Original Article

Identification of (1E, 3E)-1, 3-bis [(2-hydroxy-1-naphthyl) methylene] Urea as Mutated MAP Kinase P38 Inhibitor through Reverse Pharmacophore Mapping Approach: Green Synthesis, Characterisation and *in silico* Docking analysis

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ABSTRACT

Introduction: Investigations on Schiff bases are one of the current pharmaceutical research trends due to their broad-spectrum biological activities and unique structural features such as intramolecular hydrogen bond formation, unsaturated C-N bond, the high mobility of hydrogen-bonded proton and pseudo aromatic ring formation. **Aim:** Current work is an attempt to discover the therapeutic potential of such structurally related Schiff bases compounds1-[(E)-[6-[(E)-(2-hydroxy-1-naphthyl) methyleneamino]-2-pyridyl] iminomethyl] naphthalen-2-ol; **P(a)**, (E)-1-(2-methoxy-1-naphthyl)-N-[6-[(E)- (2-methoxy-1- naphthyl) methyleneamino]-2-pyridyl] methanimine; **P(b)**, (E)-1-(1-naphthyl)- N-[6-[(E)-1-naphthylmethyleneamino]-2-pyridyl] methanimine; P**(c)** and (1E,3E)-1,3-bis [(2-hydroxy-1-naphthyl)methylene]urea; **P2(a)**. **Materials and Methods:** Reverse pharmacophore approach was used to identify the Mutated MAP Kinase P38 as the potent target for these selected compounds. The molecular docking studies were performed by using the glide module of Schrödinger Software suite and the Molecular Dynamics simulations were performed by using GROMACS 5.1 with OPLS force field. The *in silico* ADMET studies for all the compounds were performed using the online server SwissADME. The interesting results obtained from docking, dynamic simulation and ADMET properties of P(a), P(b), P(c) and P2(a) led to the synthesis and characterisation of these compounds. **Results:** The docking and simulation studies showed the Schiff base **P2(a)** has the highest binding affinity. The ADMET profile inclusive of oral-bioavailability and physicochemical properties shown by this P2(a) proves that it is the most pertinent lead molecule for a novel drug design. **Conclusion:** Hence, this work identifies the potential drug-like molecule (1E, 3E)-1,3-bis[(2-hydroxy-1-naphthyl) methylene] urea; P2(a) as Mutated MAP Kinase P38 inhibitor and provides the scope of advance *in vivo* studies to further explore the therapeutic potential of such compounds.

Key words: Schiff bases, Reverse PharmMapper, Molecular docking, Kinase inhibitor, Dynamics simulation, ADMET Studies.

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INTRODUCTION

Schiff bases have received considerable attention in the current medicinal and pharmaceutical chemistry research areas due to their broad-spectrum biological activities like antimicrobial,[1,2] anticancer[3,4] analgesic[5-8] anti-inflammatory[5,7,9] antioxidant,[10] anti-

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tubercular activities.[11] Schiff base molecules containing 2, 6 diamino pyridine and naphthalene rings are reported as exhibiting excellent biological activities like antibacterial activities, antifungal properties, anticancer activities and their metal coordination behaviors have been reviewed and reported.[12-16] Therefore, as a continuation of our investigations in biological activities of Schiff bases derived from naphthaldehyde; four structurally related Schiff bases 1-[(E)- [6-[(E)- (2-hydroxy-1-naphthyl) methyle neamino]-2-pyridyl] iminomethyl] naphthalen-2-ol; P(a), (E)-1-(2-methoxy-1-naphthyl)-N-[6-[(E)-(2-methoxy-1-naphthyl)methyleneamino]-2-pyridyl]methanemine; P(b), (E)-1-(1-naphthyl)-N-[6-[(E)-1-naphthylmethyl-eneamino]-2-pyridyl] methanimine; P(c) and (1E,3E)-1,3-bis[(2-hydroxy-1-naphthyl)methylene]urea; P2(a)[17] were chosen (Figure 1) for *in silico* studies. The reverse PharmMapper method which is one of the most efficient target fishing strategies was applied for the initial screening.

MATERIAL AND METHODS

Pharm Mapper analysis

Pharm Mapper is a web-based tool[18] for potential drug target prediction against any given small molecules using a 'reverse' pharmacophore mapping method. This is a highly efficient mapping technique which has the high-throughput ability and identifies the potential target candidates from the database within a very short time. Pharm Mapper is a tool for identifying targets for a novel synthetic compound, a newly isolated natural product, a compound with known biological activity or an existing drug whose mechanism of action is unknown.[19] Pharm Mapper screening was used to identify the potential target for the Schiff base P(c).

