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Exploring 7-Azaindole Containing Heterocyclic Derivatives with Pyrimidine Scaffolds as Potent Bromodomain 4 Inhibitors - An *In-Silico* Approach

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ABSTRACT

The present work was envisioned to design a series of novel 7-Azaindole derivatives containing pyrimidine scaffolds for its inhibition towards BRD4. *In-silico* studies such as docking, ADMET, calculation of molecular properties and Toxicity studies were performed using Accelery's Discovery studio 3.5. Most of the designed compounds were found to have better binding towards BRD4 through hydrogen bonding with amino acids such as **ARG 150**, **SER 148**, **ASP 129**, **ILE 263** and pi interactions with better –C-Docker interaction energy. Most of the compounds were in the expected range of the other above mentioned *in-silico* studies. Among all the designed ligands, A16 possess 3, 4, 5-trimethoxy phenyl substituent found to have better binding affinity with BRD4 with all the virtual studies values within the expected range.

INTRODUCTION

Cancer is one of the dreadful disease which is having a serious consequence throughout the world. Bromodomain containing protein family has been emerged as an important target in cancer research. Bromodomain is a protein domain, constitutes 110 amino acids which are responsible for the recognition of acetylated lysine of histones. They are epigenetic readers and the family mainly consists of BRD1, BRD2, BRD3, BRD4 and Bromodomain Extra Terminal (BRDT).[1] The structural features of all the bromodomains include two N-terminal bromodomains and an extra C-terminal domain (ET).[2] Among the family, BRD4 has been extensively concentrated more in research field and it has been implicated in various types of cancers such as breast, cervical, ovarian, bone, etc.[3] The structural features of BRD4 constitutes a highly conserved N-terminal bromodomain; BD1 and BD2, an extra terminal domain and a C-terminal domain.[4] By interacting with acetylated chromatin and non-histone proteins, BRD4 has been involved mainly in the regulation of DNA replication, transcription and cell cycle progression. BRD4 structure mainly consists of four α helices (αZ, αA, αB, and αC) which are separated by various loop regions of ZA and BC which forms the hydrophobic cavity which is responsible for the recognition of acetylated lysines of histones.[5] BRD4 inhibitors could block the interactions between BRD4 and acetyl lysine binding site and should be best in mimicking acetyl lysine and competing with acetyl lysine binding site to bind with BRD4. BC loop which constitutes a hydrophobic region is also playing an important role in BRD4 binding affinities.[6]

Azaindoles have gained a considerable interest among medicinal chemists owing to their physicochemical and pharmacological properties. As a bio isostere of purine or indole, they were found to facilitate solubility enhancement, reduction of lipophilicity, favorable target binding and improvement of ADME as well as toxicity parameters. Because of its unique ability to accept and donate hydrogen bonds, [7] 7-azaindole moiety has been found to exhibit a wide variety of pharmacological actions such as anticancer, [8] anti-inflammatory, [9] antimicrobial[10,] etc. Pyrrolopyridine structure constitutes a pyrole and pyrimidine ring mainly have four isomers and among them (4, 5, 6 and 7 azaindole), 7-azaindole found to exhibit various pharmacological activities. In addition, the binding between the ligand and the receptor can be enhanced by the bioisosteric replacement of indole ring with an azaindole ring by the formation of an extra hydrogen bond and thus an increase in the pharmacological activity.[11] So hereby in the present work, we designed a series of 7-azaindole derivatives and

explored their binding interaction with the bromodomain containing protein 4 and thus inhibition. This work was mainly planned to design derivatives of 7-azaindole for its binding affinity towards BRD4 and also to evaluate *in-silico* parameters such as ADME and toxicity.

MATERIALS AND METHODS

Virtual screening studies are mainly performed to identify potential lead compounds from a set of database of inactive compounds. The main aim of virtual screening is to reduce the number of ligands to be screened experimentally and thus by reducing the number of false positives. Docking is mainly accomplished to study the effect of binding interaction between the ligand and the receptor and can generate good poses of ligand in an active site of the receptor.

Molecular properties

Molecular properties considered to be an important criterion during designing of ligands. In the present paper, molecular properties such as molecular weight, A log P, H acceptors, H donors, molecular fractional polar surface area were calculated[12] using Accelery's Discovery studio 3.5 and the results are depicted in table 1.

