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New Nitrogen Based Azole Derivatives: Synthesis, Characterization, and *In-Vitro* Anticancer, Antibacterial Activity with Focus on Molecular Docking Studies

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Abstract

A series of new nitrogen-based Azole derivatives **I-(4a-4l)** have been synthesized **by** conventional method. The structures of these compounds were confirmed by FT-IR, ^{1}HNMR and Mass spectral analysis and Physical characterization. Compounds were evaluated for their in vitro antibacterial and anticancer activities. In Step-I, thiazolidine-2,4-dione(1a) prepared by cyclization between thiourea with chloroacetyl chloride. Compound 1a reacts with 4-aminobenzaldehyde by knoevenagel condensation to form 5-(4-aminobenzylidene) thiazolidine-2,4-dione(2a) in step-II. Substituted benzaldehydes reacts with compound 2a via Schiff's base mechanism to form 5-(4-((3,4-dimethoxy benzylidene) amino) benzylidene) thiazolidine-2,4-dione (3a-3j) in step-III. In final step, compounds 3a-3j undergo mannich bases reaction with piperazine or morpholine to give title derivatives **I-(4a-4l)**. Compounds **I-4b, I-4h** and **I-4l** exhibited excellent antibacterial activity when comparison with standard. Compounds **I-4f (IC_{50} 26.615±0.001)** and **I-4l (IC_{50} 24.136±0.012),** exhibited excellent anticancer activity against MCF-7 cell lines. Finally, molecular docking studies was performed by AUTODOCK Vina software by using EGFR receptor with Pdb Id:1M17. Compounds **I-4a, I-4f, I-4j** and **I-4l** showed good binding score.

Keywords

Thiazolidine-2,4-dione**,** 4-amine benzaldehyde, Molecular docking, MTT, MCF-7 Cell lines, Anticancer and Antibacterial activities.

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INTRODUCTION:

Medicinal chemistry is a chemistry grounded discipline involving aspects of birth, medical and knowledge. It's concerned with the invention, discovery, design, identification, and physic of biologically active amalgams, the interpretation of their mode of action at the molecular place, and the construction of the relationship between chemical structure and pharmacological exercise**.** Azole and its by-products, a class of well-known nitrogen and Sulphur containing heterocyclic admixtures, absorb an important position in medicinal and Thiazolidine chemistry with a wide range of bioactivities [1-3]. Triazoles spinoffs have a long history of use in agrochemicals as manures and manures and in pharmaceutical sedulousness as antipyretic and anti-

inflammatory. Cancer is the second leading cause of death globally and was responsible for around 8.8 million deaths in the year 2015. Globally, nearly 1 in 6 deaths is due to cancer. Approximately 70% of deaths from cancer occur in low and middle-income countries. Presently in India, it is a major cause of morbidity and mortality.

Cancer and associated diseases are increasing rapidly among Indian women, primarily because of low awareness and late detection. Data showed that India accounts for the third highest number of cancer cases among women after China and the United States, with a 4.5-5% growth rate annually [4-7]. The presence of three nitrogen hetero-atoms in five-membered ring systems defines an interesting class of compounds, the triazole. Triazoles also known as pyrrodiazole, containing a five membered di-unsaturated ring structure composed of three nitrogen atoms and two carbon atoms at non-adjacent positions having molecular formula $C_2H_3N_3$. The compound triazole was first identified by Fischer in 1878. But, in 1885, Bladin first gave the name of triazole to the carbon nitrogen ring system and described derivatives of triazoles [8-10]. Triazoles exist as two isomers –1,2,3- triazoles and 1,2,4-triazoles.

In the present paper, we have focused on synthesis of nitrogen based new azole derivatives were synthesized via civilization, Knoevenagel reaction, Schiff's and Mannich bases. The structure of these synthesized compounds was confirmed by means of IR, Mass and ^{1}H NMR analysis. In addition, molecular docking studies and the preliminary antibacterial activity and anticancer activities were tested. The synthetic route of target compounds in showed in Scheme. In continuation of our antibiotic research, we have also evaluated the antibacterial and invitro anticancer activities of this new compounds against *Staphylococcus aureus, Bacillus subtilis* (Gram positive) and *Escherichia coli, Salmonella paratyphi* (Gram negative) were chosen based on their Pharmacological-clinical importance [11].

MATERIALS AND METHOD:

Each reactant and solvent utilised were of the maximum purity achievable and of analytical reagent grade (AR). All the necessary solvents and reagents have been bought from Hychem Laboratories and SD Fine. Using silica gel plates with TLC techniques, the physical characteristics of the new synthesized compounds were determined. The mobile phase that is utilized in the above procedure is n-hexane: ethyl acetate (8:2Auto dock software was employed to carry out the molecular docking studies. Shimadzu LC-MS with positive mode had been employed for recording the mass spectra, and the 1HNMR spectra were captured at 300 MHz utilising the solvent DMSO-d6. subsequently, a Shimadzu FT-IR Spectrophotometer had been employed to record the structure's FTIR spectra in KBr pellets in the 4000-400 cm-1 range.

General Procedures [12-13].

Step: I. General procedures for thiazolidine-2, 4-Dione(1a). The equimolar quantity (1:1) of chloroacetic acid (56.4 g, 0.6mol) in 60 ml of water was added to the solution of thiourea (45.6 g, 0.6mol) in 60 ml of water. The mixture was stirred for 15 min. and precipitates were obtained after cooling. Then add slowly 60 ml of concentrated hydrochloric acid from a dropping funnel. Once the mixture got converted to solution form, it was refluxed for 8-10 hour at 100-110°C. On cooling, the contents of the flask solidified to a cluster of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was purified by recrystallization from ethyl alcohol.

