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In Vitro Inhibitory Activity of Itraconazole on Proliferation of T47D and MCF-7 Cancer Cell Lines

Jakkanaboina Tilak Vijay[1], Kandimalla Vivek Babu[2], Addepally Uma[3]
[1]*Centre for Biotechnology, Institute of Science &Technology, Jawaharlal Nehru Technological University, Hyderabad, Telungana, India; J. TilakVijay*
[2]*Manager-R&D, Biological E Limited, Kolthur, Shameerpet, Hyderabad, Telungana, India*
[3]*Centre for Biotechnology, Institute of Science &Technology, Jawaharlal Nehru Technological University, Hyderabad, Telungana, India*

Abstract: Cobicistat, Lomitapide, Itraconazole, Plerixafor, Azilsartanmedoxomil and Zafirlukast were selected to evaluate their inhibitory properties against ERα in vitro using MTT assay.T47D and MCF-7 cancer cells selected in the study are known to overexpress ERα.MTT assay relies on the ability of live but not dead cells to reduce a water-soluble yellow dye MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a water-insoluble purple formazan product. In this study, a cell-based assay was used to demonstrate the potency of the five drugs in comparison with standard drug, tamoxifen. The methodical investigational stages in MTT assay are carried out which determined the potential cytotoxicity of drugs at different concentrations. Cell viability gradually decreased in a dose-dependent manner. The IC_{50} values were found to be in the range 11.27 – 23.48 □g/ml and I_{max} in the range 53.12 – 93.02 % for T47D and 11.48 – 18.46 □g/ml and I_{max} in the range 54.71 – 90.42 % for MCF-7 cell lines, respectively. Itracozanole exhibited almost similar inhibitory nature in comparison with Tamoxifen when tested on T47D and MCF-7 cell lines. Therefore, it can be emphasized that Itraconazole, which is a triazole antifungal agent which inhibits cytochrome P-450-dependent enzymes has the ability to inhibit T47D and MCF-7 breast cancer cell lines in a dose dependent manner.

Keywords: T46D, MCF-7, breast cancer, MTT assay

I. INTRODUCTION

The study of cell proliferation and cell viability requires the accurate quantification of the number of viable cells in a cell culture. Therefore, assays for calculating cell viability are necessary for optimizing cell culture conditions, evaluating cell growth factors and nutrients, discovering novel antibiotics and anti-cancer drugs, evaluating toxic effects of environmental pollutants and cell mediated toxicity and studying programmed cell death (apoptosis).MTT Cell Proliferation Assay provides a colorimetric format for measuring and monitoring cell proliferation. Cells can be plated and then treated with compounds or agents that affect proliferation. Cells are then detected with the proliferation reagent, which is converted in live cells from the yellow tetrazole MTT to the purple formazan form by a cellular reductase. Berridge MV *et al* (2005)[1] The major advantage of MTT over other assays which use isotope incorporation as an end point is that the substrate and the product absorb at different wavelengths, such that no washing steps are required prior to solubilization of the MTT-formazan product. The product is used as an estimate of cell survival after an exposure to the drug. Certain drugs which are specific against a particular disease were found to be effective against other disease conditions as well, for example, Pioglitazone, a drug used for type 2 diabetes, may prevent recurrent stroke and heart attacks in people with insulin resistance but without diabetes Kernan WN et al. (2016)[2] Meng Lee et al. (2017)[3]. Several studies indicate that persons with type-2 diabetes are at higher risk of cancer of the pancreas, liver, endometrium, breast, colon, rectum and urinary bladder Smith U & Gale EA *et al* (2010)[4], however, the use of metformin was associated with decreased risk of the occurrence of various types of cancers, especially of pancreas and colon and hepatocellular carcinoma JacekKasznicki et al.(2014)[5]. Evidence suggested that metformin might reduce breast cancer incidence in postmenopausal women Chlebowski RT *et al* (2012)[6]. In another study, by screening already approved drugs, researchers identified calcium channel blockers, which are used to treat hypertension, can efficiently stop cancer cell invasion *in vitro* Guillaume Jacquemet et al (2016)[7]. Preliminary investigations revealed that Gleevec blocked the progression and development of rheumatoid arthritis in laboratory mice Ricardo T. Paniagua et al (2006)[8]. On the other hand, common air borne allergies can be attenuated by using cancer drug Ibrutinib, for patients with certain types of leukemia and

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lymphoma Jennifer A. Regan et al (2017)[9]. It has been reported that an Alzheimer's drug DBZ was found to reduce esophageal cancer in rats and could be an effective way to treat Barrett's esophagus

Therefore, in this context, 2035 FDA approved drugs from DrugBank database subjected to molecular docking resulted in top 5 drugs viz., Cobicistat, Lomitapide, Itraconazole, Plerixafor, Azilsartanmedoxomil and Zafirlukast showed better binding affinities than ERα bound tamoxifen [unpublished data]. Hence, in this study, these five drugs were selected to evaluate their inhibitory properties against ERα *in vitro* expressed in T47D and MCF-7 breast cancer cell lines.

