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Molecular docking studies and in vitro screening of new dihydropyridine derivatives as human MRP1 inhibitors

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ABSTRACT

The overexpression of multidrug resistance protein 1 (MRP1) by tumor cells results in multidrug resistance (MDR) to structurally unrelated anticancer drugs. Circumvention of MDR by combination of chemosensitizers with antitumor compounds is a new field of investigation in cancer chemotherapy. Much effort has been put-in recently to identify the modulators/inhibitors of MRP1 to overcome the MDR. 1,4-Dihydropyridine (DHP) derivatives are indicated to be a new class of MRP1 inhibitors in cancer treatment. Molecular docking studies were carried out on 48 newly synthesized DHP derivatives with the crystal structure of MRP1 to gain some structural insights on the binding mode and possible interactions with the active site of MRP1 (NBD1). The 10 top-ranked molecules were selectively evaluated, experimentally for their MRP1 inhibitory effect using the insect cell membrane MRP1 ATPase assay. The inhibitory capacity (IC_{50} concentrations) of the test compounds was compared with the reported IC_{50}- or the K_i-concentrations for benzbromarone, a standard MRP1 inhibitor. Amongst the compounds tested, compounds **IA_1** and **IIA_5** were found to exhibit a potent MRP1 inhibitory action with IC_{50} values of 20 ± 4 and 14 ± 2 μM (mean ± SD), respectively as compared to benzbromarone (IC_{50} = 4 μM). The compound **IIA_5**, in particular was found to be more potent than **IA_1** in accordance with the docking results. These new DHP derivatives possess promising characteristics for their development as MDR reversal agents.

1. Introduction

The human multidrug resistance protein 1 (MRP1/ABCC1) is a 1531 amino acid containing ATP-binding cassette (ABC) transporter involved in cancer cell multidrug resistance (MDR phenotype). It is a membrane-bound, energy-dependent efflux pump which is structurally and functionally related to the MDR protein, P-glycoprotein (P-gp/ABCB1).[1] MRP1 is overexpressed in the membranes of cancer cells and actively extrudes various cytotoxic agents out of tumor cells, leading to a decrease in cellular drug concentrations. MRP1 consists of two cytosolic nucleotide-binding domains, NBD1 (N-terminal), NBD2 (C-terminal) and three transmembrane domains, TMD0, TMD1, and TMD2 (Fig. 1). The TMDs are mainly involved in substrate recognition and transport while the NBDs are responsible for ATP binding/hydrolysis, which energize substrate transport.[2]

In humans, MRP1 is widely expressed in all tissues except the liver, where together with other ABC transporters, it plays a significant role in tissue defense from toxic agents.[3,4] Like P-gp, MRP1 confers resistance on cancer cells by using the energy of ATP binding and hydrolysis to efflux anticancer drugs. However, contrary to P-gp, MRP1 can transport a range of anionic substrates as such or as glutathione conjugate (GSH)/glucuronide/sulfate.[5] Originally cloned from a doxorubicin-selected lung cancer cell line, MRP1 has since been found to be expressed in a range of solid and hematological tumors, and has been demonstrated to transport a wide array of structurally diverse substrates.[6,7] With the exception of taxanes, most anticancer drugs viz., doxorubicin, vincristine, etoposide and methotrexate that can be substrates of P-gp, are also MRP1 substrates although in the latter case they may require metabolism to glutathione conjugates prior to transport. Energy-dependent efflux of such drugs reduces intracellular concentrations in tumor cells and hence their cytotoxicity, thereby confers resistance.

MDR is a tremendous problem in the treatment of many types of cancer. In the clinical setting, patients who overexpress multidrug resistance proteins such as P-gp and MRP1 in their

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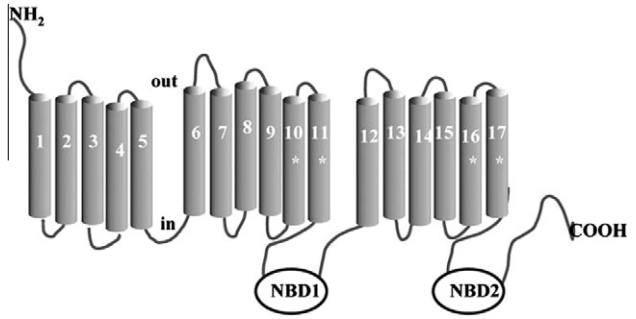


