ORIGINAL RESEARCH ARTICLES

IN SILICO EVALUATION OF PYRAZOLE AND TRIAZINE CONTAINING PYRIDOPYRIMIDINES

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ABSTRACT

Cancer, seen as one of the highly prevelant life endangering diseases today, is mainly associated with the lifestyle led by the population at risk. The superficial observation seen in cancer is the uncontrollable growth and dispersion of abnormal cells. Statistically, the number of patients who receive chemotherapy has been soaring high, which has drawn attention towards development of more reliable and less toxic cytotoxic agents. In the present work, hybrid structure of pyridopyrimidine substituted with pyrazole and triazine in individual turns is studied. Pyridopyrimidines are *ortho* fused bicyclic heterocyclic structures constituted by the amalgam of a pyridine and a pyrimidine ring. *In silico* studies with molecular docking was performed to filter best compounds out the 16 different derivatives. Most of the compounds showed satisfactory results for the *in silico* screening. In case of docking, among all the screened compounds, 1d, 1o, 2d and 2j showed best affinity towards 2EUF while 1n, 1o, 2g and 2h showed best binding towards 5FWK. On that account, these compounds may have a potential cytotoxic effect and hence could be utilized for further studies and explorations of their properties.

Keywords: Cancer, pyrazole, triazine, pyridopyrimidines, *In silico*, docking

INTRODUCTION

Cancer, seen as one of the highly prevelant life endangering diseases today, is mainly associated with the lifestyle led by the population at risk. The superficial observation seen in cancer is the uncontrollable growth and dispersion of abnormal cells. Cancer is often linked with a number of risk factors which mainly include many external and internal aspects such as tobacco, radiations, hormones, mutations and so on[1]. However, there is a diverse, more complex nature to the source of cancer which is not completely inferred[2,3]. Heterocyclic compounds occupy a very prominent position in the family of organic compounds[4]. Their role is being explored extensively due to their synthetic utility and an advance in the field of medicinal chemistry[5]. Fused pyridine and pyrimidine rings utilized in the present study have been explored for an array of benefits which proved their account to dig into it further.

In the present work, hybrid structure of pyridopyrimidine substituted with pyrazole and triazine in individual turns is studied. The fact that pyridopyrimidines are put forth among the pharmaceutical products, has laid the building block for its further exploration in organic chemistry. Pyridopyrimidines are *ortho* fused bicyclic heterocyclic structures constituted by the amalgam of a pyridine and a pyrimidine ring[6]. They have made advances in various aspects of medicine like anti-cancer[7], anti-viral[8], CNS[9], anti-inflammatory[10], fungicidal[11], anti-bacterial[12] and anti-microbial[13] therapies. Organic as well as medicinal chemists have paid special interest towards synthesizing these pyridopyrimidines due to their medicinal and biological characteristics[14].

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Pyrazole is a 2 nitrogen containing 5 membered aromatic ring whereas triazine is a 3 nitrogen containing 6 membered aromatic ring. In the past studies conducted on these structures, they put forth an extensive assemblage of functionality and steriochemical complexity. The data compiled of works done on these structures and their derivatives offered drug like properties and physiological as well as pharmacological aspects that created opportunities to render their full potential[15,16].

Evidently enough, the thought of bringing these structures together could unfold previously unknown and valuable facet regarding their potentials. Hence, in this study, the pyrazole and triazine containing pyridopyrimidine with various substituents has been taken. Substitution was carried out on the benzene ring at the 6th position of the pyridopyrimidine nucleus which has been worked on with the intention to augment the anti cancer attribute of the resulting molecule.

Some drugs having a pyridopyrimidine ring structure have extended to the market such as palbociclib (Fig. 1, HR positive breast cancer treatment), piritrexim isethionate (Fig. 2, bladder and urethral cancer treatment) and pipemidic acid (Fig. 3, antibiotic that kills both gram-negative and gram-positive bacteria), while many other are in various stages of development[17].

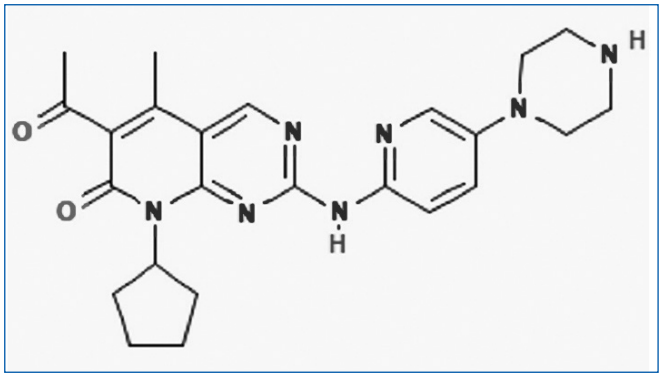

Fig. 1: Structure of palbociclib

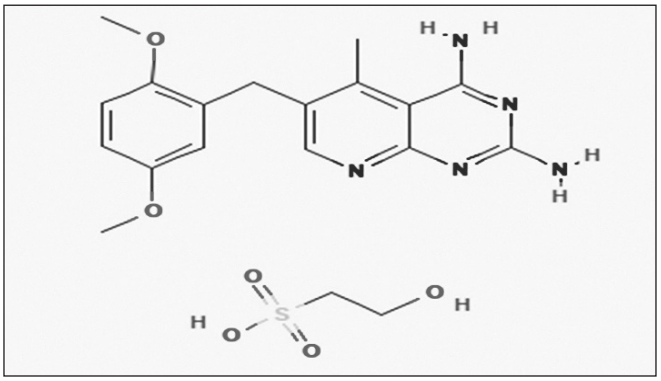

Fig. 2: Structure of piritrexim isethionate

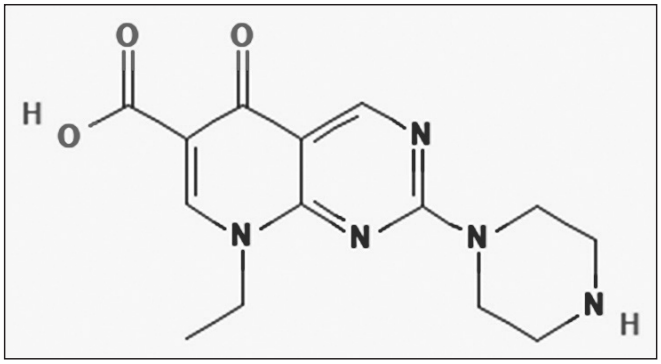

Fig. 3: Structure of pipemidic acid

With this intention in mind, some novel pyrazole and triazine containing pyridopyrimidines were synthesized with various substituents on the phenyl ring (Fig.4 and Fig.5) and their anti cancer potenial was evaluated using in *silico* models. The substituents taken in hand for this study include pyrazole and triazine containing pyridopyrimidines.

