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ORIGINAL PAPER

In silico identification of viper phospholipaseA2 inhibitors: validation by in vitro, in vivo studies

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Abstract Snake venom, particularly of vipers from the Indian subcontinent, contains Phospholipase A2 (PLA2) as one its constituents which is widely implicated in hemorrhagic, cardiac arrest and death. Development of inhibitors of the protein can facilitate the weakening or annihilation of the venom toxicity and save many human lives. In the present communication, our studies relate to the design and development of structure-based ligands as inhibitors of PLA2 of Viper venom. The study involves the computational approach towards evaluating a library of molecules comprising of natural products, and synthetic molecules through docking studies on the venom protein PDB ID: 1OXL (a dimer, available in the literature). In silico experiments have resulted in the identification of several of them as PLA2 inhibitors. The inhibitory effect of PLA2 by these compounds is attributed to a great extent to their interaction with the residues Phe 46 and Val47 of chain B of the target protein and hence these two residues are identified as the key contributor for the said activity. In order to validate the in silico findings, a selected panel of compounds have been tested by in vitro and in vivo experiments against the venom, which has led to the observance of significant corroboration between the wet lab and in silico findings, validating thereby the in silico approach used in the present study.

Keywords Binding affinity · Docking · In silico · Phospholipase A2 · Snake venom

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Introduction

Snakebites claim more than a hundred thousand lives a year the world over, and the mainstay remedy is serotherapy. The preparation of the anti-sera is not only time consuming, expensive, fraught with storage problems (cold chain), hypersensitive reactions, it is also not abundantly available (especially in the rural areas) in the Asian subcontinent in general, and more so in India where 40% of the total world's mortality is reported [1, 2]. Therefore, development of an effective antidote that is readily available, elicits a minimum of hypersensitive reactions and avoids cold storage problems is much required. Inhibition of venom PLA2s, (its vital components) is of pharmacological and therapeutic interest because of their involvement for a wide variety of inflammatory /pharmacological disorders including neurotoxicity, hemolytic, hemorrhagic, coagulant/anticoagulant and platelet effects [3, 4].

Several investigators [5, 6] have reported extracts or pure natural products isolated from various plant sources as possible anti-venom agents [7, 8]. Studies have also been carried out to understand their catalytic action and various ligands as inhibitors of the enzymatic action have been developed [9, 10]. Besides this, several other investigators have reviewed the isolation of natural as well as synthetically prepared chemicals as PLA2 inhibitors of snakes and mammals [11–14].

One of the strategies for the identification and development of effective snake venom antidotes is the exploration of scores of chemical compounds bearing different functionalities and their testing by in vitro/in vivo methods. However, this approach is time- consuming, expensive and above all would require a large number of animals for proof of efficacy. The other rational approach is the use of computer techniques, e.g., molecular modeling which would involve virtual selection through systematic study of ligand-receptor interactions, creation of in silico libraries of compounds, and identification of the best hit/s. Moreover, Molecular modeling techniques are widely used in the chemical, pharmaceutical and agrochemical industries for the success stories associated with them, which include development of cyclooxegenase-2 inhibitor [15] (COX-2 inhibitor), K^+ channel blocker [16], acetyl cholinesterase inhibitor [17] (AchE inhibitor), imatinib moving target [18], fullerene derivatives as HIV-1 PR inhibitors [19], and TbGalE inhibitors [20].

In our earlier studies, one of us [21–24] has demonstrated venom neutralization property of a plant isolate and the extract. Phospholipase A2 (PLA2) forms one of the major components of snake venoms in general and displays a range of adverse biological activities [25]. Therefore, the development of inhibitors of phospholipaseA2 is an area of importance. Working in this direction, we have directed our efforts for the exploration and identification of PLA2 inhibitors through computational approach and wet lab validation of the in silico identified inhibitors by in vitro and in vivo experiments. The protein target for the in silico studies involved PLA2 from Viper russelli co-crystallized with 2-carbamoylmethyl- 5-propyl-octahydro-indol-7-yl-acetic acid (PDB ID: 1OXL).

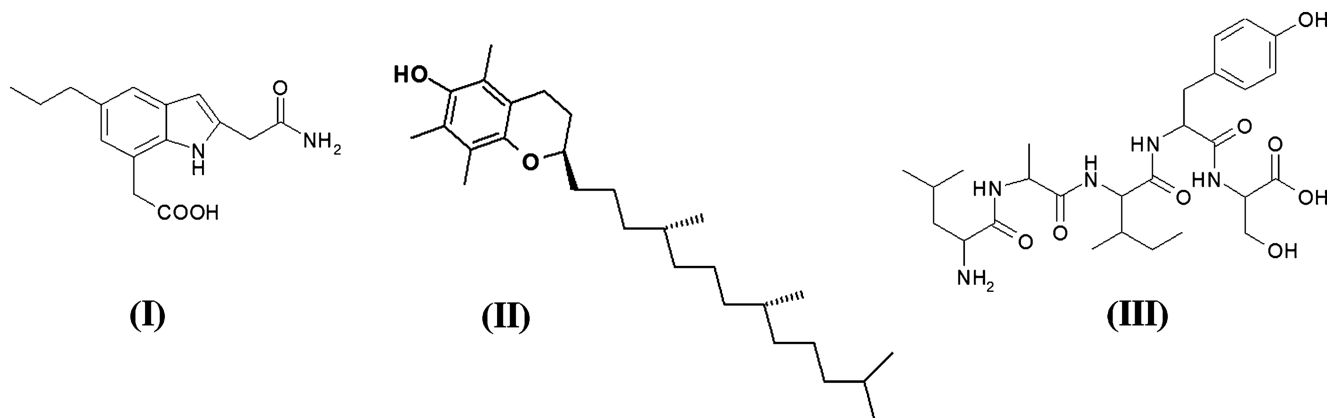
Materials and methods

3D crystal structure of Viper PLA2 co-crystallized with three different inhibitors (PDB IDs 1OXL [26], 1KPM [27] and 1JQ8 [28]) were downloaded from the Protein Data Bank. The three inhibitors namely 2-carbamoylmethyl- 5-propyl-octahydro-indol-7-yl-acetic acid (I), vitamin E (II) and a designed pentapeptide (III) in the protein-ligand complex are shown in Scheme 1.

