QSAR and Docking Studies on 1,1-Dioxo-2H-benzothiadiazines Acting as HCV NS5B Polymerase Inhibitors

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Abstract: Quantitative structure-activity relationship (QSAR) and molecular modeling studies have been made on a large series of 1,1-dioxo-2H-benzothiadiazines acting as HCV NS5B polymerase inhibitors. A multiple linear regression analysis has pointed out that the polymerase inhibition activity of compounds would be largely controlled by their polarizability, van der Waals volume, and the presence of sulfur and nitrogen atoms in them. The docking study indicated that increasing the number of H-bond donors and acceptors in the molecules as well as putting some hydrophobic groups at proper sites in them would be highly advantageous for their activity.

Keywords: Benzothiadiazines, NS5B polymerase inhibitors, Quantitative structure-activity relationship study.

1. INTRODUCTION

Hepatitis C virus (HCV), a member of the *Flaviviridae* family of viruses, is dangerously affecting a major portion of world population, often leading to liver transplantation [1]. It leads to a chronic state that persists for decades and eventually leads to several fatal diseases such as cirrhosis, liver failure or liver cancer [2]. So far neither any anti-HCV vaccine nor any drug could be developed to treat HCV infection and the new standard of care involves the combination of a protease inhibitor with pegylated α-interferon and the oral nucleoside anti-viral agent ribavirin [3-5]. The clinical benefit of this treatment is limited and some undesirable side effects are associated with these therapies [6, 7]. These limitations have drawn serious attention of medicinal chemists to search for an effective antiviral therapy that targets the viral and host proteins. For the synthesis and replication of the viral RNA, an NS5B protein of HCV that is an RNA dependent RNA polymerase residing at the seat-terminal domain of the polypeptide of several structural and non-structural proteins has been found to be responsible. It contains the catalytic machinery responsible for viral RNA synthesis [8]. Since it is essential for viral replication and since its crystal structure has been well studied, it has been recognized as the most suitable protein target for HCV drug discovery which has been clinically validated [9-11]. Recently, several classes of non-nucleoside allosteric NS5B polymerase inhibitors have been reported. In a recent study, some authors reported 1,1-dioxo-2H-benzothiadiazine analogues to act as HCV NS5B polymerase inhibitors [12-16]. In order to find more potent and effective drugs in this class, we have attempted here QSAR and docking studies on these compounds.

2. MATERIALS AND METHODS

We combined all the series of 1, 1-dioxo-2H-benzothiadiazine analogues studied by various authors [12-16] as shown in Tables **1** and **2** along with their NS5B polymerase inhibition activity in terms of $\log(1/IC_{50})$, where IC_{50} refers to molar concentration of the compound leading to 50% inhibition of HCV RNA replication. Several physicochemical and topological parameters were calculated for them to perform multiple linear regression (MLR) analysis on these compounds. The relevant parameters that were found to produce significant QSAR model are also given in Tables **1** and **2**. These parameters were calculated using Dragon version 6. In order to perform multiple regression analysis on these compounds, the whole series of compounds was divided into two subsets, the training set comprising of 39 compounds and the test set comprising of 13 compounds. Table **1** lists the compounds of training set and Table **2** those of test

