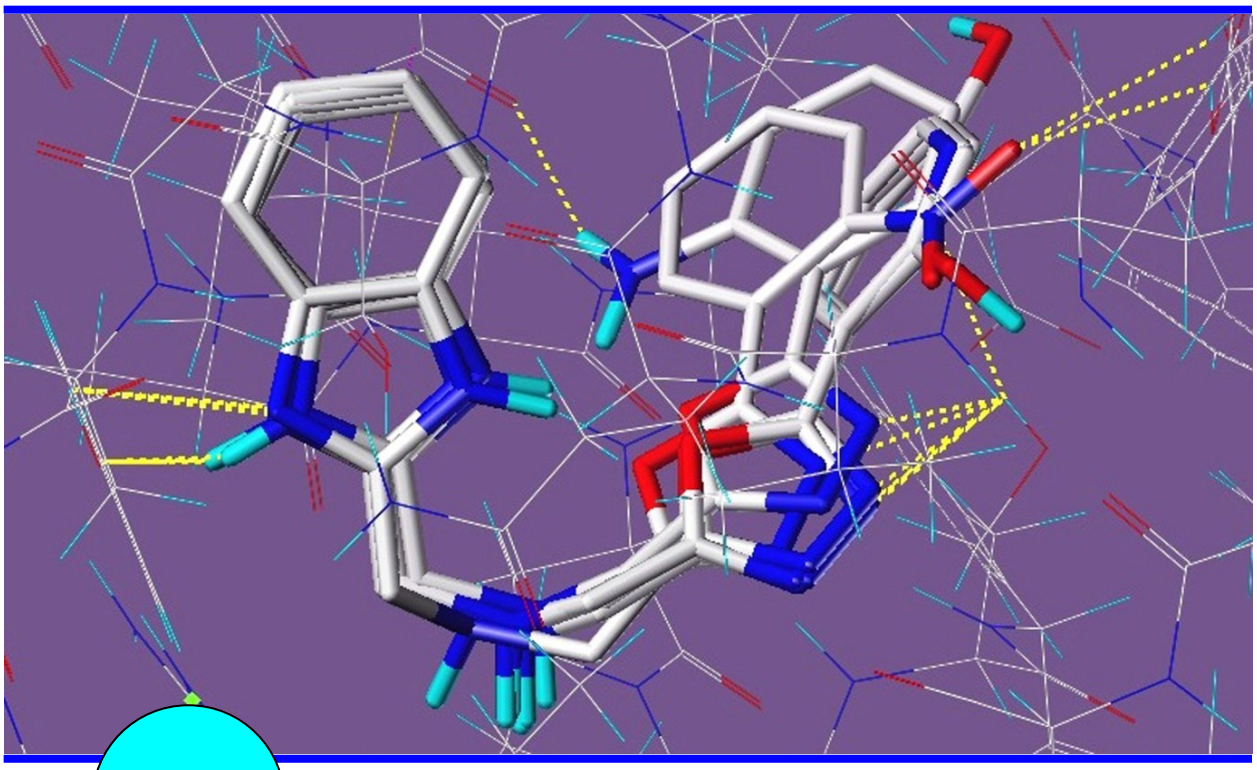

BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2016; 11: 67-74**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index; **ISSN:** 1991-0088

In silico and antithrombotic studies of 1,3,4-oxadiazoles derived from benzimidazole

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Article Info

Received: 3 July 2015
Accepted: 21 September 2015
Available Online: 18 December 2015

DOI: 10.3329/bjp.v11i1.23981

Cite this article:
Vishwanathan B, Gurupadayya BM, Sairam KV. *In silico* and antithrombotic studies of 1,3,4-oxadiazoles derived from benzimidazole. Bangladesh J Pharmacol. 2016; 11: 67-74.

Abstract

In the present study, a series of 1,3,4-oxadiazole derivatives (**4a-4k**) derived from benzimidazole were docked onto factor Xa (PDB: 1NFY) protein using SYBYLX 2.1. and also evaluated for *in vitro* clot lysis for thrombolytic activity. The synthesized molecules were also screened for *in silico* ADME studies. The molecular docking studies highlighted that the molecules showed high affinity towards 1NFY with higher docking score and the *in silico* ADME results were promising and indicated that the molecules holds great potential as a drug candidate. The thrombolytic evaluation was performed for decrease in solid clot weight by the clot lysis study at a concentration of 6.25, 12.5 and 25 µM strengths, respectively. The results of *in vitro* clot lysis for thrombolytic evaluation revealed that the tested compounds **4a-4k** exhibited significant clot lysis with respect to negative control phosphate buffered saline and in comparison to the reference drug streptokinase (30,000 IU). Among all the tested compounds, compound **4j**, **4d** and **4g** exhibited potent thrombolytic activity with EC_{50} value of 16.2, 18.1 and 23.7 µM, respectively. The thrombolytic efficacy investigation highlights that the synthesized compound **4j** could be considered for further clinical studies to ascertain its possible hit as thrombolytic agents.