Molecular Docking Studies

Docking study was mainly carried out to analyze the interaction between the receptor and the designed ligands and the study was carried out using Discovery studio 3.5. Docking mainly constitutes protein preparation, ligand preparation and receptor ligand interaction using different programmes. In the present paper, C-Dock programme with CHARMm forcefield was executed for running docking.

a. Protein preparation

The X-ray crystallographic structure of BRD4 (PDB id: 4HY3), was downloaded from RCSB Protein Data Bank with a resolution of 2.8Å. For the preparation of protein, 110 amino acids with A chain was retained, water molecules were deleted and hydrogens were added.[13] The protein was prepared by employing **MACROMOLECULES** tool in the Discovery studio. The prepared protein was subjected to minimization using CHARMm forcefield.

b. Ligand preparation

The structures of the designed ligands were drawn in ChemDraw and were uploaded in Discovery studio in mol format. They were subjected to ligand preparation and minimization using SMALL MOLECULES[13] tool in the Discovery studio.

c. Molecular Docking

The minimized ligands were docked into the active site of the receptor using CHARMm force field by executing C-Docker programme in Discovery studio. C-Docker energy, C-Docker interaction energy, Hydrogen bonding and the amino acids involved in binding with the receptor[14] were extensively studied and the results are depicted in table 2.

ADME Parameters

The series of designed 7-azaindole derivatives were subjected to ADME studies in Discovery studio 3.5. Various factors such as absorption, BBB, solubility level, hepatotoxicity, CYP2D6 binding and plasma protein binding[15] were predicted and the results are given in table 3.

Toxicity parameters

Virtual toxicity studies for the designed compounds were carried out using TOPKAT module in Discovery studio. NTP carcinogenicity, Ames mutagenicity, Developmental toxicity and Skin irritation parameters[16] were calculated and tabulated in table 4.

RESULTS

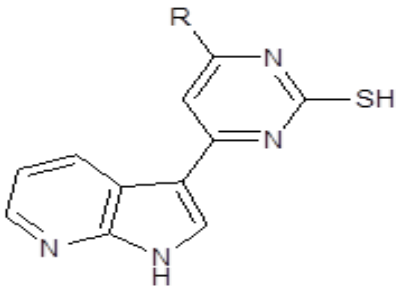

Figure No. 1: General structure of the designed 7-azaindole derivative

Table No. 1: General structure of the designed ligands with list of substituents

Compound code	R substituent	compound code	R substituent
A1	4-flouro phenyl	A12	3-ethoxy-4-methoxy phenyl
A2	3-flouro phenyl	A13	3,4-dimethoxy phenyl
A3	2-flouro phenyl	A14	2,4-dimethoxy phenyl
A4	2-nitro phenyl	A15	2-naphthyl
A5	3-hydroxy phenyl	A16	3,4,5-trimethoxy phenyl
A6	3-methoxy phenyl	A17	Pyridine-2-yl
A7	4-ethoxy phenyl	A18	Thiophene-2-yl
A8	2,4-dihydroxy phenyl	A19	Anthracene-2-yl
A9	3-nitro phenyl		
A10	4-nitro phenyl		

Table No. 2: Molecular properties of the designed 7-azaindole derivatives

Compound code	No. of hydrogen bond donor	No. of hydrogen bond acceptor	A logP	Mol weight	Molecular fractional Polar surface area
A1	2	4	4.373	322.359	0.316
A2	2	4	4.373	322.359	0.316
A3	2	4	4.373	322.359	0.316
A4	2	6	4.062	349.367	0.426
A5	3	5	3.926	320.368	0.378
A6	2	5	4.151	334.395	0.321
A7	2	5	4.500	348.422	0.306
A8	4	6	3.683	336.368	0.427
A9	2	6	4.062	349.367	0.426
A10	2	6	4.062	349.367	0.426
A11	2	6	4.135	364.421	0.317
A12	2	6	4.483	378.448	0.304
A13	2	6	4.135	364.421	0.317
A14	2	6	4.135	364.421	0.317
A15	2	4	5.984	404.486	0.252
A16	2	7	4.118	394.447	0.314
A17	2	5	3.445	305.357	0.368
A18	2	4	4.121	310.397	0.427
A19	2	4	5.076	354.428	0.284

