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Synthesis, structure–activity relationship and molecular docking of cyclohexenone based analogous as potent non-nucleoside reverse-transcriptase inhibitors

Muhammad Faizan Nazar [a,*], Muhammad Imran Abdullah [b], Amir Badshah [c], Asif Mahmood [d], Usman Ali Rana [e], Salah Ud-Din Khan [e]

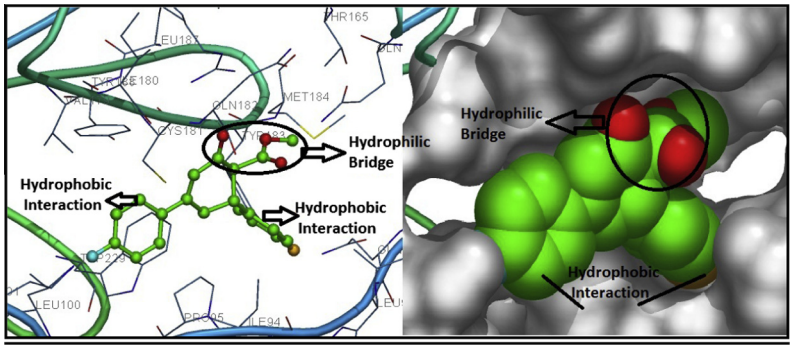
[a] Department of Chemistry, University of Gujrat, Gujrat 50700, Pakistan
[b] Institute of Chemistry, University of the Punjab, Lahore 54590, Pakistan
[c] Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
[d] Department of Chemistry, University of Sargodha, Sargodha 40100, Pakistan
[e] Sustainable Energy Technologies (SET) Center, College of Engineering, PO-Box 800, King Saud University, Riyadh 11421, Saudi Arabia

HIGHLIGHTS

- A convenient route for the synthesis of eight new cyclohexenone derivatives from Robinson annulation is described.
- Micro- and spectral analysis have been effectively operated to confirm the molecular structures of cyclohexenones.
- These cyclohexenones displayed potential activity against reverse transcriptase.
- The docking protocol and bioassay studies of as-synthesized cyclohexenones are in good agreement.

GRAPHICAL ABSTRACT

On the basis of rational drug design synthesis principles eight novel and potential cyclohexenone based anti-reverse transcriptase analogous have been successfully synthesized and evaluated in detail.



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ABSTRACT

The chalcones core in compounds is advantageously chosen effective synthons, which offer exciting perspectives in biological and pharmacological research. The present study reports the successful development of eight new cyclohexenone based anti-reverse transcriptase analogous using rational drug design synthesis principles. These new cyclohexenone derivatives (CDs) were synthesized by following a convenient route of Robinson annulation, and the molecular structure of these CDs were later confirmed by various analytical techniques such as ^{1}H NMR, ^{13}C NMR, FT-IR, UV–Vis spectroscopy and mass spectrometry. All the synthesized compounds were screened theoretically and experimentally against reverse transcriptase (RT) and found potentially active reverse transcriptase (RT) inhibitors. Of the compounds studied, the compound 2FC4 showed high interaction with RT at non-nucleoside binding site, contributing high free binding energy (ΔG −8.01 Kcal) and IC_{50} (0.207 μg/ml), respectively. Further results revealed that the compounds bearing more halogen groups, with additional hydrophobic

* Corresponding author at: Office # 09, Department of Chemistry, Institute of Chemical and Biological Sciences, University of Gujrat, Gujrat 50700, Pakistan. Tel.: +92 3016942411; fax: +92 533643167.
E-mail addresses: faizan_qau@yahoo.com, faizan.nazar@uog.edu.pk (M.F. Nazar).

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character, offered superior anti-reverse transcriptase activity as compared to rest of compounds. It is anticipate that the present study would be very useful for the selection of potential reverse transcriptase inhibitors featuring inclusive pharmacological profiles.

Introduction

The emergence of drug-resistant mutants has received the world's attention to develop new antivirals with better drug resistance and pharmacokinetic profiles [1–3]. In recent years, the research on the treatment and prevention of HIV/AIDS remain a challenging endeavor for researchers. The HIV reverse transcriptase (RT) is the major target for the antiviral therapies [4–6]. HIV is highly error prone and low fidelity DNA polymerase enzyme [7,8]. Due to this property, the mutations are common during the reverse transcription. Furthermore, the HIV has two RNA genomes, which help in facile production of recombinant progeny [9,10]. Currently, two main classes of drugs are the inhibitors of reverse transcriptase enzyme that is nucleoside/nucleotides reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [11–14]. NRTIs inhibit the RT through active sites, while the NNRTIs bind to separate away from the active site [15–18].

Among a variety of potent bioactive organic molecular systems, the chalcones core in compounds is advantageously chosen effective synthons, which offer exciting perspectives in biological and pharmacological research. Cyclic chalcones are carriers of different types of biological activity. The motive for their preparation is a variety of medical effects. From a chemical point of view, an important feature of chalcones is the ability to act as activated unsaturated systems in conjugated addition reactions of carbanions in the presence of basic catalysts [19,20]. Eddington et al. found that the enaminones are known to possess a variety of medicinal properties including anticonvulsant, antimalarial, anti-inflammatory and cardiovascular effect [21]. Tomita and Iwatsubo also reported a number of γ-secretase inhibitors to reduce a β levels in mouse brains and biological fluids by oral administration [22]. However, several γ-inhibitors have shown unwanted side effects [23,24]. Thus, the discovery and development of new inhibitors without side effects have been continuously challenging and demanding tasks for medicinal chemists. The 4-hydroxy-2-cyclohexenone has been used as a building block in the synthesis of several bioactive compounds such as the anti-cholesterol agents, compactin and ML-236A and the immunosuppressant FK-506 [25–27]. Cyclohexenone carboxylates have known to possess anti-cancer [28], anti-HIV [29–31], anti-fungal [32], anti-tumor [33,34], anticonvulsant [21,35] and antitubercular [36] activity. A series of novel cyclohexenone derivatives have been synthesized, which are used in the treatment of neurological disorders [37] as well as efficient fluorescent probes for biomembranes [38–40].

