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In Silico Anti-HIV and Anticoagulant Activity of [60] Fullerene Conjugated Coumarin and P-Coumaric Acid Isolated from Endophytic Fungi, Alternaria Species-1

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Abstract

Biological compounds conjugated nanoparticles exhibiting many biological activities. Coumarin and p-coumaric acid have been implicated to alleviate multiple diseases and we have isolated from endophytic fungi Alternaria species-1 of Crotalaria pallida. In the present study, we are interested in assessing the anti-HIV and anticoagulant properties of coumarin and p-coumaric acid by molecular interaction studies. We have used coumarin, p-coumaric acid, coumarin conjugated fullerene, p-coumaric acid conjugated fullerene, fullerene individually. Two coagulant and nine HIV-1 proteins were selected for molecular docking studies. We report that p-coumaric acid has greater interaction with coagulant proteins followed by coumarin and fullerene. Among HIV-1 proteins higher interaction was observed with p-coumaric acid especially HIV-1 gp120. However, upon coagulating fullerene to coumarin and p-coumaric acid, coumarin-fullerene showed significantly greater interaction with coagulant proteins and all HIV-1 proteins, compared to p-coumaric acid-fullerene and fullerene. Our in silico study, thus identifies nanoparticles synthesized by fullerene conjugated to naturally occurring coumarin and p-coumaric acid as a potential, safe and cost effective alternative strategy to treating HIV or its use as an anticoagulant.

Keywords: Alternaria species-1; Coumarin; P-coumaric acid; Molecular docking; Anticoagulant; Anti-HIV

Introduction

Coumarins are the benzopyrone compounds belonging to flavonoid groups of secondary metabolites in plants. Presently there are more than 1300 coumarinsthat have been identified in plants, bacteria, and fungi. Plants and their endophytic fungal species are producing pharmaceutically important phytochemicals among which coumarin(s) are one of the important constituents. Coumarin(s) possess many biological activities such as anticancer [1,2], anti-HIV[3,4], neuroprotective [5,6], antioxidant [7-9] and anti-inflammatory [10,11].

Crotalaria pallid Aiton (Fabaceae) is an erect annual or short-lived perennial herb of 1.5 m or more tall. The plant has been used as traditional medicine; its roots have been used to treat swelling of the joints and its leaves as vermifuge [12]. Endophytes are ubiquitous organisms, bacteria or fungi, occurring within plant tissues, distinct from the epiphytes that live on plant surfaces [13]. The endophytes inherit the characteristics of host plants in secreting or production of the secondary metabolites [14-16]. These inherited properties of endophytes are beneficial industrially in the production of important secondary metabolites. Our team has identified coumarin(s) producing endophytic fungal species from Crotalaria pallida. Among them, the leaf endophytic fungi Alternaria species-1 is able to produce coumarin and p-coumaric acid. These coumarins were characterized by UV, NMR, XRD, FTIR and they have exhibited anticancer activity [17,18]. The present investigation was aimed to identify the role of these coumarin(s) and [60] fullerene conjugated coumarin and p-coumaric acid on HIV-1 replicating enzymes/ proteins

and coagulant proteins. The results will help us to know the importance of nanoparticles associated with coumarins possible mechanism in inhibiting the target proteins and it definitely help us in designing drug for HIV-1.

Materials and Methods

Collection of Coumarin and P-Coumaric Acid

Collected the coumarin and p-coumaric acid which were previously isolated from endophytic fungi Alternaria species-1 of Crotalaria pallida. They were characterized by UV, FTIR, XRD and 13C NMR [17,18].

Selection of Ligends

Structure of coumarin and p-coumaric acid was obtained from NCBI Pubchem database. The pharmacokinetics properties were screened utilizing Pre-ADMET device. Sedate likeliness, ADME profile and lethality examination were anticipated for all the two ligands.

Designing of Fullerene Coumarin and p-coumaric Acid

Using Chemdoodle, we draw the fullerene conjugated coumarin and p-courmaric acid nanoparticles. The coumarin and p-coumaric acid firmly attached at position of 59 and 60 carbon atoms of the compound. [60] Fullerene attached coumarin and p-coumaric acid and [60] fullerene were used to screen against selected coagulant and HIV-replicating enzymes or proteins separately.

Selection of Receptors

The most important coagulant proteins such as factor XA - cation inhibitor complex (2jkh) and structure of a bacterial homolog of vitamin K epoxide reductase (3kp9) were selected based on their functions. The most important HIV-1 replicating enzymes HIV-1 protease (1aid), HIV-1 gp120 (1gc1), HIV-1 reverse transcriptase (1ikv), HIV-1 integrase (1qs4), cyclin t1-tat-tar RNA (2w2h), HIV-1 active site (3bvb), Wild type HIV-1 protease (3ekv), HIV-1 reverse transcriptase in complex (3mee), fully glycosylated HIV-1 gp120 (4rqs) were selected based on their functions. The three dimensional structures of these receptors were accessible in their local shape in PDB database. The three dimensional directions of the chose receptors were recovered from PDB database (Table 1 and Table 2).

PDB number	Name
2jkh	factor HA - cation inhibitor complex
3kp9	Structure of a bacterial homolog of vitamin k epoxide reductase

Table 1. Coagulant proteins selected for in silico study

PDB number	**Name**
1aid	Structure of a non-peptide inhibitor complexed with hiv-1 protease: developing a cycle of structure-based drug design
1gc1	HIV-1 gp120 core complexed with cd4 and a neutralizing human antibody
1i**k**v	k103n mutant HIV-1 reverse transcriptase in complex with efivarenz
1qs4	Core domain of hiv-1 integrasecomplexed with mg++ and 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2h-tetrazol-5-yl)-propenone
2w2h	Structural basis of transcription activation by the cyclin t1-tat-tar rna complex from eiav
3bvb	Crystal structure of hiv-1 active site mutant d25n and inhibitor darunavir
3ekv	Crystal structure of the wild type hiv-1 protease with the inhibitor, amprenavir
3mee	HIV-1 reverse transcriptase in complex with tmc278
4rqs	Crystal structure of fully glycosylated hiv-1 gp120 core bound to cd4 and 17b fab

Table 2. HIV enzymes and proteins were chosen for in silico activity

Multi-Receptor Docking

Molecular docking is performed to concentrate the receptor-ligand association which is viewed as the reason for structure based medication revelation. Docking studies were performed via iGEMDOCKv4.2. The reactant and restricting site of the objective has been identified via AutoGrid. The structure and synthetic properties of the dynamic destinations permit the acknowledgment and authoritative of the ligand. Around 2.5 million bioactive adaptations were created by 10 emphases and the best compliances were screened based on most minimal restricting vitality produced in the grouping histogram. The communications of 10-hydroxycamptothecine with selected receptors were further compared with and the association of those receptors with their normal ligands. The ascertained docking vitality was contrasted and measured trial restricting vitality connected with known atoms for every receptor.

