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# Pharmacophore-Based Design of New Chemical Scaffolds as Translational Readthrough-Inducing Drugs (TRIDs)

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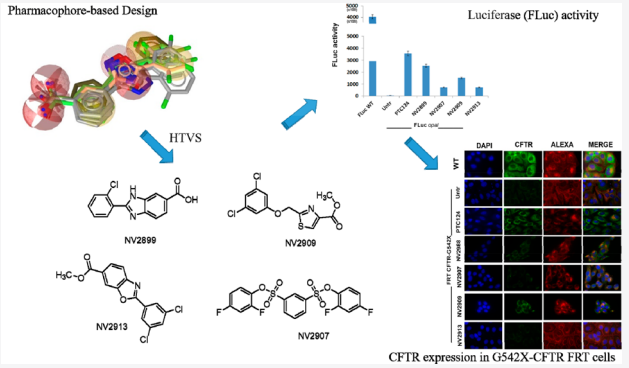
**ABSTRACT:** Translational readthrough-inducing drugs (TRIDs) rescue the functional full-length protein expression in genetic diseases, such as cystic fibrosis, caused by premature termination codons (PTCs). Small molecules have been developed as TRIDs to trick the ribosomal machinery during recognition of the PTC. Herein we report a computational study to identify new TRID scaffolds. A pharmacophore approach was carried out on compounds that showed readthrough activity. The pharmacophore model applied to screen different libraries containing more than 87000 compounds identified four hit-compounds presenting scaffolds with diversity from the oxadiazole lead. These compounds have been synthesized and tested using the Fluc reporter harboring the UGA PTC. Moreover, the cytotoxic effect and the expression of the CFTR protein were evaluated. These compounds, a benzimidazole derivative (NV2899), a benzoxazole derivative (NV2913), a thiazole derivative (NV2909), and a benzene-1,3-disulfonate derivative (NV2907), were shown to be potential new lead compounds as TRIDs, boosting further efforts to address the optimization of the chemical scaffolds.




**KEYWORDS:** *Pharmacophore modeling, cystic fibrosis, premature termination codons, nonsense mutation, HTVS*



In recent years many efforts have been dedicated to personalized medicinal approaches to genetic disease. In this context, with cystic fibrosis being a largely diffused genetic pathology, researchers have focused on the therapy of the basis genetic defect. Approximately 10%−15% of the cystic fibrosis (CF) cases are due to nonsense-mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR).[1] The presence of a nonsense mutation, giving rise to a premature termination codon (PTC) in the mRNA, produces a truncated protein that is rapidly degraded, so the patients lack the functional protein and suffer a hardest form of the disease. Therapies targeting this specific genetic defect have been addressed and, besides aminoglycosides, heterocyclic scaffolds play the main roles.[2−6]

Concerning patients with nonsense mutations, in the last ten years, the only pharmaceutical option was the translational readthrough of the PTC in order to bypass the PTC and restore to a sufficient extent the expression of a functional protein. Aminoglycosides (e.g., gentamicin, tobramycin, paromomycin) had been previously studied to this aim. They suppress the normal proof-reading function of the ribosome allowing the translation and lead to the insertion of a near-cognate amino acid at the PTC site.[7,8] However, severe side effects have been reported[9] by prolonged treatments with aminoglycosides including renal, auditory, and vestibular toxicities, that have limited their widespread clinical use as translational readthrough-inducing drugs (TRIDs). In this context, recently a new aminoglycoside ELX-2, showing potential activity as a TRID and less side effects, has been launched in phase II clinical trials.[10]

Previously, in 2007 Ataluren (aka PTC124) was proposed by PTC Therapeutics as able to promote the readthrough of premature but not normal termination codons.[11] The small molecule Ataluren is a diaryl-1,2,4-oxadiazole, is less toxic than aminoglycosides, and has been suggested as a potential treatment of genetic disorders caused by nonsense mutations, particularly those involving the UGA premature codon.[12] Results of the phase II and III trials showed improvement in markers of CFTR function but no improvements in sweat chloride levels or nasal potential difference.[13] PTC Therapeutics concluded phase III clinical trials in 2014 for CF and

