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Synthesis and molecular docking studies of novel phenothiazine chalcone derivatives as T3151 mutated Bcr-Abl kinase inhibitors

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ABSTRACT

Six novel phenothiazine chalcone derivatives 2-7 were prepared and characterized by Spectral analysis. Molecular docking studies were carried out in order to assess the affinity between the synthesized compounds 2-7 and T315I mutated Bcr-Abel kinase protein. Compounds 4 and 7 showed very good docking scores and thereby serve as novel templates as T315I mutated Bcr-Abel kinase inhibitors.

KEY WORDS: Chronic myelogenous leukemia, Phenothiazine, Chalcone, Condensation, Molecular docking.

1. INTRODUCTION

Chronic myelogenous leukemia (CML) is a hematological stem cell disorder caused due to unregulated myeloid cell growth in the bone marrow, and due to the accumulation of more white blood cells. Abelson tyrosine kinase (ABL) is important in cell growth and proliferation and is normally under control (Noronha, 2008). However, many CML patients contain the ABL gene from chromosome 9 fused with the breakpoint cluster (BCR) gene from chromosome 22, developing a short chromosome known as the Philadelphia chromosome, and it is responsible for the production of BCR-ABL, an active tyrosine kinase that causes uncontrolled cellular proliferation. An ABL inhibitor, imatinib, was presently used as first line therapy (Mughal, 2013; Desogus, 2015). However, more number of clinical relapse has been observed due to long term treatment with imatinib (Noronha, 2008). A majority of these patients have several point mutations in and around the ATP binding pocket of the ABL kinase domain in BCR-ABL (Michele, 2007; Jorge, 2011). In order to overcome the resistance of mutated BCR-ABL to imatinib, few second generation inhibitors like nilotinib were developed (Noronha, 2008; Karthigai Priya, 2015; Muhammad, 2015, 2016). All of the BCR-ABL mutants are inhibited by the nilotinib like second generation inhibitors except the T315I mutant. Now the development of third generation inhibitors to target the T3151 mutation are in progress (Priya, 2015, Muhammad, 2015).

In the post-genomic period, rational drug design or cancer aims to discover small molecules that changes the activity of key targets important for carcinogenesis. This work mainly aims to identify novel small molecular inhibitors against important molecular targets involved in leukemia (Katritzky, 1981). Presently i*n silico* methods are used to identify the lead molecule. Virtual screening, showed a great promise in drug discovery, plays a vital role in identifying active leads from a large number of compounds (Kapetanovic, 2008). It reduces the chemical space size and allows us to focus on the identification of promising molecules for lead discovery and optimization.

This prompted us to undertake the present research work to try some of the phenothiazine derivatives synthesized in our laboratory to address the resistance of mutated BCR-ABL to first and second generation inhibitors such as imatinib and nilotinib. If the present work is encouraging, it is expected that more number of drugs are available for the treatment of patients with the mutated BCR-ABL-T3151. The success of these inhibitors will also have greater implication in other diseases driven by kinases.

2. MATERIALS AND METHODS

General: All compounds synthesized were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectral data and elemental analyses. Melting points were determined using a BUNA melting point instrument and are uncorrected. IR spectra were recorded on a Shimadzu Affinity-I FT-IR spectrometer. NMR spectra were measured at 400 MHz on a Bruker-400 spectrometer using TMS as internal standard and DMSO-d_6 as solvent. Elemental analyses was carried out using a Perkin Elmer 240C Elemental Analyzer. 2-acetyl phenothiazine has been obtained from Sigma Aldrich and all the chemicals used in this study were of AR grade and purchased from M/s High Media, India. The Molecular Docking study was carried out using Argus 4.0.1 and Pymol 1.5 software.

Active Site Prediction: The Active sites of tyrosine kinase Receptor (PDB ID: 2V7A with imatinib) along with area and volume of binding pocket was determined with Meta Pocket 2.0 Finder program (http://metpocket.eml.org.) (Zengming Zhang, 2011).

Preparation of the Protein: The three dimensional crystal structure of the T3151 Abl mutant in complex with the aura kinase inhibitor was retrieved from the Protein Data Bank (http://www.rscb.org/pdb) (Modungo, 2007). The complexes attached to the receptor molecule, the hetero atoms and the unwanted water molecules were removed and finally hydrogen atoms were merged to the target receptor molecule using Argus Lab.

