

## Synthesis, Biological Evaluation and Molecular Modeling Studies of Novel Chromone/Aza-Chromone Fused $\alpha$ -Aminophosphonates as Src Kinase Inhibitors

S Bapat<sup>1,2</sup>, N Viswanadh<sup>1,3</sup>, M Mujahid<sup>1</sup>, A N Shirazi<sup>4</sup>, R K Tiwari<sup>4</sup>, K Parang<sup>4</sup>, M Karthikeyan<sup>1,3</sup>,  
M Muthukrishnan<sup>1,3</sup> and Renu Vyas<sup>5\*</sup>

<sup>1</sup>CSIR- National Chemical Laboratory, Dr Homi Bhabha Road, Pune, Maharashtra, India- 411008

<sup>2</sup>Dr D Y Patil Biotechnology and Bioinformatics Institute, Tathawade, Pune, Maharashtra, India- 411008

<sup>3</sup>Academy of Scientific and Innovative Research (AcSIR), New Delhi 110025, India

<sup>4</sup>Chapman University School of Pharmacy, Harry and Diane Rinker Health Science Campus, Irvine, CA92618, USA

<sup>5</sup>MIT school of Bioengineering Science and Research, Loni, Kalbhori, Pune-412201, India

*Received 05 March 2018; revised 22 October 2018; accepted 15 December 2018*

A series of novel chromone/aza-chromone fused  $\alpha$ -aminophosphonate derivatives were synthesized in good yields using silica chloride as the catalyst. All the synthesized compounds were tested for their c-Src kinase inhibitory activity. Aza-chromone compound showed Src kinase inhibition with an IC<sub>50</sub> value of 15.8  $\mu$ M. The compounds were subjected to molecular docking and dynamics simulations to study the atomic level interactions with an unphosphorylated proto-oncogenic tyrosine protein kinase Src (PDB code 1Y57) as well as phosphorylated tyrosine protein kinase Src (PDB code 2H8H). Docking and molecular dynamic results revealed phosphorylated Src tyrosine kinase protein better results than unphosphorylated tyrosine Src kinase protein. Chemoinformatics study revealed the compounds had lead like properties. Machine learning (SVR) models were built to study the structure activity correlations. A CC of 0.835 was obtained when the SVR model was applied to the 17 synthesized compounds. It is envisaged that the work will provide guidelines for future drug design efforts for Src kinase inhibitors.

**Keywords:** Phosphorylated cSrc kinase, Chromone Compounds, Correlations, Tyrosine Kinase Protein, Proto-Oncogenic

### Introduction

Src tyrosine kinase overexpression is regularly implicated in number of cancer diseases, such as colon, lung, breast, prostate, ovary and pancreas<sup>1</sup>. There has been raising interest in the development of Src kinase inhibitors in recent years, both for their use as research probes, delineating the specific functions of these kinases, and as potential anticancer/related therapeutics. Chromone is well recognized as a privileged motif, due to its abundance in various natural products with wide range of biological properties that include anti-bacterial, antifungal, anti-cancer, anti-HIV and anti-ulcer agents<sup>2,3</sup>. Similarly,  $\alpha$ -aminophosphonates and  $\alpha$ -amino phosphonicacids constitute an important group of naturally occurring compounds that are considered as isoelectronic analogues of the natural  $\alpha$ -amino acids. Recently, coumarin and chromone derivatives have also been evaluated for their Src kinase inhibition<sup>4</sup>. However, chromone/azachromone fused  $\alpha$ -aminophosphonate

conjugates have not been studied as Src kinase inhibitors. In this context, and in view of our long-standing interest in the chemistry of privileged chromone motif for various biological applications<sup>5</sup>, in the present study, we report the synthesis of chromone/azachromone fused  $\alpha$ -amino phosphonate conjugates. This study also provides insights on the structure activity relationship as well as the Src kinase interaction with these compounds using molecular modeling and molecular dynamics simulation studies.