In silico studies

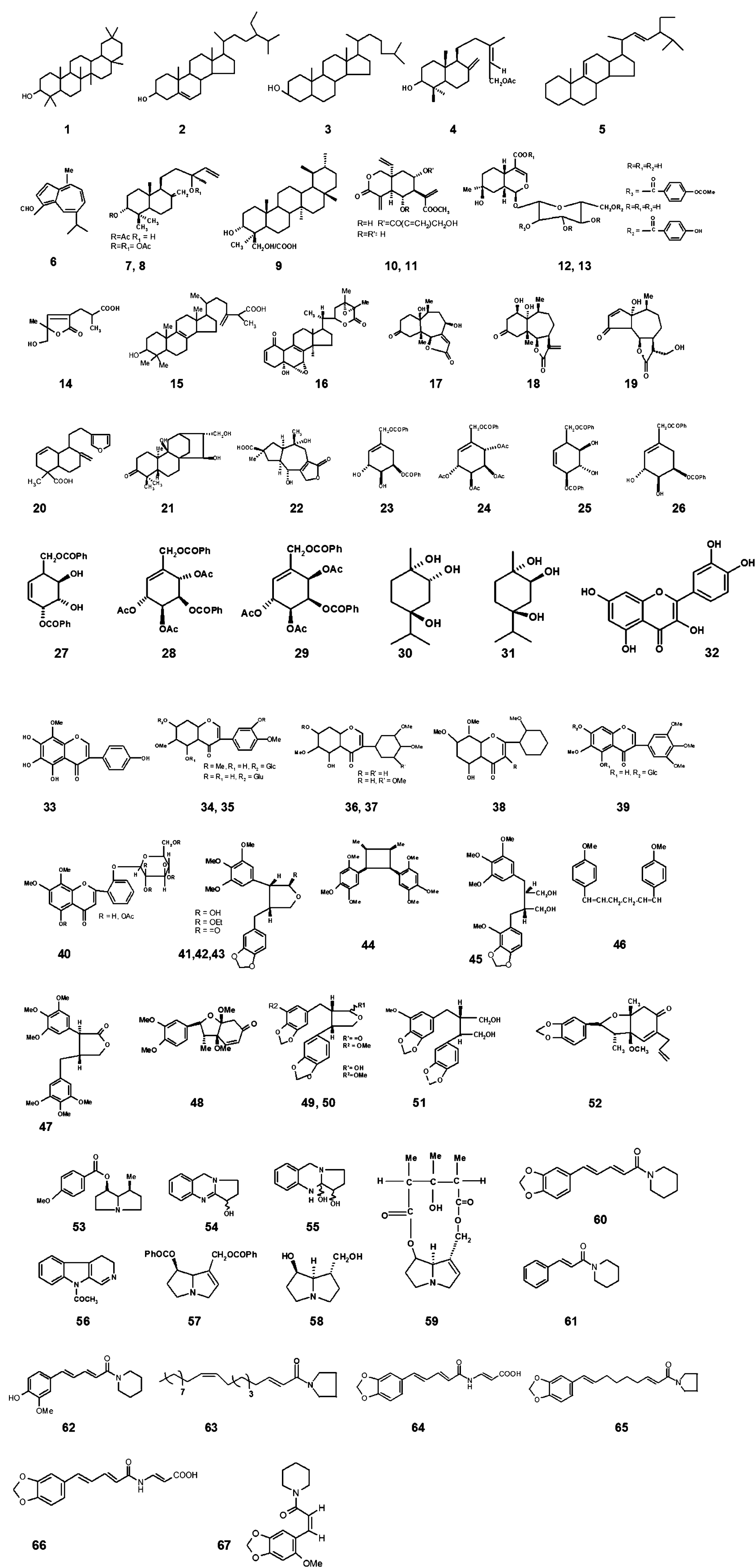
The whole docking operation used in the present study could be stated as follows. Firstly, the downloaded crystal complex structure of PLA2 was opened using the software MOE. Upon checking and correcting the bond orders, bond lengths and missing residues, hydrogen atoms were added to the complex; since the crystal structure did not contain hydrogen atoms. Except for hydrogen atoms, all other atoms were fixed at their crystallographic positions and the complex was minimized (time taken ~ 1 hr) to maintain an optimal distance (based on the chosen force field) between the hydrogen atoms. Secondly, a grid map with 40 × 60 × 40 points and a spacing of 0.375Å was calculated in order to evaluate the binding energies between the inhibitors and the target protein. The non-bonded cut-off was set at 7Å, assuming that atoms beyond this will not have a profound impact on the shape or properties of the docking site. By establishing cutoffs that isolate the pertinent atoms and interactions, the non-interacting parts of the protein (i.e., beyond 7Å) were deleted. Docking score using software MOE was obtained by simulated annealing algorithm [29].

