Potential Agents for Treating Cystic Fibrosis: Cyclic Tetrapeptides That Restore Trafficking and Activity of del-F508-CFTR

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Potential Agents for Treating Cystic Fibrosis: Cyclic Tetrapeptides That Restore Trafficking and Activity of ΔF508-CFTR


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Supporting Information

ABSTRACT: Cystic fibrosis (CF) is a loss-of-function disease caused by mutations in the CF transmembrane conductance regulator (CFTR) protein, a chloride ion channel that localizes to the apical plasma membrane of epithelial cells. The most common form of the disease results from the deletion of phenylalanine-508 (ΔF508), leading to the accumulation of CFTR in the endoplasmic reticulum with a concomitant loss of chloride flux. We discovered that cyclic tetrapeptides, such as 11, 14, and 15, are able to correct the trafficking defect and restore the cell surface activity of ΔF508-CFTR. Although this class of cyclic tetrapeptides is known to contain inhibitors of certain histone deacetylase (HDAC) isoforms, their HDAC inhibitory potencies did not directly correlate with their ability to rescue ΔF508-CFTR. In full HDAC profiling, 15 strongly inhibited HDACs 1, 2, 3, 10, and 11 but not HDACs 4–9. Although 15 had less potent IC50 values than reference agent vorinostat (2) in HDAC profiling, it was markedly more potent than 2 in rescuing ΔF508-CFTR. We suggest that specific HDACs can have a differential influence on correcting ΔF508-CFTR, which may reflect both deacetylase and protein scaffolding actions.

KEYWORDS: Cyclic peptides, CFTR, cystic fibrosis, HDAC inhibition
trafficking, while HDAC7 knockdown also improved function.\(^\text{19}\)

Herein, we report on cyclic tetrapeptides, structurally related to the HDAC inhibitor natural product apicidin (3), that correct the maturation of ΔF508-CFTR from the ER, resulting in robust cell surface channel activity. Our data suggest potential involvement of both epigenetic and nonepigenetic processes related by protein acetylation pathways that adjust the proteostatic environment of the cell to promote favorable ΔF508-CFTR folding and function.

Conformationally biased cyclic tetrapeptides\(^\text{20,21}\) and cyclic pseudotetrapeptides\(^\text{22}\) related to apicidin (3) have potent HDAC inhibitory properties. Considering the connection between HDAC inhibition and the improvement of ΔF508-CFTR trafficking, we screened our collection of >40 derivatives (see Table S1 in the Supporting Information), many of which inhibited class I HDACs in the nanomolar range, for their ability to restore the function of ΔF508-CFTR. Our collection of cyclic tetrapeptides was designed to cover a broad range of pharmacophoric configurations related to the natural product apicidin (3). The inclusion of β-amino acids (4–17) or triazoles in the backbone (18 and 19) biased the macrocycles toward conformational homogeneity.\(^\text{20–22}\) Amino acids were varied at each of the four positions to survey different stereochemistries, backbone amide alkylations, side chain identities, and zinc coordinating groups. The tetrapeptides of interest (Chart 1; 4–19) were synthesized as described previously (Scheme S1 in the Supporting Information).\(^\text{20–22}\)

Compounds were screened for their ability to restore ΔF508-CFTR function in CF bronchial epithelial cells expressing the halide-sensitive YFP-H148Q/I152L fluorescent reporter system (Table 1 and Figure 1).\(^\text{23}\) Briefly, the fluorescence of this YFP mutant is quenched in the presence of halides, with iodide being the most potent. The rapid influx of extracellular iodide following activation with cAMP is dependent on the presence of a functional CFTR halide channel at the cell surface. The extent and rate of quenching indicate the level of CFTR and/or its activity at the cell surface.