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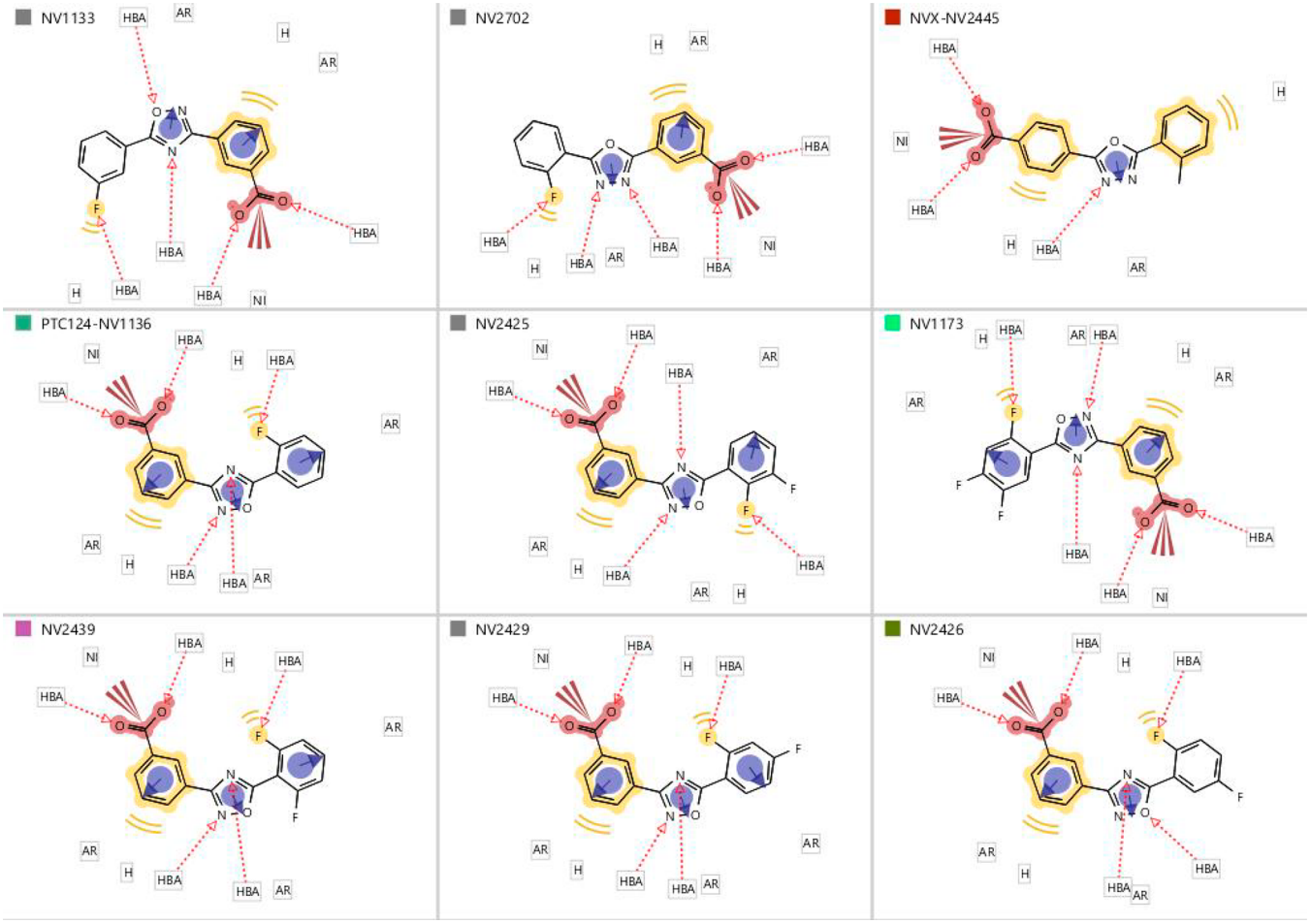


**Figure 1.** Pharmacophore features for the actives data set: HBA, H-bond acceptor; AR, aromatic; H, hydrophobic; NI, negative ionic.

Duchenne Muscular Dystrophy (DMD), in order to evaluate the long-term safety of Ataluren. At the conclusion of this study, Ataluren has been approved for DMD while for CF it was evidenced that, although cystic fibrosis patients who received this treatment had beneficial effects, patients taking chronic inhaled tobramycin did not show the same benefits, allowing the researchers to hypothesize that the two drugs were competing at the level of the ribosome.

Additional confirmation phase III clinical study resulted in the approval of the drug under the trade name Translarna for DMD patients, while the trial for CF patients was suspended in April 2017 due to conflicting results.[13,14]

Considering Ataluren's failure to be the prospective lead compound as a TRID and its biological target still not being clear[15] prompted us to further studies on the topic in order to find a valuable substitute to Ataluren.

In this context, we identified a set of small molecules containing an oxadiazole heterocyclic core by means of virtual screening on public and *in-house* libraries and cell-based experimental studies.[16,17] The compound NV2445 (Figure 1) showed the most promising activity both in FLuc assays and in cells expressing a nonsense-CFTR-mRNA.[18,19] Notwithstanding these efforts with the aim to identify an effective alternative to Ataluren, new and more potent TRIDs are necessary for the treatment of the disease. In this paper, we performed an *in silico* design focusing on the identification of new chemical scaffolds not related with 1,2,4- or 1,3,4-oxadiazoles. We carried out a pharmacophore-based modeling study exploiting available experimental data on our previous identified compounds.[16−18,20] As starting point of our analysis, we considered the 24 active compounds in FLuc assay over the 61 synthesized compounds. Nine of the most promising compounds have a carboxylic group (Ataluren and NV2445, included); for this reason, we decided to identify these nine compounds as the active data set (Figure 1).

