In-silico investigation of CREB-binding protein on Castration-Resistant Prostrate cancer: Insight from Molecular Dynamic Simulations and Computer Aided Drug Discovery

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A thesis submitted to the College of Health Sciences, University of KwaZulu-Natal, Westville, in fulfillment of the requirements of the degree of Master of Medical Sciences

Supervisor

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This is the thesis in which the chapters are written as a set of discrete research publications, with an overall introduction and final summary.

This is to certify that the contents of this thesis are the original research work of **Ms Oluwayimika Eunice Akinsiku.**

As the candidate's supervisor, I have approved this thesis for submission.

Supervisor:

Signed: ---------- Name: Prof. Mahmoud E. Soliman Date: ----------

PREFACE

This thesis is divided into six chapters, including this one:

Chapter 1

This is an introductory chapter that addresses the background, rationale and relevance of the study as well as the proposed aim and objectives. The general outline and structure of the thesis concludes this chapter.

Chapter 2

This chapter comprises of a comprehensive review on prostate cancer, its epidemiology, symptoms and treatment. It also covers details on the Androgen Receptor, its structure and function, castration-resistant prostate cancer (CRPC) and its mechanism. Recent studies into CBP/P300 as a potential drug target are also discussed in the later part.

Chapter 3

This chapter conceptualizes computer-aided drug design by discussing a various molecular modeling and molecular dynamic techniques and applications. The computational tools needed to investigate comparative enzymatic structural/conformational characteristics as well as methods used to analyze binding affinity are elucidated upon.

Chapter 4

(Published work- this chapter is presented in the required format of the journal and is the final version of the accepted manuscript)

This chapter entitled, "exploring the role of Asp1116 in selective Drug targeting of CREB-cAMP-responsive element-binding protein Implicated in Prostate Cancer" presents Asp 1116 as the culprit behind the selective targeting of Y08197 at the bromodomain active site using molecular dynamic simulation. MM/PBSA further revealed a similar inhibitory effect between Y08197 and an FDA-drug, CPI-637. Findings also showed the selective affinity of Y08197 to CBP while being compared to another bromodomain, BRPF1. This article has been published in Combinatorial Chemistry and High Throughput Screening

Chapter 5

(Submitted manuscript- this chapter is presented in the required format of the journal and is a final version as the submitted manuscript)

This chapter is titled "Update and Potential Opportunities in CBP [cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-binding protein] Research using Computational Techniques", a review manuscript expounding the functions and interactions of the CREB-binding protein in diseases especially cancer. Various computational researches further prove its potency as a therapeutic target as stated in this review. This project un-doubtfully emphasizes and encourages the spotlight on CREB-binding protein for continuous drug target.

Chapter 6

This is the final chapter that proposes future work and concluding remarks.

ABSTRACT

Prostate cancer has evolved over the years despite various treatment and therapy. One recent threat of the prostate cancer is the Castration- Resistant Prostate Cancer (CRPC). The CRPC is an advanced form of prostate cancer. Despite therapies involving chemical, surgical or hormonal treatment, the cancer cells in CRPC have been discovered to continue growth and development at an alarming rate. This forms the basis for this research. One novel drug (Y08197) aimed towards CBRB-binding protein was reported to have anti- cancer properties and therapeutic effect on CRPC. Their research based on in vivo and in vitro experiments, revealed CREB-binding protein as a therapeutic target. This study majored in a search for the culprit behind this miracle. We used molecular dynamics simulation to gain insights into the mechanistic and selective targeting of the novel drug as well as its similar inhibitory effect to an FDA drug (CPI-637). Also, since little is known about the structural and inhibitory properties of CREB-binding protein, we also commenced a review study to look into its inhibitory history. The use of computational techniques such as molecular modeling, molecular docking, virtual screening protocols and molecular dynamics allows the evaluation and assessment of potential leads compounds. These in-silico techniques as stated above are cost-effective and efficient in research and pivot to fast track drug discovery process. Herein, we used molecular dynamics simulation to gain insights into the mechanistic and selective targeting of Y08197 at the bromodomain active site. Molecular Mechanics/ Poisson-Boltzmann Surface Area (MM/PBSA) analysis revealed a similar inhibitory effect between Y08197 and CPI-637. Furthermore, in exploring the selective affinity of Y08197 towards CBP in combination with Bromodomain and PHD finger-containing protein 1(BRPF1), our result highlighted Asp1116 as the ‘culprit’ residue responsible for this selective targeting. Conclusively, the implementation of the information extracted in this study, can be replicated in future structure based CBP inhibitors and pharmacological research implicated in carcinogenesis.

DECLARATION 1 – PLAGIARISM

I, Oluwayimika Eunice Akinsiku, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original work.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a. Their words have been re-written, but the general information attributed to them has been referenced.

b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.

5. This thesis does not contain text, graphics or tables copied from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references section. A detail contribution to publications that form part and/or include research presented in this thesis is stated (include publications submitted, accepted, in press and published).

Signed **O.E AKINSIKU**

DECLARATION 2 – LIST OF PUBLICATION

1. Oluwayimika E. Akinsiku, Opeyemi Soremekun, Fisayo A. Olotu and Mahmoud Soliman (2020), "Tapping on the crucial role of Asp1116 in selective Drug targeting of CREB-cAMP-responsive element-binding protein Implicated in Prostate Cancer", Combinatorial Chemistry & High Throughput Screening-Accepted (published).

Contributions

Oluwayimika E. Akinsiku: contributed to the project by performing all the experimental work and manuscript preparation and writing.

Opeyemi Soremekun: contributed by performing post dynamic analysis, writing of manuscript and creation of all graphs and images

Fisayo A. Olotu: contributed by reading through manuscript before submission.

Mahmoud E.S Soliman: supervisor.

Appendix A: pdf version of the publication

2. Oluwayimika Akinsiku, Opeyemi S. Soremekun, and Mahmoud E. S. Soliman (2020). “Update and Potential Opportunities in CBP [cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-binding protein] Research using Computational Techniques” The Protein Journal (published).

Contributions

Oluwayimika E. Akinsiku: contributed to the project by performing all the experimental work and manuscript preparation and writing.

Opeyemi Soremekun: contributed by manuscript proof-reading and editing

Mahmoud E.S Soliman: supervisor

RESEARCH OUTPUT

A. PUBLICATIONS

1. Oluwayimika E. Akinsiku, Opeyemi Soremekun, Fisayo A. Olotu and Mahmoud Soliman (2020), "Tapping on the crucial role of Asp1116 in selective Drug targeting of CREB-cAMP- responsive element-binding protein Implicated in Prostate Cancer", Combinatorial Chemistry & High Throughput Screening-Accepted (published).
2. Oluwayimika E. Akinsiku, Opeyemi Soremekun and Mahmoud Soliman (2020) "Update and Potential Opportunities in CBP [cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-binding protein] Research using Computational Techniques" The Protein Journal (published).

B. CONFERENCES

1. Poster Presentation: "Tapping on the crucial role of Asp1116 in selective Drug targeting of CREB-cAMP- responsive element-binding protein Implicated in Prostate Cancer" - Annual Research Symposium, college of health sciences, Nelson R Mandela School of Medicine Campus, UKZN, South Africa, 1st November 2019

2. Poster Presentation: "Tapping on the crucial role of Asp1116 in selective Drug targeting of CREB-cAMP- responsive element-binding protein Implicated in Prostate Cancer" - National CHPC Conference, Century City Cape Town, South Africa, 1st December 2019

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LIST OF ABBREVIATION

3D	Three- Dimension
α	alpha
β	beta
ΔG	free binding energy
Å	Angstrom
AR	androgen receptor
ARE	Androgen response element
AF-1	Activation function-1
AF-2	Activation function-2
AMBER	Assisted Model Building with Energy Refinement
BRD	bromodomain
BET	Bromodomain and Extra-Terminal family
CADD	Computer-Aided Drug Design
cAMP	cyclic Adenosine Monophosphate
CBP	CREB- binding protein
CHARMM	Chemistry at Harvard Macromolecular Mechenics
CREB	c-AMP response element-binding protein
CRPC	Castration-Resistant Prostate Cancer
DNA	deoxyribonucleic acid
DBD	deoxyribonucleic acid binding domain
DHT	Dihydrotestosterone
FF	Force Field

GAFF	Generalized AMBER Force Field
GROMACS	GROningen Machine for Chemical Simlations
HATs	Histone acetyltransferase
HP	Hamiltonian Operator
HM	Homology Modeling
HRPC	Hormone Refractory Prostate Cancer
LBD	Ligand binding domain
KE	Kinetic Energy
LH	Luteinizing Hormone
MD	molecular dynamics
MM	molecular mechanics
MM/GBSA	Molecular Mechanics/Generalized Born Surface Area
MM/PBSA	Molecular Mechanics/Poisson-Boltzmann Surface Area
NAMD	Nanoscale Molecular Dynamics
NTD	N- terminal domain
NLS	Nuclear localization signal
NMR	Nuclear Magnetic Resonance
ns	nanoseconds
PCA	Principal Component Analysis
PDB	Protein data bank
PE	Potential Energy
PES	Potential energy surface
PKA	Protein Kinase A

PSA	Prostate- Specific Antigen
RESP	Restrained Electrostatic Potential
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
RNA	Ribonucleic acid
SW1/SNF	Switch/Sucrose Non-Fermentable
TSA	Thermal Shift Assay
RoG	Radius of Gyration
VS	Virtual screening
QM	Quantitative Modeling
QSAR	Quantitative structure-activity relationship

LIST OF AMINO ACIDS

Three Letter Code	Amino Acids
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic Acid
Cys	Cysteine
Gln	Glutamine
Glu	Glutamic Acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tryrosine
Val	Valine

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CHAPTER ONE

1.1 BACKGROUND AND RATIONALE TO STUDY

The incidence of prostate cancer has been at its peak in the past few decades, with millions being diagnosed yearly with it. It is a significant health concern accompanied by excellent research in the search for treatments. Although Prostate cancer is extensively prevalent in older adults above 70years of age, it is the second most recurring and fifth lethal cancer in the world today [1, 2]. Most American men are at higher risk; hence the American cancer society predicts about 174 650 new cases and 31 620 deaths in 2019. Despite great success from current research, a five-year survival rate has been established with patients treated from prostate cancer [3, 4].

The primary cause of prostate cancer has not been identified yet; however, just like every other cancer cell, its onset begins with abnormal cell growth and division in the prostate gland [3]. Since the 1990s, the anatomy of the prostate gland, which is a sized walnut organ in males only responsible majorly to produce semen in sperm, has been studied. Typically, the prostate functions with the biosynthesis of androgens produced within the testicles. Many inhibitors such as cyproterone acetate, flutamide, nilutamide, bicalutulamide and enzalutamide, developed towards the treatment of prostate cancer aimed at the blockage of the androgens to its receptors, Androgen Receptor (AR). In other cases, castration of the testes is adopted to inhibit androgen biosynthesis. However, it is discovered that after the intake of these drugs, the cancer cells seem to develop a "backdoor pathway" for the continuation of androgen supply via intratumoral synthesis. In this case, the tumor developed is referred to as "castration-resistant prostate cancer (CRPC). Therefore, drugs that can target the intra-tumoral androgen biosynthesis offer the most promise.

Studies by Penning 2014, clearly reports the redundancy of the intra-tumoral androgen biosynthesis pathway, further expatiating that blocking or targeting one enzyme might result in a by-cut of that pathway to another, hence making the drug ineffective [5]. Although scientist has thought to combine drugs targeting multiple pathways and an anti-androgen to increase effectiveness, drug resistance continues to develop.

Critical insight into the molecular function of c-AMP response element- binding protein (CREB)-binding protein (CBP) bromodomain protein in the transcriptional activity of the prostate cancer cell, launch a new therapeutic strategy for developing drug targets. It turns out that CBP/P300 has been implicated in the activation of androgens and plays an oncogenic role in prostate cancer [6–11]. To further juxtapose this point, a small novel inhibitor (GNE-049), was developed to target CBP/P300 bromodomain in vitro and in vivo and the following results were obtained [12]:

- CBP/P300 is necessary for AR target gene expression
- CBP/P300 is involved in androgen response
- When CBP/P300 is targeted, the co-activation of the AR function is impaired.
- CBP/P300 shows antitumor activities in vivo.

It is on these bases that many novel inhibitors are developed to target CBP/P300 in the treatment of CRPC [13].

One promising inhibitor is Y08197, a novel and selective CBP/P300 bromodomain inhibitor recently report to exhibit high selectivity for CBP/P300 over other bromodomains and effectively inhibit AR-regulation genes as well as induce a G0/G1 phase arrest and apoptosis in 22Rv1 prostate cancer cells [14]. Although the paper covers the alpha screen assay, thermal stability shift assay (TSA), and statistical analysis, yet no in-silico experiment was revealed, moreover the reason for this selectivity was not discussed.

The effectiveness of the drug discovery process can be attributed to the breakthrough in computer-aided drug design. Its methods and software resources enable the fast track of drug discovery and mechanics [15, 16]. Its therefore not surprising that its effective use of these computational techniques would shed more light on intriguing questions from the novel CBP/P300 inhibitor, Y08197. These computational techniques include molecular modeling and docking [17, 18] virtual screening [19] identification of pharmacophoric hot spots, and molecular dynamic simulations [20] allows millions of compounds to be screened hence obtaining a lead target with the best binding pose. Moreover, the dynamics of interaction between the protein and ligand are observed, and data plots are obtained [21] [22]. With this in view, the concept of "shooting in the dark" is eliminated and thereby reducing the drug discovery timeline.

1.2 AIMS AND OBJECTIVES FOR THIS STUDY

This thesis's primary purpose is to burrow deep to view the specificity of inhibition of Y08197 against CBP while observing the ligand specificity of the compounds test against CBP and BRPF1, another bromodomain but of lowest affinity to Y08197 with the aid of computational studies.

In achieving this, outlined are the following objectives:

1. To create a concise route map to investigate the binding affinity of Y08197 against CBP compared to BRPF1 by:

    1.1. Obtaining the crystalized structure of CBP and BRPF1 from the protein data bank

    1.2. Modifying missing residues and deleting co-crystallized molecules with the aid of

a modeler.

1.3. Drawing out the 2-D structure of the ligand (Y08197) and (CPI-627) with the aid of Marvin Sketch suite.

1.4. Optimizing the ligands for further molecular docking with the aid of Avogadro software

1.5. Docking the ligands into the binding pockets of the protein target to observe binding pose energies (Autodock Vina).

2. To investigate the proposed binding pose of interaction between Y08197 and the bromodomains (CBP and BRPF1) as well as CPI-627 against CBP. This is achieved by:

2.1. Performing about 200ns molecular simulation on the following systems: apo-CBP, apo-BRPF1, CBP-Y08197, BRPF1-Y08197, and CBP- CPI-637 (AMBER).

2.2. Performing post molecular dynamic analysis utilizing a set of proposed parameters specific to the binding site to evaluate its simulations.

2.3. Implementing per-residue energy decomposition analysis on all systems based on the Molecular Mechanics/ GB Area Surface Method (MM/GBSA) approach to identify the amino acid residues which form the highest contributions to the overall binding free energy.

1.3 NOVELTY AND SIGNIFICANCE TO THIS STUDY

The progression of prostate cancer to its advanced level, castration-resistant prostate cancer (CRPC), raises demand in research for treatment [23]. It has been reported that one in four men diagnosed with prostate cancer eventually dies of the diseases [24]. Despite much drug therapy, the puzzle remains "what pathway in the androgen backdoor synthesis to target that would once-off inhibit the growth of prostate cancer cells in CRPC patient?" Hence, the search for novel compounds that could effectively target proteins and enzymes.

The treatment of Castration-resistant prostate cancer (CRPC) continues to be an evolving aspect for research, especially with the aid of computer-aided drug design. However, before this, the breakthrough in the treatment of CRPC has been from in vitro studies. Recent studies have shown that targeting the CBP bromodomain of the AR transcriptional activity pathway could propose a positive therapeutically outcome that could inhibit the growth of the cancer cells [6–11]In a recent publication by lee Ji [25], it was reported that the novel compound Y08197 effectively targets CBP/P300, a co-activator of the AR transcriptional pathway, and inhibits the growth of cancer cells. In this study, we used *in silico* techniques to verify its selective inhibitory properties and decipher the main reason for this inhibition. This will aid in understanding in depth the mechanism of action of this potential drug against its binding target, thereby enhancing treatment. This is significant to current research because it could totally eradicate the cancer cells in the patients and save lives globally.

To this end, the work presented in this thesis remains fundamental for the advancement of research toward targeted drug design/delivery against castration-resistant prostate cancer.

REFERENCE

1. Bray F, Ferlay J, Soerjomataram I (2018) Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. 394–424. https://doi.org/10.3322/caac.21492
2. Smith MR, Saad F, Egerdie B, et al (2019) Sarcopenia during ADT for PC. Prostate Cancer Prostatic Dis 16:1077–1083. https://doi.org/10.14740/wjon1191
3. Turner B, Drudge-Coates L (2010) Prostate cancer: risk factors, diagnosis and management. Cancer Nurs Pract 9:29–36. https://doi.org/10.7748/cnp2010.12.9.10.29.c8126
4. Facts C (2019) Joinpoint Trends in Cancer Incidence Rates for Selected Sites in Two Age Groups, US, 1995-2015 35 Figure S6. Trends in Cancer Death Rates for Selected Sites
5. Penning TM (2014) Androgen biosynthesis in castration-resistant prostate cancer. Endocr Relat Cancer 21:. https://doi.org/10.1530/ERC-14-0109
6. Fu M, Wang C, Reutens AT, et al (2000) p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 275:20853–20860. https://doi.org/10.1074/jbc.M000660200
7. Fu M, Rao M, Wang C, et al (2003) Acetylation of Androgen Receptor Enhances Coactivator Binding and Promotes Prostate Cancer Cell Growth. Mol Cell Biol 23:8563–8575. https://doi.org/10.1128/mcb.23.23.8563-8575.2003
8. Ianculescu I, Wu DY, Siegmund KD, Stallcup MR (2012) Selective roles for cAMP response element-binding protein binding protein and p300 protein as coregulators for androgen-regulated gene expression in advanced prostate cancer cells. J Biol Chem 287:4000–4013. https://doi.org/10.1074/jbc.M111.300194

9. Zhong J, Ding L, Bohrer LR, et al (2014) P300 acetyltransferase regulates androgen receptor degradation and pten-deficient prostate tumorigenesis. Cancer Res 74:1870–1880. https://doi.org/10.1158/0008-5472.CAN-13-2485

10. Comuzzi B, Nemes C, Schmidt S, et al (2004) The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. J Pathol 204:159–166. https://doi.org/10.1002/path.1609

11. Debes JD, Sebo TJ, Lohse CM, et al (2003) p300 in Prostate Cancer Proliferation and Progression. Cancer Res 63:7638–7640

12. Jin L, Garcia J, Chan E, et al (2017) Therapeutic targeting of the CBP/p300 bromodomain blocks the growth of castration-resistant prostate cancer. Cancer Res 77:5564–5575. https://doi.org/10.1158/0008-5472.CAN-17-0314

13. Filippakopoulos P, Knapp S (2014) Targeting bromodomains: Epigenetic readers of lysine acetylation. Nat Rev Drug Discov 13:337–356. https://doi.org/10.1038/nrd4286

14. Zou L jiao, Xiang Q ping, Xue X qian, et al (2019) Y08197 is a novel and selective CBP/EP300 bromodomain inhibitor for the treatment of prostate cancer. Acta Pharmacol Sin 1–12. https://doi.org/10.1038/s41401-019-0237-5

15. Adcock SA, McCammon JA (2006) Molecular dynamics: Survey of methods for simulating the activity of proteins. Chem Rev 106:1589–1615. https://doi.org/10.1021/cr040426m

16. Kollman P (1993) Free Energy Calculations: Applications to Chemical and Biochemical Phenomena. Chem Rev 93:2395–2417. https://doi.org/10.1021/cr00023a004

17. Joseph-McCarthy D, Alvarez JC (2003) Automated generation of MCSS-derived pharmacophoric DOCK site points for searching multiconformation databases. Proteins Struct Funct Genet 51:189–202. https://doi.org/10.1002/prot.10296

18. Verdonk ML, Cole JC, Hartshorn MJ, et al (2003) Giftgas over Byen. Civilbefolkningens Beskyttelse Under Den Næste krig. Proteins 52:609–623. https://doi.org/10.1002/prot.10465

19. Song CM, Lim SJ, Tong JC (2009) Recent advances in computer-aided drug design. Brief Bioinform 10:579–591. https://doi.org/10.1093/bib/bbp023

20. Eweas AF, Maghrabi IA, Namarneh AI (2014) Advances in molecular modeling and docking as a tool for modern drug discovery. Der Pharma Chem 6:211–228

21. Hua X-M (1997) Monte Carlo simulation of Comptonization in inhomogeneous media. Comput Phys 11:660. https://doi.org/10.1063/1.168615

22. Patrikeev GA (1971) Macromolecular mechanics. Polym Mech 7:183–192. https://doi.org/10.1007/BF00855851

23. Bellmunt J, Oh WK (2010) Review: Castration-resistant prostate cancer: New science and therapeutic prospects. Ther Adv Med Oncol 2:189–207. https://doi.org/10.1177/1758834009359769

24. Ritch C, Cookson M (2018) Recent trends in the management of advanced prostate cancer. F1000Research 7:1–7. https://doi.org/10.12688/f1000research.15382.1

25. Zou L jiao, Xiang Q ping, Xue X qian, et al (2019) Y08197 is a novel and selective CBP/EP300 bromodomain inhibitor for the treatment of prostate cancer. Acta Pharmacol Sin 40:1436–1447. https://doi.org/10.1038/s41401-019-0237-5

CHAPTER TWO

2.1 BACKGROUND ON PROSTATE CANCER

2.1.1 INTRODUCTION

Living things are made up of billions of cells that grow, divide, and die under-regulated cellular conditions. Cells of every part of the human body have various functions and are well organized into tissues, tissues into organs and organs into the system. This emphasizes the significance of the cell in that every disease can be traced to a cellular problem. In the case of cancer, the cells begin to grow and divide out of control. This cellular madness could begin from one cell dividing into two, two into four, four into eight, to mention a few. Not long is a tumour formed. Tumours can be either benign or malignant, depending on how dangerous it becomes. A malignant tumour can migrate via blood vessels to other parts of the body, a process called metastasis. When cancer cells begin to multiply, they impair cellular function and hence cause death [1–3].

According to WHO, cancer is a cardinal cause of death globally, with statistics of 7.4million deaths in 2004. The American cancer society recently predicted 1.7million new cases expected to be diagnosed in 2019. From ancient Egypt to date, the disease "cancer and its treatment" has been studied. From 1991 to 2016, there has been a considerable decline in cancer deaths due to research progression, especially on the four most common cancer types; lung, colorectal, breast, and prostate [4]. Figure 2.1 shows an estimated cancer rate report from 1930 till 2010.

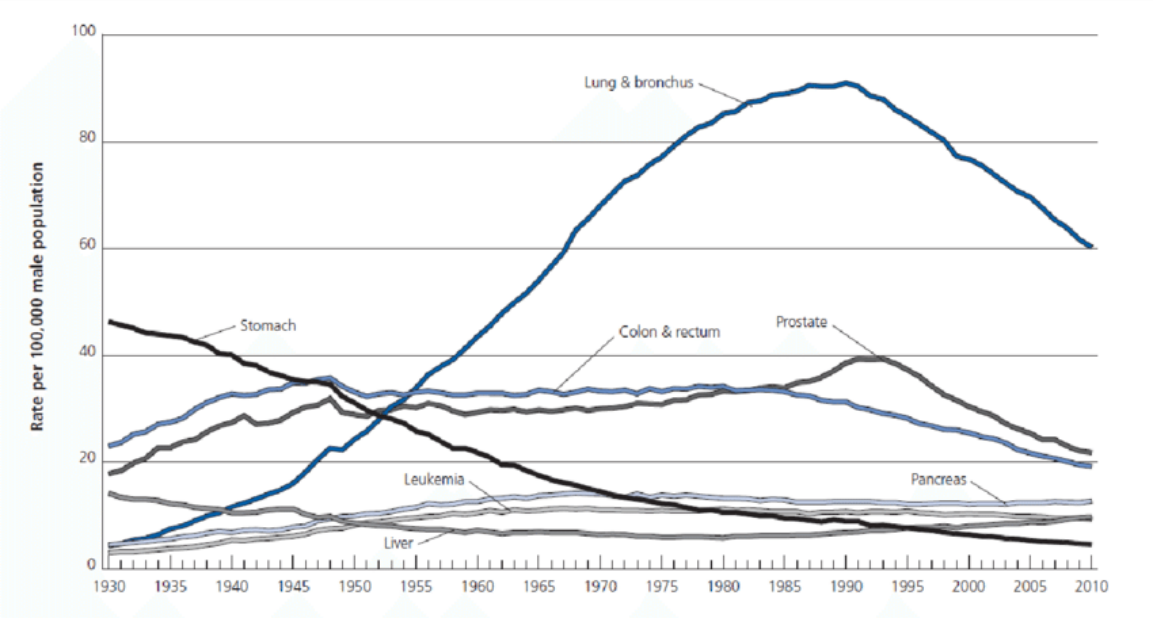

Figure 2.1: An estimated cancer rate report [5]

Of all the cancers, prostate cancer is most frequent in males and second death leading cancer in the world today [6, 7]. Abnormal proliferation that occurs in the prostate gland is an onset for prostate cancer. The prostate gland is a vital organ in males, culpable for semen production, which lies in the prostate fluid [8]. The male anatomy diagram as seen in figure 2.2, shows the various organ associated with the male reproductive system. The prostate fluid contains an enzyme that functions to keep the semen liquid and serves as a measuring stick to diagnose diseases associated with the prostate gland. The enzyme is called a prostate-specific antigen. The prostate is found anterior to the rectum, below the bladder [9].

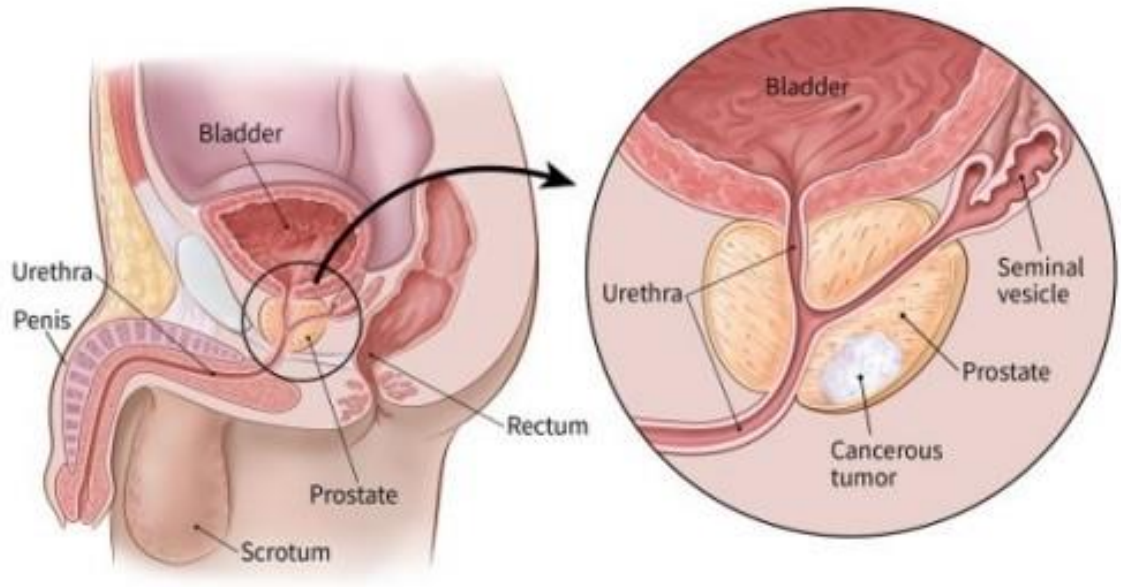

Figure 2.2 The male anatomy of the prostate gland [7]

Prostate cancer begins with a tumour in the prostate gland. Treatment usually depends on the extent of tumour growth. Various therapies have been adopted over the years that include radiotherapy, chemotherapy, immunotherapy, hormonal therapy, cryotherapy, and surgery.

2.1.2 EPIDEMIOLOGY

Prostate cancer, most notably as found only in males, is the second most recurring and fifth lethal cancer in the world today. For some reason not yet apparent, 74% of prostate cancer cases are most prevalent in blacks than in whites. The American cancer society estimated that 164 690 new cases would be diagnosed in 2018, 174 650 new cases newly diagnosed as well as 31 620 deaths in 2019 will occur. Of course, prostate cancer is when the prostate gland in males begins to divide rapidly out of control [6].

2.1.3 SYMPTOMS

The symptoms of prostate cancer could be like other male associated diseases. However, prostate cancer is confirmed via biopsy. Increased levels of PSA indicate the presence of prostate cancer. Prostate cancer symptoms m diagnosed as well as 31 620 deaths in 2019 will occur. Of course, prostate cancer is when the prostate gland in males begins to divide rapidly out of ay include the following [10]:

- Frequent urination or frequent urge to urinate
- Blood in urine
- Pain during urination
- Pain during sex/ejaculation
- Edema
- Back pain
- Weight loss
- Unstable bowel

2.1.4 TREATMENT

Treatment varies in administration depending on the stage of cancer progression. Most of the treatment therapies aim at the AR pathway. Androgen-deprivation remains the mechanism of treatment against prostate cancer. These treatments include:

- Radiotherapy:
- Immunotherapy:
- Chemotherapy:
- Cryotherapy:

- Surgery:
- Hormonal therapy:

2.2 THE ANDROGEN RECEPTOR

The androgen receptor is primarily responsible for mediating the function of the prostate gland, and hence it is a biomarker to the onset of prostate cancer. The androgen receptor belongs to the steroid hormone receptor family that intracellular transduces signals from steroids such as testosterone and dihydrotestosterone activating series of cascade reactions. Androgens are produced in the testis, ovaries, and adrenal glands; however, the significant androgens in males are testosterone, dihydrotestosterone, and androstenedione [11–13].

Research shows that the prostate cancer cells require androgen throughout its stages for development, but especially at its early stage; hence AR continues to be a major bull's eye for therapy. Testosterone produced by the brain's pituitary gland, supervised by luteinizing hormone (LH), is concerted to dihydrotestosterone (DHT) by 5α-reductase. The dihydrotestosterone equally enters the cell. However, DHT fastens directly to AR with affinity twice that of testosterone and dissociates five times faster. Androgen response element (ARE)s in the nucleus is stimulated as receptor dimers are initiated. Transcription of genes follows which promotes prostate-specific antigen (PSA), growth, and survival ultimately [14–17]. The figure 2.3 explains the reaction followed by the entering of testoterone in the cell. Testoterone is reduced to DHT by a cytochrome P450 called 5α-reductase. DHT binds to AR and results in a conformational change. AR enters the nucleus and activates AREs in the gene promoter region which sponsors transcription and gene expression such as the FADS1 gene.