In the present study, we have synthesized eight new 3,5-diaryl-6-carbethoxy cyclohexenones (2FO, 2FB, 2FC4, 2FE, 2FM2, 2FM3, 2FM4 and 2FF). We have performed the docking and bioassay experiments in order to determine the 3,5-diaryl-6-carbethoxy cyclohexenone like compounds as a non-nucleoside reverse transcriptase inhibitors (NNRTIs). To increase drug like properties of our compounds, we have introduced benzene ring, which contained high hydrophobic groups like fluoro, chloro and ethoxy that assist the drug molecule to make strong hydrophobic interaction with reverse transcriptase (RT). We have also changed the position of substituent groups on benzene ring in order to find the substituent effect on free binding energy (ΔG). The different interacting parts of drug are shown in Fig. 1.

Experimental section

Reagents

The reagents; ethyl acetoacetate, 3-Bromobenzaldehyde, 2-Chlorobenzaldehyde 4-Chlorobenzaldehyde, 2-Methoxybenzaldehyde, 3-Methoxybenzaldehyde, 4-Methoxybenzaldehyde, 4-Ethoxybezaldehyde and 4-Fluoroacetophenone were purchased from Fluka (Germany). The liquid reagents were distilled at their boiling points and the solid reagents were characterized by recording their melting points. No further purification was required. Sulfuric acid and hydrochloric acid (37%) were obtained from Stedee Ltd. The solvents chloroform, ethyl acetate, absolute ethanol and pet ether were purchased from Sigma Aldrich (Germany). All solvents were used after necessary purification and drying according to the standard procedures. The dried solvents were stored over molecular sieves (4 Å).

Compound characterization techniques

The R_f values were calculated by using precoated silica gel aluminum backed plates Kiesel gel $60F_{254}$ Merck (Germany) using ethylacetate:pet-ether (1:4) as developing solvents. Melting points of the compounds were determined in open capillaries using Gallenkamp melting point apparatus and are uncorrected. The FTIR spectral data were recorded on Bio-Rad Merlin Spectrophotometer using KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker (300 MHz) AM-250 spectrometer in $CDCl_3$ solution using TMS as internal standard. EIMS was recorded on Agilent mass spectrometer. Purity of each compound was ascertained by thin layer chromatography. The purification of synthesized compounds was achieved mostly through recrystallization, the use of solvent extraction, or by making preparative thin layer chromatography or column chromatography, whenever required.

General synthetic methods

The synthetic strategy for the titled compounds is outlined in the following Scheme 1. Furanyl-containing chalcone analog 2 (3 mmol) and ethyl acetoacetate (0.39 g, 0.40 mL, 3 mmol) were refluxed for 2 h in 10-15 mL ethanol in the presence of 0.5 mL 10% NaOH. The reaction mixture was then poured with good stirring into 200 mL ice-cold water and kept at room temperature

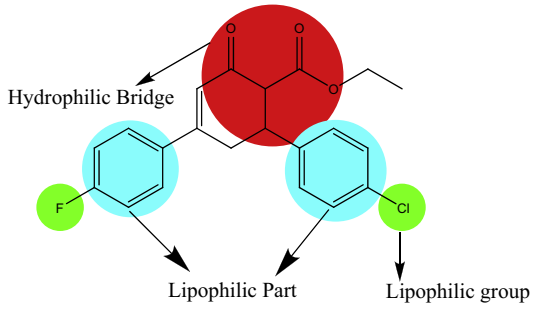


Fig. 1. Representing the different interacting part of cyclohexenone.

until the reaction product separated as a solid, which was filtered off and recrystallized from ethanol.

The molar ratio, physical, FTIR, ^{1}H NMR, ^{13}C NMR and EIMS data for these compounds are given below.

Ethyl 4-(4-fluorophenyl)-6-phenyl-2-oxocyclohex-3-enecarboxylate (2FO)

Fluffy white solid, Yield 3.0 g (66%); m.p.: 106–107 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.08 (t, J = 7.2 Hz, 3H), 4.09 (q, J = 7.2 Hz, 2H), 3.04 (dd, J_1 = 2.3 Hz, J_2 = 16.6, 1H), 2.90–3.01 (m, 1H), 3.77 (dd J_1 = 2.9 Hz, J_2 = 7.0, 2H), 6.53 (s, 1H), 7.56 (dd J_1 = 2.5 Hz, J_2 = 6.9, 2H), 7.10 (dd J_1 = 2.3 Hz, J_2 = 6.8, 2H), 7.2 (m, 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.25, 169.33, 162.40, 157.06, 143.50, 143.10, 133.55, 128.50, 128.50, 128.28, 128.18, 127.21, 126.69, 126.21, 116.20, 115.85, 61.05, 59.18, 43.25, 35.85, 14.03. IR (KBr, cm^{-1}): 1164, 1508, 1599, 1663, 1731, 2990. MS (EI): m/z (%) = 266(37), 162(100), 134(60), 51(5), 39(4), 238(10).

