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RESEARCH ARTICLE
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"SYNTHESIS, INSILICO STUDY & ANTI-PROLIFERATIVE ACTIVITY OF NEW DERIVATIVES OF TRI-ARYL IMIDAZOLE"

Sandeep P. Gadhwe[1*], Rajesh B. Patil[1], Chetan S Shirbhate[2], Govind Kankawale[2]

[1*,2]Department of Pharmaceutical Chemistry Sinhgad College of Pharmacy, Pune-411041, (Maharashtra), India.
[3,4]Department of Pharmaceutical Quality assurance Sinhgad College of Pharmacy, Pune-411041, (Maharashtra), India.

***Correspondence Author:** Mr. Sandeep p. Gadhwe
*Department of Pharmaceutical Quality Assurance Sinhgad College of Pharmacy, Pune-411041, (Maharashtra), India. Email: sandeepgadhwe@gmail.com, Orchid id: 0000-0001-7332-4506
Ph: +919765416989.

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Abstract
This study focuses on the process of Docking, Synthesis, and Characterization of novel chemotherapeutic agents with the potential Anticancer Activity. The aim is to discover new compounds for the anticancer activity using MCF7 cell lines on Breast Cancer that exhibit promising properties for Cancer Treatment. Imidazole offers better pharmacodynamic characteristics and directly affect membranes at high concentrations, independent of sterols and sterol esters. Recent developments in imidazole derivatives for drug development show improved efficacy & lower toxicity. The docking approach to assess the binding affinity and interaction between compounds & Protein. Radziszewski synthesis scheme with substituted benzyls and quinoline aldehyde employed to create imidazole compounds. The synthesized Compounds are subsequently analyzed using various techniques like NMR (1H), TLC, FT-IR, Mass Spectral data, Biological evaluation to assess their ability to inhibit cancer cell growth or induce cancer cell death. The findings obtained from this study seek to make a valuable contribution to the advancement of novel chemotherapeutic agents with improved anticancer properties.

Keywords: Triaryl imidazole, MTT assay, docking.

1.INTRODUCTION

2,4,5-Triaryl-1H-imidazole compounds have gained significant importance for their diverse biological activities and applications in synthetic chemistry. Imidazole, a crucial substructure found in numerous natural products and pharmacologically active compounds, including cimetidine for ulcers, omeprazole as a proton pump inhibitor, and flumazenil as a benzodiazepine antagonist, is of particular interest.[1] Trifenagrel4, a 2,4,5-triaryl-1H-imidazole, effectively reduces platelet aggregation in both animals and humans.[2] Various synthetic strategies have been developed over the years, such as the pioneering synthesis of 2,4,5-triphenyl imidazole by Radziszewski and Japp in 1882 and the proposed use of nitriles and esters.[3] More recently, several methods utilizing different catalysts have been reported for synthesizing 2,4,5-triaryl-1H-imidazoles, but they often require

prolonged reaction times and specialized conditions. Therefore, there is a need for the development of new methods to efficiently synthesize these derivatives. In 1980, Breslow discovered the accelerated Diels-Alder reaction in water, leading to increased interest in utilizing water or aqueous media for various organic transformations.[4] Boric acid (BO_3H_3 or $B[OH]_3$) has emerged as an excellent catalyst in organic synthesis due to its advantages, such as water solubility, ease of handling, affordability, and eco-friendly nature.[5] Moreover, ultrasound has gained popularity as an alternative energy source in organic synthesis, enabling reactions to occur under milder conditions and shorter reaction times, resulting in higher yields.[6] Imidazole derivatives, with their presence in natural products and pharmacologically active compounds, remain an intriguing class of heterocyclic compounds.

Imidazole derivatives have been extensively studied in the literature and have been found to possess a wide range of pharmacological activities. They exhibit anti-fungal and anti-bacterial properties, effectively targeting fungal and bacterial infections. These derivatives also have anti-inflammatory and analgesic effects, providing relief from inflammation and pain. Furthermore, they show anti-tubercular activity, combating tuberculosis-causing bacteria. In the field of mental health, imidazole derivatives hold promise as anti-depressants, aiding in mood elevation.[7] Their anti-cancer properties make them potential candidates for cancer treatment. Additionally, imidazole derivatives display anti-viral activity, fighting against viral infections. These findings underscore the diverse pharmacological potential of imidazole derivatives.

Efficient and straightforward synthetic methods are crucial for common organic compounds in organic synthesis. Imidazole derivatives, which possess two nitrogen atoms and occur naturally in Vitamin B12, histamine, and histidine, play a significant role as heterocyclic structures. These derivatives exhibit diverse biological activities, including antimicrobial, anti-inflammatory, antiviral, glucagon antagonist, fungicidal, and antithrombotic properties. As a result, several methods have been developed for synthesizing 2,4,5-triaryl-substituted imidazole derivatives using catalysts such as Sulfanilic acid, trifluoroacetic acid, sodium bisulfite, hetero-polyacid, chitosan sulfuric acid, oxalic acid, acetic acid, phosphomolybdic acid, A-MFGO, and silica sulfuric acid.[8] Microwave technology has emerged as a valuable tool in recent years for synthesizing heterocyclic compounds. This method offers advantages such as shorter reaction times, safety, high yields, efficiency, and environmental friendliness. A cost-effective and simple procedure has been implemented for the preparation of 2,4,5-triaryl-substituted imidazole, while microwave-assisted techniques have been employed for synthesizing specific derivatives of these compounds.[9]

