UNRAVELING THE MOLECULAR MECHANISM OF ALLYL ISOTHIOCYANATE (AITC) THROUGH NETWORK PHARMACOLOGICAL APPROACH; PROTEIN-LIGAND INTERACTION STUDY AND ITS VALIDATION ON BIOLOGICAL SYSTEM

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ABSTRACT

The framework of this report is based on the interpretation of the communication path/process amid in Allyl isothiocyanate interactome to analyze the molecular pharmacology of Allyl isothiocyanate (AITC). So, here we undertake to implement the alterations in the biological system by activation of AITC at a molecular level, yielding future direction for Bladder cancer therapy by the changes in malignant behavior at drug-related modules. We constructed the AITC's PPI networks through STRING with Cytoscape work benches; the modulation was done using MCODE. The ClueGO modular enrichment interpreted the transformation in metabolic behavior implicating signaling pathways that linked closely to the metabolic process. Different network properties and modules were analyzed based on Degree in Sub-networks yielding top-ranked biomarker genes TP53 and EP300. DAVID yields 80 enriched pathways most of them correlated to disease groups in humans, particularly carcinogenesis. A molecular docking study reveals good binding scores with the best key regulatory bimolecular genes and obtained final hub proteins are validated through Survival analysis. We were able to highlight important pathways involved in BC and also validate the key regulatory proteins by analyzing their relation can provide insight into effective medication management in bladder cancer.

Keywords: AITC, Network Pharmacology, Hub Validation, Anti-cancer, TP53 gene, EP300 gene.

ABBREVIATIONS:

AITC: Allyl isothiocyanate; ITCs: Isothiocyantes; BC: Bladder cancer; PINs: Protein interaction networks; STRING : Search tool for Retrieving the Interacting Genes; MCODE : Molecular COmplex DEtection; ClueGO: Clue Gene Ontologies; DEG: Differentially Expressing Gene list; DAVID database: Database of Annotation Visualization and Integrated Discovery tool; UC: Urological cancer type; MVAC : Methotrexate, Vinblastine Sulfate, Doxorubicin Hydrochloride (Adriamycin) Cisplatin; BITC: Benzyl isothiocyante; SFN: sulforaphane; PEITC : phenethyl isothiocyante; PPIs: protein proteins interacting networks; GO: gene ontology classes; KEGG tool: kyoto encyclopedia of the gene and genome; DC parameter: degree centrality parameter; cytoNCA: cyto network centrality analysis; PDB: protein data bank; CP: cyclo Phosphamide; RCSB: Research collaborator structural bioinformatics; TP53: tumor protein 53; EP300: E1A (adenovirus early region 1A) –associated protein p300; PDBQT form: protein-data bank partial charge (Q) & atom type (T) ; mol file: molecular files; ChEBI: chemical database of European bioinformatics institute; chEMBL: chemical database of bioactive molecules maintained by European Molecular Biology Laboratory; SPDBV: Swiss PDB viewver; MF: molecular function; BP: biological process; H-bond: hydrogen bond; KM plot: Kaplan Meier plot; FDR: false discovery rate; BH: Benjamini Hochberg.

INTRODUCTION

Globally, about 600 000 people each year are spotted with bladder cancer. It is recognized as the most expensive type and diagnosed with higher chances of recurrence condition. So it became the most challenging and expensive medication. Even though the radical operation is performed, BC showed higher recurrence rates and became potentially metastatic. So BC is diversely found in treatment strategies (Mett*set al*., 2000; Siegel *et al*.,

2019). When coming to treatment strategies in BC, only a few are approved as influential as MVAC therapy, Cisplatin+gemcitabine combinational therapy and chemotherapy (Platinum based) also, and immune checkpoint inhibitors, such as pembrolizumab (Von der Maase*et al*., 2000; Tripathiand Plimack, 2018; Wang*et al*., 2019). But unfortunately, these treatment methods show adverse side effects like Neutropenia, immune-related abnormalities, and renal dysfunction, so now facing difficulties in therapeutical effects in bladder cancer (Von der Maase*et al*., 2000; Tripathiand Plimack, 2018; Tan*et al.,* 2019). Even though, an improved prognosis for BC patients, the survival prolongation is very far from satisfactory, particularly for patients with advanced metastatic conditions. Presently anticancer drugs for urothelial cancer are under development. And few are in the trial (Zhang *et al*., 2021; Ventola, 2017). Although, there are little data available on the safety and adverse effects of newly invented drugs.

Based on these facts and artificially producing cancer drugs, the therapeutic strategies focus on natural compounds governing the specific interest in cancers. There is consent knowledge of using natural plant products in disease eradication to improve living quality and prophecy in cancer studies. In contrast, Cruciferous veggies are growing in most regions worldwide as staple foods are the main source of Isothiocyanates (ITCs). Researchers depicted their benefits in inflammation, cancer prevention, and various disease models. Although some of the reviews depicted the anti-cancer efficiency of isothiocyanates, relatively very less comprehensive information was found on molecular mechanisms, especially in the bladder cancer cell (Abbaoui*et al*., 2017; Abbaoui*et al*., 2018; Leone*et al*., 2017). The broad information is explicit on members of isothiocyanates such as - Phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC), the sulforaphane (SFN) also Allyl isothiocyanate (AITC), the malignant behavior and potency of anti-cancer modules need to be explored. So this study may provide future direction to bladder cancer patients.