Ethyl 4-(4-fluorophenyl)-6-(3-bromophenyl))-2-oxocyclohex-3-enecarboxylate (2FB)

Fluffy white solid, Yield 2.8 g (63%); m.p.: 118–119 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.07 (t, J = 7.1 Hz, 3H), 4.08 (q, J = 7.2 Hz, 2H), 3.04 (dd, J_1 = 2.3 Hz, J_2 = 17.5, 1H), 2.87–2.99 (m, 1H), 3.75 (dd J_1 = 3.0 Hz, J_2 = 6.8, 2H), 6.53 (s, 1H), 7.56 (dd J_1 = 2.3 Hz, J_2 = 6.7, 2H), 7.12 (dd J_1 = 2.2 Hz, J_2 = 6.8, 2H), 7.25–7.45 (m, 4H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.41, 168.93, 162.48, 157.05, 143.16, 133.58, 133.53, 130.51, 128.99, 128.54, 128.31, 128.20, 127.90, 123.86, 116.26, 115.97, 61.22, 59.32, 43.66, 35.90, 14.03. IR (KBr, cm^{-1}): 635, 1165, 1530, 1603, 1665, 1734, 2985. MS (EI): m/z (%) = 344(15), 162(100), 134(50), 51(3), 39(2), 316(2), 237(5).

Ethyl 4-(4-fluorophenyl)-6-(4-chlorophenyl)-2-oxocyclohex-3-enecarboxylate (2FC4)

Fluffy white solid, Yield 2.9 g (64%); m.p.: 121–122 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.10 (t, J = 7.2 Hz, 3H), 4.10 (q, J = 7.1 Hz, 2H), 3.04 (dd, J_1 = 2.4 Hz, J_2 = 16.0, 1H), 2.89–2.99 (m, 1H), 3.70 (dd J_1 = 3.0 Hz, J_2 = 7.0, 2H), 6.53 (s, 1H), 7.55 (dd J_1 = 2.4 Hz, J_2 = 6.8, 2H), 7.09 (dd J_1 = 2.3 Hz, J_2 = 6.2, 2H), 7.28 (dd J_1 = 1.2 Hz, J_2 = 4.5, 2H), 7.35 (dd J_1 = 2.1 Hz, J_2 = 6.6, 2H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.55, 169.02, 162.46, 157.45, 139.38, 133.93, 133.62, 133.62, 128.71, 128.61, 128.60, 128.30, 127.29, 127.29, 116.25, 115.96, 61.19, 58.33, 43.44, 36.00, 14.03. IR (KBr, cm^{-1}): 756, 1155, 1503, 1593, 1655, 1736, 2982. MS (EI): m/z (%) = 300(32), 162(100), 134(50), 51(2), 39(2), 272(5), 237(6).

Ethyl 4-(4-fluorophenyl)-6-(4-ethoxyphenyl)-2-oxocyclohex-3-enecarboxylate (2FE)

Fluffy white solid, Yield 2.7 g (67.5%); m.p.: 119–120 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.09 (t, J = 7.1 Hz, 3H), 4.09 (q, J = 7.1 Hz, 2H), 3.05 (dd, J_1 = 2.3 Hz, J_2 = 16.0, 1H), 2.85–2.95 (m, 1H), 3.60 (dd J_1 = 3.1 Hz, J_2 = 6.9, 2H), 6.54 (s, 1H), 7.54 (dd J_1 = 2.3 Hz, J_2 = 6.9, 2H), 7.10 (dd J_1 = 2.4 Hz, J_2 = 6.6, 2H), 6.85 (dd J_1 = 2.1 Hz, J_2 = 6.6, 2H), 7.23 (dd J_1 = 3.0 Hz, J_2 = 6.6, 2H), 1.43 (t, J = 7.2 Hz, 3H), 4.09 (q, J = 7.1 Hz, 2H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.17, 169.33, 162.46, 162.40, 157.41, 140.92, 133.84, 133.80, 128.70, 128.29, 128.29, 127.24, 116.25, 116.18, 115.96, 114.72, 63.47, 60.99, 59.86, 43.38, 36.38, 14.85, 14.03. IR (KBr, cm^{-1}): 1045, 1148, 1516, 1614, 1661, 1734, 2980. MS (EI): m/z (%) = 310(50), 162(100), 134(20), 51(2), 39(2), 282(3), 237(3).

Ethyl 4-(4-fluorophenyl)-6-(2-methoxyphenyl)-2-oxocyclohex-3-enecarboxylate (2FM2)

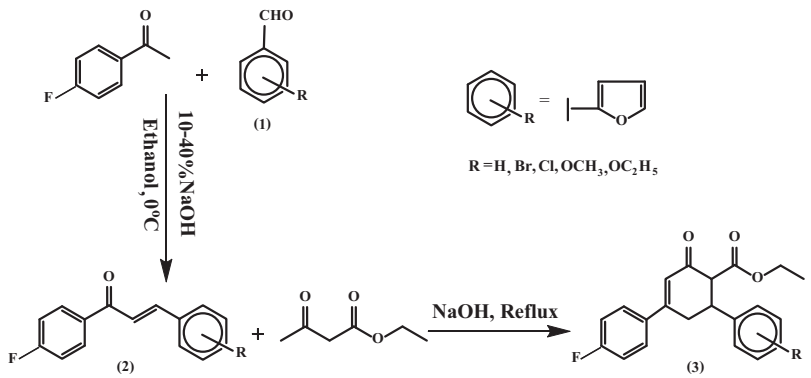
Fluffy white solid, Yield 2.8 g (62%); m.p.: 121–122 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.07 (t, J = 7.3 Hz, 3H), 4.10 (q, J = 7.0 Hz, 2H), 3.06 (dd, J_1 = 2.2 Hz, J_2 = 16.5, 1H), 2.81–2.90 (m, 1H), 3.65 (dd J_1 = 3.0 Hz, J_2 = 6.8, 2H), 6.54 (s, 1H), 7.56 (dd J_1 = 2.3 Hz, J_2 = 6.9, 2H), 7.09 (dd J_1 = 2.3 Hz, J_2 = 6.7, 2H), 7.10 (m, 4H), 3.81 (s, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.80, 169.20, 162.39, 157.42, 155.80, 142.50, 133.73, 133.70, 128.27, 128.18, 127.30, 127.00, 120.80, 116.19, 115.85, 114.00, 61.06, 59.27, 55.26, 43.75, 36.16, 14.03. IR (KBr, cm^{-1}): 1049, 1136, 1507, 1605, 1655, 1737, 2982. MS (EI): m/z (%) = 296(30), 162(100), 134(40), 51(2), 39(2), 268(2), 237(2), 119(55), 91(10), 65(5).

