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Can inhibitors of HIV integrase work on HCV polymerase?

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Abstract

As members of the flavivirus family, hepatitis C viruses (HCVs) are a group of small, single-stranded, positive sense RNA viruses. It has been estimates that over 170 million people are currently affected by HCV. To date, interferon-α alone or in combination with ribavirin is the only approved therapies for the treatment of HCV infections, and both are associated with low efficacy and various side effects. A recent approach to this problem is the development of novel inhibitors that interrupt the normal functions of enzymes/proteins encoded by the HCV genome. Under this perspective, development of inhibitors targeting the HCV polymerase activity has been explored and, only recently, approximately 200 compounds including alkyl-, phenyl-, pyrrole- and thiophene-substituted diketoacids have been evaluated in the HCV NS5 polymerase essay. Interestingly, some of the diketoacids also inhibits hepatitis B virus polymerase, and HIV reverse transcriptase.

Quite recently, some of us have synthesized a novel series of diketoacid derivatives, which proved to be active on the HIV integrase. Accordingly, in this work we performed a detailed molecular modeling on the interactions and binding energies of these inhibitors with the key enzyme involved in the HCV genome replication, i.e. the virially encoded RNA-dependent RNA polymerase (RdRp).

Introduction

Hepatitis C (HCV) is a RNA virus with a genomic size of 9.4 kb. Since the advent of serological essays for HCV in 1990, it has been shown to be the major etiological agent for post-transfusion and sporadic non-A, non-B hepatitis worldwide. In USA alone, there are 175 000 cases of newly documented hepatitis C per year, and the number of HCV carriers has been estimated to be 4 millions. Unlike hepatitis B, which is associated with chronicity in approximately 5% of adult infections, more than 80% of HCV-infected individuals develop chronic hepatitis. Moreover, a significant proportion of individuals (20-50%) is at risk of developing cirrhosis and hepatocellular carcinoma The known modes of HCV transmissions are transfusions (including contaminated immunoglobulin preparations), occupational exposure and intravenous drug abuse.

HCV is a single stranded RNA virus within the *Flaviviridae* family. The structural proteins (E1 and E2) are encoded at the 5' end, followed by the non-structural proteins (NS2 to NS5B) that have various functions, including a helicase, or protease (NS3), and a RNA polymerase (NS5).

To date, IFN-alpha monotherapy and, more recently, combination therapy of ribivarin plus intron-A (Rebetron) are the only FDA approved agents that have demonstrated efficacy in the treatment of HCV infections. However, several steps in the HCV replicative pathway can be the targets for selective antiviral interventions. For instance, development of inhibitors targeting the HCV polymerase activity has been explored and, only recently, approximately 200 compounds including alkyl-, phenyl-, pyrrole- and thiophene-substituted diketoacids have been evaluated in the HCV NS5 polymerase

essay. Interestingly, some of the diketoacids also inhibits hepatitis B virus polymerase, and HIV reverse transcriptase.

Under these perspectives, in this work we used molecular modeling methods to derive QSARs for diketoacid analogues already synthesized and known as active in the replication of the HIV viruses, to carry out design of new, potential inhibitors for the HCV using computer models of the receptors and rational structure-based molecular design methods, and to apply computer assisted combinatorial chemistry approaches to design and optimize virtual libraries of enzyme inhibitors and perform *in silico* screening and scoring of the drug candidates.

Computational details

The 3-D structure of the RNA-dependent RNA polymerase was taken from PDB (code 1C2P). All ionizable residues were kept in the standard ionization state at neutral pH. The protein relaxation was performed using the SANDER module of AMBER 6.0, with the force field by Cornell et al. The GB/SA continuum solvation model was employed to mimic a water environment. The inhibitors by Merck® and the diketoacid analogues were subjected to an initial energy minimization using Discover, followed by a conformational search using a combined molecular mechanics-molecular dynamics simulated annealing protocol.

300 Monte Carlo/Simulated Annealing (MC/SA) runs performed using AutoDock 3.0 for the docking of each diketoacid to the protein. The lowest interaction energy structure was refined using a quenched molecular dynamics method. The best energy configuration was solvated by a cubic box (extended 10 Å from the protein) of TIP3P water molecules with periodic boundary conditions. Each system gradually heated to 298 K and then

equilibrated for 25 ps at 298 K, followed by 400 ps of data collection runs (2 fs integration time step).

Results and Discussion

In this work, we performed a detailed molecular modeling on the interactions and binding energies of inhibitors by Merck® with one of the key enzyme involved in the HCV genome replication (the virially encoded RNA-dependent RNA polymerase - RdRp) to derive a IC50 prediction for diketoacids analogues synthesized by our group. Starting from the experimental IC50 values for inhibitors by Merck®, we modeled and docked these inhibitors obtain the relevant ΔG binding to the RdRp. Accordingly, we plotted the simulated DG values against the corresponding IC50, and obtained a good linear relationship, as illustrated in Figure 1.

