

Design, Synthesis, and Evaluation of a novel Curcumin–Isoniazid Bis-Hydrazone Conjugate as an HIV-1 Reverse Transcriptase Inhibitor

Sathish Kumar Angadi¹

Kakatiya University

Md. Sanobar¹

Kakatiya University

Pasula Janakiramulu¹

Kakatiya University

Estari Mamidala

drestari@kakatiya.ac.in

Kakatiya University

Research Article

Keywords: curcumin,isoniazid bis-hydrazone ,HIV-1 reverse transcriptase ,antiviral drug discovery ,medicinal chemistry

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11341028/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The development of structurally novel HIV-1 reverse transcriptase (RT) inhibitors remains important for addressing the challenges associated with drug resistance and the limited chemical diversity of existing antiretroviral agents. In the present study, a novel curcumin–isoniazid bis-hydrazone conjugate was synthesized by condensation of curcumin with isoniazid and characterized using spectroscopic techniques. The conjugate was obtained as a yellow-orange solid in 45% yield, with its structural characteristics supported by FTIR, ^1H NMR, ^{13}C NMR and ESI-MS analyses. The inhibitory activity against recombinant HIV-1 RT was evaluated using a colorimetric reverse transcriptase assay. The conjugate exhibited concentration-dependent inhibition, increasing from $38.65 \pm 3.25\%$ at $2.56 \mu\text{M}$ to $87.59 \pm 1.23\%$ at $164.29 \mu\text{M}$, with an estimated IC_{50} of $10.6 \mu\text{M}$ ($6.44 \mu\text{g/mL}$). Nevirapine, used as the reference inhibitor, showed an IC_{50} of $6.5 \mu\text{M}$ under the experimental conditions. Molecular docking against the Y181C mutant HIV-1 RT structure (PDB ID: 1JLB) showed a predicted binding energy of $-8.1 \text{ kcal mol}^{-1}$ for the conjugate, compared with $-6.9 \text{ kcal mol}^{-1}$ for nevirapine. The conjugate formed hydrogen-bond interactions with Arg172 and Gln161, together with multiple additional contacts within the binding pocket. These findings identify the curcumin–isoniazid bis-hydrazone conjugate as a novel HIV-1 RT inhibitory scaffold and provide a basis for further structural optimization and biological evaluation.

Introduction

Human immunodeficiency virus type 1 (HIV-1) infection remains a major global health challenge despite substantial advances in antiretroviral therapy. Combination antiretroviral therapy has transformed HIV infection into a manageable chronic condition; however, the emergence of drug-resistant viral variants, long-term treatment requirements, adverse effects and limitations associated with existing drug classes continue to drive the search for new antiviral agents [1]. Among the viral proteins targeted by antiretroviral drugs, HIV-1 reverse transcriptase (RT) is particularly important because it catalyzes the conversion of the viral RNA genome into DNA, an essential step in the HIV replication cycle. Its central role in viral replication and its well-characterized structural features have made RT one of the most extensively investigated targets for anti-HIV drug discovery [2].

HIV-1 RT is a heterodimeric enzyme composed of p66 and p51 subunits and possesses both RNA- and DNA-dependent DNA polymerase and RNase H activities. Inhibition of its polymerase function prevents the formation of proviral DNA and consequently interferes with viral replication. Two major classes of RT inhibitors, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), are important components of antiretroviral therapy. Unlike NRTIs, NNRTIs generally inhibit RT through binding to an allosteric pocket adjacent to the polymerase active site. Structural studies of RT–inhibitor complexes have provided detailed insights into the molecular basis of inhibitor recognition, binding and resistance and continue to support structure-based drug design [2–3]. Despite the clinical success of NNRTIs, the relatively low genetic barrier to resistance associated with several members of this class remains an important limitation. Amino-acid substitutions within or around the NNRTI-binding pocket can alter ligand accommodation and reduce inhibitor susceptibility. Consequently, the discovery of structurally diverse NNRTIs capable of establishing favorable interactions with RT while retaining activity against resistant variants remains an active area of medicinal chemistry research. Recent developments have explored diverse chemical scaffolds, including diarylpyrimidines, indoles, DABOs, HEPT analogues and quinoline-based compounds, together with scaffold hopping, molecular hybridization and other structure-based strategies to improve potency and overcome resistance [4].

Molecular docking and other computational approaches have increasingly become valuable components of contemporary HIV-1 RT inhibitor discovery. Docking can provide insights into the orientation of a ligand within the RT binding pocket and identify potential hydrogen-bonding, hydrophobic and other non-covalent interactions that may contribute to ligand recognition. [2] highlighted the increasing application of molecular docking in the characterization and design of HIV-1 RT inhibitors. More recent studies have combined docking with molecular dynamics, machine learning and other computational approaches to identify and optimize potential RT inhibitors [5]. Recent experimental studies further demonstrate the value of integrating biochemical and computational approaches in RT inhibitor discovery [6]. identified a novel small-molecule inhibitor with a non-nucleoside mode of action and demonstrated its activity against HIV-1, including selected drug-resistance-associated variants; molecular docking was subsequently used to investigate its interaction with RT [7]. Similarly, recent computational and experimental investigations have continued to identify new chemical scaffolds with predicted or demonstrated RT inhibitory properties, highlighting the potential of structure-guided approaches for expanding the chemical space of HIV-1 inhibitors [8].