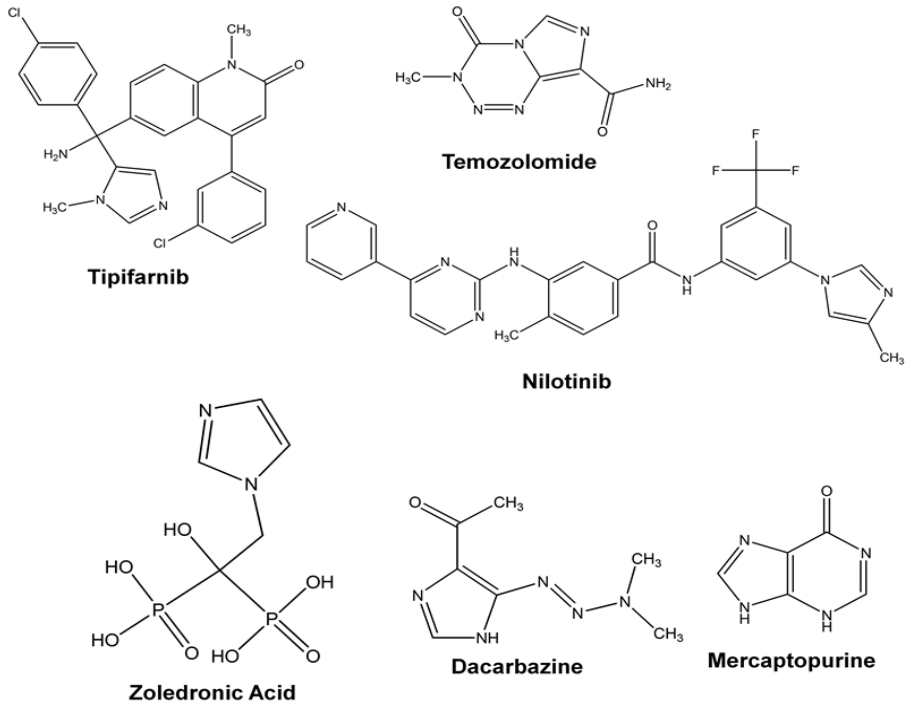


Figure 1.1 Standards marketed drugs containing imidazole moiety

Imidazole holds significant biological importance as it is incorporated into various essential biological molecules. The amino acid histidine, with its imidazole side chain, plays a vital role in proteins and enzymes, particularly in the structure and binding functions of hemoglobin. Imidazole is also involved in the formation of histamine, a common compound in allergic reactions. In terms of applications, imidazole is utilized in the purification of His tagged proteins through immobilized metal affinity chromatography (IMAC). It acts as an eluent, freeing the His-tagged proteins from nickel coordination.1 Imidazole is also employed in buffer preparation, chelation of divalent cations, and oral treatment of psoriasis and seborrheic dermatitis. In drug discovery, imidazole derivatives serve as a crucial synthetic strategy for various pharmacological agents. Additionally, imidazole is found in fungicides, antifungal medications, antiprotozoal drugs, antihypertensive medications, theophylline (a CNS stimulant), and anticancer medication such as mercaptopurine.

2. EXPERIMENITAL

2.1 Material and Instruments

All the chemicals and reagents used in the experiment were of LR grade and met the standard quality requirements. Melting points were determined using a scientific melting point apparatus with open capillaries, and the values obtained were not corrected. The 1H-NMR and 13C-NMR spectra were recorded in CDCl3 using a BRUKER-300 MHz spectrometer, and the chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) used as an internal standard. Mass spectra were obtained using a Bruker Compass Data Analysis 4.2 Impact HD spectrometer. The IR spectra of the synthesized compounds were recorded on a Bruker Alpha-T ATR FT-IR spectrophotometer using potassium bromide discs.

2.2 Methods

All well-known methodology was followed for synthesis of benzoin and benzil. For synthesis of new triaryl derivatives, we adopted following methodology for getting the product to improve synthesis followed by column chromatography for purification.

2.2.1 General Procedure for Synthesis of Substituted Tri-Aryl Imidazole from Benzil

In the given reaction procedure, a solution containing 1.42 g of ceric ammonium nitrate (10 mol%), 0.80 mg of ammonium acetate (40 mmol), and 0.67 mg of substituted benzyl (10 mmol) is prepared in a mixture of ethanol and water (20 ml, 1:1 V/V). To this solution, 0.5 mg of 4-quinoline carbaldehyde (12 mmol) is added. The reaction mixture is then heated to 65°C and monitored by TLC (thin-layer chromatography) until the reaction is complete. After completion, a precipitated solid is obtained. The reaction mixture is cooled to room temperature and poured onto ice-water (50 ml) to induce precipitation. Filtration is employed to collect the resulting product, which is 2, 4, 5-triaryl-1H-imidazole. The collected product is then washed and dried.

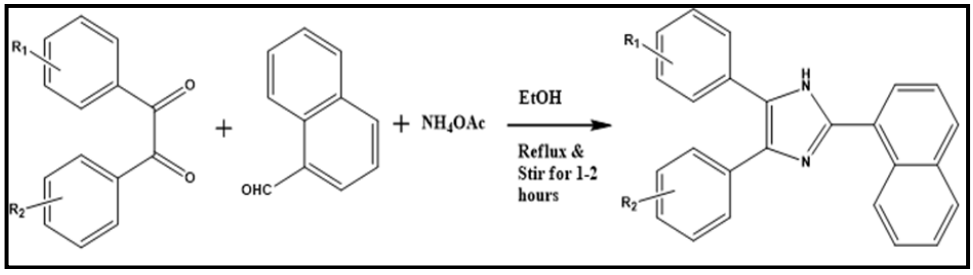


Fig. No.2.1 General Scheme for Synthesis of New substituted Tri-Aryl imidazole From Benzoin.

2.3 Docking study

In this procedure, docking simulations were performed to investigate the possible mode of action of differently substituted triaryl substituted imidazole derivatives. The protein with PDB id: 8DU6 was downloaded from www.rcsb.org and used for the docking studies. The ligands with codes TI1 to TI6 were employed for the docking simulations. Auto-dock vina 1.2.0 was utilized for the docking

simulations. Two-dimensional structures of the compounds were drawn using Marvin Sketch 5.6.0.0 and converted into three-dimensional (3D) geometry. The 3D molecules' geometry was optimized through energy minimization using UCSF Chimera 1.8, with the addition of Gasteiger charges and a combination of steepest descent and conjugate gradient geometry. The protein was processed by removing water and other residues and further optimized through energy minimization in Chimera software. Polar hydrogen was added to the protein structure using MGL tools 1.5.4. Flexible docking was performed by setting all torsion angles for the compounds as free. A grid box was prepared to cover the active site of the protein. The results of the docking simulations were analyzed in terms of estimated binding free energy, estimated inhibition constant (Ki), and interactions of the ligands with residues at the active site.