ADMET Test

The ADME/toxicity parameters compliance was evaluated by screening through ADMETSAR, a commercial tool. The ADMETSAR is system pharmacology or system chemical biology and toxicology platform designed for the assessment of would be therapeutic indications, off-target effects and potential toxic end points of natural products. In the studied work, this database/tool was used to predict and evaluate the human metabolism compliance, toxicity risk assessment and mode of action by using standard experimental data.

Results and Discussion

Selection of Receptors

Proteins were selected based on their functions in coagulation of blood and replication of HIV-1. Two coagulant and nine HIV-1 replicating proteins were selected based on functional role in signalling pathways. Using Chemdoodle, [60] fullerene with coumarin and p-coumaric acid were drawn and represented in Figure 1 and 2. The chemical structures were obtained from pubchem and 3D structures were downloaded using software (www.mn-am.com/online_demos/corina_demo).

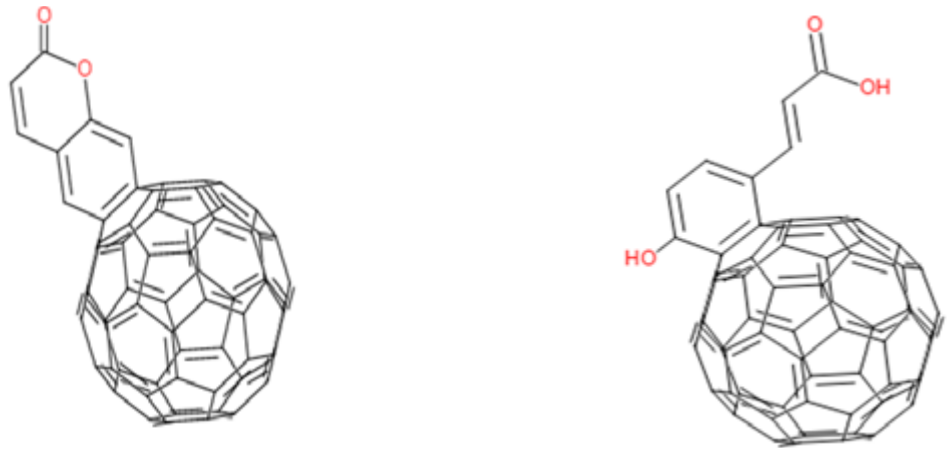
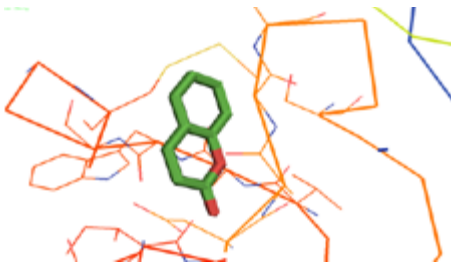
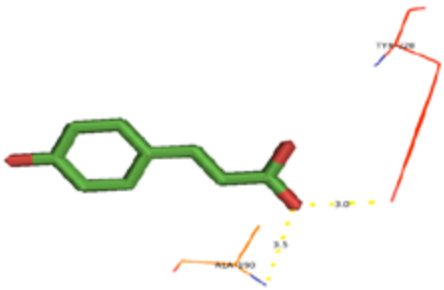


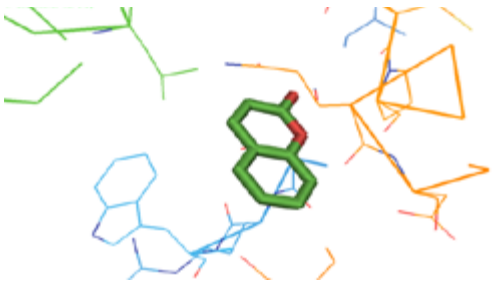
Figure1. [60] Fullerene derivatives used for the attachment of a preformed coumarin (A) and p-coumaric acid (B)



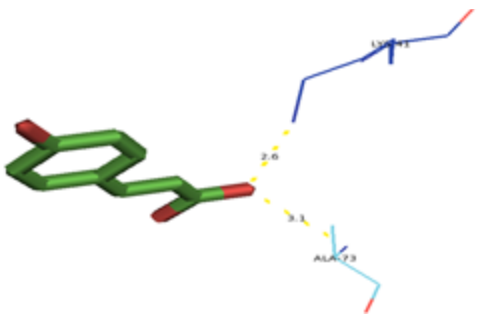
2jkh+ Coumarin (-78.37)



2jkh+p-coumaric acid (-80.99)



3kp9+Coumarin (-64.00)



3kp9+p-coumaric acid (-68.50)

In order to predict anticoagulant activity, the factor HA-cation inhibitor complex exhibited highest interaction with [60] fullerene coumarin (-136.30) compared with [60] fullerene p-coumaric acid (-133.50), how ever the coumarin (-80.37) and p-coumaric acid (-80.99) alone have not shown more interaction with same protein. The [60] fullerene have shown high interaction (-103.49) (Table 3-5) (Figure 3). The other protein we have used for in silico anticoagulant activity was structure of a bacterial homolog of vitamin K epoxide reductase; it is universally used for anticoagulant studies (Figure 3). Anticoagulant properties of plant products have been used on these proteins to know their activity level [19,20]. Similar type of in silico research was done by Kolyadko*et al*. [21], Iyer*et al*. [19] using other bioactive compounds.

Table 3: Molecular docking studies between coumarin and p-coumaric acid with coagulant proteins.