II. MATERIALS AND METHODS

A. Drugs For In Vitro Screening

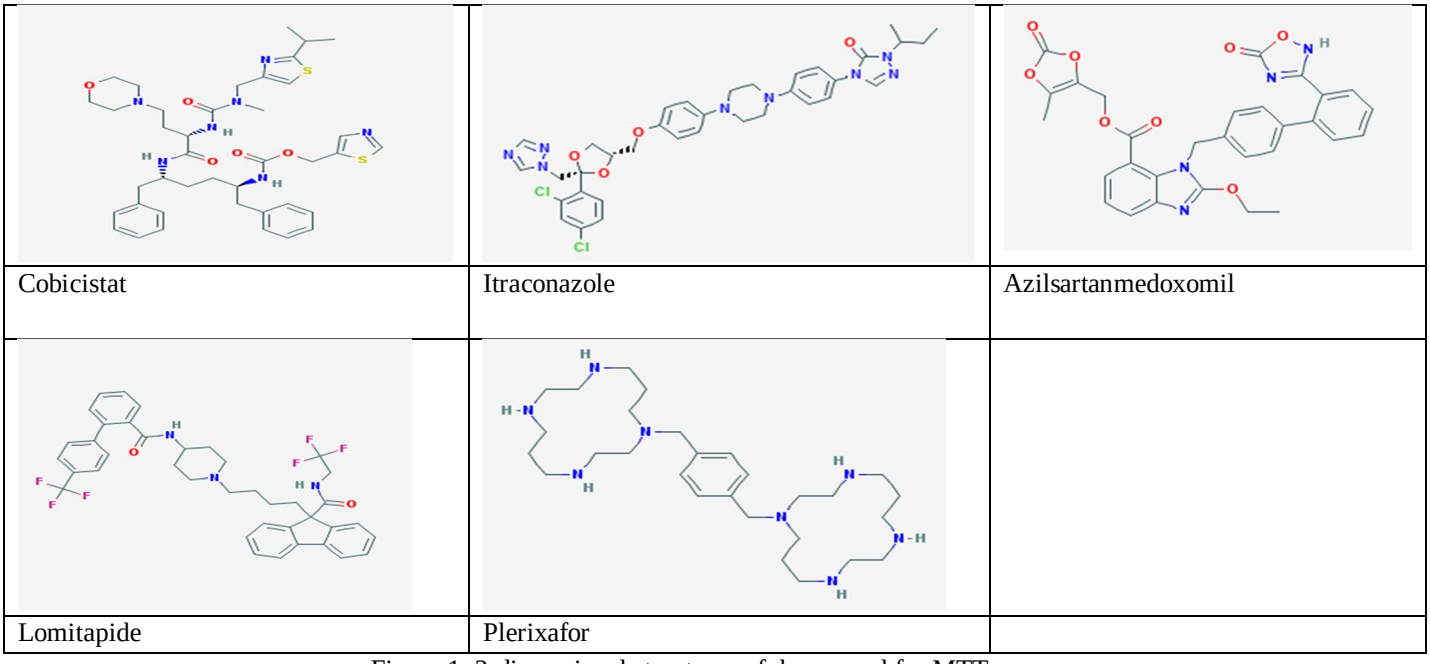
Using computer-aided drug design tools, a robust QSAR modelwas generated that explained the structure-activity relationships (SARs) of ERα inhibitors in a quantitative manner. Followed by this, a molecular docking analysis on 2035 FDA approved drugs resulted in 15 drugs which showed better binding affinities than ERα bound tamoxifen. Finally, top five drugs viz., Cobicistat, Lomitapide, Itraconazole, Plerixafor, Azilsartanmedoxomil and Zafirlukast were selected to evaluate their inhibitory properties against ERα *in vitro*.