### Methodology

#### Synthesis

Synthesis of the targeted compounds is shown in Figure 1. Commercially available 2-hydroxyacetophenone/2- aminoacetophenone was subjected under Vilsmeier condition as per the reported procedure to obtain the key intermediate 3- chromone carboxaldehyde/ 3- azachromone carboxaldehyde. Further, these aldehydes on condensation with different amines and diethyl phosphite in presence of silica

\*Author for Correspondence  
E-mail: renu.vyas@mituniversity.edu.in

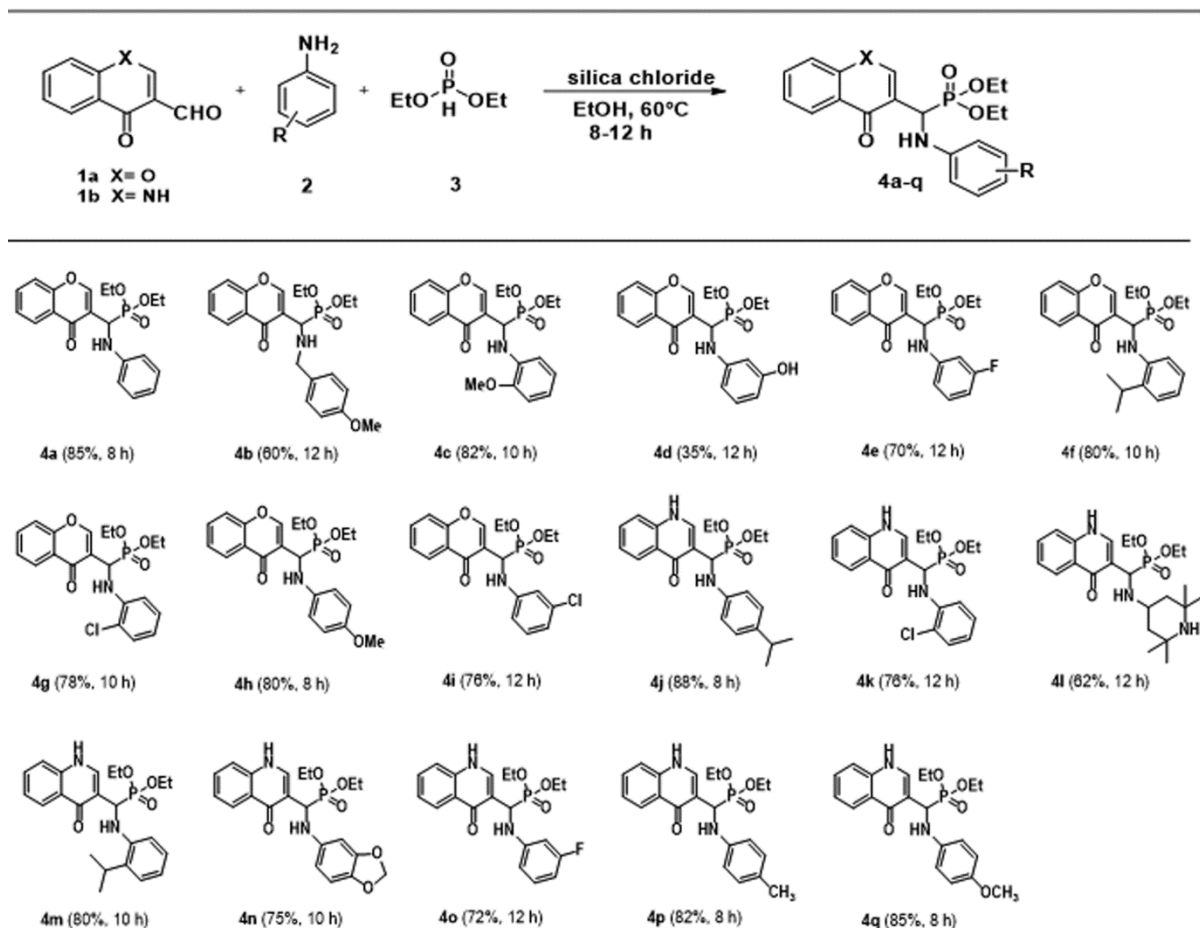


Fig. 1 — Silica chloride- catalyzed one pot three component synthesis of  $\alpha$ -aminophosphonates (4a-4q)

chloride provided chromone/azachromone fused  $\alpha$ -aminophosphonates<sup>6,7</sup>.

Most of the compounds were obtained in good yields with reaction time of 8-12 hours. It should be noted that when this three component reaction was performed in the absence of silica chloride, no product formation was observed. The structures of the compounds were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectral analysis<sup>8</sup>.

