MICROWAVE ASSISTED SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING STUDIES AND POSSIBLE BIOLOGICAL ACTIVITIES OF SOME NOVEL AZOLE DERIVATIVES

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Abstract

The present work deals with the sequence of Indazole, Triazole and Indole-2-one fused novel Azole derivatives **MNSR-3(a-l)** was synthesized by microwave assisted method, and were screened as potential anticancer, antibacterial activities and molecular docking studies. All of the newly synthesized compounds structurally characterized on the basis of IR, ^{1}HNMR and Mass spectral analysis. Further, were screened for antibacterial activity was carried out by agar diffusion (Cup plate) method by using Streptomycin as standard and anticancer activity against MCF-7 cell lines by MTT assay method. The results showed that some of the compounds **MNSR-3c** and **3i** exhibited good anticancer activity and **MNSR-3c, 3f, 3h** and **3l** are exhibited potential antibacterial activity by comparing standard drug. Additionally, the molecular docking studies of Indole, Triazole and Indole-2-one fused novel Azole derivatives was carried out to explain putative bonding interaction between the active site of EGFR enzyme and potent inhibitors by Schrodinger suite.

Keywords: Indazole, Triazole, Isatin, Antibacterial and Anticancer Activities, MCF-7, Doxorubicin, Streptomycin, EGFR and Schrodinger suite.

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1.INTRADUCTION

Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological importance. A series of azoles have been synthesized using an appropriate synthetic route and characterized by elemental analysis and spectral data. In recent times, microwave assisted organic reactions have received great interest and become very popular due to their enhanced reaction rate, eco-friendly nature, safety, and higher yields. Thus, keeping in view the advantages of this technique [1]. The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. The process of establishing new drugs is exceedingly complex and involves the talents of people from a variety disciplines, including Chemistry, Biochemistry, Physiology, Pharmacology, Pharmaceutics and Medicine [2-3]. Many Azole derivatives find important applications in the field of agriculture, medicine and industry. Azoles are parts of the larger family of sulphur and nitrogen containing organic compounds and metal their complexes which display a broad range of biological activity [4-8], finding applications ant-tumor, antibacterial, antifungal and antiviral agents. 1,2,4-triazole, pyrazole, thiazole, imidazole, oxazole and Indazole is also exhibiting excellent bioactivities have particularly multifarious uses in agriculture, medicine and industry. Azole derivatives, such as ketoconazole and bifonazole, are well-established antifungal drugs.

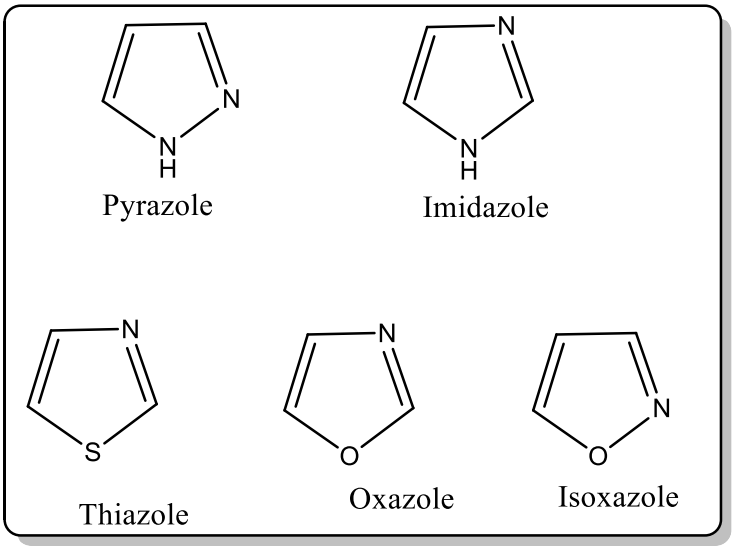

Figure.1 Example for Azole rings

Indole is an aromatic heterocyclic organic compound with the formula C_8H_7N. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered pyrrole ring[9-11]. Indole is widely distributed in the natural environment and can be produced by a variety of bacteria. Indole is also known as benzopyrrole which contains benzenoid nucleus and has 10 π-electrons (two from lone pair on nitrogen and double bonds provide eight electrons) which makes them aromatic in nature. Similar to the benzene ring, electrophilic substitution occurs readily on indole due to excessive π-electrons delocalization. Indazole, also called isoindazole, is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and pyrazole. Indazole is an amphoteric molecule which can be protonated to an indazolium cation or deprotonated to an indazolate anion. The corresponding pKa values are 1.04 for the equilibrium between indazolium cation and indazole and 13.86 for the equilibrium between indazole and indazolate anion [12-14]. Molecular docking is generally used to detect the protein-ligand orientation and interaction. The quality of any docking results depends on the starting structure of both the protein and the potential ligand[15-18]. The protein and ligand structure need to be prepared to achieve the best docking results. It includes the following steps, 1. Preparation of receptor & ligand files. 2. Calculation of affinity maps by using a 3D grid around the receptor & ligand. 3. Defining the docking parameters and running the docking simulation

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

The present work is based on Mannich and Schiff's base reactions. All the chemicals were used in this

research work were obtained from A.R grade and procured from the Merck and LOBA chemicals. The synthesized compounds melting points were determined by Thieles tube method by using liquid paraffin as a solvent. IR spectra were recorded by Thermo Nicolet Nexus 670 FTIR spectrometer and ^{1}HNMR were recorded on Bruker DPX-200 Hz using DMSO-d6 and chemical shifts (δ ppm) are recorded in parts per million downfield from internal reference Tetramethyl silane (TMS). Mass spectra had been recorded by the use of Schimadzu LCMS-8030 mass spectrophotometer and all the spectra had been interpreted. The recoated Silica Gel G plates were used to found the progress of reaction as well as to assessment the purity of the compounds: n-Hexane: Ethyl acetate (7:3). The molecular docking studies were carried out by using Schrodinger Suite Software.

2.2. General synthetic procedure

Step-I: 5-substituted-1-((3-methyl-1H-indazol-1-yl)methyl)indoline-2,3-dione 1(a-1): Take a solution of substituted isatin derivatives(0.1 mole) in ethanol (10 mL), were added formaldehyde (0.11 mole, 1 ml) and 3-methyl Indazole (0.11 mole) in an open vessel containing a Teflon coated stir bar. The mixture was heated in microwave at the power of 160 watts for 3-5mins. The reaction mixture was kept overnight in refrigeration and the product thus obtained was filtered, passed through column [silica gel, ethyl acetate: hexane (2:8)] to yield pure 1(a-l) products.