Pharmacophore models have been generated using LigandScout 4.3 by Inte:Ligand GmbH.[21,22] The data set compounds have been randomly split into a training and a test set (80%−20%). Before models generation, conformers' generation and alignment have been performed. The pharmacophore features have been identified in a ligand-based mode, and ten different pharmacophore models have been identified. The better one (Model 1) showed a score of 0.90 out of 1 and for each compound matched 7 to 10 features. Model 1 consists of three aromatic features, two hydrophobic features, 5 H-bond acceptor features, and one negative ionic feature (Figure 2).

In order to refine and validate the model we employed the data set of active compounds previously identified by us and a data set of 450 decoys generated using the DUD-E tool.[23,24] For the validation of the pharmacophore model related to its insight power, we considered the enrichment factor (EF) and the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The validation test identified 29 hits, all the 24 actives and five decoys. Figure 2 (bottom) displays the ROC plot, the AUC, and the EF factor values at


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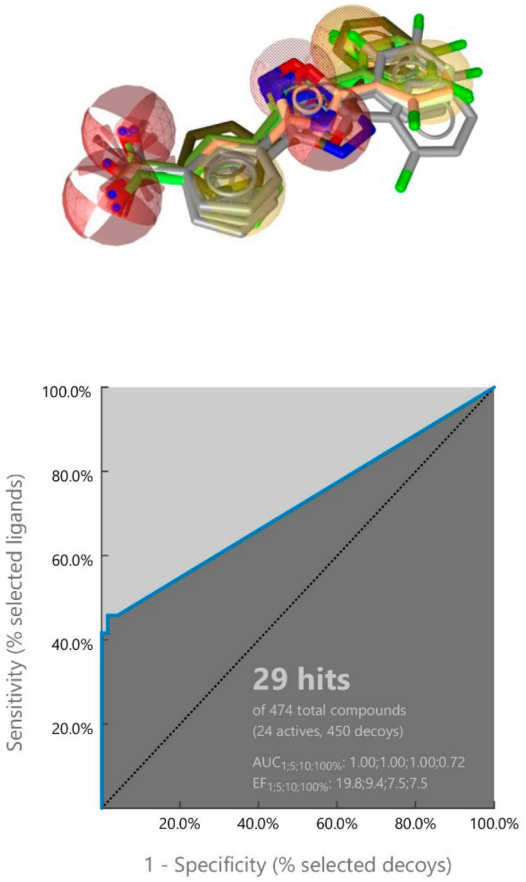



**Figure 2.** (top) 3D representation of the best model (Model 1 legend: red sphere, H-bond acceptor; red arrows, negative ionizable area; yellow sphere, hydrophobic interactions; blue donuts, aromatic ring); (bottom) ROC curve for the Model 1 (legend: AUC, area under the curve; EF, enrichment factor).

1%, 5%, 10%, and 100% of the best pharmacophore model. As shown in Figure 2, the early enrichment (EF 1%) is equal to 19.8 with an AUC value of 1.00 demonstrating that our pharmacophore model was able to discriminate between active and inactive compounds. Overall, the model has a preference for active compounds with an AUC value of 1 in the first 10% and AUC = 0.72 at 100%, and an EF value of 7.5 in the first 10% until 100% showing a good accuracy for new hits identification.

In the end, this model was used as a query to screen 5 different databases: (1) an OTAVA commercial library containing 2775 potential mRNA binders; (2) the Drugbank library updated to January 2018 containing 8721 compounds; (3) the Maybridge hit discover library containing 52146 compounds; (4) an *in-house* library of 1829 small molecules designed by the Almerico group;[25−28] (5) an *in-house* library of about 26000 small molecules, consisting of aromatic pentatomic heterocycles with two heteroatoms or more, their benzo-condensed analogs, and their open chain precursors, designed by the Pace and Pibiri group.[18] The overall used library contained about 87000 compounds. The search yielded 23 hits compounds. Among these, four hits were chosen among the most synthetically accessible. They do not contain the oxadiazole core (Figure 3).

The hit compounds were easily obtained in one or two steps from commercially available compounds according to Scheme 1. Thus, the benzimidazole derivative **NV2899** was prepared by cyclocondensation in DMF between 3,4-diaminobenzoic acid and **2**, obtained in turn from chlorobenzaldehyde **1** and metabisulfite (Scheme 1A).