Step: II. General procedure for the synthesis of 5-(4-aminobenzylidene) thiazolidine-2,4-dione(2a). A mixture of 2,4-thiazolidinedione 1a (0.01mol), 4-amino benzaldehyde (0.01mol), glacial acetic acid (25mL) and fused sodium acetate (0.18g) was refluxed for 1hr with occasional shaking. Cool, then the reaction mixture was cooled to room temperature and it was poured in water (250mL), the product obtained was filtered, washed with water, alcohol and ether and was recrystallized with glacial acetic acid.

Step: III. **5-((E)-4-(((E)-4-substitutedbenzylidene) amino) benzylidene) thiazolidine-2,4-dione (3a-3e).** Compound **2a** (0.01 mol) was taken in a mixture of substituted benzaldehyde (0.01 mol) and glacial acetic acid (5 mL) and Ethanol 30ml, then the reaction mixture was refluxing for 1-2hrs. The progress of the reaction was monitored by TLC (n-Hexane: EtoAc 4:1). The reaction mixture was cooled to room temperature. A solid was obtained, which was filtered off and washed with hexane and recrystallized from methanol to give crystalline solid.

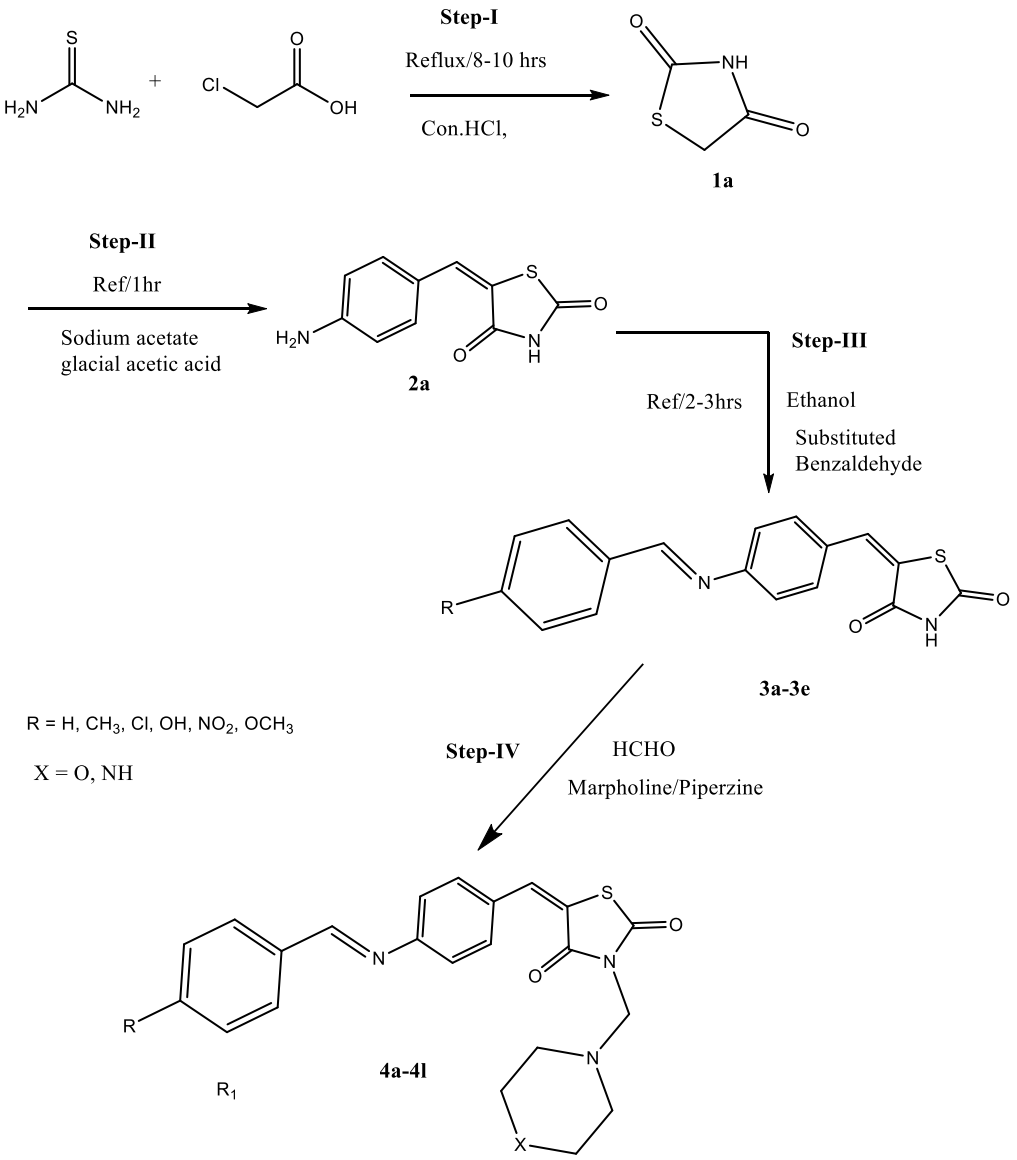


Figure.1. Schematic representation of new nitrogen-based Azole derivative

Step: IV. General procedure for the synthesis of 5-((E)-4-(((E)-4-substituted benzylidene) amino) benzylidene)-3-(-(piperazin/morpholino methyl) thiazolidine-2,4-dione(4a-4j).

A mixture of equimolar quantity of 5-((E)-4-(((E)-4-substituted benzylidene) amino) benzylidene) thiazolidine-2,4-dione (3a-3e) (0.01 mol) in alcohol (30 ml) was stirred with aq. formaldehyde (0.02 mol) at room temperature for 1hr. The reaction mixture was then heated under reflux with an appropriate secondary amine (0.02 mol) for about 3 hrs. The alcohol was removed; the residue was cooled and left overnight in a refrigerator. The resultant product was triturated with crushed ice. The product was filtered under suction, washed 2 or 3 times with small portions of ice-cold water and dried. The compound was purified by recrystallization from aq. alcohol.

