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RESEARCH ARTICLE

A SIMPLE ONE-POT SYNTHESIS OF PYRIMIDO[4,5-*C*]PYRIDAZINES AND IN SILICO STUDIES OF HUMAN AKT1 INHIBITORS

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Abstract

A simple one-pot and efficient synthetic method for the synthesis of pyrimido[4,5-c]pyridazine by a multicomponent reaction. The synthesis compounds are study for their inhibitory activities towards AKT1 pathways by using in silico method. It was found that all compounds have binding energy lower than -7.9 kcal/mol and compound 3a is the most active with binding energy -9.65 kcal/mol, which have been performed using autodock vina.

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Introduction:-

Substituted pyridazines structures have been describe as a biologically active compounds with therapeutic agents and are the subunits of multiple classes of natural products(Rimaz et al., 2015). The compounds are important in therapeutic use because of their inhibitory properties of p38R(Duffy et al., 2011), phosphodiesterase 5 (PDE5)(Giovannoni et al., 2006; Rimaz et al., 2010), monoamine oxidase(Altomare et al., 1998), selective human A1 adenosine receptor ligands(Giovannoni et al., 2010), AKT1(Kettle et al., 2012), and Dihydropteroate Synthase(DHPS) inhibitor (Hazrathoseyni et al., 2016).

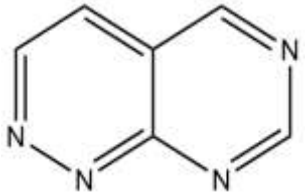

Figure 1:- Pyrimido[4,5-*c*]pyridazine

There are several synthetic routes have been reported in the literature which are of different reaction pathways, some of them are multi-component reactions. The Hoffmann reaction on 6-methylpyridazine-3,4-dicarboxamide gave a mixture of 3-methylpyrimido[4,5-*c*]pyridazine-5,7-dione and an acid(Nakagome et al., 1968) with other derivatives of pyrimido[5,4-*c*]pyridazine, and pyrimido[4,5-*c*]pyridazine-5,7(6H,8H)-diones and its derivatives from 3-chloro-4-pyridazinecarbonitrile via aminocarbonitriles are also reported(Haider et al., 1988). Derivatives of pyrimido[4,5-*c*]pyridazines have been synthesis from 6-acetyl-3-amino-2,5-diphenyl-2,5-dihydropyridazine-4-carbonitrile as precursor with various reactants obtained quantitatively the desired products(Duffy et al., 2011).Bhuyan and co-workers reported a simple and mild methods from reactions of 6-hydrazino uracils and acetylenedicarboxylates at room temperature(Bhuyan et al., 1999) while derivatives of pyridazine-3-carboxalate react towards urea or thiourea as binucleophile reagent afford pyrimido[4,5-*c*]pyridazines derivatives(El Rady &Barsy, 2006).Novel class of tri-

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cyclic derivatives of 1,2-dihydro-pyrimido[4,5-*c*]pyridazines from 4-chloro-5-methyl-2-methylsulfanyl-pyrimidine was reported by Sabatand co-workers (Sabat et al., 2006) which show Lck kinase inhibitor in nanomolar level.

AKT is a serine threonine kinase that facilitates numerous biological functions such as cell proliferation, survival, glucose metabolism, protein synthesis, genome stability, and inhibition of apoptosis in response to different growth factors and extracellular stimuli. There are three AKT isoforms in humans: AKT1, AKT2 and AKT3 which share common structure and a similar mechanism of activation. For regulating cell growth and division AKT1 plays major role, AKT2 plays an important role in cellular energy and metabolism (Shariati &Meric-Bernstam, 2019). There are several clinical trials are progress to test the efficacy of AKT pathway inhibitors for treating cancer. Ipatasertib(Saura et al., 2017) and Inhibitor VIII (IQO)(Wu et al., 2010) are the effective inhibitor of AKT1 and the crystal structure of AKT1 complexed to inhibitor VIII (PDB: 3O96) have been reported in the literature.

Hence, our synthesize new derivatives of pyrimido[4,5-*c*]pyridazine compounds are testing and evaluating them as potential AKT1 inhibitors.