Natural-product-derived scaffolds represent an attractive source of structural diversity for medicinal chemistry because they contain multiple functional groups capable of participating in hydrogen bonding, hydrophobic interactions and other molecular recognition processes. Curcumin, a naturally occurring diarylheptanoid, has attracted considerable interest because of its broad biological activities and chemically versatile structure. However, its direct pharmaceutical development is limited by factors including relatively poor physicochemical and pharmacokinetic properties. Structural modification and molecular hybridization of curcumin with biologically relevant pharmacophores therefore represent potential strategies for generating new derivatives with altered biological and molecular properties. In the present study, curcumin was combined with isoniazid, a hydrazide-containing pharmacophore, through bis-hydrazone formation to generate a structurally distinct conjugate.

In the present study, we synthesized and characterized a novel curcumin–isoniazid bis-hydrazone conjugate and evaluated its inhibitory activity against recombinant HIV-1 RT using a colorimetric enzyme assay. Nevirapine was used as a reference RT inhibitor. In parallel, molecular docking was performed using the Y181C mutant HIV-1 RT structure (PDB ID: 1JLB) to investigate the predicted binding mode and protein–ligand interactions of the newly synthesized conjugate. The objective of this study was to determine whether the newly designed conjugate exhibits measurable HIV-1 RT inhibitory activity and to provide a molecular-level rationale for its interaction with the RT binding pocket. The combined experimental and computational findings provide an initial assessment of this conjugate as a novel chemical scaffold for further HIV-1 RT inhibitor development.

Results and discussion

Synthesis of the curcumin–isoniazid bis-hydrazone conjugate

The target conjugate was prepared by condensation of curcumin with two equivalents of isoniazid under acidic reflux conditions in ethanol. The transformation was accompanied by formation of a yellow–orange precipitate. Following isolation and purification, the reported isolated yield was 45%. The reaction scheme is summarized in **Fig. 1**. The synthetic design converts the carbonyl functionalities of the curcumin scaffold into hydrazone linkages bearing isonicotinoyl groups, thereby generating a structurally distinct conjugate relative to the parent curcumin scaffold.

¹H NMR characterization

The ¹H NMR spectrum recorded in CD₃OD at 400 MHz showed resonances at δ 7.377, 6.985, 6.789 and 6.622 ppm, corresponding predominantly to aromatic and conjugated proton environments (**Figure S1**). A resonance at δ 3.896 ppm was assigned to methoxy protons. Additional signals at δ 4.899 and 3.304 ppm were reported but were treated cautiously because of possible exchangeable and/or solvent-associated contributions. Signals at δ 2.001 and 1.226 ppm were also observed in the aliphatic region.

¹³C NMR characterization

The ¹³C NMR spectrum recorded at 100 MHz in CD₃OD contained resonances at δ 149.229, 148.060, 132.095, 130.402, 128.899, 121.384, 116.555, 110.277 and 100.105 ppm, assigned to aromatic, heteroaromatic and conjugated carbon environments (**Figure S2**). The resonance at δ 56.333 ppm was assigned to methoxy carbon. Signals at δ 49.213 and 48.787 ppm were considered solvent-related in the patent interpretation. No prominent resonance was reported in the δ 180–200 ppm region. Elemental analysis gave C 65.12%, H 5.30%, N 13.81% and O 15.77%, consistent with the reported molecular formula C₃₃H₃₂N₆O₆.

Mass spectrometric and FTIR characterization

ESI-MS provided a positive-ion signal at m/z 609. These signals were reported to be consistent with a neutral molecular mass of approximately 608 Da (**Figure S4**). The FTIR spectrum displayed broad O–H/N–H stretching bands at 3350.45, 3340.43 and 3256.12 cm^{–1}, aromatic C–H stretching at 3050.23 cm^{–1}, and aliphatic C–H stretching at 2942.25 and 2890.52 cm^{–1}. Bands at 1656.28 and 1610.67 cm^{–1} were interpreted as consistent with C=O/C=N and conjugated skeletal vibrations. Additional bands at 1455.67, 1120.32 and 1054.21 cm^{–1} were reported in the fingerprint region (**Figure S3**).

Based on the combined spectroscopic and analytical characterization, the synthesized compound was assigned the IUPAC name N'-[(1E,3Z,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-(2-isonicotinoylhydrazineyl)hepta-1,6-dien-3-ylidene]isonicotinohydrazide.

Inhibition of HIV-1 reverse transcriptase

The curcumin–isoniazid bis-hydrazone conjugate exhibited a concentration-dependent inhibition of HIV-1 reverse transcriptase (RT). As shown in **Table 1**, the percentage inhibition increased progressively with increasing concentration of the conjugate, from 38.65 ± 3.25% at 2.56 μM to 87.59 ± 1.23% at 164.29 μM. Intermediate concentrations of 5.13, 10.27, 20.54, 41.07, and 82.15 μM produced 45.04 ± 1.23%, 49.65 ± 4.30%, 57.80 ± 1.63%, 67.73 ± 2.21%, and 76.24 ± 6.84% inhibition, respectively (**Fig. 2**).

Nevirapine (NVP), included as the reference reverse transcriptase inhibitor, also demonstrated a concentration-dependent inhibitory response. Its HIV-1 RT inhibition increased from 40.78 ± 2.68% at 2.56 μM to 83.69 ± 2.68% at 164.29 μM. The corresponding inhibition values at 5.13, 10.27, 20.54, 41.07, and 82.15 μM were 47.52 ± 2.21%, 56.03 ± 1.63%, 58.51 ± 1.84%, 68.44 ± 0.61%, and 73.05 ± 2.68%, respectively (**Fig.2**). Thus, both the synthesized conjugate and NVP showed increasing inhibition of HIV-1 RT across the tested concentration range.

