VirusDis. (April–June 2019) 30(2):207–213
https://doi.org/10.1007/s13337-019-00516-7

ORIGINAL ARTICLE

Novel inhibitors against wild-type and mutated HCV NS3 serine protease: an in silico study

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Received: 10 December 2018 / Accepted: 13 March 2019 / Published online: 21 March 2019

Abstract In 2011, the FDA approved boceprevir as a hepatitis C virus (HCV) NS3 serine protease inhibitor. The sustained virological response rate for treatment with this approved compound is considerably low. Patients have not responded as much as expected to boceprevir therapy. In this in silico study, modified boceprevir compounds are suggested and tested on wild-type HCV NS3 protease and 19 mutated HCV NS3 proteases using molecular docking. Results reveal the superiority of two of the proposed modified compounds to boceprevir. One of which appears to be more potent than boceprevir itself concerning activity against wild-type NS3 and most of the examined mutated NS3 proteases.

Keywords Boceprevir · Fluorinated sulfonamide · HCV · Molecular modeling · Mutated NS3 protease · Protein–ligand docking

Introduction

Hepatitis C virus (HCV) affects more than 180 million people worldwide [15]. 10% of Global HCV infection is in Egypt [1]. It was first identified in 1989 [8]. The HCV-infected liver is susceptible to the development of cirrhosis and hepatocellular carcinoma [15, 27]. Ribavirin with interferon was the routine therapeutic regimen for HCV until 2011 when the FDA approved boceprevir [2, 9].

HCV proteins include both structural and non-structural proteins [11, 27]. The protease NS3 and the RNA-dependent RNA polymerase NS5B are the most targeted non-structural proteins [10, 13, 20]. Viral proteases are essential proteins that regulate the life cycles of many viruses, including HCV. Within liver cells, the HCV polyprotein is cleaved by viral and host cell proteases to form active viral proteins [11]. HCV NS3 protease has catalytic triad amino acids (His57, Asp81 and Ser139) which play an essential role in the activity of NS3 protease and polyprotein cleavage process. NS3 protease inhibitors prevent the polyprotein cleavage process and hence stop viral maturation.

Dual therapy with ribavirin and interferon has a wide range of side effects that could result in the cessation of the therapeutic regimen. Drug designers are working to develop anti-HCV drugs that act on a specific protein [24, 27]. In particular, the inhibition of viral protease will block the viral life cycle of HCV. The FDA approved the drug boceprevir in 2011 as the first direct-acting antiviral against NS3 serine protease [22]. Despite this drug's potency, the sustained virological response rate for boceprevir treatment is low due to the high mutation rate of HCV. In addition to its many side effects so it had been discontinued by US FDA. Many other drugs are approved through the US FDA as HCV inhibitors. Some of these are

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13337-019-00516-7) contains supplementary material, which is available to authorized users.

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telaprevir, dasabuvir, velpatasvir, pibrentasvir, pegylated interferon with ribavirin, ledipasvir, sofosbuvir and simeprevir [14]. All of these drugs show a potent effect against HCV, mainly when the treatment consists of a combination of two anti-HCV. The percentage of its treatment efficiency may reach to 93%, but it is still limited due to its very high cost. Such as cocktail treatment with ledipasvir and sofosbuvir [7, 14] At present, six HCV genotypes have been defined; these genotypes exhibit approximately 70% sequence similarity and have been classified into more than 100 subtypes [9]. There are many clinical and experimental studies to investigate the effect of HCV protease inhibitors on the NS3 protease mutations. These studies show that NS3 protease mutations are resistant to HCV protease inhibitors and that treatment failure for patients is associated with the development of resistance-associated mutations (RAMs) in the NS3 gene [6, 25]. Theoretical and modeling methods are also used to study the binding affinity of protease inhibitors with mutant NS3 protease [17, 19]. The mutations reduce the binding affinity of inhibitors to NS3/4A protease. Theoretically, some macrocyclic inhibitors are potent against some NS3 protease mutations and have good scores. In an individual patient, different virus strains are likely to present; therefore, studying the effects of these mutations is necessary for comprehensive drug design in the context of treating HCV [15].

