Synthesis, *In Vitro* and *In Silico* NS5B Polymerase Inhibitory Activity of Benzimidazole Derivatives

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Abstract: Hepatitis C virus (HCV) NS5B polymerase is the key replicating protein of the virus and thus an attractive target for drug development. Here we report on the synthesis and biological evaluation of a new series of benzimidazole derivatives as HCV NS5B inhibitors. This yielded compound **6b** and **6d** bearing 2-(2-benzyloxy)phenyl and 2-(4-methylbenzyloxy)phenyl moieties, respectively, as promising leads. Binding mode of compound **6d** in allosteric pocket (AP)-1 of NS5B will form the basis for future structure-activity relationship optimization.

Keywords: HCV, NS5B polymerase, Benzimidazole derivatives, RNA-dependent RNA polymerase.

INTRODUCTION

Hepatitis C virus (HCV) is the principal etiological agent of chronic hepatitis C infection [1, 2], affecting 170-200 million people worldwide [2, 3]. Nearly 80% of the infected individuals develop chronic liver disease, 5-20% of which progresses to liver cirrhosis and hepatocellular carcinoma [3, 4]. The current standard of treatment against HCV infection consists of combination therapy with pegylated interferon and ribavirin. This treatment, however, is limited in efficacy against the most prevalent HCV genotype (1a/b) and is also associated with significant side effects, thus limiting patient compliance. In industrialized nations, HCV infection has become the major cause of orthotopic liver transplants [3, 5].

Viral polymerases are key enzymes of the viral replication machinery and as such represent significant targets for therapeutic intervention. Viral polymerase inhibitors have been clinically validated against human immunodeficiency virus (HIV), cytomegalovirus (CMV), herpes simplex virus (HSV), and hepatitis B virus (HBV), among others. In context of HCV, its non-structural protein 5B (NS5B) harbors the RNA-dependent RNA polymerase (RdRp) activity, crucial for replicating the viral RNA genome [6-8]. Further, HCV NS5B has no functional equivalent in the host and thus represents a promising and attractive target for development of novel anti-HCV agents [9, 10].

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HCV NS5B has been extensively characterized both biochemically and structurally. Similar to other polymerase, the 66 kDa NS5B exhibits the characteristic "right hand" architecture with fingers, thumb and palm domains [7,9,10]. Rational drug-design approach has heavily relied on NS5B crystallographic analysis for designing and developing NS5B inhibitors, broadly classified as nucleoside or non-nucleoside inhibitors (NI or NNI, respectively). The former represent rNTP substrate mimics which cause chain termination upon incorporation into a growing RNA chain and thus function as competitive inhibitors [7, 9-14]. By contrast, NNIs consist of diverse small molecule scaffolds which bind to one of the five distinct allosteric pocktes (AP) on NS5B and inhibit at the initiation phase of the polymerase reaction [10, 14]. Although several small molecule inhibitors of NS5B have been identified in recent years with promising *in vitro* activities, they have proved unsatisfactory in clinical trials due to toxicity related issues or emergence of resistant mutant HCV [7, 9-15]. Therefore, development of novel and efficacious antiviral therapeutics targeting HCV are highly warranted.

Based on QSAR models, we recently predicted that non-nucleosides such as benzimidazole-coumarin conjugates (**1**), thiouracil derivatives (**2**), and pyrimidine nucleosides (**3** and **4**) (Fig. **1**) have common structural fragments [16-18]. Multiple regression analysis on the benzimidazole-coumarin derivatives (**1,** Fig. **1**) [16] revealed the following equation:

$$\log(1/EC_{50}) = 0.415(\pm 0.104)ClogP + 3.232(\pm 0.463)$$

$$n = 18,\ r = 0.904,\ r_{cv}^2 = 0.77,\ s = 0.22,\ F_{1,17} = 71.29 \quad (8.40) \quad (1)$$

