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Synthesis, *In Silico* and *In Vitro* anticancer screening of some novel nitrogen bridged imidazopyrimidines

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ABSTRACT

Novel derivatives of Imidazo[1,2-a] pyrimidines(3a-3n) were synthesized by the ring opening of 4-Arylidine-2-Phenyl-Oxazole-5-Ones with 2-Amino Pyrimidines. The compounds obtained were identified and characterized by their physical, spectral and elemental analysis data. In vitro anti-proliferative activity of some of the synthesized compounds was carried out on A549 (Lung cancer) cell lines by employing MTT assay. In silico screening of these derivatives was carried out by molecular docking techniques using Schrodinger(Glide) and Auto dock softwares to find potent B-Raf Kinase inhibitors among the synthesized compounds. It was observed that compounds 3d and 3h with IC 50 values < 10 µM demonstrated good anti-proliferative activity on cell lines and are comparable with the respective docking G-Scores and mmGBSA values.

Keywords: Imidazopyrimidinyl benzamide derivatives, 4-arylidene oxazolones, anti-proliferative activity.

INTRODUCTION

Rafkinases are the proto-oncogenes that work at the entry point of the mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK) pathway which connects cell-surface receptors to nuclear transcription factors and thereby impinge on the vital functions of the neoplastic cells such as immortalization, cell proliferation, insensitivity to growth-inhibitory signals, metastasis, angiogenesis and evasion of apoptosis[1,2]. B-Raf is a member of the Raf-kinase family of growth signal transduction protein kinases. This pathway is hyperactivated in 30% of all human tumors including prevalent cancers of the colon and lung. The molecular mechanisms underlying the role of Raf kinase in tumourigenesis and the opportunities for therapeutic intervention led to the discovery of new class inhibitors as anti neoplastic agents.

Imidazo[1,2-a]pyrimidines are the bridge-head nitrogen heterocycles, which have been reported for a tremendously broad range of useful pharmacological activities. Literature survey states that these molecules are claimed to have antibacterial, antifungal, anthelmintic, antiviral, antiprotozoal, anti-inflammatory, anticonvulsant, anxiolytic, hypnotic, local anaesthetic, anticancer[3], bronchodilatory, immunomodulatory and most extensively, gastrointestinal and antiulcer activities[4,5]. Drugs like Zolpidem and Fasiplon which are imidazo pyrimidine derivatives have been placed in the market as hypnotic and anxiolytic drugs respectively.

After a thorough search of the literature it was observed that the parent imidazo [1,2-a]pyrimidine skeleton is a very handy scaffold. On the other hand, in case of imidazo[1,2-a]pyrimidin-2-ones only a few activities are reported and are therefore less exploited. Taking into account of these observations in the present work it is desired to explore the role C-3 substitutions in comparision to other known scaffolds by introducing benzyl and amide substitutions to observe the effect on anti-proliferative activity and hence we designed and synthesized some novel imidazo[1,2-a] Pyrimidine-2-ones and were evaluated for their *in vitro* and *in silico* antiproliferative activity.

MATERIALS AND METHODS

All the solvents and 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 silica gel plates. Visualization was accomplished with UV light (256 nm) and iodine chamber. Purification of synthesized compounds was done by re-crystallization process. Melting points were determined 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 Varian CFT-20 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 and Cl of all compounds synthesized were within ± 0.4 % of theoretical values.

General procedure for the synthesis of Benzoyl glycine(1): Glycine (0.33 mol) was dissolved in 10% sodium hydroxide solution in a conical flask and benzoyl chloride (0.385 mol) was added in five portions to the solution. The solution was transferred to beaker and few grams of crushed ice were added to the solution and Conc.HCl was added slowly with stirring until the mixture was acidic to litmus. The resulting precipitate of benzoyl glycine which was contaminated with little benzoic acid was collected upon a buchner funnel, and washed with cold water. The resulting solid was boiled with 10 ml of CCl_4 for 10 minutes to extract any benzoic acid present, and was filtered under gentle suction. The obtained product was recrystallised from boiling water.

General procedure for the synthesis of 4-Arylidene-2-phenyl oxazole-5(4H)-ones (2a-j):
A mixture of aromatic aldehyde (0.25 mol),benzoyl glycine(0.25 mol), acetic anhydride(0.75 mol) and anhydrous sodium acetate (0.25 mol) were placed in a 500ml 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 100^0C.

Synthesis of N-1 substituted 2,3-dihydro imidazo[1,2-a]pyrimidine benzamide derivatives(3a-j): A mixture of 4-Arylidene 2-phenyl oxazole-5(4H)-one (1mmol) and 2-Amino pyrimidine (1mmol) was added to flask containing ethylene glycol (5ml) and heated to 120^0C. 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 recrystallised from ethanol.