Since the mutations in hepatitis C NS3/4A protease cause boceprevir resistance [19], this study introduces certain modifications to boceprevir which are tested against wild-type NS3 protease and 19 mutated NS3 proteases. These modifications to boceprevir are based on the addition of cyclopropyl ring and extending sulfonyl or carbonyl group at position P4. This is because of the excellent Van Der Waals interactions that were reported between the cyclopropyl ring and terminal NH groups of Arg155 using x-ray crystallography [4]. The extended sulfonyl or carbonyl group at position P4 would help in forming stable H-bonds with Cys159 and hence give better inhibition [5]. Moreover, in our previous QSAR study on these modified compounds, the addition of functional groups to boceprevir gave the compounds good inhibition properties [21, 22]. This study is as a trial to overcome the high cost of combination or cocktail treatments by improving the inhibition activity of boceprevir. Experimentally, the sulfonamide group is shown to have inhibition activity against HCV replication and NS3 protease [16]. Molecular docking is performed using SCIGRESS 3.0 software. The docking scores are compared for each modification and each mutation.

Materials and methods

Building the molecular structure

All calculations are performed on Dell Precision T3500 workstation in biophysics department, science faculty, Cairo university using SCIGRESS 3.0 molecular modeling software. Suggested modified boceprevir compounds are sketched, optimized and prepared for the docking study using SCIGRESS 3.0 software tools, as previously reported [22]. The optimization is performed in two steps, beginning with the use of the classical mechanic's force field MM3 and followed by the application of the semi-empirical quantum mechanical parameterization method 3 (PM3) [26]. After optimization, the vibrational spectrum is calculated to ensure that the optimized compounds are real. This calculation is performed at the same level used for computation [21]. The optimized structures are now ready for the docking study.

The structure of HCV NS3 serine protease is retrieved from the Protein Data Bank (www.pdb.org) [3]. Using the 4A92 structure, we can dock different modified compounds into the active sites of the wild-type and mutated proteases. This docking is performed after the removal of water molecules, the addition of missing hydrogens and the removal of ligands from the protein structure using SCIGRESS 3.0 software tools.

Generation of NS3 mutations

Nineteen mutations of the wild-type protease are generated in silico using SCIGRESS 3.0 software. The mutated proteins are geometrically optimized using the MM3 force field. The NS3 serine protease mutations are as follows: A156S, A156T, A156V, D168A, D168E, D168G, D168I, D168V, F43S, Q41R, R155K, R155Q, S138T, T54A, T54S, V36A, V36G, V36L and V36M [12, 25].

Molecular docking

Docking calculations are performed using the default docking options for SCIGRESS 3.0 software installed on a Dell Precision T3500 workstation. The ligands and active site residues are flexible to allow the molecular arrangements that occur during recognition and binding [12]. The Potential Mean Force (PMF04) [18] scoring function is utilized with a grid spacing of 0.25. A genetic algorithm is used with a population size of 50 and a maximum of 3000 generations (crossover rate 0.8, mutation rate 0.3 and convergence 1.0). The maximum number of iterations for local search is 20, with a rate of 0.06.

Docking calculations are performed three times for each ligand and mean values are compared via Duncan multiple range tests conducted using the SPSS 19.0 software package. The protein–ligand interactions profile is calculated using the PLIP server (https://projects.biotec.tu-dresden.de/plip-web/plip/) [23].

