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Increasing Frataxin Gene Expression with Histone Deacetylase Inhibitors as a Therapeutic Approach for Friedreich's Ataxia

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Abstract

The genetic defect in Friedreich's ataxia (FRDA) is the expansion of a GAA·TCC triplet in the first intron of the *FXN* ene, which encodes the mitochondrial protein frataxin. Previous studies have established that the repeats reduce transcription of this essential gene, with a concomitant decrease in frataxin protein in affected individuals. Since the repeats do not alter the *FXN* protein coding sequence, one therapeutic approach would be to increase transcription of pathogenic *FXN* genes. Histone posttranslational modifications near the expanded repeats are consistent with heterochromatin formation and *FXN* gene silencing. In an effort to find small molecules that would reactivate this silent gene, histone deacetylase inhibitors were screened for their ability to up-regulate *FXN* gene expression in patient cells and members of the pimelic 2-aminobenzamide family of class I histone deacetylase inhibitors were identified as potent inducers of *FXN* gene expression and frataxin protein. Importantly, these molecules up-regulate *FXN* expression in human neuronal cells derived from patient induced pluripotent stem cells and in two mouse models for the disease. Preclinical studies of safety and toxicity have been completed for one such compound and a phase I clinical trial in FRDA patients has been initiated. Further medicinal chemistry efforts have identified improved compounds with superior pharmacological properties.

Keywords

Friedreich's ataxia; histone deacetylase inhibitor; heterochromatin; neurodegenerative disorder

Introduction

Most individuals affected with Friedreich's ataxia (FRDA) carry a homozygous mutation consisting of the expansion of GAA•TTC trinucleotide repeats within the first intron of the frataxin (*FXN*) gene (Campuzano *et al.*, 1996), which results in inhibited gene expression. A few FRDA subjects (about 4%) are compound heterozygotes for the expanded repeat mutation in one allele and a different loss-of-function mutation (missense, nonsense, indel) in the other one (Cossée *et al.*, 1999). Frataxin amounts in affected individuals range between 5 and 35% of the levels in healthy individuals, while heterozygous subjects with no sign of disease have approximately 50% (Campuzano *et al.*, 1997). The degree of frataxin

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expression reduction correlates with the length of the expanded GAA•TTC sequences and with the severity of clinical symptoms.

Although the exact function of frataxin is still under debate, available evidence supports a role in the biogenesis of iron-sulfur (Fe-S) clusters in mitochondria (Tsai & Barondeau, 2010). Fe-S clusters are essential cofactors for a variety of enzymes localized in all cellular compartments (Rouault & Tong, 2008). Frataxin deficiency results in impaired activities of Fe-S enzymes, altered cellular iron metabolism with iron accumulation in mitochondria, decreased mitochondrial energy production, and increased oxidative stress (these aspects of FRDA pathogenesis are treated in detail in other review articles in this issue, e.g. González-Cabo and Palau, 2012). To counteract these abnormalities, antioxidants, iron chelators and stimulants of mitochondrial biogenesis have been proposed as therapeutics (Pandolfo, 2013). However, no clear results supporting a benefit of any of these drugs have so far been obtained in randomized controlled trials. While continuing investigation of these therapeutics is warranted, the alternative of restoring frataxin expression in affected cells appears an appealing approach to slow down or stop disease progression, and stabilize or reduce the severity of disabilities. Gene replacement and protein replacement can in principle be used for this purpose. However, while preliminary studies in cell cultures and animal models do support their validity (Hebert and Whittom, 2007; Fleming *et al.*, 2005; Vyas *et al.*, 2012), further development depends on the resolution of many general problems in the field, in particular targeted delivery, controlled expression, and, for gene therapy, potential genotoxicity. An alternative is offered by the fact that, unlike the situation in diseases caused by coding repeat expansions (e.g. Huntington's disease or the autosomal dominant spinocerebellar ataxias), or by mutations resulting in a truncated or dysfunctional protein, in FRDA the protein encoded by the disease gene is structurally normal. Boosting the expression of the endogenous frataxin genes is therefore an appealing therapeutic approach. Here, we review the progress in the development of drugs targeting the frataxin gene silencing caused by the GAA•TTC repeat expansion. But first, we summarize the evidence that Friedreich's ataxia is a gene silencing disease with an epigenetic basis.