B. Cell Culture

T47D and MCF-7 cancer cells selected in the study are known to overexpress ERα. The cells weremaintained in Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose and 2 mM L-glutamine, and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

C. MTT Assay

Plated and cultured T47D and MCF-7 cells (100 µL per well) in a clear bottom 96-well tissue culture plates. The number of cells were 10^5 cells per well.Added test samples (Cobicistat, Lomitapide, Itraconazole, Plerixafor, Azilsartanmedoxomil and Zafirlukast) with concentrations ranging from 2.5 to 160 µg/ml (2.5, 5, 10, 20, 40, 80 and 160 µg/ml) in triplicate after 24 hr of seeding and incubated the cells for 48 hr period of time. A volume of 20 µL culture medium was used for all test samples. After incubation, the medium was removed and cells were washed with PBS twice. Further, 15 µL MTT reagent was added in each well and made up with PBS medium to a final concentration of 0.5 mg/ml. The volume of the reagent adjusted depending on the volume of the cell culture. Cells were incubated for 3 hours at 37°C until intracellular purple formazan crystals are visible under microscope. Removed MTT reagent and added 100 µL of the DMSO to each well and mixed gently on an orbital shaker for one hour at room temperature Morgan DML. *et al* (1998)[10] Absorbance was measured at OD570nm for each well on an absorbance plate reader.



Cobicistat	Itraconazole	Azilsartanmedoxomil
Lomitapide	Plerixafor	

Figure 1: 2-dimensional structures of drugs used for MTT assay.

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D. Inhibitory Data

The 2-dimensional structures of all drugs are given in Fig. 1. Mean OD values of each test compound were corrected by subtracting with the mean OD of blanks. Relative percent inhibition activity is expressed as:

%inhibition = 100 – (corrected mean OD of sample x 100 / corrected mean OD of control)

The mean concentrations of each compound that generated 50 % inhibitions (IC_{50}) were calculated Boyd MR & Paull KD.(1995)[11] and the dose-response curve of the growth-inhibiting effect of test compounds on cancer was determined using Origin 2019 software to determine the IC_{50} (µg/ml). All experiments were performed in triplicate.

III. RESULTS AND DISCUSSION

MTT assay is widely used for accurate determinations of drug sensitivity and as the end-point in many drug screening assays Plumb JA *et al* (1989)[12].In this study, a cell-based assay was used to demonstrate the potency of the five drugsin comparison with standard drug, tamoxifen. The methodical investigational stages in MTT assay are carried outwhich determined the potential cytotoxicity of drugs at different concentrations. It has been evidenced from data that a decreasing absorbance at 540 nm was observed in the well which received increasing concentration of the drug in comparison to the control cells without any treatment. A decreased absorbance was observed in wells treated with drug suggesting cytotoxicity Alley MC *et al* (1986)[13] Mosmann T. (1983)[14]. IC_{50} is the concentration of the tested drug able to cause the death of 50% of the cells and can be predictive of the degree of cytotoxic effect. The lower the value, the more cytotoxic is the substance. Table 1 and Figure 2, 3 shows the comparison of the IC_{50} of five drugs in comparison with tamoxifen against human T47D and MCF-7 cancer cell lines.

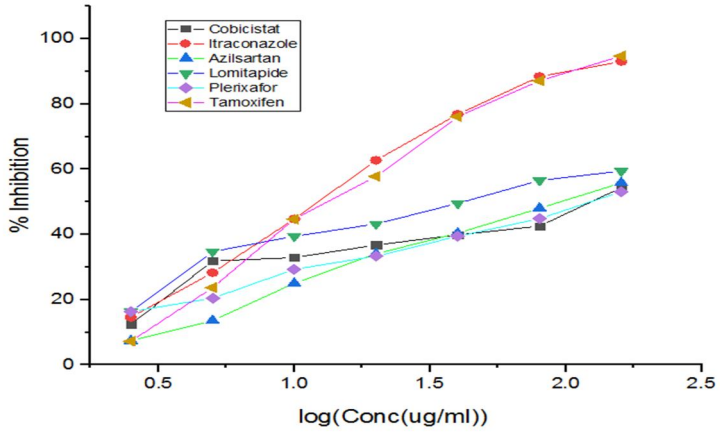

Figure 2: Comparison of the dose-response curve of five drugs with tamoxifen against T47D cancer cell lines.