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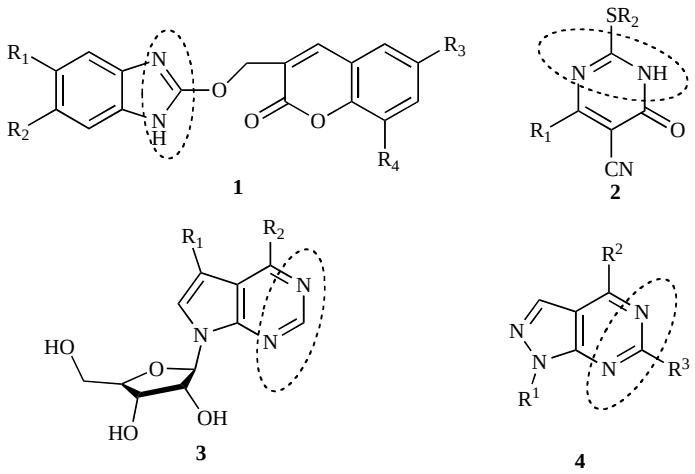


Fig. (1). Structures of benzimidazoles (**1**), thiouracil (**2**), pyrrolopyrimidine nucleosides (**3**), and 6-hydrazinopurine 2′-methyl ribonucleosides (**4**) showing the common structural fragment.

Table 1. Physicochemical Properties of the Synthesized Compounds (5a-i, 6a-d).

Compd no.	Mol Wt	MP (°C)	Mol Formula	clogP	Elemental Analysis (Calcd/Obsd)
5a	263	295	$C_{13}H_8Cl_2N_2$	5.00	C, 59.34/59.30; H, 3.06/3.11; N,10.65/10.64; Cl,26.95/26.96
5b	230	234	$C_{13}H_8F_2N_2$	3.91	C, 67.82/67.79; H, 3.50/3.60; F, 16.51/16.46; N, 12.17/12.19
5c	271	370	$C_{18}H_{13}N_3$	4.26	C, 79.68/79.64; H, 4.83/4.81; N, 15.49/15.43
5d	195	273	$C_{12}H_9N_3$	2.32	C, 73.83/73.87; H, 4.65/4.62; N, 21.52/21.51
5e	237	278	$C_{15}H_{15}N_3$	3.94	C, 75.92/75.90; H, 6.37/6.34; N,17.71/17.66
5f	236	241	$C_{16}H_{16}N_2$	5.09	C, 81.29/81.31; H, 6.82/6.80; N, 11.85/11.87
5g	300	350	$C_{20}H_{16}N_2O$	4.89	C, 79.98/79.95; H, 5.37/5.33; N,9.33/ 9.36; O, 5.33/5.35
5h	334	393	$C_{20}H_{15}ClN_2O$	5.61	C,71.75/71.76; H, 4.52/4.51; Cl, 10.59/10.61; N, 8.37/8.40; O, 4.78/4.81
5i	314	374	$C_{21}H_{18}N_2O$	5.39	C, 80.23/80.21; H, 5.77/5.79; N, 8.91/8.95; O, 5.09/5.10
6a	264	241	$C_{18}H_{20}N_2$	5.64	C, 81.78/81.73; H, 7.63/7.61; N, 10.60/10.50
6b	328	350	$C_{22}H_{20}N_2O$	5.43	C, 80.46/80.45; H, 6.14/6.24; N, 8.53/8.59; O, 4.87/4.85
6c	362	392	$C_{22}H_{19}ClN_2O$	6.15	C, 72.82/72.80; H,5.28/5.38; Cl, 9.77/9.67; N, 7.72/7.75; O, 4.41/4.43
6d	314	374	$C_{23}H_{22}N_2O$	5.93	C, 80.67/80.62; H, 6.48/6.50; N, 8.18/8.28; O, 4.67/4.62