#### Biological evaluation

Phosphate esters, carboxylic acids, and tetrahedral intermediates are present in the metabolic and signaling pathways. Phosphonates have the ability to structurally mimic the above organic compounds, thus serving as a small-molecule inhibitor of multiple processes<sup>9</sup>. Phosphonates are used as enzyme inhibitors, potent antifungal, herbicidal, antiparasitic, or antimicrobial agents. Glyphosate is the active ingredient in commercial herbicide and inhibits 5-enolpyruvylshikimic acid 3-phosphate synthase

(ESPS) which is critical in the biosynthesis of aromatic amino acids in plants<sup>10</sup>. All the synthesized compounds were screened for their Src kinase inhibitory activity. The minimum inhibitory concentration (MIC:  $\mu\text{g}/\text{mL}$ ) was determined for each compound. Most of the compounds failed to produce very good inhibition homomeric compounds 4c & 4o showed moderate activity with  $\text{IC}_{50} = 40.6$  &  $63.6$   $\mu\text{g}/\text{mL}$ . One of the compound 4j showed good Src kinase inhibition with  $\text{IC}_{50}$  value of  $15.8$   $\mu\text{g}/\text{mL}$ .

#### Cell culture assay and cell proliferation assay

Kinases play a key role in a number of physiological processes and their inhibitors are seen to exhibit anticancer activity against various human cancer cell lines<sup>11</sup>. Kinase inhibition assays are widely used enzyme inhibition screening assays. Similarly binding constant assay and TR-FRET assay have seen to have potencies against both Src kinase. Human ovarian adenocarcinoma cell line SK-OV3 (ATCC no. HTB-77) and human colon adenocarcinoma HT-29

(ATCC no. HTB-38) were obtained from American Type Culture Collection. Cells were grown on 75 cm<sup>2</sup> cell culture flasks with EMEM (Eagle's minimum essential medium), supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO<sub>2</sub>, 95% air at 37 °C. Cell proliferation assay was carried out using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA).

#### Preparation of macromolecule

The protein targets retrieved from RCSB Protein Data Bank were unphosphorylated proto-oncogenic tyrosine protein kinase Src (PDB code 1Y57) and phosphorylated tyrosine protein kinase Src (PDB code 2H8H) which served as docking receptors. The proteins were fixed for errors in atomic representations and optimized using Protein Preparation Wizard Maestro v10.3<sup>12</sup>. The bond orders were assigned to residues, hydrogen atoms were added at pH 7.0. Minimization was carried out using OPLS 2005 force field with a RMSD cutoff value of 0.3 Å. Preparation of ligands. The 2D structures of all compounds i.e. 4c, 4i, 4j, staurosporine and PP2 were drawn and analyzed by Marvin view. The compounds were converted to 3D structure (.pdb) using LigPrep tool<sup>13</sup>. LigPrep is a Schrödinger suite tool which is used to generate 3D structures from 2D structures, search tautomers, isomers for compounds and carry out energy minimization by applying the OPLS 2005 force field.

#### Molecular docking

The molecular docking was performed and analyzed via the Glide v6.8 docking tool<sup>14</sup>. The receptor grid was centered based on the active site of the protein using receptor grid generation tool. Ligands prepared using LigPrep were flexibly docked in grid box using Monte Carlo based simulation algorithm. An extra precision (XP) method was employed that generated binding poses based on energy. The favourably docked molecules were ranked according to the Glide Score.

#### Molecular dynamics simulation

The docked c-Src kinase proteins with compound 4j were subjected to molecular dynamics simulation with the CHARMM 36 force field in GROMACS 4.6 tool<sup>15</sup>. The simulation was minimized using 5000 steps of Steepest Descent method. The system was

later equilibrated at 300 Kelvin temperature and 1.06 pressures for 200 ps. The time step set was 0.2 fs. The final production was run for 20ns and the distance, RMSF and hydrogen bond trajectories were calculated for the protein-ligand system.

#### Structure activity correlation models

The 2D descriptors and Principal components were computed in MOE CCG computing package. The SVR model was developed using the RapidMiner data mining program<sup>16</sup>. The construction, validation of the SVR model was partitioned into three subsets in 70:20:10 ratio and the model was built by employing the  $\epsilon$ -SVR algorithm and radial basis function (RBF) kernel function, and validated using five-fold validation technique. The algorithm utilizes three parameters, viz. cost function ( $C$ ), kernel gamma ( $\gamma$ ) and width of the loss function ( $\epsilon$ ).

