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Synthesis, antiproliferative activity and *in silico* studies of new 2,4,5-triaryl-1H-imidazole derivatives

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Abstract:

Cancer and especially a breast cancer is a devastating health burden. Numerous approved therapies have proven beneficial in the treatment of breast cancer. However, resistance, serious adverse effects and remission due to limited efficacy needed a pursuit of newer effective agents. Substituted imidazole derivatives have been reported to possess antiproliferative activity. The present work is about the synthesis of a library of novel 2,4,5-triaryl-1H-imidazole derivatives. The synthesized compounds were screened for their anticancer activity against cancer cell line (MCF-7) by the MTT assay and many of them showed significant activity. Three compounds among the series have exhibited better antiproliferative activity due to presence of chromen-4-one and indole moiety attachment at 2^{nd} position compared to the standard drug anastrazole. Furthermore, an in-silico molecular docking study has been performed against cyclin-dependent kinase to know the binding modes of these molecules and to further help the design of more promising agents. The compounds with chromen-4-one, quinoline-4-one and indolyl substituents referred to as CTI1, CTI3, and GTI9, respectively have been reported to possess promising anticancer activity.

Keywords Triaryl imidazole, Anticancer, MTT assay, Docking, Anastrazole

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1. Introduction

The incidence of cancer is on the rise in India as well as around the globe. Earlier oral, breast, and cervical cancers constituted a major burden of cancer globally. However, the recent statistics suggest that the cases of lung, colorectal, breast, and prostate cancers are increasing manifold. In 2023, it is projected that there will be approximately 1,958,310 new cases of cancer and 609,820 cancer-related deaths in the United States.[1] The statsestics of 2020 showed over 2.3 million new cases and 685,000 deaths from breast cancer worldwide in 2020.[2] Several approved drugs (Figure 1), especially having a imidazole core, are used the treatment of breast cancer. Apart from this, the midazole derivatives possess antimicrobial, anticancer, and anti-inflammatory properties, making them potential drugs.[3,4] Further, triaryl imidazoles, a class of imidazole derivatives, exhibit broad-ranging biological activities. They have shown potential as anticancer agents, with studies demonstrating their ability to inhibit tumor growth and induce apoptosis.[5–7] Some of the approved drugs conataning imidazole core include Dacarbazine, Temzolomide, Zoledronic acid, Mercaptopurine, Nilotinib, and Tipifarnib.[8]

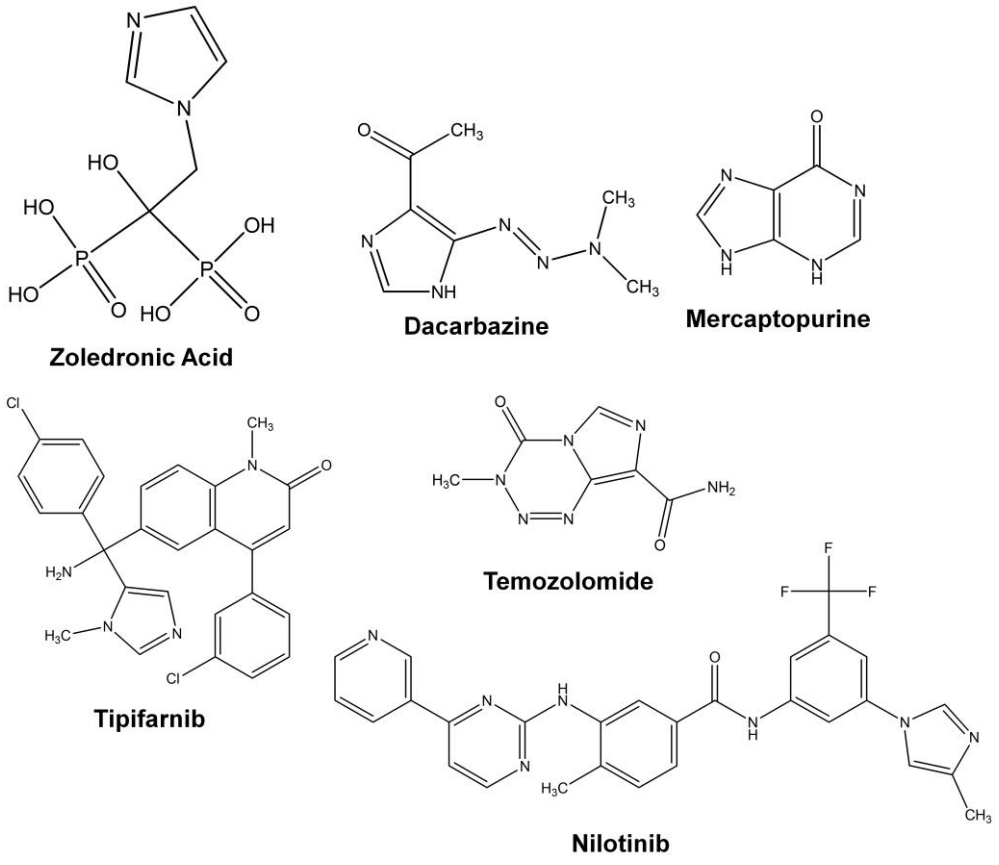

Figure 1. Some of the standards marketed drugs containing imidazole moiety

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Intestinally, imidazoles and benzimidazoles derivatives have been found effective as tubulin-modulators for anti-cancer therapy.[9] The 2,4,5-triaryl-1H-imidazole derivatives have been reported effective in non-small cell lung cancers and breast cancer.[10] The imidazole and fused imidazole derivatives have been shown to modulate various targets, including microtubules, tyrosine and serine-threonine kinases, histone deacetylases, p53-Murine Double Minute 2 (MDM2) protein, poly (ADP-ribose) polymerase (PARP), G-quadraplexes, and other targets.[11,12] Especially, the triaryl imidazole have been reported having a potential in inhibiting CDKs (cyclin-dependent kinases) and other kinases for anticancer activity.[13] CDKs are a family of serine/threonine kinases that play a crucial role in controlling cell cycle transcription. Recent studies have explored the synthesis and evaluation of imidazole-based CDK inhibitors as promising anticancer agents.[14]