Completed.I-4a: 5-((4-benzylidene) amino) benzylidene)-3-(morpholine-1-ylmethyl) thiazolidine-2,4-dione. IR (*ν* cm-1): 3087(C-H Str in Ar-H), 2940, 2828(-C-H Str in aliphatic), 2329(CSC Str in thiazoilidine ring), 1721(-C=O Str in thiazolidine ring), 1613(-C=N Str in imine), 1581(-C=CH Str), 1410(-C-C Str in Ar-C), 1040(-C-N Str). **^{1}H-NMR(DMSO) *δ*** 9.7804(s, 1H, Imine proton), 8.8549(s, 1H, Benzylidene proton), 7.9204-7.9107(d, 2H, Ar-H), 7.8862-7.8765(d, 2H, Ar-H), 7.6905-7.6801(d, 2H, Ar-H), 7.6087-7.6037(t, 3H, Ar-H), 4.4865(s, 2H, -N-CH_2-N proton), 2.6834(t, 4H, morpholine), 2.2732(t, 4H, morpholine protons). **Mass(m/z):** 407.13(M^+); 408.21(M^++1, 100%).

Completed.I-4b: 5-((4(4-methyl benzylidene) amino) benzylidene)-3-(morpholine-1-ylmethyl) thiazolidine-2,4-dione. IR (*ν* cm-1): 3071(C-H Str in Ar-H), 2979, 2896, 2799(-C-H Str in aliphatic),

2354(CSC Str in thiazoilidine ring), 1716(-C=O Str in thiazolidine ring), 1616(-C=N Str in imine), 15834(-C=CH Str), 1414(-C-C Str in Ar-C), 1068(-C-N Str**). ^{1}H-NMR(DMSO)** ***δ*** 9.4022(s, 1H, Imine proton), 8.8054(s, 1H, Benzylidene proton), 7.8983-7.8903(d, 2H, Ar-H), 7.6674-7.6603(d, 2H, Ar-H), 7.6287-7.6102(d, 2H, Ar-H), 7.5993-7.5983(d, 2H, Ar-H), 4.4532(s, 2H, -N-CH_2-N proton), 2.6872(t, 4H, morpholine), 2.3984(t, 4H, morpholine protons), 1.9087(s, 3H, Ar-CH_3 protons). **Mass(m/z):** 421.15(M+); 422.04(M^++1, 100%).

Completed.I-4c: 5-((4(4-nitro benzylidene) amino) benzylidene)-3-(piperazine-1-ylmethyl) thiazolidine-2,4-dione. IR (*ν* cm-1): 3214(-NH Str in piperazine ring), 3015(C-H Str in Ar-H), 2917, 2845(-C-H Str in aliphatic), 2311(CSC Str in thiazoilidine ring), 1715(-C=O Str in thiazolidine ring), 1614(-NO Str in Ar-NO_2), 1576(-C=N Str in imine), 1403(-C=CH Str), 1311(-C-C Str in Ar-C), 1003(-C-N Str). **^{1}H-NMR(DMSO)** ***δ*** 11.5632(s, 1H, -NH in piperazine), 9.5984(s, 1H, Imine proton), 8.9043(s, 1H, Benzylidene proton), 7.9987-7.9032(d, 2H, Ar-H), 7.7998-7.7904(d, 2H, Ar-H), 7.5998-7.5897(d, 2H, Ar-H), 7.5694-7.5478(d, 2H, Ar-H), 4.3625(s, 2H, -N-CH_2-N proton), 2.8321(t, 4H, morpholine), 2.2453(t, 4H, morpholine protons). **Mass(m/z):** 451.23(M^+); 452.31(M^++1, 100%).

Completed.I-4d: 5- ((4(4-chloro benzylidene) amino) benzylidene) -3-(morpholine-1-yl methyl) thiazolidine-2,4-dione. IR (*ν* cm-1): 3214(-NH Str in piperazine ring), 3015(C-H Str in Ar-H), 2917, 2845(-C-H Str in aliphatic), 2311(CSC Str in thiazoilidine ring), 1715(-C=O Str in thiazolidine ring), 1614(-NO Str in Ar-NO_2), 1576(-C=N Str in imine), 1403(-C=CH Str), 1311(-C-C Str in Ar-C), 1003(-C-N Str). ^{1}H-NMR(DMSO) *δ* 11.5632(s, 1H, -NH in piperazine), 9.5984(s, 1H, Imine proton), 8.9043(s, 1H, Benzylidene proton), 7.9987-7.9032(d, 2H, Ar-H), 7.7998-7.7904(d, 2H, Ar-H), 7.5998-7.5897(d, 2H, Ar-H), 7.5694-7.5478(d, 2H, Ar-H), 4.3625(s, 2H, -N-CH_2-N proton), 2.8321(t, 4H, morpholine), 2.2453(t, 4H, morpholine protons).

Table.1. Physical Characterization of new nitrogen base Azole derivatives-I-4(a-l).