Step-II:4-amino-5-(p-tolyl)-2,4-dihydro-3H-1,2, 4-triazole-3-thione (2a-2c): A mixture of substitu ted benzoic acid (0.01 mol) and thio carbohydrazide (0.015 mol) were taken open vessel containing a Teflon coated stir bar. The mixture was heated in microwave at the power of 180 watts for 5mins. The completion of the reaction was confirmed by TLC. The reaction mixture was cooled; the precipitate was filtered and washed with water. The product was recrystallized with ethanol.

Step-III: MNSR-3a: 1-((3-methyl-1H-indazol-1-yl) methyl)-3-((3-phenyl-5-thioxo-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one.

To take a equimolar quantity of compound (1(a-l)) (0.01mol) and 4-amino-5-(p-tolyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (2a-2c) (0.01mol) in an open vessel containing a Teflon coated stir bar. Then add absolute ethanol (20ml) and 10ml of glacial acetic acid. The reaction was carried out under MW irradiation at 160W for 2-3 min. Then after the completion of the reaction mixture was cooled to room temperature and the solvent was distilled. The obtained product was recrystallized using ethanol. The progress of the reaction was predicted by TLC (n-Hexane: Ethyl acetate 7:3).

Compound. MNSR-3a: 1-((3-methyl-1H-indazol -1-yl)methyl)-3-((3-phenyl-5-thioxo-4,5-dihydro -1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3395 (NH *Str* in triazole), 3093(C-H *Str* in Ar-H), 2956, 2859(C–H *Str* in aliphatic -CH-), 1713(C=O *Str* in indole), 1615(-C=N *Str*), 1548(-C=CH *Str*), 1266(-SO_2 *Str*), 1025(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.6210(s, 1H, -NH proton in triazole), 7.8710-7.8162(t, 3H, Ar-H protons), 7.6835-7.6719(d, 2H, Ar-H protons), 7.6528-7.6334(d, 2H, Ar-H protons), 7.5831-7.5788(t, 3H, Ar-H protons), 7.5585-7.5182(t, 2H, Ar-H protons), 7.1459-7.1331(d, 2H, Ar-H protons), 4.4585(s, 2H, -CH_2- protons), 2.1026(s, 3H, -CH_3 in indazoel). Mass (LC-MS): m/z 465. 14(M), 466.21(M + 1, 100%).

Compound. MNSR-3b: 5-methyl-1-((3-methyl-1H-indazol-1-yl)methyl)-3-((3-phenyl-5-thioxo-4,5-dihydro -1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3295(NH *Str* in triazole), 3053(C-H *Str* in Ar-H), 2996, 2769(C–H *Str* in aliphatic -CH-), 1717(C=O *Str* in indole), 1611(-C=N *Str*), 1507(-C=CH *Str*), 1268(-SO_2 *Str*), 1011(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.3130(s, 1H, -NH proton in triazole), 8.3714(s, 1H, Ar-H protons), 7.9792-7.8913(d, 2H, Ar-H protons), 7.8795-7.8487(t, 2H, Ar-H protons), 7.7977-7.7848(d, 2H, Ar-H protons), 7.6989-7.6849(d, 2H, Ar-H protons), 7.6060-7.5189(t, 3H, Ar-H protons), 7.4948-7.4817(t, 2H, Ar-H protons), 4.5632(s, 2H, -CH_2- protons), 2.2343(s, 3H, -CH_3 in indazoel), 2.1105(s, 3H, -CH_3 in indole). Mass (LC-MS): m/z 479.15(M), 480.32(M + 1, 100%).