For dose–response analysis, the test concentrations were transformed to log₁₀ concentration and plotted against the corresponding percentage inhibition values. The IC₅₀ estimated from the concentration–response analysis was 10.6 μM (6.44 μg/mL) for the curcumin–isoniazid bis-hydrazone conjugate, whereas NVP showed an IC₅₀ of 6.5 μM (1.73 μg/mL). The complete concentration–response data and log₁₀-transformed concentrations are presented in **Table 1**.

Table 1 Inhibition of HIV-1 reverse transcriptase by the curcumin–isoniazid bis-hydrazone conjugate and nevirapine (NVP). Values are expressed as mean ± SD (n = 3).

Test concentration (μM)	$\log_{10}$ concentration	Conjugate HIV-1 RT inhibition (%)	NVP HIV-1 RT inhibition (%)
2.56	0.408	38.65 ± 3.25	40.78 ± 2.68
5.13	0.71	45.04 ± 1.23	47.52 ± 2.21
10.27	1.012	49.65 ± 4.30	56.03 ± 1.63
20.54	1.313	57.80 ± 1.63	58.51 ± 1.84
41.07	1.614	67.73 ± 2.21	68.44 ± 0.61
82.15	1.915	76.24 ± 6.84	73.05 ± 2.68
164.29	2.216	87.59 ± 1.23	83.69 ± 2.68
IC_{50}	—	$10.6 \mu\text{M}$ ($6.44 \mu\text{g/mL}$)	$6.5 \mu\text{M}$ ($1.73 \mu\text{g/mL}$)

Molecular docking analysis

Molecular docking was performed to investigate the predicted binding mode of the curcumin–isoniazid bis-hydrazone conjugate within the HIV-1 reverse transcriptase (RT) binding site. Nevirapine (NVP), a known HIV-1 RT inhibitor, was included as the reference compound under the same docking conditions. The docking results are summarized in **Table 2**, and the predicted binding mode and protein–ligand interactions of the conjugate are illustrated in **Fig. 3**.

Table 2 Molecular docking scores and predicted interactions of the curcumin–isoniazid bis-hydrazone conjugate and nevirapine with HIV-1 reverse transcriptase

Sl. No.	Compound	Binding energy (kcal mol^{-1})	No. of H bonds	H-bond interacting residues	Other interacting residues
1	Curcumin–isoniazid bis-hydrazone conjugate	–8.1	2	Arg172, Gln161	Ile382, Ile180, His96, Pro95, C
2	Nevirapine (NVP)	–6.9	1	Gln91	Glu89, Leu92, Gln161, Val90,

The curcumin–isoniazid bis-hydrazone conjugate exhibited a predicted binding energy of $-8.1 \text{ kcal mol}^{-1}$, which was more favorable than that obtained for NVP ($-6.9 \text{ kcal mol}^{-1}$). The more negative docking score suggests a stronger predicted interaction of the conjugate with the modeled HIV-1 RT binding site under the applied docking conditions. The conjugate formed two hydrogen-bond interactions, involving Arg172 and Gln161, which may contribute to stabilization of the ligand within the binding pocket.

In addition to hydrogen bonding, the conjugate showed interactions with several residues distributed around the binding cavity, including Ile382, Ile180, His96, Pro95, Gly99, Val381, Ile94, Tyr183, Leu92, Leu100, Gln91, Val179, Lys101, Lys103, Tyr188, Cys181, Gln182, and Thr165. The interaction map shown in Figure 3 indicates that the conjugate is accommodated within an extensive binding environment comprising both hydrophobic and polar residues of the HIV-1 RT binding pocket. Figure 3 also illustrates the location of the conjugate within the three-dimensional binding site and the corresponding two-dimensional protein–ligand interaction map generated using BIOVIA Discovery Studio Visualizer. The multiple contacts observed around the ligand, together with the two hydrogen-bond interactions, may contribute to the favorable predicted binding energy of the conjugate.

The reference inhibitor NVP showed a docking energy of $-6.9 \text{ kcal mol}^{-1}$ and formed one hydrogen bond with Gln91. Other residues surrounding the docked NVP included Glu89, Leu92, Gln161, Val90, Gln182, Tyr183, Ile94, Thr165 and Pro95. Thus, under the same docking protocol, the curcumin–isoniazid bis-hydrazone conjugate showed a more favorable predicted binding energy and a greater number of hydrogen-bond interactions than NVP.

The docking analysis suggests that the curcumin–isoniazid bis-hydrazone conjugate can be accommodated within the HIV-1 RT binding region and establishes multiple contacts with residues lining the binding pocket. The predicted binding energy of $-8.1 \text{ kcal mol}^{-1}$, compared with $-6.9 \text{ kcal mol}^{-1}$ for NVP, provides a computational rationale for the experimentally observed HIV-1 RT inhibitory activity of the conjugate. However, these docking results should be regarded as supportive computational evidence of a favorable binding mode rather than direct proof of the inhibition mechanism.

HIV-1 reverse transcriptase (RT) remains an important target for antiretroviral drug discovery because of its essential role in the conversion of viral RNA into DNA. Although combination antiretroviral therapy has substantially improved the clinical management of HIV infection, the emergence of drug-resistant variants continues to create a need for structurally diverse RT inhibitors. In particular, the non-nucleoside RT inhibitor (NNRTI) binding pocket has been extensively exploited for structure-based drug discovery, and recent studies continue to emphasize the importance of developing new chemotypes capable of maintaining favorable interactions with RT despite resistance-associated mutations [9].