Introduction

Arterial thromboembolism is the most common cause of cardioembolic events including myocardial infarction, ischemic stroke and limb gangrene. Thus, arterial thromboembolism is obviously the leading causes of morbidity and mortality world-wide (Murray and Lopez, 1997).

The management of ischemic heart diseases is now flanked by newer, more aggressive forms of therapy, which includes the early administration of thrombolytic drugs. Clinical research have focused on developing techniques that directly restore nutritive myocardial perfusion, in particular, drugs capable of lysing the intracoronary thrombus (Meschia et al., 2002).

The treatment of acute myocardial infarction has changed during the past decade as newer approaches have become accessible, as prevention of complications has been the cornerstones for treatment (Godfrey et al., 2011). Thrombolytic agents are used to lyse already formed blood clots in clinical settings where ischemia may be fatal (acute mycardial infarction, pulmonary embolism, ischemic stroke, and arterial thrombosis). This method seems to be most attractive because of its simplicity, which makes it suitable for the vast majority of patients with acute myocardial infarction. Thus, highlighting the clinical advantage of thrombolytic agents, this process of clot dissolution is accomplished by the enzyme plasmin that digests the fibrin strands of the clot.

Thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, streptokinase etc., play a crucial role in

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the management of arterial thromboembolism. The t-PA like streptokinase and urokinase which are widely used as thrombolytic drugs have marked clinical drawback. These agents have a narrow therapeutic index and require continuous monitoring. Also, these agents have significant risk of hemorrhage, and produce anaphylactic reaction and lacks specificity. These entire therapeutic shortcomings of presently available streptokinase and urokinase and other t-PA indicate the need for better thrombolytic agents with clinical advantage (Sobel, 1984).

In the previous study, we had reported the synthesis and characterization of 1,3,4-oxadiaozle derivatives derived from benzimidazole **4a-4k** (Figure 1), from (1*H*-benzo [*d*] imidazol-2-yl) methanamine (Vishwanathan and Gurupadayya, 2014). The 1,3,4-oxadiazoles; *N*-[(1*H*-benzo [*d*] imidazol-2-yl) methyl] (5-substituted-1,3,4-oxadiazol-2-yl) methanamine (**4a-4j**) were prepared from nucleophilic addition of aryl/heteroaryl/aliphatic carboxylic acids with to yield 2-[(1*H*-benzo [*d*] imidazol -2-yl) methylamino] acetohydrazide in presence of phosphorous oxychloride. The acetohydrazide derivative was prepared by condensation of ethyl 2-[(1*H*-benzo [*d*] imidazol-2-yl)methylamino]acetate with hydrazine monohydrate. The ester derivative was prepared by condensation of the starting material (1*H*-benzo [*d*] imidazol-2-yl) methanamine with ethyl 2-chloroacetate in the presence of anhydrous potassium carbonate. The starting material (1*H*-benzo [*d*]imidazol-2-yl) methanamine was synthesized by nucleophilic addition of benzene-1,2-diamine with glycine in the presence of hydrochloric acid. The compound 5-{[(1*H*-benzo[*d*]imidazol-2-yl)methylamino]methyl}-1,3,4-oxadiazole-2-thiol (**4k**) was prepared by condensation of the acetohydrazide derivative with carbon disulfide and potassium hydroxide.

In continuation of the work, in the present investigation, we herein report the *in silico* ADME studies, molecular docking results of the synthesized compounds onto factor Xa (1NFY) in order to visualize the importance of possible antithrombotic efficacy and the *in vitro* clot lysis efficacy of these eleven 1,3,4-oxadiazole derivatives **4a-4k** (Table I).

Materials and Methods

In silico ADME studies

A computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, topological polar surface

