Mechanistic Evidence to Support the Anti-hepatitis B Viral Activity of Multifunctional Scaffold & Conformationally Restricted Magnolol

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RESEARCH ARTICLE

Mechanistic Evidence to Support the Anti-hepatitis B Viral Activity of Multifunctional Scaffold & Conformationally Restricted Magnolol

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Abstract Magnolol, phenolic bioactive phytomolecule, is a potential antihepatitis B viral agent. Current study is to mechanistically analyze the probable site of action for magnolol. Magnolol has been docked with EF3-CaM adenylyl cyclase (1PK0), deoxycytidine kinase (2NOA), human nucleoside diphosphate kinase (3FKB), human hepatitis B viral capsid (1QGT), hepatitis B X-interacting protein (3MSH) proteins using GRIP docking methodology, and compared with reference ligands like adefovir diphosphate (active metabolite of adefovir), lamivudine, tenofovir monophosphate (active metabolite of tenofovir) and tenofovir diphosphate (active metabolite of tenofovir). Results revealed its preferential interactability towards 2NOA i.e. deoxycytidine kinase, which raise up its chance to get metabolized into phosphorylated analogs, providing further impetus for discovery and clinical development of semi-synthetic analogs of magnolol. Out of all the virtually designed magnolol derivatives, Leadgrow_ML1449 have superior binding affinities towards all the test proteins except EF3-CaM adenylyl cyclase.

Keywords Magnolol · Antihepatitis B viral activity · Docking studies · GRIP docking · Natural antiviral agent

Introduction

Being a treasure house of various therapeutic phytochemicals, plants have been reported to be an integrated part of traditional medicinal system. 70–95 % population of most of the developing countries depends on these traditional medicines for primary care [1]. The history of antiviral therapy essentially starts in 1977 when the first, and still 'Gold standard', antiviral compound, acyclovir, was found to inhibit DNA replication of herpes simplex virus at concentration far below those affecting cellular DNA synthesis [2–4]. Herbal formulations and bioactive phytoconstituents are reported to have potential antiviral activity [5–7].

Magnolol, a multifunction bioactive phyto-compound (Fig. 1) can be isolated from Chinese medicinal plants *Magnolia officinalis* and *Streblus asper*. Magnolol is a small polyphenolic molecule with low toxicity. In preclinical experiments, magnolol was found to have anti-oxidative, anti-inflammatory, antiviral, anti-tumorigenic, anti-diabetic, anti-microbial, anti-neurodegenerative and anti-depressant properties. Magnolol can also effectively regulate pain control, hormonal signalling, gastrointestinal and uterus modulation as well as provide cardiovascular and liver protective effects [6, 8]. Literature revealed that magnolol potentially inhibited 50 % of the expression of HBsAg and HBeAg by Hep G2.2.15 cells at the concentration below 25 μM, while lamivudine (3TC, standard anti-hepatitis B agent) inhibit these expressions (50 %) at concentration of 200–400 μM, henceforth our natural bioactive phytomolecule magnolol should be the preferred anti-hepatitis B viral agent [6]. In purview of this, author tried to mechanistically analyze the probable site of anti-HBV action using various proteins like EF3-CaM adenylyl cyclase (1PK0), deoxycytidine kinase (2NOA), human nucleoside diphosphate kinase (3FKB), human hepatitis B viral capsid (1QGT), hepatitis B X-interacting protein (3MSH) and compared it with standard antiviral agents like adefovir diphosphate (active metabolite of adefovir), lamivudine, tenofovir monophosphate (active metabolite of tenofovir) and tenofovir diphosphate (active metabolite of tenofovir).

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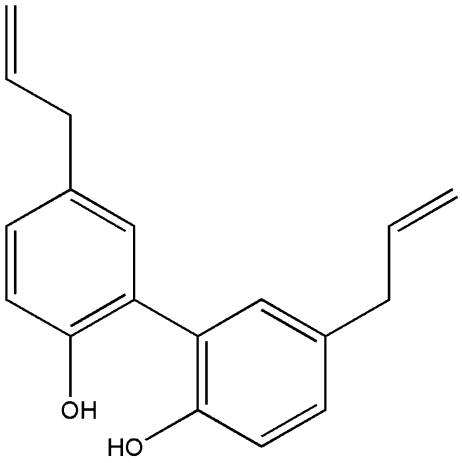

Fig. 1 Structure of magnolol, a bioactive phytoconstituents

Materials and Methods

Proteins Used

EF3-CaM adenylyl cyclase (1PK0), deoxycytidine kinase (2NOA), human nucleoside diphosphate kinase (3FKB), human epatitis B viral capsid (1QGT), Hepatitis B X-interacting protein (3MSH) were taken from the RSCB protein data bank [9–13].