Frataxin deficiency is due to transcriptional silencing

Two early reports documented that expanded GAA•TTC repeats cause transcriptional silencing of the *FXN* gene (Bidichandani *et al.*, 1998; Ohshima *et al.*, 1998). In the first study, plasmids containing various numbers of repeats were shown to cause a length- and orientation-dependent inhibition of reporter gene expression (Ohshima *et al.*, 1998). In the second study, the activity of the endogenous *FXN* gene was analyzed, with the result that patients who are homozygous for this expansion have a marked deficiency in *FXN* mRNA (Bidichandani *et al.*, 1998). This study also documented that the repeats interfere with in vitro transcription, again in a length- and orientation-dependent manner, with both prokaryotic and eukaryotic RNA polymerases. No evidence has been obtained for aberrant splicing of the primary transcript; that is, generation of the mature messenger RNA from the primary transcript is not affected by the repeats (Bidichandani *et al.*, 1998; Ohshima *et al.*, 1998). Although an effect of the repeats on RNA splicing from a reporter construct has been reported, this study did not extend the results to the endogenous *FXN* transcript in patient cells (Baralle *et al.*, 2008). There is also no evidence that long GAA-repeat intron 1 RNA is stable and could lead to an RNA-toxicity disease, such as found in myotonic dystrophy or fragile X-associated tremor/ataxia (reviewed in (Orr & Zoghbi, 2007)). Together, both early papers (Bidichandani *et al.*, 1998; Ohshima *et al.*, 1998) presented strong evidence that the loss of frataxin protein in FRDA is due to repression of *FXN* transcription by the GAA•TTC repeats, and the data presented were fully consistent with the negative correlation between repeat length and age of onset and severity of the disease in patients.

Having established that RNA transcription is impaired by the GAA•TTC repeats, the question remained as to how the repeats interfere with RNA polymerase at the *FXN* gene. An impressive series of papers from Wells and colleagues documented that expanded GAA•TTC repeats adopt unusual DNA structures in vitro, such as triplexes and "sticky" DNA (Sakamoto *et al.*, 1999; Wells, 2008). These structures could very well impede the progress of RNA polymerase II through the repeats and lead to stalled or aborted transcription. Another model is that a DNA-RNA triplex formed at the repeats is responsible for blocking transcription elongation (Grabczyk & Usdin, 2000a; 2000b). While the biochemical results on DNA structure are fully consistent with the observed correlation between repeat length, triplex/"sticky" DNA formation and the age at onset and severity of disease, confirmation of the role of such structures in *FXN* gene silencing must await experimental evidence that expanded GAA•TTC repeats exist in a non-B DNA structure (DNA or DNA-RNA triplexes, or "sticky" DNA) at the chromosomal *FXN* in patient cells. Chemical probing and triplex-specific antibody-based approaches are needed to resolve this issue.

Repeat induced heterochromatin formation at pathogenic *FXN* alleles

An alternative, but not mutually exclusive, mechanism for silencing pathogenic *FXN* alleles is epigenetic gene silencing through heterochromatin. Heterochromatin is characterized by histone hypoacetylation, histone H3 lysine 9 and lysine 27 methylation, and the association of histone deacetylase enzymes, specific histone methyltransferases and heterochromatin proteins, such as members of the HP1 family and polycomb group proteins. The first report in support of an epigenetic silencing mechanism in FRDA came from Festenstein and colleagues (Saveliev *et al.*, 2003), who showed that a transgene containing GAA•TTC repeats was silenced in vivo, in a manner reminiscent of position effect variegated gene silencing. In this study, repeat-induced silencing was augmented by over-expression of the heterochromatin protein HP1, and the silenced transgene was packaged into condensed chromatin, as evidenced by resistance to nuclease digestion (Saveliev *et al.*, 2003). Interestingly, the repeats could be located outside of the transcribed region of the transgene, suggesting that models for a block in transcription elongation due to non-B DNA structures need to be reconsidered. While this study pointed to heterochromatin formation by the GAA•TTC repeats as the mechanism for *FXN* silencing in FRDA (Saveliev *et al.*, 2003), these authors did not examine the chromatin structure of endogenous *FXN* alleles in FRDA patient cells.

