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Original article

In silico Molecular Docking Study of Repensine and Bentysrepinine against HBV DNA Polymerase

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ABSTRACT

Bentysrepinine (Y101), a derivative of repensine, is a novel di-peptide structure isolated from *Dichondra repens*. *In vitro* and *in vivo* tests exhibited that bentysrepinine markedly inhibited DNA-HBV and cccDNA activities. The binding mode of Y101 and repensine with DNA polymerase was driven by hydrophobic interactions. This might provide novel recognition of inhibitory effect of Y101 against HBV, though its inhibition mechanism needs to be validated by bio-assay at cellular level and of polymerase activity. Preliminary docking study suggested that Y101 might be able to inhibit HIV inverse transcriptase, also have the potential to interact with DNA polymerase and HCV NS5B polymerase.

Key words

bentysrepinine; hepatitis B virus; molecular docking; polymerase; repesnine

1. Introduction

Hepatitis B virus (HBV) infection remains a major public health threat with more than 400 million humans chronically infected worldwide at the risk of developing end-stage liver disease and hepatocellular carcinoma (Lee, 1997; Lin et al, 2004). Approximately, 80% of HBV carriers have different levels of hepatocyte destruction, which may develop into liver cirrhosis and hepatocellular carcinoma. HBV causes acute and chronic infections of the liver and is responsible for 1.2 million deaths annually (Kane, 1995; Lavanchy, 2004).

Nowadays, at least two different treatment options, including interferon and nucleoside analogs, such as lamivudine, adefovir dipivoxil, and entecavir, are considered as antiviral therapy for chronic hepatitis B infection (Dienstag et al, 1999). Although various treatment options exist for chronic HBV infection, none is entirely satisfactory.

The development of new anti-HBV agents is focused on discovering diverse compounds with either novel chemical structures or natural products and their structure modification or novel anti-HBV targets and the mechanisms to discover new drugs. *Dichondra repens* Forst. (*matijin* in Chinese), an ethnic herbal medicine widely spread in Guizhou province of China, has been used in the treatment of hepatitis for very

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long time. The whole plant is presented in Figure 1. Repensine (*Matijinsu*, Figure 2), a novel compound with a di-peptide structure, was isolated from *D. repens* and exhibited strong anti-HBV activity. On the basis of tremendous amount of research efforts, domestic and US patents applied related to repensine study have been granted and issued.

Figure 1 Whole plant of *D. repens*

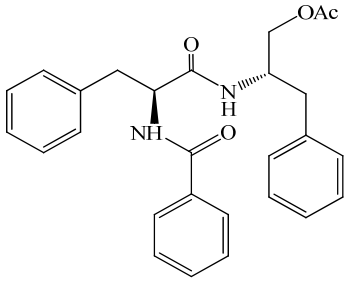

Figure 2 Chemical structure of repensine

The anti-HBV activity of repensine and its derivatives had been reported in our previous studies (Liang et al, 2002; Xu et al, 2009). Some derivatives showed the mark inhibition on the replication of HBV DNA. In order to enhance its antiviral activity, chemical structure modification was performed using repensine as template, over 200 derivatives were then synthesized, and further researches on leading compound were made for the discovery of more potent anti-HBV derivatives (Qiu et al, 2011).

The antiviral activity essay of over 200 derivatives demonstrated that bentysrepinine (Y101, Figure 3) was able to inhibit DNA-HBV and cccDNA with potent activity. In addition, it has also been proved to be beneficial to the patients with liver injury. Therefore, Y101 was chosen as candidate for preclinical study.

This study aimed to find out the difference between repensine and Y101 by utilizing molecular docking technology,

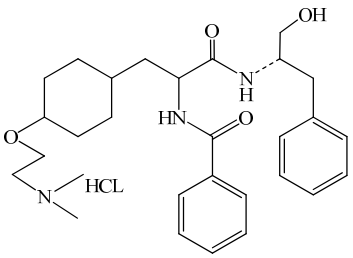

Figure 3 Chemical structure of bentysrepinine (Y101)

and to further explore the potential inhibition mechanism of Y101 and repensine against HBV DNA polymerase, on the basis of binding mode of both compounds.