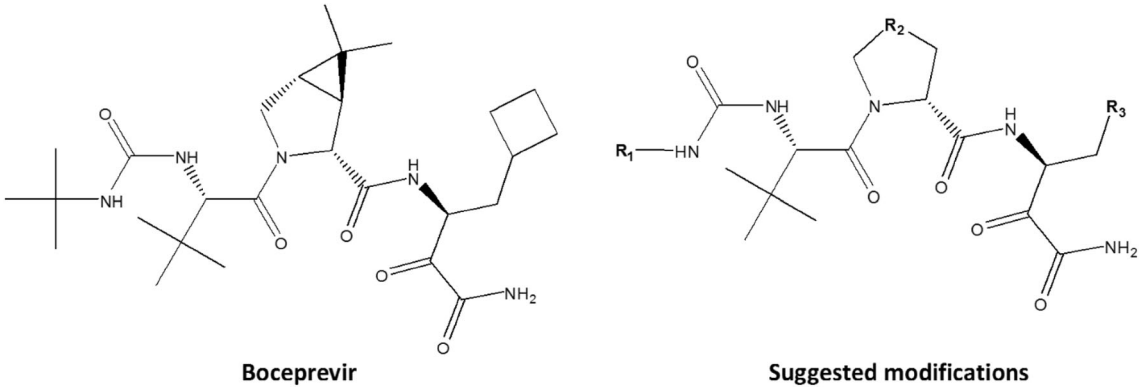

Fig. 1 Structures of the parent compound boceprevir and the suggested modified compounds. R_1, R_2 and R_3 represent the positions where groups are added to the parent compound. These groups are listed in Table S1

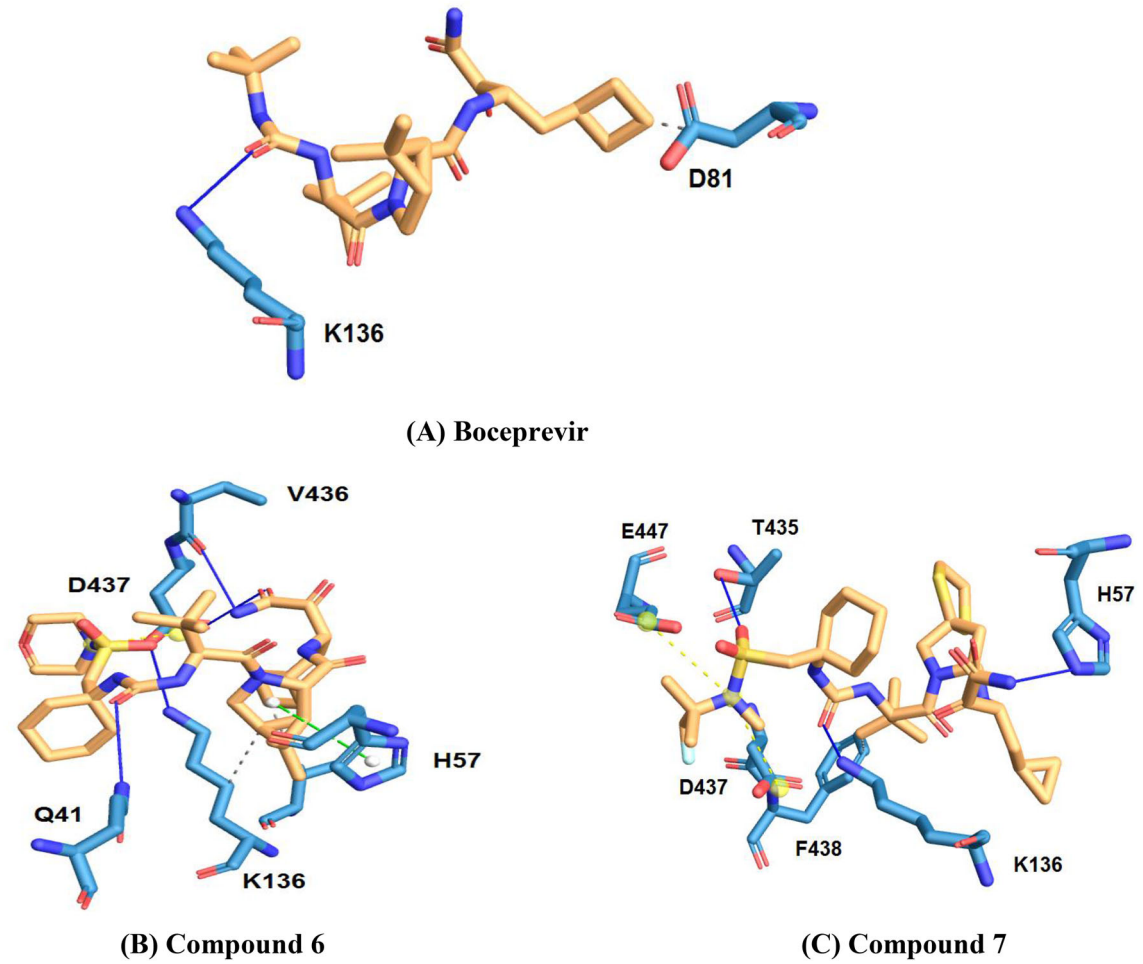

Fig. 2 Docking diagram showing the modes of interaction with wild-type NS3 for boceprevir (**a**), compound 6 (**b**) and compound 7 (**c**). The solid blue line is a hydrogen bond, dashed gray line is a hydrophobic interaction, dashed yellow line is a salt bridge and dashed green line is a pi-stacking interaction