Natural products acquire huge potential activity in disease management with their therapeutic applications and Allyliosthiocyanate (AITC) is one of the natural sources that stand among them. Cruciferae-membered vegetables like cabbage, kale, and Broccoli are a huge source of glucosinolates (Sulfur-containing compounds), however, a hydrolyzed product of glucosinolates called Isothiocyanates which are found most biologically active. The specific mode of action of AITC is still inconclusive. This motivates us to choose AITC as case material in our study as it is an important class of isothiocyanates. Earlier studies exhibit the highly pleiotropic effects of AITC as the anticancer drug was better explained (Zhang, 2010), anti-inflammatory (Moilanen*et al*., 2012; Kanyasiri and Xiao, 2015), anti-microbial (Shofran*et al*., 1998), anti-oxidant (Maneshand Kuttan, 2003), cardio-protective (Wazand Matouk, 2022), radio-protective (Manesh, and Kuttan 2006). So the gained knowledge asserts that AITC as a fine food ingredient is also potent in medical treatments. Numerous studies reveal the effects of AITCs in many aspects but the puzzle remained inextricable. The multiple traits exhibited by AITC showed its efficacy to interact with huge interactome molecules as key regulatory molecular targets and thus act in bio-signaling pathways (Bo *et al.,* 2016). So it intended to explore new therapeutic targets for AITC as key regulatory genes. Bioinformatics is a multidisciplinary area that links *invivo* and *invitro*phases through the retrieved data and may reveal perspectives of functional aspects in each gene connected in a network (Hiroaki, 2002). A complete set of the proteome in an individual get involved in biological process and signaling events, and more importantly, each protein expression regulate the entire interactome. Their act-in alone is rarely found and may achieve by PPI's multi-target and effects signaling cascades (Nietzsche *et al.,* 2016; Gitter and Anthony, 2011). Protein-protein interactions are rudimentary actions for many signaling pathways (Morell *et al*., 2009; Dhasmana *et al.,* 2020). Gene Ontology classification offers well distinguished Biological processes, molecular functions, protein class, and cellular components of future biomarkers. It is the most accepted statistical operation that links proteins and cellular systems (Lascorz *et al.,* 2011). Sustaining the probability of the above declared in silicon approaches in view, the modular identification in a big interactome and their GO investigation offers an assortment of competent techniques in targeting disease-gene products.

MATERIALS AND METHODS

PPI's complex network analysis; Pathway enrichment

Biomolecular targets of AITC were identified from kinds of literature by text mining and public databases. Through the STRING (V11.0) network channel, the interacting profiles were sorted (Linhoff*et al*., 2009). All associations available in STRING (Szklarczyk*et al*., 2019) with confidence were set to 0.7.; Cytoscape program (V3.7.2) with various plugins of Cytoscape was applied to the unification and visualization platform (Tsolis *et al*., 2015; Bader and Hogue, 2003). MCODE is applied for modulating large protein clusters in the network. The term modulation is referred to as identifying a functionally active set of genes in a big interactome of protein-protein interaction network (PIN), they are densely interconnected regions. For instance, the cluster in a big PIN means, the product of part in pathways that represent protein families (Huang, 2007). Each cluster generated by Cytoscape

software is a highly denser molecule by size and tightly held together in complex thus giving high-scored modules. Here, the parameters to find functionally active clusters were set with K-core value set up to≥3 followed by Node score cutoff set to ≥0.2, Maximum depth from seed=100 as default, and Degree cutoff is 2. Upon obtaining the generated modules, Gene ontology and Pathway enrichment were carried out by the ClueGO plugin of Cytoscape (V2.5.1) within modules corresponding to KEGG as well as Reactome database, the threshold frequency P<0.05 is set accomplished by two-sided hypergeometric test; Bonferroni correction was applied.

Functional enrichment analysis of Key modules

ClueGo a Cytoscape plugin was used for functional enrichment and thus uploaded a list of genes (From MCODE clusters) to get classes of ontology and pathway enrichment. Thus, run the represented ontology classification of genes compared to the reference list to prove statistical importance (Gabriela *et al*., 2009). The results can be displayed in charts having a link to retrieve gene list for each category-features and its % ontology while setting down statistically significant p-value with <0.05.

Sub-network construction

In a pharmacological PPI network, the sub-networks were constructed with the help of MCODE and cytoNCA. Here subnetworks detect all feasible interactions of selected genes. Consequently, cytoNCA is for centrality analysis in Cytoscape, it identifies the most crucial genes in the PPI network. Here we sorted DEGs based on Degree ranking. So, the genes which bear top degree centrality score first in sub-networks (Zhuang *et al*., 2015).

Detection of Pathway enrichment analysis in sub-networks A and B

The DAVID database to discover functional protein representation (Dennis *et al*., 2003) and KEGG representation for pathway analysis were uploaded to the DAVID workbench (https://david.ncifcrf.gov/) for Pathway enrichment analysis with specified P-value <0.05, to get the list of pathways directly associated to the cellular system.

Molecular-Dock study

Docking is a conventional tool in bioinformatics research, especially in new drug inventions. In structure-aided drug designing, docking plays an essential role in attaining knowledge of protein-ligand interaction. This implies knowing the ligand behavior at the atomic level of interpretation and its visualization. The compound AITC with good cancer-inhibiting activity was tested for a binding score for both TP53 and EP300 receptors. The 3D structures were retrieved from the Protein data bank, docking was done with mgltools-win32-1.5.6 with AutoDock Vina (Tsolis *et al*., 2015).

Preliminary draft for Dock: The Phyto compound- AITC as the potential anticancer effect was considered as a ligand in this study. A drug – Cyclo Phosphamide (CP) was considered a Standard drug for comparative study.

Protein preparation: 'TP53' & 'EP300' proteins in three-dimensional structures were downloaded from PDB (Rose *et al*., 2017) using (PDB: 6MXZ) & (PDB: 6WQX) for TP53 and (PDB: 6V8B) for EP300 and similar for Cyclophosphamide (CP-standard drug) respectively. DockPrep is used for geometry optimization which is a built-in tool. The prepared structures were then used for mgltools-win32-1.5.6. The charges and H_2 atoms were added to proteins and saved in PDBQT.

Preparation of ligand: For compounds AITC and Cyclophosphamide, the mole files were obtained through ChEBl (CHEBI: 73224). Open Babel software was used to convert the mol file to pdbqt form. Energy minimization was applied for compound - AITC using SPDBV- 4.10, the molecular docking analysis was performed with the AutoDock Vina tool. Before the dock, protein, and ligands get optimized followed by the dock to express the different bonding interaction and their binding affinity scores. Here the grid box was set at -21.306, 17.86, and -5.407 with x, y, z points (center) and 22,20,26 accordingly at x,y,z points (size), the exhaustiveness found 10 for TP53(pdb:6MXZ). For TP53(pdb:6WQX), the grid box resolution was set at 29.636, -8.58, and -13.413 along x, y, z points (center)& 30, 28, and 30 accordingly at the x, y, z points (size). For receptor EP300 with AITC, the grid box set at 30.005, 13.333, and -12.083along at x,y,z points(center), accordingly 38,34, and 38 for the x, y, z points at the center to the distinct particular binding site to carryout docking; however the standard drug CP was docked for the binding site of the receptor, thus obtained calculations were then compared at results of AITC.