The synthesis of triaryl imidazole derivatives has been reported by variety of synthetic approaches. The use of lactic acid as a promoter[15], a one-pot condition, a three-component reaction under solvent-free conditions using $Ni_{0.5}Zn_{0.5}Fe_2O_4$ nanoparticles[16], and several other methods including the condensing benzyl/benzoin, aldehydes and ammonium acetate using different catalysts such as HY/silica gel, acidic Al_2O_3, AcOH, $ZrCl_4$, ionic liquid, iodine, NH_4OAc, $Yb(OTf)_3$, $NiCl_2.6H_2O$, sodium bisulphate, PEG-400, boric acid, CAN Fe_3O_4 nanoparticle and poly(AMPS-co-AA)[17].

The current investigation describes synthesis of new triaryl imidazole derivatives with conventional method.

2. Materials and methods

2.1. Materials and Instruments

All reagents and chemicals used were of LR grade and standard quality. Melting points were determined on scientific melting point apparatus in open capillaries and were uncorrected. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ using BRUKER-300 MHz spectrometer and chemical shifts are given in units as parts per million, downfield from TMS (tetramethylsilane) as an internal standard. Mass spectra were obtained on a Bruker Compass Data Analysis 4.2 Impact HD spectrometer. The IR spectra of the synthesized compounds were recorded on Bruker Alpha-T ATR FT-IR spectrophotometer in potassium bromide discs.

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2.2 General procedure for synthesis of Tri-aryl substituted Imidazole

A mixture of Benzil/Benzoin (10 mmol), appropriate aromatic aldehyde (10 mmol), and NH_4OAC (60 mmol) in 10 ml ethanol were taken in RBF, stirred and refluxed for 1hr. The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled to room temperature and poured on crushed ice. The obtained crude solid product was filtered, washed with water and dried. Further, purification was done by recrystallization using ethanol to get the corresponding 2,4,5-triaryl-1H-imidazole.

2.3 Docking study on 2-aryl-4,5-substituted diphenyl-1H-imidazole

Docking simulation was performed to establish possible mode of action of the developed differently substituted triaryl substituted 1H-imidazole derivatives. Cyclin-dependent kinase- (PDB id: 5IEY dated: 24/05/23) downloaded from www.rcsb.org was used for docking studies. The co-crystal ligand having code 6AE belongs to sulphonamide derivatives inhibitor bound at the binding site of 5IEY. Autodock vina 1.2.0 was used to perform the docking simulations. Marvin Sketch 5.6.0.0 (2011) was used to draw two-dimensional structures of the compounds which were converted into three-dimensional (3D) geometry. Geometry of 3D molecules was optimized through energy minimization using UCSF Chimera 1.8[18], during which Gasteiger charges were added and energy minimization was carried out with combination of steepest descent and conjugate gradient geometry search criteria until gradient converses to 0.05 and 0.01, respectively. The protein was processed by removing water and other nonstandard residues. The resulting clean protein was further optimized by energy minimization in UCSF Chimera with Amber ff12SB force field and similar geometry search criteria. During docking simulation polar hydrogen was added to protein structure with MGLtools1.5.4. All the torsion angles for the compounds were set free so as to perform flexible docking. Grid box of size 40 × 46 × 40 with 0.375 A° spacing was defined along x, y and z axis, which was large enough to cover active site of protein. The results of docking simulations were analyzed in terms of estimated binding free energy in kcal/mol, estimated inhibition constant (Ki) in μM and interactions of ligands with residues at active site.

2.4. Cell Viability Assay\ MTT Assay of test compounds and their analysis [19]

Cell survival can be monitored by various methods, including cell viability reagents, which use the reducing power of living cells to measure proliferation and establish relative cytotoxicity in different species and sample types. Trypsinization of adherent cell culture was

achieved. Around 8,000 cells/well in a 96-well plate were seeded with the appropriate cell culture medium. later, the cells were treated with different concentrations of the test compound in different concentrations along with control and positive control, drug. The analysis was repeated in triplicate. The cell viability was measured using the MTT assay after 24 hours. The cells were incubated with MTT solution, centrifuged, and dissolved the resulting crystals in DMSO. The absorbance was measured using a micro plate reader at 540 nm and calculated the percentage of cell viability.

2.4.2 Statistical Analysis of Cell viability Assay

Statistical treatment (One way ANOVA, Graph pad Prism ver. 8) given to the cell viability assay results where, IC_{50} values are calculated based on mean taken from triplicates results of compounds and MTT assay % cell viability vs concentrations plot was done by comparing result % of standard drug with % of test compounds along with control. Anastrozole is used as a standard drug used for activity.[20]

3. Results and discussion

3.1. Chemistry

The scheme of synthesis is shown in Figure 2 and Table 1. In the synthesized derivatives, the imidazole ring shows characteristic absorption bands at 1600-1700 cm-1 (C=N stretching), 1400-1500 cm-1 (C=C stretching), and 600-800 cm-1 (C-H bending) in IR. While ^{1}H NMR spectrum showed a characteristic signals at 7.0-8.5 ppm (aromatic protons), 4.5-5.5 ppm (imidazole proton), and 2.0-3.5 ppm (methylene protons), all of which are typical for imidazole ring. The aryl groups show signals depending on their substitution patterns and coupling constants. Mass spectroscopic analysis reveals that imidazole ring shows characteristic fragmentation patterns, such as loss of NH_3, C_2H_4, or C_3H_4N.