Table No. 3: Docking results of designed 7-azaindole derivatives towards BRD4 (PDB id 4HY3)

Ligand code	(-) CDOCKER Energy	(-) CDOCKER interaction Energy	Interactions Ligand Residue	H-bond distance in Å	Interacting amino acids
A1	-0.9594	16.9817	-	-	-
A2	-0.8270	17.5817	-	-	-
A3	-3.3514	17.5110	-	-	-
A4	-5.9380	21.8923	-	-	-
A5	1.5189	18.9588	SH group attached to pyrimidine ring	2.2604	ASP 129
A6	-142.8410	-12.5202	-	-	-
A7	-23.0308	6.4407	-	-	-
A8	-150.8350	-14.1694	-	-	-
A9	-154.0560	-20.2549	-	-	
A10	-8.3492	16.3300	-	-	-
A11	0.3556	29.5587	NH of azaindole	2.4126	SER 148
			SH group attached to pyrimidine ring	2.1255	ILE 263
A12	-2.4533	26.7606	-	-	-
A13	5.3643	29.3863	NH of azaindole	2.1382	ARG 150
A14	-90.2605	-3.0851	-	-	
A15	-39.3467	7.2233	SH group attached to pyrimidine ring	2.1490	SER 148
A16	1.3120	31.1855	-	-	-
A17	6.8229	21.0165	-	-	-
A18	4.0758	16.9730	SH group attached to pyrimidine ring	2.1237	ASP 129
A19	-1.9427	26.6695	-	-	-

Table No. 4: ADME studies of the designed 7-azaindole derivatives

Ligand code	Absorption level	solubility level	BBB level	Hepatotoxicity	CYP 2D6 binding	Plasma protein binding (PPB level)
A1	0	1	1	true	false	true
A2	0	1	1	true	false	true
A3	0	1	1	true	false	true
A4	0	1	2	true	false	false
A5	0	2	2	true	false	false
A6	0	2	1	true	false	true
A7	0	1	1	true	false	true
A8	0	2	2	true	false	false
A9	0	1	2	true	false	true
A10	0	1	2	true	false	false
A11	0	1	1	true	false	true
A12	0	1	1	true	false	true
A13	0	1	1	true	false	true
A14	0	1	1	true	false	true
A15	1	0	0	true	false	false
A16	0	1	2	true	false	false
A17	0	2	2	true	false	false
A18	0	2	1	true	false	false
A19	0	1	1	true	false	false

Table No. 5: *In-silico* toxicity studies using TOPKAT for the designed 7-azaindole derivatives

Ligand code	NTP Carcinogenicity call (female rat)	NTP Carcinogenicity call (male rat)	Ames mutagenicity	Developmental toxicity	Skin irritation
A1	No	No	Yes	No	None
A2	No	No	Yes	No	None
A3	No	No	Yes	No	None
A4	No	No	Yes	No	Mild
A5	No	No	Yes	No	None
A6	No	No	Yes	No	None
A7	No	No	No	No	None
A8	No	No	Yes	No	None
A9	No	No	Yes	No	Mild
A10	No	No	Yes	No	Mild
A11	No	No	Yes	No	None
A12	No	No	No	No	None
A13	No	No	Yes	No	None
A14	No	No	Yes	No	None
A15	No	No	Yes	No	None
A16	No	No	No	No	None
A17	No	No	Yes	No	None
A18	No	No	Yes	No	None
A19	No	No	Yes	No	None