Survival analysis of Hubs:

The tool KM Plotter was used here to elucidate survival rates in BC patients (Nagy *et al*., 2021). About 405 different BC samples (out of 7489 of all carcinoma types), and default parameters were chosen. In the tool, we input genes TP53 and EP300, and case studies were divided into 2 groups upper quartile and lower quartile with one set as default as "median". In the current study, the patients with gene expression groups above median values were referred to as "Higher expression" groups alternatively the patients screened with lower gene expression than the median were referred to as "Lower expression" groups. This log system integrates the Log-rank method of the Kaplan-Meier method, also univariate and multivariate Cox regression analysis which are crucial steps in survival rate detection.

RESULTS AND DISCUSSION

PPI's complex network analysis; Pathway enrichment

Here, in the "Molecular Complex Detection" analyzed Protein interaction network, 37 clusters were obtained with several nodes and edges. By assessing each MCODE result cluster, ranging from 1 to 37 nodes attributed to the MCODE cluster, 10 clusters were selected as major node involving was shown **(Fig.1).** These MCODE node statuses contain a list of cluster genes involved with one "seed" proteins namely - CEBPB, SSTR3, TAF9, GPHB5, IKBKB, CALCRL, EDN1, CASP10, ADRA2A, and GSTA2. Since all genes are not biologically involved and their functional biology is still not detected. Results gave molecular dense networks from huge protein interaction profiled data. It detected tightly bound phases of networks in protein networks as complex modules. It separates seed vertex along with their higher molecular weights in by outward traversing and initially beyond this, it weights every molecule based on their neighborhood densities (local). MCODE thus filtered every module with less than 2-core. Among 37 clusters, the vertices weighing lower molecular weights were removed, so provided 10 clusters of highly interconnected regions **(Fig.1)**. It has the advantage of fine turn-over the network of our interest without consideration of the mother network, this directed mode has an advantage than graph cluster methods. Then interconnectivity in modular proteins was detected.

Functional enrichment analysis of Key modules

ClueGO or CluePedia – a plug-in from Cytoscape assists to view the gene ontology features of genes from different datasets. Here, ClueGO visualized the gene ontology classes in selected protein complex networks. The gene ontology main classes- Molecular functions as well as biological process are terms of functional classification depicted in the PPI network **(Fig. 2a).** Here the kappa score was set to ≥ 0.4 (primary criteria) with Benjamini-Hochberg correction. This statistical operation is based on a hyper geometric test with two-sided with $p \leq 0.05$. Molecular function as well as biological process of DEG's obtains from complex protein network were enriched in reactive O_2 species response (GO term:0000302), protease binding (GO term:0002020), leukocyte activation participated in inflammatory response (GO term:0002269), acute inflammatory response (GO:0002526), DNA polymerase activity by RNA-direction(GO term:0003964), MAP kinase activation (GO term:0004707), regulation of Gluconeogenesis (GO:000611), G-coupled signaling pathway (GO term:0007187) and negative regulation tumor factor necrosis signaling pathway (GO term:0010804) **(Fig.2b).**Thus, KEGG pathway analysis and REACTOME path analysis showed sorted DEG's were predominantly expressed in central carbon metabolism in cancer (KEGG05230), intrinsic pathway for apoptosis (R-HAS:109606), Cytochrome P450-arranged by substrate type (R-HAS:211897), Regulation of TP53 Activity through Acetylation (R-HAS:6804758), Amine ligand-binding receptors (R-HAS:375280), Adipocytokine signaling pathway (KEGG:04920) and cytoprotection by HMOX1 (R-HAS:9707564) showed **(Fig. 2b).**Thus results from ClueGO, explained the DEG's alter the metabolic behavior in signaling pathways which directly linked to metabolic functions thus avoid raising complications such as tumor progression and metastasis.

Sub-network construction

The sub-networks obtained from MCODE modular genes, the genes bearing the highest degree parameter in sub-networks A & B are highlighted in red color **(Fig.3a & Fig. 3b).** By these parameters, we identified EP300 and TP53 as hubs that formed the core network. TP53 is called a tumor-suppressing gene and its mutation is recorded in some cancers (Olivier *et al*., 2010). TP53 mutation is associated with the main hallmarks of cancer such as cell proliferation, cell migration, growth in anchorage dependence, muscle invasion, apoptosis, tube colony formation, cell survival rate, and angiogenesis (Gutschner and Diederichs, 2012; Di Como *et al*., 1999) so it identified as a potential biomarker in various cancer (Termam *et al*., 2000). In sub-network type analysis TP53 ranked first in the protein-protein interaction network in degree parameter. The core gene TP53 is enriched with various biological functions such as ATP binding, kinase activity, PO^{4-} binding, MAPK signaling, etc., so it is recommended in BC

inhibition. The gene EP300 (E1A binding protein p300) encodes the functional protein for transcriptional regulation by histone acetylation. The gene EP300 shows its effect of about 4.12% in several cancer types and for bladder cancer - bladder urothelial cancer (Zhu *et al*., 2020) showing a variety of prevalence in molecular alteration.