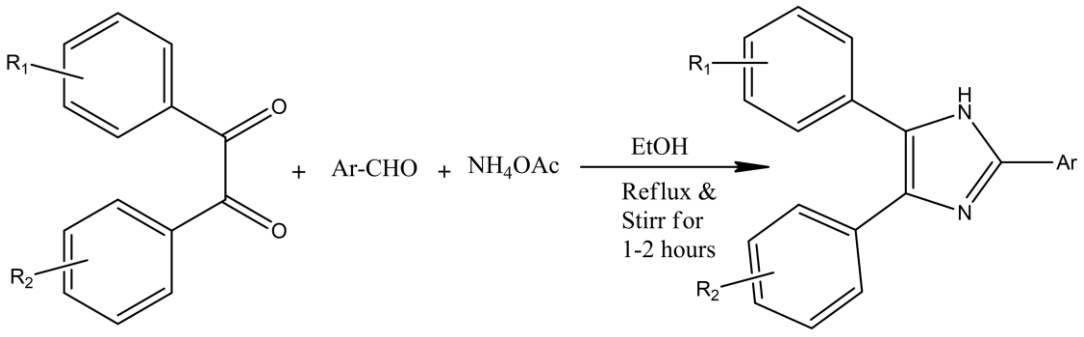

Figure 2 : General Reaction scheme of Substituted Triaryl Imidazole

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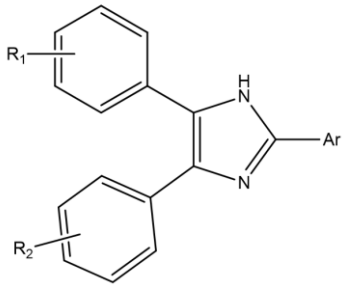
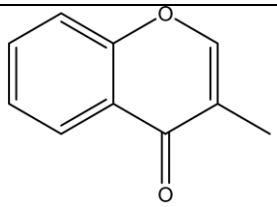
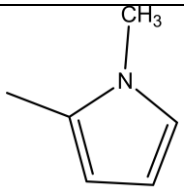
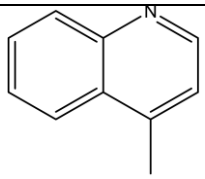
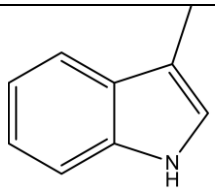
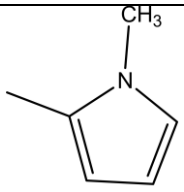
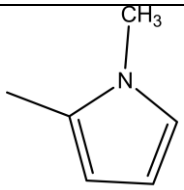
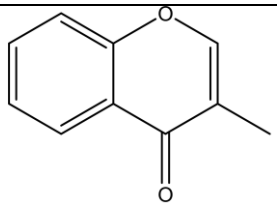
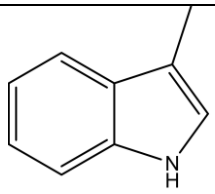
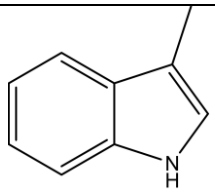
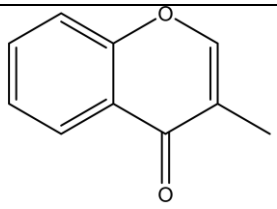
Table 1: Data of various substitutions, Reaction variables of New Triaryl Imidazoles derivatives (CTI1-GTI10)



Compound Name	-R1	-R2	-Ar	MW	Time (Min.)	% Yield	M.P.
CTI1	-CH3	-CH3		392.45	80 Min	52%	127 °C
CTI2	-OCH3	-OCH3		359.42	90 Min	48%	122 °C
CTI3	-OCH3	-OCH3		407.46	75 Min	62%	152°C
CTI4	-OCH3	-OCH3		395.16	95 Min	63%	148 °C
CTI5	-Cl	-2OCH3		393.87	70 Min	59%	136 °C

GTI6	4-CH_3	4-CH_3		327.42	70 Min	44 %	148 °C
GTI7	4-OCH_3	4-OCH_3		424.45	85 Min	38 %	130 °C
GTI8	4-CH_3	4-CH_3		363.45	75 Min	46 %	150 °C
GTI9	2-Cl	3-OCH_3, 4-OCH_3		429.90	120 Min	49 %	134 °C
GTI10	2-Cl	3-OCH_3, 4-OCH_3		458.89	105 Min	46 %	118 °C

All compounds were characterized by melting point (MP), infrared (IR), ^{1}H NMR, ^{13}C NMR, and mass spectroscopy (MS). (**CTI1-GTI10**)

1) **3-[4,5-bis(4-methylphenyl)-1H-imidazol-2-yl]-4H-chromen-4-one (CTI-1):** IR(cm-1): 3399 (N-H), 1596 (C=N), 1654(C=C), 1730(C=O), 2914 (CH3), 1033 (C-N), 3056(Ar- H), 1477(Ar-C-C), 876 (Ar-C-H). ^{1}H NMR 500 MHz(CDCl3): δ 0.880(s, 2H), 3.090(CDCl3), 1.593(s,7H), 7.141(1H), 7.488(s,1H), 7.552(1H), 7.602(1H), 3.090(1H), 6.988(s,1H), 7.743(1H), 8.406(s,1H), 9.101(1H), 11.750(s,1H). M.P. 122-127°C.

2) **4,5-bis(4-methoxyphenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-imidazole (CTI-2):** IR(cm-1): 3613(N-H), 1648(C=C), 1550(C=N), 1212(C-N), 1743(C=O), 2913(-CH3), 868(Ar-C-H), 1413(Ar-C-C). ^{1}H NMR 500 MHz(CDCl3): δ 2.170(s,1H),3.884(CDCl3), 1.593(s,7H), 1.254(s,7H), 0.880(2H). M.P. 120-124°C.

