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NITROGEN BRIDGED IMIDAZO PYRIMIDINE ACETAMIDES: SYNTHESIS, MOLECULAR DOCKING, TOXICITY PREDICTION AND ANTI-PROLIFERATIVE STUDIES

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ABSTRACT
In the present investigation new series of imidazo[1,2-a]pyrimidines were designed and synthesized by the reaction of various 4-Substituted aryl 2-methyl oxazole 5-ones(**2a-2n**) with the 2-amino pyrimidine. The formation of imidazo[1,2-a] pyrimidine acetamides(**4a-n**) were further confirmed by the physical, IR, NMR and mass spectra and elemental analysis. All the compounds (**4a-4n**) were screened for anti proliferative activity by employing MTT assay on A549 lung cancer cell lines. From the *in vitro* antiproliferative screening results it was concluded that compounds **4n(**IC_{50} value 1.4 µmol**), 4c(**IC_{50} value 2.1 µmol**),** and **4i (**IC_{50} value 2.6 µmol**),** are potent antiproliferative agents,whereas, the other compounds tested were less potent than the standard drug (Dabrafenib IC_{50} value 0.003 µmol). Molecular docking studies of these compounds were carried out to assess the ability of these compounds to bind and inhibit the B-Raf kinase using Autodock and Schrodinger. In the docking studies it was observed that compounds 4n, 4c, 4h and 4i are good ligands for inhibition of the B-raf kinase. All the designed compounds were subjected to molecular property prediction by using OSIRIS property explorer for liphophilicity, solubility and drug likeness. The toxicity profile of the molecules was also predicted to identify potentially toxic compounds, druglikeness and pharmacokinetic parameter such as percentage absorption in the series before being tested in other biological models.

KEYWORDS: Imidazopyrimidinyl acetamide derivatives, 4-arylidene oxazolones, anti-proliferative activity. Osiris molecular property explorer.

INTRODUCTION
Fusion of pyrimidine with different heterocyclic scaffolds gives rise to a new set of hybrid heterocycles with enhanced activities.[1] These heterocycles when incorporated with sulphur and nitrogen in the core structure demonstrated a number of pharmacological and biological activities. Even though the remarkable pharmacological properties of various fused pyrimidines like purines, pteridines, quinazolines, pyridopyrimidines, triazolopyrimidines, pyrazolopyrimidines, pyrimidoazepines, furopyrimidines and pyrrolopyrimidines were studied and reported[2,3], the literature suggests that very few work has been done on substituted imidazo[1,2-a] pyrimidines. In this context, imidazo[1,2-a]pyrimidines were found to be interesting skeletons[4] for designing, synthesizing and screening for its possible anticancer activity as Raf Kinase inhibitors.

B-raf kinase is the regulating protein kinase, play a vital role in cell division, differentiation and secretion through MAP kinase/ERK pathway.[5] The inherited and acquired mutation of B-raf gene is associated with several cancers in humans including lymphomas, melanomas, papillary thyroid carcinoma, non-small-cell lung carcinoma, colorectal cancer and adenocarcinoma of the lung.

In recent years, the B-RAF kinase was considered to be a potential and high yield novel anticancer target. Moreover, most of the pharmaceutical firms are developing specific inhibitors of mutated B-raf protein for anticancer use. Vemurafenib (RG7204 or PLX4032), licensed by the US Food and Drug Administration as Zelboraf for the treatment of metastatic melanoma, is the current state-of-the-art example for why active B-Raf

inhibitors are being pursued as drug candidates. Vemurafenib is biochemically interesting as a mechanism to target cancer due to its high efficacy and selectivity. More general B-raf inhibitors include GDC-0879, PLX-4720, Sorafenib Tosylate dabrafenib and LGX818.[6]

MATERIALS AND METHODS

Chemistry

The solvents were purified according to the standard procedures and all commercial chemicals used were of synthetic grade from SD fine chemicals Ltd., E.Merck and Aldrich chemicals. Completion of the reactions was monitored by thin layer chromatography (TLC) using E-Merck 0.25 mm precoated silica gel plates and eluting solvents were indicated in the procedures. Visualization was accomplished with UV light (256 nm) and iodine chamber. Purification of synthesized compounds was done by re-crystallization process. Melting points determinations were performed in a kofler hot-stage melting point apparatus and are uncorrected. The IR spectra were recorded on Schimadzu FTIR spectrophotometer by using 1% potassium bromide discs. ^{1}H-NMR spectra were determined on a Bruker-400 NMR spectrometer using DMSO-d6 or $CDCl_3$ with TMS as the internal standard. Mass spectra was recorded Quadrapole mass spectrophotometer. Elemental analyses for C, H, N, O, Br and Cl of all compounds synthesized were within ± 0.4% of theoretical values.