Molecular docking

The docking studies for Schiff base molecules were carried out by Glide program of Schrödinger Software suite.[20] Schrödinger is commercial software that facilitates the finding of interactions by docking of a molecule at the active site of the target protein. The reverse docking server Pharm Mapper showed a large positive Z'-score of 2.916 with PDB id: 2GTN protein.[21] Hence, this protein was selected as the target to perform docking studies on all the Schiff base molecules. The active site in the target protein was determined by analyzing the binding pattern of (2-(2, 6-difluorophenoxy)-n-(2-fluorophenyl) - 9-isopropyl-9h-purin-8-amine) [LIE] which is co-crystallized and available in the 2GTN was used as control. The residues Gly110, Met109, Thr106, Ala51, Leu75, Leu86, Asp112, Val30, Phe169, Asn115, Val38, Asp168 and Gly71 were observed as the interacting residues and assisted in the binding of a ligand with the protein as shown in Figure 2. Prior to docking, the target protein was prepared by protein preparation wizard to add hydrogen, correct structural errors, add missing atoms and remove extra water and other hetero molecules attached to the protein. SMILE formats of all synthetic Schiff base molecules were taken as input to the Ligprep module to prepare ligand molecules for docking studies.

The grid was created on the target protein with all residues of the active site with van der Waals (VdW) radii scaling factor of 1, partial cutoff charge 0.25 and grid center at -2.250954, -0.742516 and 19.284213 coordinates. Total grid point at the inner box and outer boxes were 10x10x10 and 27x27x27 respectively. The docking was performed on the receptor grid using

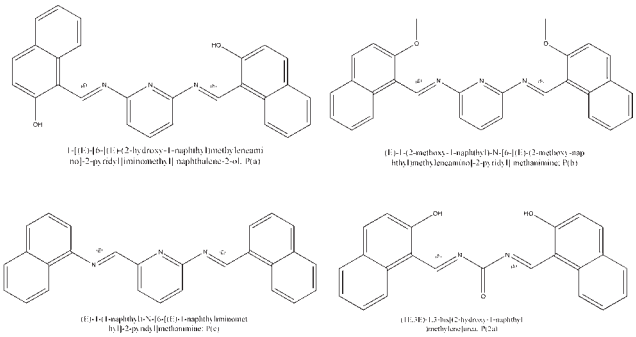

Figure 1: Structure of P(a), P(b), P(c) and P2(a).

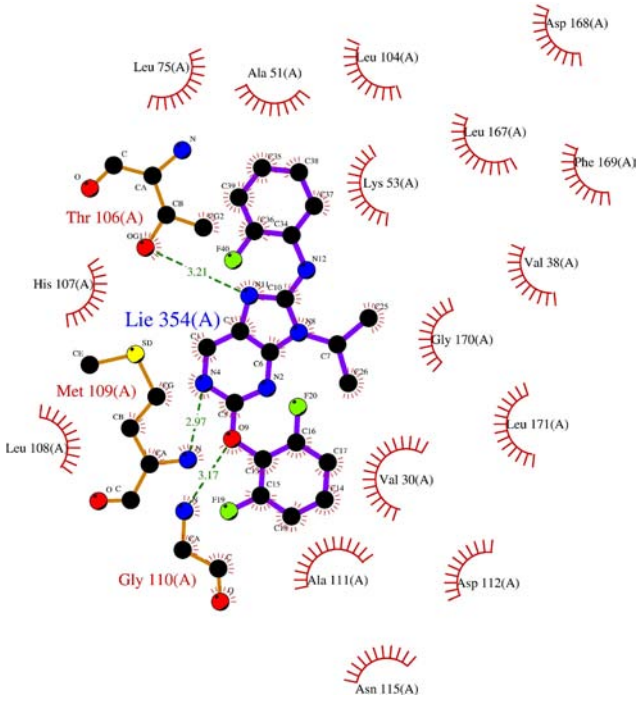

Figure 2: Active site residues of 2GTN from PDB Sum.

VdW scaling factor 0.80 and 0.15 charge cutoff. The standard precision was used on flexible ligand sampling with sample nitrogen inversion and sample ring conformations. OPLS (Optimized Potential for Liquid Simulation) methods were used for energy calculation of docked molecular conformations.

