Bioorganic & Medicinal Chemistry Letters 20 (2010) 4427–4431

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Active site binding modes of dimeric phloroglucinols for HIV-1 reverse transcriptase, protease and integrase

Pawan Gupta [a], Rajender Kumar [b], Prabha Garg [b,*], Inder Pal Singh [c]

[a] Centre for Pharmacoinformatic, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Mohali, Punjab 160 062, India
[b] Computer Centre, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Mohali, Punjab 160 062, India
[c] Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Mohali, Punjab 160 062, India

ARTICLE INFO

Article history:
Received 30 March 2010
Revised 3 June 2010
Accepted 9 June 2010
Available online 12 June 2010

Keywords:
HIV-1 reverse transcriptase
HIV-1 integrase
HIV-1 protease
Dimeric phloroglucinols
Docking

ABSTRACT

In a recent report on anti-HIV activity of dimeric phloroglucinol compounds, seven out of twenty one compounds were shown to possess good activity in HIV infected human CD4+ T cell line. The seven active compounds were docked into the active sites of HIV-1 reverse transcriptase (RT), integrase (IN), and protease (PR). Two compounds which have RT inhibitory activity exhibited H-bonding interaction with Lys101. Compounds **1**, **5,** and **6** exhibited good binding interactions with catalytic residues Asp64 and Asp116, while compounds **5** and **7** showed binding with PR (Asp25, Gly27, and Asp29). We propose here that compound **5** may be a dual inhibitor acting against both IN and PR. The docking results gave the information about active site binding modes of dimeric phloroglucinols interacting with HIV proteins.

Human immunodeficiency virus type 1 (HIV-1) causes acquired immunodeficiency syndrome (AIDS). The *pol* gene of HIV-1 encodes three key enzymes to propagate its life cycle—(1) reverse transcriptase (RT), also known as RNA-dependent DNA polymerase, that is, used by the virus to transcribe the viral genomic RNA to proviral DNA, which is then integrated into the host genome, (2) integrase (IN), the enzyme used for catalyzing 3′ ends preprocessing of the viral DNA which is covalently ligated to the host chromosomal DNA and (3) protease (PR), enzyme used for the processing of the new virulent viral particles. These three enzymes are potential drug targets for chemotherapeutics.[1–4]

Phloroglucinols are known to occur naturally in certain plant genera *Dryopteris, Aspidium, Mallotus, Hypericum, Eucalyptus*, and *Helichrysum*. The phloroglucinol compounds have shown a diverse range of biological activities including anti-HIV activity.[5]

Recently, anti-HIV activity of dimeric phloroglucinol derivatives has been reported.[6] These compounds have two monomeric units joined through a methylene linkage with variation in the acyl chain on both the phloroglucinol nuclei or with different substituents on the linked methylene bridge. Out of twenty one, seven dimeric phloroglucinol derivatives showed good anti-HIV activity in a human CD4+ T cell line (Fig. 1). The compounds **2** and **3** exhibited highest anti-HIV activity. All the seven active compounds were also screened against RT; compounds **3** and **4** exhibited good inhibitory activity against RT.[6]

These seven compounds were used for docking studies. The objective of the present study is to explore the binding mode of dimeric phloroglucinol derivatives with HIV-1 reverse transcriptase, protease, and integrase using computational docking methodology. Two compounds that showed RT inhibitory activity were docked into the active site of HIV-1 RT enzyme to validate the concept. The other compounds which did not show RT inhibitory activity were selected for docking studies against the active sites of HIV-1 PR and IN.