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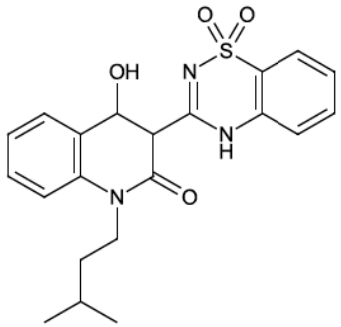
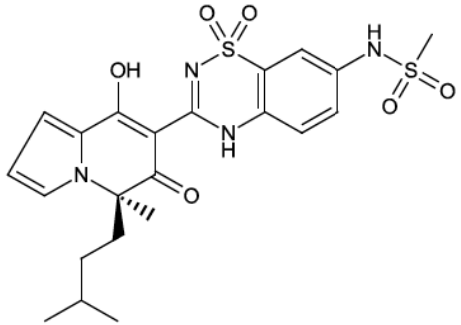
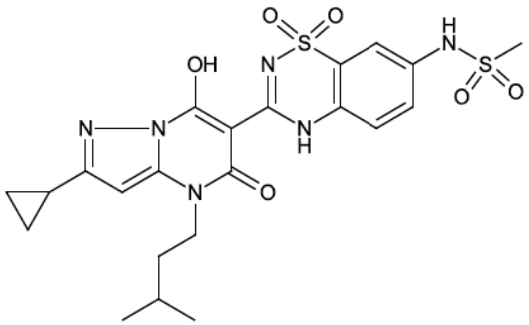
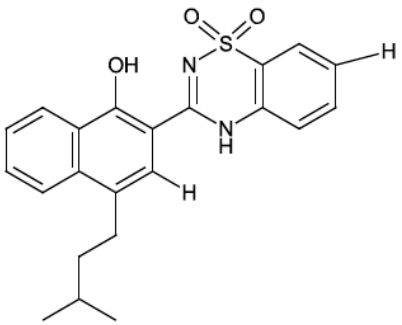
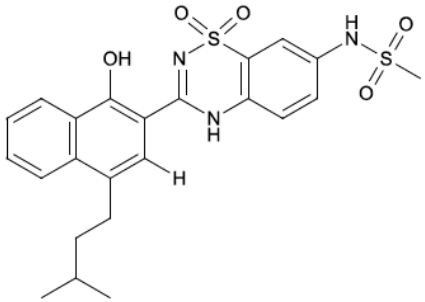
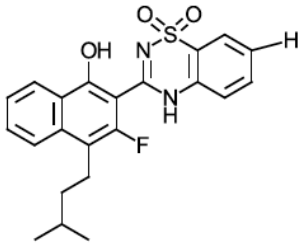
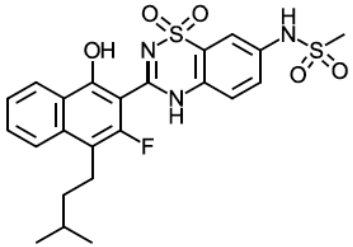
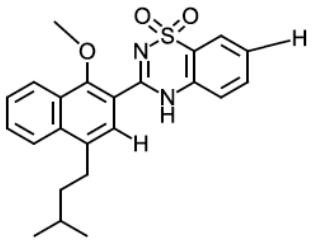
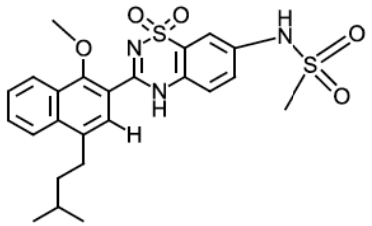
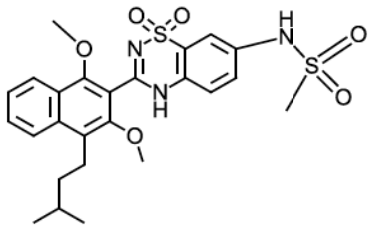
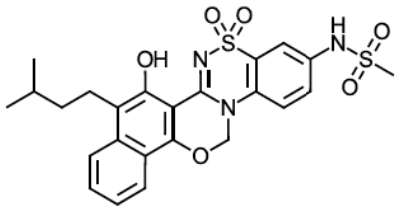
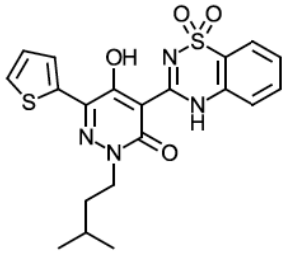
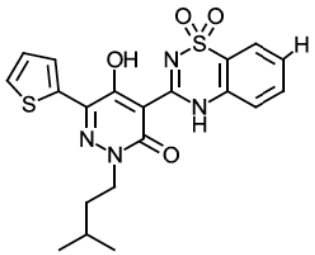
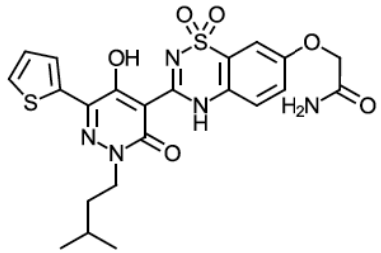
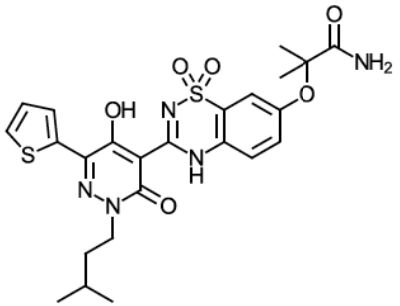
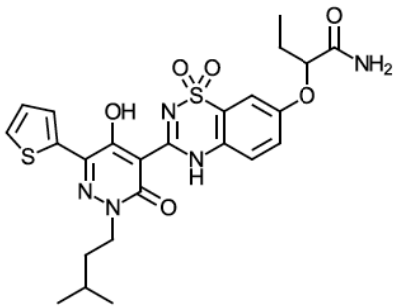
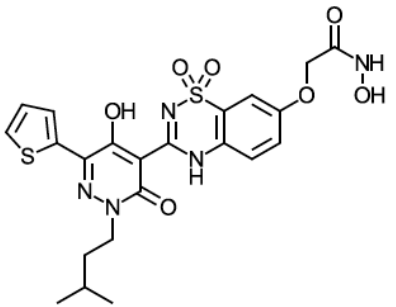
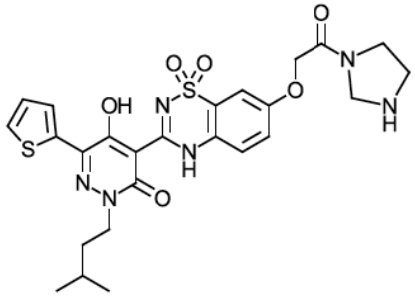
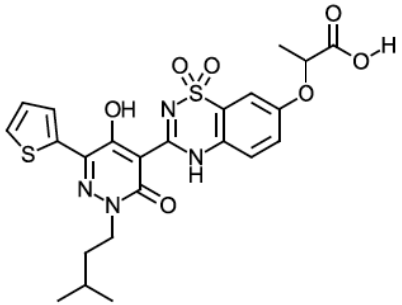
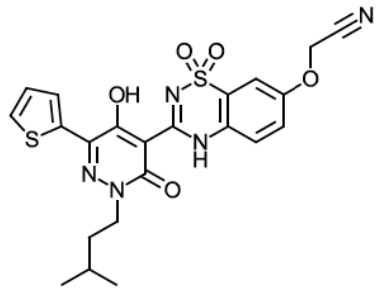
Table 1: Training Set of 1,1-Dioxo-2H-Benzothiadiazine Analogues and Their Physicochemical Parameters and HCV NS5B Polymerase Inhibition Activities

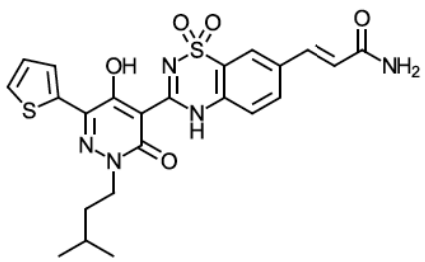
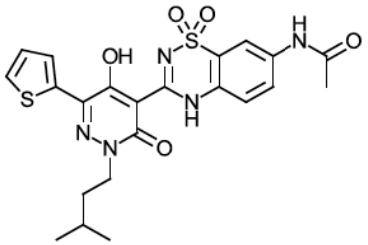
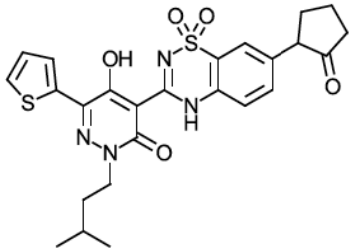
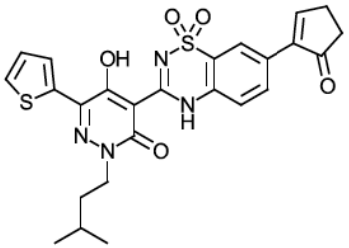
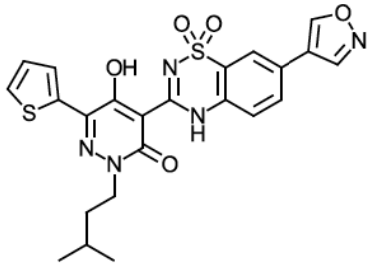
No.	Compound	GATS2p	GATS8s	GATS8i	GATS8v	R8u	SaaN	NssS	NaaN	log(1/IC$_{50}$)	
										Obsd[a]	Cald Eq. 1
1		0.931	1.033	1.055	1.119	1.051	0	1	4	7.50	7.44
2		1.005	1.421	1.07	1.19	0.831	0	2	4	6.82	6.78
3		1.05	1.192	0.958	1.018	0.824	4.495	2	5	8.05	8.01