2D images of the best poses from docking results

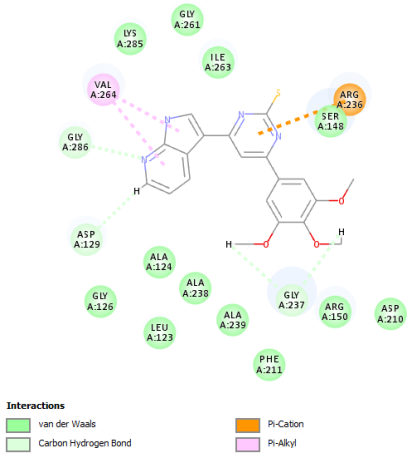

Figure No. 2: Binding interaction of A16 with 4HY3

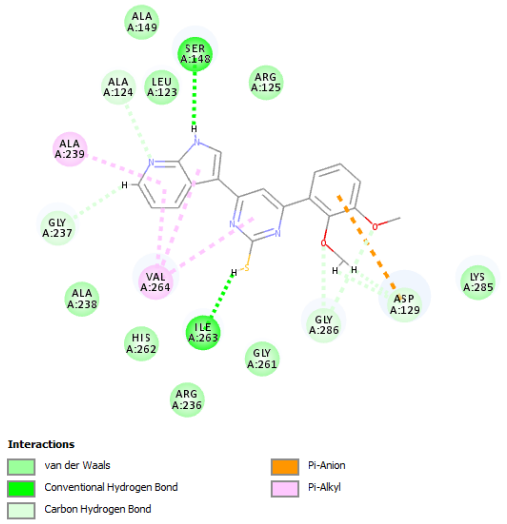

Figure No. 3: Binding interaction of A11 with 4HY3

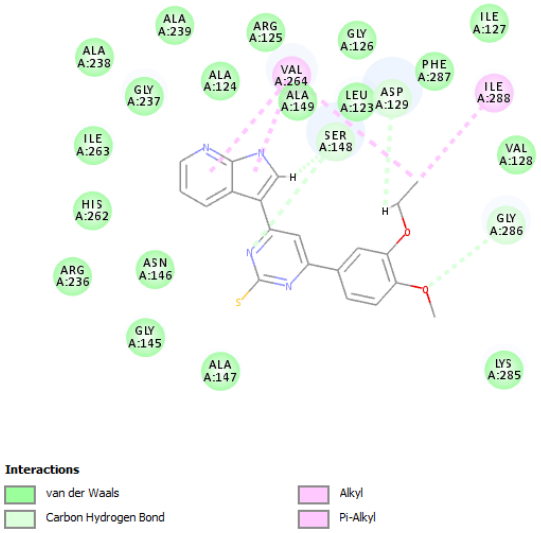

Figure No. 4: Binding interaction of A12 with 4HY3

DISCUSSION

A series of novel pyrimidine derivatives were designed on the basis of their binding affinity towards Bromodomain 4 (4HY3). Molecular properties of all the designed compounds were calculated and were subjected to ADME, toxicity and molecular docking studies using Accelery's Discovery studio 3.5. From the results obtained, it was well clear that all the designed 7-Azaindole derivatives were found to possess better molecular properties. From the molecular properties, it was found that all the designed compounds were found to possess good number of hydrogen bond donor and acceptors. All the ligands possess number of

hydrogen bond acceptor in the range of 4 to 7 and number of hydrogen bond donor in the range of 2 to 4. The designed ligands were observed to be having an A log P value in the range of 3.4 to 5.9 and molecular fractional polar surface area in the range of 0.25 to 0.42.

In order to explain the possible binding mode of the ligands with in the binding site of the receptor, a molecular docking study was performed and found to have better interaction with the receptor binding cavity. Docking results of the designed ligands were depicted in table no: 3. The ligands such as **A16**, **A11**, **A12** and **A13** which possess the substituents such as **3,4,5 trimethoxy phenyl, 2,3-dimethoxy phenyl, 3,4-dimethoxy phenyl and 3-ethoxy-4-methoxy phenyl** were found to have better binding with C-Docker interaction energy of **-31.1855, -29.5587, -29.3863** and **-26.7606**. The main amino acids involved in binding were found to be **SER 148, ILE 263** and **ARG 150**. Besides hydrogen bonding, several other interactions such as pi-alkyl and pi-stacked was also observed. All most all the compounds were found to have hydrogen bond interaction with amino acids such as **ASP 129, ARG 150, SER 148 and ILE 263**. The C-Docker interaction energy of the designed ligands were found to be in the range of -6.4407 to -31.1855. In the ligands, which possess better C-Docker interaction energy, NH of azaindole and SH attached to pyrimidine ring found to form hydrogen bonding with the receptor with a distance in the range of 2.1255 to 2.4126. The 2D images of the best compounds are depicted in figure 2 to 5.