In the present study, the newly synthesized curcumin–isoniazid bis-hydrazone conjugate demonstrated appreciable and concentration-dependent inhibition of HIV-1 RT in the biochemical enzyme assay. RT inhibition increased from $38.65 \pm 3.25\%$ at $2.56 \mu\text{M}$ to $87.59 \pm 1.23\%$ at $164.29 \mu\text{M}$, with an estimated IC_{50} of $10.6 \mu\text{M}$ ($6.44 \mu\text{g/mL}$). The progressive increase in inhibition with increasing compound concentration supports a concentration–response relationship and indicates that the conjugate directly interferes with the activity of recombinant HIV-1 RT under the experimental conditions employed. Importantly, the present assay measures enzyme inhibition rather than inhibition of HIV-1 replication in cells; therefore, the observed activity should be interpreted as biochemical RT inhibitory activity and not yet as cellular antiviral efficacy.

The activity observed for the conjugate is consistent with the continuing interest in structurally diverse small molecules targeting HIV-1 RT. Recent reviews have highlighted structure-based design, molecular docking and chemical scaffold diversification as important strategies for developing new NNRTIs and addressing resistance associated with conventional RT inhibitors [10]. A recent study by further demonstrated the value of combining biochemical RT assays with cellular antiviral studies and molecular docking to establish the mechanism of newly identified RT inhibitors [11, 12]. Thus, the present biochemical findings provide an initial experimental basis for considering the curcumin–isoniazid conjugate as a new RT-targeting chemical scaffold.

The comparison with nevirapine provides additional context for the observed activity. Under the conditions used in the present study, NVP produced concentration-dependent RT inhibition, with an estimated IC_{50} of 6.5 μ M (1.73 μ g/mL), whereas the conjugate showed an IC_{50} of 10.6 μ M. Thus, NVP displayed greater potency in the present biochemical assay. Nevertheless, the conjugate achieved 87.59% inhibition at 164.29 μ M, demonstrating substantial inhibition at the upper end of the tested concentration range. The difference in activity between the new conjugate and NVP also emphasizes that a favorable structural interaction with RT does not necessarily translate directly into equivalent enzymatic potency. Recent NNRTI research has similarly demonstrated that changes in molecular architecture can substantially influence binding interactions, resistance profiles and biological activity [13].

The molecular docking results provide a structural rationale for the experimentally observed RT inhibition. Docking of the conjugate into the HIV-1 RT structure (PDB ID 1JLB, Y181C mutant) produced a predicted binding energy of -8.1 kcal mol $^{-1}$, compared with -6.9 kcal mol $^{-1}$ for NVP under the same docking protocol. The more negative docking score indicates a more favorable *predicted* ligand–receptor interaction for the conjugate in the modeled binding site. However, docking scores should not be interpreted as direct measurements of experimental binding affinity or enzymatic potency. Rather, they provide a computational assessment of the relative favorability of the predicted binding poses. Recent HIV-1 RT studies similarly use molecular docking as a complementary tool for rationalizing experimental RT inhibition and identifying important protein–ligand interactions [14].

The interaction analysis further supports the predicted accommodation of the conjugate within the RT binding region. The conjugate established two hydrogen-bond interactions involving Arg172 and Gln161, together with additional contacts involving Ile382, Ile180, His96, Pro95, Gly99, Val381, Ile94, Tyr183, Leu92, Leu100, Gln91, Val179, Lys101, Lys103, Tyr188, Cys181, Gln182 and Thr165. These interactions indicate that the relatively extended conjugate can occupy a broad region of the modeled binding cavity and establish contacts with both polar and hydrophobic residues. The presence of several contacts may help stabilize the predicted ligand orientation and may contribute to the favorable docking score.

The interaction pattern is particularly noteworthy because several residues identified in the docking analysis, including Tyr181-related and Tyr188-region residues, Lys101, and other residues lining the NNRTI-associated pocket, have been extensively discussed in the context of HIV-1 RT inhibitor recognition and resistance. Structural studies have established that mutations within and around the NNRTI binding pocket can alter hydrophobic contacts, steric complementarity and ligand accommodation [15]. The use of the Y181C mutant structure in the present computational analysis is therefore relevant to evaluating whether the conjugate can adopt a favorable binding orientation in a structurally altered RT pocket. Nevertheless, because only a single mutant structure was examined, the present docking results cannot establish activity against the broader spectrum of NNRTI-resistant RT variants.

Interestingly, the conjugate showed a greater number of hydrogen-bond interactions than NVP in the docking analysis. NVP formed one hydrogen bond with Gln91, whereas the conjugate formed two hydrogen bonds with Arg172 and Gln161, in addition to multiple other contacts. This observation provides a plausible structural explanation for the favorable docking score of the conjugate. Similar relationships between hydrogen-bonding patterns, additional hydrophobic contacts and experimentally determined RT inhibition have been reported in recent structure-based HIV-1 RT inhibitor studies. For example, recent work on a novel non-nucleoside RT inhibitor demonstrated that multiple hydrogen bonds and additional van der Waals, hydrophobic and π -interactions contributed to its predicted binding mode and were considered alongside experimental inhibition data [14].

