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Research article

Computational approach to study the synthesis of noscapine and potential of stereoisomers against nsP3 protease of CHIKV

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ABSTRACT

Chikungunya fever is a major public health issue in India affecting millions of people and occurs due to Chikungunya. Chikungunya virus (CHIKV) is a single stranded RNA virus from the family of Togaviridae and genus alpha virus. It contain three structural proteins: glycosylated E1 and E2, embedded in the viral envelope, and a non-glycosylated nucleocapsid protein. Till date, researchers are working on inhibition of CHIKV but till now no cheap and effective medicine is available in the market. Therefore, the authors of this work thought of isoquinoline based noscapine to inhibit the nsP3 protease of CHIKV. The aim of the work is to understand the mechanism for the synthesis of noscapine theoretically using DFT. Further study the potential of all four isomers of noscapines {(13 (S,R), 14 (R,R), 15 (R,S) and 16 (S,S)} against nsP3 protease of CHIKV with the help of docking and MD simulation. The integrated e-pharmacophore binding affinity based virtual screening, docking and molecular dynamics simulation recognized four hits isomers as inhibition nsP3 protease of CHIKV. The docking energies of all the isomers of noscapine (13–16) with nsP3 protease CHIKV was found out to be more negative than baicalin (−8.06 kcal/mol) on selected sites. Amongst the isomers of noscapine, CMPD **13** possessed best binding affinity with four hydrogen bonding interactions. Further, ADME properties and blood-brain barrier permeability properties have been calculated. DFT studies of all the isomers of noscapine was investigated.

1. Introductions

Fever is an abnormally high body temperature usually accompanied by shivering, headache and in severe instances delirium [1, 2]. There are different types of fever, which are transmitted by mosquitoes. The main four types of fever are dengue, malaria, chikungunya and zika fever [3]. Chikungunya is a viral infection transmitted by Aedesaegypti mosquito symptoms may include fever, joint pain, fatigue, rash, muscle pain, headache. It is spread by two types of mosquitoes Aedesalbopictus and Aedesaegypti [4, 5, 6]. Chikungunya fever is a major public health issue in India affecting billions. After 2010, the infection was in a decline stage until in 2016, when a massive outbreak affected the country. CHIKV is a single stranded RNA virus from the family of Togaviridae and genus alpha virus. CHIKV contain three structural proteins: glycosylated E1 and E2, embedded in the viral envelope, and a nonglycosylated nucleocapsid protein [7]. The non-structural polyprotein is divided into four different proteins (nsP1, nsP2, nsP3, and nsP4). Non-structural protein (nsp) are necessary for the transcription and translation of viral mRNA inside the cytoplasm of host cells [8]. Current therapies for CHIKV-infected patients with arthritis/arthralgia mainly involve management of pain and inflammation using non-steroid anti-inflammatory drugs (NSAIDs), as well with fluid intake to prevent dehydration. There is no licensed antivirals or vaccines available for CHIKF, therefore, there is a vital need for the development of novel and potent drugs against CHIKV [9, 10, 11]. Noscapine is benzylisoquinoline alkaloid belongs to plant family poppy. It possesses various functional moieties. It is used for its antitussive effect by its sigma receptor agonist activity [12, 13]. Noscapine is an anti-cancer drug and the elimination half-life of noscapine is 1.5–4 h. It is

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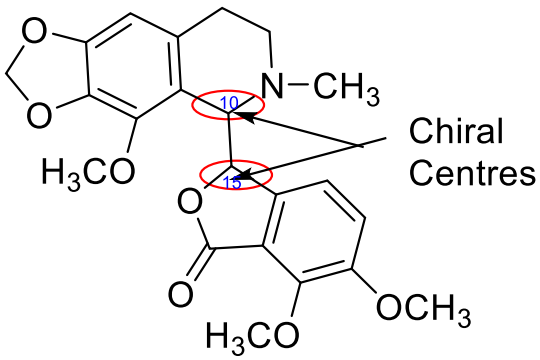


Fig. 1. Structure of noscapine showing the chiral center at 10th and 15th positions.

a tubulin-binding anti-angiogenic anticancer drug that causes cell cycle arrest and induces apoptosis in cancer cells both in vitro as well as in vivo [13, 14, 15]. The aim of the work is to understand the mechanism for the synthesis of noscapine using Density functional theory (DFT). Further, it aims to understand the potential of all four isomers of noscapines (R,R; R, S; S,R and S,S configuration on 10th and 15th number) as mentioned in Fig. 1 against nsP3 protease of CHIKV with the help of docking; absorption, distribution, metabolism and excretion (ADME); molecular dynamics (MD) simulations and molecular mechanics-generalized born surface area (MM-GBSA) analysis.

2. Experimental

In this work, authors studied the effect of four isomers of noscapine to inhibit the activity of nsP3 protease of CHIKV. First the synthetic strategy was proved based on the DFT studies. After the successful synthetic procedure, author screen them by molecular docking, ADME and DFT to get the potent one. Finally, the screened compound was refined by MD simulation and the MM-GBSA. The work is explained in Fig. 2.

2.1. Designing of ligand

Schemes 1, 2, and 3 for the synthesis of noscapine have been taken from the literature. The molecule was chosen because of its potential in different area of medical science [14, 15]. As a chemist, the biological potency of this molecule may be due to the functional group, presence of chiral carbons etc. Noscapine has two chiral carbon atoms and therefore, it has of four isomers, **13–16** (Fig. 3). The mechanism of synthesis of the noscapines was studied using DFT.

2.2. DFT studies

The mechanistic proof for the synthesis was subjected to analyze by the quantum mechanical approach via the DFT. The basic preparation of all four molecule were done in Gauss View and DFT analysis was performed by the GAUSSIAN 09 package [16, 17]. A three layer function based on the Becke's as Lee, Yang, Parr was used. 6-311 basis set was used to optimize the geometries of the molecules. On the basis of studies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) values of the isomers were taken to calculate all the physiochemical descriptors. With the help of HOMO and LUMO values, hardness (η), softness (S), chemical potential (μ), electronegativity (χ), and global electrophilicity index (ω) were calculated as given by Eqs. (1), (2), (3), (4), and (5). For a molecule, N denotes the no. of electron and E denotes the energy states [18, 19].

$$\eta = \frac{1}{2}\left(\frac{\delta^2 E}{\delta N^2}\right) = \frac{1}{2}\left(E_{LUMO} - E_{HOMO}\right) = \frac{1}{2}\left(IE - EA\right) \tag{1}$$

$$S = \frac{1}{2\eta} \tag{2}$$

$$\mu = \left(\frac{\delta E}{\delta N}\right)_v = \frac{1}{2}\left(E_{LUMO} + E_{HOMO}\right) = -\frac{1}{2}\left(IE + EA\right) \tag{3}$$