Compound	Molecular Formula	R	X	Molecular Weight (gms)	M.P (^{0}C)	%Yield
I-4a	$C_{22}H_{21}N_3O_3S$	H	O	407.13	187-189	86
I-4b	$C_{23}H_{23}N_2O_3S$	-CH_3	O	421.15	163-165	78
I-4c	$C_{22}H_{21}N_5O_4S$	-NO_2	-NH	451.13	155-157	80
I-4d	$C_{22}H_{20}N_3O_3SCl$	-Cl	O	441.09	201-203	78
I-4e	$C_{22}H_{20}N_4O_5S$	-NO_2	O	452.12	139-141	81
I-4f	$C_{23}H_{25}N_3O_4S$	-OCH_3	O	437.4	147-149	84
I-4g	$C_{23}H_{24}N_4O_3S$	-OCH_3	NH	436.16	167-169	82
I-4h	$C_{23}H_{24}N_4O_3S$	-CH_3	NH	436.16	208-210	76
I-4i	$C_{22}H_{21}ClN_4O_2S$	-Cl	NH	440.11	177-179	86
I-4j	$C_{22}H_{22}N_4O_2S$	-H	NH	406.15	151-153	81
I-4k	$C_{22}H_{22}N_4O_3S$	-HO	NH	422.14	191-193	79
I-4l	$C_{22}H_{21}N_3O_4S$	-HO	O	423.13	219-221	82

Completed.4g: 5-((4(4-methoxy benzylidene) amino) benzylidene)-3-(piperazine-1-ylmethyl) thiazolidine-2,4-dione. IR (*ν* cm-1): 3026(C-H Str in Ar-H), 2954, 28769, 2748(-C-H Str in aliphatic), 2342(CSC Str in thiazoilidine ring), 1709(-C=O Str in thiazolidine ring), 1615(-C=N Str in imine), 1538(-C=CH Str), 1410(-C-C Str in Ar-C), 1048(-C-N Str). ^{1}H-NMR(DMSO) *δ* 12.0192(s, 1H, -NH in piperazine), 9.4021(s, 1H, Imine proton), 8.6932(s, 1H, Benzylidene proton), 8.0276-8.0012(d, 2H, Ar-H), 7.9043-7.8994(d, 2H, Ar-H), 7.7032-7.7002(d, 2H, Ar-H), 7.4856-7.4102(d, 2H, Ar-H), 4.8532(s, 2H, -N-CH_2-N proton), 3.6923(s, 3H, Ar-OCH_3), 2.7320(t, 4H, morpholine), 2.5643(t, 4H, morpholine protons). **Mass(m/z):** 436.16(M+); 437.03(M^++1, 100%).

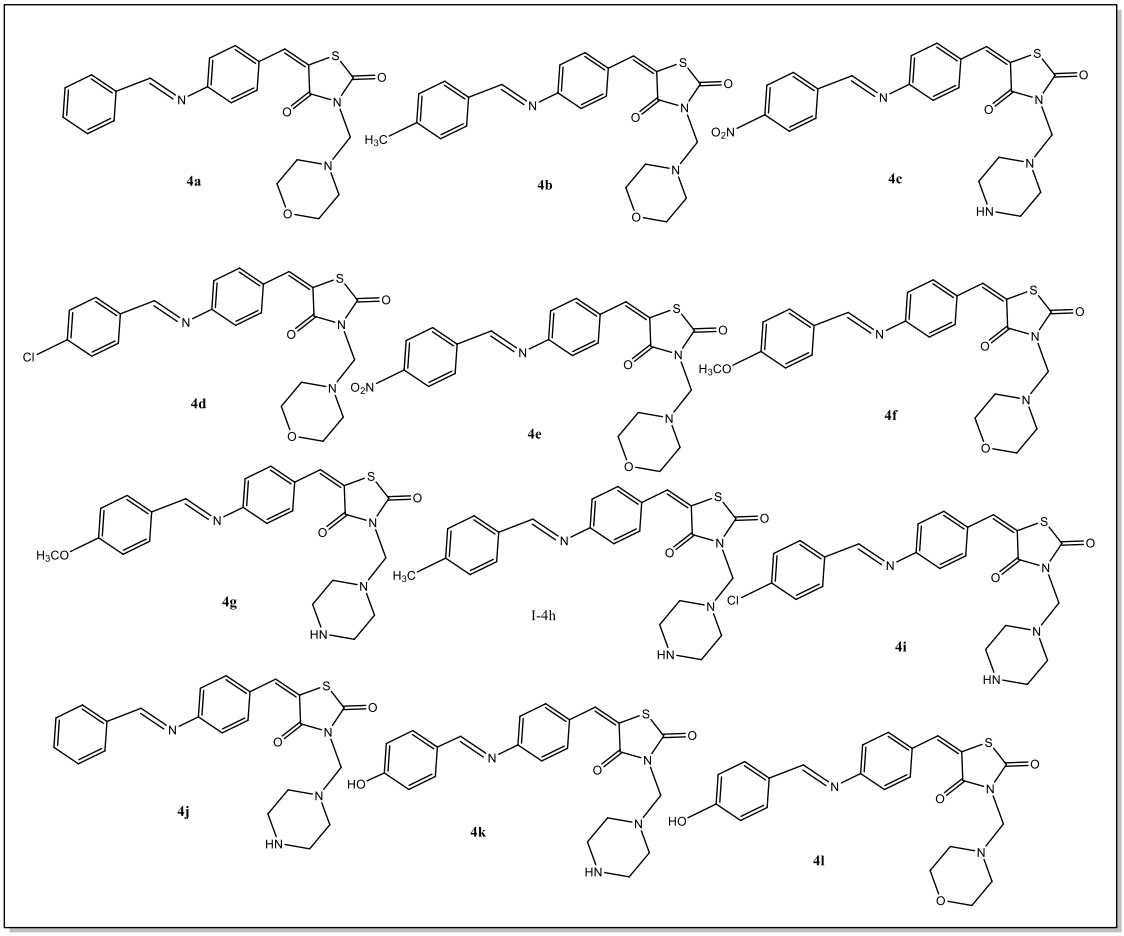


Figure.2: All the synthesized new nitrogen-based Azole derivatives-I-4(a-l)

