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Synthesis of Organo-Fluorine Acid Hydrazides as Possible Antimicrobial Agents

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Abstract: ***Organo-fluorine derivatives have occupied centre-stage of studies by chemists and pharmacologists for the pharmacological properties of various members of the group. Further to the discovery and widespread use of fluoroquinolones and their efficacy as antibacterial agents against numerous strains of resistant bacteria, these have been extensively synthesized and investigated for their antimicrobial properties. The widespread use of organo- fluorine derivatives has increased the demand for the development of practical and simple reagents and experimental strategies for the incorporation of fluorine into all types of molecular structures and this was the reasoning behind this study. The work embodied here represents the study of reaction of 2- fluoro and 4- fluoro aniline with diethyl malonate. The further synthesis of four derivatives namely, N-(2-fluoro)-phenyl malonamic acid, N-(4-fluoro)-phenyl malonamic acid amide, N-(2-fluoro)-phenyl malonamic acid hydrazide and N-(4-fluoro)-phenyl malonamic acid hydrazide. These synthesized products were further tested invitro for their antimicrobial properties. The antifungal investigation was done against Aspergillus flavus, Penicillium citrinum, and Fusarium moniliforme. The bacteria chosen for the investigation for antibacterial activity were Escherichia coli, Staphylococcus aureus and Bacillus subtilis. Out of the four derivatives mentioned N-(2-fluoro)-phenyl malonamic acid and N-(4-fluoro)-phenyl malonamic acid amide were not found to possess any antimicrobial activity were as the hydrazides, that is N-(2-fluoro)-phenyl malonamic acid hydrazide and N-(4-fluoro)-phenyl malonamic acid hydrazide showed positive antimicrobial efficacy against all strains of bacteria and fungi under observation. However, it is suggested that further investigative and subsequent studies are required for any concrete conclusive inference.***

Keywords: Organo-fluorine, reactive methylene, antimicrobial, antibacterial, antifungal, pharmacology, fluorine.

I. INTRODUCTION

Fluorinated organic compounds have been in the forefront of investigative study by chemists and pharmacologists for their pharmacological properties. Fluorine which ranks 13th in abundance and, with other fluorine containing functional groups, has established as biological substances, pharmaceuticals, agrochemicals, liquid crystals, dyes, polymers, and a wide range of consumer products. This confirms its resistance to metabolic change due to the strength of the C-F bond providing biological stability and the application of its non-stick-interfacial physical characteristics. Though fluorinated compounds are rarely found in nature and fluorine being toxic in nature, raise questions about its utility in pharmaceutics and as agro chemicals. Its introduction often remains a synthetic challenge. Organo-Fluorine Chemical Science has often occupied centre stage further to the discovery and widespread use of fluoroquinolones and their efficacy as antibacterial agents against numerous strains of resistant bacteria. Another important agent has been 5-fluoro uracil, an established anticancer agent. Most of these substances which have proven pharmacological utility are fluorinated aromatic compounds. Fluorinated compounds are of increasing interest to the pharma sector and hence means for synthesis are increasingly being made available [1]. A scientific review article published by the American Chemical Society recently has stated that presently, about 20% of the commercial pharmaceuticals are fluoro pharmaceuticals [2]. Organo fluorine compounds have been occupying centre stage is study because of evident medicinal properties. Advances in medicinal studies in organo fluorine chemistry has contributed significantly to the great advances in modern medical treatments. Literature survey has established that because of the known influence of a fluorine atom on physical, chemical, and biological phenomena therapeutic efficacy has been enhanced and further to this their pharmacological properties have been significantly improved [3]

Relevant work has continued to express that fluorinated organic compounds have established their role in drug design and development furthering rapidly because of its unique properties associated with how judiciously to introduce fluorine into a molecule to productively influence conformation, pK_a, intrinsic potency, membrane permeability, metabolic pathways, and pharmacokinetic properties too [4] The widespread use of organo-fluorine derivatives has increased the demand for the development of practical and simple reagents and experimental strategies for the incorporation of fluorine into all types of molecular structures and this was the reasoning behind this study.