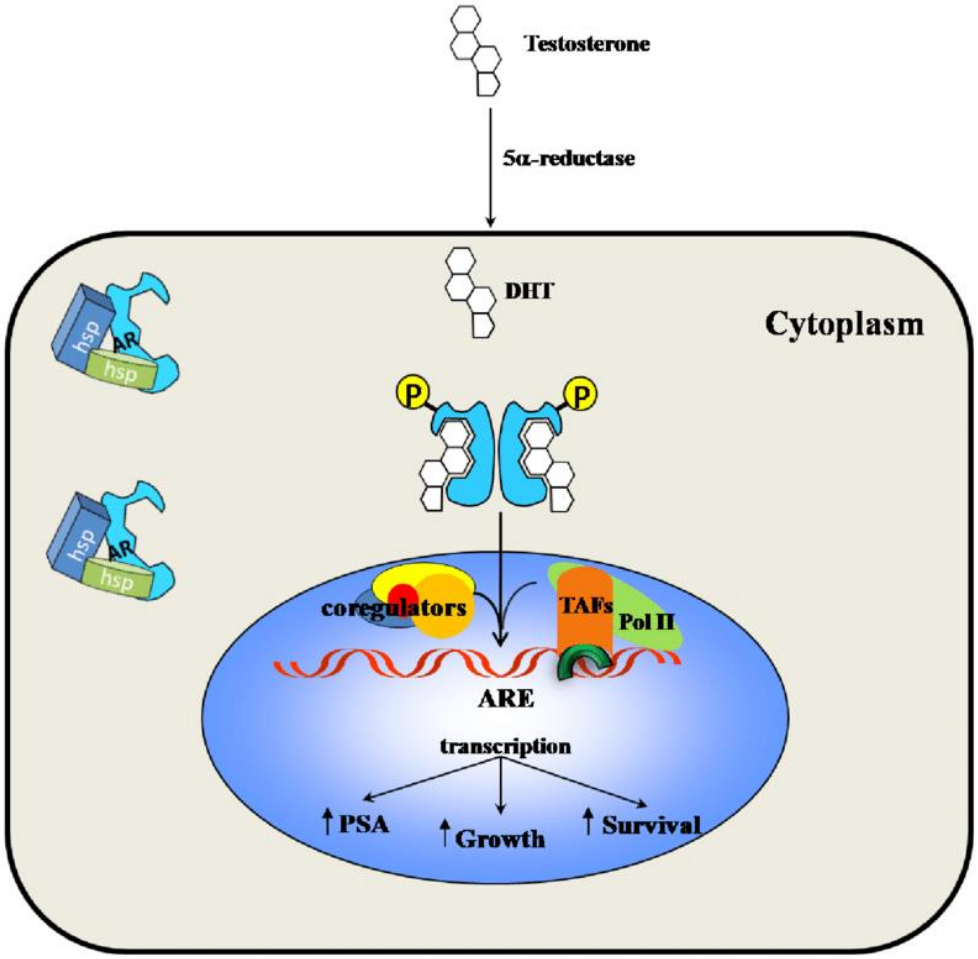

Figure 2.3 Androgen signalling pathway [8]

2.2.1 STRUCTURE

The AR structure consists of four (4) distinctive and functional domains, which are the N-terminal domain (NTD), a highly safeguarded deoxyribonucleic acid binding domain (DBD) and the ligand-binding domain (LBD). These domains are well defined in the AR. However, the hinge region holds part of a bipartite ligand-dependent nuclear localization signal (NLS) for the transport of AR in the nucleus. The hinge region is found in between the LBD and the DBD [18, 19]:

- The N-terminal domain: this domain is predominantly active. Research shows that it is capable of activating transcription with or without androgen binding. Activation function (AF-1), a transcription activation function, is found in the NTD that is maximally needed for AR activity.

- The deoxyribonucleic acid domain (DBD): the DBD is the most conserved in the AR. There are two zinc-fingers in it that enhances the binding of DNA sequences to the enhancer and promoter region of the AR-regulated genes. When this happens, the NTD and the LBD is activated to read the signal. Next, the transcription genes are either repressed or stimulated.

- The ligand-binding domain (LBD): as the name implies, the LBD promotes the binding of testosterone and DHT, defining the AR signalling pathway. Like the NTD, the LBD houses the Activation function (AF-2), which is vital for defining the co-regulator binding site. It also mediates straightforward interactions between the LBD and NTD.

- The hinge region: the hinge region contains a short sequence of amino acid that links the DBD and the LBD. Majorly the hinge region contains bipartite ligand-dependent nuclear localization signal required for AR signal transport from the cytoplasm to the nucleus.

2.2.2 FUNCTION

The function of the AR cuts across male maturity from puberty to old age. Majorly, the AR mediates the actions of androgens (testosterone and DHT) and initiating a cascade of reactions in the nucleus that involves growth and survival. However, other functions include [20, 21]:

1. AR is actively involved in the development and differentiation of urogenital structures
2. The initiation and maintenance of sperms are carried out by the AR
3. AR drives the differentiation and regulation of prostate function such as prostate-specific antigen
4. AR mediates the production and regulation of genes that are vital for cell cycle
5. AR is associated with healthy prostate development as well as in prostate cancer progression.

2.3 Castration-Resistant Prostate Cancer (CRPC)

CRPC, Castration-resistant prostate cancer, is referred to as a recently discovered stage of prostate cancer developed when AR remains active despite the blockage of androgen production by castration. In other words, these prostate cancer tumors continue to express AR-related genes hence fostering the growth of the cancer cells. It is also called hormone-refractory or androgen-independent prostate cancer. Several research types indicate that this could be a result of mutations in the AR, increased production of androgens via alternative pathways, or de novo synthesis of androgens by the cancer cells themselves. However, the molecular mechanism of this reactivation of the AR is still not clear [18] [22]. The below figure 2.4 further explains the effect of the presence and absence of AR in both normal prostate and in prostate cancer cells. Clearly, the absence of AR mediates methylation, translation of genes such as the

EGF, IL-6, IGF-1 which results in hormone refractory prostate cancer (HRPC).

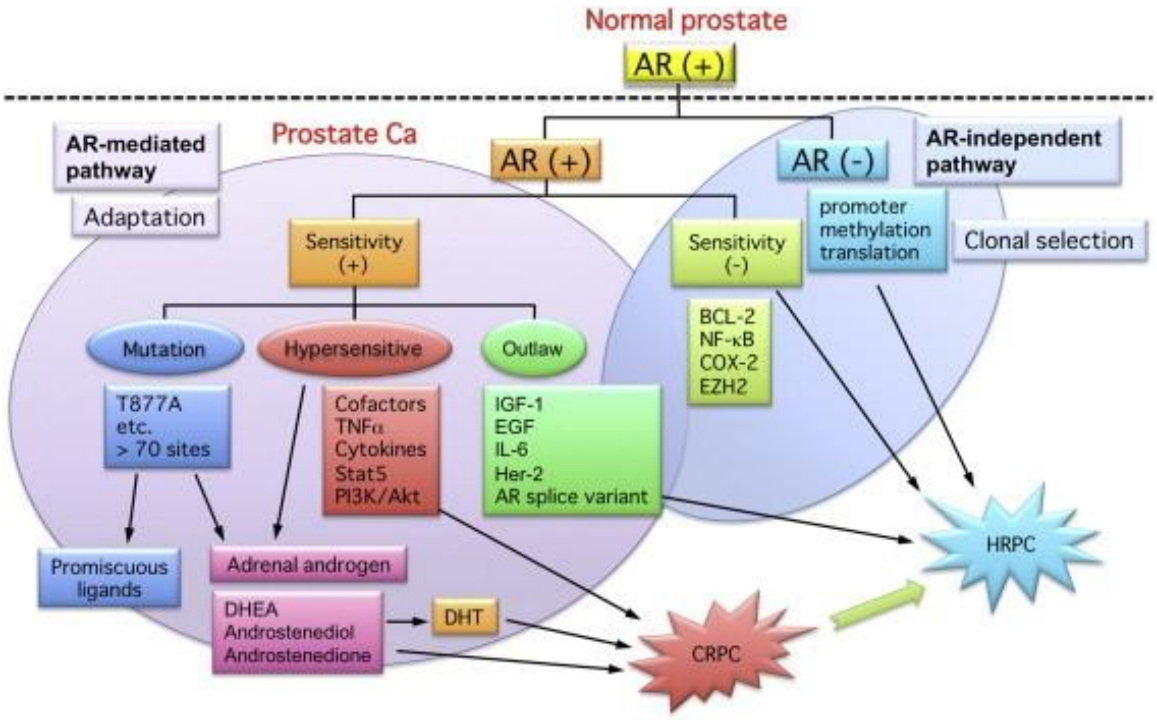

Figure 2.4 Mechanism of AR signalling both in healthy prostate and CRPC [23]

2.3.1 MECHANISM

Research progresses on the mechanism of CRPC. Some have thought stimulation begins from a shoot up of AR expression despite castration, mutations in the AR or AR-related genes or activation of signal transduction pathways due to the binding of weak androgens produced either from the adrenals or intracellular prostate cancer cells itself (intratumoral androgen production) [24]. The prostate cancer cells utilize various mechanisms of the AR signalling pathway for its growth as seen in Figure 2.5 which continues to be possible drug target for research.

In 1998, Christopher Gregory et al. published a paper stating clearly that the cause of CRPC is

an increase in the expression of androgen-related genes. The result of his experiment on castrated mice showed the expression of genes such as prostate-specific antigen (PSA) and human kallikrein-2 after 12 days despite the absence of testicular androgen [25]. It is also essential to note that although androgen continues in circulation at a superficial level of less than 50 ng/dL26 yet in CPRC, it reported activating AR amplification and expression [26]. Androgen deprivation has been the traditional treatment of prostate cancer; however, the advent of CRPC has kick-started various other treatment therapies to ensure its management.

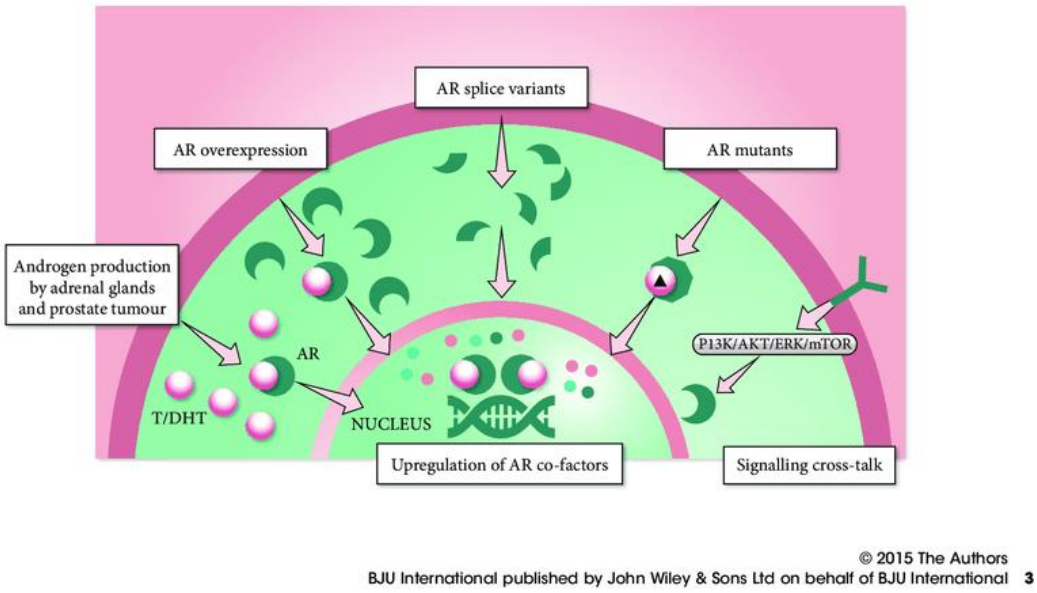

Figure 2.5 Possible mechanisms of AR transcriptional activity in CRPC [27]

2.4 CREB-BINDING PROTEIN (CBP) AS A POTENTIAL DRUG TARGET

The CREB (cAMP response element-binding protein) binding protein (CBP) is a lysine acetyltransferase protein capable of functioning as transcriptional coactivators in human cells [28]. Studies indicate that the interaction of CBP/p300 and the NTD of the androgen receptor could ensure the stability of the AR-AREs complex as well as the N/C interactions [29]. Likewise, CBP/p300 is highly expressed in advanced PCa, hence the deprivation of androgens results in the regulation of the proteins upstream [30]. Since CBP/p300 are active coactivators of AR activity, by aiming at this interaction and therefore targeting the BRD of CBP, AR signalling, and progression could be blocked. In vivo and in vitro studies have juxtaposed the fact that small molecule inhibitor of CBP/p300 shows the ability to suppress PCa growth and development [31, 32]. This formed the basis of this research in our study. More details about the CBP and its inhibition are explained in chapter four.

REFERENCE

1. López-lázaro M (2016) What Is The Main Cause Of Cancer? Cancer Stud Ther 1:2–4. https://doi.org/10.31038/cst.2016112
2. Tyagi N, Sharma GN, Shrivastava B, et al (2017) Cancer: An Overview. Int J Res Dev Pharm Life Sci 6:2740–2747. https://doi.org/10.21276/ijrdpl.2278-0238.2017.6(5).2740-2747
3. Tautu P, Wagner G (1984) Eesearch Clinical @ neology Guest Editorial An Approach to Oncological Genetics. 141–148
4. Facts C (2019) Joinpoint Trends in Cancer Incidence Rates for Selected Sites in Two Age Groups, US, 1995-2015 35 Figure S6. Trends in Cancer Death Rates for Selected Sites. Trends in Cancer Death Rates for Selected Sites," 2019, [Online]. Available: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2019/cancer-facts-and-figures-2019.pdf.
5. Bray F, Ferlay J, Soerjomataram I (2018) Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. 394–424. https://doi.org/10.3322/caac.21492
6. American Cancer Society (2016) Prostate Cancer What is prostate cancer ? Am Cancer Soc 88, cancer.org/1.800.227.2345, p. 88, 2016.
7. Brown CH (2012) Understanding prostate cancer. Pharm Times 78:. https://doi.org/10.12968/indn.2005.1.10.73853
8. Kumar VL, Majumder PK (1995) Prostate gland: Structure, functions and regulation. Int Urol Nephrol 27:231–243. https://doi.org/10.1007/BF02564756
9. Lee CH, Akin-Olugbade O, Kirschenbaum A (2011) Overview of Prostate Anatomy, Histology, and Pathology. Endocrinol Metab Clin North Am 40:565–575.

https://doi.org/10.1016/j.ecl.2011.05.012

10. Parker C (2012) Treating prostate cancer. BMJ 345:1–63. https://doi.org/10.1136/bmj.e5122

11. Wilson JD (1999) The role of androgens in male gender role behavior. Endocr Rev 20:726–737. https://doi.org/10.1210/edrv.20.5.0377

12. Pediatr C (1995) Binding Androgen Mutation of the Androgen Receptor Domain Associated with Insensitivity Syndrome Gene Ligand- the Complete Ryo Ogawa , Hideki Tomohisa Kazumichi Onigata Kanji Nagashima and Akihiro Morikawa of Pediatrics , Gunma of Medicine Japan Abstract . 4:149–154

13. Beato M, Herrlich P, Schütz G (1995) Steroid hormone receptors: Many Actors in search of a plot. Cell 83:851–857. https://doi.org/10.1016/0092-8674(95)90201-5

14. Srinivas-Shankar U, Wu FCW (2006) Drug insight: testosterone preparations. Nat Clin Pract Urol 3:653–665. https://doi.org/10.1038/ncpuro0650

15. Shang Y, Myers M, Brown M (2002) Formation of the androgen receptor transcription complex. Mol Cell 9:601–610. https://doi.org/10.1016/S1097-2765(02)00471-9

16. Dehm SM, Tindall DJ (2006) Molecular regulation of androgen action in prostate cancer. J Cell Biochem 99:333–344. https://doi.org/10.1002/jcb.20794

17. Heinlein CA, Chang C (2004) Androgen receptor in prostate cancer. Endocr Rev 25:276–308. https://doi.org/10.1210/er.2002-0032

18. Fawcett JW (1992) Spinal cord repair: Future directions. Paraplegia 30:83–85. https://doi.org/10.1038/sc.1992.29

19. Tan MHE, Li J, Xu HE, et al (2014) Androgen receptor : structure , role in prostate cancer and drug discovery. Nature Publishing Group 36:3–23. https://doi.org/10.1038/aps.2014.18

20. Yuan X, Balk SP (2009) Mechanisms mediating androgen receptor reactivation after

castration. Urol Oncol Semin Orig Investig 27:36–41. https://doi.org/10.1016/j.urolonc.2008.03.021

21. Grossmann ME, Huang H, Tindall DJ (2001) Androgen Receptor Signaling in Androgen-Refractory. Journal of National Cancer Institute, 93:1687–1697
22. Montgomery RB, Mostaghel EA, Vessella R, et al (2008) Maintenance of intratumoral androgens in metastatic prostate cancer: A mechanism for castration-resistant tumor growth. Cancer Res 68:4447–4454. https://doi.org/10.1158/0008-5472.CAN-08-0249
23. Mizokami A, Namiki M (2015) Reconsideration of progression to CRPC during androgen deprivation therapy. J Steroid Biochem Mol Biol 145:164–171. https://doi.org/10.1016/j.jsbmb.2014.03.015
24. Chandrasekar T, Yang JC, Gao AC, Evans CP (2015) Mechanisms of resistance in castration-resistant prostate cancer (CRPC). Transl Androl Urol 4:365–380. https://doi.org/10.3978/j.issn.2223-4683.2015.05.02
25. Gregory CW, Hamil KG, Kim D, et al (1998) Androgen receptor expression in androgen-independent prostate cancer is associated with increased expression of androgen-regulated genes. Cancer Res 58:5718–5724
26. Saad F, Hotte SJ (2010) Guidelines for the management of castrate-resistant prostate cancer. J Can Urol Assoc 4:380–384. https://doi.org/10.5489/cuaj.10167
27. Schalken J, Fitzpatrick JM (2016) Enzalutamide: Targeting the androgen signalling pathway in metastatic castration-resistant prostate cancer. BJU Int 117:215–225. https://doi.org/10.1111/bju.13123
28. Giotopoulos G, Chan WI, Horton SJ, et al (2016) The epigenetic regulators CBP and p300 facilitate leukemogenesis and represent therapeutic targets in acute myeloid leukemia. Oncogene 35:279–289. https://doi.org/10.1038/onc.2015.92
29. Comuzzi B, Nemes C, Schmidt S, et al (2004) The androgen receptor co-activator CBP

is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. J Pathol 204:159–166. https://doi.org/10.1002/path.1609

30. Dasgupta S, Lonard DM, O'Malley BW (2014) Nuclear Receptor Coactivators: Master Regulators of Human Health and Disease. Annu Rev Med 65:279–292. https://doi.org/10.1146/annurev-med-051812-145316

31. Jin L, Garcia J, Chan E, et al (2017) Therapeutic targeting of the CBP/p300 bromodomain blocks the growth of castration-resistant prostate cancer. Cancer Res 77:5564–5575. https://doi.org/10.1158/0008-5472.CAN-17-0314

32. Xiang Q, Wang C, Zhang Y, et al (2018) Discovery and optimization of 1-(1H-indol-1-yl)ethanone derivatives as CBP/EP300 bromodomain inhibitors for the treatment of castration-resistant prostate cancer. Eur J Med Chem 147:238–252. https://doi.org/10.1016/j.ejmech.2018.01.087

CHAPTER THREE

3.1 INTRODUCTION

Computational chemistry mixes up theoretical chemistry with computer science. It is branch-off chemistry that involves the use of computational programs and tools to solve chemical problems. It is also called molecular modelling. Mostly, chemical and biological systems are modelled to understand simulation at the atomic level. Computational chemistry has been adopted in pharmaceutical chemistry, and it enhances better design and discovery of drugs targeting various diseases. Many a time, novel compounds are searched, optimized, and docked into target pockets of protein to obtain binding pose as well as observe their interactions via MD simulations [1, 2]. These computational methods include databases, quantitative structure-activity relationships (QSAR), virtual screening, and homology modelling, and molecular dynamics simulation [3] effectively increase drug design process [4]. These tools offer a promising future in drug discovery research [5].

There are two molecular modelling principles (Figure 3.1) that can be used to establish the energetics and conformational changes to the drug-target system:

- Quantum Mechanics
- Molecular Mechanics

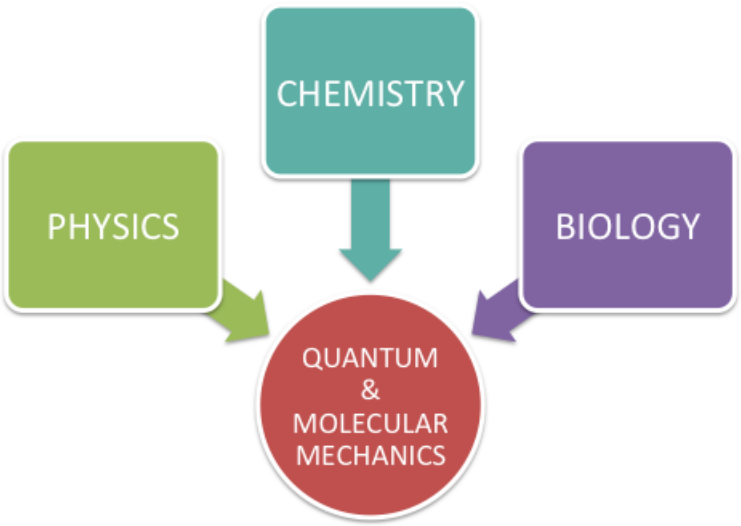

Figure 3.1. Application of quantum and molecular mechanics (prepared by the author)

Hence, this chapter begins with the description of quantum and molecular mechanics principles, where the Schrödinger's equation, born – Oppenheimer, Potential energy surface (PES) and force fields, will be discussed—followed by details on molecular dynamic simulations, the post-analysis parameters as well as the binding energies calculations. Other molecular modelling tools used in this study are also explained.

3.2 Principles of Quantum Mechanics

The principle of quantum mechanics is based on the theory of atoms and the subatomic, the structure and properties of elementary particles. It is an aspect of physics on which most of the physics theories are based on, from newton's theory of matter to Einstein's theory of gravity.

The discovery of quantum mechanics cannot be attributed to one man; as matters of fact, scientists believe the theory was not derived but postulated. A. Einstein, M. Planck, L. Broglie, N. Bohr, and W. Heisenberg, E. Schrodinger, M. Born, P. Dirac, and others all contributed to the postulation of the quantum theory.

The principle tends to explain the behaviour of particles such as an electron, proton, neutron, molecules as well as photons. Quantum mechanics cuts across the field of physics, chemistry, and biology, as seen in Figure.3.1, creating a better understanding of life and its complexity [6].

3.2.1 Schrödinger Equation

In mathematical physics, the Schrödinger equation can be represented as:

$$\boldsymbol{HP = T + PE} \qquad \textbf{Eq. 1}$$

Where:

Hamiltonian operator (HP), equals the summation of KE, kinetic energy

potential energy is PE

The operator is T

Also, HP could mean the below equation:

$$\boldsymbol{H = \left[-\frac{h^2}{8\pi^2} \sum_i \frac{1}{mj} \left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \right) \right] + \sum_i \sum_{<j} \left(\frac{e_i e_i}{r_{ij}} \right)} \qquad \textbf{Eq. 2}$$

From the above equation, it can be understood that Schrödinger's equation plays by Hamilton's

law of motion. In general, by using the Schrödinger's equation, particles expressed in wave function are calculated in quantum mechanics [7]. The Schrodinger equation can be time-dependent or time- independent [8] however, in the time-dependent equation is mostly adopted in computational chemistry. Totalling the kinetic energy plus the potential energy equals the Hamiltonian operator, as seen in the above two equations.

3.2.2 Born-Oppenheimer approximation

The born-Oppenheimer approximation elucidates that nuclear motion of a molecule and how it interferes with its electronic motion. This implies that the molecular energy is dependent on the nuclear coordinates and electron coordinates, which defines the molecular geometry. Born-Oppenheimer approximation believes that the nuclei are 1800 times heavier than the electron, and hence depending on the position of the nuclei, the electron can experience a Hamiltonian [9]. Schrodinger's equation is simplified, and the total internal energy of a molecule can be calculated using his equation.

$$\boldsymbol{T^{elec}} = \left[- \frac{\boldsymbol{h^2}}{\boldsymbol{8\pi^2 m}} \sum_{\boldsymbol{i}}^{\boldsymbol{electrons}} \left(\frac{\boldsymbol{\partial^2}}{\boldsymbol{\partial x^2}} + \frac{\boldsymbol{\partial^2}}{\boldsymbol{\partial y^2}} + \frac{\boldsymbol{\partial^2}}{\boldsymbol{\partial z^2}} \right) \right] \qquad \textbf{Eq. 3}$$

Here, for fixed nuclei electrons, the Schrödinger's equation is:

$$\boldsymbol{H_{elec\varphi elec}(r, R)} = \boldsymbol{E_{eff}}\ \boldsymbol{(R)_{\varphi elec}(r, R)} \qquad \textbf{Eq. 4}$$

3.2.3 Potential energy surface

The Potential energy surface (PES) is produced when the born- Oppenheimer's approximation operates by calculating the mathematical problem for a fixed position. Figure 3.2 shows a typical two-dimensional model of potential energy surface showing all stationary points and their resultant gradient. This is a deeper aspect of Born-Oppenheimer approximation. A PES is simply a plot that shows the interaction between the molecular energy and the molecular geometry [10]. On the PES, the most vital points are stationary points. It is on these points that the internal coordinates that produce the gradient are zero [11].

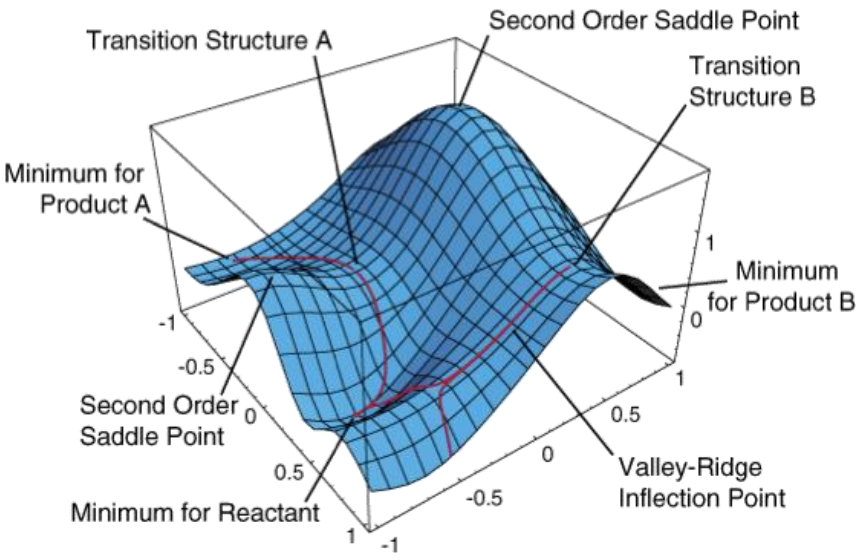

Figure 3.2. A two-dimensional potential energy surface model [12]

3.3 Principles of Molecular Mechanics

Molecular mechanics functions based on models used to mimic molecular structures such as DNA, RNA, and Proteins to predict molecular energy depending on the conformation taken up by the molecule. These make use of force field methods to conduct conformational analysis, such as the relative energies of the transitional state equilibrium between conformers [2]. The atoms in a molecular system interact via covalent bonds, creating rotations and angles. These atomic rotation and interaction aid molecular mechanics to be understood concerning identifying cellular structure, response, and function. Also, in disease prognosis and treatment, MM calculation aids research [13].

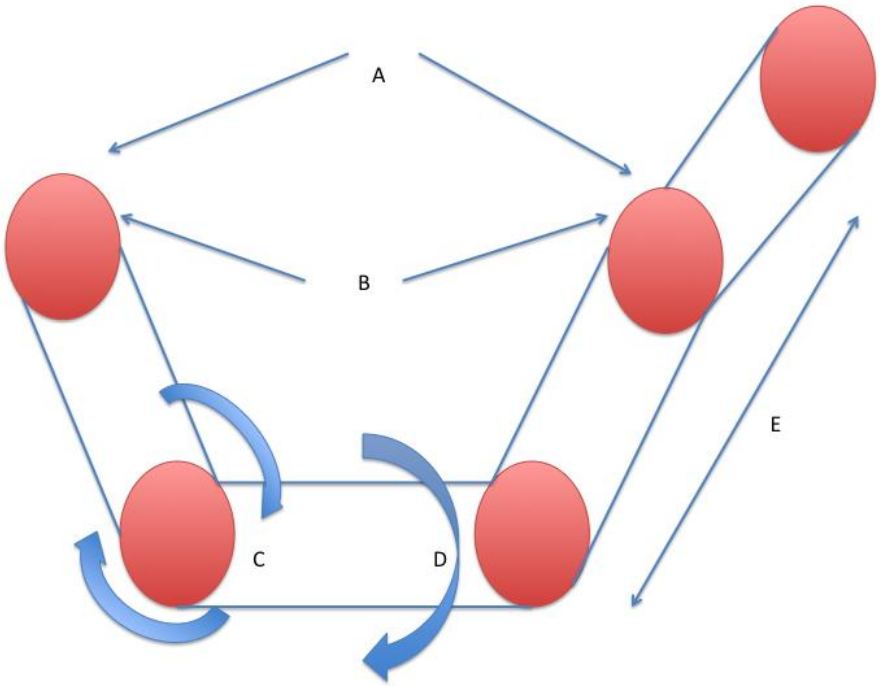

Figure 3.3 Methods of molecular mechanics (prepared by author). Where; A = Electrostatic attraction, B = Van der Waals, C = Angle bending, D = Dihedral rotation, E = Bond stretching

Molecular mechanics rests on three main principles; the principle of thermodynamic hypothesis, additively, and transferability. Since the native state of the protein is usually stable, the principle of the thermodynamic hypothesis states that the minimum potential energy corresponds to the native state of the protein. Additively sums up the individual function such as electrostatic, van-der-Waals, bond stretching, bond angle fluctuations, and rotations to give the total potential function, Ep as seen in Figure 3.3 showing the interactions among all functions.

$$E_p^{local} = \sum_{bonds} V_{BL} + \sum_{angles} V_{BA} + \sum_{dihedral} V_{DA} \qquad \textbf{Eq. 5}$$

Where VBL, VBA, VDA are bond-length, bond-angle, and dihedral potentials, respectively.

The transferability hypothesis implies that there is not a difference in the properties of the atoms in large molecules as they are in their small test form i.e., the energies derived when the bond length and angles are studied as small molecules can be transferred to their more extensive and complex molecules without modification. With this in view, complex molecules can be studied by studying them in their small atomic states [14].

Despite the enormous advantages embedded in MM, some setbacks have been identified. Firstly, MM is unable to give information about bond formation and bond breaking. Secondly, MM only tells us about the equilibrium geometries and equilibrium conformation and nothing more. Lastly, its inability to predict chemical reaction poses a major setback [6] [15,16].

3.4 The Force Field

The force field defines the molecular conformation energy of a protein in a mathematical function. Although there are several force fields developed, the widely used ones such as AMBER [17], GROMAS [18], CHARMM [19], and OPLS [20] are applied to biomolecule simulations. These force fields can also be used to support protein modelling when incorporated with carbohydrates, lipids, nucleic acids, and small molecules. With these force fields, molecular mechanics and dynamic calculations are achieved. There are different parameters in the force fields; thus, they must be adjusted to give results of the forces acting within a molecule [21, 22]. In our study, we used Generalized AMBER Force Field (GAFF) to express the ligands in terms of its parameters. A detailed discussion about the AMBER force field is provided in chapter 4.