The results of compound screening are reported in Table 1 as a fold increase in the rate of quenching relative to DMSO control. Several compounds were able to restore ΔF508-CFTR activity at concentrations of 1 μM, with 9, 11, and 15 being more potent than apicidin (3) (Table 1). Cyclic tetrapeptide 15, our most potent corrector of ΔF508-CFTR function, showed greater effectiveness than the archetypal HDAC inhibitor vorinostat (2) in the iodide flux assay, at a 5-fold lower concentration (Figure 1a).\(^\text{23}\) The level of ΔF508-CFTR correction activity for 15 was nearly 40%, which is a notable, clinically relevant value. The activity seen with 15 is due to restoration of ΔF508-CFTR since its effect was sensitive to the CFTR specific inhibitor CFInh-172 (Figure 1b).\(^\text{24}\)

To further support the view that these cyclic tetrapeptides restore CFTR activity, we monitored the trafficking of the ΔF508-CFTR protein to the cell surface by Western blot analysis (Figures 2 and S1 in the Supporting Information). During trafficking, wild-type CFTR and ΔF508-CFTR are glycosylated to give rise to proteins referred to as band B in an immunoblot analysis,\(^\text{23}\) which are further processed in the Golgi to generate the slower migrating band C glycoform that indicates the protein has reached the cell surface (Figures 2 and S1 in the Supporting Information).\(^\text{19,23}\) Band C was observed in the blots for most of the active cyclic tetrapeptides, which supports the restoration of cell surface CFTR activity by overcoming the trafficking defect associated with ΔF508-CFTR.

To gain additional insight into the relationship of HDAC inhibition to ΔF508-CFTR rescue, it is important to determine the HDAC isoform profile for the 11 known zinc-dependent isozymes according to the current state-of-the-art method. Unnatural trifluoroacetylated substrates have been developed to assay the class Ia HDACs (4, 5, 7, and 9),\(^\text{25,26}\) which have markedly lower deacetylase activity against standard acetylated substrates than HDACs from class I (1, 2, 3, and 8), class IIb (6 and 10), and class IV (11).\(^\text{27}\) Profiling by using these novel substrates revealed that the class Ia isoforms are rarely targeted effectively by known reference HDAC inhibitors at pharmacologically relevant concentrations. Accordingly, we had the HDAC

![Chart 1. Cyclic Tetrapeptides and Pseudotetrapeptides](chart1.png)
Table 1. Results for ΔF508-CFTR Rescue and Selected HDAC Inhibition a

<table>
<thead>
<tr>
<th>compd</th>
<th>CFTR b (at 1 μM)</th>
<th>IC 50 values (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDAC1</td>
<td>HDAC3</td>
</tr>
<tr>
<td>4</td>
<td>60 ± 10</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>IA</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>60 ± 10</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>IA</td>
<td>120</td>
</tr>
<tr>
<td>8</td>
<td>IA</td>
<td>ND a</td>
</tr>
<tr>
<td>9</td>
<td>IA (140 ± 10 at 1 μM)</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>IA</td>
<td>&gt;10 μM</td>
</tr>
<tr>
<td>11</td>
<td>120 ± 10</td>
<td>47</td>
</tr>
<tr>
<td>12</td>
<td>70 ± 10</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>50 ± 20</td>
<td>50</td>
</tr>
<tr>
<td>14</td>
<td>100 ± 10</td>
<td>12</td>
</tr>
<tr>
<td>15</td>
<td>140 ± 30</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>IA</td>
<td>200</td>
</tr>
<tr>
<td>17</td>
<td>IA</td>
<td>6500</td>
</tr>
<tr>
<td>18</td>
<td>80 ± 10</td>
<td>7</td>
</tr>
<tr>
<td>19</td>
<td>80 ± 10</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>22</td>
</tr>
<tr>
<td>2 a 3</td>
<td>30 (100 at 5 μM)</td>
<td>41</td>
</tr>
</tbody>
</table>

a New compounds were purified by HPLC and characterized by MS and 1H NMR (see the Supporting Information). HDACs 1, 3, and 8 are from class I; HDAC 6 is from class IIb. HDAC inhibition is given as IC 50 values, which are the means of at least two experiments performed in duplicate, unless otherwise noted; ND, not determined. Some HDAC inhibition data were previously published by us (refs 20–22). The ΔF508-CFTR values are reported as fold increase over DMSO background (rate constant = 8.0 × 10−4 sec−1) in rate of fluorescence decay based on iodide conductance with lung epithelial cells (CFBE41o−) expressing ΔF508-CFTR, at 1 μM test compound, unless otherwise noted. Data are means of two experiments performed in duplicate; IA, inactive at 1 μM. In complex with nuclear receptor corepressor 2 (NCOR2). Weak HDAC inhibition: IC 50 = 2700 nM in a HeLa cell nuclear extract assay. Not performed in duplicate. IC 50 values are from BPS Bioscience (ref 28).