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II. HISTORY OF SYNTHESIS OF REACTIVE METHYLENE ORGANIC DERIVATIVES

Considerable work has been done in the late past and has continued over the decades, regarding the study of the reaction between primary aromatic amines and ethyl malonate by heating a mixture of freshly distilled aniline and ethyl malonate, the method by which Freund [**5]** prepared malonanilide. Later on Rugheimer and Hoffman [6] studied the reaction between ethyl malonate and o-,m- and p-toluidines. Whitely [**7]** took up the study of ethyl malonate with mono amines and prepared various derivatives from the products obtained. Chattaway and Mason [8] later worked on the reaction between many halogen substituted derivatives of anilines and ethyl malonate. During the course of their study they prepared p:p'- dichloromalon dianilide, ethyl-p-chloro-malonanilate and malon-p-chloranilic acid from p- chloroaniline and ethyl malonate. They also condensed 2:4 – dichloro and 2:4:6 – trichloroanilines with ethyl malonate and isolated the corresponding dianilide, anilate and anilic acid. Later Chattaway and Olmstead [9] gave a convenient method for preparing malon anilic acid and the three malon toluidic acids (o-, m-, p-) by the condensation of malonic ester with the corresponding primary amines and then hydrolysing the mono ester formed. The work was pursued and further extended Ahluwalia, Haq and Ray [10]. Following the above method various other workers prepared several substituted malonic acids. Ittyerah and Pandya [11] made a light modification and Olmsteads method and thus improved the yields of malon o-, m-and p- toluidic acids .Later Chellapa and Ittyerah [12] prepared 1:3: 4-xylidic acid by the condensation of freshly distilled 1:3: 4-xylidine with pure ethyl malonate.

III. METHOD OF SYNTHESIS OF REACTIVE METHYLENE ORGANO FLUORINE DERIVATIVES

The work embodied here represents the study of reaction of 2- fluoro and 4- fluoro aniline with diethyl malonate. The usual method adopted was to mix the freshly distilled amine (1 mol.) and diethyl malonate (2 mol.) in a round bottomed flask and refluxed with an upright air condenser of suitable length to allow the alcohol formed during the reaction to escape and the ester to flow back. The time period for refluxing the amine and diethyl malonate was varied to obtain the maximum yield of the acid.in the course of the reaction can be explained by the following illustration 1:

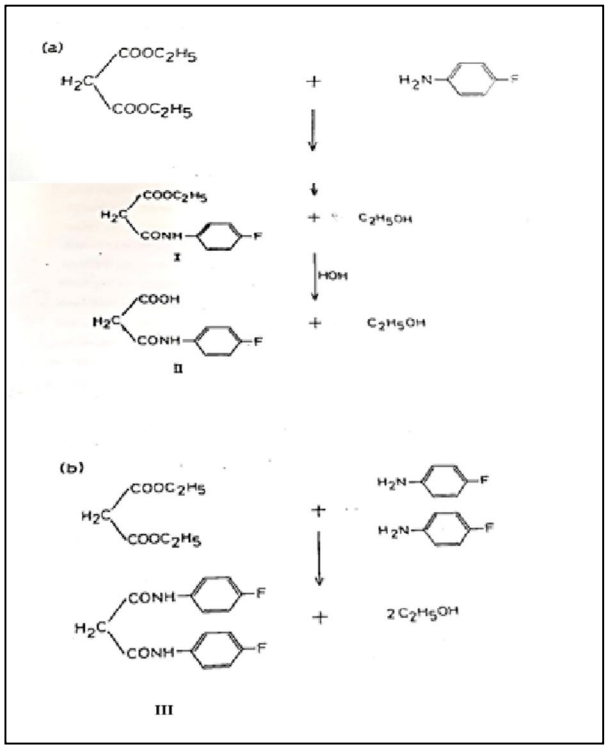

Fig 1.

It is evident from the above scheme that three products (I), (II) and (III) are possible. All the three products have been isolated by condensing diethyl malonate with 2-fluoro and 4-flouro anilines. Experimental conditions for obtaining the maximum yield of the product (II) have been worked out. The molecular ratio of 1:2 of the amine and the ester gave the maximum yields. Another factor that governed the yields was the time and the way of heating. Vigorous heating produced more of the dianilide (III) and less of the acid (II). On the contrary very gentle ebullition favoured better and purer yields of the acids.

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Among the new products obtained are N-(2-fluoro)-phenyl and N-(4-fluoro)-phenyl malonamic acids, their ethyl esters, malon-di-2-fluoro and malon-di-4-fluoro anilides. The ester (I) were used in preparing the corresponding acid amides by treating them with liquor ammonia. I.R.(KBr) spectra of N-(2-fluoro)-phenyl and N-(4-fluoro)-phenyl malonamic acids, ethyl-N-(4-fluoro)-phenyl malonamate and N-(4-fluoro)phenyl malonamic acid amide were taken and the following information has been obtained.