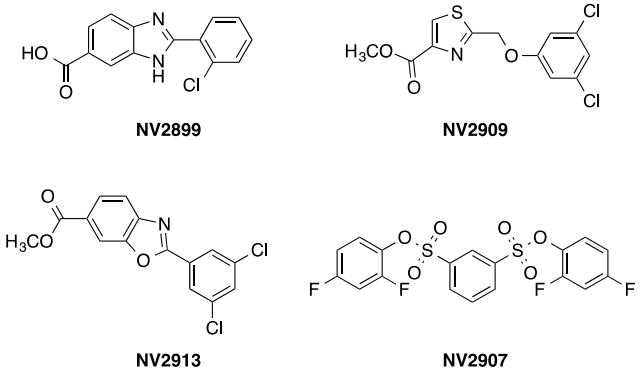
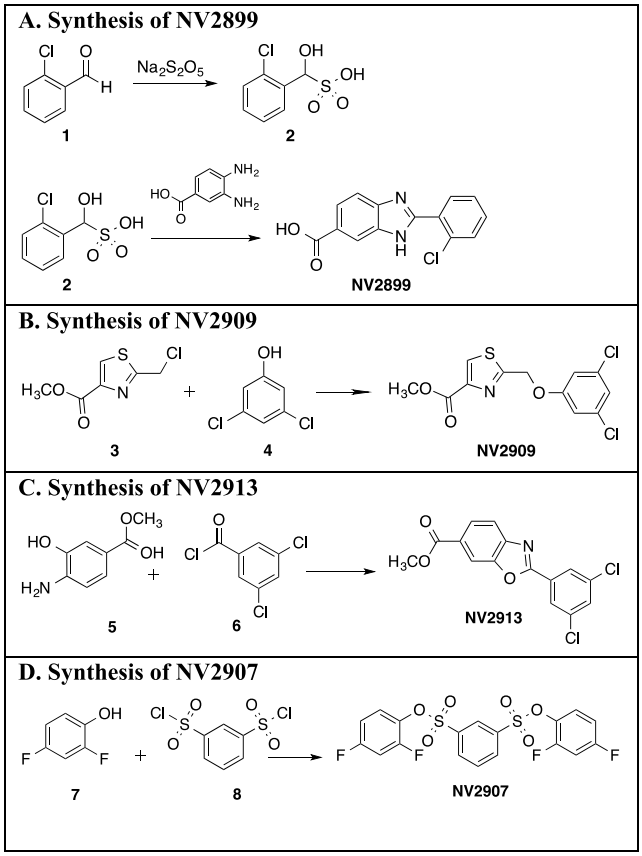



**Figure 3.**

**Scheme 1**




**NV2909**, a 2-(3,5-dichlorophenoxymethyl)-thiazole derivative, was synthesized in one step from the corresponding chloromethyl-thiazole **3** and 3,5-dichlorophenol **4** in acetonitrile in the presence of a base (Scheme 1B).

Also, the 2-(3,5-dichlorophenyl)benzoxazole **NV2913** was prepared from 3,5-dichlorophenyl acyl chloride **5** and the aryl ester **6**, likely through a not isolated amido intermediate (Scheme 1C).



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As shown in Scheme 1D, **NV2907** was obtained from 2,4-difluorophenol **7** and 1,3-disulfonyl chloride **8**, in DCM and potassium carbonate as a base.

To evaluate the activity of the newly synthesized small molecules in promoting the readthrough of PTCs, we used the FLuc cell-based assay.[16,17] To this aim HeLa cells were transfected transiently with the plasmids pFLuc-WT (control) and pFLuc-opal (UGA stop mutation).[29] After transfection, Hela cells were treated for 24 h with the compounds (NV2907, NV2909, NV2899, NV2913) and subsequently the activity of the Fluc protein was measured. HeLa cells transfected with the pFluc-WT plasmid were used as positive control and showed high levels of luciferase activity (Figure 4). As negative control pFluc-opal transfected HeLa cells were used, and these cells did not show any activity. Moreover, when these pFluc-opal cells were treated with Ataluren and the four different compounds, we observed an increase of luciferase activity (Figure 4).

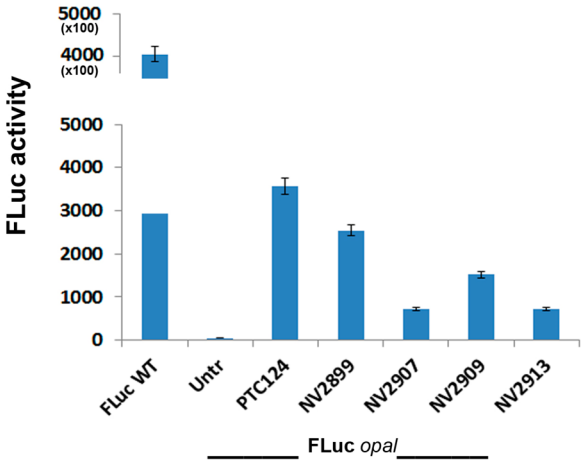


**Figure 4.** Histogram showing luciferase (FLuc) activity after 24 h of exposition to PTC124 and the new identified compounds NV2899, NV2907, NV2909, NV2913 (all at the concentration of 12 $\mu$M) in HeLa FLuc-opal transfected cells.