Figure 1. A topology model of MRP1. The transmembrane helices labeled with a star are involved in substrate recognition.

tumors are usually not responsive to associated anticancer agents.[8] Thus, many of these patients progress to an advanced stage disease and have poorer prognoses. It has been hypothesized that inhibition of MDR transporters can restore sensitivity to some oncolytics, allowing patients with drug-resistant tumors to become responsive to their drug therapy.[9] This hypothesis is presently being tested in the clinic for P-gp.[10] However, the search for MRP1 inhibitors (or modulators/chemosensitizers) to overcome MRP1/ABCC1-mediated MDR just began a few years ago and few potent and selective modulators have been investigated.[11,12]

In this regard, 1,4-dihydropyridine (DHP) calcium channel blockers are shown to be a new class of MDR inhibitors in cancer treatment. In analogy to verapamil, some members of this group viz., nifedipine and nimodipine were identified as potent MDR antagonists (Fig. 2).[13] In particular, this family of compounds has been studied extensively as P-gp inhibitors.[14] Contrary to it, very few DHPs have been shown to modulate drug efflux mediated by MRP1,[15–17] although the binding site(s) within MRP1 remain unknown. However, the clinical use of such calcium antagonists remains a therapeutic problem due to their strong vasodilatory activity, and new drugs with no calcium antagonistic activity are required to overcome the MDR in cancer patients.

The MDR-active compounds, in general are aromatic in nature, highly lipophilic[18] and possess a monocationic or dicationic side chains.[19] Most of these compounds also possess a tertiary nitrogen function in the form of a cation at physiological pH.[20] It is a very important finding that DHPs without or a weak calcium antagonistic activity possess the MDR reversal property.[21] Structure–activity relationship of DHP calcium channel antagonists suggests that the ability to overcome MDR varies considerably with the nature of 3,5-substituents. That is, the MDR-reversal property of DHPs is mostly dependent on their 3,5-substitution pattern. Ester groups at 3- and 5-positions are critical for Ca^{2+} channel blocking activity. Therefore, replacement of these groups with amides can significantly reduce their cardiovascular side effects.[22] Further, it is an established fact that the enzymatic hydrolysis of corresponding amides will be relatively slower than the esters themselves, making their stay time longer in biological systems. In addition, the presence of an heteryl group at 4-position of DHP was found to be effective in increasing MDR reversing activity while decreasing Ca^{2+} channel blocking activity.[23] Thus, in view of the above and in continuation of our work on novel DHP carboxamides,[24–28] our group has quite recently designed, synthesized, and characterized some new 1,4-DHP derivatives (Table 1) possessing the structural requirements for MDR reversal.[29,30] In this communication, we present the molecular docking studies of this new compound library on the crystal structure of MRP1–NBD1 as this particular domain showed the highest affinity for 1,4-DHPs amongst other domains of the complex protein, MRP1. The ten top-ranked molecules were evaluated for their MRP1 inhibitory effect, experimentally by the MRP1 ATPase assay kit, in vitro. The docking results

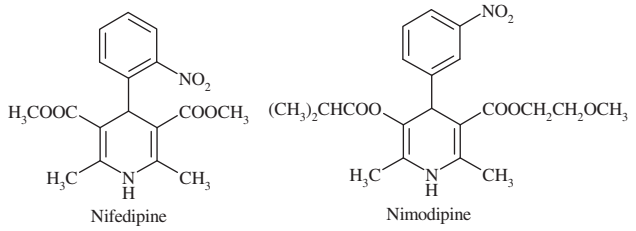
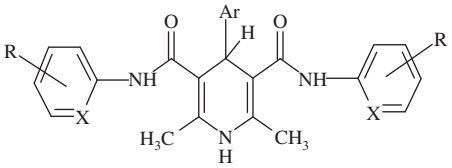


