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Synthesis of C-methyl chalcones as HIV-integrase inhibitors—computational approach

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Abstract Eight new 3-methyl chalcones (3a-h) were synthesized for active inhibition against HIV-integrase basing on docking studies on a series of titled compounds. The selected integrase (IN) enzyme is taken as an attractive target for therapeutic drug design. The protease, reverse transcriptase, and IN were the three viral enzymes encoded within the HIV *pol* gene and translated as a polyprotein. Retroviral IN is an enzyme produced by a retrovirus (such as HIV) that enables its genetic material to be integrated into the DNA of the infected cell. Basing on the literature and computational study new analogs of C-methyl chalcones were identified as antiretroviral drugs. Prominent G-score was obtained for compounds **3e** (-6.86604), **3g** (-6.8236), and **3h** (-6.58996) out of the estimated series of analogs.

Keywords C-methyl chalcones · HIV-integrase · Docking studies

Introduction

HIV-1 integrase (IN) is a multidomain enzyme which is required for the integration of viral DNA into the host genome. It is one of the three enzymes of HIV, the others being the Reverse Transcriptase and the Protease. It is an attractive target for therapeutic drug design (Chiu and

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G. Trimurtulu Laila Impex R&D Center, Jawahar Autonagar, Vijayawada 520007, Andhra Pradesh, India Davies, 2004). The HIV genome consists of single-standard RNA like other retroviruses. The viral RNA is released into the host cell following fusion of the viral particles to the cell membrane during infection (Sierra et al., 2005). This viral RNA serves as a template for the synthesis of a double-stranded DNA copy of the viral RNA (cDNA) bearing long terminal repeats by the HIV-encoded reverse transcriptase. The conversion of the viral RNA into cDNA is necessary for making new viral RNA copies and for transcribing the virally encoding genes. Transcription of the viral cDNA also requires its insertion into a host chromosome (Semenova et al., 2010). In recent years drug discovery inventions are now turning toward allosteric IN inhibitors which should be devoid of cross-resistance with IN strand transfer inhibitors. An attempt was made to evaluate their mechanism of action basing on docking studies of these molecules in the IN pocket.

One of the chalcone (2-methoxy-3-methyl-4,6-dihydroxy-5-(3'-hydroxy)-cinnamoyl benzaldehyde) isolated from the *Desmos* spp. (Wu *et al.*, 2003) had showed potent anti-HIV activity with EC₅₀ values of 0.022 and 2.30 μ g/mL and therapeutic indexes of 489 and 45.2, respectively. Basing on this report, in the present investigation an attempt was made to develop a new series of chalcone compounds by docking approach and was also aimed to synthesize the compounds.

Results and discussions

From the G-score (Table 1) it was clearly understood that when there was only an *m*-substitution (either +I group) without any other substitutions or any halogen substitution on ligand, the ligand interacts more effectively with the protein. The molecule **3e** with ethoxy group at *m*-position got the best dock pose with G-score -6.86. No regular hydrogen bonds were identified in this pose with any of binding site residues of 3OAY. Interestingly the molecule was able to coordinate with metal cofactor of the enzyme by its suitable positioned carbonyl and hydroxyl oxygen atoms (Fig. 1). These interactions satisfactorily drew the molecule inside the binding pocket for better fit which can be seen in the surface view of ligand-target binding site complex (Fig. 2). The compounds (**3g**) with hydroxyl group at *m*-position also got G-score -6.82, similarly the compound **3h** also showed dock score -6.58996 that may be due to hydroxyl group at *p*-position.

Experimental

Protein preparation

X-ray crystal structure of HIV IN (PDB id: 3OAY) was downloaded from www.rcsb.org. The protein (PDB: 3OAY Chain B) was prepared using the Protein Preparation Wizard. Preprocessed bond orders were assigned, hydrogens were added, metals were treated, and water molecules were deleted. Heterostate for co-crystallized ligand was generated using Epik, protonation state and optimization of H-bonding of the protein side chains were assigned using Protassign. Energy minimization was done (impref minimization) using Optimized Potentials for Liquid Simulations (OPLS) 2001 force field (Jorgensen *et al.*, 1996).