3) **4-[4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl]quinoline.(CTI-3) :** IR(cm1): 3277(N-H), 1695(C=N), 1651(C=C), 1153(C-N), 2915(-CH3), 1416(Ar-C-C),

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832(Ar-C-H). ^{1}H NMR 500 MHz(CDCl3): δ 1.637(s,14H), 0.866(1H), 3.884(CDCl3), 0.880(1H), 0.893(2H).). HRMS M+ (calcd.). 408.17. M.P. 150-155°C.

4) **3-[4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl]-1H-indole (CTI-4) :**
IR(cm-1): 3186(N-H), 1694(C=N),1652(C=C), 1159(C-N), 2925(-CH3), 1520(Ar-C-C), 826(Ar-CH). ^{1}H NMR 500 MHz(CDCl3): δ 1.227-1.295(s,6H), 3.884(CDCl3), 3.851(2NH), 3.814(s,8H), 2.032(s,5H). M.P. 146-151°C.

5) **4-(2-chlorophenyl)-5-(3,4-dimethoxyphenyl)-2-(1-methyl-1H-pyrrol-2-yl)-1H-imidazole (CTI-5)**: IR(cm-1): 3284(N-H), 1695(C=N), 1650(C=C), 1140(C-N), 1742(C=O), 2922(-CH3), 1461(Ar-C-C), 859(Ar-CH), 808(R-Cl). ^{1}H NMR 500 MHz(CDCl3): δ 1.254(s,6H), 3.725(cl), 3.980(CDCl3) 7.534(3H), 7.607(s,3H), 7.904 (1H), 7.908 (2H), 7.418(2H), 7.261(1H). ^{13}C NMR 125 MHz(CDCl3): δ 77.253(C3), 56.098(C3), 24.051(c4), 134.26, 133.94, 130.73, 127.80, 126.30, 126.30, 121.60, 111.14, 110.75, 108.31, 107.82(C3). HRMS M+ (calcd.) 394.14. M.P. 134-138 °C.

6) **2-(1-methyl-1H-pyrrol-2-yl)-4,5-di-p-tolyl-1H-imidazole (GTI 6):**
IR: 2900 (N-CH_3), 1653.63 (C=C Stretch), 1522 (Ar. N), 980.58 (=CH Out of Plane), 1653 (C=N Stretch), 3316 (NH Stretch), 818.15 (C-H Out of Plane), 2891.29 (CH Stretch), 1461.70 (CH_2 & CH_3 Alkanes), 1317.49 (Ar. N Stretch). ^{1}H NMR 500 MHz ($CDCl_3$): δ7.849 (s, 5H), 7.866 (s, 6H), 7.291-7.307 (m, 6H), 7.258 ($CDCl_3$), 2.433 (s, 9H), 6.727 (s, 1H). M.P. 146-149 °C.

7) **3-(4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)-4H-chromen-4-one (GTI 7):**
IR: 1644.54 (C=C Stretch), 1342.55 (Ar. N), 676.74 (=CH Out of Plane), 3504.77 (NH Stretch), 749.85 (C-H Out of Plane), 3059.09 (CH Stretch), 1461.62 (CH_2 & CH_3 Alkanes), 1003.63 (C-O Stretch), 1072.41 (C-N Stretch), 1256.00 (-CH_3), 1693.88 (C=O Stretch). ^{1}H NMR 500 MHz ($CDCl_3$): δ7.262 (s, 1H),7.955 (s, 8H), 7.412 (s, 2H), 7.503 (s, 2H), 1.254 (m, 6H), 7.262 ($CDCl_3$), 8.328 (w, 1H). HRMS M+ 425.15 (Calcd. 425.15, 28.5%). M.P. 128-132 °C.

8) **3-(4,5-di-p-tolyl-1H-imidazol-2-yl)-1H-indole (GTI 8):**
IR: 3311.59 (NH Stretch), 1653.03 (C=C Stretch), 1264.15 (Ar. N), 696.67 (=CH Out of Plane), 1171.33 (C-N Stretch), 1694.56 (C=N), 2039.82 (N=C), 799.64 (C-H Out of Plane), 2932.02 (CH Stretch), 1465.41 (CH_2 & CH_3 Alkanes), 1420.80 (Ar. C-C Stretch). ^{1}H NMR 500 MHz ($CDCl_3$): δ7.848 (s, 6H), 7.376 (s, 7H), 7.258 ($CDCl_3$), 1.254 (s, 3H), 1.968 (s, 3H), 7.593 (s,1H), 7.153 (s, 1H). ^{13}CNMR (125MHz, $CDCl_3$) δ77.272 ($CDCl_3$), 21.931, 29.702, 21.281, 129.712, 119.519. HRMS M+ 364.1821 (Calcd. 364.18, 28.5%). M.P. -148152 °C.

9) **3-(4-(2-chlorophenyl)-5-(3,4-dimethoxyphenyl)-1H-imidazol-2-yl)-1H-indole (GTI 9):**
IR: 1415.54 (Ar. C-C Stretch), 730.60 (=CH Out of Plane), 1685.98 (C=N Stretch), 2022.54 (N=C), 3332.58 (NH Stretch), 860.57 (C-H Out of Plane), 2885.58 (CH Stretch), 1509.18 (CH_2 & CH_3 Alkanes), 1333.75 (Ar. N Stretch), 1236.58 (C-O

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Stretch), 1129.46 (C-N Stretch), 1630.95 (NH Out of Plane), 3020.03 (=CH Stretch), 808.00 (C-Cl). ^{1}H NMR 500 MHz ($CDCl_3$): δ7.227 (s, 10H), 7.448 (s, 2H), 7.260 ($CDCl_3$), 1.979 (s, 5H), 3.975 (m, 1H), 7.603 (s, 1H), 7.322 (s, 1H). M.P. 132-136 °C.