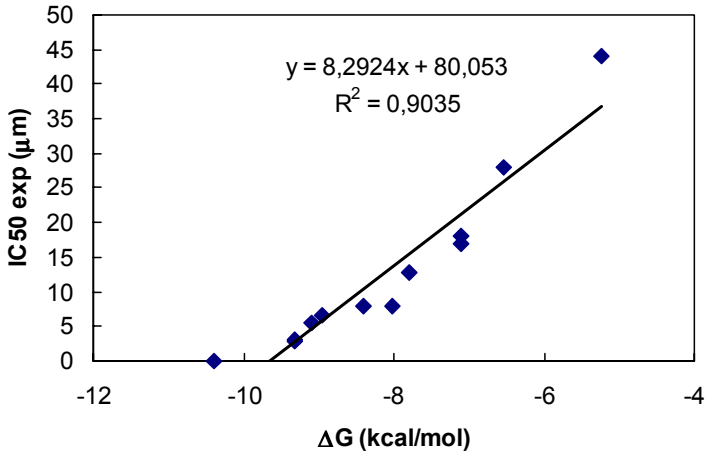

Figure 1. Linear relationship obtained by simulation of Merk® diketoacids.

We then used the simulated ΔG of binding and the above relationship to estimate the IC50 values for a series of 61 diketoacids analogues synthesized by our group. The corresponding results are reported in Table 1.

Table 1. Simulated ΔG for diketoacid analogues and the corresponding IC 50 obtained with the linear relationship reported in Figure 1.

No.	ΔG (kcal/mol)	IC50 (μm)	No.	ΔG (kcal/mol)	IC50 (μm)
rds_1788	-7,39	18,8	rds_1687	-4,88	39,6
rds_1792	-7,15	20,8	rds_1688	-5,58	33,8
rds_1793	-8,30	11,2	rds_1779	-4,40	43,6
rds_1794	-8,28	11,4	rds_1780	-4,03	46,6
rds_1624	-6,45	26,6	rds_1781	-4,50	42,7
rds_1625	-7,16	20,7	rds_1759	-4,94	39,1
rds_1626	-0,14	78,9	rds_1760	-2,61	58,4
rds_1628	-0,36	77,1	rds_1763	-4,01	46,8
rds_1612	-4,93	39,2	rds_1764	-5,32	35,9
rds_1591	-7,41	18,6	rds_1778	-0,62	74,9
rds_1606	-1,24	69,8	rds_1823	-2,73	57,4
rds_1607	-0,02	79,9	rds_1826	-3,65	49,8
rds_1610	-3,13	54,1	rds_1827	-4,41	43,5
rds_1611	-3,74	49	rds_1754	-6,25	28,2
rds_1599	-2,08	62,8	rds_1786	-7,81	15,3
rds_1600	-3,67	49,6	rds_1771	-6,01	30,2
rds_1738	-6,35	27,4	rds_1755	-4,46	43,1
rds_1707	-7,29	19,6	rds_1787	-7,23	20,1
rds_1711	-1,63	66,5	rds_1680	-7,95	14,1
rds_1712	-3,65	49,8	rds_1716	-5,07	38
rds_1713	-0,48	76,1	rds_1692	-2,96	55,5
rds_1714	-4,31	44,3	rds_1693	-2,19	61,9
rds_1715	-1,32	69,1	rds_1698	-3,78	48,7
rds_1639	-6,10	29,5	rds_1699	-3,32	52,5
rds_1640	-6,60	25,3	rds_1703	-1,62	66,6
rds_1643	-4,81	40,2	rds_1736	-3,99	47
rds_1644	-5,69	32,9	rds_1737	-2,32	60,8
rds_1790	-8,28	11,4	rds_1704	-0,83	73,2
rds_1791	-9,18	3,89	rds_1743	-5,36	35,6
rds_1675	-7,23	20,1	rds_1744	-7,76	15,7
rds_1683	-7,00	22			
rds_1684	-3,61	50,1			

Figure 2a and b show the molecular models of two diketoacid potential inhibitors characterized by the two outstanding IC50 values docked to the protein active site. Figure 3a and b show the details of the interactions between the residues involved in the binding of rds_1791 and rds_1793, respectively. These docking figures reveal that, in both cases, the two drugs are well inserted into binding site. The main difference between the two molecules can be ascribed to a different chemical and fisical structures of these diketoacids analogues, resulting into a different balance of the intermolecular interactions.