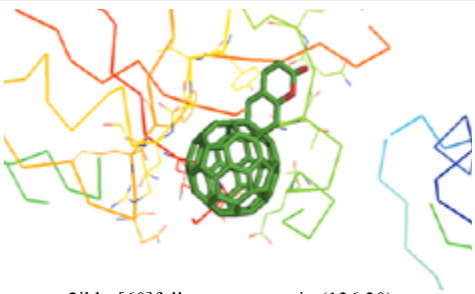
PDBs	ligand	total energy	VDW	H-bond	Interacting amino acids
2jkh	coumarin	-78.37	-78.37	0	Ala190, cys191, trp215, gly216
	p-coumaric acid	-80.99	-75.39	-5.60	Asp189, tyr228, ala190, cys191, gln192, trp215, gly216, tyr228
3kp9	coumarin	-64.00	-64.00	0	Ser62, arg63, arg63, pro210, gln213, gln213
	p-coumaric acid	-68.50	-61.51	-6.98	Lys41, ala73, val59, leu60, leu60, trp64, ala65, met118

Table 4: Molecular docking studies of [60] fullerene with coagulant proteins.

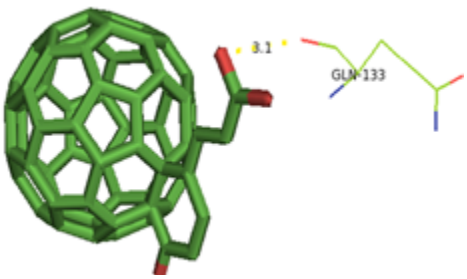
PDBs	Total energy	VDW	H-bond	Interacting amino acids
2jkh	-103.49	-103.49	0	Glu129, glu129, thr132, thr132, tyr162, val163, asp164, asp164, arg165, gln178, phe181, lys230
3kp9	-93.23	-93.23	0	Phe67, val75, leu78, gly79, leu107, ala110, phe114, thr170, thr173

Table 5: Anticoagulant activity of [60]fullerene conjugated coumarin and p-coumaric acid

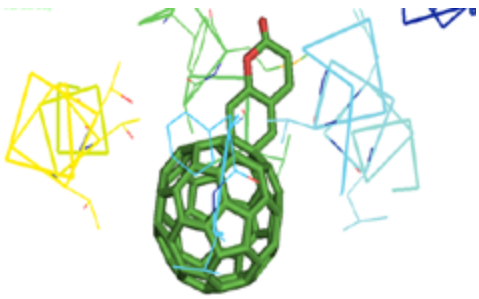
PDBs	Ligand	total energy	VDW	H-bond	Interacting amino acids
2jkh	Coumarin	-136.30	-136.30	0	Glu129, glu129, thr132, thr-132,thr-132, thr-132, gln-133, tyr-162, tyr-162, val-163, asp-164, asp-164, gln-178, phe-181, lys-230
	p-coumaric acid	-133.50	.133.50	0	glu-129, glu-129, thr-132, thr-132, thr-132, thr-132, tyr-162, val-163, asp-164, asp-164, gln-178, phe-181, lys-230
3kp9	Coumarin	-127.24	-127.24	0	Phe67, leu68, val75, gly76, leu78, gly79, leu107, ala110, met111, phe114, thr170,thr173
	p-coumaric acid	-122.40	-122.40	0	Phe67, leu68, val75, val75, leu78, gly79, leu107, ala110, met111, met111, phe114, thr170, thr173



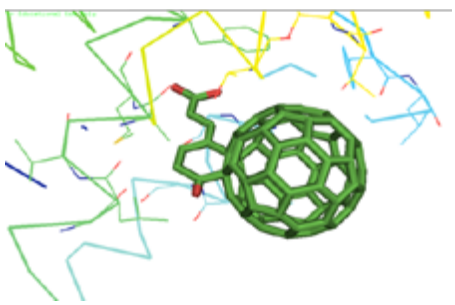
2jkh+[60]fullerene coumarin (136.30)



2jkh+[60]fullerene p-coumaric acid (-133.50)



3kp9+[60]fullerene coumarin (-127.24)



3kp9+[60]fullerene p-coumaric acid (-122.40)

Figure 3: Interaction of p-coumaric acid and coumarin alone and fullerene conjugated with different coagulant proteins