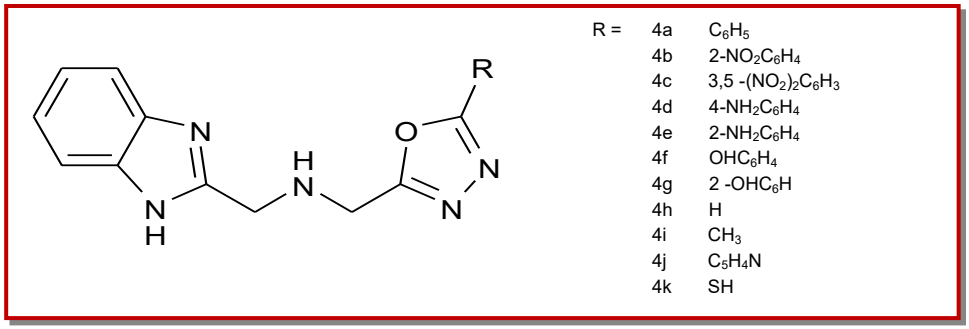

Figure 1: Synthesized 1,3,4-oxadiazole derivative from benzimidazole 4a-4k

Table I

Synthesized 1,3,4-oxadiazole derivatives from benzimidazole 4a-4k

Sl. No.	Compound	Compound
1	(1*H*-benzo [*d*] imidazol-2-yl)-*N*-{ (5-phenyl-1,3,4-oxadiazol-2-yl) methyl} methanamine	4a
2	(1*H*-benzo [*d*] imidazol-2-yl) -*N*-[{5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl} methyl] methanamine	4b
3	(1*H*-benzo [*d*] imidazol-2-yl)-*N*-[{5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl} methyl] methanamine	4c
4	4-(5-[{ (1*H*-benzo[*d*]imidazol-2-yl) methylamino}methyl]-1,3,4-oxadiazol-2-yl) benzenamine	4d
5	2-(5-[{ (1*H*-benzo[*d*]imidazol-2-yl) methylamino}methyl]-1,3,4-oxadiazol-2-yl) benzenamine	4e
6	4-(5-[{ (1*H*-benzo[*d*]imidazol-2-yl) methylamino}methyl]-1,3,4-oxadiazol-2-yl) phenol	4f
7	2-(5-[{ (1*H*-benzo[*d*]imidazol-2-yl) methylamino}methyl]-1,3,4-oxadiazol-2-yl) phenol	4g
8	*N*-[(1,3,4-oxadiazol-2-yl) methyl] (1*H*-benzo [*d*] imidazol-2-yl) methanamine	4h
9	(1*H*-benzo [*d*] imidazol-2-yl)-*N*-[(5-methyl-1,3,4-oxadiazol-2-yl) methyl] methanamine	4i
10	(1*H*-benzo [*d*] imidazol-2-yl)-*N*-[{5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl} methyl] methanamine	4j
11	5-[{ (1*H*-benzo [*d*] imidazol-2-yl) methylamino} methyl]-1,3,4-oxadiazole-2-thiol	4k

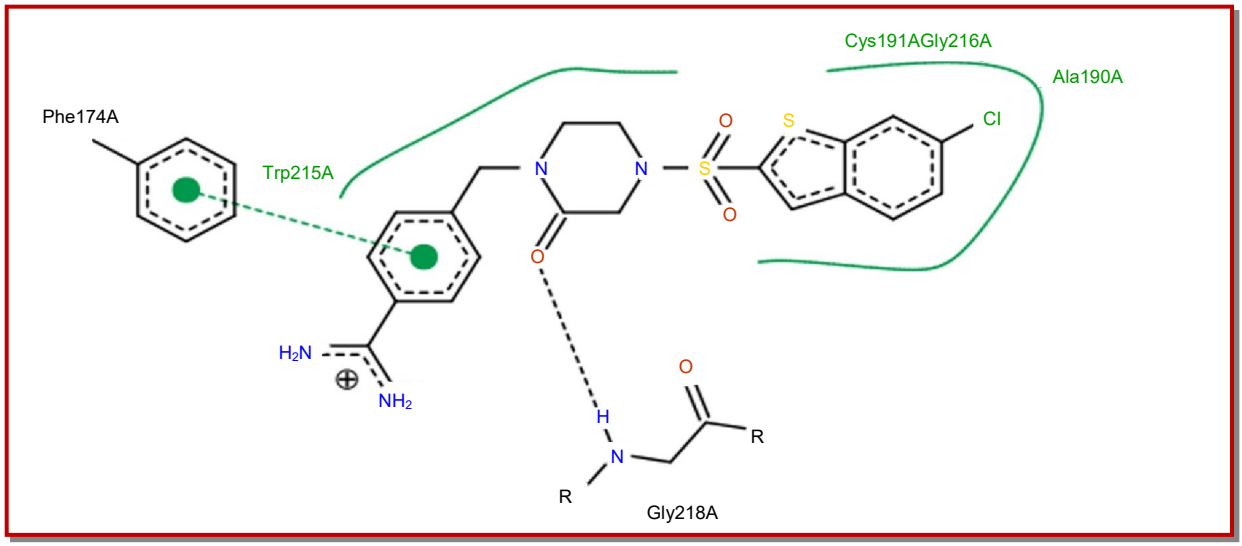

Figure 2: Graphical representation of RPR132747 interaction with protein 1NFY

area (TPSA), percentage of absorption (%ABS), the drug -likeness and drug score (representing the combined physico-chemical, pharmacokinetic and pharmaco-dynamic effects of a compound) for the synthesized 1,3,4-oxadiazoles in order to verify that these compounds exhibit good (theoretical) oral bioavailability potential. The lipophilicity and TPSA were calculated using online software; Molinspiration online property calculation toolkit (http://www.molinspiration.com), while the aqueous solubility, drug-likeness and drug score were calculated using the OSIRIS property explorer software. For the study of drug-likeness, the OSIRIS program uses a list of 5,300 molecular fragments, where the frequency of occurrence of each fragment is determined based on a collection of 3,300 drugs and 15,000 commercially available chemicals that are not drugs (Ertl et al., 2000). The %ABS was estimated according to the formula:

$$\%ABS = 109 - (0.345 \times TPSA)$$

Molecular docking studies

Crystallographic data of the factor Xa enzyme (PDB-ID: 1NFY) was used for docking studies. The x-ray crystal structure of 1NFY was downloaded from (http://www.pdb.org). The compounds were docked into 1NFY to understand antithrombotic efficacy of the designed 1,3,4-oxadiazoles. In view of the fact that 1NFY has been well established and reported for docking analysis to understand antithrombotic importance (Amin et al., 2014).

The protein structure of 1NFY is well established with hydrophobic active site and the proteins were determined at 2.1 Å resolution. The bound conformations of 4-({4-[(6-chloro-1-benzothien-2-yl)sulfonyl]-2-oxopiperazine-1-yl}methyl)benzene carboximidamide (RPR132747) was used as controls in order to define the active site in factor Xa (Figure 2).

The protein was prepared using a protein preparation module in SYBYLX 2.1, where bond orders were assigned, water and other residues were removed and the protein model was charged with AMBER7 FF99. Ligands, 1,3,4-oxadiazoles 4a-4k were drawn in Chem Draw and molecules were converted into *.sdf using Open Babel software. Using the ligand preparation utility of SYBYLX 2.1, 3D structures were generated from 2D and the ligands were energy minimized using minimize module of SYBYL X 2.1 by applying molecular mechanics force fields (MMFF94s) and charged using Gasteiger-Marsili method. Molecular docking simulations were performed in order to distinguish the basic receptor-ligand interactions. The docking experiments were carried using the SYBYL X 2.1. The optimized 3D-structures of 1,3,4-oxadiazoles 4a-4k were docked within 10 Å radius to find the most optimal binding pose of each ligand. The docking algorithm performs a series of hierarchical searches for locations of possible ligand affinity within the binding site of the enzyme. The score of the docked molecules were obtained, which indicate the affinity of the molecules towards the enzyme core.

Chemicals

The chemicals were procured from Sigma-Aldrich and were used without further purification.

In vitro thrombolytic evaluation

In vitro test adapted for screening of test compounds for clot lysis study was performed on whole blood as per the reported method (Prasad et al., 2006). The blood samples were collected from butcher house of healthy sheep (*Ovis aries*). The entire procedure was carried out at room temperature and studies were required to be completed within 3 hours after blood withdrawal as per the protocol. Phosphate buffered saline (PBS) was used as control and test compounds were screened at 6.25,

12.5 and 25 µM concentrations, respectively. The commercially available lyophilized streptokinase vial (1,500,000 IU) was diluted with PBS and mixed properly. From which streptokinase equivalent to 30,000 IU was used as a standard to observe the thrombolytic activity.

Whole blood was collected and 500 µL of blood was transferred to each of the previously weighed micro-centrifuge tubes and incubated at 37°C for 45 min for clot formation. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube with the clot was again weighed to determine the clot weight. 100 µL of diluted test compounds (6.25, 12.5 and 25 µM) for screening were added to the labeled microcentrifuge tube containing clot. 100 µL of streptokinase (30,000 IU) was added as the positive thrombolytic control and 100 µL of PBS as the negative thrombolytic control was employed. All the tubes were then again incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The percent clot lysis was calculated according to the following formula:

$$\%\ Clot\ lysis = \left[\frac{Intial\ clot\ weight - Final\ clot\ weight}{Intial\ clot\ weight}\right] \times 100$$

Statistical analysis

The mean clot lysis percentage of test compounds in different concentrations was compared with the standard streptokinase and phosphate buffered saline using the repeated measures ANOVA with Dunnet's test. Mean, standard error of mean (SEM) calculations and ANOVA test were performed using ''GraphPad Prism version 4.0'' software. The data obtained is expressed as mean ± SEM. The concentration which produced clot lysis half maximally (EC_{50}) was also determined graphically from the percentage of clot lysis at various concentrations of the test compound.

Results

In silico ADME results

Molecular weight of each ligand was within the range of 229 to 395 D. They also had moderate to high predicted oral availability based on %ABS ranging between 50 to 82%.