General procedure for the synthesis of Acetyl glycine(1)

0.5 mol of glycine was added to 150 ml of distilled water in a conical flask, stirred vigorously until the solvent gets completely dissolved.1mol of acetoyl chloride was added in one portion and stirred for 15-20 minutes. The obtained solution was crystallized in a refrigerator overnight. The precipitate was collected and washed with ice-cold water and dried at 100^{0}C. The combined filtrate and washings were also evaporated to dryness and recrystallized using boiling water. %yield: 97, mp: 208^{0}C (207-209^{0}C-Literature value).

General procedure for the synthesis of 4-Arylidene-2-acetyl oxazole-5(4H)-ones (2a-2n)

A mixture of aromatic aldehyde (0.25 mol), acetyl glycine(0.25 mol), acetyl chloride (1 mol) were placed in a 500 ml conical flask and heated on a electrical plate with constant shaking. As soon as the mixture has been liquified completely, the contents were transferred to a RB flask and was refluxed for 2 hrs. Then 100 ml of ethanol was added to the contents of the flask, and the mixture was allowed to stand overnight. The crystalline product was filtered through suction, and washed with two 25 ml portions of ice-cold alcohol and then with two 25 ml portions of boiling water and dried at 100 ^{0}C.

Synthesis of N-1 substituted 2,3-dihydro imidazo[1,2-a]pyrimidine acetamide derivatives(4a-4n)

A mixture of 4-Arylidene 2-acetyl oxazole-5(4H)-one (0.001 mol) and 2-Amino pyrimidine (0.001 mol) was added to flask containing ethylene glycol (5 ml) and heated to 120^{0}C. The progress of the reaction was monitored by TLC. At the end of the reaction, the reaction mixture was cooled to room temperature and then poured into water (50ml) and filtered to give the crude product. The obtained product was filtered through Buchner funnel and was recrystallized from ethanol. All the compounds synthesized were purified and the structures were further characterized by spectral and elemental analysis.

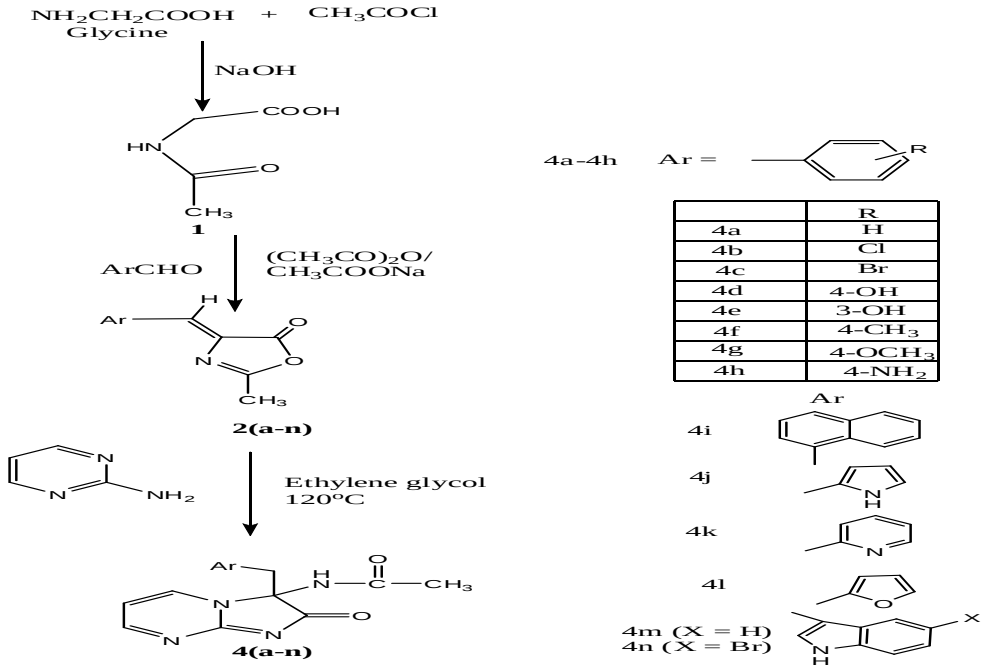

Figure 1 Synthetic scheme for the synthesis of N-substituted 2-oxo-2,3-dihydro imidazo[1,2-a]pyrimidine acetamide derivatives 4a-4n

N-(3-benzyl-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4a)
% Yield: 85%, mp(^{O}C): 266-269, IR(KBr) v (cm-1): 3343(NH); 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.61(s,1H,NH), 3.71(s,2H,-CH_2), 1.84(s,3H,-CH_3), MS m/z: 282(M+), Anal. calcd. (%) for: $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85; O, 11.34, found: C, 63.80; H, 5.02; N, 19.82; O, 11.36.