where EC_{50} refers to the molar concentration of the drug leading to 50% inhibition of HCV RNA replication and *ClogP* to calculated hydrophobicity of the molecule derived from the utilization of ChemDraw version 8.0 software. In eq. (1), *n* is the number of data points, *r* is the correlation coefficient, r_{cv}^2 is the square of the cross-validated correlation coefficient obtained from leave-one-out (LOO) jackknife procedure, *s* is the standard deviation, *F* is the *F*-ratio between the variances of calculated and observed activities, 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% confidence level. This analysis suggested that the activity of the compounds were significant function of the hydrophobicity of the molecules. In another study, a model generated by *k* Nearest Neighbor-Molecular Field Analysis (*k*NN-MFA) of the same set of benzimidazole-coumarin derivatives had revealed contribution of steric potential towards biological activity [17]. Based on these analyses, we synthesized new benzimidazole scaffolds with variation in steric fields and hydrophobicity and investigated their structural requirements for NS5B inhibition. Herein we describe the synthesis, activity investigations, and molecular modeling analysis of these compounds.

MATERIALS AND METHODS

Synthetic Methods and Spectroscopic Details

All reactions were carried out by using microwave irradiation techniques. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light, or iodine vapor. Melting points were determined using digital display melting point apparatus (uncorrected) (Table **1**). Infrared spectra were

measured on a Shimadzu FTIR-8400S using KBr plate. ^{1}H NMR spectra were determined on a Brucker 300 MHz spectrometer using TMS as an internal standard.

2-(alkoxyaryl)-1H-benzimidazoles **(5a-i):** A mixture of 1,2-phenylenediamine (0.0313 mol), 1.01 equivalents of appropriate aldehyde, and 1.01 equivalents of sodium metabisulfite was mixed and introduced in an open Erlenmeyer flask. The mixture was irradiated in a microwave oven for 24–60 s. After irradiation, the mixture was poured onto cold water. The precipitate was collected by filtration, washed with water, dried, and recrystallized.

2-(3,4-dichlorophenyl)-1H-benzo[d]imidazole **(5a):** white solid, Yield: 62%, (methanol). IR (KBr, cm^{-1}): 3065, 3049, 2918, 1586, 1572, 1494, 1477, 1387, 1320, 1073, 880, 793; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 6.21 (*dd*, 2H), 6.89-6.96 (*dd*, 2H), 7.27-7.79 (*m*, 2H), 7.98 (*s*, 1H), 9.55 (*s*, 1H).

2-(3,4-difluorophenyl)-1H-benzo[d]imidazole **(5b):** white solid, Yield: 67%, (methanol). IR (KBr, cm^{-1}): 3078, 2977, 2921, 2894, 1602, 1514, 1433, 1406, 1384, 1323, 1091, 842; ^{1}H NMR (DMSO-d_6, ppm): δ 6.38 (*dd*, 2H), 7.61 (*m*, 2H), 7.82 (*d*, 2H), 7.95 (*d*, 2H), 8.17 (*s*, 1H), 9.35-9.73 (*s*, 1H).

2-(4-(pyridine-2-yl)phenyl)-1H-benzo[d]imidazole **(5c):** white solid, Yield: 58%, (methanol). IR (KBr, cm^{-1}): 3098, 2998, 2934, 2887, 1610, 1517, 1487, 1434, 1398, 1102, 798; ^{1}H NMR (DMSO-d_6, ppm):δ 6.50 (*d*, 2H), 6.86 (*m*, 2H), 7.25-7.68 (*dd*, 6H), 7.79-8.25 (*m*, 2H), 9.55-9.73 (*s*, 1H).

2-(pyridine-4-yl)-1H-benzo[d]imidazole **(5d):** white solid, Yield: 48%, (ethanol). IR (KBr, cm^{-1}): 3099, 3055, 2975, 2921, 2869, 1587, 1523, 1473, 1438, 1325, 750; ^{1}H NMR (DMSO-d_6, ppm):δ 6.93-7.01 (*d*, 2H), 7.26-7.45 (*m*, 2H), 7.72-7.93 (*m*, 2H), 8.39 (*d*, 2H), 9.45 (*s*, 1H).

