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1,2,3-Triazolyl pyrazole derivatives as anti-cancer agents: biological evaluation and molecular docking

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ABSTRACT

*A series of newcompounds3-{5-methyl-1-[2-methyl-3-(trifluoromethyl) phenyl/substituted phenyl]-1H-1,2,3-triazol-4-yl}-1-(aryl)-1H-pyrazole-4-carbaldehydes**(5a-n)**wassynthesized by a Vilsmeier-Haackformylation reaction of 4-{(1E)-1-[2-(aryl) hydrazinylidene]ethyl}-5-methyl-1-[2-methyl-3-(trifluoromethyl)phenyl/substituted phenyl]-1H-1,2,3-triazole **(4a-n)** with Phosphorous oxychloride-DMF mixture. The newly synthesized compounds were elucidated by their spectral studies. Further, the in-vitro anti-cancer activities of the newly synthesized compounds**(5a-n)**were carried out against breast cancer cell lines MCF-7 and MDA-MB-231. The compounds **5c, 5f, 5g, 5j, 5m** and **5n** exhibits significant activities against both the cell lines MCF-7 and MDA-MB-231 with IC_{50} values in the range of 6.8-9.8 μM and 11.1-14.1 μM respectively. The anti-cancer results were further supported by the in-silico molecular docking studies for the inhibition of Epidermal growth factor receptor (EGFR) kinase (PDB ID: 2A91) and human estrogen receptor (PDB ID: 2IOK) respectively, showed minimum binding energies and good affinities towards the active pockets comparable with the standard drug Toremifene. Thus, they may be considered as good inhibitors of EGFR kinase domain (PDB ID: 2A91) and human estrogen receptor (PDB ID: 2IOK).*

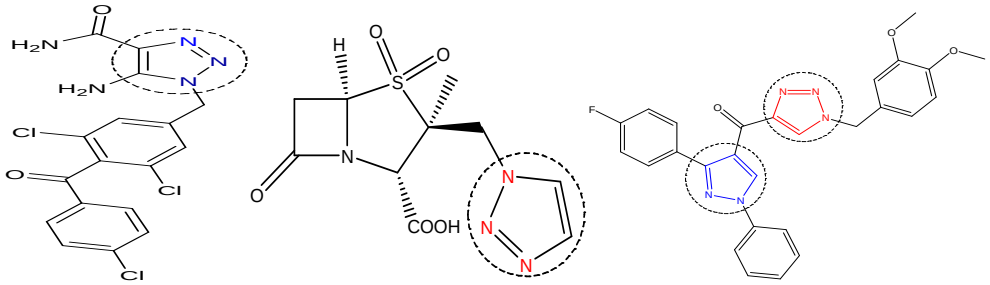
Key words: Triazole, pyrazole, anti-cancer activity, Molecular docking studies.

INTRODUCTION

The World Health Organization's cancer agency unreveals that there will be approximately 22 million new cancer cases per year within the next two decades. Also, in 2012 there were nearly 14 million new cases were estimated by International Agency for Research on Cancer (IARC), but further estimated that the figure would jump significantly due to global ageing and the vigorous spread of cancers to developing countries. Cancer, a diverse versatile group of diseases characterized by uncontrolled, quick and pathological proliferation of abnormal cells, is among one of the major worldwide health problems standing next to the cardiovascular ailment in terms of morbidity and mortality. Despite considerable significant advances in diagnostic and therapeutic techniques, the medicines used as treatments

have certain limitations and unfortunately cancer is highlighted as the primary cause of death in the future[1, 2].In world-wide breast cancer is the second most common cancer in women with nearly 1.05million new cases being estimated in the year 2011 and a rise to 1.31 million cases is predicted by 2020[3, 4].In general, breast cancer is broadly classified as endocrine receptor (i.e.estrogen-receptor or progesterone-receptor), positive or negative. Several numerous studies have revealed that Estrogen receptors (ERs) play a predominantly key role in the initiation and proliferation of breast cancer[5]. Growth factor signalling plays a major part in prostatic oncogenesis[6].Receptor protein tyrosine kinases associated with multiple factors like growth, controlling various types of cell functions like proliferation, apoptosis, differentiation, cell cycleprogression etc. EGFR family of trans-membrane proteins serves to the development and progression of multiple malignancies including prostate cancer[7, 8]. Consequently, many women develop metastatic breast carcinoma, disease which is preliminarily incurable and the prognosis has changed little over the past decade[9]. Among all the current therapeutic methods, chemotherapy is one of the best commonly used treatments worldwide to cure and prevent various types of cancers[10]. Currently, combination chemotherapy with flexible action mechanisms is one of the methods that are being adopted to treat breast cancer are often associated with side effects and the development of drug resistance in cancer cells, whereby the patients majorly succumb totheir disease within span of 2 years of diagnosis. Hence, there is a great need for the development of novel small molecules as anticancer agents that are safer with the great potential to effectively manage the different subtypes of breast cancer[11, 12].

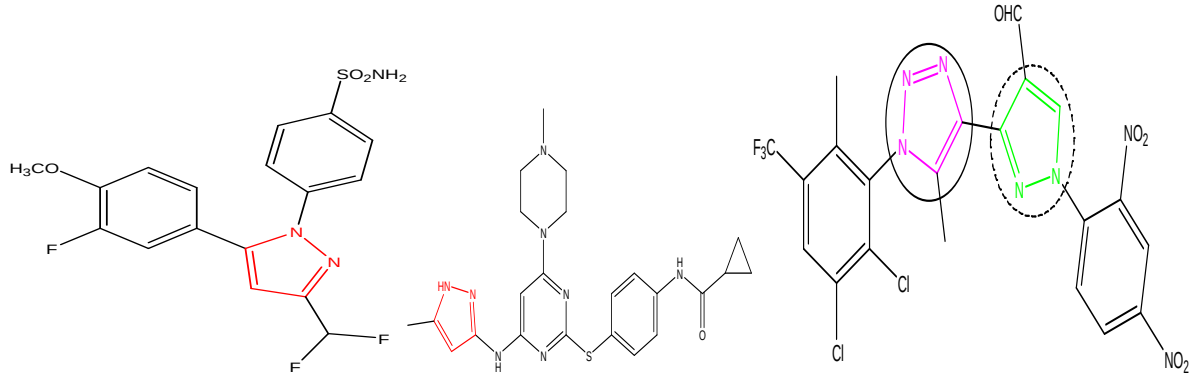
On the other hand, N-Heterocyclic compounds are very important in drug design[13, 14].Among these, 1,2,3-Triazole derivatives have occupied an unique important role not only in organic chemistry but also in medicinal chemistry due to their synthetic ease by well-known click chemistry and attractive features as well as numerous pharmacological activities[15, 16]. 1,2,3-triazoles are stable, high dipole moment and has the capability of forming hydrogen bonding, which could be favourable in the bio molecular target binding[17]. 1,2,3-Triazole is one of the important key structural units found in a numerous variety of bioactive molecules as anti-microbial[18, 19], anti-viral[20], anti-tubercular[21] and anti-inflammatory agents[22]. Several 1,2,3-triazole containing drug molecules including tazobactum[23],carboxyamidotriazole[24] are now available in the market. In recent year, researchers are increasingly focusing on their anti-cancer activity[22 - 27]. Similarly, pyrazole moiety is great importance to chemists as well as biologists as its derivatives found to exhibit extensive range of biological and pharmacological activities, including effects as anti-microbial[28, 29], anti-inflammatory[28], anti-oxidant[30], analgesic[31],anti-convulsant[31], anti-cancer[32, 33]etc. Pyrazoles are reported to exert their anti-cancer activity through inhibition of various bio molecular targets such as epidermal growth factor (EGF), tumour growth factor (TGF) and different kinases that are invariably significant for the management of cancer[34, 35]. Moreover, the combination of 1,2,3-triazoleand pyrazole scaffolds in single molecular frame work illustrated enhanced activities as shown by examples in **Figure 1.**



CAI (Carboxyamidotriazole)
(Anti-biotic)

Tazobactam
(Anti-cancer)

(Anti-cancer)



Deracoxib (Anti-inflammatory) **Tozasertib (Anti-cancer)** **(Anti-microbial)**

Figure 1: Some examples of biologically active 1,2,3-Triazole,pyrazole and 1,2,3-Triazole-pyrazolepharmacopores

In addition, the computational biology and bioinformatics play a major role in designing the drug molecules and potentially speed up the drug discovery process. Molecular docking of the drug molecule with the receptor (target) invariably gives significant information about drug receptor interactions and is commonly used to find out the binding orientation of drug molecules to their protein targets in order to predict the affinity and activity[36].

Against the above background, the efforts are mainly concentrated on establishing 1,2,3-triazole scaffold integrated with pyrazole framework to describe the relevance enhancement in pharmacological activity. Based on these interesting biological activity profiles of triazoles and pyrazoles analogues, we are inspired and made an effort to synthesize some new number of 1,2,3-triazolebearing pyrazoles as key nucleus and evaluate their *in-vitro* anti-cancer potential with the hope of improving its biological activities as multipurpose agents which earlier reported to exhibits an excellent antimicrobial activities by our research group[29]. Most of the compounds exhibit good biological activities. Further, *in-silico* molecular docking studies of the compounds were also carried out to support the *in-vitro* anti-cancer results.

MATERIALS AND METHODS

2.1 Experimental

The solvents used were of analytical grade and the reagents were used was procured. All melting points were examined in open capillary method and were uncorrected. IR spectra were recorded in Shimadzu FT-IR 157 spectrometer by KBr disc method. ^{1}H NMR spectra were recorded either on a Perkin-Elmer EM-390 or on a Bruker WH-200 (400 MHz) in CDCl3 or DMSO-*d6* as solvent, using tetramethylsilane (TMS) internal standard and chemical shifts and coupling constants were expressed by δ (ppm) and Hz respectively. The Mass spectrum of the compounds was determined on a Jeol SX 102/Da-600 mass spectrometer/ Data System using Argon/Xenon (6KV, 10 mA^0) as a FAB gas with accelerating voltage 10 KV and spectra were recorded at room temperature. The elemental analyses (CHN) were carried on CHNS-O-analyser Flash EA 1112 series. The reaction progresses were monitored by TLC on pre-coated silica gel G plates. The spectral data's of all new synthesized compounds were inconsistent with proposed structure and microanalysis within ± 0.4 of the calculated values.

2.2. General procedure for the synthesis of 3-{5-methyl-1-[2-methyl-3-(trifluoromethyl)phenyl/substituted phenyl]-1H-1,2,3-triazol-4-yl}-1-(aryl)-1H-pyrazole-4-carbaldehyde (5a-n)

Vilsmeier-Haack reagent was prepared by slow drop wise addition Phosphorous oxy chloride (3 mmol) to DMF (10 ml) at 0°C and stirred at same temperature for 30 min under inert nitrogen atmosphere. To this mixture 4-{(1E)-1-[2-(aryl) hydrazinylidene]ethyl}-5-methyl-1-[2-methyl-3-(trifluoromethyl)phenyl/substituted phenyl]-1H-1,2,3-triazole (1mmol) ***(4a-n)*** was added lot wise at 0°C, then the reaction mixture was heated to 60°C-70°C for 4-5 hours. The reaction progress was monitored by TLC. After the complete reaction by TLC, the reaction mass was quenched into crushed ice and water (80 ml). The reaction mixture was then neutralized with 10% aqueous ammonia solution, whereupon the solid product separated out was filtered, suck dried and washed with excess of cold water, dried and recrystallized from ethanol to afford the pure product ***(5a-n).***

3-{1-[2,3-dichloro-6-methyl-5-(trifluoromethyl)phenyl]-5-methyl-1H-1,2,3-triazol-4-yl}-1-phenyl-1H-pyrazole-4-carbaldehyde (5a)

IR (KBr) γ/cm-1: 3086 (Ar-H), 1671 (C=O), 1582 (C=N), 1185 (C–F) and 708 (C–Cl). 1H-NMR: (400 MHz, CDCl3): *δ* 2.19 (s, 3H, CH3 of triazole), 2.51 (s, 3H, CH3 of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl), 7.51-7.53 (t, 3H, *J* = 8.0 Hz, Ar-H of Phenyl), 7.77-7.79 (d, 2H, *J* = 8.0 Hz, Ar-H of Phenyl), 7.90 (s, 1H, Ar-H of 2,3-dichloro-6-trifluoromethyl Phenyl), 8.60 (s, 1H, Ar-H of pyrazole), 10.82 (s, 1H, CHO of pyrazole). 13C NMR: (100 MHz, CDCl3): *δ* 9.62 (CH3 of triazole), 13.66 (CH3 of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl), 120.62, 124.22, 127.05, 128.25, 128.33, 129.11, 129.88, 131.02, 131.13, 131.42, 133.65, 134.02, 135.17, 136.49, 137.54, 137.75, 146.65, 187.80 (CHO of pyrazole). MS: m/z = 480.27 (M+), 482.27 (M++2). Anal. calcd. for C21H14Cl2F3N5O: Calculated: C(52.52%), H(2.94%), N(14.58%) ; Found C(52.45%), H(2.92%), N(14.52%).