***In vitro* anticancer activity:**
The Anticancer activity of the synthesized compounds was studied by MTT assay A549 (Lung cancer) cell lines. A549 (Lung cancer), cell lines were seeded 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 200, 100, 50, 10 µg/mL. The above cells were incubated for 48 hours at 37^oC 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^oC 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^oC 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 readings were averaged and validity of the test samples was compared with DMSO control.

Molecular docking studies

Molecular docking studies on B-raf Kinase enzyme was carried out in order to assess the anti cancer potency of all the synthesized compounds. Schrodinger Glide[6] and Autodock[7] software were used in the docking studies. The B-Raf kinase structure (PDB: 3IDP) was downloaded from RCSB Protein data bank and 3D structure was refined by removing the inhibitor molecule from the active site, then water molecules were deleted and polar hydogens and gestiger charges were added. The 3D structures of all the ligands were prepared in Ligprep and energies were minimized. The protein grid was defined which reflects the active site of the B-Raf kinase.

In Autodock, co-ordinates X, Y and Z were set to 6.153 24.264 and 34.675 respectively at 56-56- 56 points and Lamarkian Genetic Algorithm (LGA) was used for protein rigid-ligand flexible docking calculations. The maximum number of energy evaluations before the termination of LGA run was 250000 and the maximum number of generations was 27000 for each ligand. Total numbers of GA runs were set to 10 and other docking parameters were set to the software default values. The docking analysis was done by considering the low energy confirmation among the top 10 docked confirmations and binding energy was taken as parameter to assess the potency of the ligands and results are presented in Table. The Schrodinger G-score and MMGBSA values were also tabulated.

SCHEME

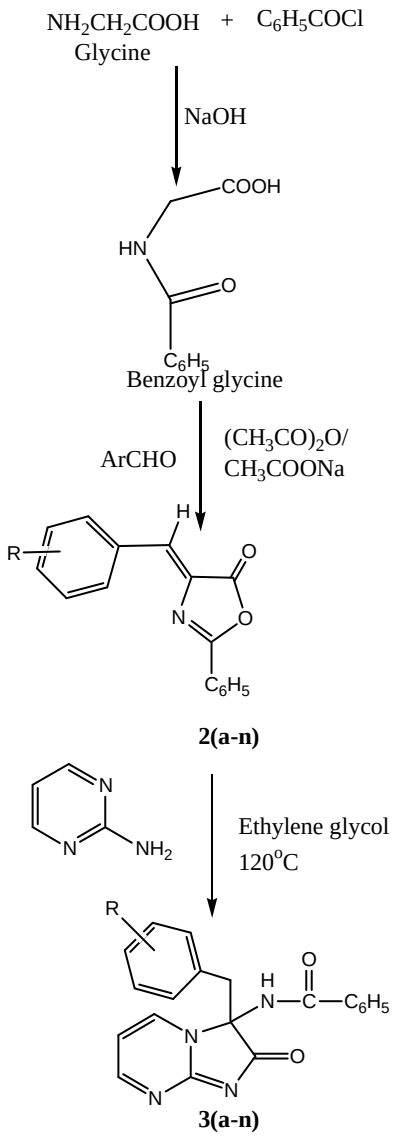

Table 1 Spectral data of N-1 substituted 2,3-dihydro Imidazo[1,2-a]Pyrimidine Benzamide derivatives 3a-3j

Compound	R	IR(KBr Disc in cm^{-1})	^{H}NMR(200MHz, $CDCl_3$) δ
3a	**H**	3243(NH); 2939(CH)	9.63(s,1H,NH),3.71(s,2H,-CH_2)
3b	**Cl**	3243(NH); 2939(CH)	9.77(s,1H,NH),3.73(s,2H,-CH_2)
3c	**Br**	3243(NH); 2939(CH)	9.67(s,1H,-NH),3.74(s,2H,-CH_2)
3d	**4-OH**	3224(NH);3391(OH);951(CH)	9.77(s,1H,-NH),3.73(s,2H,-CH_2)
3e	**3-OH**	3243(NH); 3395(OH); 2939(CH)	9.63(s,1H,-NH),3.71(s,2H,-CH_2)
3f	**4-CH_3**	3233(NH); 2939(CH)	9.77(s,1H,-NH),3.74(s,2H,-CH_2)
3g	**4-OCH_3**	324(NH); 2941(CH); 2998; 2938	9.55(s,1H,-NH),2.39(s,3H,-OCH_3)3.71(s,2H,-CH_2)
3h	**4-NH_2**	3157(NH)3243(NH); 2939(CH)	9.63(s,1H,-NH),3.71(s,2H,-CH_2)
3i	**Naphthyl**	3241(NH); 2938(CH)	9.77(s,1H,-NH),3.73(s,2H,-CH_2)
3j	**pyrol-2-yl**	3243(NH); 2939(CH)	9.63(s,1H,-NH),3.71(s,2H,-CH_2)
3k	**2-pyridyl**	3248(NH); 2942(CH)	9.77(s,1H,-NH),3.73(s,2H,-CH_2)
3l	**2-furyl**	3246(NH); 2939(CH)	9.63(s,1H,-NH),3.71(s,2H,-CH_2)
3m	**3-indolyl**	3368(indole NH); 3247(NH); 2936(CH)	9.77(s,1H,-NH),3.70(s,2H,-CH_2)
3n	**5-bromo3-indolyl**	3243(NH); 2924(CH)	9.63(s,1H,-NH),3.81(s,2H,-CH_2)