Molecular dynamics simulation

The Molecular Dynamics simulations for each protein-ligand system were performed at 300 K using GROMACS 5.1[22] with OPLS force field. The initial topologies were generated using PRODRG server[23] and tweaked using in-house tools after optimizing it with MOPAC.[24] The topology parameters of MAP kinase P38 and the Schiff bases ligands were generated using GROMACS program and PRODRG server respectively to generate a protein-ligand complex system. A dodecahedron box of 1 nm was created around the complex periphery with periodic boundary condition using *gmxeditconf*. This box was filled with Tip4P water model to create real-life simulation of protein using *gmx solvate*. The system was neutralized by the addition of Na^+ and Cl^- ions using *gmxgenion*. The resultant system was subjected to 1000 steps of minimization to achieve a stable conformation of the complex and to remove the internal clashing of complex and water molecules. Using NVT, the system was systematically heated from 0 to 300 K with 100ps equilibration conditions and periodic boundary condition. The second equilibration was done on the resultant system of NVT with NPT conditions for 100ps. The equilibration protein backbone atoms were restrained and side chain as well as ligand molecule were allowed to move freely. After equilibration, all the four systems were subjected to 20ns of simulation at 300K with Verlet cutoff scheme along with particle-mesh Ewald method[25] at 300 K.

Chemistry

An efficient green protocol by using Microwave irradiation has been reported for the synthesis of the Schiff bases P(a), P(b), P(c) and P2(a).[26] This method provided a better Yield and faster reaction without using hazardous and toxic dehydrating agents like H_2SO_4, $TiCl_4$ and $MgSO_4$-PPTS as compared to the classical method.[27] Moreover, the quantity of solvent used in this reaction is considerably less compared to the conventional synthesis.

All the reagents required for the reaction were sourced from Aldrich Chemicals and were chemically pure. The solvents were freshly distilled before use. Microwave synthesis was carried out in a Technika domestic microwave oven with model number WD904 at power 300W. LC-MS analysis was performed using WATERS LCMS Model (SQD-2 with H Class UPLC) at Azyme Biosciences, Bangalore, India. ESI HRMS was done using Agilent 6520 Q-TOF at Central Drug Research Institute, Lucknow, India. Infra-red spectra of complexes were recorded in KBr pellets with a JASCO FTIR in the range of 4000-400 cm^{-1}. ^{1}H-NMR (400 MHz) were recorded with JEOL 400-MHZ NMR Spectrophotometer (with Multiple Probe Facility) using TMS as the internal reference.

Synthesis of 1-[(E)-[6-(E) -(2-hydroxy-1-naphthyl) methyleneamino]-2 pyridyl] iminomethyl] naphthalene 2-ol, P(a): A mixture of 2,6 Diamino pyridine (0.500g, 0.458 mmol) and 2-Hydroxy-1- naphthaldehyde (1.5g, 0.916) in 2ml of ethanol was irradiated in a microwave oven for 6 min. The reaction mixture was then dissolved in 10ml of hot ethanol and stirred for 5 h and cooled to get crystalline reddish-brown solid as the product...

Synthesis of (E)-1-(2-methoxy-1-naphthyl)-N-[6-[(E)-(2-methoxy-1-naphthyl) methylene amino]-2-pyridyl] methanimine P (b): The reaction was carried by using the same protocol used for the synthesis of P(a). A mixture of 2,6 Diamino pyridine (0.500g, 0.458 mmol) and 2-Methoxy-1- naphthaldehyde (1.7g, 0.916) in 2ml of ethanol was irradiated in a microwave oven for 6 min. The reaction mixture was then dissolved in 10ml of hot ethanol, stirred for 7 h and cooled to get the product as crystalline bright yellow colored powder.

Synthesis of (E)-1-(1-naphthyl)-N-[6-[(E)-1-naphthylmethyleneamino]-2-pyridyl] methanimine, P(c): The reaction was carried by using the same protocol used for the synthesis of P(a). 2,6 Diamino pyridine (0.500g, 0.458 mmol) and 1-naphthaldehyde (1.4g, 0.916) was mixed with 2ml of ethanol and the mixture was irradiated in a microwave oven for 4 min. The resultant mixture was then dissolved in 10ml of hot ethanol, stirred for 7 h and cooled to get the product as bright yellow colored powder.

Synthesis of (1E, 3E)-1,3-bis[(2-hydroxy-1-naphthyl) methylene]urea, P2(a): The same protocol which was used for the synthesis of P(a) is used here. Urea (0.200g, 3.3 mmol) and 1-naphthaldehyde (1.1g, 6.6 mmol) was mixed with 2ml of ethanol and the mixture was irradiated in a microwave oven for 8 min. The resultant mixture was then dissolved in 8ml of hot ethanol, stirred for 7 h and cooled to get bright yellow colored fine powder as product.