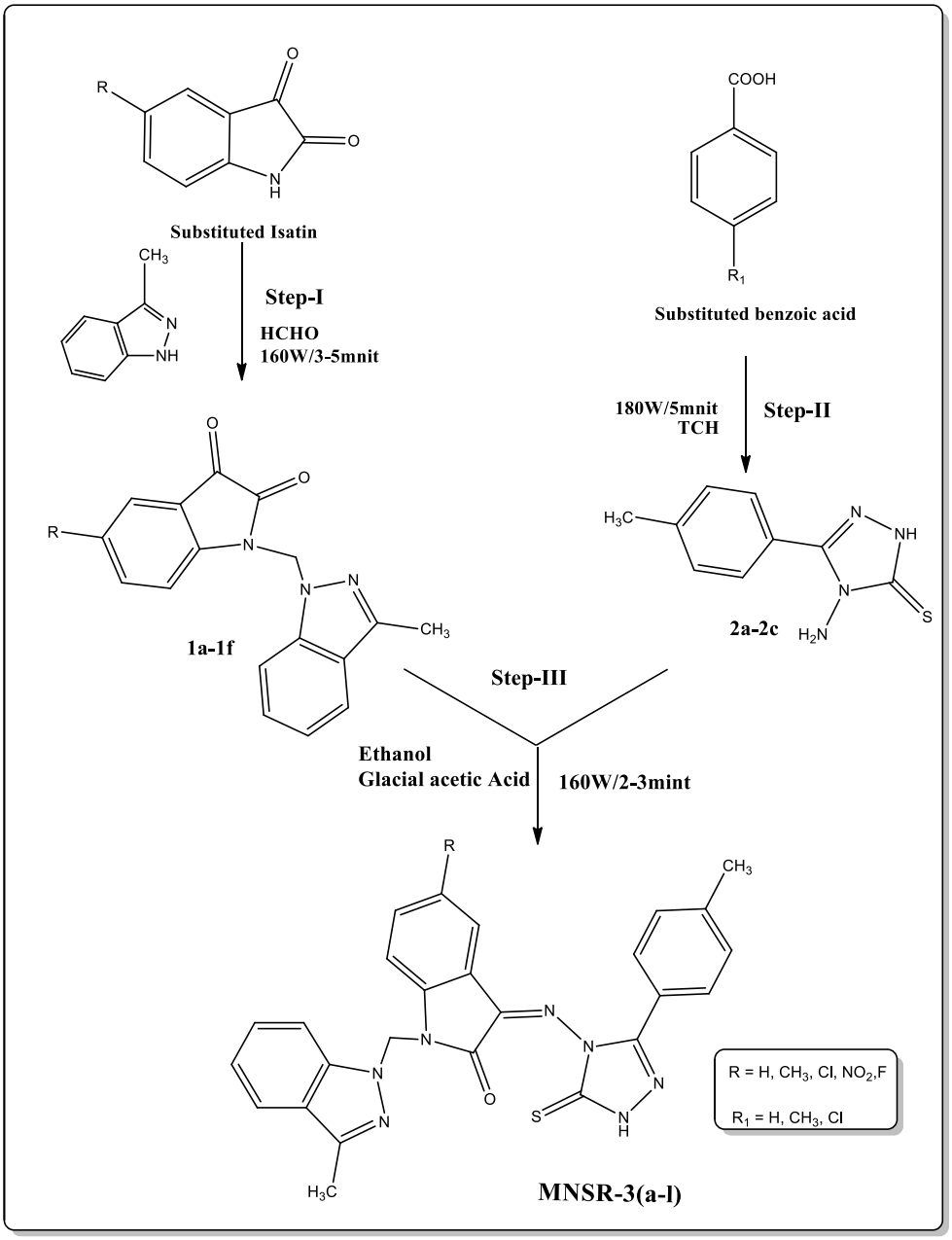

Figure 2. Synthesis scheme for the Indazole, Triazole and Indole-2-one fused novel Azole derivatives (Scheme-I, MNSR-3(a-l)

Compound. MNSR-3c: 5-chloro-1-((3-methyl-1H-indazol-1-yl)methyl)-3-((3-phenyl-5-thioxo-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3283(NH *Str* in triazole), 3097(C-H *Str* in Ar-H), 2988, 2795(C–H *Str* in aliphatic -CH-), 1712(C=O *Str* in indole), 1612(-C=N *Str*), 1520(-C=CH *Str*), 1248(-SO_2 *Str*), 1081(C-N *Str*), 804(-Cl *Str*, Ar-Cl). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 10.6512(s, 1H, -NH proton in triazole), 8.1532(s, 1H, Ar-H protons), 8.1317-8.0972(d, 2H, Ar-H protons), 8.0651-8.0145(d, 2H, Ar-H protons), 7.9476-7.9012(d, 2H, Ar-H protons), 7.8938-7.8048(t, 3H, Ar-H protons), 7.7898-7.6708(t, 2H, Ar-H protons), 4.7494(s, 2H, -CH_2- protons), 2.0326(s, 3H, -CH_3 in indazoel). Mass (LC-MS): m/z 499.10(M), 500.38(M + 1, 100%), 501.71(M + 2, 30%).

Compound. MNSR-3d: 5-fluoro-1-((3-methyl-1H-indazol-1-yl)methyl)-3-((3-phenyl-5-thioxo-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3281(NH *Str* in triazole), 3097(C-H *Str* in Ar-H), 2988, 2795(C–H *Str* in aliphatic -CH-), 1710(C=O *Str* in indole), 1612(-C=N *Str*), 1541(-C=CH *Str*), 1248(-SO_2 *Str*), 1081(C-N *Str*), 807(-F *Str*, Ar-F). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.0429(s, 1H, -NH proton in triazole), 8.5652(s, 1H, Ar-H protons), 8.2216-8.2062(d, 2H, Ar-H protons), 8.1498-8.1231(d, 2H, Ar-H protons), 8.0599-8.0302(d, 2H, Ar-H

protons), 7.8515-7.8368(t, 2H, Ar-H protons), 7.7862-7.6902(t, 3H, Ar-H protons), 4.5505(s, 2H, -CH_2- protons), 2.1728(s, 3H, -CH_3 in indazoel). Mass (LC-MS): m/z 483.13(M), 484.28(M + 1, 100%), 485.65(M + 2, 30%).

Compound. MNSR-3e: 1-((3-methyl-1H-indazol-1-yl)methyl)-5-nitro-3-((3-phenyl-5-thioxo-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3205(NH *Str* in triazole), 3056(C-H *Str* in Ar-H), 2980, 2885(C–H *Str* in aliphatic -CH-), 1701(C=O *Str* in indole), 1636(-NO_2 *Str* Ar-NO_2), 1612(-C=N *Str*), 1542(-C=CH *Str*), 1244(-SO_2 *Str*), 1099(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.6067(s, 1H, -NH proton in triazole), 7.8048(s, 1H, Ar-H protons), 7.6872-7.6353(d, 2H, Ar-H protons), 7.5364-7.5279(d, 2H, Ar-H protons), 7.4895-7.4348(t, 3H, Ar-H protons), 7.3963-7.8963(t, 2H, Ar-H protons), 6.7264-6.5832(d, 2H, Ar-H protons), 4.5649(s, 2H, -CH_2- protons), 2.2840(s, 3H, -CH_3 in indazoel). Mass (LC-MS): m/z 510.17(M), 511.29(M + 1, 100%).

Compound. MNSR-3f: 1-((3-methyl-1H-indazol-1-yl)methyl)-3-((5-thioxo-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3248(NH *Str* in triazole), 3098(C-H *Str* in Ar-H), 2976, 2883(C–H *Str* in aliphatic -CH-), 1718(C=O *Str* in indole), 1603(-C=N *Str*), 1522(-C=CH *Str*), 1268(-SO_2 *Str*), 1099(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.3452(s, 1H, -NH proton in triazole), 8.2324-8.1903(d, 2H, Ar-H protons), 7.9932-7.8754(d, 2H, Ar-H protons), 7.7685-7.6012(d, 2H, Ar-H protons), 7.5876-7.3543(d, 2H, Ar-H protons), 7.2093-7.1873(t, 2H, Ar-H protons), 6.9875-6.8751(t, 3H, Ar-H protons), 4.6543(s, 2H, -CH_2- protons), 2.3874(s, 3H, -CH_3 in indazoel), 1.9873(s, 3H, Ar-CH_3). Mass (LC-MS): m/z 479.15(M), 480.13(M + 1, 100%).

Compound. MNSR-3g: 5-methyl-1-((3-methyl-1H-indazol-1-yl)methyl)-3-((5-thioxo-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3332(NH *Str* in triazole), 3087(C-H *Str* in Ar-H), 2965, 2843(C–H *Str* in aliphatic -CH-), 1711(C=O *Str* in indole), 1625(-C=N *Str*), 1520(-C=CH *Str*), 1287(-SO_2 *Str*), 1076(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 10.8972(s, 1H, -NH proton in triazole), 8.3094(s, 1H, Ar-H protons), 8.2512-8.2024(d, 2H, Ar-H protons), 8.1943-8.0984(d, 2H, Ar-H protons), 7.8985-7.7893(d, 2H, Ar-H protons), 7.5674-7.5003(d, 2H, Ar-H protons), 7.2093-7.1983(t, 2H, Ar-H protons), 4.4563(s, 2H, -CH_2- protons), 2.3874(s, 3H, -CH_3 in indazoel), 2.1982-2.0943(s, 6H, Ar-$(CH_3)_2$. Mass (LC-MS): m/z 493.17(M), 494.32(M + 1, 100%).