1-(4-chlorophenyl)-3-{1-[2,3-dichloro-6-methyl-5-(trifluoromethyl)phenyl]-5-methyl-1H-1,2,3-triazol-4-yl}-1H-pyrazole-4-carbaldehyde (5b)

IR (KBr) γ/cm-1: 3081 (Ar-H), 1678 (C=O), 1576 (C=N), 1181 (C–F) and 710 (C–Cl). 1H-NMR: (400 MHz, CDCl3): *δ* 2.12 (s, 3H, CH3 of triazole), 2.45 (s, 3H, CH3 of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl), 7.44-7.46 (d, 2H, *J* = 8.0 Hz, Ar-H of 4-chloro phenyl), 7.66-7.68 (d, 2H, *J* = 8.0 Hz, Ar-H of 4-chloro phenyl), 7.84 (s, 1H, Ar-H of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl), 8.50 (s, 1H, Ar-H of pyrazole), 10.75 (s, 1H, CHO ofpyrazole). 13C NMR: (100 MHz, CDCl3): *δ* 9.61 (CH3 of triazole), 13.67 (CH3 of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl), 120.61, 124.23, 127.06, 128.24, 128.32, 129.10, 129.86, 131.04, 131.15, 131.44, 133.67, 134.05, 135.18, 136.49, 137.54, 137.75, 146.65, 187.84 (CHO of pyrazole). MS: m/z = 514.7 (M+), 516.7 (M++2). Anal. calcd. for C21H13Cl3F3N5O: Calculated: C(49.00%), H(2.55%), N(13.61%) ; Found C(48.91%), H(2.52%), N(13.53%).

3-{1-[2,3-dichloro-6-methyl-5-(trifluoromethyl)phenyl]-5-methyl-1H-1,2,3-triazol-4-yl}-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carbaldehyde (5c)

IR (KBr) γ/cm-1: 3071 (Ar-H), 1665 (C=O), 1568 (C=N), 1167 (C–F) and 711 (C–Cl). 1H-NMR: (400 MHz, DMSO-d6): *δ* 2.09 (s, 3H, CH3 of triazole), 2.35 (s, 3H, CH3 of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl), 8.4 (s, 2H, Ar-H of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl and 2,4-dinitro phenyl), 8.700-8.717 (d, 1H, *J* = 6.8 Hz, Ar-H of 2,4-dinitro phenyl), 8.940-8.957 (d, 1H, *J* = 6.8 Hz, Ar-H of 2,4-dinitro phenyl)), 9.42 (s, 1H, Ar-H of pyrazole), 10.64 (s, 1H, CHO of pyrazole). 13C NMR: (100 MHz, CDCl3): *δ* 8.74 (CH3 of triazole), 14.33 (CH3 of 2,3-dichloro-5-trifluoromethyl-6-methyl phenyl), 121.31, 124.04, 125.21, 125.34, 127.72, 129.66, 129.90, 129.96, 132.13, 132.19, 135.16, 135.48, 135.84, 136.04, 136.26, 137.11, 143.84, 146.56, 147.92, 187.31 (CHO of pyrazole). MS: m/z = 570.1 (M+), 572.1 (M++2). Anal.calcd. for C21H12Cl2F3N7O5: Calculated: C(44.23%), H(2.12%), N(17.91%) ; Found C(44.12%),H(2.08%), N(17.82%).

Spectral details of the other compounds have been presented in the supplementary file.

2.3In-vitro anti-cancer activity

Cytotoxicity of all the synthesized compounds ***(5a-n)*** were determined on the basis of measurement of in vitro growth inhibition of 96 well plates tumour cell lines by cell-mediated reduction of tetrazolium salt to water insoluble formazan crystals using Toremifene as a standard. The cytotoxicity was assessed against MCF-7and MDA-MB-231 human breast adenocarcinoma cell lines and non-tumorous breast cell line MCF-10A (human mammary epithelial cell line)using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide) colorimetric assay[37, 38]. Estrogen receptor (ER)-positive MCF-7 and ER negative MDA-MB-231 breast cancer cells, non-tumorous MCF-10A and VERO cell lines were procured from National Centre for Cell Science, Pune, Maharashtra, India. The IC_{50} values (50% inhibitory concentration) were calculated from the absorbance data for the dose-response curves plotted. IC_{50} values (in μM)were shown as mean ± SD of three independent experiments.

2.4 Molecular docking studies

The most common drugs currently used for the treatments of breast cancer were Tamoxifen, Raloxifene, Toremifene[39].Ingestion of this drug was based on interfering with either estrogen production or estrogen action which causes several side effects such as blood clots, strokes, uterine cancer, or cataracts[40]. The side effects of these drugs make the need for the necessity of new improved drugs.

To explore and support the anti-cancer activity, molecular docking of the synthesized compounds ***(5a-n)*** were carried out using Epidermal growth factor receptor (EGFR) kinase(PDB ID: 2A91) and human estrogen receptor

(PDB ID: 2IOK). It has become increasingly evident that the impaired deactivation of Receptor tyrosine kinases (RTKs) may bea mechanism in cancer[41]. Further, normal cancer cells have receptors that linked to circulating estrogen and progesterone. Estrogen and progesterone bind to the receptors that involved with growth factors (e.g. oncogenes and mutated tumour suppressor genes) to cause cancer cell growth[42]. Based on the literature it is evident that the drug Toremifene has been used to target the human estrogen receptor[43]. Bearing above facts, docking studies of the synthesized compounds ***(5a-n)*** was evaluated against human estrogen receptor and tyrosine kinase receptor which are known to be responsible for causing breast cancer. Further, we selected human estrogen receptor (PDB ID: 2IOK) and the tyrosine kinase receptor (RTK) namely, Epidermal growth factor receptor (EGFR) kinase domain (PDB ID: 2A91) as a biological targets for the synthesized compounds.

For macromolecular docking studies, the chemical structures of synthesized ligands ***(5a-n)***,metal complexes and standard Toremifene were retrieved and drawn using Chem. Draw ultra. The 3D optimization was done in Chem. Draw 3D ultra software and stored as .pdb file. Hex docking was carried out by setting suitable parameters as represented in **Table 1**and this docking score can be interpreted as interaction energy. More negative E–Total energy valueimplies that there exists a strong interaction between drug and receptor and that leads to receptor inhibition activity. Molecular docking work was performed with the Hexmolecular modeling package version 8.0. The three dimensional crystal structure of Epidermal growth factor receptor (EGFR) kinase (PDB ID: 2A91) and human estrogen receptor (PDB ID: 2IOK) was used throughout the work. The ligands were converted to 2Dand 3D energy-minimized conformations using Hex 3D Ultra 8.0., respectively and visualized the conformation by using Acceryl Discovery Studio 3.1 Client. The catalytic active sites identification of human estrogen receptor along with area and volume of binding pocket was conducted with Computed Atlas of Surface Topography of Proteins (Castp) program(http://cast.engr.uic.edu)[44].

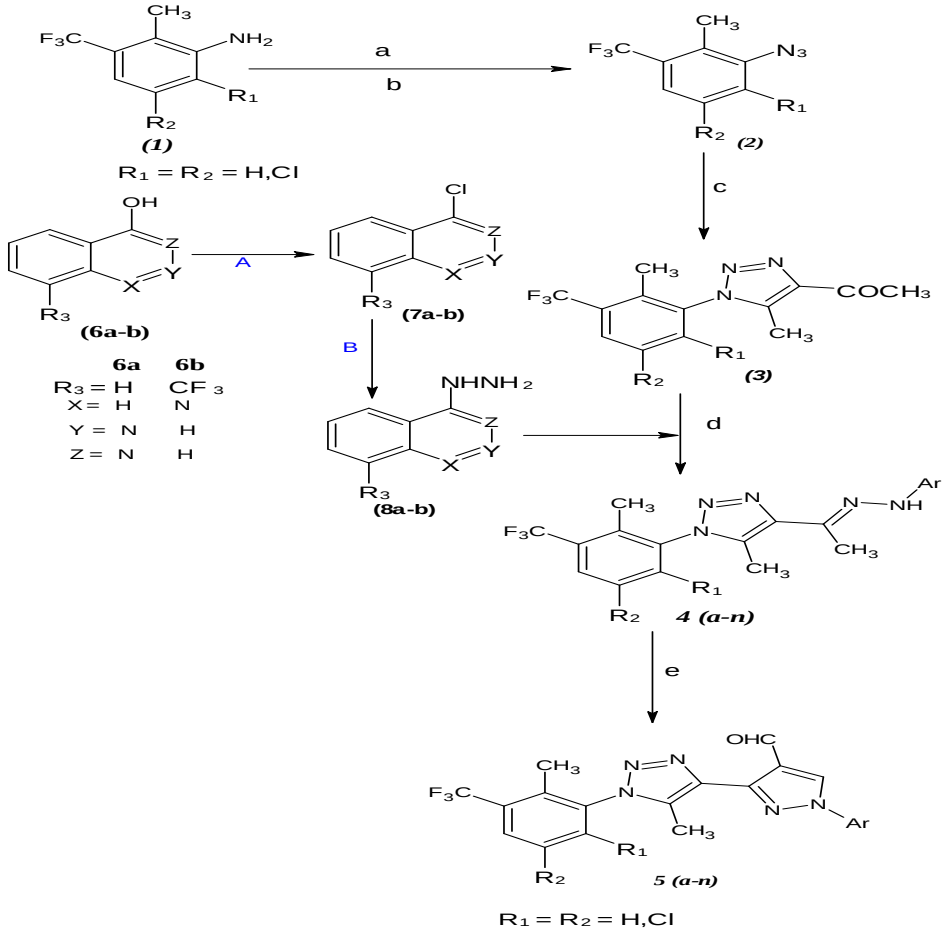
Table 1:Parameters used for docking studies

Correlation type	Shape onlyGrid Dimension	0.6
Receptor range	180	
Ligand Range	180	
Twist range	360	
Distance Range	40	

RESULTS AND DISCUSSION

3.1. Chemistry

The synthetic pathway for the title compounds, 3-{5-methyl-1-[2-methyl-3-(trifluoromethyl) phenyl/substituted phenyl]-1H-1,2,3-triazol-4-yl}-1-(aryl)-1H-pyrazole-4-carbaldehyde ***(5a-n)***has outlined in **Scheme 1,** and were synthesized by the Vilsmeier-Haack formylation of 4-{(1E)-1-[2-(aryl) hydrazinylidene]ethyl}-5-methyl-1-[2-methyl-3-(trifluoromethyl)phenyl/substituted phenyl]-1H-1,2,3-triazole ***(4a-n)*** with Phosphorous oxy chloride and DMF mixture with good yields (70-75%). The key starting material 4-{(1E)-1-[2-(aryl) hydrazinylidene] ethyl}-5-methyl-1-[2-methyl-3-(trifluoromethyl) phenyl/substituted phenyl]-1H-1,2,3-triazole ***(4a-n)*** were synthesized by multi-step reactions from ***(1)*** following literature procedures [45]. The intermediate ***(3)*** was synthesized by 1,3-dipolar cylclo addition reaction between ***(2)*** and acetyl acetone in presence of sodium methoxide in methanol.