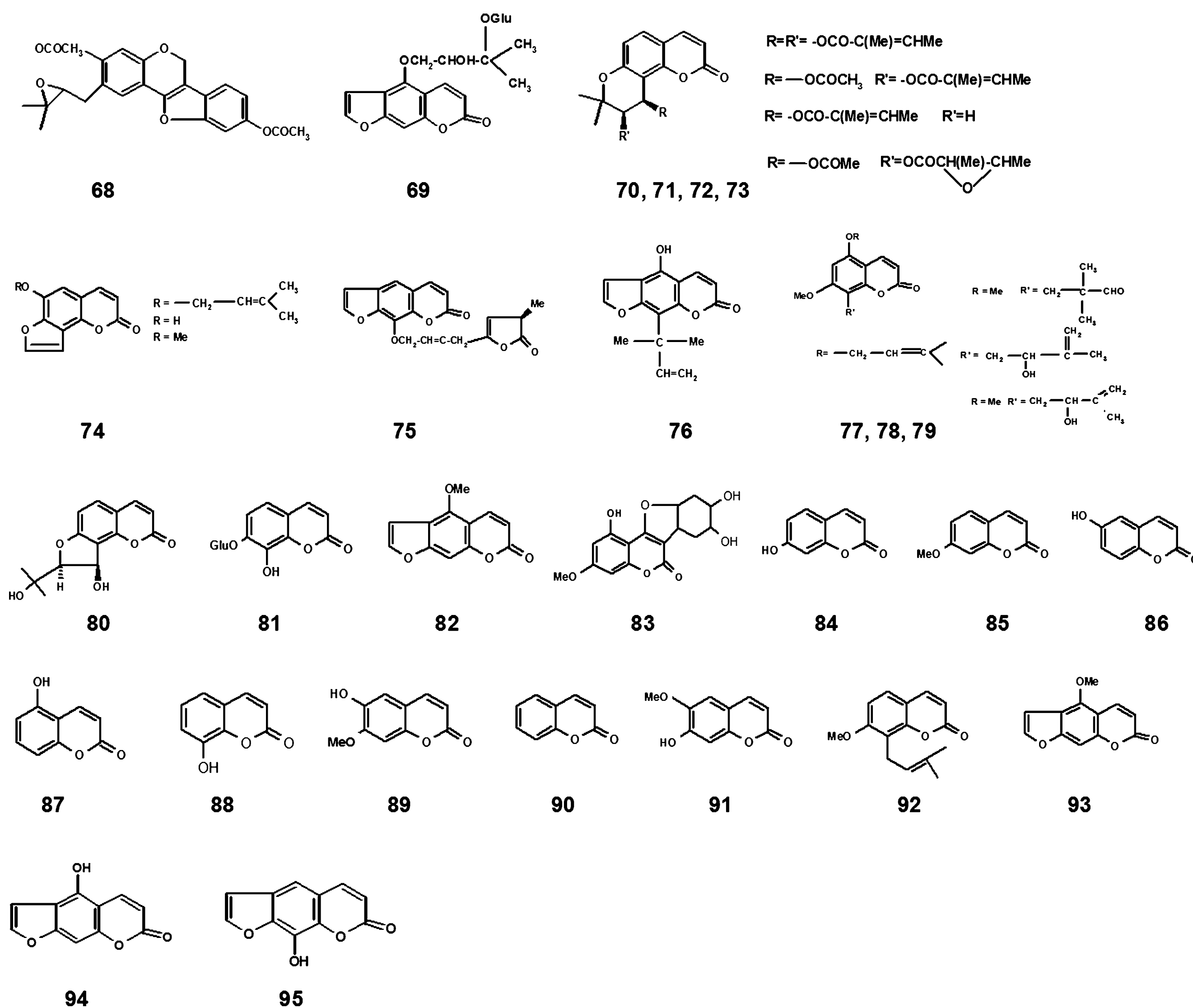
In MOE, docking score is a summation of van der Waals interaction energy, and electrostatic energy (both taking place between the ligand and the protein) as well as that of the solvation energy and the ligand energy in the complex. In order to attain the best binding mode of the ligand (most stable conformation of the docked molecule), each docking experiment consisted of 25 runs and each run in turn of six cycles with the iteration limit set at 8000 and the initial temperature of 1000 K [30]. The conformation with the strongest predicted binding affinity to the target protein



Scheme 1 Reported inhibitors of *viper russelli* venom PLA2

Scheme 2 Compounds 1-67, taken as ligands, comprised of 20 steroids/terpenoids, nine polyhydroxy cyclohexenes, 12 lignans, seven alkaloids and eight amides from IIIM repository





Scheme 3 Compounds 68-95, taken as ligands, included coumarins from institutes' repository as well as from the literature

(based on the lowest docking score) was extracted from the optimized inhibitor-PLA2 complex.

All the calculations were done using the force field MMFF94. The receptor and ligand preparations and docking experiments were performed on SGI Fuel workstation and Wipro Xeon based server.

Compound library creation for docking studies

The ligand database comprised of: (i) natural molecules (from the repository of our institute), (ii) some of the natural products reported to have snake venom neutralization activity [6], (iii) synthetic molecules and (iv) a dataset of in silico created virtual molecules. The compounds were drawn, optimized, and saved in moe format.