Ligand preparation

The structure of the compounds were drawn by using chemsketch (ACDLABS 12.0) and converted to 3D structure with the help of 3D optimization tool. By using the LigPrep (2.3) module (Ligprep, Version 2.3, 2009), the drawn ligand was geometry optimized by using the OPLS-2005 (Cornell *et al.*, 1995; Duan *et al.*, 2003) force field with the Steepest Descent followed by truncated Newton

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Conjugate gradient protocol. Partial atomic charges were computed using the OPLS-2005 force field. The LigPrep is a utility in Schrodinger software suite that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers and steric isomers, and geometry minimization of ligands. Finally, 32 poses had been prepared with different tautomeric and steric features for docking studies.

Docking

Docking studies were performed using Docking protocol of GLIDE v5.5 from Schrödinger Suite 2009 (Glide, Version 5.5, 2009). GLIDE scoring function was used to calculate the binding affinity. The prepared target structure was used for docking simulations. Initially a receptor grid, where the ligand has to be docked with the receptor was set by picking the centroid of the co-crystallized inhibitor present at the active site. It creates a grid box and the size of the grid box was limited to 20 Å. The output of different conformations of the docked complexes (poses) was set to a maximum of 20. Then the molecules were docked at the active site of 3OAY. The poses generated were ranked based on G-score. The poses with better G-score were further analyzed for interactions. The best docked pose was selected as the one with the lowest G-score; the more negative the G-score, the more favorable the binding. Further, the dock score was compared with that of the reference substrate.

Synthesis of chalcones

Chemicals and equipment

Acetophenone (synthesized in our laboratory) and other chemicals used were of AR grade (Merck). Melting points were taken in open glass capillary by using Remi melting point apparatus and were uncorrected. ¹H NMR spectra were recorded on Brucker 400 MHz and ¹³C NMR was on

Compound	\mathbf{R}^1	R^2	R ³	R^4	R ⁵	G-score
a	OCH ₃	Н	OCH ₃	Н	Н	-5.83644
b	Cl	Н	Н	Н	Н	-6.73129
c	OCH ₃	Н	OCH ₃	Н	OCH ₃	-6.50991
d	Н	-O-CH ₂ -O-		Н	Н	-6.46386
e	Н	OC_2H_5	Н	Н	Н	-6.86604
f	Н	Н	OC_2H_5	Н	Н	-6.41563
g	Н	OH	Н	Н	Н	-6.8236
h	Н	Н	OH	Н	Н	-6.58996

Table 1 Synthesized chalcones (3a-h)

All these compounds were first time prepared by us



Brucker 100 MHz. Mass spectra were recorded on LC/MS Agilent LC1100 series.

General method for synthesis of chalcones

Chalcones (3a-d) were synthesized by using Claisen condensation (Scheme 1-Ia) and chalcones (3e-h) were prepared by using the hot condensation process (Scheme 1-Ib). To a solution of acetophenone (0.5 g, 0.00238 mol) and substituted benzaldehydes (2a-h) (1 eq) in ethanol (10 mL), aqueous potassium hydroxide (0.599 g, 4.5 eq in 0.5 mL water) is added. The mixture is stirred at room temperature for 24 h. To this, ice-cold water was added and the separated solid was filtered and dissolved in ethyl acetate, dried over sodium sulfate, and concentrated under vacuum.

3-(2,4-Dimethoxyphenyl)-1-(2-hydroxy-4,6-dimethoxy-3methylphenyl)prop-2-en-1-one (**3a**) This compound was obtained as a yellow solid. mp. 195–197 °C, yield 75 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 14.29 (1H, s, OH at C-2), 8.10 (1H, d, J = 15.6 Hz, H-β), 7.89 (1H, d, J = 15.6 Hz, H-α), 7.54 (1H, d, J = 8.4 Hz, H-6'), 6.52 (1H, m, H-5'), 6.4 (1H, s, H-3'), 5.97 (1H, s, H-5), 3.88

(6H, s, OCH₃ at C-4, 6), 3.84 (3H, s, OCH₃ at C-2'), 3.35 (3H, s, OCH₃ at C-6'), 2.30 (3H, s, CH₃ at C-3). ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 193.4 (C=O), 164.2 (C-2), 163.1 (C-4), 162.7 (C-6), 160.9 (C-4'), 160.1 (C-2'), 137.8 (C-β), 130.2 (C-1'), 125.8 (C-α), 117.8 (C-6'), 106.5 (C-5'), 106.1 (C-3), 105.5 (C-1), 98.4 (C-3'), 86.4 (C-5), 55.7, 55.5 (OCH₃ at C-4, 6), 55.4, 55.3 (OCH₃ at C-4', 2'), 7.28 (CH₃ at C-3). MS: m/z (M–H)⁻ at 357.2, mass-358.2. M.F. $C_{20}H_{22}O_{6}$.