10) **3-(4-(2-chlorophenyl)-5-(3,4-dimethoxyphenyl)-1H-imidazol-2-yl)-4H-chromen-4-one(GTI 10):**
IR: 1408.63 (Ar. C-C Stretch), 735.08 (=CH Out of Plane), 1997.17 (N=C), 3442.62 (NH Stretch), 680.21 (C-H Out of Plane), 2887.61 (CH Stretch), 1358.43 (Ar. N Stretch), 1002.06 (C-O Stretch), 1082.82 (C-N Stretch), 3118.74 (=CH Stretch), 803.55 (C-Cl), 1562.01 (C=C Stretch), 1780.98 (C=O Stretch). ^{1}H NMR 500 MHz ($CDCl_3$): δ7.745 (s, 3H), 7.469 (s, 4H), 7.350 (s, 1H), 7.285 (s, 4H), 3.744 (s, 5H), 3.710 (s, 1H), 7.263 ($CDCl_3$), 7.318 (s, 1H). M.P. 118-123 °C.

3.2 Docking Study:

In order to get insights into mode of action of the heterocyclic group bearing 2, 4,5-triaryl imidazole derivatives, we carried out docking simulations using Autodock vina with MGL Tools. The results of docking studies are shown in Table 2. The docking results are in good agreement with the experimental results. The compounds **CTI1, CTI3, GTI8,** and **GTI9** suggested that these compounds forms key interactions with important residues at the binding site of 5IEY kinase.

Table 2: PROTEIN LIGAND PROFILER RESULTS

Sr. No	Comp. ID	Binding Free Energy (Kcal/mol)	Key Interactions	
			Hydrogen Bonds	Hydrophobic Interactions
1.	CTI1	-10.489	ASP86A, ILE10A	VAL18A, ALA31A, PHE80A, PHE82A, GLN131A, LEU134A, ALA144A.
2.	CTI2	-8.343	--	VAL18A, ALA31A, ASP86A, LYS89A, LEU134A, ALA144A, GLU12A.
3.	CTI3	-9.867	GLU12A, LYS89A	ILE10A, ILE10A, VAL18A, ALA31A, PHE82A, PHE82A, ASP86A, LEU134A, ALA144A.
4.	CTI4	-9.323	GLU12A, ASP86A, LYS89A	ILE10A, ILE10A, VAL18A, ALA31A, PHE82A, PHE82A, GLN131A, LEU134A, ALA144A
5.	CTI5	-8.725	ASP86A	ILE10A, VAL18A, VAL18A, ALA31A, ASP86A, LEU134A,

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				LEU134A.
6.	GTI6	-9.752	--	ILE10A, VAL18A, ALA31A, PHE80A, ASP86A, LYS89A, GLN131A, LEU134A, ALA144A
7.	GTI7	-9.983	GLU12A, ASP86A	VAL18A, ALA31A, PHE82A, GLN131A, LEU134A, ALA144A
8.	GTI8	-11.288	ILE10A	ILE10A, VAL18A, ALA31A, PHE80A, GLN85A, LYS89A, LEU134A, ALA144A.
9.	GTI9	-10.025	ILE10A, GLN131A	VAL18A, ASP86A, LEU134A, LEU134A.
10.	GTI10	-9.687	GLN131A	ILE10A, ILE10A, VAL18A, ALA31A, LYS89A, LYS89A, LEU134A.
11.	6AE (Co-crysal ligand)	-8.261	THR14A, THR14A, LYS33A, YS129A, ASP145A, ASP145A ASP145A	ILE10A, VAL18A, ALA31A, PHE80A, PHE80A, LEU134A.

The key interactions observed with the co-crystal compound and compounds CTI1 CTI3 and GTI9 are shown in Figure 4-7.