Docking studies of dimeric phloroglucinol derivatives were performed using AUTODOCK4.2 program[7] on crystal structure of HIV-1 RT, IN, and PR enzymes, their PDB IDs are 3FFI, 1QS4[8], and 1HSG,[9] respectively. All compounds were built using SYBYL7.1 molecular modeling package[10] installed on a Silicon Graphics Fuel Work station running IRIX 7.1. Tripos force field, Gasteiger Huckel, partial atomic charges[11] and Powell's conjugate gradient method were used for minimization of all molecules with 0.05 Kcal/mol energy gradient convergence criterion.[12] All protein structures were prepared for docking using AutoDock Tool. Co-crystallized ligands and all water molecules were removed from crystal proteins while a magnesium ion (Mg^{2+}) at the active site of HIV-1 IN (1QS4) was maintained. Polar hydrogen's were added and non-polar hydrogen's were merged, finally Kallman united atom charge and atom type parameter was added. Grid map dimensions were set surrounding active site and also significant portion of the surrounding

* Corresponding author. Tel.: +91 1722292016; fax: +91 1722214692.
E-mail address: prabhagarg@niper.ac.in (P. Garg).

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doi:10.1016/j.bmcl.2010.06.057

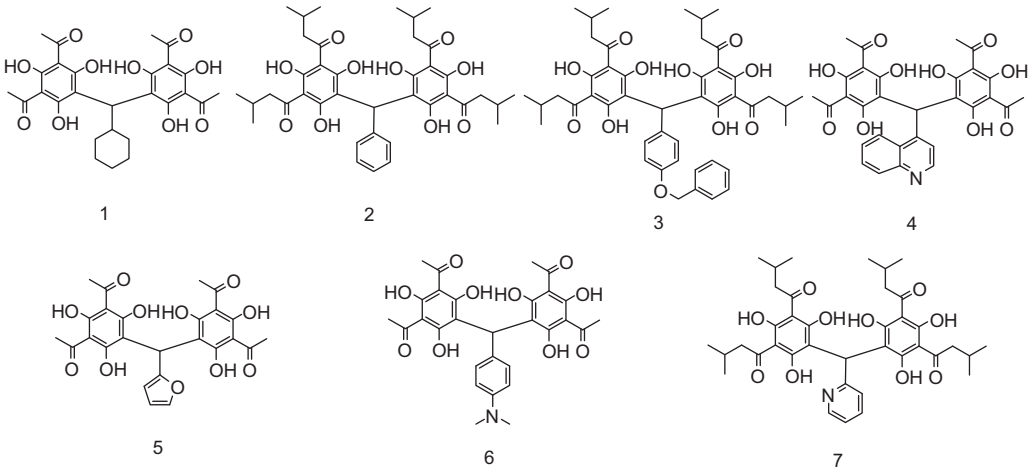

Figure 1. Structures of anti-HIV dimeric phloroglucinols.[6]

surface. Lamarckian genetic search algorithm was employed and docking run was set to 30. All other parameters were set to default value: maximum number of energy evaluation 25,000,00 per run; maximum number of generation in the genetic algorithm was increased to 27,000.[7]

First, docking studies of co-crystallized ligands of these proteins 3FFI, 1QS4, and 1HSGA were performed and it was found that all the co-crystallized ligands superimposed well with experimentally determined ligands (Table 2 and Figs. 10–12 in Supplementary data). These set of parameters of docking protocol were further used for our studies. Among the seven compounds, two dimeric phloroglucinol compounds **3** and **4** showed 87.75% and 80.46% inhibition against RT.[6] The H-bonding interactions exhibited by the compounds **3** and **4** with Lys101 was already reported to be important for inhibition.[13] The docking results of RT are shown in Table 1. The middle portion of compounds **3** (benzyloxyphenyl) and **4** (quinolyl) were found near to Lys103, Ser105, Val106, Pro225, Phe227, Leu234, Pro236, and Tyr318 (Figs. 2 and 3). These residues also exhibited hydrophobic interactions strengthening the binding of inhibitor to the active site. Both the compounds **3** and **4** have similar aromatic rings on both sides of the linker methylene bridge that interacted with different residues of active site. The acyl groups on the phloroglucinol nuclei in compounds **3** and **4** are surrounded by Pro95, His96, Pro97, Val106, Thr107, Val108, Tyr181, Tyr188, Phe227, Trp229, and Leu234 which also exhibited hydrophobic interactions (Figs. 2 and 3). Similarly, another acylated aromatic ring of compounds **3** and **4** also exhibited hydrophobic interaction with residues Pro97, Leu100, Lys101, Lys102, Lys103, Asn136, Gly138, Val179, Ile180, Gly190, and Ser191 (Figs. 2 and 3). Some of these interactions are also supported by published results.[14,15] The substituents on compound **4**, the acetyl and quinolyl groups have low steric volume than compound **3** with isovaleryl and benzyloxyphenyl (Fig. 1). This may be possible reason for higher RT inhibitory activity of compound **3** compared to **4**.