The present findings are also consistent with the broader direction of contemporary HIV-1 RT inhibitor research, in which biochemical inhibition, structural modeling and molecular docking are increasingly integrated to identify new scaffolds. Recent investigations have reported novel RT inhibitors in which docking was used to explain experimentally observed activity and to explore interactions with wild-type or mutant RT structures [13]. In addition, a recent study of α -amyrin combined an HIV-1 RT biochemical assay with docking and reported dose-dependent enzyme inhibition together with favorable predicted interactions in the RT binding region, illustrating the value of combining experimental and computational approaches for preliminary identification of RT-targeting compounds [16].

Taken together, the *in vitro* and *in silico* findings provide complementary evidence for the biological relevance of the curcumin–isoniazid bis-hydrazone scaffold. The concentration-dependent biochemical inhibition demonstrates that the compound can inhibit recombinant HIV-1 RT, while the docking analysis indicates that the conjugate can adopt a favorable orientation within the modeled RT binding region and establish multiple stabilizing contacts. The stronger predicted docking score relative to NVP should, however, be regarded as a structural observation rather than evidence that the conjugate is more potent than NVP, because the experimental IC_{50} of the conjugate was higher than that of NVP in the present assay.

Materials and methods

Chemicals and reagents

Curcumin and isoniazid were used as the starting materials for the synthesis of the curcumin–isoniazid bis-hydrazone conjugate. Ethanol was used as the reaction solvent, while acetic acid was used as the acid catalyst. Cold ethanol and distilled water were used for washing the crude product. The crude product was purified by silica-gel column chromatography using n-hexane/ethyl acetate (8:2, v/v) as the eluent. All reagents used for the synthesis were of analytical grade and were obtained from reliable commercial suppliers. n-Hexane and ethyl acetate were purchased from BLD Pharmatech Co., Ltd.

Synthesis of the curcumin–isoniazid bis-hydrazone conjugate

Curcumin (368 mg, 1 mmol) was dissolved in 15 mL of ethanol under continuous stirring. Separately, isoniazid (274 mg, 2 mmol) was dissolved in 10 mL of ethanol. The isoniazid solution was added dropwise to the curcumin solution with constant stirring, followed by addition of 2–3 drops of acetic acid. The reaction mixture was refluxed at 60–80 °C for 6 h. The reaction progress was monitored by thin-layer chromatography (TLC) using n-hexane/ethyl acetate (8:2, v/v) as the solvent system. After completion, the mixture was cooled to room temperature and the resulting yellow–orange precipitate was collected by vacuum filtration, washed with cold ethanol and distilled water, and dried under vacuum. The patent specification reports subsequent silica-gel column purification using n-hexane/ethyl acetate (8:2, v/v), affording the target conjugate as a yellow–orange solid in 45% yield.

Spectroscopic characterization

The synthesized Curcumin–isoniazid bis-hydrazone conjugate was characterized by ESI-MS, FTIR, ¹H NMR, and ¹³C NMR.

Yellow-orange solid. Yield: 45%. ¹H NMR (400 MHz, CD₃OD) δ 7.377, 6.985, 6.789, 6.622, 4.899, 3.896 (OCH₃), 3.304, 2.001, 1.226. ¹³C NMR (100 MHz, CD₃OD) δ 149.229, 148.060, 132.095, 130.402, 128.899, 121.384, 116.555, 110.277, 100.105, 56.333 (OCH₃), 49.213, 48.787. ESI-MS m/z 609 [M + H]⁺ and 607 [M – H][–], consistent with a molecular mass of approximately 608 Da. FTIR (KBr) ν_{max} 3350.45–3256.12 (O–H/N–H), 3050.23 (aromatic C–H), 2942.25–2890.52 (aliphatic C–H), 1656.28 and 1610.67 cm^{–1} (C=O/C=N). Molecular formula: C₃₃H₃₂N₆O₆.

In vitro HIV-1 reverse transcriptase inhibition assay

The inhibitory activity of the synthesized curcumin–isoniazid bis-hydrazone conjugate against HIV-1 reverse transcriptase (HIV-1 RT) was evaluated using a Reverse Transcriptase Assay, colorimetric kit (Cat. No. 11 468 120 910; Roche Diagnostics GmbH), according to the manufacturer's instructions. Nevirapine (NVP) was used as the reference HIV-1 RT inhibitor. The assay is based on the incorporation of digoxigenin (DIG)- and biotin-labeled nucleotides into newly synthesized DNA by reverse transcriptase. The resulting labeled DNA is captured on streptavidin-coated microplate wells, detected using an anti-DIG antibody conjugated to peroxidase (Anti-DIG-POD), and visualized using ABTS substrate. The intensity of the developed color is directly proportional to reverse transcriptase activity.

For inhibition studies, 4–6 ng of recombinant HIV-1 RT was diluted in 20 µL of the kit-provided lysis buffer and transferred to individual reaction tubes. A reaction containing lysis buffer without HIV-1 RT was used as the negative control. The synthesized curcumin–isoniazid bis-hydrazone conjugate and NVP were prepared separately at the required test concentrations, and 20 µL of each concentration was added to the corresponding reaction tube containing HIV-1 RT. Subsequently, 20 µL of the reverse transcriptase reaction mixture was added to each tube. The reaction mixture contained the template/primer and nucleotides required for DNA synthesis according to the kit protocol. The reaction mixtures were incubated for 1 h at 37 °C to allow interaction of the test compound or reference inhibitor with HIV-1 RT and subsequent reverse-transcription activity.