Table 2. Physical data of N-1 substituted 2,3-dihydro Imidazo[1,2-a]Pyrimidine Benzamide derivatives 3a-3n

Compound	Yield (%)	M.P(^{0}C)	Mol. wt	Mol.Formula	Analysis Found(Calculated)				
					C	H	N	Cl/Br	O
3a	74	254-246	344	$C_{20}H_{16}N_4O_2$	69.7	4.68	16.27	-	9.2
3b	67	294-295	378	$C_{20}H_{15}ClN_4O_2$	63.4	3.99	14.79	9.36	8.45
3c	65	299-300	423	$C_{20}H_{15}BrN_4O_2$	56.75	3.57	13.24	18.8	7.5
3d	75	245-246	360	$C_{20}H_{14}N_4O_3$	66.66	4.48	15.55	-	13.3
3e	79	245-246	360	$C_{20}H_{14}N_4O_3$	66.66	4.48	15.55	-	13.3
3f	74	259-261	358	$C_{21}H_{18}N_4O_2$	70.38	5.06	15.63	-	8.93
3g	71	263-265	374	$C_{21}H_{18}N_4O_3$	67.37	4.85	14.96	-	12.82
3h	55	255-257	359	$C_{20}H_{17}N_5O_2$	66.8	4.77	19.49	-	8.9
3i	68	289-291	394	$C_{24}H_{18}N_4O_2$	73.08	4.60	14.2	-	8.11
3j	61	260-262	333	$C_{18}H_{15}N_5O_2$	64.86	4.54	21.1	-	9.6
3k	68	256-258	354	$C_{19}H_{15}N_5O_2$	66	4.38	20.2	-	9.27
3l	65	296-298	334	$C_{18}H_{14}N_4O_3$	64.66	4.22	16.76	-	14.36
3m	74	289-291	383	$C_{22}H_{17}N_5O_2$	68.92	4.47	18.27	-	8.35
3n	74	296-298	462	$C_{22}H_{16}N_5O_2Br$	57.16	3.49	15.5	17.2	6.92

RESULTS AND DISCUSSION

Synthesis

Schemes 1 depicts the synthetic route used to obtain the **N-1 substituted 2,3-dihydro Imidazo[1,2-a]Pyrimidine Benzamide derivatives 3a-3j**. Synthesis of compounds **3a-j** started with the condensation of Benzoyl glycine (1) with a series of aromatic / heterocyclic aldehydes to yield 4-Arylidine-2-Phenyl-Oxazole-5-Ones (**2a-2j)** in the presence of acetic anhydiride and sodium acetate. Ring opening of oxazolones with 2-Amino pyrimidine in the presence of ethylene glycol resulted in N-Substituted Imidazo[1,2-a] Pyrimidines (**3a-j**) in quantitative yields. The formation of synthesized compounds was confirmed by TLC and physical data and results are presented in Table and the structure of all the compounds was characterized by IR and ^{1}H-NMR spectroscopy and the results are tabulated in Table 1 and 2.

In vitro anticancer activity:

The Anticancer activity of the synthesized compounds was studied by MTT assay A549 (Lung cancer) cell lines. The compounds demonstrated cytotoxic activities with the IC 50 values ranging from 2 to 27µg.mol respectively. The IC 50 values of all compounds was tabulated in Table 3.

In silico screening

The molecular docking of the title compounds was done into the active site of Raf Kinase (PDB : 3IDP). The G-score values were ranging between -6.0 to -9.0. More negative Glide Score indicates the better interaction of the inhibitor with the target protein. The compounds were positioned in a similar orientation to that of Dabrafenib and showed strong hydrogen bonding interactions similar to that of Dabrafenib. The binding site of the protein is the hydrophobic pocket comprising of amino acids Asp-594, ILe 592, Thr 529, Phe 595. Compounds 3d and 3h were within the active site of the enzyme and embedded within cleft formed by the aminio acid residues Cys 532, Thr 529, Phe 595, Leu 514, Lys 483 and Val 471. Further, compound 3d (Figure 1) formed one strong H bond with the Cys 532 with bond length of 1.008Å and compound 3h (Figure 2) formed another hydrogen bond with the amino

acid residue Thr 529 with a bond length of 1.012Å which are crucial for inhibition of the B-Raf kinase enzyme. However, a polar interaction was observed between 1N of compound 3h formed and Phe 595. The G-scores and mmGBSA values and binding energies of low energy conformations of the title compounds were tabulated in Table 3.