Active site formed by these hydrophobic residues and multiple ring system inhibitors showed that hydrophobic interaction is

Table 1
H-bonding and Mg^{2+} interactions observed between compounds and active site of RT, PR, and IN

Compd	RT H-boning (Å)	PR H-boning (Å)	IN H-boning (Å)	[a]Mg^{2+} – interaction (Å)
1	–	OH–O=C Gly27′ (2.19) O–HN Asp29 (2.18)	OH–O=C Asp64 (2.08) OH–O=C Asp64 (1.93) C=O–HN His67 (1.98) C=O–HN Asn155 (1.86) OH–NH Asp116 (2.21)	HO–Mg^{2+} (3.40) HO–Mg^{2+} (3.41) HO–Mg^{2+} (3.20) C=O–Mg^{2+} (3.9) C=O–Mg^{2+} (3.70)
2	–	C=O–HN Ile50 (2.12) C=O–HN Ile50′ (1.64)	OH–O=C Asp64 (2.23) C=O–HNAsn155 (2.02)	HO–Mg^{2+} (3.50) HO–Mg^{2+} (2.67) C=O–Mg^{2+} (3.11)
3	OH–O=C Lys101 (2.06)	–	–	
4	OH–O=C Lys101 (1.90)	–	–	
5	–	OH–O=C Asp25′ (2.09) OH–O=C Gly27 (1.96) C=O–HN Asp29 (2.22)	OH–O=C Asp64 (1.87) OH–O=C Cys65 (2.06) C=O–HN His67 (1.89) OH–O=C Asp116 (1.92) C=O–HN Asn155 (1.64)	HO–Mg^{2+} (3.20) HO–Mg^{2+} (3.97) HO–Mg^{2+} (3.18)
6	–	OH–O=C Asp25′ (1.81) OH–O=C Asp25 (2.05)	OH–O=C Cys65 (1.96) C=O–HN His67 (1.79) OH–O=C Asp116 (1.89) C=O–HN Asn155 (1.57)	HO–Mg^{2+} (2.76) HO–Mg^{2+} (3.66)
7	–	OH–O=C Asp25′ (2.16) OH–O=C Gly27′ (2.07) OH–O=C Gly27 (1.71) C=O–HNAsp29 (2.14)	OH–O=C Glu92 (2.07) C=O–HN His67 (1.89)	HO–Mg^{2+} (2.15) C=O–Mg^{2+} (2.91)

The value in parentheses is H-bonding distance between donor/acceptor atom of ligand and protein acceptor/donor atoms and ′ indicate B chain of PR.
[a] Distance between highly electronegative atom of ligand and Mg^{2+}.