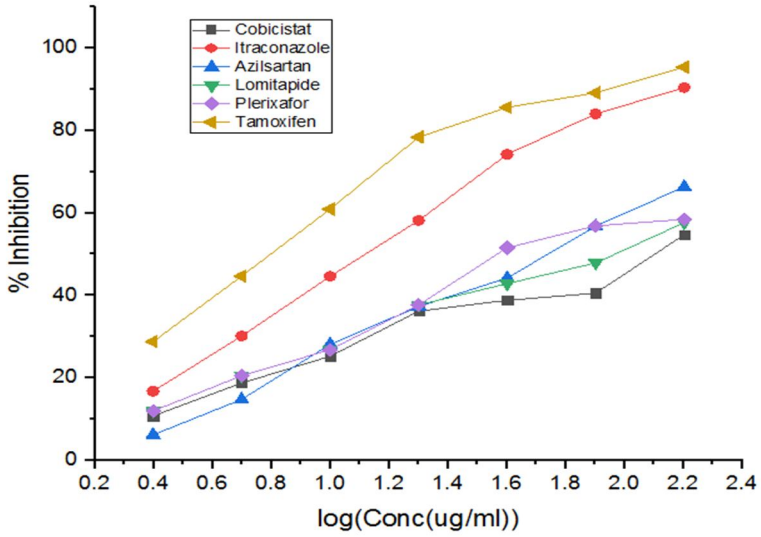

Figure 3: Comparison of the dose-response curve of five drugs with tamoxifen against MCF-7 cancer cell lines.

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Table 1: Comparison of the IC_{50} values of five drugs with tamoxifen.

	IC_{50}ug/ml	
Drug	T47D	MCF-7
Cobicistat	13.77	16.09
Itraconazole	11.27	11.48
Azilsartan	16.58	18.46
Lomitapide	18.54	17.12
Plerixafor	23.48	16.15
Tamoxifen	6.07	6.42

Cancer cells were exposed to the selected drugs for 48 h and cell viability was evaluated by MTT assay. It was found that cell viability gradually decreased in a dose-dependent manner. The IC_{50} values were found to be in the range 11.27 – 23.48 □g/ml and I_{max} in the range 53.12 – 93.02 % for T47D and 11.48 – 18.46 □g/ml and I_{max} in the range 54.71 – 90.42 % for MCF-7 cell lines, (Tables 2 and 3) respectively.

Table 2: Inhibitory effects of five drugs on growth of T47D cells cultured *in vitro*.

Conc.	% Inhibition					
(□g/ml)	Cobicistat	Itraconazole	Azilsartan	Lomitapide	Plerixafor	Tamoxifen
2.5	12.46	14.64	7.42	16.39	16.39	7.26
5	31.86	28.31	13.61	34.75	20.45	23.68
10	32.91	44.74	25.04	39.44	29.33	44.72
20	36.76	62.75	34.06	43.22	33.34	57.81
40	39.89	76.83	40.37	49.55	39.45	76.14
80	42.57	88.42	48.04	56.56	44.86	87.13
160	54.37	93.02	55.83	59.44	53.12	94.72
			IC_{50} (□g/ml)			
	13.77	11.27	16.58	18.54	23.48	6.07

Table 3:Inhibitory effects of five drugs on growth of MCF-7 cells cultured *in vitro*.

Conc.	% Inhibition					
(□g/ml)	Cobicistat	Itraconazole	Azilsartan	Lomitapide	Plerixafor	Tamoxifen
2.5	10.73	16.71	6.14	11.91	11.91	28.75
5	18.71	30.13	14.73	20.48	20.48	44.68
10	25.2	44.61	28.11	26.79	26.79	60.94
20	36.16	58.18	37.29	37.66	37.66	78.4
40	38.77	74.25	44.19	42.84	51.56	85.64
80	40.53	84.04	56.79	47.88	56.88	89.15
160	54.71	90.42	66.32	57.66	58.45	95.41
			IC_{50} (□g/ml)			
	16.09	11.48	18.46	17.12	16.15	6.42

The morphology of the cells treated with the drugs appeared significantly different as compared to untreated control cells, which could probably due to the growth inhibitory and cell death initiating ability of the screeneddrugs.As is evidenced from figures 2 and 3, Itracozanole exhibited almost similar inhibitory nature in comparison with Tamoxifen when tested on T47D cell lines. However, against MCF-7 cell lines, the percent variation at the highest tested dose, 160 ug/ml concentration was found to be only 5%.
At a tested concentration of 40 ug/ml dose, none of the drugs surpassed 70% inhibition except Itracozanoleand Tamoxifen in both T47D and MCF-7 cells. Therefore, from the data it can be emphasized that Itraconazole has the ability to suppress and inhibit the growth characteristics of T47D and MCF-7 cell lines which are found to be overly expressed in breast cancers.