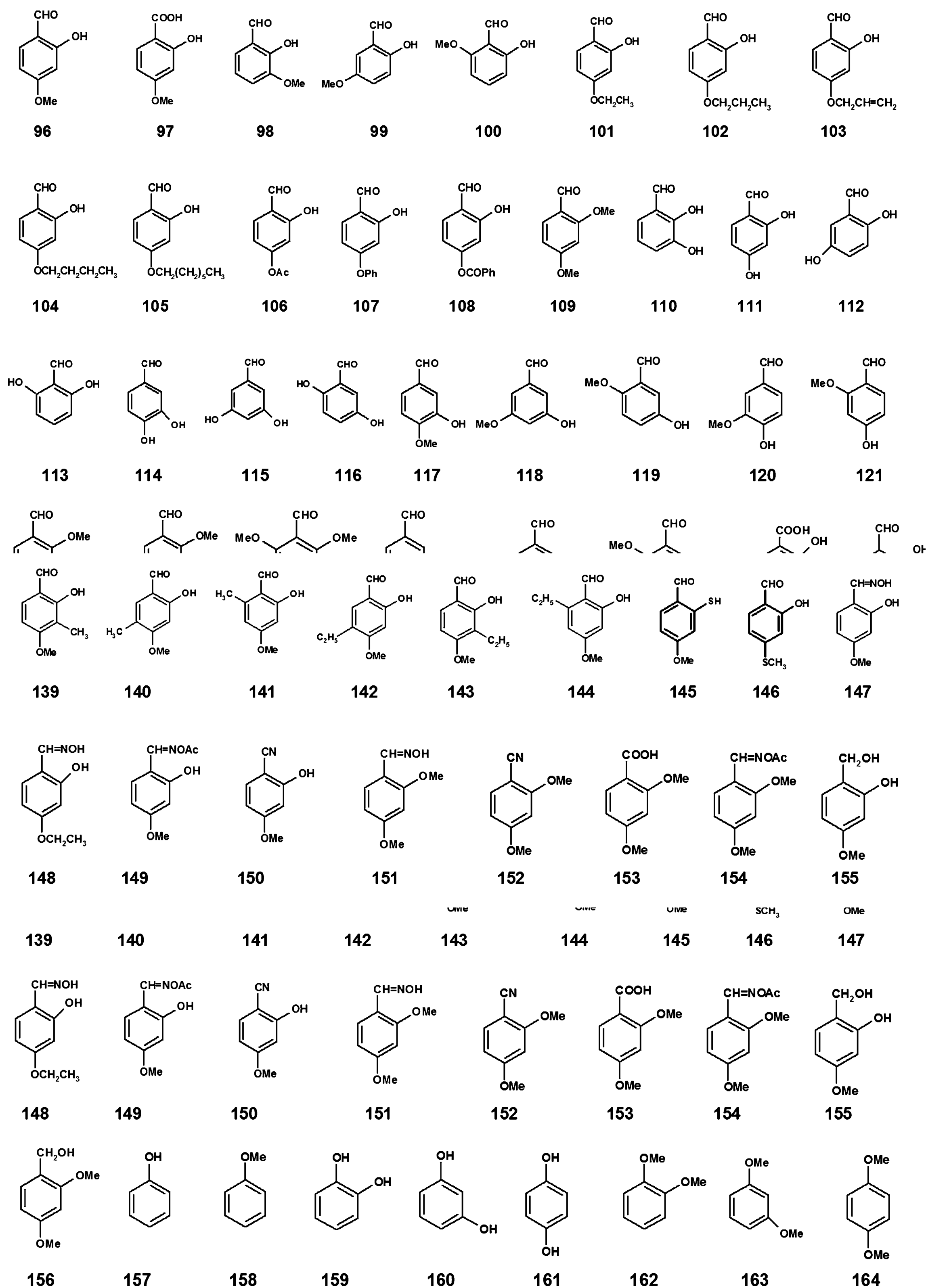
The binding energy (MOE dock score) of the known inhibitor, i.e., indole derivative with PLA2 was taken as a standard for the comparative studies on our comprehensive ligand database.

Based on the estimated binding energy, the ligand molecules were categorized into "better" and "poor" binders. For validation of the results from in silico studies, wet lab experiments were carried out with a few selected compounds from each category (Better and Poor binders).

Compounds 1-67, taken as ligands, comprised of 20 steroids/terpenoids, nine polyhydroxy cyclohexenes, 12 lignans, seven alkaloids and eight amides from IIIM repository (Scheme 2).

Compounds 68-95, taken as ligands, included coumarins from institutes' repository as well as from the literature (Scheme 3).

Compounds **96-166**, taken as ligands, all aromatic in nature, comprised of the few natural phenolic compounds,



Scheme 4 Compounds **96-166**, taken as ligands, all aromatic in nature, comprised of the few natural phenolic compounds, and the rest being synthetic molecules encompassing phenols, phenolic aldehydes/ acids/esters/oximes and nitriles

and the rest being synthetic molecules encompassing phenols, phenolic aldehydes/ acids/esters/oximes and nitriles. The rationale for docking of the phenolic compounds on PLA2 was based on the fact that one of us earlier reported the potent anti-snake venom activity of 2-hydroxy-4-methoxy benzoic acid 97 isolated from *Hemidesmus indica* [24], and secondly we had in our hands natural product **96** i.e., 2-hydroxy-4-methoxy benzaldehyde [31, 32] isolated earlier by us from the rhizomes of *Janakia arylpatra.* The natural product **96** is known to exhibit tyrosinase inhibitory property [31], potent antifeedant activity [our unpublished data], and is also reported for its effect on rat-1/HER cells and NIH3T3-β-platelet derived growth factor cells [33, 34]. In addition to compounds 96 and 97, we took several other benzaldehydic compounds, bearing free phenolic group/s or their alkyloxy derivatives, and also compounds that lacked formyl substituent's but carried hydroxyl, cyano, oxime, thiol, nitro groups as substituent's (Scheme 4).

In vitro studies

For in vitro and in vivo experiments, lyophilized polyvalent snake venom antiserum (as reference serum) was obtained from Bengal Chemicals and Pharmaceuticals Private Ltd. Kolkata, India. Before use, the antiserum was dissolved in 0.9% saline.