## Results and Discussion

#### Synthesis

Syntheses of the targeted  $\alpha$ -aminophosphonate derivatives (4a-q) were prepared through a three component reaction involving aldehydes, amines and phosphites employing Kabachnik-Fields reaction<sup>17</sup> condition. To the best of our knowledge, no previous reports using this catalyst for such reaction have been reported. All the synthesized compounds have been evaluated for their c-Src kinase inhibitory activity. The IC<sub>50</sub> value ( $\mu$ M) was determined for each compound. Staurosporine and PP2 were employed as the positive controls. Out of the seventeen compounds screened, three compounds (4c, 4j, 4o) showed moderate Src kinase inhibition in the range of IC<sub>50</sub> values between 15.8-63.6  $\mu$ M. In particular, compound 4j, was identified as the potent inhibitor (IC<sub>50</sub>=15.8  $\mu$ M) in the series.

#### Molecular docking analysis

With an objective to explore the binding potential of the synthesized and tested molecules for Src tyrosine kinase, we performed docking studies as shown in Table 1. Among the 17 structures, compounds 4c, 4i and 4j were mainly investigated further for docking simulations since they exhibit higher inhibitory activity as ascertained from their MIC data. Human c-Src tyrosine kinase is a multi-domain protein consisting of 535 amino acids possessing SH3, SH2 domains followed by a short N-terminal and C-terminal regulation segment<sup>18</sup>. In the unphosphorylated structure, the SH2 and SH3

Table 1 — Molecular docking analysis of phosphorylated and unphosphorylated c-Src kinase tyrosine protein with selected compounds. The binding energies were calculated using Glide v6.8 docking tool. - : No docking score was obtained

Sr. no	Compound	Unphosphorylated Protein: 1Y57		Phosphorylated Protein: 2H8H	
		Amino acids involved in intermolecular interactions	GLIDE Score (kcal/mol)	Amino acids involved in intermolecular interactions	GLIDE Score (kcal/mol)
1	4c	Lys295 Asp404	-4.5	Lys295 Ala390	-5.0
2	4i	Asp404	-4.4	Lys295	-4.6
3	4j	Lys295 Ala390	-5.4	Asn391 Lys295	-6.5
4	4p	-	-	Ala390 Ser345	-6.10
5	4m	-	-	Lys295 Ala390	-4.18
6	Staurosporine	Met341	-8.3	-	-
7	PP2	Met341	-7.6	-	-

domains lie at a right angle to each other, with only SH3 domain in contact with the N-terminal lobe<sup>19</sup>. The SH3 domain is bound between SH2 and kinase domains in this inactive conformation<sup>20</sup>. The C-terminal tail folds back to N-terminal, and the active site is exposed as it is not blocked by the activation loop<sup>21</sup>. Upon phosphorylation of Tyr527 in C-terminal or Tyr 416 in the activation loop, the SH2 and SH3 domains lie parallel to the N and C lobes forming an open and closed cleft that determines the access to the catalytic site<sup>22</sup>. Phosphorylation of Tyr 527 down regulates the kinase activity and phosphorylation of Tyr 416 is deemed necessary for exhibiting full kinase activity. The binding site in c-Src protein is an ATP pocket present in the N-lobe region<sup>21</sup>. Compound 4j and 4c were bound to the Lys295 residue present in the C-terminal region of the pocket that acts as an important site for catalysis, binding of a compound to the terminal inhibits further kinase activity. Docking against the un-phosphorylated protein (1Y57) showed that 4j performed better with -5.4 kcal/mol binding energy as compared to 4c and 4i which displayed -4.5 and -4.4 kcal/mol binding energy respectively and formed only a single hydrogen acceptor bond with Asp404. It is to be noted here that the compound 4j displayed a similar good docking score of -6.5 kcal/mol with the phosphorylated protein (2H8H) as well. All the synthesized compounds were docked into the ATP binding site which is located at a cleft between the N and C-terminal lobes, flanked by the hinge region, P-loop, helix  $\alpha$  C and the activation loop. The docked orientations of the 4j compound with respect to the native ligands imatinib derivative in unphosphorylated protein (1Y57) and anilino

quinazoline derivative with phosphorylated protein (2H8H) in the pocket site are depicted in Figures 2a and 2b respectively. It is to be noted that the isopropyl aniline and methyl groups of compound 4j and 4p respectively fit in the pocket regions, whereas the isopropyl aniline group of compound 4m was observed to protrude out of the pocket region thereby leading to a lower binding efficiency. In the docked conformation of 4p, the methyl group did not fit well into the active site cavity. These observations indicate that the *para*-orientation of the isopropyl group in the aniline ring in compound 4j may have an important role in anchoring it within the active site of the receptor.