Figure 2. Chemical structures of some 1,4-dihydropyridine MDR inhibitors

Table 1
The compound library designed and synthesized for MRP-1 inhibition



S. No.	Compound	X	R	Ar	Mol. wt
1	**IA_1**	–CH–	H	2-Furyl	413.46
2	**IA_2**	–CH–	H	2-Pyridyl	424.47
3	**IA_3**	–CH–	H	2-Thienyl	429.45
4	**IA_4**	–CH–	H	2-Imidazolyl	s413.44
5	**IA_5**	–CH–	H	2-Pyrrolyl	412.46
6	**IA_6**	–CH–	H	4-ClC_6H_4	457.98
7	**IA_7**	–CH–	H	4-FC_6H_4	441.48
8	**IA_8**	–CH–	H	4-$C_2H_5C_6H_4$	451.53
9	**IB_1**	–CH–	4-CH_3	2-Furyl	441.51
10	**IB_2**	–CH–	4-CH_3	2-Pyridyl	452.52
11	**IB_3**	–CH–	4-CH_3	2-Thienyl	457.50
12	**IB_4**	–CH–	4-CH_3	2-Imidazolyl	441.49
13	**IB_5**	–CH–	4-CH_3	2-Pyrrolyl	440.51
14	**IB_6**	–CH–	4-CH_3	4-ClC_6H_4	486.03
15	**IB_7**	–CH–	4-CH_3	4-FC_6H_4	469.53
16	**IB_8**	–CH–	4-CH_3	4-$C_2H_5C_6H_4$	479.59
17	**IC_1**	–CH–	2-CH_3	2-Furyl	441.51
18	**IC_2**	–CH–	2-CH_3	2-Pyridyl	452.52
19	**IC_3**	–CH–	2-CH_3	2-Thienyl	457.50
20	**IC_4**	–CH–	2-CH_3	2-Imidazolyl	441.48
21	**IC_5**	–CH–	2-CH_3	2-Pyrrolyl	440.51
22	**IC_6**	–CH–	2-CH_3	4-ClC_6H_4	486.03
23	**IC_7**	–CH–	2-CH_3	4-FC_6H_4	469.53
24	**IC_8**	–CH–	2-CH_3	4-$C_2H_5C_6H_4$	479.59
25	**IIA_1**	–N–	H	2-Furyl	415.44
26	**IIA_2**	–N–	H	2-Pyridyl	426.47
27	**IIA_3**	–N–	H	2-Thienyl	431.44
28	**IIA_4**	–N–	H	2-Imidazolyl	415.45
29	**IIA_5**	–N–	H	2-Pyrrolyl	414.46
30	**IIA_6**	–N–	H	4-ClC_6H_4	459.97
31	**IIA_7**	–N–	H	4-FC_6H_4	443.44
32	**IIA_8**	–N–	H	4-$C_2H_5C_6H_4$	453.50
33	**IIB_1**	–N–	5-Cl	2-Pyridyl	495.42
34	**IIB_2**	–N–	5-Cl	2-Thienyl	500.43
35	**IIB_3**	–N–	5-Cl	2-Imidazolyl	484.44
36	**IIB_4**	–N–	5-Cl	2-Pyrrolyl	483.45
37	**IIB_5**	–N–	5-Cl	2-Furyl	484.43
38	**IIB_6**	–N–	5-Cl	4-ClC_6H_4	528.96
39	**IIB_7**	–N–	5-Cl	4-FC_6H_4	512.43
40	**IIB_8**	–N–	5-Cl	4-$C_2H_5C_6H_4$	522.49
41	**IIC_1**	–N–	6-CH_3	2-Pyridyl	454.52
42	**IIC_2**	–N–	6-CH_3	2-Thienyl	459.50
43	**IIC_3**	–N–	6-CH_3	2-Imidazolyl	443.51
44	**IIC_4**	–N–	6-CH_3	2-Pyrrolyl	442.51
45	**IIC_5**	–N–	6-CH_3	2-Furyl	443.50
46	**IIC_6**	–N–	6-CH_3	4-ClC_6H_4	487.96
47	**IIC_7**	–N–	6-CH_3	4-FC_6H_4	471.46
48	**IIC_8**	–N–	6-CH_3	4-$C_2H_5C_6H_4$	481.73

were compared to biological data with an aim of obtaining the SAR profile of these compounds which may lead to some useful information for the rational design of new MRP1 inhibitors.

2. Materials and methods

2.1. Molecular docking study

The protein sequence and crystal structure of MRP1–NBD1 (residues 642–871) was downloaded from Protein Data Bank (PDB ID: 2cbz).[31] It was visualized in Spdb viewer and hydrogen moieties were added and minimized. The Protparam analysis revealed this protein to be acidic in nature due to the presence of higher number of negatively charged moieties (Asp + Glu).[32] The primary protein analysis revealed that this protein is stable and mostly conserved.

The 2cbz protein active site analysis was done by using Q-site finder online server by feeding the pdb file as the input file. This software displayed ten different binding sites on MRP1–NBD1. Amongst these 10 binding sites, the binding site-1 was found to be acidic (negative charge), having large volume for ligand binding and hence exhibited good interaction with the designed ligands and standard drugs (*verapamil* and *amlodipine*) which are basic in nature. Hence docking studies were performed at this site (Fig. 3).