Direct neutralization effect of ligand molecules on PLA2 activity of the *Vipera russelli* venom (VRV) was assayed by egg yolk coagulation method [35]. In this in vitro method, the ligand molecules (at 1 and 5 mg concentration) were incubated with fixed amount of venom, i.e., 10 μg and the results obtained were based on triplicate experiments. For PLA2 activity experiments, egg yolk (obtained from

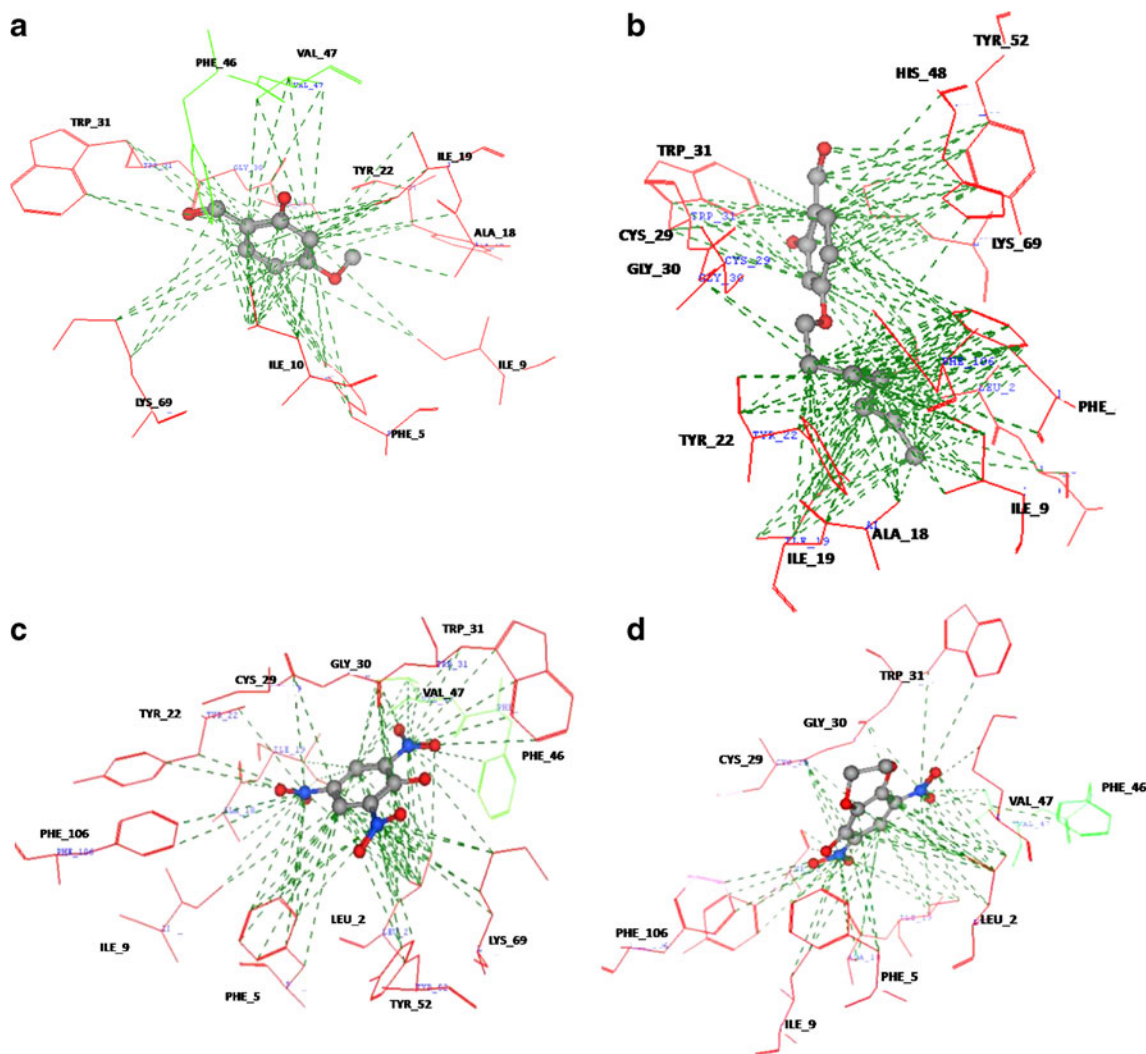


Fig. 1 (**a**–**d**) Interaction of compounds 96, 105, 164 and 165 with the receptor PLA2 (PDB ID 1OXL)

commercially available eggs), EDTA, Tris HCl buffer, sodium chloride and calcium chloride from Sigma except otherwise stated were used. The assay solutions of 0.5% EDTA, 50 mM Tris HCl buffer (pH 7.5), 2% sodium chloride, 1% calcium chloride and normal saline were prepared freshly.

For carrying out the experiment, 2.0 ml of egg yolk suspension and 2.0 ml of test material (venom or venom compound) were mixed in the test tube and incubated at 37 °C for 1 hr. After incubation, the time required for coagulation of egg yolk was recorded by placing the test tube containing test materials on the boiling water bath. A blank was run with normal saline instead of test material. One unit enzyme activity was defined as the amount of venom, which increased the coagulation time of the egg yolk control by one minute. Neutralization of the enzyme activity was estimated on the different amount of compound mixed with the same amount of venom taken as 5 μg/ml in the present study. The venom-compound mixture was incubated at 37 °C for 1 hr. Centrifuged at 2000 rpm for 30 minutes; supernatant was tested in a total of 0.2 ml for the enzyme neutralization activity.

In vivo studies

For in vivo study, male albino mice of body weight about 18-20gms were used after acclimatization to the laboratory condition. The study plan was approved by the Animal Ethics Committee of IIIM (formerly Regional Research Laboratory) Jammu, India. All animals were cared for and handled in accordance with the guideline of the ethical committee.