N-(2-fluoro)-phenyl and N-(4-fluoro)-phenyl malonamic acids, (spectrum 1&2) showed absorptions at 3250cm-1(-NH stretching vibrations), 1710cm-1(-COOH group), 1650cm-1(-COCH2 group) and 1200cm-1 (C-F bond). The I.R. spectrum(3) of ethyl 1-N-(4-fluoro)-phenyl malonamate shows absorptions at 3260 cm-1 (NH stretching vibrations, 1725 cm-1 (-COOC2H5 group), 1650 cm-1 (-COCH2 group) and 1150cm-1(C-F bond). The I.R. spectrum(4) of N-(4-fluoro)-phenyl malonamic acid amide shows absorptions at 3270cm-1(-NH stretching vibrations), 1680 cm-1 (-CONH2 group) , 1650cm-1 (-COCH2 group) and 1250 cm-1 (C-F bond). In this work undertaken further an attempt has been made to prepare acid hydrazides of N-(2-fluoro)- phenyl and n- (4-fluoro)- phenyl malonamic acids. For preparing the same Ethyl-N- (2- fluoro)-phenyl malonamate (2 gms) was dissolved in ethanol (15 ml) and was treated with 3ml. of hydrazine hydrate (99%) and refluxed on a boiling water bath for half an hour . On cooling, the hydrazide separated out as a white precipitate. This was filtered dried and recrystallised from ethanol. On analysis it was found to be N-(2-fluoro)-phenyl malonamic acid hydrazide, yield 1.70 gm (90.63%), M.P. 129°C (Found C 50.84 %: H, 4.01%, N, 19.04%, C9H10N3O2F requires C, 51.18%, H, 4.73%, N, 19.90%. N-(4-fluoro)-phenyl malonamic acid hydrazide was prepared following similar method. (1.) & (2.) were initially prepared and then later (3.) and (4)

1) N-(2-fluoro)-phenyl malonamic acid
2) N-(4-fluoro)-phenyl malonamic acid amid
3) N-(2-fluoro)-phenyl malonamic acid hydrazide
4) N-(4-fluoro)-phenyl malonamic acid hydrazide

IV. EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED PRODUCTS

A study of the voluminous literature reveals the immense utility of reactive methylene derivatives as effective antimicrobial agents. Thus, it was thought worthwhile to assess the in vitro antifungal and antibacterial activities of some of the compounds synthesized during the course of work. A number of products synthesized were tested for their antifungal and antibacterial properties. The evaluation of a few of the products are enlisted here in this paper. The antifungal investigation was done against Aspergillus flavus, Penicillium citrinum, and Fusarium moniliforme. The bacteria chosen for the investigation for antibacterial activity were Escherichia coli, Staphylococcus aureus and Bacillus subtilis. The following compounds were tested for their in vitro antibacterial and antifungal activity. (details follow)

1) N-(2-fluoro)-phenyl malonamic acid
2) N-(4-fluoro)-phenyl malonamic acid amide
3) N-(2-fluoro)-phenyl malonamic acid hydrazide
4) N-(4-fluoro)-phenyl malonamic acid hydrazide

The filter paper disc method [13] was used for the screening of the compounds for the antimicrobial activity. Standard size discs of whatman filter paper no 3 with a diameter of 6.5 mm were sterilized by dry heat at 140 °C for one hour and were saturated with the solution of the compound. these were air dried at room temperature to remove any residual solvent which might interfere with the determination. The, discs were then placed on the surface of the sterilized solidified culture medium that had been inoculated with the test organism (using a sterile cotton swab). The thickness of the culture medium was kept equal in all the petri dishes (20 ml.). For each of the experiments a petri dish inoculated with the organism but without a disc containing the compound was taken and incubated as control. These were incubated at 28±1 °C for 5-6 days in case of fungi and at 35 °C for 24 hours in case of bacteria after which the zone of inhibition or depressed growth was measured.

A. Culture Medium For Bacterial Growth

Nutrient Agar

Peptone	= 10.0 gms
Beef extract	= 3.0 gms
Sodium Chloride	=5 gms
Agar Agar	=20.0 gms(0.85 BDH Agar Agar)
Distilled water	=1000 ml
pH	=7.6-7.8

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B. Culture Medium For Fungal Growth

Czapek's Dox Agar
Sodium Nitrate =2.0 gms
K2HPO4 =1.0 gms
MgSO4.7H2O =0.5 gms
KCl =0.5 gms
Sucrose =30.0 gms
Agar Agar =20.0 gms(0.85 BDH Agar Agar)
Distilled water =1000 ml
Chlroremphenicol = 30.0 gms
pH = 6.4-.8

C. Solubility Data

100 mg of the compound was dissolved in 100 ml of the solvent (1000 ppm). With this three more dilutions (750 ppm, 500 ppm and 250 ppm) were made. All the compounds were dissolved ethanol, except for the coumarins which dissolved in DMF Dimethylformamide.