3.5 Molecular Dynamics Simulations

The atoms of the protein or complex (i.e., the protein-ligand complex) consistently produce motions that generate a trajectory of all the particles as a function of time. This explains the molecular dynamic simulation of a system [23]. Although there are other simulation methods such as Monte Carlo and Brownian dynamics, Molecular dynamics is the most broadly used and specific type of simulation methods. While the former depends on generating large numbers of configurations through probabilities moving from one state to the other in a specific statistical manner and simulation of large macromolecules respectively, the latter can calculate individual molecules in a system. Furthermore, different dynamic quantities that cannot be calculated by Monte Carlo is done by MD [23, 24]. With the use of MD simulations, the test for experimental observations of molecules such as proteins is carried out to check whether its

prediction matches theoretical models. Newton's equation is used in molecular dynamics calculations, which stands based on MM principles [25]. The end-result is represented as a trajectory which explains the particle position and velocity varies with time [26]. Hence, this is obtained by the force determinant (Fi) for every particle as a function of time. Conclusively, Ft equals the negative gradient of PE.

$$\boldsymbol{F_I} = -\frac{\boldsymbol{U}}{\boldsymbol{r_i}} \qquad \textbf{Eq. 6}$$

Where, The potential function (U), The position of the particle (r),

According to the Laws of Motion by Newton,

The acceleration of a particle (a) is calculated as follows:

$$\boldsymbol{a_i} = -\frac{\boldsymbol{F_i}}{\boldsymbol{m_i}} \qquad \textbf{Eq. 7}$$

The integral of acceleration due to change in time together with the change in position gives the integral of velocity due to change in time. This is called the velocity change, and it is denoted as:

$$\boldsymbol{dv} = \int \boldsymbol{adt}, \qquad \textbf{Eq. 8}$$

$$\boldsymbol{dr} = \int \boldsymbol{vdt}, \qquad \textbf{Eq. 9}$$

Conclusively, the velocity (v) and momenta (p) of the given atom defines the kinetic energy of the particle, which is:

$$\boldsymbol{K(v)} = \frac{\boldsymbol{1}}{\boldsymbol{2}}\sum_{\boldsymbol{i=1}}^{\boldsymbol{N}} \boldsymbol{m_i v_i} \qquad \textbf{Eq. 10}$$

$$\boldsymbol{K(p)} \;=\; \frac{\mathbf{1}}{\mathbf{2}}\sum_{\boldsymbol{i=1}}^{\boldsymbol{n}}\frac{\boldsymbol{P_i^2}}{\boldsymbol{m_i}} \qquad \textbf{Eq. 11}$$

Where, The given Cartesian coordinates are q, The atoms momenta are p, The potential energy function is U (q) vi (t) i.e., velocity is the first derivatives of the positions over a change in time:

$$\boldsymbol{V_i(t)} \;=\; \frac{\boldsymbol{d}}{\boldsymbol{dt}}\boldsymbol{q_i(t)} \qquad \textbf{Eq. 12}$$

Here $qi\ (t)$ is the positions of the atom at a specific time, t. These atoms move to new positions based on the first atom coordinates of a specific system, contemporary velocity, and position of the particle at a given time t. Hence, the conformations newly obtained and the system's temperature changes concerning the kinetic energy in direct proportionality.

3.6 System Stability of Simulated Systems

3.6.1 System Convergence

System convergence is a word used to explain the dynamics of proteins that occur during the unfolding of the protein structure based on bond types and bond angle vibrations. This process is necessary for an MD trajectory to be accurate and reproducible; hence the simulated system must show a state of equilibrium indicating a state of final energetics and conformational plateau [27]. The protein-ligand system, therefore, depicts an energetically stable conformation at this plateau.

3.6.2 Root Mean Square Deviation (RMSD)

RMSD may be calculated by the spatial differentiation between two static structures of the same trajectory. The RMSD of a trajectory is denoted as:

$$\boldsymbol{RMSD} = \left(\frac{\sum_N \left(\boldsymbol{R_i} - \boldsymbol{R_1^0}\right)^2}{\boldsymbol{N}}\right)^{\frac{1}{2}} \qquad \textbf{Eq. 14}$$

Where: N depicts the total of atoms in a system, Ri represents the position of the vector of the Cα atom of particle i is the conformational reference which is obtained by calculating after aligning the structure to an initial conformation (O). The least-square fitting protocol is used for this calculation.

The average RMSD of a complex may be defined by taking the average structural deviation over the number of frames in each trajectory and can be calculated for the receptor, ligand, and complex within a simulated system [28].

3.6.3 Radius of Gyration (RoG)

The radius of the system's gyration explains the root mean square distance of the atoms from their common centroid/center of gravity. The RoG allows for the estimation of compactness of a protein complex along a trajectory. The RoG of a complex may be based on the following reaction:

$$\boldsymbol{r^2 gyr} = \frac{\left(\sum_{i=1}^{n} \boldsymbol{w_i}(\boldsymbol{r_i} - \boldsymbol{r^-})^2\right)}{\sum_{i-1}^{n} \boldsymbol{w_i}} \qquad \textbf{Eq. 15}$$

Where: The position of the ith atom is ri.Center weight of atom I is r

The average RoG is obtained by dividing the average with the number of frames in a trajectory [29].

3.7 Conformational Features of System

3.7.1 Root Mean Fluctuation (RMSF)

The root mean fluctuation (RMSF) of a protein measures residue's Cα atom fluctuation based on the average protein structure along the system's trajectory. This extends to postulate the flexibility of regions of a protein based on the computed RMSF [30]. To calculate the standardized RMSF, the following equation is applied:

$$\boldsymbol{sRMSF = \frac{(RMSF_i - \overline{RMSF})}{\sigma(RMSF)}} \qquad \textbf{Eq. 16}$$

Where: the subtraction of the average RMSF from the RMSF of the ith residue gives the RMSFi. When the result is divided by the RMSF's standard deviation, the resultant standardized RMSF is obtained.

The above method differs from RMSD and RoG as it is computed as the total residue fluctuation along the trajectory and is not analysed at every frame in the trajectory.

3.7.2 The Principal Component Analysis

The principal component analysis (PCA) is a covariance-matrix-based mathematical method which simplifies the magnitude of the data generated from an MD simulation to comprehend

the correlated motions. Usually the PCA technique is measured when the displacement of the atom and the protein loop dynamics of a biomolecular system is to be calculated.

The application of PCA in a MD simulation is known as "essential dynamics" as only fundamental motions of a data set are isolated from the millions of conformational snapshots. The conformational motions are then filtered from largest to smallest fluctuations and graphically depicted using a covariance matrix [31]. The new set of defined coordinates are defined as the principal components of the data set and ordered such that the first 3-4 principal components have similar fluctuations as observed in the trajectory [32].

3.8 Thermodynamic Calculations

3.8.1 The binding free-energy calculations

The binding free energy calculation is a vital endpoint approach, which provides essential information regarding the binding mechanism between the enzyme and the ligand, integrating both enthalpy and entropic contributions. Estimation of the binding free energy leads to the development of various algorithms and approaches, including free energy perturbation, thermodynamic integration, linear interaction energy, and molecular docking calculations, to mention a few [33, 34]. In recent computational studies, free energy calculations have aided substantially. These have provided detailed knowledge about protein structure determination and protein-protein complexes as well as drug design [35, 36]. Molecular Mechanics/ Poisson-Boltzmann Surface Area (MMPB-SA) and the Molecular Mechanics/Generalized Born Surface Area (MM/GB-SA) approaches approach are conventional methods commonly which is utilized for calculating the free binding energy of compounds ranging from small compounds called ligands to biological complex macromolecules [37, 38].

Both MM/GB-SA and MM/PB-SA depends on the simulations of molecular complex, i.e. complex of ligand and protein to calculate within a force field, the austere statistical-mechanical binding free energy [40]. Both methods reveal approving use, which could represent their lack of calculations and modular nature that originates from training sets copulating unchanging solvation models that combines with MM calculations despite the abrupt changes [39]. Avid accuracy and computational effort are displayed by each of the approaches between the experimental scores and rigorous alchemical approximated methods. These could then be set side by side to replicate and justify the experimental data [40]. Both methods are also used to modify the free energy decomposition (FED). The FED thoroughly range into different groups, based on the atom groups or types of interactions generated by the collisions [41].

The MM-PBSA, unlike the MM/GB-SA, employs a harder algorithm and, at the same time, replaces the MM/GB-SA model of electrostatics in water [42,43]. Nevertheless, the calculations involving protein-drug interaction such as carbohydrates [44] and nucleic acids [45], is more favourable with MM-GBSA than MM-PBSA [46]. Binding free- energy calculations is also be used to intensify virtual screening results and the docking outcome of therapeutics drugs [47]. Highlighting the MM/GB-SA, the binding free energy between the ligand and receptor is calculated as follows [48]:

$$\boldsymbol{G_{bind} = G_{complex} - G_{receptor} - G_{ligand}} \qquad \textbf{Eq. 17}$$

$$\boldsymbol{\Delta G_{bind} = \Delta E_{mm} + \Delta G_{gbsa} - T_{entropy}} \qquad \textbf{Eq. 18}$$

$$\boldsymbol{\Delta E_{mm} = \Delta E_{int} + \Delta E_{vdw} + \Delta E_{eel}} \qquad \textbf{Eq. 19}$$

$$\boldsymbol{\Delta G_{gbsa} = \Delta G_{ebg} + \Delta G_{esurf}} \qquad \textbf{Eq. 20}$$

Where, The energy of the MM of a system in an empty space is ΔEmm, The free energy of the solvent is ΔGgbsa, T, entropy is TΔS, The total fused internal energy (ΔEInt) is ΔEmm,

The polar contributions that sum up the generalized born model (ΔGegb) together with the non-polar contributions (ΔGesurf), is made up of the non-bonded van der Waals (ΔEvdw) and electrostatic (ΔEeel) also ΔGgbsa [37] [49] [50].

One advantage of the dynamic analysis of binding affinity is that it determines the inhibitory activity of each inhibitor approximately [51,52]. While using the Molecular Mechanics/Generalized Born Surface Area binding energy calculation, the actual ligand binding conformation can be found before the approximate value of the binding energy [53]. In this research, we used MM/GB-SA approach to predict the ligand-protein binding free energies.

3.9 Molecular Modelling Tools used in this study

3.9.1 Marvin Sketch Suite

Marvin Sketch is advanced software used primarily to draw chemical structures. These structures can be edited into file types such as MOL, MOL2, SDF, RXN, RDN, In Chi, CML, PDB, etc. other editing functions include 3D editing, 2D cleaning, and conformer generation, Copy and paste between different editors, Fog effect in 3D viewing mode, Creating and editing molecule sets (without a database), Structure annotation to mention a few. Moreover, with Marvin sketch, structure-based calculations can be called using the calculation plugin section

[54]. In this study, Marvin's sketch was used to draw out the ligand compounds and prepared before molecular docking. More details are discussed in chapter 4.

3.9.2 Molecular Docking

Molecular docking regularly operates to pinpoint precise ligands-protein conformations and to approximately calculate the vigour of the protein-ligand interaction in structure-based drug design. Drug candidates or inhibitors could be recognized in the macromolecules' active site using docking. Examples are receptor, nucleic acid, or enzyme with identified known conformations [55]. The resultant binding energy complex formed by the ligand and the receptor is:

$$\boldsymbol{E_{binding} = E_{target} + E_{ligand} - E_{target-ligand}} \qquad \textbf{Eq. 21}$$

In recent times, many molecular docking programs have been developed for academic and commercial purposes [56], such as Dock [57], AutoDock [58], GOLD [59], FlexX [60], GLIDE [61], ICM [19], PhDOCK [62], Surflex [63], and so on. These programs could be sectioned into four categories based on its fragment, evolution, stochastic Monte Carlo, and the shape-complementary methods [64]. Of each of the classifications, there is a requirement for separate details, for example, there are four computational steps they all share in common: (1) rigid and simplified body search (2) determination of the portion(s) of interest; (3) docked-structures modification and (4) superlative models selection, respectively [56]. Every method is ideal for docking problems, yet when these computational methods are combined, the reliability and accuracy of results are improved[65]. Docking features in two types: (1) flexible docking, (2) rigid docking. These complex molecules, as well as ligands, are kept unmovable and fixed in rigid docking; however, flexible docking is the hallmark of the complexes (macromolecule or

ligands or both).

Herein this study, the methods adopted for docking is the advanced version of Auto Dock, Auto Dock Vina [66]. The application of Molecular docking varies in different areas such as virtual screening which involves hit identification, drug discovery which involves lead optimization, biological activity predictions, binding- site identification (blind docking), structure-function studies, protein-protein interaction, de-orphaning of a receptor, enzymatic reaction mechanisms and protein engine.

REFERENCE

1. Kore PP, Mutha MM, Antre R V., et al (2012) Computer-Aided Drug Design: An Innovative Tool for Modeling. Open J Med Chem 02:139–148. https://doi.org/10.4236/ojmc.2012.24017
2. Lewars EG (2016) Computational chemistry: Introduction to the theory and applications of molecular and quantum mechanics: Third Edition 2016
3. Ekins S, Mestres J, Testa B (2007) In silico pharmacology for drug discovery: Methods for virtual ligand screening and profiling. Br J Pharmacol 152:9–20. https://doi.org/10.1038/sj.bjp.0707305
4. Talele T, Khedkar S, Rigby A (2010) Successful Applications of Computer Aided Drug Discovery: Moving Drugs from Concept to the Clinic. Curr Top Med Chem 10:127–141. https://doi.org/10.2174/156802610790232251
5. Song CM, Lim SJ, Tong JC (2009) Recent advances in computer-aided drug design. Brief Bioinform 10:579–591. https://doi.org/10.1093/bib/bbp023
6. Senn HM, Thiel W (2009) QM/MM methods for biomolecular systems. Angew Chemie - Int Ed 48:1198–1229. https://doi.org/10.1002/anie.200802019
7. Bahrami M, Großardt A, Donadi S, Bassi A (2014) The Schrödinger-Newton equation and its foundations. New J Phys 16, pp. 1-18:. https://doi.org/10.1088/1367-2630/16/11/115007
8. Barde NP, Patil SD, Kokne PM, Bardapurkar PP (2015) Deriving time dependent Schrödinger equation from Wave-Mechanics, Schrödinger time independent equation, classical and Hamilton-Jacobi equations. Leonardo Electron J Pract Technol 14:31–48
9. Terme D, Beitrag S, Grund D, Standpunkte V (1927) Born Oppenheimer. Gridbridge 24:480–484
10. Sutcliffe B, Woolley RG (2013) Advances in Quantum Methods and Applications in

Chemistry, Physics, and Biology. 27, pp. 3-372:. https://doi.org/10.1007/978-3-319-01529-3

11. https://opentextbc.ca/chemistry/chapter/7-5-strengths-of-ionic-and-covalent-bonds
12. http://www.chem.wayne.edu/~hbs/chm6440/PES.html , 11 Nov. 2013
13. Bao G, Suresh S (2003) Cell and molecular mechanics of biological materials. Nat Mater 2:715–725. https://doi.org/10.1038/nmat1001
14. Shattuck T, Shattuck TW (2014) Department of Chemistry Colby College. https://doi.org/10.13140/2.1.2228.5440
15. F. Jensen and F. Jensen, "Introduction to Computational Chemistry Second Edition," by John Wiley & Sons, Inc., no. January 1989, 2014
16. Swope WC, Andersen HC, Berens PH, Wilson KR (1982) A computer simulation method for the calculation of equilibrium constants for the formation of physical clusters of molecules: Application to small water clusters. J Chem Phys 76:637–649. https://doi.org/10.1063/1.442716
17. Case DA, Cheatham TE, Darden T, et al (2005) The Amber biomolecular simulation programs. J Comput Chem 26:1668–1688. https://doi.org/10.1002/jcc.20290
18. Oostenbrink C, Villa A, Mark AE, Van Gunsteren WF (2004) A biomolecular force field based on the free enthalpy of hydration and solvation: The GROMOS force-field parameter sets 53A5 and 53A6. J Comput Chem 25:1656–1676. https://doi.org/10.1002/jcc.20090
19. MacKerell AD, Bashford D, Bellott M, et al (1998) All-atom empirical potential for molecular modeling and dynamics studies of proteins. J Phys Chem B 102:3586–3616. https://doi.org/10.1021/jp973084f
20. Jorgensen WL, Maxwell DS, Tirado-Rives J (1996) Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic

liquids. J Am Chem Soc 118:11225–11236. https://doi.org/10.1021/ja9621760

21. Guvench O, MacKerell AD (2008) Comparison of protein force fields for molecular dynamics simulations. Methods Mol Biol 443:63–88. https://doi.org/10.1007/978-1-59745-177-2_4

22. Darian E, Gannett PM (2005) Application of molecular dynamics simulations to spin-labeled oligonucleotides. J Biomol Struct Dyn 22:579–593. https://doi.org/10.1080/07391102.2005.10507028

23. Hua X-M (1997) Monte Carlo simulation of Comptonization in inhomogeneous media. Comput Phys 11:660. https://doi.org/10.1063/1.168615

24. Neyts EC, Bogaerts A (2013) Combining molecular dynamics with monte carlo simulations: Implementations and applications. Theor Chem Acc 132:1–12. https://doi.org/10.1007/s00214-012-1320-x

25. Hirst JD, Glowacki R, Baaden M (2014) Molecular simulations and visualization : introduction and overview. Faraday Discuss 00:1–14. https://doi.org/10.1039/C4FD90024C

26. Böhm H-J, Stahl M (2003) The Use of Scoring Functions in Drug Discovery Applications , Reviews in Computational Chemistry, Vol 18, pp.41 – 87. 2003. DOI: 10.1002/0471433519.ch2

27. Amadei A, Ceruso MA, Di Nola A (1999) On the convergence of the conformational coordinates basis set obtained by the Essential Dynamics analysis of proteins' molecular dynamics simulations. Proteins Struct Funct Genet 36:419–424. https://doi.org/10.1002/(SICI)1097-0134(19990901)36:4<419::AID-PROT5>3.0.CO;2-U

28. Krieger E, Nabuurs SB, Vriend G (2003) Chapter 25: homology modeling. Struct

Bioinforma 44:507–521. https://doi.org/10.1007/978-1-61779-588-6

29. Lobanov MY, Bogatyreva NS, Galzitskaya O V. (2008) Radius of gyration as an indicator of protein structure compactness. Mol Biol 42:623–628. https://doi.org/10.1134/S0026893308040195

30. Bornot A, Etchebest C, De Brevern AG (2011) Predicting protein flexibility through the prediction of local structures. Proteins Struct Funct Bioinforma 79:839–852. https://doi.org/10.1002/prot.22922

31. Martinez AM, Kak AC (2001) PCA versus LDA. IEEE Trans Pattern Anal Mach Intell 23:228–233. https://doi.org/10.1109/34.908974

32. Tan Y, Hanson JA, Chu J, Yang H (2014) VIP MSM UBER WIP PCA ESSENTIAL Protein Dynamics pp. 193-226. 2014.

33. Kamerlin SCL, Haranczyk M, Warshel A (2009) Progress in Ab initio QM/MM free-energy simulations of electrostatic energies in proteins: Accelerated QM/MM studies of pKa, redox reactions and solvation free energies. J Phys Chem B 113:1253–1272. https://doi.org/10.1021/jp8071712

34. Kollman P (1993) Free Energy Calculations: Applications to Chemical and Biochemical Phenomena. Chem Rev 93:2395–2417. https://doi.org/10.1021/cr00023a004

35. Gohlke H, Kiel C, Case DA (2003) Insights into protein-protein binding by binding free energy calculation and free energy decomposition for the Ras-Raf and Ras-RalGDS complexes. J Mol Biol 330:891–913. https://doi.org/10.1016/S0022-2836(03)00610-7

36. Hou T, Chen K, McLaughlin WA, et al (2006) Computational analysis and prediction of the binding motif and protein interacting partners of the Abl SH3 domain. PLoS Comput Biol 2:0046–0055. https://doi.org/10.1371/journal.pcbi.0020001

37. Genheden S, Ryde U (2015) The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Expert Opin Drug Discov 10:449–461. https://doi.org/10.1517/17460441.2015.1032936

38. Wang J, Hou T, Xu X (2006) Recent Advances in Free Energy Calculations with a Combination of Molecular Mechanics and Continuum Models. Curr Comput Aided-Drug Des 2:287–306. https://doi.org/10.2174/157340906778226454

39. Hou T, Wang J, Li Y, Wang W (2011) Assessing the Performance of the MM_PBSA and MM_GBSA Methods. 1. The Accuracy.pdf. Journal of Chemical I nformation and Modelling 51:69–82

40. Zoete V, Meuwly M, Karplus M (2005) Study of the insulin dimerization: Binding free energy calculations and per-residue free energy decomposition. Proteins Struct Funct Genet 61:79–93. https://doi.org/10.1002/prot.20528

41. Hou T, Li N, Li Y, Wang W (2012) Characterization of domain-peptide interaction interface: Prediction of SH3 domain-mediated protein-protein interaction network in yeast by generic structure-based models. J Proteome Res 11:2982–2995. https://doi.org/10.1021/pr3000688

42. Tsui V, Case DA (2000) Theory and applications of the Generalized Born solvation model in macromolecular simulations. Biopolymers 56:275–291. https://doi.org/10.1002/1097-0282(2000)56:4<275::AID-BIP10024>3.0.CO;2-E

43. Onufriev A, Bashford D, Case DA (2000) Modification of the generalized born model suitable for macromolecules. J Phys Chem B 104:3712–3720. https://doi.org/10.1021/jp994072s

44. Feig M, Brooks CL (2002) Evaluating CASP4 predictions with physical energy functions. Proteins Struct Funct Genet 49:232–245. https://doi.org/10.1002/prot.10217

45. Wang J, Deng Y, Roux B (2006) Absolute binding free energy calculations using

molecular dynamics simulations with restraining potentials. Biophys J 91:2798–2814. https://doi.org/10.1529/biophysj.106.084301

46. Sun H, Li Y, Tian S, et al (2014) Assessing the performance of MM/PBSA and MM/GBSA methods. 4. Accuracies of MM/PBSA and MM/GBSA methodologies evaluated by various simulation protocols using PDBbind data set. Phys Chem Chem Phys 16:16719–16729. https://doi.org/10.1039/c4cp01388c

47. Aalten DMFV, Findlay JBC, Amadei A, Berendsen HJC (1995) Essential dynamics of the cellular retinol-binding protein evidence for ligand-induced conformational changes. Protein Eng Des Sel 8:1129–1135. https://doi.org/10.1093/protein/8.11.1129

48. Decherchi S, Masetti M, Vyalov I, Rocchia W (2015) Implicit solvent methods for free energy estimation. Eur J Med Chem 91:27–42. https://doi.org/10.1016/j.ejmech.2014.08.064

49. Cappel D, Hall ML, Lenselink EB, et al (2016) Relative Binding Free Energy Calculations Applied to Protein Homology Models. J Chem Inf Model 56:2388–2400. https://doi.org/10.1021/acs.jcim.6b00362

50. Huang YMM, Chen W, Potter MJ, Chang CEA (2012) Insights from free-energy calculations: Protein conformational equilibrium, driving forces, and ligand-binding modes. Biophys J 103:342–351. https://doi.org/10.1016/j.bpj.2012.05.046

51. Chang CEA, Chen W, Gilson MK (2007) Ligand configurational entropy and protein binding. Proc Natl Acad Sci U S A 104:1534–1539. https://doi.org/10.1073/pnas.0610494104

52. Wright DW, Hall BA, Kenway OA, et al (2014) Computing clinically relevant binding free energies of HIV-1 protease inhibitors. J Chem Theory Comput 10:1228–1241. https://doi.org/10.1021/ct4007037

53. Fang WS, Zhang J (2009) Current Topics in Medicinal Chemistry: Editorial. Curr Top

Med Chem 9:1597. https://doi.org/10.2174/156802609789941942

54. Marvin 17.21.0, ChemAxon (https://www.chemaxon.com)

55. Vijesh AM, Isloor AM, Telkar S, et al (2013) Molecular docking studies of some new imidazole derivatives for antimicrobial properties. Arab J Chem 6:197–204. https://doi.org/10.1016/j.arabjc.2011.10.007

56. Cross JB, Thompson DC, Rai BK, et al (2009) Comparison of several molecular docking programs: Pose prediction and virtual screening accuracy. J Chem Inf Model 49:1455–1474. https://doi.org/10.1021/ci900056c

57. Ewing TJA, Makino S, Skillman AG, Kuntz ID (2001) DOCK 4.0: Search strategies for automated molecular docking of flexible molecule databases. J Comput Aided Mol Des 15:411–428. https://doi.org/10.1023/A:1011115820450

58. Morris GM, Goodsell DS, Huey R, Olson AJ (1996) Distributed automated docking of flexible ligands to proteins: Parallel applications of AutoDock 2.4. J Comput Aided Mol Des 10:293–304. https://doi.org/10.1007/BF00124499

59. Verdonk ML, Cole JC, Hartshorn MJ, et al (2003) Giftgas over Byen. Civilbefolkningens Beskyttelse Under Den Næste krig. Proteins 52:609–623. https://doi.org/10.1002/prot.10465

60. Warren GL, Andrews CW, Capelli AM, et al (2006) A critical assessment of docking programs and scoring functions. J Med Chem 49:5912–5931. https://doi.org/10.1021/jm050362n

61. Friesner RA, Banks JL, Murphy RB, et al (2004) Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. J Med Chem 47:1739–1749. https://doi.org/10.1021/jm0306430

62. Joseph-McCarthy D, Alvarez JC (2003) Automated generation of MCSS-derived pharmacophoric DOCK site points for searching multiconformation databases.

Proteins Struct Funct Genet 51:189–202. https://doi.org/10.1002/prot.10296

63. Jain AN (2003) Surflex: Fully automatic flexible molecular docking using a molecular similarity-based search engine. J Med Chem 46:499–511. https://doi.org/10.1021/jm020406h

64. Scheraga HA, Khalili M, Liwo A (2007) Protein-Folding Dynamics: Overview of Molecular Simulation Techniques. Annu Rev Phys Chem 58:57–83. https://doi.org/10.1146/annurev.physchem.58.032806.104614

65. Vajda S, Kozakov D (2009) Convergence and combination of methods in protein-protein docking. Curr Opin Struct Biol 19:164–170. https://doi.org/10.1016/j.sbi.2009.02.008

66. O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry 31 (2010) 455-461*

CHAPTER FOUR

Exploring the crucial role of Asp1116 in selective Drug targeting of CREB-cAMP-responsive element-binding protein Implicated in Prostate Cancer

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4.0 Abstract

The selective targeting of CREB-cAMP-responsive element-binding protein (CBP) has recently evolved as a vital therapeutic approach for curtailing its aberrant upregulation associated with the development of prostate cancer. Inhibition of CBP has therefore been discovered to be an important therapeutic option in androgen receptor signalling pathway mediated prostate cancer. Y08197, a novel selective inhibitor of CBP has shown promising therapeutic outcome in prostate carcinogenesis over non-selective analogues, CPI-637. Herein, we used molecular dynamics simulation to gain insights into the mechanistic and selective targeting of Y08197 at the bromodomain active site. Molecular Mechanics/ Poisson-Boltzmann Surface Area (MM/PBSA) analysis revealed similar inhibitory effect between Y08197 and CPI-637. Furthermore, in exploring the selective affinity of Y08197 towards CBP in relative to Bromodomain and PHD finger-containing protein 1(BRPF1), our findings highlighted Asp1116 as the 'culprit' residue responsible for this selective targeting. Upon binding, Asp1116 assumed a conformation that altered the architecture of the bromodomain active site, thereby orienting the helices around the active site in a more compacted position. Interestingly, in addition to some specific structural perturbations mediated by Asp1116 on the dynamics of CBP, our study revealed that the strong hydrogen bond interaction (N-H···O) elicited in CBP-Y08197 sequestered Y08197 tightly into the CBP bromodomain active site. Conclusively, the inhibition and selective pattern of Y08197 can be replicated in future structure-based CBP inhibitors and other bromodomain implicated in carcinogenesis.

Keywords: CBP; BRPF1; Prostate Cancer, Y08197, Molecular Dynamic Simulation

4.1 Introduction

Prostate cancer is regarded as the second most frequent and the fifth death leading cause of cancer in males worldwide today [1]. In 2018, the International Agency for Research on Cancer estimated that almost 1.3million new cases of prostate cancer and 359 000 associated deaths will occur worldwide [1]. Researches into the carcinogenesis of prostate cancer have implicated aberration in some proteins such as androgen receptor (AR) [2]. Androgen-deprivation therapy has been has shown high therapeutic outcome, however, clinical progression after 2 to 3 years suggested an unregulated signalling of mutations or an alternatively sliced AR that is no longer dependent on androgen binding to effect its activation [3]. This is called Castration Resistant Prostate Cancer (CRPC) or Androgen Independent Prostate Cancer (AIPC) or Hormone Refractory Prostate Cancer (HRPC) [4]. Therefore, recent research is aimed towards the development of drug therapies that target CRPC. Due to the role AR play in the prostate carcinogenesis, different drugs have been developed as target against the AR [5], such as abiraterone [6] and the second-generation antagonists, enzalutamide [3], bicalutamide [7] and apalutamide [8] . Despite the successes recorded by these drugs, drug resistance still emanates, this necessitated continuous research into developing and design of inhibitors that can circumvent this (Rachel A Davey and Mathis Grossmann, 2016). Strategies designed in combating prostate cancer involved targeting several pathways such as androgen synthesis, androgen receptor splice variants, androgen receptor coactivators, PI3K-AKT pathway, WNT pathway, DNA repair and so on (Wank K. et al). One of such proteins that have gained attention in the last few years and has been regarded as a promising target is the CREB (cAMP-response element binding protein)- binding protein (CBP).