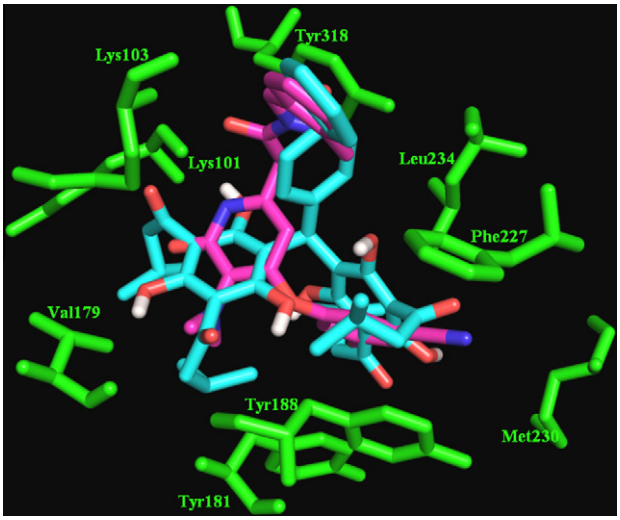

Figure 2. Docking interactions of compound **3** into the active site of RT (3FFI). Green color sticks for active site residues; cyan color sticks for docked conformation of compound **3**; magenta color sticks for co-crystallized ligand (generated by Pymol program; www.pymol.org).

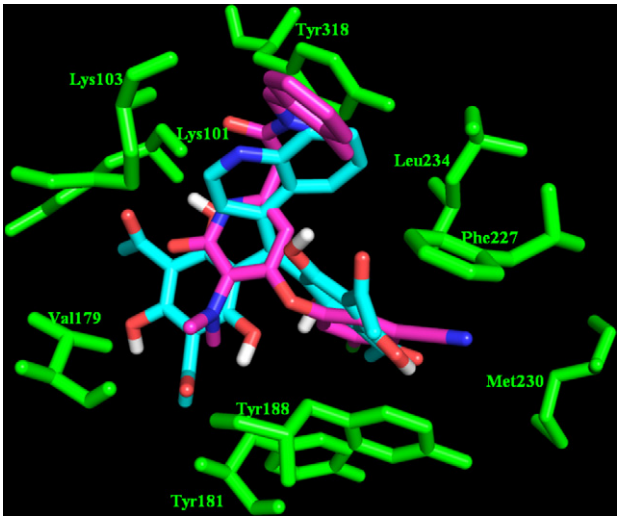

Figure 3. Docking interactions of compound **4** into the active site of RT (3FFI). Green color sticks for active site residues; cyan color sticks for docked conformation of compound **4**; magenta color sticks for co-crystallized ligand (generated by Pymol program; www.pymol.org).

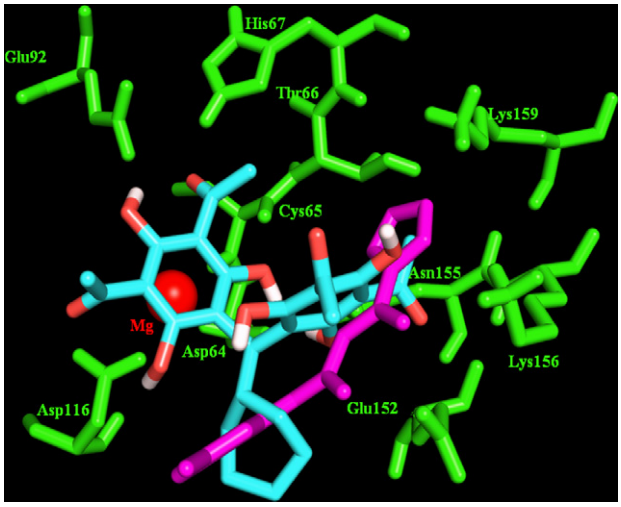

Figure 4. Docking interactions of compound **1** into the active site of IN (1QS4). Green color sticks for active site residues; cyan color sticks for docked conformation of compound **1**; magenta color sticks for co-crystallized ligand; red ball for magnesium ion (generated by Pymol program; www.pymol.org).