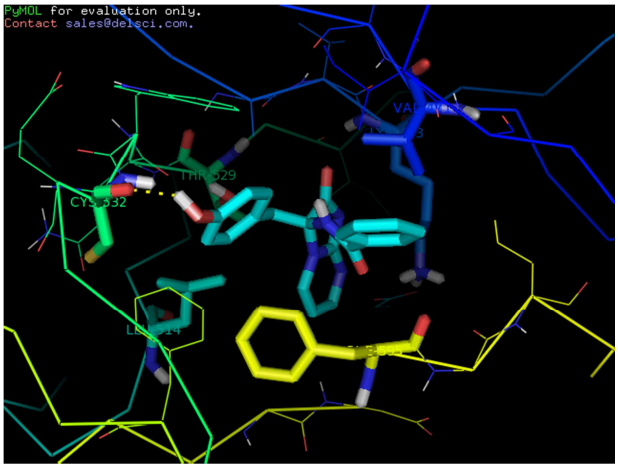

Figure 1 The binding mode of the compound (3d) within the active site of B-Raf kinase

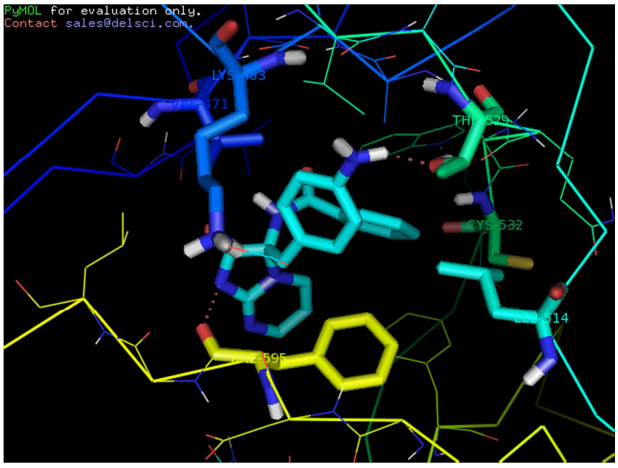

Figure 2 The binding mode of the compound (3h) in the active site of B-Raf kinase hydrogen bonding interactions with amino acid residue Thr-529

Table 3 *In silico* and *in vitro* screening of N-Substituted Imidazo[1,2-a] Pyrimidines derivatives (3a-n)

Compound	Binding Energy[Autodock]	G-Score	MM-GBSA	IC 50 value (µMol) A549
3a	-8.20	-8.3	-53.09	10
3b	-8.78	-8.2	-57.437	25
3c	-8.57	-	-	54
3d	-8.28	-8.8	-61.008	3.4
3e	-8.32	-7.5	-49.94	34
3f	-8.88	-7.8	-56.107	5.5
3g	-8.44	-8.0	-51.73	26
3h	-7.43	-8.7	-55.99	2.1
3i	-8.63	-6.3	-47.207	10.3
3j	-8.35	-7.1	-51.05	4.2
3k	-8.32	-	-	6.0
3l	-8.12	-7.8	-52.14	17.5
3m	-8.66	-7.7	-51.95	9.8
3n	-9.28	-5.2	-34.8	12.2
Dabrafenib	-13.08	-9.1	-70.52	0.003

CONCLUSION

A novel series of N-Substituted Imidazo[1,2-a] Pyrimidines derivatives (3a-j) were designed and synthesized in search of potent anti-proliferative agents. The synthesized compounds showed good to excellent activity against A549 lung cancer cell lines. Particularly compounds 3d, 3h demonstrated cytotoxic activities with the IC 50 values of 3.4 and 2.1µg mol respectively , 3h being the best among the synthesized compounds. The designed molecules were also screened for their *in silico* cytotoxic activity by studying its docking parameters as Raf kinase inhibitors. All the compounds demonstrated good binding interaction with B-raf kinase G-scores and mmGBSA values. Particularly compounds **3d, 3h** were found to have highest G-scores and mmGBSA values of -8.8, -61.008 and -8.7, -55.99 respectively. The results obtained with *in vitro* cytotoxic studies were comparable with the *in silico* studies. Thus N-Substituted Imidazo[1,2-a] Pyrimidines derivatives can be considered as a better scaffolds for discovering new anti-proliferative agents.

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