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Isatin Derivatives as Novel Inhibitors of HIV Integrase/LEDGF Interaction

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ABSTRACT: Series of Novel isatinderivatives were investigated forinhibitionof HIVIntegrase/ Lens epithelium derived growth factor (LEDGF)protein-protein interaction by using ALPHA screen technique. Hypothetical binding modes of the selected compound in HIV integrase were generated using GLIDE docking tool.Isatin derivatives (SP III-5H and SPIII-NA) inhibits HIV IN/LEDGF interaction and SPIII-5H more potent compound (15.1 μM) in this series. Molecular modeling studies indicate that the SPIII-5H can bind within the active site of HIV integrase (DDE) and thus interrupt the binding of HIV integrase with LEDGF.

KEYWORDS: Isatin; HIV Integrase; LEDGF; ALPHA screen technique; Protein-Protein interaction.

Introduction

AIDS is a fatal pathogenic disease caused by retrovirus Human Immunodeficiency Virus (HIV). No effective vaccine is available till now to combat HIV/AIDS. The only available option is chemotherapy(HAART) that can reduce the viral load and improve the quality of life of HIV/AIDS patients. Present therapeutic agents are suffering with emergence of resistance and thus demanding novel targets to sustain the treatment and enhance the life span of the infected population[1]. HIV integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration[2]. Review of literature have demonstrated that HIV-1 Integrase interacts with a cellular lens epithelium-derived growth factor (LEDGF/p75) and that this viral/cellular interaction plays an important role for tethering HIV-1 pre integration complexes (PICs) to transcriptionally active units of host chromatin[3-6]. Small molecule inhibitors of HIV IN/LEDGF have emerged as promising new class of antiviral agents for the treatment of HIV/AIDS[7,8]. Present study is to investigate the novel isatin derivatives as potential inhibitor of HIV Integrase-LEDGF/p75 protein-protein interaction.

Materials and Methods

Compounds: Isatin derivatives were synthesized by reaction between isatin derivatives with sulphadimidin in presence of glacial acetic acid[9].

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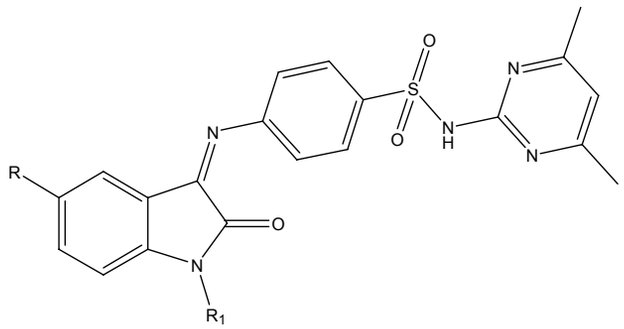
Structure of Isatin Derivatives



Compounds	R	R_1
SPIII-5Br	Br	H
SPIII-5Cl	Cl	H
SPIII-5H	H	H
SPIII-5Me	$-CH_3$	H
SPIII-NA	H	$-COCH_3$
SPIII-5HI-BZ	H	$-COC_6H_5$

Molecular Modeling

(a) Protein preparation: The crystal structure of the dimeric CCD of HIV-1 IN in complex with quinoline derivative **3** (PDB code 3LPU) was used in this study. Standard protocol of GLIDE docking tool available from Maestro 9.3.5 was used to dock the compounds under study.

(b) Ligand preparation: Ligprep 2.3 was used to prepare these compounds before docking. During this process, the OPLS_2005 force field was chosen and possible ionization states at the pH range of 5.0–9.0 were generated.

HIV IN /LEDGF assay: The HIV-1 IN CCD was expressed and purified as described[10]. The IBD of p75 (residues 347–442) containing a GST tag was prepared as previously reported[11]. The LEDGF/p75–IN AlphaScreen assay was developed as described elsewhere[12]. Reactions were performed in a 25 µL final volume in 384-well ProxiPlates (PerkinElmer). The buffer was composed of 25 mM HEPES, pH 7.3, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT, and 0.1% BSA. The His6-tagged HIV IN CCD was added to a final concentration of 40 nM and incubated with test compounds at varying concentrations (0.1–100 µM) and room temperature for 30 min. Subsequently, the remaining components containing a GST-tagged LEDGF/ p75 IBD (final concentration, 40 nM), nickel chelate acceptor beads (final concentration, 8 µg/mL), and glutathione donor beads (final concentration, 8 µg/mL) were added to the well. Proteins and beads were incubated at room temperature for 2 h. Incubation was performed in the dark to avoid direct light exposure. The plates were measured with an EnVisionMultilabel Plate Reader (PekinElmer), with the final emission ranging from 520 to 620 nm.