#### Molecular dynamics study

Molecular dynamics studies were performed for further probing the dynamicity and fluctuations of ligand at the atomic level in the active sites of the unphosphorylated protein (1Y57) and phosphorylated protein (2H8H) hereafter referred to as T1 and T2 respectively. Figure 3a shows the RMSD trajectories of T1 and T2. In the case of T1, the early peak in RMSD of compound 4j during the initial time scale of simulation indicates the relaxation of the compound. It reached equilibration after 3ns, reflecting its stability in the pocket region. The RMSD trajectory for T2 was stable with a rise at 3ns, but is observed to be relaxing back at 13ns. In order to study the behaviour of the compound in a dynamic environment, the root mean square fluctuation (RMSF) of protein-compound complex was performed. The RMSF trajectory (Figure 3b) was more stable in case of T2 as compared to T1; this can be attributed to the open

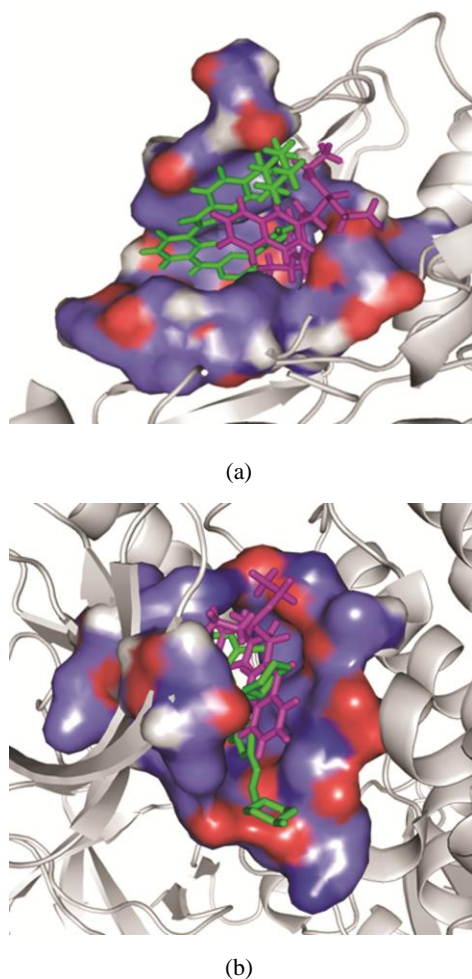


Fig. 2 — (a) Surface view of Compound 4j (pink) and native ligand (green) in the binding pocket of receptor 1Y57 (Unphosphorylated protein)  
(b) Surface view of Compound 4j (pink) and native ligand (green) in the binding pocket of receptor 2H8H (Phosphorylated protein)

conformation of T1 causing high fluctuations in the loop regions. The H-bond distances were calculated between compound and amino acid residues involved in intermolecular interactions and molecular dynamics simulations. The amino group of Lys295 and oxygen atom of compound 4j moved apart for T1, whereas quinoline group of Ala390 and isopropyl aniline group of the compound 4j separated in T2 during the course of the simulation, thereby disrupting the hydrogen bond which was formed during docking. Rhythmic fluctuation of compound, the amino group of Lys295 and oxygen group of Ala390 led to increase in distance, causing dissociation of the hydrogen bond formed during docking. In compound 4p, the amino group of Ala390 and oxygen atom of Ser345 moved apart for T2 during the course of the simulation, thereby disrupting the hydrogen bond which was formed during docking. The results revealed that compound 4p moved apart from its interacting residues similar to compound 4j. The H-bond distance between the compound 4p and interacting residue were very high as compared to 4j, indicating loss of bonding interactions in the active site region. A hydrogen bond distance profile was calculated between binding pocket residues and compound 4j in the entire MD simulation. The heteroatoms of the compound formed strong level via electrostatic bonds (1 - 2.5 Å) between Leu273, the atomic molecular dynamics simulations. Some interesting observations were noted while comparing the simulation results between T1 and T2. In the case of T2, the HB1 atom of compound 4j and H22 atom of Ala293 which were at a distance of 2.6 Å initially moved apart to a distance of 8.3 Å during simulations.

