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Title: *In Silico Discovery of Potential Novel CRISPR-Cas12a Inhibitors*

Abstract

CRISPR technology is becoming increasingly popular for use in gene editing, but the precise control of CRISPR-Cas systems is critical for it to be both successful and safe. Two classes of CRISPR-Cas exist: Class 1 and class 2 which are further divided into types characterized by distinct organization of the effectors and unique signature proteins. Class 2 includes the prevalent type II, with Cas 9 being its effector, whereas Cas 12(a-c) and Cas 13 are the effectors for the rare types V and VI. Cas 9 and Cas 12 proteins are homologous to nucleases, and share a domain that belongs to the RuvC-like endonuclease family. Further, the structures of several Cas9 proteins, Cas12a (Cpf1) and Cas12b (C2c1) have been resolved complexed with guide RNA, target DNA, and trascRNA. Even though CRISPR-Cas9 was the first CRISPR tool to be utilized for gene editing, CRISPR-Cas12a is believed to be more precise. In addition, small molecule inhibitors of Cas9 have been identified, whereas nothing has been reported for Cas12a to date.

Even though external and internal controls preventing CRISPR systems from causing unintended off-site effects exist, internal controls are limited by the challenges of genetic engineering. In contrast, external controls can be relatively straightforward through the discovery of small molecule inhibitors. Natural antiCRISPR (Acr) proteins inhibit Cas effectors, thus functioning as a defense mechanism against the CRISPR-Cas system. The purpose of this study was to identify inhibitors of CRISPR-Cas12a that would result in similar actions as the Acr proteins. Using virtual screening, we targeted the same binding site of the Acr proteins on Acidaminococcus sp. Cpf1 (AsCpf1), a CRISPR-Cas12a species. Private and commercially available compound libraries were first reduced using Rapid Elimination of Swill (REOS) filters and then prepared generate accurate, low energy 3-dimensional (3D) conformations. Subsequently, Glide docking was employed in order to identify hits based on energetics and visual inspections. The resulting top 20% of structures were further evaluated in terms of their ability to cross the blood brain barrier (logP, hydrogen bond donors and acceptors, polar surface area, pKa, number of rotatable bonds, and molecular weight). Experimental evaluation of the potential hits is underway.