Docking Studies

The 2D structure of magnolol was built using Chem Draw Ultra 8.0 and then converted into the 3D with the help of VLife MDS 4.3 software. The 3D structure was then energetically minimized up to the rms gradient of 0.01 using merck molecular force field (MMFF). Conformers of magnolol were then generated using systemic method based on the selection of rotatable bonds. The active site selection was done by either choosing the cavity and if available, co-crystallized reference ligand e.g. lamivudine, in case of deoxycytidine kinase. The docking simulation was done using GRIP batch docking methodology and magnolol was virtually docked with EF3-CaM adenylyl cyclase, deoxycytidine kinase, human nucleoside diphosphate kinase, human hepatitis B viral capsid, hepatitis B X-interacting receptor proteins and compared with the PLP score of reference ligand (drug/active metabolite) like adefovir diphosphate, lamivudine, tenofovir monophosphate and tenofovir diphosphate. The parameters fixed for

Table 1 Docking studies of magnolol, virtual analogs and reference ligands with various targeted proteins

Molecules under docking study	Docking with potential targets				
	Crystal structure of the EF3-CaM complexed with PMEApp (1PK0)	Human hepatitis B viral capsid (HBCAG) (1QGT)	The structure of deoxycytidine kinase complexed with lamivudine and ADP (2NOA)	Structure of NDPK H122G and tenofovir-diphosphate (3FKB)	Crystal structure of hepatitis B X-interacting protein at high resolution (3MSH)
Magnolol	−74.02	−81.14	−104.87	−77.26	−69.45
MLR1	−57.56	−29.48	11.63	−41.03	−38.90
MLR2	−59.29	4.52	52.60	−35.72	−37.39
MLR3	−60.03	−52.73	15.48	−60.56	−44.87
MLR4	−71.27	−59.03	44.47	−56.35	−45.33
MLE1	−57.83	−47.38	30.09	−53.95	−44.87
MLE2	−59.58	−40.24	33.24	−54.19	−44.16
MLE3	−56.14	−34.58	7.64	−55.80	−36.96
MLE4	−62.27	−6.54	58.79	−59.72	−48.46
Leadgrow_ML0403	−71.53	−95.47	−120.77	−92.05	−81.04
Leadgrow_ML1449	**−69.54**	**−100.14**	**−133.48**	**−105.57**	**−88.93**
Leadgrow_ML1635	−68.38	−94.87	−106.13	−99.99	−77.98
Adefovir diphosphate	−87.30	ND	ND	ND	ND
Lamivudine	ND	ND	−79.06	ND	ND
Tenofovir mono phosphate	ND	ND	ND	−76.97	ND
Tenofovir diphosphate	ND	ND	ND	−75.77	ND

Bold indicates the best binding affinity out of all

ND not done

GRIP docking simulation were like this— number of placements: 50, rotation angle: 10°, ligand wise results: 10, exhaustive method, scoring function: PLP score. By rotation angle, the ligand gets rotated for different poses. By placements, the method will check all the 50 possible placements into the active site pocket and will result out few best placements out of 50. The method also highlights the best placement of magnolol which is having best (minimum) score, different for each receptor. More negative the PLP score after GRIP docking, better will be affinity of ligand for the targeted protein. This particular placement of magnolol was selected for the interactive analysis to determine the amino acid residues of the active site pocket and then checked for various interaction of magnolol with receptors like hydrogen bonding, hydrophobic bonding and van der Waal's interaction, aromatic/pi-staking, charge interaction [14–17].