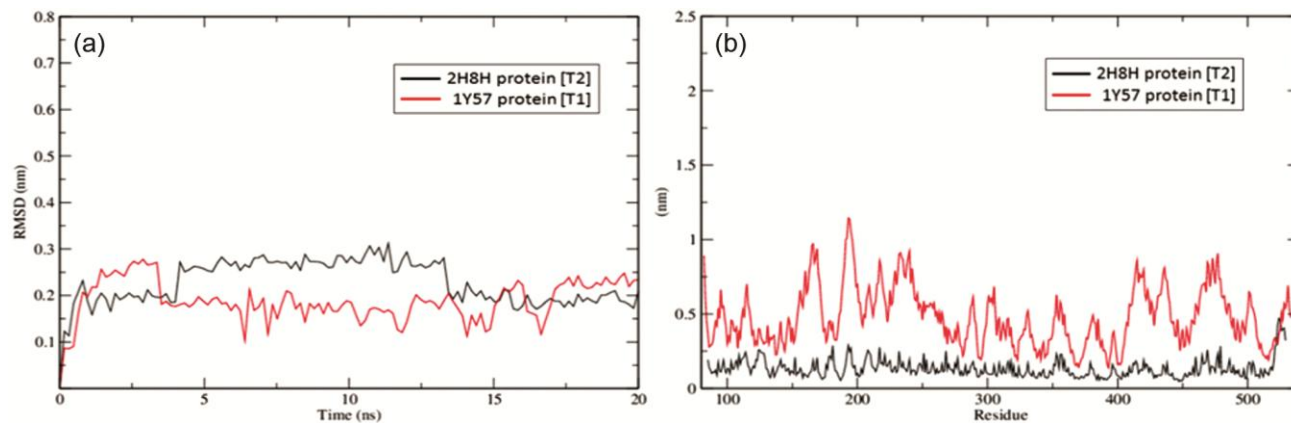


Fig. 3 — (a) Root Mean Square Deviation of compound trajectory for 2H8H (T2) and 1Y57 (T1) protein. The RMSD value indicates stability of compound molecules in the pocket region. (b) Root Mean Square Fluctuation (RMSF) of protein- compound complex for 2H8H (T2) and 1Y57 (T1) protein

Table 2 — Statistical results obtained for known Src kinase inhibitors using SVR model ( $R^2=0.914$ )

Data set	CC	SVR analysis	
		MAPE	RMSE
Total	0.956655	0.176301	4.087586
Training set	0.945926	0.238429	4.333623
Test set	0.983458	0.06091	4.450468
Validation set	0.999823	0.002656	0.001257

Table 3 — Statistical results obtained for 17 compounds using SVR model ( $R^2=0.835$ )

Data set	CC	SVR analysis	
		MAPE	RMSE
Total	0.835001	0.36019	50.99193
Training set	0.956363	0.371753	4.141958
Test set	0.756247	0.451966	84.40276
Validation set	0.833723	0.557296	95.83819

#### Active site analysis

Dynamics studies revealed that Leu273, Gly274 and Val281 from Glycine rich and P-loop region; Lys393 from Activation loop and Ala293 from  $\beta 3$  of the N-terminal lobe may help in understanding the residues playing crucial role in ligand binding. Targeting the N-terminal lobe can inhibit the regulatory mechanism in Src kinases. Inhibiting Leu393 present in the regulatory activation loop, may inactivate tyrosine phosphorylation in Src and other kinases. The inhibition studies varies between phosphorylated and unphosphorylated proteins due to certain conformational changes in the active site which render (i) displacement of the C helix, which removes the catalytically important residue from the active site cleft, (ii) change in the activation loop adopting an  $\alpha$ -helical conformation, which precludes binding of peptide substrates and also sequesters Tyrosine residue, making it inaccessible for phosphorylation, and (iii) constrain in the orientation of the N and C lobes which may not be optimal for catalysis. Thus carrying out a molecular modelling studies against phosphorylated and unphosphorylated cSrc kinase protein using synthesized chromone derivatives will help us to develop a better cSrc kinase inhibitor.

