.^{16–19}

Riluzole (**1**, Scheme 1) is known to have highly variable exposure following oral dosing in humans, a phenomenon not observed in animal models.^{20–23} This has been attributed to oxidation of the exocyclic amine by heterogeneously expressed isozymes of the CYP1A2 gene. The *N*-hydroxy metabolite is quickly glucuronidated to an inactive conjugate and eliminated via the renal pathway (Scheme 1).

The oxidation process on the exocyclic amine leads to significant first pass liver metabolism resulting in low exposure

Received: April 24, 2018

Accepted: June 15, 2018

Published: June 15, 2018

Scheme 1. Metabolism of Riluzole

levels for patients with high CYP1A2 isozyme activity and low overall drug response rates. In order to circumvent this variability of exposure, a prodrug approach was envisioned to mask the exocyclic amine of riluzole with a covalent conjugate that afforded the following desired properties: gut stability, systemic absorption, liver microsome stability, and release of parent drug in the blood fast enough to overcome metabolic clearance of the prodrug.^{24–26} Details of our initial approach, involving conjugation of riluzole to single amino acids and other plasma enzyme labile constructs, have been previously published.² We now describe new dipeptide prodrugs of riluzole that culminated in the discovery of dipeptide **6** (FC-3423) and related compounds (see Table 1). FC-3423 imparts

Table 1. Riluzole Dipeptide Stability in Buffer (pH = 7.4), Fresh Rat Serum, Simulated Gastric Fluid (SGF), and Simulated Intestinal Fluid (SIF)

No.	AA1-AA2	pH 7.4 buffer t _{1/2} (h)	Rat Plasma t _{1/2} (h)	SGF t _{1/2} (h)	SIF t _{1/2} (h)
2		<0.1	<0.1	ND	ND
3		<0.1	<0.1	>4.0	<1.0
4		<0.25	<0.25	>4.0	<<1.0
5		>24	>2.0 ^a	>4.0	>4.0
6	 FC-3423	>24	>2.0 ^a	>4.0	>4.0
7		0.20	0.25	2.5	<<1.0
8		>24	>>2.0	ND	ND
9		ND	2.0	>4.0	<<1.0
10		>>2.0	>>2.0	>4.0	>4.0

^a ~30% reduction in prodrug after 2 h.

a high bioavailability of riluzole and shows high potency in mouse melanoma xenograft studies. Such peptide conjugates to the exocyclic amine of riluzole were expected to be labile to cleavage by blood peptidases leading to release of riluzole. It was expected that if peptidase mediated hydrolysis occurred, two modes of cleavage are possible: dipeptide cleavage to release riluzole in a single step and/or cleavage of the N-terminus to a mono-peptide derivative, which may further hydrolyze producing riluzole via a two-step process (Scheme 2). In the case of dipeptides, an alternative cleavage process

Scheme 2. Dipeptide Conjugates of Riluzole Convert to Riluzole Following Enzymatic and/or Chemical Methods *in Vivo*

involves cyclization of the dipeptide to a diketopiperazine (DKP), following neutralization of the terminal amine acid-addition salt, leading to drug release (Scheme 2). Dipeptide ester prodrugs have been attempted previously with limited success due the fast nature of dipeptide cyclization and drug release leading to gut exposure before prodrug absorption as well as potential shelf stability issues.^{27–30} The amino-benzothiazole was expected to be an ideal candidate for release following DKP formation since its leaving group properties are significantly slower than esters. Conversely, the chemical degradation process was expected to occur more easily than release of a standard amine. Dipeptide constructs were also expected to be easily prepared using well-known synthetic chemistry.

