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Identification of a novel inhibitor for the Bromodomain (BRD4) through a receptor-based drug discovery approach

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ABSTRACT

Recently, the demands on the drug discovery process have increased drastically because of the need to apprehend a novel target which might be both pertinent to cause disease and chemically tractable. The emergence of bioinformatics and computer strategies have given room to analyse conditions at the molecular level. The present work was to perform a molecular docking analysis and ADMET study of different δ-carboline derivatives with bromodomain (BRD4) receptor using receptor-based drug discovery approach. Based on the literature, 60 compounds were designed and subjected to molecular docking for the inhibition of brd4 receptor. The results showed that Compound 34 received the highest binding affinity with BRD4 receptor. Hence eight compounds were selected based on docked pose determined using AutoDock/Vina with the minimal energy of above -5.1. Then ADMET study was carried, in that, all the eight compounds had middle to high BBB permeability. During metabolism, all compounds except compounds 37, 42 and 47 showed no inhibition of CYP2C99 in the liver. Analysis of drug-likeness profile showed almost all compounds eligible in CMC rule, violation rule of CMC, MDDR rule with the value of 1 and violations of WDI showed 0 value. Such findings strongly implied that derivatives of δ-carboline could serve as lead molecules to inhibit BRD4, and this could lead to the future development of the right candidate for cancer research.

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INTRODUCTION

Colorectal cancer (CRC) was a low incidence a few centuries ago, but is now predominant cancer and represents about 10% in the developed countries. The increase of CRC in developed countries can be attributed to modern nutritional practices and enhanced risk factors. Many treatments have been designed to provide the patient with extra opportunities for primary and metastatic CRC, which includes laparoscopic surgery, radiotherapy, neoadjuvant and palliative treatments. These treatments, however, had restricted effects on cure rates and long-term survival of the patients (Kuipers *et al.*, 2015; Tang *et al.*, 2017). Drug resistance was practically acknowledged at the outset in CRC treatment.

The significance of drug resistance was demonstrated by intensive studies in causes of low sensitivity medications such as fluoropyrimidines, irinotecan and oxaliplatin (Panczyk, 2014). Indoles derivatives are the most versatile compounds used for the synthesis of most of the FDA-approved drugs, ranked 9^{th} in the Top 25 most frequently approved nitrogen heterocycles in the FDA drugs. In recent decades there is an emphasis on the synthesis of indole derivatives. This emphasis is due to the endless architectural possibilities of the polycyclic structure by adding various fused heterocyclic scaffolds to achieve promising novel heterocycles. In many rational designs, the flexibility of the Indole framework can be observed in a broad spectrum of biological goals from topoisomerase inhibitors to G2/M inhibitors (Martins *et al.*, 2015). Bromodomains (BRDs), which regulate gene transcription (Bason *et al.*, 2011; Das *et al.*, 2014) on histone at chromatin-modifying enzymes (Galdeano and Ciulli, 2016; Pérez-Salvia and Esteller, 2017). The protein acts as a gatekeeper for both the mitotic and the cell cycle (Conery *et al.*, 2016; Zhan *et al.*, 2015). Contrary to most epigenetic modifiers, during mitosis BRD4 stays attached to chromosomes. It uniquely connects to the late mitosis and early G1 by binding to acetylated H3 and H4 bookmark genes. For cell cycle progression, BRD4 is also essential and leads to G1 Arrest (Doroshow *et al.*, 2017). Carbolines (pyridoindoles) are a heterocyclic group of compounds with a broad spectrum of biological activity (Lin *et al.*, 2016; Kumar *et al.*, 2008). The least researched four classes of compounds relative to their α, β, and γ analogues are the δ -carbolines (5H-pyrido[3,2-b]indole). This class of heterocycles has not yet discovered effective medicines. However, several compounds of this carboline class were relatively diverse in terms of biological activity. A large group has had powerful antimuscarinic, antihyperglycemic, antimalarial, antiplasmodial, antifungal, anticryptococcal, antiviral and antitumor activity. Crytolepine and its analogues have been recently revealed to be cytotoxic to melanoma cells and lung cancer cells. This research, therefore, discusses the identification of a novel δ-carboline derivatives to inhibit BRD4 protein by receptor-based drug discovery strategy, and ADMET to comprehend receptor (BRD4 proteins) binding patterns of δ-carboline to acquire more selective and potent medicines for the new possible CRC therapy.