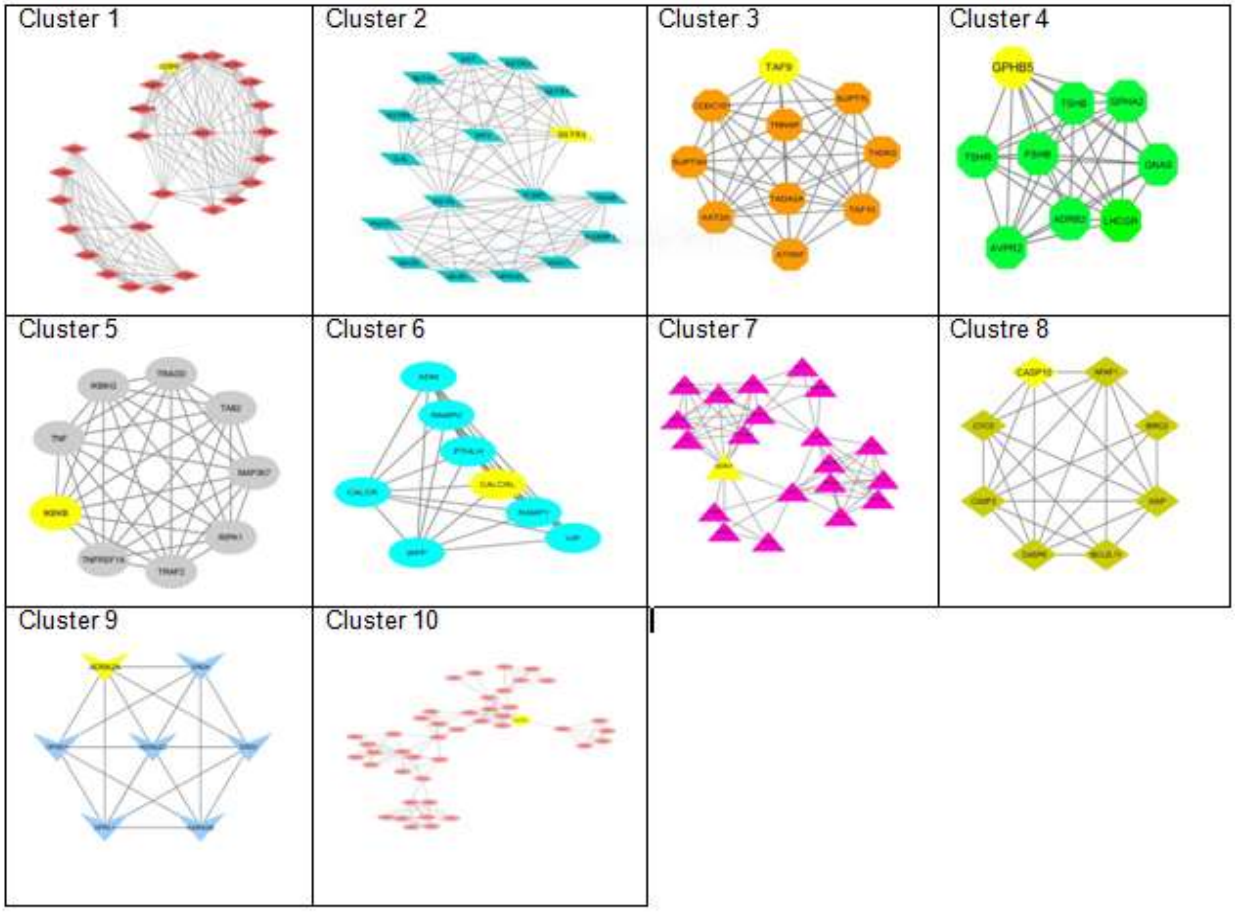

Fig.1. Predicted complex modules by MCODE; 1-10 clusters. The seed proteins which are responsible for forming the clusters are highlighted in yellow colour as CEBPB, SSTR3, TAF9, GPHB5, IKBKB, CALCRL, EDN1, CASP10, ADRA2A, and GSTA2.

Fig. 2a. ClueGO in detailed Gene ontology classes (Molecular function and Biological process).

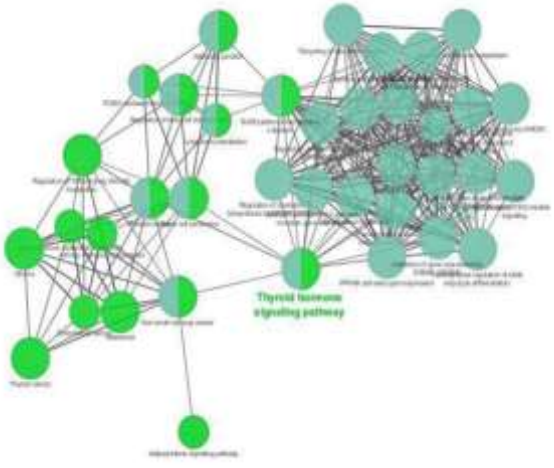

Fig. 2b. ClueGO Pathway enrichment

Fig. 2. In CluGO gene ontology classes, the green colour classification represents Molecular function (MF) and the blue colour in the graph chart is of Biological process (BP) in **Fig. 2a.** In ClueGO Pathway enrichment; the top enriched items and from KEGG were displayed using nodes interaction. Enrichment by Gene Ontology (GO) terms was visualized using the ClueGo/CluePedia plug-in from Cytoscape. Vital molecular functions (MF) and biological processes (BP) involved in the DEGs are shown with the specific gene interactions. The MF and BP enrichment analyses are inferred from the modular genes by MCODE clusters. The connectivity of the GO terms network is described by functional nodes and edges that are shared between the DEGs with a kappa score of 0.4. The enrichment shows only significant GO terms (p-value ≤0.05). The values of p≤0.05 indicate the node size. The node color code indicates the specific functional class that they are involved in. The colour represents various MF and BP involved in the enrichment analysis. The colored fonts indicate the most important functional GO terms that define the names of MF and BP of each group in **Fig. 2b.**