The chemical library (chemical structures of 1,4-DHPs) was designed using ACD Chemsketch software (version 11.0) and saved the molecules as in MDL (mol format). These mol formatted chemical ligands were given as the input file for Accelrys Discovery Studio (version 2.4) for energy minimization and subjected to subsequent docking by the ligand fit method, for each molecule. The top ranked poses for each ligand were retained and analyzed with the MRP1 protein structure. The top 10 ligands which exhibited the highest dock score were chosen for further evaluation (in vitro studies).

2.2. MRP1 ATPase assay

2.2.1. Chemicals

Dimethyl sulfoxide (DMSO, ACS reagent, spectrophotometric grade, $\geqslant$99.9%) was purchased from Sigma–Aldrich (St. Louis, MO, United States).

2.2.2. Assay

MRP1 ATPase assay kit containing the purified membrane suspension from Sf9 (*Spodoptera frugiperda*) insect cells, expressing high levels of the human MRP1 transporter protein, was purchased from Solvo Biotechnology (Budapest, Hungary). The colorimetric assay for measuring transporter-associated ATPase activity was performed as per the instructions of the manufacturer, which is a modified method of Sarkadi et al.[33]

Two variants of this assay were employed in our studies. The *activation assay* measured the increase in vanadate-sensitive ATPase activity in the presence of a range of test compound concentrations through spectrophotometric quantification of the amount of inorganic phosphate generated as a product of ATPase-mediated conversion of ATP to ADP. This assay gave a measure of the ability of an agent to stimulate transporter activity (from basal levels), a characteristic common to substrates of such ATPase transporters. In the *inhibition assay*, ATPase activity was maximally stimulated using a saturating concentration of an activator of the transporter being studied. MRP-1 ATPase activity stimulated by 10 mM *N*-ethyl maleimideglutathione (NEM-GS) mix, and the decrease in this maximum vanadate-sensitive ATPase activity was measured in the presence of a range of test compound concentrations. Agents which inhibit MRP-1 function will typically reduce the ATPase activity of the pump, leading to a decrease in the activator-stimulated production of inorganic phosphate. All the MRP-1 ATPase studies were carried out in the presence of 2 mM glutathione. Benzbromarone (3,5-dibromo-4-hydroxyphenyl)-(2-ethyl-3-benzofuranyl) methanone, a specific MRP1 inhibitor was used as a reference drug.[34]

Positive control was performed in the presence of an ATPase activator, NEM-GS (10 mM). In the activation assay, the maximal stimulatory effect of the test compounds is expressed on a scale where 0% is the basal ATPase activity and 100% is defined as the activity observed in presence of the reference activator, NEM-GS. In the ATPase inhibition assays, membrane was incubated with NEM-GS in the presence or absence of increasing concentrations of test compounds (1–3000 μM). Here, IC_{50} is defined as the concentration of the test compounds that inhibits by 50% the maximally stimulated ATPase. The affinity of test compounds is evaluated from their inhibition constant K_i which was calculated as follows:

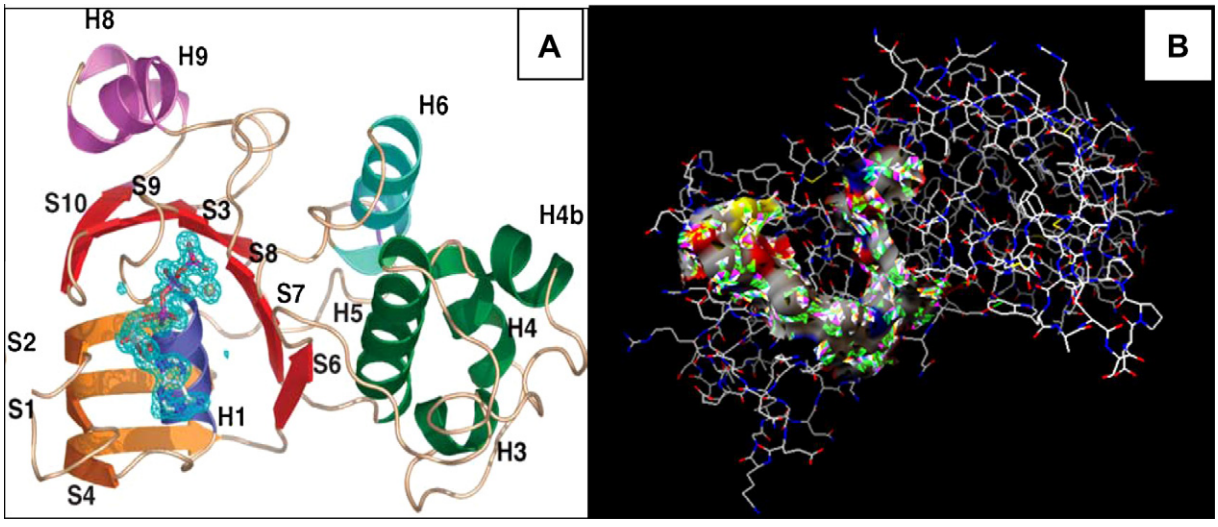


Figure 3. Three-Dimensional (3D) structure of nucleotide binding domain (MRP1–NBD1). (A) Stereo ribbon representation of MRP1–NBD1––The F1-like ATP-binding core is shown in *red*, the specific ABC-β sub-domain in *orange*, the central helix H1 in *blue*, helix H6 in *cyan*, helices H8 and H9 in *magenta* and the ABC-α sub-domain in *green*. (B) Active site of MRP1–NBD1 selected for docking.

$$K_i = \frac{IC_{50} \times K_{act}}{K_{act} + (S^\circ)}$$

where IC_{50} is determined for the test compounds for MRP1 inhibition assay, (S°) is the concentration of the reference activator used in the inhibition assay, and K_{act} is the concentration of the reference activator that allowed half maximum ATPase activation of the corresponding transporter, with K_{act}(NEM-GS/MRP1) = 500 μM.[35]

3. Results and discussion

3.1. Molecular docking analysis

The Protparam results confer that the physico-chemical properties of complex protein (MRP1) and nucleotide binding domain (MRP1–NBD1) were relatively similar and that MRP1–NBD1 represents the binding properties of complex protein due to higher binding affinity towards 1,4-dihydropyridine derivatives.

All the series of molecules were evaluated in silico (Fig. 4A) and observed the rank order of each series of molecules. The fitness scores of molecules (1,4-DHPs) along with their relative rank order are presented in Table 2.

In the series **IA–C**, the compound **IA_1** was found to be relatively potent with high fitness score (Fig. 4B). In the series **IIA–C**, the compound **IIA_5** was shown to be the most potent molecule with relatively highest dock score amongst all the molecules docked (Fig. 5A). This molecule displayed three hydrogen bond interactions with MRP1–NBD1 viz., one between the C-3 substituted carbamoyl nitrogen of DHP and *Glu*694 (distance = 3.10 Å), another between the C-5 substituted carbamoyl nitrogen of DHP and *Tyr*710 (distance = 3.27 Å) and the third between the tertiary nitrogen of 2-pyridyl substituent at C-5 of DHP and *Arg*780 (distance = 3.17 Å) (Fig. 5B).

3.2. MRP1 ATPase assay

In this study, we tested the ability of the top-ranked 10 molecules in docking studies to influence ATPase activity on isolated Sf9 cell membranes expressing human MRP1 transporter. In the activation study, it was observed that only two test compounds, **IA_1** and **IIA_5** induced inhibition on MRP1 basal ATPase activity at concentrations above 10 μM. In theory, it is assumed that good substrates activate transporter's basal ATPase activity, whereas slowly transported substrates rather inhibit it.[36] In agreement with this information, the compounds **IA_1** and **IIA_5** have been shown to be slowly transported substrates of MRP1. In addition, in the inhibition study compounds **IA_1** and **IIA_5** were able to inhibit the