The natural as well as synthetic compounds were dissolved in 5% DMSO-water and mixed with the venom solution. The pure compound was dissolved in 0.9% normal saline (warm) and mixed with the venom solution. The LD_{50} (intra venous and subcutaneous) of *Daboia russelli* (LD_{50} 2.28 μg and 4.0 μg) was determined along with minimum hemorrhagic activity.

The toxicity of snake venom was assessed [36] by injection of different concentration of venom in 0.2 ml physiological saline into tail vein of male albino mice 18-20 gm. To assess the in vitro antagonism, various doses of venom were mixed with a fixed amount of compound. The mixture incubated at 37 °C for 1 hr, and centrifuged at 2000 rpm for 10 min. The supernatant was injected *intra venous* (*i.v.*) into male albino mice (18-20 gm), six mice per

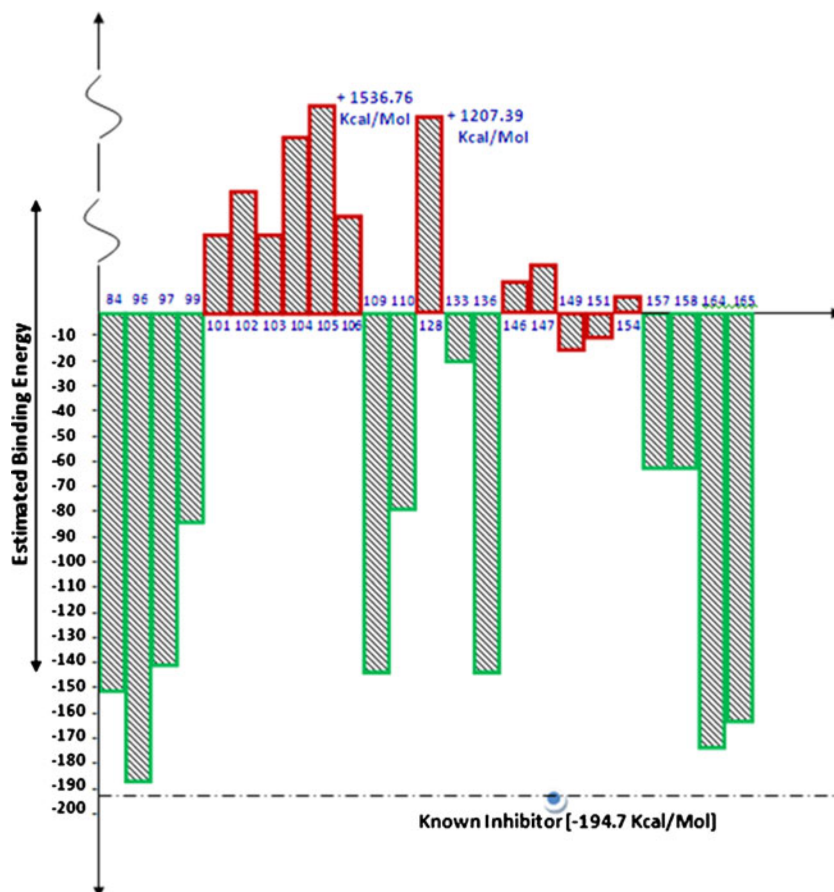


Fig. 2 Graphical representation of estimated binding energies of some of the compounds from the ligand database. (Numbers in blue on x-axis denotes the structure no.)

Table 1 PLA2 neutralizing activity (in vitro) by egg yolk coagulation method

S. No	Treatment group (venom 5 μg/ml) venom	Weight of the compound (mg)	Coagulation time in min.	Fold protection
	Normal saline alone*		39.5	
	Compound No.		0.44	
1	84	1 mg	16.45	2.4
		5 mg	4.76	8.3
2	96	1 mg	10.2	3.8
		5 mg	1.1	35.9
3	97	1 mg	14.7	2.7
		5 mg	4.6	8.6
4	98	1 mg	21.8	1.8
		5 mg	8.5	4.6
5	99	1 mg	23.3	1.7
		5 mg	13.3	2.9
6	101	1 mg	7.3	5.41
		5 mg	1.93	20.5
7	105	1 mg	28.5	1.4
		5 mg	22.2	1.7
8	109	1 mg	17.9	2.2
		5 mg	8.3	4.7
9	119	1 mg	25.3	1.5
		5 mg	14.5	2.7
10	125	1 mg	26.6	1.5
		5 mg	17.2	2.3
11	128	1 mg	27.2	1.4
		5 mg	17.5	2.2
12	133	1 mg	24.5	1.6
		5 mg	14.3	2.7
13	136	1 mg	13.9	2.8
		5 mg	5.3	7.4
14	137	1 mg	14.2	2.8
		5 mg	4.6	8.6
15	138	1 mg	14.7	2.7
		5 mg	5.0	7.9
16	147	1 mg	26.6	1.5
		5 mg	17.2	2.3
17	155	1 mg	29.3	1.3
		5 mg	20.0	1.9
18	158	1 mg	25.2	1.5
		5 mg	16.4	2.4
19	159	1 mg	27.1	1.4
		5 mg	14.8	2.6
20	165	1 mg	9.2	4.3
		5 mg	3.9	10.1
21	166	1 mg	26.3	1.5
		5 mg	15.8	2.5
22	167	1 mg	31.5	1.25
		5 mg	11.2	3.5
23	168	1 mg	35.9	1.1
		5 mg	8.78	4.5

dose. The median lethal dose (LD_{50}) was calculated 24 hrs after injection of the venom-compound mixture. Lethal toxicity was also assessed by *subcutaneous* (*s.c)* injection of various doses of venom. The neutralizing potency of each compound was assessed by *s.c* injection of 1-5LD_{50} of venom at 0 hr.,0.5 hr and 1.0 hr into groups of six mice followed immediately afterward by the various doses of compound (i.v).