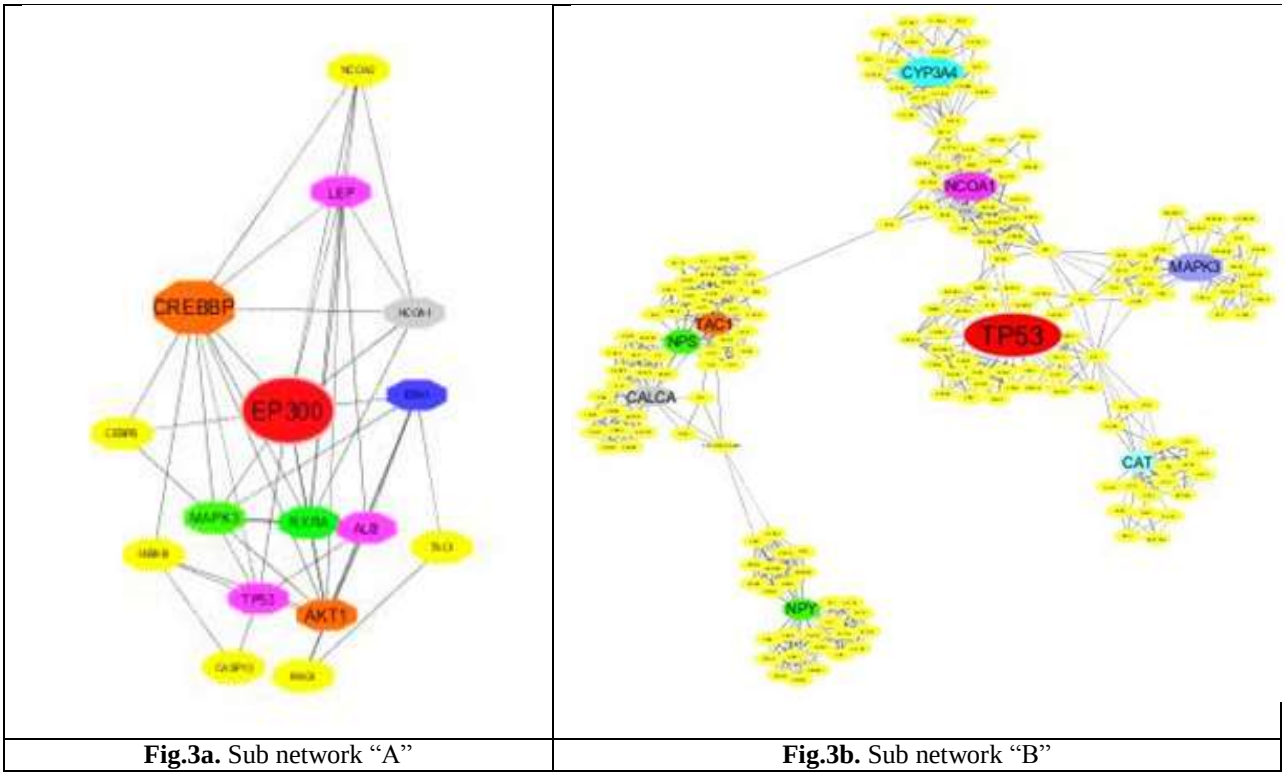

Fig.3a. Sub network "A" **Fig.3b.** Sub network "B"

Fig.3. In Sub network "A" generated in Cytoscape, EP300 ranked first in degree topology. CREBBP stands next to it. Followed by AKT1, CDN1, TP53, ALB, MAPK3, RXRA, LEP, and NCOA ranked in 10th score in the topological parameter in **Fig. 3a.** Whereas in Sub network "B" generated by Cytoscape having a dense network of DEGs from Modules. Here, TP53 comes under the first rank in Degree, and TAC1 stands next to it. Followed by MAPK3, NCOA1, CYP3A4, CALCA, TAC1, NPS, NPY, and CAT as the highest rank scores based on Degree in **Fig. 3b.**

Detection of Pathway enrichment analysis in sub-networks A and B

DAVID classification of pathway enrichment analysis showed 56 regulating pathways **(Table 1)**, where the hubs TP53 and EP300 are majorly involved in biological classes (Hu *et al*., 2009; Yuan *et al*., 2022). Results obtained from the KEGG enrichment pathway with our targeted genes closely regulated by the compound AITC were actively involved in 80 signaling pathways however 31 among them are involved in cancer-assisted terms as follows: cancer-MicroRNAs, transcriptional misregulation, glioma, cell cycle, and PI3K-Akt pathways shown in **Table 1**. Here, the two key proteins TP53 and EP300 underwent functional pathway enrichment analysis. From this

study, 80 pathways were generated (from Hsa04919 to Hsa05202), with their Gene IDs, Gene ontology terms, and % score involved.

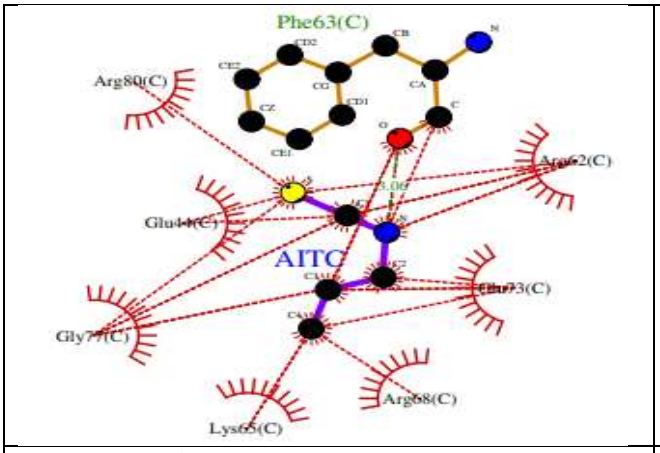

Fig.4a. AITC-TP53 interaction

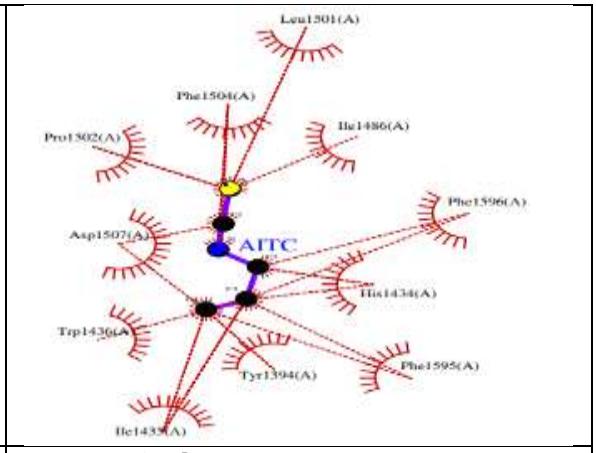

Fig.4b. AITC-EP300 interaction

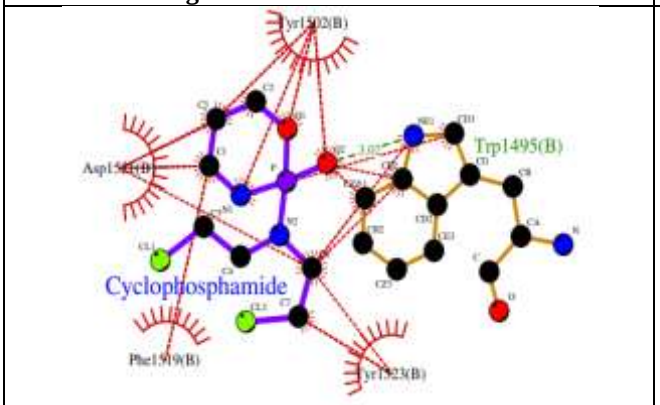

Fig.4d. CP-TP53 interaction

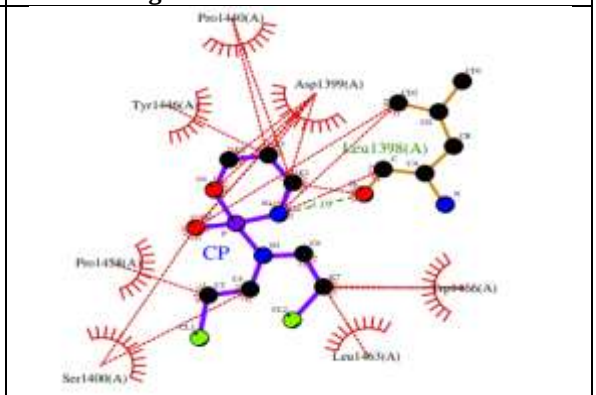

Fig.4d. CP-EP300 interaction

Fig.4. Molecular docking analysis of AITC and CP against TP53 (PDB ID: 6MXZ) and EP300 (PDB ID: 6V8B), from Fig.4a to Fig.4d (Detailed interaction profile).