4-(1H-benzo[d]imidazol-2-yl)-N,N-dimethylbenzenamine **(5e):** white solid, Yield: 59%, (methanol). IR (KBr, cm^{-1}): 3072, 3028, 2975, 2931, 1583, 1528, 1479, 1402, 1379, 1093, 837; ^{1}H NMR (DMSO-d_6, ppm): δ 1.17 (*s*, 6H), 6.44-7.16 (*m*, 4H), 7.51-7.69 (*d*, 2H), 7.80-8.12 (*d*, 2H), 10.98 (*s*, 1H).

2-(4-isopropylphenyl)-1H-benzo[d]imidazole **(5f):** white solid, Yield: 73%, (methanol). IR (KBr, cm^{-1}): 3121, 3046, 2983, 2920, 2893, 2858, 1590, 1515, 1436, 1406, 1321, 827; ^{1}H NMR (DMSO-d_6, ppm): δ 1.29 (*d*, 6H), 3.00-3.17 (*m*, 1H), 7.03-7.24 (*dd*, 4H), 7.76 (*m*, 4H), 9.84-9.94 (*s*, 1H).

2-(2-(benzyloxy)phenyl)1H-benzo[d]imidazole **(5g):** white solid, Yield: 55%, (ethanol). IR (KBr, cm^{-1}): 3099, 3064, 2958, 2929, 2891, 1602, 1514, 1461, 1440, 1367, 1247; ^{1}H NMR (DMSO-d_6, ppm): δ 5.17-5.43 (*s*, 2H), 6.52-6.75 (*m*, 4H), 7.15 (*d*, 2H), 7.52 (*m*, 4H), 7.74-7.89 (*d*, 3H), 9.81 (*s*, 1H).

2-(2-(4-chlorobenzyloxy)phenyl)1H-benzo[d]imidazole **(5h):** white solid, Yield: 70%, (methanol). IR (KBr, cm^{-1}): 3113, 3071, 2987, 2920, 2885, 1594, 1523, 1492, 1080, 757; ^{1}H NMR (DMSO-d_6, ppm): δ 5.93-5.95 (*s*, 2H), 6.56-6.78 (*d*, 4H), 7.32-7.35 (*m*, 2H), 7.55-7.58 (*d*, 2H), 9.58 (*s*, 1H).

2-(2-(4-methylbenzyloxy)phenyl)1H-benzo[d]imidazole **(5i):** white solid, Yield: 69%, (ethanol). IR (KBr, cm^{-1}): 3037, 2979, 2918, 2853, 1584, 1498, 1458, 1377, 1320, 761; ^{1}H NMR (DMSO-d_6, ppm): δ 2.33-2.55 (*s*, 3H), 5.89-5.97 (*s*, 2H), 6.86-6.95 (*dd*, 4H), 6.98-7.14 (*m*, 4H), 7.17-7.25 (*m*, 4H), 9.01 (*s*, 1H).

1-ethyl-2-(alkoxyaryl)-1H-benzimidazoles **(6a-d):** were obtained by using an excess of alkylating agent iodoethane.

1-ethyl-2-(4-isopropylphenyl)-1H-benzo[d]imidazole **(6a):** cream solid, Yield: 74%, (ethanol); ^{1}H NMR (DMSO-d_6, ppm): δ1.24 (d, 6H), 1.67 (t, 3H), 3.14-3.20 (*m*, 1H), 3.78 (*m*, 2H), 7.11-7.14 (*dd*, 4H), 7.56 (*m*, 4H), 9.87-9.89 (*s*, 1H);

2-(2-(benzyloxy)phenyl)-1-ethyl-1H-benzo[d]imidazole **(6b):** red solid, Yield: 69%, (methanol); ^{1}H NMR (DMSO-d_6, ppm): δ1.65 (*t*, 3H), 3.89-3.93 (*m*, 2H), 5.27-5.33 (*s*, 2H), 6.62-6.74 (*m*, 4H), 7.05 (*d*, 2H), 7.56 (*m*, 4H), 7.77-7.89 (*d*, 3H), 9.71 (*s*, 1H).