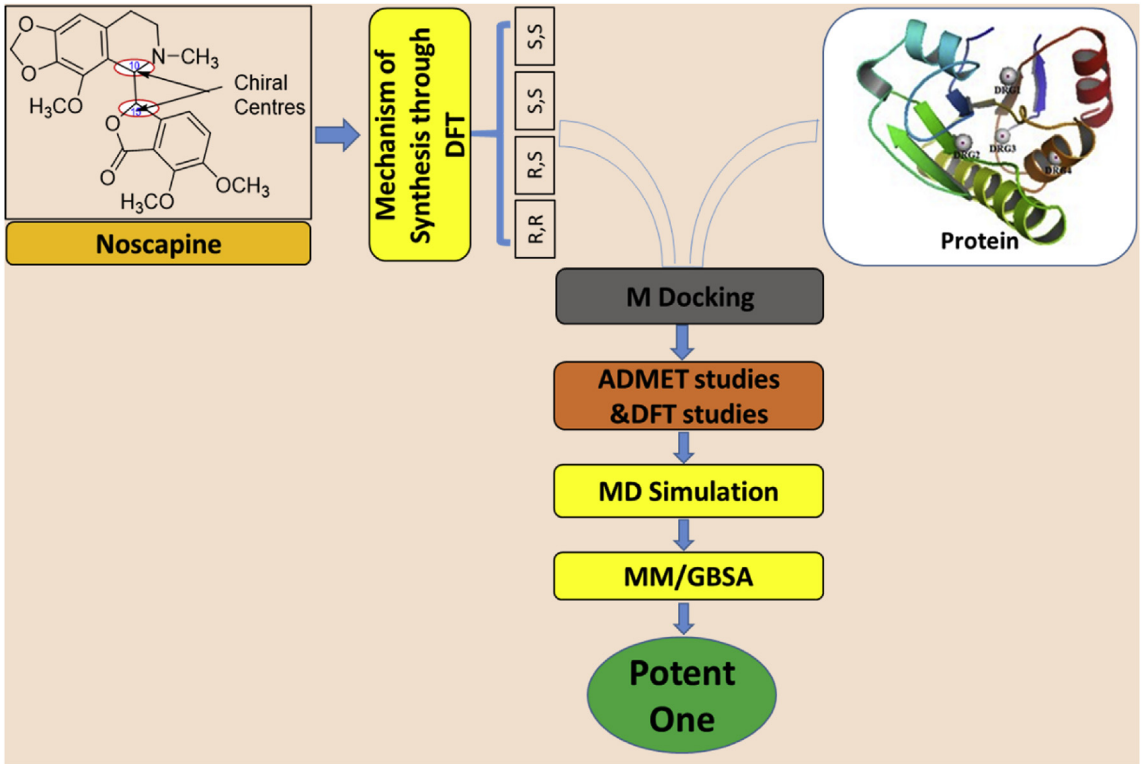
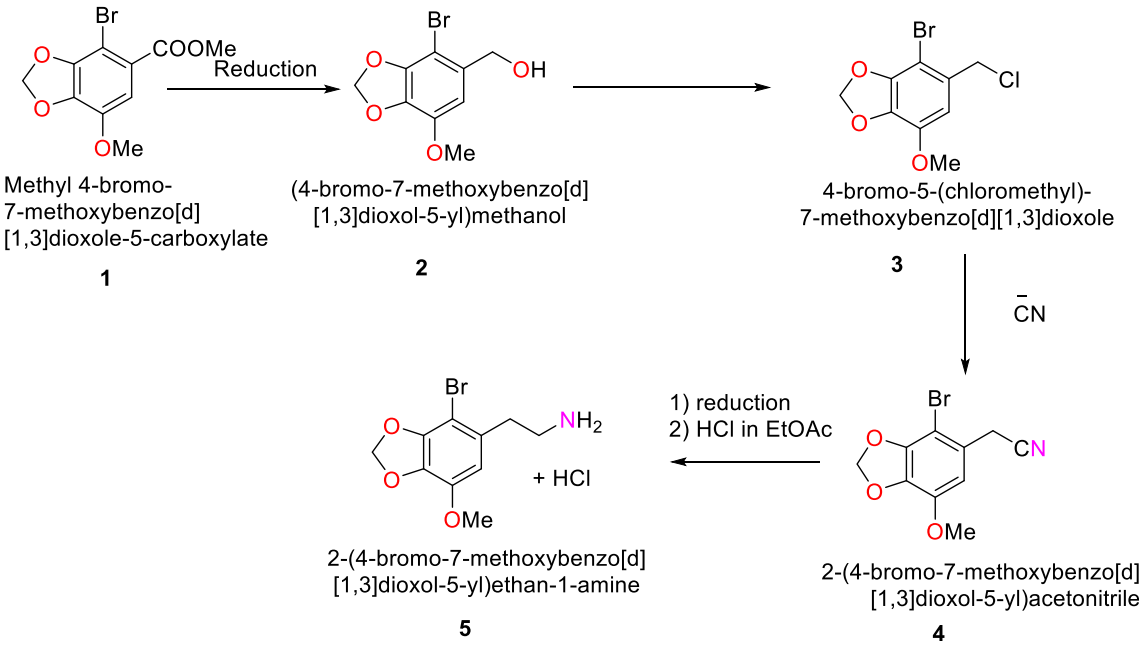
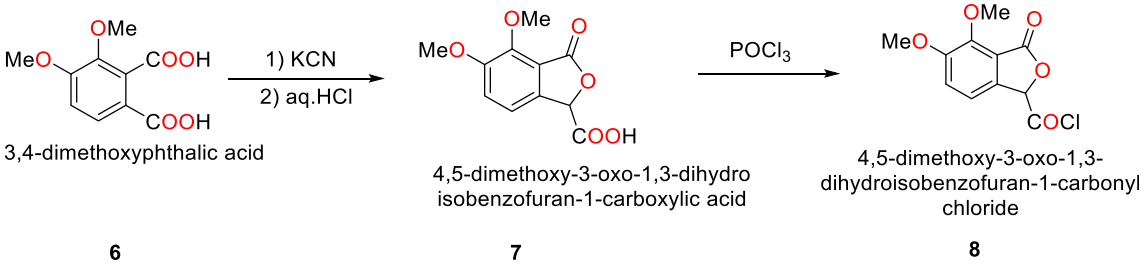


Fig. 2. The schematic representation of the experimental methodology.



Scheme 1. Synthesis of 2-aeylethylamine containing blocking group.



Scheme 2. Synthesis of phthalide-3-carboxylic acid.

$$\chi = -\mu = \left(\frac{\delta E}{\delta N}\right)_v = -\frac{1}{2}\left(E_{LUMO} + E_{HOMO}\right) = \frac{1}{2}\left(IE + EA\right) \quad (4)$$

$$\omega = \left(\frac{\mu^2}{2\eta}\right) \quad (5)$$

2.3. Docking preparation

Preparation of receptor and ligand are the important step before the docking. The preparation of ligand is performed with the help of Marvin Chemsketch. Herein, the geometry optimization and addition of explicit solvent molecules were checked. Receptor is prepared with UCSF Chimera 1.11.2 in dock prep module [20]. Removal of solvents, adding hydrogen, replacing incomplete residues using Dunbrack rotamer library, etc. Finally, the prepared receptor and ligand were used for docking.

2.4. Binding site prediction

The PDB of nsP3 protease of CHIKV has been taken from Research Collaboratory for Structural Bioinformatics (RCSB) and the code is 3GPO [21]. It is a tetramer and one chain was taken for the binding site prediction. The automated version of active site prediction (AADS) of Supercomputing Facility for Bioinformatics & Computational Biology Centre (SCFBio) of the Indian Institute of Delhi was used to determine the number of active sites in the chain of nsp3 protease of CHIKV [22].

2.5. Docking

Molecular docking is a computational technique based on the atomic level interaction of the ligand into the active binding site of the receptor [23]. Molecular docking between the optimized ligand and prepared nsP3 protease of CHIKV was performed by the ParDOCK server provided by the Supercomputing Facility for Bioinformatics & Computational Biology Centre of the Indian Institute of Delhi [24]. All the four isomers of noscapine were docked on the all sites determined by AADS. ParDOCK is a computational tool used for rigid docking of a molecules against a receptor. The post screening of the potent molecule was also performed with the help of ParDOCK. ParDOCK performed the rigid docking of the ligand in binding cavity of protein. Herein, author studies the conformations of four Noscapines via the rigid docking. The scoring function of ParDOCK can be understood by Eq. (6) [24].