2. Methods

2.1 Homology modeling

The sequence of HBV polymerase, coded as gi59451 was downloaded from NCBI sequence database (http://www.ncbi. nlm.nih.gov). Sequence analysis showed that residues 354 to 694 were responsible for the RT activity of HBV. The known HIV-1 RT structure with PDB code 1RTD was selected as template to build the structure of HBV polymerase. Sequence alignment suggested that the functionally important residues were highly conserved for these two polymerases. The prediction of the 3D conformation of the optimal protein structure was carried out on Swiss model server (Guex et al, 2009; Kiefer et al, 2009). The coordinates of the original ligand thymidine-5'-triphosphate (TTP), DNA double-strand, and two magnesium ions from 1RTD were added in the obtained protein structure.

2.2 Molecular dynamics

The Gromacs 4.5.4 (Hess et al, 2008) software with AMBER99SB force filed was used for all simulations. The topology parameter of TTP was obtained using RESP method at HF/6-31G (d, p) level. The whole system was placed in a box with an edge of 2.5 nm and solvated with SPC water molecules with shell of 1.2 nm. Sodium ions were added to neutralize the system. The system was minimized firstly and subsequently MD simulation was performed for 500 ps with a time step of 1 fs. The V-rescale method was used for temperature coupling and the reference temperature was set as 300 K.

2.3 Molecular docking

The structure of HBV DNA polymerase from homology modeling was adopted as receptor, and processed using Protein Prepare wizard in Schrödinger program. The original ligand of protein structure (PDB code: 1RTD) TTP was used as the docking center with a box size of 10 Å. Except for TTP, repensine and Y101, lamivudine was also selected as docking ligand as it showed the good inhibitory activity against HBV DNA polymerase. Lamivudine was modified into triphosphate form since nucleostide reverse transcriptase inhibitor (NRTI) must undergo intracellular conversion to the mono-, di-, and active triphosphates through the action of cellular kinases (Daga et al, 2010). The structures of ligands were prepared using LigPrep module: using Epik to determine possible ionization state at pH 7.0 ± 2.0 and adding metal binding states. The OPLS-2005 forcefield was used to produce the low-energy conformer of ligands. Molecular docking computation was carried out using Glide module with default parameters. All these calculations were carried out in Schrödinger software (Friesner et al, 2004).

3. Results

3.1 Homology modeling of HBV DNA polymerase

Due to the reason that the experimental structure of HBV DNA polymerase was not available, homology modeling method was used to predict its 3D structure. Since reverse transcription activity of HBV was associated with residues 354 to 694, primary sequence alignment demonstrated that important functional amino acid residues of polymerases of HBV and HIV-1 were highly conservative (Das et al, 2001). Thus, homology modeling of HBV polymerase was performed using the known structure of HIV-1 reverse transcriptase (PDB code: 1RTD) as template. Sequence alignment from residues Cys312 to Val686 between HBV and template is shown in Figure 4.

The prediction of 3D protein structure was completed on Swiss model server. The resulting active site of HBV is presented in Figure 5.