The first such report came from Herman et al. (Herman *et al.*, 2006), and subsequently other laboratories have used similar chromatin immunoprecipitation methods to monitor the histone modifications on the endogenous *FXN* alleles in cell lines derived from Friedreich's ataxia patients and in patient primary cells (peripheral lymphocytes) (Herman *et al.*, 2006; Greene *et al.*, 2007; Rai *et al.*, 2010; Kim *et al.*, 2011; Kumari *et al.*, 2011). The general consensus from these studies is that the first intron of active *FXN* alleles in cells from unaffected individuals is enriched in acetylated histones H3 and H4, compared with the inactive alleles in Friedreich's ataxia cells. Additionally, lysine 9 of histone H3 (H3K9) is highly methylated in Friedreich's ataxia cells compared with the normal cells. Along with hypoacetylation, trimethylation of H3K9 is a hallmark of heterochromatin, and provides the binding site for heterochromatin protein HP1 (Saveliev *et al.*, 2003) (Figure 1). Although these studies were conducted in Friedreich's ataxia lymphoid cells, the same epigenetic differences between active and inactive *FXN* alleles have also been found in the affected tissues (brain and heart) from mouse models for the disease (Al-Mahdawi *et al.*, 2008; Rai *et al.*, 2008) and in Friedreich's ataxia autopsy brain, cerebellum, and heart (Al-Mahdawi *et al.*, 2008). Ongoing studies have also documented similar heterochromatin marks on pathogenic *FXN* alleles in neurons derived from patient induced pluripotent stem cells (Soragni et al.,

unpublished). Recent reports have also suggested that the chromatin changes associated with pathogenic *FXN* alleles prevent transcript elongation by RNA polymerase II through expanded GAA•TTC repeats (Punga & Bühler, 2010; Kim *et al.*, 2011; Kumari *et al.*, 2011), although one report suggested that both transcription initiation and elongation may be affected (Kumari *et al.*, 2011).

Taken together, there is now a strong body of evidence supporting the notion that the repeats induce silencing through changes in the chromatin landscape of the *FXN* gene. Just how the repeats signal heterochromatin formation is still a subject of debate. Perhaps a non-B DNA structure is the key signal for recruitment of the cellular machinery for heterochromatin formation. Alternatively, one report has implicated the chromatin insulator protein CTCF in repeat-induced silencing (De Biase *et al.*, 2009). CTCF is depleted from pathogenic *FXN* alleles in FRDA cells, but just how the repeats cause this depletion remains a mystery.

Histone Deacetylase Inhibitors to Correct Frataxin Deficiency in Friedreich's Ataxia