Completed.I-4e: 5-((4(4-nitro benzylidene) amino) benzylidene)-3-(morpholine-1-ylmethyl) thiazolidine-2,4-dione. IR (ν cm-1): 3214(-NH Str in piperazine ring), 3015(C-H Str in Ar-H), 2917, 2845(-C-H Str in aliphatic), 2311(CSC Str in thiazoilidine ring), 1715(-C=O Str in thiazolidine ring), 1614(-NO Str in Ar-NO_2), 1576(-C=N Str in imine), 1403(-C=CH Str), 1311(-C-C Str in Ar-C), 1003(-C-N Str). ^{1}H-NMR(DMSO) δ 11.5632(s, 1H, -NH in piperazine), 9.5984(s, 1H, Imine proton), 8.9043(s, 1H, Benzylidene proton), 7.9987-7.9032(d, 2H, Ar-H), 7.7998-7.7904(d, 2H, Ar-H), 7.5998-7.5897(d, 2H, Ar-H), 7.5694-7.5478(d, 2H, Ar-H), 4.3625(s, 2H, -N-CH_2-N proton), 2.8321(t, 4H, morpholine), 2.2453(t, 4H, morpholine protons).

Completed.4f: 5-((4(4-methoxy benzylidene) amino) benzylidene)-3-(morpholine-1-ylmethyl) thiazolidine-2,4-dione. IR (ν cm-1): 3004(C-H Str in Ar-H), 2976, 2875, 2756(-C-H Str in aliphatic), 2326(CSC Str in thiazoilidine ring), 1713(-C=O Str in thiazolidine ring), 1608(-C=N Str in imine), 1569(-C=CH Str), 1421(-C-C Str in Ar-C), 1057(-C-N Str). ^{1}H-NMR(DMSO) δ 9.3874(s, 1H, Imine proton), 8.6854(s, 1H, Benzylidene proton), 7.9845-7.8944(d, 2H, Ar-H), 7.5867-7.5125(d, 2H, Ar-H), 7.4876-7.4312(d, 2H, Ar-H), 7.3984-7.3193(d, 2H, Ar-H), 4.5986(s, 2H, -N-CH_2-N proton), 3.8603(s, 3H, Ar-OCH_3), 2.8213(t, 4H, morpholine), 2.2854(t, 4H, morpholine protons). **Mass(m/z):** 437.14(M+); 438.05(M^++1, 100%).

Completed.4h: 5-((4(4-methylbenzylidene) amino) benzylidene)-3-(piperazine-1-ylmethyl) thiazolidine-2,4-dione. IR (ν cm-1): 3103(C-H Str in Ar-H), 2978, 2883, 2792(-C-H Str in aliphatic), 2302(CSC Str in thiazoilidine ring), 1712(-C=O Str in thiazolidine ring), 1618(-C=N Str in imine), 1529(-C=CH Str), 1421(-C-C Str in Ar-C), 1055(-C-N Str). ^{1}H-NMR(DMSO) δ 11.8973(s, 1H, -NH in piperazine), 9.5892(s, 1H, Imine proton), 8.8032(s, 1H, Benzylidene proton), 7.9984-7.9134(d, 2H, Ar-H), 7.7845-7.7612(d, 2H, Ar-H), 7.5894-7.5213(d, 2H, Ar-H), 7.3823-7.2993(d, 2H, Ar-H), 4.7435(s, 2H, -N-CH_2-N proton), 2.9432(t, 4H, morpholine), 2.4323(t, 4H, morpholine protons), 1.9873(s, 3H, Ar-CH_3). **Mass(m/z):** 420.16(M+); 421.21(M^++1, 100%).

Completed.4i: 5-((4(4-chlorobenzylidene) amino) benzylidene)-3-(piperazine-1-ylmethyl) thiazolidine-2,4-dione. IR (ν cm-1): 3210(C-H Str in Ar-H), 2988, 2856, 2745(-C-H Str in aliphatic), 2310(CSC Str in thiazoilidine ring), 1723(-C=O Str in thiazolidine ring), 1620(-C=N Str in imine), 1548(-

C=CH Str), 1435(-C-C Str in Ar-C), 1084(-C-N Str), 812(-Cl, Str in Ar-Cl). **^{1}H-NMR(DMSO):** δ 12.0192(s, 1H, -NH in piperazine), 9.6023(s, 1H, Imine proton), 8.8564(s, 1H, Benzylidene proton), 7.8945-7.7903(d, 2H, Ar-H), 7.6843-7.5874(d, 2H, Ar-H), 7.4302-7.4021(d, 2H, Ar-H), 7.398-7.3023(d, 2H, Ar-H), 4.6843(s, 2H, -N-CH_2-N proton), 2.8794(t, 4H, morpholine), 2.4564(t, 4H, morpholine protons). **Mass(m/z):** 440.11(M+); 441.04(M^++1, 100%), 442.12(M^++2, 30%).

Completed.4j: 5-((4(benzylidene) amino) benzylidene)-3-(piperazine-1-ylmethyl) thiazolidine-2,4-dione. IR (ν cm-1): 3095(C-H Str in Ar-H), 2967, 2843(-C-H Str in aliphatic), 2367(CSC Str in thiazoilidine ring), 1703(-C=O Str in thiazolidine ring), 1605(-C=N Str in imine), 1593(-C=CH Str), 1406(-C-C Str in Ar-C), 1056(-C-N Str). **^{1}H-NMR(DMSO)**: δ 11.8453(s, 1H, -NH in piperazine), 9.6854(s, 1H, Imine proton), 8.7943(s, 1H, Benzylidene proton), 7.789-7.6897(d, 2H, Ar-H), 7.5986-7.5435(d, 2H, Ar-H), 7.3984-7.3021(d, 2H, Ar-H), 7.2803-7.2091(t, 3H, Ar-H), 4.5102(s, 2H, -N-CH_2-N proton), 2.8034(t, 4H, morpholine), 2.1092(t, 4H, morpholine protons). **Mass(m/z):** 406.15(M+); 407.16(M^++1, 100%).