Compound. MNSR-3h: 5-chloro-1-((3-methyl-1H-indazol-1-yl)methyl)-3-((5-thioxo-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3286(NH *Str* in triazole), 3087(C-H *Str* in Ar-H), 2998, 2834(C–H *Str* in aliphatic -CH-), 1716(C=O *Str* in indole), 1628(-C=N *Str*), 1554(-C=CH *Str*), 1265(-SO_2 *Str*), 1065(C-N *Str*), 798(-Cl *Str* in Ar-Cl). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 12.0943(s, 1H, -NH proton in triazole), 8.4875(s, 1H, Ar-H), 8.2983-8.1894(d, 2H, Ar-H protons), 8.0984-8.0023(d, 2H, Ar-H protons), 7.8785-7.7765(D, 2H, Ar-H protons), 7.6752-7.4756(d, 2H, Ar-H protons), 7.2763-7.1922(t, 2H, Ar-H protons), 4.4503(s, 2H, -CH_2- protons), 2.1786(s, 3H, -CH_3 in indazoel), 1.9983(s, 3H, Ar-CH_3). Mass (LC-MS): m/z 513.11(M), 514.01(M + 1, 100%), 515(M + 2, 30%).

Compound. MNSR-3i: 5-fluoro-1-((3-methyl-1H-indazol-1-yl)methyl)-3-((5-thioxo-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3267(NH *Str* in triazole), 3064(C-H *Str* in Ar-H), 2987, 2854(C–H *Str* in aliphatic -CH-), 1707(C=O *Str* in indole), 1618(-C=N *Str*), 1534(-C=CH *Str*), 1283(-SO_2 *Str*), 1082(C-N *Str*), 806(-F *Str* in Ar-F). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 10.8721(s, 1H, -NH proton in triazole), 8.2094(s, 1H, Ar-H), 8.1982-8.0224(d, 2H, Ar-H protons), 7.9843-7.8332(d, 2H, Ar-H protons), 7.7732-7.6532(d, 2H, Ar-H protons), 7.4532-7.3046(d, 2H, Ar-H protons), 7.1982-7.0213(t, 2H, Ar-H protons), 4.6754(s, 2H, -CH_2- protons), 2.3872(s, 3H, -CH_3 in indazoel), 2.0943(s, 3H, Ar-CH_3). Mass (LC-MS): m/z 497.14(M), 498.12(M + 1, 100%), 499.54(M + 2, 30%).

Compound. MNSR-3j: 5-nitro-1-((3-methyl-1H-indazol-1-yl)methyl)-3-((5-thioxo-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one. IR (ν cm−1): 3247(NH *Str* in triazole), 3099(C-H *Str* in Ar-H), 2978, 2878(C–H *Str* in aliphatic -CH-), 1708(C=O *Str* in indole), 1645(-NO_2 Str), 1619(-C=N *Str*), 1534(-C=CH *Str*), 1298(-SO_2 *Str*), 1076(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.8953(s, 1H, -NH proton in triazole), 8.5643(s, 1H, Ar-H), 8.3764-8.2984(d, 2H, Ar-H protons), 8.1903-8.1203(d, 2H, Ar-H protons), 7.9853-7.8235(d, 2H, Ar-H protons), 7.7654-7.6234(d, 2H, Ar-H protons), 7.5643-7.4563(t, 2H, Ar-H protons), 4.5432(s, 2H, -CH_2- protons), 2.2341(s, 3H, -CH_3 in indazoel), 2.0543(s, 3H, Ar-CH_3). Mass (LC-MS): m/z 524.14(M), 525.21(M + 1, 100%).

Compound. MNSR-3k: 3-((3-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1H-pyrazol-4-yl)imino)-1-((3-methyl-1H-indazol-1-yl)methyl)indolin-2-one. IR (ν cm−1): 3281(NH *Str* in triazole), 3029(C-H *Str* in Ar-H), 2976, 2845(C–H *Str* in aliphatic -CH-), 1703(C=O *Str* in indole), 1624(-C=N *Str*), 1502(-C=CH *Str*), 1286(-SO_2 *Str*), 1087(C-N *Str*), 801(-Cl *Str* in Ar-Cl). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.9043(s, 1H, -NH proton in triazole), 8.3123-8.3092(d, 2H, Ar-H), 8.0543-8.0021(d, 2H, Ar-H protons), 7.7843-7.6784(d, 2H, Ar-H protons), 7.5633-7.4532(d, 2H, Ar-H protons), 7.3784-7.2198(t, 2H, Ar-H protons), 7.1094-7.0321(t, 2H, Ar-H protons), 4.3902(s, 2H, -CH_2- protons), 2.4634(s, 3H, -CH_3 in indazoel). Mass (LC-MS): m/z 499.10(M), 450.21(M + 1, 100%), 501(M + 2, 30%).

Table:1. Physical data of Indazole, Triazole and Indole-2-one fused novel Azole derivatives (MNSR-3(a-l)

Compound	Molecular Formula	R_1	R	Molecular Weight (gms)	M.P (^{0}C)	%Yield
MNSR-3a	$C_{25}H_{19}N_7OS$	H	H	465.14	219-221	85
MNSR-3b	$C_{26}H_{21}N_7OS$	-CH_3	-H	479.15	233-235	81
MNSR-3c	$C_{25}H_{18}N_7OSCl$	-Cl	-H	499.10	173-175	79
MNSR-3d	$C_{25}H_{18}N_2OSF$	-F	-H	383.13	265-267	86
MNSR-3e	$C_{25}H_{18}N_8O_3S$	-NO_2	-H	510.12	201-203	82
MNSR-3f	$C_{26}H_{21}N_7OS$	H	-CH_3	479.15	187-189	88
MNSR-3g	$C_{27}H_{23}N_7OS$	CH_3	-CH_3	493.17	167-169	76
MNSR-3h	$C_{26}H_{20}N_7OSCl$	-Cl	-CH_3	513.11	231-233	79
MNSR-3i	$C_{26}H_{20}N_7OS$	-F	-CH_3	497.14	243-246	84
MNSR-3j	$C_{26}H_{20}N_8O_3S$	-NO_2	-CH_3	524.14	179-181	82
MNSR-3k	$C_{25}H_{18}N_7OSCl$	-H	-Cl	499.10	159-162	85
MNSR-3l	$C_{26}H_{20}N_7OSCl$	CH_3	-Cl	513.11	209-211	80