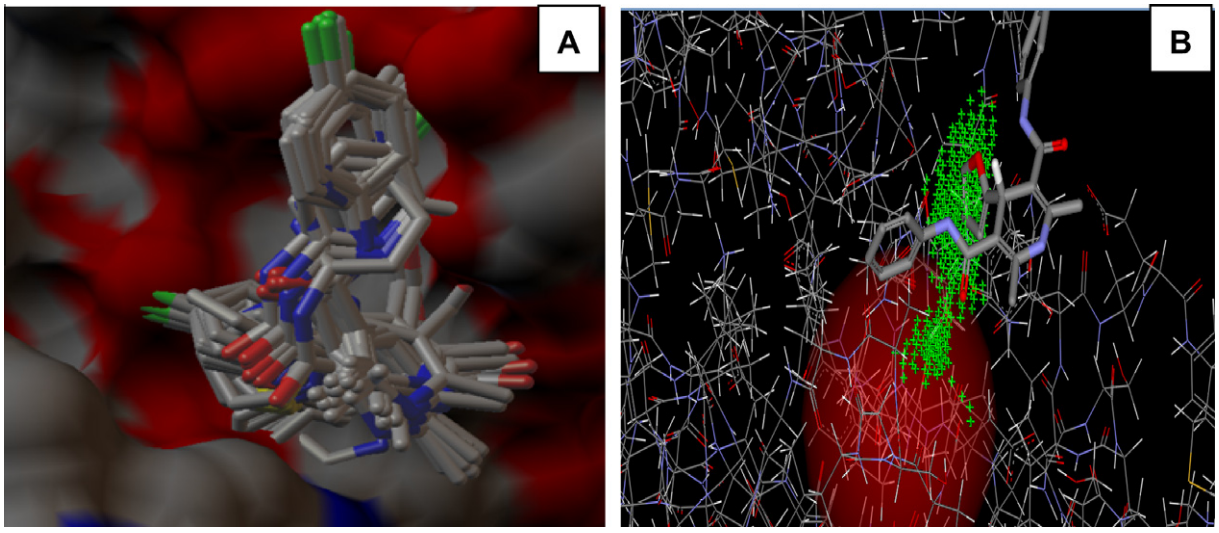


Figure 4. (A) Binding orientation of new 1,4-DHPs at active site of MRP1–NBD1. (B) Docking interaction of **IA_1** with active site of MRP1–NBD1.

Table 2
Discovery Studio Dock score of 1,4-DHPs

Rank order	Compound	IUPAC names	Discovery Studio Dock Score	RMSD
1	**IIA_5**	4-(2-Pyrrolyl)-2,6-dimethyl-3,5-bis-*N*-(pyridin-2-yl)carbamoyl-1,4-dihydropyridine	31.547	1.871
2	**IA_1**	4-(2-Furyl)-2,6-dimethyl-3,5-bis-*N*-(phenyl)-carbamoyl-1,4-dihydropyridine	27.874	1.658
3	**IA_6**	4-(4-Chlorophenyl)-2,6-dimethyl-3,5-bis-*N*-(phenyl)carbamoyl-1,4-dihydropyridine	26.132	1.999
4	**IA_2**	4-(2-Pyridyl)-2,6-dimethyl-3,5-bis-*N*-(phenyl)-carbamoyl-1,4-dihydropyridine	24.827	5.326
5	**IIA_1**	4-(2-Furyl)-2,6-dimethyl-3,5-bis-*N*-(pyridin-2-yl)-carbamoyl-1,4-dihydropyridine	24.465	1.799
6	**IB_4**	4-(2-Imidazolyl)-2,6-dimethyl-3,5-bis-*N*-(4-methylphenyl)carbamoyl-1,4-dihydropyridine	21.2	3.457
7	**IA_5**	4-(2-Pyrrolyl)-2,6-dimethyl-3,5-bis-*N*-(phenyl)-carbamoyl-1,4-dihydropyridine	19.672	5.31
8	**IA_3**	4-(2-Thienyl)-2,6-dimethyl-3,5-bis-*N*-(phenyl)-carbamoyl-1,4-dihydropyridine	18.296	1.885
9	**IA_4**	4-(2-Imidazolyl)-2,6-dimethyl-3,5-bis-*N*-(phenyl)carbamoyl-1,4-dihydropyridine	18.08	4.894
10	**IIA_4**	4-(2-Imidazolyl)-2,6-dimethyl-3,5-bis-*N*-(pyridin-2-yl)carbamoyl-1,4-dihydropyridine	17.916	2.189
[a]	Amlodipine	(*RS*)-3-Ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate	37.515	6.177
[a]	Verapamil	(*RS*)-2-(3,4-Dimethoxyphenyl)-5-{[2-(3,4-dimethoxyphenyl)ethyl]-(methyl)amino}-2-prop-2-yl-pentanenitrile	33.322	3.422

[a] Standard drugs.