2-(2-(4-chlorobenzyloxy)phenyl)-1-ethyl-1H-benzo[d]imidazole **(6c):** brown solid, Yield: 56% (methanol); ^{1}H NMR (DMSO-d_6, ppm): δ 1.70-1.73 (*t*, 3H), 3.90-3.94 (*m*, 2H), 5.91 (*s*, 2H), 6.58-6.77 (*d*, 4H), 7.42-7.44 (*m*, 2H), 7.58-7.60 (*d*, 2H), 9.88 (*s*, 1H).

2-(2-(4-methylbenzyloxy)phenyl)-1-ethyl-1H-benzo[d]imidazole **(6d):** cream solid, Yield: 87%, (methanol: water, 8:2); ^{1}H NMR (DMSO-d_6, ppm): δ1.69 (*t*, 3H), 2.46-2.49 (*s*, 3H), 3.78 (*q*, 2H), 5.96-5.99 (*s*, 2H), 6.80-6.93 (*dd*, 4H), 7.13.-7.24 (*m*, 4H), 7.30-7.35 (*m*, 4H), 9.21 (*s*, 1H).

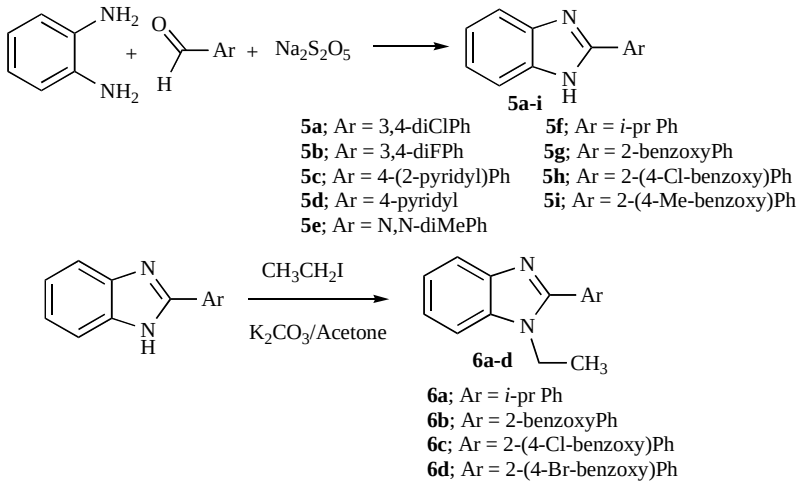
Biological Evaluation

In Vitro Screening of Inhibitors The anti-NS5B activity of the candidate compounds was evaluated *in vitro* by the NS5B RNA dependent RNA polymerase (RdRp) inhibition assay as described previously [19, 20]. This reaction utilized poly rA/U_{12} as the template-primer (TP) and recombinant HCV NS5BCΔ21 (genotype 1b) carrying N-terminal His-tag and C-terminal 21-amino acid deletion, as the source of enzyme. Aurintricarboxylic acid (ATA), a validated NS5B inhibitor previously identified by us was included as a positive reference control [20]. To identify candidates belonging to a wider range of structural scaffolds, preliminary screening was conducted at a concentration of 100 μM for each compound. Reactions were initiated by the addition of 1.0 mM $MnCl_2$ and terminated by the addition of ice-cold 10% (v/v) trichloroacetic acid (TCA) containing 0.5 mM pyrophosphate. The amount of radioactive UTP incorporated into nascent RNA was subjected to trichloroacetic acid (TCA) precipitation, spotted on a GF-B filter and quantified on a liquid scintillation counter (Packard). Activity of NS5B in the absence of the inhibitor was set at 100% and that in the presence of the inhibitor was calculated relative to this control.

Molecular Docking

Crystal structures of NS5B polymerase in complex with tetracyclic indole (PDB ID: 2dxs) [21], representing AP-1 pocket was used in the present study to perform the entire virtual screening operation.

The docking approach was validated by determining 'the lowest energy pose' (binding conformation) predicted by the FlexX [22], versus the experimental binding mode as deter-



Scheme 1. Synthesis of compounds **5a-i** and **6a-d.**

mined by X-ray crystallography. Towards, this end, we removed the afore-mentioned crystallographic bound inhibitors from their binding sites, minimized and then re-docked them into their respective binding sites, on HCV NS5B polymerase. To establish the specificity of the FlexX methodology, the dataset was docked into the binding pocket, AP-1 of NS5B.