The coumarin, p-coumaric acid, [60] fullerene coumarin, [60] fullerene p-coumaric acid and [60] fullerene were tested against 09 different HIV-1 replicating enzymes and proteins individually. The coumarin, p-coumaric acid, [60] fullerene coumarin, [60] fullerene p-coumaric acid and [60] fullerene were tested against 09 different HIV-1 replicating enzymes and proteins individually. The [60] fullerene attached coumarin have shown highest interactions with crystal structure of fully glycosylated hiv-1 gp120 core bound to CD4 and 17b fab (4rqs) (-168.39), structural basis of transcription activation by the cyclin t1-tat-tar RNA complex from eiav (2w2h) (-166.35), Structure of a non-peptide inhibitor complexed with hiv-1 protease: developing a cycle of structure-based drug design (1aid) (-129.47), HIV-1 gp120 core complexed with cd4 and a neutralizing human antibody (1gc1) (-133.74), k103n mutant HIV-1 reverse transcriptase in complex with efivarenz (1ikv) (-128.18), core domain of HIV-1 integrasecomplexed with mg++ and 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2h-tetrazol-5-yl)-propenone (1qs4) (-102.24) followed by p-coumaric acid showed interaction with same proteins by -166.35, -120.35, 125.65, -122.88, -117.2, -100.37 respectively. Whereas the [60] fullerene attached p-coumaric acid have more interaction with HIV-1 reverse transcriptase in complex with tmc278 (3mee) (-133.02) followed by coumarin (-125.96). Without [60] fullerene, the p-coumaric acid has exhibited highest interaction with all the HIV-1 enzymes and proteins tested. The highest interactions was noticed with 4rqs (-88.29), 1ikv (-78.10), 2w2h (-77.20), 1gc1 (-75.76), 3mee (-74.08), 1qs4 (-69.30), 1aid (-56.32). The [60] fullerene alone has shown more interaction with 4rqs (-134.78) followed by 3mee (-102.05), 1gc1 (-100.5), 1aid (-98.01), 1ikv (-95.47), 1qs4 (-80.86) (Table 6-8) (Fig. 4, 5). Our results are confirmatory with the in silico findings of [22-25]. Inhibition of gp120-CD4 (4rqs) interaction or virus-host cell fusion thus appears to be an attractive strategy to prevent HIV-1 infection. The 2w2h protein is important to leading to activation of viral transcription through the hyperphosphorylation of RNA polymerase II and inhibits the HIV-1 replication. HIV-1 protease cleaves the Gag and Gag-Pol viral poly-proteins, allowing the virus to efficiently infect new host cells. When a mature HIV-1 virion infects a susceptible target cell, interactions of the envelope glycoprotein with the co-receptors on the surface of the cell brings about a fusion of the membranes of the host cell and the virion. Our tested compounds have showed interaction with the HIV-1 proteins with high biding energy, hence they may inactivated no functions of these ultimately they able to inhibits the HIV-1 replication. Al-Amri*et al.* [20] have used novel anticoagulant peptides targeting blood coagulation factor VIIa in silico method. [26, 27] have reported the plant extracts role in in silico anticoagulant activity. Choi et al.,28 have reported that phenyl coumarin have inhibited the HIV-1 vpr through virtual screening. Partially purified plant coumarins have shown potential inhibition of HIV-1 replicating enzymes in in vitro condition [29, 30]. The coumarins are potent in inhibition of HIV-1 polymerase and reverse transcriptase activities [31, 32] have listed the phytochemicals role in HIV-1 management. [60] Fullerene-coumarin and p-coumaric acid represents simple chemically diverse example of a bio-nano conjugate. Both nanoparticles and coumarinscontribute significant functions in combination. Barron [33] have explained the [60] fullerene conjugate dramatically modify both cellular uptake and transdermal transport, effecting cell viability and other functions. Interestingly the [60] fullerene conjugated coumarin have exhibited highest interaction with all the HIV-1 enzymes and proteins which we tried. It confirms that the nanoparticle attached coumarins are potent in attaching proteins and enzymes, leading to inactivation or inhibition of the same. Ma *et al*. [34] have mentioned the importance of nanotechnology in development of novel anti-HIV compounds in AIDS treatment. Bakry*et al.* [35] and Nayak*et al.* [26] have highlighted the importance of fullerenes in drug delivery to target cells. We have performed the drug likeliness of the both the ligands (coumarin and p-coumaric acid) using ADMETSAR. Both the compounds are non-carcinogens and they can be used as drug to treat diseases (Table 9). We have also checked the drug likeliness of [60] fullerene and showed as non-carcinogen (Table 9). The [60] fullerene conjugated coumarin and p-coumaric acid have shown more potent in interacting with coagulant and HIV-1 proteins and enzymes and these are crucial for coagulation and replication of HIV-1. Our results, projects that, [60] fullerene conjugated coumarin and p-coumaric acid can be used as inhibitor of coagulation and HIV-1 and it conveys the importance of nanoparticles with natural products. We have observed that coumarin and [60] fullerene conjugated coumarin binds to 2jkh and 3kp9, which are coagulant proteins but to an entirely distinct set of amino acids. Similar distinction in interaction was exhibited by HIV replicating enzyme proteins with coumarin vs. [60] fullerene conjugated coumarin with an exception of 4rqs which shared common amino acid binding regions (supplement Table 1). Hence, based on the obtained results we conclude that the attachment of coagulant and HIV proteins with coumarin and/or [60] fullerene conjugated coumarin were different based on amino acids binding region. Thus, this opens up a broad area of research to analyse modulations brought about by natural molecules like coumarin and study their differences with their nanoparticle conjugates that show higher binding affinity. The fullerene conjugated p-coumaric acid exhibited more interaction with protease, gp120, reverse transcriptase of HIV-1proteins. These interactions may leads to formation of a stable complex which is inhibitory in functionally. Eventually, ever in silico results propose potential anti-HIV properties of both coumarin and p-coumaric acid. Parallely, we have shown inhibitory stable complex formation between coagulant proteins such as Vit K epoxide reductase and factor X, substantially our claims of coumarin and p-coumaric acid as anti-coagulant. For illustration purposes, refer to the graphical abstract (Figure 6).

pdb	total energy	VDW	H-bond	Interacting amino acids
1aid	-98.01	-98.01	0	Asp25, gly27, gly48, gly49, ile50, ile84, asp25, gly27, gly48, ile50, ile84
1gc1	-100.51	-100.51	0	Thr166, thr166, glu167, glu167, gln168, asp169, asp169, ser170, ser170, val178, his179, his179, thr180, thr180
1ikv	-95.47	-95.47	0	His361, trp406, gln500, gln500, tyr501, gly504, ilr505, ilr505, thr1419, pro1420, pro1420, po1421
1qs4	-80.85	-80.85	0	Ile182, ile182, lys185, lys186, lys186, arg187, arg187, gly190
2w2h	-85.48	-95.48	0	Arg5, arg5, lys6, asn7, asn8, arg11, phe146, glu147, glu147
3bvb	-68.6	-68.6	0	Gly49, gly51, gly52, pro79, thr80, pro81, pro81
3ekv	-70.4	-70.4	0	Gln2, ile3, ile3, thr4, thr4, lys7, arg8, pro9, leu10
3mee	-102.09	-102.09	0	Lys22, lys22, gln23, asn57, thr131, asn137, pro140, pro140, gly141, arg143
4rqs	-134.78	-134.78	0	Pro44, arg45, arg45, val2, lys101, his102, trp103, trp103, lys101, his102, his102, trp103, trp103

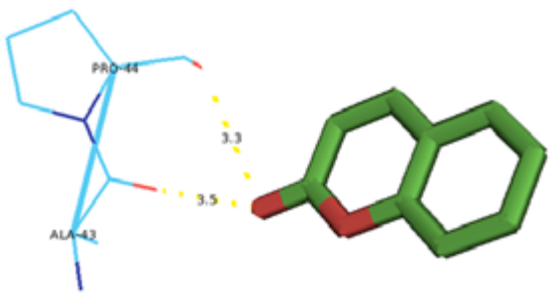
Table 6: Molecular docking studies of [60] fullerene with HIV-1 replicating enzymes and proteins