Compound R_1=R_2=Cl	***5a***	***5b***	***5c***	***5d***	***5e***	***5f***	***5g***
Ar	phenyl	4-Cl-phenyl	2,4-$(NO_2)_2$ phenyl	2,4,6-$(Cl)_3$ phenyl	Phthalazinyl	8-CF_3- quinyl	4-$(NO_2)_2$ phenyl

Compound R_1=R_2=H	***5h***	***5i***	***5j***	***5k***	***5l***	***5m***	***5n***
Ar	phenyl	4-Cl-phenyl	2,4-$(NO_2)_2$ phenyl	2,4,6-$(Cl)_3$ phenyl	Phthalazinyl	8-CF_3- quinyl	4-$(NO_2)_2$ phenyl

a. *$NaNO_2$/ HCl/ 0-5°C* ***b.*** *NaN_3/ H_2O/ 0-5°C* ***c.*** *$NaOCH_3$/ Acetyl acetone/ Methanol/ 50°C* ***d.*** *$ArNHNH_2$/* ***(8a-b)****/ Ethanol/ H_2SO_4/ 78°C* ***e.*** *DMF/ $POCl_3$/ 70°C (Vilsmeier-Haack reagent).*
A. $POCl_3$/ MDC/ 40-50°C B. $NH_2NH_2.H_2O$/ Ethanol/ 78°C.

Scheme 1: Synthetic scheme for the compounds***(5a-n)***

Structures of all the synthesized compounds ***(5a-n)*** were established on the basis of their spectral (IR, NMR and Mass) and elemental (C, H and N) analyses. Analytical and spectral data of all the synthesized molecules were in full agreement with the proposed structures and also discussed for a representative compound ***5c:*** The IR spectrum of ***(5c)*** showed absorption peak at 3071 cm^{-1}assigned to aromatic C-H stretch. The peak for C=N was observed at 1568 cm^{-1}. The peak at 1665 and 1211 cm^{-1}were assigned to C=O and C-O stretch respectively. The medium absorption at 1167, 715 $cm^{-1}$was due to the presence of C-F and C-Cl bonds. The absence of the absorption bands at 3274 $cm^{-1}$and 1620 cm^{-1}corresponding to –NH2 and –C=O stretching frequencies in the compounds clearly revealed the formation of 1,2,3-Triazolyl pyrazole aldehydes ***(5a-n)***. The ^{1}H NMR spectrum of ***(5c)*** showed two singlet's at δ (ppm) 2.09 and 2.35 were assigned to two methyl protons of 1,2,3-triazole ring and 2,3-dichloro-6-methyl-5-

(trifluoromethyl) phenyl respectively. The two singlet signals at δ 9.42 and 10.64 were assigned to aromatic and aldehydic protons of pyrazole ring respectively along with other characteristic signals. Two distinct doublets at *δ* 8.70 and 8.94 (*J* = 6.8 Hz) and a singlet at δ 8.4 integrating for four protons were assigned to protons of 2,4-dinitro phenyl and 2,3-dichloro-6-methyl-5-(trifluoromethyl) phenyl moiety. The signals due to -NH protons of the corresponding hydrazones were disappeared. The ^{13}C NMR spectrum of ***(5c)*** showed signals at *δ* (ppm) 8.74, 14.33 and 187.31 corresponds to two methyl carbons of 1,2,3-triazole and 2,3-dichloro-6-methyl-5-(trifluoromethyl) phenyl ring and aldehydic carbon of pyrazole ring respectively along with other characteristic signals. The IR, ^{1}H NMR and ^{13}C NMR spectra of other compounds have shown similar characteristic properties. The physicochemical data of the compounds ***(5a-n)*** were presented in **Table 2.**

Table 2: Characterization data of the compounds *(5a-n)*

Compd.	Ar	Mol. formula	Mol. Wt	M.P (°C)	Yield (%)	C,H,NAnalysis (%) Found (calculated)		
						C	H	N
R_1,R_2=Cl								
5a	Phenyl	$C_{21}H_{14}Cl_2F_3N_5O$	480.2	185-187	71	52.45 (52.52)	2.92 (2.94)	14.52 (14.58)
5b	4-Cl-phenyl	$C_{21}H_{13}Cl_3F_3N_5O$	514.7	193-195	73	48.91 (49.00)	2.52 (2.55)	13.53 (13.61)
5c	2,4-dinitro phenyl	$C_{21}H_{12}Cl_2F_3N_7O_5$	570.2	225-227	70	44.12 (44.23)	2.08 (2.12)	17.82 (17.91)
5d	2,4,6-trichloro phenyl	$C_{21}H_{11}Cl_5F_3N_5O$	583.6	220-222	75	43.25 (43.22)	1.92 (1.90)	12.03 (12.00)
5e	Phthalazinyl	$C_{23}H_{14}Cl_2F_3N_7O$	532.3	228-230	71	51.85 (51.90)	2.62 (2.65)	18.40 (18.42)
5f	8-CF_3- Quinyl	$C_{25}H_{14}Cl_2F_6N_6O$	599.3	252-254	70	50.02 (50.10)	2.29 (2.35)	13.92 (14.02)
5g	4-nitro phenyl	$C_{21}H_{13}Cl_2F_3N_6O_3$	525.2	218-220	74	48.10 (48.02)	2.52 (2.49)	16.08 (16.00)
R_1,R_2=H								
5h	Phenyl	$C_{21}H_{16}F_3N_5O$	411.4	175-177	74	61.22 (61.31)	3.85 (3.92)	17.10 (17.02)
5i	4-Cl-phenyl	$C_{21}H_{15}ClF_3N_5O$	445.8	185-187	73	56.48 (56.57)	3.30 (3.39)	15.62 (15.71)
5j	2,4-dinitro phenyl	$C_{21}H_{14}F_3N_7O_5$	501.4	215-217	70	50.40 (50.31)	2.90 (2.81)	19.62 (19.56)
5k	2,4,6-trichloro phenyl	$C_{21}H_{13}Cl_3F_3N_5O$	514.7	212-214	72	49.07 (49.00)	2.62 (2.55)	13.70 (13.61)
5l	Phthalazinyl	$C_{23}H_{16}F_3N_7O$	463.4	224-226	74	59.55 (59.61)	3.43 (3.48)	21.12 (21.16)
5m	8-CF_3- Quinyl	$C_{25}H_{16}F_6N_6O$	530.4	246-248	70	56.52 (56.61)	2.98 (3.04)	15.78 (15.84)
5n	4-nitro phenyl	$C_{21}H_{15}F_3N_6O_3$	456.4	216-218	72	55.35 (55.27)	3.30 (3.31)	18.38 (18.41)

3.2. In-vitro anti-cancer activity

The anti-cancer activity evaluation of newly synthesized compounds ***(5a-n)*** revealed that, most of the tested compounds exhibited moderate to excellent ranges of cytotoxic activity against the tested breast cancer cell lines MCF-7 and MDA-MB-231 respectively. (**Table 3**) Among them, the compounds ***5c, 5f, 5g, 5j, 5m*** and ***5n*** exhibited significant activities against both the cell lines MCF-7 and MDA-MB-231 with IC_{50} = 6.8-9.8 µM and 11.1-14.1µM respectively comparable with the standard drug Toremifene (IC_{50} = 7.0µM and 13.0 µM respectively). Similarly the derivatives ***5b, 5d, 5e, 5i, 5k*** and ***5l*** shown good activities against tested MCF-7 and MDA-MB-231 cell lines with IC_{50} = 10.3-13.8 µM and 14.5-17.2 µM respectively when compared to the standard drug Toremifene. However, compounds***5a***,***5b*** and ***5g*** showed equal cytotoxicity on normal breast MCF-10A cell line with IC_{50} values 21.9, 23.7 and 25.3 µM respectively. The compounds ***5a*** and ***5h*** with IC_{50} values 22.8, 27.6 µM (for MDA-MB-231 cell line) and 16.6, 18.8 µM (for MCF-7 cell line)were non-significant and are unable to show any cytotoxic activity towards the panel of human breast cancer cell lines MCF-7 and MDA-MB-231.

The structure-activity relationship of the compounds ***5 (a-n)*** revealed that the compounds ***5c,5f, 5g, 5j, 5m*** and ***5n*** containing 2-methyl-3-trifluoromethyl-5,6-dichloro/dihydro phenylsubstituent's in 1st position of the 1,2,3-triazole ring and 2,4-dinitro phenyl, 8-trifluoromethyl quinyl, 4-nitro phenyl substituent's in the 2nd position of pyrazole ring respectively were found to be more potent anti-cancer agents while the compounds ***5b, 5d, 5e, 5i, 5k*** and ***5l***

bearing 2-methyl-3-trifluoromethyl phenyl substituent in 1st position of the 1,2,3-triazole ring and 4-chloro phenyl, 2,4,6-trichloro phenyl and pthalazinyl substituent's in the 2nd position of pyrazole ring respectively were also exhibits good anti-cancer activity against MCF-7 and MDA-MB-231 cell lines than the remaining compounds. Structure-activity relationship (SAR) further reveals that the presence of multi electron-withdrawing, lipophilic and electronegative groups like fluorine, chlorine, nitro and trifluoromethyl in phenyl and heterocyclic rings and the electron-donating groups like phthalazinyl, quinyl were generally may be more beneficial than the less substituted or un-substituted groups in the phenyl and heterocyclic rings.