Spectral characterisation of the Schiff bases

1-[(E)-[6-(E)-(2-hydroxy-1-naphthyl) methyleneamino]-2-pyridyl] iminomethyl] naph-

thalene 2-ol, P (a): Yield: 85%. M.p: 280-285°C. IR (KBR, ν, cm^{-1}): 1616 (C=N), 1347 (phenolic C-O); 830 (CH). ^{1}H NMR (400MHz, $CDCl_3$, δ, ppm): 15.17(d, 2H, OH), 10.03 (d, 2H, HC=N), 8.170 (d, 2H, Ar-H), 7.82. (d, 2H, Ar-H), 7.79-7.76(m,1H, Ar-H), 7.67-7,65 (m, 2H, Ar-H), 7.58-7.54(m, 2H, Ar-H),7.37-7.36 (m, 2H, Ar-H), 7.03 (d, 2H, Ar-H), 6.94(d, 2H, Ar-H). EI-MS: m/z [M+H] +: 418.1

(E)-1-(2-methoxy-1-naphthyl)-N-[6-[(E)-(2-methox-1-naphthyl) methylene amino]-2-pyridyl] methanimine P(b): Yield: 80%. M.p: 260-265°C. IR (KBR, ν, cm-1): 1592 (C=N), 1247 (methoxy C-O), 807 (CH). ^{1}H NMR (400MHz, CDCl3, δ, ppm): 10.9 (s, 2H, HC=N); 9.27(d, 2H, Ar-H), 8.06 (d, 2H, Ar-H), 7.98-7.95 (m, 2H, Ar-H), 7.91 (d, 2H, Ar-H), 7.78-7.76 (m, 2H, Ar-H), 7.67-7.65 (m, 2H, Ar-H), 7.44-7.40 (m, 1H, Ar-H), 7.30 (d, 2H, Ar-H), 4.059 (s, 6H, -OCH3). EI-MS: m/z [M+H] +:447.2

(E)-1-(1-naphthyl)-N-[6-[(E)-1-naphthylmethyleneamino]-2-pyridyl] methanimine, P(c): Yield:70%. M.p: 215-220°C. IR (KBR, ν, cm^{-1}): (C=N) 1578, (CH) 813. ^{1}H NMR (400MHz, $CDCl_3$, δ, ppm): 10.172 (s, 2H, HC=N),9.30(d, 2H,),8.88 (d, 2H, Ar-H),8.6(d, 2H, Ar-H),7.9(d, 2H, Ar-H),7.95-7.93(m, 2H, Ar-H),7.78-7.75(m, 4H, Ar-H),7.55-5.53(m, 2H, Ar-H),7.31(d, 2H, Ar-H). EI-MS: m/z [M-H]: 384.4

(1E, 3E)-1, 3-bis [(2-hydroxy-1-naphthyl) methylene] urea; P2(a): Yield: 98.7 %. M.P: 187 °C. IR (KBR, ν, cm^{-1}): 1597 (C=N), 3072 (OH, broad), 750 (C-H). ^{1}H NMR (400 MHz, DMSO-D6, δ ppm): 13.2 (s, 2H, OH) 9.01 (s, 2H, HC=N), 8.25-8.22 (m, 2H, Ar-H), 7.79 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 7.29-7.27 (m, 2H, Ar-H), 7.16-7.13 (m, 2H, Ar-H), 6.59 (d, 2H, Ar-H). EI-MS: m/z [M+H] + 369.3

In-silico physicochemical and Absorption, Distribution, Metabolism, Excretion and Toxicity properties.

The *in silico* ADMET study for the four compounds were performed using the online server SwissADME which reads the mol file and converts it to Single Line Input Line Entry System (SMILES) to predict the Physicochemical Properties, Lipophilicity, Water Solubility, Pharmacokinetics and Druglikeness.[28]

RESULTS AND DISCUSSION

Pharm Mapper analysis

Potential protein was identified using reverse pharmacology mapping via Pharm Mapper the top potential receptors based on Z' score and their association with cancer pathway were selected. The reverse docking server Pharm Mapper showed a large positive Z'-score of 2.916 with 2GTN protein. *In vitro* studies of the compounds containing tridentate Schiff base bearing a simple 2',4'-dihydroxyacetophenone functionality and ethylenediamine as the bridging ligand with RCHO moiety have demonstrated anti-proliferative activity.[29] Thus, our results very well correlate with the experimental evidence and found that Mitogen-activated Kinase protein (Protein ID: 2GTN) exhibited excellent interactions with the base compound P(c).