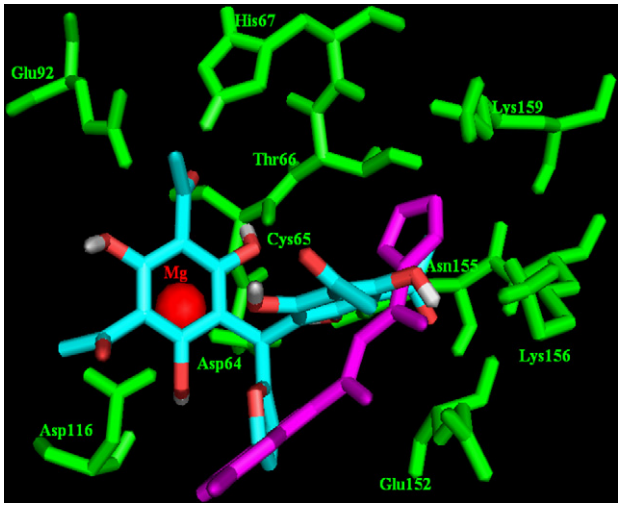

Figure 5. Docking interactions of compound **5** into the active site of IN (1QS4). Green color sticks for active site residues; cyan color sticks for docked conformation of compound **5**; magenta color sticks for co-crystallized ligand; red ball for magnesium ion (generated by Pymol program; www.pymol.org).

important for inhibition. Both compounds were well superimposed on co-crystallized ligand (Figs. 2 and 3) suggesting that these docked conformations may be the most favorable conformation of these inhibitors.

The compounds **1**, **2**, and **5**–**7** docked (1QS4) near to Mg^{2+} which was found to be essential for HIV-1 IN activity (Figs. 4–6 and Supplementary data Figs. 13 and 14).[16–19] The H-bonding interactions exhibited by these molecules are with Asp64, Cys65, His67, Glu92, Asp116, and Asn155. The docking results of HIV-1 IN are shown in Table 1. Most of the compounds consistently exhibited H-bonding to catalytic residues (Asp64 and Asp116), except compound **7**. These residues are important for integrase activity.[1–3,8,20–27] The substituents on methylene of compounds **1** (cyclohexyl), **2** (phenyl), **5** (2-furyl), **6** (*N*,*N*-dimethylaminobenzene) and **7** (pyridyl) are surrounded by Gln62, Leu63, Ile141, Pro142 (the residues Ile141-Asn144 are missing in PDB ID 1QS4 and analyzed by overlapping PDB ID BIS[24] with 1QS4) and Gln148 (Figs. 4–6 and Supplementary data for Figs. 13 and 14). These residues form moderately lipophilic binding pocket and show hydrophobic interactions with these substituents.[25] One of the phloroglucinol nucleus located near to Thr66, Glu152, Asn155, Lys156, and Lys159 exhibited hydrophobic interactions to these residues.

The highly electronegative atoms (oxygen of –OH and –C=O groups) formed coordination bond to the metal ion and chelated it (Table 1 for distance between nearby highly electronegative atom and Mg^{2+}). Interestingly, cation–π interaction exhibited by these molecules between Mg^{2+} and π electrons of one of the aromatic rings also strengthen the binding of inhibitors to the active site of HIV-1 IN. The isovaleryl group of compounds **2** and **7** showed hydrophobic interactions to the active site residues (Cys65, Thr66, His67, Asp116, Asn117, Gly118, Asn120, Glu152, Asn155 and Lys159) (Figs. 13 and 14 in Supplementary data). The docking analysis revealed that compounds bound differently to the active site than 5-CITEP (co-crystallized ligand of 1QS4), because of structural diversity. On the basis of docking studies,