Generally, a compound needs a score less than 5 for lipophilicity, the lipophilicity data suggested that the compounds were optimally lipophilic in nature ranging from -0.47 to 1.92. All the compounds (except compound 4c) also showed a TPSA of less than 140 Å, indicating a good permeability of the drug in the cellular plasma membrane.

Most of the clinical available drugs have Log S higher than -4.00. The compounds exhibited a Log S value greater than -4.00, ranging between -0.81 and -3.78 (except compound **4c**). A positive value for drug-likeness indicates that the compound contains predominantly fragments that are often present in most currently available drugs. Only compound **4c**, the 3,5-dinitrophenyl derivative was an exception in the *in silico* studies as it was exhibiting a TPSA value of 171 Å, Log S value of -4.24 and one Lipinski violation.

The drug score combines drug-likeness, lipophilicity, solubility, molecular weight and the risk of toxicity into a single numerical value that can be used to predict a global value for each compound as a potential new drug candidate, a positive value of drug scores indicate that the molecules contain predominant pharmacophoric groups, which are often found in pharmaceuticals.

The results from the calculations show that the compounds gave values for drug-likeness between -8.39 to 2.7. All compounds showed positive values in the drug score calculation, the values ranged from 0.37 to 0.91. The results highlights that the designed 1,3,4-oxadiazole have potential as new drug candidates. Table II represents the calculated %ABS, TPSA and Lipinski parameters of the synthesized compounds.

Molecular docking results

The docking study of the 1,3,4-oxadiazoles onto the active site of the enzyme factor Xa (1NFY) were significant, as all the eleven 1,3,4-oxadiazoles were exhibiting significantly higher docking score and considerable lower crash score in comparison to the co-crystallized ligand. The ligand, RPR132747 exhibited a docking and crash score of 6.2033 and -2.2025 Kcal/M respectively, with the main hydrophobic interactions with the surrounding residues Cys191, Gly216, Ala190 and Trp215, strongly contributed to the stabilization. The hydrogen and the nitrogen of the imine function exhibited hydrogen bond interaction with the residues Cys191.H and Cys191.O at a distance of 2.887 and 1.988 Å, respectively. The proton of the amino group exhibited hydrogen bond interaction with the residue Trp215 at a distance of 2.512 Å. The oxygen of the keto function of piperazin-2-one moiety exhibited hydrogen bond interaction with the residue Gly218 at a distance of 2.617 Å. None of the compounds exhibited crash score of greater than -4.5 Kcal/M. A crash score greater than -4.5 Kcal/M, indicates inappropriate penetration of the ligand into the binding site of 1CQE resulting in the decreased forces of interaction with the amino acid.

The docking study revealed that the 1,3,4-oxadiazole derivatives, possessed high affinity towards 1NFY (Figure 3). The 1,3,4-oxadiazoles derived from benzimi-

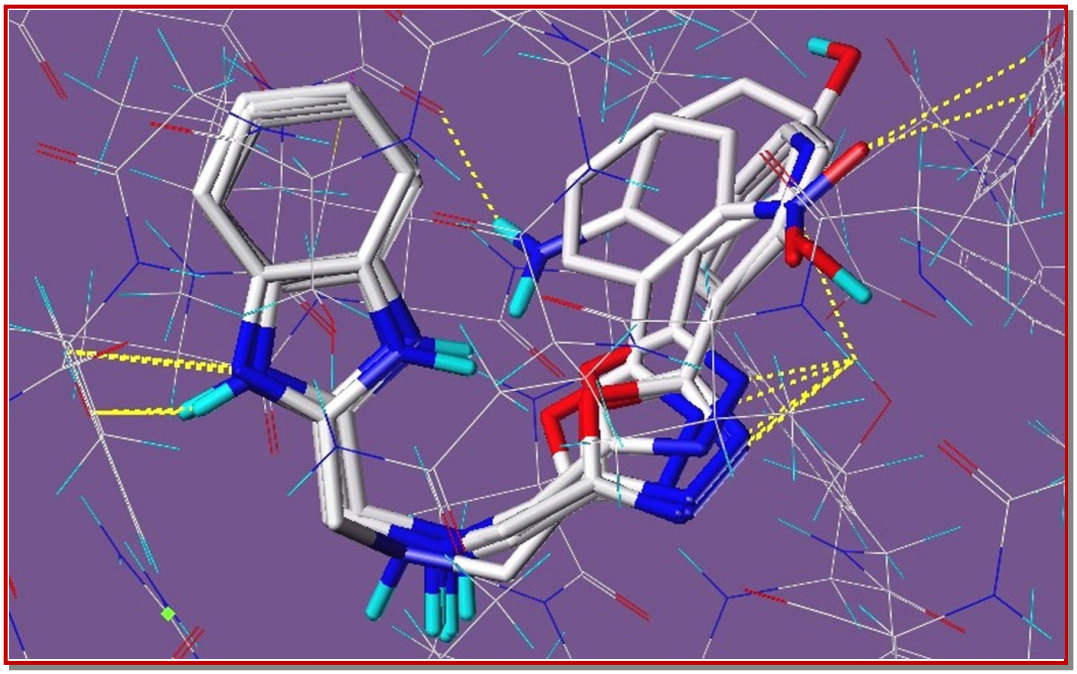

Figure 3: Energy minimized and conformationally analyzed structures aligned against common atoms interacting with protomol 1NFY