Materials and Methods:-

All Chemicals were purchased from Sigma-Aldrich and Merck. Reactions were checked with TLC using appropriate solvent systems, and chromatograms were visualized using solution of iodine spray. Melting points of all the compounds were recorded on the electrically heated instruments and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 983 Spectrophotometer and ^{1}H NMR Spectra were recorded on 300 MHZ F.T NMR Spectrophotometer using $CDCl_3$ as solvent. The chemical shifts are expressed in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.

Experimental Procedure

General procedure for the synthesis of pyrimido[4,5-c]Pyridazines.

To a stirring solution of sodium tertiary butoxide (0.03 moles) in benzene, active methylene compound i.e. methyl acetoacetate, diethyl malonate (0.015 mole) was added followed by the addition of aromatic 1,2-diketones (benzil, p-methoxybenzil, furil) (0.015 moles), which resulted in the formation of a gelly solid mass (stirring uneffective). Ethanol (10 mL) was added to dissolved the solid mass followed by the addition of hydrazine and then urea or thiourea. The reaction mixture was stirred at room temperature. When the reaction is completed (monitored by TLC), the reaction mixture was worked up with benzene, dried over anhydrous sodium sulphate and the solvent evaporated. The product after keeping in the fridge overnight formed a crystalline solid which was purified by recrystallisation from ethanol.

Analytical Data

5-methyl-3,4-diphenylpyrimido[4,5-c]pyridazine-7-thiol (3a)

White crystalline solid, 80%, M.P: 137 °C. ^{1}H NMR: δ 1.6 (s, 3H), 6.3 (s, 1H), 7.3-7.5 (m, 8H), 7.9 (m, 2H). IR (cm^{-1}): 1073, 701, 1630, 2879, 3050.

3,4-di(furan-2-yl)-5-methylpyrimido[4,5-c]pyridazine-7-thiol (3b)

Brown crystalline solid, 75%, M.P: 164 °C. ^{1}H NMR: δ 1.7 (s, 3H), 6.6 (m, 3H), 7.6-7.7 (m, 4H). IR (cm^{-1}): 697, 1322, 1594, 2875.

7-mercapto-3,4-diphenylpyrimido[4,5-c]pyridazin-5-ol (3c)

White crystalline solid, 85%, M.P: 129 °C. ^{1}H NMR: δ 7.3-7.9 (m, 10H), 6.3 (s, 2H). IR (cm^{-1}): 689, 1120, 1636, 3070.

5-methyl-3,4-diphenylpyrimido[4,5-c]pyridazin-7-ol (3d)

Yellow crystalline solid, 80%, M.P: 110-112 °C. ^{1}H NMR: δ 1.7 (s, 3H), 6.3 (s, 1H), 7.3-7.9 (m, 10H).IR (cm^{-1}): 1121, 1623, 2881, 3055.

4-(4-methoxyphenyl)-3-phenylpyrimido[4,5-c]pyridazine-5,7-diol (3e)

Yellow crystalline solid, 85%, M.P: 208-210 °C. ^{1}H NMR: δ 4.0 (s, 3H), 7.2-7.6 (m, 5H), 8.0-8.5 (m, 4H).IR (cm^{-1}): 1112, 1065, 1616, 3060.

In Silico Analysis

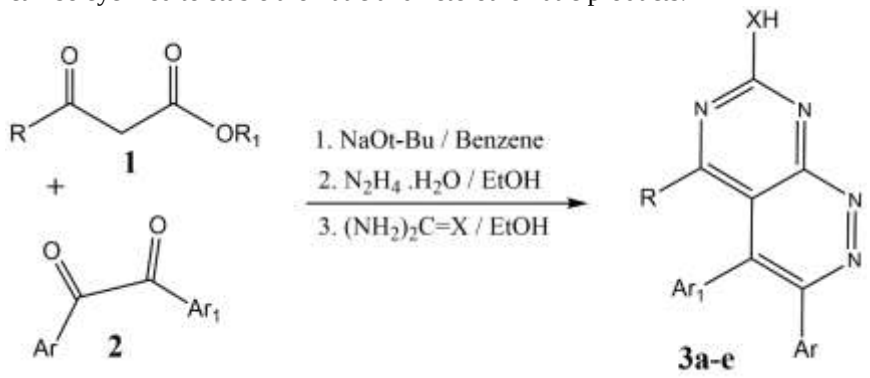
Molecular docking investigation carried out using the autodock vina(Trott & Olson, 2009). The crystal structure of AKT1 is retrieved from the RSCB protein data bank (PDB id: 3O96). The protein preparation was done in Molegro molecular viewer(Bitencourt-Ferreira & de Azevedo, 2019) by removing co-crystallized ligand, cofactors and embedded water molecules. It was further process by adding polar hydrogens and assigning Kollman charges in