Results and discussion

Wild-type NS3 docked with the modified compounds

In our previous study of modified boceprevir compounds [22], eight modifications were tested in silico for stability and reactivity using quantitative structure–activity relationship (QSAR) models. In the current work, molecular docking of the eight compounds to the active site of NS3 protease (PDB ID 4A92) is performed. Figure 1 shows the structures of both boceprevir and the modified compounds, with three modification positions: R_1, R_2 and R_3. Table S1 (In supplementary materials) represents the structures of the added groups (at R_1, R_2 and R_3). This table also shows the average docking scores and the amino acids involved in H-bond formation between the ligands and the active site of the protein. The average docking score for boceprevir is -94.55 ± 2.41. Notably, except for compounds 2 and 4, these compounds have better average docking scores than the parent compound (boceprevir). Compounds 6 and 7 have the best average docking scores among the modified compounds (compared to boceprevir's average docking

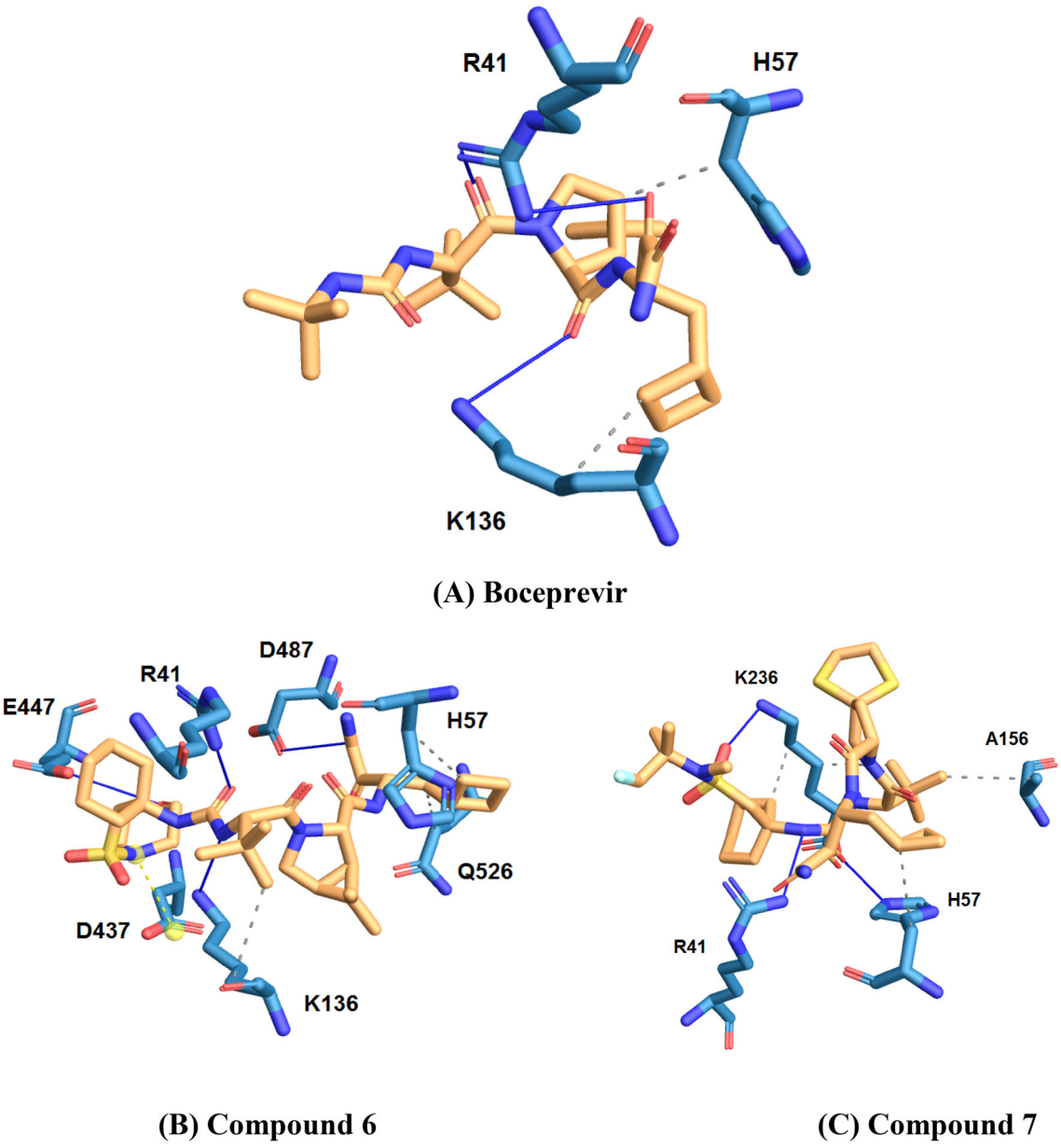

Fig. 3 Docking diagram showing the modes of interaction with mutated NS3 (Q41R) boceprevir (**a**), compound 6 (**b**) and compound 7 (**c**). The solid blue line is a hydrogen bond, dashed gray line is a hydrophobic interaction, dashed yellow line is a salt bridge and dashed green line is a pi-stacking interaction

score value based) as well as better interactions with the active site of NS3. As listed in table S1 and shown in Fig. 2, boceprevir forms only one H-bond with Lys136 (Fig. 2a). Compound 6 produces four H-bonds in docking interactions with Gln41, Lys136, Val436 and Asp437 (Fig. 2b). Three H-bonds formed between compound 7 and NS3 protease at His57, Lys136 and Thr435 (Fig. 2c). Figure 2 also shows the hydrophobic interaction, salt bridge and pi-stacking. Compound 6 (Fig. 2b) produces two hydrophobic interactions with Val132 and Lys136, one salt bridge at Asp437 and one pi-stacking with His57. On the other hand, in Fig. 2c, compound 7 forms one hydrophobic interaction with Phe438 and two salt bridges with Asp437 and Glu447. These results exhibit good agreement with those of a prior study [22], which suggested that compound 7 was the best of the investigated modified boceprevir compounds based on examinations of QSAR models.

NS3 protease mutations against boceprevir and modified boceprevir compounds