EXPERIMENTAL METHODS

Preparation of target protein

The 3D structure of the BRD4 receptor (PDB ID: 4NUD - 1.20 Å resolution) was retrieved from the structural database (https://www.rcsb.org/structure/4NUD) (Berman *et al.*, 2002; Morris *et al.*, 2009; Zhang *et al.*, 2013) . The protein structure was processed by using Autodock/vina (Trott and Olson, 2010).

Preparation of lead compounds

60 (Compounds 1-60) structurally diverse of δ-Carbolines side chains were selected for construction of a virtual library of the lead compound. The chemical structure of δ-Carbolines side chains was prepared by ChemBioDraw Ultra Version 12.0. The conformational energies of the inhibitors have been reduced. Minimalized structures were then subjected to docking experiments using Autodock/Vina.

Virtual screening and docking

The virtual library comprising of 60 compounds were used as lead and PDB ID: 4NUD as the target for this docking study. The 3D structures of all the compounds and co-crystal of BRD4 were then docked using AutoDock/Vina (Morris *et al.*, 2009; Seeliger and de Groot, 2010). For AutoDock docking, more specific preparations have been made using AutoDock Tools (ADT). For each residue, the structure of the receptor was built with gasteiger loads attached to each atom. The active site has been determined using the grid boxes used in ADT (x, y, z axes were $102\times94\times124$ ~ 0.375 Å). The findings below 1.0 Å in RMSD were clustered together, and the binding energy was matched with the receptor structure for further study.

ADMET studies

PreADMET online tool (PreADMET, 2020) used to assess the drug-likeness of all the selected compounds. PreADMET measures the drug-like value of substances based on the Lipinski rule of five. 2D structures were built using ChemBioDraw (Daina *et al.*, 2014; Waseem *et al.*, 2017). Molfile data has been applied to predict drug properties.

RESULTS AND DISCUSSION

Binding nature of BRD4

Based on the literature survey, BRD4 receptor was selected for the study. This BRD4 was known for controlling the growth and metastasis of cancer. BRD4 proteins conduct regulatory transcription under normal conditions, though the transcription of several oncogenes (Fu *et al.*, 2015). AutoDock/Vina was used to predict binding affinity in molecular docking studies of δ-Carbolines with receptors as well as to distinguish potent lead compounds widely used.

Table 1: Important interactions between the residues of BRD4 and selected leadcompounds

Lead Molecule	Affinity Kcal/mol	Hydrophilic interactions	Ionic interactions	Hydrophobic interactions
Compound 5	-5.8	Glu151, Tyr137, Asn140	Lys141	Pro142, Val147
Compound 17	-5.6	Tyr98	Lys102	Ile101, Pro104, Pro46, Pro45
Compound 29	-5.1	Asn44, Tyr98	NII*	Pro45, Val90, Ala89
Compound 34	-6.2	Asn44, Try98	NII*	Ala89, Val90, Pro45
Compound 37	-6.0	Asn93, Try98	NII*	Ala89
Compound 39	-6.0	Try98	Lys99	Pro104, Pro45
Compound 42	-6.1	NHI#	Glu49	Pro45, Pro46
Compound 47	-5.9	Asn140, Tyr137	Lys141	Val147, Pro142

Table 2: ADME properties of the selected compounds

Lead	BBB	Caco2	CYP (I)/CYP 2C9	HIA	MDCK	PPB	SP	Value		SKlog	
								D	P	S buffer	S pure
1	0.3	20.6	Non	95.9	1.7	50.1	-4.5	0.4	0.4	-3.1	-2.3
2	0.3	21.0	Non	96.0	2.9	57.7	-4.5	0.8	0.8	-3.5	-2.5
3	0.0	21.1	Non	96.0	11.2	67.8	-4.4	1.3	1.3	-3.8	-3.0
4	0.0	21.7	Non	96.0	32.3	83.1	-4.4	2.2	2.2	-4.7	-3.9
5	0.0	21.6	Inhibitor	96.0	23.5	86.0	-4.2	2.1	2.1	-4.7	-4.1
6	0.2	21.9	Non	96.0	24.3	85.8	-4.2	2.0	2.0	-4.2	-4.1
7	0.0	20.9	Inhibitor	96.7	8.4	75.8	-4.4	1.5	1.5	-4.7	-3.7
8	0.0	19.7	Inhibitor	96.7	2.3	78.4	-4.5	1.1	1.1	-3.7	-3.2