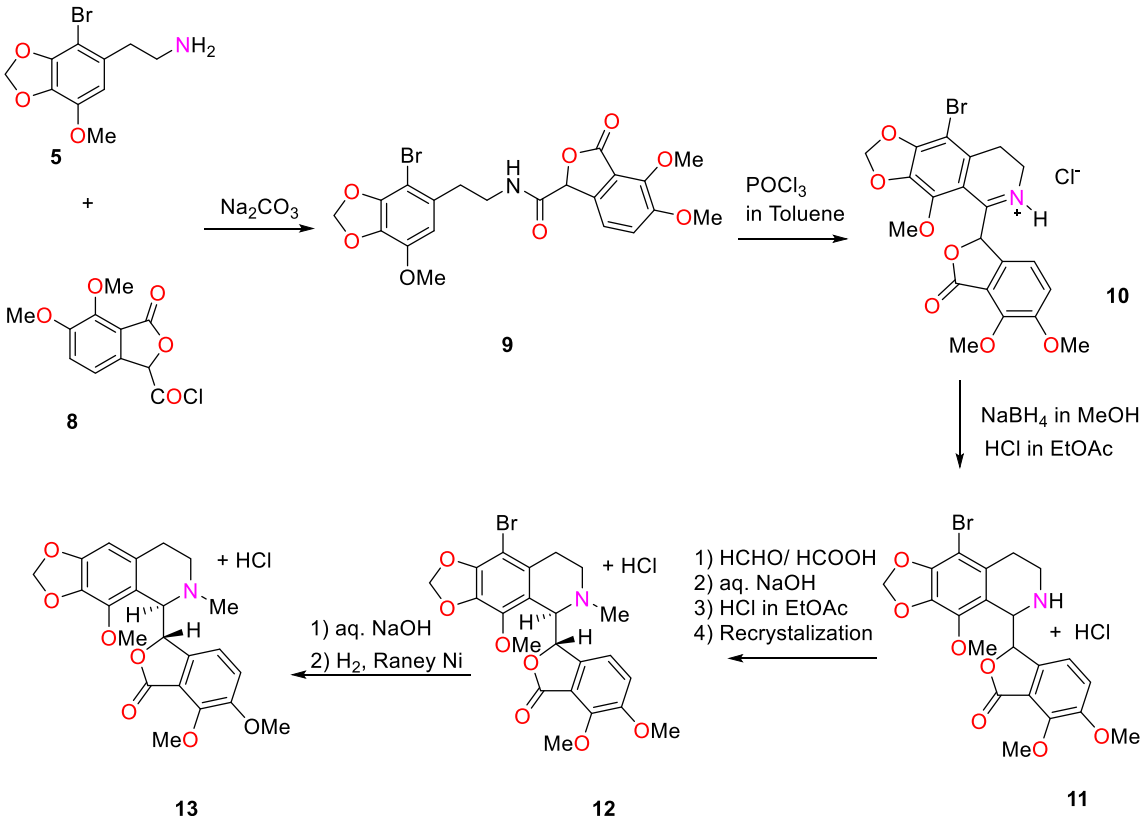
$$E = \sum E_{el} + E_{vdw} + E_{hpb} \quad (6)$$

where, E represents total non-bonded energy, E_{el} represents electrostatic contribution, E_{vdw} represents van der Waals interaction and E_{hpb} represents hydrophobic term.

The post dock modelling was performed through the Discovery Studio visualizer client 2019 [25].

2.6. ADME properties

The pharmacokinetic behavior of molecule is important to consider it as the drug. Bioavailability score in term of lipophilicity and aqueous



Scheme 3. Regioselective synthesis of (S,R) isomer of noscapine (13).

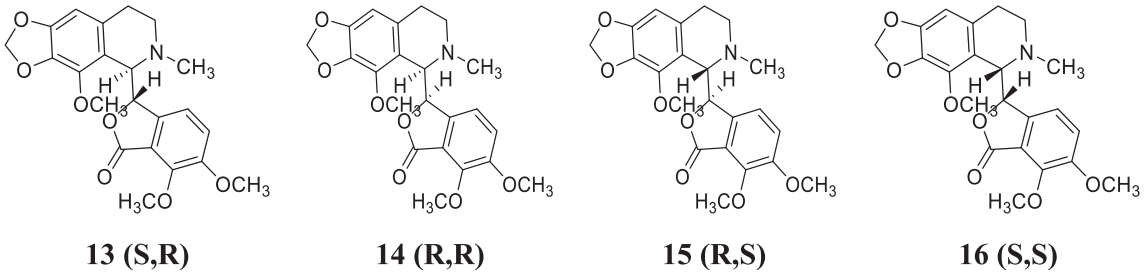


Fig. 3. List of the stereoisomers of the noscapine.

solubility for molecule to be the promising drug is more important [26]. The ADME properties of the all four ligand molecule were calculated through although the online server http://swissadme.ch/ [27].

2.7. Molecular dynamics

MD Simulations is a best method for studying the physical movements of atoms and molecules in a system. The trajectories of atoms and molecules are resolved by numerically solved Newton's equations of motion (F = ma) for a system of interacting particles, where forces within the particles and their potential energies are often calculated by using interatomic potentials or molecular mechanics (MM) force fields**.** [28, 29] MD simulations of 13–16 selected binding site of nsP3 protease of CHIKV were performed by using AMBER18 with the ff14SB force field and TIP3P 8.0 model were employed to produce the force field paramete15 of nsP3 protease and water molecules [30]. The molecular structures of **13-16** were optimized at the AM1 level and BBC charges were assigned to atoms of four inhibitors by using the Antechember module in AMBER18 suit. Generally Amber force field (GAFF) was applied to produce the force field parameters of 13–16 (46). MD simulation in solvent system were minimized by the steepest descent minimization of 2000 steps followed by the conjugate gradient minimization of 5000 steps to remove those unfavorable factors. The system was heated from 0 to 300 K under a softly heating process of 1 ns at constant volume and subsequently equilibrated for another 1 ns at 300 K. Finally, MD production is consist of 20000000 steps (nstlim) with a 2fs time step (dt) giving for 40ns. The reference temperature 300K (temp0 = 300.0) using Berendsen coupling algorithm to maintain constant temperature and pressure 1 bar was maintained with a temperature coupling time of 2ps (tautp = 2.0) is required. The time step was set to 2fs, print energy output every 500 steps (ntwr = 500) and save coordinates every 500 (ntwx = 500) in amber input files and were used for 40ns MD Simulations. Trajectories analyses were performed using the CPPTRAJ modules [31].

2.8. MM/GBSA studies

Molecular mechanics Generalized born surface area (MM-GBSA) is methods for fast calculations of binding free energies. MM-GBSA method was applied for the estimation binding free energies of protein-ligand