The docking calculations for boceprevir and the two best compounds (compounds 6 and 7) are performed with different mutated NS3 proteases. The NS3 protease mutations are A156S, A156T, A156V, D168A, D168E, D168G, D168I, D168V, F43S, Q41R, R155K, R155Q, S138T, T54A, T54S, V36A, V36G, V36L and V36M. From supplementary materials, Table S2 lists the average score of these docking interactions and the involved amino acids in H–bonds interaction. The resistance of mutated NS3 protease against studied compound is predicted by the increase in average docking score values. Also, the inhibition activity of studied compounds to inhibit the activity of mutated NS3 protease is indicated by the decrease in the value of average docking score. According to average docking score values, mutated NS3 protease (Q41R) produces good docking interaction with boceprevir (− 118.51 kcal/mol), compound 6 (− 126.44 kcal/mol) as well as compound 7 (− 122.97 kcal/mol). Figure 3 and table S2 represent the involved amino acids that form H-bonds between (Q41R) NS3 protease mutation and boceprevir, ligand 6 or ligand 7. Boceprevir forms two H-bonds with mutated NS3 (Q41R) at Arg41 and Lys136. Also, it forms two hydrophobic interactions with His57 and Lys136 (Fig. 3a). Q41R mutated NS3 produces four H-bonds, three hydrophobic interactions and one salt bridge with compound 6 (Fig. 3b). The four H-bonds are formed with Arg41, Lys136, Gul447 and Asp487. His57, Lys136 and Gln526 form three hydrophobic interactions with compound 6. Finally, one salt bridge forms with Asp437. As shown in Fig. 3c, compound 7 forms three H-bonds and three hydrophobic interactions. The three H-bonds are with Arg41, His57 and Lys136, while the three hydrophobic interactions are with His57, Lys136 and Ala156. Figure 4 shows a complete summary of the docking score values calculated for the docking of boceprevir and modified boceprevir compounds to wild-type and mutated NS3 proteases. This figure is statistical prediction about which compound will give the best docking score with all studied mutations. Comparisons of total docking score for boceprevir (− 2042.961 kcal/mol) and all other examined compounds indicate that the best total docking score (the most negative value) is reported for compound 6 (− 2240.116 kcal/mol) and that compound 4 has the worst total docking score of − 1906.909 kcal/mol. Figure 5 shows the average docking scores of compounds 6 and 7 compared with those of boceprevir for wild-type and mutated NS3. Compound 6 (Fig. 5a) shows better average docking scores than boceprevir for wild-type and mutated