Following the enzymatic reaction, 60 µL of each reaction mixture was transferred to the wells of the streptavidin-coated microplate modules supplied with the kit. The plate was covered with an adhesive foil and incubated for 1 h at 37 °C. The reaction solutions were then removed, and the wells were washed five times with 250 µL of washing buffer, with each washing step maintained for approximately 30 s. Subsequently, 200 µL of Anti-DIG-POD working solution (200 mU/mL) was added to each well, and the plate was incubated for a further 1 h at 37 °C. The wells were again washed five times with 250 µL of washing buffer for 30 s per wash.

Finally, 200 µL of ABTS substrate solution was added to each well, and the plate was incubated at 15–25 °C for approximately 10–30 min, allowing development of the green-colored reaction product. Absorbance was measured using a microplate ELISA reader at 405 nm, with approximately 490 nm as the reference wavelength. The assay signal is directly proportional to HIV-1 RT activity; therefore, inhibition by the test compound or reference inhibitor was determined by comparison with the corresponding HIV-1 RT control reaction in the absence of inhibitor.

The curcumin–isoniazid bis-hydrazone conjugate was evaluated at 2.56, 5.13, 10.27, 20.54, 41.07, 82.15 and 164.29 µM, corresponding to 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL, respectively. NVP was evaluated at the concentrations used in the comparative inhibition experiments. All inhibition experiments were performed in triplicate.

Calculation of HIV-1 RT inhibition

The percentage inhibition of HIV-1 RT activity was calculated using the following equation:

$$\text{RT inhibition (\%)} = (1 - A_{\text{sample}} - A_{\text{blank}} / A_{\text{control}} - A_{\text{blank}}) \times 100$$

where A sample is the absorbance obtained in the presence of the test compound or NVP, A control is the absorbance of the HIV-1 RT reaction in the absence of inhibitor, and A blank is the background absorbance.

Determination of IC₅₀

The percentage inhibition values obtained for the curcumin–isoniazid bis-hydrazone conjugate and NVP were used to construct concentration–response curves. The corresponding inhibitor concentrations were transformed to log₁₀ concentration and plotted against the percentage inhibition of HIV-1 RT activity. The IC₅₀ value, defined as the concentration of inhibitor required to inhibit 50% of HIV-1 RT activity, was determined from the fitted concentration–response curve.

Statistical analysis

All experiments were performed in triplicate, and the HIV-1 RT inhibition values are expressed as mean $\pm$ standard deviation (SD). The percentage inhibition values obtained at each concentration were used for dose–response analysis. The corresponding concentrations were transformed to $\log_{10}$ concentration and plotted against the percentage inhibition of HIV-1 RT activity. The IC_{50} value was determined from the fitted concentration–response curve, representing the concentration of the test compound required to inhibit 50% of HIV-1 RT activity.

***In silico* molecular docking study**

Protein preparation

The crystal structure of the Y181C mutant HIV-1 reverse transcriptase (HIV-1 RT) in complex with nevirapine (NVP) was retrieved from the Protein Data Bank (PDB ID: 1JLB) through the RCSB Protein Data Bank (<https://www.rcsb.org/structure/1JLB>). The protein structure was prepared for molecular docking by retaining the relevant HIV-1 RT chain and removing crystallographic water molecules and the co-crystallized ligand, nevirapine. Hydrogen atoms were subsequently added to the protein structure. Kollman charges and Gasteiger charges were assigned during the preparation of the receptor structure, and the resulting protein was saved in PDBQT format for docking using AutoDock Vina [9]. To assess the ability of the docking protocol to reproduce the experimentally observed ligand-binding mode, the co-crystallized nevirapine was removed from the protein and subsequently redocked into the prepared HIV-1 RT binding site under the same docking conditions used for the test compound.

Ligand preparation

The structure of the curcumin–isoniazid bis-hydrazone conjugate was drawn using ChemSketch and saved in SDF format. The ligand structure was subsequently converted to PDBQT format using Open Babel for molecular docking [10]. The prepared ligand was then subjected to docking against the HIV-1 RT binding site.

Molecular docking simulation

Molecular docking was performed using AutoDock Vina to investigate the binding mode and predicted binding affinity of the curcumin–isoniazid bis-hydrazone conjugate toward HIV-1 RT. The docking search space was defined using a grid box centered at $x = 2.124$, $y = -32.411$, and $z = 26.631$ Å, with dimensions of $40 \times 40 \times 40$ Å along the x -, y -, and z -axes, respectively. The same docking parameters were applied to the redocking of nevirapine and to the test compound to facilitate comparison of their predicted binding modes and binding affinities.

The docking poses were ranked according to their predicted binding energies, expressed in kcal/mol, with more negative values indicating energetically more favorable predicted binding. The binding interactions of the docked compounds with HIV-1 RT were subsequently analyzed and visualized using BIOVIA Discovery Studio Visualizer [11, 17]. The interactions were examined with particular attention to hydrogen bonds and other non-covalent interactions involving residues within the predicted ligand-binding site.

Redocking validation

As an internal validation of the docking protocol, the crystallographic ligand nevirapine was redocked into the HIV-1 RT binding pocket after removal from the original PDB structure. The predicted binding pose was compared with the experimentally determined orientation of the co-crystallized ligand. The redocking results were used to assess whether the selected receptor preparation and docking parameters could reproduce the known binding mode before evaluating the curcumin–isoniazid bis-hydrazone conjugate.