2.4 Detection Method

Melting points were determined through an open capillary method using a scientific melting point apparatus, without any corrections made. The IR spectra of the synthesized compounds were recorded on a Bruker Alpha-T ATR FT-IR spectrophotometer. The 1H-NMR and 13C-NMR spectra were acquired using a BRUKER-300 MHz spectrometer, with CDCl3 as the solvent and chemical shifts expressed in parts per million (ppm) relative to the internal standard, Mass spectra were generated using a Bruker Compass Data Analysis 4.2 Impact spectrometer.

2.5 MTT Assay of test compounds and their analysis

Cell viability can be assessed using various techniques, such as cell viability reagents that utilize the metabolic activity of living cells to evaluate their proliferation and determine relative cytotoxicity across different species and sample types. To perform the experiment, adherent cell cultures were 8,000 cells per well were seeded in a 96-well plate with the appropriate cell culture medium. The cells were then treated with different concentrations of the test compound, as well as control and positive control drugs. This analysis was conducted in triplicate. After 24 hours, cell viability was assessed using the MTT assay. The cells were incubated with MTT solution, followed by centrifugation and dissolution of the resulting crystals in DMSO. The absorbance of the solution was measured at 540 nm using a microplate reader, and the percentage of cell viability was calculated based on the obtained measurements.

2.6 Statistical Analysis of Cell viability Assay

The cell viability assay results were subjected to statistical analysis using GraphPad Prism version 8 and One-way ANOVA. The IC50 values were determined by calculating the mean of triplicate results for each compound. A plot of percentage cell viability versus concentrations was generated by comparing the percentage of the standard drug with that of the test compounds and the control. Anastrozole was employed as the standard drug for activity comparison.

3.RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis method is depicted in Figure 2.1 and the associated data can be found in Table 3.1. The synthesized derivatives exhibit distinct absorption bands in the infrared (IR) spectrum, which can be attributed to the imidazole ring. These bands appear in the range of 1600-1700 cm-1 for C=N stretching, 1400-1500 cm-1 for C=C stretching, and 600-800 cm-1 for C-H bending. In the 1H NMR spectrum, characteristic signals are observed at 7.0-8.5 ppm for aromatic and quinoline protons, 4.5-5.5 ppm for imidazole protons, and 2.0-3.5 ppm for methylene protons, all of which are typical for the imidazole ring. The aryl groups exhibit signals that vary depending on their substitution patterns and coupling constants. Mass spectrometric analysis demonstrates specific fragmentation patterns for the imidazole ring, such as the loss of NH3, C2H4, or C3H4N.

3.1.1 Characterization data for benzoin synthesis. (1st Step)

3.1.1.1. Synthesis of 2-hydroxy-1-(2-nitrophenyl)-2-phenylethanone. (Comp 11): Rf 0.60, R(cm^{-1}): 3728.60(C-OH), 3000.28 (Ar-C-H), 1699(C=O), 1523(C-NO_2), HRMS M+1 (Calcd.) 265. M.P. 138°C. Having % Yield 60%.

3.1.1.2. Synthesis of 2-hydroxy-1-(3 hydroxy-4-methoxyphenyl)-2-phenylethanone. (Comp 12): Rf:0.70, IR(cm^{-1}) 3868.60(C-OH), 2994(Ar-C-H), 1780(C=O), 1270.19(C-O), HRMS M+1 (Calcd.) 256.18. M.P. 130°C. Having % Yield 60%.

3.1.1.3. Synthesis of 1-(4-(dimethyl-amino) phenyl)-2-hydroxy-2-phenylethanone. (Comp 13): Rf 0.805, IR(cm^{-1}): 3855(C-OH), 1313.71(Ar-C-N), 1676.8(C=O), 1937.7(C-H), HRMS M+1 (Calcd.) 252.61. M.P. 288°C. Having % Yield 50%.

3.1.1.4. Synthesis of (2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-phenylethanone) (Comp 14): Rf 0.655, IR(cm^{-1}):3870(C-OH), 1462.37(Ar-C-H), 1670(C=O), 1233.15(C-O), HRMS M+1 (Calcd.) 261.03. M.P. 110°C. Having % Yield 60%.

3.1.1.5. Synthesis of 2-hydroxy-1-(4-nitrophenyl)-2-phenylethanone. (Comp 15): 2-hydroxy-1-(4-nitrophenyl)-2-phenylethanone: Rf: 0.60, IR(cm^{-1}) 3728.60(C-OH), 3000.28(Ar-C-H), 1699(C=O), 1523(C-NO, HRMS M+1 (Calcd.) 265. M.P. 138°C. Having % Yield 60%.

3.1.1.6. Synthesis of 2-hydroxy-1-(3-hydroxyphenyl)-2-phenylethanone. (Comp 16): 2-hydroxy-1-(3-hydroxyphenyl)-2-phenylethanone: Rf value: 0.60, IR(cm^{-1}):3728.60(C-OH), 3000.28(Ar-C-H), 1699(C=O), 1523(C-NO), HRMS M+1 (Calcd.) 265. M.P. 138°C. Having % Yield 60%.

3.1.2 Characterization data for benzil synthesis. (2nd Step)

3.1.2.1. Synthesis of 1,2-bis(2-nitrophenyl) ethane-1,2-dione. **(Comp 21)**: Rf :0.60, IR(cm^{-1}) 2813.18(Ar-C-H), 1854.90(C=O), 1525.30(C-NO2), HRMS M+1 (Calcd.) 256.18. M.P. 78°C. Having % Yield 40%.