Preparation of the ligands: The structures of phenothiazine derivatives synthesized in our laboratory 1-6 were drawn using Chemdraw 8.0 software and converted to Pymol format with the standard settings and then made available for docking studies.

Molecular Docking: The molecular docking program Aurgus Lab software which is most commonly available software was used to perform the virtual screening. Dockings were carried out under 'Standard default settings' mode. All the parameters used in Argus lab docking were selected by default. Calculation type was set to "dock" mode and "flexible mode" was selected for the ligand. The docking results were studied using PYMOL (TM) software, which allows visualization of the ligand-protein docking and calculation of several descriptors like feasible hydrogen bonding between the protein and the ligand. The scores were calculated and presented in the table. Least energy indicated the easy binding character of ligand and receptor.

Synthesis of the compounds

General procedure: Compound 2 was prepared as reported and characterised by us earlier.

(*E*)-5-(4-(3-oxo-3-(10H-phenothiazin-2-yl)prop-1-en-1-yl)benzylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (3)

To a suspension of Barbituric acid (1.2 mmol) in methanol (50 ml), 2-acetyl phenothiazine chalcone **2**(1mmol), catalytic amount of piperidine(10 drops) and glacial acetic acid (10 drops) were added.The mixture was stirred and refluxed overnight (12-16 hrs).After cooling, the solvent was evaporated, dried. The compound was purified by silica gel column chromatography ($CHCl_3$: methanol) 9.5:0.5.

Yield:57%; m.p: 182 ºC, UV(λ_{max} nm): 448.50, 319.50, 247.50; IR (KBr) cm^{-1}: 3348 (NH), 1651 (C=O), 1581 (C=C), 1554, 1523, 1473, 1435, 1400, 1330, 1307, 1188, 1072, 1006, 975, 941, 798, 736; ^{1}H-NMR (400MHz $CDCl_3$) δ: 7.29 (1H, d, *J*=1.6 Hz, H-1'), 7.02-6.98 (1H, td, *J*= 8, 1.6 Hz, H-3'), 7.07 (1H, d, *J*= 8 Hz, H-4'), 6.93-6.90 (1H, dd, *J*= 8, 1.6Hz, H-6'), 6.78-6.74 (1H, td, *J*= 8, 1.6Hz, H-7'), 7.63-7.60 (1H, dd, *J*= 8, 1.6 Hz, H-8'), 6.67-6.64 (1H, dd, *J*= 8, 1.6 Hz, H-9'), 7.83-7.80 (4H, m, H-3, 2",6", H-1'''), 7.67 (3H, m, H-2, 3",5") 8.78 (1H, s, NH); ^{13}C-NMR: 112.87 (C-1'), 136.74(C-2'), 122.70 (C-3'), 130.64 (C-4'), 126.10 (C-6'), 114.58 (C-7'), 126.23 (C-8'), 115.17 (C-9'), 141.06(C-9a'), 114.58 (C-5a'), 142.13 (C-10a'), 123.76 (C-4a'), 187.94(C = O), 122.09 (C-2), 142.32 (C-3), 133.97(C-1"), 127.97 (C-2", 6"), 131.87 (C-3", 5"), 122.59 (C-4"), 145.21(C-1'''), 165.32, 165.78 and 161.12 (C=O). MS *m/z* = 468.3 $[M+H]^+$; Anal.Calcd. for $C_{26}H_{17}N_3O_4S$: C, 66.80; H, 3.67; N, 8.99; Found: C, 66.20; H, 3.43; N, 9.29

(*E*)-5-(4-(3-oxo-3-(10H-phenothiazin-2-yl)prop-1-en-1-yl)benzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (4)

To a suspension of ThioBarbituric acid (1.2 mmol) in methanol (50 ml), 2-acetyl phenothiazine chalcone**2**(1mmol), catalytic amount of piperidine(10 drops) and glacial acetic acid (10 drops) were added.The mixture was stirred and refluxed overnight (12-16 hrs).After cooling, the solvent was evaporated. The compound was purified by silica gel column chromatography ($CHCl_3$: methanol) 9.5:0.5.