Ethyl 4-(4-fluorophenyl)-6-(3-methoxyphenyl)-2-oxocyclohex-3-enecarboxylate (2FM3)

Fluffy white solid, Yield 2.9 g (63%); m.p.: 110–111 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.08 (t, J = 7.0 Hz, 3H), 4.03 (q, J = 6.9 Hz, 2H), 3.05 (dd, J_1 = 2.3 Hz, J_2 = 17.0, 1H), 2.82–2.89 (m, 1H), 3.67 (dd J_1 = 3.0 Hz, J_2 = 6.9, 2H), 6.53 (s, 1H), 7.54 (dd J_1 = 2.4 Hz, J_2 = 7.0, 2H), 7.08 (dd J_1 = 2.4 Hz, J_2 = 6.8, 2H), 7.20 (m, 4H), 3.83 (s, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.97, 169.30, 162.42, 160.40, 157.42, 142.54, 133.76, 133.71, 129.50, 128.29, 128.18, 119.33, 116.20, 115.91, 111.50, 110.40, 61.07, 59.28, 55.30, 43.88, 36.25, 14.03. IR (KBr, cm^{-1}): 1055, 1144, 1513, 1601, 1653, 1735, 2985. MS (EI): m/z (%) = 296(50), 162(100), 134(50), 51(2), 39(4), 268(6), 237(4), 119(10), 91(8), 65(4).

Ethyl 4-(4-fluorophenyl)-6-(4-methoxyphenyl)-2-oxocyclohex-3-enecarboxylate (2FM4)

Fluffy white solid, Yield 3.0 g (65%); m.p.: 109–110 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.07 (t, J = 6.9 Hz, 3H), 4.03 (q, J = 7.0 Hz, 2H), 3.05 (dd, J_1 = 2.2 Hz, J_2 = 17.5, 1H), 2.79–2.89 (m, 1H), 3.67 (dd J_1 = 3.0 Hz, J_2 = 6.8, 2H), 6.53 (s, 1H), 7.55 (dd J_1 = 2.3 Hz, J_2 = 6.9, 2H), 7.09 (dd J_1 = 2.3 Hz, J_2 = 6.9, 2H), 7.24 (dd J_1 = 2.1 Hz, J_2 = 6.6, 2H), 6.89 (dd J_1 = 2.1 Hz, J_2 = 6.6, 2H), 3.80 (s, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.95, 169.25, 162.43, 157.42, 154.43, 142.32, 133.77, 133.74, 128.29, 128.20, 126.99, 126.99, 119.33, 119.33,



Scheme 1. General methodology for the synthesis of cyclohexenone derivatives.

116.21, 115.89, 61.08, 59.29, 55.26, 43.92, 36.30, 14.03. IR (KBr, cm^{-1}): 1065, 1144, 1498, 1609, 1655, 1734, 2982. MS (EI): *m*/*z* (%) = 296(90), 162(100), 134(70), 51(2), 39(3), 268(2), 237(5), 119(3), 91(10), 65(5).

Ethyl 4-(4-fluorophenyl)-6-(2-furanyl)-2-oxocyclohex-3-enecarboxylate (2FF)

Light pink solid, Yield 1.2 g (61%); m.p.: 77–78 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.09 (t, J = 7.1 Hz, 3H), 4.09 (q, J = 6.9 Hz, 2H), 3.04 (dd, J_1 = 2.2 Hz, J_2 = 17.0, 1H), 2.79–2.85 (m, 1H), 3.68 (dd J_1 = 2.9 Hz, J_2 = 6.9, 2H), 6.54 (s, 1H), 7.61 (dd J_1 = 2.4 Hz, J_2 = 6.8, 2H), 7.08 (dd J_1 = 2.2 Hz, J_2 = 6.8, 2H), 7.24 (dd J_1 = 2.1 Hz, J_2 = 6.6, 2H), 6.89 (dd J_1 = 2.1 Hz, J_2 = 6.6, 2H), 5.5–6.09 (m, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 193.70, 169.10, 162.38, 153.45, 141.50, 141.34, 133.65, 133.50, 128.28, 128.13, 127.03, 116.20, 115.79, 110.00, 105.90, 61.09, 59.58, 43.50, 36.20, 14.03. IR (KBr, cm^{-1}): 1149, 1509, 1595, 1673, 1733, 2945. MS (EI): *m*/*z* (%) = 256(45), 162(100), 134(90), 51(10), 39(20), 228(90), 119(70), 91(50), 65(5).

On the basis of aforementioned results, the structures of cyclohexenone derivatives are given in Table 1.