3.1.2.2. Synthesis of 1,2-bis(3-hydroxy-4-methoxyphenyl) ethane-1,2-dione. (Comp 22): Rf 0.85, IR(cm^{-1}) 2794.57(Ar-C-H), 1693.48(C=O), 3425.37(C-H) 1299(-OCH_3), HRMS M+1 (Calcd.) 257.18. M.P. 180°C. Having % Yield 54%.

3.1.2.3. Synthesis of 1,2-bis(4-(dimethyl-amino) phenyl) ethane-1,2-dione. (Comp 23): Rf 0.75, IR(cm^{-1}) 2881.93(Ar-C-H), 1691.30(C=O), 3515.32(H-N), HRMS M+1 (Calcd.) 254.07. M.P. 123°C. Having % Yield 70%.

3.1.2.4. Synthesis of 1,2-bis(4-hydroxy-3-methoxyphenyl) ethane-1,2-dione. (Comp 24): Rf 0.81 IR(cm^{-1}) 1269.3(-O-CH_3), 1686(C=O), 3199.39(C-OH), HRMS M+1 (Calcd.) 257.03. M.P. 197°C. Having % Yield 67%

3.1.2.5. Synthesis of 1,2-bis(4-nitrophenyl) ethane-1,2-dione. (Comp 25): Rf 0.65 IR(cm^{-1}):-2822(-O-H), 1695(C=O), 1282(C-NO_2), HRMS M+1 (Calcd.) 255.22. M.P. 127°C. Having % Yield 58%

3.1.2.6. Synthesis of 1,2-bis(4-hydroxyphenyl) ethane-1,2-dione. (Comp 26): Rf 0.61, IR(cm^{-1}):-2744(C-H), 1888(C=O), 3444(C-OH), HRMS M+1 (Calcd.) 226.23. M.P. 171°C. Having % Yield 61%.

3.1.3 Characterization Data for Synthesis of Triaryl Imidazole. (3rd Step)

3.1.3.1 Synthesis of 4-[4,5-bis(3-nitrophenyl)-4H-imidazol-2-yl]quinoline (TI1): Rf 0.82, IR(cm^{-1}):1526.65(C-NO_2), 3430.51(N-H), 1381.86(-CH_2). $_{13}$C NMR 250MHz($CDCl_3$):- δ 7.26(-CH) 7.80(-CH), 8.15(-CH) , HRMS M+ (Calcd.) 392.14. M.P. 120-124°C, With % yield is 60%.

3.1.3.2 Synthesis of 2-[4-(2-hydroxy-3-methoxyphenyl)-2-(quinolin-4-yl)-4H-imidazol-5-yl]-6-methoxyphenol (TI2): Rf 0.75, IR(cm^{-1}):- 2799.38(C-H), 3307.18(N-H), 1412.61(-CH_2), 768.24(Di-Substituted). $_{13}$C NMR 250MHz($CDCl_3$):- δ 3.65(-CH_3) 4.70(-OH), 7.06(-CH) , HRMS M+ (Calcd.) 393.40. M.P. 230-234°C, With % yield is 72%.

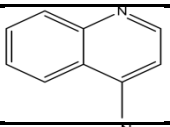
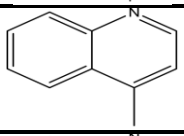
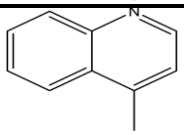
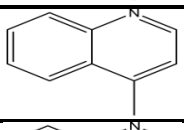
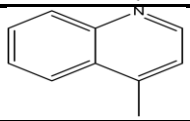
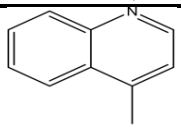
3.1.3.3 Synthesis of 3-{4-[3-(dimethyl-amino)phenyl]-2-(quinolin-4-yl)-4H-imidazol-5-yl}-N,N-dimethylaniline (TI3): Rf 0.78, IR(cm^{-1}):- 3350.31(N-H), 2891.86(C-H), 1413.47(C-CH_3), 750.08(Mono-Substituted).). $_{13}$C NMR 250MHz($CDCl_3$):- δ 3.99(-CH_3) 4.80(-OH), 7.84(-CH) , HRMS M+ (Calcd.) 390.33. M.P. 180-185 °C, With % yield is 54%.

3.1.3.4 Synthesis of 5-[4-(3-hydroxy-4-methoxyphenyl)-2-(quinolin-4-yl)-4H-imidazol-5-yl]-2-methoxyphenol (TI4): Rf 0.72, IR(cm^{-1}):- 3854.85(C-OH), 3514.33(N-H), 1231.97(-OCH_3), 2882.63(C-H))1 $_{13}$C NMR 250MHz($CDCl_3$):- δ 3.99(-CH_3) 5.76(-OH), 7.42(-CH) , HRMS M+ (Calcd.) 393.21. M.P. 240-245 °C, With % yield is 66%.

3.1.3.5 Synthesis of 4-[4,5-bis(4-nitrophenyl)-4H-imidazol-2-yl]quinoline. (TI5): Rf 0.67, IR(cm^{-1}):- 3501.14(N-H), 3059.89(-CH_3), 2778.03(C-H), 2016.18(C=C) $_{13}$C NMR 250MHz($CDCl_3$):- δ 7.81(-CH), 2.50(-OH), 3.32(-CH3) , HRMS M+ (Calcd.) 392.41. M.P. 105-109°C, With % yield is 48%.

3.1.3.6 Synthesis of 4-[4-(4-hydroxyphenyl)-2-(quinolin-4-yl)-4H-imidazol-5-yl]phenol (TI6): Rf 0.91, IR(cm^{-1}):-3437(-OH),2091.40(C=C), 3498.41(N-H), 2930.33(-CH_3). $_{13}$C NMR 250MHz ($CDCl_3$):- δ 2.49(-OH) 3.36(-CH_3), 7.44(-CH) , HRMS M+ (Calcd.) 392.14. M.P 140-148 °C, With % yield is 63%.

Table No.3.1 Characterization data for synthesis of Triaryl Imidazole from Benzil.