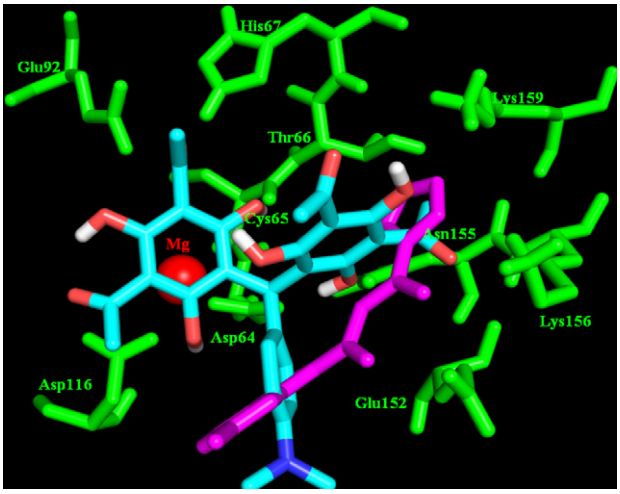

Figure 6. Docking interactions of compound **6** into the active site of IN (1QS4). Green color sticks for active site residues; cyan color sticks for docked conformation of compound **6**; magenta color sticks for co-crystallized ligand; red ball for magnesium ion (generated by Pymol program; www.pymol.org).

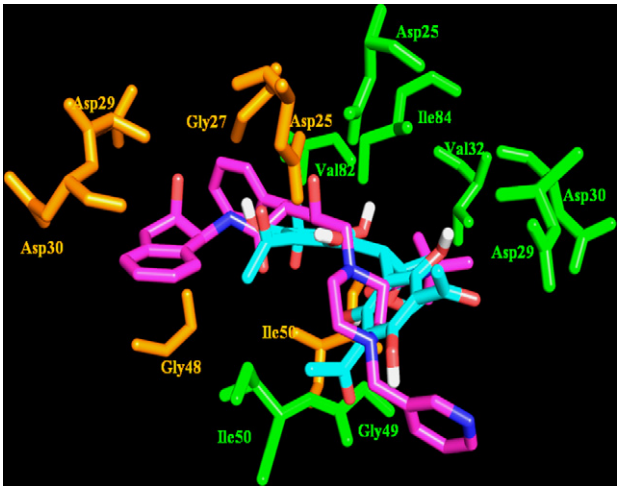

Figure 8. Docking interactions of compound **5** into the active site of PR (1HSG). Green color sticks represent chain A residues; orange color sticks represent chain B residues; cyan color sticks for docked conformation of compound **5**; magenta color sticks for co-crystallized ligand (generated by Pymol program; www.pymol.org).

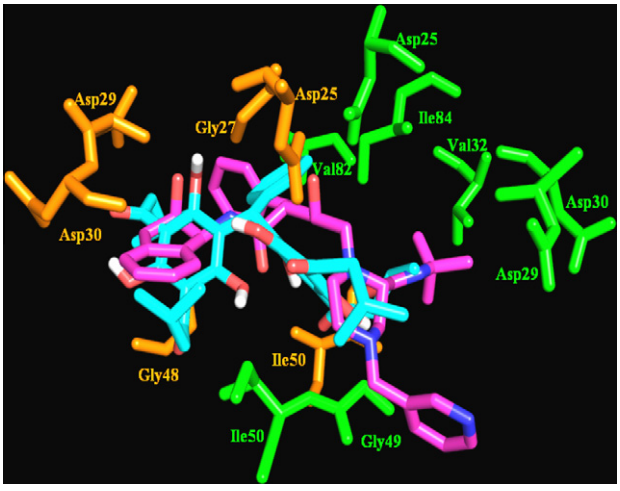

Figure 7. Docking interactions of compound **2** into the active site of PR (1HSG). Green color sticks represent chain A residues; orange color sticks represent chain B residues; cyan color sticks for docked conformation of compound **2**; magenta color sticks for co-crystallized ligand (generated by Pymol program; www.pymol.org).