N-(3-(4-chlorobenzyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4b)
% Yield: 86%, mp(^{O}C): 292-295, IR(KBr) v (cm-1): 3453(NH); 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.75(s,1H,NH), 3.73(s,2H,-CH_2), 1.84(s,3H,-CH_3), MS m/z: 316(M+), Anal. calcd. (%) for: $C_{15}H_{13}ClN_4O_2$: C, 56.88; H, 4.14; Cl, 11.19; N, 17.69; O, 10.10, found: C, 56.84; H, 4.18; Cl, 11.23; N, 17.65; O, 10.10.

N-(3-(4-bromobenzyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4c)
% Yield: 75%, mp(^{O}C): 296-298, IR(KBr) v (cm-1): 3546NH); 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.63(s,1H,-NH), 3.75(s,2H,-CH_2), 1.8(s,3H,-CH_3), MS m/z: 361(M+), Anal. calcd. (%) for: $C_{15}H_{13}BrN_4O_2$: C, 49.88; H, 3.63; Br, 22.12; N, 15.51; O, 8.86, found C, 49.91; H, 3.60; Br, 22.10; N, 15.52; O, 8.87.

N-(3-(4-hydroxybenzyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4d)
% Yield: 79%, mp(^{O}C): 268-270, IR(KBr) v (cm-1): 3330(NH); 3391(OH); 2951(CH), ^{1}H NMR (DMSO) δ (ppm): 9.75(s,1H,-NH), 3.73(s,2H,-CH_2) 1.83(s,3H,-CH_3), MS m/z: 298(M+), Anal. calcd. (%) for: $C_{15}H_{14}N_4O_3$: C, 60.40; H, 4.73; N, 18.78; O, 16.09, found C, 60.60; H, 4.51; N, 18.81; O, 16.06.

N-(3-(3-hydroxybenzyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4e)
% Yield: 77%, mp(^{O}C): 254-255, IR(KBr) v (cm-1): 3429(NH); 3395(OH); 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.61(s,1H,-NH), 3.71(s,2H,-CH_2),1.82(s,3H,-CH_3), MS m/z: 298(M+), Anal. calcd. (%) for: $C_{15}H_{14}N_4O_3$: C, 60.38; H, 4.75; N, 18.75; O, 16.12, found C, 60.42; H, 4.71; N, 18.70; O, 16.17.

N-(3-(4-methylbenzyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4f)
% Yield: 79%, mp(^{O}C): 264-268, IR(KBr) v (cm-1): 3233(NH); 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.7(s,1H,-NH), 3.74(s,2H,-CH_2), 2.21(s,3H,-CH_3) 1.82(s,3H,-CH_3), MS m/z: 296(M+), Anal. calcd. (%) for: $C_{16}H_{16}N_4O_2$: C, 64.85; H, 5.44; N, 18.91; O, 10.80, found C, C, 64.79; H, 5.46; N, 18.93; O, 10.82

N-(3-(4-methoxybenzyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4g)
% Yield: 65, mp(^{O}C): 276-278, IR(KBr) v (cm-1): 3421(NH); 2941(CH); 2998; 2938, ^{1}H NMR (DMSO) δ (ppm): 9.6(s,1H,-NH), 2.39(s,3H,-OCH_3), 3.72(s,2H,-CH_2), MS m/z: 312(M+), Anal. calcd. (%) for: $C_{16}H_{16}N_4O_3$: C, 61.53; H, 5.16; N, 17.94; O, 15.37, found C, 61.55; H, 5.14; N, 17.98; O, 15.33.

N-(3-(4-aminobenzyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4h)
% Yield: 72, mp: 242-245, IR(KBr) v (cm-1): 3251(NH), 3243(NH), 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.65(s,1H,-NH), 5.32(s,2H,-NH_2), 3.71(s,2H,-CH_2), 1.84(s,3H,-CH_3), MS m/z: 297(M+), Anal. calcd. (%) for: $C_{15}H_{15}N_5O_2$: C, 60.58; H, 5.10; N, 23.58; O, 10.75, found C, 60.60; H, 5.09; N, 23.56; O, 10.76.

N-(3-(naphthalen-1-ylmethyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3yl)acetamide(4i)
% Yield: 71, mp(^{O}C): 254-258, IR(KBr) v (cm-1): 3123(NH); 2938(CH), ^{1}H NMR (DMSO) δ (ppm): 9.72(s,1H,-NH), 3.72(s,2H,-CH_2), 1.84(s,3H,-CH_3), MS m/z: 332(M+), Anal. calcd. (%) for: $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.86; O, 9.63, found C, C, 68.70; H, 4.88; N, 16.84; O, 9.66.

