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NOVEL SMALL-MOLECULE COMPOUNDS WHICH INHIBIT STRAND TRANSFER 2 ACTIVITY OF HIV-1 INTEGRASE

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OBJECTIVE: Integration of the proviral DNA into the host genome is essential event in the human immunodeficiency virus type 1 (HIV-1) replication life cycle. Therefore, integrase (IN), which plays crucial role in this integration event, has been the attractive target of anti-HIV drugs. Up to now, a number of inhibitory compounds have been reported, yet none has been successful in clinical treatment usage. In this study we attempted to find new IN inhibitory compounds, and screened a small molecule-compound library.

METHODS: In-house strand-transfer assay was constructed to screen IN inhibitory compounds. In brief, biotinylated 31 bp donor DNA was mixed with recombinant IN, followed by incubation with digoxigenin (DIG), labelled 29 bp target DNA and the test compound. After 1 h incubation at 37°C, integrated product was captured by streptavidin-coated 96 well plate, and quantified by alkaline phosphatase-conjugated anti-DIG antibody and CSPD chemiluminescence detection system. Lineweaver-Burk plot analyses and intercalation assays were performed to clarify the mechanism of inhibitions. To evaluate *in vitro* virus replication suppressions, single replication assays using HeLa/CD4/LTR-EGFP cell line were performed.

RESULTS: We tested 12000 small-molecule compounds and discovered one compound, carbazole derivative, with potent strand-transfer inhibitory activity. To analyse structural determinants of the strand transfer inhibitory activity, we chemically synthesized 15 derivatives with different side chains on the carbazole structural backbone. Among these 15 compounds, eight derivatives have shown potent strand-transfer inhibitions. IC_{50}s of these eight compounds ranged from 0.78 to 5.3 μM. The result of Lineweaver-Burk plot analyses indicated the carbazole derivatives as competitive inhibitor of strand transfer.

No intercalation activities were observed. In HeLa/CD4/LTR-EGFP cell culture assay, IC_{50}s of the eight compounds ranged from 0.49 to 1.92 μM. However, these eight derivatives demonstrated cytotoxicity (CC_{50}=1.97 to 5.04 μM) in this HeLa cell culture.

CONCLUSIONS: We have successfully found novel small-molecule IN inhibitory compounds carbazole derivatives. Though their strong cytotoxicity may limit carbazole derivatives to be used in clinical at this moment, it can be the lead compound for developing novel IN inhibitors. In addition, analysing IN inhibitory mechanisms of carbazole may give more detailed information of HIV-1 IN structure and function.

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