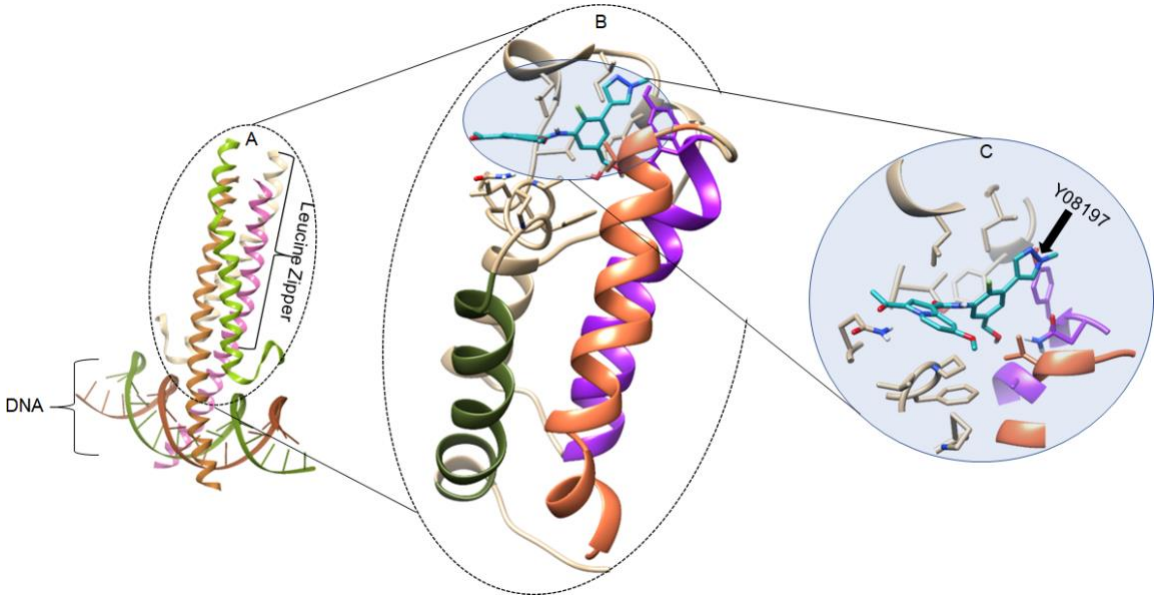

Figure 4.1: 3D crystallography structure of CBP in complex with a B-DNA (A) and Y08197 (B). Y08197 occupying the active site of CBP (prepared by author)

CREB-BCBP) is a bromodomain-containing protein and serves as co-activator in transcription during androgen signalling pathway. In 2004, Barbara Comuzzi, reported from an experiment conducted that not only was CBP up regulated despite the withdrawal of androgen but clearly stated it should be further investigated for therapeutic drug target for CRPC patients [7]. Hence, Ling-jiao Zou recently published that targeting the bromodomain of CBP, with a selective inhibitor, Y08197, a novel 1-(indolizin-3-yl) ethanone derivative, inhibited the CBP bromodomain with an IC50 value at 100.67 ± 3.30 nM [9]. Although, the experiment was carried out on different bromodomain, Y08197 seem to selectively inhibit CBP amidst others [9]. As such, in this in silico studies, we aim to burrow deep to view the simulation of this reaction as well as observe the ligand specificity of the compounds test against CBP and BRPF1, another bromodomain but of lowest affinity to Y08197.

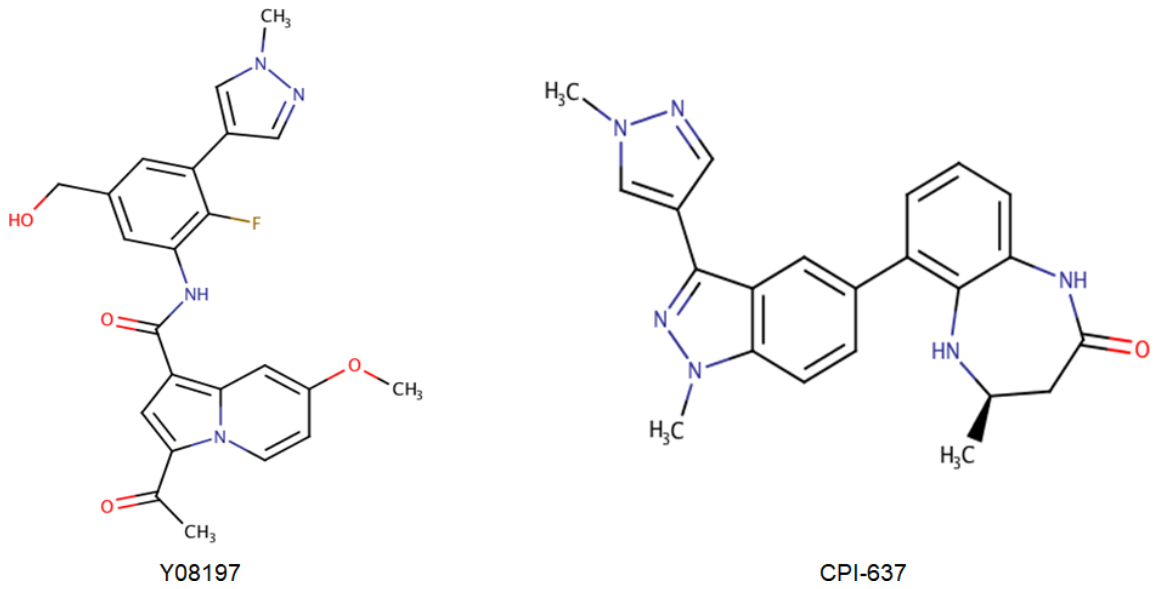

Figure 4.2: 2D chemical structures of Y08197 and CPI-637

4.2 Materials and Methods

4.2.1 Starting structures preparation and MD Simulation

The starting structure of CBP and BRPF1 was obtained from Protein Data Bank. CBP with PDB ID (6FR0) [10] and BRPF1 with PDB ID (5MWH) [11] were retrieved. Molecules that were co-crystallized with the protein were deleted and missing residues were added with the aid of modeller [12]. B3LYP/6-311++G(d,p) [13] level of Gaussian16 [14] was employed to carry out ligand optimization. Afterwards, molecular docking was done using the optimised structures with the aid of UCSF Chimera [15]. FF14SB module [16] [17] of the AMBER forcefield was employed in carrying out MD simulation. The Generalized amber Force Field (GAFF) and Restrained Electrostatic Potential (RESP) were used in describing the atomic charges of the ligands. Leap variant present in Amber 14 was used for system neutralization and hydrogen atoms addition [17]. The system was kept solvated with an orthorhombic box of TIP3P water molecules surrounding all protein atoms at a distance of 9Å [13]. System minimization was carried out firstly with a 2000 steps minimization using a restraint potential of 500kcal/mol. Secondly, we used a 1000 steps full minimization process without restrain, afterwards, the system was gradually

heated at a temperature of 0k to 300k at 50ps for simulation time. The system solutes are kept at a potential harmonic restraint of 10 kcal mol- 1Å −2 and collision frequency of 1.0 ps-1. Equilibration succeeded heating at an estimate of 500 ps of each system. Temperature at 300k, number of atoms and pressure at 1bar (isobaric-isothermal ensemble, NPT using Berendsen barostat) were all kept constant. The simulation time was set at 200 ns with each SHAKE algorithm to narrow the hydrogen atom bonds. Each step of the simulation was run for 2fs and an SPFP precision model was adopted. The simulations were kept at constant temperature and pressure (NPT), and Langevin thermostat at collision frequency of 1.ops-2. PTRAJ variant of Amber14 was adopted for further analysis which included root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF) and Radius of Gyration [18]. The data plots were then made with ORIGIN analytical tool and visualization done using UCSF Chimera [19].

4.2.2 *Binding free energy estimation*

The Molecular Mechanics/ Poisson-Boltzmann Surface Area (MM/PBSA) was employed in the estimation of differential binding of CPI and Y08197 [20]. MM/PBSA is an end-point energy estimation used in the prediction of binding affinities of ligands and their corresponding protein target. MM/PBSA is mathematically described as:

$$\Delta G_{bind} = G_{complex} - (G_{receptor} + G_{inhibitor}) \quad (1)$$

$$\Delta G_{bind} = \Delta G_{gas} + \Delta G_{sol} - T\Delta S \quad (2)$$

$$\Delta G_{gas} = \Delta E_{int} + \Delta E_{ele} + \Delta E_{vdW} \quad (3)$$

$$\Delta G_{sol} = \Delta G_{ele,sol(GB)} - \Delta G_{np,sol} \quad (4)$$

$$\Delta G_{np,sol} = \gamma SASA + \beta \quad (5)$$

ΔG_{gas} represents the total gas phase energy calculated by intermolecular energy (ΔE_{int}), electrostatic energy (ΔE_{elel}) and van der Waals energy (ΔE_{vdW}). ΔG_{sol} represent the solvation energy, $T\Delta S$ represent entropy change. $\Delta G_{ele,sol(PB)}$ describes polar desolvation energy while $\Delta G_{np,sol}$ describes the non-polar desolvation energy. γ is the surface tension proportionality constant and is set to 0.0072 kcal/(mol-1. Å-2), β is a constant equal to 0 and SASA is the solvent accessible surface area (Å2).

4.2.3 Energy Decomposition

To explore the energy contribution of each residue in the active site to the with apalutamide, binding free energy decomposition was done. MM/PBSA methodology was explored in the per-residue free energy decomposition.

4.3 Results and Discussion

4.3.1. CBP and BRPFI perturbatory effect upon CPI-637 and Y08197 binding

To understand the structural perturbation of CBP and BRPF1 upon CPI and Y08197 binding, we used Root mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Radius of Gyration (RoG) to characterize the structural events in the proteins in the course of the simulation. In the course of the 200 ns simulation run, RMSD values of Cα atoms of CPI-CBP, Y08197-CBP and Y08197-BRPF1 were estimated in relative to the starting structure. As illustrated in Fig. 3A, all the systems, attained structural stability after 20 ns. The three systems had similar motional movements with an average RMSD value of 0.90Å, 1.15Å and 1.05Å respectively. Furthermore, we also explore the conformation of the active site, this is to have an insight into the effect of the ligands in relative to the RMSD of the whole system. From Fig 3B, the active site is observed to be well stable, this provided a suitable environment for the ligand to interact with the residues making up the active site. Furthermore, we investigated the stability of the ligand, although both ligands had similar motional movement and stability, CPI was observed to have higher stability when compared to Y08197.

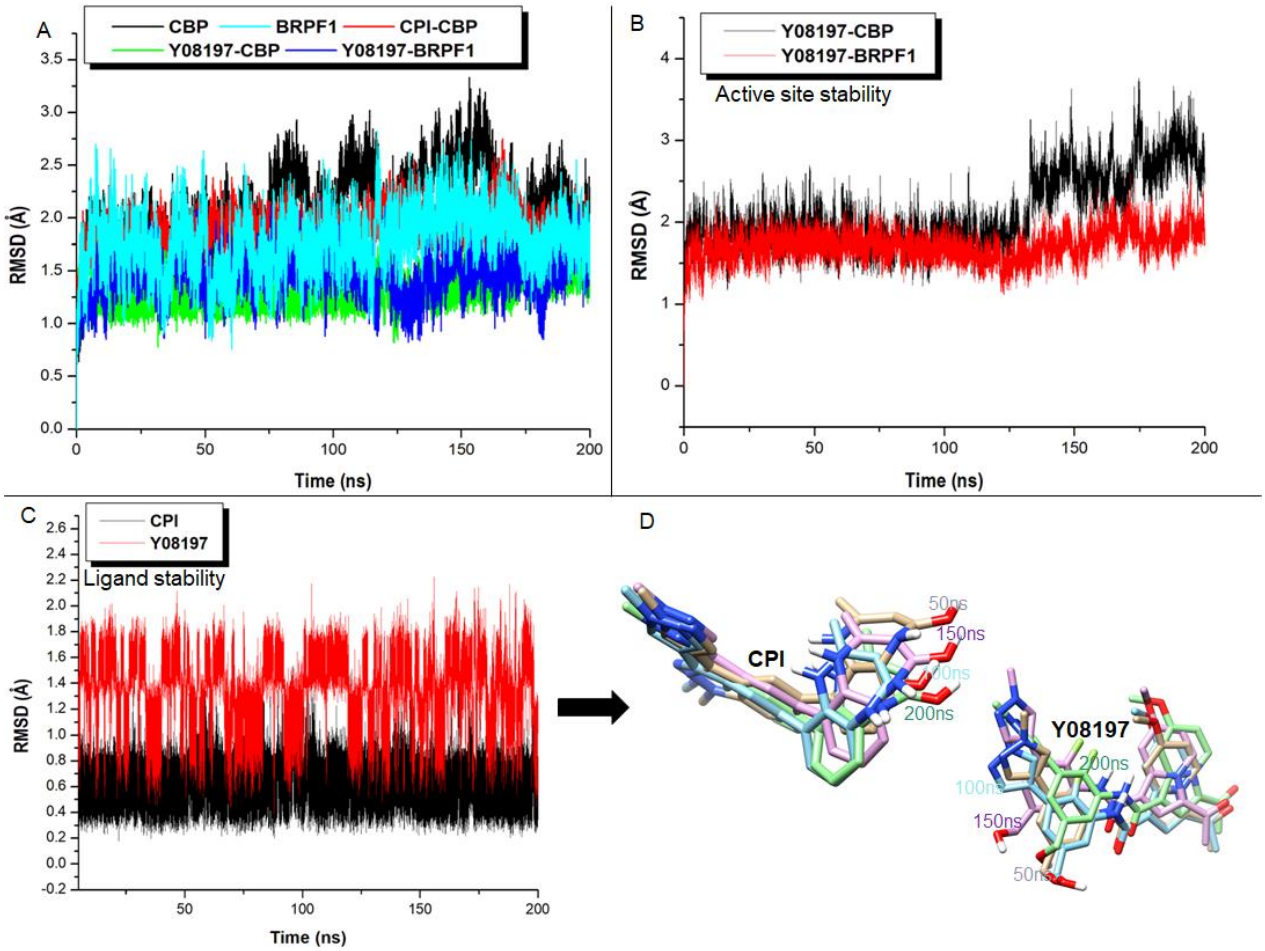

Figure 4.3: Conformational analysis plot showing stability and atomistic motions among CBP (**black**), BRPF1 (**cornflower**), CPI-CBP (**red**), Y08197-CBP (**green**) and Y08197-BRPF1(**red**) systems **[A].** C-α RMSD plot showing the active site stability and atomistic motions of Y08197-CBP (**black**) and Y08197-BRPF1(**red**) **[B].** Stability and atomistic motion plot of the ligands CPI (**black**) and Y08197 (**red**) **[C].** Snapshot of the superimposed ligands at 50ns, 100ns, 150ns and 200ns **[D].**

Table 4.1: The finally equilibrated values of RMSD (FE-RMSD) for each system.

	Average RMSD (Å)				
Regions	**CBP**	**BRPF1**	**CPI-CBP**	**Y08197-CBP**	**Y08197-BRPF1**
Protein	0.87	0.86	0.90	1.15	1.05
Active site	0.84	0.80	0.62	0.57	0.99

4.3.2. Hydrogen Bond Analysis

The strength of protein-ligand binding is greatly influenced by hydrogen bonds contributed by residues, especially those found in the active site [21]. We therefore, estimated the hydrogen bonds of the equilibrated trajectories of the systems and computed direct hydrogen bonds between **CPI**, **Y08197** and the proteins. At the final simulation step (200ns) it was observed that O-H···O in the CPI-CBP system contributed by ASN87 had occupancy of 34. The CBP-**Y08197** had two hydrogen bond interactions contributed by ASN87 and ASP35. The N-H···O of ASN87 had a higher occupancy when compared to N-H···O of ASP35. Furthermore, BRPF1-**Y08197** had an extra H-bond provided by ASN81; this bond had the highest occupancy among the H-bond interactions found among the systems. This extra H-bond could provide an insight into the selective binding of Y08197 to CBP.

Table 4.2: Direct Hydrogen bond between CBP-CPI, CBP-Y08197 and BRPF1-Y08197

Complexes	direct hydrogen bonds						
	Donor			acceptor		distance (Å)	Occupancy (%)
CBP-**CPI**	**CPI**	HE	NE	ARG92	N5	2.91	12.80
	CPI	HE	NE	ARG92	N5	2.91	12.80
	CPI	H3	N2	ASN87	OD1	2.88	34.00
	ASN87	HD21	ND2	**CPI**	O1	2.90	12.30
	CPI	H20	O1	ASN87	OD1	2.78	13.00
CBP-**Y08197**	ASN87	HD21	ND2	**Y08197**	O2	2.89	30.79
	ASP35	H	N	**Y08197**	O1	2.89	10.9
BRPF1-**Y08197**	Y08197	H2	O2	**ASN24**	OD1	2.76	10.03
	ASN81	HD21	ND2	**Y08197**	O4	2.85	63.00

4.3.3. Analysis of Binding Free Energy

MM/PBSA has found useful application in the drug design space used in the estimation of binding affinity between ligands and biomolecules. MM/PBSA was therefore used in the estimation of the total binding free energy (ΔG_{bind}) and other energy components between Y08197/CPI and CBP/BRPF1. The estimated ΔG_{bind} increases from Y08197-BRPF1 > Y08197-CBP > CPI-CBP. The same increasing trend was observed when the binding affinity after MD simulation was compared to the docking score. However, of note is that, there is no much difference in the binding affinity between CPI-CBP (-32.06Kcal/mol) and Y08197-CBP (-32.00Kcal/mol). This result agrees with the work of Zou et al., which suggested that the inhibitory effect CPI and Y08197 is similar. From the computed result, it was observed that van der Waals and electrostatic interactions promoted ligand-protein interactions, binding was disfavoured by polar solvation ($\Delta G_{ele,sol(GB)}$). The nonpolar solvation ($\Delta G_{np,sol}$) was also seen

to favour the binding.

Table 4.3: Calculated binding Free-Energy (in kcal/mol) of the Studied Complexes.

Complexes	Energy						
	ΔE_{vdW}	ΔE_{ele}	ΔG_{gas}	$\Delta G_{ele,sol(G}$	$\Delta G_{nonpol,so}$	ΔG_{sol}	ΔG_{bind}
Y08197-BRPF1	-36.40 (±0.06)	-18.04 (±0.11)	-54.44 (±0.14)	26.84 (±0.08)	-4.28 (±0.01)	26.84 (±0.08)	-27.60 (±0.07)
Y08197-CBP	-41.92 (±0.05)	-4.50 (±0.09)	-46.42 (±0.10)	19.70 (±0.080)	-5.30 (±0.01)	14.43 (±0.08)	-32.00 (±0.05)
CPI-637-CBP	-39.53 (±0.06)	-13.23 (±0.11)	-52.77 (±0.12)	25.87 (±0.09)	-5.16 (±0.01)	20.71 (±0.09)	-32.06 (±0.05)

In order to explore the contribution of each residue present at the active site to the protein-ligand interactions, the energy decomposition of the residues was computed as depicted in fig 4. Leu1109, Pro1110, Leu1120, Arg1173 and Val1174 were found to be the interacting residues between CBP and CPI. Leu1109, Phe1110, Leu1120, Ile1122, Asn1168, Val1174 were found to be the interacting residues between CBP and Y08197.

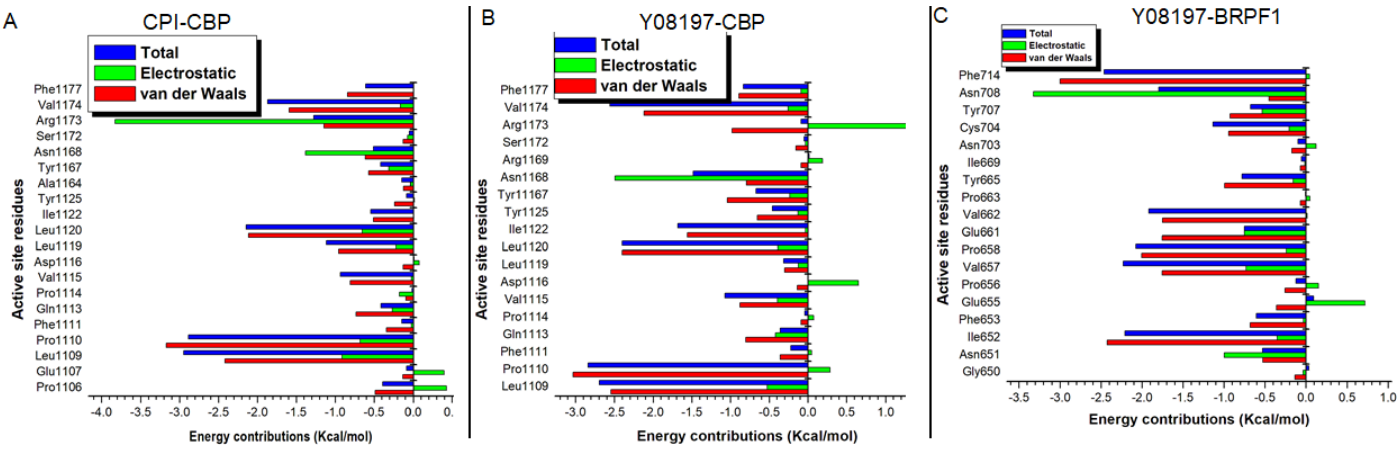

Figure 4.4: Individual energy contributions of crucial site residues of CBP and BRPF1. Per-residue decomposition plot showing energy contributions of interactive active site residues of CPI-CBP (**A**), Y08197-CBP (**B**) and Y08197-BRPF1 (**C**).

Though ASP1116 did not contribute high van der Waals and electrostatic interaction to the binding between CBP and Y08197, however, as discussed above, it has a high hydrogen bond contribution to this binding. While, Val657, Pro658, Val662, Phe714 were the interacting residues with energy contribution more than -1Kcal/mol responsible for the binding between BRPF1 and Y08197. Zou et al., have found out that Y08197 selectively target CBP as against other bromodomain containing proteins [9] In order to have insight into the mechanism of this selective targeting, we selective targeting of Y08197 on CBP, we explore the ligand interaction between Y08197 and the proteins (CBP and BRPF1). Although the active sites of CBP and BRPF1 have some common residues. However, the extra electrostatic and van der Waals energy contributions provided by Val115, Asn1168, Pro1110 added to this selectivity. Most importantly Asp1116; as discussed in the hydrogen bond analysis, the hydrogen bond between N-H···O in Asp1116 is peculiar only to CBP (Fig 5).

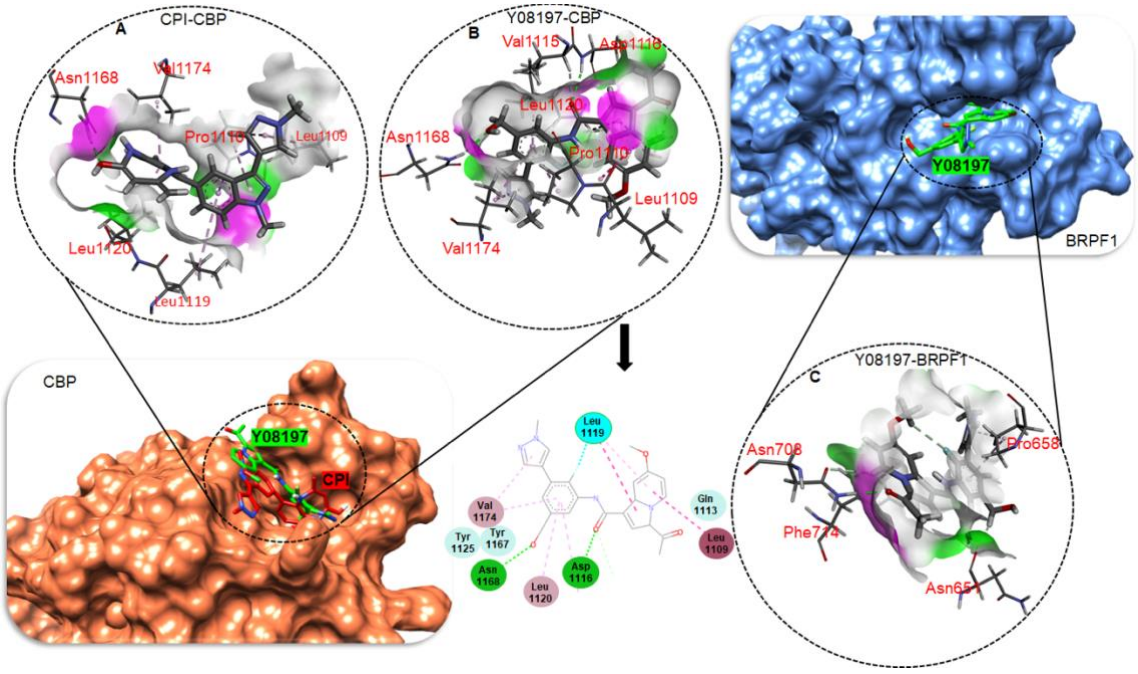

Figure 4.5: 3D structure of the ligand interaction between CPI and CBP (**A**) and YO1897-CBP (**B**) highlighting the molecular interactions of key residues and reactive moieties.

Conclusion

The mortality rate of prostate cancer has become a major concern globally. Different therapeutic strategies have been targeted towards some proteins that are implicated in cancer. One of such proteins is the CREB-cAMP- responsive element-binding protein (CBP), which have gained attention in the drug design space. Y08197, a novel inhibitor that has recently been reported to selectively target CBP. We explored this selective targeting and discovered that the Asp1116 is an important residue that facilitates this targeting. This is evidenced by the array of results that pointed to Asp1116 as the "chief culprit". Asp1116, can therefore be explored in designing CBP inhibitors that possess more potency and perhaps less toxicity.

Conflict of interest

The authors declare none.

Acknowledgement

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REFERENCE

(1) Bray, F.; Ferlay, J.; Soerjomataram, I. Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. **2018**, 394–424. https://doi.org/10.3322/caac.21492.

(2) Kaarbø, M.; Klokk, T. I.; Saatcioglu, F. Androgen Signaling and Its Interactions with Other Signaling Pathways in Prostate Cancer. **2007**, 1227–1238. https://doi.org/10.1002/bies.20676.

(3) Penticuff, J. C.; Kyprianou, N. Pathophysiology of Castration-Resistant Prostate Cancer. **2016**, 5–23. https://doi.org/10.1007/978-3-319-31341-2.

(4) Bellmunt, J.; Oh, W. K. Castration-Resistant Prostate Cancer : New Science and Therapeutic Prospects. **2010**, 189–207. https://doi.org/10.1177/1758834009359769.

(5) Racca, F.; Programme, S. RESISTANT PROSTATE CANCER TREATMENT (MCRPC).

(6) Yuan, X.; Balk, S. P. Mechanisms Mediating Androgen Receptor Reactivation after Castration. *Urologic Oncology: Seminars and Original Investigations* **2009**, *27* (1), 36–41. https://doi.org/10.1016/j.urolonc.2008.03.021.

(7) Comuzzi, B.; Nemes, C.; Schmidt, S.; Jasarevic, Z.; Lodde, M.; Pycha, A.; Bartsch, G.; Offner, F.; Culig, Z.; Hobisch, A. The Androgen Receptor Co-Activator CBP Is up-Regulated Following Androgen Withdrawal and Is Highly Expressed in Advanced Prostate Cancer. *Journal of Pathology* **2004**, *204* (2), 159–166. https://doi.org/10.1002/path.1609.

(8) Rathkopf, D. E.; Scher, H. I. Apalutamide for the Treatment of Prostate Cancer. *Expert Review of Anticancer Therapy* **2018**, *18* (9), 823–836. https://doi.org/10.1080/14737140.2018.1503954.

(9) Zou, L. jiao; Xiang, Q. ping; Xue, X. qian; Zhang, C.; Li, C. chang; Wang, C.; Li, Q.;

Wang, R.; Wu, S.; Zhou, Y. lai; et al. Y08197 Is a Novel and Selective CBP/EP300 Bromodomain Inhibitor for the Treatment of Prostate Cancer. *Acta Pharmacologica Sinica* **2019**, No. December 2018, 1–12. https://doi.org/10.1038/s41401-019-0237-5.

(10) Pawe, S.; Nevado, C.; Ca, A. Binding Motifs in the CBP Bromodomain: An Analysis of 20 Crystal Structures of Complexes with Small Molecules. **2018**, 8–13. https://doi.org/10.1021/acsmedchemlett.8b00286.

(11) Zhu, J.; Zhou, C.; Caflisch, A. AC SC. *European Journal of Medicinal Chemistry* **2018**. https://doi.org/10.1016/j.ejmech.2018.05.037.

(12) Eswar, N.; Webb, B.; Marti-Renom, M. A.; Madhusudhan, M. S.; Eramian, D.; Shen, M.-Y.; Pieper, U.; Sali, A. Comparative Protein Structure Modeling Using MODELLER. *Current protocols in protein science / editorial board, John E. Coligan ... [et al.]* **2007**, *Chapter 2* (November), Unit 2.9. https://doi.org/10.1002/0471140864.ps0209s50.

(13) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *The Joufile:///C:/Users/HP/Desktop/CTLF/Calculating Structures and Free.pdfrnal of Chemical Physics* **1983**, *79* (2), 926–935. https://doi.org/10.1063/1.445869.

(14) Weedbrook, C.; Pirandola, S.; Cerf, N. J.; Ralph, T. C. Gaussian Quantum Information.

(15) Yang, Z.; Lasker, K.; Schneidman-Duhovny, D.; Webb, B.; Huang, C. C.; Pettersen, E. F.; Goddard, T. D.; Meng, E. C.; Sali, A.; Ferrin, T. E. UCSF Chimera, MODELLER, and IMP: An Integrated Modeling System. *Journal of Structural Biology* **2012**, *179* (3), 269–278. https://doi.org/10.1016/j.jsb.2011.09.006.

(16) David A. Case. AmberTools12 Reference Manual. *Russell The Journal Of The Bertrand Russell Archives* **2012**, 535.

(17) Salomon-ferrer, R.; Case, D. A.; Walker, R. C. An Overview of the Amber Biomolecular Simulation Package. **2012**, *00* (February), 1–13. https://doi.org/10.1002/wcms.1121.

(18) Roe, D. R.; Cheatham III, T. E. PTRAJ and CPPTRAJ: Software for Processing and Analysis of Molecular Synamics Trajectory Data. *J Chem Theory Com* **2013**, *9* (7), 3084–3095. https://doi.org/10.1021/ct400341p.

(19) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera - A Visualization System for Exploratory Research and Analysis. *Journal of Computational Chemistry* **2004**, *25* (13), 1605–1612. https://doi.org/10.1002/jcc.20084.

(20) Kollman, P. A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; et al. Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. *Accounts of Chemical Research* **2000**, *33* (12), 889–897. https://doi.org/10.1021/ar000033j.

(21) Zhao, H.; Tang, S.; Xu, X.; Du, L. Hydrogen Bonding Interaction between Atmospheric Gaseous Amides and Methanol. **2017**, 1–16. https://doi.org/10.3390/ijms18010004.

CHAPTER FIVE

Update and Potential Opportunities in CBP [cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-binding protein] Research using Computational Techniques

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5.0 ABSTRACT

CBP [cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-binding protein] is one of the most researched proteins for its therapeutic function. Several studies have identified its vast functions and interactions with other transcription factors to initiate cellular signals of survival. In cancer and other diseases such as Alzheimer's, Rubinstein-taybi syndrome, and inflammatory diseases, CBP has been implicated and hence an attractive target in drug design and development. In this review, we explore the various computational techniques that have been used in CBP research, furthermore we identified computational gaps that could be explored to facilitate the development of highly therapeutic CBP inhibitors.

Keywords: CREB, Molecular Dynamic Simulation, CREB Inhibitors, Bromodomains

5.1 INTRODUCTION

The CREB (cyclic adenosine monophosphate (cAMP) response element-binding protein) Binding Protein (CBP), is a protein encoded by the CREBBP gene. CBP is a bromodomain-containing protein which emphasises its functionality in identifying acetylated lysine in histone proteins while also acting as effectors in signal associated with acetylation [1]. This class of protein has been reported to play a significant role in many biological and physiological processes, including transcription, differentiation, and apoptosis, whose activity is regulated by phosphorylation [1]. It's unique structure is made up of domains that catalyses transcription process initiated in cell growth, gene expression and differentiation as shown in figure 1.