Table 3: Drug likeness prediction of selected compounds

Compound	CMC	Lead Rule Violation Fields	Lead Rule	Lead Like Rule Violations	Rule of Five	WDI Rule
1	Qualified	AlopP 98	V*	1	Suitable	In 90% cutoff
2	Qualified	AlopP 98	V*	1	Suitable	In 90% cutoff
3	Qualified	AlopP 98	V*	1	Suitable	In 90% cutoff
4	Qualified	AlopP 98	S#	0	Suitable	In 90% cutoff
5	Qualified	-	S#	0	Suitable	In 90% cutoff
6	Qualified	-	S#	0	Suitable	In 90% cutoff
7	Qualified	-	S#	0	Suitable	In 90% cutoff
8	Qualified	AlopP 98	V*	1	Suitable	In 90% cutoff

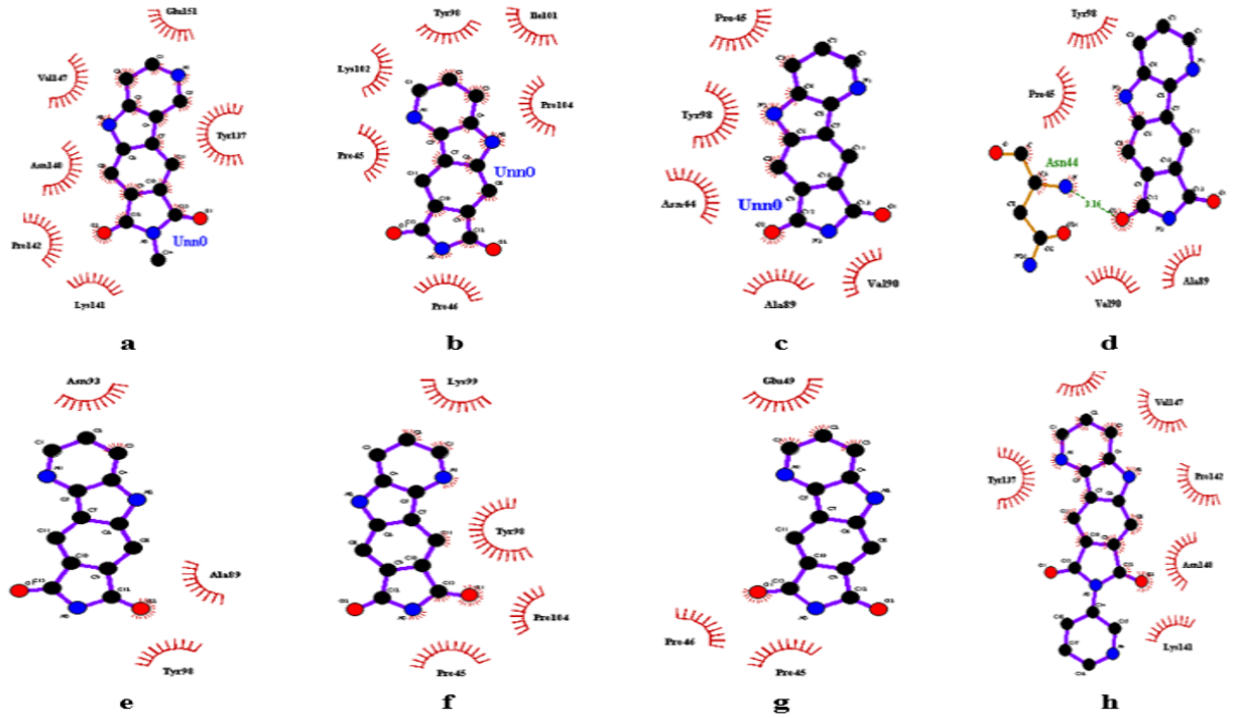

Figure 1: The interaction of the designed compounds, a) 5 b) 17 c) 29 d) 34 e) 37 f)39 g) 42 and h) 47 within the catalytic pocket of 4NUD