Yield: 62%; m.p: 167 ºC, , UV (λ_{max} nm): 448.50, 319.50, 247.50; IR (KBr) cm^{-1}: 3348 (NH), 1651 (C=O), 1581 (C=C), 1554, 1523, 1473, 1435, 1400, 1330, 1307, 1188, 1072, 1006, 975, 941, 798, 736; ^{1}H-NMR (400MHz $CDCl_3$) δ: 7.29 (1H, d, *J*=1.6Hz, H-1'), 7.02-6.98 (1H, td, *J*=8, 1.6 Hz, H-3'), 7.07 (1H, d, *J* = 8 Hz, H-4'), 6.93-6.90 (1H, dd, *J*= 8, 1.6 Hz, H-6'), 6.78-6.74 (1H, td, *J*=8, 1.6Hz, H-7'), 7.63-7.60 (1H, dd, *J*=8, 1.6 Hz, H-8'), 6.67-6.64 (1H, dd, *J*=8, 1.6 Hz, H-9'), 7.83-7.80 (4H, m, H-3, 2",6", H-1'''), 7.67 (3H, m, H-2, 3",5") 8.78 (3H, s, NH); ^{13}C-NMR: 112.87 (C-1'), 136.74(C-2'), 122.70 (C-3'), 130.64 (C-4'), 126.10 (C-6'), 114.58 (C-7'), 126.23 (C-8'), 115.17 (C-9'), 141.06(C-9a'), 114.58 (C-5a'), 142.13 (C-10a'), 123.76 (C-4a'), 187.94(C = O), 122.09 (C-2), 142.32 (C-3), 133.97(C-1"), 127.97 (C-2", 6"), 131.87 (C-3", 5"), 122.59 (C-4"); 146.09(C-1'''), 166.52, 165.86 (C=O) and 181.28(C=S). MS *m/z* = 484.2 $[M+H]^+$ Anal.Calcd. for $C_{26}H_{17}N_3O_3S_2$: C, 64.58; H, 3.54; N, 8.69; Found: C, 64.58; H, 3.54; N, 8.69

(*E*)-1-(10H-phenothiazin-2-yl)-3-(4-((E)-(phenylimino)methyl)phenyl)prop-2-en-1-one (**5**)**:** To a solution of 2-acetyl phenothiazine chalcone **2** (0.01 mol) in chloroform (10 ml) is added 0.01ml of aniline and the mixture was refluxed for 30 min. After cooling, the solvent was evaporated and the resulted solid was washed with 2 ml of dil.HCl and water, filtered and dried. The purity of the compound**5** was checked by TLC using chloroform as the solvent.

Yield: 70%, m.p: 143 ºC, UV (λ_{max} nm): 432.50, 315.50, 248.50; IR (KBr) cm^{-1}: 3348 (NH), 1651 (C=O), 1581 (C=C), 1554, 1523, 1473, 1400, 1330, 1307, 1188, 1072, 1006, 975, 798, 73; ^{1}H-NMR (400MHz $CDCl_3$) δ: 7.31 (1H, d, *J* = 1.6 Hz, H-1'), 7.70-7.68 (1H, dd, *J* = 8, 1.6 Hz, H-3'), 7.10 (1H, d, *J* = 8 Hz, H-4'), 6.93-6.91 (1H, dd, *J* = 8, 1.6 Hz, H-6'), 6.79-6.75 (6H, td, *J* = 8, 1.6 Hz, H-7', and phenyl ring protons), 7.01-6.98 (1H, td, *J* =8 , 1.6 Hz, H-8'), 6.67 (2H, m, H-9' and H-1'''), 7.80 (1H, d, *J*=16Hz, H-2), 7.98 (1H, d, *J*= 16 Hz, H-3), 8.74 (1H, t, *J*= 8 Hz, H-2"), 8.31-8.25 (2H, m, H-4", 6"), 7.74 (1H, t, *J*=8Hz, H-5"), 8.75 (1H, s, NH); MS *m/z* = 484.2 $[M+H]^+$. Anal.Calcd. for $C_{28}H_{20}N_2OS_3$: C, 77.75; H, 4.66; N, 6.48; Found C, 76.98; H, 5.61; N, 6.91

(Z)-4-((E)-3-oxo-3-(10H-phenothiazin-2-yl)prop-1-en-1-yl)benzaldehyde oxime (**6):** To a solution of the chalcone **2** (0.01 mol) in chloroform (10 ml) is added 0.01ml of hydroxylamine and the mixture was refluxed for 30 min. After cooling, the solvent was evaporated and the resulted solid was washed with water, filtered and dried.