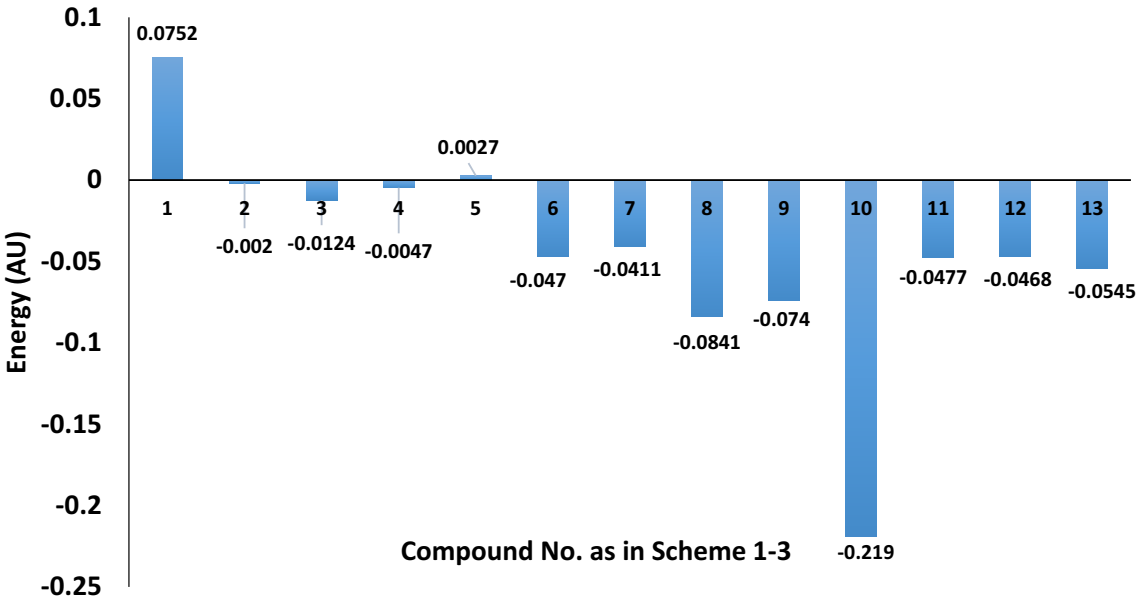


Fig. 4. Understanding the pattern of the energy of the molecules as in Schemes 1, 2, and 3 to understand the feasibility of the synthesis of 13.

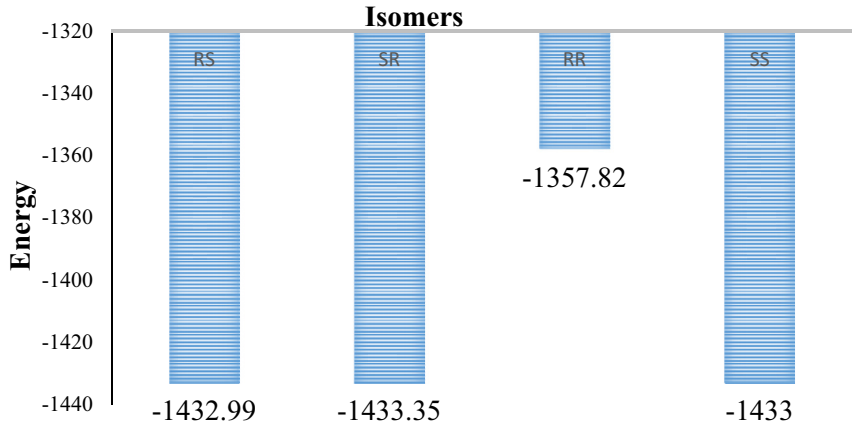


Fig. 5. Showing the relative energy of the isomers.

complex (pl_complex) and evaluates the influence of structural difference on binding ability of ligand to protein. Binding free energies are determined by the given equation [32, 33].

$$\Delta G_{bind} = \Delta G_{pl\_complex-wt} - \Delta G_{p-wt} - \Delta G_{l-wt} - \Delta G_{gas}$$

where ΔG_{bind} is binding free energy between ligands and protein; $\Delta G_{pl\_complex-wt}$, ΔG_{p-wt} and ΔG_{l-wt} represent the change in free energies of pl_complex, protein and ligand in water while ΔG_{gas} describe a change of free energy induced by binding of ligand to protein in gas phase. AMBER18 suit was used to perform the MM-GBSA analysis [30].

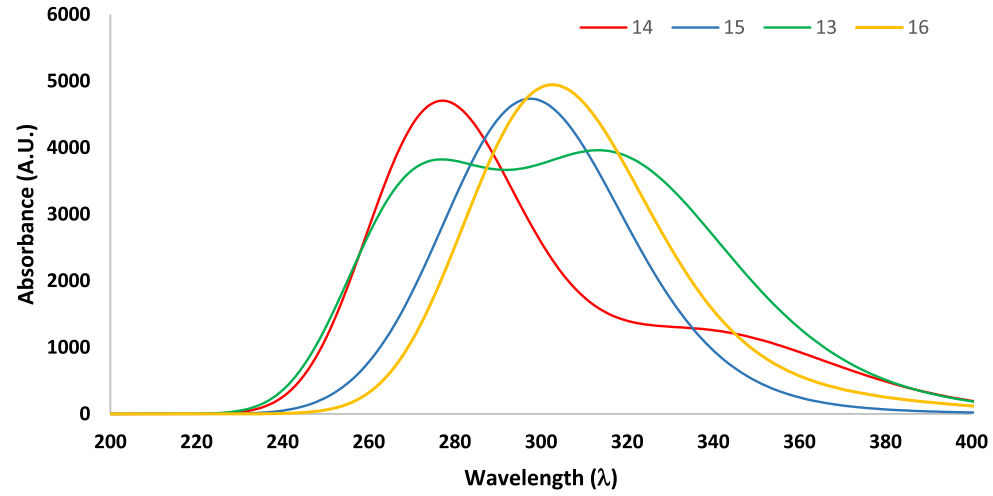
3. Result & discussion

3.1. Design of scheme for the synthesis of noscapine

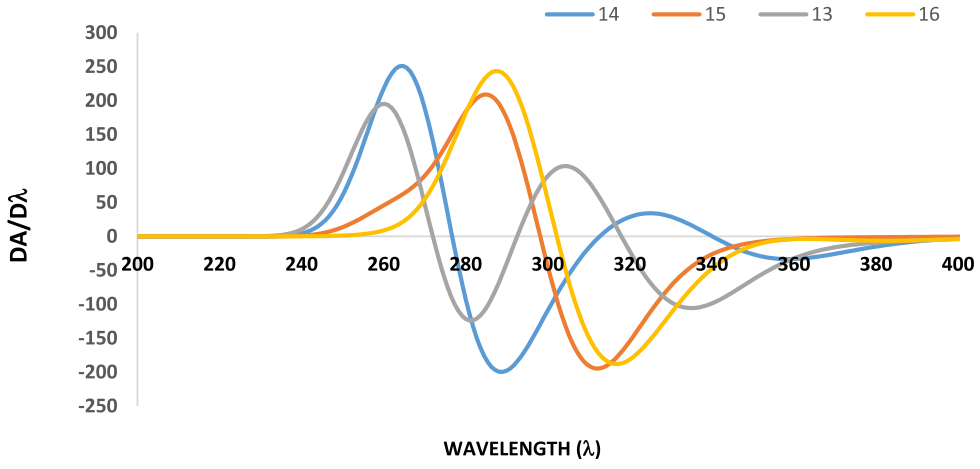
Schemes 1, 2, and 3 for the synthesis of noscapine have been taken from the literature [13, 14, 15]. The molecule was chosen because of its potential in different area of medical science [34]. As a chemist, the biological potency of this molecule may be due to the functional group, parent moiety stereoisomer **13** or presence of chiral carbons. Noscapine has two chiral carbons, therefore, it has of four isomers, **13–16**. Literature discussed the biological potency of one of the isomer of noscapine, **15** against the cancer. In the present work, all four isomers have been taken and studied their potential against nsP3 protease of CHIKV. Till date, no one has studied the mechanism for the synthesis of noscapine and all its isomers using computational tools.