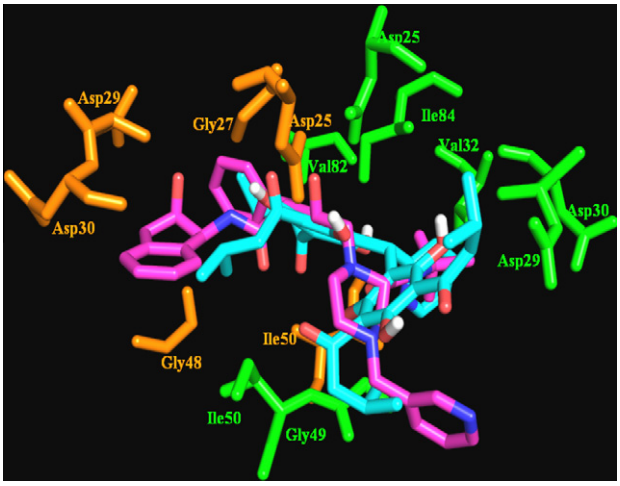

Figure 9. Docking interactions of compound **7** into the active site of PR (1HSG). Green color sticks represent chain A residues; orange color sticks represent chain B residues; cyan color sticks for docked conformation of compound **7**; magenta color sticks for co-crystallized ligand (generated by Pymol program; www.pymol.org).

we observed that compounds **1**, **5** and **6** exhibited good binding interactions (with catalytic residues Asp64 and Asp116) than compounds **2** and **7**. So, these compounds might have good HIV-1 IN inhibitory activity (Table 1). These docking interactions are supported by published results.[18,21,22,26–31]

The docking results of HIV-1 PR showed H-bonding interactions with active site residues described in Table 1. The H-bonding interactions exhibited by residues Asp25, Asp25′, Gly27, Gly27′ Asp29, Gly48′, Ile50, and Ile50′.[29,32,33] The cyclohexyl, 2-furyl, *N,N*-dimethylaminobenzene, and pyridyl groups of compounds **1** and **5–7** are surrounded by Asp29, Asp30, Val32, Ile47, Gly48 and Gly49 (Figs. 8 and 9 and Supplementary data for Figs. 15 and 16).

These residues exhibited hydrophobic interactions with the substituents of compounds **1** and **5–7**. One of the phloroglucinol nucleus of compounds (**1** and **5–7**) is surrounded by residues Gly27′, Asp25′, Ala28′, Gly49′, Ile50′, Pro81 and Val82. Similarly, the other phloroglucinol nucleus is surrounded by residues Gly27, Asp28, Asp29, Arg8′ Leu23′ and Pro81′ (Figs. 7–9 and Supplementary data for Figs. 12 and 13). These residues also imparted hydrophobic interactions to the aromatic rings of compounds. Interestingly, compound **2** oriented differently into the active site than compounds **1**, **5–7** (Fig. 9) possibly resulting in higher anti-HIV activity of compound **2**. This compound exhibited H-bonding interactions with Ile50 and Ile50′ (Table 1). The phenyl group of this compound is surrounded by residues Val32, Pro81, Val82 and Ile84 which showed hydrophobic interactions. One phloroglucinol ring is surrounded by Ile50, Ile50′ Val82 and Ile84, while other by Asp29′, Val32′, Ile47′, Gly48′ and Ile50. These residues showed hydrophobic interactions that imparted tight binding to the active site of PR. On the basis of docking results, we observed that compounds **5** and **7** exhibited good binding interactions (Asp25′, Gly27 and Asp29) to the active site of HIV-1 PR than compounds **1**, **2** and **6** (Table 1). These compounds might have anti-HIV-1 PR inhibitory activity.

The docking studies of dimeric phloroglucinol derivatives have shown good binding interactions into the active site of HIV-1 RT, IN and PR. Compounds **3** and **4** showed good binding pose with RT protein that can be helpful to understand the mechanism of binding of these compounds to the active site of RT.

We concluded from docking studies that compounds **1**, **5** and **6** showed good binding interactions to the IN protein, while compounds **5** and **7** showed with PR protein. It was also found that compound **2** is differently oriented into the active site of PR, may be because of high anti-HIV activity or bind to the active site by alternative mechanism of viral inhibition.[6] Overall results of docking analysis found that compound **5** exhibited good binding mode in both protein IN and PR.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.057.

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