PDBs	Ligand	Total energy	VDW	H-bond	Interacting amino acids
1aid	Coumarin	-50.87	-50.87	0	Arg14, arg14, ile15, pro63, val64
	p-coumaric acid	-56.32	-51.98	-4.33	Asn83, glu34, pro81, val82, asn83
1gc1	Coumarin	-65.33	-65.33	0	Leu260, leu261, gly263, ser264, thr450, ser481, glu482
	p-coumaric acid	-75.76	-68.24	-7.52	Ser365, lys35, thr45, thr45, lys46, gly47
1ikv	Coumarin	-69.12	-69.12	0	Gln91, tyr181, thr1139, pro1140, pro1140
	p-coumaric acid	-78.10	-71.68	-6.41	Phe1077, arg1078, arg1078, asn1081, lys1154, met1184, ile1411, ile1411
1qs4	Coumarin	-60.79	-60.79	0	Lys185, gly197, glu198, glu198, pro109
	p-coumaric acid	-69.30	69.30	0	Glu85, glu87, lys103, arg107, glu87, lys103, arg107
2w2h	Coumarin	-70.78	-70.78	0	Gln56, asn257, trp258, trp258, arg259, ala260, cys261, gln262
	p-coumaric acid	-77.20	-66.70	-10.5	Asn257, arg259, ala260, gln50, gln56,asn257, trp258, trp258, cys261, gln262
3bvb	Coumarin	-54.25	-54.25	0	Ala28, asp29, asp29, asp30, asp30, ile47, gly48
	p-coumaric acid	-62.33	-58.83	3.5	Gly48, asp29, asp30, asp30, ile47, ile47
3ekv	Coumarin	-53.57	-53.57	0	Arg14, pro63, pro63, ile64, glu65
	p-coumaric acid	-65.35	-53.38	-11.9757	Ile15, gly16, gly17, arg14, pro63, pro63, ile54
3mee	Coumarin	-67.96	-67.96	0	Val381, trp24, pro25, pro25, ser134, ser134, ile135, asn137
	p-coumaric acid	-74.08	-67.08	-7	Gln161, met184, gln91, gln91, gln161, tyr181, tyr183
4rqs	Coumarin	-80.67	-80.67	0	His102, his102, trp103, trp103, his102, his102, trp103
	p-coumaric acid	-88.29	-84.81	-3.48	Ala43, ala43, trp103, trp103, gly104, gln105, trp103, trp103, gly104, gln105

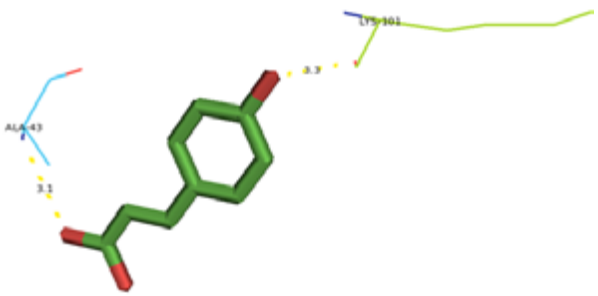
Table 7: Molecular docking studies between [60]fullerene conjugated coumarin and p-coumaric acid with HIV-1 replicating enzymes and proteins

Table 8: Molecular docking studies between [60] fullerene conjugated coumarin and p-coumaric acid with HIV-1 replicating enzymes and proteins

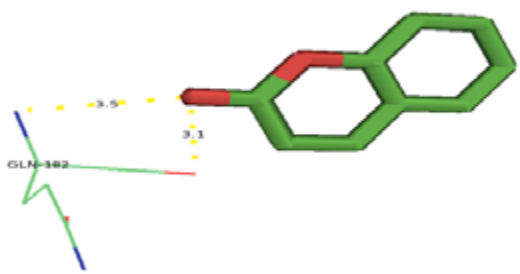
PDBs	Ligand	Total energy	VDH	H-bond	Interacting amino acids
1aid	coumarin	-129.47	-129.47	0	Asp25, gly27, gly48, gly49, ile50, asp25, ile47, gly48, gly49, ile50, ile54, ile84
	p-coumaric acid	-125.65	-125.65	0	Asp25, gly27, gly48, gly49, ile50, asp25, ala28, val32, gly48, gly49, ile50, thr80, pro81, val82, ile84
1gc1	coumarin	-133.74	-133.74	0	Thr166, glu167, glu167, gln168, asp169, asp169, ser170, ser168, ser168, trp169, thr175, val178, his179, his179, thr180, thr180
	p-coumaric acid	-122.88	-122.88	0	Pro40, phe83, thr166, glu167, glu167, gln168, asp169, asp169, ser170, val178, val178, his179, his179, thr180, thr180
1ikv	coumarin	-128.18	-128.18	0	His361, trp406, gln500, gln500, tyr501, gly504, ile505, ile505, thr1419, pro1420, pro1420, pro1421, pro1421
	p-coumaric acid	-117.24	-117.24	0	Gly93, ile94, his96, gln269, lys350, glu378, glu378, val381, ile382
1qs4	coumarin	-102.98	-102.98	0	Thr115, asp116, asp116, gly140, gln148, glu152, glu157, lys188
	p-coumaric acid	-100.30	-100.30	0	Phe181, ile182, ile182, lys185, lys185, lys186, arg187, arg187, trp132
2w2h	coumarin	-132.97	-132.97	0	Arg5, arg5, lys6, asn7, asn8, arg11, trp12, phe146, glu147
	p-coumaric acid	-120.76	-120.76	0	Arg5, arg5, lys6, lys6, asn8, arg11, gly145, phe146, phe146, glu147
3bvb	coumarin	-97.976	-97.97	0	Pro79, pro79, thr80, pro81, pro81, gly51, gly52, phe53
	p-coumaric acid	-97.728	-97.72	0	Asp29, asp30, ile72, gly73, thr74, thr74, asn88, asn88, thr91, gln92
3ekv	coumarin	-96.64	-96.64	0	Ile50, gly51, gly52, phe53, pro79, pro79, thr80, pro81, pro81
	p-coumaric acid	-91.17	-91.178	0	Pro79, pro79, thr80, thr81, pro81, gly49, gly51, gly52
3mee	coumarin	-125.96	-125.96	0	Lys22, lys22, gln23, asn57, asn137, pro140, gly141, arg143
	p-coumaric acid	-133.02	-133.02	0	Lys20, val21, lys22, lys22, gln23, gln23, asn57, thr131, asn137, pro140
4rqs	coumarin	-168.39	-168.39	0	Ala43, pro44, arg45, arg45, val2, val2, lys101, his102, his102, trp103, gly104, gln105, lys101, his102, his102, trp103, gly104, gln105
	p-coumaric acid	-166.35	-166.35	0	Gln42, ala43, arg45, arg45, his102, his102, trp103, trp103, gly104, gln105, gln105, gln105