3.2. Study the mechanism for synthesis of noscapine through DFT

In modern days, computational techniques were frequently applied to solve the various problems in drug designing. Such a computational technique is the density function theory based on the quantum mechanical approach to do the same [35, 36]. The mechanism of synthesis of noscapine is studied through DFT. The energies of reactants, intermediates and product were calculated through DFT. The energies are given in Fig. 3 for the graphical correlations of the molecules **1–13** as in Schemes 1, 2, and 3. The energy of **1** is positive, shows the highly reactive nature. **CMPD1** on reduction converted to alcohol **2** seems to be quite stable. **2** further converted to the chloro-derivatives **3** by the SN^2 mechanism. Further, the substitution of chlorine by cyanide ion gave **CMPD4**. Then, **4** undergo for reduction of cyanide into the amino group to give **5**. The Phthalic acid derivative **6** undergo dehydration followed by the oxidation to form **7**. The acid group of **7** gets converted into acyl chloride **8** by reacting $POCl_3$. Then **5** and **8** serve as the starting material



(i)



(ii)

Fig. 6. (i) UV-Visible spectra and (ii) 1st derivative UV-Visible spectra of compounds 13-16.

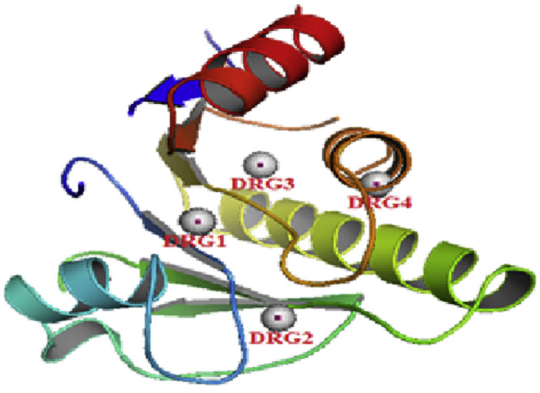


Fig. 7. Number of binding sites in the ns3 protease of CHIKV.

Table 1
Docking energy score of the four sites for the isomers of noscapine, **13-16**.

S. No.	Isomers	Drug sites	Binding Affinity
1.	14	1	-8.94
2.	14	2	-8.90
3.	14	3	-8.93
4.	14	4	-8.24
5.	15	1	-9.27
6.	15	2	-9.02
7.	15	3	-8.02
8.	15	4	-7.66
9.	13	1	-8.91
10.	13	2	-9.57
11.	13	3	-8.73
12.	13	4	-9.64
13.	16	1	-8.09
14.	16	2	-8.64
15.	16	3	-9.15
16.	16	4	-8.79

for the synthesis of Noscapine. **5** and **8** undergo elimination reaction by releasing one molecule of HCl. The energy of **5** and **8** are 0.0027 and -0.0841 AU respectively while the energy of **9** is -0.074 AU. It indicates the feasibility of formation of intermediate **9**. CMPD **9** undergo cyclization in presence of $POCl_3$ in toluene indicate the most stability as chelation increases stability which is also supported by the huge decrease in energy having value -0.219 AU. **10** converted to **11** by the reduction and the energy of **11** is -0.0477 AU. Further, the asymmetric methylation

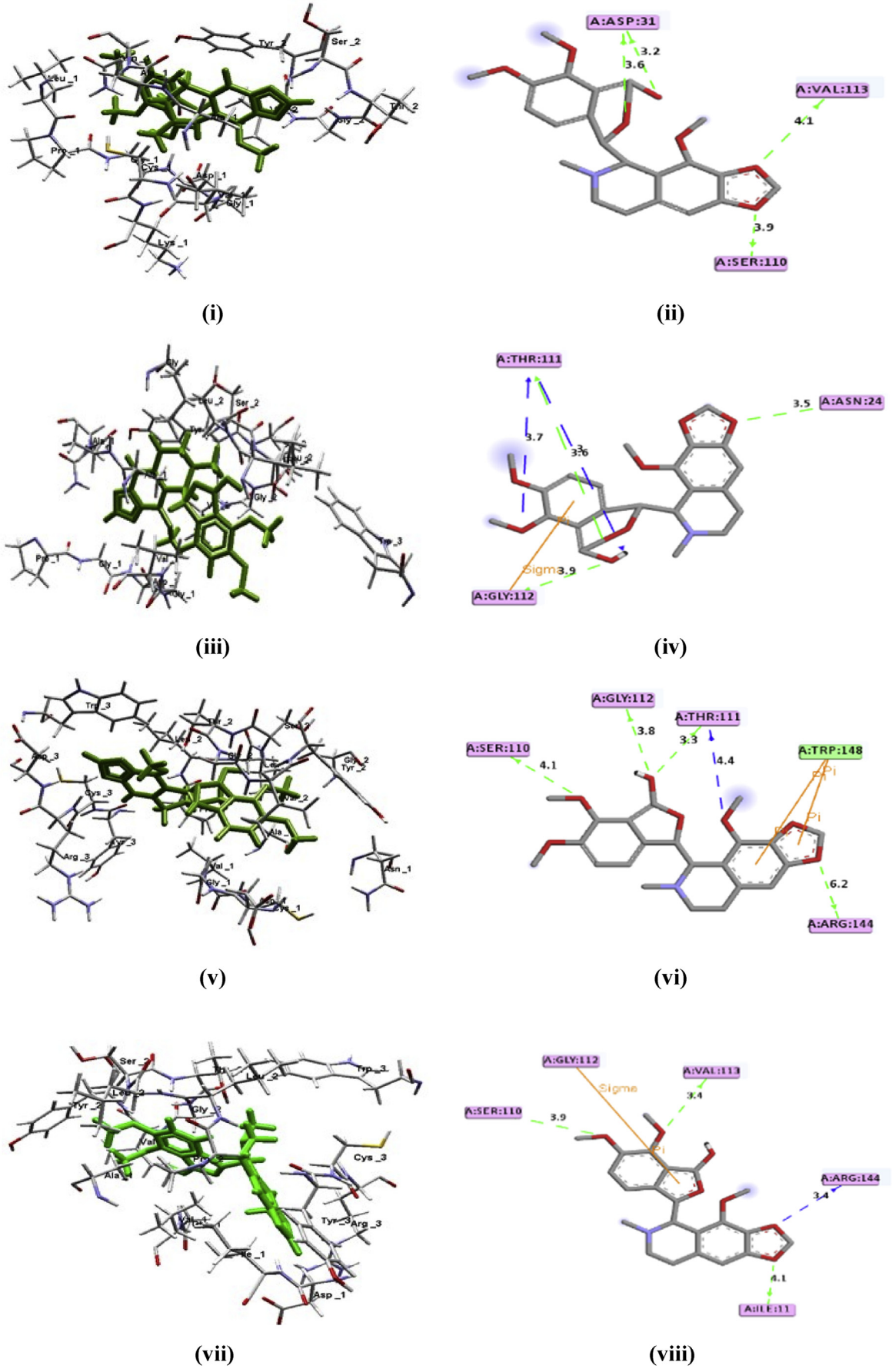


Fig. 8. (i), (iii), (v), (vii) for the 3D-poses and (ii), (iv), (vi), (viii) for 2D-poses for the interaction of 13–16 with the amino acids of nsP3 protease of CHIKV.

Table 2
Interaction of 13–16 with different amino-acids of nsP3 protease of CHIKV.

CMPD	Π-σ Interactions		Π- Π Interactions		H-Bond Interactions	
	Amino acid	Distance (Å)	Amino acid	Distance (Å)	Amino acid	Distance (Å)
13	-	-	-	-	ASP-31 VAL-113 SER-110	3.2, 3.6 4.1 3.9
14	GLY-112	2.73	-	-	THR-111 GLY-112 ASN-24	3.7, 3.6, 3.0 3.9 3.5
15	-	-	TRP-148	5.20, 4.27	SER-110 GLY-112 THR-111 ARG-144	4.1 3.8 3.3, 4.4 6.2
16	GLY-112	2.80	-	-	SER-110 VAL-113 ARG-144 ILE-11	3.9 3.4 3.4 4.1

Table 3
ADME properties of all the isomers of noscapine (13–16).