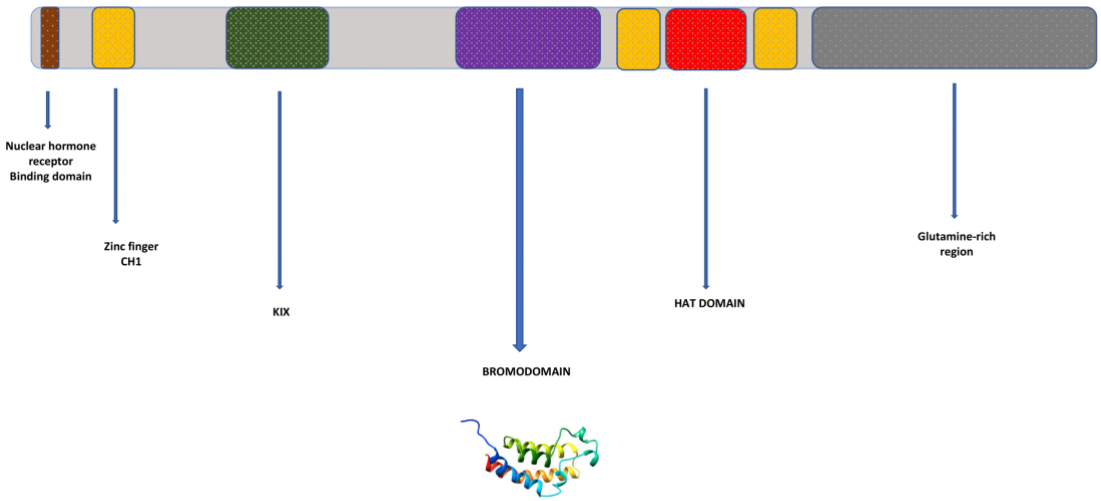

Figure 5.1: CBP and its interacting domains

The histone acetyltransferase (HATs) domain, also part of the CREB binding protein is necessary for protein-protein interactions, histone and non-histone alike such as NCOA3 and FOXO1. In 1993, p300, a Switch/Sucrose Non-Fermentable (SWI/SNF) complexes binding protein family was identified, sharing similarity with CBP, with its bromodomain, HATs domain and the cysteine-histidine region [2]. Although, CBP are coactivators of transcription, they do not interact with the promoter element. Instead, they are mobilized to promoters by protein-protein interaction [1] [3] [4]. The CREB binding protein has a binding domain called the KIX (kinase inducible domain) or the CREB binding domain [3]. This CREB (cAMP-response element-binding protein) unit within CBP controls the rate of transcription when phosphorylated at Ser-133 residues through protein kinase A which triggers the transcription activity of CBP [5]. The transactivation domain of CREB is bipartite, which consist of a glutamine-rich constructive activated site called Q2 and kinase-inducible domain (KID), and are directly in response to gene expression [6]. Despite the phosphorylation interaction between cAMP-dependent PKA and CREB, it is still unknown whether phosphorylation on the amino acid Ser-133 elicit CREB-CBP complexation. The mechanism of interaction is still not precise, either direct or allosteric [5].

5.2 BROMODOMAIN: WHAT ABOUT IT?

Wetlaufer defined protein domains as stable units of protein structure, possessing structural and evolutionary functions that fold autonomously [1]. Bromodomains (BRDs) are parts of a given protein sequence (approximately 110 amino acids) that recognizes lysine acetylation of N- terminal histones during gene transcription [1]. They are responsible for histone acetylation, chromatin remoulding, and transcription activation [7]. John Wetlaufer Tamkun first proposed the discovery of bromodomain-proteins while studying the drosophila gene Brahma [8]. PCAF,

histone acetyltransferase (HATs) KAT2B was the first 3-dimensional structure of BRD to be solved using NMR spectroscopy in 1999 [7]. Bromodomains are also called histone code readers [9] [10]. Of all the proteins in the human proteome, there are 61 BRDs, and based on their structure-function relationship, they are grouped into eight subfamilies [1]. These BRDs all have four α- helices linked by loops of different lengths (a, b, c and z) with which it interacts with acetylated lysine residues. These helices are coiled up in a left-handed α- helical fold. Between helix b and c and helix z and a, there are two loops forming a hydrophobic pocket [11]. The differences shown in the binding of bromodomains are due to the differences in sequence beyond the residues bound directly with acetyl-lysine binding [11–13] Although each protein is specific with its structure yet 48 of the more than 61 BRDs contain the asparagine residue at the acetyl-lysine binding site (KAc recognition position) while the remaining 13 have a tyrosine, threonine or an aspartate in the same position. The latter is called atypical BRDs [14]. There are eight subgroups of the BRDs classified in accordance to their amino acid sequence similarities as seen in figure 2 below (*Classification of the different classes of BET Proteins*).

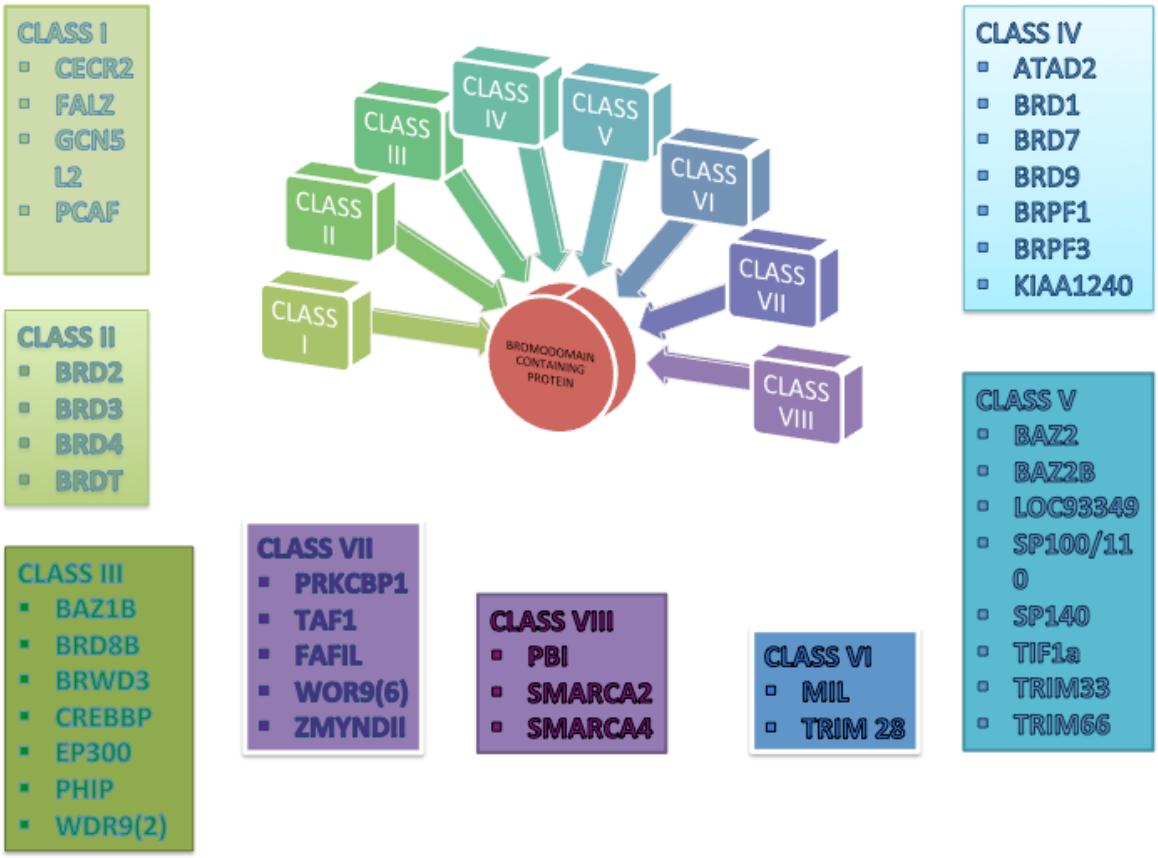

Figure 5.2: Classification of the different classes of BET Proteins (prepared by the author)

They are the BET family, histone acetyltransferases HATs (GCN5, PCAF), methyltransferases (MLL, ASH1L), ATP-dependent chromatin-remodelling complexes (BAZ1B), helicases (MARCA), nuclear-scaffolding proteins (PB1) and transcriptional coactivators (TRIM/TIF1, TAFs) transcriptional mediators (TAF1) [12]. Specific sub-groups have gained more attention compared to others; this is partly due to the development of inhibitors targeting BRDs. Of all the BRDs, the BET (bromodomain and extra-terminal family) BRDs (BRD2, BRD3, BRD4, and BRDT) are most researched and has over 206 PBD structures available today [12].

5.3 CREB- BINDING PROTEIN (CBP)

CBP is a nuclear protein of Mr 265K that bounds to phosphorylated cAMP-regulated transcription factor CREB, this fusion allows CBP to function as protein kinase A-regulated transcriptional activator [15] [16]. Both CBP and p300, its analogous, shares a few functional domains in common which constitute their similarities: (1) they are BRDs which are commonly found in human HATs; (2) they both have domains of the three cysteine-histidine namely CH1, CH2, and CH3; (3) they both have the KIX domain; and (4) an ADA2-homology domain [17]. On a quick database check on STRING, CBP is shown to interact with the following proteins as shown in the figure 3.

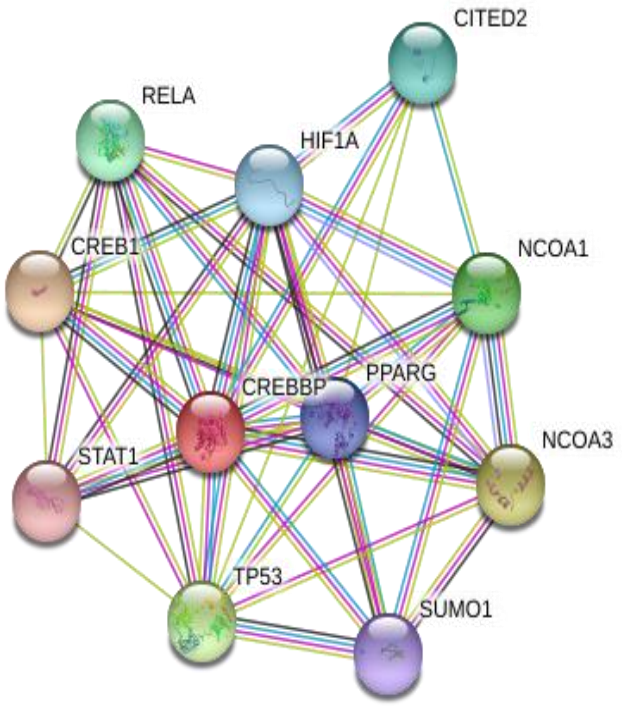

Fig 5.3: A database report from STRING showing the functional interactions of CREBBP with other proteins.

Such proteins include NCOA3, TP53, NCOA1, RELA, CITED2, HIIF1A, PPARG, SUMO1 and STAT1. Meanwhile, Intact database reports a more detailed interactions of 790 binary

proteins. Despite the broad structural similarities, Ho Man Chan and Nicholas Thangue attest to the unique characteristics of CBP and p300 [18]. Also, both CBP and p300 are phosphorylated at the different amino acid sites; CBP is phosphorylated at serine 436, an amino acid absent in p300 [19] which is absent in the latter In 1996, p300 and CBP were reported to function as histone acetyltransferases (HATs). CBP especially was discovered to possess intrinsic histone acetyltransferase activity even though it lacked conserved motifs found in regular acetyltransferases. With this property in view, it is only direct to suggest that it modulates cell cycle progression. It is demonstrated to acetylate nucleosomes associated with PCAF [20] [21]. CBP has been shown to play a vital role in gene expression. A study reported CBP as a HAT capable of acetylating nuclear factor-4 (HNF-4) of liver cells at lysine residues inside the nuclear localization sequence [22]. CBP continues to be of great interest in the development and design of drugs CBP plays an extensively role at the molecular level, such as, cellular growth, histone acetylation, and transcription of some factors amidst other unique functions. For example, CBP/p300 brings about the assembly of multi-protein complexes, which serves as molecular scaffolds [18]. CBP, along with other transcription factors, are known to regulate the overall process involved in the cell, including gene transcription [23]. It is essential to the point that in transforming viral proteins such as E1A from adenovirus, CBP is a prerequisite target [24]. Also, another review suggests that CBP/p300 proteins are targets for adenovirus E1A oncoprotein indicating its vital role in cell cycle regulation [4]. Observations by Ait-Si-Ali et al. reported that HAT is involved in the cell cycle by the phosphorylation of CBP by cyclin-E-CDK2 in the C- terminal region of the protein hence stimulating HAT activity [25]. Moreover, the results indicated that E1A activates the CBP HAT enzyme on the binding, which then results in a conformational change in its domain, leading to an increased catalytic activity. CBP interacts with viral oncoproteins such as p53 to cause loss of cell growth or growth suppression. p53 interacts with a carboxyl-terminal region of CBP

and activate genes involved in DNA damage and block cellular differentiation such as p21, murine double minute (MDM-2), BAX and cyclin G [26] [27].

5.4 CREB-BINDING PROTEIN (CBP) AND THE ONSET OF DISEASES

CBP's function in cancer was first identified in the translocation of chromosome t(12;22) q(13;12) . Studies have shown that CREB is involved in all stages of tumour development, in addition to its being a proto-oncogene. A statistic of patients with prostate cancer, lung cancer, acute leukaemia, and breast cancer showed overexpression and over activation of CBP [28]. Also, the inhibition of cell proliferation and induction of apoptosis was observed in the downregulation of CBP, which suggests that it as a prospective target for cancer therapy [29]. Although the involvement of CBP in cancer development is not explicit yet, CBP directly controls genes critical to cell progression, growth, and metastasis. CBP has also been identified in the development of embryos and cancer [20]. In Alzheimer's disease, CBP activator (CREB1), together with CBP, enhances memory formation and learning [30]. However, in certain circumstances, increase in CREB function can also alter cognitive performance. A publication by Wei Tang et al., aimed to search the function of CREB1 in the onset of Alzheimer’s diseases (AD) [30]. The result implicated CREB1 and CBP as the culprit in the pathophysiology of Alzheimer’s disease (AD), yet further research could be done on a much larger population to confirm these observations [30]. A research was conducted to analyse the function of CBP in inflammatory diseases. It turned out that few studies have been reported in line with rheumatoid arthritis (RA) synovial fibroblasts (SF). Results showed that the inhibition of CBP has an anti-inflammatory effect, while p300 showed both pro and anti-inflammatory functions [31].

5.5 VARIOUS ATTEMPT TO TARGET CBP

Recently, Hammitzsch et al., developed a CBP inhibitor (CBP 30) to block Th17 responses in human autoimmune diseases. Th17 has been proven to be very vital to various human autoimmune diseases. In the above research, the inhibitor blocked the bromodomain of the coactivator CBP, showing remarkable results [32]. Although the inhibitor was tested with about 43 bromodomain binding protein, excellent result that far exceeds even the known JQ1 (a BET inhibitor) was observed. In castration-resistant prostate cancer (CRPC), an advanced prostate cancer, CBP, and its homolog p300 are highly expressed. Given this, various therapy is aimed towards blocking the activity of CBP. In a recent study, YO8197, a selective inhibitor of CBP bromodomain was explored in terms of its antitumor activity against prostate cancer cell lines in vitro [33] of which further in silico studies by akinsiku et al., proved the mechanistic and selective targeting of Y08197 at the bromodomain site. Asp 116 was identified as the culprit responsible for the selective targeting [34]. Another CBP inhibitor, C646 has been investigated against neuroepithelial cell proliferation [35]. This study by Bai et al., further justified the abnormality in NE-4C cells of CBP in high glucose. With the administration of C646 to the diabetic induced mouse, the results indicated that the levels of acetylation were reduced. Conclusively, it was evident that C646 could effectively impede the increase of histone H4 acetylation and neuro-epithelial cell proliferation [35][36]. Statistics reports that 1% of pregnant women are affected by diabetes and might have congenital heart disorder and neural tube defects (NTDs) in the child born [37]. Figure 4 shows 2D- structure of CREB-BP inhibitors as discussed.

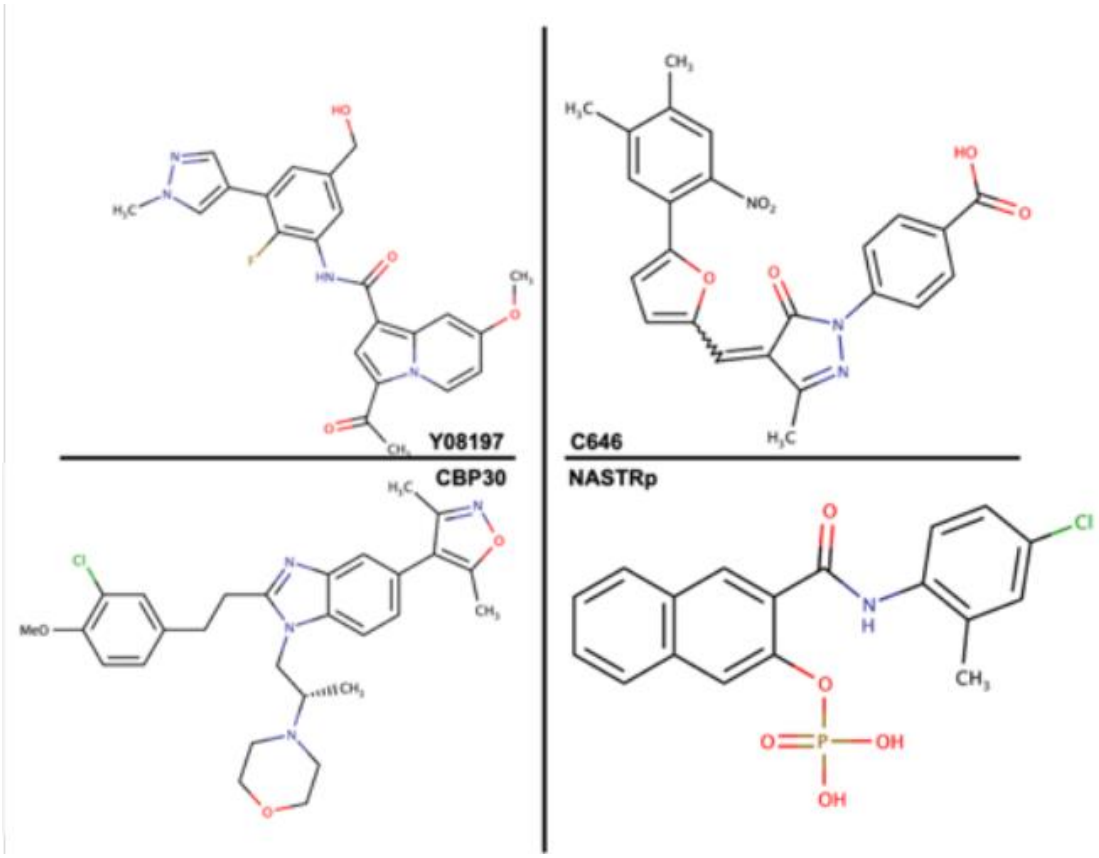

Figure 5.4: 2D Structures of CREB inhibitors (as prepared by the author)

Recent research proved that NASTRp is effective in inhibiting cancer cells via cell arrest [38]. Since mutant KRAS drives the activation of CAMP responsive element-binding (CREB), it is only appropriate to devise an inhibitor that can effectively do such through RAF/MEK/ERK signalling pathway inducing apoptosis in cancer cells [39]. Compound DC_CP20, a new CBP BRD inhibitor, discovered through a time-resolved fluorescence energy transfer (TR-FRET)-based high throughput screening of about 20 000 libraries of compounds [40]. An IC50 of 744.3nM was demonstrated when bound with the acetylated lysine of CBP BRD. Moreover, with the aid of molecular docking, the binding affinity was further juxtaposed, being bound tightly in the inner Kac-binding pocket competitively. The compound proves an inhibitory

property to human leukaemia MV4-11 cells at cellular levels. These promising results pose a further study in the development of drug therapies for CBP- related cancers [41]. Studies have shown the frequent occurrence of SPOP (speckle-type POZ protein), a mutated gene in primary prostate cancer (Pca) in about 10 to 15% range [38]. A study by Yuqian Yan et al., identified an unknown mutation called Q165P at the cliff of the SPOP math domain [42]. The effect of this mutation is that it halts the dimerization of SPOP, and consequently substrate degradation. Furthermore, unlike F133V, the former is highly sensitive to the known BET inhibitor, JQ1. In vivo and in vitro experiments carried out revealed a novel BET and CBP inhibitor, NEO2734, is effective against the JQ1-resistant SPOP hotspot mutant, which could proceed further to clinical trials for effective anti-cancer therapy against SPOP-mutated PCa patients [42].

5.6 COMPUTER-AIDED TECHNIQUES IN STUDIES OF CREB-BINDING PROTEIN

Over the years, traditional strategies used in drug development and design pipeline have been complemented with computational software and methods. These tools include; pharmacophore modelling, molecular docking, virtual screening, molecular dynamics (MD) simulation, Quantitative Structure-Activity Relationship (QSAR), and homology modelling. Computer-aided drug design techniques have been effective over the years in finding new drugs from genomic and proteomic initiatives. These new techniques have effectively reduced cost and increased drug discovery. Molecular docking have been adopted over the years and involve ligand-receptor orientation to find the best conformation of fitness that would trigger a biological response. Some popular docking programs are FlexX [43], GOLD [44], AutoDock [45], GLIDE [46], DOCK [47] [48], HEX SERVER [49], Surflex [50], Patchdock [51] among others.

The importance of molecular dynamics (MD) simulation cannot be overemphasized, especially with its coherent contribution to the interplay between computational and experimental techniques. These step-by-step techniques effectively reveal the dynamic behaviour of the proteins at timescales intervals, the stability of the protein structure, and the ligand's binding interactions. Other properties such as conductivity, dipolar moment, density, thermodynamic parameters, entropies, amidst others. are observed [52–55]. MD simulation programs include CHARMM [56], NAMD [57], GROMACS [58], AMBER [59], among others. We searched some published papers with an emphasis on the computational methods that have been adopted in CREB research. A paper by Woo Lee published in 2015 reports the anti-cancer properties of Naphthol AS-TR phosphate (NASTRp), a novel CREB-CBP Complex inhibitor with many functions. Among all compounds, NASTRp showed the best effect, especially in biological assays. In this research, computational tools were employed in conducting a database search of compounds with possible chemical properties. Using the DBslnfilter, compounds were screened under properties such as no 3D coordinates, mixtures, isotopes, Molecular Weight <100, or Molecular Weight >500, metals. In this structural database are approximately 600,000 compounds that also contain about 50 chemical databases [60]. These compounds are usually downloaded in the SDF file format [61], followed by a database search command investigation on each compound to identify any two-dimensional similarity. Compounds were screened using PubChem, after which a four-processor MIPS R16000 Silicon Graphics Tezro was used to conduct modelling calculations. The results were then combined into 3-D SLNs. All Compounds not containing carboxylates, phosphates, and sulfonamides were eliminated using the hit list manager. The PDB ID: IKDX represents the KIX domain coordinates. This result from taking the average of the NMR structures with the phoenix Elbow [62] the resultant produces the KIX and NASTRp coordinates. The docking calculations were obtained using HEX 6.3 [63]. The result indicated that out of the calculations of the top ten docking scores,

NASTRp was shown to have the best binding score. Although molecular simulation wasn't carried out to accompany the experiment yet, the results indicate NASTRp as a potential anti-cancer drug. Researchers over the years have shown great interest in investigating CBP as a potential drug target, as shown in some few works demonstrated in advanced MD simulations.

Table 5.1: A table showing the various drugs experimentally designed to target CBP for different diseases with necessary details.

S/N	DRUGS	EXPERIMENTS	DISESAES TARGETED	RESULTS	REF
01	CBP 30	In vitro	Human autoimmune diseases	Inhibited IL-17A secretion via Th cells from healthy donors	[32]
02	Y08197	In vitro	Castration Resistance Prostrate Cancer	Affected the downstream signalling transduction, inhibiting expression of AR-related genes	[33]
03	C646	In vitro In vivo	Neuroepithelial Cell Proliferation	Rescued increased H4k5/k8/k12/k16 acetylation levels	[35]
04	NASTRp (Naphthol AS-TR phosphate)	In vitro	Lung adenocarcinoma	Inhibited oncogenic cells via cell cycle arrest and also initiated downregulations of Atg5-12 and Atg7	[39]
05	Compound DC_ CP20	In silico	Human Leukaemia	Inhibited the proliferation of human leukaemia MV4-11 cells and downregulated the	[40]

				expression of c-Myc in the cells	
06	NEO2734	In vitro In vivo	Prostate Cancer	Inhibition of cell growth with a significant effect compared to a combination of JQ1 and CPI-637	[42]
07	Nicur	In silico	Gastrointestinal Epithelial cells	Blocked CBP HAT activity and down regulates p53 activation upon cellular responses	[65]

Md simulation was conducted to decipher the mechanism of the selective inhibitor CBP30 against its target CBP/p300 bromodomain. It was discovered that the specific residue for CBP, Arg1173/1137, was accountable for the selective binding to CBP30 through hydrogen bond interactions and cation–π. In order to prove the result, four (4) system was set up; the apo-CBP, CBP-CBP30 complex, apo- p300, p300-CBP30 complex. Observing the interactions, CBP30 ring B formed a contact collision with the Arg1173 side-chain of Apo-CBP, meanwhile forming a favourable cation–π between the holo-CBP. For as long as 93% simulation time, the cation–π interaction was preserved. CBP, both contact and cation–π interaction reflected in apo-p300 and CBP 30, yet another H-bond is seen between CBP30 O3 and Arg1137 NH1 atoms of holo-p300. With these results, a greater understanding is known of the mechanism of CBP30 against BET and non-BET bromodomains [64]. Vincek et al., 2018, identified a CBP inhibitor, NiCur, and further proved its ability to block the activity of CBP HAT as well as the regulation of p53 activation upon genotoxic stress downstream via computational studies [65]. NiCur was docked using Autodock-4 [45] into the active site of the CBP HAT and poses

generated showed its binding affinity. A group of researchers reviewed the result of docking fragment-based high throughput ligands in rigid binding targets of the N-terminal BRD of BRD4 and CREBBP bromodomain [64]. In silico screening was aided with the newly developed procedure based on fragment for high throughput docking of large libraries of compounds. These compounds are called anchor-based library tailoring (ALTA) [45]. Of over 2 million compounds decomposed using the DAIM program [66], approximately 97 fragments with either hydrogen bond donor or acceptor and a ring were parameterized using MATCH [67]. These compounds, with the use of SEED [68] [69], were docked into two structures of CBP. Only 4000 fragments survived the double filtering stage, of which the best compounds continued the docking process in the ALTA procedure using AutoDock Vina [45]. Poses were minimized with CHARMM . Remarkably, only 20 compounds emerged the best in terms of their interaction with the asparagine residue in the binding target. Since the aim of the experiment involved its definition of the stability of the interaction, 100ns molecular simulation was carried out with each docked pose. It was reported that the ethylbenzene derivatives showed greater efficiency and binding selectivity compared to other CBP bromodomain inhibitors (SGC-CBP30) [70] and I-CBP112 [71] reported by others.

CONCLUSION

This study proves the progression of CREB-BP from concept to computational research. Its unique properties have been evaluated through times and have been a significant target, especially in cancer drug development. Various inhibitors have been identified, and the investigation continues to emerge in its progression to being drugs for diseases. Having looked into examples of studies in which MD simulation and docking were adopted, it is quite evident that more progress is likely to be seen in this continuous study.