3-(2-Chlorophenyl)-1-(2-hydroxy-4,6-dimethoxy-3-methylphenyl)prop-2-en-1-one (**3b**) This compound was obtained as a yellow solid, mp. 184–185 °C, yield 55 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 13.95 (1H, s, OH at C-2), 8.12 (1H, d, J = 15.6 Hz, <u>H</u>-β), 7.84 (1H, d, J = 16.0 Hz, <u>H</u>-α), 7.685 (1H, m, H-4'), 7.43 (1H, m, H-5'), 7.32 (2H, m, H-3', 6'), 5.99 (1H, s, H-5), 3.93 (3H, s, OCH₃ at C-4), 3.90 (3H, s, OCH₃ at C-6), 2.03 (3H, s, CH₃ at C-3). ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 192.7 (C=O), 164.3 (C-2), 163.8 (C-4), 161.1 (C-6),137.4 (C-β), 135.3 (C-2'), 134.1 (C-1'), 130.5 (C-6'), 130.2 (C-4'), 127.8 (C-3'), 127.6 (C-5'), 126.9 (C-α), 106.4 (C-3), 106.3 (C-1), 86.4 (C-5), 55.8, 55.5 (OCH₃ at C-4, 6), 7.2 (CH₃ at C-3). MS: m/z (M+H)⁺ at 333.1, mass-332.1, M.F. C₁₈H₁₇O₄Cl.

Scheme 1 Synthesis of chalcones (3a-h). Reagents and conditions: *Ia* KOH/EtOH, 24 h. *Ib* KOH/EtOH, 24 h, Δ at 50 °C



1-(2-Hydroxy-4,6-dimethoxy-3-methylphenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (*3c*) This compound was obtained as a yellow solid, mp. 187–190 °C, yield 72 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 14.42 (1H, s, OH at C-2), 8.32 (1H, d, J = 15.6 Hz, H-β), 8.21 (1H, d, J = 15.6 Hz, H-α), 6.13 (2H, s, H-3', 5'), 5.98 (1H, s, H-5), 3.92 (3H, s, OCH₃ at C-4'), 3.90 (6H, s, OCH₃ at C-2', C-6'), 3.88 (3H, s, OCH₃ at C-4), 3.85 (3H, s, OCH₃ at C-6), 2.02 (3H, s, CH₃ at C-3). ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 194.5 (C=O), 164.2 (C-2), 162.8 (C-4), 162.8 (C-6), 161.6 (C-2', 6'),160.9 (C-4'), 133.8 (C-β), 127.5 (C-α), 107.3 (C-1'), 106.9 (C-1), 106.1 (C-3), 90.7 (C-3', 5'), 86.5 (C-5), 55.7 (OCH₃ at C-2', 6'), 55.6, 55.4, 55.3 (OCH₃ at C-4', 4, 6), 7.3 (CH₃ at C-3). MS: m/z (M+H)⁺ at 389.2, mass-388.15, M.F. C₂₁H₂₄O₇.