Physiochemical descriptors	Isomers			
	13	14	15	16
Log S	-2.61	-4.14	-4.14	-4.14
Heavy atoms	30	30	30	30
MW (g/mol)	413.42	413.42	413.42	413.42
No. of rotational bonds	4	4	4	4
No. H-bond accepto15	8	8	8	8
Num. H-bond donors	0	0	0	0
Log Po/w (iLOGP)	0	3.56	3.29	3.53
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Log K_p in cm/s	-8.29	-6.90	-6.90	-6.90
tPSA($Å^2$)	58.62	75.69	75.69	75.69
Bioavailability Score	0.55	0.55	0.55	0.55
Synthetic accessibility	5.36	4.31	4.31	4.31

of **11** gave **12** having energy -0.0468 AU and it is comparable to **11**. Finally, **12** undergo reduction in presence of dihydrogen and raney nickel to get the desired product **13** having energy -0.0545 AU. From the energy value of **11, 12** and **13**, it is clear that **13** is most stable.

Based on Fig. 4, wherein the energy was plotted against the compound and it clearly shows the feasibility of the synthesis of compound **13**.

3.3. DFT studies of the isomers of noscapine

Herein, the authors tried to study the behavior of isomers of noscapine towards the chemical synthesis on the aspect of theoretical evaluation [37]. Author's proposal was to study the last steps of synthesis in the term of stability of product where actually chirality play a key role. As per expectation, authors found that the synthesis of isomer (R,R) is difficult as its energy is higher than the energy of others. The energies of product is given in Fig. 5. It is clearly evident that R,S, S,R, and S,S have almost similar energy value.

3.4. UV-visible spectra of CMPD 13-16

The UV-Visible spectra of the isomers of noscapine (13–16) were determined using td-DFT calculation. It was found that they give different λmax for the 13–16 and λmax values of the compound 13, 14, 15 and 16 are 323, 311, 271; 340, 276 and 271 nm respectively as in Fig. 6. The oscillator strength for the 13 is 0.0491, 0.0437 and 0.0844; for 14 is 0.0283 and 0.0689, for 15 is 0.107 and for 16 is 0.1031 respectively.

3.5. Docking

Nowadays, computer added drug designing (CADD) plays the key role in development of the new potent drug molecule. CADD uses the different tools to screen a library of molecule within the limited time frame [38, 39]. Herein, authors used the rigid docking to retain the original conformation of the ligand. Before the docking of noscapine, authors were interested to find the all binding pocket in nsP3 protease of CHIKV. Fig. 7 shows the total four available binding pocket. All four noscapines were docked against the all four binding pocket of the nsP3 protease of CHIKV. The results of docking are mentioned in Table 1. The docked view of all the isomers in the most active sites in 2D- and 3D-are given in Fig. 8. The interaction of the isomers of noscapine, **13–16** with nsP3 protease is given in Table 3. Noscapine, **13–16** shows hydrogen bonding, pi-pi and pi-σ interactions with nsP3 protease of CHIKV as in Fig. 8.

Based on the docking score of **13-16** against the all four binding pockets of the nsP3 protease of CHIKV, the isomer **13** shows the best binding affinity at the fourth drug site of nsP3 protease of CHIKV. The binding energy value of the fourth site for **13** is -9.64 kJ/mol (Table 1). The binding energy values for top score for the other three isomers **14**, **15** and **16** are -8.94, -9.27 and -9.15 kJ/mol respectively. The docking score

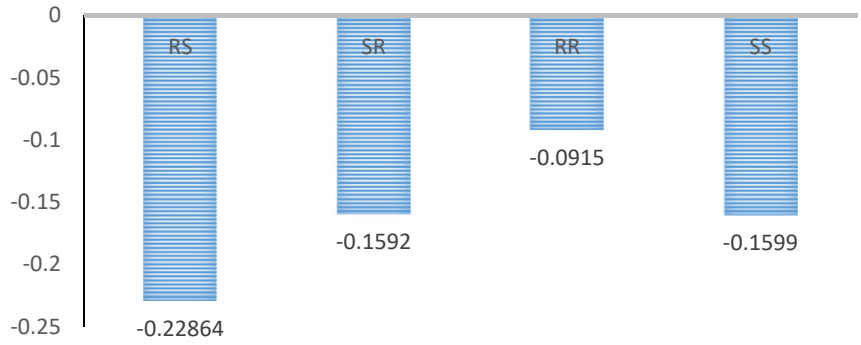


Fig. 9. Plot for the ΔE of isomers of noscapine (13–16).

Table 4
Physio-chemical descriptors of the isomers of noscapine, 13-16.

S. No.	*A*	I	*μ*	*S*	*η*	*X*	*ω*
13	0.05454	0.21375	-0.13415	6.28101	0.07961	0.13415	0.11301
14	0.07800	0.16954	-0.12377	10.92418	0.04577	0.12377	0.16734
15	-0.00093	0.22771	-0.11339	4.37368	0.11432	0.11339	0.05623
16	0.04579	0.20568	-0.12574	6.25429	0.07995	0.12574	0.09887