Docking protocol

The docking studies were performed by using the autodock docking software. The structures of the studied compounds were drawn using ChemBio Ultra 11.0 and later the energies of all compounds were minimized by using Gaussian 09 at B3LYP/6-31(d). The Gasteiger–Huckel method was used to assign the charges to the ligand. The RT structure was obtained from RCSB Protein data Bank (PDB ID 1jlb). In order to prepare the enzymes, the hydrogens atoms were added at a pH range of (6.5–8.1). By the aid of AutoDock tools the salvation parameters and Kollman united atom type charges were added. The autogrid program was used to generate the affinity (gird) maps of 20 × 20 × 20 Å gird points and 0.375 Å spacing [41–43].

Bioassay conditions and enzyme inhibition

The commercial kit (RETRO SYS™ kit) was used to determine the IC_{50} inhibition values for the reverse transcriptase (RT). For this purpose, the compound under study was diluted in the form of reaction mixture and pre-incubated for 30 min at 33 °C. To this mixture, the standardized amount of RT was added, which initiated the reaction by incorporation of bromo-deoxyuridine (BrdUMP). Here, the incorporation of (BrdUMP) from RT depends on the level of inhibition. The reaction was stopped by washing the plate and the amount of product was quantified using RT product tracer, which has the capacity to inclusively bind the incorporated (BrdUMP). The excess amount of tracer was determined by the observation of color due to the reaction between alkaline phosphate and p-nitrophenyl phosphate (pNPP). The incorporated (BrdUMP) product in the absence of inhibitor was also measured, whereas the IC_{50} values of inhibition were calculated using the above results. The mechanism of action of RETRO SYS™ kit has been shown in Fig. 2.

Results and discussion

Synthesis of cyclohexenone

The reaction of chalcones and their heterocyclic analogs with ethyl acetoacetate in the presence of basic condition underwent Michael addition followed by internal aldol condensation to produce cyclohexenone, as outlined in Scheme 1. The organic compounds were separated out by pouring the reaction mixture into water. All compounds precipitated after being kept for several days. The solid products were separated by filtration, dried and recrystallized from ethanol. The yields of the cyclocondensation were good, varying from 61.0% to 67.5%. Structural analysis of the newly synthesized cyclohexenone was done by analytical and spectral data. The IR spectra of these compounds revealed a sharp strong absorption band at *ca.* 1731–1737 cm^{-1} that can be correlated with the presence of the ester function in the structure of cyclohexenone. Furthermore, another sharp strong absorption band at *ca.* 1653–1673 cm^{-1} can be assigned to the conjugated carbonyl group. The bands at *ca.* 2945–2990 cm^{-1} can be assigned to the OH group belonging to enol form and the hydrogen bonded carbonyl of ester group can be related with the IR band at *ca.* 1500–1600 cm^{-1}.

The results from ^{1}H NMR studies were successfully correlated to the data from the IR analysis. One of the representative compounds of cyclohexenone series, ethyl 4-(4-fluorophenyl)-6-(4-chlorophenyl)-2-oxocyclohex-3-enecarboxylate (2FC4), has been described here in details. In the ^{1}H NMR spectrum of (2FC4), the ethyl protons resonated as a triplet and a quartet at *ca.* 1.10 ppm and *ca.* 4.10 ppm, integrating for three and two protons respectively. The characteristic signal in the ^{1}H NMR spectrum of compound (2FC4) is however the singlet of the vinylic proton in the position C-3 of the cyclohexenone rings, that resonated at approximately 6.53 ppm integrating for one proton, and confirms that the intramolecular cyclocondensation subsequent to the Michael addition actually took place. The signal due to the C-5 methylene protons appeared as two doublets at *ca.* 3.04 ppm and at *ca.* 3.70 ppm respectively, thereby indicating that they are diastereotropic protons. The signal due to C6–H appeared as a multiplet in the range of 2.87–2.99 ppm integrating for one proton. As for the protons in the aromatic region, the ^{1}H NMR spectrum allowed the assignments of the protons in the furane ring at 7.09 ppm, 7.28 ppm, 7.35 ppm and 7.55 ppm. The number of other aromatic protons on the aryl moiety integrated in the ^{1}H NMR spectra of compound (2FC4) was in good agreement with the factual one.

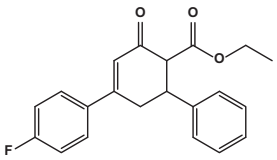
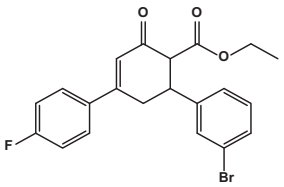
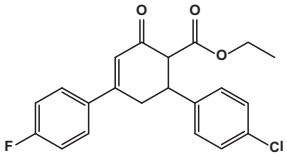
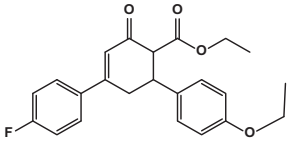
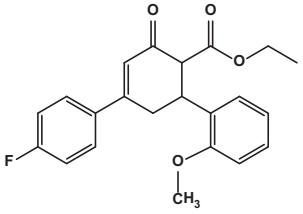
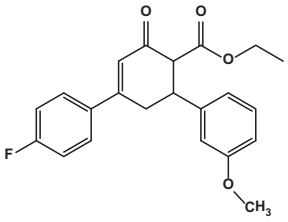
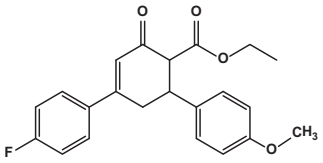
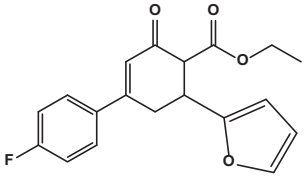
The ^{13}C NMR spectrum of (2FC4) shows a characteristic peak at 169.02 ppm, which confirms carbonyl carbon of the ester group. A peak at *ca.* 193.55 ppm further confirmed the presence of the carbonyl carbon of ketone. While the peak at *ca.* 133.62 ppm showed the presence of vinylic carbon.