Results

Isatin derivatives (SP III-5H, SPII-5Cl, SPIII-5Me, SPIII-5Br, SPIII-NA and SPIII-5H-BZ) were investigated for inhibitionof HIV Integrase/LEDGF interaction (Fig 1, Table 1) and compounds SPIII-5H and SPIII-NA inhibits HIV IN/LEDGF interaction at the concentration of 15.1 and 26.4 µM, respectively.

Table 1 HIV Integrase and HIV IN/LEDGF inhibitory activity of Isatine derivatives.

	HIV IN IC_{50} (µM)		LEDGF-IN
Compound	**3' Proc.**	**ST Proc.**	**α-screen (µM)**
SPIII-5H	9 ± 1	6 ± 1	15 ± 1
SPIII-NA	16 ± 5s	9 ± 2	26 ± 4
SPIII-5Br	32 ± 5	11 ± 5	>100
SPIII-5Cl	67±11	46±11	>100
SPIII-5Me	>100	>100	>100
SPIII-5H-BZ	>100	95 ± 5	>100

The results are IC50 ±S.D, *n* = 3 for HIV-1 IN inhibitory activity

[a]Concentration required to inhibits 3' processing reaction,

[b]Concentration required to inhibits 3' processing reaction,

[c]Concentration required to inhibits HIV IN/LEDGF interaction.

SPIII-5H more potent compound (IC_{50}: 15.1µM) in this series. Molecular modeling studies indicate that the ligand makes Hydrogen Bonds (HB) with protein backbone near residues Glu170, His171 and Thr174 and interactions mimic interactions of Integrase Binding Domain (IBD) and thus can block binding of IBD in LEDGF/p75. Molecular modeling studies indicate that the isatine derivative SPIII-5H can bind within the active site of HIV integrase (DDE) and thus interrupt the binding of HIV integrase with LEDGF. Isatin are the novel class of inhibitors of HIV IN/LEDGF interaction (protein-protein) and this lead molecule along with the residues identified through modeling stuides is suitable for further molecular modifications.

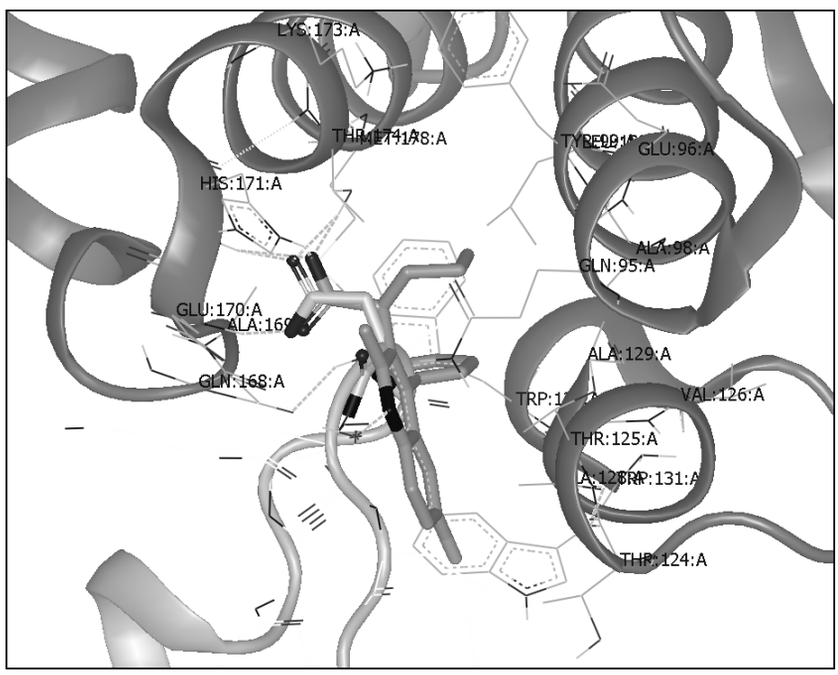


Fig. 1(A) Cartoon representation of the integrase core dimer with Quinoline derivative superimposed with IBD-IN core complex (2B4H, gray).