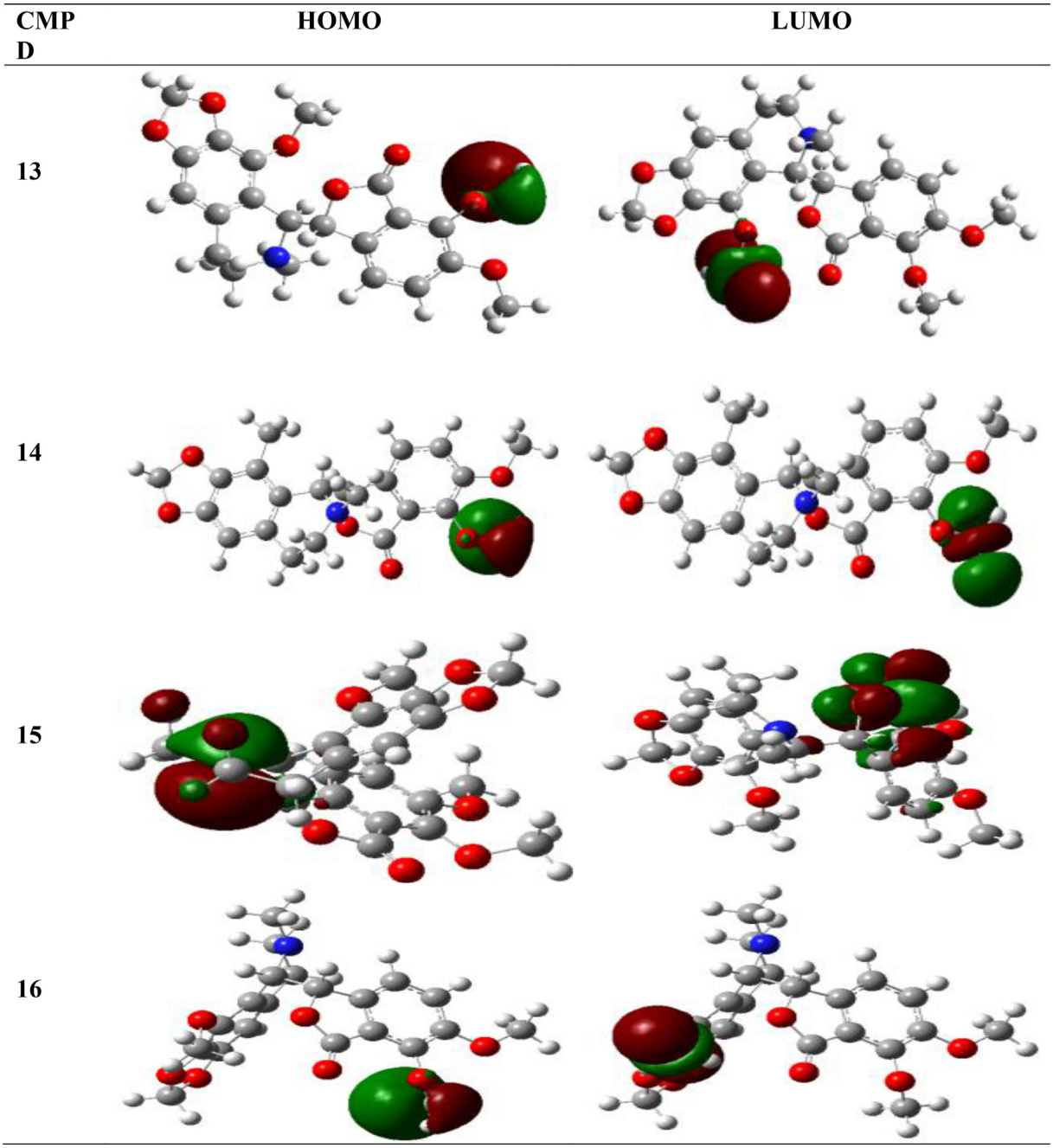


Fig. 10. The E_{HOMO}, E_{LUMO}, E & ΔE of isomers of noscapine (13–16) using DFT.

reveals the potency of **13** over the others. As per the literature, several research groups choose the 1st/2nd binding site. But in this work, it was found that different sites can show stronger interactions. **14** and **15** showed best binding on site 1st while 13 and 16 showed best binding with the site 3rd and site 4th respectively.

The docked pose analysis of the top scorer of isomers, **13–16** are given in Fig. 8. From the dock pose, it is found that **13** form Hydrogen (H) bonding with ASP-31 (3.2, 3.6), VAL-113 (4.1) and SER-110 (3.9). **14** form H-bonding with THR-111 (3.7, 3.6, 3.0), GLY-112 (3.8), THR-111 (3.3, 4.4) and ARG-144 (6.2) (Table 2).

CMPD13 forms H-bonding interactions with SER-110 (3.9), ASP-31 (3.2, 3.6), VAL-113 (4.1). **15** forms H-bonding interactions with SER-

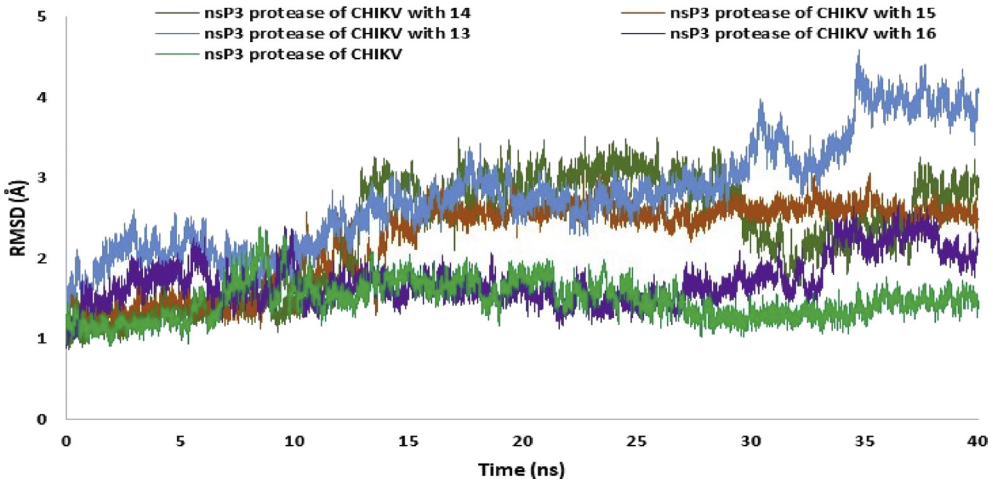


Fig. 11. MD Simulation of nsP3 protease of CHIKV and with 13–16.

110 (4.1), GLY-112 (3.8), THR-111 (3.3, 4.4) and ARG-144 (6.2). **16** forms H-bonding interactions with SER-110 (3.9), VAL-113 (3.4), ARG-144 (3.4) and ILE-11 (4.1). Beside this **14** and **16** forms Π-σ interactions with GLY-112 (2.73) and GLY-112 (2.80) respectively, while only **15** forms the Π- Π Interactions with TRP-148 (5.20, 4.27).

3.6. ADME properties

The bioavailability of the molecule is most important. The biological properties have been calculated and discussed on the basis of TPSA, chemical structure, milogP (partition coefficient) and Lipinski's "Rule of Five" states that most of the molecules with good membrane permeability will have LogP ≤5, molecular weight ≤500, the number of hydrogen bond acceptors ≤10, and the number of hydrogen bond donors ≤5 [40, 41, 42]. All the parameters 13–16 were reported in Table 3. From the data, it is understood that except Log P value and synthetic accessibility all the remaining data is same. It means on changing the absolute configuration on the chiral center, a change in the hydrophobicity is found/observed. It is also found that 13–16 follow Lipinski's rule with no isolation.

3.7. DFT study of the isomers of noscapine

On changing absolute configuration on the chiral carbon, the chemical properties changes mainly due to the ΔE value. ΔE comes from the difference of energy of LUMO and HOMO [43]. Further, the chemical

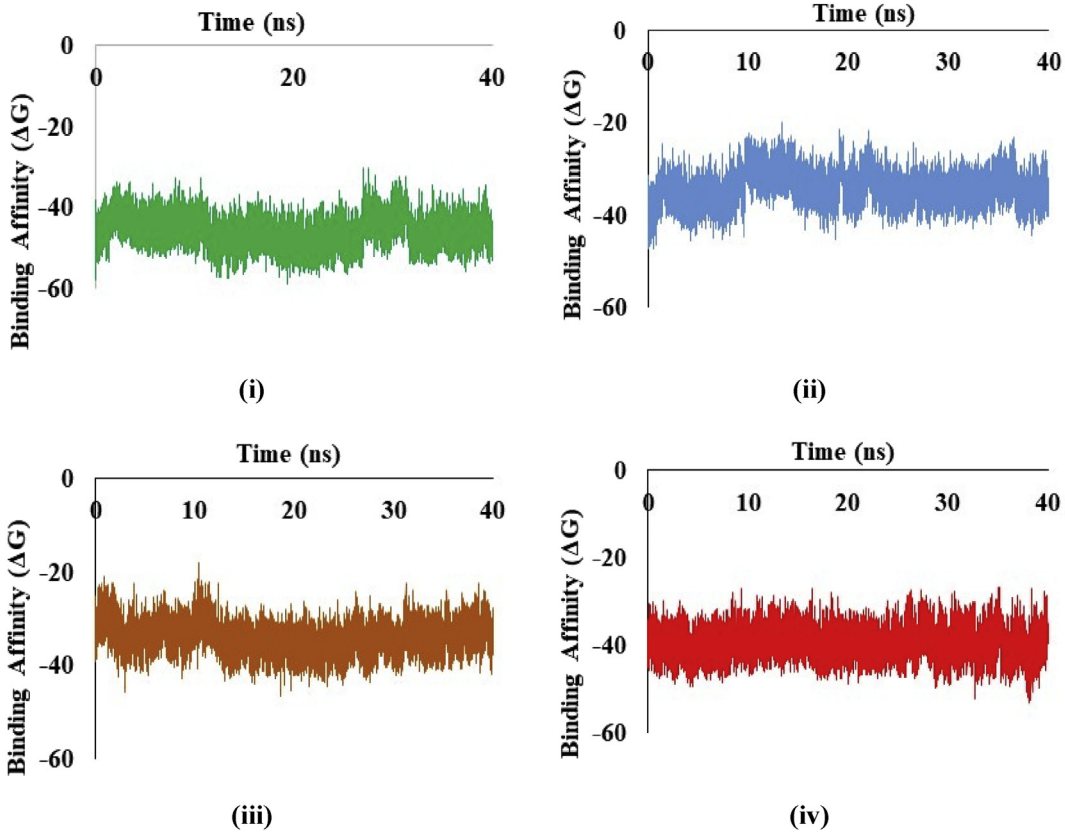


Fig. 12. (i)-(iv) Plot for the binding energy between the nsP3 protease of CHIKV with 13–16 respectively.