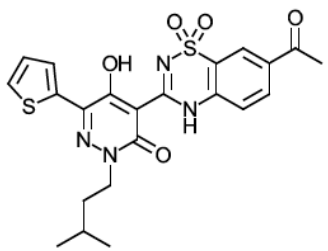
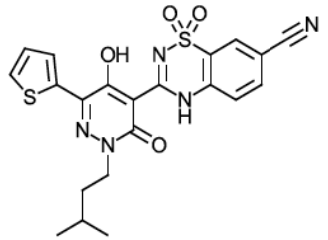
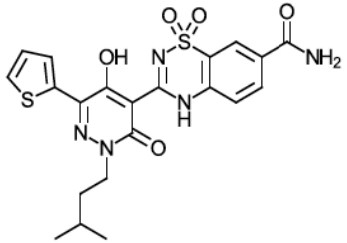
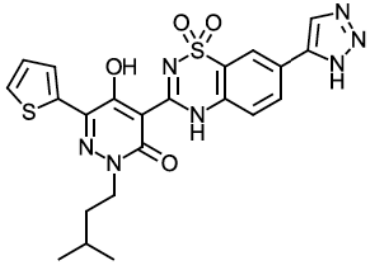
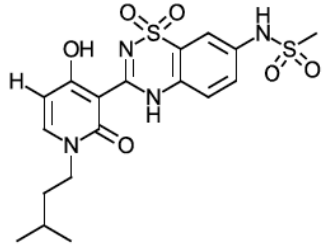
4		0.892	1.227	1.133	1.162	0.906	0	1	5	5.08	5.49
5		1.003	1.249	1.024	1.116	0.829	0	2	6	6.82	6.75
6		0.893	1.071	1	1.188	0.897	0	1	6	6.96	7.00
7		1.003	1.154	0.935	1.137	0.81	0	2	7	7.51	7.63

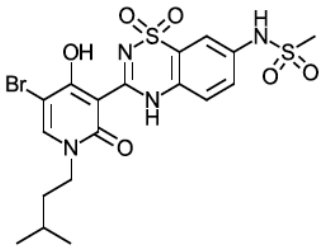
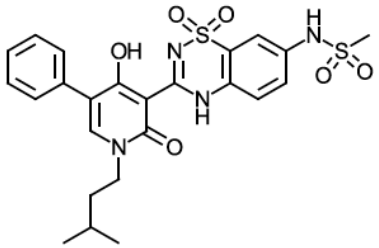
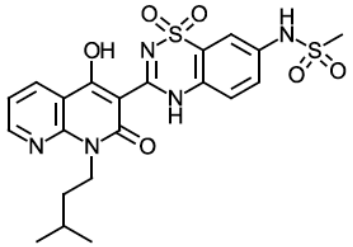
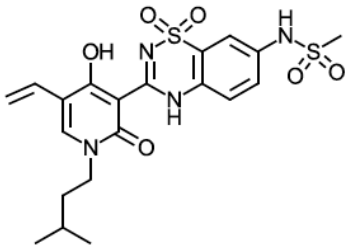
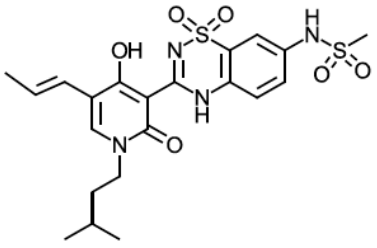
8		0.825	1.584	1.066	1.128	1.131	0	1	5	4.57	4.57
9		0.945	1.401	0.979	1.094	0.976	0	2	6	7.32	6.34
10		0.9	1.393	0.954	1.107	1.249	0	2	7	6.20	6.82
11		0.939	1.132	1.083	1.176	0.938	0	1	7	6.31	6.31
12		0.923	1.107	1.023	1.083	0.89	0	1	7	6.28	5.95

13		0.832	1.159	1.059	1.107	0.656	0	1	4	5.42	4.98
14		0.885	1.152	1.054	1.137	0.73	0	1	3	5.89	6.12
15		0.907	0.972	1.023	1.111	0.72	0	1	4	7.16	6.73
16		0.908	1.471	0.917	1.066	0.782	0	1	4	5.80	5.62

17		0.906	1	1.026	1.105	0.696	0	2	4	7.00	6.88
18		0.866	0.983	1.016	1.095	0.687	0	2	4	6.47	6.61
19		0.92	1.152	0.971	1.076	0.754	0	1	4	6.43	6.44
20		0.892	1.05	0.999	1.102	0.678	0	1	4	6.24	6.34

21		0.905	0.987	0.996	1.096	0.675	0	1	4	6.64	6.64
22		0.898	1.346	0.995	1.103	0.715	0	2	4	5.96	5.85
23		0.92	1.438	0.949	1.059	0.647	0	1	4	4.96	5.16
24		0.891	1.437	0.933	1.062	0.694	0	1	4	5.28	5.21
25		0.866	1.186	1.003	1.109	0.627	3.644	1	5	5.68	5.80

26		0.867	1.014	1.036	1.097	0.642	0	1	4	5.60	5.85
27		0.867	1.066	1.012	1.128	0.694	0	1	4	6.15	6.21
28		0.881	0.997	1.036	1.124	0.657	0	1	4	6.24	6.25
29		0.88	1.19	0.996	1.127	0.629	7.357	1	5	6.54	6.60
30		1.02	1.349	0.974	1.09	0.583	0	2	3	6.64	6.75

31		0.885	1.382	1.009	1.302	0.585	0	2	3	6.82	6.76
32		1.001	1.237	1.008	1.096	0.7	0	2	4	6.48	6.87
33		0.993	1.225	1.042	1.197	0.756	4.239	2	5	7.64	7.81
34		1.005	1.288	1.042	1.179	0.701	0	2	3	7.02	7.27
35		1.005	1.347	0.962	1.122	0.714	0	2	3	7.46	7.32