N-(3-((1H-pyrrol-3-yl)methyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3yl)acetamide(4j)
% Yield: 87, mp(^{O}C): 306-310, IR(KBr) v (cm-1): 3456(NH), 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.65(s,1H,-NH), 3.71(s,2H,-CH_2), 1.87(s,3H,-CH_3), MS m/z: 271(M+), Anal. calcd. (%) for: $C_{13}H_{13}N_5O_2$: C, 57.56; H, 4.83; N, 25.82; O, 11.80, found C, 57.49; H, 4.86; N, 25.84; O, 11.82.

N-(2-oxo-3-(pyridin-3-ylmethyl)-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4k)
% Yield: 69, mp(^{O}C): 322-326, IR(KBr) v (cm-1): 3339(NH);2942(CH), ^{1}H NMR (DMSO) δ (ppm): 9.78(s,1H,-NH), 3.73(s,2H,-CH_2), 1.81(s,3H,-CH_3), MS m/z: 283(M+), Anal. calcd. (%) for: $C_{14}H_{13}N_5O_2$: C, 59.36; H, 4.63; N, 24.72; O, 11.30, found C, 59.30; H, 4.60; N, 24.79; O, 11.34.

N-(3-(furan-3-ylmethyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4l)
% Yield: 73, mp(^{O}C): 306-309, IR(KBr) v (cm-1): 3246(NH); 2939(CH), ^{1}H NMR (DMSO) δ (ppm): 9.62(s,1H,-NH), 3.75(s,2H,-CH_2), 1.82(s,3H,-CH_3), MS m/z: 272(M+), Anal. calcd. (%) for: $C_{13}H_{12}N_4O_3$: C, 57.35; H, 4.44; N, 20.58; O, 17.63, found C, 57.38; H, 4.40; N, 20.59; O, 17.64.

N-(3-((1H-indol-3-yl)methyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-)acetamide(4m)
% Yield: 81, mp(^{O}C): 365-366, IR(KBr) v (cm-1): 3368(indole NH); 3234(NH); 2936(CH), ^{1}H NMR (DMSO) δ (ppm): 9.77(s,1H,-NH), 3.70(s,2H,-CH_2), 1.80(s,3H,-CH_3), MS m/z: 321(M+), Anal. calcd. (%) for: $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79; O, 9.96, found C, 63.50; H, 4.75; N, 21.68; O, 10.07.

N-(3-((5-bromo-1H-indol-3-yl)methyl)-2-oxo-2,3-dihydroimidazo[1,2-a]pyrimidin-3-yl)acetamide(4n)

% Yield: 77, mp(^{O}C): 374-378, IR(KBr) v (cm-1): 3243(NH); 2924(CH), ^{1}H NMR (DMSO) δ (ppm):10.85(s,1H,-NH), 9.61(s,1H,-NH), 3.61(s,2H,-CH_2), 1.78(s,3H,-CH_3), MS m/z: 399(M+), Anal. calcd. (%) for: $C_{17}H_{14}BrN_5O_2$: C, 51.02; H, 3.53; Br, 19.96; N, 17.50; O, 8.00, found C, 50.99; H, 3.57; Br, 19.97; N, 17.47; O, 8.02.

***In vitro* antiproliferative activity**

The synthetic imidazo[1,2-a] pyrimidine derivatives(4a-4n) were tested for their antitumor activities against A549 (Lung cancer) cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide(MTT) assay method. A549 (Lung cancer), cell lines were seeded in 96-well plates at a density of 1x10^4 cells (cell number was determined by Trypan blue exclusion dye method) per each well in 100 µl of DMEM supplemented with 10% fetal bovine serum. After 12 hrs seeding, above media was replaced with fresh DMEM supplemented with 10% FBS then 10µl sample from above stock solutions were added to each well in triplicates which gives final concentration of 300, 200, 100, 50, 10 µg/mL. The above cells were incubated for 48 hours at 37^{o}C with 5% CO_2. After 48 hours incubation, the above media was replaced with 100 µl of fresh DMEM without FBS and to this 10 µl of MTT (5 mg dissolved in 1 ml of PBS) was added and incubated for 3 hours at 37^{o}C with 5% CO_2. After 3 hours of incubation the above media was removed with multi channel pipette, then 200 µl of DMSO was added to each well and again incubated at 37^{o}C for 15 minutes. Finally the plate was read at 570 nm using spectrophotometer. The absorbance is directly proportional to the number of non viable cells. The averages of the absorbance values were recorded by reading the control wells that were considered as 100%. The value of absorbance achieved from the compounds and solvent wells were proportionated to control values, and the percentages of their cell viability were determined. The IC_{50}values were determined for all the tested compounds (4a-4n) and standard Dabrafenib. The IC_{50} values obtained in the MTT assay of synthesized compounds are tabulated and presented in table 1.