ADMET properties like absorption level, solubility level, Blood-Brain Barrier level (BBB), hepatotoxicity, CYP2D6 binding and Plasma Protein Binding (PPB) were evaluated and the results were depicted in table no: 4. From the results, it was well understood that all the designed ligands possess BBB permeability of very high, high and medium. The ligand A15, which possess the substituent as 2-naphthyl found to have a very high range of BBB permeability with a value of 0 and remaining ligands were found to have medium to high range of BBB permeability. While considering the absorption ability of the designed ligands, it was observed that A15 found to have moderate absorption and all the remaining ligands were found to have good absorption level. Regarding *in-silico* solubility studies, among the designed ligands, A15 with 2-naphthyl substitution with extremely low solubility. The ligands which possess substituents such as 4-flouro phenyl, 3-flouro phenyl, 2-flouro phenyl, 2-nitro phenyl, 4-ethoxy phenyl, 3-nitro phenyl, 4-nitro phenyl, 2,3-dimethoxy phenyl, 3,4-dimethoxy phenyl, 3-ethoxy-4-methoxy phenyl, 3,4,5- trimethoxy phenyl and anthracene-2-yl found to be observed with very low solubility and the ligands which has 3-hydroxy phenyl, 3-

methoxy phenyl, 2,4-dihydroxy phenyl, pyridine-2-yl, thiophene-2-yl found to possess low solubility. By analyzing CYP2D6 inhibition, it was well understood that all the designed ligands were non inhibitors of CYP2D6. The ligands which possess the substituents such as 3-floro phenyl, 2-flouro phenyl, 4-floro phenyl, 3-methoxy phenyl, 4-ethoxy phenyl, 3-nitro phenyl, 2,3-dimethoxy phenyl, 3-ethoxy-4-methoxy phenyl, 3.4-dimethoxy phenyl and 2,4-dimethoxy phenyl found to possess an ability of high PPB.[17]

Toxicity studies of the designed ligands were carried out using TOPKAT module in the Discovery Studio 3.5. From the results, it was understood that among the designed 7-Azaindole derivatives, the ligands which possess the substituents such as 4-ethoxy phenyl, 3-ethoxy-4-methoxy phenyl and 3, 4, 5-trimethoxy phenyl were found to exhibit as non-mutagens in Ames mutagenicity study. The designed ligands were found to be virtually non-toxic and except the nitro derivatives, all the other ligands were found to have no skin irritation.[18]

From the above results, it was clear that the designed ligands can be a series of good BRD4 inhibitors. From the docking results, by the formation of hydrogen bond interaction, pi interactions with the receptors binding site, the above designed 7-Azaindole derivatives could be promising leads in anticancer drug discovery. From the other *in-silico* studies, it was well clear that, as most of the designed ligands fall within the expected range of ADMET and Toxicity studies, the designed series of ligands could be druggable as BRD4 inhibitors.

CONCLUSION

A series of 7-Azaindole derivatives with pyrimidine scaffolds were designed for its inhibition towards Bromodomain-4. Docking studies revealed the binding interactions of the designed ligands with the acetylated lysine of histone tail and from the other in-silico studies such as ADMET, Toxicity and Molecular properties calculation, it was clear that the ligand, which has **3,4,5-trimethoxy phenyl substituent (A16)** found to have better binding affinity with a C-Docker interaction energy of -31.1855 and with the expected *in-silico* results in ADMET and Toxicity studies and thus would be a better BRD4 inhibitor.