Table 5
Calculated binding free energies drug-target complex, target, drug and differences of drug-target complex in kcal/mol of the 13-16.

Energy Component	Differences of 14		Differences of 15		Differences of 13		Differences of 16	
	Average	Std. Err. of Mean	Average	Std. Err. of Mean	Average	Std. Err. of Mean	Average	Std. Err. of Mean
BOND	0.00	0.00	0.00	0.00	-0.00	0.00	0.00	0.00
ANGLE	0.00	0.00	-0.00	0.00	-0.00	0.00	0.00	0.00
DIHED	-0.00	0.00	-0.00	0.00	0.00	0.00	-0.00	0.00
VDWAALS	-49.36	0.02	-48.53	0.02	-58.93	0.02	-54.34	0.015
EEL	-12.21	0.02	-17.92	0.03	-22.66	0.03	-8.19	0.05
1-4 VDW	0.00	0.00	-0.00	0.00	-0.00	0.00	0.00	0.00
1-4 EEL	-0.00	0.00	0.00	0.00	-0.00	0.00	-0.00	0.00
EGB	32.05	0.02	37.11	0.03	41.22	0.02	28.44	0.04
ESURF	-4.95	0.001	-4.89	0.001	-5.81	0.001	-5.76	0.001
Ggas/ΔG_{gas}	-61.57	0.03	-66.44	0.04	-81.59	0.03	-62.54	0.05
Gsolv/ΔG_{solv}	27.10	0.02	32.22	0.03	35.41	0.02	22.68	0.04
TOTAL/ΔG_{total}	-34.47	0.02	-34.22	0.02	-46.18	0.01	-39.85	0.02

potential, affinity, softness have been studied which comes from the E_{HOMO} and $E_{LUMO.}$ DFT method was used for the calculations of HOMO-LUMO gap (ΔE), E_{HOMO}, E_{LUMO} and total energy (E) [44]. The HOMO-LUMO energy gap are quantum mechanical descriptors which play a major role in chemical interactions. The energy gaps between HOMO and LUMO helps to characterize the chemical reactivity and kinetic stability of molecules [45]. If any molecule have small energy gap that is more polarizable and is generally associated with a high chemical reactivity, low kinetic stability and is also termed as soft molecule. The HOMO orbital is primarily acts as an electron donor and the LUMO orbitals is largely acts as the electron acceptor [46]. The energy of all the isomers calculated from the DFT calculation are found similar except **14**. But the value of ΔE of all the isomers differs significantly and **15** has lowest change in energy while the **14** has the highest ΔE value as in Fig. 9. Plot for energy and change in energy explained the significance of absolute configuration. Further, ionization, affinity, softness and other parameters were calculated as in Table 5.

The physiochemical descriptors for **13, 14, 15** and **16** were calculated from the DFT result. The values for chemical potential (μ), global softness (S), global hardness (η), electronegativity (χ), and electrophilicity index (ω) are given in Table 4. Hardness defines the polarizability of the compound or the disturbance of electronic cloud in an electric field. Hardness describes the resistance towards the mechanical deformation and the softness is the reciprocal of the hardness [47]. Chemical potential is defined as the escaping tendency of the electrons from a system. Literature reported that the electron flow from high chemical potential to the low chemical potential [48]. Electronegativity may be defined as to attract the electron by the molecule. The electronegativity of the molecule may be defined as the drop in energy of molecule when some amount of electronic charge is added to the system [49]. Electrophilicity index is defined as the ability of molecule to accept electron. It also measures the decrease in energy of the molecule due to transfer of electron from donor to acceptor [50].

The electronic distribution of frontier molecular orbitals were also performed to understand the effect of configuration, whether it changes or not. It is very interesting that only in case of 15, HOMO is centered on nitrogen of isoquinoline ring, while for the rest of the isomers it centered on the methoxy part of the benzofuran ring. The pattern of LUMO is observed different for 13–16. For 13, it is centered on the methoxy group of isoquinoline part, for 14 it is centered on methoxy group of benzofuran ring, for 15 it is centered on core benzofuran ring and for 16 it is centered on core of isoquinoline ring. The frontier orbital pictures are given in Fig. 10.

3.8. MD simulation

MD simulations analysis of macromolecular system is useful to study the interaction between ligand and receptor [51]. The inhibition of protein, folding unfolding and stability can be explain very well with the help of MD simulations. Root mean square deviation (RMSD) measures the deviation of mean atomic position by square rooting it. RMSD of backbone of protein play key role to provide the inhibition/stability [52]. MD simulation analysis of 13–16 with nsP3 protease of CHIKV was performed. MD simulations of 13, 14, 15 and 16 on binding sites 3, 1, 1, and 4 respectively were performed and given in Fig. 11. The RMSD values was plotted versus the simulation time in nanoseconds (ns) to distinguish whether the simulations had reached to minimum deviations. Based on the results obtained in docking of isomers of noscapine on all four binding sites, MD simulation was performed to study the inhibition of nsP3 protease of CHIKV using 13–16. The value of RMSD for SS isomer is found lowest with little deviation while **13** isomers showed maximum deviation having higher RMSD value than that of **16**.

3.9. Binding energy for the complex in nsP3 protease of CHIKV with 13–16 through MM-GBSA calculations

Generalized born surface area methods is more prominent and accurate compare to the docking algorithms. MM-GBSA analysis of the all MD output was performed to get the precise values of solvation, binding energy, etc. [53] Binding energy analysis for the inhibition of nsP3 protease of CHIKV on selected sites using 13–16 is given in Fig. 12. For all isomers, the binding energy values is negative. These negative values corroborate the docking result. Although the binding energy is recorded for the 40 ns at the interval of 2 fs and an average value is given in Table 5. The average binding energy for 13–16 with nsP3 protease of CHIV are -46.18, -34.47, -34.22 and -39.85 kcal/mol respectively.

Results shows an interesting thing that **13** showed the best binding with nsP3 protease of CHIKV although longer deviation were seen in the RMSD pattern. Even, **16** isomer also showed good binding energy indicates that **13** and **16** have the strong ability to inhibit the nsP3 protease of CHIKV.

4. Conclusion

In this work, the mechanism for the synthesis of noscapine was studied using DFT. Then, *in silico* biological activity was performed to check the biological potency of noscapine isomers against the nsP3 protease of CHIKV. Docking results indicate the supremacy of **13** over the other isomers. Physiochemical descriptors from DFT result shows the acceptable result in the context of flow. Further. MD simulations were performed for the nsP3 protease of CHIKV with and without isomers of noscapine, 13–16 on selected sites based by docking. MD result reveals more deviation in case of 13 but for 16, it is in the acceptable range. Further, the binding was calculated using MM-GBSA to study the potency of **13** and **16** for the inhibition of nsP3 protease of CHIKV. MM-GBSA result again shows the highest value of binding energy for the 13.

Declarations

Author contribution statement

Prashant Singh, Kamlesh Kumari: Conceived and designed the experiments; Wrote the paper.

Durgesh Kumar, Vijay Kumar Vishvakarma, Parul Yadav: Performed the experiments.

Abhilash Jayarak: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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