Molecular docking studies

Molecular docking studies were carried out for all the synthesized imidazo pyrimidine acetamides (4a-4n) on V600E B-Raf kinase enzyme to assess the anti cancer potency. To carry out docking studies SCHRÖDINGER (glide)[7] and Autodock[8] were used. From the RCSB protein data bank the target protein V600E mutant B-Rafkinase(PDB ID:3IDP) was downloaded. From the co-crystal structure the inhibitor molecule was removed and the inhibitor free protein was used in further docking calculations. This resultant protein structure was refined, water molecules were removed and polar hydrogens, Gestiger charges were added and the co-ordinates were written in PDBQT file format. The 3D structures of all the compounds 4a-4n were prepared in Ligprep tool and energies were minimized. PDBQT's of protein and ligands were used to perform docking in AUTODOCK 4.2.0. Docking was carried out as per the reported procedure in our earlier article[9] using AUTODOCK and SCHRODINGER. The results of docking calculations in terms of binding energy k.cal/mole for AUTODOCK and G.score, mmGBSA values for SCHRODINGER are presented in Table 2.

Molecular properties and toxicity prediction

The molecular properties of the synthesized compounds 4a-4n were calculated using Osiris property explorer, The molecular properties such as molecular weight, C log P, log S, Number of rotatable bonds, hydrogen bond donors(HBD), hydrogen bond acceptors(HBA), polar surface area(PSA) and drug likeness (drugscore) were selected for the study. The two dimensional structures of individual compounds are combined to a single sdf file using openbabel[10], then the above mentioned descriptors were selected to generate the selected molecular properties. Further, from the log S values obtained, the percentage absorption of all the compounds was calculated from the correlation ABS = 109-0.345PSA. The calculated molecular properties are presented in table 3. The genotyoxicity and reproductive toxicity of the compounds were also predicted using Osiris and the data is presented in table 2.

RESULT AND DISCUSSION

Synthesis

The N-substituted 2-oxo-2,3-dihydro imidazo[1,2-a]pyrimidine acetamide derivatives 4a-4n were synthesized from the systematic synthetic route depicted in **figure 1**. The Acetyl glycine(**1**) was prepared from the reaction between acetoyl choride and glycine. The compound 1 was then treated with various aryl and hetero aryl aldehydes to afford 4-Arylidine-2-methyl-Oxazole-5-Ones(2a-2n), the completion of the reaction was confirmed by TLC and formation of the compounds was confirmed by melting point, IR, mass and NMR spectra. The strong IR absorption peak at frequency 1252 corresponds to C-O-C stretching, confirm the formation of compound 2a. All the compounds synthesized in the second step **2a-2n** were treated with 2 amino pyrimidine in ethylene glycol at 120^{o}C yielded the target compounds N-substituted 2-oxo-2,3-dihydro imidazo[1,2-a] acetamide derivatives 4a-4n. All the compounds 4a-4n were characterised by melting point, physical and spectral data. The elemental analysis of the compounds gave the percentage composition of each element in the molecule and the experimental values were comparable with the calculated values. The compounds 4a-4n were obtained in good yields and were stable.

***In vitro* antiproliferative activity**

All the synthesized N-substituted 2-oxo-2,3-dihydro imidazo[1,2-a]pyrimidine acetamide derivatives 4a-4n were screened for *in vitro* anticancer activity on the A549 human lung cancer cell lines employing MTT

assay. The IC_{50} values were calculated from the log concentrations of each compound and presented in table 1. The IC_{50} values were compared with the standard drug B-Raf Kinase inhibitor Dabrafenib (IC_{50} 0.003µmol). All the tested compounds **4a-4n** showed anticancer activity with IC_{50} value ranging from 1.4 µmol to 55 µmol. Compound 4n exhibited high potency with an IC_{50} value of 1.4 µmol amongst the screened compounds. Whereas, compounds **4c**, **4d**, **4h** and **4i** showed remarkable anticancer activity with a IC_{50} value of 2.1 µmol, 6.4 µmol, 3.2 µmol and 2.6 µmol respectively. However, the remaining compound showed moderate anticancer activity. The antiproliferative data further revealed the importance of aryl and heteroaryl moiety on the 3rd position of the imidazopyrimidine ring system. The high potency exhibited by compound 4n probably due to the presence of bicyclic aromatic ring which may contribute sufficient hydrophobicity and the presence of at least one H bond donor or acceptor also is important and this could also be observed in compound **4i**. The hydrogen bond donors at 4th position in case of phenyl ring could be important for activity of 4d and 4h.

