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Triazole compounds – Potentials in the treatment of cystic fibrosis

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Triazole compounds – Potentials in the treatment of cystic fibrosis

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AIMS

1. To determine the influence of certain triazole compounds on ΔF508-CFTR protein and mRNA expression.

2. To determine the effects of triazole compounds on chloride ion channel activation in ΔF508-CFTR expressing cell lines.

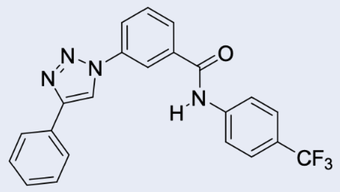
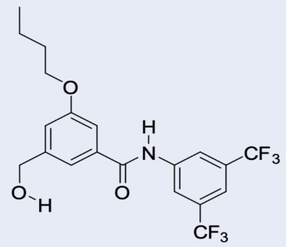
INTRODUCTION

CFTR and Cystic Fibrosis: Cystic fibrosis (CF) is a genetic disease that is most common among Caucasians of Northern European origin. CF is caused by mutations in a membrane protein CFTR (**C**ystic **F**ibrosis **T**ransmembrane-conductance **R**egulator) impairing its chloride ion channel function. ΔF508 is the most common CFTR mutation affecting over 70% of CF cases. Our laboratory has shown that ΔF508-CFTR mutation can be partially reversed by physical-chemical means [1]. CFTR can be visualized in 3-electrophoretic forms in western blotting. Band-C (M_r ~160 kDa) is a plasma membrane bound fully glycosylated form; band-B (M_r ~130 kDa) is an endoplasmic reticulum bound unglycosylated form, and band-A (M_r ~120 kDa) is a newly synthesized apoprotein form. Immature CFTR band-B is prominently visible in ΔF508-CFTR expressing cell lines, whereas mature CFTR band-C is prominently visible in wild type-CFTR expressing cell lines. Apoprotein CFTR band-A is rarely visible in either cell types.

Triazole Compounds: Synthetic anion carriers have shown to augment the chloride ion channel function in cell lines expressing ΔF508-CFTR [2]. Triazole, a heterocyclic type of compounds, synthesized in Talukdar lab (IISER, Pune, India), known for their ability to bind and facilitate chloride influx in cultured cell lines are used in this study to determine their effects on ΔF508-CFTR upregulation. PJ and ABS series compounds with different chemistries can form Cl^- ion channels in cellular vesicles [3].

MATERIALS

Table-1

Triazole Compounds	Properties
	**PJ-08** Triazole-((4-trifluoromethyl)phenyl)-benzamide Mr 410 Solubility: DMSO
	**ABS-094** ((3,5-bis (trifluoromethyl) phenyl) benzamide-alcohol M_r 463 Solubility: Ethanol

METHODS

1. CFBE cells (a human lung epithelial cell line) transfected with ΔF508-CFTR gene was treated with various concentrations of triazole compounds (see table-1 above).
2. Positive control: CFBE cells transfected with wild type or ΔF508 CFTR treated with 5 mM sodium butyrate [1].
3. Negative control: CFBE cells expressing ΔF508-CFTR was treated with vehicle alone (ethanol or DMSO) to the optimal concentration.
4. Treatment: Cells were treated with the reagents when they reached ~75% confluency followed by incubation at 27°C for 60 hrs.
5. Immunoblotting: Cell lysates were prepared, and assayed for protein concentrations. Known protein concentrations were electrophoresed on a 7.5% SDS-PAGE and transferred to nitrocellulose membranes. Expression levels of CFTR and loading control Actin-α was measured by chemiluminescence using c400 image analyzer (Azure Biosystems).

RESULTS

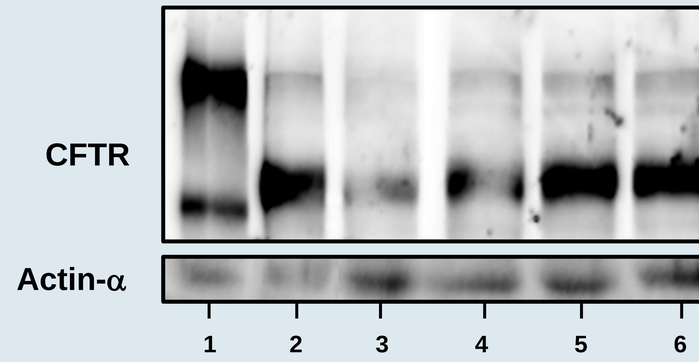


FIGURE 1: Effects of PJ-08 on ΔF508-CFTR expression. ΔF508-
cells were treated with 2.5 μM (lane 4), 5 μM (lane 5), or 10
described in methods. CFBE cells transfected with wild type (l
(lane 2 treated with 5mM sodium butyrate were used as positi
treated with vehicle (DMSO) alone was used as negative cont
samples from positive controls (lanes 1,2) and 25 μg protein sam
3) and PJ-08 treated samples (lanes 4-6) were electrophoresed an
polyclonal anti-CFTR antibody (upper panel) or with monoclona
(lower panel). *PJ-08 treatment significantly increased immature C*
increase in mature CFTR band-C as well when compared with the

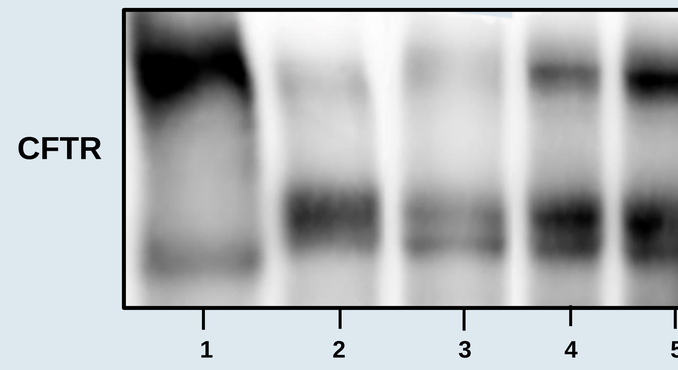


FIGURE 2: Effects of ABS-094 on ΔF508-CFTR expression. ΔF508
cells were treated with ethanol alone (lane 3, as negative contro
μM (lane 5) ABS-094. CFBE cells expressing wild type (lane 1) c
treated with 5mM sodium butyrate were used as positive contro
control samples (lanes 1, 2) and 25 μg of vehicle and ABS-094 tre
5) were immunoblotted with anti-CFTR antibody as described in
treatment has increased band-C and band-B CFTR in a concentrati

REFERENCES

FUTURE DIRECTIONS

ACKNOWLEDGEMENT

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