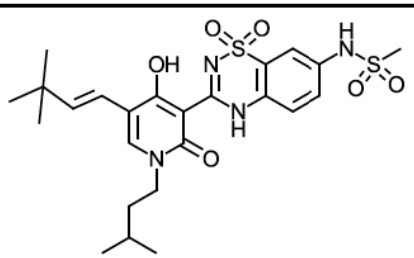
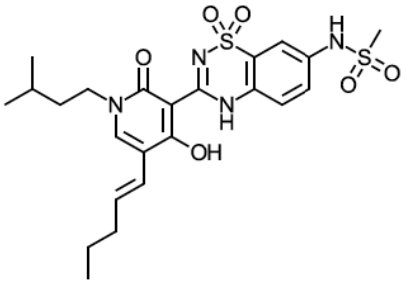
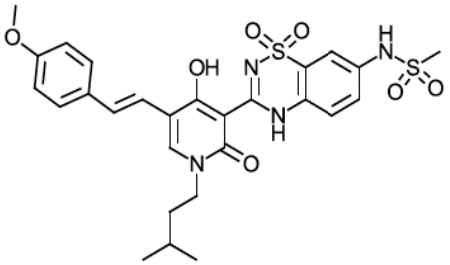
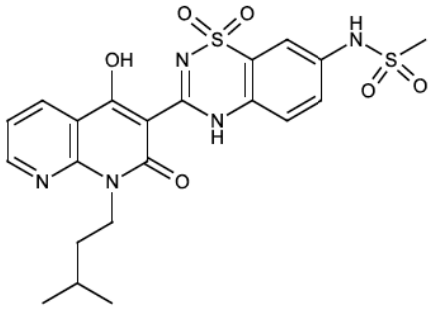
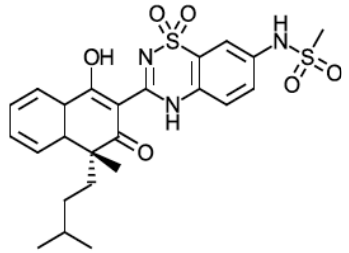
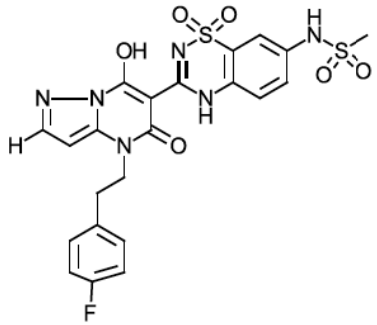
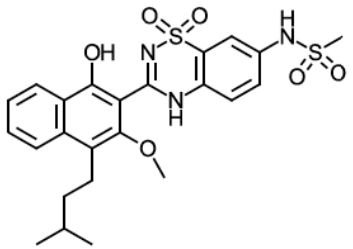
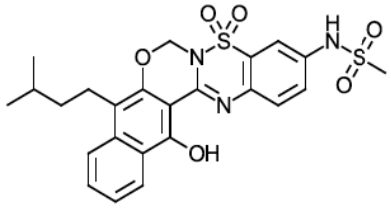
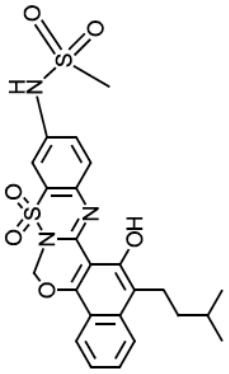
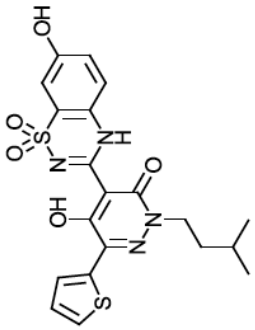
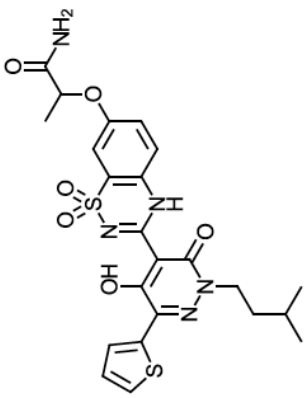
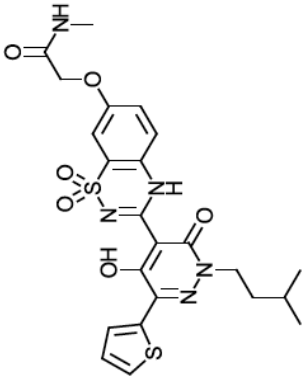
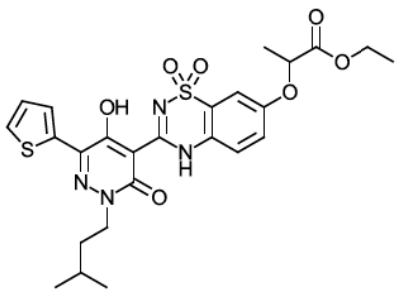
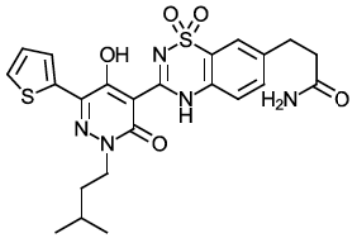
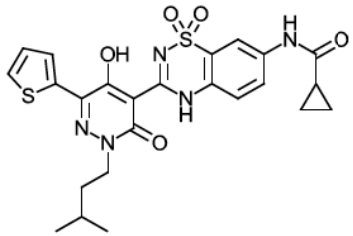
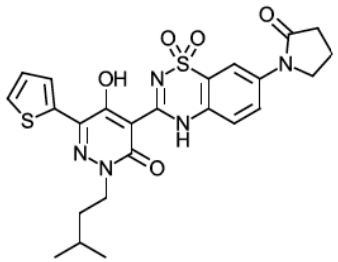
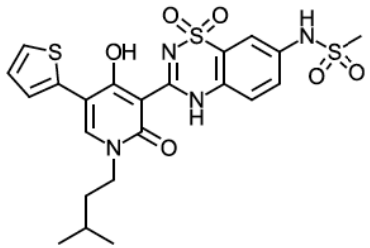
36		0.968	1.17	1.22	1.217	0.594	0	2	3	6.32	6.01
37		1.036	1.324	1.009	1.117	0.604	0	2	3	6.98	6.95
38		0.97	1.283	1.09	1.108	0.574	0	2	5	4.90	5.33
39		0.993	1.225	1.042	1.197	0.756	4.239	2	5	8.16	7.81