Autodock tools. The ligands were prepared in ChemBio3D and optimized using the MMF94 force field. The grid parameters were determined based on the native ligand IQO. The grid is centered on IQO, making sure all the residues of the binding cavity are encompassed (center at x=9.657, y=-7.762, z=10.604, spacing = 0.375 Å). The exhaustiveness parameter for analyzing the binding affinity was set to 10 modes. The re-docking of crystal compounds confirmed the validation of the docking parameters. The analysis of docking result was carried out using the pymol and Discovery studio visualizer.

Results and Discussion:-

Chemistry

The carbonyl group (C=O) in its various forms (aldehydes, ketones, carboxylic acids and its derivatives) is the most important functional unit in organic synthesis. The versatility of the carbonyl functions in organic synthesis is based on its capability to undergo a wide variety of bond forming reactions both at the carbonyl carbon atom and at the sites influenced by it. Condensation reactions between different carbonyl compounds or with diketones often leads to products which can be cyclized to stable aromatic and heteroaromatic products.



	R(1)	R(3)	Ar	Ar_1	X
a)	CH_3	CH_3	Phenyl	Phenyl	S
b)	CH_3	CH_3	Furyl	Furyl	S
c)	OEt	OH	Phenyl	Phenyl	S
d)	CH_3	CH_3	Phenyl	Phenyl	O
e)	OEt	OH	Phenyl	Anisyl	O

Scheme 1:- Synthesis of pyrimido[4,5-*c*]pyridazine.

The use of carbonyl and dicarbonyl compounds in the synthesis of pyrimido[4,5-*c*]pyridazine has been reported(Bhuyan et al., 1999). In this work, a new convenient route for the synthesis of pyrimido[4,5-*c*]pyridazine starting from an easily available starting materials. In this present investigation pyrimido[4,5-*c*]pyridazines was synthesized from active methylene compounds (methyl acetoacetate, diethylmalonate) by condensation with aromatic 1,2-diketones (benzil, p-methoxybenzil, furil) and further cyclisation by hydrazine and urea or thiourea as shown in **Scheme 1**. In all these cases solid products are obtained.

The ^{1}H-NMR spectrum of the compound 3a shows a singlet signal at δ 1.6 ppm indicating the presence of CH_3 group, a singlet at δ 6.3 ppm due to SH group, which was removed on deuteration and a multiplet signal at δ 7.3 – 7.5 and δ 7.9 indicates hydrogens of the aromatic rings. While in compound 3b, the ^{1}H-NMR signal of thiol (SH) is overlapping with the aromatic hydrogen signal at δ 6.6 ppm showing multiplet signals.

In silico analysis

Docking analysis was perform on all the synthesis compounds at the AKT1 enzyme active sites and compared with the co-crystallized compound IQO(also called inhibitor VIII)(Rehan et al., 2014) and Ipatasertib (under phase III clinical trial)(Shariati &Meric-Bernstam, 2019). All the compounds have their binding energies less than -7.9 kcal/mol with the lowest binding energy with compound **3a** at -9.65 kcal/mol. All the compounds interact the protein with hydrogen bonding through Ser205 and Lys268 but compound **3e** interact with Val271 in addition to the above interactions.

Table 1:- Docking Scores and interactions.