3-(Benzo[d][1,3]dioxol-5-yl)-1-(2-hydroxy-4,6-dimethoxy-3-methylphenyl)prop-2-en-1-one (3d) This compound was obtained as a yellow solid, mp. 188–190 °C, yield 85 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 14.12 (1H, s, OH at C-2), 7.71 (2H, brs, H-α, β), 7.35 (1H, m, H-5'), 7.10 (1H, s, H-8'), 7.02 (1H, m, H-6'), 6.82 (1H, s, H-5), 6.01 (2H, brs, H-2'), 3.90 (6H, s, OCH₃ at C-4, 6), 2.03 (3H, s, CH₃ at C-3). ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 192.8 (C=O), 164.2 (C-2), 163.4 (C-4), 161.0 (C-6), 149.4 (C-4'), 148.3 (C-9'),141.9 (C-β), 130.2 (C-7'), 126.1 (C-α), 124.8 (C-6'), 108.6 (C-5'), 106.6 (C-8'), 106.3 (C-1), 106.1 (C-3), 101.4 (C-2'), 86.4 (C-5), 55.8, 55.4 (OCH₃ at C-4, 6), 7.2 (CH₃ at C-3). MS: m/z (M+H)⁺ at 343.2; mass-342.2, M.F. C₁₉H₁₈O₆.

3-(3-Ethoxyphenyl)-1-(2-hydroxy-4,6-dimethoxy-3-methylphenyl) prop-2-en-1-one (**3e**) This compound was obtained as a yellow solid, mp. 92–94 °C, yield 80 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 13.9 (1H, s, OH at C-2), 7.8 (1H, d, J = 15.6 Hz, H- β), 7.6 (1H, d, J = 16.0 Hz, H- α), 7.36 (1H, m, H-5'), 7.24 (1H, s, H-2'), 7.30 (1H, d, J = 7.2 Hz, H-6'), 7.01 (1H, d, J = 7.6 Hz, H-4'), 6.29 (1H, s, H-5), 4.11 (2H, q, J = 7.2 Hz, O-C<u>H</u>₂-CH₃), 3.98 (3H, s, OC<u>H</u>₃ at C-4), 3.93 (3H, s, OC<u>H</u>₃ at C-6), 1.93 (3H, s, C<u>H</u>₃ at C-3), 1.37 (3H, t, J = 6.8 Hz, -CH₂-

C<u>H</u>₃). ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 192.5 (C=O), 163.7 (C-2), 162.7 (C-4),161.1(C-6), 158.9 (C-3'), 141.7 (C-β), 136.3 (C-1'), 130.1 (C-6'), 127.1 (C-α), 120.4 (C-5'), 116.6 (C-4'), 114.1 (C-2'), 105.6 (C-1), 104.2 (C-3), 87.6 (C-5), 63.1 (O-CH₂-CH₃), 56.2, 55.8 (OCH3 at C-4, 6), 14.5 (-CH₂-CH₃), 7.2 (CH₃ at C-3). MS: *m/z* at 343.2; mass-342.2, M.F. C₂₀H₂₂O₅.

3-(4-Ethoxyphenyl)-1-(2-hydroxy-4,6-dimethoxy-3-methylphenyl)prop-2-en-1-one (3f) This compound was obtained as a yellow solid, mp. 96–98 °C, yield 85 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 13.94 (1H, s, OH at C-2), 7.81 (1H, d, J = 16.0 Hz, H-β), 7.73 (1H, d, J = 15.6 Hz, H-α), 7.69 (2H, d, J = 8.8 Hz, H-2', 6'), 7.62 (2H, d, J = 8.4 Hz, H-3', 5'), 6.28 (1H, s, H-5), 4.12 (2H, q, J = 7.8 Hz, $-OCH_2-CH_3$), 3.92 (3H, s, OCH_3 at C-4), 3.90 (3H, s, OCH_3 at C-6), 1.99 (3H, s, CH_3 at C-3), 1.37 (3H, t, J = 6.8 Hz, $-OCH_2-CH_3$), ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 192.3 (C=O), 163.5 (C-2), 162.9 (C-4), 161.5 (C-6), 160.4 (C-4'), 142.3 (C-β), 135.3 (C-1'), 130.3 (C-2', 6'), 127.3 (C-α), 120.4 (C-3', 5'), 106.6 (C-3), 105.0 (C-1), 87.5 (C-5), 63.3 ($-OCH_2-CH_3$), 55.9, 55.8 (OCH3 at C-4, 6), 14.5 ($-CH_2-CH_3$), 7.1 (CH_3 at C-3). MS: m/z (M+H)⁺ at 343.2; mass-342.2, M.F. C₂₀H₂₂O₅.