Table 2: Test Set of 1,1-Dioxo-2H-Benzothiadiazine Analogues and Their Physicochemical Parameters and HCV NS5B Polymerase Inhibition Activities

No.	Compound	GATS2p	GATS8s	GATS8i	GATS8v	R8u	SaaN	NssS	NaaN	log (1/IC_{50})	
										Obsd	Cald, Eq. 1
1		1.02	1.369	0.996	1.115	0.922	0	2	3	8.52	7.61
2		1.031	1.202	0.929	1.044	0.654	4.028	2	6	7.74	7.44
3		0.953	1.295	1.03	1.18	1.162	0	2	7	7.43	7.26
4		0.939	1.132	1.083	1.176	0.938	0	1	7	6.31	6.29

5		0.939	1.094	1.004	1.068	0.896	0	1	7	5.89	6.17
6		0.894	1.088	1.068	1.138	0.701	0	1	4	6.47	5.98
7		0.923	1.23	0.966	1.086	0.755	0	1	4	7.21	6.28
8		0.872	0.989	1.001	1.08	0.727	0	2	4	7.04	6.74

9		0.91	1.225	0.991	1.084	0.714	0	1	4	5.20	5.87
10		0.922	0.986	1.014	1.093	0.661	0	1	4	5.47	6.57
11		0.955	1.268	1.171	1.125	0.702	0	2	4	5.10	5.33
12		0.896	1.434	0.964	1.075	0.674	0	1	4	5.14	5.04
13		0.959	1.288	0.954	1.085	0.682	0	2	4	7.44	6.62

set. The test set compounds were arbitrary selected such that they have appreciable structural and activity differences among them.

3. RESULTS AND DISCUSSION

When a regression analysis was performed on the compounds of training set, a significant QSAR model as shown by Eq. 1 was obtained. In this equation, GATS2p, GATS8s, GATS8i, and GATS8v, are Geary autocorrelation of *lag* 2 and 8 weighted by polarizability (p), I-state (s), ionization potential (i), and van der Waals volume (v), respectively, and R8u is R autocorrelation of lag 8/unweighted, SaaN is sum of atom type E-state indices, NssS is number of atoms of type ssS (sulfur atoms preceded by two single bonds), and NaaN is number of atoms of type aaN (nitrogen atoms preceded by two aromatic bonds).

$$\log(1/IC_{50}) = 7.994(\pm2.470)GATS2p - 3.601(\pm0.758)GATS8s - 7.644(\pm2.275)GATS8i + 6.919(\pm2.510)GATS8v + 2.781(\pm0.973)R8u + 0.141(\pm0.066)SaaN + 0.387(\pm0.302)NssS - 0.234(\pm0.123)NaaN + 1.665(\pm3.592)$$
$$n = 39, r = 0.946, r^2_{cv} = 0.814, r^2_{pred} = 0.664, s = 0.315, F_{8,30} = 31.77 \quad (3.17) \quad (1)$$

Among the statistical parameters, n is the number of data points, r is the correlation coefficient, r^2_{cv} is the square of the cross-validated correlation coefficient obtained from leave-one-out (LOO) jackknife procedure, s is the standard deviation, $F_{8,30}$ is the Fischer ratio between the variances of calculated and observed activities, where the subscript *8* refers to number of variables (k) and *30* to degree of freedom (n-k-*1*), and the data within the parentheses with ± sign are 95% confidence intervals. The figure within the parenthesis for F is the standard F-value at 99% level. The values of these statistical parameters exhibit that the correlation obtained is quite significant. The internal validity of the correlation was judged by the value of its r^2_{cv} which is calculated as:

$$r^2_{cv} = 1 - [\Sigma_i (y_{i,\,obsd} - y_{i,\,pred})^2 / \Sigma_i (y_{i,obsd} - y_{av,obsd})^2] \quad (2)$$

where $y_{i,obsd}$ and $y_{i,pred}$ are the observed and predicted (from LOO) activity values of compound i, respectively, and $y_{av,obsd}$ is the average of the observed activities of all compounds used in the correlation. The correlation is supposed to be valid if $r^2_{cv} > 0.60$. From this point of view, the correlation expressed by Eq. 1 seems to be quite valid. However, the predictive ability of any correlation equation is judged by predicting the activity of the compounds in the test set using it and calculating the value of r^2_{pred}, which is defined as:

$$r^2_{pred} = 1 - [\Sigma_i (y_{i,obsd} - y_{i,pred})^2 / \Sigma_i (y_{i,obsd} - y_{av,obsd})^2] \quad (3)$$

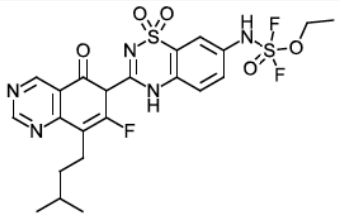
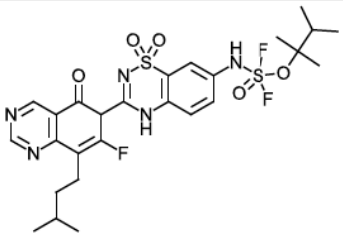
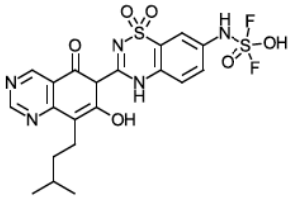
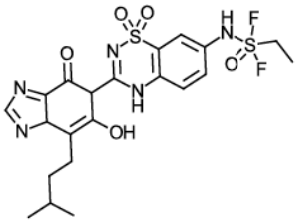
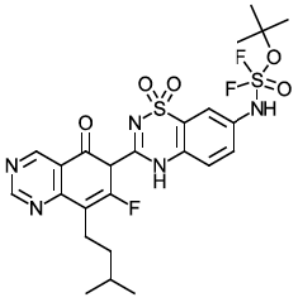
where $y_{i,obsd}$ and $y_{i,pred}$ refer to the observed and predicted (from equation obtained) activity of compound i in the test set and $y_{av,obsd}$ is same as in Eq. 2. The value of $r^2_{pred} > 0.50$ indicates the good predictive ability of the model.