FRT CFTR wild type cells were used to determine the possible cytotoxic effects of NV2907, NV2909, NV2899, and NV2913 and to visualize the impact on cell proliferation. In the graphs in Figure 5 are reported the percentages of dead cells and proliferating cells after treatment with 12 $\mu$M NV2907, NV2909, NV2899, and NV2913. PTC124 at 12 $\mu$M was used as control. Our results showed similar increase in dead cells at 24−48−72 h in all samples analyzed (Figure 5 top). We also evaluated the expression of the CFTR protein after treatment with the selected compounds. FRT cells stably transfected with the pTracer vectors containing either the WT-CFTR(CFTR-WT) or the G542X-CFTR human cDNA were used. The expression of the CFTR WT and G542X-CFTR after treatment with 12 $\mu$M of NV2899, NV2907, NV2909, and NV2913 was detected by immunofluorescence microscopy. After 24 h of treatment with the two compounds NV2899 and NV2909, we observed CFTR expression in G542X-CFTR FRT cells. In contrast CFTR expression was not revealed in G542X-CFTR FRT untreated cells (negative control) (Figure 6).

In conclusion, our studies, with the aim to identify a valuable substitute of Ataluren for the treatment of nonsense mutation in CFTR gene, allowed us to find out new promising chemical scaffolds as TRIDs. Following a ligand-based pharmacophore approach together to a virtual screening protocol, several small molecules capable to allow the *in vitro* expression of the gene pFLuc-opal (UGA stop mutation) were discovered. These molecules have different scaffolds from the known 1,2,4- and 1,3,4-oxadiazoles. In particular, the identified compounds are a benzimidazole derivative (NV2899), a benzoxazole derivative (NV2913), a thiazole derivative (NV2909), and a benzene-1,3-disulfonate derivative (NV2907). These compounds showed low cytotoxic effect and displayed the expression CFTR protein in transfected cells. We envision that these molecules could to be potential lead compounds as TRIDs, and further studies will be performed in order to optimize these scaffolds.

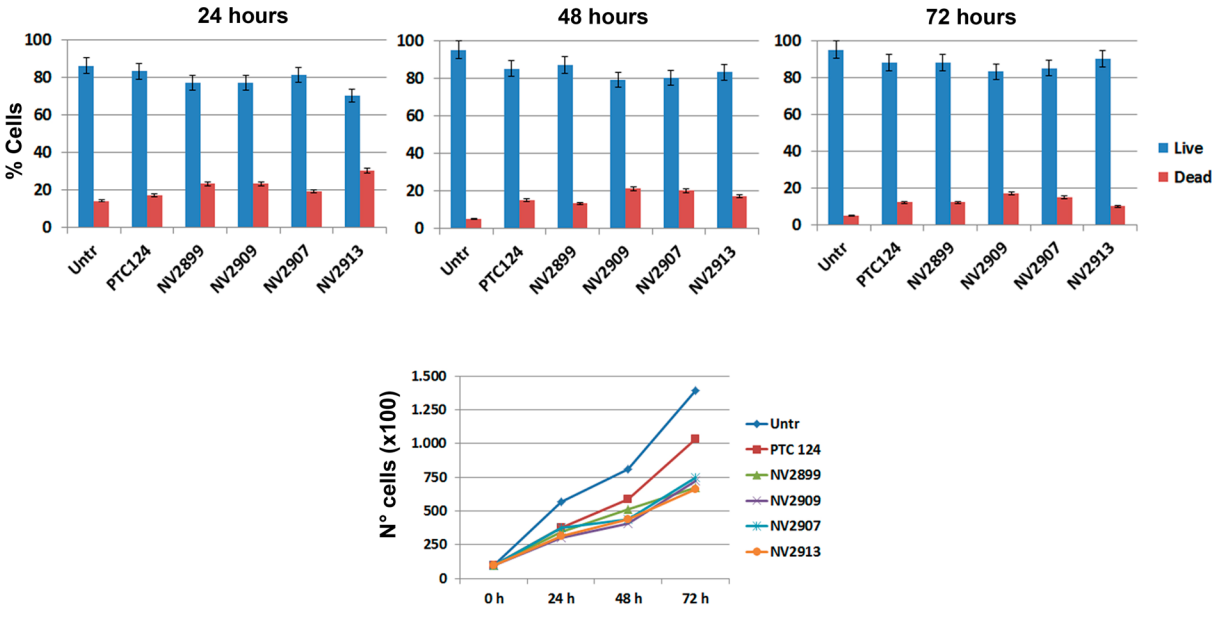


**Figure 5.** (top) Histograms showing live and dead cells at 24−48−72 h post treatment with the indicated compounds. (bottom) Graphs showing cell proliferation at the same time intervals (24−72 h).