Results and Discussion

Docking studies revealed that magnolol have capability to target more than one key mechanism to act as anti-HBV agent, but preferentially interacting with deoxycytidine kinase (Table 1). In case of EF3-CaM adenylyl cyclase (1PK0), magnolol have van der Waal's interactions with Arg329A, Lys346A, Val350A, His351A, Lys353A, Asn583A, Phe586A, Glu588A amino acid residues while having hydrophobic interactions with His351A, Lys353A and Glu588A amino acid residues of 1PK0. In addition to these, magnolol also have aromatic interactions/pi-staking with His351A of EF3-CAM adenylyl cyclase. Adefovir diphosphate, active metabolite of reference drug have van der Waal's interactions with Arg329A, Lys346A, Leu348A, Lys353A, Ser354A, Lys372A, Ala490A, Asp493A, Gly547A, Thr548A, His577A, Gly578A, Thr579A, Glu580A and Asn583A amino acid residues while having hydrophobic interactions with Leu348A, Gly547A, Thr548A, His577A, Gly578A, Asn583A amino acid residues of 1PK0. Apart from these, adefovir diphosphate do have aromatic interactions/pi-staking with His577A, charge interactions with Asp493A and hydrogen bonding with Arg329A, Lys346A, Ser354A, Lys372A and Thr548A amino acid residues of EF3-CAM adenylyl cyclase. In case of human Hepatitis B viral capsid (1QGT), magnolol have van der Waal's interactions with Gln57C, Ala58C, Cys61C, Gln57D, Leu60D, Cys61D and Glu64D amino acid residues while having hydrophobic interactions with Gln57C, Ala58C, Cys61C, Gln57D, Ala58D, Leu60D, Cys61D and Glu64D amino acid residues of 1QGT. In addition to these, magnolol also have hydrogen bonding with Gln57D amino acid residue of human Hepatitis B viral capsid. In case of deoxycytidine kinase co-crystallized with lamivudine and ADP (2NOA), magnolol have van der Waal's interactions with Ile30A, Glu53A, Trp58A, Leu82A, Met85A, Tyr86A, Phe96A, Gln97A, Arg104A, Arg128A, Asp133A and Phe137A amino acid residues while having hydrophobic interactions with Ile30A, Val55A, Ala100A and Phe137A amino acid residues of 2NOA. In addition to these, magnolol also exerts aromatic interaction with Trp58A, Phe96A and Phe137A amino acid residues of deoxycytidine kinase. Likewise, lamivudine (3TC) have van der Waal's interactions with Ile30A, Glu53A, Val55A, Trp58A, Leu82A, Met85A, Tyr86A, Phe96A, Gln97A, Arg104A, Arg128A, Asp133A, Phe137A and Arg194A amino acid residues while having hydrophobic interactions with Ile30A, Val55A, Leu82A, Met85A, Ala100A, Asp133A and Phe137A amino acid residues of deoxycytidine kinase. Apart from these, lamivudine also have charge interactions with Asp133A & hydrogen bonding with Gln97A and Arg128A of deoxycytidine kinase. In case of human nucleoside diphosphate kinase (3FKB), magnolol have van der Waal's interactions with Thr98D, Val116D, Gly117D and Asn119D amino acid residues while having hydrophobic interactions with Thr98D, Val116D and Gly117D amino acid residues of 3FKB. Additionally, magnolol do have aromatic interactions with Phe64D