Ramachandran plot suggested that the built structure had a good quality. Most of the residues were in favored region and additional allowed region. Only limited residues in the disallowed region were shown in Figure 5B. Hence, the protein structure built from homology modeling was reasonable and could be used for further docking calculations.

```
HBV_poly  312                 CWWLQFR NSKPCSDYCL SLIVNLREDW GPCDDHGEHH
1RTD-hom  18                  GPKVKQW PLTEEKIKAL VEICTEMEKE G---------
                              ------- ---------* --*----*-- *

HBV_poly  349   IRIPRT--PA RVRGGVFLVD KNPHNTAESR LVVDFSQFSR GNYRVSWPKF
1RTD-hom  46    KISKIGPENP YNTPVFAIKK KDS---TKWR KLVDFRELNK R--TQDFWEV
                ------  -- ---------- *--    ---* --***----- -  -------

HBV_poly  397   AVPNLPSLTN LLSSNLSWLS LDVSAAFYHI PLHPAAMPHL LVGSSGLSRY
1RTD-hom  91    QL-GIPHPAG LKKK-KSVTV LDVGDAYFSV PLD------- ----------
                -- --*---- *--- -*--- ***--*---- **.

HBV_poly  447   VARLSSNSRY FNNQHGTMQN LHDSCSRNLY VSLLLLYQTF GRKLHLYSHP
1RTD-hom  122   ---------E DFRKYTAFTI ---------- ---------- -PSINNETPG
                         - ----------                       ---------

HBV_poly  497   IILGFRKIPM AVGLSPFLLA Q-FTSAICSV VRRAFPHCLG FSYMDDVVLG
1RTD-hom  142   IRYQYNVLPQ GWKGSPAIFQ SSMTKILEPF RK-QNPDIVI YQYMDDLYVG
                *-------*- ----**---- - -*------ -- --*---- --****---*

HBV_poly  546   AK-SVQHRES LYTAVTNFLL SLGIHLNPNK TKRWGYSLNF MGYIIGSWGT
1RTD-hom  191   SDLEIGQHRT KIEELRQHLL RWGLTTPDKK H-QKEPPFLW MGYELHPDK-
                -- ------- --------** --*------* - -------- ***------

HBV_poly  595   LPQDHIVQKI KHCFRKLPVN RPIDWKVWQR IVGLLGFAAP FTQCGYPALM
1RTD-hom  239   -WTVQPIVLP E--KDSWTVN -DI-----CK LVGKLNWASQ I--YPGIKVR
                 --------- -  -----**  -*     -- -**-*--*-- -  -------

HBV_poly  645   PLYACIQAKQ AFTFSPTYKA FLSKQYMNLY PVARQRPGLC QV
1RTD-hom  278   QLCKLLRGTK -ALTEV--IP LTEEAELELA ENREILKEPV HG
                -*--------  -----  -- --------*- ---------- --
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Figure 4 Sequence alignment of HBV DNA polymerase with template 1RTD

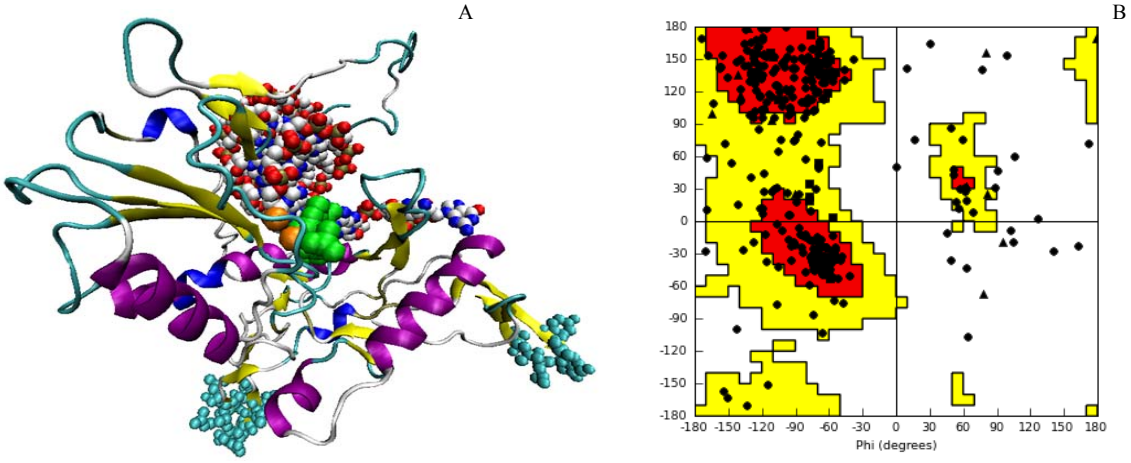

Figure 5 3D protein structure predicted by Swiss model (A) and Ramachandran plot of predicted protein structure (B)

ligand TTP in green sphere model, magnesium ions in orange; residues 436−450 and 476−482 in cyan spheres

3.2 Molecular dynamics of HBV DNA polymerase