1-(2-Hydroxy-4,6-dimethoxy-3-methylphenyl)-3-(3-

hydroxyphenyl)prop-2-en-1-one (**3g**) This compound was obtained as a yellow solid, mp. 160–165 °C, yield 76 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 13.99 (1H, s, OH at C-2), 9.67 (1H, s, OH at C-3'), 7.82 (1H, d, J = 15.6 Hz, H-β), 7.62 (1H, d, J = 15.6 Hz, H-α), 7.28 (1H, t, J = 8.0 Hz, H-5'), 7.16 (1H, d, J = 7.6 Hz, H-6'), 7. 09 (1H, s, H-2'), 6.87 (1H, d, J = 7.6 Hz, H-4'), 6.30 (1H, s, H-5), 4.0 (3H, s, OCH₃ at C-4), 3.93 (3H, s, OCH₃ at C-6), 1.93 (3H, s, CH₃ at C-3). ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 192.4 (C=O), 163.6 (C-2), 162.9 (C-4), 161.1 (C-6), 157.7 (C-3'), 142.1 (C-β), 136.1 (C-1'), 130.1 (-C-6'), 127.2 (C-α), 119.6 (C-5'), 117.6 (C-4'), 114.3 (C-2'), 105.5 (C-3), 104.2 (C-1), 87.6 (C-5), 56.2, 55.8 (OCH3 at C-4, 6), 7.3 (CH₃ at C-3). MS: *m*/z (M-H)⁻at 313.2; mass-314.2, M.F. C₁₈H₁₈O₅.

1-(2-Hydroxy-4,6-dimethoxy-3-methylphenyl)-3-(4-

hydroxyphenyl)prop-2-en-1-one (*3h*) This compound was obtained as a yellow solid, mp. 105–110 °C, yield 75 %.

¹H NMR (CDCl₃, 400 MHz, δ in ppm): 13.94 (1H, s, OH at C-2), 10.09 (1H, s, OH at C-4'), 7.80 (1H, d, J = 15.6 Hz, H-β), 7.60 (1H, d, J = 15.6 Hz, H-α), 7.28 (2H, d, J = 8.0 Hz, H-2', 6'), 6.86 (2H, d, J = 7.6 Hz, H-3', 4'), 6.27 (1H, s, H-5), 3.92 (3H, s, OCH₃ at C-4), 3.90 (3H, s, OCH₃ at C-6), 1.92 (3H, s, CH₃ at C-3). ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 192.4 (C=), 163.5 (C-2), 162.5 (C-4), 161.5(C-6), 157.9 (C-4'), 142.2 (C-β), 136.3 (C-1'), 130.1 (C-2',6'), 127.2 (C-α), 119.6 (C-3',5'), 105.1 (C-3), 103.8 (C-1), 87.1 (C-5), 55.9, 55.8 (OCH₃ at C-4, 6), 7.1 (CH₃ at C-3). MS: m/z (M+H)⁺ at 315.2; mass-314.2 M.F. C₁₈H₁₈O₅.

References

Chiu TK, Davies DR (2004) Structure and function of HIV-1 integrase. Curr Top Med Chem 4(9):965–977

- Cornell W, Cieplak P, Kollmann PA (1995) A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. J Am Chem Soc 117:5179–5197
- Duan Y, Wu C, Kollmann P et al (2003) A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. J Comput Chem 24(16):1999–2012

Glide, Version 5.5, (2009) Schrodinger, LLC, New York, NY http://www.rcsb.org/pdb/home/home.do

- Jorgensen WL, Maxwell DS, Tirado-Rives J (1996) Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. J Am Chem Soc 118:11225–11236
- Ligprep, Version 2.3, (2009) Schrodinger, LLC, New York, NY
- Semenova E, Marchand C, Pommier Y (2010) HIV-1 integrase inhibitors: update and perspectives. Laboratory of Molecular Pharmacology, Center for Cancer Research, NationalCancer Institute, National Institutes of Health, Bethesda, pp 4255–20892
- Sierra S, Kupfer B, Kaiser R (2005) Basics of the virology of HIV-1 and its replication. J Clin Virol 34:233–244
- Wu J-H, Wang X-H, Yi Y-H et al (2003) Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus Desmos. Bioorg Med Chem Lett 13(10):1813–1815