#### Structure activity correlation model

We extracted  $IC_{50}$  data from literature for the reported Src kinase inhibitors and computed 2D molecular descriptors and principal components (Table 2)<sup>23</sup>. The 2D PCA plot displays the chemical space occupied by the newly synthesized compounds with respect to the known Src inhibitor space. A CC

of 0.835 was obtained when the 17 synthesized compounds were applied to the model (Table 3). The  $IC_{50}$  of compound 4j predicted was similar to the value predicted by experimental methods. The clues obtained from the structure correlations studies and help in fine tuning the presently synthesized compounds for further enhancing the bioactivity.

#### Chemoinformatics Analysis

A drugability check was performed for all the 17 synthesized compounds. Lipinski rule of 5 predictions were performed using the Screening Assistant 2 tool<sup>24</sup>. The notion that these compounds could be further developed as anti-cancer compounds was further assisted by ADME properties predicted using PreADMET software<sup>25</sup>. The selected compound 4j prioritized in this study fulfilled the above criteria indicating that it may be further developed in an oral dosage form. TPSA (Topological polar surface area) results indicated satisfactory values for all the 17 compounds<sup>26</sup>. Thus most synthesized compounds predicted favorable ADME predictions. LAZAR (Lazy structure activity relationships) software detects carcinogenic properties based on the similarities in functional group with carcinogenic compounds present in the Lazar database<sup>27</sup>.

#### Conclusion

In summary, a series of chromone/azachromone fused  $\alpha$ -aminophosphonate conjugates have been synthesized using silica chloride as a new catalyst. All the synthesized compounds were evaluated for their c-Src kinase inhibitory activity and one of the compounds 4j was found to be effective with an  $IC_{50}$

value of 15.8  $\mu\text{M}$ . The stability and convergence of dynamics results for the phosphorylated and unphosphorylated proteins with 4j compound in the simulations establishes its potential as an efficient inhibitor. Thus docking and dynamics studies revealed the compound to be more effective against the phosphorylated form of Src kinase. Results also showed that Leu273, Gly274 and Val281 from Glycine rich and P-loop region; Lys393 from Activation loop and Ala293 from  $\beta 3$  of the N-terminal lobe could be considered as potential binding sites for cSrc kinase proteins.

### Acknowledgements

Financial support from the CSIR Network projects (BSC0121, CSC0130, and CSC0108) is gratefully acknowledged. RV thanks Director of MIT school of Bioengineering science and research, MIT ADT University for infrastructure and support.

