Synthesis and Molecular Docking Study of Novel Chromeno-
chromenones as Anti-HIV-1NNRT Inhibitors

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1. Experimental
4. Molecular docking score.
5. Spectra of corresponding products.

1. Experimental

General details

All solvents were used as commercial anhydrous grade without further purification. Aluminium sheets 20 x 20cm, Silica gel 60 F254, Merck grade was used for thin layer chromatography to determine progress of reaction. Melting points were determined in open capillary tube and are uncorrected. $^1$H and $^{13}$C NMR spectra were recorded on a Brucker AV-300 MHz spectrometer in CDCl$_3$ solvent. Mass spectra were taken on Polaris-Q Thermoscientific GC-MS.


Methods and materials to guide the lead optimization strategy and rationalize the SARs, modeling study was performed to examine the possible binding conformations of our newly synthesized compounds and their interaction mode with RT, using Glide (Glide 5.8, Schrodinger, 2012). Structure-based docking studies were carried out to investigate the intermolecular interaction between the ligand and the targeted enzyme. The coordinates of the non-nucleoside binding site were taken from the crystal structure of HIV-1 reverse transcriptase (RT) in complex with TMC278 (Rilpivirine) (PDB code: 2ZD1). Docking study of all the molecules from 7-(1H-indol-3-yl)-6H,7H-chromeno[4,3-b]chromen-6-one and 5-(6-oxo-6H,7H-chromeno[4,3-b]chromen-7-yl)-6-hydroxypyrimidine-2,4(1H,3H)-dione series was carried out with enzyme reverse transcriptase PDB ID: 2ZD1. The ligands were prepared by using LigPrep (LigPrep 2.5, Schrodinger, 2012). The protein was refined using the protein preparation wizard present in Maestro 9.3 (Maestro 9.3, Schrodinger, 2012). All the water molecules were deleted. H atoms were added to the protein, including the protons necessary to define the correct ionization and tautomeric states of the amino acid residues. Prime interface module incorporated in Maestro was used to add the missing residues of the
side chain. Each structure minimization was carried out with the impact refinement module, using the OPLS-2005 force field to alleviate steric clashes potentially existing in the structures. Minimization was terminated when the energy converged or the root mean square deviation reached a maximum cutoff of 0.30 Å. To find out active site grid was prepared using grid generation panel of glide with the default settings. Grid is prepared for defining the binding site of native ligand on the receptor. The ligand was selected to define the position and size of the active site (Friesner et al., 2004; Halgren et al., 2004). Glide XP docking was used for docking purposes.


From the docking studies, docking score of compound 4i, 4d and 4a was found to be -10.696, -10.660, -10.431 respectively. All molecules from Chromeno-Chromene series were docked into the non-nucleoside inhibitor-binding pocket (NNIBP) of HIV-1 RT. As illustrated in Figs. 1(a, b and c), the indolyl and chromen-chromene moiety of compound 4a, 4d, and 4i of the 7-(1H-indol-3-yl)-6H,7H-chromeno[4,3-b]chromen-6-one ring interacts through CH-p/hydrophobic interactions into the hydrophobic binding pocket, surrounded by the aromatic portion of Leu234, Val106, Phe227, Val179 and Ile180. From the two dimensional Fig. 1 (1a, 1b and 1c) and three dimensional view Fig. 2 (2a,2b and 2c), it is observed that Lys101, 102, and 103 is juxtaposed for better interaction with the aromatic ring of indolyl moiety. The indolyl nucleus moiety at C-7 and chloro/methoxy substitutions on heteroaryl ring of the chromeno-chromemone ring (compound 4a, 4d and 4i) makes π-π interaction into the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of Phe227 and Trp229 residue respectively.
Figure 1: Two dimensional view of the binding interaction of the most active compounds, 4a (1a), 4d (1b) and 4i (1c) with active site of HIV-1 reverse transcriptase (RT) in complex with TMC278. 

Abbreviations: VAL, valine; LEU, leucine; GLY, glycine; ASP, aspartate; SER, serine; ALA, alanine; LYS, lysine; ILE, isoleucine; HIE, histidine epsilon H; MET, methionine; THR, threonine.

Figure 2: Three dimensional view of the binding interaction of the most active compounds, 4j(2a), 4k (2b) and 4r (2c) with active site of HIV-1 reverse transcriptase (RT) in complex with TMC278.

The decrease in activity of compound 4p oxo-chromeno[4,3-b]chromen-7-yl)-6-hydroxyopyrimidine-2,4(1H,3H)-dione derivatives was due to lack of π-π interaction with Phe227 and Trp229 but C=O group of barbituryl moiety and aromatic ring of chromene moiety form the hydrogen bond interactions with the backbone of Lys101 residue (as shown in Figure-3).

The Docking score of Native ligand and all compounds are shown in Table 3. Docking score of compound 4i, 4d and 4a was found to be -10.696, -10.660, and 10.431 respectively while docking score of native ligand was found to be -13.413 which confirm that these compounds might have potent RT inhibition activity. Further, in silico binding studies suggested that inhibitors possessing π-π interaction with the aromatic side chains of Phe227 and Trp229 improves the inhibitor selectivity for RT and thus helps in further drug design attempts to obtain potent Chromeno-Chromene derivatives.
In conclusion, a convenient and environmentally green methodology for the synthesis of 7-(1H-indol-3-yl)-6H,7H-chromeno[4,3-b]chromen-6-one and 5-(9-chloro-6-oxo-6H,7H-chromeno[4,3-b]chromen-7-yl)-6-ine hydroxypyrimid2,4(1H,3H)-dione derivatives via the three-component reactions of 4-hydroxycoumarin, substituted salicylaldehydes, and indole/barbutiric acid has been developed. The attractive features of this protocol are simple reaction procedure, easy product separation, and purification. L-Proline has adaptability for the synthesis of a broad range of 7-(1H-indol-3-yl)-6H,7H-chromeno[4,3-b]chromen-6-one and 5-(9-chloro-6-oxo-6H,7H-chromeno[4,3-b]chromen-7-yl)-6-ine hydroxypyrimid 2,4(1H,3H)-dione derivatives in moderate to high yields. To the best of our knowledge, the use of L-Proline is the first report on the synthesis of 7-(1H-indol-3-yl)-6H,7H-chromeno[4,3-b]chromen-6-one and 5-(9-chloro-6-oxo-6H,7H-chromeno[4,3-b]chromen-7-yl)-6-ine hydroxypyrimid2,4(1H,3H)-dione derivatives.

Docking score of compound 4i, 4d and 4a was found to be -10.696, -10.660, -10.431 respectively, while of native ligand was found to be -13.413 which confirms that these compounds might have potent RT inhibition activity. The decrease in activity of compound 4p was due to lack of π-π interaction with Phe227 and Trp229. This study suggested that inhibitors possessing π-π interaction with the aromatic side chains of Phe227 and Trp229 improves the inhibitor selectivity for RT.

References:
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3. Spectras of corresponding products

IR, $^1$H-NMR, $^{13}$C-NMR and GCMS of product 4c
GC-MS Report
Sample HMK-B6

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IR, $^1$H-NMR, $^{13}$C-NMR and GCMS of product 4g
IR, $^1$H-NMR, $^{13}$C-NMR and Mass of product 4m
GC-MS Report
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Intensity

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