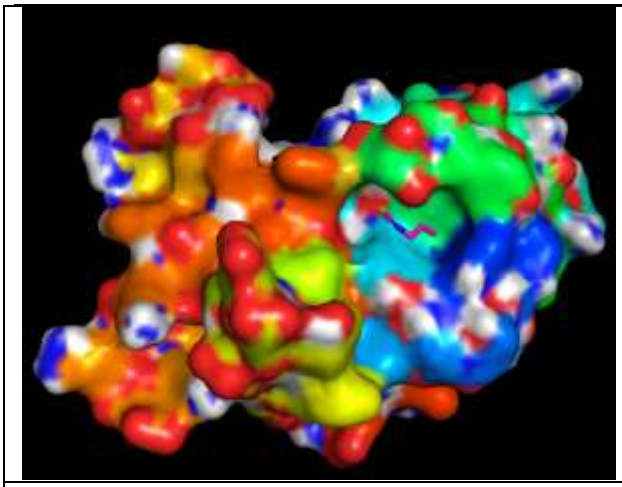

Fig. 5. Docking generated by AutoDock Vina software, TP53 domains (PDB ID: 6MXZ) with ligand – Allyl isothiocyante.

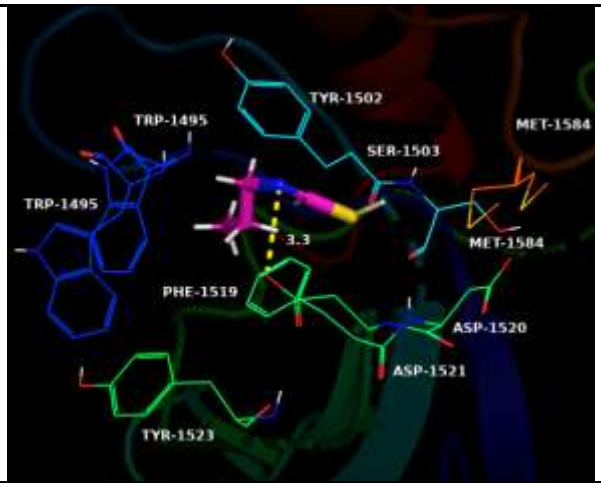

Fig. 6. Details of interaction between Allyl isothiocyante and amino acid present in protein TP53 domains (PDB ID:6MXZ).

Fig.5 and Fig.6. Molecular docking analysis of TP53domains with ligand- AITC; Detailed interaction profile.

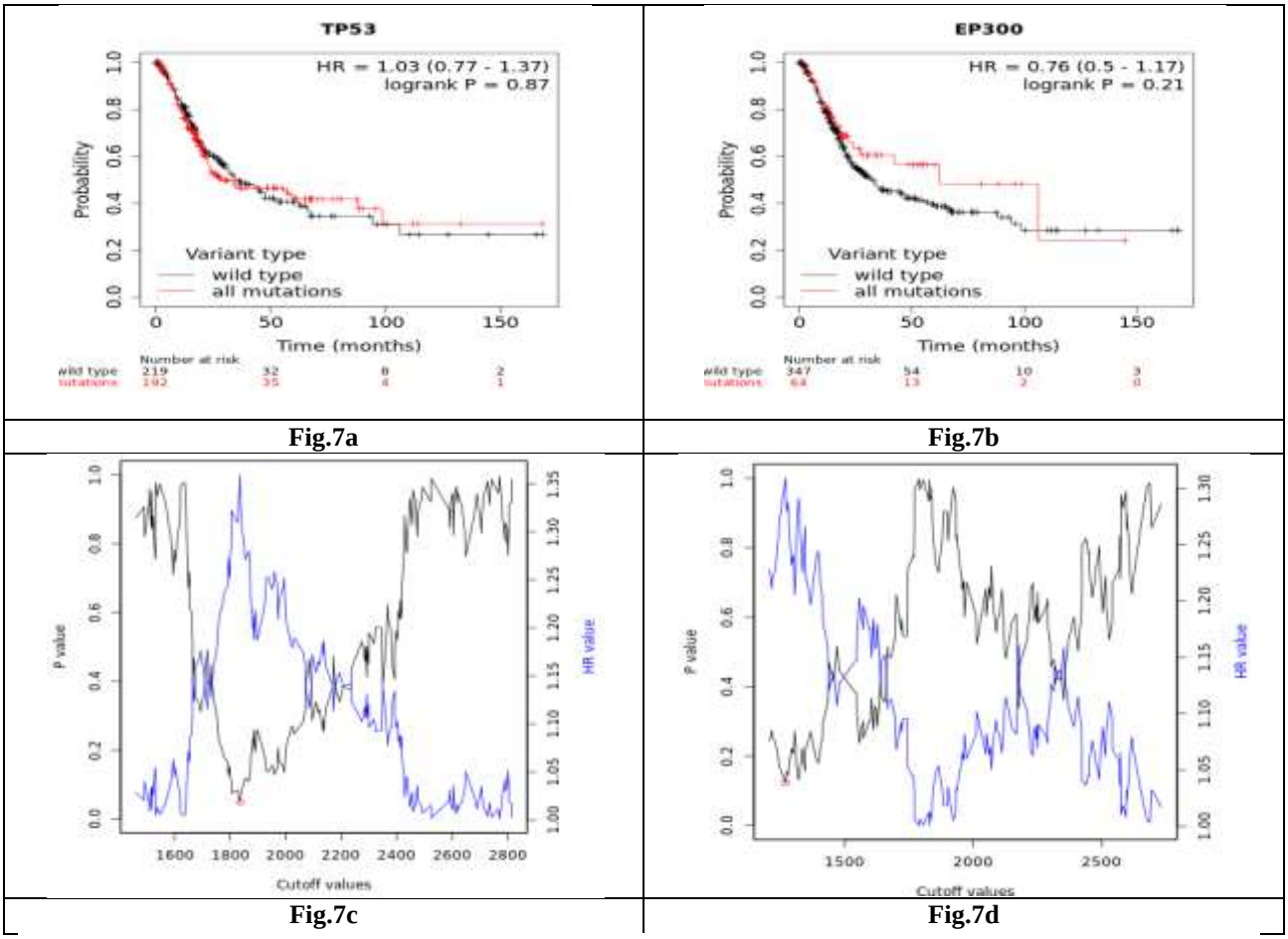

Fig.7a **Fig.7b**

Fig.7c **Fig.7d**

Fig.7. Kaplan–Meier survival curves showing validation of the two-gene models TP53 and EP300 based on clinical characteristics in **Fig. 7a** and **Fig. 7b.** Survival analysis of obtained biomarker genes TP53 **(Fig.7a)** and EP300 **(Fig.7b)** are found to validated biomarker genes for anti-cancer studies. In Tp53, HR > 1, this indicates that the low expression group has a higher chance of survival. The Cutoff value used in the analysis is 1270, with significance vs. cutoff values between lower and upper quartiles of expression in **Fig.7c** and **Fig. 7d.**