The data of the antifungal screening is shown in table below:

Table I

S.No	Name of the compound	Concentration in ppm	Sensitivity A.flavus	Sensitivity P. citrinum	Sensitivity F.moniliforme
1.	N-(2-fluoro)-phenyl malonamic acid	250 500 750 1000	-negative -negative -negative -negative	-negative -negative -negative -negative	-negative -negative -negative -negative
2.	N-(4-fluoro)-phenyl malonamic acid amide	250 500 750 1000	-negative -negative -negative -negative	-negative -negative -negative -negative	-negative -negative -negative -negative
3.	N-(2-fluoro)-phenyl malonamic acid hydrazide	250 500 750 1000	+ positive + positive + positive + positive	+ positive + positive + positive + positive	+ positive + positive + positive + positive
4.	N-(4-fluoro)-phenyl malonamic acid hydrazide	250 500 750 1000	+ positive + positive + positive + positive	+ positive + positive + positive + positive	+ positive + positive + positive + positive

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The data of the antibacterial screening is shown in table below:

Table II

S.No	Name of the compound	Concentration in ppm	Sensitivity with zone of inhibition in mm. E.coli	Sensitivity with zone of inhibition in mm S.aureus	Sensitivity with zone of inhibition in mm B.subtilis
1.	N-(2-fluoro)-phenyl malonamic acid	250 500 750 1000	R R R R	R R R R	R R R R
2.	N-(4-fluoro)-phenyl malonamic acid amide	250 500 750 1000	R R R R	R R R R	R R R R
3.	N-(2-fluoro)-phenyl malonamic acid hydrazide	250 500 750 1000	10 10 10 15	10 15 20 30	10 15 25 40
4.	N-(4-fluoro)-phenyl malonamic acid hydrazide	250 500 750 1000	10 10 20 25	10 15 20 30	10 15 25 40

*R- Resistant

The table of preliminary antimicrobial activity indicates clearly that though products No 1 & 2 did not show any antimicrobial activity at all the concentrations but product no 3 & 4 that is the malonamic acid hydrazides did show activity against all the microbes both fungi and bacteria.

V. REACTIVE METHYLINE ORGANO FLUORINE DERIVATIVES AS PROMISING ANTIMICROBIAL AGENTS

The Emphasis earlier in this study has been sufficiently focussed on organo fluorine compounds. Similarly, reactive methylene group has its own significance in heterocyclic chemistry. Heterocyclic compounds have proven their worthiness as various pharmacotherapeutic agents. Benzoxazole is an important member in the class of heterocyclic compounds with proven medicinal chemistry. It has been incorporated in many therapeutic compounds making it a versatile heterocyclic compound carrying a wide spectrum of biological activities. In one of the recent studies conducted, the molecular structures of synthesized benzoxazole derivatives which were confirmed by physicochemical and spectral means, which were further evaluated for their in vitro biological potentials. They were found to possess promising in vitro antimicrobial potential and showed promising anticancer activity against human colorectal cancer cell line. It was hence concluded that these compounds may prove to be important for further development of novel antimicrobial and anticancer agents [14]**.** Another research article recently published confirmed malonic acid derivatives as potential antimicrobial agents [15]**.** In the present study too malonic acid ester has been used as a starting material and condensed with fluoro anilines to prepare the reactive methylene organo fluorine derivatives.

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VI. RESULTS AND DISCUSSION

During the course of study Diethyl malonate was taken as a starting material along with 2 and 4-fluoroaniline. The method of synthesis which has been earlier described in the paper resulted in the yield of a number of derivatives out of which 4 products have been discussed here which have been tested for their antimicrobial activity against a few strains of fungi and bacteria. The antifungal investigation was done against Aspergillus flavus, Penicillium citrinum, and Fusarium moniliforme. The bacteria chosen for the investigation for antibacterial activity were Escherichia coli, Staphylococcus aureus and Bacillus subtilis. The filter paper disc method was used for determination of antimicrobial activity. Out of the four derivatives mentioned N-(2-fluoro)-phenyl malonamic acid and N-(4-fluoro)-phenyl malonamic acid amide were not found to possess any antimicrobial activity whereas, the hydrazides, that is N-(2-fluoro)-phenyl malonamic acid hydrazide and N-(4-fluoro)-phenyl malonamic acid hydrazide showed positive antimicrobial efficacy against all strains of bacteria and fungi under observation.