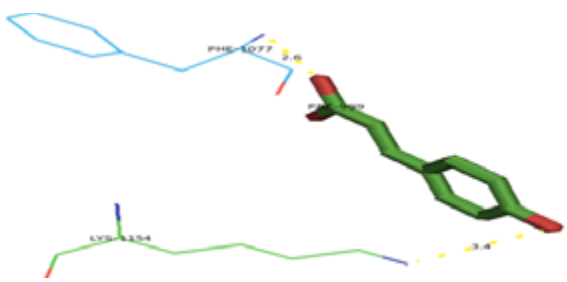
4rqs+coumarin (-80.67)



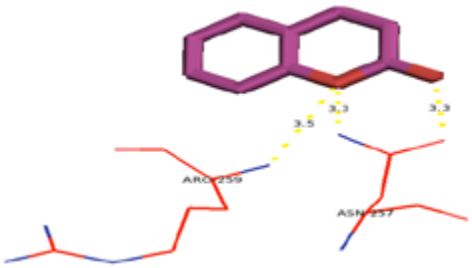
4rqs+p-coumaric acid (-88.29)



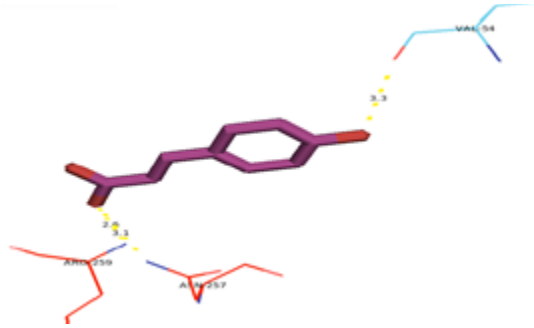
1ikv+coumarin (-69.12)



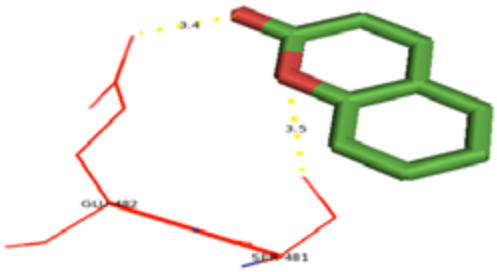
1ikv+p- coumaric acid (-78.10)



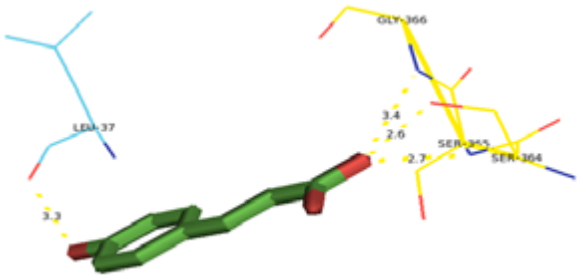
2w2h+ coumarin (-70.78)



2w2h+p- coumaric acid (-77.20)

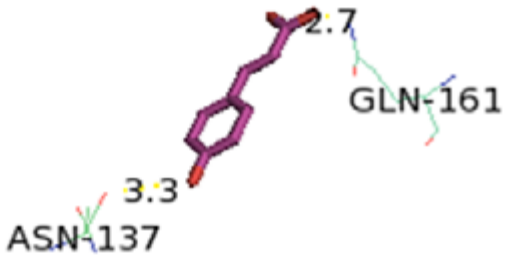


1gc1+coumarin (-65.33)



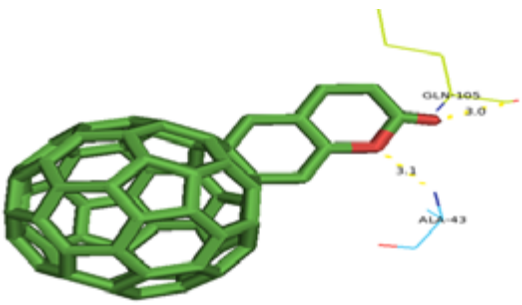
1gc1+p-coumaric acid (-75.76)

3mee+coumarin (-67.96)

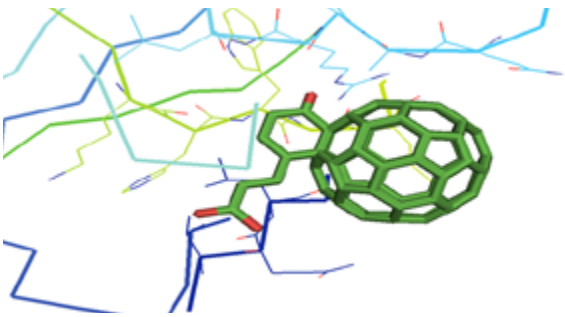


3mee+p-coumaric acid (-74.08)

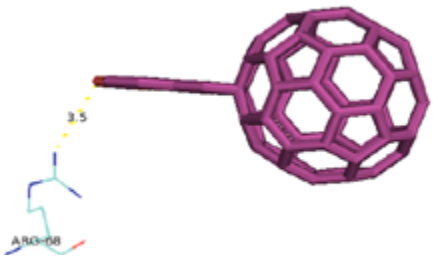
Figure 4: Interaction of p-coumaric acid and coumarin with different HIV-1 replicating enzymes and proteins



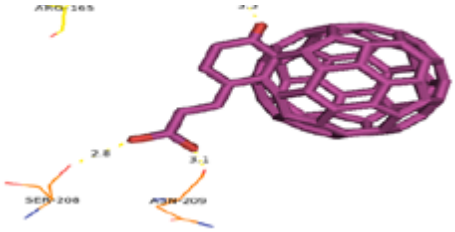
4rqs+[60]fullerene coumarin (-168.39)



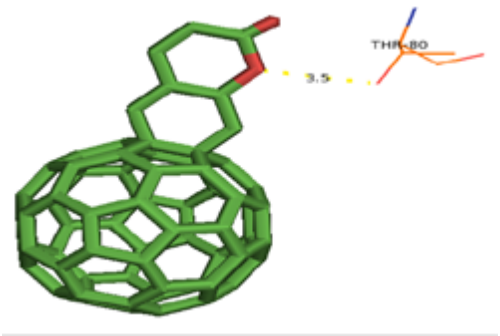
4rqs+[60]fullerene p-coumaric acid (-166.35)