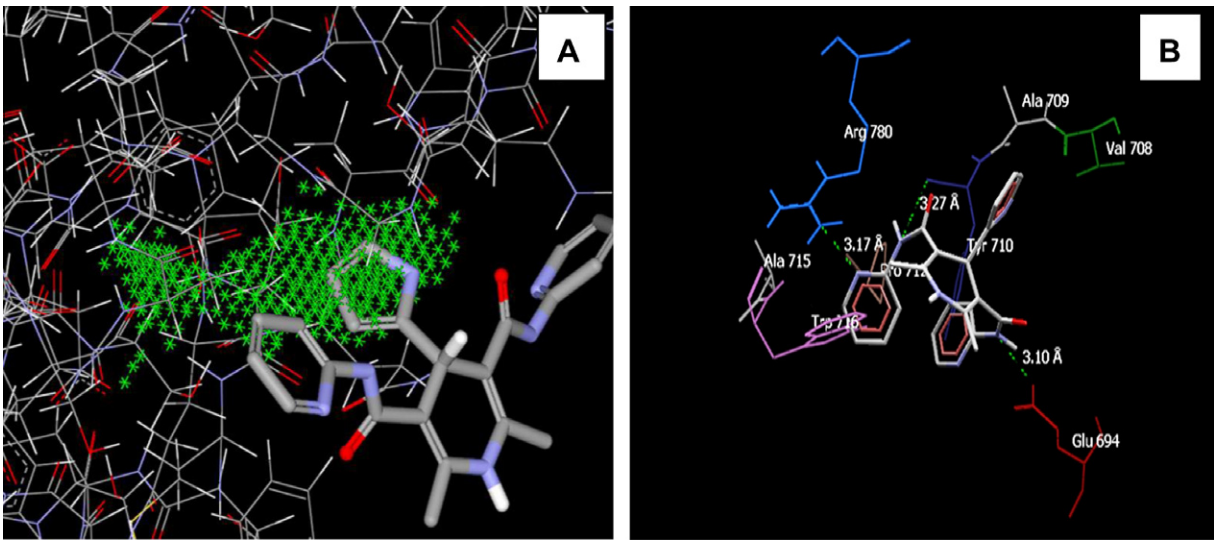


Figure 5. (A) Docking interaction of **IIA$_5$** with active site of MRP1–NBD1. (B) 3D representation of **IIA$_5$** with selected amino acid residues at the active site of MRP1–NBD1. Hydrogen bonds are indicated with green dashes. Hydrogen atoms are omitted for clarity.

Table 3
Effect of the compounds **IA$_1$** and **IIA$_5$** on MRP1 ATPase activity in membrane vesicles prepared from transduted Sf9 cells

Compound	Effect on basal ATPase (μM)	IC_{50} for activated ATPase (μM)	Calculated K_i (μM)
IA$_1$	Inhibition[a]	20 ± 4	0.95
IIA$_5$	Inhibition[a]	14 ± 2	0.66
Benzbromarone	—	4 ± 1	0.19

[a] Slight inhibition starting at concentrations above 10 μM.

NEM-GS-activated MRP1 ATPase with respective IC_{50} values of 20 ± 4 and 14 ± 2 μM (mean ± SD of three experiments, Table 3) as comparable to a standard MRP1 inhibitor, benzbromarone (IC_{50} = 4 μM). However, the rest of the eight molecules with a relatively small difference in docking scores as compared to compounds **IA$_1$** and **IIA$_5$**, failed to show significant inhibition at the concentrations tested. This clearly indicates that a small difference in the docking score is resulting in the greater difference in the MRP1 inhibitory potency. Probably further studies may be required to account for this observation.

The inhibition study data indicates the % of MRP1 inhibited by the test compound at a given concentration. The percentage of MRP1 inhibition observed by the test compounds **IA$_1$** and **IIA$_5$** at 30 μM concentration was found to be 52.82% and 69.82%, respectively, (Fig. 6A and B). The maximum% inhibition of MRP1 was observed to be 78.01 at 100 μM of the compound **IIA$_5$**. These results clearly indicate that both the test compounds **IA$_1$** and **IIA$_5$** specifically interact with MRP1 and inhibit it in comparison to the reference drug, benzbromarone.

Thus, taking into account the efficacy of the compounds **IA$_1$** and **IIA$_5$** to inhibit the ATPase-activated by NEM-GS, the calculated K_i for compounds **IA$_1$** and **IIA$_5$** were ~0.95 and 0.66 μM, respectively, for MRP1 which in turn, indicate the relative affinity of the compounds to this specific protein. The 0.66 μM value of K_i testifies the stronger affinity of the compound **IIA$_5$** for MRP1 over the other effective compound **IA$_1$** (K_i = 0.95 μM), and interestingly, it is comparable to the standard, benzbromarone (K_i = 0.19 μM).