Our results revealed that the mass spectrum of (2FC4) also agrees well with the proposed structure that was evidenced from the observation of characteristic peaks. However, the molecular ion peak was found absent in this case. The fragment peak at *m*/*z* 300 could be ascribed to the loss of ester moiety, while the base peak appeared at *m*/*z* 162 could be attributed to the result of Retro-Diels–Alder fission of cyclohexene ring. The peaks appeared at *m*/*z* 272 and 237 are deemed to the loss of carbon monoxide and the side chain respectively.

Docking studies

The docking experiments were performed using the viral enzyme reverse transcriptase (RT) with newly synthesized cyclohexenone based compounds and compared for the interaction energies with reference inhibitor of RT (Nevirapine). The RT structure with its inhibitor, Nevirapine, was obtained from RCSB Protein data base (PDB ID 1jlb). The active site co-ordinates for the docking of RT were taken from X-ray structure of RT-Nevirapine complex, which was (*X* 2.25), (*Y* −35.45) and (*Z* 23.94) respectively. The RT is a unique enzyme, which is responsible to generate the complementary (cDNA) from a RNA template in HIV virus. The RT is needed for the replication of retro-viruses, for example HIV. The

Table 1
Structures and R_f values of cyclohexenone derivatives.

Compound	Structure	Molecular formula	Molecular weight	[a]R_f values × 100
2FO		$C_{21}H_{19}FO_3$	338.37	57
2FB		$C_{21}H_{18}BrFO_3$	417.27	59
2FC4		$C_{21}H_{18}ClFO_3$	372.82	60
2FE		$C_{23}H_{23}FO_4$	382.42	55
2FM2		$C_{22}H_{21}FO_4$	368.14	49
2FM3		$C_{22}H_{21}FO_4$	368.14	53
2FM4		$C_{22}H_{21}FO_4$	368.14	56
2FF		$C_{19}H_{17}FO_4$	328.39	61

[a] Solvent for R_f values = pet-ether:ethylacetae (4:1).

field template showed a good similarity of the most active 2FC4 compound with drug Nevirapine, as shown in Fig. 3.

From theoretical evaluation, one can notice that cyclohexenone based compounds do not break at any point of the Lipinski's rule of five, making them promising lead for drug candidates [44]. Fig. 3 predicts the 3D model of RT complexed with 2FC4, which shows a great affinity with reverse transcriptase. The result of the docking experiment support the postulation that compounds 2FC4 may act as potent inhibitor of the RT. The 3D docking model shows that the cyclohexenone ring act as hydrophilic bridge, while the halogens containing benzenes ring interacts with non-nucleoside hydrophobic site of reverse transcriptase, as shown in Fig. 4.

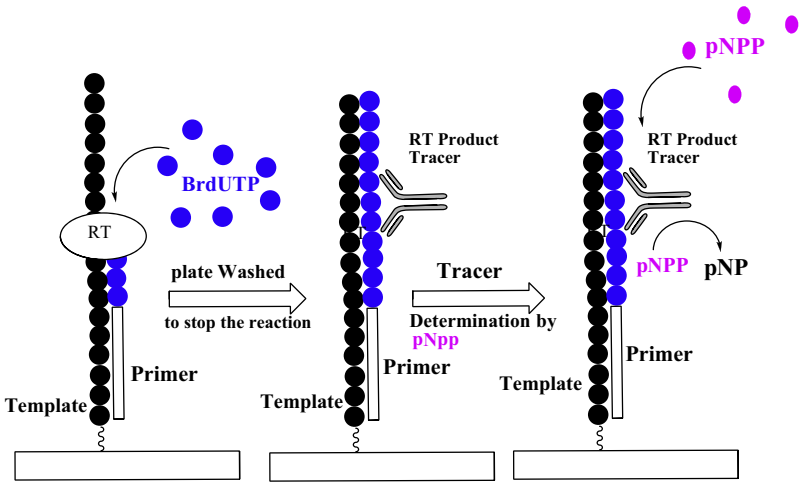


Fig. 2. Mechanism of action of RETRO SYS™ kit for RT bioassay.

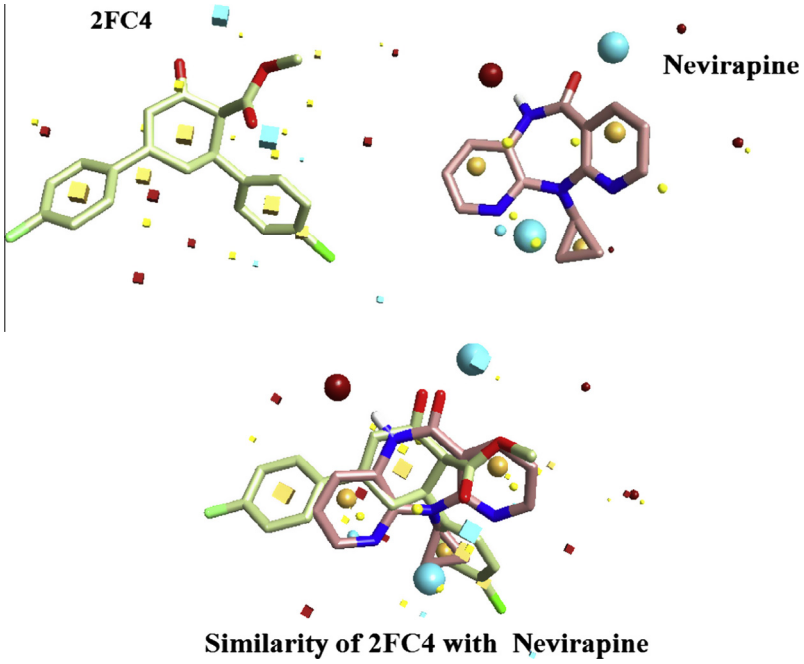


Fig. 3. Representing the similarity of 2FC4 with Nevirapine calculated by field templater.