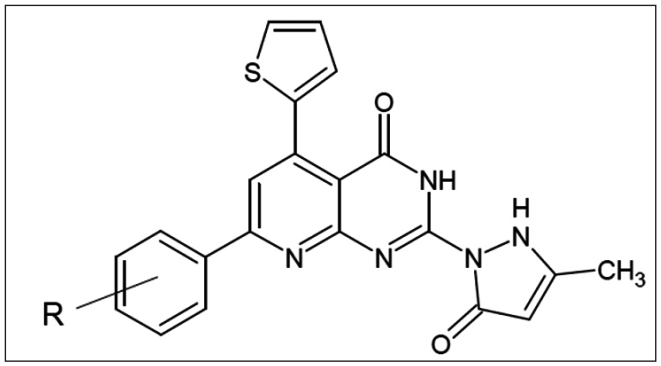

Fig. 4: Pyrazole containing pyridopyrimidine

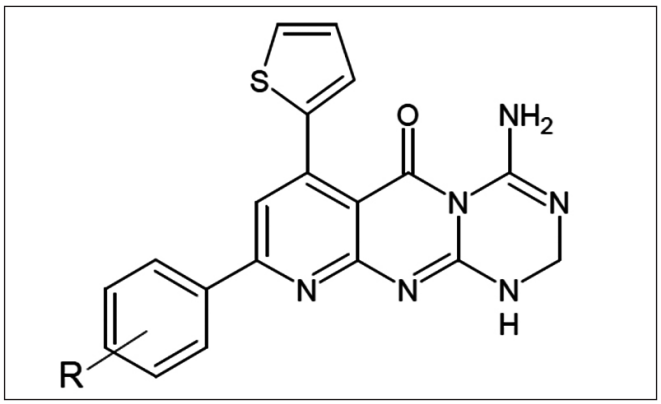

Fig. 5: Triazine containing pyridopyrimidine

MATERIALS AND METHODS

In silico studies

Various *in silico* studies were carried out such as determining the drug likeness like Lipinski's Rule of Five and bioactivity scoring, ADMET properties were assayed

Table I: Lipinski's rule of 5 and physicochemical parameters of substituted pyrazole containing pyridopyrimidines (1a-p)

Sl. No.	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	Volume
1a	3.14	116.67	30	417.45	8	3	0	3	342.54
1b	4.90	96.44	31	470.34	7	2	0	3	361.60
1c	3.35	116.67	30	417.43	8	3	0	3	342.54
1d	2.85	136.90	31	433.45	9	4	0	3	352.56
1e	4.40	96.44	30	480.35	7	2	0	3	352.41
1f	3.12	116.67	30	417.45	8	3	0	3	352.54
1g	3.08	136.90	31	433.45	9	4	0	3	350.56
1h	2.49	130.58	33	479.54	9	2	0	4	382.52
1i	4.09	96.44	30	415.48	7	2	0	3	351.09
1j	4.49	96.44	33	469.45	7	2	0	4	365.82
1k	3.78	96.44	30	419.44	7	2	0	3	339.46
1l	3.58	142.27	32	446.65	10	2	0	4	357.86
1m	2.69	122.47	30	416.47	8	4	0	3	345.81
1n	4.44	96.44	31	429.50	7	2	0	3	367.65
1o	2.67	122.47	30	416.47	8	4	0	3	345.81
1p	3.55	142.27	32	446.45	10	2	0	4	357.86

Table II: Lipinski's rule of 5 and physicochemical parameters of substituted triazine containing pyridopyrimidines (2a-p)

Sl. No.	miLogP	TPSA	natoms	MW	nON	nOHNH	Nviolations	nrotb	Volume
2a	2.42	118.43	28	390.43	8	4	0	2	318.53
2b	4.18	98.20	29	443.32	7	3	0	2	337.58
2c	2.63	118.43	28	290.43	8	4	0	2	318.53
2d	2.13	138.66	29	406.43	9	5	0	2	326.55
2e	3.68	98.20	28	453.32	7	3	0	2	328.40
2f	2.40	118.43	28	390.43	8	4	0	2	318.53
2g	2.37	138.66	29	406.43	9	5	0	2	326.55
2h	1.77	132.34	31	452.52	9	3	0	3	358.51
2i	3.35	98.20	28	388.46	7	3	0	2	327.07
2j	3.77	98.20	31	442.43	7	3	0	3	341.81
2k	3.06	98.20	28	392.42	7	3	0	2	315.44
2l	2.86	144.03	30	419.43	10	3	0	3	333.85
2m	1.98	124.23	28	389.44	8	5	0	2	321.80
2n	3.72	98.20	29	402.48	7	3	0	2	343.63
2o	1.95	124.23	28	389.44	8	5	0	2	321.80
2p	2.83	144.03	30	419.43	10	3	0	3	333.85

Table III: Bioactivity scores of substituted pyrazole containing pyridopyrimidines (1a-p)

Sl. No.	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1a	-0.13	-0.52	0.18	-0.59	-0.79	-0.07
1b	-0.13	-0.53	0.14	-0.69	-0.79	-0.16
1c	-0.15	-0.57	0.15	-0.63	-0.82	-0.12
1d	-0.14	-0.55	0.17	-0.56	-0.80	-0.10
1e	-0.26	-0.61	0.10	-0.81	-0.91	-0.17
1f	-0.13	-0.52	0.20	-0.59	-0.80	-0.08
1g	-0.10	-0.56	0.20	-0.65	-0.80	-0.08
1h	-0.16	-0.64	0.12	-0.58	-0.59	0.02
1i	-0.19	-0.60	0.12	-0.72	-0.83	-0.15
1j	-0.08	-0.43	0.19	-0.50	-0.69	-0.09
1k	-0.15	-0.56	0.19	-0.69	-0.82	-0.12
1l	-0.28	-0.56	0.02	-0.75	-0.87	-0.20
1m	-0.13	-0.51	0.23	-0.76	-0.74	-0.04
1n	-0.16	-0.59	0.13	-0.67	-0.83	-0.14
1o	-0.13	-0.52	0.25	-0.76	-0.73	-0.03
1p	-0.27	-0.57	0.04	-0.75	-0.87	-0.19

Table IV: Bioactivity scores of triazine containing pyridopyrimidines (2a-p)

Sl. No.	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
2a	-0.07	-0.19	0.34	-0.55	-0.55	0.15
2b	-0.08	-0.22	0.29	-0.66	-0.56	0.04
2c	-0.10	-0.25	0.31	-0.59	-0.58	0.09
2d	-0.09	-0.24	0.32	-0.51	-0.57	0.10
2e	-0.21	-0.29	0.26	-0.78	-0.68	0.04
2f	-0.08	-0.19	0.37	-0.54	-0.56	0.15
2g	-0.05	-0.25	0.36	-0.61	-0.57	0.12
2h	-0.12	-0.35	0.26	-0.54	-0.36	0.21
2i	-0.15	-0.30	0.26	-0.69	-0.61	0.04
2j	-0.03	-0.13	0.34	-0.46	-0.47	0.11
2k	-0.10	-0.24	0.33	-0.65	-0.58	0.09
2l	-0.24	-0.26	0.16	-0.71	-0.65	-0.00
2m	-0.09	-0.19	0.38	-0.71	-0.52	0.15
2n	-0.13	-0.29	0.27	-0.64	-0.62	0.05
2o	-0.08	-0.20	0.40	-0.71	-0.52	0.17
2p	-0.23	-0.27	0.18	-0.17	-0.65	0.01