Table 3: The in-vitro cytotoxic activity of *(5a-n)*on cancer cells by MTT assay at 48h. of exposure

	IC_{50}[a]in µM		
	MDA-MB-231	**MCF-7**	**MCF-10A**
5a	22.8±0.2	16.6±0.4	21.9±0.3
5b	15.1±0.4	10.7±0.3	>100
5c	14.1±0.3	8.8±0.2	>100
5d	14.9±0.2	10.9±0.3	>100
5e	17.2±0.3	13.8±0.2	23.7±0.2
5f	11.3±0.2	7.1±0.1	>100
5g	13.3±0.4	9.2±0.2	>100
5h	27.6±0.3	18.8±0.3	>100
5i	15.9±0.4	10.3±0.3	>100
5j	13.3±0.4	9.8±0.2	>100
5k	14.5±0.2	10.7±0.2	>100
5l	15.3±0.3	12.9±0.2	25.3±0.1
5m	11.1±0.3	6.8±0.3	>100
5n	12.7±0.2	7.9±0.2	>100
Toremifene[b]	13.0±0.2	7.0±0.1	>100

[a]*Data presented is the mean ± SD value of three independent determinations.*
[b] *Positivecontrol.*

3.3 Molecular docking studies

Docking studies of the synthesized compounds ***(5a-n)*** were evaluated against Epidermal growth factor receptor (EGFR) kinase (PDB ID: 2A91) and human estrogen receptor(PDB ID: 2IOK) which are known to be responsible for causing anti-cancer. In the present studyan attempt was made to evaluate their anti-cancer property, so we selected human estrogen receptor (PDB ID: 2IOK) and tyrosine kinase receptor (RTK) (PDB ID: 2A91) which are involved in causing breast cancer. Toremifene drug was used as standard for our docking studies which was known to be potential inhibitor of human estrogen receptor. **Figure 2** and **3.** The obtained docking scores (binding interaction energy) were tabulated in **Table 4.** For human estrogen receptor (PDB ID: 2IOK), all the synthesized compounds ***(5a-n)*** exhibited more and comparable binding interaction energy (E-total value) ranging from -120.88 to -190.19 kjmol^{-1}against the receptor with least docking scores compare to standard drug Toremifene (-101.94kjmol^{-1}). Similarly, the binding interaction energy (docking scores) of the synthesized compounds ***(5a-n)*** for tyrosine kinase receptor (PDB ID: 2A91) ranging from -215.86 to -342.86kjmol^{-1}are comparable with the standard Toremifene (-249.93 kjmol^{-1}). Among all the compounds docked, compounds ***5a, 5b, 5c, 5f, 5j*** and ***5m*** showed comparatively least E-total values -159.17, -155.04, -163.69, -190.19, -150.08 and -168.58 kjmol^{-1}respectively have significantly more inhibiting ability towards human estrogen receptor (PDB ID: 2IOK) while the derivatives ***5c, 5f, 5g, 5j, 5k, 5m*** and ***5n*** with comparatively least E-total values -288.46, -312.14,-311.56, -282.51, -283.27, -342.36 and -283.87 kjmol^{-1}respectively exhibits significantly more inhibiting ability towards tyrosine kinase receptor (PDB ID: 2A91). Consequently, these molecules (ligands) ***(5a-n)*** binds to the active site of the receptors like through hydrogen bond, hydrophobic and other interactions with variety of amino acids of the active site there by potentially inhibits the cancer causing property of the receptor. **Table 4.** Further, the docking studies of the ligand molecules ***(5a-n)*** with human estrogen receptor (PDB ID: 2IOK) and tyrosine kinase receptor (PDB ID: 2A91) enzymes revealed that all the compounds exhibited the bonding with various amino acids in the active pockets. The estimated binding affinity of ligands***5(a-n)*** with the complex hydrogen network and other interactions with a plethora of amino acids includes ARG1515, ASN1519, ILE452, SER1512, LEU509, ILE451, LEU1509, ARG515,LEU511 and HIS21, MET10, TYR282, ALA419, GLU40, TYR282, LEU415, VAL35, VAL4,CYS5.etc. which are present in active sites of the human estrogen receptor (PDB ID: 2IOK) and tyrosine kinase receptor (PDB ID: 2A91) enzymes respectively, gives a clue about the importance of hydrogen bond formation and other interactions for effective enzyme binding. For example, ligands (compounds) ***5a*** and ***5b*** exhibits hydrogen bond and other interactions (binds)with amino acid residues of human estrogen receptor (PDB ID: 2IOK) enzyme were represented byILE452::B:SER1512:LEU509::LEU1511:ILE452::SER1512:A:LEU509:ILE451:ILE451::ARG1515::LEU1509,

ARG515:ARG1515:ILE451:ILE452:LEU511:LEU1509:LEU1509,LEU1511:LEU1509:LEU511andLEU1511::SER 1512::ARG1515::ILE452:SER1512::LEU509::LEU1511:ILE452:SER1512::LEU509::ASN1519::ILE451:ILE451: O:ARG1515:LEU1509::ILE451,ILE452:LEU511::LEU1509::ARG515:LEU1509:LEU1511:LEU1509:ARG1515L EU511 respectively, thereby potentially inhibits the cancer causing property of the receptor. Similarly, ligands (compounds)***5a*** and ***5b*** exhibits hydrogen bond and other interactions (binds) with amino acid residues oftyrosine kinase receptor (PDB ID: 2A91) were given by TYR282::GLU40::TYR282::GLU40::LEU415::VAL35:VAL4:CYS5:TYR282:ALA419 and TYR282::HIS21::GLU40:TYR282:HIS21::GLU40:OE2::LEU415::VAL35:VAL4:CYS5:MET24::LEU25:HIS21:T YR282,ALA419 respectively, thereby potentially inhibits the cancer causing property of the receptor. The other ligands (compounds) ***(5a-n)*** of the series showed similar interactions as shown in **Figure 4** and **5.** From the data in **Table 4,** it can be concluded that all compounds potentially inhibits the human estrogen receptor (PDB ID: 2IOK) and tyrosine kinase receptor (RTK) (PDB ID: 2A91) and especially, most of the compounds ***(5a-n)*** were found to be potential inhibitors of both the receptors. The obtained results may provide enough explanation and good compromise between docking scores and *in-vitro* results of anti-cancer activity.

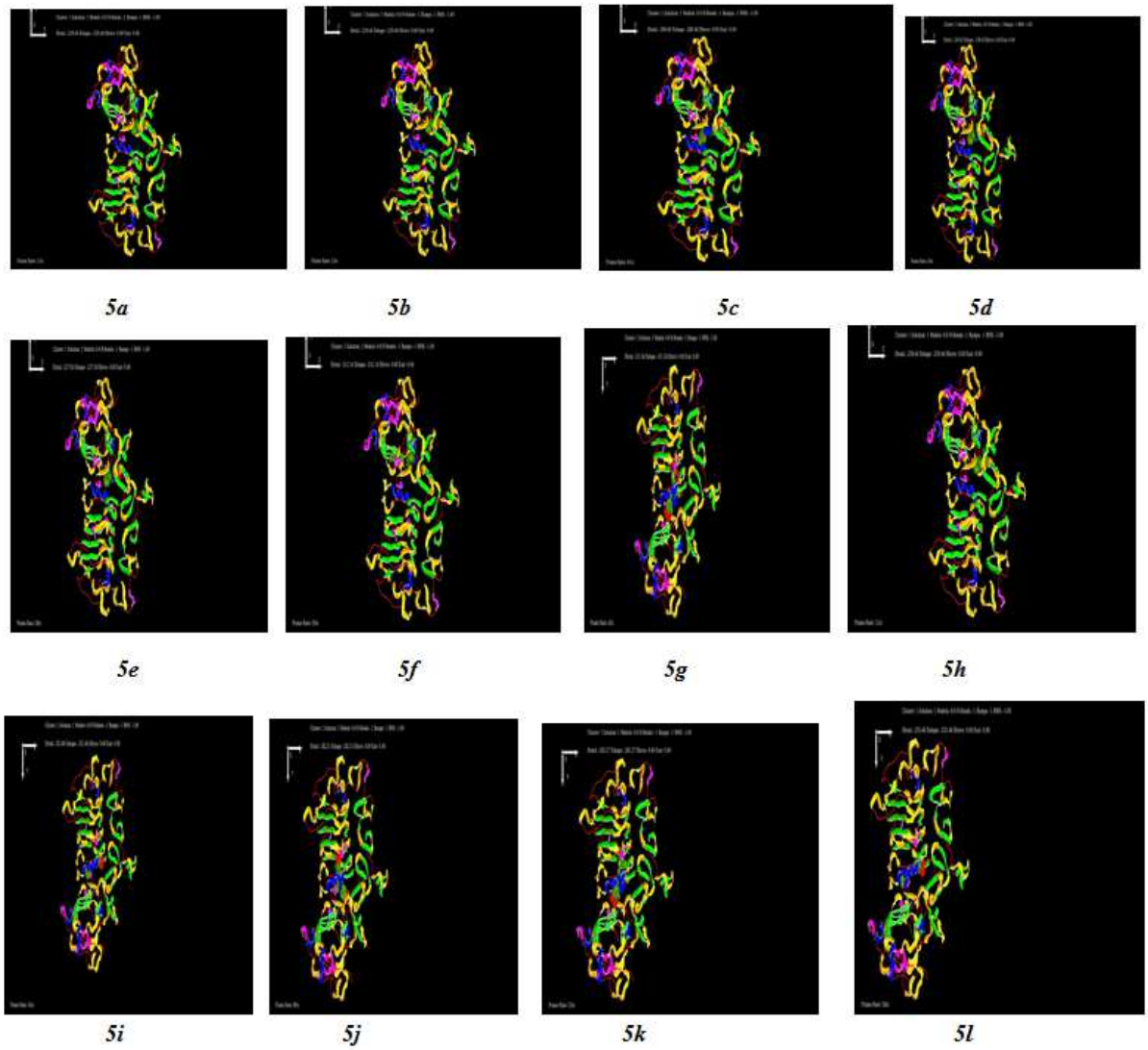
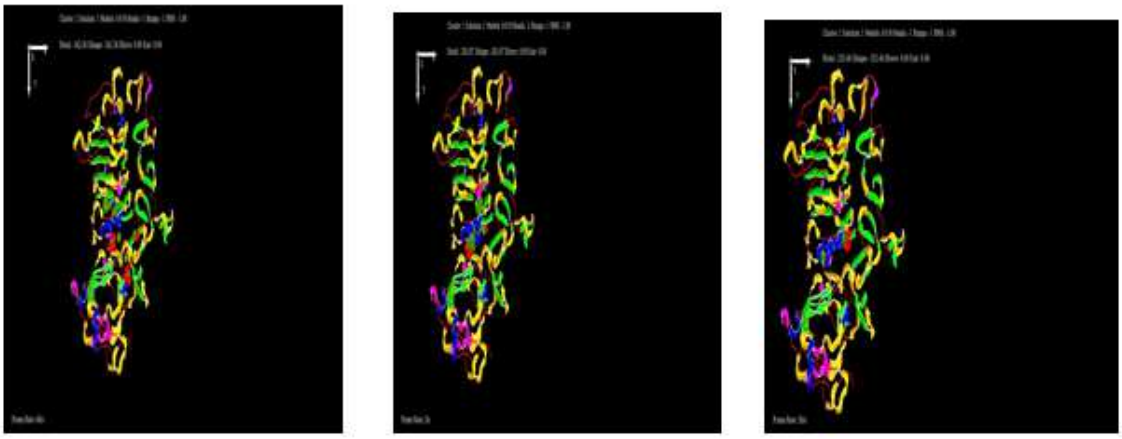
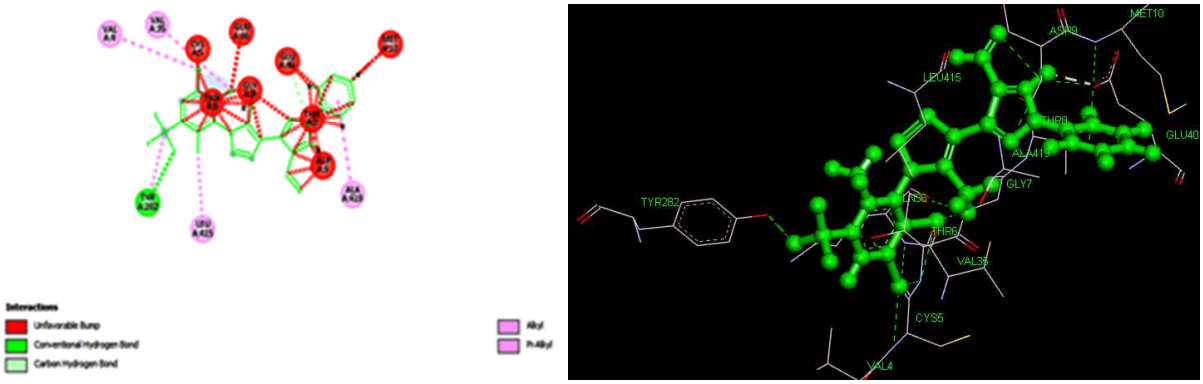


Figure 2: Docking and binding interaction energies of the compounds*(5a-n)*with receptor PDB ID: 2A91