RESULTS AND DISCUSSION

Chemistry

Synthesis of benzimidazole derivatives was carried out as described in Scheme **1**. For compounds **5a-i**, the synthesis was initiated with 1,2-phenylenediamine and substituted aldehydes, using microwave-assisted synthesis procedure. Compounds **5f-i** were subsequently alkylated with iodoethane to give good yield. All the compounds gave satisfactory elemental analysis (Table **1**). IR and ^{1}H NMR spectra were consistent with the assigned structures.

HCV NS5B Polymerase Inhibition and Analysis of SAR

With the objective of identifying novel HCV NS5B inhibitors, we explored the anti-NS5B activities of the synthesized benzimidazole derivatives employing recombinant HCV NS5B (genotype 1b) and *in vitro* NS5B RdRp inhibition assay, as described previously [19, 20]. Preliminary screening was conducted at 100 μM compound concentration.

Of the thirteen benzimidazole derivatives, eight compounds exhibited 5-30% inhibition of HCV NS5B at 100 micromolar (μM) concentrations, while the remaining five had no effect. A systematic analysis of the substitutions on C2 and N3 positions of compounds **5a-i** and **6a-d** is listed in Table **2**. The lack of NS5B inhibition by compounds, **5b, 5g, 5h** and **5i** appears to be correlated with the unsubstituted N of the benimidazole ring. Similarly, compounds **5a-i**, having no substitution at N, exhibited weaker inhibition compared to compounds **6a-d** having N-ethyl substitution. Introduction of lipophilic substituents such as Cl or isopropyl on the phenyl ring slightly increased their activity (**5a, 5f**). On the other hand, the presence of 3,4-difluoro group (**5b**) resulted in an inactive compound. Introduction of 2-pyridine at the *para*-position of the phenyl ring (**5c**) as well as changing the benzene ring to heterocyclic ring like 4-pyridine (**5d**) also did not improve the activity of the compounds.

Further, neither benzoxy substituent at the *ortho* position of the phenyl ring (**5g**) nor substitution of Cl, or Me on the benzoxy part (**5h, 5i**), contribute in any positive way towards HCV NS5B polymerase inhibition. To improve the activity of this series of compounds, we introduced the alkyl group on the benzimidazole nucleus. All the alkylated derivatives thus obtained, except **6c**, exhibited 2-fold increase in activity (**6a, 6b, 6d**). Compounds **6b** and **6d** were found to be the most active with 24.2% inhibition, thus suggesting the importance of *N*-alkylation with 2-benzoxyphenyl substituent at the benzimidazole ring.

The phenyl ring at C-2 position was found to be more active than the six-membered heterocycle such as 4-pyridine. Furthermore, replacement of the phenyl substituent by a six-membered heterocyclic moiety resulted in decreased inhibitory activity. The bulky substituents at the C-2 position of the benzimidazole ring resulted in compounds with good inhibitory potency. It is evident from the 3D *k*NN-MFA model that steric fields have some influence on HCV NS5B polymerase inhibition [17]. Based on this model, the NS5B inhibitory activity of the set of compounds is predicted, and for compounds **6b** and **6d** the *in vitro* results coincide with the predicted one. Similarly, eq. (1) was used to predict the activity based on Clog*P* of each compound.

Molecular Modeling

The FlexX docking program is an automated, fast method for posing ligands into protein binding sites, based on incremental construction algorithm [22-24]. Molecular docking computations were carried by running the FlexX module on CPU Intel(R) Xeon CPU 3.06 GHz, RAM Memory 2 GB, under the OS Enterprise Linux 3.0.