Comp. Name	-R1	-R2	-Ar	MW	Time (Min.)	% Yield	M.P.
TI1	2-NO_2	2-NO_2		437.41	70 Min	60%	120-124 °C
TI2	3-OH, 4-OCH_3	3-OH, 4-OCH_3		439.46	70 Min	72%	230-234 °C
TI3	4-(2CH_3-N)	4-(2CH_3-N)		433.23	85 Min	54%	180-185 °C
TI4	3-OCH_3, 4-OH	3-OCH_3, 4-OH		439.15	90 Min	66%	240-245 °C
TI5	4(-NO_2)	4(-NO_2)		437.41	75 min	48%	105-109 °C
TI6	4(-OH)	4(-OH)		379.30	70 Min	63%	140-148 °C

3.2 DOCKING RESULTS

All compounds protein (**8du6)** ligand interaction was studied but the compounds showed good binding interaction and energies are **TI1** with -10.399 & hydrophobic interactions LUE346A, LUE349A, ALA350A, TRP383A, LEU387A, PHE404A, ILE424A, PHE425A, LUE525A. Secondly **TI4** with -11.393 & Hydrogen bond interaction ARG394A, hydrophobic interactions LEU346A, ALA350A, TRP383A, PHE404A, LEU525A. **TI3** with binding energy -8.315 & hydrophobic interactions LEU346A, ALA350A, TRP383A, LEU384A, LEU387A, LEU525A, VAL533A. All these compounds show good results when compared with standard ligand attached with protein i.e TQF binding energy -6.635 with hydrogen bonding ASP351A, LYS529A and hydrophobic interaction LEU346A, THR347A, ALA350A, TRP383A, LEU525A. Testimonial images of 2D and 3D of TI1-TI6 interaction with protein shown below.

Table No.3.2 protein ligand interaction results

Sr. No	Comp. Id	Binding free Energy(kcal/mol)	Hydrogen bond	Hydrophobic Interactions
1	TI1	-6.244	-	LEU346A, THR347A, ALA350A, LEU384A, MET388A, PHE404A, LEU525A
2	TI2	-10.399	-	LUE346A, LUE349A, ALA350A, TRP383A, LEU387A, PHE404A, ILE424A, PHE425A, LUE525A
3	TI3	-8.315	-	LEU346A, ALA350A, TRP383A, LEU384A, LEU387A, LEU525A, VAL533A
4	TI4	-11.393	ARG394A	LEU346A, ALA350A, TRP383A, PHE404A, LEU525A
5	TI5	-7.159	ASN532A	LEU346A, THR347A, ALA350A, TRP383A, LEU525A, MET528A
6	TI6	-7.754	-	LEU346A, THR347A, ALA350A, TRP383A, LEU384A, LEU525A
7	TQF	-6.635	ASP351A, LYS529A	LEU346A, THR347A, ALA350A, TRP383A, LEU525A,