Compound. MNSR-3l: 3-((3-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1H-pyrazol-4-yl)imino)-5-methyl-1-((3-methyl-1H-indazol-1-yl)methyl)indolin-2-one. IR (ν cm−1): 3309(NH *Str* in triazole), 3012(C-H *Str* in Ar-H), 2965, 2809(C–H *Str* in aliphatic -CH-), 1720(C=O *Str* in indole), 1610(-C=N *Str*), 1576(-C=CH *Str*), 1299(-SO_2 *Str*), 1065(C-N *Str*), 805(-Cl *Str* in Ar-Cl). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.9054(s, 1H, -NH proton in triazole), 8.3092(s, 1H, Ar-H), 8.1983-8.0213(d, 2H, Ar-H protons), 7.8943-7.7643(d, 2H, Ar-H protons), 7.6985-7.5984(d, 2H, Ar-H protons), 7.3874-7.2765(d, 2H, Ar-H protons), 6.8954-6.7865(t, 2H, Ar-H protons), 4.3876(s, 2H, -CH_2-protons), 2.3476(s, 3H, -CH_3 in indazoel), 2.1093(s, 3H, Ar-CH_3). Mass (LC-MS): m/z 513c .11(M), 514.01(M + 1, 100%), 515(M + 2, 30%).

2.3. Pharmacological activity

Anticancer Activity: Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The in vitro anti-cancer activity of test compounds was carried out by MTT Assay against the MCF-7 Cell lines [18-20]. The cell viability was once appraising by means of the MTT Assay with three impartial experiments with six different concentrations of compounds in triplicates. The novel Azole derivatives were evaluated for cytotoxicity against MCF-7 (human breast cancer cells) cell lines by MTT assay method with Doxorubicin as standard drug and results were mention in the Table.No.2.

Antibacterial Activity: Novel azole derivatives [1-((3-methyl-1H-indazol-1-yl)methyl)-5-substituted-3-((3-phenyl-5-thioxo-4,5-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one] **MNSR-3(a-l)** were screened for antibacterial activity by agar diffusion-Cup plate method[21-23]. The zone of inhibition was recorded against Staphylococcus *aureus, Bacillus subtilis* (Gram positive) and *Escherichia coli, Salmonella paratyphi, Pseudomonas* (Gram negative) bacteria. Determine the zone of inhibition in mm. Finally, the results were recorded in Table No.3.

Molecular Docking Studies. In drug design, molecular docking studies play an important role in mechanistically by placing a molecule into the binding site of the target molecule. I have docked the synthesized Indazole, Triazole and Indole-2-one fused novel Azole compounds into active site of the epidermal growth factor receptor (EGFR) was retrieved from the Protein databank website with PDB Id: 1M17 by using the Ligprep tool of Schrodinger suite [24-25]. Furthermore, structure-ally optimized protein shape was once used to observe protein-ligand interactions of the dataset ligands the use of Glide Xp docking protocol. The

Glide scores of the dataset ligands have been recorded in Table.No.4.

3. RESULTS AND DISCUSSION

Synthesis: The series of synthesized indazole, triazole and Indole-2-one fused novel Azole compound show satisfactory analysis for the proposed structures, and which were confirmed on the basis of their FT-IR, LC-MASS and ^{1}H NMR spectral data. In this synthetic process, a series of novel azole derivatives were synthesized by treating substituted Isatin with indazole and followed by Schiff‘base reactions with 4-amino-5-phenyl- triazole their potent antibacterial and anticancer activities.In the present work the effort is made to develop a convenient method for the synthesis of azole derivatives (Scheme-I, MNSR-3a-3l).

Spectral data: Spectral characterization of indazole, triazole and Indole-2-one fused novel Azole derivatives was performed by IR spectroscopy. Practically, in all the compounds are showing the aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3002-3097 cm^{-1} and 2900-2743 cm^{-1}. All the compounds have been show strong absorption in the region of 1696-1723cm^{-1} is found to be presence of C=O stretching frequency and in most of the compounds the C=C stretching of the aromatic ring is around 1545-1535cm^{-1} respectively. The Ar-Cl stretching is showing the strong absorption in the region 793-827 cm^{-1} and few compounds containing -NO_2 group shows peaks due to stretching of –NO_2 group is observed at around 1622-1654cm^{-1} respectively. Similarly, the ^{1}HNMR (DMSO-d6) spectra of azole derivatives are showed a singlet at 10.01-12.021 for -NH in triazole proton and singlet at 8.78-9.67 for methylene protons. Some of the compounds are showing triplet at 3.32-3.698 for –OCH_3 in methoxy protons. All these compounds have aromatic protons were found between δ 8.456-6.793 ppm as singlet, doublet and triplet protons.

Anticancer activity: indazole, triazole and Indole-2-one fused novel Azole compounds were screened for cytotoxic activity against one cancer cell like human breast cancer cells (MCF7) by using MTT assay method, with doxorubicin as a standard drug. All the results (Table 2) proposed that MCF-7 cell lines were susceptible to the evaluated compounds showed IC_{50}values in the range of **18.03 ± 0.354 µg to 71.43 ± 0.321µg (Scheme-I)** against MCF7 cell line Cell line. From the results, the compounds **MNSR-3c (21.71±0.204µg)**, **MNSR-3i (18.03± 0.354µg)** showed good activity against one cell lines, whereas, remaining of the compounds showed moderate activity against MCF-7 cell lines.