Conclusion

These findings demonstrate that the curcumin–isoniazid bis-hydrazone conjugate is a novel small-molecule scaffold with appreciable in vitro HIV-1 reverse transcriptase inhibitory activity. The conjugate exhibited concentration-dependent inhibition of HIV-1 RT, increasing from $38.65 \pm 3.25\%$ at $2.56 \mu\text{M}$ to $87.59 \pm 1.23\%$ at $164.29 \mu\text{M}$, with an estimated IC_{50} of $10.6 \mu\text{M}$. Molecular docking further indicated a favorable predicted binding mode within the HIV-1 RT binding pocket, with a binding energy of $-8.1 \text{ kcal mol}^{-1}$ and hydrogen-bond interactions involving Arg172 and Gln161, together with multiple additional contacts. Although the conjugate showed lower experimental potency than nevirapine, its distinct chemical scaffold and favorable predicted interactions support its potential as a lead structure for further optimization. Further cellular, selectivity, resistance-profile and mechanistic studies are warranted to establish its antiviral potential and therapeutic relevance.

Declarations

Data availability The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Patent disclosure A patent application covering the curcumin–isoniazid bis-hydrazone conjugate and the process for its preparation was filed with the Indian Patent Office on 5 September 2026, under Application No. 202641108061.

Ethics approval No human participants or animal experiments were involved in the work described in this manuscript.

Conflict of interest The authors declare no conflict of interest.

Funding No external funding information was provided.

Author Contribution

E.M.: Conceptualization, design of the study, project administration, supervision, data analysis, writing—original draft, writing—review and editing. S.K.A.: Synthesis and characterization of the compound, experimental investigation, data curation, writing—original draft. M.S.: Biological evaluation, HIV-1 reverse transcriptase inhibition studies, data collection, data analysis, writing—review and editing. P.J.: Molecular docking studies, computational analysis, visualization, data interpretation, writing—original draft. All authors have read and approved the final manuscript.

References

1. World Health Organization. HIV drug resistance: brief report 2024. Geneva: World Health Organization. 2024. <https://www.who.int/publications/i/item/9789240086319>. Accessed October 11, 2026.
2. Singh AK, Kumar A, Arora S, Kumar R, Verma A, Khalilullah H, Jaremko M, Emwas AH, Kumar P. Current insights and molecular docking studies of HIV-1 reverse transcriptase inhibitors. *Chem Biol Drug Des*. 2024;103:e14372.
3. Ragedi MT, Naidu D, Oyeneyin OE, Mkhwanazi NP, Tukulula M. New 3-[(tetrazol-5-yl)methine-N-benzylamine]-1H-indole derivatives: synthesis, anti-HIV activity and molecular docking studies. *Eur J Med Chem*. 2025;118301.
4. Li J, Ye B, Gao S, Liu X, Zhan P. The latest developments in the design and discovery of non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of HIV. *Expert Opin Drug Discov*. 2024;19(12):1439–56.
5. Chandra P, Ganguly S, Deb PK, Ghosh M. Design of novel imidazole derivatives as potential non-nucleoside reverse transcriptase inhibitors using molecular docking and dynamics strategies. *Curr Pharm Des*. 2025;31:65–82.
6. Yu KL, Shin Y, Kim DE, Kim JA, Kang JE, Singh P, Lee KW, Park CM, Kwon H, Kim S, Bae S, Yoon CH. Identification of a novel small-molecule inhibitor of the HIV-1 reverse transcriptase activity with a non-nucleoside mode of action. *Virology*. 2025;22:65.
7. Sayyed SK, Quraishi M, Rajasekharan SK, Sonawane T, Ravichandran V. Zinc C293 as a novel scaffold with dual activity against HIV-1 integrase and reverse transcriptase. *Comput Biol Chem*. 2025;119:108573.
8. Danladi S, Adeniyi AA, Sani ZI, Temitope A. In-silico identification of phytochemical compounds from various medicinal plants as potent HIV-1 non-nucleoside reverse transcriptase inhibitors utilizing molecular docking and molecular dynamics simulations. *Biol Methods Protoc*. 2025;10(1):bpaf081.
9. Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and AutoDock/Vina. *J Comput Aided Mol Des*. 2010;24:417–22.
10. O’Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: an open chemical toolbox. *J Cheminform*. 2011;3:33.
11. BIOVIA, Dassault Systèmes. Discovery Studio Visualizer, version 2016. San Diego, CA, USA: Dassault Systèmes; 2016.
12. Zhang K, Zhang YJ, Li M, Pannecouque C, De Clercq E, Wang S, Chen FE. Deciphering the enigmas of non-nucleoside reverse transcriptase inhibitors (NNRTIs): A medicinal chemistry expedition towards combating HIV drug resistance. *Med Res Rev*. 2025;45(2):426–83.
13. Rahman SMA, Singh G, Khan MS, Balasubramaniam AK, Monga V. Recent developments of pyrimidine appended HIV-1 non-nucleoside reverse transcriptase inhibitors. *Bioorg Chem*. 2025;157:108273.
14. Machado GP, Costa PAG, Leite DI, da Costa LJ, Fernandes TVA, Pimentel LCF, Boechat N, Bastos MM. New potential HIV-1 inhibitors, analogues to efavirenz, containing the isatin core. *Bioorg Med Chem*. 2026;139:118685.
15. Kunwar A, Rai A, Bhambri S, Jha PC. Rational scaffold discovery for HIV-1 reverse transcriptase inhibitors via integrated in silico approaches. *Mol Divers*. 2026;1–18.
16. Mamidala E, Munipally P. Inhibitory potential of α -amyrin from *Calotropis procera* against HIV-1 reverse transcriptase: insights from in silico and in vitro assays. *Silico Pharmacol*. 2025;13:201.
17. Wang Z, Wang W, Gao Z, Gao H, De Clercq E, Pannecouque C, Chen CH, Kang D, Zhan P, Liu X. Structure-based design, synthesis, and biological characterization of indolylarylsulfone derivatives as novel human immunodeficiency virus type 1 inhibitors with potent antiviral activities and favorable drug-like profiles. *J Med Virol*. 2024;96(8):e29830.