The docking revealed the top ten positions for each ligand (Figure 1). Among all the studied lead compounds, compound 5, 17, 29, 34, 37, 39, 42, 47 were found to be the best with minimal energy (Figure 2). The highest binding affinity with minimal energy was used for the comparison (Table 1). The highest binding affinity was obtained for compound 34 followed by compound 42 > compound 37 and 39 > compound 47 > compound 5 > compound 17 > compound 29 with affinity kcal/mol of -6.2, -6.1, -6.0, -6.0, -5.9, -5.6 and -5.1., and all molecules exhibited the good binding shown in Figure 2. The interactions between the compounds and the amino acids of Glu151, Tyr137, Lys141, Pro142, Asn140, Val147, Tyr98, Ile101, Pro104, Pro46, Pro45, Lys102, Pro45, Val90, Ala89, Asn44, Tyr98, Pro45, Ala89, Val90, Asn44, Pro45, Tyr98, Asn93, Ala89, Tyr98, Lys99, Tyr98, Pro104, Pro45, Glu49, Pro45, Pro46, Val147, Pro142, asn140, Lys141, Tyr137, Trp81, and Glu78 which is specifically involved in the catalytic enzyme mechanism. Hydrogen bonding and hydrophobic interactions stabilised the ligand complex. All of the top poses created by the tool exhibited binding to the amino acids in the pocket. The top pose with the lowest binding affinities and high docking scores is typical in most docking programs. The indole side chain displayed robust hydrophobic and hydrophilic interactions with non-polar and polar residues indicated an improvement in binding affinity (Table 1). By understanding the results, we selected eight compounds based on pose generated by Vina and generated the best results with the minimal energy of above -5.1. Hydrogen, hydrophobic and ion interactions with BRD4 consequently stabilise the structure. Usually, a particular pose with low binding energy has a strong affinity for a long time and is thus the strongest docked conformation.

ADMET prediction

Pre ADMET predicts that all eight compounds are medium to high BBB based on the Cbrain/ Cblood ratio (Table 2). P-glycoprotein is located on the surface of endothelial cells of BBB that selectively permits the drugs. The selected compounds are tightly bound to plasma proteins and have GI absorption of cacao-2 celline and preferred for the oral dosage form. No compound inhibits the CYP2C19, CYP2C9, CYP2D6, CYP3A4 and all the others are except CH 37, CH 42 and CH 47 that showed inhibition of CYP2C99 in the liver during metabolism. CH42 and CH47 are strong substrates for CYP3A4 metabolism, and all other compounds showed weak for the metabolism of CYP3A4.

Drug likeness profile of the selected compounds

All the eight compounds were taken for the drug-likeness profile study. This profile checks the compounds pharmacological and toxicity properties. Lipinski's rule showed drug suitability for all compounds with the number of hydrogen bond < 5, hydrogen bond acceptor < 10, MW below 500, ClogP