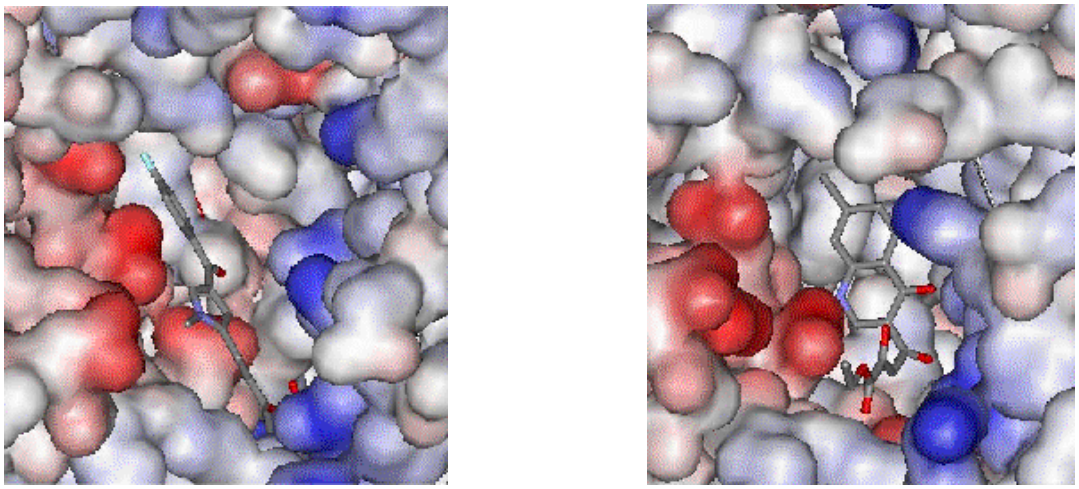

Figure 2a and b. Inhibitors rds_1791 (left) and rds_1793 docked into the RdRp binding pocket.

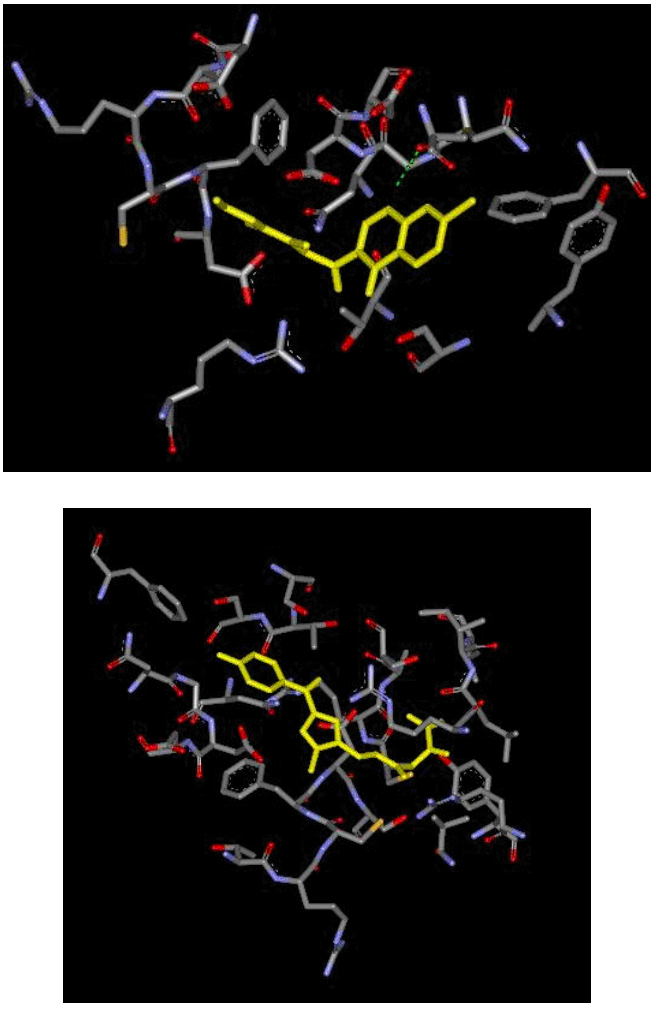

Figure 3a and b. Details of the interactions between the residues involved in the binding of rds_1791 (right) and rds_1793 (left) to RdRp active site.

Conclusion

In this work we applied a detailed molecular simulation protocol to estimate the free energy of binding of 12 Merck® dikeotacid analogue RdRp inhibitors. These ΔGs have been correlated with the corresponding experimentally available IC50 values, obtaining a fairly good linear relationship (R^2=0.903). We finally used the simulated ΔG of binding and the obtained linear relationship to estimate the IC50 values for a series of 61 diketoacids analogues synthesized by our group.

Interestingly, the IC50 predicted values for diketoacid analogue inhibitors are of the same order of most of Merck® functional molecules. On the basis of these results, we are currently checking the difference between the activity behavior *in silico* and in vitro (by means of enzimatic test), and applying computer assisted combinatorial chemistry approaches to design and optimize virtual libraries of potential RdRp inhibitors based on diketoacid structures.

Acknowledgments

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