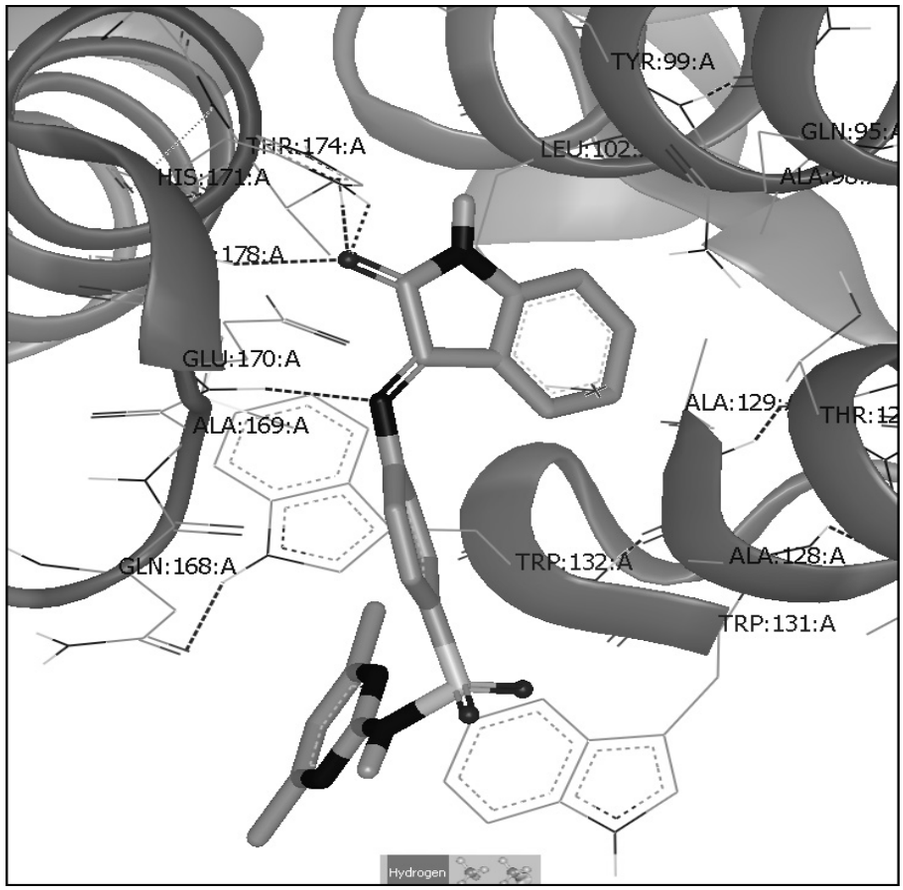


Fig. 1(B) Docking pose of SP-III5H in 3LPU.

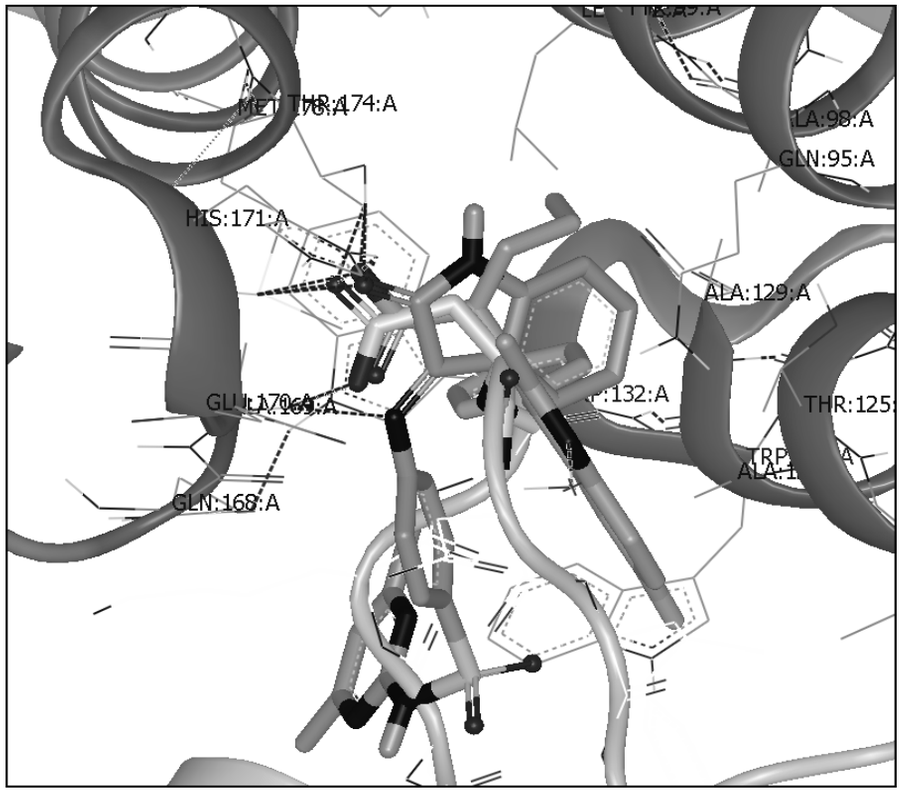


Fig. 1(C) Superposition of Quinoline, IBD and SP-III 5H 3LPU crystal structure.

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