The X-ray crystal structure of NS5B polymerase in complex with JTP_1000 (PDB ID: 2dxs) [21], obtained from the RCSB Protein Data Bank (PDB), was used to dock the conformational library of each ligand. Multiple low energy binding modes were observed for all the docked compounds. The

Table 2. Binding Energy, Predicted activity as per eq. (1) and *k*NN MFA Model Described in Text and Experimental HCV NS5B Inhibitory Activity of 2-(alkoxyaryl)1*H*-benzimidazoles (5a-i) and 1-ethyl-2-(alkoxyaryl)1*H*-benzimidazoles (6a-d)

Compd no.	Predicted Activity as per Eq (1)	Predicted Activity Using *k*NN MFA	FlexX Binding Score (kcal/mol)	% Inhibition[a]
5a	5.307	5.884	-25.66	7.1
5b	4.855	5.723	-25.11	0
5c	4.999	5.934	-25.43	6.2
5d	4.195	6.023	-25.46	7.5
5e	4.867	5.478	-27.10	14.6
5f	5.344	6.129	-26.43	10.9
5g	5.261	5.738	-20.13	0
5h	5.560	6.112	-24.17	0
5i	5.469	5.098	-22.34	0
6a	5.573	6.463	-21.56	14.6
6b	5.485	6.241	-27.11	22.4
6c	5.784	5.938	-18.34	0
6d	5.693	6.472	-27.10	24.2

[a]Percent inhibition of HCV NS5B RdRp activity was determined at 100 μM concentration of the indicated compound. The data represents an average of two independent measurements in duplicate. NS5B RdRp activity in the absence of the inhibitor was taken as 100 percent after subtraction of residual background activity.

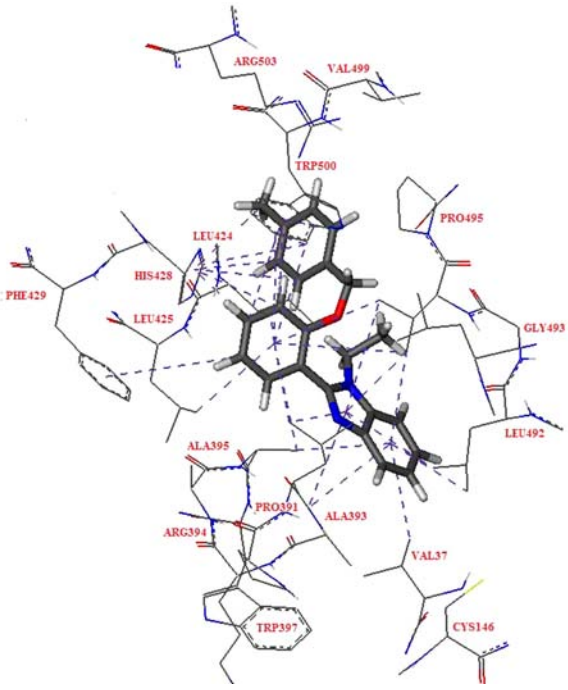


Fig. (2). Docked conformation of compound **6d** within allosteric pocket (AP)-1 of HCV NS5B (PDB ID: 2dxs). Active site amino acid residues are represented as wire, while the inhibitor is shown as stick model.

docked conformations were then used to analyze the binding interactions. By default, FlexX produces 30 docked structures for each benzimidazole derivative. The conformation with the lowest docking energy in the most populated cluster was selected as the possible 'active' conformation against the 2dxs active site. In the present study, all compounds were successfully docked into the 2dxs site. Docking poses with the lowest binding energy shows more negative score for tighter binding at the receptor site (Table **2**).

Binding modes of all the benzimidazole analogues (**5a-i, 6a-d**) were fairly in good agreement with the structure-activity discussion as described above. These binding interactions were found similar to the previously studied binding mode of benzimidazole derivative [25] with residues identified as His428, Pro495, Val494, Leu392, Ala395, and Phe429. The benzimidazole ring forms hydrophobic contacts with residues Val37, Ala395, Thr399, Leu492, and Cys146. The 2-(4-Me-benzoxy)phenyl substituent attached to C-2 position of the benzimidazole ring is located in a deep hydrophobic pocket formed by His428, Leu392, Leu425, Phe429, Pro495, Pro496, Val499, and Arg503. It is noteworthy to mention that on one side of the phenyl ring there is sufficient room to accommodate a large hydrophobic substituent. The 4-Me-benzoxy substituent fits nicely into a narrow hydrophobic pocket formed by His428, Pro495, Leu497, Val499, and Arg503. The ethyl group at N-3 position of the benzimidazole ring shows binding interactions with Leu489, Gly493, and Val494. The binding mode of the most active compound **6d** is displayed in Fig. (**2**).