Figures

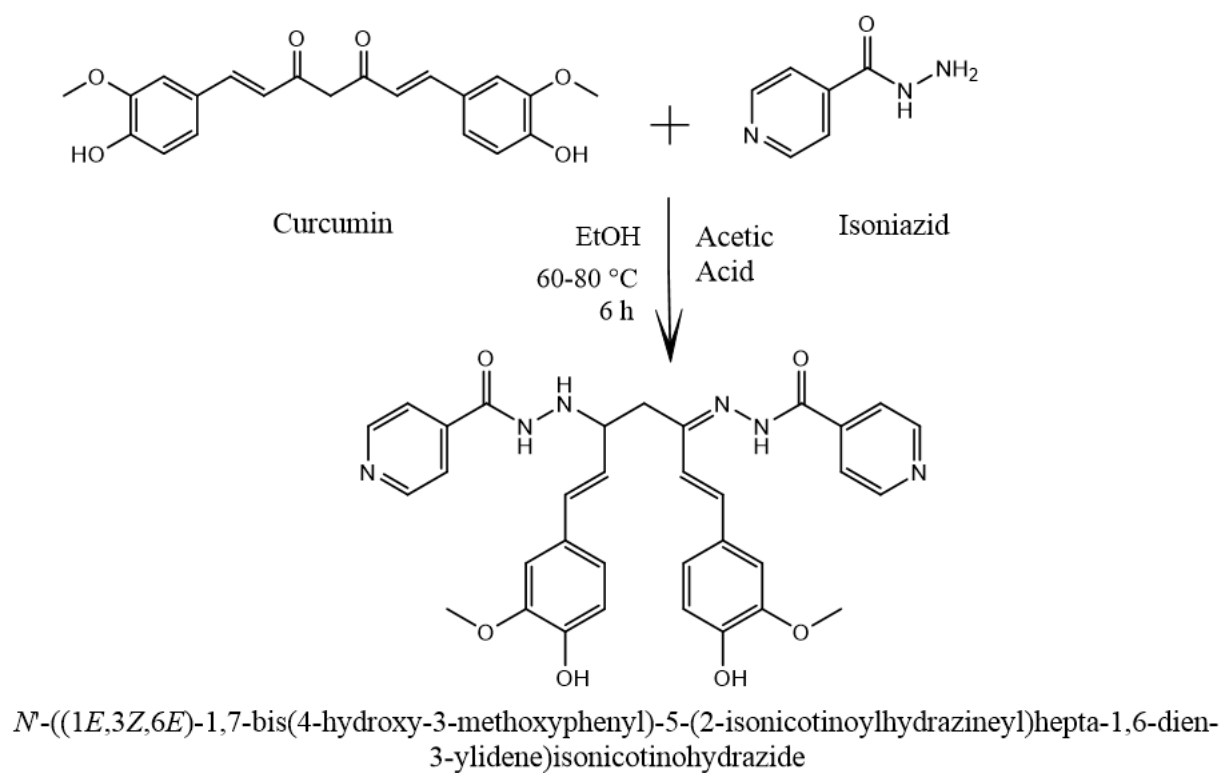


Figure 1

Figure of synthetic scheme of novel curcumin–isoniazid bis-hydrazone conjugate from curcumin and isoniazid.

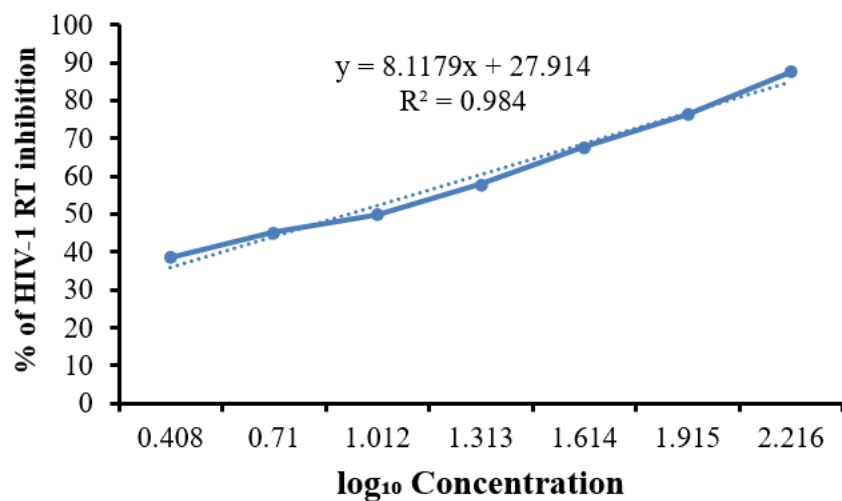


Figure 2

Concentration-dependent inhibition of HIV-1 reverse transcriptase by the curcumin–isoniazid bis-hydrazone conjugate. The percentage of RT inhibition was plotted against the $\log_{10}$ concentration of the test compound.