Table.2: Test compounds treated with MCF-7 cells showing the IC_{50} values

S. No	SAMPLE NAME	IC_{50} (µg)
		MCF7
1	**MNSR-3a**	71.43± 0.321
2	**MNSR-3c**	**21.7**± 0.104
3	**MNSR-3d**	**30.21**± 0.251
4	**MNSR-3f**	39.86± 0.043
5	**MNSR-3i**	**18.03**± 0.354
6	**MNSR-3j**	54.36± 0.193
7	**MNSR-3k**	68± 0.021
8	**MNSR-3l**	61.7± 0.154
9	**Doxorubicin**	12.02± 0.215

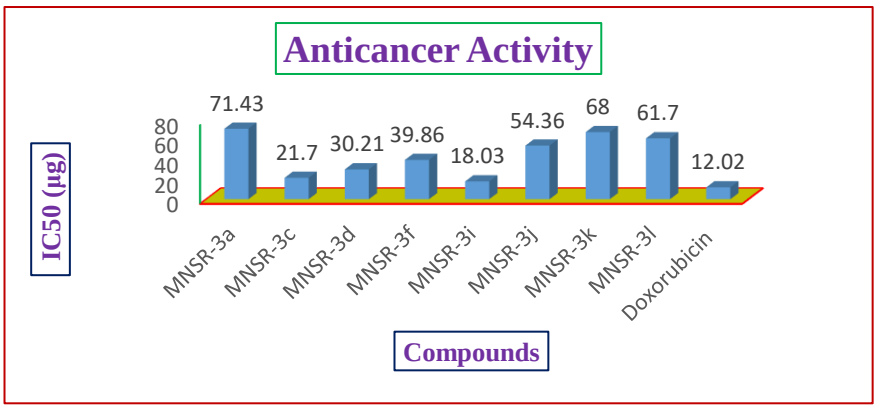

Figure 4: Graphical representation of Anticancer activity of Indazole, Triazole and Indole-2-one fused novel Azole derivatives (Scheme-I, MNSR-3(a-l) on MCF-7 Cell lines.

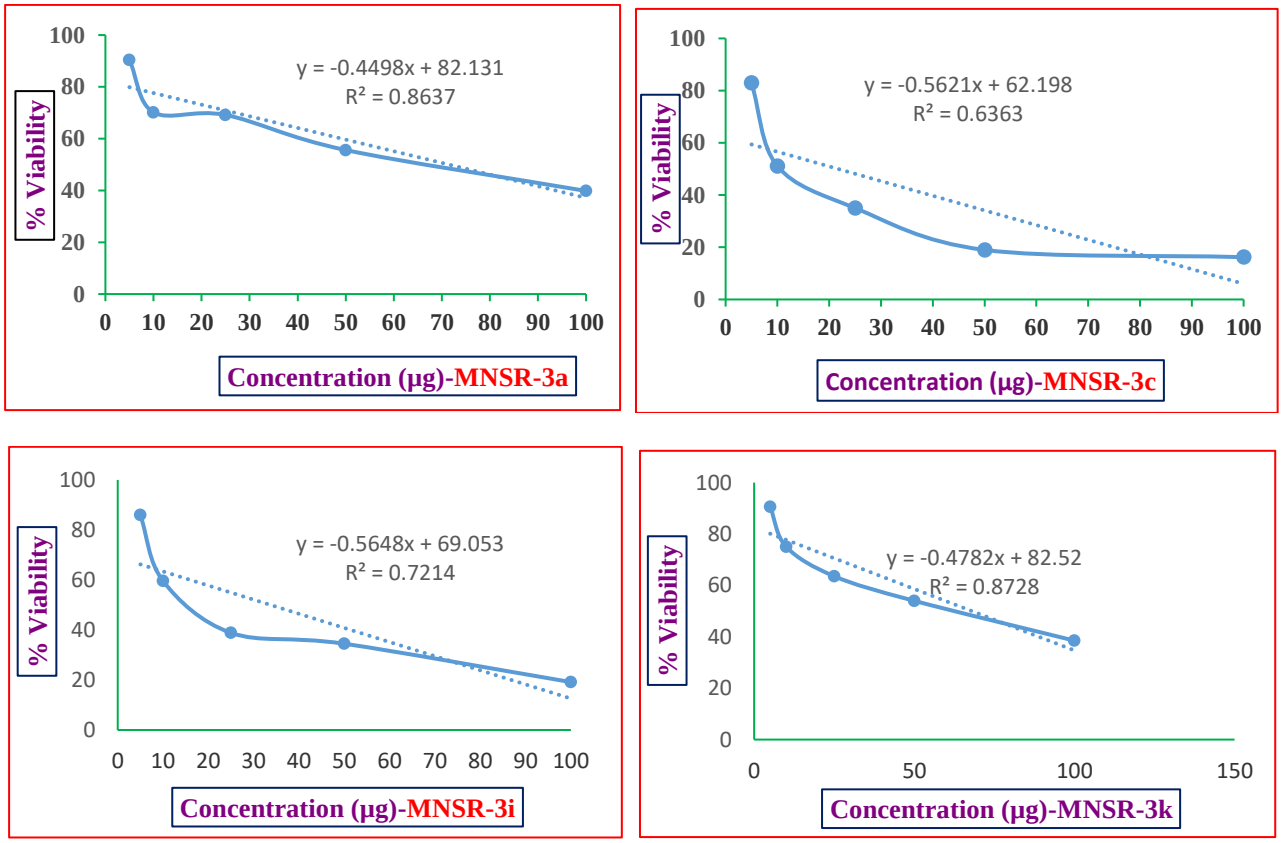

Figure 5: Graphical representation of Indazole, Triazole and Indole-2-one fused novel Azole derivatives-IC_{50} values

Antibacterial activity: The antibacterial activity of all the synthesized compounds MNSR-3(a-l), Scheme-I) is performed by cup plate method (diffusion technique). The streptomycin used as a standard drug against *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive) and *Escherichia coli*, *Salmonella paratyphi*, *Pseudomonas* (Gram negative). Most of the synthesized compounds showed significant antibacterial activity. The compound **MNSR-3c** (**24***mm against *S.aureus* and **22***mm against *E.coli*), **MNSR-3f** (**26***mm against *S.aureus* and **25***mm against *E.coli* and 24* mm against *Sal.paratyphi)*, **MNSR-3l** (**21***mm against *S.aureus*, **23***mm against *Bas.subtilis* and 25* mm against *Sal.paratyphi)* were showed good activity.