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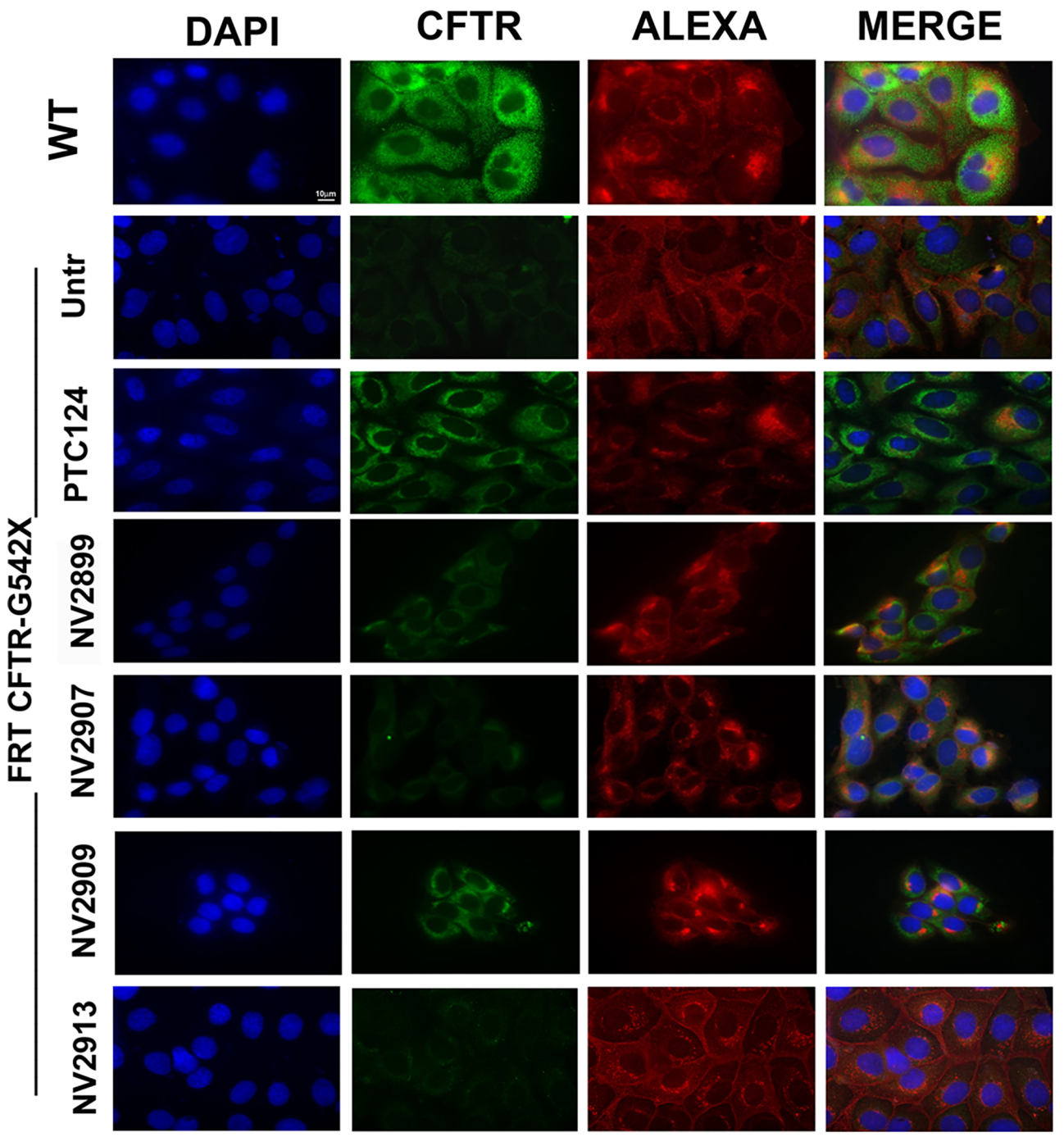


**Figure 6.** Immunofluorescence analysis to visualize CFTR (green-Ab570) expression in G542X-CFTR FRT cells treated with the indicated compounds for 24 h. Cell membrane was stained in red and nuclei in blue with DAPI.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.9b00609.