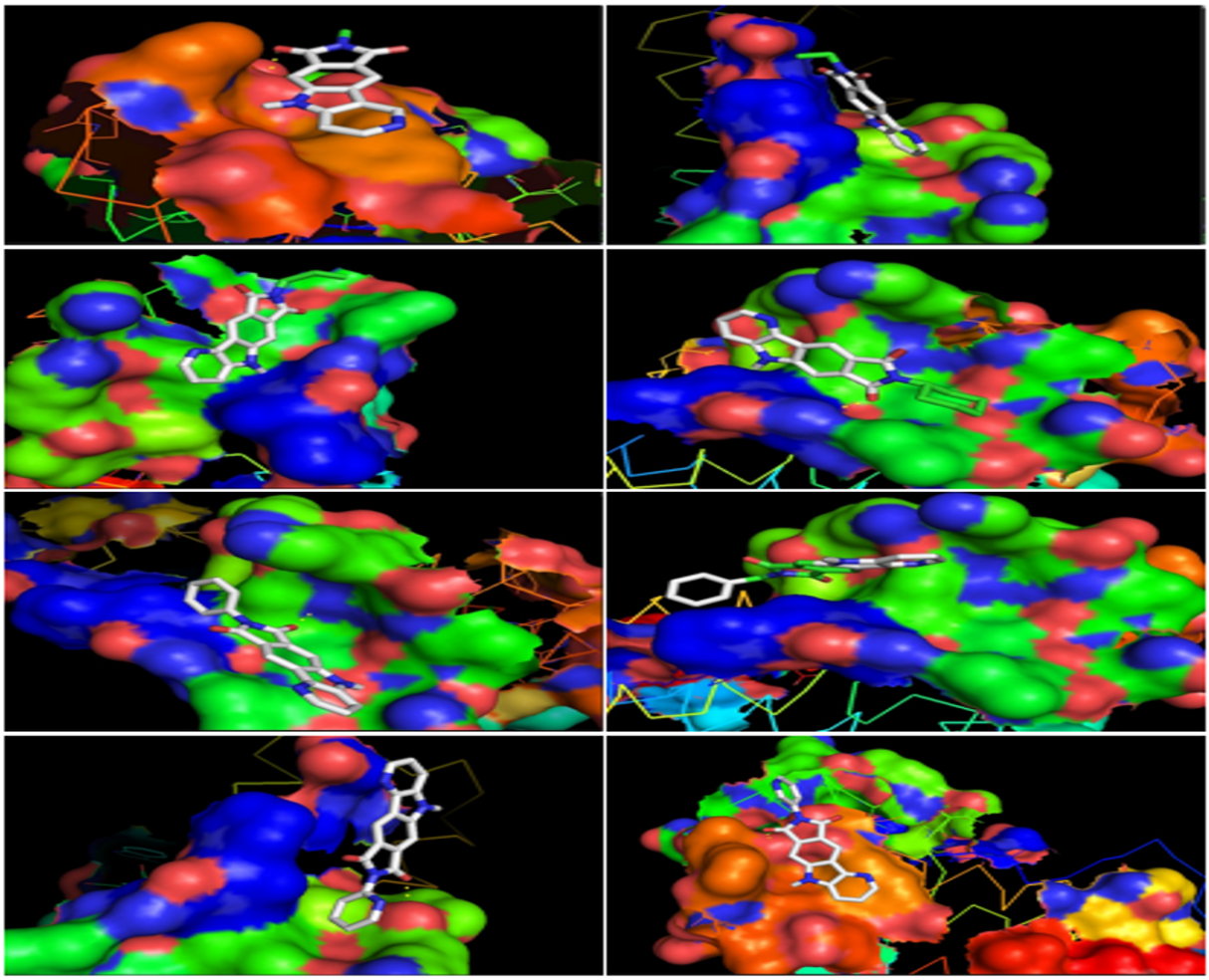

Figure 2: The best binding posesof the compounds, a) 5 b) 17 c) 29 d) 34 e) 37 f) 39 g) 42 h) 47 within thecatalytic pocket of 4NUDThe best binding posesof the compounds, a) 5 b) 17 c) 29 d) 34 e) 37 f) 39 g) 42 h) 47 within thecatalytic pocket of 4NUD

with below 5. Compound CH 15, CH17, CH29 and CH47 showed <<0.1μm is suitable for its binding affinity nature except for Compounds CH34, CH37, CH39, and CH47. Almost all the compounds qualified in CMC rule, CMC rule of violation (0), MDDR rule with structure with the value of 1 and WDI violations showed 0 value (Table 3).

CONCLUSION

The above work demonstrated that the compounds 5,17,29,34,37,39,42 and 47 belonging to δ-Carbolines derivatives would work against BRD4 by blocking their binding site. Therefore, in MYC transcription, these molecules play an essential role in interfering with signal transduction of chromatin by inhibiting the acetyl-lysine domains involved in transcriptional initiation and elongation.

Conflict of Interest

The authors declare no conflict of interest.

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