### References

- Bhise S B, Nalawade A D & Wadhawa H, Role of protein tyrosine kinase inhibitors in cancer therapeutics, *IJBB*, **41**(6) (2004) 273-283.
- Prada E A, & Auernhammer C J, Targeted therapy of gastroenteropancreatic neuroendocrine tumours: preclinical strategies and future targets. *Endocr Connect*, **7**(1) (2018) R1-R25.
- Amin S A, Adhikari N, Shukla V, Jha T & Gayen S, Structural findings of pyrazolo [1, 5-a] pyrimidine compounds for their Pim-1/2 kinase inhibition as potential anticancer agents. *IJBB*, **54**(1) (2017) 32-46.
- Gaspar A, Matos M J, Garrido J, Uriarte E, Borges F, Chromone: a valid scaffold in medicinal chemistry, *Chem rev*, **114** (9) (2014) 4960-4992.
- Keri R S, Budagumpi S, Pai R K, Balakrishna R G, Chromones as a privileged scaffold in drug discovery: A review, *Eur J Med Chem*, **78** (2014) 340-374.
- Mohadeszadeh M, & Iranshahi M, Recent Advances in the Catalytic One-Pot Synthesis of Flavonoids and Chromones, *Mini Rev Med Chem* **17**(14) (2017) 1377-1397.
- Mujahid M, Yogeewari P, Sriram D, Basavanag U, Díaz-Cervantes E, Córdoba-Bahena L, Robles J, Gonnade R, Karthikeyan M & Vyas R, Spirochromone-chalcone conjugates as antitubercular agents: synthesis, bio evaluation and molecular modeling studies. *RSC Adv*, **5** (129) (2015) 106448-106460
- Mujahid M, Gonnade R, Yogeewari P, Sriram D & Muthukrishnan M, Synthesis and antitubercular activity of amino alcohol fused spirochromone conjugates, *Bioorg Med Chem Lett* **23** (5) (2013) 1416-1419.
- Yu C, Qian L, Ge J, Fu J, Yuan P, Yao S C & Yao S Q, Cell-Penetrating Poly (disulfide) Assisted Intracellular Delivery of Mesoporous Silica Nanoparticles for Inhibition of miR-21 Function and Detection of Subsequent Therapeutic Effects, *Ang Chem Int Edi* **55**(32) (2016) 9272-9276.
- Cervantes D P, Bachem C C, Lwanga E E H & Yang X X, PhD Thesis, Glyphosate and AMPA Concentrations in Two Types of Agroecosystems and in the Natural Vegetation of Hopelchen, Mexico, 2016.
- Brandvold K R, Santos S M, Breen M E, Lachacz E J, Steffey M E, & Soellner M B, Exquisitely specific bisubstrate inhibitors of c-Src kinase, *ACS Chem Bio*, **10**(6) (2015) 1387-1391.
- Bhachoo J, & Beuming T, Investigating Protein–Peptide Interactions Using the Schrödinger Computational Suite, *Mod Pep-Pro Int: Met Pro*, (2017), 235-254.
- Release, S. (2016). 3: LigPrep. *Schrödinger, LLC, New York, NY*.
- de Ruyck J, Brysbaert G, Blossey R, & Lensink M F, Molecular docking as a popular tool in drug design, an in silico travel, *Adv Appl Bioinform Chem*, **9** (2016) 1.
- Paissoni C, Spiliotopoulos D, Musco G, & Spitaleri A, GROMACS 2.1: A GROMACS tool to perform MM/PBSA and computational alanine scanning, *Comput. Phys. Commun*, **186** (2015) 105-107.
- Hofmann M & Klinkenberg R, RapidMiner: Data mining use cases and business analytics applications, CRC Press 2013.
- Kumar K S, Krishna B S, Reddy C B, Reddy M V N & Reddy C S, Solvent-free synthesis of  $\alpha$ -aminophosphonates: Cellulose-SO<sub>3</sub>H as an efficient catalyst, *Arab Jour of Chem*, **10** (2017) S368-S375.
- Roskoski Jr R, Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors, *Pharmacol Res*, **94** (2015) 9-25.
- Espada J & Martin-Perez J, An Update on Src Family of Nonreceptor Tyrosine Kinases Biology, In *Int Rev Cell Mol Biol*, **331**, (2017) 83-122, Academic Press.
- Foda Z H, Shan Y, Kim E T, Shaw D E & Seeliger M A, A dynamically coupled allosteric network underlies binding cooperativity in Src kinase, *Nat Commun*, **6** (2015) 5939.
- Banerjee M, Duan Q & Xie Z, SH2 ligand-like effects of second cytosolic domain of Na/K-ATPase  $\alpha 1$  subunit on Src kinase, *PLoS One*, **10**(11) (2015) e0142119.
- Morando M A, Saladino G, D'Amelio N, Pucheta-Martinez E, Lovera S, Lelli M & Gervasio F. L, Conformational selection and induced fit mechanisms in the binding of an anticancer drug to the c-Src kinase, *Sci. Rep*, **6** (2016) 24439.
- (a) Vyas R, Goel P, Karthikeyan M, Tambe S, Kulkarni B, Pharmacokinetic modeling of Caco-2 cell permeability using genetic programming (GP) method, *Lett. Drug Desig. Discov*. **11** (9) (2014) 1112-1118; (b) Vyas R, Bapat S, Goel P, Karthikeyan M, Tambe S S, Kulkarni B D, Application of Genetic Programming (GP) formalism for building disease predictive models from protein-protein interactions (PPI) data, *IEEE/ACM Trans Comput Biol Bioinform*, **15**(1) (2016) 27-37.
- Le Guilloux V, Arrault A, Colliandre L, Bourg S, Vayer P, Morin-Allory L, Mining collections of compounds with Screening Assistant 2, *J Cheminform*, **4** (1) (2012) 20.
- PreADMET, <http://preadmet.bmdrc.org/> (Accessed February 12, 2017).
- Tripathi M, Khan S I, Thakur A, Ponnann P, Rawat D S, 4-Aminoquinoline-pyrimidine-aminoalkanoles: synthesis, in vitro antimalarial activity, docking studies and ADME predictions, *New J Chem*, **39** (5) (2015) 3474-3483.
- LAZAR Toxicity predictions, <https://lazar.in-silico.de/predict> (Accessed August 31, 2017).