Completed.4k: 5-((4(4-hydroxybenzylidene) amino) benzylidene)-3-(piperazine-1-ylmethyl) thiazolidine-2,4-dione. IR (ν cm-1): 3576(-OH Str in Ar-OH); 3246(-NH Str in piperazine ring); 3025(C-H Str in Ar-H), 2903, 2876(-C-H Str in aliphatic), 22984(CSC Str in thiazoilidine ring), 1713(-C=O Str in thiazolidine ring), 1612(-C=N Str in imine), 1532(-C=CH Str), 1428(-C-C Str in Ar-C), 1087(-C-N Str). **^{1}H-NMR(DMSO):** δ 12.8763(s, 1H, Ar-OH); 12.1032(s, 1H, -NH in piperazine), 9.8432(s, 1H, Imine proton), 8.9954(s, 1H, Benzylidene proton), 8.1053-8.0453(d, 2H, Ar-H), 7.9543-7.9102(d, 2H, Ar-H), 7.5432-7.4092(d, 2H, Ar-H), 7.4653-7.4102(d, 2H, Ar-H), 4.8623(s, 2H, -N-CH_2-N proton), 2.9574(t, 4H, morpholine), 2.6432(t, 4H, morpholine protons). **Mass(m/z):** 422.14(M+); 423.21(M^++1, 100%).

Completed.4l: 5-((4(4-hydroxybenzylidene) amino) benzylidene)-3-(piperazine-1-ylmethyl) thiazolidine-2,4-dione. IR (ν cm-1): 3562(-OH Str in Ar-OH); 3286(-NH Str in piperazine ring), 3087(C-H Str in Ar-H); 2903, 2789(-C-H Str in aliphatic); 2316(CSC Str in thiazoilidine ring), 1709(-C=O Str in thiazolidine ring); 1589(-C=N Str in imine), 1429(-C=CH Str), 1389(-C-C Str in Ar-C), 1034(-C-N Str). **^{1}H-NMR(DMSO)** δ 112.6536(s. 1H. Aromatic-OH); 11.8543(s, 1H, -NH in piperazine), 9.4975(s, 1H, Imine proton), 9.0342(s, 1H, Benzylidene proton), 7.9854-7.9102(d, 2H, Ar-H), 7.8453-7.7843(d, 2H, Ar-H), 76978-7.6213(d, 2H, Ar-H), 7.3423-7.3013(d, 2H, Ar-H), 4.8241(s, 2H, -N-CH_2-N proton), 3.0232(t, 4H, morpholine), 2.5323(t, 4H, morpholine protons). **Mass(m/z):** 423.13(M^+); 424.15(M^++1, 100%).

Pharmacological activity

Antibacterial activity [14]: The antibacterial activity of the extensively synthesis of new nitrogen-based Azole derivatives was tested using the disk plate (Agar diffusion) method (Scheme-I, I-3a-3l). Gram-positive bacteria such as *Staphylococcus aureus, Bacillus subtilis,* and Gram-negative *Escherichia coli and Salmonella paratyphi* have been employed as test organisms to evaluate this antibacterial activity. This method consisted of coating the Petridishes with inoculated fluid agar medium to a constant thickness. Core borer was utilized to create the bores, which were then filled with test and standard drugs (Streptomycin) and inoculated at 37±1^{o}C for a single hour. The drug will diffuse in to the agar medium are prevents the growth of microbes and produce a clear zone of inhibition Table.2.

Anticancer Activity [15-16]: MTT assay was employed on human breast cancer celles-MCF-7 to determine the potential anticancer activity of new nitrogen-based Azole derivatives. This invitro assay quantifies cell viability and proliferation, providing a measure of the compound's impact. The experiment involved testing six different concentrations of each compound, repeated three time for reliability. Doxorubicin was used as a standard for comparison, and the resulting cytotoxic data is summarized in Table-3.

Molecular Docking Studies [17-18]. The current study incorporates the use of the insilico molecular modelling tool Autodock Vina. The receptor grid that was generated will helps in locating the protein active site and preparing the grid for the ligands to be docked in the shape and properties of the receptor are represented on a grid by many different sets of fields that provide progressively more precise scoring of the ligand poses. The binding energies of mentioned analogs, further clarify the design of potential drug candidates against EGFR Protein. The three-dimensional (3D) structure of Epidermal Growth Factor Receptor tyrosine kinase (PDB ID: 1M17) was downloaded from Brook heaven Protein Data Bank (https://www.rcsb.org) and saved as a Brookhaven protein data bank file and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valences, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using AUTODOCK suite of MGL Tools.

RESULTS AND DISCUSION.

Chemistry: The new nitrogen-based Azole derivatives are developed by a four-step process

under conventional method. The yield of the synthesized compound was found to be in the range from **78-86%** (Scheme-I). The present work is based on Mannich, Schiff's base, cyclisation and Knoevenagel reactions which involve thiazolidine-2,4-di one with 4-amino benzaldehyde to give compound 2a. Then it reacts with substituted benzaldehydes via Schiff's base to form I-3(a-3) compounds, followed by Mannich base reaction with morpholine and pipezine to get title compounds Scheme-I (4a-4l). All are showing satisfactory analysis for the proposed structures, and which were confirmed on the basis of their FT-IR, LC-MASS and 1H NMR spectral data.