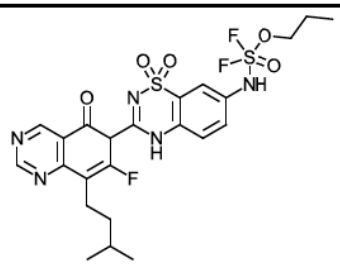
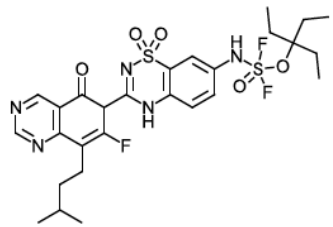
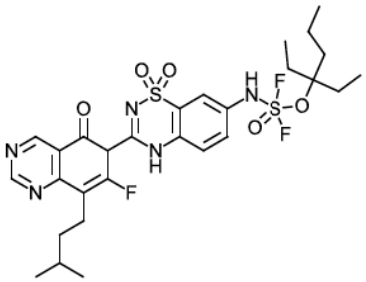
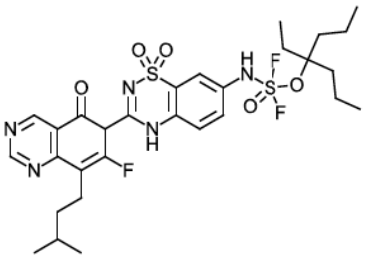
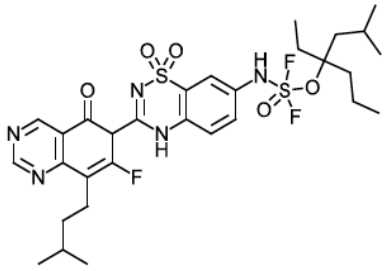
Now from all yardsticks, Eq. 1 is found to represent a very significant QSAR model having good predictive ability. Its predictive ability can be judged by comparing the activity values of both training and test set compounds with their corresponding observed values as shown in Tables **1** and **2** and by observing Figure **1** showing the plot of calculated values vs observed activities of the compounds of both the sets. Since the model has good predictive ability, we have used it to predict some new compounds of the series having high potency. As can be seen from Table **3**, all the predicted compounds have activity higher than any compound in the existing series. However, this model has little mechanistic value, we can only infer from this model that polarizability and van der Waals volume of the molecules and the presence of sulfur and nitrogen atoms in them will be conducive to the activity.

HCV has six major genotype classes, with genotypes 1 and 2 being the most prevalent in US, Europe and Japan. Genotypes 1, 2, and 3 are found worldwide. Genotype 1 is the most common HCV genotype in the United States and genotype 1b has been most widely studied. N-1-alkyl-4-hydroxyquinolon-3-yl benzothiadiazines class of HCV NS5B polymerase inhibitors have been found to bind near the active site of the enzyme in the palm domain [17]. Optimization of this class of compounds had led to an analogue **I**, containing a methylsulfonylamino D-ring substituent that increased enzyme inhibition potency of compound in the low nanomolar range [18]. From this observation, Donner *et al*. [16] explored aminimum core requirement where the replacement of A-ring and appending small aromatic ring or alkenyl group at 5-position of B-ring, such as compound **II,** could be optimal, resulting in inhibitors with low nanomolar potencies. As can be seen in Tables **1** and **2**, we have taken both types of compounds, containing 3 rings as well as 4 rings.

In a similar study, Zhou *et al*. [15] showed that a co-crystal structure of compound, such as **III**, bound to NS5B protein had the interactions with the enzyme as shown in Figure **2**, where there were several tightly bound water molecules. The key structural features in this binding were several hydrogen bonds and a deep

Table 3: Proposed Compounds Belonging to the Series of Table 1 and Their Predicted Activity

No.	Structure	GATS8v	GATS8p	GATS8i	GATS8s	R8u	SaaN	NssS	NaaN	Pred log(1/IC$_{50}$)
1		1.182	0.803	0.934	0.904	0.809	7.919	5	2	8.84
2		1.18	0.832	0.949	0.856	0.782	7.956	5	2	9.04
3		1.153	0.842	0.885	0.925	0.807	7.994	5	2	9.25
4		1.114	0.987	0.914	1.066	0.826	0	3	2	8.81
5		1.117	0.815	0.897	0.849	0.822	7.937	5	2	9.00

6		1.215	0.826	0.962	0.904	0.838	7.93	5	2	9.12
7		1.209	0.875	0.974	0.858	0.779	7.97	5	2	9.38
8		1.177	0.893	0.968	0.855	0.714	7.979	5	2	9.18
9		1.099	0.91	0.92	0.816	0.786	7.989	5	2	9.49
10		1.032	0.904	0.879	0.784	0.897	7.996	5	2	9.72