Table.No.4 Antibacterial activity of Indazole, Triazole and Indole-2-one fused novel Azole derivatives-MNSR-3(a-l)

	Zone of Inhibition (in mm)			
	Staphylococcus aureus	***Bacillus subtilis***	***E. Coli***	***Salmonella paratyphi***
MNSR-3a	10	14	12	15
MNSR-3b	13	18	09	9
MNSR-3c	**24***	16	**22***	13
MNSR-3d	15	13	12	17
MNSR-3e	10	16	19	18
MNSR-3f	**26***	10	25*	**24***
MNSR-3g	17	09	11	09
MNSR-3h	**24***	**22***	15	**23***
MNSR-3i	14	09	10	11
MNSR-3j	19	09	11	15
MNSR-3k	09	15	09	14
MNSR-3l	**21***	**23***	12	**25***
Streptomycin	30	31	29	30

All values are expressed as % Inhibition; Bore size = 6mm; Concentration of test compounds is 100µg/mL

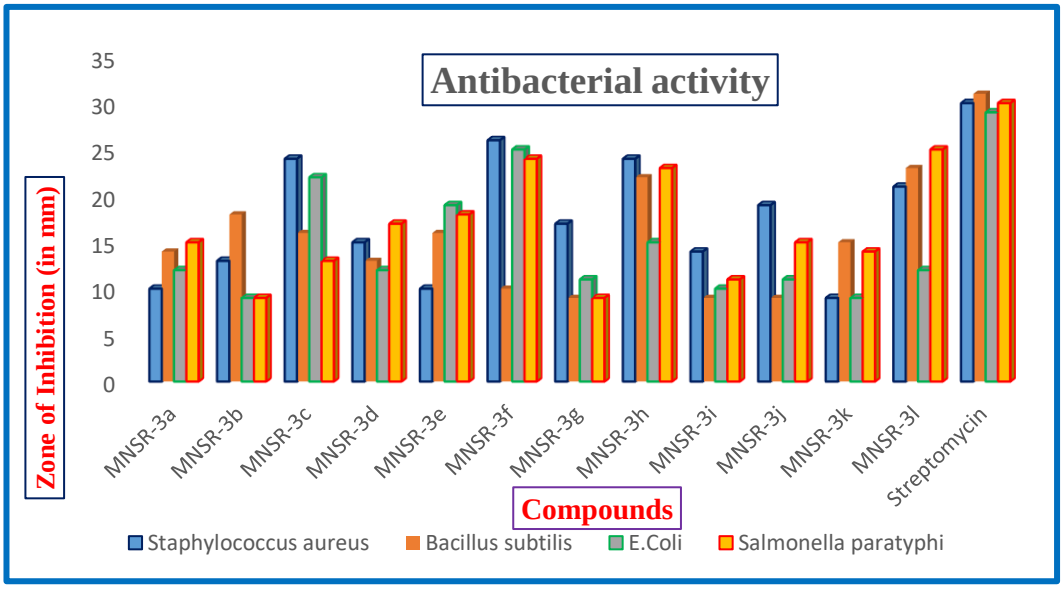

Figure 6: Graphical representation of Antibacterial activity of Indazole, Triazole and Indole-2-one fused novel Azole derivatives (MNSR-3(a-l)

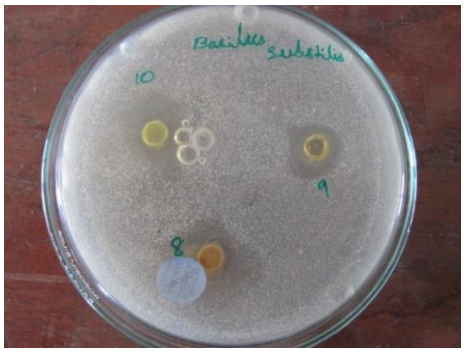

Figure.7: Photographs of Antibacterial activity

Molecular Docking studies: Among the docked ligands, compound **MNSR-3c** reported highest dock score of **-4.31** with Glide binding energy of **-44.079** Kcal/mol. Dock scores of all the compounds ranged from **-4.31** (compound **MNSR-3c**) to -1.908 (compound **MNSR-3j**). MET 769, CYS 773 and LYS 721 are the most common amino acids with H-bonds. Pi-Pi stacking was observed between compounds MNSR-3c or MNSR-3j (methyl indole nucleus) and LYS 721. Halogen bond was observed between compound MNSR-3c and CYS 773.

Table.No.5: Insilco EGFR inhibition of Indazole, Triazole and Indole-2-one fused novel Azole compounds Glide dock sores of the dataset ligands

Compound No	Dock score XP GScore	No of H-bonds	Interacting amino acids	H-bond lengths (Å)	Emodel energy	Glide energy
MNSR-3c	-4.31	1	LYS 721	1.87	-42.634	-44.079
MNSR-3i	-4.053	2	MET 769	1.90, 2.74	-60.663	-37.873
MNSR-3f	-3.746	1	MET 769	1.89	-64.746	-42.948
MNSR-3a	-3.574	1	LYS 721	2.38	-59.097	-39.705
MNSR-3e	-3.214	0	-	-	-54.088	-44.445
MNSR-3d	-3.175	0	-	-	-49.642	-40.252
MNSR-3k	-3.301	1	CYS 773	2.10	-64.406	-44.089
MNSR-3b	-3.051	1	CYS 773	2.11	-53.74	-43.342
MNSR-3g	-2.928	0	-	-	-60.905	-46.118
MNSR-3l	-3.017	0	-	-	-59.512	-41.394
MNSR-3h	-2.453	0	-	-	-55.309	-40.366
MNSR-3j	-1.908	0	-	-	-49.142	-44.876