Compounds	Binding affinity (Kcal/mol)	Hydrogen Bonds	Residue involved in other interactions
3a	-9.65	Ser205, Lys268	Trp80, Leu210, Leu264, Lys268, Val270, Ile290
3b	-7.98	Ser205, Lys268	Trp80, Leu210, Leu264, Lys268, Val270, Asp292
3c	-8.90	Ser205, Lys268	Trp80, Leu210, Leu264, Lys268, Val270
3d	-9.35	Ser205, Lys268	Trp80, Leu210, Leu264, Lys268, Val270, Ile290
3e	-9.41	Ser205, Lys268, Val271	Trp80, Leu210, Leu264, Lys268, Val270, Ile290
Ipatasertib	-10.17	Tyr272, Thr211, Ser205	Gln79, Trp80, Leu264, Val270, Arg273
IQO	-12.4	Ser205, Lys268, Tyr272	Ile84, Trp80, Leu210, Leu264, Lys268, Val270, Arg273, Asp292

All the compounds including the reference compounds shows π-π interactions with Trp80, they are also stabilized at the interface by hydrophobic π-alkyl, π-σ and π-cation interactions. The possible active sites of the protein by using the co-crystallized ligand are Ser205, Trp80, Lys268, Tyr272, Ile84, Val270, Asp292 and Arg273. Based on the active sites, the synthesis compound **3a** is having a good interaction with the active residues in this protein.

The nitrogen atom of the pyrimidine ring established a hydrogen bonding with Ser205 and Lys268. The surface model of binding in (Figure 2) shows that the polar interactions are shown as red colour and the non-polar interactions are shown as greycolour in the pocket of active sites.

The compound **3e** also have a good interaction with the active sites showing the polar interactions with Ser205, Lys268 with nitrogen atom of the pyrimidine ring and Val271 with methyl group and other non-polar hydrophobic interactions (Figure 3).

The synthesis compounds **3a** and **3e** have a good posing in the active sites cavity of the protein which was shown at the overlay structure of these compounds with the reference compound (Figure 4).

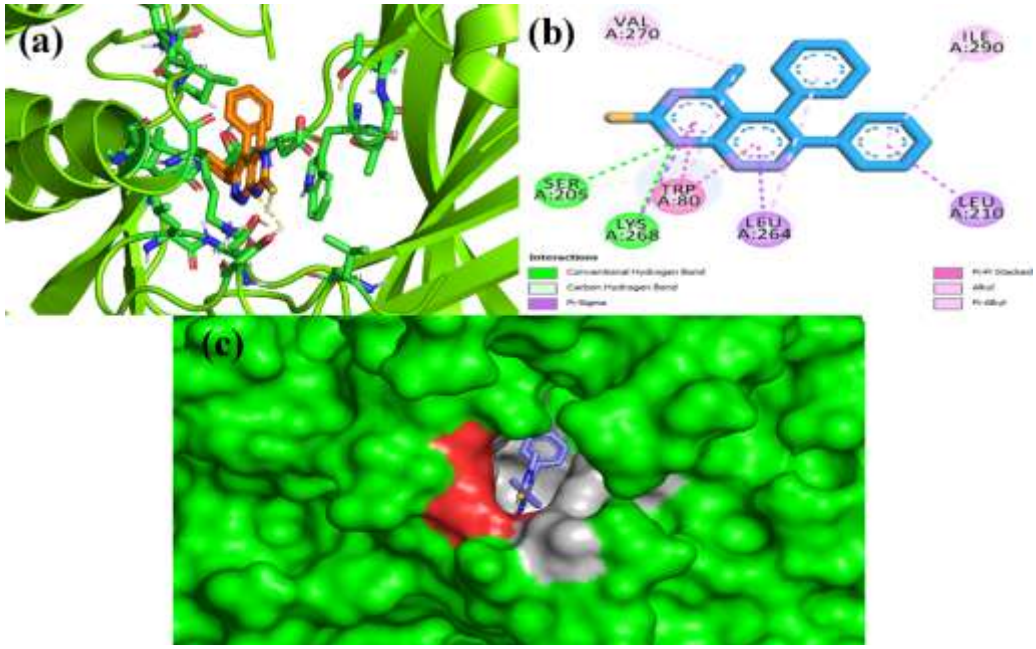


Figure 2:- (a) binding mode of compound 3a with AKT1 protein 3O96 (b) 2D representation of compound 3a with AKT1 protein (c) surface model of binding of compound 3a with AKT1 protein showing polar interaction with red colour and non-polar interaction with grey colour.