Molecular docking

Ligand-receptor interactions of a particular class of compounds can be improved by making careful changes in the functional groups of the core structure of these molecules. The Schiff base molecules of interest possessed functional groups which can play significant roles in hydrogen bonding, hydrophic and pi-pi interactions. The hydroxyl groups on the naphthalene residue were expected to act as hydrogen bond donors, whereas the methoxy groups were expected to function as hydrogen bond acceptors. Hydrophobic interactions were also predicted due to the presence of naphthalene rings.

A close analysis of docking figures suggests that the molecules are appropriately embedded into the protein active site where one of the naphthalene rings in all the Schiff bases (Figure 3) seemed to be out from the active site and these are the solvent accessible surface of the molecules. This may be attributed to the unique conformation of these molecules provided by the bulkier naphthalene rigs. In order to minimize the steric hindrance, these molecules are expected to undergo C-C bond rotation and adopt a conformation where one of the naphthalene rings is perpendicular to the plane of other parts of the molecules. Molecules P2(a) and P(a) provided a hydrogen bond donor site as expected due to the presence of the ortho hydroxyl group. However, P2(a) exhibited better docking scores due to an additional oxygen atom from the urea moiety. All the four Schiff bases being Salen type compounds exhibited varying docking proficiency. The increased potency of P2(a) and P(a) can be due to the involvement of hydroxyl group present in their structures. Previous reports on the role of hydroxy groups in protein docking are in agreement with our findings. Ling Qiu *et al*. have explained the importance of hydroxyl group of a well-known intravenous anesthetic.[30]

All residues which were found interacting with the co-crystallized ligand LIE were also found to be involved at the same binding site of P2(a), P(a), P(b) and P(c) molecules. Table 1 shows the various parameters

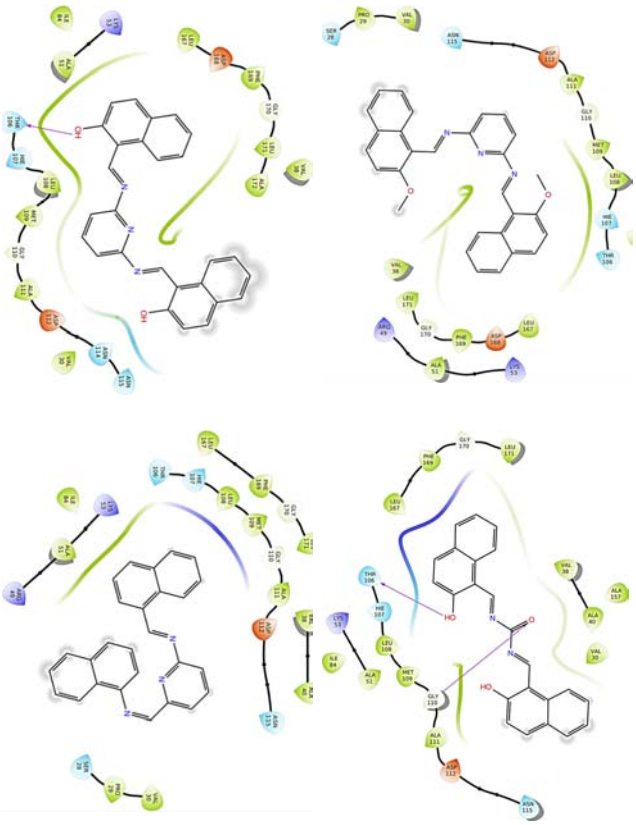

Figure 3: Docking interaction of P(a):1, P(b):2. P(c):3 and P2(a):4

that were analyzed while comparing the docking studies. As per parameters of docking, it was found that P2(a) is the best docked molecule with docking score -7.84 and glide score -7.84 among all other Schiff base molecules. It is important to note that the interactions of Schiff bases with the amino acid residues were not exclusively hydrophobic; as some of the residues (Arg 49, His 107) in the vicinity of the ligands were polar in nature. On the other hand, Schiff base molecules P(a) and P2(a) were able to form hydrogen bonds with Thr 106. P2(a) exhibited additional hydrogen bonding with Gly110 due to the C=O functionality from urea which plays a considerable role in stabilizing of Schiff base – protein complex (Figure 3). The docking results were in agreement with the experimental results from PDB Sum of 2GTN.[23,31] The formation of a hydrogen bond is conserved in both the co-crystallized molecule LIE and ligands P2(a) and P(a) with Thr 106 (Figure 2 and 3).

Molecular Dynamics Simulation

The obtained docking results of Schiff base Protein-Ligand complex allowed us to propose a general binding mode of the ligands and to determine residues involved in the ligand recognition. Although molecular docking offers reasonable binding structures for investigated

Table 1: Docked conformation parameters for P2(a), P(a), P(b) and P(c) with the target protein.