Molecular docking studies

All the molecules were docked in the binding site of V 600E B-Raf kinase (PDB ID 3IDP) using Autodock and Schrodinger (Glide) docking programs. The docking results revealed that amongst the synthesized compounds, compound **4n** was found to be potent than the other compounds, Whereas compound **4c,4d,4h** and **4i** were found to be moderately potent and the remaining compounds were weakly active. The protein ligand interactions of each of the synthesized also compound was also studied in their docked or least energy conformation.

Table 1 *In vitro* proliferative activity and predicted toxicity of synthesized imidazo[1,2-a] pyrimidine acetamides (4a-4n)

Compound	IC_{50} value (µMol)	Geno toxicity#	Reproductive toxicity#
4a	14	Negative	Negative
4b	24	Negative	Negative
4c	**2.1**	Negative	Negative
4d	6.4	Negative	Negative
4e	55	Negative	Negative
4f	46	Negative	Negative
4g	30	Negative	Negative
4h	3.2	Negative	Negative
4i	**2.6**	Negative	Positive
4j	26	Negative	Negative
4k	54	Negative	Negative
4l	50	Negative	Negative
4m	12	Negative	Negative
4n	**1.4**	Negative	Negative
Dabrafanib	0.003	Negative	Negative

#Toxicity was predicted by using Osiris property explorer.

Table 2 Molecular docking results of synthesized imidazo[1,2-a]pyrimidine acetamides (4a-4n)

Compound	Binding Energy# [kcal/mole]	G-Score$	mm-GBSA$
4a	-7.53	-8.2	-41.55
4b	-7.58	-7.6	-55.39
4c	-8.13	-8.2	-49.50
4d	-7.69	-8.1	-55.79
4e	-7.69	-6.8	-56.01
4f	-7.16	-7.7	-39.32
4g	-7.76	-7.4	-41.765
4h	-8.12	-7.9	-51.814
4i	-8.33	-9.0	-40.93
4j	-7.28	-7.9	-51.814
4k	-6.86	-6.9	-41.0763
4l	-7.04	-5.1	-54.6
4m	-7.89	-5.1	-50.02
4n	-9.10	**-8.9**	-63.238
Dabrafanib	-13.08	-9.1	-70.52

*#Results obtained with Autodock, $ Results with Schrodinger-Glide, * Results of in vitro anticancer studies on A549 cell lines.*

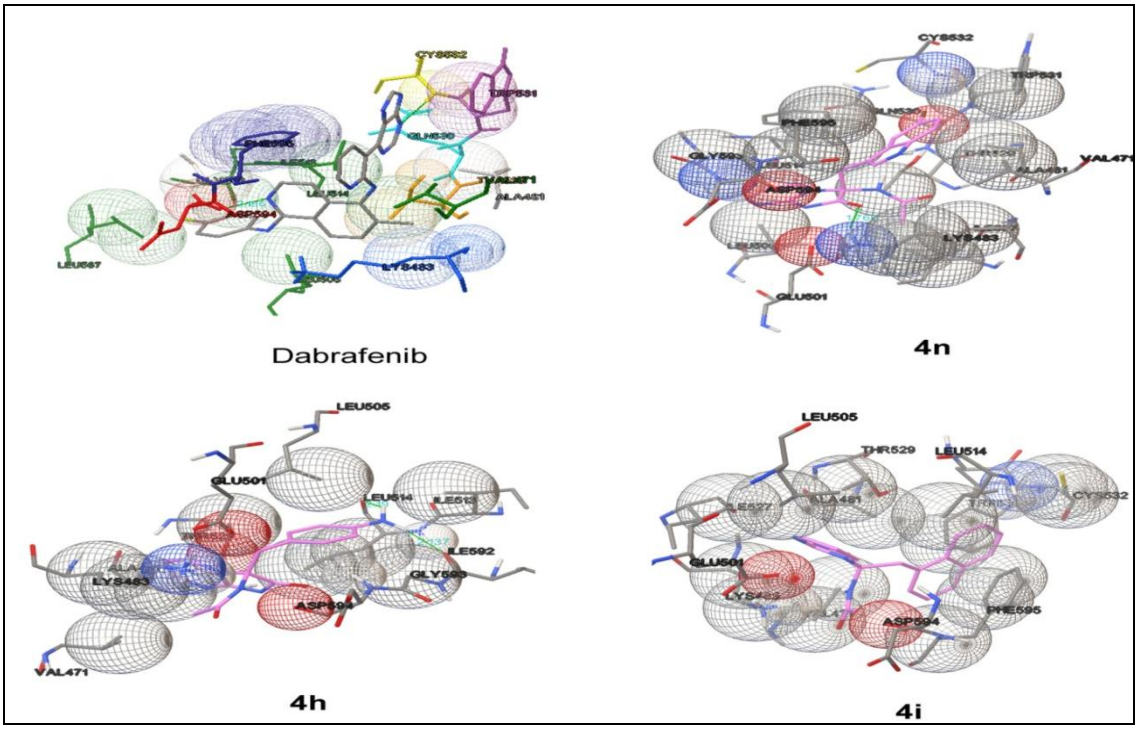

Figure 2. Three dimensional representation of binding mode of compounds Dabrafenib, 4n, 4h and 4i(purple) in V-600E B-raf kinase(PDBID 3IDP) binding site and interacting amino acid residues