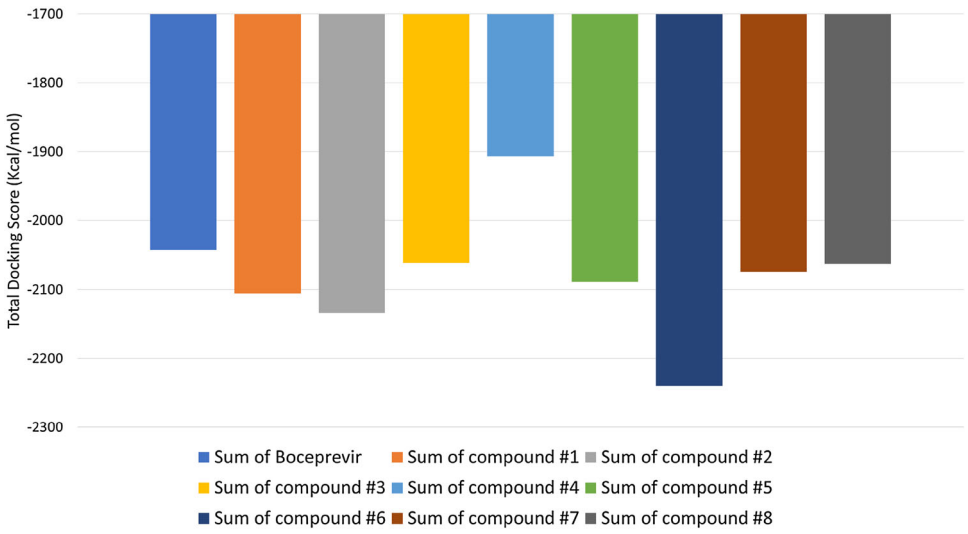

Fig. 4 The total sums of the docking score values (in kcal/mol) calculated for wild-type and mutated NS3 for each ligand (boceprevir and the eight modified compounds). The superiority of most of the modified compounds to boceprevir is evident. The best total sum is reported for compound 6