Molecular-Dock study

Molecular docking analysis of TP53 and EP300 with AITC ligand and CP was performed. Both compounds were ranked in molecular docking based on their binding energy values. The interpreted results were checked between ligand AITC and CP with receptors- TP53, EP300, and the detailed result of the interaction profile **(Table 2).** Lower binding and free energy values along with H_2 bond formation are considered for validation in the study. Results indicate that amino acid residues Arg80, Glu44, Gly77, Lys65, Arg68, Glu73, and Arg62 were involved in hydrophobic bond formation and Phe63 was involved in the formation of Hydrogen bonds with bond length 3.06 A°. For EP300, the amino acid residues Pro1502; Asp1507, Trp 1436, Ile1435, Tyr1394, Phe1595, His1434, Phe1596, Ile1486, Leu1501, and Phe1504 involved in hydrophobic interaction but no formation of hydrogen bonds. The bond distances between ligands AITC and CP with receptors TP53 and EP300 were shown **(Fig. 4a to Fig. 4d)**. Results of TP53 with AITC (-5 kcal/ mol) and EP300 (-4.0 kcal/mol) showed better inhibition by exhibiting interactions. From the present study, it can be suggested that TP53 is a better inhibitor than EP300 by its binding affinity and H-bond formation with the active pocket **(Fig. 5).** Detailed interaction between Allyl isothiocyanate and amino acid present in protein TP53 domains (PDB ID: 6MXZ) was shown **(Fig.6)**. However, both serve better hallmarks for bladder cancer by their results showing values nearby standard drug Cyclophosphamide and may use in cancer treatment.

Survival analysis of Hubs

In the current study, obtained samples were n=485 (BC). Where n is the number of patients with available clinical data, the KM-Plotter was applied for the survival test. The parameters were set to default and restricted the sample data for a subtype of cancer i.e., BC. The prognostic ability of each final hub gene i.e., TP53 and EP300 is checked to distinguish the higher expression groups and lower expression groups **(Fig.7a to Fig.7d)**. For TP53, the expression range of the probe is 119 – 7996. Restrict analysis to subtypes and restrict analysis based on cellular content is selected all as default resulting P-value is 0.1233 and FDR IS 100%. In Median survival, the Low expression cohort (months) is found to be 55.67 and the High expression cohort (months) is found as 32.47. For EP300, the Cutoff value used in the analysis is 1835. Expression range of the probe id 232-6646. Here the best cutoff was checked and Censored at the threshold was checked. No restriction for the analysis of subtypes like gender, race, stage, grade, and mutation burden and no restriction for cellular content like Mesenchymal stem cellular contents, Regulatory T-cell count, CD8+memory cell, and Type-2 T-helper cell type, etc., For EP300 P value, is found as 0.0495 and FDR is over 50% and Computed Median survival as Low expression cohort (months) is 62.3 and High expression cohort (months) is 25.93. Kaplan Meier survival plot shows non-treated TP53 biomarker has prognostic capability by expressing lower groups having more chance of survival compared with higher expressed groups whereas the treated EP300 biomarker has the prognostic ability by showing higher expression in groups has more chance in survival rate compared to low-expressed groups.

Table 1. GO functional pathway enrichment analysis showing 56 Pathways with their associated genes (data generated by DAVID).

GO ID	GO Term	%	Associated genes found
Hsa04919	Thyroid hormone signaling pathway	22.6	[EP300,TP53,AKT1,CREBBP,MAPK3,NCAO 1,RXRA]
Hsa05161	Hepatitis B	22.6	[EP300,TP53,AKT1,CREBBP,CASP10,IKBK B,MAPK3]
Hsa05215	Prostate cancer	19.4	[EP300, TP53, AKT1, CREBBP, IKBKB, MAPK3]
Hsa05152	Tuberculosis	19.4	[EP300, AKT1, CEBPB, CREBBP, CASP10, MAPK3]
Hsa04068	FoxO signaling pathway	19.4	[EP300, AKT1,CREBBP, IKBKB]
Hsa04151	PI3K-Akt signaling pathway	19.4	[TP53,AKT1,IKBKB,IRS1,MAPK3,RXRA]
Hsa04024	cAMP siganling pathway	19.4	[EP300, AKT1, CREBBP, EDN1, GPHB5, MAPK3]
Hsa04935	Growth hormone synthesis, secretion and action	19.4	[EP300, AKT1, CREBBP, IRS1, MAPK3, SSTR3]
Hsa05167	Kaposi-sarcoma associated herpes virus infection	19.4	[EP300, TP53, CREBBP, IKBKB, MAPK3, MAPK3]
Hsa05166	Human T-cell leukemia virus 1 infection	19.4	[EP300, TP53, AKT1, CREBBP, IKBKB, MAPK3,]
Hsa05206	MicroRNAs in cancer	19.4	[EP300,TP53, CREBBP, IKBKB, IRS1, MAPK3]
Hsa05165	Human papilloma virus infection	19.4	[EP300, TP53, AKT1, CREBBP, IKBKB, MAPK3]
Hsa05166	HTLV-infection	17.8	[EP300, TP53, IKBKB, CREBBP, AKT1]
Hsa04066	HIF-1 signaling pathway	16.1	[EP300, AKT1, CREBBP, EDN1, MAPK3]
Hsa05160	Hepatitis C	16.1	[TP53, AKT1, IKBKB, MAPK3, RXRA]
Hsa04210	Apoptosis	16.1	[TP53, AKT1,CASP10, IKBKB, MAPK3]
Hsa05164	Influenza A	16.1	[EP300, AKT1, CREBBP, IKBKB, MAPK3]
Hsa04722	Neurotrophin signaling pathway	16.1	[TP53, AKT1,IKBKB, IRS1, MAPK3]
Hsa04071	Sphingolipid signaling pathway	16.1	[TP53, AKT1, KNG1, MAPK3, OPRD1]
Hsa05417	Lipid and atherosclerosis	16.1	[TP53, AKT1, IKBKB, MAPK3, RXRA]
Hsa05418	Fluid shear stress and atherosclerosis	16.1	[TP53,AKT1,EDN1, GSTA2, IKBKB]
Hsa05206	Micro RNAs in cancer	14.2	[EP300, TP53, IKBKB, CREBBP]
Hsa05168	Herpes simples infection	14.2	[EP300, TP53, IKBKB, CREBBP]