The structure of HBV DNA polymerase and TTP complex is shown in Figure 6, from which we could see that the hydrogen bond between TTP and nucleic acid base was retained during the MD simulation. Figure 7A is the root mean square deviation (RMSD) of backbone atoms from the initial structure. The RMSD increases rapidly to 0.4 nm within the first 50 ps, due to the optimization of interactions within the protein structure. The RMSD is well equilibrated after 300 ps and fluctuates at about 0.52 nm. Figure 7B shows that the root mean square fluctuation (RMSF) was calculated for the backbone atoms. Most of the residues have a RMSF less than 0.3 nm. The most flexible regions are residues 436−450 and 476−482, and these residues are far from the active pocket (Figure 5A). Therefore, our HBV DNA polymerase homology model has acceptable stability and can be used for molecular docking calculations.

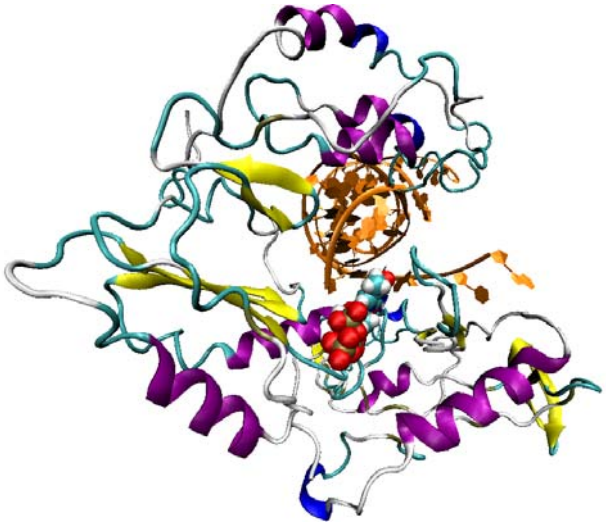

Figure 6 Structure of HBV DNA polymerase after 500 ps molecular dynamics

Water molecules are hidden for clarity. Images were produced by VMD software (http://www.ks.uiuc.edu)

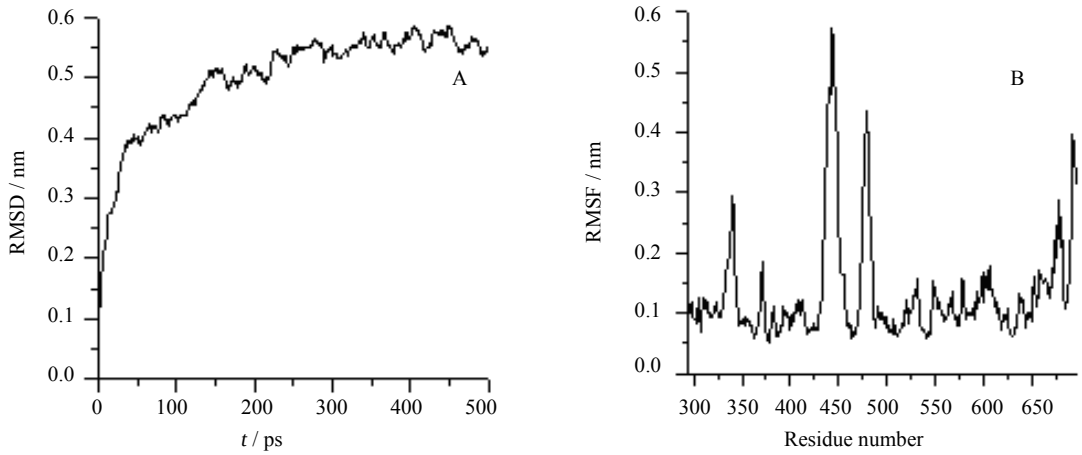

Figure 7 RMSD (A) and RMSF (B) of backbone of HBV DNA polymerase

3.3 Docking results of Y101 and repensine with HBV DNA polymerase

The binding modes between receptor and ligands were predicted by using Glide module. Table 1 lists the docking results of four ligands. From Table 1 we can see that the glide scores of Y101 and repensine are similar and a little lower than those of TTP and lamivudine. As for lamivudine, the Comlomb interaction dominates the whole interaction, while van der Waals interactions play an equally important role for Y101 and repensine. The hydroxyl group of Y101 creates a hydrogen bond with Asp366, and the benzene ring of Y101 forms π-π interaction with Phe423. All these cause lower interaction energy of Y101 than repensine. From Figure 8a we can see that lamivudine fits well in the active pocket of HBV DNA polymerase. The phosphate groups interact with two Mg^{2+} via electrostatic interaction; its ring structure interacts with the complementary DNA base in base pair type; its ring parallels with the neighbor DNA base and forms π-π stacking. Y101 and repensine do not have nucleic base analog, so the interaction mode of them with HBV DNA polymerase is quite different from that of NRTIs. The carbonyl oxygen atom of

Y101 coordinates with the Mg^{2+} to stabilize the system and the three benzene groups to occupy the hydrophobic pocket can be seen from Figure 8b.