Spectral data: All are showing 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. The Ar-Cl stretching is showing the strong absorption in the region 784-824 cm-1 and few compounds containing -NO_2 group shows peaks due to stretching of –NO_2 group is observed at around 1602-1638 cm-1, -SO stretching absoreved around 1278-1363cm-1 respectively. Similarly, the 1HNMR (DMSO-d6) spectra of azole derivatives are showed a singlet at 10.00-12.056 for -NH in proton in piperazine ring. Some of the compounds are showing a single at 12.76-12.87 for Ar-OH protons and singlet at 8.70-9.2 All these compounds have aromatic protons were found between δ 8.209-6.896 ppm as singlet, doublet and triplet protons. All the methoxy and methyl protons were showing as singlet at 3.598-3.986 and 1.9987-2.3856 respectively.

Antibacterial activity: The antibacterial activity of all the synthesized compounds **(I-4(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, Klebsiella pneumonia* (Gram negative). Most of the synthesized compounds showed significant antibacterial activity. The compound **(Scheme-I**) **I-4b**, **I-4h**, and **I-4l**-are showing most potent activity against all four microorganisms.

Table.2. Antibacterial activity of Compounds I-4(a-l).

Compound	Zone of Inhibition (in mm)			
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumoniae***
Streptomycin	**33**	**31**	**30**	**32**
I-4a	12	13	10	14
I-4b	**25**	**22**	**24**	**23**
I-4c	14	10	13	10
I-4d	12	16	17	10
I-4e	10	17	12	11
I-4f	20	19	15	14
I-4g	15	9	13	9
I-4h	**22**	**24**	**20**	**23**
I-4i	9	14	11	17
I-4j	14	10	9	9
I-4k	12	9	10	14
I-4l	**27**	**24**	**25**	**23**

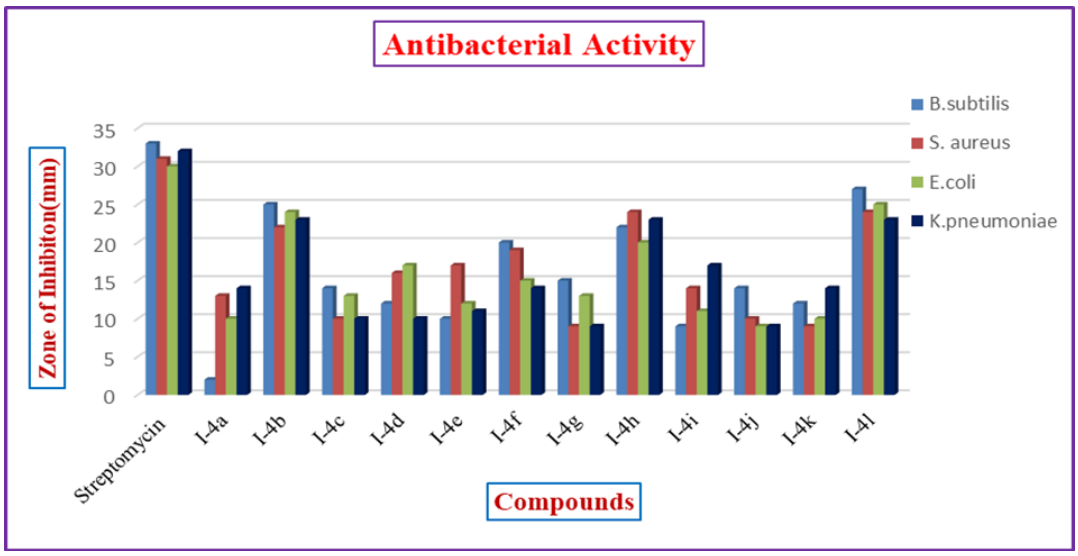


Figure.3. Graphical representation of antibacterial activity of compounds: I-4(a-l)- Zone of Inhibition (in mm).

Anticancer activity: Nitrogen based new Azole compounds were screened for cytotoxic activity against one cancer cell like human breast cancer cells (MCF-7) by using MTT assay method, with doxorubicin as a standard drug. All the results proposed that MCF-7 cell lines were susceptible to the evaluated compounds showed IC_{50} values in the range **of 24.136± 0.012µg** to **59.125± 0.031µg** (**Scheme-I, I-4(a-l)**) against MCF7 cell line Cell line. From the results, the compounds **I-4l(24.136 ± 0.321µg/ml), I-4f(26.615±0.321µg/ml)** were showed good activity against one cell lines, whereas, remaining of the compounds showed moderate activity against MCF-7 cell lines.

Table.3. Cytotoxic Activity of Compounds: 4a-4l on MCF-7 Cell line

Compounds	MCF-7 Cell line (IC_{50})
I-4a	50.352± 0.043
I-4c	59.125± 0.031
I-4f	**26.615± 0.001***
I-4h	39.005± 0.032
I-4j	54.268± 0.312
I-4l	**24.136± 0.012***
Doxorubicin	**12.586± 0.010**