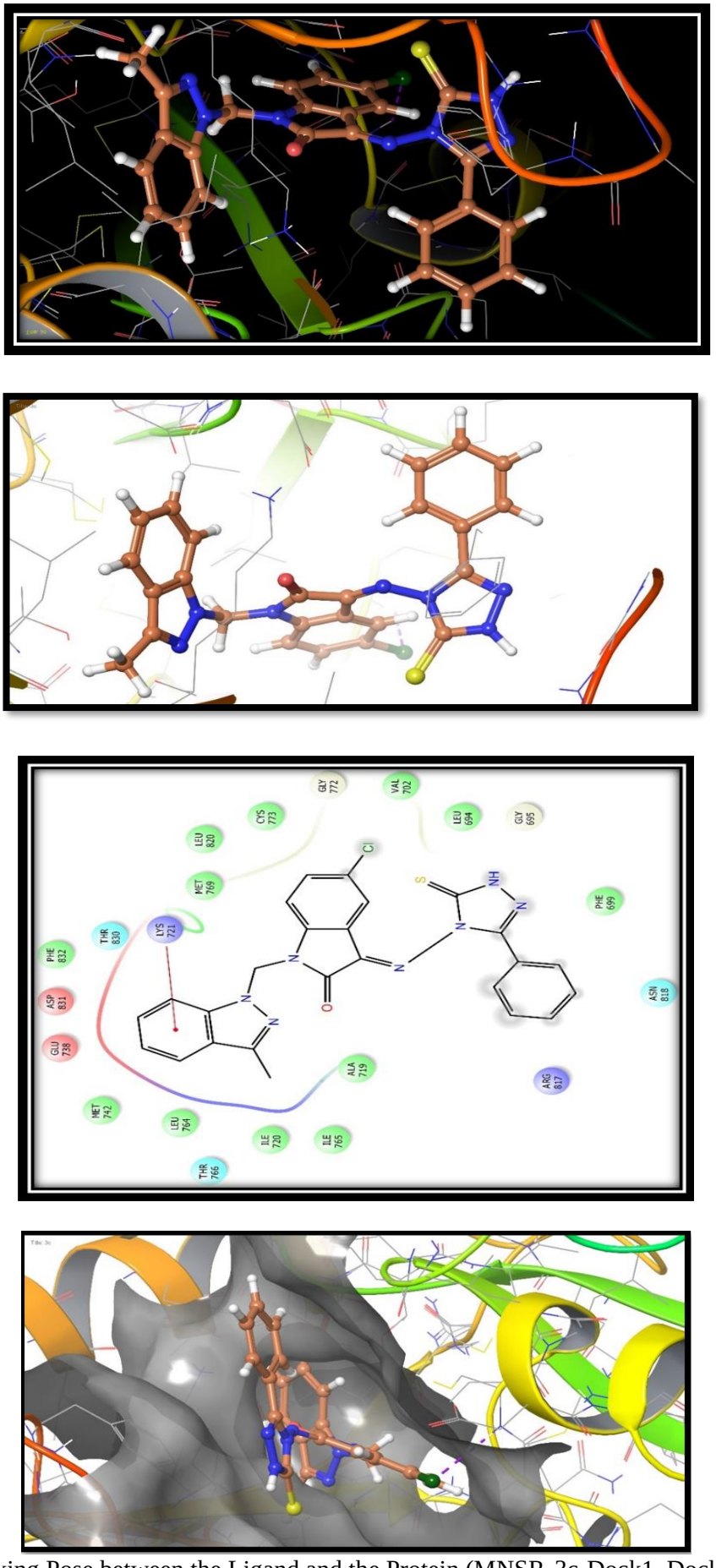

Figure.8. Docking Pose between the Ligand and the Protein (MNSR-3c-Dock1, Dock-2 and 2d, 3d)

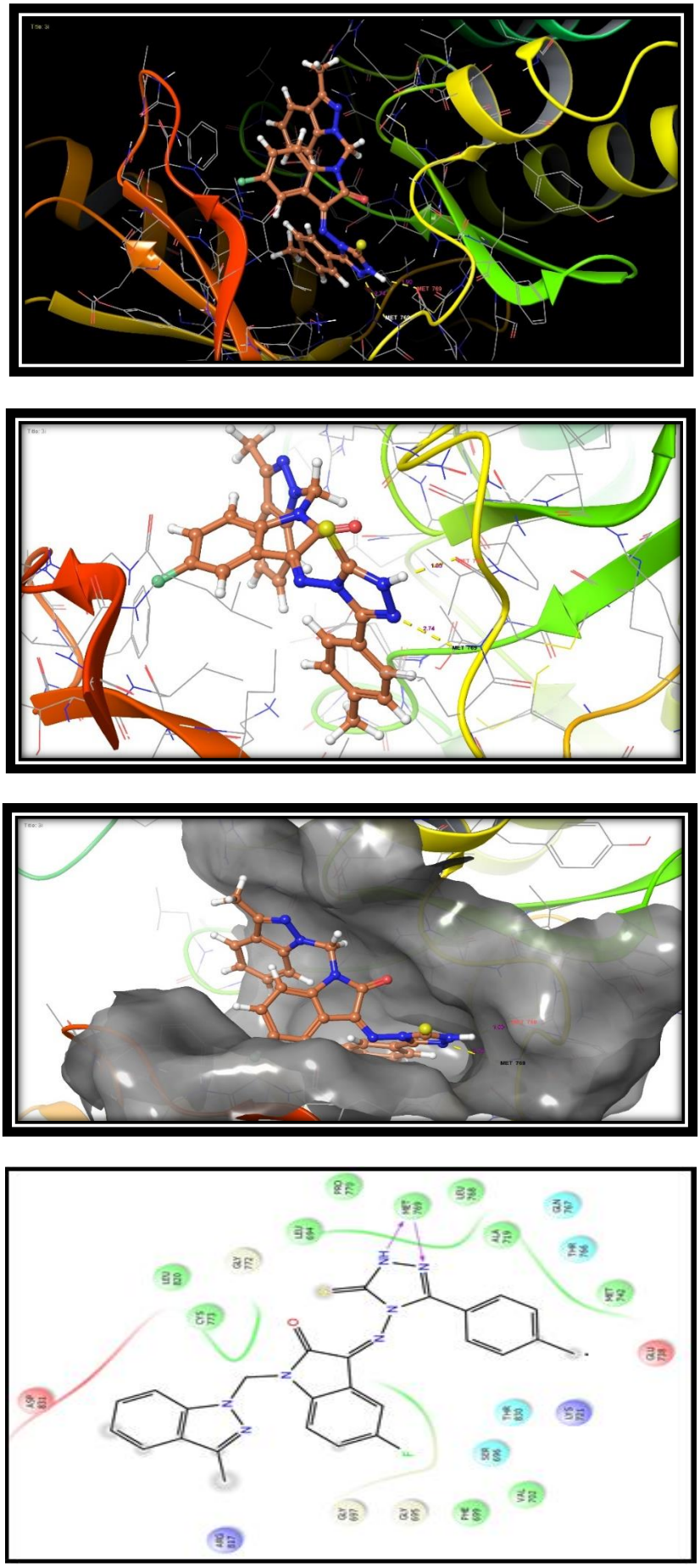

Figure.9. Docking Pose between the Ligand and the Protein (MNSR-3i-Dock1, Dock-2 and 2d, 3d)

5. CONCLUSIONS:

The novel indazole, triazole and Indole-2-one fused Azole compounds are developed by a three step process under microwave assisted method. The yield of the synthesized compound was found to be in the range from **76-88%.** The structures of the newly synthesized compounds (Scheme-I, MNSR-3(a-l)) were elucidated by IR, ^{1}H-NMR, Mass spectroscopy along with physical data. All the synthesized compounds screened for the anticancer activity, Antibacterial and Molecular docking studies of the prepared compounds by standard protocol available in literature. In conclusion, the present study highlights the importance of the derivatives of Novel Azole derivative's containing various substitutions are features responsible for the various biological activities and may serve as a lead molecule for further modification to obtain clinically useful novel entities**.**

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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