The minimum hemorrhagic dose (MHD) of venom (defined as the least amount of venom which when injected *intradermally* (i.d) into mice results in a hemorrhagic lesion of 10 mm diameter 24 hrs later) was measured [36]. Neutralization of the hemorrhagic activity was estimated by mixing a fixed amount of compound with different amounts of venom. The compound-venom mixture was incubated at 37 °C for 1 hr, centrifuged at 2000 rpm×10 min, and 0.1 mL of supernatant injected (*i.d*). The hemorrhagic lesion was estimated after 24 hrs. To assess the antihemorrhagic activity of venom in vivo, various amount of venom were injected (*i.d*) followed immediately by the compound (i.v) and the hemorrhagic lesion measured after 24 hrs.

Results and discussion

For the present study, three different crystal structures of PLA2 (1OXL, 1KPM, 1JQ8) were used. The protein–inhibitor complex of 1OXL was chosen as the standard with no bias towards the other two inhibitors. It was found that the in silico docking conformation of the co-crystallized inhibitor matches exactly (least RMS deviation) with that of the conformation of the inhibitor in the crystal structure, using the MMFF94 force field for docking. A dock score of -194.6 kcal mol^{-1} was recorded for the reported indole inhibitor. Compounds **1-67** showed very high dock energy scores (+14,00000 kcal mol^{-1} to 160 kcal mol^{-1}) which when compared to the dock score of known inhibitor (−194.6 kcal mol^{-1}) indicated their very poor affinity for the selected target and were, therefore, abandoned.

In contrast to compounds **1-67** where very high dock scores were observed for all the compounds, docking results of compounds **68-95** were encouraging and some of the docked molecules (benzopyran-2-ones) such as compounds **84, 86-92**, exhibited dock energies in the range -30 kcal mol^{-1} to −153.23 kcal mol^{-1}. Nature of the substituent in the aromatic moiety of the coumarin and its positional isomers exerted significant influence on the activity, e.g., coumarin **90** devoid of any substituent showed binding energy (BE) of −53 kcal mol^{-1} and introduction of a functional group such as a hydroxyl group (C-7 hydroxyl, compound **84**) resulted in far better affinity as seen from its dock score of −153.31 kcal

Table 2 Properties of snake venoms in male albino mice

Venoms	LD_{50} (iv)/ 20 g mice (μg)	LD_{50} (sc)/ 20 g mice (μg)	Minimum hemorrhagic dose (μg)	Minimum defibrinogenating dose (μg)	Minimum hemorrhagic dose (μg/ml)
Daboia russelli	2.24 (1.4 – 3.4)	40 (26.5 – 60.2)	5	2.5	1

Results are express as mean of six observations. Figures in parentheses are the fiducial limits

ND: not done, LD_{50} : lethal dose 50

mol^{-1}. Presence of hydroxyl group at positions 5, 6, and 8 (compounds **86-88**) showed binding energies in the range −40 to - 80 kcal mol^{-1} far lower than observed for compound **84** showing thereby the influence of positional isomers on the binding affinity of the venom protein. Presence of methoxyl group instead of OH at position 7 (compound **85**) had a significant effect on the binding properties and it showed low affinity and binding energy of +218 kcal mol^{-1} was observed. Compounds **89, 91, 92** with additional substituent's in the coumarin moiety also resulted in low binding affinity, e.g., compound 89 showed −131.01 kcal mol^{-1} while compound 91 displayed binding energy of −30 kcal mol^{-1} . Furano-coumarins **93-95** were also found with high dock scores to indicate the poor binding affinity of ligand to the venom protein [+281 to +366 kcal mol^{-1}].

Molecular docking of compounds **96,** and **97** showed a strong affinity of compound **96** for the protein as could be seen by its binding energy of −187.4 kcal mol^{-1}, close to the known PLA2 inhibitor (binding energy −194.3 kcal mol^{-1}). Compound **97** showed comparatively less affinity and dock score of −141.84 kcal mol^{-1} could be observed. In compound **96**, replacement of C-2 hydroxyl by methoxyl group (compound **109**) also showed lower binding affinity than parent molecule (binding energy of −144.75 kcal mol^{-1}). Likewise, higher homologs like C-4 ethyloxy (compound101), butyloxy (**104**), heptyloxy (**105**) or C-4 acyl or benzoate derivatives (**106**, **108**) showed very poor binding affinity and steep increase in the binding energies was observed (e.g., **101** BE +138.42 kcal mol^{-1}, **105** BE +1536.76 kcal mol^{-1}, **106** BE +254.8 kcal mol^{-1}, **108** +562.45 kcal mol^{-1}). Incorporation of NO_2 substituent in compound **96** at C-3,C-5 and C-6 position (compounds **136, 137** and **138**) showed lower binding affinity and BE energy observed in the range from −120 to −143 kcal mol^{-1}. Changes in compound **96** by displacement of methoxyl from C-4 to C-3,C-5 (compounds **99**–**100**) or displacement of C-2 OH to other positions (C-3, C-5, C-6) in association with a free OH or OMe groups (compounds **110**–**121**) as well as C-2 methyl ether (compounds **122**–**127**) showed far less binding affinity than **96**. These results apparently showed the influence of positional isomers on the inhibitory activity, an effect that was also observed for coumarins **84**–**88** as described earlier.