Yield: 70%, m.p: 134 ºC, IR (KBr) cm^{-1}: 3348 (NH & OH), 1651 (C=O), 1581 (C=C), 1554, 1523, 1473, 1400, 1330, 1307, 1188, 1072, 1006, 975, 798, 73; ^{1}H-NMR (400MHz $CDCl_3$) δ: 7.31 (1H, d, *J*=1.6Hz, H-1'), 7.70-7.68 (1H, dd, *J*= 8, 1.6Hz, H-3'), 7.10 (1H, d, *J*=8Hz, H-4'), 6.93-6.91 (1H, dd, *J*=8, 1.6Hz, H-6'), 6.79-6.75 (1H, td, *J*=8,

1.6Hz, H-7'), 7.01-6.98 (1H, td, *J*=8, 1.6Hz, H-8'), 6.67 (1H, d, *J*=8, 1.6Hz, H-9'), 7.80 (1H, d, *J*=16Hz, H-2), 7.98 (1H, d, *J*=16Hz, H-3), 8.74 (1H, t, *J*=8Hz, H-2"), 8.31-8.25 (3H, m, H-4", 6", H-1'''), 7.74 (1H, t, *J*=8Hz, H-5"), 8.75 (1H, s, NH); ^{13}C-NMR: 112.86 (C-1'), 136.57 (C-2'), 122.08 (C-3'), 134.92 (C-4'), 130.31 (C-6'), 114.58 (C-7'), 126.07 (C-8'), 115.14 (C-9'), 141.03 (C-9a'), 114.58 (C-5a'), 141.11 (C-10a'), 124.04 (C-4a'), 187.85 (C = O), 124.37 (C-2), 142.18 (C-3), 136.53 (C-1"), 122.82 (C-2", 6"), 132.21(C-4''), 148.39 (C-1'"), 126.21 (C-5"), (C-3"). MS *m/z* = 373.6 $[M+H]^+$. Anal.Calcd. for $C_{22}H_{16}N_2O_2S$: C, 70.95; H, 4.33; N, 7.52; Found; C, 71.58; H, 4.16; N, 8.45.

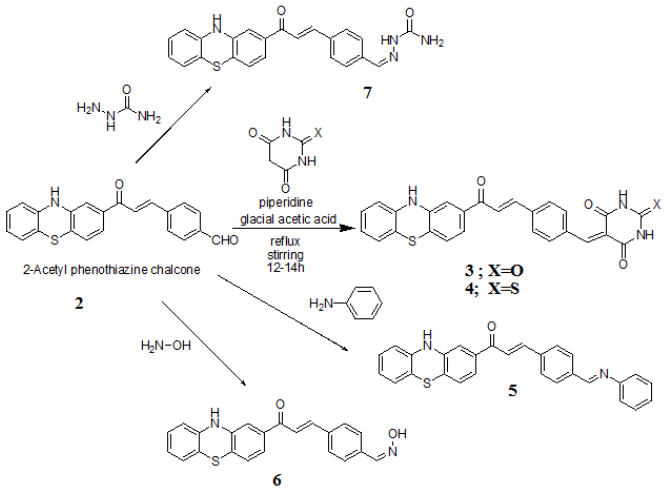
(Z)-2-(4-((E)-3-oxo-3-(10H-phenothiazin-2-yl)prop-1-en-1-yl)benzylidene)hydrazinecarboxamide (7): To a solution of the chalcone **2** (0.01 mol) in chloroform (10 ml) added 0.01ml of semicarbazideand the mixture was refluxed for 30 min. After cooling, the solvent was evaporated and the resulted solid was washed with water, filtered and dried to yield the compound 6.