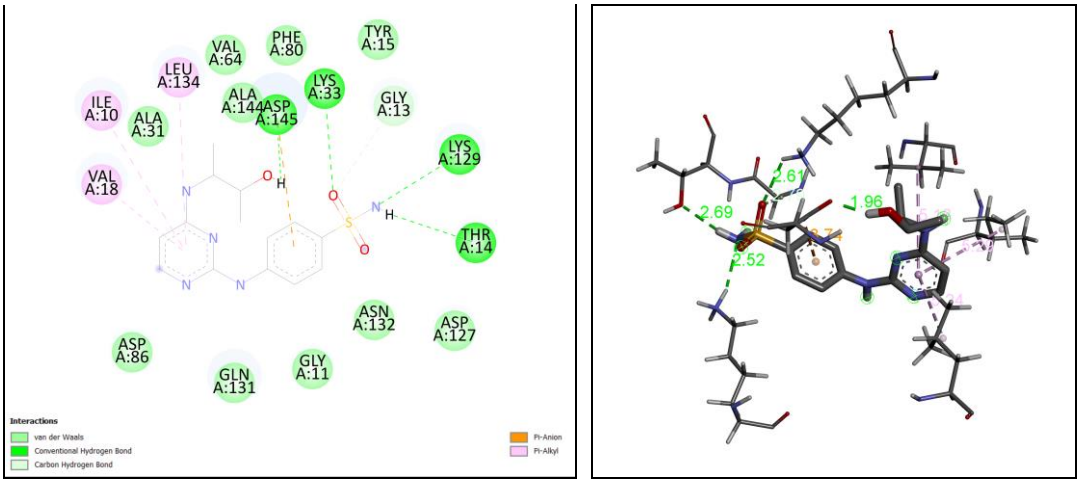

Figure 4. Binding interactions of 6AE Co-crystal 2D and 3D Structure

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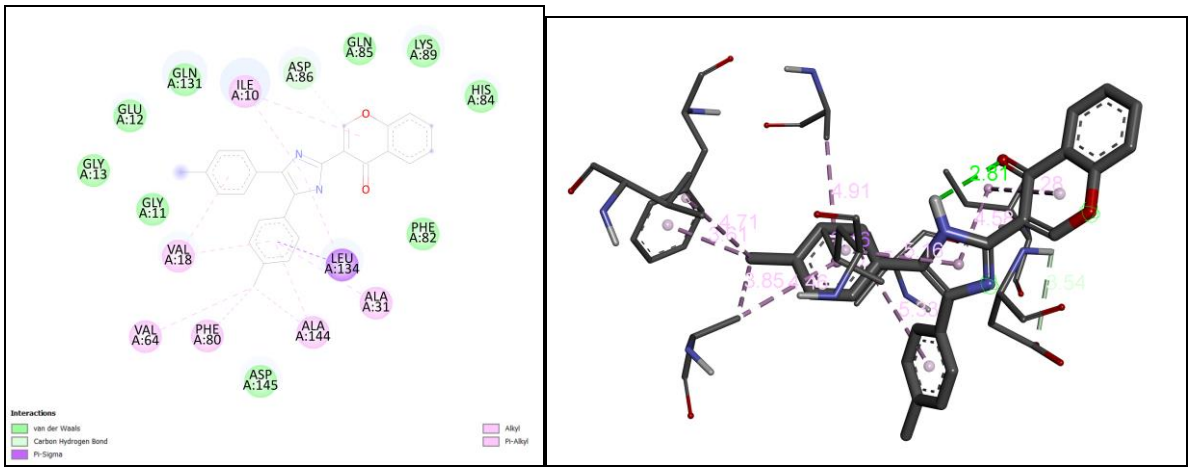