The initial target compounds designed and prepared were those with a conformational bias leading to DKP formation and the release of riluzole. These included dipeptides with cyclization promoters proline,³¹ sarcosine,³² and 1-amino-isobutyrate (α,α -dimethyl glycine) in the C-terminal position bonded directly to the exocyclic amine of riluzole. N-Substituted glycines were evaluated on the N-terminus prioritizing starting material availability and lack of chirality. Unsubstituted glycine in the N-terminal position was used for direct comparison to the substituted analogues in the *in vitro* assays. The preparation of target molecules is shown in Scheme 3. Riluzole was coupled to an N-protected amino acid

Scheme 3. Compound Preparation^a

^aConditions: (a) Boc-protected amino acid 1 (AA₁), HATU, DIPEA, DMF; (b) HCl/dioxane or TFA/DCM; (c) Boc-protected amino acid 2 (AA₂), EDC, HOAt, DIPEA, DMF.

using the HATU coupling reagent followed by deprotection with TFA or HCl in dioxane. Subsequent dipeptide formation was achieved using EDCI coupling followed by standard deprotection of the Boc-protected final intermediate.

Test compounds were initially analyzed for their ability to form riluzole in pH 7.4 buffer, fresh rat serum, and simulated gastric (SGF) and intestinal (SIF) fluids (Table 1). The data indicate that N-terminal glycine itself and derivatives with small alkyl N-substituted glycines were labile in buffer, plasma, and SIF such that the compounds were likely going through a fast chemical cyclization to DKPs and riluzole, and not remaining intact long enough for GI transit and absorption into the blood *in vivo* (see Table 1, entries 2–4, 7, and 9). Compounds with N-*t*-butylglycine coupled to D-(prolyl)-riluzole and (1-aminoisobutryl)riluzole (Table 1, entries 8 and 10) were highly stable in rat serum and buffer showing almost no reduction in prodrug levels after 2 h. This suggests that compounds of this type are unlikely to form riluzole through enzyme cleavage or by chemical degradation. The N-*t*-butylglycyl and N-isopropylglycyl derivatives coupled to N-sarcosylriluzole (Table 1, entries 5 and 6) had modest lability in rat serum with approximately 30% loss of prodrug after 2 h. The compounds were stable in buffer, SGF, and SIF with half-lives >4 h. In addition to this, **6** was stable in human, mouse, rat, and dog liver microsomes ($t_{1/2} > 60$ min), displayed high Caco-2 permeability with little chance of efflux (P_{app} A-B = 13.8 nm/s, P_{app} B-A = 24.1 nm/s, efflux ratio = 1.7) and had <10% inhibition of CYPs 1A2, 2D6, and 3A4 (testosterone and midazolam) at 10 μ M. The *in vitro* profile of **6** supported transitioning to IV and PO pharmacokinetics to observe *in vivo* prodrug clearance and appearance of riluzole.

N-*t*-Butylglycylsarcosylriluzole **6** (FC-3423) was administered to rats (IV and PO) and monitored for prodrug and riluzole levels up to 8 h post-administration. The prodrug displayed excellent PK parameters and was efficiently converted to riluzole (Table 2, IV parameters; Table 3, PO parameters). Figure 1 (upper panel) shows the graph of prodrug and riluzole combined plasma concentration over time upon IV administration indicating a steady decline in prodrug as riluzole appears. The IV PK of riluzole from **6** compares well

Table 2. Pharmacokinetic Parameters of Compound 6 (FC-3423) and Riluzole from Compound 6, after Intravenous Administration of Compound 6 in Male Sprague–Dawley Rats at 2.8 mg/kg

Parameters (units)	Riluzole		FC-3423	
	Mean	SD	Mean	SD
C_{max} (ng/mL)	187	67.5	1729	232
T_{max} (h)	0.78	1.06	0	0
$t_{1/2}$ (h)	7.83	3.63	1.93	0.03
AUC_{last} (h-ng/mL)	1137	641	1526	157
Cl (L/h/kg)			1.78	0.2
V_{ss} (L/kg)			3.57	0.32

Table 3. Pharmacokinetic Parameters of Compound 6 (FC-3423) and Riluzole from Compound 6, after Oral Administration of Compound 6 in Male Sprague–Dawley Rats at 14.6 mg/kg

Parameter (units)	Riluzole		FC-3423	
	Mean	SD	Mean	SD
C_{max} (ng/mL)	1587	150	213	39
T_{max} (h)	8	0	1.8	2
$t_{1/2}$ (h)	ND	ND	3.8	0.40
AUC_{last} (h-ng/mL)	20518	1437	2259	305