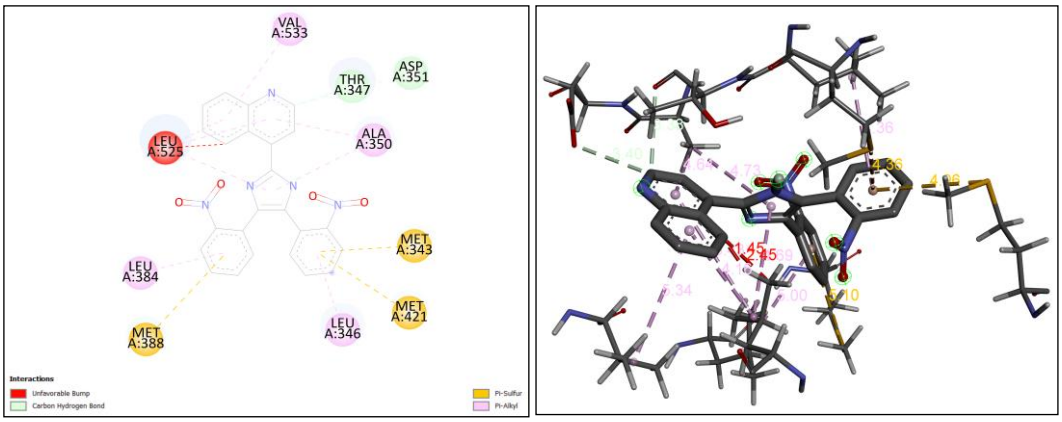


Fig No.3.2.1 Binding interactions 3D and 2D Structure of TI1

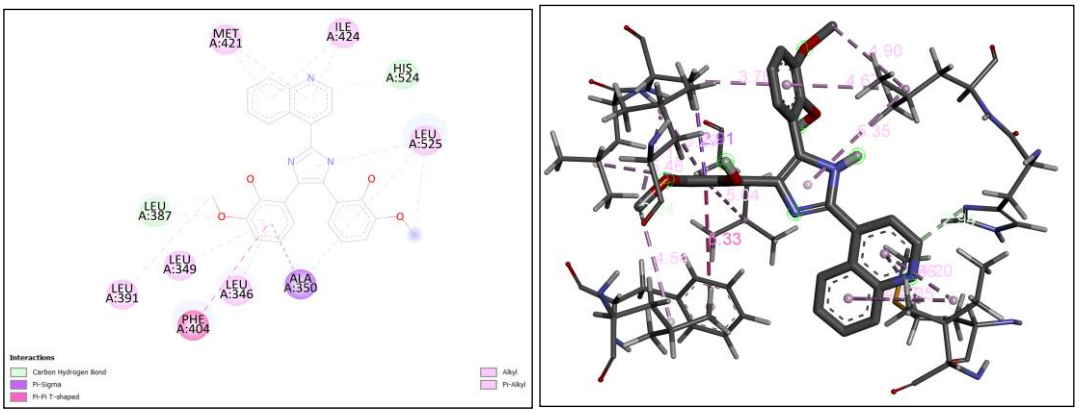


Fig No.3.2.2 Binding interactions 3D and 2D Structure of TI2

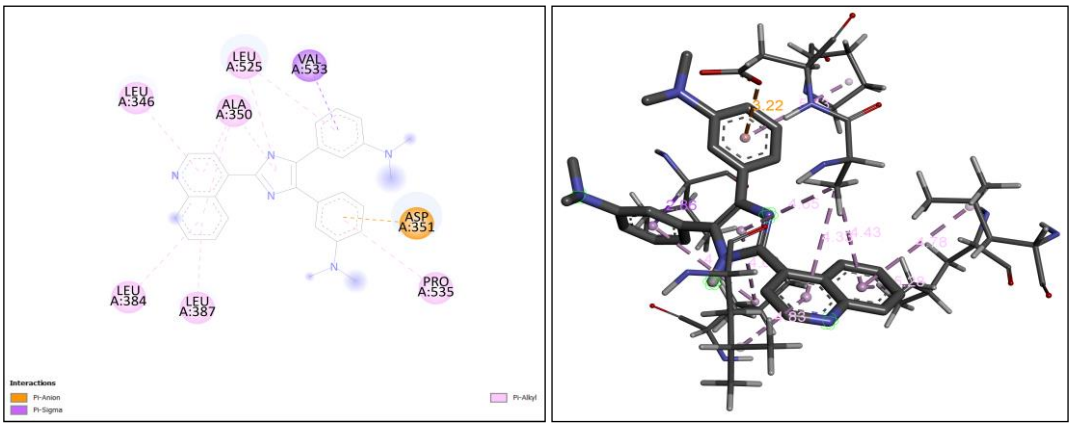


Fig No.3.2.3 Binding interactions 3D and 2D Structure of TI3

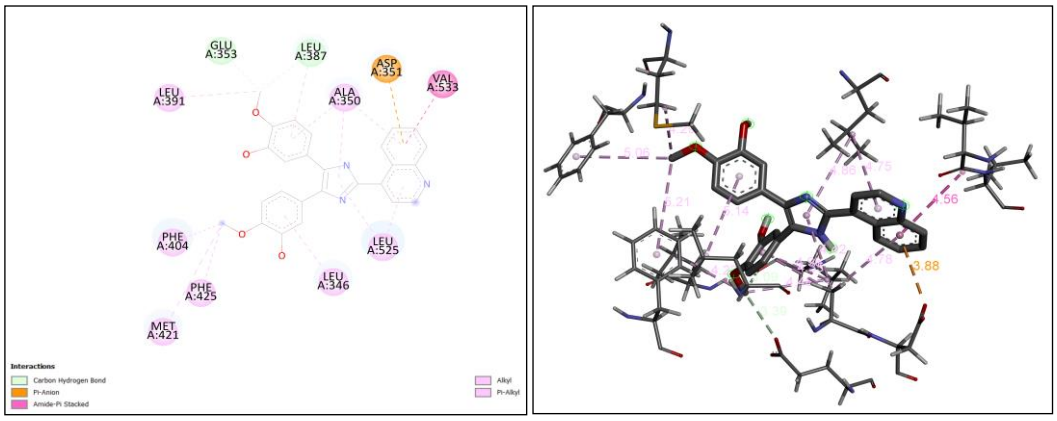


Fig No.3.2.4 Binding interactions 3D and 2D Structure of TI4

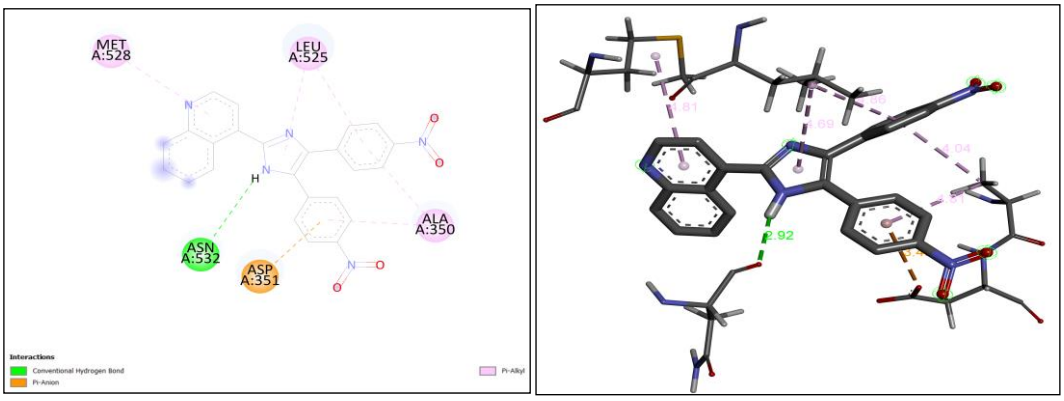


Fig No.3.2.5 Binding interactions 3D and 2D Structure of TI5

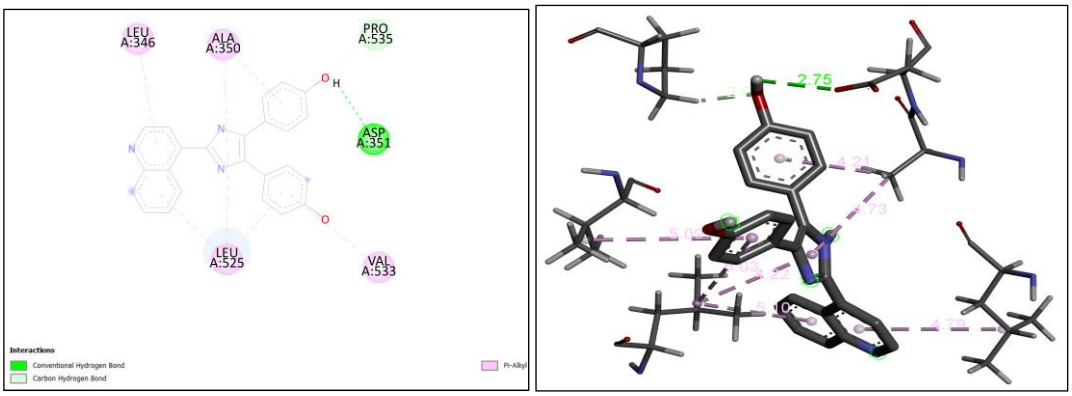


Fig No.3.2.6 Binding interactions 3D and 2D Structure of TI6

3.3 MTT ASSAY STATISTICAL ANALYSIS

Table No.3.3.1 Tabel of IC50 Values of synthesized TI Compounds.