Table V: Absorption parameters of pyrazole substituted pyridopyrimidines (1a-p)

Sl. No.	Caco-2 Permeability (log cm/s)	MDCK Permeability (cm/s)	Pgp-inhibitor	Pgp-substrate	HIA	F 20%	F 30%
1a	-4.924	1.6e-05	0.004	0.0	0.012	0.788	0.211
1b	-4.932	1.7e-05	0.474	0.0	0.003	0.005	0.007
1c	-4.97	2.1e-05	0.007	0.0	0.01	0.628	0.21
1d	-5.06	1.1e-05	0.004	0.0	0.017	0.987	0.765
1e	-4.923	1.8e-05	0.991	0.0	0.019	0.003	0.091
1f	-4.979	1.7e-05	0.004	0.0	0.013	0.878	0.313
1g	-5.095	1.4e-05	0.005	0.0	0.016	0.976	0.7
1h	-5.032	6e-06	0.014	0.011	0.006	0.001	0.002
1i	-4.898	2.3e-05	0.874	0.0	0.007	0.009	0.037
1j	-4.964	2e-05	0.097	0.0	0.004	0.011	0.006
1k	-4.85	2e-0.5	0.863	0.0	0.006	0.003	0.224
1l	-4.844	3.6e-05	0.624	0.0	0.008	0.003	0.009
1m	-4.907	1.4e-05	0.034	0.001	0.009	0.014	0.105
1n	-4.929	2.4e-05	0.557	0.001	0.005	0.032	0.014
1o	-4.921	1.5e-05	0.039	0.001	0.01	0.013	0.159
1p	-4.864	3.7e-05	0.676	00	0.009	0.003	0.01

Table VI: Absorption parameters of triazine substituted pyridopyrimidines (2a-p)

Sl. No.	Caco-2 Permeability (log cm/s)	MDCK Permeability (cm/s)	Pgp-inhibitor	Pgp-substrate	HIA	F 20%	F 30%
2a	-4.82	2.1e-05	0.001	0.014	0.011	0.912	0.589
2b	-4.815	4.4e-05	0.273	0.145	0.003	0.004	0.009
2c	-4.789	2.5e-05	0.001	0.008	0.008	0.813	0.603
2d	-4.88	1.6e-05	0.001	0.018	0.014	0.994	0.957
2e	-4.825	3.2e-05	0.937	0.001	0.009	0.002	0.039
2f	-4.877	2.4e-05	0.001	0.014	0.013	0.941	0.692
2g	-4.911	1.6e-05	0.002	0.006	0.017	0.988	0.955
2h	-5.446	2.3e-05	0.1	0.013	0.009	0.003	0.152
2i	-4.792	3.7e-05	0.016	0.771	0.005	0.01	0.138
2j	-4.871	3e-05	0.014	0.11	0.004	0.003	0.004
2k	-4.791	3.9e-05	0.256	0.409	0.004	0.002	0.091
2l	-4.793	0.000227	0.069	0.023	0.006	0.002	0.005
2m	-5.026	4e-05	0.002	0.957	0.007	0.015	0.363
2n	-4.789	4.8e-05	0.012	0.965	0.004	0.006	0.022
2o	-5.048	4.8e-05	0.002	0.961	0.008	0.018	0.311
2p	-4.823	0.000234	0.062	0.025	0.007	0.002	0.005

and also the preferred orientation of the ligand interacting with the protein was determined by docking studies using software like schrodinger[18].

Determination of Lipinski's rule of five

In the living system behaviour of the molecules is influenced by number of factors where physico-chemical properties and the presence of various pharmacophoric features is one of the important criteria. In case of optimization of bioactive compounds to therapeutically beneficial molecules, the importance of oral bioavailability cannot be overstated. All these factors are important interpreters of good oral BA. Lipinski's rule of 5 is mainly used to verify molecular properties, essential and related to PK of the drug molecule[19,20,21].

All the structures were drawn using Chemsketch software. Smiles notations are generated for each structure. By using molinspiration software MW, log P, number of Hydrogen bond donors/acceptors, TPSA and nrotb were determined.

Analysis of electronic parameters and ADME properties of targeted pyridopyrimidines

It is mainly by using Qikprop of Schrodinger 2018-3 Suite Device Maestro 11.7.012. QikProp is a rapid, precise, accessible software package to predict ADME properties. QikProp determines physically considerable descriptors and pharmaceutically appropriate properties of organic molecules[22,23].

Evaluation of toxicity of targeted pyridopyrimidines

One of the major issues in drug development is toxicity. In many stages of drug development, evaluation of safety and toxicity must be done. In many cases, toxic effects are identified in the later stage of the development. Hence *in silico* toxicity determination is playing an important role in the initial stage[24-26]. Toxicity prediction was mainly done using admetLAB database, which is freely available.

Molecular docking studies

Molecualar docking is primarily pursued to find out the preferred orientation of one molecule with another to get a better ligand molecule to particular protein with minimum energy. Molecular docking was performed by utilizing Glide module of Schrodinger 2018-3 suitedevice Maestro 11.7.012. Crystal structures with good resolution of all the target proteins were taken from Protein Data Bank (PDB)[27, 28].

RESULTS AND DISCUSSION

The study focused on carrying out the *in silico* screening of various physico chemical properties of the substituted pyrazole and triazine containing pyridopyrimidines. The parent structure of pyrazole and triazine containing pyridopyrimidines was substituted with 16 different substituents giving rise to a total of 32 substituted pyridopyrimidine moieties which were screened *in silico*. SMILES were generated for each structure using ChemSketch, then utilized for the further studies using various other softwares. The intital work done was to determine the drug likeness properties of the molecules. Molinspiration is a free online software which aids in estimating the compliance with Lipinski's Rule of Five and also evaluating the bioactivity scores. Compliance with Lipinski's Rule of 5 eases in concluding the oral bioavailability of the molecules. All the compounds assayed were found to obey the Lipinski's rule without any violations. All the compounds were found to have molecular weight less than 500 daltons. The log p values of the entire derivatives ranged from 2.67- 4.90. As a result of this, the compounds were expected to have good intestinal absorption. Among the derivatives of 1a-p, derivative with 3- amino group(1o) has log p of 2.67 being more hydrophillic while 2,4-Cl(1b) has log p 4.90 being more lipophilic. Among the series 2a-p, molecule with 4-methyl sulphonyl(2h) attached to it has log p 1.77 whereas 2,4-Cl(1 b) has logp 4.18 being on hydrophilic and lipophilic side, respectively. Total number of hydrogen bond acceptors and donors are good oral bioavailibity predictors. Number of hydrogen bond donors and acceptors of the entire screened compounds were within the permitted range i.e., not more than 10 and 5, respectively. tPSA is related to the hydrogen bonding potential of the compounds and a good descriptors to characterize drug absorption, bioavailability, Caco-2 permeability and BBB penetration. Among all the screened compounds, most of them showed tPSA lower and within the permitted range of below 140 A^0 except 1l, 1p, 2l and 2p i,e., meta and para nitro substituents. The molecular flexibility is measured using the number of rotatable bonds and gives a good descriptor of absorption and bioavailability of drugs. All the derivatives of these compounds were found to have less than 10 rotatable bonds and hence can be depicited to be less flexible. All the compounds were found to have very good physico-chemical properties and may have promising oral bioavailability (Table I and Table II).