Figure 1. Upper panel: Average plasma concentrations of compound **6** (FC-3423) and riluzole, from **6**, over time following intravenous administration of **6** (2.8 mg/kg) to male Sprague–Dawley rats. Lower panel: Average plasma concentrations of riluzole over time following intravenous administration of riluzole (1.0 mg/kg) to male Sprague–Dawley rats.

with riluzole itself following IV administration (Figure 1, lower panel). When administered orally to rats, **6** was well absorbed ($\%F = 70$) and produced a steady exposure of riluzole over time (Figure 2, upper panel). As in the case of IV administration of **6** the appearance of riluzole following oral administration of **6** compares favorably with the PK of riluzole itself following PO administration (Figure 2, lower panel).

Compound **6** was administered orally, once daily for 21 days (4.5 mg/kg and 14.6 mg/kg), to nude mice with tumors generated following human melanoma C8161 cell line inoculation.¹⁴ The data, shown in Figure 3, demonstrates robust tumor growth suppression at doses as low as 4.9 mg/kg p.o. During the course of the 21-day treatment period, selected animals tested positive for riluzole plasma exposure 2 h post-dosing, indicating challenged animals were exposed to drug. Riluzole prodrug **6** (FC-3423) is as effective as riluzole (7.5 mg/kg) at an equimolar dose (14.8 mg/kg) and a 3-fold lower dose (4.9 mg/kg), suggesting that prodrug delivery of riluzole provides greater efficacy than for dosing of riluzole itself.

We have designed a type IIb prodrug that passes through the gut and the liver unchanged and is then converted to riluzole

Figure 2. Upper panel: Average plasma concentrations of compound 6 (FC-3423) and riluzole, from 6, over 24 h following oral administration of 6 (14.6 mg/kg) to male Sprague–Dawley rats. Lower panel: Average plasma concentrations of riluzole over 8 h following oral administration of riluzole (7.5 mg/kg) to male Sprague–Dawley rats.

via peptidase enzymes, capable of cleaving the peptidic moieties. All available data in multiple species (rat data only shown) supports bioavailability and conversion to riluzole following oral administration. Riluzole has been shown to suppress the growth of melanoma in preclinical *in vivo* testing as well as for some patients when administered orally. However, the exposure of riluzole in humans has significant variability, due to Cyp1A2 oxidation mediated by the first pass through the liver. Compound 6 (FC-3423) is not a substrate for any of the major Cyp enzymes suggesting that exposure

variability in humans should be low. In addition to this, 6 is readily converted to riluzole following exposure to serum and suppresses melanoma tumor growth in mice. Based on *in vitro* and *in vivo* profiles, FC-3423 (6) demonstrates a further proof of concept that riluzole can be a successfully delivered therapeutic agent administered in the form of a prodrug.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00189.

Experimental procedures, NMR and LC/MS data for all compounds, and HRMS data for final products (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: jpelletier@fc-cdci.com. Phone: 1-610-405-0472.

*E-mail: areitz@fc-cdci.com. Phone: 1-215-589-6435.

ORCID

Jeffrey C. Pelletier: 0000-0002-0397-0681

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under award number R44CA156781. We thank Biohaven Pharmaceutical Holding Company, Ltd. for support and encouragement, especially Vlad Coric, Rob Berman, and Kim Gentile. We thank Jim Goydos and Ann Silk of Rutgers University, for their support and encouragement. We also thank Marie Loughran, Hong Ye, and Mark McDonnell of FCCDC for their contributions to this research.

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Figure 3. FC-3423 exhibits an increase in potency when compared to riluzole (7.5 mg/kg) in a mouse xenograft model of melanoma. C8161 human melanoma cells (10^6) were inoculated in the flanks of nude mice, and when the tumor volumes reached 40 mm³, the mice were divided randomly into groups with similar tumor volumes: Vehicle (Veh, DMSO), riluzole (Ril, 7.5 mg/kg), and FC-3423 (4.9 mg/kg and 14.8 mg/kg). Mice were treated daily by oral gavage. Tumor volume (mm³) mean $\pm$ SD of 12 mice per group; $P < 0.001$ for riluzole or FC-3423 groups (regardless of dose) compared to Veh.

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