Figure 5. Binding interactions of CTI1 2D and 3D Structure

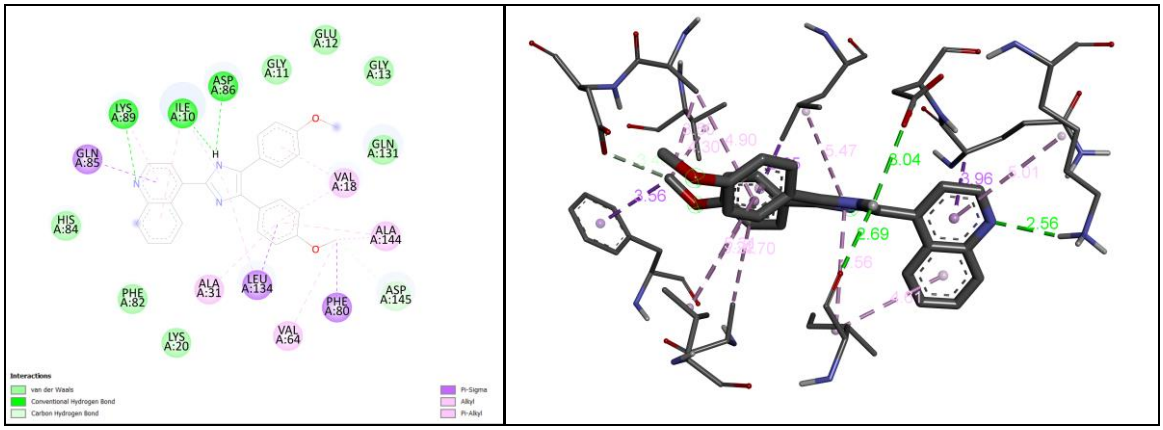

Figure 6. Binding interactions of CTI3 2D and 3D Structure

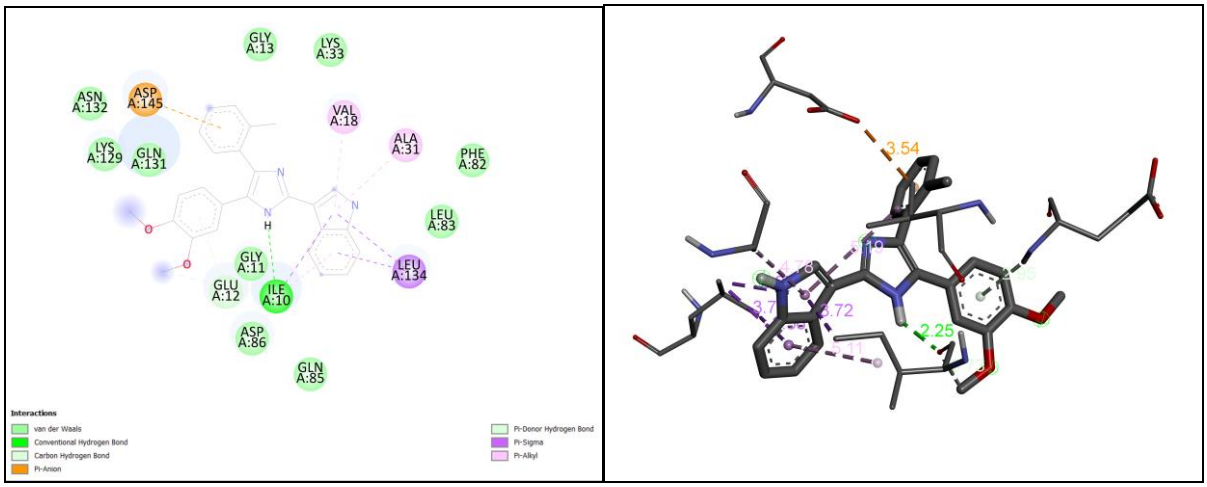

Figure 7. Binding interactions of GTI9 2D and 3D Structure

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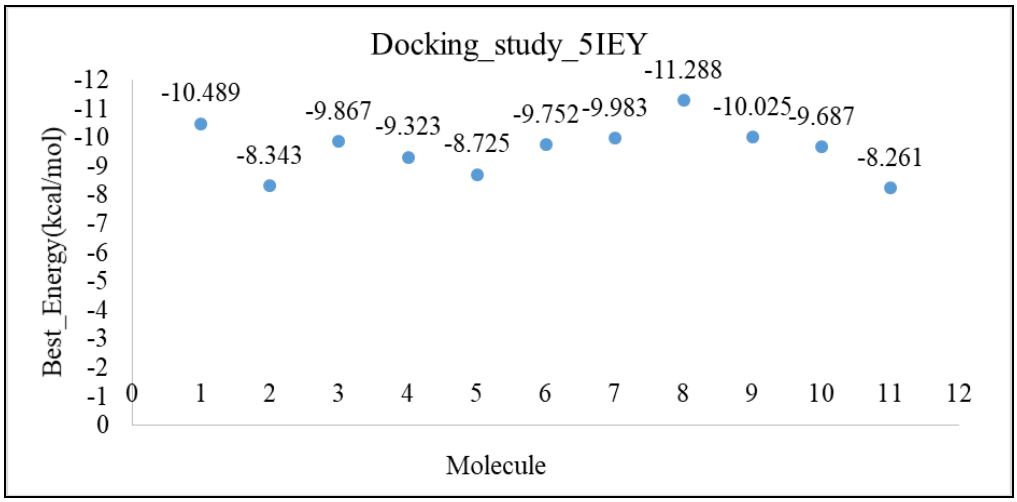

Figure 8. DOCKING STUDY 5IEY

3.3. Cell Viability Assay\ MTT Assay Statistical analysis

All the values were expressed as the mean ± SEM and were subjected to One-Way Analysis of Variance (ANOVA) followed by Tukey's test, where $P < 0.0001$ was considered as statistical significant. All compounds are active against the control (MCF7 Cell lines) at lowest Concentration except **GTI7** which is active only in higher concentration producing significant linear response. The results are shown in Table 3 and Figure 9 and 10. All concentrations of compound **CTI1, CTI3, GTI8, GTI9** are showing significant result for cytotoxic activity compared to the control and standard drug. All these compounds are highly active than standard drug at their lowest concentration (20µM). From above compounds **GTI8** have lowest IC_{50} value. On other hand, the compounds which have most significant results, lower IC50 values and linearly active concentrations are **CTI1, CTI3, GTI9.** Compounds **CTI2, CTI4, CTI5** and **GTI10** are linearly active and significant with their linear concentrations compared to control but their lowest concentration (20 µM) is not significant when compare to the lowest concentration of standard drug.

Compounds	IC50
CTI1	29.99
CTI2	117.9
CTI3	<10
CTI4	<10
CTI5	20.63
GTI6	132.2
GTI7	22.43
GTI8	<10
GTI9	<10
GTI10	13.3

Table 3: IC50 Values of all Tri-aryl Imidazoles

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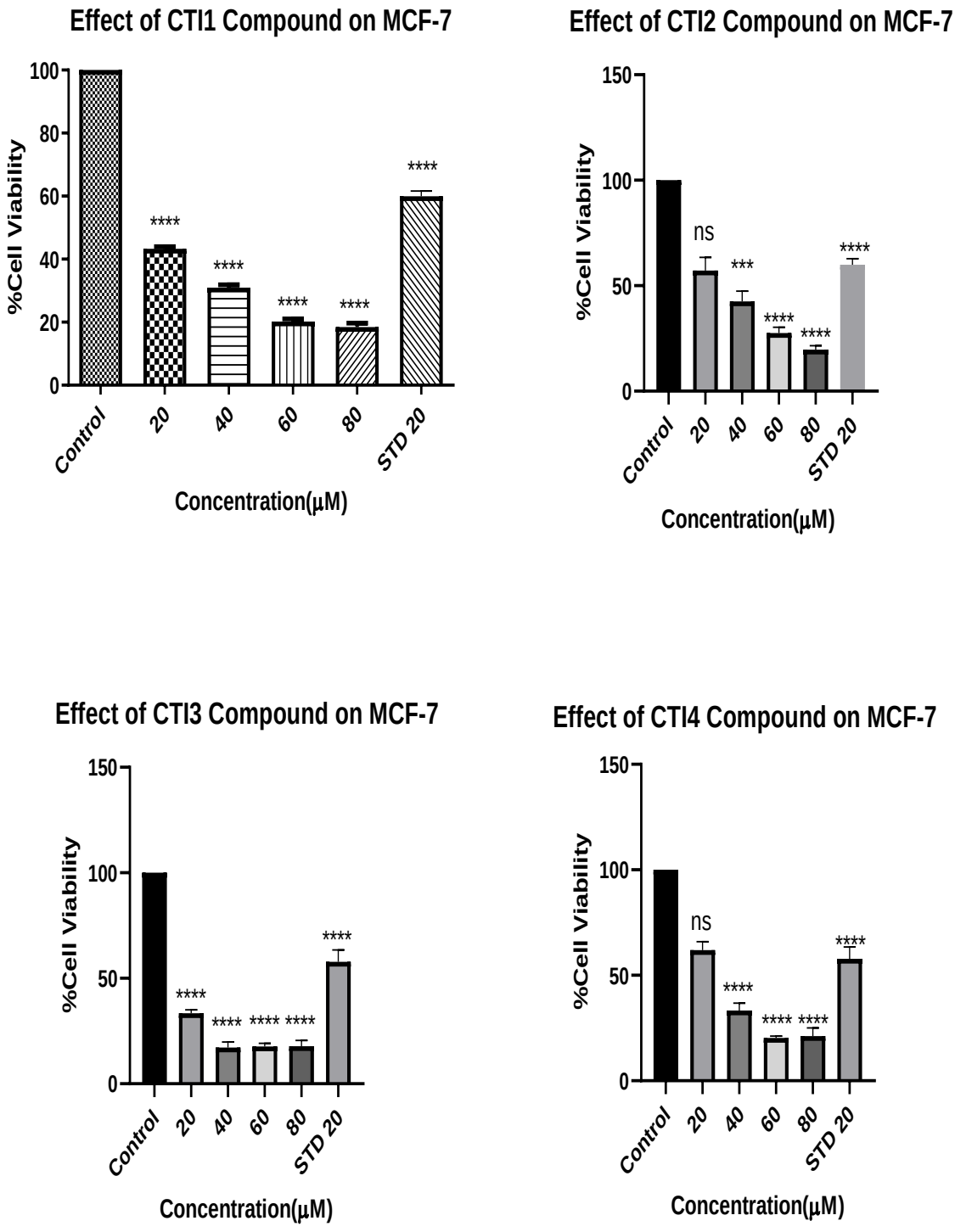