Table II

Predicted ADME, Lipinski parameters and molecular properties of the synthesized compounds

Sl No.	Compound	% ABS	TPSA	n-ROTB	n-HBA	n-HBD	*mi* logP	Mol. weight	n violations	LogS	Drug Likeness	Drug Score
1	4a	82	79.6	5	6	2	1.9	305.3	0	-3.3	2.5	0.8
2	4b	66	125.5	6	9	2	1.8	350.3	0	-3.8	-5.2	0.4
3	4c	50	171.3	7	12	2	1.8	395.3	1	-4.2	-8.4	0.4
4	4d	73	105.7	5	7	4	1.4	320.4	0	-3.4	-0.3	0.6
5	4e	73	105.7	5	7	4	1.0	320.4	0	-3.4	1.0	0.7
6	4f	75	99.9	5	7	3	1.7	321.3	0	-3.0	2.0	0.8
7	4g	75	99.9	5	7	3	1.4	321.3	0	-3.0	1.0	0.8
8	4h	77	92.5	5	7	2	0.8	306.3	0	-2.5	2.7	0.9
9	4i	82	79.6	4	6	2	0	229.2	0	-1.2	1.9	0.9
10	4j	82	79.6	4	6	2	-0.5	243.3	0	-0.8	1.9	0.9
11	4k	82	79.6	4	6	2	0.5	261.3	0	-2.1	-0.4	0.7

dazole; 4a-4k exhibited a docking score in the range of 6.6126 to 8.7926 Kcal/M. Compound **4d** (4-aminophenyl) exhibited the highest docking score (8.7926 Kcal/M) among all the 1,3,4-oxadiazoles and compound **4k** (mercapto) with the least docking score (6.6126 Kcal/M).

Compound **4d** (4-aminophenyl) generated a good docking score of 8.7926 Kcal/M and a crash score of -0.9615 Kcal/M. The hydrogens of the amino group at position C_2 of the phenyl moiety were involved in hydrogen bond interaction with Gly218 and Asp189 residues at a distance of 2.374 and 1.967 Å, respectively. The 1*H* hydrogen of the benzimidazole moiety and the hydrogen of the amino bridge between the benzimidazole and 1,3,4-oxadiazole were involved in hydrogen bond interaction with Tyr99 residue at a distance of 2.034 and 2.420 Å, respectively. While, the nitrogen at position 3 of the benzimidazole moiety was exhibiting hydrogen bond interaction with Ser195 residue at a distance of 2.083 Å. The oxygen of the 1,3,4-oxadiazole moiety was involved in hydrogen bond interaction with Gly216 residue at a distance of 2.301 Å. All the 1,3,4-oxadia-

zoles generated crash score less than -4.5 Kcal/M indicating that the 1,3,4-oxadiazoles exhibited appropriate penetration onto the binding site of 1NFY. The crash score of the all designed 1,3,4-oxadiazoles were in the range of -0.2259 to -1.6941 Kcal/M significantly less than that of the control ligand RPR132747 (-2.2025 Kcal/M).

Followed by **4d**, compound **4c** (3,5-dinitrophenyl) generated a docking score of 8.3500 Kcal/M, the 1*H* of the benzimidazole moiety showed hydrogen bond interaction with Ser214 and Ser195 residues at a distance of 2.626 and 2.723 Å, respectively. The nitrogen at position 3 and 4 were involved in hydrogen bond interaction with Ser195 residue at a distance of 1.981 and 2.695 Å, respectively. Compound **4g** (4-hydroxyphenyl) exhibited a docking score of 8.1578 Kcal/M, the oxygen of 1,3,4-oxadiazole was involved in hydrogen bond interaction with Gly216 residue at a distance of 1.894Å, The hydrogen of 1*H* benzimidazole moiety showed hydrogen bond interaction with Gly216 residue at a distance of 1.869Å, The oxygen and the hydrogen of the hydroxy group at position C_4 of the phenyl moiety were exhibiting hydrogen bond interaction with Cys191 residue at a distance of 2.703 and 1.966 Å, respectively.