The docking pose of compound **4n** figure 2 reveals that Oxygen atom on imidazopyrimidine forms a H bond with Lys 483 with a bond distance of 1.766A, Bromo indole is in interaction with key amino acid residue Cys 532, Trp 531, Gly 530, other important interaction are with residues Gly 593, Phe 595 and Leu 514. However, the best docking pose of the compound 4c with binding energy -8.13 interacted well with the enzyme and forms H bond with Lys 48 with a bond distance of 0.786 A, Glu 501, Leu 505, Thr 529 are in close proximity with imidazopyrimidine ring and the para bromo phenyl group with Gly 593.

In Compound 4d with the binding energy (kcal/mole) and G-score of -7.69, -8.1 respectively the 4-methoxy phenyl group forms a H bond with Ilu 592, with a bond distance of 2.011 A, Glu 501, Leu 505, Thr 529, Ala 481 are in close proximity with imidazopyrimidine ring. compound 4h with binding energy and G-score -7.28, -7.9 respectively, forms two hydrogen bond with Leu 514 and Ile 592 with a bond distance of 2.19 and 2.13 A respectively and the imidazopyrimidine ring is in interaction with Leu 505, Glu 501, Lys 483 and Ala 481.

Compound 4i with binding energy and G-score of -8.33, -9.0 respectively, forms an important interaction with Cys 532 that is similar with the standard compound and Asp 594, phe 595 forms hydrophobic interactions with Napthyl ring, Glu 501, Leu 505, Lys 483, Ala 481 are in close proximity with imidazopyrimidine ring. However Oxygen atom on imidazopyrimidine of compound 4j with binding energy and G-score of -7.28, -7.9 respectively, forms hydrogen bond with Glu 501 with a bond distance 1.783 A and the imidazopyrimidine ring is in interaction with Leu 505, Lys 483 and Ala 481, imidazole ring interaction with Ile 527. The docking studies of the compounds 4a-4n revealed that the para substituted benzyl group, napthyl methyl and bromo indole group at 3 position of imidazopyrimidine ring favours the interactions necessary for the inhibition of B-Raf kinase.

Molecular property and toxicity prediction

The molecular properties of the newly synthesized compounds 4a-4n were calculated by using Osiris property explorer. Some of the calculated values of some basic molecular descriptors such as logP, log S, molecular weight, polar surface area, number of hydrogen bond donors and number of hydrogen bond acceptors in molecule membrane hydrophobicity and bioavailability were predicted. The Lipinski's rule of five[11] was adopted to sort out the drug likeness of the synthesized compounds. In this screening all the synthesized compounds complied with the Lipinski rule and none of the compounds amongst synthesized (4a-an) violated the rules and were predicted as potential therapeutic compounds. The log S solubility parameter corresponds to good absorption (logS >_4).[12]

All the compounds demonstrated logS values ranging from - 4.45 to -1.57 which predicts its extent of absorption. Molecular polar surface area is also an important parameter for the prediction of drug transport property. (PSA<140). This value is used to estimate the percentage absorption by the expression ABS=109-0.345PSA.[13] The PSA values of the new molecules are

in the range of 74.13—100.15 which are in the prescribed limits for the drug like candidates. The logP values are ranged from -0.603 to 1.869 while the logS values were between - 4.45 to -1.57 both the set of values are within the accepted ranges for drug like molecules as described. All the synthesized compounds were predicted to have an percentage absorption of 74-83 which may reflect on *in vivo* activity. Moreover, all the synthesized compounds have rotatable bonds 3-4 and 6-7Hydrogen bond acceptors and 1-2 hydrogen bond donors. Compounds 4a-n exhibited positive drug likeness ranging from 0.4618 to 2.345 which represent that the molecule consists of fragments that are commonly found in marketed drugs. On the basis of drug likeness, compound 4C and 4N were predicted to be promising druggable candidates. Further, the result of molecular docking and *in vitro* antiproliferative studies supports the accuracy of prediction. The toxicity of the compounds was also predicted using Osiris, none of the compounds amongst the synthesized compounds showed genotoxicity which further supports the drug like features in the molecules. This toxicity prediction would be useful for the selection of compounds to test in animal models.