On carrying out bioactivity scoring using Molinspiration software, scores towards activities such as GPCR ligand, ion channel modulator, kinase inhibitor, nuclear

receptor ligand, protease inhibitor and kinase inhibitor were evaluated (Range- -2 to +2). All the derivatives showed promising activity as kinase inhibitors.Some derivatives also showed potential as enzyme inhibitor. Kinase inhibitors play a role in the phosphorylation which can turn protein on or off and thereby affect the level of activity and function (Table III and Table IV).

Caco-2 cells provide with a model for gut-blood barrier and predictions limit for non-active transport. The human colon adenocarcinoma cell line is an alternative approach for human intestinal epithelium. Since all the derivatives of 1a-p were found to have a value greater than -5.15 log cm/s (Table V), they can be expected to show appreciable intestinal permeability while in 2a-p, except 2 h and 2 m (Table VI) all others fit the reference value. MDCK cell lines are attributed as gold standard for evaluating the uptake efficiency into the body and the effect of blood brain barrier. Except the derivative 1h from the series 1a-p, all other compounds showed excellent MDCK permeability of greater than 2e-06 cm/s. Pgp, for P-glycoprotein is an important efflux transporter of the ATP binding cassette encoded by the gene ABCB1. Modulation of P-glycoprotien mediated transport can be exploited for specific therapeutic advantages and hence is a great measure. The values displayed are the probability of the compounds being either inhibitors or substrates of Pgp such as the values closer to 1 indicate the compounds are either inhibitors or substrates while values closer to 0 indicate they are non-inhibitors or non-substrates. As evident, compounds are clearly not substrates for Pgp, while some compounds show promising inhibitors activity. For any drug administered orally, oral bioavailability is one of the most important pharmacokinetic parameters that needs to be measured. For both, F20% and 30% values are the probability of being bioavailable at 20% and 30% ranging between 0 to 1. Values closer to 1 indicate the probability of being bioavailable at the respective percentage (Table V and Table VI).

PPB has a direct influence on the oral bioavailability because greater the drugs bound to the to serum protein, lesser the free concentration. The more that is bound, less efficiently it can traverse cellular membranes. The plasma protein binding of the derivatives was found to be slightly higher than the acceptable range, hence, measures should be taken to reduce it. Volume distribution (VD) tells about the distribution estimate in the body fluid and its uptake in the tissues and all derivatives were found to have excellent VD between the range of 0.4-20 L kg^{-1}. Fu is the fraction of the drugs unbound in the plasma and the screened compounds were found to be extensively bound to plasma since they have values of lesser than 5%. Lesser the drugs bound to plasma, more it's available for the target actions (Table VII and Table VIII).

Table VII: Distribution properties of substituted pyrazole containing pyridopyrimidines (1a-p)

Sl. No.	PPB (%)	VD	BBB Penetration	Fu (%)
1a	99.79	0.417	0.012	0.965
1b	101.0	0.298	0.018	0.772
1c	99.90	0.402	0.009	0.885
1d	99.66	0.413	0.005	1.309
1e	100.0	0.433	0.027	0.769
1f	99.70	0.428	0.013	1.087
1g	99.79	0.378	0.003	1.181
1h	97.58	0.496	0.008	1.852
1i	100	0.359	0.02	0.806
1j	100.2	0.331	0.043	0.765
1k	100.1	0.441	0.021	0.843
1l	133.3	0.359	0.042	0.766
1m	99.49	0.559	0.017	0.960
1n	100.1	0.339	0.016	0.779
1o	99.50	0.589	0.016	1.014
1p	100.2	0.375	0.04	0.841

Table VIII: Distribution properties of substituted triazine containing pyridopyrimidines (2a-p)

Sl. No.	PPB (%)	VD	BBB Penetration	Fu (%)
2a	95.90	0.434	0.136	2.441
2b	98.20	0.33	0.732	1.138
2c	96.19	0.46	0.141	2.329
2d	96.66	0.553	0.027	2.427
2e	96.55	0.465	0.745	2.520
2f	95.72	0.546	0.111	2.537
2g	96.7	0.489	0.026	2.150
2h	94.75	0.626	0.252	3.600
2i	96.37	0.418	0.668	2.076
2j	96.70	0.582	0.696	2.123
2k	96.14	0.427	0.672	2.298
2l	96.85	0.583	0.572	1.838
2m	93.72	0.669	0.286	3.812
2n	96.77	0.406	0.461	1.741
2o	93.85	0.757	0.195	3.493
2p	97.05	0.695	0.357	1.942

The process of drug transformation, also called as metabolism, is mainly of two types based on the nature of occurrence i.e., Phase 1 and Phase 2 reactions. Most of the drugs administered are broken down by the enzymes belonging to the family of cytochrome P450 and are mainly present in the liver. Presently, mainly action of 5 isoenzymes have been studied on the compounds which include CYP 1A2, 3A4, 2C9, 2C19 and 2D6. The compounds were studied to see whether they were substrates or inhibitors of the coenzymes. The values presented are interpreted by the probability of the compound being an inhibitor or a substrate. Values closer to 0 indicate non-substrates or non-inhibitors while values closer to 1 indicate substrate or inhibitor properties (Table IX and Table X).

Clearance is an integral pharmacokinetic parameter that all together tells about the volume of distribution, the half-life and frequency of a drug. The compounds showed poor clearance. The half-life of the drug is a combination of clearance and volume of distribution. Most of the derivatives under study showed a promising half-life of below 0.3 (Table XI).

Toxicity evaluation of the derivatives was carried out. hERG stands for human ether a go-go gene, blockage of which may lead to certain complications. Results revealed an undesirable output and hence should be carefully reviewed. Human hepatotoxicity test (H-HT) and drug induced liver injury (DILI) test is carried out to rule out the possible liver damage due to the drugs, which is one of the major concerns leading to the withdrawal of drugs from the market and AMES toxicity test is done as a test for mutagenicity, as it corresponds to carcinogenecity. Rat oral acute toxicity is mainly performed to extrapolate the results obtained in mammals to their similarities in the humans for their safety evaluation. Maximum recommended dose tells about the tolerated dose for the required action without leading to toxicity. Adverse reaction of the drug towards the skin is also studied for dermatologically useful drugs, as a result of which skin sensitization test is carried out. the compounds showed poor results for the human hepatotoxicity (H-HT), Drug induced liver injury (DILI), respiratory toxicity, and maximum recommended daily dose (FDAMDD). On the other hand the compounds showed a desirable outcome against AMES mutagenicity, rat oral acute toxicity, skin sensitization, carcinogenicity and eye corrosion/ irritation hence avoiding any unpredictable side effects on these target (Table XII and Table XIII).