The 1*H* of the benzimidazole and hydrogen of the amino bridge were exhibiting hydrogen bond interaction with Ser195 and Gly216 residues respectively. The oxygen and the nitrogens of 1,3,4-oxadiazole were exhibiting hydrogen bond interaction with Gly216 and Ser195 residues, respectively. These were the common and major interaction exhibited by **4a**-**4k** series of 1,3,4-oxadiazoles. The other 1,3,4-oxadiazoles with docking score >7 Kcal/M were **4d** (4-aminophenyl), **4j** (pyridin-3-yl), **4g** (2-hydroxyphenyl), **4i** (methyl), **4b** (2-nitrophenyl) and **4a** (phenyl) exhibiting a docking score of 7.8387, 7.7295, 7.4938, 7.4188, 7.3991 and 7.164 Kcal/M, respectively. The docking score of 1,3,4-oxadiazoles; **4a**-**4k** along with their crash score and polar score are presented in Table III.

Thrombolytic activity

The newly synthesized 1,3,4-oxadiazole derivatives **4a**-**4k** were evaluated for *in vitro* thrombolytic activity by clot lysis study. The magnitude of the compounds activity was determined in comparison with the thrombolytic activity of streptokinase.

The mean EC_{50} values of the tested compounds were in the range of 74.8 to 16.2 µM. The mean percentage clot lysis values ± SEM of tested compounds **4a**-**4k** at the different test strength and the mean EC_{50} values ± SEM are presented in Table IV.

The result of thrombolytic evaluation highlighted that compound **4j** (pyridin-3-yl), **4d** (4-aminophenyl), **4g** (2-hydroxyphenyl) and **4k** (mercapto) exhibited potent clot lysis efficacy than that of the standard streptokinase (46.57%) at the test dose strength of 25 µM with an percentage clot lysis of 56.2, 53.0, 51.0 and 47.0%, respectively in comparison to the other set of oxadiazole derivatives. The statistical analysis of the cumulative clot lysis data exhibited by **4a**-**4k** at all three test concentration highlighted that compound **4d**-**4k** exhibited a significance value of $p<0.001$ with respect to

Table III

Docking score of 1,3,4-oxadiazole derivatives 4a-4k towards 1NFY

Sl. No.	Compound	Total score[a]	Crash score[a]	Polar score[a]
1	RPR132747	6.2	-2.2	0.8
2	4a	7.2	-0.7	1.7
3	4b	7.4	-0.5	4.1
4	4c	8.4	-0.4	3.6
5	4d	8.8	-0.9	4.2
6	4e	7.8	-1.2	1.3
7	4f	7.5	-0.7	3.7
8	4g	8.2	-1.0	1.6
9	4h	7.7	-0.9	1.5
10	4i	6.7	-0.4	3.6
11	4j	7.4	-0.7	3.4
12	4k	6.6	-0.3	3.6

[a]Data expressed in Kcal M^{-1} as obtained from the docking utility of SYBYL 2.1 software

Table IV

***In vitro* thrombolytic activity data of the compounds 4a-4k**

Sl No.	Compounds[a]	Mean percentage clot lysis ± SEM[bc]			Mean EC_{50} value ± SEM (μM)[c]
		6.25 μM	12.5 μM	25 μM	
1	Control	2.0 ± 0.2	2.6 ± 0.7	2.6 ± 0.3	
2	4a	12.0 ± 0.3***	16.0 ± 0.6***	22.5 ± 0.4***	74.8 ± 1.1
3	4b*	14.4 ± 0.3***	20.5 ± 0.8***	30.9 ± 0.1***	46.7 ± 0.2
4	4c*	20.8 ± 1.5***	24.1 ± 0.2***	28.9 ± 0.8***	74.2 ± 1.4
5	4d***	43.7 ± 0.7***	47.0 ± 0.3***	53.0 ± 0.3***	18.8 ± 0.3
6	4e***	21.7 ± 0.8***	30.9 ± 0.8***	45.0 ± 0.6***	28.9 ± 0.3
7	4f***	28.9 ± 0.5***	32.6 ± 0.4***	40.6 ± 0.7***	40.3 ± 1.7
8	4g***	33.0 ± 0.8***	40.1 ± 0.9***	51.0 ± 0.2***	23.7 ± 0.2
9	4h***	28.1 ± 0.4***	32.6 ± 0.4***	40.5 ± 0.5***	39.4 ± 1.2
10	4i***	20.9 ± 0.3***	34.6 ± 0.7***	39.3 ± 1.0***	35.4 ± 1.7
11	4j***	40.9 ± 0.6***	49.0 ± 0.2***	56.2 ± 0.6***	16.2 ± 0.3
12	4k***	25.2 ± 1.0***	34.5 ± 0.7***	47.0 ± 0.5***	27.3 ± 0.7
13	Streptokinase (30,000 IU)		46.6 ± 0.4		--------

