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Investigation of the phenyl dihydrouracil molecules as CRBN ligands for the PROTAC development

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Background/Aim. CRBN is the most widely used E3 ligase for PROTACs development due to the large variety of its glutarimide ligands, which have good drug-like properties and low molecular weight. However, because of the racemization and low stability of glutarimides, the process of drug development faces many challenges [1–2]. This work aims to study and analyze the set of more stable phenyl dihydrouracil molecules as promising binders for CRBN. These investigations will be helpful during the development of PROTACs. **Methods.** Fluorescence polarization (FP) method was used to evaluate the binding of small molecules to the CRBN protein [3]. **Results.** The set of 5 phenyl dihydrouracils (PDHU) with different modifications (substituents and short linkers) installed at the *ortho*, *meta*, *para* positions of the phenyl group was provided by the Chemistry Department of Enamine. The indicated compounds have been tested in the fluorescence polarization assay, where competitive binding with CRBN was observed for the Cy5-Thalidomide probe and compounds. DDB1/CRBN protein (17 nM) and Cy5-Thalidomide (10 nM) weredilutedin 50 mMTris-HClpH 7.5, 1 mM DTT, 0.01% BSA, 0.01% Triton X-100 assay buffer. Corning(Cat#4514) black plate sand 20 μL reaction volume were used. The complete reaction was incubated for 2.5 hours. The fluorescent signal was read with a BMG PHERAstar FSX platereader. The plate had positive control (0% inhibition, bound probe) and negative control (100% inhibition, unbound probe), n=32 both. The compounds were tested in dose-response. For this experiment, the samples were diluted 3-fold from 100 μM, 8 concentration points in total (n=4). On the same plate we also had pomalidomide titrationin of the same concentration range, which was used as anassay control. The IC_{50} values were determined using GraphPadPrism 10 software, the curves were normalized and fitted by the 4PL model. The measured IC_{50} values for the tested compounds were lower than 12 μM. Small molecule 1 (CH_3 in *para* position and acid linker in *meta* position) demonstrated IC_{50} value 0.252 μM which is similar to topomalidomide (0.245 μM). **Conclusions.** The IC_{50} value of tested phenyl dihydrouracil Compound 1 is similar to the well-known Cereblon-glutarimide ligand pomalidomide, which indicates that phenyl dihydrouracils might be a good replacement for the Thalidomide derivatives with the potential to be used during CRBN-based PROTACs development.

Keywords: CRBN, PROTAC, phenyl dihydrouracil, pomalidomide, fluorescence polarization.

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