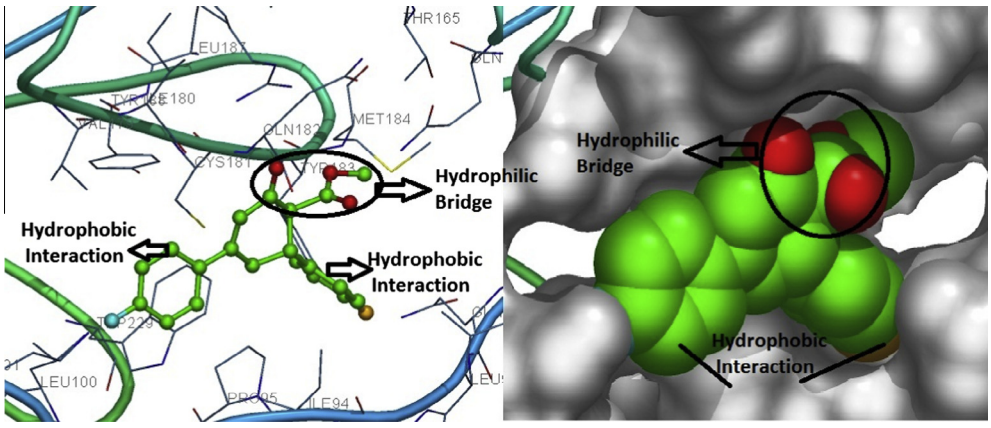


Fig. 4. Docking model derived for 2FC4 with the catalytic portion of reverse transcriptase.

Table 2
The docking data of cyclohexenones with reverse transcriptase (RT).

Compound	Free energy of binding (ΔG) Kcal/mol	Docking energy Kcal/mol	Inhibition constant (Ki) μM
2FO	−5.93	−6.86	45.06
2FB	−7.78	−7.98	66.75
2FC4	**−8.01**	**−8.86**	**440.00**
2FE	−6.82	−7.73	54.05
2FM2	−4.05	−5.46	1.07
2FM3	−4.42	−5.66	3.08
2FM4	−6.13	−6.05	120.03
2FF	−4.02	−5.09	1.13
Nevirapine	**−7.88**	**−8.01**	**350.96**

The bold data represents the docking protocol of reference reverse transcriptase inhibitor 'Nevirapine' and our synthesized cyclohexenones derivative (2FC4) showing reverse transcriptase inhibition in comparison with Nevirapine.

We have used the AutoDock to determine the docked energy, free energy of binding (ΔG) and the inhibition constant (Ki) compared with reference Nevirapine (Table 2).

The calculated interaction energies of all cyclohexenone based compounds are in negative, which shows that 2FO, 2FC4, 2FE and 2FM4 are potent inhibitor of reverse transcriptase. The 2FC4 is the most potent compound as compared to other cyclohexenones and reference standard Nevirapine with a free energy of binding ∼ (ΔG −8.01 Kcal/mole) (Table 2). The chain residue of RT to which the most potent inhibitor 2FC4 binds are PRO95, HIS96, LEU100, LYS101, VAL179, CYS181, ILE382, as shown in Figs. 5 and 6.

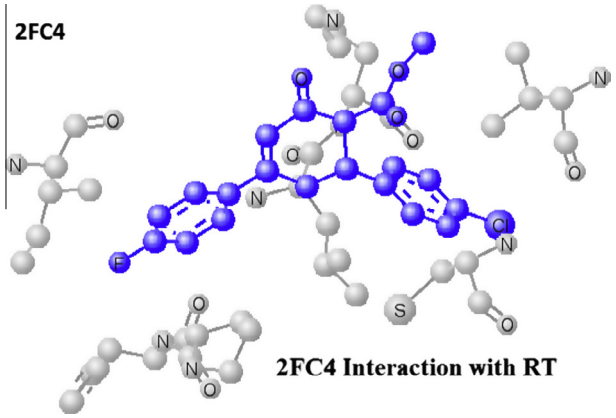


Fig. 5. Predicted 3D binding conformations of 2FC4 with catalytic portion of reverse transcriptase (RT).

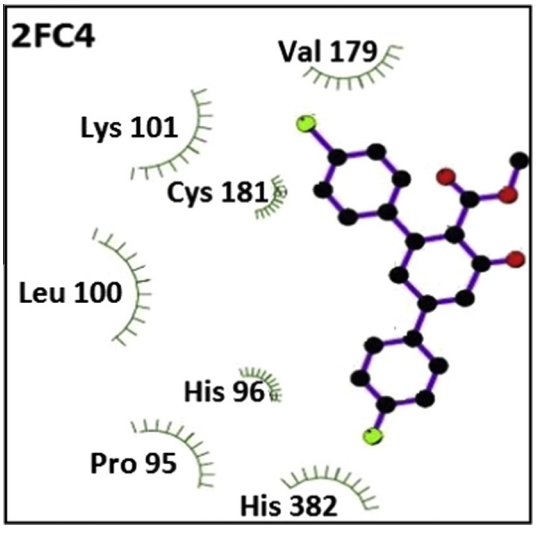


Fig. 6. Predicted binding conformations of cyclohexenone based 2FC4 with catalytic portion of reverse transcriptase (RT) showing the interacting amino acid with 2FC4.