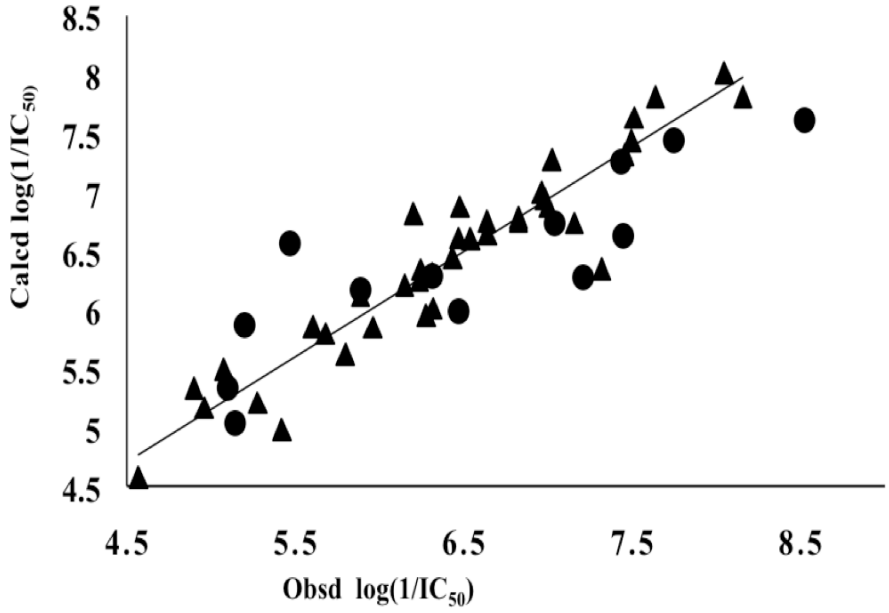
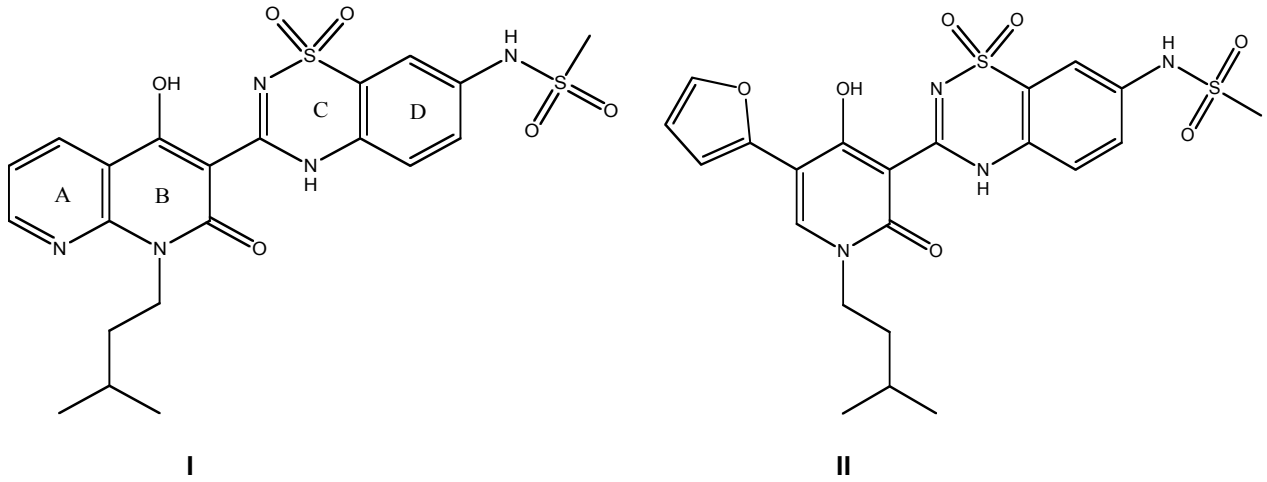
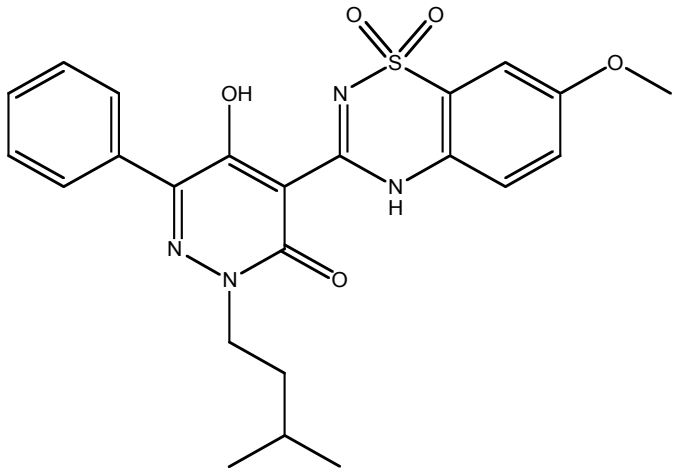


Figure 1: A plot of calculated activities vs observed activities for the training (▲) as well as test set compounds (●).





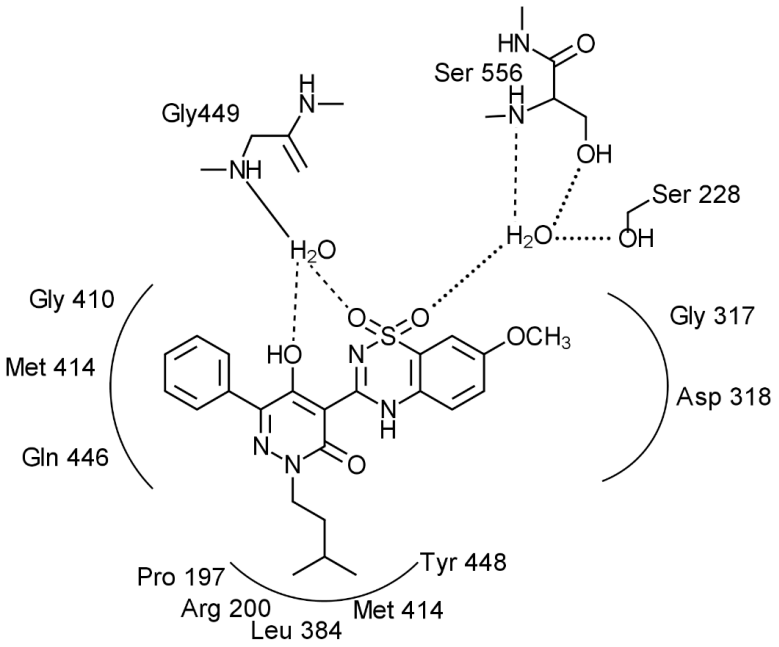
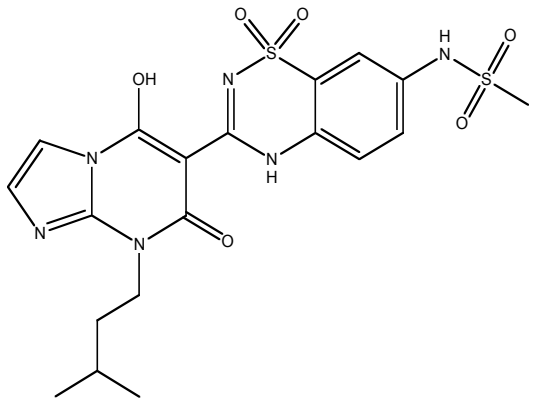


Figure 2: Schematic diagram of compound **III** bound to palm domain of NS5B polymerase [15]. Dashed lines show the H-bindings.

hydrophobic pocket engulfing isoamyl group at N-2 position of the pyridazinone ring. Additionally, there were two shallow pockets, one to accommodate 6-phenyl ring and the other to accommodate 7'-methoxy group. The geometry of the former suggested that a thiophene moiety could be better accommodated there than the phenyl moiety. The 7'-methoxy group seemed to be suboptimal due to a lack of appropriate filling.