[a]Cumulative mean data was analyzed by Dunnet's test compared with the negative control. n = 3; (***) equals p<0.001, (**) equals p<0.01, (*) equals p<0.05; [b]Results are expressed as the mean values from three parallel experiments ± S.E.M; [c]Individual conc. data was analyzed by Dunnet's test compared with the negative control. n = 3; (***) equals p<0.001, (**) equals p<0.01, (*) equals p<0.05

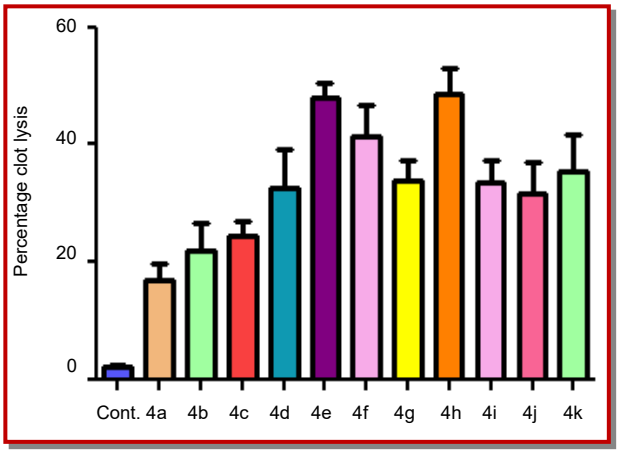

Figure 4: Mean percentage cot lysis exhibited by 4a-4k

control. Compound **4b** and **4c** exhibited a significance value of p<0.05 highlighting the confidence interval of only 95.0% with respect to control (Figure 4).

Discussion

The docking studies towards thrombolytic efficacy indicated all the eleven 1,3,4-oxadiazoles have better affinity to inhibit FXa (1NFY) enzyme with a overall higher docking score in comparison to the control ligand RPR132747 and all the designed 1,3,4-oxadiazoles exhibited considerably lower crash score than that of the control ligand RPR132747, highlighting the potential of the designed 1,3,4-oxadiazoles as anti-thrombotic agents. The *in silico* ADME studies highlighted that the designed 1,3,4-oxadiazoles have potential as drug candidate with appropriate values towards Lipinski parameters for biological efficacy and also with optimal values required for pharmacokinetic importance.

The results of the *in vitro* thrombolytic activity were encouraging as the tested compounds exhibited substantial clot lysis activity. All the eleven tested 1,3,4-oxadiazole derivative exhibited moderate to potent clot lysis, with mean percentage clot lysis value ranging from 12.0 to 56.2% in comparison to 46.6% clot lysis exhibited by the reference standard streptokinase (30,000 IU).

The compounds **4a**-**4k** at the testing strength of 6.25 μM exhibited a mean clot lysis ranging from 12.0 to 43.7%. Compound **4d** (4-aminophenyl) exhibited the highest thrombolytic activity among the series with mean clot lysis value of 43.7% and compound **4a** (phenyl) exhibited the least activity with mean clot lysis value of 12.0%. The statistical analysis of the compounds thrombolytic activity highlighted that the compounds **4a**-**4k** exhibited a significance value of p<0.001 highlighting

the confidence interval of 99.9% with respect to control. At the testing strength of 12.5 µM, compounds **4a**-**4k** exhibited a mean clot lysis ranging from 16.0 to 49.0%. Compound **4j** (pyridin-3-yl) exhibited a mean clot lysis value of 49.0% being the highest and **4a** (phenyl) being the least with mean clot lysis value of 16.0%. The statistical analysis of the compounds thrombolytic activity highlighted that the compounds **4a**-**4k** exhibited a significance value of $p<0.001$ with respect to control. At 25 µM testing strength, compounds **4a**-**4k** exhibited clot lysis ranging from 22.5 to 56.2%. Compound **4j** (pyridin-3-yl) exhibited the highest thrombolytic property with mean clot lysis value of 56.2% and compound **4a** (phenyl) exhibiting the least, with a mean clot lysis value of 22.5%. The statistical analysis of the compounds thrombolytic activity highlighted that the compounds **4a**-**4k** exhibited a significance value of $p<0.001$ with respect to control.

Conclusion

Thrombolytic evaluations were highly significant and the compounds exhibited potent clot lysis activity. Compound **4j** (pyridin-3-yL) was most potent thrombolytic agent with a mean EC_{50} value 16.2 µM.

Financial Support

Self-funded

Ethical Issue

All the animal experimental procedures and protocols adapted in the study were reviewed and approved by the Institutional Animal Ethics Committee. The experimental procedures and protocols were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Govt. of India.

Conflict of Interest

Authors declare no conflict of interest

Acknowledgement

We express our gratitude to the JSS College of Pharmacy, Mysore and JSS University for providing us all necessary facilities.

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