Figure 9. ANOVA of MTT assay analysis % cell viability vs Concentrations of test compounds CTI1-CTI4)

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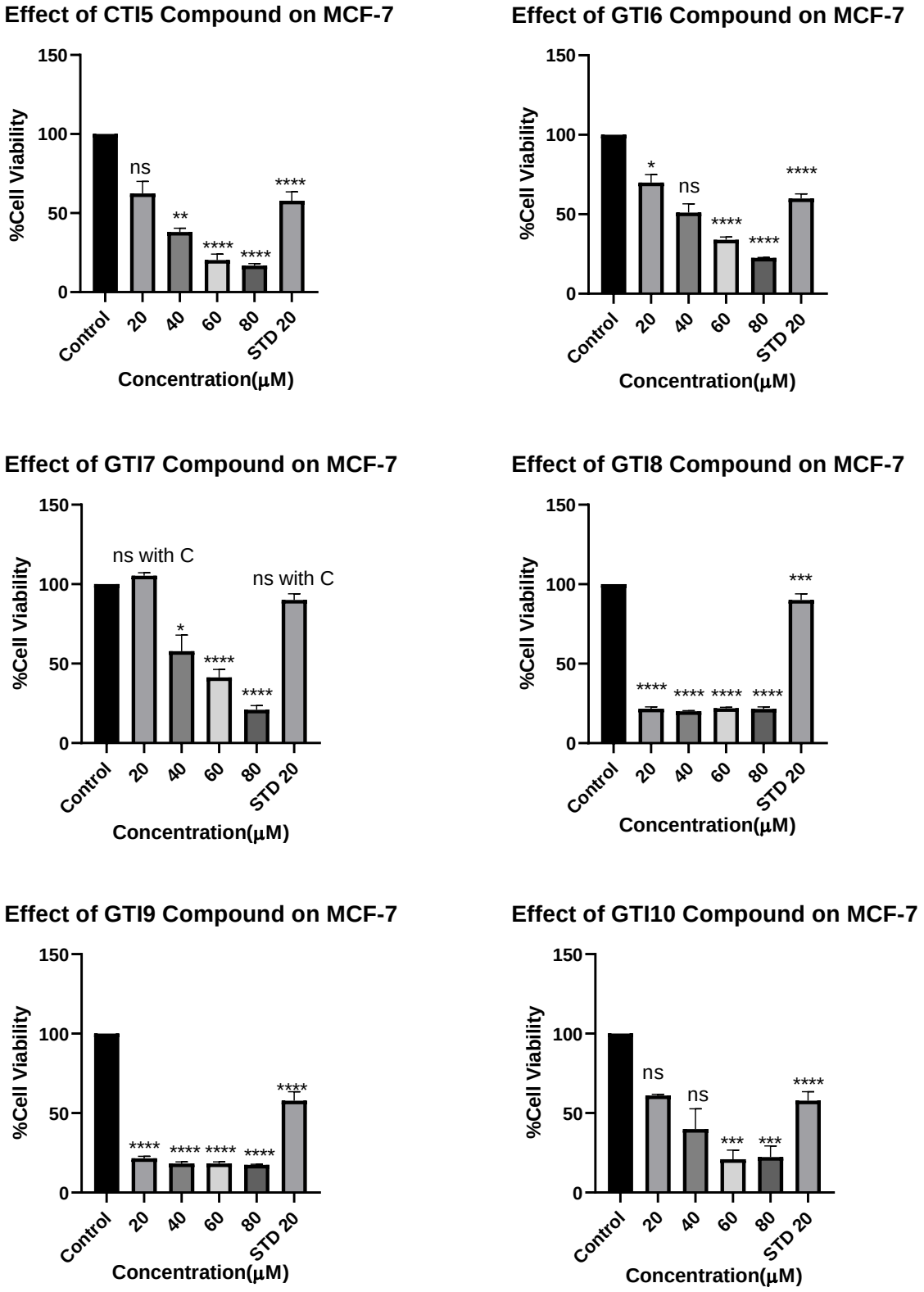

Figure 10. ANOVA of MTT assay analysis % cell viability vs Concentrations of test compounds CTI5-GTI10)

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*(Note: Each value mean presents the ± S.E.M. of three observations by ANOVA followed by Tukey's test, only 20µM Concentration of GTI7 statistically not significant as compared to Normal control group. 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 (Anastrazole). ns=not significant.)*

4. Conclusion

In summary, the newly synthesized 2-aryl-4,5-substituted diphenyl-1H-imidazole **(CTI-GTI10)** were evaluated for anticancer MTT assay on MCF7 cell lines. Though most of the compounds showed good cytotoxicity by significantly affecting the growth of the cancer cells, compounds **CTI1, CTI3, GTI9** was shown the best activity in causing cytotoxicity. All these compounds also gave the good docking score as well as lowest IC_{50} values compared to other test compounds. Overall, the title compounds **CTI1, CTI3, GTI9** can be looked upon as potential leads for further development & investigations.

6. Conflict of Interest

The authors claim there is no conflict of interest.

7. References

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