Yield: 71%, m.p: 162 ºC, IR (KBr) cm^{-1}: 3348 (NH), 1651 (C=O), 1581 (C=C), 1554, 1523, 1473, 1435, 1400, 1330, 1307, 1188, 1072, 1006, 975, 941, 798, 736; ^{1}H-NMR (400MHz $CDCl_3$) δ: 7.29 (1H, d, *J*=1.6Hz, H-1'), 7.02-6.98 (1H, td, *J* = 8, 1.6Hz, H-3'), 7.07 (1H, d, *J*=8Hz, H-4'), 6.93-6.90 (1H, dd, *J*=8, 1.6Hz, H-6'), 6.78-6.74 (1H, td, *J*=8, 1.6Hz, H-7'), 7.63-7.60 (1H, dd, *J*=8, 1.6Hz, H-8'), 6.67-6.64 (1H, dd, *J*=8, 1.6Hz, H-9'), 7.83-7.80 **(**4H, m, H-3, 2",6" and H-1'''), 7.67 (3H, m, H-2, 3",5") 8.78 (1H, s, NH); ^{13}C-NMR: 112.87 (C-1'), 136.74(C-2'), 122.70 (C-3'), 130.64 (C-4'), 126.10 (C-6'), 114.58 (C-7'), 126.23 (C-8'), 115.17 (C-9'), 141.06(C-9a'), 114.58 (C-5a'), 142.13 (C-10a'), 123.76 (C-4a'), 187.94(C = O), 122.09 (C-2), 142.32 (C-3), 133.97(C-1"), 127.97 (C-2", 6"), 131.87 (C-3", 5"), 132.59 (C-4") and 163.74(C=O); MS *m/z* = 415.32 $[M+H]^+$. Anal. Calcd. for $C_{23}H_{18}N_4O_2S$: C, 66.65; H, 4.38; N, 13.52; Found; C, 66.38; H, 4.16; N, 13.33.

3. RESULTS AND DISCUSSION

Synthesis of the compounds: From medicinal chemistry point of view, phenothiazines are novel groups of condensed three ring heterocycles. Phenothiazine derivatives and their hetero analogues compounds containing 1, 4-thiazine structural fragment exhibit diverse biological activities. Phenothiazine derivatives that contain amino alkyl substituents at the thiazine nitrogen atom are used as antipsychotic and antihistamine drugs. In the present work to start with a series of phenothiazine derivatives has been prepared and characterized.

Claisen-Schmidt condensation (Scheme 1) of equimolar quantities of phenothiazinyl methyl ketone 1 with terethaldehyde in the presence of alcoholic alkali yield the chalcone 2. The compound 2 is condensed with barbituric acid, thiobarbituric acid, semicarbazide, hydrazine hydrate and semicarbazide to form the phenothiazine derivatives 3-7. The analytical data, reaction conditions and the yield of the product 3-7 were given in the experimental section.



Scheme.1. Synthesis of compounds 3-7

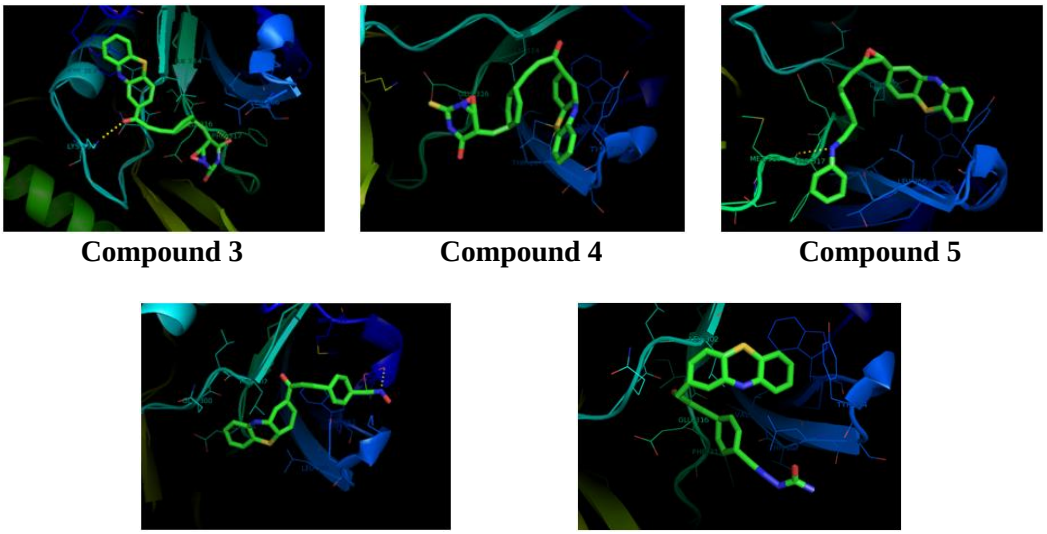
For the compounds 3-7, IR spectra showed characteristic absorption bands to show the presence of carbonyl group at 1651cm^{-1}, C=C at 1600cm^{-1} and NH stretching at 3336.85 cm^{-1}. For all the synthesized compounds, the signals for the aromatic carbons and protons were assigned using known effects of substituents, position, multiplicities and integral values. In ^{1}H-NMR spectra for the compound 3-7 H-2 and H-3 are found to be trans protons where δ value appears between δ 7.30 and 7.77 and the coupling constant *J* value is 16Hz. NH proton appeared as a singlet at δ 8.79. In **3**, the singlet at δ 10.05 is due to CHO group and all the aromatic protons appeared between δ 6.50-8.28. The ^{13}C –NMR signals were assigned based on their positions and intensities. The ^{13}C-NMR spectrum of chalcone were recorded in $CDCl_3$and spectral signals were in good agreement with the proposed structures; C-1 (i.e) C=O group shows the presence at δ 187.92. In 3 the aldehyde carbon appeared at δ 192.59 and all the aromatic carbon or unsaturated C=C appeared between100-160. Characteristic molecular ion peaks were observed in the mass spectra of the chalcone and shown in reported in the experimental section.