Molecule Name	Docking score	Glide ligand efficiency	Glide ligand efficiency sa	Glide ligand efficiency ln	Glide gscore	Glide lipo	glide rewards	Glide evdw	Glide ecoul	Glide erotb	Glide emodel	Glide energy	Glide einternal	Glide confnum	Glide posenum
p2(a)	-7.839	-0.28	-0.85	-1.809	-7.839	-2.841	-1.948	-42.45	-6.89	0.568	-67.998	-49.339	6.489	13	231
P(a)	-6.915	-0.216	-0.686	-1.549	-6.915	-2.785	-1.28	-39.382	-5.693	0.454	-59.94	-45.075	2.927	23	249
P(b)	-6.367	-0.187	-0.607	-1.407	-6.367	-3.07	-0.928	-48.412	-2.337	0.403	-68.106	-50.749	2.166	152	214
P(c)	-6.308	-0.21	-0.653	-1.433	-6.308	-3.064	-1.347	-41.874	-2.181	0.524	-59.239	-44.055	1.82	28	374

ligands, the MD simulation can account for further investigation of binding modes of ligands and also explains the effects of ligand binding on the conformation and stability of the protein. The Protein-Ligand complex with the least binding energy was obtained from the docking program and was subjected to molecular dynamics simulation. The P(a), P(b), P(c) and P2(a) ligands bound to protein were simulated to check the stability of protein backbone.

The RMSD plot (Figure 4) shows the relative stability based on the motion of the protein backbone when bound with Schiff bases. P2(a), which shows the best binding mode among all the Schiff bases, also shows the lowest deviation from the X-ray structure and tends to converge after 10 ns. Similarly, P(a) starts with a higher fluctuation and shows a slightly high fluctuation between 3.5 ns and 4.5 ns becomes more converged subsequently. After 8ns, it shows a very stable motion and may be a good candidate for further studies. P(c) and P(b) show a very high fluctuation in their backbone throughout. This may be due to steric clashes between the drug and the active site region residues.

Fluctuations in similar fashion can also be observed in the Root Mean Square Fluctuation (RMSF) plot (Figure 5) where the residues constituting the active site (between residues 35-105) show a relatively lower fluctuation in P2(a) and P(a) than that of other complexes. Molecular dynamics results suggest that both the Schiff base complexes can interact with map kinase p38, without affecting the secondary structure. All the molecular docking and molecular dynamics results were well consistent with the experimental data. Structural information regarding binding mode and the effects of Schiff bases on the protein stability and structure were reported here. The results obtained from the docking, ADMET properties and molecular dynamic (MD) simulation studies of P(a), P(b), P(c) and P2(a) led to the synthesis and characterization of these molecules for further investigations.

In-silico physicochemical and ADMET properties

ADMET study provides the significant data for deciding a right preclinical drug candidate by screening them based on their absorption, distribution, metabolism, excretion and toxicity. The *in-silico* physicochemical and ADMET properties for all four synthetic Schiff base molecules are shown in Table 2. Although none of the Schiff bases are Violating Lipinski's rule; compound P2(a) is observed to have the most favorable physicochemical and ADMET properties such as number of rotatable bonds, H-bond acceptors, H-bond donors and Log P values with non-violation of all the rules including Lipinski, Ghose, Veber, Egan and Muegge violations rules.

Table 2: The *in-silico* physicochemical and ADMET properties.

	Molecule	P2(a)	P(a)	P(b)	P(c)
Physicochemical Properties	Canonical SMILES	O=C(/N=C/c1c(O)ccc2c1cccc2)/N=C/c1c(O)ccc2c1cccc2	Oc1ccc2c(c1/C=N/c1cccc(n1)/N=C/c1c(O)ccc3c1cccc3)cccc2	COc1ccc2c(c1/C=N/c1cccc(n1)/N=C/c1c(OC)ccc3c1cccc3)cccc2	c1cc(/C=N/c2cccc3c2cccc3)nc(c1)/N=C/c1cccc2c1cccc2
	Formula	C23H16N2O3	C27H19N3O2	C29H23N3O2	C27H19N3
	MW	368.4	417.4	445.5	385.4
	Heavy atoms	28	32	34	30
	Aromatic heavy atoms	20	26	26	26
	Fraction Csp3	0	0	0.07	0
	Rotatable bonds	4	4	6	4
	H-bond acceptors	5	5	5	3
	H-bond donors	2	2	0	0
	Iso Electric Point	2.8	3.4	-	-
	Refractivity	109.2	132	141	127.3
	Polarizability	43.2	50	53.9	49
	Log P	4.5	7	7.3	7.7
	Pka	7.8/8.4 (Two Protonation Sites)	8.32/8.9 (Two Protonation Sites)	-1.6	0.4/-1.2
	MR	112.5	130.6	139.6	126.6

Table 2: Cont'd.