The close contact of fluoro and chloro containing benzene ring of 2FC4 with PRO95, LEU100, VAL179 and CYS181 residue (3.84, 3.48, 3.38 and 3.10 Å respectively) suggested hydrophobic interaction. The oxygen of cyclohexenone make interaction with LYS101 residue (3.14 Å) suggested polar interaction. On the other hand –F of benzene ring make interaction with ILE382 residue (3.15 Å) suggested halogen bond interaction. These interactions stabilized the most potent compound 2FC4 in the active site of RT as contributing favorable free binding energy (ΔG −8.01 Kcal/mol).

Molecular descriptors-based SAR studies of cyclohexenone derivative

For the comprehension of three-dimensional microscopic interactions and binding between a ligand and a receptor, a detailed analysis in structure–activity relationship (SAR) is important in drug design and synthesis. A number of chemical parameters are reported to be responsible for their molecular interactions. Although many reports on the structure activity relationships based on the biological properties have been the subject of a large number of investigations [45,46]. Calculations were performed by software package, namely ACDlabs, in order to obtain a quantitative molecular description of reported cyclohexenone (2FO, 2FB, 2FC4, 2FE, 2FM2, 2FM3, 2FM4 and 2FF) with RT and its structural properties. Following are the molecular descriptors and their values are tabulated in Table 3.

(1) Octanol–water partition coefficient (Log*P*).
(2) Bioconcentraion factor (BCF).
(3) Polar surface area (PSA).

Log*P* (Table 2) values are compatible with those described as a predictive indicator of a drug's capacity for membrane penetration [47]. The fluoro, chloro, bromo, and ethoxy groups containing compounds (2FB, 2FC4, 2FE) are more active ones in the series of 8 compounds (Table 2). Their Log*P* values (5.25, 5.43 and 5.11 respectively) are higher than the other compounds. The octanol–water partition coefficient (Log*P*) is representative of steric interactions and in the present study it showed a good correlation with the free binding energy (ΔG) and inhibition constant (Ki) values (Table 2) of cyclohexenone. A direct correlation of the free binding energy [(ΔG), Log*P* and *K*] values of cyclohexenone was indicative of the fact that cyclohexenone with a higher Log*P* are expected to be more active as is reflected by the halogenated cyclohexenone 2FC4 (Table 3).

The theoretical data indicates that the halogen group is somewhat more active against reverse transcriptase (RT) than methoxy and ethoxy. This difference can be attributed to the hydrophobic property that bromo and chloro are more hydrophobic than meth-

Table 3
Calculated molecular descriptors.

Compounds	R	Log *P*	Log Koc	Log BCF	No. of H-donor	No. of H-acceptor	PSA
2FO	–F	4.66	3.9	3.3	0	3	43.17
2FB	2-Br	5.25	4.3	3.9	0	3	43.07
2FC4	4-Cl	5.43	4.4	3.8	0	4	52.6
2FE	4-OC_2H_5	5.11	4.2	3.7	0	4	52.6
2FM2	2-OCH_3	3.90	3.8	3.6	0	4	52.01
2FM3	3-OCH_3	4.01	4.5	4.8	0	4	52.67
2FM4	4-OCH_3	4.57	3.9	3.2	0	4	52.80
2FF	Furan	3.82	3.5	2.7	0	4	56.51

Table 4
The inhibitory analysis of cyclohexenone against reverse transcriptase (RT).

Compounds	2FO	2FB	2FC4	2FE	2FM2	2FM3	2FM4	2FF	Nevirapine
IC_{50} (μg/ml)	3.521	0.989	0.207	1.413	2.731	1.875	1.590	3.879	0.610

oxy and in turn it has greater binding energy to bind with biomolecule and inhibit their life cycle and growth.

Reverse transcriptase (RT) inhibitory assay

To understand the mechanism by which the cyclohexenone derivatives induced the reverse transcriptase (RT) inhibitory activity. The inhibitory activity of the compound were performed against the reverse transcriptase by using a commercial kit (RETRO SYS™ RT activity kit), the result were shown in Table 4. The compound 2FC4 with strong inhibition activity effectively inhibited the reverse transcriptase (RT) with IC_{50} of 0.207 μg/ml. There was good agreement between the docking experiment and reverse transcriptase (RT) Ki values, indicating that the cyclohexenone based compounds act as inhibitors for the reverse transcriptase (RT).

Conclusions

The present study revealed that the cyclohexenone (2FB, 2FC4 and 2FE) are comparatively more active against reverse transcriptase (RT) than (2FO, 2FM2, 2FM3, 2FM4 and 2FF). Theoretically, the compound 2FC4 is found potentially active against RT. The inhibition of RT depends on the hydrophobic effect and electronic effects of a substituent; while it is the electronic effect of chloro group, which increases the inhibition by increasing stability of enzyme-inhibitor complex with free binding energy (ΔG −8.01 Kcal/mole) and MIC values (IC_{50} 0.207 μg/ml). This is also supported by the calculated Log *P* values of studied compounds. The docking and bioassay studies are also supported results and tell us that the compound 2FC4 is the most potent inhibitor of reverse transcriptase.

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