Docking studies: T315I mutated Bcr-Abl kinase structure was derived from PDB and used as a target for docking simulation to investigate if the compounds 2-7 have a similar mechanism as Bcr-Abl kinase inhibitors. Docking was performed using Argus lab software and the the crystal structure of protein T315I mutated Bcr-Abl kinase was refined from the crude PDB structure and then saved as mole file to be used for docking simulation. Compound 2-7 were constructed on ChemDraw8.0. Structure and the 2D structure of the selected compounds were converted to their 3D form, and then energy minimized and saved as mol. The observed negative value for the docking score energy (Table 1) indicate the binding affinity of these compounds into T315I mutated Bcr-Abl kinase , which may give a reasonable explanation for their high activity as third generation inhibitors.

Table.1.Docking scores, H-bonded amino acids and the amino acids interacting in the hydrophobic pocket.

Compound	Score	H-bonding	Amino acid residue in the hydrophobic pocket
2	-10.9211	LYS 294	LYS 219, ILE 235,ILE 314, LEU 266, GLU 316
3	-11.0901	LYS 294, PHE 317	LYS 219, ILE 235,ILE 314, LEU 266, GLU 316
4	-12.0787	-	GLU 316, ILE 314, THR 267, TRP 361, TYR 264
5	-11.019	MET 318	PHE 317, VAL 268, LEU 266,TRP-261,LEU302
6	-11.0301	ASP 341	GLN-300,LEU302,GLU316,TRP-261,LEU266,MET237
7	-12.0519	-	PHE312,THR267,VAL268,TYR-264,TRP-261,LEU-302

Docking scores, H-bonded amino acids and the surrounding amino acids in the hydrophobic binding pocket were shown in the table 1 and in the Fig1. It is evident that both hydrophilic and hydrophobic interactions are present between the compounds **2-7** and the T315I mutated Bcr-Abl kinase. Among all the compounds tested compounds **4** and **7** have remarkably good scores which shows a strong binding affinity between the molecules and the protein. Energetic analysis in Table 1 demonstrated the lower binding energy of inhibitors **4** and **7** with T315I mutated Bcr-Abl kinase are under the participation of Van der Waals forces and hydrophobic interaction during the process of enzyme-inhibitor interaction. The interacting amino acids are in the hinge region of the protein where the ATP biding site is present.



Compound 3 **Compound 4** **Compound 5**

Compound 6 **Compound 7**

Figure.2.3D image of compound 3-7 Binding with active site of 2V7A

4. CONCLUSION

Six novel phenothiazine chalcone derivatives **2-7** were synthesized and identified by IR, NMR and Mass spectral data. Molecular docking studies were carried out between the compounds **3-7** and T315I mutated Bcr-Abl kinase to assess whether these molecules can behave as T315I mutated Bcr-Abl kinase inhibitors. Compounds **4** and **7** showed docking scores of 12.0787 and 12.0507 K cal/mol and thereby suggests to serve as novel templates as T315I mutated Bcr-Abl kinase inhibitors for further studies.

5. ACKNOWLEDGMENTS

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