Replacement of C-2 OH in compound **96** by thiol group (compound **145**, BE +205.78 kcal mol^{-1})) or C- 4 methoxyl by SCH_3 group (compound **146**, BE +213.46 kcal mol^{-1}) also resulted in low binding affinity. When benzaldehydic compounds bearing mono substituent in the form of OH or OMe group at C-2 to C-5 position (compounds **129**–**134**), were docked on to the protein, the inhibitory effect in terms of binding affinity was found significantly low and binding energies in the range +1207 to -90.57 were recorded. Docking of mono- and dihydroxybenzenes (compounds **157**–**164**) was done to explore their inhibitory property and all the compounds showed comparatively better binding affinity than compounds **129**–**134** and binding energies observed in the range of −27 to −80 kcal mol^{-1}. When phenolic compounds **165** and **166** bearing three and two nitro groups respectively were docked, the molecules exhibited very high binding affinity and binding energy of −160.12 and −147.87 kcal mol^{-1} was recorded.

From the whole lot of natural products screened, compound **96** proved to be the best hit followed by compounds **84** and **97**. Compounds **109**, **136**, **137** and **155** prepared by derivatisation of phenolic compound **96**

Table 3 *Vipera russeellii* venom induced lethal and hemorrhagic action neutralizations by the compounds in male albino mice in vitro

Compounds	μg	*Daboia russelli* μg (LD_{50})	*Daboia russelli* μg (MHD)
96	100	22(9.8)	20(4)
134	100	12(5.8)	20(4)
147	100	NP	NP

Venom -compound incubated 37^0 C/ 60 min, injected i.v (lethal) and i.d (hemorrhage) activity

The values in parentheses are the number of LD_{50} / MHD. Results are expressed as mean of ten observations

NP=No Protection; LD_{50}=Lethal dose 50=2.28 μg of *Vipera russelli* venom; MHD=Minimum hemorrhagic dose=5 μg

Table 4 *Vipera russeellii* venom induced lethal and hemorrhagic action neutralizations by the compounds in male albino mice in vivo

	μg	*Daboia russelli* μg (LD_{50})	*Daboia russelli* μg (MHD)
96	100	150(3.75)	20(4)
134	100	100(2.5)	5(1)
147	100	NP	NP

Venom injected subcutaneous s.c (lethal) and intradermal i.d (hemorrhage) followed immediately by the compound intra venous

The values in parentheses are the number of LD_{50} / MHD. Results are expressed as mean of ten observations. NP=No protection; LD_{50}=Lethal dose 50=2.28 μg of *Vipera russelli* venom; MHD=Minimum hemorrhagic dose=5 μg

(natural), and synthetically prepared compounds 164 and 165 showed best binding affinity for the protein. In docking study, the amino acids within the active site of PLA2 involved the interaction of the ligand with amino acid residues Leu2, Phe5, Ile9, Ala18, Ile19, Tyr22, Cys29, Gly30, Trp31, Lys 69 of chain 1 and residues Phe46 and Val47 of the chain 2 of the PLA2 dimer. The molecular interactions of the some good and poor protein binders are shown in Fig. 1. It was interesting to note that wherever the residues Phe46 and Val47 of chain two of the protein were involved in the interaction with the ligand, the binding affinity was found to be good and this general pattern was observed for all the compounds identified as good binders. The interaction of these two residues was found absent for molecules that exhibited high dock energy scores, i.e., poor binding affinity. This observance led to the identification of Phe46 and Val47 as pivotal residues for the binding affinity for ligands to be PLA2 inhibitors.

Based on the docking results, the database of ligand compounds were segregated into two categories: (i) Better binders, and (ii) poor binders. A graphical representation of some of the molecules (including the best hit and also the best known inhibitor) is shown in Fig. 2. In vitro and in vivo studies were carried out to correlate the wet labs results with the in silico derived results and establish the validity of the in silico methodology. For in vitro studies, in the control study, one unit of VRV activity was found to be 0.4 μg, which increased the coagulation time by one minute (Control 0.9% saline where the coagulation time was found to be 37±1.0 seconds). After optimization, 10 μg dose of venom was selected for the study of neutralizing effect of test molecules. The compound **96** (5 mg dose) showed significant protection to the degree of 36 folds when compared to venom control. The other compounds found to possess moderate protection were **84**, **97**, **101**, **136**, **137**, **138** and **165** (see Table 1).

For in vivo validation studies, compounds **96** and **134** were selected which showed high binding affinity, and compound **147** which showed very low binding affinity, and snake venom induced lethal and hemorrhagic action neutralization studies against *Viper russelli* venom in male albino mice were carried out. The LD_{50} (intra venous and subcutaneous) of *Daboia russelli* was determined along with minimum hemorrhagic and minimum defibrinogenating. From the results as given in Tables 2, 3 and 4, 100% inhibition could be observed for best scoring molecule **96** , followed by **134** while poor or no inhibition in terms of neutralization was observed for compound **147** (found to be poor binder in our in silico experiments).

Conclusions

Identification of potent inhibitors of Phospholipase A2 (of *Viper russelli*) has been achieved by docking studies, that involved natural products from the institute's repository as well as phenolic and substituted benzaldehydes on the crystallized structure of protein PLA2 (1OXL) of *Viper russelli* venom. The evaluated dock scores have allowed the identification of several molecules as PLA2 inhibitors with majority of these belonging to phenolic and substituted benzaldehydic compounds. Moreover, the docking studies also revealed the pivotal role of the residues Phe46 and Val47 of chain B of the target protein through binding interactions with the ligand molecules for the latter to act as PLA2 inhibitor. Results derived from the in silico studies (that led to the identification of PLA2 inhibitors) have been validated by using egg yolk coagulation method (in vitro) and venom neutralization studies on albino mice (in vivo), which showed a good proximity between the in silico and wet lab results. The interesting results reported in the present study offers a promising platform for more detailed in vivo experiments especially for the exploration of the neutralization activity against other venoms such as that of cobra (*Naja kaouthia*), wasp and spider. Further exploratory work using naturally occurring phenolic compounds and structure activity relationship studies would also form an interesting study. Efforts in that direction are being initiated.

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