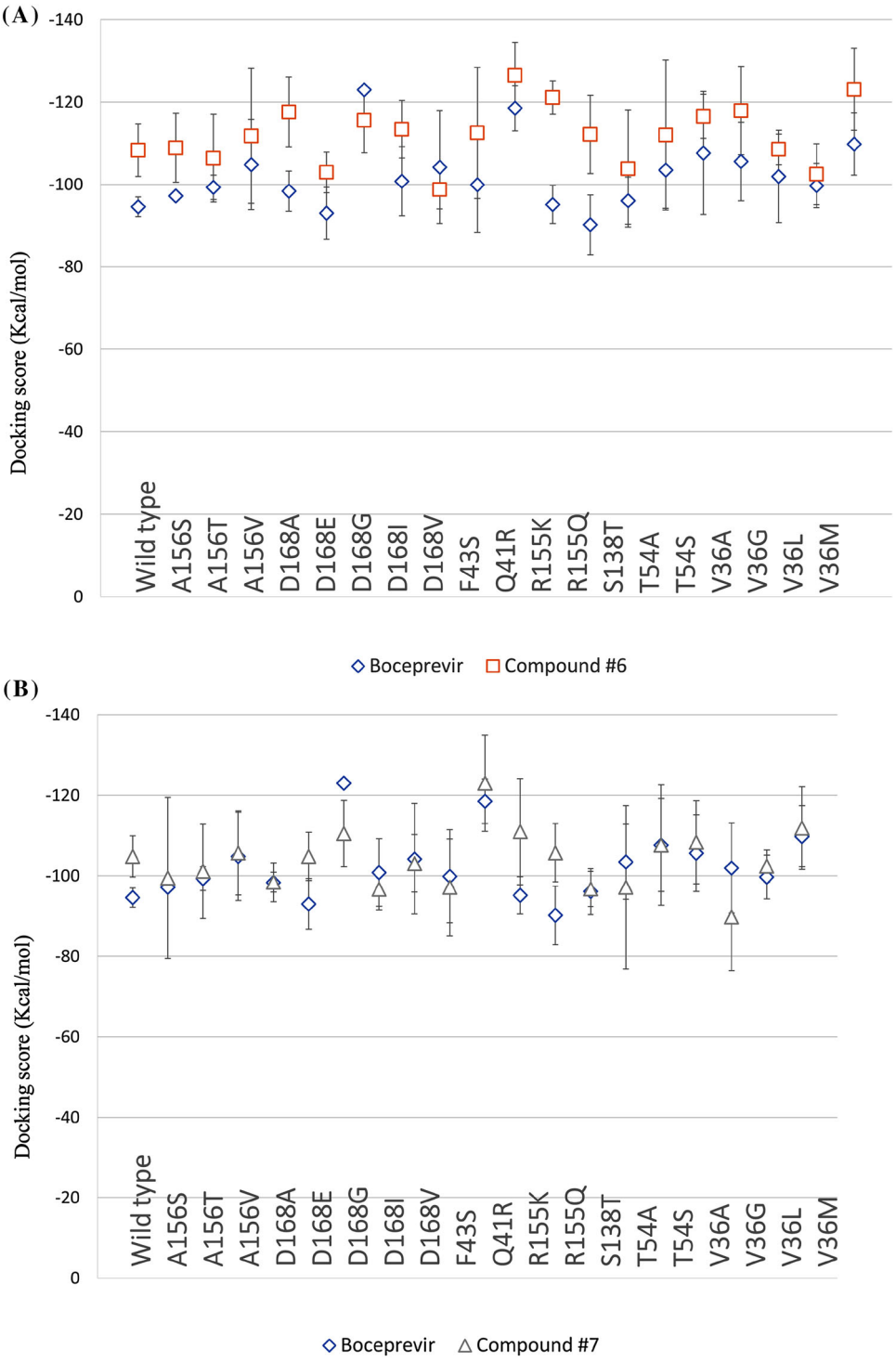

Fig. 5 The average docking score values (kcal/mol) calculated for boceprevir, compound 6 and compound 7 for each of the tested wild-type and mutated NS3 variants. Error bars represent the standard deviations of the mean values

NS3, except for NS3 with the D168G or D168V mutations. Based on the standard deviations of the mean values (error bars), the docking scores for compound 6 and boceprevir significantly differ for the wild-type, A156S, D168A, R155K and R155Q forms of NS3, whereas the mean docking scores for compound 7 (Fig. 5b) and boceprevir only significantly differ for the wild-type and R155Q forms of NS3. From these results, it can be concluded that compound 6 and, to a lesser extent, compound 7 would have better interaction potencies than boceprevir with the NS3 active site for both wild-type NS3 and most of the mutated NS3 proteases.

Based on the results of this study can be concluded that the molecular principles confirmed an increased resistance

of boceprevir due to mutations in HCV NS3 protease [25]. In contrast, the compounds suggested in the present work reduce the resistance against mutated NS3 protease. The present molecular docking study on modified boceprevir compounds suggests that it would be precious to experimentally examine the activities of compounds 6 (with a tetrahydropyranated sulfonamide at position R_1) and 7 (with a fluorinated sulfonamide at position R_1, a 1,3-dithiolane ring at position R_2 and a cyclopropane at position R_3) against wild-type and mutated NS3 proteases due to their high interaction propensities.

Compliance with ethical standards

Conflict of interest All the authors declare that there are no conflicts of interest related to this work.

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