Numerous studies have shown that small molecule inhibitors of the histone deacetylase (HDAC) enzymes are able to revert silent heterochromatin to an active chromatin conformation, and restore the normal function of genes that are silenced in various human diseases, including neurodegenerative and neuromotor diseases (Di Prospero & Fischbeck, 2005; Kazantsev & Thompson, 2008). Eighteen histone deacetylase enzymes have been identified in the human genome, including the zinc-dependent (class I, class II, and class IV) and the NAD^+-dependent enzymes (class III or sirtuins). Histone deacetylase enzymes 1, 2, 3, and 8 belong to class I, showing homology to the yeast enzyme RPD3. Class II is further divided into class IIa (histone deacetylase enzymes 4, 5, 7, and 9) and IIb (histone deacetylase enzymes 6 and 10), according to their sequence homology and domain organization. Histone deacetylase enzyme 11 is the lone member of class IV. The sirtuins (class III) are related to the yeast silent information regulator 2 protein and are involved in regulation of metabolism and aging. Several small molecule chemical families have been shown to act as histone deacetylase inhibitors (Cole, 2008). These include small carboxylates (such as sodium butyrate, valproic acid (VPA), and sodium phenylbutyrate), hydroxamic acids (such as trichostatin A, TSA, suberoylanilide hydroxamic acid, SAHA, and suberoyl bishydroxamic acid, SBHA), benzamides (such as MS-275), epoxyketones (trapoxins), and cyclic peptides (including apicidin and depsipeptide). While the development of HDAC inhibitors as anti-cancer therapeutics is quite advanced, and two such compounds have been approved by the FDA (SAHA, under the generic name of vorinostat, and the cyclic peptide romidepsin) (Marks, 2010), interest in the development of HDAC inhibitors for neurodegenerative and neuromotor diseases has increased in the past few years, based on an increasingly recognized role of epigenetic modifications in neurodegeneration (Kazantsev & Thompson, 2008).

Based on our finding of epigenetic silencing marks associated with pathogenic *FXN* alleles, we asked whether HDAC inhibitors could reverse silencing in FRDA lymphoblasts and primary lymphocytes (Herman *et al.*, 2006). We screened a small collection of the HDAC inhibitors discussed above and found that only 2-aminobenzamides (Fig. 1) were active in restoring *FXN* gene expression in FRDA cells. While each of the HDAC inhibitors tested (at their reported IC50 value for HDAC inhibition), including TSA, SAHA, SBHA and VPA increased the fraction of total acetylated histones in FRDA cells,,only 2-aminobenzamides increased acetylation at *FXN* intron 1 and *FXN* gene expression (Herman *et al.*, 2006). Chromatin immunoprecipitation experiments also showed that the 2-aminobenzamide HDAC inhibitor **4b** increased acetylation at particular lysine residues of histones H3 and H4 within intron 1 of *FXN* (H3-K14, H4-K5, and H4-K12) (Herman *et al.*, 2006), while SAHA

and TSA did not. Taken together, our results suggest that a specific histone deacetylase, or histone deacetylase-protein complex, is involved in *FXN* silencing and 2-aminobenzamide histone deacetylase inhibitors selectively target this protein in cells. Alternatively, a different mechanism of action of these inhibitors could account for our results (see below).

These results were initially obtained with lymphoblast cell lines (Herman *et al.*, 2006), but have been extended to primary lymphocytes from FRDA patients (Rai *et al.*, 2010), and to mouse models (Rai *et al.*, 2008; 2010; Sandi *et al.*, 2011). We have recently shown increases in *FXN* gene expression in human FRDA neuronal cells derived from patient induced pluripotent stem cells (Soragni et al., unpublished). Importantly, the HDAC inhibitors increase the levels of frataxin protein in both cellular and animal models, paralleling the observed changes in *FXN* messenger RNA. These findings demonstrate that expanded GAA repeats in the primary *FXN* RNA transcript do not interfere with RNA processing. Additionally, we find that the 2-aminobenzamide HDAC inhibitors only increase *FXN* expression in patient cells, with minor effects on the normal alleles (Soragni *et al.*, 2012), indicating that these compounds are likely acting on the mechanism of silencing induced by the repeats. Members of this compound class have increased levels of *FXN* messenger RNA in lymphocytes from >100 FRDA patients, and the level of *FXN* messenger RNA in their lymphocytes is generally increased to at least that of lymphocytes from carrier siblings or parents. Since heterozygous individuals do not exhibit symptoms of Friedreich's ataxia, we believe our compounds have elicited a therapeutically useful increase in *FXN* messenger RNA in patient cells.

Which HDAC isoform is the target of our 2-aminobenzamide inhibitors?