Table IX: Metabolism properties of substituted pyrazole containing pyridopyrimidines (1a-p)

Sl. No.	CYP1A2 inhibitor	CYP1A2 substrate	CYP2C19 inhibitor	CYP2C19 substrate	CYP2C9 inhibitor	CYP2C9 substrate	CYP2D6 inhibitor	CYP2D6 substrate	CYP3A4 inhibitor	CYP3A4 substrate
1a	0.96	0.649	0.797	0.05	0.808	0.151	0.471	0.728	0.805	0.447
1b	0.935	0.836	0.801	0.057	0.86	0.1	0.282	0.644	0.842	0.669
1c	0.956	0.728	0.746	0.052	0.865	0.122	0.471	0.658	0.854	0.475
1d	0.957	0.707	0.7	0.045	0.832	0.188	0.556	0.69	0.845	0.302
1e	0.952	0.742	0.75	0.058	0.873	0.079	0.235	0.62	0.87	0.56
1f	0.956	0.821	0.773	0.051	0.857	0.143	0.49	0.683	0.888	0.388
1g	0.948	0.738	0.573	0.047	0.826	0.159	0.444	0.427	0.789	0.354
1h	0.71	0.902	0.123	0.058	0.059	0.031	0.114	0.031	0.106	0.902
1i	0.916	0.865	0.847	0.058	0.865	0.169	0.276	0.828	0.78	0.751
1j	0.881	0.924	0.804	0.059	0.917	0.093	0.245	0.587	0.89	0.533
1k	0.954	0.817	0.738	0.056	0.807	0.09	0.231	0.741	0.757	0.559
1l	0.857	0.538	0.728	0.053	0.765	0.149	0.24	0.65	0.743	0.528
1m	0.955	0.442	0.78	0.054	0.873	0.075	0.332	0.731	0.89	0.426
1n	0.853	0.941	0.871	0.059	0.885	0.161	0.352	0.853	0.881	0.812
1o	0.955	0.681	0.802	0.054	0.87	0.064	0.348	0.659	0.924	0.4
1p	0.906	0.729	0.811	0.054	0.858	0.12	0.32	0.564	0.874	0.478

Table X: Metabolism properties of substituted triazine containing pyridopyrimidines (2a-p)

Sl. No.	CYP1A2 inhibitor	CYP1A2 substrate	CYP2C19 inhibitor	CYP2C19 substrate	CYP2C9 inhibitor	CYP2C9 substrate	CYP2D6 inhibitor	CYP2D6 substrate	CYP3A4 inhibitor	CYP3A4 substrate
2a	0.947	0.247	0.407	0.063	0.13	0.212	0.395	0.451	0.63	0.101
2b	0.933	0.609	0.636	0.068	0.297	0.085	0.704	0.428	0.453	0.153
2c	0.936	0.23	0.301	0.063	0.126	0.193	0.557	0.333	0.583	0.104
2d	0.926	0.235	0.228	0.057	0.111	0.295	0.5	0.33	0.629	0.078
2e	0.937	0.336	0.563	0.068	0.265	0.079	0.737	0.355	0.418	0.124
2f	0.937	0.29	0.288	0.062	0.114	0.143	0.509	0.345	0.646	0.095
2g	0.924	0.209	0.192	0.058	0.129	0.22	0.356	0.236	0.553	0.082
2h	0.924	0.571	0.281	0.094	0.072	0.134	0.18	0.257	0.565	0.209
2i	0.897	0.536	0.631	0.105	0.228	0.102	0.657	0.611	0.514	0.157
2j	0.903	0.555	0.628	0.079	0.29	0.078	0.715	0.329	0.653	0.148
2k	0.945	0.418	0.56	0.067	0.168	0.089	0.62	0.587	0.495	0.133
2l	0.891	0.38	0.576	0.066	0.14	0.17	0.319	0.371	0.587	0.121
2m	0.943	0.19	0.513	0.066	0.183	0.072	0.448	0.395	0.744	0.104
2n	0.819	0.527	0.63	0.115	0.214	0.086	0.739	0.615	0.592	0.175
2o	0.934	0.243	0.435	0.065	0.175	0.063	0.436	0.305	0.735	0.099
2p	0.896	0.468	0.576	0.067	0.165	0.099	0.349	0.273	0.662	0.117

Table XI: Excretion characteristics of substituted pyrazole and triazine containing pyridopyrimidines (1a-p & 2a-p)

Sl.no	CL	T ½	Sl.no	CL	T ½
1a	1.908	0.3817	2a	3.864	0.15
1b	1.925	0.083	2b	2.818	0.021
1c	1.599	0.295	2c	2.536	0.084
1d	1.604	0.569	2d	4.858	0.179
1e	1.688	0.172	2e	2.407	0.052
1f	2.036	0.147	2f	4.203	0.178
1g	1.214	0.37	2g	1.86	0.1
1h	1.552	0.479	2h	0.894	0.034
1i	2.248	0.174	2i	2.595	0.053
1j	2.123	0.099	2j	2.713	0.025
1k	2.051	0.115	2k	2.579	0.035
1l	1.815	0.167	2l	2.123	0.061
1m	2.751	0.194	2m	3.581	0.067
1n	2.403	0.154	2n	2.694	0.039
1o	2.722	0.251	2o	3.494	0.081
1p	1.91	0.229	2p	2.296	0.081

Docking studies were carried out by taking two different proteins with the PDB ID: 2EUF and 5FWK. 2EUF is a X-RAY structure of human CDK6-Vcyclin in complex with an inhibitor, whereas 5FWK is an electron microscopy structure of Hsp90-Cdc37-Cdk4 complex. Cyclin dependent kinases (CDKs) take part in handling cell cycle and hence can be treated as defined targets for therapeutic intervention in proliferative diseases like cancer. The substituted pyrazole and triazine containing pyridopyrimidines were investigated whether these derivatives binding mode correlated with the CDK inhibitors by docking against the target proteins. Docking was carried out in the groove of the binding site present in EUF and 5FWK. The binding affinity of these derivatives was expressed in terms of docking score. For the purpose of comparison, palbociclib, a drug developed by Pfizer was considered as the standard. This drug was the first CDK4/6 inhibitor to be approved as an anti-cancer therapy. It has a docking score of -10.278 and -6.973 against 2EUF and 5FWK, respectively. All the pyrazole containing pyridopyrimidines (1a-p) displayed docking score within the range of -7.06 to -2.80 and -7.02 to -4.52 against 2EUF and 5FWK, respectively. On the other hand, all the substituted triazine containing compounds (2a-p) showed a docking score in the range of -9.22 to -6.17 and -7.76 to -5.59 towards 2EUF and

Table XII: Toxicity properties of substituted pyrazole containing pyridopyrimidines (1a-p)