Wang *et al*. [13] obtained a co-crystal structure of compound **IV,** one of the most active compounds studied by them**,** in complexwith HCV NS5B polymerase genotype 1b D21N-His-tagged at 1.9 Å resolution (PDB code 3H98). This structure showed that the ligand binds to the enzyme at an allosteric site located in the palm domain of the polymerase, where the ligand core is anchored in the binding site *via* several hydrogen bonds and water-mediated contacts with the protein as shown in Figure **3**. The water molecule is involved in several polar interactions with one of the sulfonamide oxygen atoms of the benzothiadiazine and the backbone amide nitrogen of Ser556, as well as the side chain OH of Ser288. An additional ordered water molecule, located within 3.3 Å of the second benzothiadiazine sulfonamide oxygen, bridges interactions between the enolic OH of the pyrimidine ring with the backbone amide nitrogen of Gly449. The methyl sulfonamide substituent on the benzothiadiazine ring is involved in additional polar interactions with the protein and the amide NH of this group is within hydrogen bonding distance of the Asp318 side chain, while one of the sulfonamide oxygens is within 3 Å or less of two hydrogen-bond donors on the protein: the backbone NH of Asp318 and the side-chain N atom of Asn291. The isopentyl substituent on the pyrimidone ring occupies a deep hydrophobic pocket formed by the side chains of Pro197, Leu384, Met414, Tyr415, and Arg200.



IV

In the same NS5B polymerase, we docked all the predicted compounds of Table **3** using FlexX software. Figure **4** shows the docked structure of the most active compound (shown below as **V**) among the predicted ones. This figure shows that in addition to having several hydrogen bondings − a few mediated by water molecules − compound also has hydrophobic interactions with two deep pockets of the polymerase through its two alkyl chains, one with its alkyl chain at

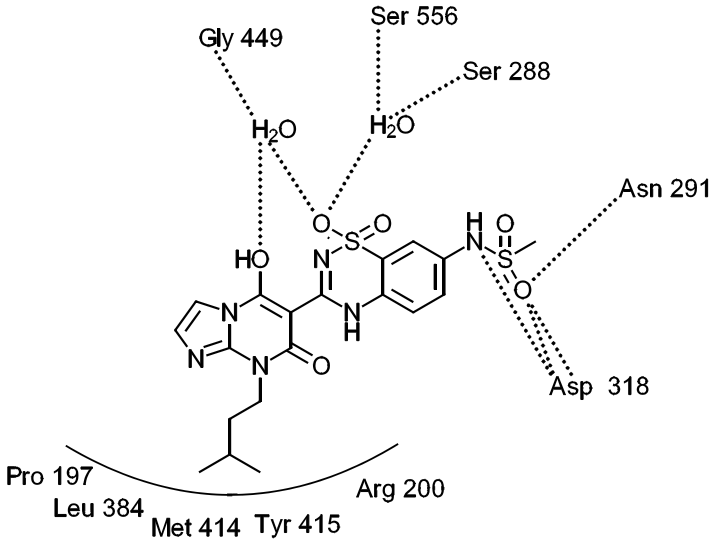
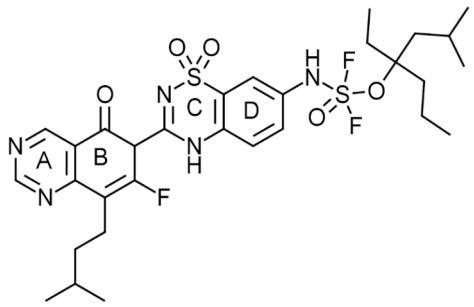


Figure 3: A schematic representation of X-ray structure of compound **IV** bound to HCV NS5B polymerase at 1.9 Å resolution. Hydrogen bonds and polar interactions discussed in the text are shown as dashed lines.



V

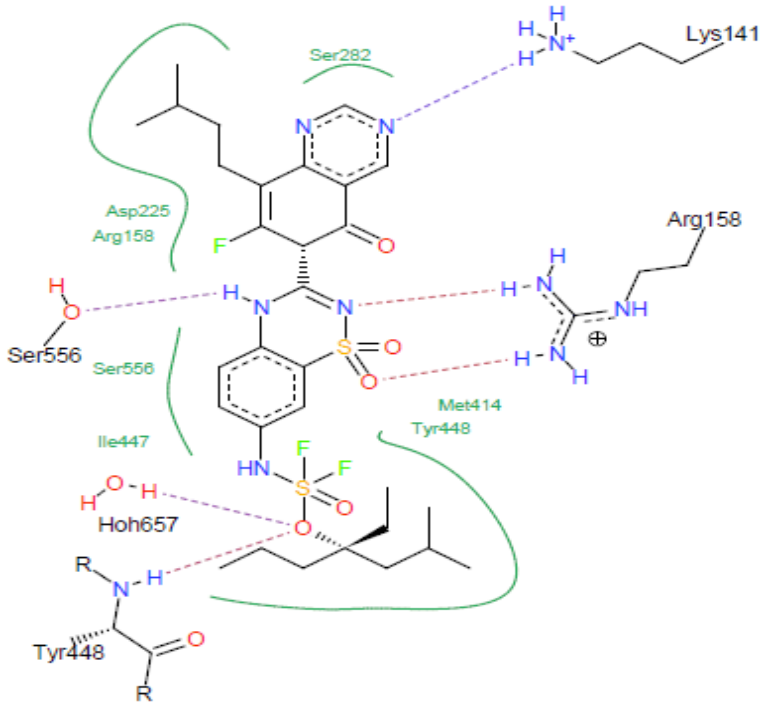


Figure 4: A representation of docked structure of compound **V**, the most active compound of the predicted ones (Table **3**), in the NS5B polymerase (PDB code 3H98).

ring B and one with its alkyl chain attached to the oxygen atom of sulfonamide group of ring D. These two alkyl chains are deeply engulfed in deep pockets of the enzyme. The increased activity of this compound may be due to these hydrophobic interactions. There is one more shallow pocket in the enzyme, formed by Ser556 and Ille447 residues, in which D-ring of the molecule may have some interaction. Thus it is seen that an alkyl chain attached to the oxygen of sulfonamide group is conducive to the activity.

4. CONCLUSION

The study showed that HCV NS5B polymerase inhibition activity of 1,1-dioxo-2H-benzothiadiazines class of compounds may be influenced by polarizability, van der Waals volume of the molecules, and the presence of sulfur and nitrogen atoms in them. The HCV NS5B polymerase has been shown to offer many sites for hydrogen bondings as well as a few for hydrophobic interactions. Therefore, increasing the number of H-bond donors and acceptors in the molecules as well as putting some hydrophobic groups at proper sites in them are suggested to be advantageous to their activity.

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