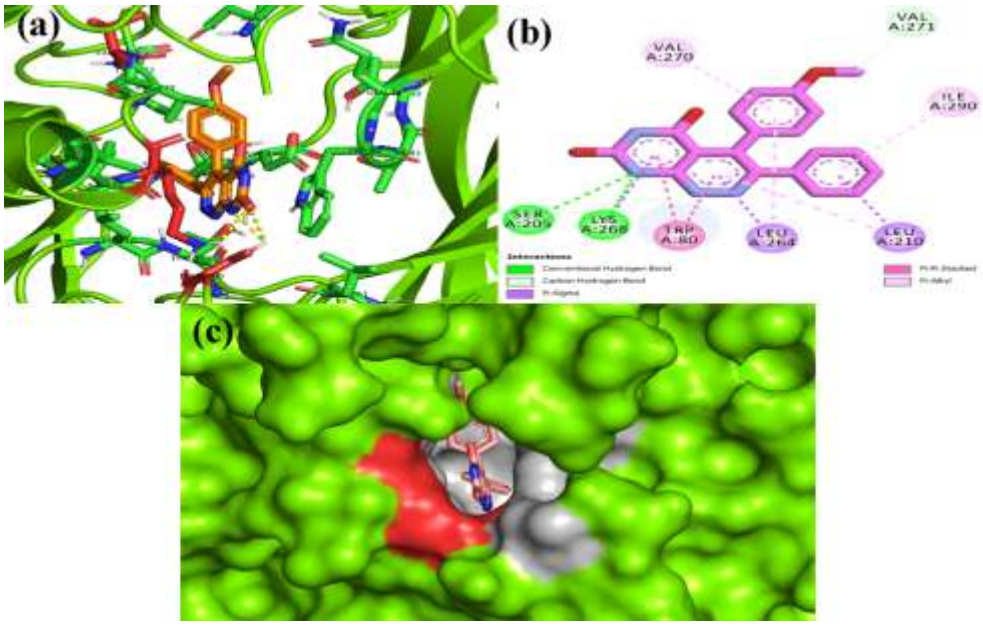


Figure 3:- (a) binding mode of compound 3e with AKT1 protein (PDB:3O96) (b) 2D representation of compound 3e with AKT1 protein (c) surface model of binding of compound 3e with AKT1 protein showing polar interaction with red colour and non-polar interaction with grey colour.

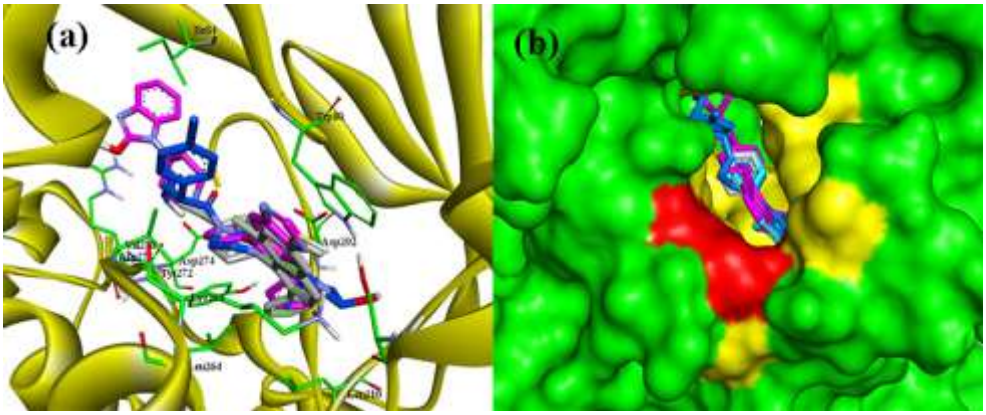


Figure 4:- Overlay of compound 3a(cyan), 3e(grey), IQO(magenta) and Ipasatertib(blue).

Conclusion:-

The chemical procedures summarised provided incredibly easy and simple methods to obtain different derivatives of pyrimido[4,5-c]pyridazine. The synthesized compounds were analysed as potential AKT1 inhibitory activity, and the results indicated that compound 3a had well inhibitory effect than the other compounds. Further research is in progress to increase our understanding of the SAR of this inhibitory activity.

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