Figure 9 shows the hydrophobicity surface of HBV DNA polymerase with Y101 and repensine, respectively. Except the two Mg^{2+} coordinated by residues Asp418 and Asp540, the active pocket is mainly made up by hydrophobic residues, such as Val419, Ala421, Ala422, Phe423, Tyr424, Tyr424, Met506, Met439, Asn571, and Asn571. Arg376 may be important as it is involved in hydrogen bond interaction for both repensine and lamivudine docking modes. Apparently, hydrophobicity surface confirms again that the binding mode of Y101 and repensine with HBV DNA polymerase was driven by hydrophobic interaction. This might provide novel recognition of inhibitory effect of Y101 against HBV, though its inhibition mechanism needs to be validated by bio-assay at cellular level and of polymerase activity.

3.4 Docking results of Y101 and repensine with other targets

Docking calculations of Y101 and repensine with HIV integrase (PDB code: 3NF7), HIV inverse transcriptase (PDB code: 3NF7), and HCV NS5B polymerase (PDB code: 3U4O) were carried out in an identical approach. The findings suggested that these two compounds might have the potential to inhibit HCV NS5B polymerase. The results are presented in Table 2.

Table 1 Docking results between ligands and HBV DNA polymerase

Ligands	Glide scores	Van der Waals interactions	Coulombic interactions	Glide energies	Numbers of hydrogen bond
repensine	−10.59	−41.74	−40.66	−82.40	3 (Phe423, Arg376)
Y101	−10.08	−43.10	−44.50	−87.60	2 (Ala421, Asp366)
TTP	−9.85	−38.98	−43.07	−82.04	3 (Ala422, Arg376)
lamivudine	−9.20	−33.10	−41.76	−74.86	4 (Ala422, Arg376)

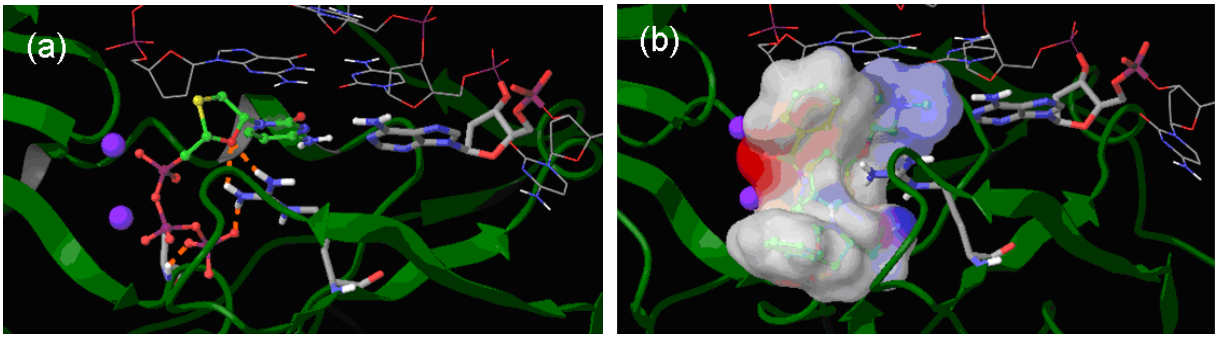

Figure 8 Docked poses of lamivudine (a) and Y101 (b)

Ligands are colored in green-carbon scheme and others in gray-carbon scheme. Images were produced by Schrödinger software

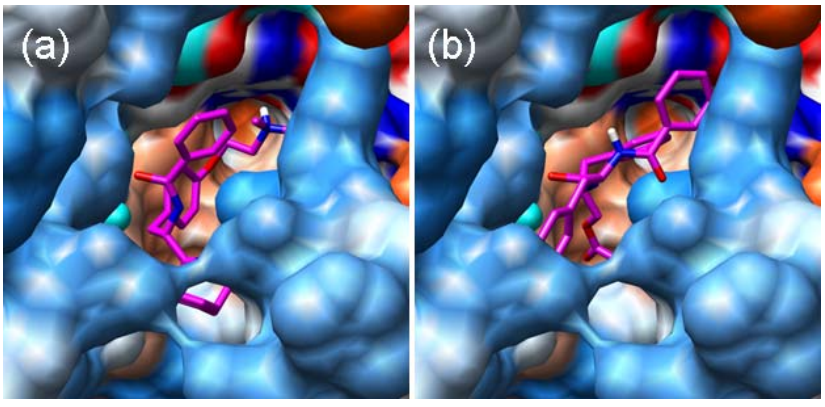

Figure 9 Hydrophobicity surface of HBV DNA polymerase with Y101 (a) and repensine (b)

Images produced using UCSF Chimera software (Pettersen et al, 2004); hydrophilic region in light blue, hydrophobic region in orange

4. Discussion

Repensine, a novel compound with a di-peptide structure, was isolated from *D. repens* and exhibited the strong antiviral activity of HBV. After chemical structure modification of repesnine, authors synthesized about 200 derivatives. Antiviral activity study of Y101, a derivative of them, was demonstrated in *in vitro* and *in vivo* tests. Y101 is a new drug with independent intellectual property rights in China, and has now completed preclinical studies which proved that Y101 inhibited

Table 2 Docking results of Y101 and repensine with other targets