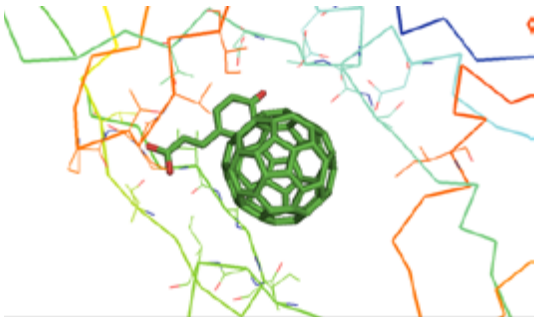
2w2h+[60]fullerene coumarin (-132.97)



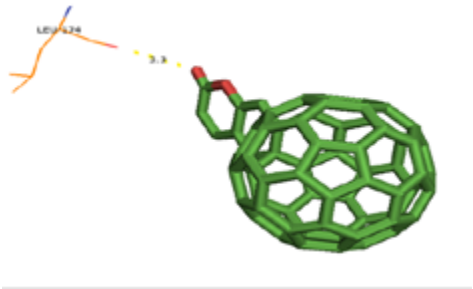
2w2h+[60]fullerene p-coumaric acid (-120.76)



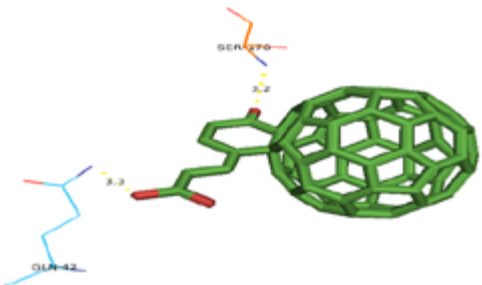
1aid+[60]fullerene coumarin (-129.47)



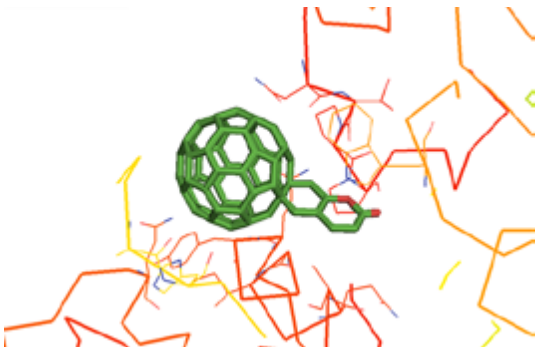
1aid+[60]fullerene p-coumaric acid (-125.65)



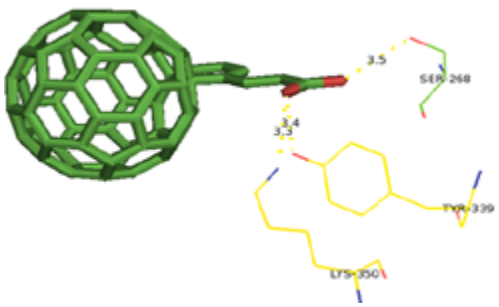
1gc1+[60]fullerene coumarin (-133.74)



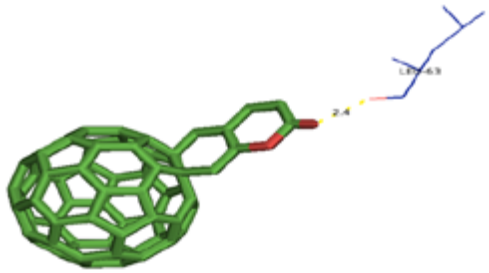
1gc1+[60]fullerene p-coumaric acid (-122.88)



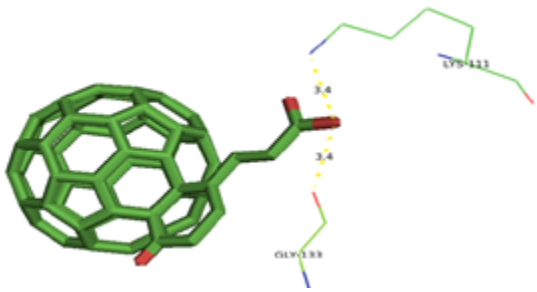
1ikv+[60]fullerene coumarin (-128.18)



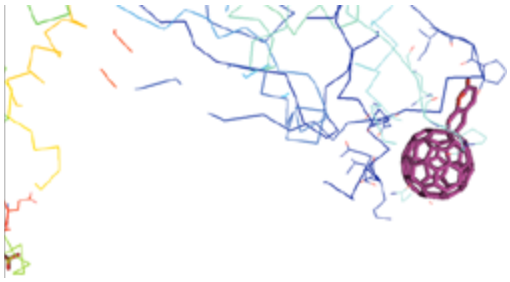
1ikv+[60]fullerene p-coumaric acid (-117.24)



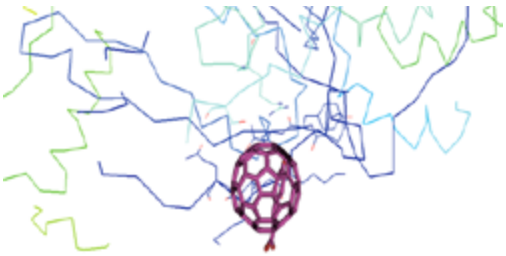
1qs4+[60]fullerene coumarin (-102.98)



1qs4+[60]fullerene p-coumaric acid (-100.30)



3mee+[60]fullerene coumarin (-125.96)



3mee+[60]fullerene p-coumaric acid (-133.02)

Figure 5: Interaction of [60]fullerene conjugated with p-coumaric acid and coumarin with different HIV-1 replicating proteins/ enzymes