Comp. Id	IC_{50}
Standard	16.99
TI1	38.28
TI2	14.62
TI3	27.87
TI4	56.28
TI5	24.09
TI6	45.12

The given data represents the IC_{50} values of seven compounds. The standard compound exhibits the highest potency, with an IC_{50} value of 16.99, indicating it has a strong inhibitory effect on a biological or biochemical function. On the other hand, compounds **T2** and **T5** also show notable inhibitory activity, with IC_{50} values of 14.62 and 24.09, respectively. Compounds **T3** and **T6** demonstrate moderate inhibitory effects, displaying IC_{50} values of 27.87 and 45.12, respectively. However, compounds T1 and T4 have relatively lower potency, with IC_{50} values of 38.28 and 56.28, respectively. These IC50 values provide valuable insights into the compounds' effectiveness and can aid in further studies and applications in various fields of research and medicine.

❖ **One-Way ANOVA test graph of synthesized Ti compounds & standard Drug.**

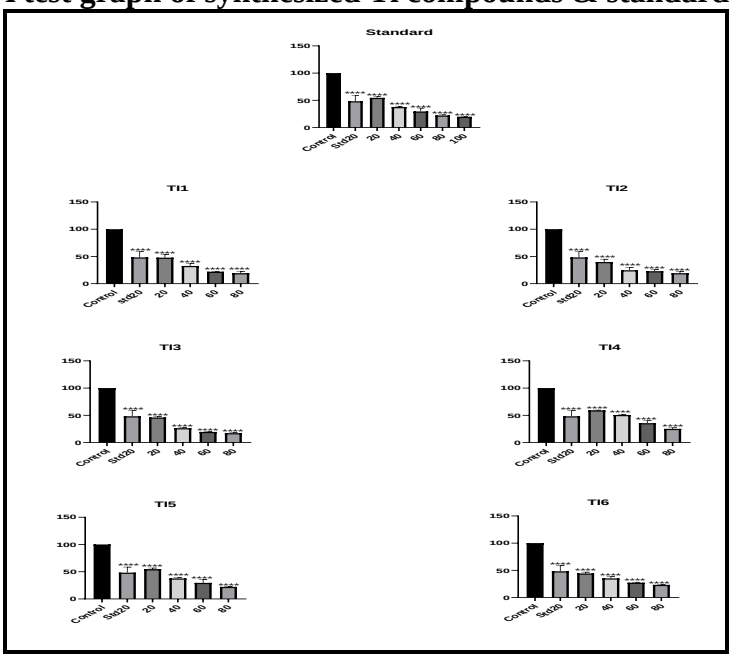


Fig. No.3.3.3 Graphical representation of ANOVA results of Standard and TI's compounds

ANOVA by Tukey's test, it's shown that almost all compounds are active according to linear concentrations concern with respect to control group (100% cell growth) but if we compare 20μM concentration **T1, T3 & T6** show a statistically significant with Standard 20μM concentration So, we can say that **T1, T3, and T6** is as potent as the standard drug or may be more potent than the standard drug in the control group, Which is showing a higher potential to kill Carcinogenic cell and have a good result as Anticancer agents.

On another hand **T4 &T5** are showing a significant difference between 20μM cons of the standard drug, only higher doses was significant with standard drug conc. So we can say that the compounds T4 &T5 have moderate Potential as compared to the standard drug in the control group. Compound T2 has much significant difference from a 20 μM of Standard drug which shows the result that T2 shows fewer results as compared to the standard one but it shows its potential activity against the cancer cell.

All synthesized Tri-Substituted imidazole's having the Potential to kill cancer cells and be developed as the best Anticancer drug in the future, In order to fully investigate the therapeutic potential of these synthesized compounds and to contribute to the development of new cancer treatment options, additional research, such as in vivo tests and clinical trials, needs to be done.

(Note:-Each value mean presents the ± S.E.M. of three observations by ANOVA followed by all other concentrations Significant as compared to Control.

*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 statistical significance as compared to standard marketed drug lowest concentration. ns=not significant.)

3.4 TOXICITY RESULTS OF SYNTHESIZED TRI-SUBSTITUTED IMIDAZOLE.

Pro tox-II uses cutting-edge computational methods and modelling methodologies to give synthesized imidazole's ability to anticipate and assess toxicity parameters. Pro tox-II software is the industry-leading solution for toxicity studies, setting a new standard in comprehensive and efficient toxicological analysis.

Table No.3.4.1 Predicted LD50 & toxicity Class, log P value of synthesized

Sr. No	Compound Id	Predicted LD 50	Predicted Toxicity Class	Molecular Weight	Molecular Reflectivity	Topological Surface area	Partitions Coefficient (Log p)
1.	T1	4000mg/kg	5	437.41	134.85	129.25	4.96
2.	T2	400mg/kg	4	439.46	134.24	96.53	3.53
3.	T3	600mg/kg	4	433.55	145.62	44.09	4.23
4.	T4	480mg/kg	4	439.46	134.24	96.53	3.53
5.	T5	1190mg/kg	4	371.52	119.72	12.47	6
6	T6	600mg/kg	4	379.41	121.25	78.07	3.51

The data consists of six compounds with their respective predicted LD50 values, predicted toxicity classes, molecular weight, molecular reflectivity, topological surface area, and partition coefficient (Log P). Among these compounds, T5 stands out as the most promising candidate due to its relatively high LD50 value of 1190mg/kg, indicating lower toxicity. It also belongs to toxicity class 4, suggesting a moderate level of toxicity. Moreover, T5 has a lower molecular weight of 371.52 and a higher partition coefficient (Log P) of 6, which indicates good solubility. These combined characteristics make T5 a favorable option with potentially lower toxicity and suitable physicochemical properties.