Hsa05212	Pancreatic cancer	12.9	[TP53,AKT1, IKBKB, MAPK3]
Hsa05211	Renal cell carcinoma	12.9	[EP300, AKT1, CREBBP, MAPK3]
Hsa05220	Chronic myeloid leukemia Yuan	12.9	[TP53, AKT1, IKBKB, MAPK3]
Hsa05222	Small cell lung cancer	12.9	[TP53, AKT1,IKBKB, RXRA]
Hsa04916	Melanogenesis	12.9	[EP300,CREBBP, EDN1,MAPK3]
Hsa04630	Jak-STAT signaling pathway	12.9	[EP300, AKT1, CREBBP, LEP]
Hsa05203	Viral carcinogenesis	12.9	[EP300, TP53, CREBBP, MAPK3]
Hsa04010	MAPK signaling pathway	12.9	[TP53, AKT1, IKBKB, MAPK3]
Hsa05224	Breast cancer	12.9	[TP53, AKT1, MAPK3, NCOA1]
Hsa05226	Gastric cancer	12.9	[TP53, AKT1, MAPK3, RXRA]
Hsa05225	Hepatocellular carcinoma	12.9	[TP53, AKT1, GSTA2, MAPK3]
Hsa05163	Human cytomegalo virus infection	12.9	[TP53, AKT1, IKBKB, MAPK3]
Hsa05131	Shigellosis	12.9	[TP53, AKT1, IKBKB, MAPK3]
Hsa01524	Platinum drug resistance	12.9	[TP53, AKT1, GSTA2, MAPK3]
Hsa05223	Non-small cell lung cancer	12.9	[TP53, AKT1, MAPK3, RXRA]
Hsa05200	Pathways in cancer	11	[EP300, TP53, AKT1, CREBBP, EDN1, GSTA2, IKBKB, KNG1, MAPK3, NCOA1, RXRA]
Hsa05169	Epstein-Barr virus infection	10.7	[TP53, EP300, CREBBP]
Hsa04310	Wnt signaling pathway	10.7	[EP300, TP53, CREBBP]
Hsa05202	Transcriptional misregulation in cancer	10.7	[TP53, CEBPB,RXRA]
Hsa05216	Thyroid cancer	9.7	[TP53, MAPK3, RXRA]
Hsa05213	Endometrial cancer	9.7	[TP53, AKT1, MAPK3]
Hsa05210	Colorectal cancer	9.7	[TP53, AKT1, MAPK3]
Hsa05230	Central carbon metabolism in cancer	9.7	[TP53, AKT1, MAPK3]
Hsa05214	Glioma	9.7	[TP53, AKT1, MAPK3]
Hsa04720	Long-term potentiation	9.7	[EP300, CREBBP, MAPK3]
Hsa05218	Melanoma	9.7	[TP53,AKT1, MAPK3]
Hsa04520	Adherens junction	9.7	[EP300, CREBBP, MAPK3]
Hsa04350	TGF-beta signaling pathway	9.7	[EP300, CREBBP, MAPK3]
Hsa04922	Glucagon signaling pathway	9.7	[EP300, AKT1, CREBBP]
Hsa04110	Cell cycle	9.7	[EP300,TP53, CREBBP]
Hsa04211	Longevity regulating pathway	9.7	[TP53,AKT1,IRS1]
Hsa01522	Endocrine resistance	9.7	[TP53,AKT1,MAPK3]
Hsa05162	Measles	9.7	[TP53,AKT1,IKBKB]

Table 2. Molecular docking analysis of AITC and Cyclophosphamide (CP) against TP53 and EP300.

Compounds	**Affinity (kcal/mol)**	**Protein-ligand interaction**			
		Number of H-bond	**H-bond interactions**	**Distance (A)**	**Hydrophobic interactions**
AITC + TP53	-5.0	1	Phe63;	3.06;	Arg80; Glu44; Gly 77; Lys65; Arg68; Glu73; Arg62
AITC+EP300	-4.0	-	-	-	Pro1502; Asp1507; Trp 1436; Ile1435; Tyr1394, Phe1595, His1434, Phe1596; Ile1486; Leu1501; Phe1504
CP+TP53	-6.3	1	Trp1495;	3.2;	Tyr1502;Tyr1523; Asp1521; Phe1519;
CP+EP300	-5.6	1	Leu1398;	3.19;	Pro1440; Tyr1446; Asp1399; Pro1458; Ser1400; Leu1463; Trp1466;

CONCLUSIONS

Through the network pharmacological approach, the most crucial biomarkers for bladder cancer therapy which are possibly involved in the pathogenesis of cancer and the patient's prognosis obtained were validated. The protein interaction profile framework was developed to show clearly the hubs may be part of the regulation in cell-cycle, a major role in DNA repair and mitosis part. This study discovered validation of biomarkers for bladder cancer – EP300 and TP53, and their huge impact on the human cellular metabolic system, this would achieve complete experimental data for new research scientists to develop a treatment for BC and their dynamics.

Altogether, the discovery in this study uncovered extensive use of AITC as an anti-BC medication in future aspects from the molecular point of view. This study helps with how AITC causes its anti-cancerous effect in the human body.

Ethical approval

Not applicable

Availability of the data materials

Databases obtained and analyzed in the present study are available in [https: //string.db. org, https://www.cytoscape. org, https://david.ncifcrf.gov//ClueGOPlugin and/or CluePedia app at http://cytoscape.org/apps/cluego and/or http://cytoscape.org/apps/cluepedia license key obtained from Gabriela Bindea, Laboratory of integrative Cancer Immunology INSERUM UMRS de I'Ecolede Medicine-75006 (Paris). France. URL:http;//www.ici.upmc.fr and Passwords for running LigPlot+ programs were downloaded from Roman with academic version v.2.1.

Fund & Consent for publication

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Author's contribution

All authors listed in this article participated in the research. The manuscript was approved by all authors.

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