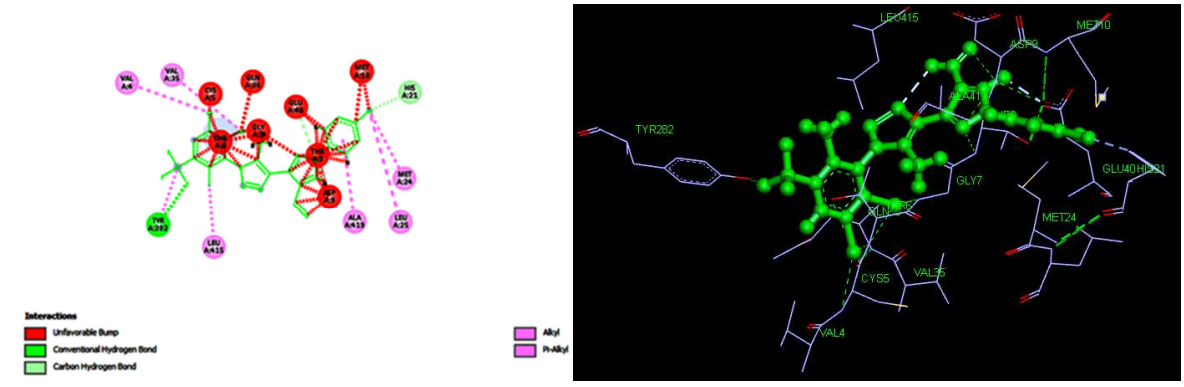


5m ***5n*** ***Toremifene***

Figure 3: Docking and binding interaction energies of the compounds(5a-n)with receptor PDB ID: 2IOK



5a ***5a***



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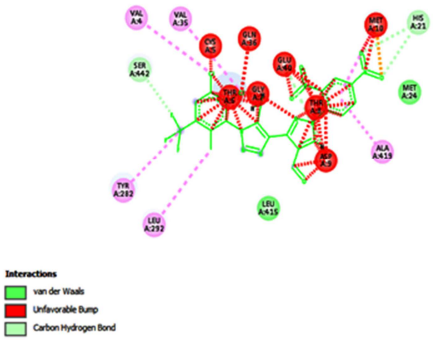

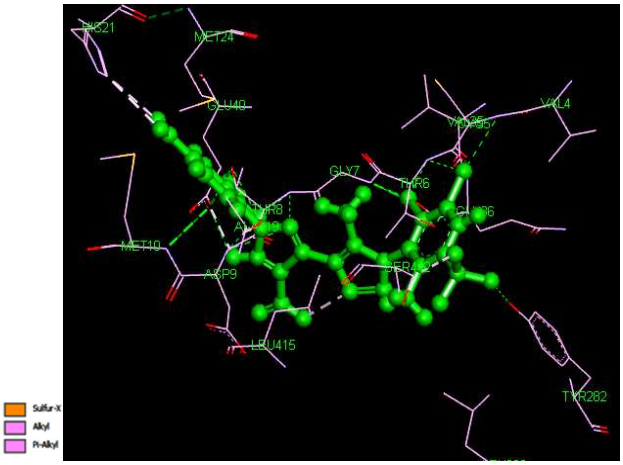

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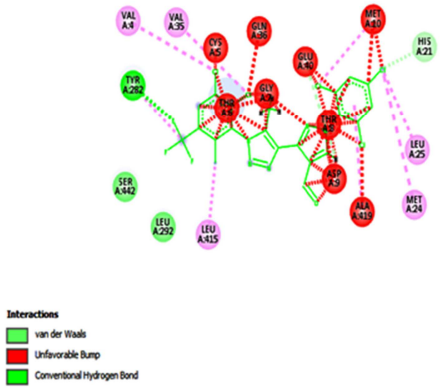
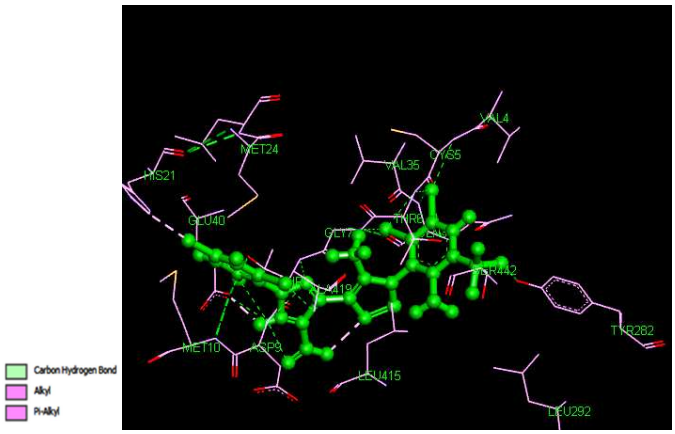
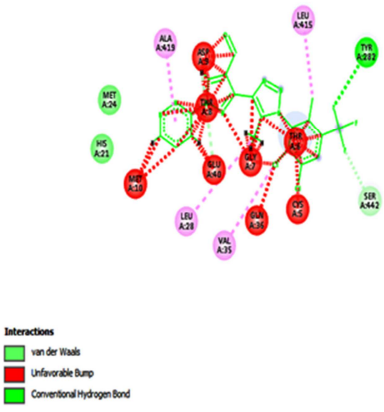
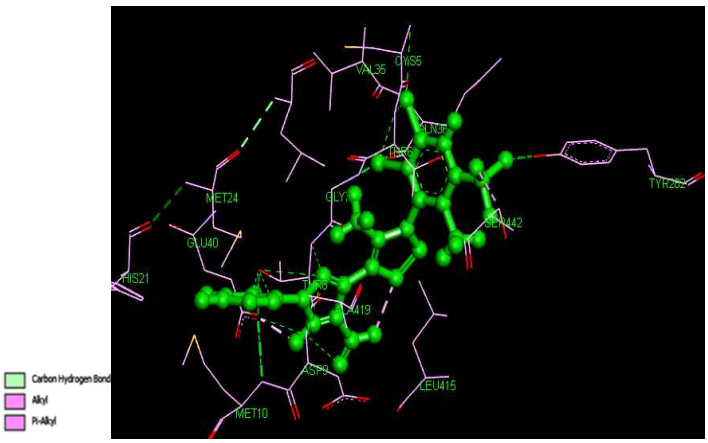


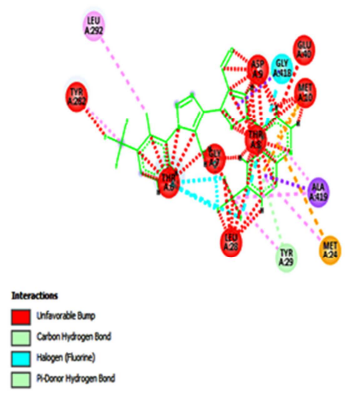


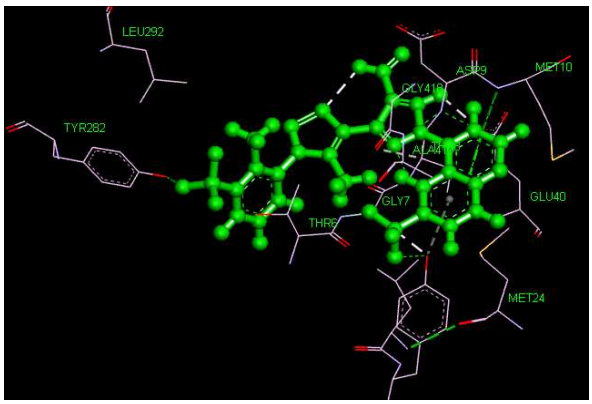
5d ***5d***



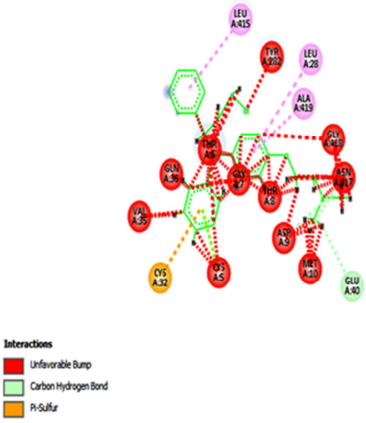


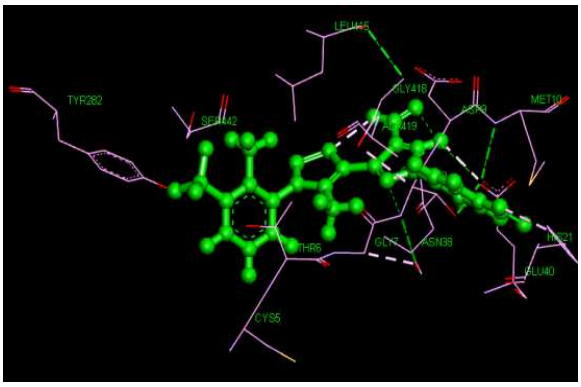
5e ***5e***





5f ***5f***





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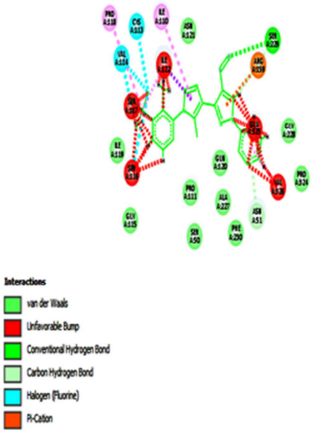
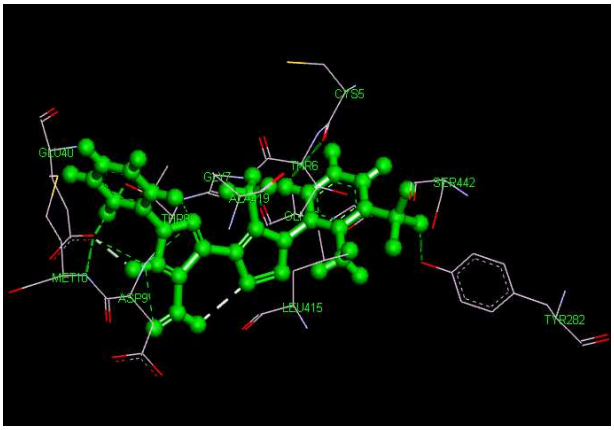
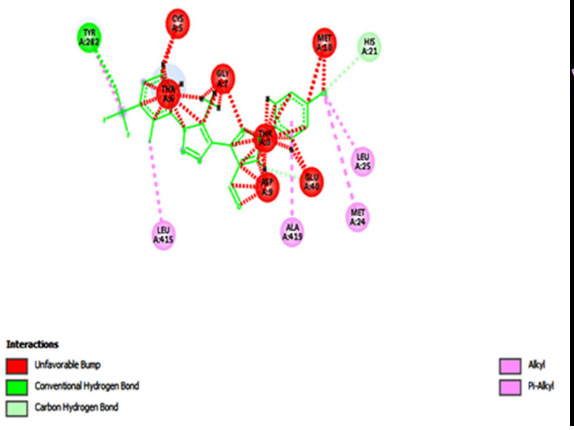
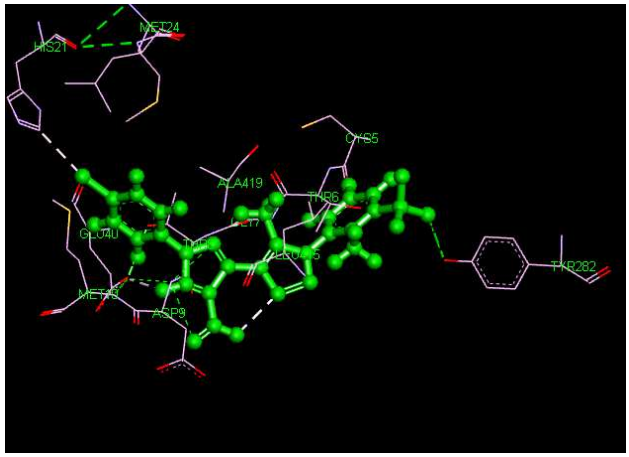