Sl. no	hERG blockers	H-HT	DILI	AMES toxicity	Rat oral acute toxicity	FDAMDD	Skin Sensitization	Carcinogen city	Eye Corrosion	Eye Irritation	Respiratory toxicity
1a	0.499	0.55	0.985	0.165	0.016	0.352	0.148	0.149	0.003	0.032	0.973
1b	0.424	0.828	0.986	0.11	0.024	0.72	0.07	0.084	0.003	0.018	0.966
1c	0.274	0.439	0.987	0.118	0.014	0.211	0.1	0.078	0.003	0.035	0.973
1d	0.357	0.329	0.986	0.203	0.015	0.597	0.142	0.07	0.003	0.041	0.953
1e	0.379	0.576	0.984	0.05	0.023	0.768	0.125	0.062	0.003	0.023	0.976
1f	0.507	0.408	0.984	0.119	0.015	0.777	0.177	0.069	0.003	0.028	0.974
1g	0.138	0.317	0.989	0.215	0.013	0.21	0.089	0.074	0.003	0.058	0.949
1h	0.765	0.854	0.994	0.019	0.157	0.795	0.031	0.76	0.003	0.008	0.564
1i	0.409	0.58	0.988	0.19	0.027	0.364	0.084	0.137	0.003	0.02	0.97
1j	0.64	0.847	0.985	0.09	0.036	0.89	0.043	0.049	0.003	0.017	0.972
1k	0.585	0.768	0.985	0.279	0.026	0.839	0.066	0.24	0.003	0.02	0.961
1l	0.592	0.713	0.985	0.991	0.02	0.604	0.497	0.717	0.003	0.026	0.969
1m	0.475	0.756	0.984	0.598	0.019	0.57	0.144	0.326	0.003	0.03	0.977
1n	0.336	0.64	0.988	0.206	0.035	0.497	0.058	0.162	0.003	0.018	0.972
1o	0.513	0.634	0.982	0.409	0.013	0.87	0.164	0.134	0.003	0.027	0.974
1p	0.631	0.734	0.983	0.989	0.018	0.818	0.547	0.592	0.003	0.022	0.971

Table XIII: Toxicity properties of substituted triazine containing pyridopyrimidines (2a-p)

Sl. no	hERG blockers	H-HT	DILI	AMES toxicity	Rat oral acute toxicity	FDAMDD	Skin Sensitization	Carcinogen city	Eye Corrosion	Eye Irritation	Respiratory toxicity
2a	0.641	0.623	0.825	0.38	0.132	0.509	0.29	0.631	0.003	0.022	0.954
2b	0.887	0.782	0.883	0.219	0.084	0.884	0.204	0.48	0.003	0.013	0.984
2c	0.545	0.665	0.903	0.262	0.105	0.306	0.187	0.562	0.003	0.023	0.957
2d	0.578	0.524	0.914	0.296	0.062	0.696	0.316	0.496	0.003	0.024	0.95
2e	0.807	0.497	0.818	0.121	0.099	0.905	0.278	0.489	0.003	0.017	0.955
2f	0.58	0.585	0.764	0.208	0.113	0.889	0.313	0.461	0.003	0.02	0.956
2g	0.372	0.609	0.97	0.411	0.064	0.168	0.167	0.531	0.003	0.028	0.957
2h	0.911	0.907	0.985	0.054	0.192	0.886	0.114	0.773	0.003	0.008	0.943
2i	0.786	0.751	0.78	0.49	0.178	0.758	0.245	0.59	0.003	0.016	0.946
2j	0.844	0.843	0.729	0.489	0.266	0.928	0.203	0.257	0.003	0.013	0.939
2k	0.827	0.925	0.71	0.609	0.266	0.901	0.174	0.636	0.003	0.014	0.923
2l	0.804	0.842	0.833	0.992	0.179	0.592	0.384	0.857	0.003	0.019	0.929
2m	0.767	0.756	0.827	0.638	0.202	0.695	0.244	0.796	0.003	0.024	0.956
2n	0.763	0.812	0.739	0.5	0.33	0.733	0.189	0.631	0.003	0.016	0.945
2o	0.74	0.687	0.782	0.47	0.141	0.902	0.274	0.651	0.003	0.022	0.959
2p	0.775	0.832	0.865	0.989	0.165	0.84	0.385	0.806	0.003	0.017	0.934

Table XIV: Docking studies of substituted pyrazole and triazine containing pyridopyrimidines (1a-p & 2a-p)

Pyrazole containing pyridopyrimidines				Triazine containing pyridopyrimidines			
2EUF		5FWK		2EUF		5FWK	
1a	-6.392	1a	-5.902	2a	-8.763	2a	-6.991
1b	-3.74	1b	-6.841	2b	-8.043	2b	-7.032
1c	-6.332	1c	-6.148	2c	-7.241	2c	-5.596
1d	-7.063	1d	-5.57	2d	-9.229	2d	-7.027
1e	-3.818	1e	-6.567	2e	-7.875	2e	-7.274
1f	-6.334	1f	-6.129	2f	-7.788	2f	-7.146
1g	-3.967	1g	-6.192	2g	-6.17	2g	-7.76
1h	-5.699	1h	0.007	2h	-7.892	2h	-7.326
1i	-5.865	1i	-6.151	2i	-7.903	2i	-7.157
1j	-6.182	1j	-5.74	2j	-8.905	2j	-6.971
1k	-6.751	1k	-6.354	2k	-8.504	2k	-7.284
1l	-5.152	1l	-4.526	2l	-8.531	2l	-6.725
1m	-5.183	1m	-5.585	2m	-7.622	2m	-7.289
1n	-2.806	1n	-7.026	2n	-7.282	2n	-7.07
1o	-7.033	1o	-6.928	2o	-8.471	2o	-7.218
1p	-6.095	1p	-6.118	2p	-8.439	2p	-7.266
Palbocilcib	-10.278	Palbocilcib	6.973	Palbocilcib	-10.278	Palbocilcib	6.973

5FWK, respectively. Among the compounds docked to 2EUF, 1d (2,4 OH) and 1o (3 amino) having docking scores of -7.06 and -7.03, respectively, belonging to the pyrazole containing pyridopyrimidines and 2d(2,4 OH) and 2j (3 triflurомethyl) with -9.22 and -8.90 docking scores, respectively, belonging to triazine containing pyridopyrimidines are considered to have excellent binding energies and affinity towards the protein. On the other hand, compounds docked to 5FWK revealed the docking scores of -7.02 and -6.92 for 1n (2,4 dimethyl) and 1o (3-amino), respectively, having pyrazole ring and -7.76 and -7.32 for 2g (2,6 OH) and 2h (4 methyl sulfonyl), respectively, having triazine containing pyridopyrimidines are recognized to have appreciable binding ability to the target. Docking score of all these potentially binding compounds were comparable with standard palbociclib.