Materials and methods (PDF)

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

TRIDs,translational readthrough inducing drugs; PTC,premature termination codon; CF,cystic fibrosis; CFTR,cystic fibrosis transmembrane conductance regulator; FLuc,firefly luciferase

## ■ REFERENCES

(1) Wilschanski, M. Class 1 CF Mutations. *Front. Pharmacol.* **2012**, *3*, 117−119.
(2) Son, J. H.; Zhu, J. S.; Phuan, P. W.; Cil, O.; Teuthorn, A. P.; Ku, C. K.; Lee, S.; Verkman, A. S.; Kurth, M. J. High-Potency Phenylquinoxalinone Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Activators. *J. Med. Chem.* **2017**, *60* (6), 2401−2410.
(3) Ye, L.; Hu, B.; El-Badri, F.; Hudson, B. M.; Phuan, P. W.; Verkman, A. S.; Tantillo, D. J.; Kurth, M. J. Δf508-CFTR Correctors: Synthesis and Evaluation of Thiazole-Tethered Imidazolones, Oxazoles, Oxadiazoles, and Thiadiazoles. *Bioorg. Med. Chem. Lett.* **2014**, *24* (24), 5840−5844.
(4) Coffman, K. C.; Nguyen, H. H.; Phuan, P. W.; Hudson, B. M.; Yu, G. J.; Bagdasarian, A. L.; Montgomery, D.; Lodewyk, M. W.; Yang, B.; Yoo, C. L.; et al. Constrained Bithiazoles: Small Molecule Correctors of Defective Δf508-CFTR Protein Trafficking. *J. Med. Chem.* **2014**, *57* (15), 6729−6738.
(5) Ponzano, S.; Nigrelli, G.; Fregonese, L.; Eichler, I.; Bertozzi, F.; Bandiera, T.; Galietta, L. J. V.; Papaluca, M. A European Regulatory Perspective on Cystic Fibrosis: Current Treatments, Trends in Drug Development and Translational Challenges for CFTR Modulators. *Eur. Respir. Rev.* **2018**, *27* (148), 170124−170134.
(6) Liessi, N.; Cichero, E.; Pesce, E.; Arkel, M.; Salis, A.; Tomati, V.; Paccagnella, M.; Damonte, G.; Tasso, B.; Galietta, L. J. V.; et al. Synthesis and Biological Evaluation of Novel Thiazole- VX-809 Hybrid Derivatives as F508del Correctors by QSAR-Based Filtering Tools. *Eur. J. Med. Chem.* **2018**, *144*, 179−200.
(7) Burke, J. F.; Mogg, A. E. Suppression of a Nonsense Mutation in Mammalian Cells in Vivo by the Aminoglycoside Anthiotics G-418 and Paromomycin. *Nucleic Acids Res.* **1985**, *13* (17), 6265−6272.
(8) Manuvakhova, M.; Keeling, K.; Bedwell, D. M. Aminoglycoside Antibiotics Mediate Context-Dependent Suppression of Termination Codons in a Mammalian Translation System. *RNA* **2000**, *6* (7), 1044−1055.
(9) Prayle, A.; Smyth, A. R. Aminoglycoside Use in Cystic Fibrosis: Therapeutic Strategies and Toxicity. *Curr. Opin. Pulm. Med.* **2010**, *16* (6), 604−610.
(10) Rowe, S. M.; Mutyam, V.; Alroy, I.; Huertas, P. WS01.3 Translational Read-through of CFTR Nonsense Mutations and Inducement of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Function by ELX-02 Treatment. *J. Cystic Fibrosis* **2018**, *17*, S2.
(11) Welch, E. M.; Barton, E. R.; Zhuo, J.; Tomizawa, Y.; Friesen, W. J.; Trifillis, P.; Paushkin, S.; Patel, M.; Trotta, C. R.; Hwang, S.; et al. PTC124 Targets Genetic Disorders Caused by Nonsense Mutations. *Nature* **2007**, *447* (7140), 87−91.
(12) Kerem, E.; Hirawat, S.; Armoni, S.; Yaakov, Y.; Shoseyov, D.; Cohen, M.; Nissim-Rafinia, M.; Blau, H.; Rivlin, J.; Aviram, M.; et al. Effectiveness of PTC124 Treatment of Cystic Fibrosis Caused by Nonsense Mutations: A Prospective Phase II Trial. *Lancet* **2008**, *372* (9640), 719−727.
(13) Kerem, E.; Konstan, M. W.; De Boeck, K.; Accurso, F. J.; Sermet-Gaudelus, I.; Wilschanski, M.; Elborn, J. S.; Melotti, P.; Bronsveld, I.; Fajac, I.; et al. Ataluren for the Treatment of Nonsense-Mutation Cystic Fibrosis: A Randomised, Double-Blind, Placebo-Controlled Phase 3 Trial. *Lancet Respir. Med.* **2014**, *2* (7), 539−547.
(14) Aslam, A.; Jahnke, N.; Remmington, T.; Southern, K. W. Ataluren and Similar Compounds (Specific Therapies for Premature Termination Codon Class I Mutations) for Cystic Fibrosis. *Paediatr. Respir. Rev.* **2017**, *24*, 32−34.
(15) Raymer, B.; Bhattacharya, S. K. Lead-like Drugs: A Perspective. *J. Med. Chem.* **2018**, *61*, 10375−10384.
(16) Pibiri, I.; Lentini, L.; Tutone, M.; Melfi, R.; Pace, A.; Di Leonardo, A. Exploring the Readthrough of Nonsense Mutations by Non-Acidic Ataluren Analogues Selected by Ligand-Based Virtual Screening. *Eur. J. Med. Chem.* **2016**, *122*, 429−435.
(17) Pibiri, I.; Lentini, L.; Melfi, R.; Gallucci, G.; Pace, A.; Spinello, A.; Barone, G.; Di Leonardo, A. Enhancement of Premature Stop Codon Readthrough in the CFTR Gene by Ataluren (PTC124) Derivatives. *Eur. J. Med. Chem.* **2015**, *101*, 236−244.
(18) Pibiri, I.; Lentini, L.; Melfi, R.; Tutone, M.; Baldassano, S.; Ricco Galluzzo, P.; Di Leonardo, A.; Pace, A. Rescuing the CFTR Protein Function: Introducing 1,3,4-Oxadiazoles as Translational Readthrough Inducing Drugs. *Eur. J. Med. Chem.* **2018**, *159*, 126−142.
(19) Tutone, M.; Pibiri, I.; Lentini, L.; Pace, A.; Almerico, A. M. Deciphering the Nonsense Readthrough Mechanism of Action of Ataluren: An *in Silico* Compared Study. *ACS Med. Chem. Lett.* **2019**, *10* (4), 522−527.
(20) Campofelice, A.; Lentini, L.; Di Leonardo, A.; Melfi, R.; Tutone, M.; Pace, A.; Pibiri, I. Strategies against Nonsense:

Oxadiazoles as Translational Readthrough-Inducing Drugs (TRIDs). *Int. J. Mol. Sci.* **2019**, *20* (13), 3329−3347.
(21) Wolber, G.; Langer, T. LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters. *J. Chem. Inf. Model.* **2005**, *45* (1), 160−169.
(22) Almerico, A. M.; Tutone, M.; Lauria, A. In-Silico Screening of New Potential Bcl-2/Bcl-Xl Inhibitors as Apoptosis Modulators. *J. Mol. Model.* **2009**, *15*, 349−355.
(23) Mysinger, M. M.; Carchia, M.; Irwin, J. J.; Shoichet, B. K. Directory of Useful Decoys, Enhanced (DUD-E): Better Ligands and Decoys for Better Benchmarking. *J. Med. Chem.* **2012**, *55* (14), 6582−6594.
(24) Perricone, U.; Wieder, M.; Seidel, T.; Langer, T.; Padova, A.; Almerico, A. M.; Tutone, M. A Molecular Dynamics-Shared Pharmacophore Approach to Boost Early-Enrichment Virtual Screening: A Case Study on Peroxisome Proliferator-Activated Receptor $\alpha$. *ChemMedChem* **2017**, *12* (16), 1399−1407.
(25) Almerico, A. M.; Tutone, M.; Lauria, A. Receptor-Guided 3D-QSAR Approach for the Discovery of c-Kit Tyrosine Kinase Inhibitors. *J. Mol. Model.* **2012**, *18* (7), 2885−2895.
(26) Lauria, A.; Patella, C.; Diana, P.; Barraja, P.; Montalbano, A.; Cirrincione, G.; Dattolo, G.; Almerico, A. M. A Synthetic Approach to New Polycyclic Ring System of Biological Interest through Domino Reaction: Indolo[2,3-e][1,2,3]Triazolo[1,5-a]Pyrimidine. *Tetrahedron Lett.* **2006**, *47* (13), 2187−2190.
(27) Tutone, M.; Chinnici, A.; Almerico, A. M.; Perricone, U.; Sutera, F. M.; De Caro, V. Design, Synthesis and Preliminary Evaluation of Dopamine-Amino Acid Conjugates as Potential D1 Dopaminergic Modulators. *Eur. J. Med. Chem.* **2016**, *124*, 435−444.
(28) Tutone, M.; Lauria, A.; Almerico, A. M. Theoretical Determination of the pKa Values of Betalamic Acid Related to the Free Radical Scavenger Capacity: Comparison Between Empirical and Quantum Chemical Methods. *Interdiscip. Sci.: Comput. Life Sci.* **2016**, *8* (2), 177−185.
(29) Auld, D. S.; Lovell, S.; Thorne, N.; Lea, W. A.; Maloney, D. J.; Shen, M.; Rai, G.; Battaile, K. P.; Thomas, C. J.; Simeonov, A.; Hanzlik, R. P.; Inglese, J. Molecular Basis for the High-Affinity Binding and Stabilization of Firefly Luciferase by PTC124. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (11), 4878−4883.


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