5h ***5h***



5i



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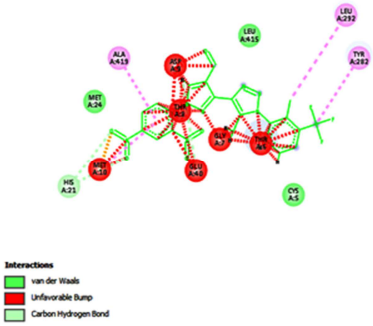

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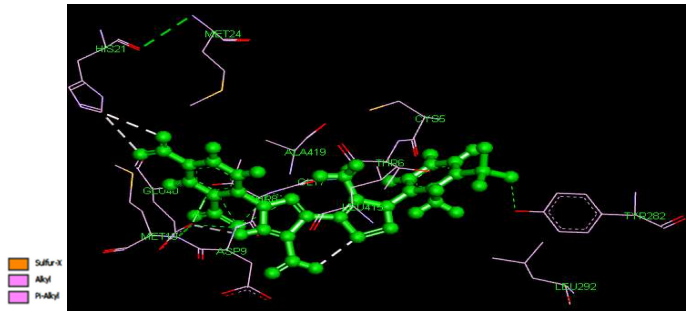

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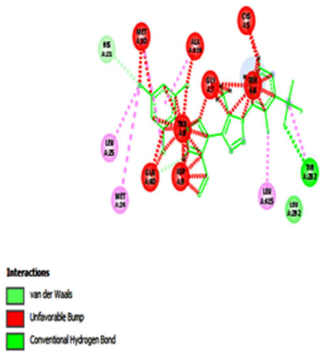

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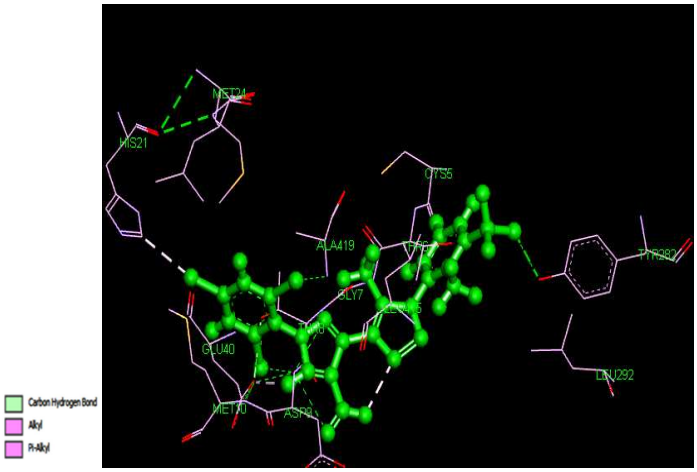

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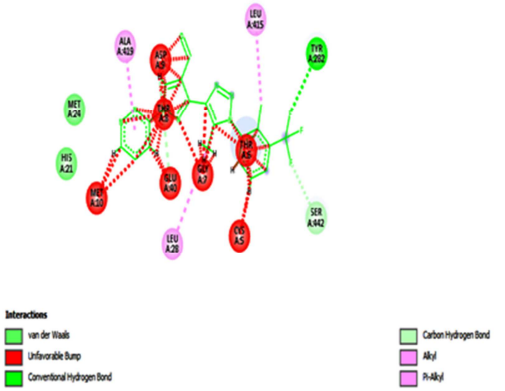
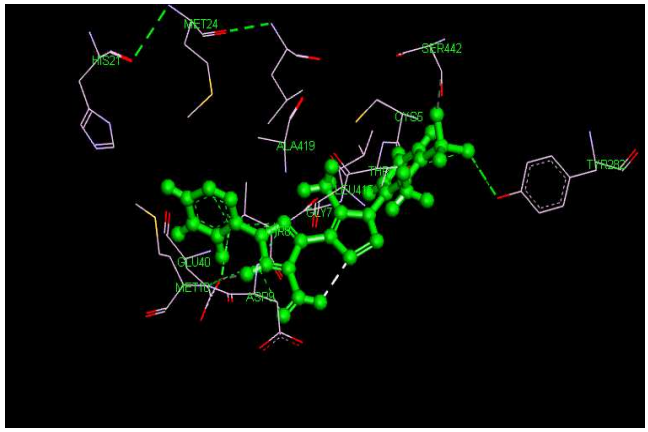
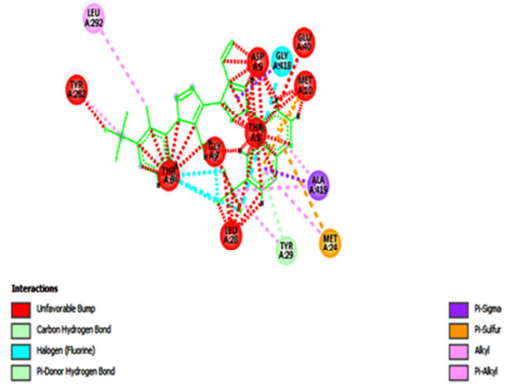
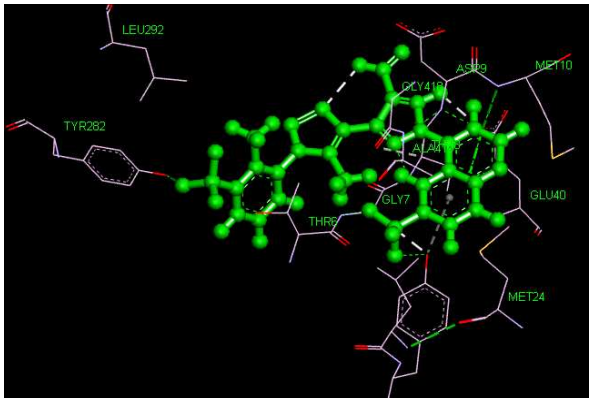


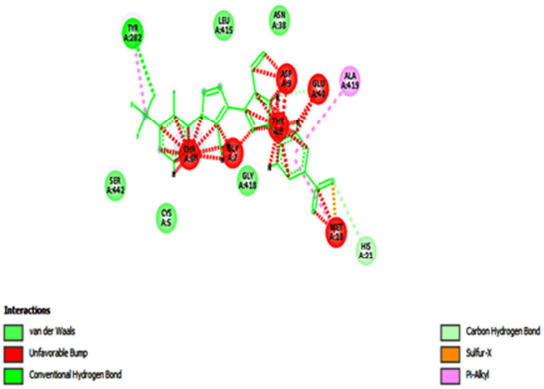


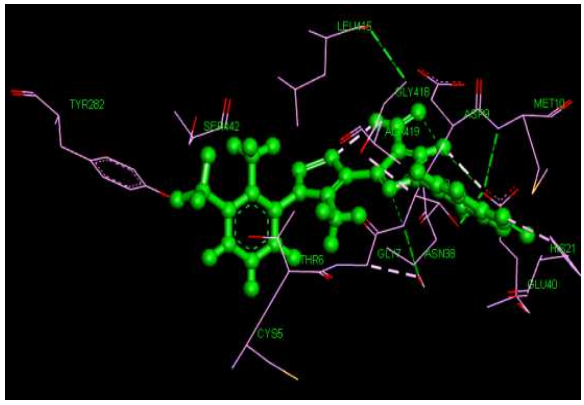
5l ***5l***



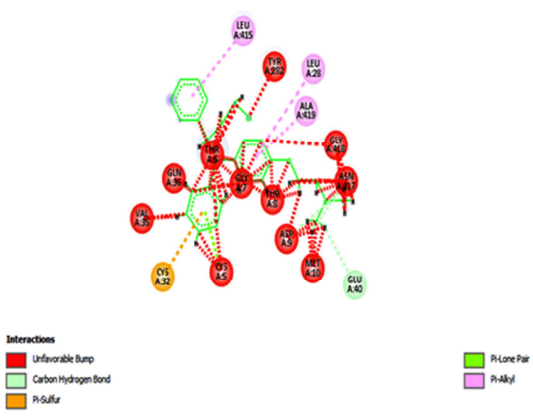


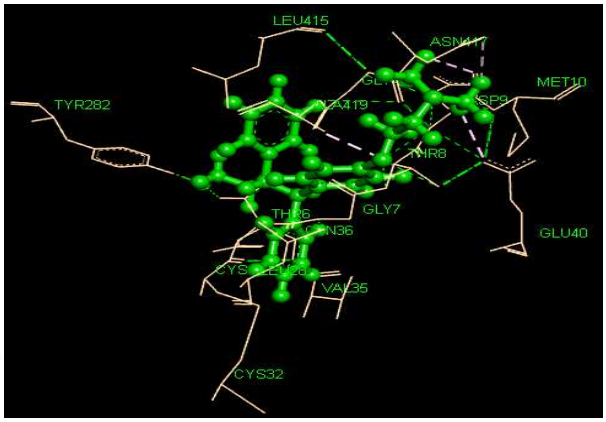
5m ***5m***





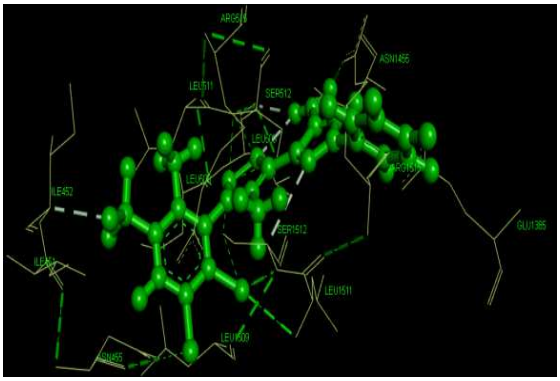
5n ***5n***

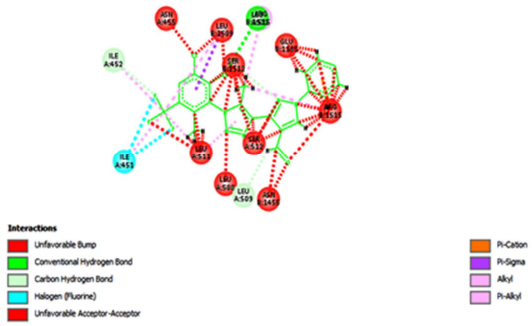




Toremifene ***Toremifene***

Figure 4:2D and 3D interactions of the compounds*(5a-n)* with the amino acid residues of the receptor PDB ID: 2A91