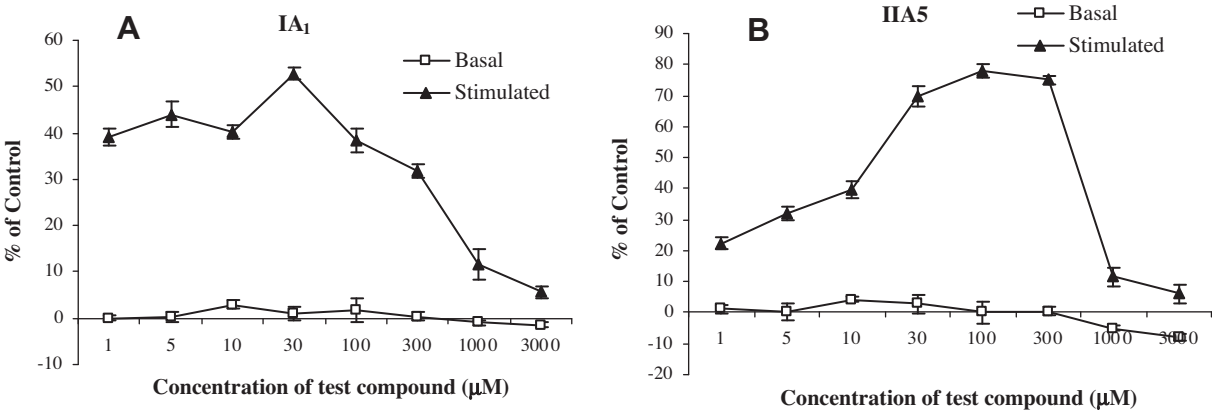


Figure 6. Modulatory effect of test compounds **IA$_1$** and **IIA$_5$** on ATPase activity in isolated Sf9 cell membranes expressing human MRP1 was tested in the 1–3000 μM concentration range for their capability to alter the ATPase activity in isolated membrane vesicles. The action of **IA$_1$** and **IIA$_5$** on MRP1 was checked in an *activation assay* (□) and in an *inhibition assay* (▲). The 'activation assays' were performed on basal ATPase activity, which was 5.2 nmol Pi/mg protein/min for MRP1. In the 'inhibition assays', NEM-GS at 10 mM for MRP1 was used as the reference activator. The maximal stimulatory effect of this activator corresponded to ATPase activity of 9.6 nmol Pi/mg protein/min. The ATPase activities were expressed on a scale where 0% corresponded to the basal activity and 100% was defined as the activity observed in the presence of NEM-GS. In the inhibition assays, IC_{50} was defined as the concentration of test compounds that inhibits by 50% the maximally stimulated ATPase. Data presented were means ± SD of three experimental points of one representative experiment out of the three.

4. Conclusion

In molecular docking studies, an active site on MRP1–NBD1 for 1,4-DHPs could be successfully identified for the first time. Novel 1,4-DHPs synthesized in our laboratory were evaluated in silico for their MRP1 inhibitory activity. Among the three series of molecules evaluated, seven 4-aryl/heteroaryl-3,5-bis-*N*-(substituted phenyl)-2,6-dimethyl-1,4-DHPs (**IA_{1-6}**, **IB_4**) and three 4-aryl/heteroaryl-3,5-bis-*N*-(substituted 2-pyridyl)-2,6-dimethyl-1,4-DHPs (**IIA_1**, **IIA_4**, and **IIA_5**) were found to be relatively more potent and comparable to the standards, verapamil and amlodipine. These molecules were specifically selected for MRP1 inhibitory effect by MRP1 ATPase kit (Solvo Biotechnology, Hungary), experimentally. In conclusion, the present findings identify the test compounds **IA_1** and **IIA_5** as inhibitors of MRP1 (ABCC1) mediated substrate transport. Compound **IIA_5** was shown to be relatively more potent than **IA_1** as noted from the docking results. Thus the docking results could show an interesting correlation between docking scores and experimental binding data which could be employed to develop a pharmacophore model to help the interpretation of the observed affinities and to design new MRP1 antagonists. Further studies on P-gp inhibitory effect of these molecules and their structural analogues is in progress, in our laboratory.

Conflicts of interest

The authors hereby declare that none of them have any financial/commercial obligations or conflicts with any individual or organization.

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