Molecule		P2(a)	P(a)	P(b)	P(c)
Lipophilicity	TPSA	82.2	78	56	37.6
	iLOGP	3.6	3.6	3.8	4
	XLOGP3	4.9	5.9	6.5	6.6
	WLOGP	5	6.3	6.9	6.8
	MLOGP	3.5	4.3	4.7	4.6
	Silicos-IT Log P	5.2	6.3	7.4	7.3
	Consensus Log P	4.5	5.3	5.8	5.9
	ESOL Log S	-5.5	-6.5	-6.9	-6.7
Water solubility	ESOL Solubility (mg/ml)	1.11E-03	1.35E-04	5.55E-05	6.43E-05
	ESOL Solubility (mol/l)	3.02E-06	3.24E-07	1.24E-07	1.67E-07
	ESOL Class	Moderately soluble	Poorly soluble	Poorly soluble	Poorly soluble
	Ali Log S	-6.44	-7.32	-7.54	-7.21
	Ali Solubility (mg/ml)	1.35E-04	1.98E-05	1.30E-05	2.37E-05
	Ali Solubility (mol/l)	3.67E-07	4.75E-08	2.91E-08	6.16E-08
	Ali Class	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble
	Silicos-IT LogSw	-7.2	-9.35	-10.73	-10.53
	Silicos-IT Solubility (mg/ml)	2.34E-05	1.85E-07	8.28E-09	1.14E-08
	Silicos-IT Solubility (mol/l)	6.34E-08	4.44E-10	1.86E-11	2.95E-11
	Silicos-IT class	Poorly soluble	Poorly soluble	Insoluble	Insoluble
Pharmacokinetics	GI absorption	High	High	Low	Low
	BBB permeant	No	No	No	No
	Pgp substrate	No	No	No	No
	CYP1A2 inhibitor	No	No	No	Yes
	CYP2C19 inhibitor	No	No	Yes	Yes
	CYP2C9 inhibitor	No	No	Yes	No
	CYP2D6 inhibitor	No	No	No	No
	CYP3A4 inhibitor	No	No	Yes	No
	log Kp (cm/s)	-5	-4.6	-4.3	-3.9
Druglikeness	Lipinski violations	0	1	1	1
	Ghose violations	0	2	2	1
	Veber violations	0	0	0	0
	Egan violations	0	1	1	1
	Muegge violations	0	1	1	1
	Bioavailability Score	0.5	0.5	0.5	0.5
Other Properties	PAINS alerts	0	0	0	0
	Brenk alerts	1	1	1	1
	Leadlikeness violations	2	2	2	2
	Synthetic Accessibility	3.1	3.4	3.6	3.4

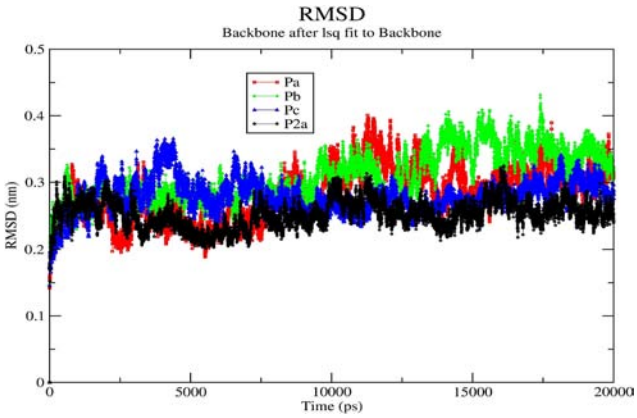

Figure 4: Time dependence of the Root Mean Square Deviation for the backbone atoms of protein during the simulation in the presence of Schiff base. P(a): Red, P(b): Green, P(c): Blue and P2(a): Black.

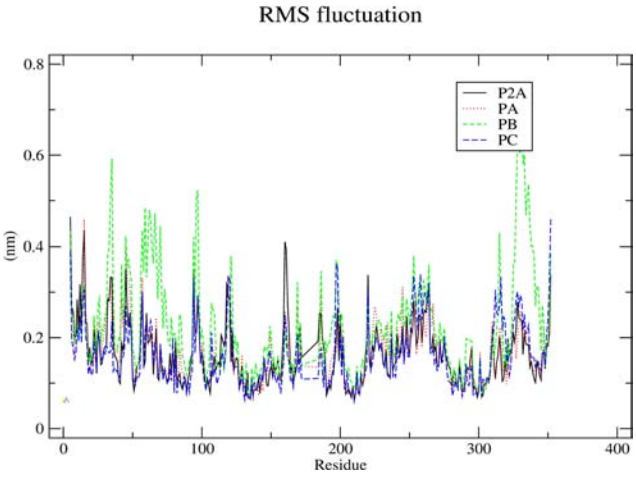

Figure 5: Root mean square fluctuation plot with 2GTN (P2(a) : P2A, P(a) : PA, P(b) : PB and P(c) : PC).