Table 3 **Molecular properties and toxicity profile of the newly synthesized imidazo[1,2-a] pyrimidine acetamides (4a-4n) calculated by OSIRIS property explorer.**

Molecule Name	MW	LogP	LogS	HBA	HBD	nrotb	PSA	Druglikeness	%ABS
4A	282.3	0.6745	-2.841	6	1	3	74.13	2.251	83.42
4B	316.7	1.2805	-3.577	6	1	3	74.13	2.302	83.42
4C	361.1	1.3997	-3.675	6	1	3	74.13	0.461	83.42
4D	298.3	0.3288	-2.545	7	2	3	94.36	2.265	76.44
4E	298.3	0.3288	-2.545	7	2	3	94.36	2.265	76.44
4F	296.3	1.0184	-3.185	6	1	3	74.13	2.251	83.42
4G	312.3	0.6045	-2.859	7	1	4	83.36	2.294	80.24
4H	297.3	-0.0028	-2.917	7	2	3	100.15	2.243	74.44
4I	332.3	1.8689	-4.447	6	1	3	74.13	2.251	83.42
4J	271.2	-0.603	-1.865	7	2	3	89.92	2.345	77.97
4K	283.2	-0.3264	-2.046	7	1	3	87.02	2.251	78.97
4L	272.2	-0.1908	-2.499	7	1	3	87.27	2.063	78.89
4M	321.3	0.7139	-3.366	7	2	3	89.92	2.345	77.97
4N	400.2	1.4391	-4.2	7	2	3	89.92	0.555	77.97
Dabrafenib	518.5	4.722	-7.001	7	1	5	135.45	-4.100	62.26

CONCLUSION

In the present study, a new series of imidazo[1,2-a]pyrimidine acetamides 4a-4n were designed and synthesized. The structures of the compounds were characterized by their ^{1}H NMR, IR and mass spectral data. The *in vitro* antiproliferative studies showed that compounds 4n, 4c, 4d and 4i were potent anticancer compounds, Further, molecular docking studies on B-Rafkinase reveals the binding pattern of designed molecules and also predict the *in silico* potency. Molecular properties of the designed compounds were predicted by using Osiris property explorer for important descriptors and along with this genotoxicity, reproductive toxicity were also predicted. This data revealed that the entire compound synthesized could satisfy the conditions required to consider as potential therapeutic candidate. Upon integration of the above data, clearly demonstrates that compounds 4n, 4c, 4h and 4i are potential anticancer agents among the synthesized compounds.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. *Vachala SD, Bhargavi B, Keloth KS.* Fused pyrimidines: The heterocycle of diverse biological and pharmacological significance. *Der Pharma Chemica 2012*; *4*(*1*): *255-265.*
2. Adrien A, Advances in Heterocyclic Chemistry, United Kingdom Ed., 32, Academic Press, *London*, 1982; 30.
3. Selvam TP, James CR, Dniandev PV, Valzita SK. A mini review of pyrimidine and fused pyrimidine marketed drugs. Research in Pharmacy 2012; 2(4): 01-09.
4. Nitesh S, Vaibhav J, Ranjan P, Somnath K, Sarita D, Neha T, Purusottam M, Garima P, Maneesh K, Dipon D, Shakti RS, Sumit S, Sankar K G, Chanakya NK, Prasad VB, Combretastatin A-4 inspired novel 2-aryl-3-arylamino-imidazo-pyridines/pyrazines as tubulin polymerization inhibitors, antimitotic and anticancer agents ***Med. Chem. Commun***, 2014; **5**: 766-78.
5. Kolch W, Kotwaliwale A, Vass K, Janosch P. The role of Raf kinases in malignant transformation Expert Rev Mol Med. 2002 Apr 25; 4(8): 1-18.
6. Holderfield M, Deuker MM, McCormick F, McMahon M. Targeting RAF kinases for cancer therapy: BRAF mutated melanoma and beyond. Nat Rev Cancer 2014 Jul; 14(7): 455–467.

7. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. J. Med. Chem 2006; 49: 6177–6196.
8. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell Ds, Olson AJ. Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. *J. Comp. Chem* 2009; 16: 2785-91.
9. Sruthi K, Sumakanth M, Mahendra Kumar CB, Naresh K. Synthesis, In Silico and In Vitro anticancer screening of some novel nitrogen bridged imidazopyrimidines. Der pharma chemica 2016; 8: 185-90.
10. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. *J. Cheminf.* 2011; *3*: 33.
11. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 1997; 23: 3-25.
12. Tyrchan C, Blomberg N, Engkvist O, Kogej T, Muresan S. Physicochemical property profiles of marketed drugs, clinical candidates and bioactive compounds. Bioorg Med ChemLett 2009; 19: 6943-7.
13. Wang RX, Fu Y, Lai LH. A new atom-additive method for calculating partition co-efficients. J Chem Inf Comput Sci 1997; 37: 615-21.