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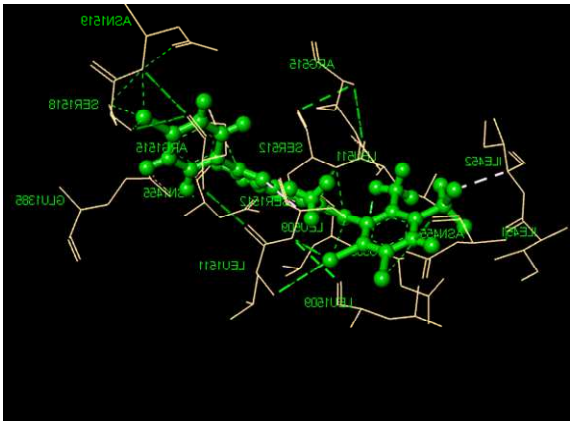
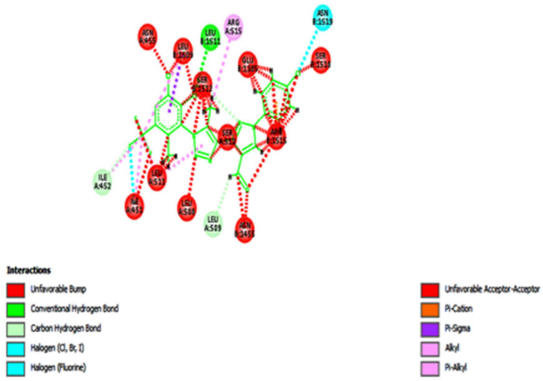
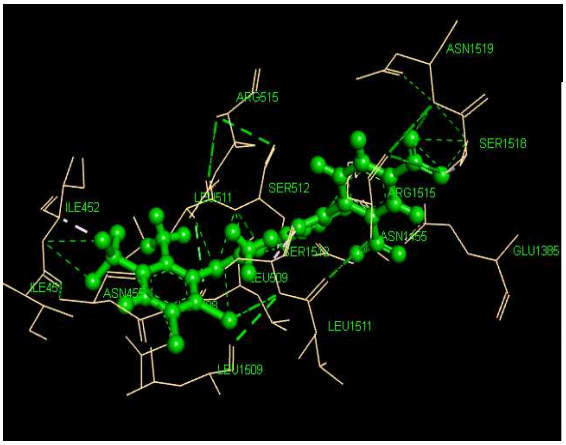
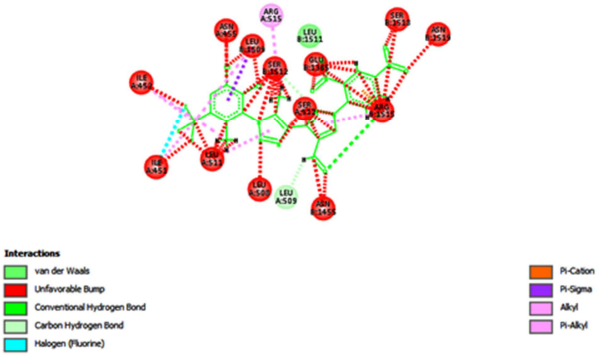




5b ***5b***





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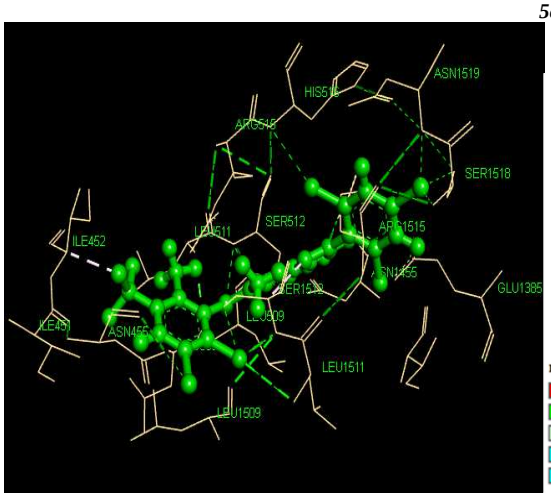
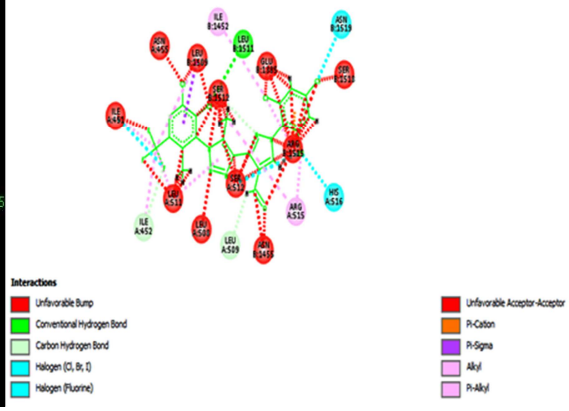
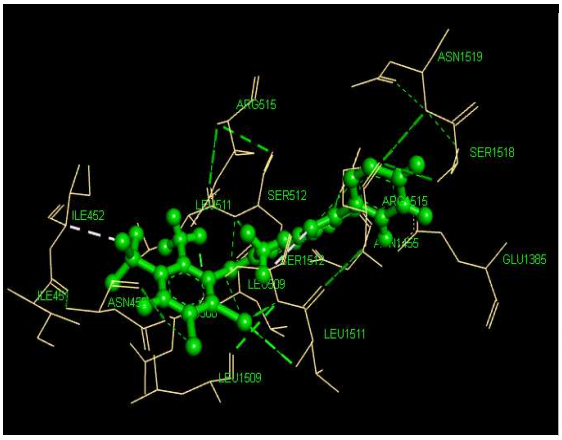
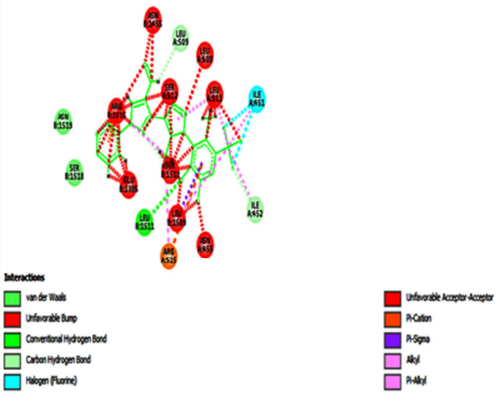


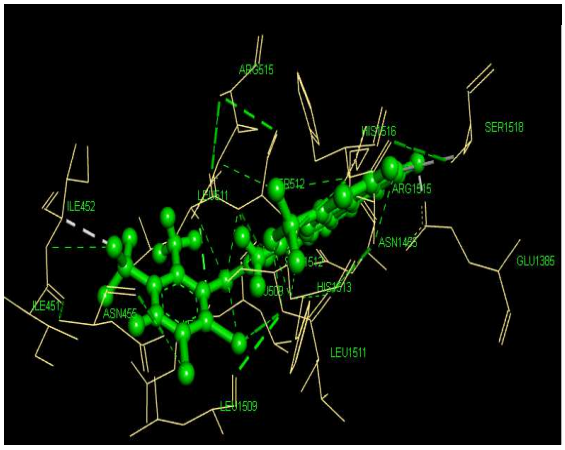


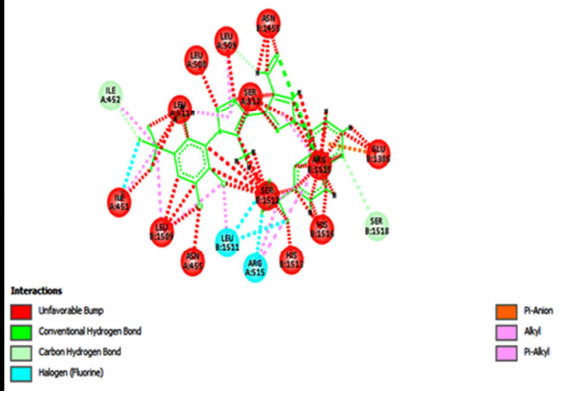
5d ***5d***





5e ***5e***





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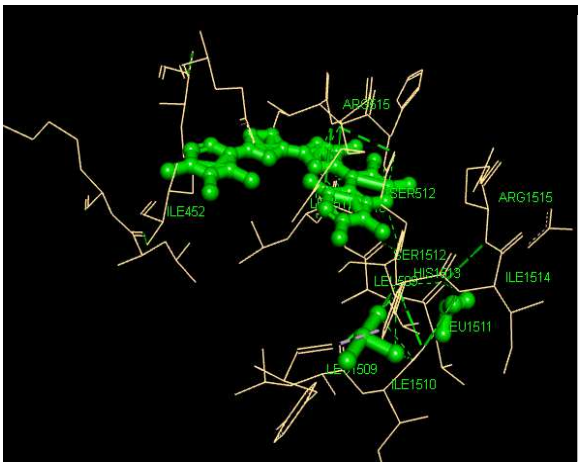

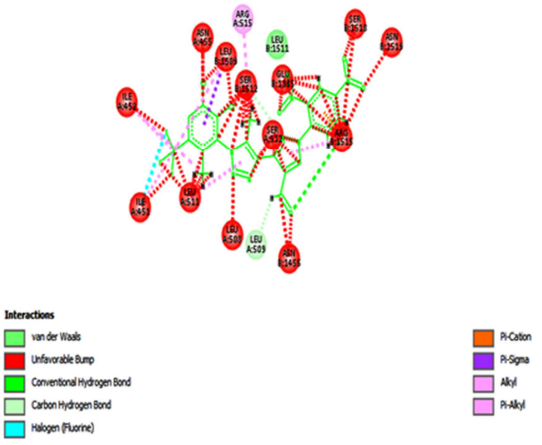

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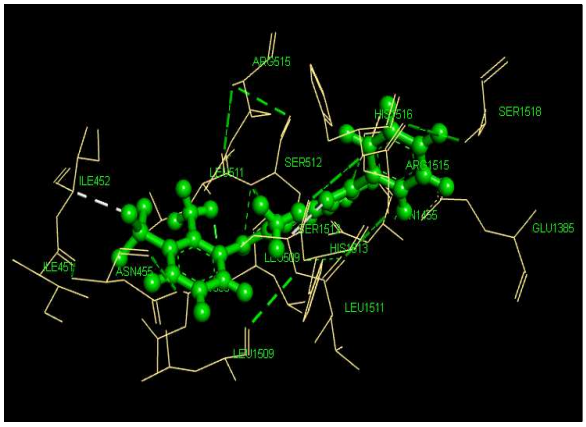

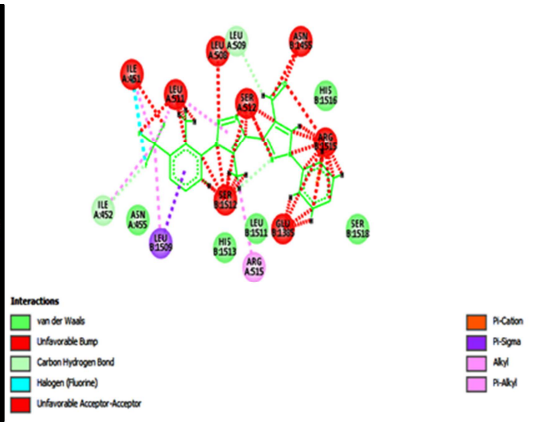

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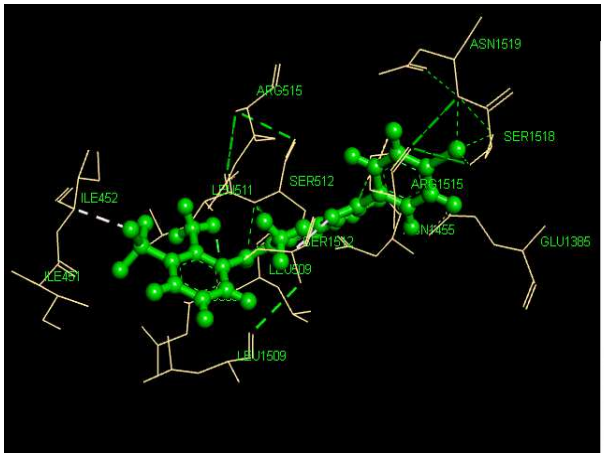