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REFERENCES

1. Deeney JT, Belkina AC, Shirihai OS, Corkey BE, Denis GV. BET bromodomain proteins Brd2, Brd3 and Brd4 selectively regulate metabolic pathways in the pancreatic β-cell. PLoS One. 2016; 11(3): e0151329.
2. Wu SY, Chiang CM. The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation. J. Biol. Chem. 2007; 282(18):13141-5.
3. Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. Mol. oncol. 2007;1(1):19-25.
4. Shi J, Wang Y, Zeng L, Wu Y, Deng J, Zhang Q, Lin Y, Li J, Kang T, Tao M, Rusinova E. Disrupting the interaction of BRD4 with diacetylated Twist suppresses tumorigenesis in basal-like breast cancer. Cancer cell. 2014; 25(2): 210-25.
5. Filippakopoulos P, Knapp S. Targeting bromodomains: epigenetic readers of lysine acetylation. Nat. Rev. Drug Discov. 2014; 13(5): 337-56.
6. Romero FA, Taylor AM, Crawford TD, Tsui V, Côté A, Magnuson S. Disrupting acetyl-lysine recognition: progress in the development of bromodomain inhibitors. J. med. chem. 2015; 59 (4):1271-98.
7. Mérour JY, Buron F, Plé K, Bonnet P, Routier S. The azaindole framework in the design of kinase inhibitors. Molecules. 2014;19 (12):19935-79.
8. Ruiz J, Rodríguez V, De Haro C, Espinosa A, Perez J, Janiak C. New 7-azaindole palladium and platinum complexes: crystal structures and theoretical calculations. In vitro anticancer activity of the platinum compounds. Dalton Trans. 2010; 39 (13): 3290-301.
9. Mushtaq N, Saify ZS, Noor F, Takween S, Akhtar S, Arif M, Khan KM. Synthesis and pharmacological activities of 7-azaindole derivatives. Pak. J. Pharm. Sci. 2008; 21 (1): 36-9.
10. Saifya ZS, Moazzam SM, Nisa M, Haider S, Aryne A, Khanum M, Khan SA, Ahmed A, Arshad N, Ghani M. 7-Azaindole derivatives as potential antibacterial agents. Biol. Sci.-PJSIR. 2009; 52(1):1-7.
11. Chowdhury S, Sessions EH, Pocas JR, Grant W, Schröter T, Lin L, Ruiz C, Cameron MD, Schürer S, LoGrasso P, Bannister TD. Discovery and optimization of indoles and 7-azaindoles as Rho kinase (ROCK) inhibitors (part-I). Bioorg. Med. Chem. Lett. 2011; 21(23): 7107-12.
12. Ramajayam R, Tan KP, Liu HG, Liang PH. Synthesis, docking studies, and evaluation of pyrimidines as inhibitors of SARS-CoV 3CL protease. Bioorg. Med. Chem. Lett. 2010; 20(12): 3569-72.
13. Yang LL, Li GB, Yan HX, Sun QZ, Ma S, Ji P, Wang ZR, Feng S, Zou J, Yang SY. Discovery of N6-phenyl-1H-pyrazolo [3, 4-*d*] pyrimidine-3, 6-diamine derivatives as novel CK1 inhibitors using common-feature pharmacophore model based virtual screening and hit-to-lead optimization. Eur. J. med. chem. 2012; 56:30-8.
14. Modi P, Patel S, Chhabria MT. Identification of some novel pyrazolo [1, 5-a] pyrimidine derivatives as InhA inhibitors through pharmacophore-based virtual screening and molecular docking. J. Biomol. Str. Dynamics. 2019; 37(7):1736-49.
15. Radi M, Dreassi E, Brullo C, Crespan E, Tintori C, Bernardo V, Valoti M, Zamperini C, Daigl H, Musumeci F, Carraro F. Design, synthesis, biological activity, and ADME properties of pyrazolo [3, 4-*d*] pyrimidines active in hypoxic human leukemia cells: a lead optimization study. J. med. chem. 2011; 54(8): 2610-26.
16. Srinivasan S, Girija R, Aruna S. Molecular docking studies on mycobacterial tuberculosis-6-oxopurine phosphoribosyltransferase EMRB with pyrimidine derivatives. J. Comp. Methods Mol. Des. 2015; 5(4):120-8.
17. Venkataramana CH, Sravani KR, Singh SS, Madhavan V. *In-silico* ADME and toxicity studies of some novel indole derivatives. J. App. Pharm. Sci. 2011;1(10):159.
18. PS P, TO SK, NB PR, KM M. Synthesis, *in vitro* antibacterial, toxicity and molecular docking anticancer activity of novel *N*-[(2-chloroquinolin-3-yl) methylidene]-2-aniline Schiff Bases. Int. J. Curr. Pharm. Res. 2015; 7(3):37-46.