![Figure 1](image1.png)

Figure 1. Cyclic tetrapeptides restore cell surface CFTR activity to ΔF508-CFTR. (a) Yellow fluorescent protein (YFP)-quenching curves for CFBE41o-YFP cells alone (black open circles) or treated for 24 h with DMSO (taupe stars), 1 μM 15 (red crosses), or 1 μM vorinostat (2; blue open squares) after stimulation with forskolin and genistein. (b) YFP-quenching curves for CFBE41o(lim)-YFP cells treated for 24 h with 15 and stimulated with forskolin and genistein in the presence (pink squares) or absence (red crosses) of CFinh172. Control: DMSO in the absence (blue squares) or presence (light blue squares) of CFinh172. Abbreviations: RFU, relative fluorescence units; A.U., arbitrary units.

![Figure 2](image2.png)

Figure 2. Cyclic tetrapeptides restore trafficking to ΔF508-CFTR. Western blot analysis of CFTR glycoforms following treatment with the indicated compounds.

Clearly, siRNA silencing experiments indicate that the knockdown of HDAC7 is a relevant molecular mechanism in ΔF508-CFTR rescue. So, how might one explain the absence of HDAC7 inhibition for cyclic tetrapeptides that are active in CFTR rescue, such as 3 and 15? It is known that expression of HDAC7 is selectively down-regulated in cells treated with vorinostat (2) or the depsipeptide HDAC inhibitor romidepsin. Thus, we carried out a Western blot analysis to assess whether treatment of the lung epithelial cells with 15 would likewise suppress expression of HDAC7, secondary to the inhibition of class I HDACs. Indeed, this analysis indicated a reduction in expression of HDAC7 (see Figure S2 in the Supporting Information). Although the class Ia HDACs have much lower intrinsic deacetylase activity against known acetylated lysine substrates, they are able to bind ε-N-Ac-Lys residues on histone tails with affinities comparable to class I and class IIb HDACs. HDAC7 could therefore function in cells as an ε-N-Ac-Lys-binding protein or could act on as yet undetermined substrates, to exert its influence in the process of ΔF508-CFTR rescue, rather than by functioning directly as a deacetylase.

Our results suggest avenues for further structure–activity optimization. First, CFTR activity varied considerably for a subset of compounds that differ by only a single amino acid substitution, for example, altering position four resulted in increased activity in the following order: 4 (Ala) < 14 (Pro) < 11 (Phe). Second, replacement of the naphthyl group in our most active compound, 15, with indole, as in 4, led to substantial...
reduction of activity. Further variations at these two positions could yield more potent compounds. Finally, given that the collection of active compounds contains cyclic tetrapeptides with different stereochemistry and different backbone subunits, via β-amino acids or disubstituted 1,2,3-triazoles, our lead compounds offer a basis for future improvements.

In summary, we have discovered that certain cyclic tetrapeptides possess notable activity in correcting ΔF508-CFTR function. The relationship between HDAC inhibition and CFTR function at the cell surface is intriguing and leads to the suggestion that ΔF508-CFTR maturation could reflect distinct steps in folding, trafficking, and chloride channel function at the cell surface that may be differentially sensitive to distinct HDAC-based biological pathways. More generally, our findings provide further impetus for focused studies on the relationship between HDAC inhibition and CF biology. In this realm, the modulation of epigenetic or non-epigenetic processes, linked by protein acetylation/deacetylation cycles that impact the functional protein environment of the cell, could offer a useful approach for treating not only CF but also other protein-misfolding diseases.33–38

ASSOCIATED CONTENT

Supporting Information. Experimental methods, compound characterization data, full table of cyclic peptides tested in the CFTR flux assay (Table S1), and Scheme S1 and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES


(28) For details about BPS Bioscience and HDAC assays: http://bpsbioscience.com/images/pdf/HDAC_profiling_Sheet.pdf. Note that the substrate used for HDAC inhibition assays in our laboratory (Biomol Fluor-de-Lys) was different than that used by BPS Bioscience (BPS 50037).