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Coding Material

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Consent to Participate

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Consent for Publication

None

REFERENCE

1. Janknecht R, Hunter T (1996) Transcriptional control: Versatile molecular glue. Current Biology 6:951–954. https://doi.org/10.1016/S0960-9822(02)00636-X
2. Dallas PB, Cheney IW, Liao D-W, et al (1998) p300/CREB Binding Protein-Related Protein p270 Is a Component of Mammalian SWI/SNF Complexes. Molecular and Cellular Biology 18:3596–3603. https://doi.org/10.1128/mcb.18.6.3596
3. Kwok RPS, Lundblad JR, Chrivia JC, et al (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature 370:223–226
4. Dorsman JC, Teunisse AFAS, Zantema A, Van Der Eb AJ (1997) The adenovirus 12 E1A proteins can bind directly to proteins of the p300 transcription co-activator family, including the CREB-binding protein CBP and p300. Journal of General Virology 78:423–426. https://doi.org/10.1099/0022-1317-78-2-423
5. Parker D, Ferreri K, Nakajima T, et al (1996) Phosphorylation of CREB at Ser-133 induces complex formation with CREB-binding protein via a direct mechanism. Molecular and Cellular Biology 16:694–703. https://doi.org/10.1128/mcb.16.2.694
6. Brindle P, Linke S, Montminy M (1993) Protein-kinase-A-dependent activator in transcription factor CREB reveals new role for CREM repressers. Nature 364:821–824. https://doi.org/10.1038/364821a0
7. Dhalluin C, Carlson JE, Zeng L, et al (1999) Structure and ligand of a histone acetyltransferase bromodomain. Nature 399:491–496. https://doi.org/10.1038/20974
8. Tamkun JW, Deuring R, Scott MP, et al (1992) brahma: A regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2 SWI2. Cell 68:561–572. https://doi.org/10.1016/0092-8674(92)90191-E
9. Strahl BD AC, Strahl BD AC (2000) The language of covalent histone modifications. Nature 403:41–5. https://doi.org/10.1038/47412

10. Taverna SD, Li H, Ruthenburg AJ, et al (2007) How chromatin-binding modules interpret histone modifications: Lessons from professional pocket pickers. Nature Structural and Molecular Biology 14:1025–1040. https://doi.org/10.1038/nsmb1338
11. Josling GA, Selvarajah SA, Petter M, Duffy MF (2012) The role of bromodomain proteins in regulating gene expression. Genes 3:320–343. https://doi.org/10.3390/genes3020320
12. Filippakopoulos P, Picaud S, Mangos M, et al (2012) Histone recognition and large-scale structural analysis of the human bromodomain family. Cell 149:214–231. https://doi.org/10.1016/j.cell.2012.02.013
13. Jeanmougin F, Wurtz JM, Le Douarin B, et al (1997) The bromodomain revisited. Trends in biochemical sciences 22:151–153. https://doi.org/10.1016/S0968-0004(97)01042-6
14. Pervaiz M, Mishra P, Günther S (2018) Bromodomain Drug Discovery – the Past, the Present, and the Future. Chemical Record 18:1808–1817. https://doi.org/10.1002/tcr.201800074
15. Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35:605–623. https://doi.org/10.1016/S0896-6273(02)00828-0
16. Eckner R, Ewen ME, Newsome D, et al (1994) Molecular cloning and functional analysis of the adenovirus E1A- associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes and Development 8:869–884. https://doi.org/10.1101/gad.8.8.869
17. Bhattacharya S, Michels CL, Leung MK, et al (1999) Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. Genes and Development 13:64–75. https://doi.org/10.1101/gad.13.1.64

18. Ho Man Chan, Nicholas B. La Thangue (2001) p300/CBP proteins: HATs for transcriptional bridges and scaffolds. Journal of cell science 114:2363–2373

19. Zanger K, Radovick S, Wondisford FE (2001) CREB binding protein recruitment to the transcription complex requires growth factor-dependent phosphorylation of its GF box. Molecular Cell 7:551–558. https://doi.org/10.1016/S1097-2765(01)00202-7

20. Bleckmann SC, Blendy JA, Rudolph D, et al (2002) Activating Transcription Factor 1 and CREB Are Important for Cell Survival during Early Mouse Development. Molecular and Cellular Biology 22:1919–1925. https://doi.org/10.1128/mcb.22.6.1919-1925.2002

21. Kornacki JR, Stuparu AD, Mrksich M (2015) Acetyltransferase p300/CBP associated factor (PCAF) regulates crosstalk-dependent acetylation of histone H3 by distal site recognition. ACS Chemical Biology 10:157–164. https://doi.org/10.1021/cb5004527

22. Soutoglou E, Katrakili N, Talianidis I (2000) Acetylation regulates transcription factor activity at multiple levels. Molecular Cell 5:745–751. https://doi.org/10.1016/S1097-2765(00)80253-1

23. Song CZ, Keller K, Murata K, et al (2002) Functional interaction between coactivators CBP/p300, PCAF and transcription factor FKLF2. Journal of Biological Chemistry 277:7029–7036. https://doi.org/10.1074/jbc.M108826200

24. Lundblad JR, Kwok RPS, Laurance ME, et al (1995) Adenoviral ElA-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. Nature 374:85–88

25. Ait-Si-Ali S, Ramirez S, Barre FX, et al (1998) Histone acetyltransferase activity of CBP is controlled by cycle- dependent kinases and oncoprotein E1A. Nature 396:184–186. https://doi.org/10.1038/24190

26. Knights CD, Catania J, Di Giovanni S, et al (2006) Distinct p53 acetylation cassettes

differentially influence gene-expression patterns and cell fate. Journal of Cell Biology 173:533–544. https://doi.org/10.1083/jcb.200512059

27. Chan HM, Narita M, Lowe SW, Livingston DM (2005) The p400 E1A-associated protein is a novel component of the p53 → p21 senescence pathway. Genes and Development 19:196–201. https://doi.org/10.1101/gad.1280205

28. Xiao X, Li BX, Mitton B, et al (2010) Targeting CREB for Cancer Therapy: Friend or Foe. Current Cancer Drug Targets 10:384–391. https://doi.org/10.2174/156800910791208535

29. Carlezon WA, Duman RS, Nestler EJ (2005) The many faces of CREB. Trends in Neurosciences 28:436–445. https://doi.org/10.1016/j.tins.2005.06.005

30. Tang W, Yang F, Lu W, et al (2017) Association study of CREB1 and CBP genes with Alzheimer's disease in Han Chinese. Asia-Pacific Psychiatry 9:1–2. https://doi.org/10.1111/appy.12274

31. Hadjipanayis A, Chen X, Lee J, et al (2019) Thu0033 Rna Profiling of Healthy and Rheumatoid Arthritis Subjects Treated With Tofacitinib Monotherapy. 284.1-284. https://doi.org/10.1136/annrheumdis-2019-eular.1928

32. Hammitzsch A, Tallant C, Fedorov O, et al (2015) CBP30, a selective CBP/p300 bromodomain inhibitor, suppresses human Th17 responses. Proceedings of the National Academy of Sciences of the United States of America 112:10768–10773. https://doi.org/10.1073/pnas.1501956112

33. Zou L, Xiang Q, Xue X, et al (2019) Y08197 is a novel and selective CBP / EP300 bromodomain inhibitor for the treatment of prostate cancer. Acta Pharmacologica Sinica 1–12. https://doi.org/10.1038/s41401-019-0237-5

34. Akinsiku OE, Soremekun OS, Olotu FA, Soliman MES (2020) Exploring the Role of Asp1116 in Selective Drug Targeting of CREBcAMP- Responsive Element-binding

Protein Implicated in Prostate Cancer. Combinatorial Chemistry & High Throughput Screening 23:178–184. https://doi.org/10.2174/1386207323666200219122057

35. Bai B, Zhang Q, Wan C, et al (2018) CBP/p300 inhibitor C646 prevents high glucose exposure induced neuroepithelial cell proliferation. Birth Defects Research 110:1118–1128. https://doi.org/10.1002/bdr2.1360

36. Zhao Z, Cao L, Reece EA (2017) Formation of neurodegenerative aggresome and death-inducing signaling complex in maternal diabetes-induced neural tube defects. Proceedings of the National Academy of Sciences of the United States of America 114:4489–4494. https://doi.org/10.1073/pnas.1616119114

37. Ornoy A, Reece EA, Pavlinkova G, et al (2015) Effect of maternal diabetes on the embryo, fetus, and children: Congenital anomalies, genetic and epigenetic changes and developmental outcomes. Birth Defects Research Part C - Embryo Today: Reviews 105:53–72. https://doi.org/10.1002/bdrc.21090

38. Barbieri CE, Baca SC, Lawrence MS, et al (2012) Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nature Genetics 44:685–689. https://doi.org/10.1038/ng.2279

39. Defects NT, Flour WF, Acid F (2018) Fortifying Flour with Folic Acid to Prevent Neural Tube Birth Defects. 23–26

40. Zhang F, Sun Z, Liao L, et al (2019) Discovery of novel CBP bromodomain inhibitors through TR-FRET-based high-throughput screening. Acta Pharmacologica Sinica. https://doi.org/10.1038/s41401-019-0256-2

41. Lee JW, Park HS, Park SA, et al (2015) A novel small-molecule inhibitor targeting CREB-CBP complex possesses anti-cancer effects along with cell cycle regulation, autophagy suppression and endoplasmic reticulum stress. PLoS ONE 10:1–16. https://doi.org/10.1371/journal.pone.0122628

42. Yan Y, Ma J, Wang D, et al (2019) The novel BET-CBP/p300 dual inhibitor NEO2734 is active in SPOP mutant and wild-type prostate cancer. EMBO Molecular Medicine 1–19. https://doi.org/10.15252/emmm.201910659

43. Rarey M, Kramer B, Lengauer T, Klebe G (1996) A fast flexible docking method using an incremental construction algorithm. Journal of Molecular Biology 261:470–489. https://doi.org/10.1006/jmbi.1996.0477

44. Verdonk ML, Cole JC, Hartshorn MJ, et al (2003) Giftgas over Byen. Civilbefolkningens Beskyttelse Under Den Næste krig. Proteins 52:609–623. https://doi.org/10.1002/prot.10465

45. Allouche A (2012) Software News and Updates Gabedit — A Graphical User Interface for Computational Chemistry Softwares. Journal of computational chemistry 32:174–182. https://doi.org/10.1002/jcc

46. Halgren TA, Murphy RB, Friesner RA, et al (2004) Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening. Journal of Medicinal Chemistry 47:1750–1759. https://doi.org/10.1021/jm030644s

47. Kuntz ID, Blaney JM, Oatley SJ, et al (1982) A Geometric Approach to Macromolecule-Ligand Interactions

48. Ewing TJA, Kuntz ID (1997) Critical evaluation of search algorithms used in automated molecular docking. Comput Appl Biosci 18:1175–1189

49. Macindoe G, Mavridis L, Venkatraman V, et al (2010) HexServer: An FFT-based protein docking server powered by graphics processors. Nucleic Acids Research 38:445–449. https://doi.org/10.1093/nar/gkq311

50. Jain AN (2003) Surflex: Fully automatic flexible molecular docking using a molecular similarity-based search engine. Journal of Medicinal Chemistry 46:499–511. https://doi.org/10.1021/jm020406h

51. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: Servers for rigid and symmetric docking. Nucleic Acids Research 33:363–367. https://doi.org/10.1093/nar/gki481

52. Hansson T, Oostenbrink C, Gunsteren WF Van (2002) Molecular dynamics simulations Hansson, Oostenbrink and van Gunsteren 191. 190–196

53. Alonso H, Bliznyuk AA, Gready JE (2006) Combining docking and molecular dynamic simulations in drug design. Medicinal Research Reviews 26:531–568. https://doi.org/10.1002/med.20067

54. Singh P, Sharma P, Bisetty K, Perez JJ (2010) Molecular dynamics simulations of Ac-3Aib-Cage-3Aib-NHMe. Molecular Simulation 36:1035–1044. https://doi.org/10.1080/08927022.2010.501797

55. Snow CD, Sorin EJ, Rhee YM, Pande VS (2005) How well can simulation predict protein folding kinetics and thermodynamics? Annual Review of Biophysics and Biomolecular Structure 34:43–69. https://doi.org/10.1146/annurev.biophys.34.040204.144447

56. Brooks BR, Bruccoleri RE, Olafson BD, et al (1983) CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. Journal of Computational Chemistry 4:187–217. https://doi.org/10.1002/jcc.540040211

57. Nelson MT, Humphrey W, Gursoy A, et al (1996) NAMD: A parallel, object-oriented molecular dynamics program. International Journal of High Performance Computing Applications 10:251–268. https://doi.org/10.1177/109434209601000401

58. Christen M, Hünenberger PH, Bakowies D, et al (2005) The GROMOS software for biomolecular simulation: GROMOS05. Journal of Computational Chemistry 26:1719–1751. https://doi.org/10.1002/jcc.20303

59. Darian E, Gannett PM (2005) Application of molecular dynamics simulations to spin-

labeled oligonucleotides. Journal of Biomolecular Structure and Dynamics 22:579–593. https://doi.org/10.1080/07391102.2005.10507028

60. Baurin N, Baker R, Richardson C, et al (2004) Drug-like annotation and duplicate analysis of a 23-supplier chemical database totalling 2.7 million compounds. Journal of Chemical Information and Computer Sciences 44:643–651. https://doi.org/10.1021/ci034260m

61. Dalby A, Nourse JG, Hounshell WD, et al (1992) Description of Several Chemical Structure File Formats Used by Computer Programs Developed at Molecular Design Limited. Journal of Chemical Information and Computer Sciences 32:244–255. https://doi.org/10.1021/ci00007a012

62. Moriarty NW, Grosse-Kunstleve RW, Adams PD (2009) Electronic ligand builder and optimization workbench (eLBOW): A tool for ligand coordinate and restraint generation. Acta Crystallographica Section D: Biological Crystallography 65:1074–1080. https://doi.org/10.1107/S0907444909029436

63. Ritchie DW, Venkatraman V (2010) Ultra-fast FFT protein docking on graphics processors. Bioinformatics 26:2398–2405. https://doi.org/10.1093/bioinformatics/btq444

64. Spiliotopoulos D, Caflisch A (2016) Fragment-based in silico screening of bromodomain ligands. Drug Discovery Today: Technologies 19:81–90. https://doi.org/10.1016/j.ddtec.2016.06.003

65. Vincek AS, Patel J, Jaganathan A, et al (2018) Inhibitor of CBP histone acetyltransferase downregulates p53 activation and facilitates methylation at lysine 27 on histone H3. Molecules 23:. https://doi.org/10.3390/molecules23081930

66. Kolb P, Caflisch A (2006) Automatic and efficient decomposition of two-dimensional structures of small molecules for fragment-based high-throughput docking. Journal of

Medicinal Chemistry 49:7384–7392. https://doi.org/10.1021/jm060838i

67. Yesselman JD, Price DJ, Knight JL, Brooks CL (2012) MATCH: An atom-typing toolset for molecular mechanics force fields. Journal of Computational Chemistry 33:189–202. https://doi.org/10.1002/jcc.21963

68. Majeux N, Scarsi M, Caflisch A (2001) Efficient electrostatic solvation model for protein-fragment docking. Proteins: Structure, Function and Genetics 42:256–268. https://doi.org/10.1002/1097-0134(20010201)42:2<256::AID-PROT130>3.0.CO;2-4

69. Majeux N, Scarsi M, Apostolakis J, et al (1999) Exhaustive docking of molecular fragments with electrostatic solvation. Proteins: Structure, Function and Genetics 37:88–105. https://doi.org/10.1002/(SICI)1097-0134(19991001)37:1<88::AID-PROT9>3.0.CO;2-O

70. Hay DA, Fedorov O, Martin S, et al (2014) Discovery and Optimization of Small-Molecule Ligands for the CBP/ p300 Bromodomains. 382:

71. Picaud S, Fedorov O, Thanasopoulou A, et al (2015) Generation of a selective small molecule inhibitor of the CBP/p300 bromodomain for Leukemia therapy. Cancer Research 75:5106–5119. https://doi.org/10.1158/0008-5472.CAN-15-0236

CHAPTER 6

6.1 CONCLUSION

Trends in cancer studies have revealed prostate cancer as the second most recurring and fifth lethal cancer in men in the world today. Despite androgen deprivation therapy (either chemical or surgical castration), it has been reported that some men show progression in androgen signalling giving rise to the present headache of prostate cancer called castration resistance prostate cancer (CRPC). Although the mechanism of this onset has not been clearly identified, research continues possible drug inhibition.

Zou et al., [1] bases the research studies included in this thesis on a recent study where a novel drug Y08197, a selective inhibitor of CBP/P300 BRD, was identified via in vitro studies with antitumor properties. In his study, the inhibitor identified with CRPC as a new therapeutic drug for the treatment of castration-resistant prostate cancer and higher selectivity over other BRDs. Hence this study aimed to burrow deep to view the specificity of inhibition of Y08197 against CBP as well as observe the ligand specificity of the compounds tested against CBP and BRPF1, another bromodomain but of lowest affinity to Y08197 with the aid of computational studies. To attain our aim, there were two particular arms to this study, which were: (a) to comprehend the mechanistic and selectiveness of Y08197 at the bromodomain active site (b) to analyse similar inhibitory effect connecting Y08197 and CPI-637.

Using various computational tools such as Chimera, Avogadro, Marvin sketch, origin to mention but a few, this research has accomplished the aim of the studies. The inhibitory out-turn between YO8197 and CPI-637 was disclosed using Molecular Mechanics/ Poisson-Boltzmann Surface Area (MM/PBSA) analysis. Besides, our findings highlighted Asp1116 as the ‘culprit’ residue responsible for the selective targeting and affinity of Y08197 towards CBP

in relative to Bromodomain and PHD finger-containing protein 1(BRPF1). The mechanism adopted upon binding, reveals that Asp1116 assumed a conformation that changes the strural design of the bromodomain active site, attuning the helices around the active site in a more closely-packed position. Interestingly, besides some specific structural perturbations mediated by Asp1116 on the dynamics of CBP, our study disclosed that the strong hydrogen bond interaction (N-HO) obtained in CBP-Y08197 sequestered Y08197 tightly into the CBP bromodomain active site.

Overall, this research study has provided indispensable acumen into the mechanism of Y08197, a selective CBP inhibitor via molecular modelling and CADD.

6.2 RECOMMENDATIONS AND FUTURE PERSPECTIVES

The study entails computational approaches used to provide methodically and cost reductive software gadget for drug design and discovery. Gadget such as Molecular dynamics (MD) simulation, Virtual screening, binding free energy assessment with the use of MM/GBSA technique executed in AMBER 14, and post-dynamic analyses. Software tools such as these were employed to regulate the binding modes of the drug and check for how stable the enzyme-binding site is. Others include how to determine ligands-active sites residues interactions and docking results verification.

6.2.1 Future Perspectives

The potential inhibitor of the study has presented promising therapeutic for the treatment of castration resistance prostate cancer. However, prospective biological testing of this compound is still required to verify this in silico studies. Also, further studies may include more

combinational therapies comparison of Y08197 and anti-androgens such as enzulamatide. It also of closer interest to uncover the great mystery of the mechanism of CRPC that is still unclear as of today

REFERENCE

1. Zou L jiao, Xiang Q ping, Xue X qian, et al (2019) Y08197 is a novel and selective CBP/EP300 bromodomain inhibitor for the treatment of prostate cancer. Acta Pharmacol Sin 1–12. https://doi.org/10.1038/s41401-019-0237-5

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RESEARCH ARTICLE

Exploring the Role of Asp1116 in Selective Drug Targeting of CREB-cAMP- Responsive Element-binding Protein Implicated in Prostate Cancer

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Abstract: ***Background*:** The selective targeting of CREB-cAMP-responsive element-binding protein (CBP) has recently evolved as a vital therapeutic approach for curtailing its aberrant upregulation associated with the development of prostate cancer. Inhibition of CBP has been discovered to be an important therapeutic option in androgen receptor signalling pathway mediated prostate cancer. Y08197, a novel selective inhibitor of CBP, has shown promising therapeutic outcome in prostate carcinogenesis over non-selective analogues such as CPI-637.

***Methods/Results*:** Herein, we used molecular dynamics simulation to gain insights into the mechanistic and selective targeting of Y08197 at the bromodomain active site. Molecular Mechanics/ Poisson-Boltzmann Surface Area (MM/PBSA) analysis revealed a similar inhibitory effect between Y08197 and CPI-637. Furthermore, in exploring the selective affinity of Y08197 towards CBP in combination with Bromodomain and PHD finger-containing protein 1(BRPF1), our findings highlighted Asp1116 as the 'culprit' residue responsible for this selective targeting. Upon binding, Asp1116 assumed a conformation that altered the architecture of the bromodomain active site, thereby orienting the helices around the active site in a more compacted position. In addition to some specific structural perturbations mediated by Asp1116 on the dynamics of CBP, our study revealed that the strong hydrogen bond interaction (N-H···O) elicited in CBP-Y08197 sequestered Y08197 tightly into the CBP bromodomain active site.

***Conclusion*:** Conclusively, the inhibition and selective pattern of Y08197 can be replicated in future structure-based CBP inhibitors and other bromodomain implicated in carcinogenesis.

Keywords: CBP, BRPF1, Prostate Cancer, Y08197, Molecular Dynamic Simulation, CBP inhibitors.

1. INTRODUCTION

Prostate cancer is regarded as the second most frequent and the fifth leading cause of cancer-related mortality in males worldwide [1]. In 2018, the International Agency for Research on Cancer estimated that almost 1.3million new cases of prostate cancer and 359,000 associated deaths will occur worldwide [1]. Prostate cancer has been linked to aberration in some proteins such as androgen receptor (AR) [2]. Androgen-deprivation therapy has shown high therapeutic outcome, yet, recurrence of pathological symptoms after 2 to 3 years suggests upregulation of signalling pathways or an alternatively spliced AR that is no longer dependent on androgen binding to affect its activation [3]. This is called Castration-Resistant Prostate Cancer (CRPC) or Androgen Independent Prostate Cancer (AIPC) or Hormone Refractory Prostate Cancer (HRPC) [4]. Therefore, recent research studies are aimed at the development of drug therapies that target CRPC. Due to the role that AR plays in prostate carcinogenesis, different drugs have been developed as a target against AR [5], such as abiraterone [6] and second-generation antagonists such as enzalutamide [3], bicalutamide [7] and apalutamide [8]. Despite the success recorded by these drugs, drug resistance still emanates, this necessitated continuous research into developing and design of inhibitors that can circumvent this [4]. Strategies designed in combating prostate cancer involve targeting several pathways such as androgen synthesis, androgen receptor splice variants, androgen receptor coactivators, PI3K-AKT pathway, WNT pathway, DNA repair, *etc* [7]. One such protein that gained attention in the last few years and has been regarded as a promising target is CREB (cAMP-response element-binding protein)- binding protein (CBP) (Fig. **1**).

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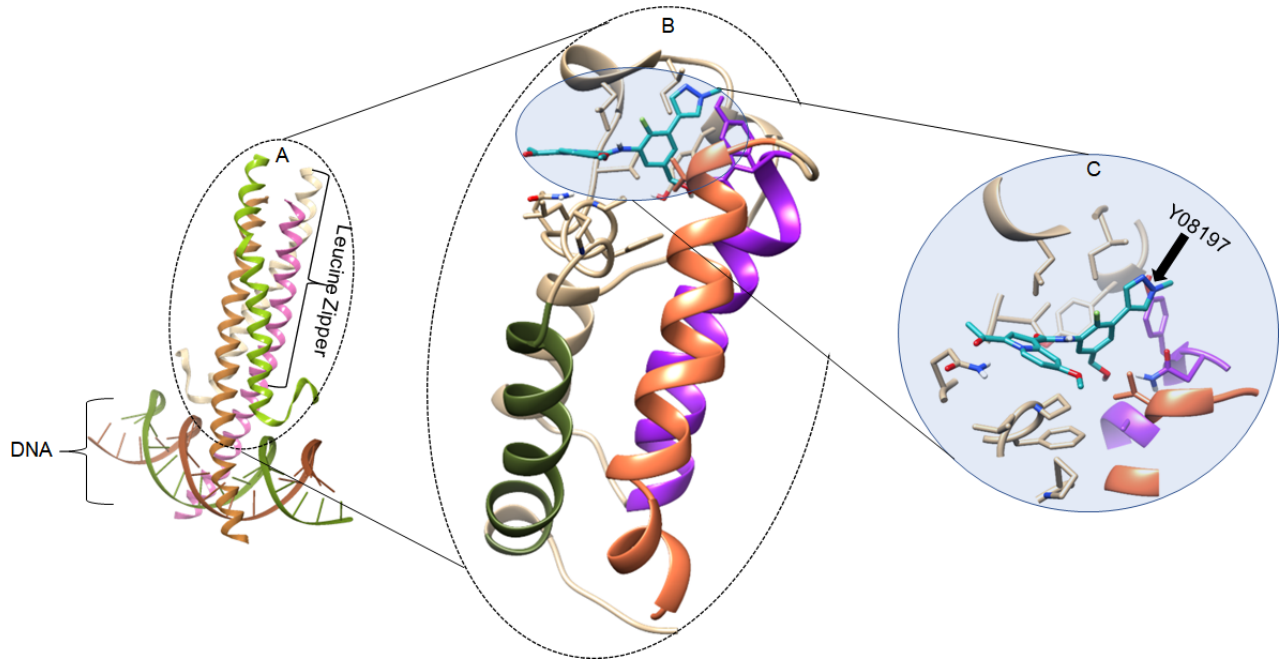

Fig. (1). 3D crystallography structure of CBP in complex with a B-DNA (A) and Y08197 (B). Y08197 occupying the active site of CBP. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

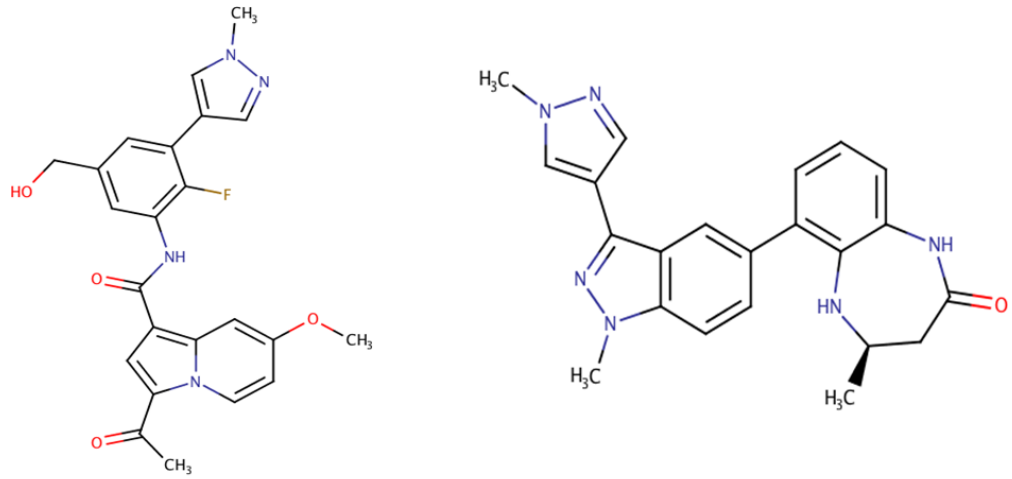

Fig. (2). 2D chemical structures of Y08197 and CPI-637. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

CBP is a bromodomain-containing protein and serves as a co-activator in transcription during androgen signalling pathway. In 2004, Barbara Comuzzi showed that CBP was upregulated upon the withdrawal of androgen, suggesting a further investigation of CBP as a future therapeutic target for CRPC patients [7]. Hence, Ling-jiao Zou recently published that targeting the bromodomain of CBP, with a selective inhibitor, Y08197, a novel 1-(indolizin-3-yl) ethanone derivative, inhibited the CBP bromodomain with an IC50 value at 100.67 ± 3.30 nM [9]. Although the experiment was carried out on different bromodomain, Y08197 appeared to selectively inhibit CBP amidst others [9]. As such, in this in silico study, we aim to extensively investigate the molecular mechanism of this reaction as well as observe the ligand specificity of the compounds against CBP and BRPF1, containing bromodomain but of lowest affinity to Y08197 (Fig. **2**).

2. MATERIALS AND METHODS

2.1. Starting Structures Preparation and MD Simulation

The initial structures of CBP and BRPF1 were obtained from Protein Data Bank with PDB ID 6FR0 [10] and 5MWH [11], respectively. Molecules that were co-crystallized with the proteins were deleted and missing residues were added with the aid of modeller [12]. B3LYP/6-311++G(d,p) [13] level of Gaussian16 [14] was employed to carry out ligand optimization. Afterwards, molecular docking of the optimised structures was carried out using UCSF Chimera [15]. FF14SB module [16, 17] of the AMBER forcefield was employed in carrying out MD simulation. The General Amber Force Field (GAFF) and Restrained Electrostatic Potential (RESP) were used in describing the atomic charges of the ligands. Leap variant present in Amber 14 was used

Table 1: The finally equilibrated values of RMSD (FE-RMSD) for each system.

	Average RMSD (Å)				
Regions	**CBP**	**BRPF1**	**CPI-CBP**	**Y08197-CBP**	**Y08197-BRPF1**
Protein	0.87	0.86	0.90	1.15	1.05
Active site	0.84	0.80	0.62	0.57	0.99

for system neutralization and hydrogen atoms addition [17]. The system was kept solvated with an orthorhombic box of TIP3P water molecules surrounding all protein atoms at a distance of 9Å [13]. System minimization was carried out firstly with a 2000 steps minimization using a restraint potential of 500kcal/mol. Secondly, we used a 1000 steps full minimization process without restrain, afterwards, the system was gradually heated at a temperature of 0k to 300k at 50ps. The system solutes are kept at a potential harmonic restraint of 10 kcal mol- 1Å −2 and collision frequency of 1.0 ps-1. Afterward, the equilibration of 500 ps was carried out. The temperature and pressure were kept constant at 300k and 1bar (isobaric-isothermal ensemble, NPT using Berendsen barostat) respectively. Each step of the simulation was run for 2fs and an SPFP precision model was adopted. The simulations were kept at constant temperature and pressure (NPT), and Langevin thermostat at collision frequency of 1.ops-2. PTRAJ variant of Amber14 was adopted for further analysis which included Root-Mean-Square Deviation (RMSD), Root-Mean-Square Fluctuation (RMSF) and Radius of Gyration [18]. The data plots were then made with ORIGIN analytical tool and visualization was done using UCSF Chimera [19].

2.2. Binding Free Energy Estimation

The Molecular Mechanics/ Poisson-Boltzmann Surface Area (MM/PBSA) was employed in the estimation of differential binding of CPI and Y08197 [20]. MM/PBSA is an end-point energy estimation used in the prediction of binding affinities of ligands and their corresponding protein target. MM/PBSA is mathematically described as:

$$\Delta G_{bind} = G_{complex} - (G_{receptor} + G_{inhibitor}) \quad (1)$$

$$\Delta G_{bind} = \Delta G_{gas} + \Delta G_{sol} - T\Delta S \quad (2)$$

$$\Delta G_{gas} = \Delta E_{int} + \Delta E_{ele} + \Delta E_{vdW} \quad (3)$$

$$\Delta G_{sol} = \Delta G_{ele,sol(GB)} - \Delta G_{np,sol} \quad (4)$$

$$\Delta G_{np,sol} = \gamma SASA + \beta \quad (5)$$

ΔG_{gas} represents the total gas phase energy calculated by intermolecular energy (ΔE_{int}), electrostatic energy (ΔE_{elel}) and van der Waals energy (ΔE_{vdW}). ΔG_{sol} represents the solvation energy, TΔS represents entropy change. $\Delta G_{ele,sol(PB)}$ represents polar desolvation energy while $\Delta G_{np,sol}$ represents the non-polar desolvation energy. γ is the surface tension proportionality constant and is set to 0.0072 kcal/(mol-1. Å-2), β is a constant equal to 0 and SASA is the solvent-accessible surface area (Å2).

2.3. Energy Decomposition

To explore the energy contribution of each residue in the active site with CPI-637 and Y08197, binding free energy decomposition was done. MM/PBSA methodology was explored in the per-residue free energy decomposition.

3. RESULTS AND DISCUSSION

3.1. CBP and BRPFI Perturbative Effect Upon CPI-637 and Y08197 Binding

To understand the structural perturbation of CBP and BRPF1 upon CPI and Y08197 binding, we used Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Radius of Gyration (RoG) to characterize the structural events in the proteins in the course of the simulation. Root Mean Square Deviation is a commonly used quantitative parameter used to estimate the similarity between two superimposed structures. RMSD can be computed for different parts of an atom, most of the time in MD simulation, the RMSD is often calculated for the Cα of the entire protein structure, for example, those found in the loop, active site and perhaps transmembrane helices [21, 22]. Many research studies have used RMSD as a measure of protein stability and equilibration [21]. Root Mean Square Fluctuation is defined as the measure of the atomic displacement of a single or a group of atoms relative to the starting or reference structures, averaged over the number of atoms [23]. Radius of Gyration (RoG) is a function used to define the distribution of atoms of a protein around its axis. The most significant parameter used in the prediction of the structural activity of a protein is RoG and distance [24]. In the course of the 200 ns simulation run, RMSD values of Cα atoms of CPI-CBP, Y08197-CBP, and Y08197-BRPF1 were estimated relative to the starting structure. As illustrated in Fig. (**3A**), all the systems attained structural stability after 20 ns. The three systems had similar motional movements with an average RMSD value of 0.90 Å, 1.15Å and 1.05Å respectively (Table **1**). Furthermore, we also explored the conformation of the active site to have an insight into the effect of the ligands relative to the RMSD of the whole system. From Fig (**3B**), the active site is observed to be well stable, this provided a suitable environment for the ligand to interact with the residues making up the active site. Furthermore, we investigated the stability of the ligand, although both ligands had similar motional movement and stability, CPI was observed to have higher stability when compared to Y08197.