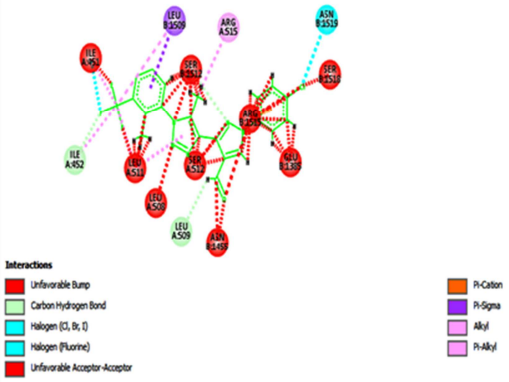

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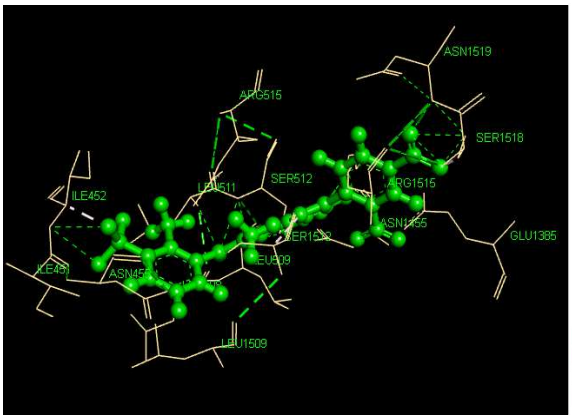
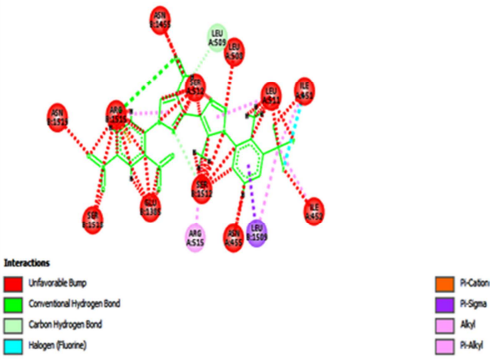
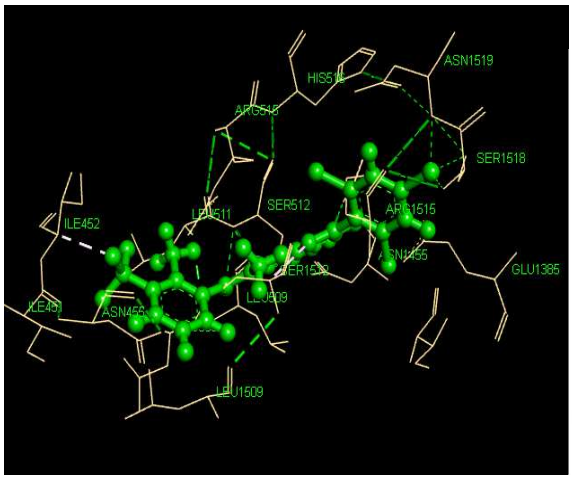
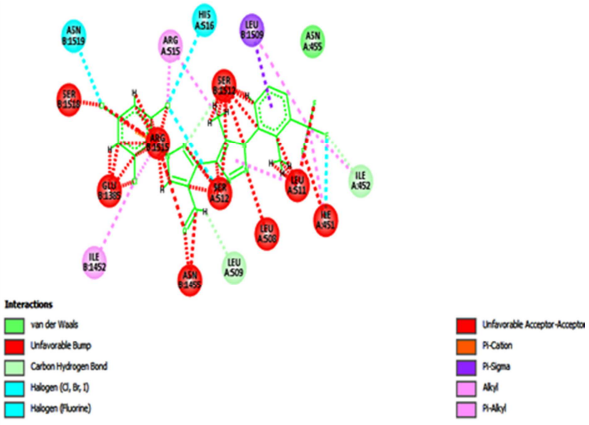




5j ***5j***





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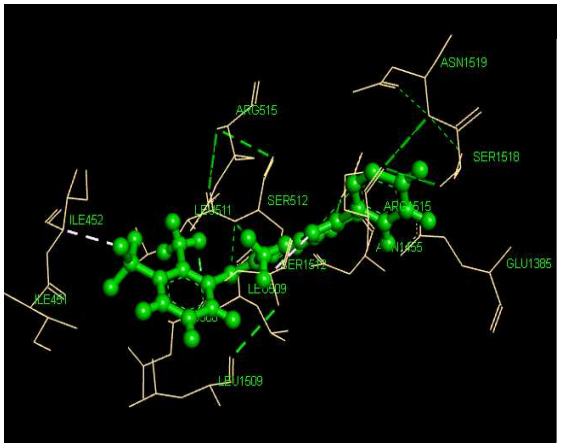
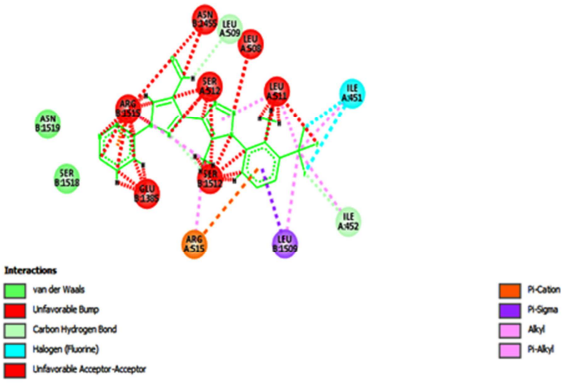
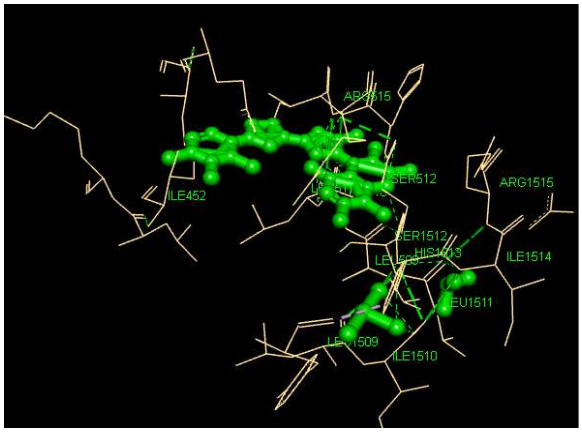
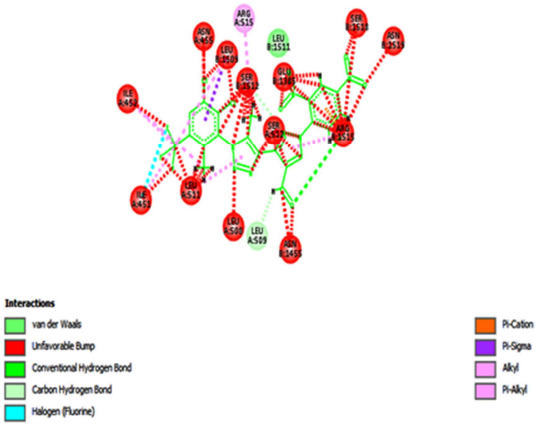


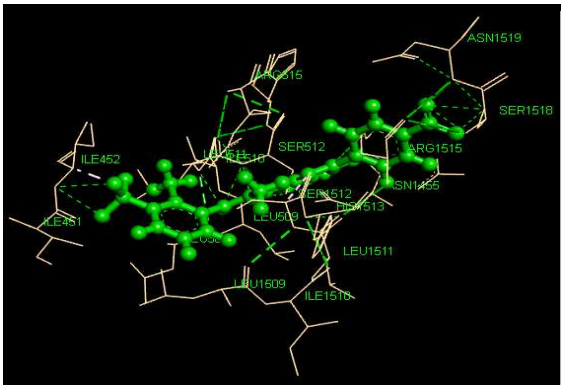


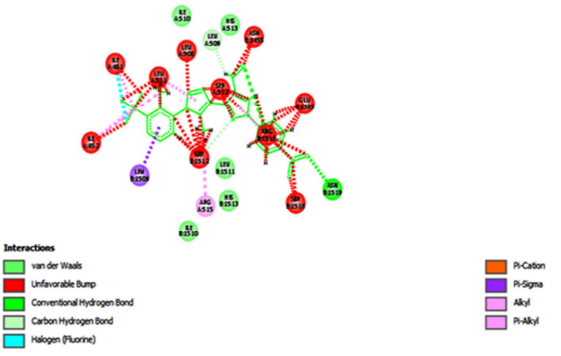
5l ***5l***



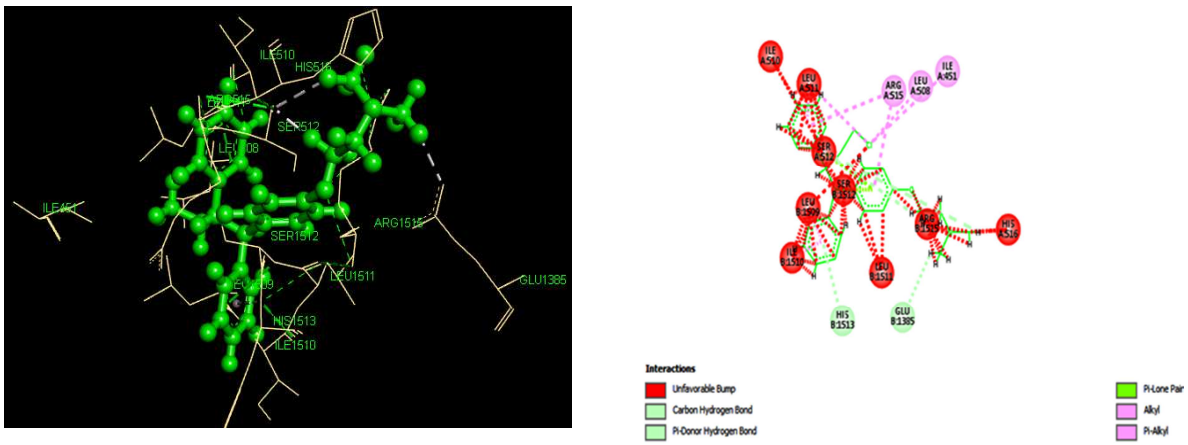


5m ***5m***





5n ***5n***



Toremifene ***Toremifene***

Figure 5:2D and 3D interactions of the compounds*(5a-n)* with the amino acid residues of the receptor PDB ID: 2IOK

Table 4: Docking scores of the synthesized compounds*(5a-n)*

Compounds	E- total values in kjmol-[1]	
	Tyrosine kinase (RTK) (2A91)	Human estrogen receptor (2IOK)
5a	- 239.44	- 159.17
5b	- 239.44	- 155.04
5c	- 288.46	- 163.69
5d	- 239.43	- 135.51
5e	- 237.93	- 143.95
5f	- 312.14	- 190.19
5g	- 311.56	- 123.80
5h	- 215.86	- 134.69
5i	- 260.05	- 133.10
5j	- 282.51	- 150.08
5k	- 283.27	- 131.99
5l	- 255.46	- 120.88
5m	- 342.36	- 168.58
5n	- 283.87	- 142.44
Toremifene	- 249.93	- 101.94

CONCLUSION

In conclusion, a series of novel new series of 3-{5-methyl-1-[2-methyl-3-(trifluoromethyl)phenyl/substituted phenyl]-1H-1,2,3-triazol-4-yl}-1-(aryl)-1H-pyrazole-4-carbaldehyde ***(5a-n)***were synthesized *via* a Vilsmeier-Haack formylation of 4-{(1E)-1-[2-(aryl)hydrazinylidene] ethyl}-5-methyl-1-[2-methyl-3-(trifluoromethyl) phenyl/ substituted phenyl]-1H-1,2,3-triazole ***(4a-n)*** in quantitative yields and evaluated for their *in-vitro* anti-cancer studies against breast cancer cell lines MCF-7 and MDA-MB-231. Among the series, compounds ***5c, 5f,5g, 5j, 5m*** and ***5n*** were found to be potent and broad spectrum anti-cancer agents comparable with respect to the standard drug Toremifene. While the compounds ***5b, 5d, 5e, 5i, 5k*** and ***5l***were also found to be good cytotoxic agents against the standard drug Toremifene. In order to support the *in-vitro* anti-cancer results, the synthesized compounds ***(5a-n)*** were docked in to the plausible target human estrogen receptor (PDB ID: 2IOK) and tyrosine kinase receptor (RTK)(PDB ID: 2A91). The docking scores or the interaction binding energies of target enzymes supported the anti-cancer inhibiting activity of the compounds ***5c, 5f, 5g, 5j, 5m*** and ***5n***. All these results could be useful to evaluate as potential anti-cancer inhibitors and further can be considered as a lead compounds for the development of anti-cancer agents for the treatment of breast cancer infection.

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