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IV. CONCLUSION

This study demonstrated that out of five drugs, Itraconazole displayed similar efficacy compared with tamoxifen and showed comparable results as proven by *in silico* computational virtual screening analysis and *in vitro* cytotoxicity assay. Finally, it is therefore stated that Itraconazole which is a triazole antifungal agent which inhibits cytochrome P-450-dependent enzymes has the ability to inhibit T47D and MCF-7 breast cancer cell lines in a dose dependent manner. The probable mechanism by which Itraconazole supresses the active cells might be due to the binding of drug to the ERa receptor as reported in our *in silico* study. This research paves a way to find more such probable compounds where certain drugs which are specific against a particular disease were found to be effective against other disease states.

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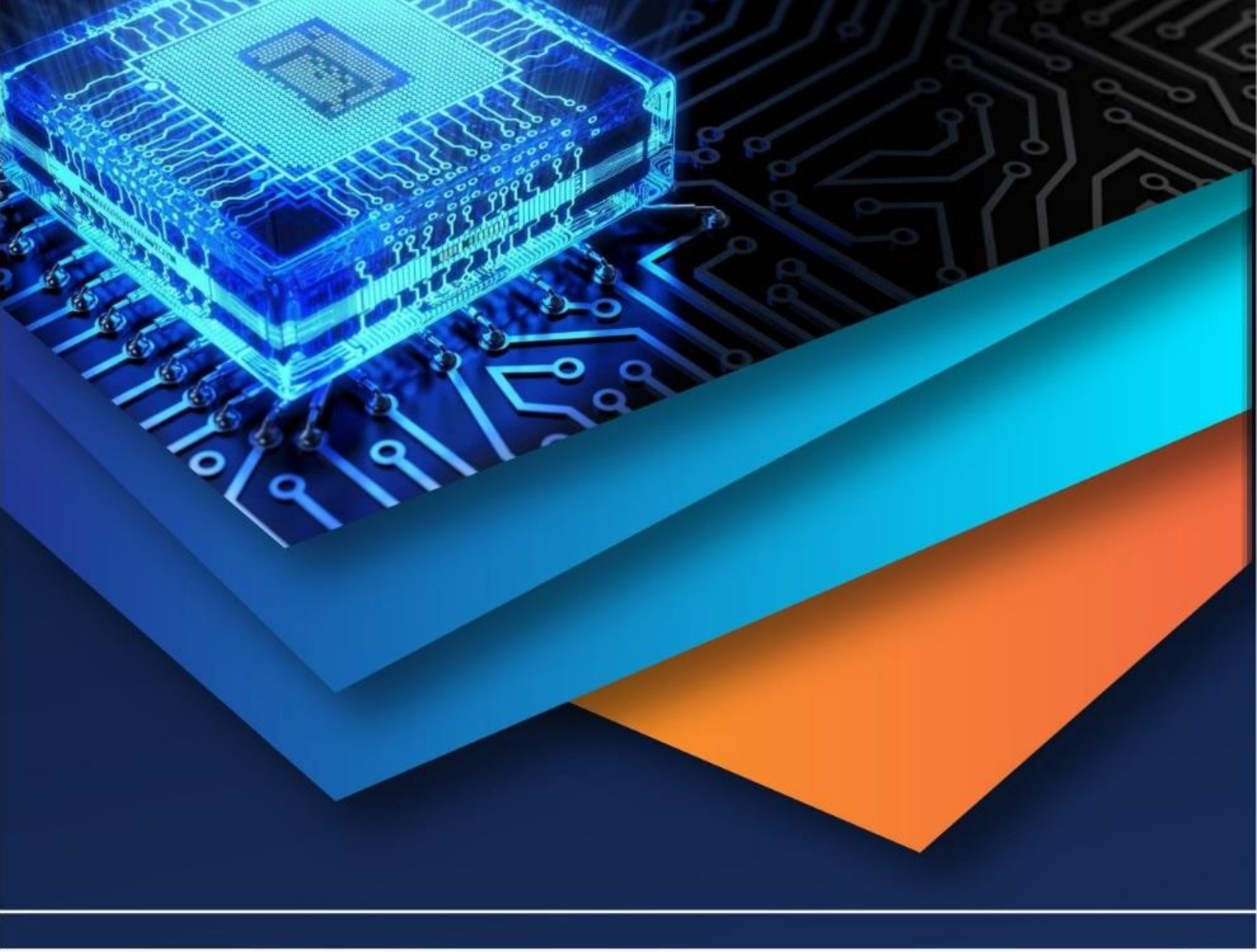

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