Receptors	Ligands	Glide scores	Glide energies	Hydrogen bond interactions
3NF7	Y101	−4.81	−42.67	1 (Lys188)
(HIV integrase)	repensine	−4.66	−46.34	2 (Lys188,Ser153)
3HVT	Y101	−7.51	−44.03	hydrophobic cavity occupied
(HIV inverse transcriptase)	repensine	−4.64	−43.85	hydrophobic cavity occupied
3U4O	Y101	−5.76	−53.47	4 (Arg386, Gln446, Gly449, Tyr448)
(HCV NS5B polymerase)	repensine	−6.91	−53.11	2 (Arg386, Asn316)

DNA-HBV and cccDNA. In 2013, it was reviewed by China Food and Drug Administration (CFDA) and approved as in the investigating new drug (IND) into clinical trials.

Clinical application of nucleoside analog therapy found that Definitions and timing of occurrence of virological forms of antiviral treatment failure during therapy of chronic hepatitis B, including primary non-response, genotypic resistance, and virological breakthrough (Ghany and Doo, 2009). Current antivirals can control but not eliminate hepatitis B virus (HBV), because HBV establishes a stable nuclear cccDNA. HBV-core protein mediated the interaction with nuclear cccDNA resulting in cytidine-deamination, apurinic/ apyrimidinic site formation and finally cccDNA degradation that prevented HBV-reactivation. Genomic DNA was not affected (Lucifora et al, 2014). That is to say, cccDNA acts as a virus protein and virus genome to generate template, if a drug is able to inhibit cccDNA, can stop the replication of the virus.

The anti-HBV mechanism of the IND in preclinical study found a larger breakthrough, which is different from the nucleoside drugs inhibiting polymerase. The IND has been significantly inhibited in the *in vitro* and *in vivo* models, also with the existing anti-HBV nucleoside drugs, non-rising the virus copy and improving the index of liver function after the withdrawal. In resistant virus strains to existing lamivudine and entecavir, it also has significant anti drug-resistance. But these advantages still need to be confirmed by clinical research.

4 Conclusion

The binding mode of Y101 and repensine with DNA polymerase has been driven by hydrophobic interaction. This might provide novel recognition of inhibition effect of Y101 against HBV, though its inhibition mechanism needs to be validated by bio-assay at cellular level and polymerase activity. Preliminary *in silico* Molecule docking study suggests that Y101 might be able to inhibit HIV inverse transcriptase, also have the potential to interact with DNA polymerase and HCV NS5B polymerase.

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