Table No.3.4.2 Activity of Synthesized compounds on Targets.

Compound ID	Hepatotoxicity (organ Toxicity)	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity (Normal Body Cell)	Aromatase	Estrogen Alpha Receptor
T1	Active	Active	Inactive	Active	Inactive	Inactive	Inactive
T2	Inactive	Active	Active	Active	Inactive	Inactive	Inactive
T3	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive
T4	Inactive	Active	Active	Active	Inactive	Inactive	Inactive
T5	Active	Inactive	Active	Inactive	Inactive	Active	Active
T6	Active	Inactive	Inactive	Active	Inactive	Inactive	Inactive

The data shows the activity of different compounds in various toxicity and receptor assays. Compounds 1, 3, and 5 are hepatotoxic, while compounds 2 and 4 exhibit potential carcinogenicity. Additionally, compounds 1, 3, and 4 impact the immune system, and all four compounds (1, 3, 4, and 5) have the potential to cause DNA mutations. None of the compounds are cytotoxic to normal cells, and they are inactive in the Aromatase and Estrogen Alpha Receptor assays. The broad range of activity in different toxicity assays for compounds 1, 3, and 5 necessitates further investigation to assess their safety and potential risks.

4. CONCLUSION

2,4,5-Triaryl Imidazoles, specifically (5-(4-nitrophenyl)-4-phenyl-4H-imidazole-2-yl)quinoline and 4-(4-phenyl-2-(quinolin-4-yl)-4H-imidazole-5-yl)phenol, can be synthesized using a reaction involving Benzaldehyde. The reaction produces two intermediate compounds called Benzoin and Benzil. When 5-(4-nitrophenyl)-4-phenyl-4H-imidazole-2-yl)quinoline reacts, it forms 2-hydroxy-1-(4-nitrophenyl)-2-phenylethanone and 1-(4-nitrophenyl)-2-phenylethane-1,2-dione, which are different forms of Benzoin and Benzil, respectively. Similarly, when 4-(4-phenyl-2-(quinolin-4-yl)-4H-imidazole-5-yl)phenol reacts, it produces 2-hydroxy-1-(3-hydroxyphenyl)-2-phenylethanone and 1-(4-hydroxyphenyl)-2-phenylethane-1,2-dione, which are different forms of Benzoin and Benzil, respectively.

The resulting triaryl imidazole compounds can easily form crystals during this process. The infrared (IR) ranges of the newly prepared triaryl imidazoles match the standard IR ranges, indicating the successful synthesis of the desired compounds. The characterization of these compounds provides valuable insights into their structural properties and potential applications in the field. The docking studies contribute to the understanding of their binding interactions and potential biological activities. Overall, this research contributes to the growing knowledge of tri-aryl imidazole derivatives and their potential significance in various fields.

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CONFLICT OF INTEREST

The authors claim there is no conflict of interest.

REFERENCES

1. Sonar, J.; Pardeshi, S.; Dokhe, S.; Pawar, R.; Kharat, K.; Zine, A.; Matsagar, B.; Wu, K.; Thore, S. An Efficient Method for the Synthesis of 2,4,5-Trisubstituted Imidazoles Using Lactic Acid as Promoter. SN Appl. Sci. **2019**, 1 (9), 1045. https://doi.org/10.1007/s42452-019-0935-0.
2. Sharghi, H.; Razavi, S. F.; Aberi, M. One-Pot Three-Component Synthesis of 2,4,5-Triaryl-1H-Imidazoles Using Mn2+ Complex of [7-Hydroxy-4-Methyl-8-Coumarinyl] Glycine as a Heterogeneous Catalyst. Catal Lett **2022**, 152 (6), 1769–1783. https://doi.org/10.1007/s10562-021-03717-2.

3. Shelke, K.; Kakade, G.; Shingate, B.; Shingare, M. MICROWAVE-INDUCED ONE-POT SYNTHESIS OF 2,4,5- TRIARYLIMIDAZOLES USING GLYOXYLIC ACID AS A CATALYST UNDER SOLVENT-FREE CONDITIONS. RJC **2008**, 1 (3), 489–494.
4. Otto, S.; Engberts, J. B. F. N. Diels_Alder Reactions in Water. Pure and Applied Chemistry **2000**, 72 (7), 1365–1372. https://doi.org/10.1351/pac200072071365.
5. Pathan, S. K.; Mahaparale, P.; Deshmukh, S.; Une, H.; Arote, R.; Sangshetti, J. Boric Acid: A Versatile Catalyst in Organic Synthesis. In Applications of Nanotechnology for Green Synthesis; Inamuddin, Asiri, A. M., Eds.; Nanotechnology in the Life Sciences; Springer International Publishing: Cham, 2020; pp 457–483. https://doi.org/10.1007/978-3-030-44176-0_17.
6. Mason, T. J. Ultrasound in Synthetic Organic Chemistry. Chem. Soc. Rev. **1997**, 26 (6), 443. https://doi.org/10.1039/cs9972600443.
7. Siwach, A.; Verma, P. K. Synthesis and Therapeutic Potential of Imidazole Containing Compounds. BMC Chemistry **2021**, 15 (1), 12. https://doi.org/10.1186/s13065-020-00730-1.
8. Pal, S.; Khan, Md. N.; Karamthulla, S.; Choudhury, L. H. Molecular Iodine Catalyzed One-Pot Multicomponent Reactions for the Synthesis of Dihydrochromeno[4,3-b]Pyrazolo[4,3-e]Pyridin-6(7H)-Ones. RSC Adv. **2013**, 3 (36), 15705. https://doi.org/10.1039/c3ra41569d.
9. Hanoon, H. D.; Radhi, S. M.; Abbas, S. K. Simple and Efficient Synthesis of 2,4,5-Triarylsubstituted Imidazole Derivatives via a Multicomponent Reaction Using Microwave Irradiation; Karbala City, Iraq, 2019; p 020005. https://doi.org/10.1063/1.5123062.