It is important to note that the active site of PDB ID: 2dxs by the crystallographic bound inhibitor versus compound **6d** overlap each other (Fig. **3**). This suggests that the benzimidazole nucleus successfully interacts at the active

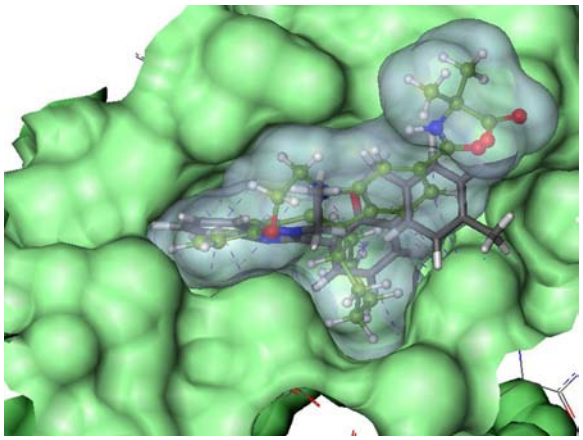


Fig. (3). Comparison of the docked compound **6d** (stick model) and the crystallographic bound JTP (ball and stick model) overlaid within the allosteric pocket (AP)-1 of NS5B (PDB ID: 2dxs). The binding surface (pocket) of the macromodel is shown in light blue color.

site but the lack of bulky substitutions at positions other than N1 may contribute negatively towards their activity. Further, the activity predicted by eq. (1) and the *k*NN MFA results are in accordance with the *in vitro* results along with few exceptions (**5b, 5g, 5h, 5i, 6c**) (Table **2**). Inhibitory activity of compounds does not appear to have significant correlation with the Clog*P* in most of the compounds. For compounds **6a**, **6b**, and **6d** the inhibitory profile shows improvement along with increased Clog*P* values. Our analysis indicates that for inhibition of NS5B polymerase RdRp activity, in addition to hydrophobicity other contributing parameters such as - steric and electrostatic fields may play an important role. The *k*NN-MFA results concluded towards variation of steric bulk at position-1, and -2 but some analogues failed to produce inhibition. Various substituents at the C-2 position of the benzimidazole ring may be inactive due to potential steric clash and the lack of hydrogen bonding at the receptor site. These findings points towards the significance of substituents at other positions of the benzimidazole nucleus.

CONCLUSION

The study reports the effects of *N*-alkylation and phenyl substitution on the benzimidazole ring for their anti-HCV activity. Preliminary screening of this series of compounds led to the identification of 2-(2-(benzyloxy)phenyl)-1-ethyl-1*H*-benzo[*d*]imidazole (**6b**) and 2-(2-(4-methylbenzyloxy) phenyl)-1-ethyl-1*H*-benzo[*d*]imidazole (**6d**) as promising NS5B inhibitor leads. Docking analysis of compound **6d** in AP-1 of NS5B provided insight for the rational design of novel inhibitors of HCV NS5B. Further SAR modifications including the effect of other substituents on the benzimidazole ring are on the way and will be reported in due course.

ACKNOWLEDGEMENTS

Support to V.M.P. in the form of resources from Meerut Institute of Engineering & Technology, Bharat Institute of Technology, Meerut, India is gratefully acknowledged. HCV NS5B inhibition studies were supported by UMDNJ Foundation grant support to N.K-B.

DECLARATION OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the writing and content of this paper.

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Received: November 15, 2011 Revised: March 29, 2012 Accepted: March 30, 2012