CONCLUSION

In the current study, a comparative inverse screening approach using Pharm Mapper is applied to identify the potential target. Molecular docking and dynamics simulation studies show the strong binding proficiency of P2(a) with Kinase protein compared to other compounds in the series. A study on ADMET confirms the oral-bioavailability of this compound. consequently, the Schiff base P2(a) can be considered as a potential lead candidate from a series of structurally similar molecules and can be taken for the preclinical studies. Hence the current study not only discovers a potential drug candidate from the salen type Schiff base family but also demonstrates an easier way to screen the drug candidates and minimizes efforts required for the chemical synthesis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

ABBREVIATIONS

ADMET: Absorption, distribution, metabolism, excretion, toxicity; **PDB id**: Protein Data Bank identification code; **LIE**: 2-(2, 6-difluorophenoxy)-n-(2-fluorophenyl) - 9-isopropyl-9h-purin-8-amine; **SMILE**: Simplified molecular-input line-entry system; **VdW**: Van der Waals; **OPLS**: Optimized Potential for Liquid Simulation; **MOPAC**: Molecular Orbital PACkage; **LC-MS:** Liquid chromatography-mass spectrometry; **FTIR**: Fourier-transform infrared spectroscopy; **^{1}H-NMR:** Proton nuclear magnetic resonance; **TMS**: Tetramethylsilane; **h:** Hour; **M.p:** Melting point; **C-C:** Carbon-Carbon bond; **RMSD**: Root Mean Square Deviation; **RSMF**: Root Mean Square Fluctuation; **MD**: Molecular dynamic.

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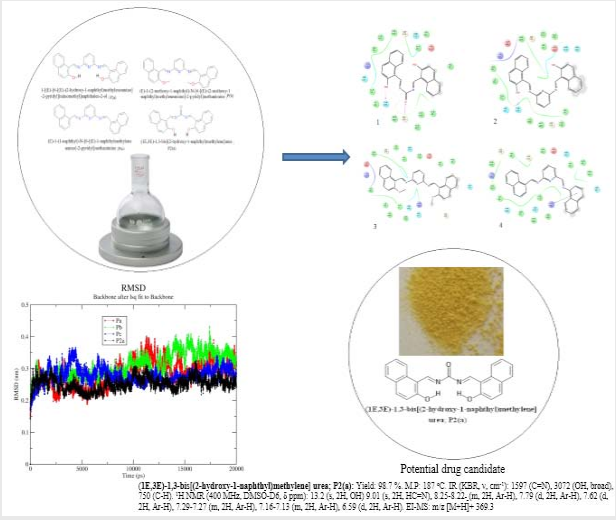
PICTORIAL ABSTRACT



(1E,3E)-1,3-bis[(2-hydroxy-1-naphthyl)methylene] urea; P2(a): Yield: 98.7 %. M.P. 187 °C. IR (KBr, ν, cm^{-1}): 1597 (C=N), 3072 (OH, broad), 750 (C-H). ^{1}H NMR (400 MHz, DMSO-D6, δ ppm): 13.2 (s, 2H, OH) 9.01 (s, 2H, HC=N), 8.25-8.22 (m, 2H, Ar-H), 7.79 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 7.29-7.27 (m, 2H, Ar-H), 7.16-7.13 (m, 2H, Ar-H), 6.59 (d, 2H, Ar-H). EI-MS: m/z [M+H]+ 369.3

SUMMARY

- A series of Salen type (Bis-substituted) Schiff Base compounds were investigated for their Kinase inhibition proficiency. The molecular interactions and the docking results were significant enough to understand the importance of strategically placed hydroxyl functional groups in these compounds. The Schiff base P2 (a), which demonstrated better docking score exhibited good ADMET properties as well. These compounds are then synthesized and characterized for the further investigations due to their interesting binding interactions with Kinase protein. Thus, the current study presents a potential drug candidate from the Schiff Bases family. Moreover, the reverse Pharm Mapper strategy used in this paper to select the appropriate target is of greater current interest among medicinal chemists.

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