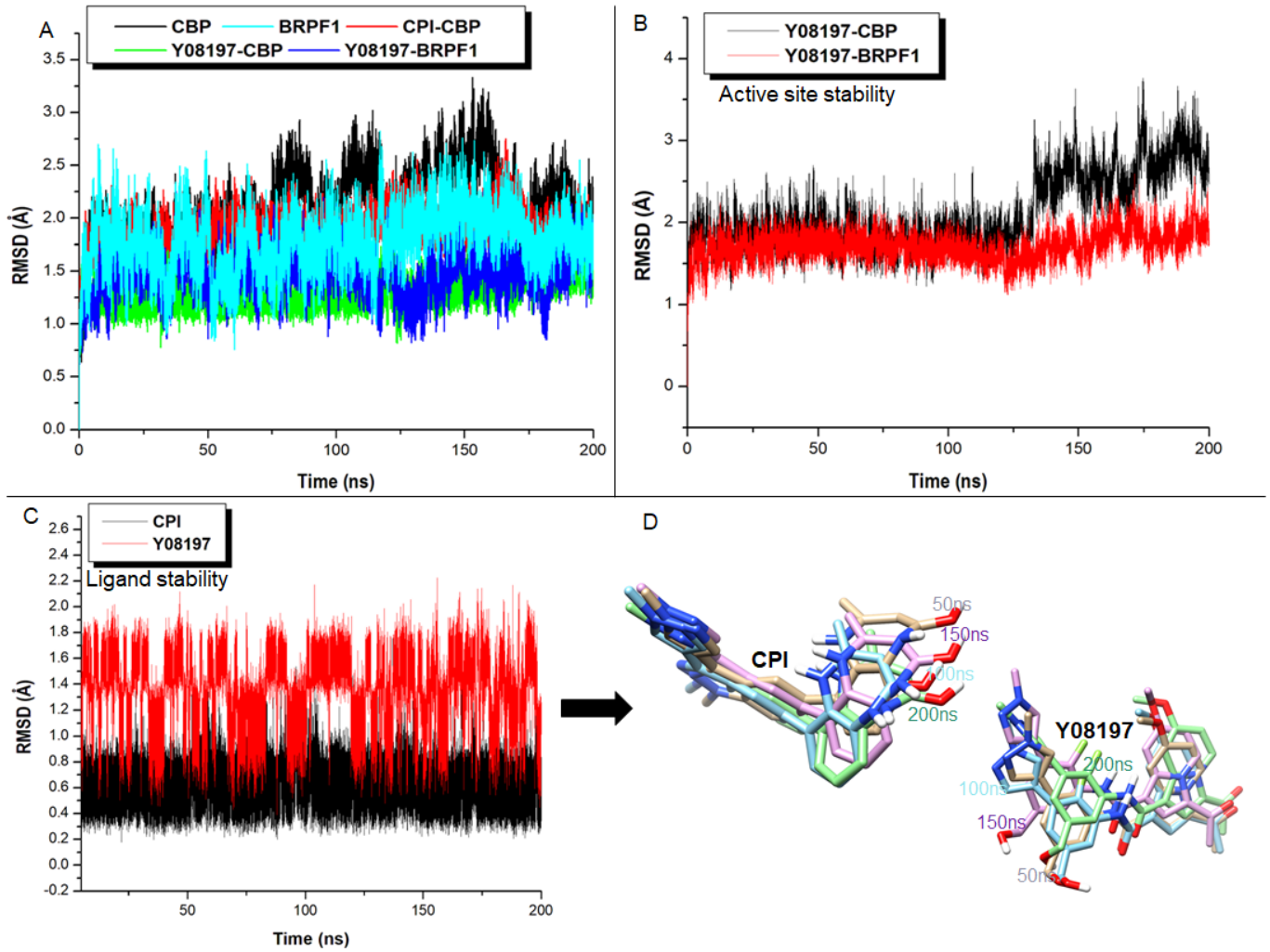

Fig. (3). Conformational analysis plot showing stability and atomistic motions among CBP (**black**), BRPF1 (**cornflower**), CPI-CBP (**red**), Y08197-CBP (**green**) and Y08197-BRPF1(**red**) systems **[A].** C-α RMSD plot showing the active site stability and atomistic motions of Y08197-CBP (**black**) and Y08197-BRPF1(**red**) **[B].** Stability and atomistic motion plot of the ligands CPI (**black**) and Y08197 (**red**) **[C].** Snapshot of the superimposed ligands at 50ns, 100ns, 150ns and 200ns **[D]**. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.2. Hydrogen Bond Analysis

The strength of protein-ligand binding is greatly influenced by hydrogen bonds contributed by residues, especially those found in the active site [25, 26]. Hydrogen bonds serve as a platform for most of the bond interactions involved in molecular recognition and protein folding. The main structural architecture of a protein is made up of alpha-helix and beta-sheet [26].

We estimated the hydrogen bonds of the equilibrated trajectories of the systems and computed direct hydrogen bonds between **CPI**, **Y08197** and the proteins. At the final simulation step (200 ns), it was observed that O-H···O in the CPI-CBP system contributed by ASN87 had an occupancy of 34. The CBP-**Y08197** had two hydrogen bond interactions contributed by ASN87 and ASP35. The N-H···O of ASN87 had a higher occupancy when compared to N-H···O of ASP35 (Table **2**). Furthermore, BRPF1-**Y08197** had an extra H-bond provided by ASN81, this bond had the highest occupancy among the H-bond interactions found among the systems. This extra H-bond could provide an insight into the selective binding of Y08197 to CBP.

3.3. Analysis of Binding Free Energy

MM/PBSA has found useful application in the drug design space used in the estimation of binding affinity between ligands and biomolecules [22, 27]. MM/PBSA was therefore used in the estimation of the total binding free energy (ΔG_{bind}) and other energy components between Y08197/CPI and CBP/BRPF1. The estimated ΔG_{bind} increases from Y08197-BRPF1 > Y08197-CBP > CPI-CBP. The same increasing trend was observed when the binding affinity after MD simulation was compared to the docking score. However, of note is that there is no much difference in the binding affinity between CPI-CBP (-32.06Kcal/mol) and Y08197-CBP (-32.00Kcal/mol) (Table **3**). This result agrees with the work of Zou et al., which suggested that the inhibitory effect CPI and Y08197 are similar. From the computed result, it was observed that van der Waals and electrostatic interactions promoted ligand-protein interactions, and binding was disfavoured by polar solvation ($\Delta G_{ele,sol(GB)}$). The nonpolar solvation ($\Delta G_{np,sol}$) was also seen to favour the binding.

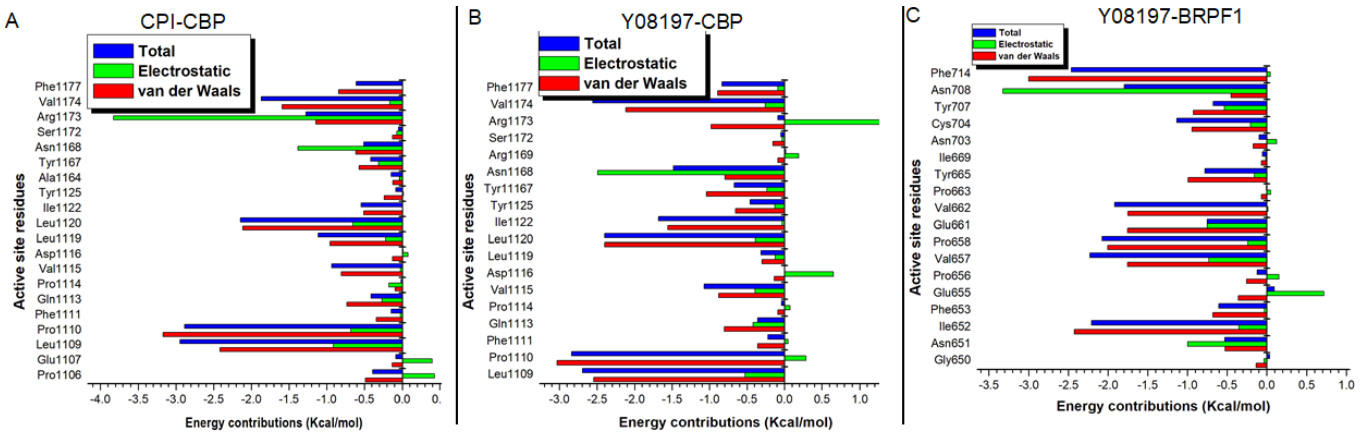

Fig. (4). Individual energy contributions of crucial site residues of CBP and BRPF1. Per-residue decomposition plot showing energy contributions of interactive active site residues of CPI-CBP (**A**), Y08197-CBP (**B**) and Y08197-BRPF1 (**C**). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

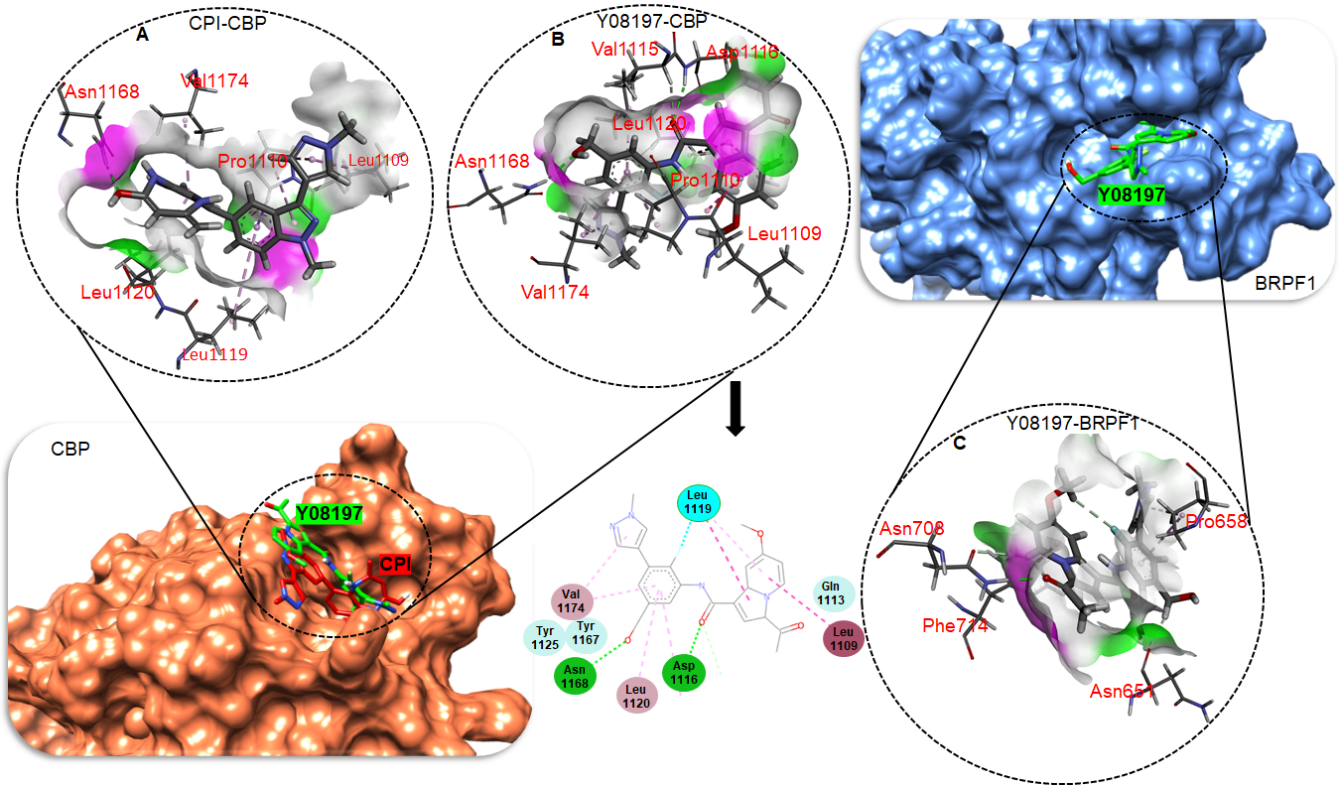

Fig. (5). 3D structure of the ligand interaction between CPI and CBP (**A**) and YO1897-CBP (**B**) highlighting the molecular interactions of key residues and reactive moieties. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

To explore the contribution of all residues present on the active site in the protein-ligand interactions, the energy decomposition of the residues was computed as depicted in Fig. (**4**). Leu1109, Pro1110, Leu1120, Arg1173, and Val1174 were found to be the interacting residues between CBP and CPI. Leu1109, Phe1110, Leu1120, Ile1122, Asn1168, Val1174 were found to be the interacting residues between CBP and Y08197.

Though ASP1116 did not contribute high van der Waals and electrostatic interaction to the binding between CBP and Y08197, however, as discussed above, it has a high hydrogen bond contribution to this binding. While, Val657, Pro658, Val662, Phe714 were the interacting residues with energy contribution more than -1Kcal/mol responsible for the binding between BRPF1 and Y08197. Zou et al., have found out that Y08197 selectively target CBP as against other bromodomain-containing proteins [9]. To have insight into the mechanism of this selective targeting, we performed selective targeting of Y08197 on CBP, and explored the ligand interaction between Y08197 and the proteins (CBP and BRPF1). Although the active sites of CBP and BRPF1 have some common residues. However, the extra electrostatic and van der Waals energy contributions provided by Val115, Asn1168, Pro1110 added to this selectivity. Most importantly Asp1116; as discussed in the hydrogen bond analysis, the hydrogen bond between N-H···O in Asp1116 is peculiar only to CBP (Fig. **5**).

CONCLUSION

The mortality rate of prostate cancer has become a major concern globally. Different therapeutic strategies have been targeted towards some proteins that are implicated in cancer. One such protein is the CREB-cAMP- responsive element-binding protein (CBP) which has gained attention in the drug design space. Y08197 is a novel inhibitor that has recently been reported to selectively target CBP. We explored this selective targeting and discovered that the Asp1116 is an important residue that facilitates this targeting. This is evidenced by the array of results that pointed to Asp1116 as the "chief culprit". Asp1116 can, therefore, be explored in designing CBP inhibitors that possess more potency and perhaps less toxicity.

AVAILABILITY OF DATA AND MATERIALS

There are no data associated with this manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This is not applicable.

HUMAN AND ANIMAL RIGHTS

This is computational research, therefore, there was no requirement for human and animal rights.

FUNDING

Not Applicable.

CONFLICT OF INTEREST

None declared..

ACKNOWLEDGEMENTS

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REFERENCE

[1] Bray, F.; Ferlay, J.; Soerjomataram, I. GLOBOCAN. *Global Cancer Statistics,* **2018**.

[2] Kaarbø, M.; Klokk, T. I.; Saatcioglu, F. *Androgen Signaling and Its Interactions with Other Signaling Pathways in Prostate Cancer.,* **2007**, 1227-1238. http://dx.doi.org/10.1002/bies.20676

[3] Penticuff, J.C.; Kyprianou, N. Pathophysiology of Castration-Resistant. *Prostate Cancer,* **2016**, •••, 5-23. http://dx.doi.org/10.1007/978-3-319-31341-2

[4] Bellmunt, J.; Oh, W. K. Castration-Resistant Prostate Cancer *New Science and Therapeutic Prospects,* **2010**, 189-207.

[5] Racca, F.; Programme, S. *RESISTANT PROSTATE CANCER TREATMENT (MCRPC)*.,

[6] Yuan, X.; Balk, S.P. Mechanisms mediating androgen receptor reactivation after castration. *Urol. Oncol.,* **2009**, *27*(1), 36-41. http://dx.doi.org/10.1016/j.urolonc.2008.03.021 PMID: 19111796

[7] Comuzzi, B.; Nemes, C.; Schmidt, S.; Jasarevic, Z.; Lodde, M.; Pycha, A.; Bartsch, G.; Offner, F.; Culig, Z.; Hobisch, A. The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. *J. Pathol.,* **2004**, *204*(2), 159-166. http://dx.doi.org/10.1002/path.1609 PMID: 15378487

[8] Rathkopf, D.E.; Scher, H.I. Apalutamide for the treatment of prostate cancer. *Expert Rev. Anticancer Ther.,* **2018**, *18*(9), 823-836. http://dx.doi.org/10.1080/14737140.2018.1503954 PMID: 30101644

[9] Zou, L. Y08197 Is a Novel and Selective CBP/EP300 Bromodomain Inhibitor for the Treatment of Prostate Cancer. *Acta Pharmacol. Sin.,* **2018**, *2019*(December), 1-12. http://dx.doi.org/10.1038/s41401-019-0237-5 PMID: 31097763

[10] Pawe, S.; Nevado, C.; Ca, A. *Binding Motifs in the CBP Bromodomain: An Analysis of 20 Crystal Structures of Complexes with Small Molecules.,* **2018**, 8-13.

[11] Zhu, J.; Zhou, C.; Caflisch, A. Structure-based discovery of selective BRPF1 bromodomain inhibitors. *Eur. J. Med. Chem.,* **2018**, *155*, 337-352. http://dx.doi.org/10.1016/j.ejmech.2018.05.037 PMID: 29902720

[12] Eswar, N.; Webb, B.; Marti-Renom, M.A.; Madhusudhan, M.S.; Eramian, D.; Shen, M-Y.; Pieper, U.; Sali, A. Comparative Protein Structure Modeling Using MODELLER.*Curr. Protoc. Protein Sci*; , **2007**, Chapter 2, . (November) Unit 2.9. http://dx.doi.org/10.1002/0471140864.ps0209s50

[13] Jorgensen, W.L.; Chandrasekhar, J.; Madura, J.D.; Impey, R.W.; Klein, M.L. Comparison of Simple Potential Functions for Simulating Liquid Water. *Joufile///C/Users/HP/Desktop/CTLF/Calculating Struct. Free. Chem. Phys.,* **1983**, *79*(2), 926-935. http://dx.doi.org/10.1063/1.445869

[14] Weedbrook, C.; Pirandola, S.; Cerf, N. J.; Ralph, T. C. *Gaussian Quantum Information.,* http://dx.doi.org/10.1103/RevModPhys.84.621

[15] Yang, Z.; Lasker, K.; Schneidman-Duhovny, D.; Webb, B.; Huang, C.C.; Pettersen, E.F.; Goddard, T.D.; Meng, E.C.; Sali, A.; Ferrin, T.E. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J. Struct. Biol.,* **2012**, *179*(3), 269-278. http://dx.doi.org/10.1016/j.jsb.2011.09.006 PMID: 21963794

[16] David, A. *Case. AmberTools12 Reference Manual*; Russell J. Bertrand Russell Arch, **2012**, p. 535.

[17] Salomon-ferrer, R.; Case, D.A.; Walker, R.C. *An Overview of the Amber Biomolecular Simulation Package.,* **2012**, *00*(February), 1-13. http://dx.doi.org/10.1002/wcms.1121

[18] Roe, D.R.; Cheatham, T.E., III PTRAJ and CPPTRAJ: Software for Processing and Analysis of Molecular Dynamics Trajectory Data. *J. Chem. Theory Comput.,* **2013**, *9*(7), 3084-3095. http://dx.doi.org/10.1021/ct400341p PMID: 26583988

[19] Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera--a visualization system for exploratory research and analysis. *J. Comput. Chem.,* **2004**, *25*(13), 1605-1612. http://dx.doi.org/10.1002/jcc.20084 PMID: 15264254

[20] Kollman, P.A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; Donini, O.; Cieplak, P.; Srinivasan, J.; Case, D.A.; Cheatham, T.E., III Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. *Acc. Chem. Res.,* **2000**, *33*(12), 889-897. http://dx.doi.org/10.1021/ar000033j PMID: 11123888

[21] Krieger, E.; Nabuurs, S.B.; Vriend, G. Homology Modeling. *Struct. Bioinforma*; , **2003**, 44, pp. 507-521.

[22] Soremekun, O.S.; Olotu, F.A.; Agoni, C.; Soliman, M.E.S. Drug promiscuity: Exploring the polypharmacology potential of 1, 3, 6-trisubstituted 1, 4-diazepane-7-ones as an inhibitor of the 'god father' of immune checkpoint. *Comput. Biol. Chem.,* **2019**, *80*(March), 433-440. http://dx.doi.org/10.1016/j.compbiolchem.2019.05.009 PMID: 31146119

[23] Martínez, L. Automatic identification of mobile and rigid substructures in molecular dynamics simulations and fractional structural fluctuation analysis. *PLoS One,* **2015**, *10*(3)e0119264 http://dx.doi.org/10.1371/journal.pone.0119264 PMID: 25816325

[24] Soremekun, O.S.; Olotu, F.A.; Agoni, C.; Soliman, M.E.S. Recruiting Monomer for Dimer Formation: Resolving the Antagonistic Mechanisms of Novel Immune Check Point Inhibitors

against Programmed Death Ligand-1 in Cancer Immunotherapy. *Mol. Simul.,* **2019**, *45*(10), 777-789. http://dx.doi.org/10.1080/08927022.2019.1593977

[25] Zhao, H.; Tang, S.; Xu, X.; Du, L. *Hydrogen Bonding Interaction between Atmospheric Gaseous Amides and Methanol.,* **2017**, 1-16.

[26] Hubbard, R. E.; Kamran Haider, M. Hydrogen Bonds in Proteins: Role and Strength. *Encycl. Life Sci,* 2010 February; http://dx.doi.org/10.1002/9780470015902.a0003011.pub2.

[27] Lawal, M.; Olotu, F.A.; Soliman, M.E.S. Across the blood-brain barrier: Neurotherapeutic screening and characterization of naringenin as a novel CRMP-2 inhibitor in the treatment of Alzheimer's disease using bioinformatics and computational tools. *Comput. Biol. Med.,* **2018**, *98*, 168-177. http://dx.doi.org/10.1016/j.compbiomed.2018.05.012 PMID: 29860210

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Update and Potential Opportunities in CBP [Cyclic Adenosine Monophosphate (cAMP) Response Element-Binding Protein (CREB)-Binding Protein] Research Using Computational Techniques

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Abstract
CBP [cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-binding protein] is one of the most researched proteins for its therapeutic function. Several studies have identified its vast functions and interactions with other transcription factors to initiate cellular signals of survival. In cancer and other diseases such as Alzheimer's, Rubinstein-taybi syndrome, and inflammatory diseases, CBP has been implicated and hence an attractive target in drug design and development. In this review, we explore the various computational techniques that have been used in CBP research, furthermore we identified computational gaps that could be explored to facilitate the development of highly therapeutic CBP inhibitors.

Keywords CREB · Molecular dynamic simulation · CREB inhibitors · Bromodomains

1 Introduction

The CREB (cyclic adenosine monophosphate (cAMP) response element-binding protein) Binding Protein (CBP), is a protein encoded by the CREBBP gene. CBP is a bromodomain-containing protein which emphasises its functionality in identifying acetylated lysine in histone proteins while also acting as effectors in signal associated with acetylation [1]. This class of protein has been reported to play a significant role in many biological and physiological processes, including transcription, differentiation, and apoptosis, whose activity is regulated by phosphorylation [1]. It's unique structure is made up of domains that catalyses transcription process initiated in cell growth, gene expression and differentiation as shown in Fig. 1. The histone acetyltransferase (HATs) domain, also part of the CREB binding protein is necessary for protein–protein interactions, histone and non-histone alike such as NCOA3 and FOXO1. In 1993, p300, a Switch/Sucrose Non-Fermentable (SWI/SNF) complexes binding protein family was identified. It was discovered to share similarity with CBP in terms of its bromodomain, HATs domain and the cysteine-histidine region [2]. Despite this similarities, they both cannot be used interchangeably. Ryan et al., researched for their differences and identified that their selectivity for lysine within the histones is the major reason for their differences [3]. Although, CBP are coactivators of transcription, they do not interact with the promoter element. Instead, they are mobilized to promoters by protein–protein interaction [1, 4, 5]. The CREB binding protein has a binding domain called the KIX (kinase inducible domain) or the CREB binding domain [4]. This CREB (cAMP-response element-binding protein) unit within CBP controls the rate of transcription when phosphorylated at Ser-133 residues through protein kinase A which triggers the transcription activity of CBP [6]. The transactivation domain of CREB is bipartite, which consist of a glutamine-rich constructive activated site called Q2 and kinase-inducible domain (KID), and are directly in response to gene expression [7]. Despite the phosphorylation interaction between cAMP-dependent PKA and CREB, it is still unknown whether phosphorylation on the amino acid Ser-133 elicit CREB-CBP complexation. The mechanism of interaction is still not precise, either direct or allosteric [6].

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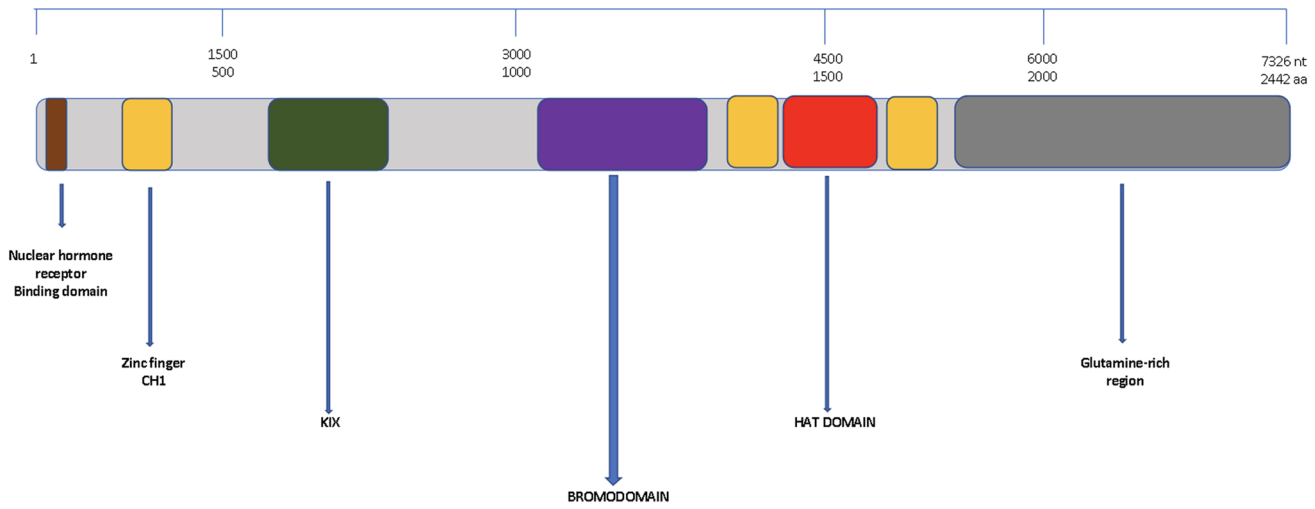

Fig. 1 CBP and its interacting domains

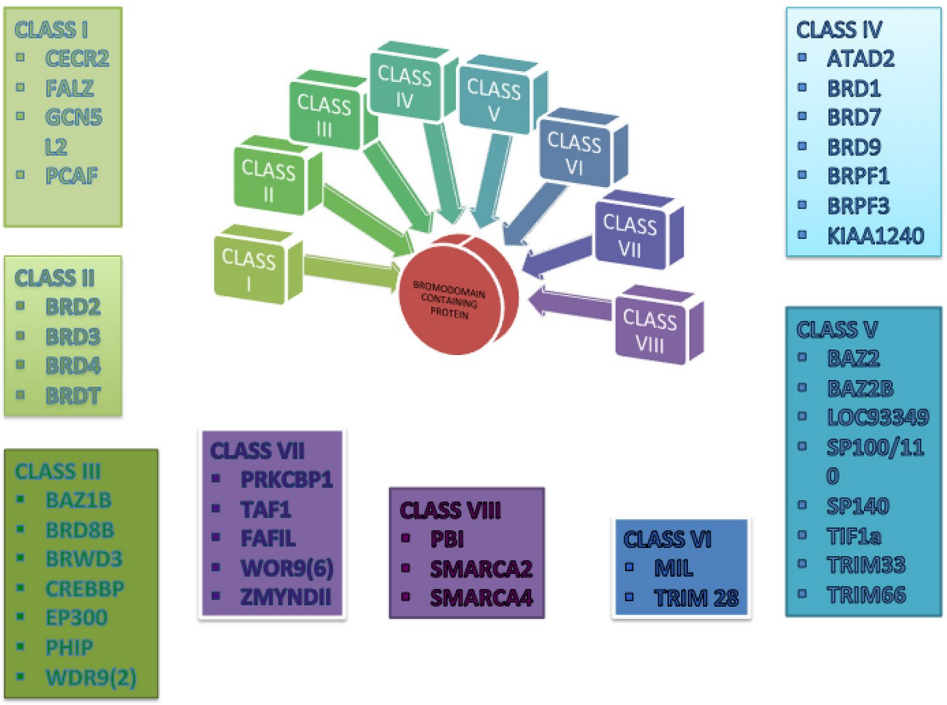

Fig. 2 Classification of the different classes of BET Proteins (prepared by the author)

1.1 Bromodomain: What About It?

Wetlaufer defined protein domains as stable units of protein structure, possessing structural and evolutionary functions that fold autonomously [1]. Bromodomains (BRDs) are parts of a given protein sequence (approximately 110 amino acids) that recognizes lysine acetylation of N-terminal histones during gene transcription [1]. They are responsible for histone acetylation, chromatin remoulding, and transcription activation [8]. John Wetlaufer Tamkun first proposed the discovery of bromodomain-proteins while studying the drosophila gene Brahma [9]. PCAF, histone acetyltransferase (HATs) KAT2B was the first 3-dimensional structure of BRD to be solved using NMR spectroscopy in 1999 [8]. Bromodomains are also called histone code readers [10, 11]. Of all the proteins in the human proteome, there are 61 BRDs, and based on their structure–function relationship, they are grouped into eight subfamilies [1]. These BRDs all have four α-helices linked by loops of different lengths (a, b, c and z) with which it interacts with acetylated lysine residues. These helices are

coiled up in a left-handed α-helical fold. Between helix b and c and helix z and a, there are two loops forming a hydrophobic pocket [12]. The differences shown in the binding of bromodomains are due to the differences in sequence beyond the residues bound directly with acetyl-lysine binding [12–14] Although each protein is specific with its structure yet 48 of the more than 61 BRDs contain the asparagine residue at the acetyl-lysine binding site (KAc recognition position) while the remaining 13 have a tyrosine, threonine or an aspartate in the same position. The latter is called atypical BRDs [15]. There are eight subgroups of the BRDs classified in accordance to their amino acid sequence similarities as seen in Fig. 2 above (*Classification of the different classes of BET Proteins*). They are the BET family, histone acetyltransferases HATs (GCN5, PCAF), methyltransferases (MLL, ASH1L), ATP-dependent chromatin-remodelling complexes (BAZ1B), helicases (MARCA), nuclear-scaffolding proteins (PB1) and transcriptional coactivators (TRIM/TIF1, TAFs) transcriptional mediators (TAF1) [13]. Specific subgroups have gained more attention compared to others; this is partly due to the development of inhibitors targeting BRDs. Of all the BRDs, the BET (bromodomain and extra-terminal family) BRDs (BRD2, BRD3, BRD4, and BRDT) are most researched and has over 206 PBD structures available today [13].

2 CREB-Binding Protein (CBP)

CBP is a nuclear protein of Mr 265 K that bounds to phosphorylated cAMP-regulated transcription factor CREB, this fusion allows CBP to function as protein kinase A-regulated transcriptional activator [16, 17]. Both CBP and p300, its analogous, shares a few functional domains in common which constitute their similarities: (1) they are BRDs which are commonly found in human HATs; (2) they both have domains of the three cysteine-histidine namely CH1, CH2, and CH3; (3) they both have the KIX domain; and (4) an ADA2-homology domain [18]. Despite the broad structural similarities, Ho Man Chan and Nicholas Thangue attest to the unique characteristics of CBP and p300 [19]. Also, both CBP and p300 are phosphorylated at the different amino acid sites; CBP is phosphorylated at serine 436, an amino acid absent in p300 [20] which is absent in the latter. On a quick database check on STRING, CBP is shown to interact with the following proteins as shown in the Fig. 3. Such proteins include NCOA3, TP53, NCOA1, RELA, CITED2, HIIF1A, PPARG, SUMO1 and STAT1. Meanwhile, Intact database reports a more detailed interactions of 790 binary proteins. In 1996, p300 and CBP were reported to function as histone acetyltransferases (HATs).