We felt that it was essential to know the cellular HDAC enzyme target of our inhibitors for future drug development. Previous studies with other benzamide-type HDAC inhibitors, such as MS-275, indicated that these compounds target the class I HDAC family (Beckers *et al.*, 2007; Bradner *et al.*, 2010). We find that the pimelic 2-amimobenzamides, exemplified by compound **106**, are also class I histone deacetylase inhibitors, with a moderate preference for HDAC3 over HDACs 1 and 2, with little activity against the other class I HDAC, HDAC8, and little or no activity against class II HDACs (Chou *et al.*, 2008). To try to understand why the 2-aminobenzamides but not other potent HDAC inhibitors, such as the hydoxamates SAHA and TSA, fail to activate *FXN* gene expression, we determined the kinetic parameters for these compounds with recombinant class I HDACs (Chou *et al.*, 2008). While the hydroxamates are rapid-on/rapid-off, classical competitive inhibitors, compound **106** inhibits HDACs 1 and 3 through a slow-on/slow-off mechanism (Chou *et al.*, 2008). Ki measurements show that HDACi **106** has a ~10-fold preference for HDAC3 over HDAC1, and this difference is reflected in proteomic profiling of the HDAC enzymes with a chemical probe version of HDAC inhibitor **106** in cell extracts (Xu *et al.*, 2009). Other class I HDAC inhibitors, such as MS-275 and related benzamides, exhibit a ~4 to 10-fold preference for HDAC1 over HDAC3 (Hu *et al.*, 2003; Beckers *et al.*, 2007; Siliphaivanh *et al.*, 2007); thus, **106** is one of the first examples of a HDACi that shows selectivity for HDAC3 over all other class I HDAC enzymes. Small interfering RNA approaches also point to a role for HDAC3 in *FXN* gene silencing in FRDA cell models (Soragni et al., unpublished).

We also examined the cellular histone deacetylase inhibition activities of SAHA and **106** in a Friedreich's ataxia lymphoblast cell line (Chou *et al.*, 2008). While both compounds show increased global acetylation of histone H3, after washing cells free of the compounds, acetylated histones are rapidly lost in the SAHA-treated cells. In contrast, acetylation persists for several hours in the **106**-treated cells, paralleling the in vitro slow dissociation described above. Thus, the active 2-aminobenzamides differ from hydroxamates in their

mechanism of inhibition of class I HDACs, and this difference could well account for the efficacy of this class of compounds in reactivation of silent *FXN* alleles in Friedreich's ataxia cells and mouse models.

Do compounds targeting other HDACs activate *FXN* gene expression?

While the pimelic 2-aminobenzamides are potent activators of *FXN* gene expression in FRDA cells, the question remained as to whether compounds with other HDAC isoform selectivities would also activate *FXN* gene expression. To address this issue, we tested compounds with different selectivity profiles in the FRDA lymphoblast cell line (Xu *et al.*, 2009). Appending a phenyl group at the 5 position of the 2-aminobenzamide ring results in a compound that is ~300-fold selective for HDACs 1 and 2 over HDAC3 (Methot *et al.*, 2008; Xu *et al.*, 2009). While this compound is a potent HDAC inhibitor in these cells, it is without effect on *FXN* gene expression. Similarly, we find that potent inhibitors of class II and class III (sirtuins) histone deacetylase also fail to activate *FXN* expression in the FRDA cells, although each of these compounds is active against known substrates (Xu *et al.*, 2009). Recent work has suggested that compounds with moderate selectivity for HDAC3 over HDACs 1 and 2 are the best activators of *FXN* gene expression (Soragni et al., unpublished).

Efficacy of Histone Deacetylase Inhibitors in Mouse Models for Friedreich's Ataxia