VII. CONCLUSION

Vast and expansive literature reveals that derivatives with fluorine containing functional groups, are proven biological substances, pharmaceuticals, agrochemicals, liquid crystals, dyes, polymers and a wide range of consumer products. After the introduction and massive usage fluoroquinolones and subsequent discovery of a large number of products in this class with proven antimicrobial efficacy, fluoro compounds did establish their importance as antimicrobial agents. Heterocyclic aromatic compounds as such have been largely studied for their usage for the benefit of mankind. In this study too, four derivatives were synthesized and tested for their antibacterial and antifungal efficacy. Out of the four two showed positive in vitro efficacy, that is N-(2-fluoro)-phenyl malonamic acid hydrazide and N-(4-fluoro)-phenyl malonamic acid hydrazide showed positive antimicrobial efficacy against all strains of bacteria and fungi under observation. However, it is suggested that further investigative and subsequent studies are required for any concrete conclusive inference.

VIII. ACKNOWLEGMENT

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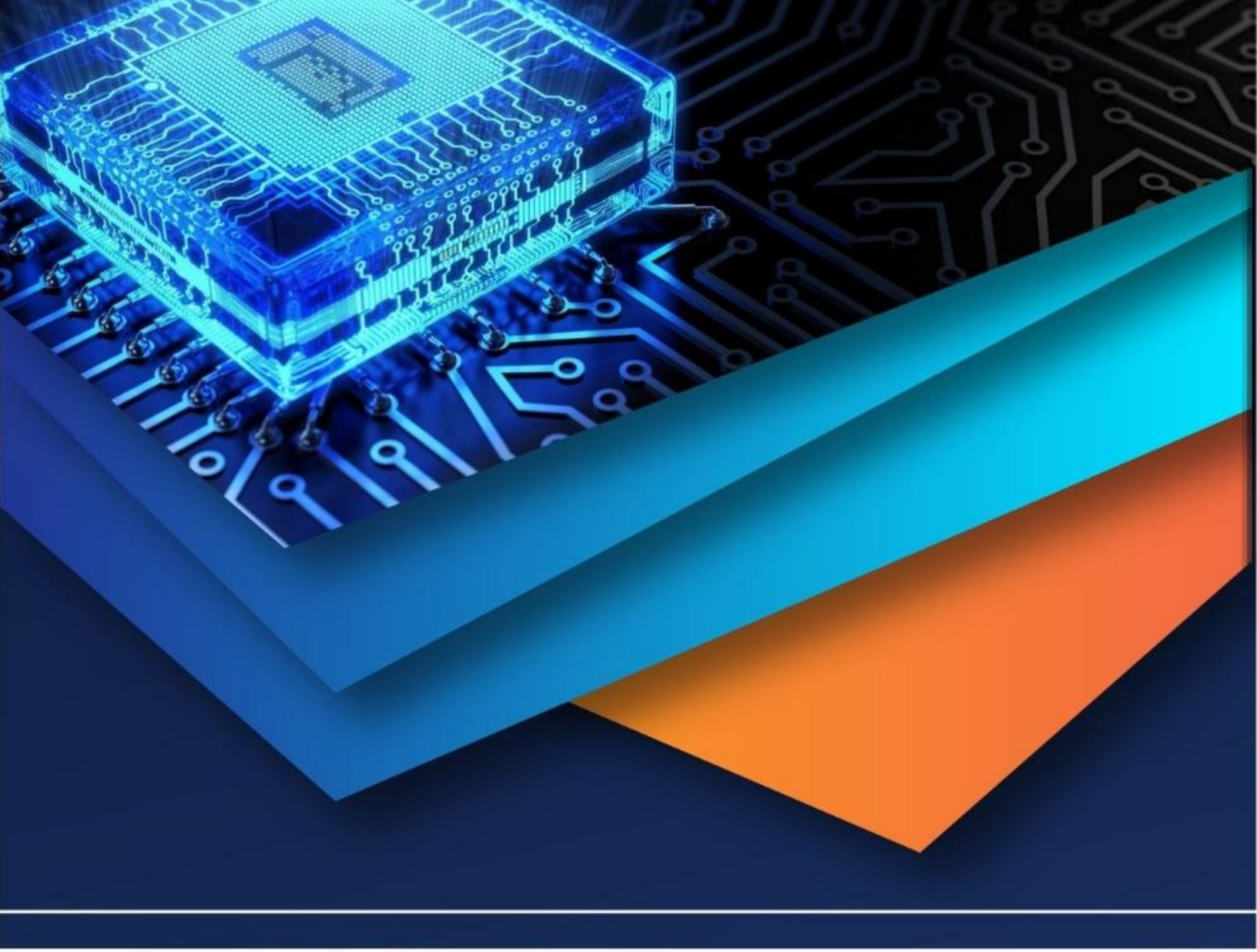

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