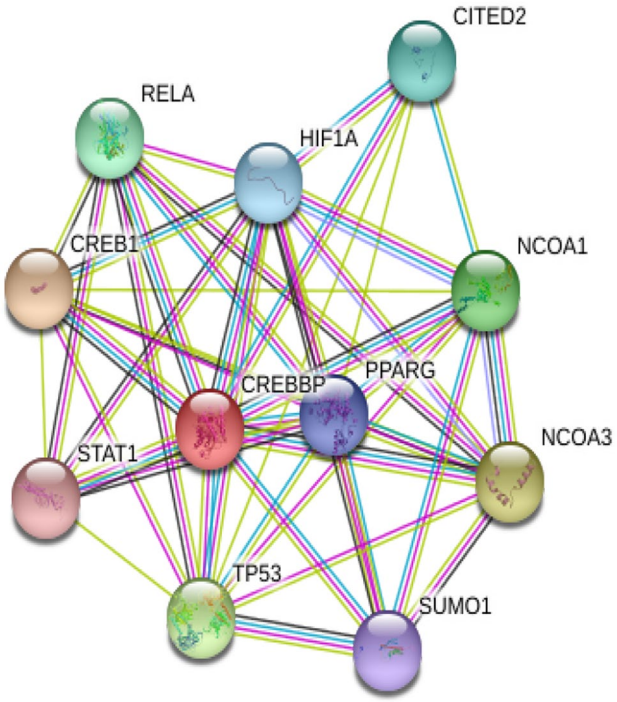

Fig. 3 A database report from STRING showing the functional interactions of CREBBP with other proteins

CBP especially was discovered to possess intrinsic histone acetyltransferase activity even though it lacked conserved motifs found in regular acetyltransferases. With this property in view, it is only direct to suggest that it modulates cell cycle progression. It is demonstrated to acetylate nucleosomes associated with PCAF [21, 22]. CBP has been shown to play a vital role in gene expression. A study reported CBP as a HAT capable of acetylating nuclear factor-4 (HNF-4) of liver cells at lysine residues inside the nuclear localization sequence [23]. CBP continues to be of great interest in the development and design of drugs CBP plays an extensively role at the molecular level, such as, cellular growth, histone acetylation, and transcription of some factors amidst other unique functions. For example, CBP brings about the assembly of multi-protein complexes, which serves as molecular scaffolds [19]. CBP, along with other transcription factors, are known to regulate the overall process involved in the cell, including gene transcription [24]. It is essential to the point that in transforming viral proteins such as E1A from adenovirus, CBP is a prerequisite target [25]. Also, another review suggests that CBP proteins are targets for adenovirus E1A oncoprotein indicating its vital role in cell cycle regulation [5]. Observations by Ait-Si-Ali et al., reported that HAT is involved in the cell cycle by the phosphorylation of CBP by cyclin-E-CDK2 in the C-terminal region of the protein hence stimulating HAT

activity [26]. Moreover, the results indicated that E1A activates the CBP HAT enzyme on the binding, which then results in a conformational change in its domain, leading to an increased catalytic activity. CBP interacts with viral oncoproteins such as p53 to cause loss of cell growth or growth suppression. p53 interacts with a carboxyl-terminal region of CBP and activate genes involved in DNA damage and block cellular differentiation such as p21, murine double minute (MDM-2), BAX and cyclin G [27, 28].

3 CREB-Binding Protein (CBP) and the Onset of Diseases

CBP's function in cancer was first identified in the translocation of chromosome t(12;22) q(13;12). Studies have shown that CBP is involved in all stages of tumour development, in addition to its being a proto-oncogene. A statistic of patients with prostate cancer, lung cancer, acute leukaemia, and breast cancer showed overexpression and over activation of CBP [29]. Also, the inhibition of cell proliferation and induction of apoptosis was observed in the downregulation of CBP, which suggests that it as a prospective target for cancer therapy [30]. Although the involvement of CBP in cancer development is not explicit yet, CBP directly controls genes critical to cell progression, growth, and metastasis. CBP has also been identified in the development of embryos and cancer [21]. In Alzheimer's disease, CBP activator (CREB1), together with CBP, enhances memory formation and learning [31]. However, in certain circumstances, increase in CREB1 function can also alter cognitive performance. A publication by Tang et al., aimed to search the function of CREB1 in the onset of Alzheimer's diseases (AD) [31]. The result implicated CREB1 and CBP as the culprit in the pathophysiology of Alzheimer's disease (AD), yet further research could be done on a much larger population to confirm these observations [31]. A research was conducted to analyse the function of CBP in inflammatory diseases. It turned out that few studies have been reported in line with rheumatoid arthritis (RA) synovial fibroblasts (SF). Results showed that the inhibition of CBP has an anti-inflammatory effect, while p300 showed both pro and anti-inflammatory functions [32].

3.1 Various Attempt to Target CBP

Recently, Hammitzsch et al., developed a CBP inhibitor (CBP 30) to block Th17 responses in human autoimmune diseases. Th17 has been proven to be very vital to various human autoimmune diseases. In the above research, the inhibitor blocked the bromodomain of the coactivator CBP, showing remarkable results [33]. Although the inhibitor was tested with about 43 bromodomain binding protein, excellent result that far exceeds even the known JQ1 (a BET inhibitor) was observed. In castration-resistant prostate cancer (CRPC), an advanced prostate cancer, CBP, and its homolog p300 are highly expressed. Given this, various therapy is aimed towards blocking the activity of CBP. In a recent study, YO8197, a selective inhibitor of CBP bromodomain was explored in terms of its antitumor activity against prostate cancer cell lines in vitro [34] of which further in silico studies by akinsiku et al., proved the mechanistic and selective targeting of Y08197 at the bromodomain site. Asp 116 was identified as the culprit responsible for the selective targeting [35]. Another CBP inhibitor, C646 has been investigated against neuroepithelial cell proliferation [36]. This study by Bai et al., further justified the abnormality in NE-4C cells of CBP in high glucose. With the administration of C646 to the diabetic induced mouse, the results indicated that the levels of acetylation were reduced. Conclusively, it was evident that C646 could effectively impede the increase of histone H4 acetylation and neuro-epithelial cell proliferation [36, 37]. Statistics reports that 1% of pregnant women are affected by diabetes and might have congenital heart disorder and neural tube defects (NTDs) in the child born [38]. Figure 4 shows 2D-structure of CREB-BP inhibitors and Table 1 explains in detail the drugs experimentally designed to target CBP as discussed.

Recent research proved that NASTRp is effective in inhibiting cancer cells via cell arrest [39]. Since mutant KRAS drives the activation of CAMP responsive element-binding (CREB), it is only appropriate to devise an inhibitor that can effectively do such through RAF/MEK/ERK signalling pathway inducing apoptosis in cancer cells [40]. Compound DC_CP20, a new CBP BRD inhibitor, discovered through a time-resolved fluorescence energy transfer (TR-FRET)-based high throughput screening of about 20 000 libraries of compounds [41]. An IC50 of 744.3 nM was demonstrated when bound with the acetylated lysine of CBP BRD. Moreover, with the aid of molecular docking, the binding affinity was further juxtaposed, being bound tightly in the inner Kac-binding pocket competitively. The compound proves an inhibitory property to human leukaemia MV4-11 cells at cellular levels. These promising results pose a further study in the development of drug therapies for CBP-related cancers [42]. Studies have shown the frequent occurrence of SPOP (speckle-type POZ protein), a mutated gene in primary prostate cancer (Pca) in about 10 to 15% range [39]. A study by Yuqian Yan et al., identified an unknown mutation called Q165P at the cliff of the SPOP math domain [43]. The effect of this mutation is that it halts the dimerization of SPOP, and consequently substrate degradation. Furthermore,

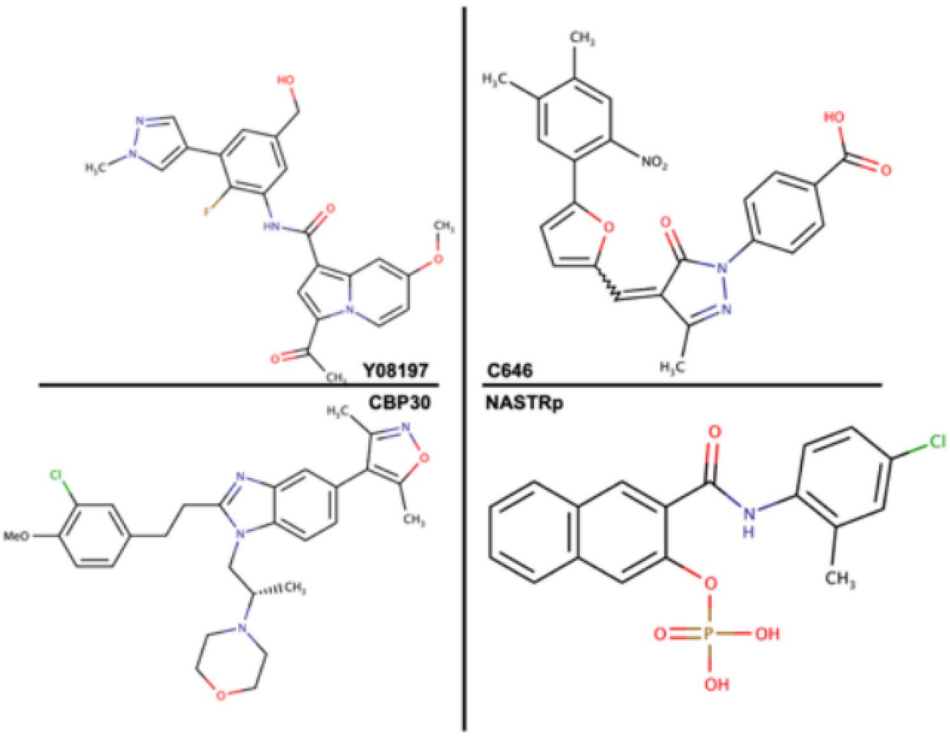

Fig. 4 2D Structures of CREB inhibitors (as prepared by the author)

Table 1 A table showing the various drugs experimentally designed to target CBP for different diseases with necessary details

S/N	Drugs	Experiments	Diseases targeted	Results	Ref
01	CBP 30	In vitro	Human autoimmune diseases	Inhibited IL-17A secretion via Th cells from healthy donors	[33]
02	Y08197	In vitro	Castration resistance prostrate cancer	Affected the downstream signalling transduction, inhibiting expression of AR-related genes	[34]
03	C646	In vitro In vivo	Neuroepithelial Cell proliferation	Rescued increased H4k5/k8/k12/k16 acetylation levels	[36]
04	NASTRp (Naphthol AS-TR phosphate)	In vitro	Lung adenocarcinoma	Inhibited oncogenic cells via cell cycle arrest and also initiated downregulations of Atg5-12 and Atg7	[40]
05	Compound DC_ CP20	In silico	Human leukaemia	Inhibited the proliferation of human leukaemia MV4-11 cells and downregulated the expression of c-Myc in the cells	[41]
06	NEO2734	In vitro In vivo	Prostate cancer	Inhibition of cell growth with a significant effect compared to a combination of JQ1 and CPI-637	[43]
07	Nicur	In silico	Gastrointestinal epithelial cells	Blocked CBP HAT activity and down regulates p53 activation upon cellular responses	[66]

unlike F133V, the former is highly sensitive to the known BET inhibitor, JQ1. In vivo and in vitro experiments carried out revealed a novel BET and CBP inhibitor, NEO2734, is effective against the JQ1-resistant SPOP hotspot mutant, which could proceed further to clinical trials for effective anti-cancer therapy against SPOP-mutated PCa patients [43].

3.2 Computer-Aided Techniques in Studies of CREB-Binding Protein

Over the years, traditional strategies used in drug development and design pipeline have been complemented with computational software and methods. These tools include; pharmacophore modelling, molecular docking, virtual

screening, molecular dynamics (MD) simulation, Quantitative Structure–Activity Relationship (QSAR), and homology modelling. Computer-aided drug design techniques have been effective over the years in finding new drugs from genomic and proteomic initiatives. These new techniques have effectively reduced cost and increased drug discovery. Molecular docking have been adopted over the years and involve ligand-receptor orientation to find the best conformation of fitness that would trigger a biological response. Some popular docking programs are FlexX [44], GOLD [45], AutoDock [46], GLIDE [47], DOCK [48, 49], HEX SERVER [50], Surflex [51], Patchdock [52] among others.

The importance of molecular dynamics (MD) simulation cannot be overemphasized, especially with its coherent contribution to the interplay between computational and experimental techniques. These step-by-step techniques effectively reveal the dynamic behaviour of the proteins at timescales intervals, the stability of the protein structure, and the ligand's binding interactions. Other properties such as conductivity, dipolar moment, density, thermodynamic parameters, entropies, amidst others. are observed [53–56]. MD simulation programs include CHARMM [57], NAMD [58], GROMACS [59], AMBER [60], among others. We searched some published papers with an emphasis on the computational methods that have been adopted in CREB research. A paper by Woo Lee published in 2015 reports the anti-cancer properties of Naphthol AS-TR phosphate (NASTRp), a novel CREB-CBP Complex inhibitor with many functions. Among all compounds, NASTRp showed the best effect, especially in biological assays. In this research, computational tools were employed in conducting a database search of compounds with possible chemical properties. Using the DBslnfilter, compounds were screened under properties such as no 3D coordinates, mixtures, isotopes, Molecular Weight < 100, or Molecular Weight > 500, metals. In this structural database are approximately 600,000 compounds that also contain about 50 chemical databases [61]. These compounds are usually downloaded in the SDF file format [62], followed by a database search command investigation on each compound to identify any two-dimensional similarity. Compounds were screened using PubChem, after which a four-processor MIPS R16000 Silicon Graphics Tezro was used to conduct modelling calculations. The results were then combined into 3-D SLNs. All Compounds not containing carboxylates, phosphates, and sulfonamides were eliminated using the hit list manager. The PDB ID: IKDX represents the KIX domain coordinates. This result from taking the average of the NMR structures with the phoenix Elbow [63] the resultant produces the KIX and NASTRp coordinates. The docking calculations were obtained using HEX 6.3 [64]. The result indicated that out of the calculations of the top ten docking scores, NASTRp was shown to have the best binding score. Although molecular simulation wasn't carried out to accompany the experiment yet, the results indicate NASTRp as a potential anti-cancer drug. Researchers over the years have shown great interest in investigating CBP as a potential drug target, as shown in some few works demonstrated in advanced MD simulations. Md simulation was conducted to decipher the mechanism of the selective inhibitor CBP30 against its target CBP/p300 bromodomain. It was discovered that the specific residue for CBP, Arg1173/1137, was accountable for the selective binding to CBP30 through hydrogen bond interactions and cation–π. In order to prove the result, four (4) system was set up; the apo-CBP, CBP-CBP30 complex, apo-p300, p300-CBP30 complex. Observing the interactions, CBP30 ring B formed a contact collision with the Arg1173 sidechain of Apo-CBP, meanwhile forming a favourable cation–π between the holo-CBP. For as long as 93% simulation time, the cation–π interaction was preserved. CBP, both contact and cation–π interaction reflected in apo-p300 and CBP 30, yet another H-bond is seen between CBP30 O3 and Arg1137 NH1 atoms of holo-p300. With these results, a greater understanding is known of the mechanism of CBP30 against BET and non-BET bromodomains [65]. Vincek et al., 2018, identified a CBP inhibitor, NiCur, and further proved its ability to block the activity of CBP HAT as well as the regulation of p53 activation upon genotoxic stress downstream via computational studies [66]. NiCur was docked using Autodock-4 [46] into the active site of the CBP HAT and poses generated showed its binding affinity. A group of researchers reviewed the result of docking fragment-based high throughput ligands in rigid binding targets of the N-terminal BRD of BRD4 and CREBBP bromodomain [65]. In silico screening was aided with the newly developed procedure based on fragment for high throughput docking of large libraries of compounds. These compounds are called anchor-based library tailoring (ALTA) [46]. Of over 2 million compounds decomposed using the DAIM program [67], approximately 97 fragments with either hydrogen bond donor or acceptor and a ring were parameterized using MATCH [68]. These compounds, with the use of SEED [69, 70], were docked into two structures of CBP. Only 4000 fragments survived the double filtering stage, of which the best compounds continued the docking process in the ALTA procedure using AutoDock Vina [46]. Poses were minimized with CHARMM. Remarkably, only 20 compounds emerged the best in terms of their interaction with the asparagine residue in the binding target. Since the aim of the experiment involved its definition of the stability of the interaction, 100 ns molecular simulation was carried out with each docked pose. It was reported that the ethylbenzene derivatives showed greater efficiency and binding selectivity compared to other CBP bromodomain inhibitors (SGC-CBP30) [71] and I-CBP112 [72] reported by others.

4 Conclusion

This study proves the progression of CREB-BP from concept to computational research. Its unique properties have been evaluated through times and have been a significant target, especially in cancer drug development. Various inhibitors have been identified, and the investigation continues to emerge in its progression to being drugs for diseases. Having looked into examples of studies in which MD simulation and docking were adopted, it is quite evident that more progress is likely to be seen in this continuous study.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

References

1. Janknecht R, Hunter T (1996) Transcriptional control: versatile molecular glue. Curr Biol 6:951–954. https://doi.org/10.1016/S0960-9822(02)00636-X
2. Dallas PB, Cheney IW, Liao D-W et al (1998) p300/CREB binding protein-related protein p270 is a component of mammalian SWI/SNF complexes. Mol Cell Biol 18:3596–3603. https://doi.org/10.1128/mcb.18.6.3596
3. Histone H, Henry RA et al (2013) Differences in specificity and selectivity between CBP and p300 acetylation of histone H3 and H3/H4. Biochemistry 52(34):5746–5759
4. Kwok RPS, Lundblad JR, Chrivia JC et al (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature 370:223–226
5. Dorsman JC, Teunisse AFAS, Zantema A, Van Der Eb AJ (1997) The adenovirus 12 E1A proteins can bind directly to proteins of the p300 transcription co-activator family, including the CREB-binding protein CBP and p300. J Gen Virol 78:423–426. https://doi.org/10.1099/0022-1317-78-2-423
6. Parker D, Ferreri K, Nakajima T et al (1996) Phosphorylation of CREB at Ser-133 induces complex formation with CREB-binding protein via a direct mechanism. Mol Cell Biol 16:694–703. https://doi.org/10.1128/mcb.16.2.694
7. Brindle P, Linke S, Montminy M (1993) Protein-kinase-A-dependent activator in transcription factor CREB reveals new role for CREM repressers. Nature 364:821–824. https://doi.org/10.1038/364821a0
8. Dhalluin C, Carlson JE, Zeng L et al (1999) Structure and ligand of a histone acetyltransferase bromodomain. Nature 399:491–496. https://doi.org/10.1038/20974
9. Tamkun JW, Deuring R, Scott MP et al (1992) brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2 SWI2. Cell 68:561–572. https://doi.org/10.1016/0092-8674(92)90191-E
10. Strahl BDAC, Strahl BDAC (2000) The language of covalent histone modifications. Nature 403:41–45. https://doi.org/10.1038/47412
11. Taverna SD, Li H, Ruthenburg AJ et al (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat Struct Mol Biol 14:1025–1040. https://doi.org/10.1038/nsmb1338
12. Josling GA, Selvarajah SA, Petter M, Duffy MF (2012) The role of bromodomain proteins in regulating gene expression. Genes 3:320–343. https://doi.org/10.3390/genes3020320
13. Filippakopoulos P, Picaud S, Mangos M et al (2012) Histone recognition and large-scale structural analysis of the human bromodomain family. Cell 149:214–231. https://doi.org/10.1016/j.cell.2012.02.013
14. Jeanmougin F, Wurtz JM, Le Douarin B et al (1997) The bromodomain revisited. Trends Biochem Sci 22:151–153. https://doi.org/10.1016/S0968-0004(97)01042-6
15. Pervaiz M, Mishra P, Günther S (2018) Bromodomain drug discovery—the past, the present, and the future. Chem Rec 18:1808–1817. https://doi.org/10.1002/tcr.201800074
16. Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35:605–623. https://doi.org/10.1016/S0896-6273(02)00828-0
17. Eckner R, Ewen ME, Newsome D et al (1994) Molecular cloning and functional analysis of the adenovirus E1A- associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev 8:869–884. https://doi.org/10.1101/gad.8.8.869
18. Bhattacharya S, Michels CL, Leung MK et al (1999) Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. Genes Dev 13:64–75. https://doi.org/10.1101/gad.13.1.64
19. Chan HM, La Thangue NB (2001) p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J Cell Sci 114:2363–2373
20. Zanger K, Radovick S, Wondisford FE (2001) CREB binding protein recruitment to the transcription complex requires growth factor-dependent phosphorylation of its GF box. Mol Cell 7:551–558. https://doi.org/10.1016/S1097-2765(01)00202-7
21. Bleckmann SC, Blendy JA, Rudolph D et al (2002) Activating transcription factor 1 and CREB are important for cell survival during early mouse development. Mol Cell Biol 22:1919–1925. https://doi.org/10.1128/mcb.22.6.1919-1925.2002
22. Kornacki JR, Stuparu AD, Mrksich M (2015) Acetyltransferase p300/CBP associated factor (PCAF) regulates crosstalk-dependent acetylation of histone H3 by distal site recognition. ACS Chem Biol 10:157–164. https://doi.org/10.1021/cb5004527
23. Soutoglou E, Katrakili N, Talianidis I (2000) Acetylation regulates transcription factor activity at multiple levels. Mol Cell 5:745–751. https://doi.org/10.1016/S1097-2765(00)80253-1
24. Song CZ, Keller K, Murata K et al (2002) Functional interaction between coactivators CBP/p300, PCAF and transcription factor

FKLF2. J Biol Chem 277:7029–7036. https://doi.org/10.1074/jbc.M108826200
25. Lundblad JR, Kwok RPS, Laurance ME et al (1995) Adenoviral ElA-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. Nature 374:85–88
26. Ait-Si-Ali S, Ramirez S, Barre FX et al (1998) Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. Nature 396:184–186. https://doi.org/10.1038/24190
27. Knights CD, Catania J, Di Giovanni S et al (2006) Distinct p53 acetylation cassettes differentially influence gene-expression patterns and cell fate. J Cell Biol 173:533–544. https://doi.org/10.1083/jcb.200512059
28. Chan HM, Narita M, Lowe SW, Livingston DM (2005) The p400 E1A-associated protein is a novel component of the p53 → p21 senescence pathway. Genes Dev 19:196–201. https://doi.org/10.1101/gad.1280205
29. Xiao X, Li BX, Mitton B et al (2010) Targeting CREB for cancer therapy: friend or foe. Curr Cancer Drug Targets 10:384–391. https://doi.org/10.2174/156800910791208535
30. Carlezon WA, Duman RS, Nestler EJ (2005) The many faces of CREB. Trends Neurosci 28:436–445. https://doi.org/10.1016/j.tins.2005.06.005
31. Tang W, Yang F, Lu W et al (2017) Association study of CREB1 and CBP genes with Alzheimer's disease in Han Chinese. Asia-Pac. Psychiatry 9:1–2. https://doi.org/10.1111/appy.12274
32. Hadjipanayis A, Chen X, Lee J, et al (2019) Thu0033 Rna profiling of healthy and rheumatoid arthritis subjects treated with tofacitinib monotherapy. 284.1-284. https://doi.org/10.1136/annrheumdis-2019-eular.1928
33. Hammitzsch A, Tallant C, Fedorov O et al (2015) CBP30, a selective CBP/p300 bromodomain inhibitor, suppresses human Th17 responses. Proc Natl Acad Sci USA 112:10768–10773. https://doi.org/10.1073/pnas.1501956112
34. Zou L, Xiang Q, Xue X et al (2019) Y08197 is a novel and selective CBP/EP300 bromodomain inhibitor for the treatment of prostate cancer. Acta Pharmacol Sin. https://doi.org/10.1038/s41401-019-0237-5
35. Akinsiku OE, Soremekun OS, Olotu FA, Soliman MES (2020) Exploring the role of Asp1116 in selective drug targeting of CREBcAMP-responsive element-binding protein implicated in prostate cancer. Comb Chem High Throughput Screen 23:178–184. https://doi.org/10.2174/1386207323666200219122057
36. Bai B, Zhang Q, Wan C et al (2018) CBP/p300 inhibitor C646 prevents high glucose exposure induced neuroepithelial cell proliferation. Birth Defects Res 110:1118–1128. https://doi.org/10.1002/bdr2.1360
37. Zhao Z, Cao L, Reece EA (2017) Formation of neurodegenerative aggresome and death-inducing signaling complex in maternal diabetes-induced neural tube defects. Proc Natl Acad Sci USA 114:4489–4494. https://doi.org/10.1073/pnas.1616119114
38. Ornoy A, Reece EA, Pavlinkova G et al (2015) Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes. Birth Defects Res Part C Embryo Today: Rev 105:53–72. https://doi.org/10.1002/bdrc.21090
39. Barbieri CE, Baca SC, Lawrence MS et al (2012) Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 44:685–689. https://doi.org/10.1038/ng.2279
40. Defects NT, Flour WF, Acid F (2018) Fortifying flour with folic acid to prevent neural tube birth defects, pp 23–26
41. Zhang F, Sun Z, Liao L et al (2019) Discovery of novel CBP bromodomain inhibitors through TR-FRET-based high-throughput screening. Acta Pharmacol Sin. https://doi.org/10.1038/s41401-019-0256-2
42. Lee JW, Park HS, Park SA et al (2015) A novel small-molecule inhibitor targeting CREB-CBP complex possesses anti-cancer effects along with cell cycle regulation, autophagy suppression and endoplasmic reticulum stress. PLoS ONE 10:1–16. https://doi.org/10.1371/journal.pone.0122628
43. Yan Y, Ma J, Wang D et al (2019) The novel BET-CBP/p300 dual inhibitor NEO2734 is active in SPOP mutant and wild-type prostate cancer. EMBO Mol Med. https://doi.org/10.15252/emmm.201910659
44. Rarey M, Kramer B, Lengauer T, Klebe G (1996) A fast flexible docking method using an incremental construction algorithm. J Mol Biol 261:470–489. https://doi.org/10.1006/jmbi.1996.0477
45. Verdonk ML, Cole JC, Hartshorn MJ et al (2003) Giftgas over Byen. Civilbefolkningens Beskyttelse Under Den Næste krig. Proteins 52:609–623. https://doi.org/10.1002/prot.10465
46. Allouche A (2012) Software news and updates Gabedit—a graphical user interface for computational chemistry softwares. J Comput Chem 32:174–182. https://doi.org/10.1002/jcc
47. Halgren TA, Murphy RB, Friesner RA et al (2004) Glide: A New Approach For Rapid, Accurate Docking And Scoring. 2. Enrichment factors in database screening. J Med Chem 47:1750–1759. https://doi.org/10.1021/jm030644s
48. Kuntz ID, Blaney JM, Oatley SJ et al (1982) A geometric approach to macromolecule-ligand interactions. J Mol Biol 161:269–288
49. Ewing TJA, Kuntz ID (1997) Critical evaluation of search algorithms used in automated molecular docking. Comput Appl Biosci 18:1175–1189
50. Macindoe G, Mavridis L, Venkatraman V et al (2010) HexServer: an FFT-based protein docking server powered by graphics processors. Nucleic Acids Res 38:445–449. https://doi.org/10.1093/nar/gkq311
51. Jain AN (2003) Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. J Med Chem 46:499–511. https://doi.org/10.1021/jm020406h
52. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res 33:363–367. https://doi.org/10.1093/nar/gki481
53. Hansson T, Oostenbrink C, Van Gunsteren WF (2002) Molecular dynamics simulations Hansson. Oostenbrink and van Gunsteren 191:190–196
54. Alonso H, Bliznyuk AA, Gready JE (2006) Combining docking and molecular dynamic simulations in drug design. Med Res Rev 26:531–568. https://doi.org/10.1002/med.20067
55. Singh P, Sharma P, Bisetty K, Perez JJ (2010) Molecular dynamics simulations of Ac-3Aib-Cage-3Aib-NHMe. Mol Simul 36:1035–1044. https://doi.org/10.1080/08927022.2010.501797
56. Snow CD, Sorin EJ, Rhee YM, Pande VS (2005) How well can simulation predict protein folding kinetics and thermodynamics? Annu Rev Biophys Biomol Struct 34:43–69. https://doi.org/10.1146/annurev.biophys.34.040204.144447
57. Brooks BR, Bruccoleri RE, Olafson BD et al (1983) CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. J Comput Chem 4:187–217. https://doi.org/10.1002/jcc.540040211
58. Nelson MT, Humphrey W, Gursoy A et al (1996) NAMD: a parallel, object-oriented molecular dynamics program. Int J High Perform Comput Appl 10:251–268. https://doi.org/10.1177/1094342096010000401
59. Christen M, Hünenberger PH, Bakowies D et al (2005) The GROMOS software for biomolecular simulation: GROMOS05. J Comput Chem 26:1719–1751. https://doi.org/10.1002/jcc.20303
60. Darian E, Gannett PM (2005) Application of molecular dynamics simulations to spin-labeled oligonucleotides. J Biomol Struct Dyn 22:579–593. https://doi.org/10.1080/07391102.2005.10507028

61. Baurin N, Baker R, Richardson C et al (2004) Drug-like annotation and duplicate analysis of a 23-supplier chemical database totalling 2.7 million compounds. J Chem Inf Comput Sci 44:643–651. https://doi.org/10.1021/ci034260m
62. Dalby A, Nourse JG, Hounshell WD et al (1992) Description of several chemical structure file formats used by computer programs developed at molecular design limited. J Chem Inf Comput Sci 32:244–255. https://doi.org/10.1021/ci00007a012
63. Moriarty NW, Grosse-Kunstleve RW, Adams PD (2009) Electronic ligand builder and optimization workbench (eLBOW): a tool for ligand coordinate and restraint generation. Acta Crystallogr D Biol Crystallogr 65:1074–1080. https://doi.org/10.1107/S0907444909029436
64. Ritchie DW, Venkatraman V (2010) Ultra-fast FFT protein docking on graphics processors. Bioinformatics 26:2398–2405. https://doi.org/10.1093/bioinformatics/btq444
65. Spiliotopoulos D, Caflisch A (2016) Fragment-based in silico screening of bromodomain ligands. Drug Discov Today: Technol 19:81–90. https://doi.org/10.1016/j.ddtec.2016.06.003
66. Vincek AS, Patel J, Jaganathan A et al (2018) Inhibitor of CBP histone acetyltransferase downregulates p53 activation and facilitates methylation at lysine 27 on histone H3. Molecules. https://doi.org/10.3390/molecules23081930
67. Kolb P, Caflisch A (2006) Automatic and efficient decomposition of two-dimensional structures of small molecules for fragment-based high-throughput docking. J Med Chem 49:7384–7392. https://doi.org/10.1021/jm060838i
68. Yesselman JD, Price DJ, Knight JL, Brooks CL (2012) MATCH: an atom-typing toolset for molecular mechanics force fields. J Comput Chem 33:189–202. https://doi.org/10.1002/jcc.21963
69. Majeux N, Scarsi M, Caflisch A (2001) Efficient electrostatic solvation model for protein-fragment docking. Proteins: structure. Funct Genet 42:256–268
70. Majeux N, Scarsi M, Apostolakis J et al (1999) Exhaustive docking of molecular fragments with electrostatic solvation. Proteins: structure. Funct Genet 37:88–105. https://doi.org/10.1002/(SICI)1097-0134(19991001)37:1%3c88:AID-PROT9%3e3.0.CO;2-O
71. Hay DA, Fedorov O, Martin S et al (2014) Discovery and optimization of small-molecule ligands for the CBP/p300 bromodomains. J Am Chem Soc 136:9308–9319
72. Picaud S, Fedorov O, Thanasopoulou A et al (2015) Generation of a selective small molecule inhibitor of the CBP/p300 bromodomain for Leukemia therapy. Cancer Res 75:5106–5119. https://doi.org/10.1158/0008-5472.CAN-15-0236