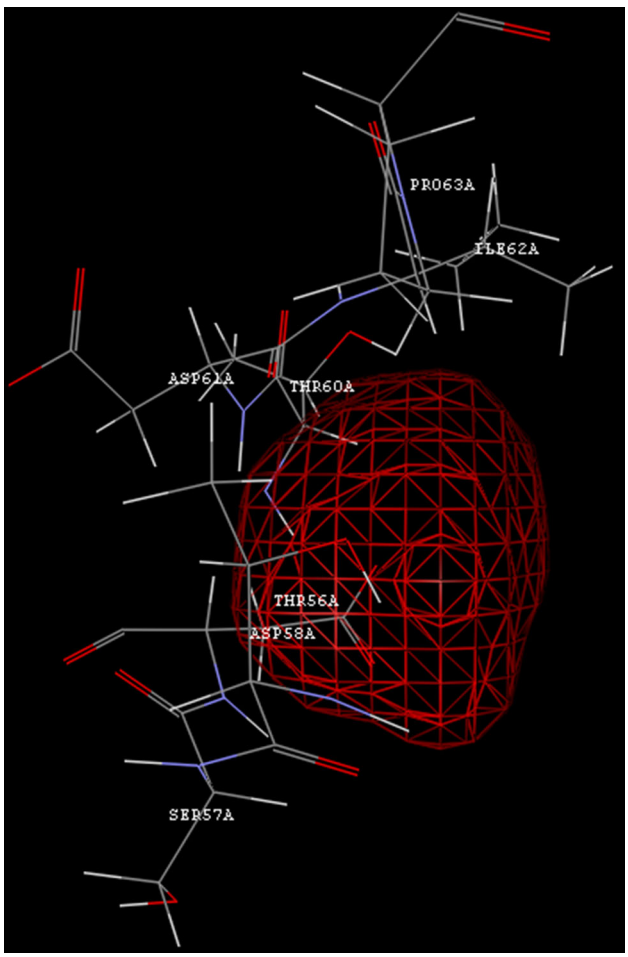

Fig. 2 Cavity no. 6 of 3MSH

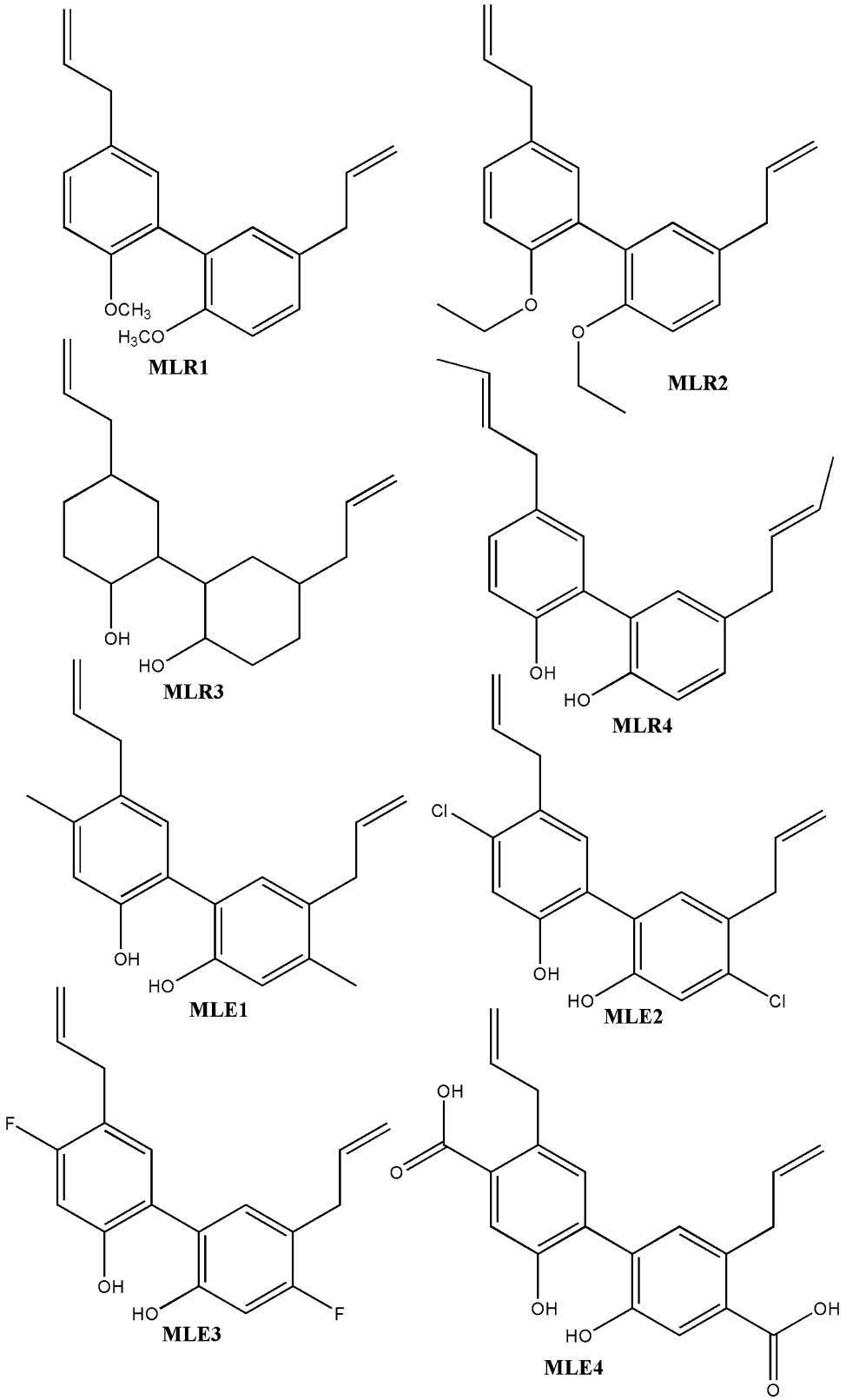

Fig. 3 Inactive MLR and MLE series of Magnolol

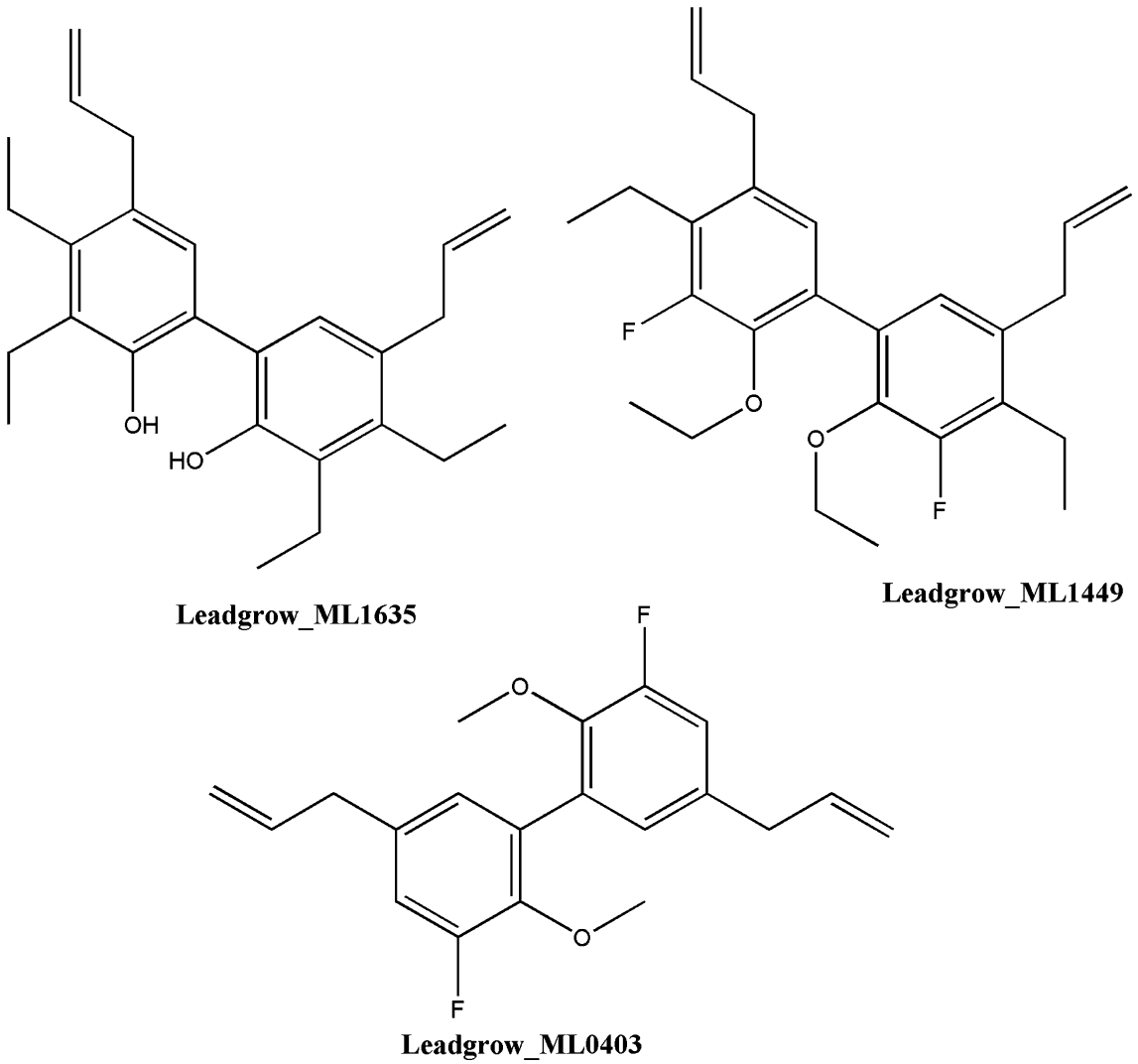

Fig. 4 Active magnolol analogs

amino acid residue of human nucleoside diphosphate kinase. Tenofovir diphosphate, active metabolite of reference drug Tenofovir, have van der Waal's interactions with Lys16D, Tyr56D, His9D, Arg62D, Phe64D, Leu68D, Arg92D, Thr98D, Arg109D, Val116D, Gly117D, Asn119D, Gly122D amino acid residues while having hydrophobic interactions with Phe64D, Leu68D, Thr98D, Val116D and Gly117D amino acid residues of 3FKB i.e. human nucleoside diphosphate kinase. In addition to these, tenofovir diphosphate also have charge interactions with Glu58D & hydrogen bonding with Lys16D, His59D and Arg92D amino acid residues of 3FKB. In case of hepatitis B X-interacting protein (3MSH), magnolol have van der Waal's interactions with Gln50A, Ala53A, Thr56A, Asp58A, Thr60A, Ile62A and Lys78A amino acid residues while having hydrophobic interactions with Ala53A, Ile62A and Lys78A amino acid residues of cavity no. 6 (Fig. 2) of hepatitis B X-interacting protein.

Magnolol i.e. 5,5′-di(prop-2-en-1-yl)biphenyl-2,2′-diol, is a conformationally restricted phytomolecule having potential anti-HBV activity, and strong affinity towards deoxycytidine kinase. Human deoxycytidine kinase (dCK) is a nucleoside kinase responsible for