The efficacy of 2-aminobenzamide HDAC inhibitors has been tested in two mouse models for FRDA (Rai *et al.*, 2008; 2010; Sandi *et al.*, 2011). Homozygous knock-in mice carrying 230 GAA•TTC triplets inserted in the endogenous frataxin gene (KIKI, $FXN^{230GAA/230GAA}$) express 66 to 75% of wild-type *Fxn* mRNA and protein in the brain and other organs (Miranda *et al.*, 2002). Similar to FRDA patient cells and tissue samples, characteristic histone modifications occur in the first intron of the *Fxn* gene in the KIKI mouse (Herman *et al.*, 2006), including increased H3-K9 trimethylation and hypoacetylation of histones H3 and H4. Treatment of KIKI mice with the HDAC inhibitor **106** (Figure 1) restored frataxin levels in the nervous system and heart to those of wild-type mice and increased histone H3 and H4 acetylation near the GAA•TTC repeat. Microarray analysis indicated that most of the differentially expressed genes in KIKI mice, compared with wild-type mice of the same genetic background, reverted toward wild-type levels, indicating correction of changes induced by frataxin downregulation. The HDAC inhibitor only increased *Fxn* mRNA levels from the knock-in allele harboring GAA repeats; as it had no effect in wild-type mice (Rai *et al.*, 2008). A second study in KIKI mice confirmed the effect of **106** and of a similar molecule, **109** on frataxin mRNA and protein. This study also showed that the elevation of frataxin mRNA and protein was sustained, persisting 48 hours after drug exposure in the case of the protein (Rai *et al.*, 2010).

Pook and colleagues investigated the long-term effects of three 2-aminobenzamide HDAC inhibitors, **106**, **109**, and **136**, in a second GAA repeat expansion mouse model (Sandi *et al.*, 2011). This mouse model harbors a yeast artificial chromosome containing the human *FXN* locus with expanded repeats. Frataxin expression from the YAC transgene is sufficient to rescue embryonic lethality when crossed into a $Fxn^{-/-}$ background, but the resulting line, called YG8R, shows reduced levels of frataxin mRNA, protein, heterochromatin formation at the repeats, motor deficits, and neuronal pathology (Al-Mahdawi *et al.*, 2008). During 5 months of treatment with the compounds **109**, **106** and **136** (Figure 2), no overt toxicity was observed. Both **109** and **106** improved motor coordination, whereas **109** and **136** increased locomotor activity. All three compounds increased global histone H3 and H4 acetylation of brain tissue, but only **109** significantly increased acetylation of specific histone residues at

the *FXN* locus. Furthermore, compound **109** significantly increased frataxin protein expression in brain tissue, improved brain aconitase enzyme activity, which is reduced in frataxin-deficient cells as a consequence of impaired iron-sulfur cluster biogenesis (Rötig et al., 1997; Tsai and Barondeau, 2010), and reduced neuronal pathology in the dorsal root ganglia (Sandi *et al.*, 2011). Taken together, the results in mouse models support the use of 2-aminobenzamides as therapeutics for Friedreich's ataxia.

Generation of Compounds with Improved Pharmacological Properties

While the 2-aminobenzamides are potent inducers of *FXN* gene expression in both cellular and animal models, this compound class suffers from two liabilities that need to be addressed for their use as human therapeutics in Friedreich's ataxia. These are less than optimal brain penetration and conversion of the active molecule into an inactive metabolic product under the acidic conditions of the stomach and in serum. For example, on subcutaneous injection of histone deacetylase inhibitor **109** in rodents, this molecule has only a 10% to 15% brain concentration compared with serum. In addition, the 2-aminobenzamides are converted to an inactive benzimidazole in vivo. Through a medicinal chemistry effort at Repligen, two structural features that individually improve brain distribution and metabolic stability were identified. These are replacement of the “left” amide with an ether, olefin, or ketone to improve brain penetration and introduction of an unsaturated linkage adjacent to the “right” amide to prevent formation of a benzimidazole metabolic byproduct (Figure 3a). On the basis of these results, we devised and synthesized a new lead compound click-1 using Cu(I)-catalyzed click chemistry (Figure 3b). This synthetic route allows for the generation of compounds containing both modifications mentioned above, but introduces a triazole into the aliphatic linker region of the standard pimelic 2-aminobenzamide scaffold. Initially, we did not know whether a heterocycle in the linker would retain activity compared to the active parent molecules, such as **4b** or **106**, etc. (Figure 2).