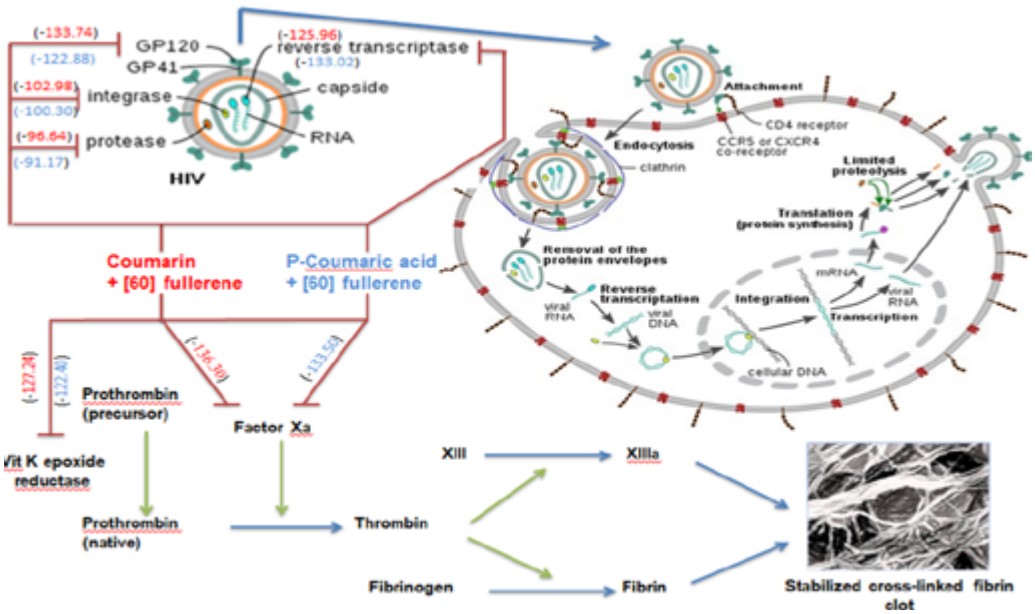


Figure 6: Graphical representation of fullerene conjugated coumarins and p-coumaric acid action on HIV-1 proteins and coagulant proteins and their possible mechanism

Table 9. ADMET Predicted profile of the courmarin, p-couramric acid of Penicilliumspecies and [60]fullerene

Property	Coumarin		p-coumaric acid		[60]fullerene	
	Value	Probability	Value	Probability	Value	Probability
Blood Brain Barrier	BBB+	0.9565	BBB+	0.5237	BBB+	0.9812
Human Intestinal absorption	HIA+	0.9912	HIA+	0.9938	HIA+	1.0000
Caco-2-permeable	CaCo2+	0.9155	CaCo2+	0.8839	CaCo2+	0.7814
P-glycoprotein-substrate	Non-Substrate	0.6697	Non-substrate	0.7196	Non-substrate	0.7761
P-glycoprotein-inhibitor I	Non-inhibitor	0.8540	Non-inhibitor	0.9812	Non-inhibitor	0.9193
	Non-inhibitor	0.8663	Non-inhibitor	0.9899	Non-inhibitor	0.9502
Renal organic cation transporter	Non-inhibitor	0.8301	Non-inhibitor	0.9091	Non-inhibitor	0.7991
Distribution						
Subcellular localization	Mitochondria	0.4995	Mitochondria	0.8227	Mitochondria	0.8227
Metabolism						
CYP450 2C9 Substrate	Non-substrate	0.7966	Non-substrate	0.7889	Non-substrate	0.8108
CYP450 2D6 Substrate	Non-substrate	0.9117	Non-substrate	0.9364	Non-substrate	0.8907
CYP450 3A4 Substrate	Non-substrate	0.7139	Non-substrate	0.7460	Non-substrate	0.7647
CYP450 1A2 Substrate	Inhibitor	0.9117	Non-inhibitor	0.9458	Inhibitor	0.7164
CYP450 2C9 Inhibitor	Non-inhibitor	0.6943	Non-inhibitor	0.9364	Non-inhibitor	0.8573
CYP450 2D6 Inhibitor	Non-inhibitor	0.9105	Non-inhibitor	0.9766	Non-inhibitor	0.8692
CYP450 2C19 Inhibitor	Non-inhibitor	0.5000	Non-inhibitor	0.9116	Non-inhibitor	0.7296
CYP450 3A4 Inhibitor	Non-inhibitor	0.8310	Non-inhibitor	0.8693	Non-inhibitor	0.8514
CYP Inhibitory Promiscuity	Low CYP inhibitory	0.8115	Low CYP inhibitory	0.8913	Low CYP inhibitory	0.8913
Excretion-Toxicity						
Human Ether-a-go-Related Gene Inhibition	Weak inhibitor	0.8702	Weak inhibitory	0.9502	Weak inhibitory	0.9402
	Non-inhibitor	0.9474	Non-inhibitor	0.9793	Non-inhibitor	0.9008
AMES Toxicity	Non-inhibitor	0.8870	Non AMES toxic	0.9521	Non AMES toxic	0.9107
Carcinogens	Non-carcinogens	0.9412	Non-carcinogens	0.8248	Non-carcinogens	0.7331
Fish Toxicity	High FHMT	0.6109	High FHMT	0.9149	High FHMT	0.9641
TetrahumenaPyriformis Toxicity	High TPT	0.9544	High TPT	0.5163	High TPT	0.9979
Honey Bee Toxicity	High HBT	0.8062	High HBT	0.8142	High HBT	0.7940
Biodegradation	Ready biodegradable	0.5884	Ready biodegradable	0.7156	Ready biodegradable	0.8252
Acute Oral Toxicity	III	0.7019	III	0.4898	III	0.7908
Carcinogenicity (Three class)	Non-required	0.5324	Non-required	0.6034	Warning	0.4645
Absorption						
Aqueous solubility	-2.7525	LogS	-2.2240	LogS	-6.7307	LogS
CaCo2 Permability	1.7653	LogPapp, cm/s	1.2009	LogPapp, cm/s	1.7738	LogPapp, cm/s
Distribution, Metabolism, Excretion, Toxicity						
Rat acute toxicity	2.4622	LD50, mol/kg	1.3698	LD50, mol/kg	1.7594	LD50, mol/kg
Fish Toxicity	1.2095	pLC50, mg/L	1.5372	pLC50, mg/L	0.0963	pLC50, mg/L
Tetrahymenapyriformis Toxicity	0.6735	pIGC50, µg/L	-0.1289	pIGC50, µg/L	1.1116	pIGC50, µg/L

Conflict of Interests

The authors confirm that this article content has no conflicts of interests.

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