the phosphorylation of deoxycytidine (dC), deoxyadenosine (dA) and deoxyguanosine (dG) to their monophosphate form. As such, dCK plays a pivotal role in the salvage pathway of deoxyribonucleosides for their ultimate conversion to triphosphorylated forms suitable for incorporation into DNA. Deficiency of DCK is associated with resistance to antiviral and anticancer chemotherapeutic agents. Conversely, increased deoxycytidine kinase activity is associated with increased activation of these compounds to cytotoxic nucleoside triphosphate

derivatives [18]. Keeping these perspectives in view, we can propose that magnolol will be having strong tendency to get converted into phosphorylated derivatives which elicit bio-isosterism to the nucleoside triphosphate than the standard antiviral agent lamivudine. And as a whole, magnolol having affinity to go and bind with all the tested proteins EF3-CaM adenylyl cyclase, deoxycytidine kinase, human nucleoside diphosphate kinase, human hepatitis B viral capsid, hepatitis B X-interacting protein, so it can be predicted by the results that magnolol will be a definite challenge to the anti-hepatitis B virus, as the resistance will not be easy against the multi-functional magnolol.

Keeping these facts in mind, few derivatives of magnolol (Figs. 3, 4) were virtually designed and tested against the test proteins to evaluate their relative binding affinities in reference to magnolol and reference ligands. Results of the docking studies were tabulated in Table 1, which reveals that MLR and MLE series of magnolol have inferior binding affinities when compared with magnolol and reference ligands. Whereas leadgrow series of magnolol i.e. Leadgrow_ML0403, Leadgrow_ML1449 and Leadgrow_ML1635 have much better binding affinity when compared with the magnolol and reference ligands, out of which Leadgrow_ML1449 exhibit improved affinity towards all test proteins except EF3-CaM. As can be observed by Table 1, Leadgrow_ML1449 have almost two fold binding affinity for deoxycytidine kinase than the standard drug lamivudine, so probably a potential antiviral candidate. Further studies are going on in purview of their synthesis and evaluation of anti-hepatitis B viral activity.

Conclusion

Magnolol have been docked with EF3-CaM adenylyl cyclase, deoxycytidine kinase, human nucleoside diphosphate kinase, human hepatitis B viral capsid, hepatitis B X-interacting proteins, and results lead us to predict magnolol as the multifunctional scaffold with potential affinity towards all the proteins comparable to that of standard antiviral agents. Based on its in vitro activity and docking results, virtual library of magnolol derivatives were generated. Out of all, Leadgrow_ML1449 exhibited superior binding affinities for test proteins when compared with magnolol and standard drugs.

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