We therefore monitored click-1 for its activity against recombinant HDACs 1, 2, and 3 in vitro, in-cell histone deacetylase activity, and activity in restoring *FXN* transcription in patient cells. We find that click-1 has comparable activity to histone deacetylase inhibitor **106** or **109** in these assays (Xu *et al.*, 2009). Blood-brain barrier penetration was measured in the rat for compound **109** and click-1. Brain penetration (as measured by brain/plasma ratio at t_{max}) was determined to be 0.15 for **109** and 0.33 for click-1 (Cmax: 800 ng/g in the brain at Tmax = 5 min post-dose [5 mg/kg IV rat]), representing a significant increase in brain penetration for click-1 compared with the standard 2-aminobenzamide **109**. The half-life of the compounds in acidic conditions was used to quantify their relative stability. At pH = 2 and 50 °C, $t_{1/2}$ = 6 hours for **109** versus $t_{1/2}$ = 33 hours for click-1, representing a 5.5-fold improvement relative to histone deacetylase inhibitor **109**. These results demonstrate that click-1 has improved acid stability and better brain penetration, through placing a double bond next to the right amide bond and removing the left amide. Based on these findings, new generations of molecules have been synthesized and are currently in preclinical testing.

Future Directions and Clinical Studies

Full preclinical assessment of a lead clinical compound (**109**/RG2833) has been accomplished and an Investigational New Drug application has been filed with the US Food and Drug Administration for initiation of a phase I clinical trial of RG2833 in man. Additionally, Repligen has received Orphan Drug status for its clinical candidate. In Europe, the European Medicine Agency (EMA) as granted RG2833 orphan drug status as well and authorized a phase I study to determine whether this drug can safely increase frataxin levels

in peripheral blood mononuclear cells (PBMCs) from FRDA patients. After further authorization from national health authorities and ethics approval, a trial has started in Italy to assess the safety of single ascending doses of RG2833, its pharmacokinetics and its effect on frataxin in PBMCs. This study is ongoing at the time of submission of this review article.

Such information will provide a proof of principle for initiating the clinical efficacy stage of clinical studies. If oral delivery of a histone deacetylase inhibitor can be effective at drug exposures that are well-tolerated, it will provide impetus to further advance the 2-aminobenzamide class of compounds to target histone deacetylase inhibition as a viable therapeutic strategy for this devastating disease.

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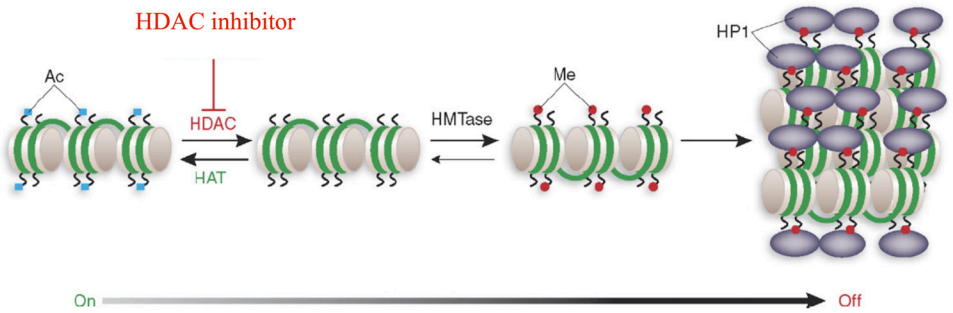

Figure 1. Putative silencing pathway in Friedreich's ataxia
Figure first published in and modified from Festenstein (2006).