Among the pyrazole containing derivatives (1a-p), binding of the ligands with the targets are illustrated as follows. In compound 1d, interaction with 2EUF takes place through hydrogen bonding of amino group of pyrazole ring and hydroxyl group of phenyl ring with ASP 163 and ASP 102 respectively and Pi-Pi stacking of thiophene ring with PHE 98 was found. In the interaction of 1o with 2EUF, hydrogen bonding of amino substituent of benzene ring and nitrogen of pyrazole ring was found with ASP 102, VAL 101 and ASP 163, respectively. Interaction of 1n with 5FWK took place through hydrogen bonding of keto and nitrogen group of pyrazole ring with LYS 53 and GLY 92, and keto group of pyridopyrimidine with GLY 109, Pi-cation and salt bridge of pyridopyrimidine with LYS 53. 1o interacts by hydrogen bonding of keto group of pyridopyrimidine, amino group of benzene ring and hydroxyl of pyrazole ring with GLY 109, ASP 88 and GLY 92 respectively, Pi-cation binding of pyrazole and salt bridge of pyridopyrimidine with LYS 53. Similarly for triazine containing pyridopyrimidines (2a-p) binding can be pictured as follows. Compound 2d interacted with target 2EUF through hydrogen bonding of the hydroxyl substituents of the benzene ring with VAL 101 and ASP 102 (Fig. 6-10). Out of all the docked derivatives, triazine containing pyridopyrimidines docked to 2EUF demonstrated prominently higher docking scores and hence could be considered for further studies to explore its potential (Table XIV).