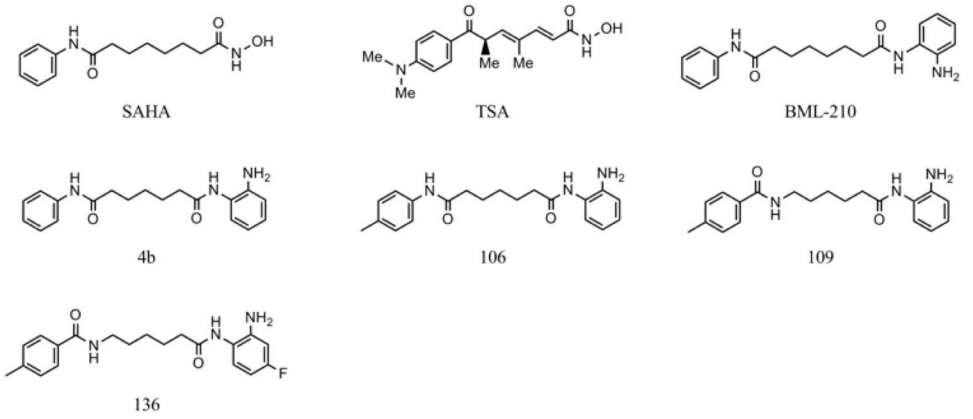

Figure 2.
Structures of the histone deacetylase inhibitors.

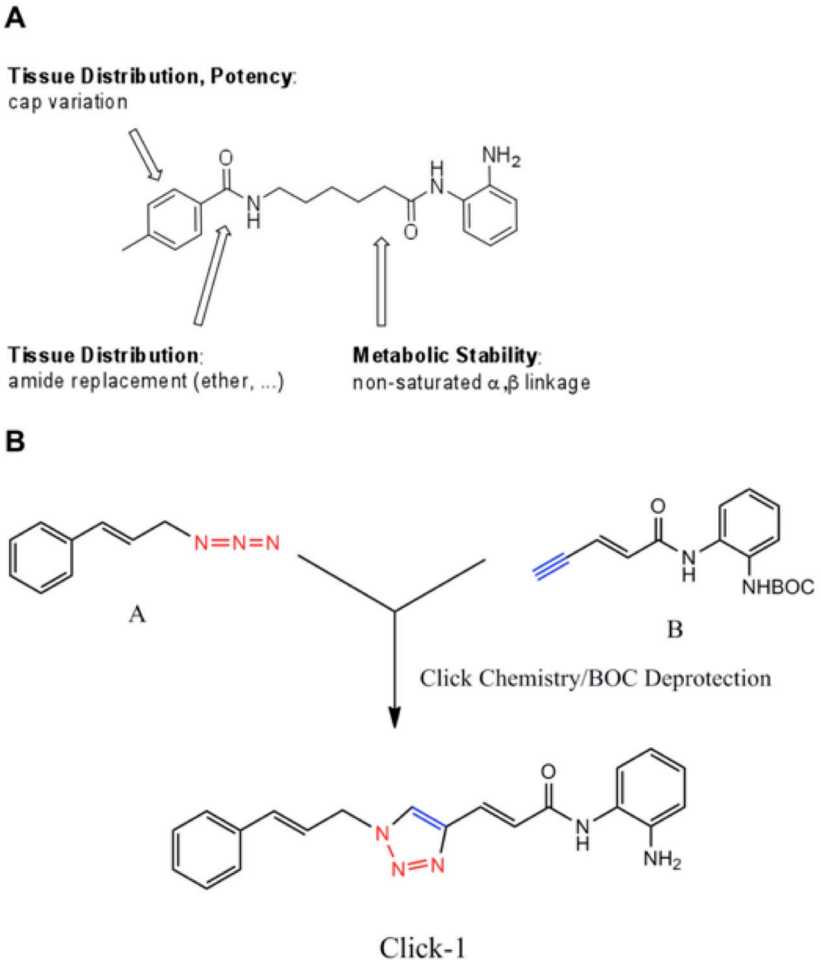

Figure 3.

Compounds with improved pharmacological properties. (A) Brain penetration can be improved by elimination of the left amide, and replacement with an ether, olefin, or ketone. Metabolic stability can be improved by introducing a non-saturated α/β linkage adjacent to the right amide, which prevents formation of a benzimidazole. (B) Synthetic route to compounds with these replacements using Cu(I)-catalyzed click chemistry, where azide A is reacted with alkyne B, and after Boc deprotection, a triazole is generated in the linker region of the histone deacetylase inhibitor.