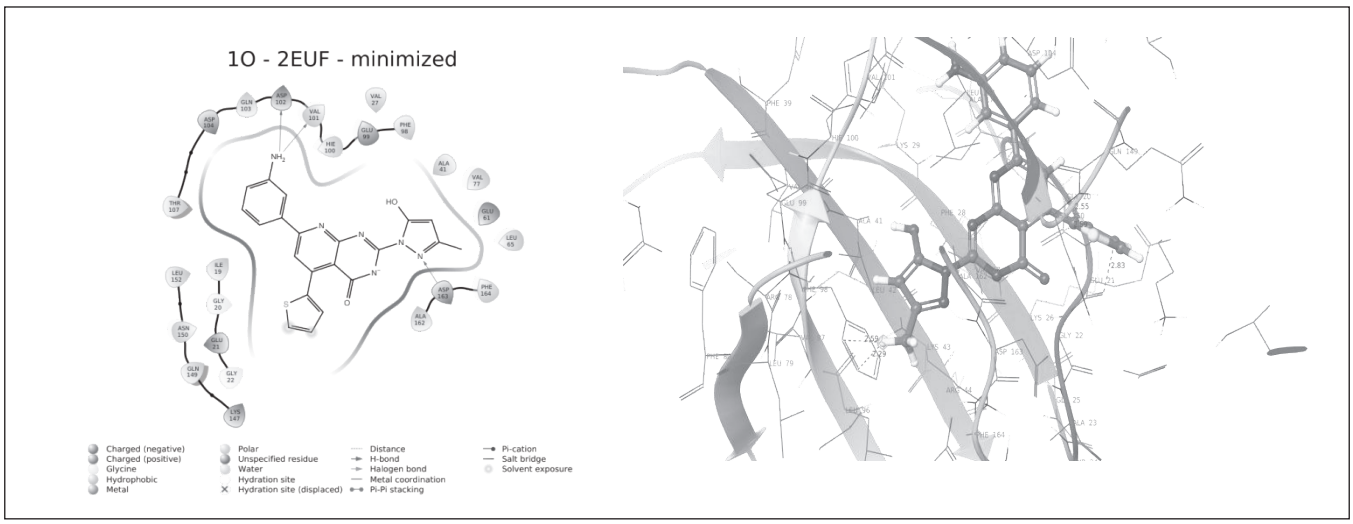

Fig. 6: Interaction of 1o with 2EUF

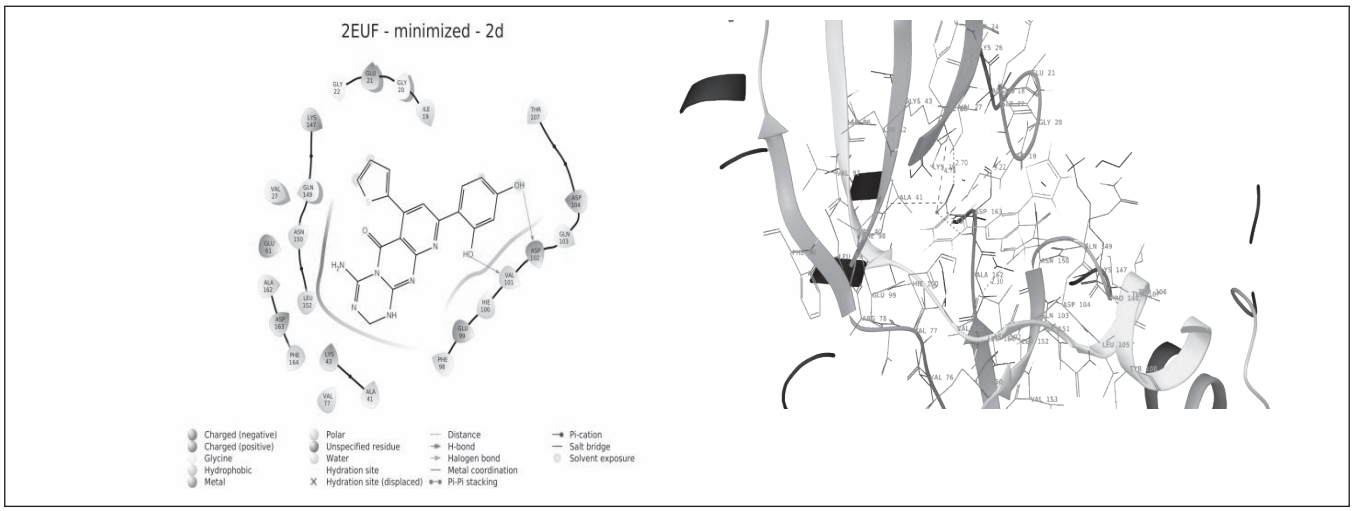

Fig. 7: Interaction of 2d with 2EUF

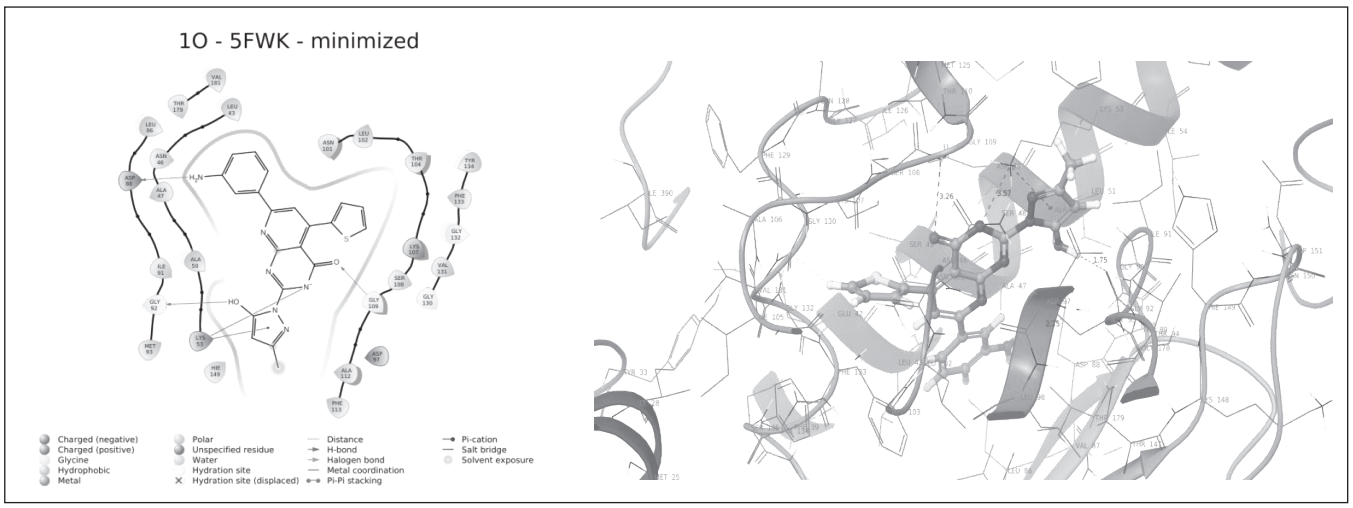

Fig. 8: Interaction of 1o with 5FWK

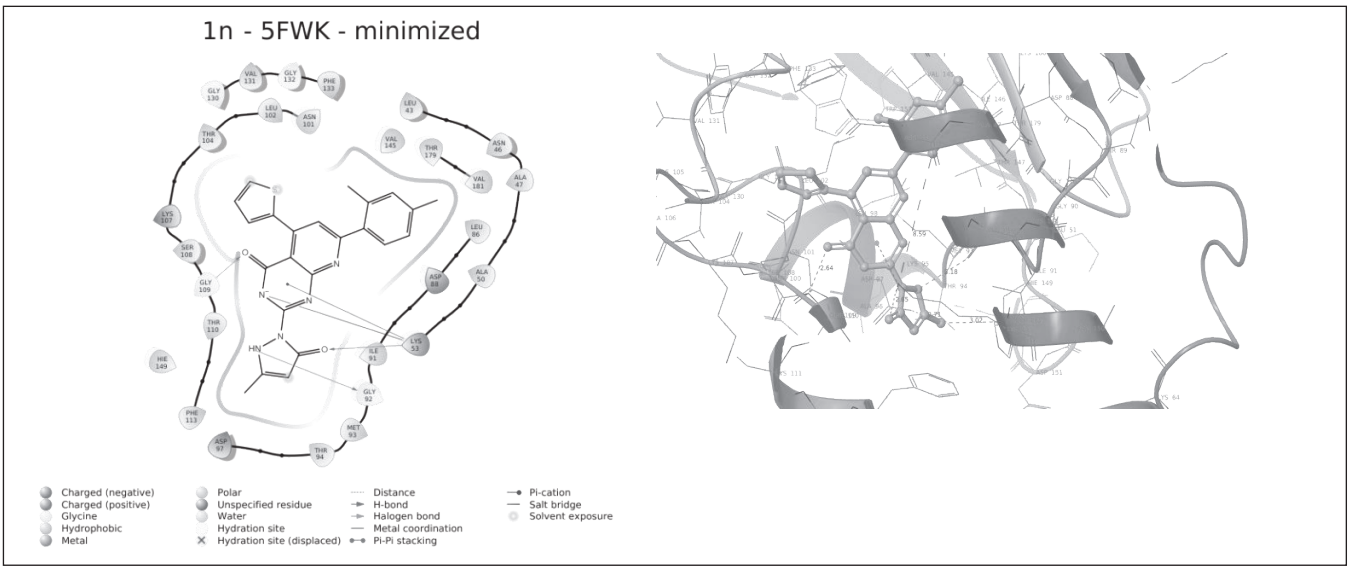

Fig. 9: Interaction of 1n with 5FWK

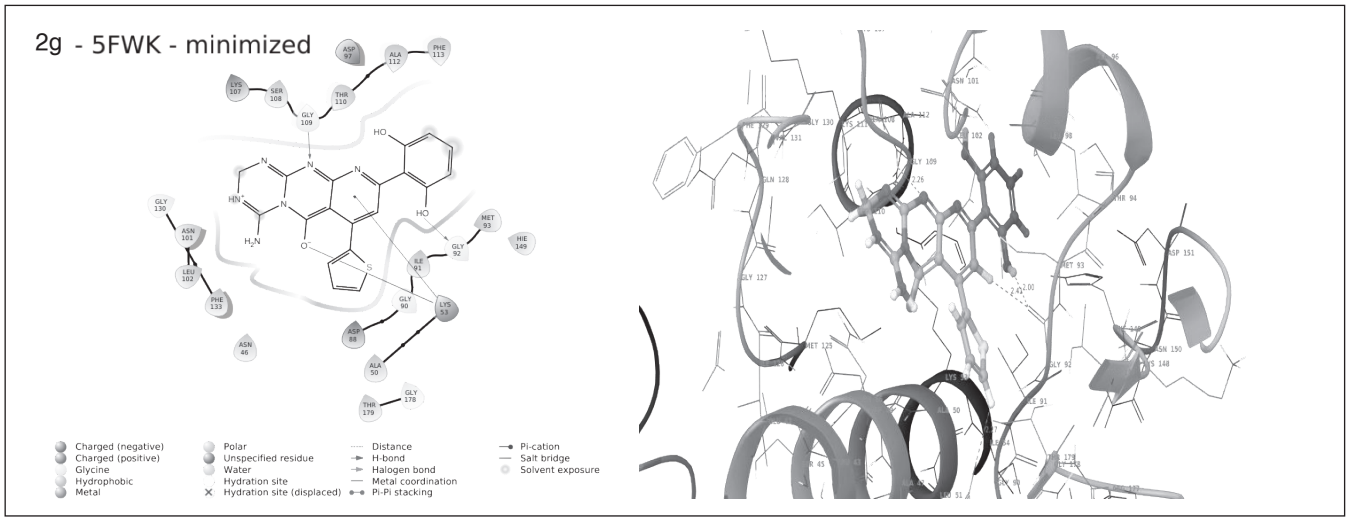

Fig. 10: Interaction of 2g with 5FWK

CONCLUSION

This study was performed with an objective to carry out the *in silico* evaluation of substituted pyrazole and triazine containing pyridopyrimidines. *In silico* studies with molecular docking was performed to filter best compounds out the 32 different derivatives. Using Molinspiration, the derivatives were examined for their conformity with Lipinski's rule of 5 and the bioactivity scoring was conducted using the same software. All the derivatives showed no violation towards Lipinski's rule of 5 and the bioactivity scores revealed the potential kinase inhibition property of the derivatives. Thereafter, the absorption, distribution, metabolism, excretion and toxicity attributes were examined using ADMETlab. The study provided an insight about the pharmacokinetics of the compounds. On carrying out the docking of the ligands with the selected protein targets i.e. 2EUF and 5FWK, docking scores were generated which the compounds that have better binding efficiency towards the proteins. Among all the screened compounds, 1d, 1o, 2d and 2j showed best affinity towards 2EUF while 1n, 1o, 2g and 2h showed best binding towards 5FWK. On that account, these compounds may have a potential cytotoxic effect and hence could be utilized for further studies and explorations of their properties.

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