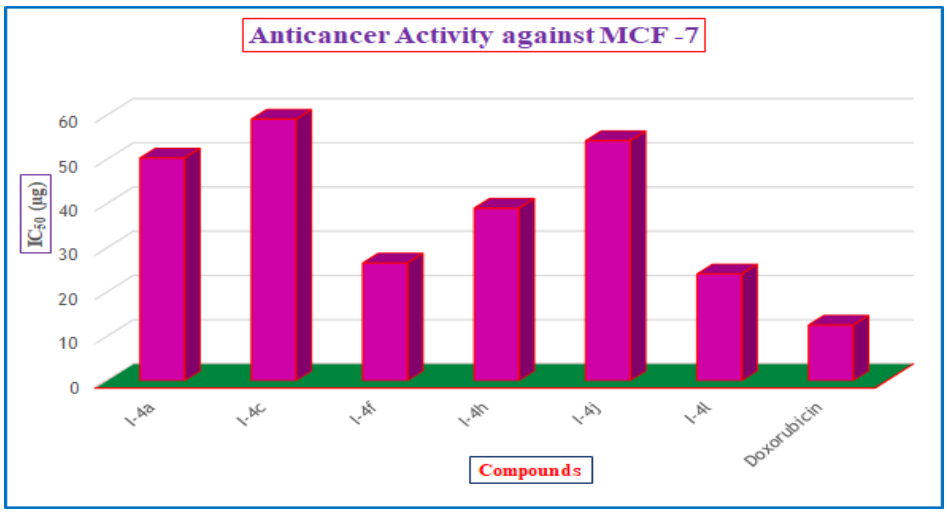


Figure.4. Graphical representation of Anticancer activity compounds: I-4(a-l)-MTT assay method.

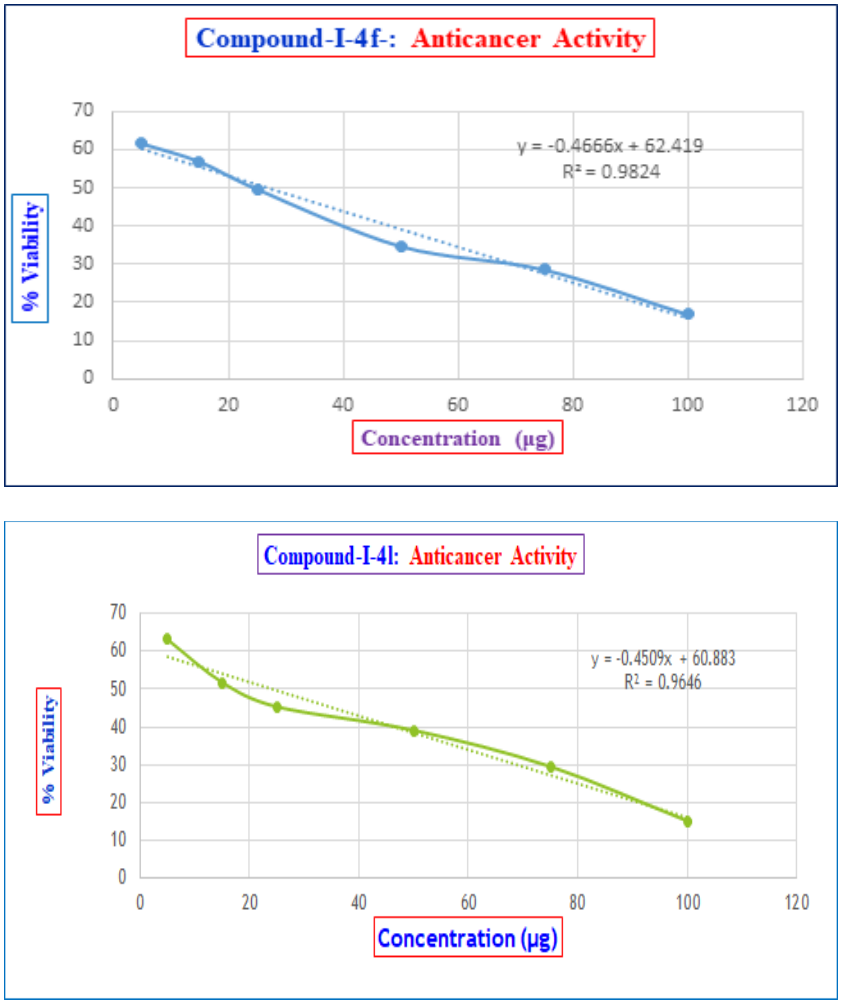


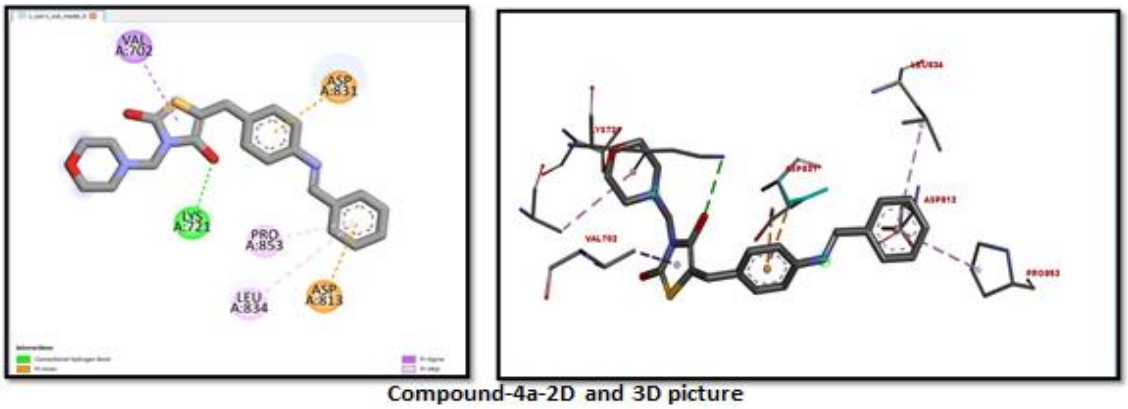
Figure.5. Linear graph representation of nitrogen based new Azole derivatives (I-4f and I-4l)-IC50 values.

Molecular docking studies: Most of the compounds **I-4f, I-4h (Scheme-I)** possess with hydrogen bonds each and have showed good interactions with amino acids. Glide dock score of the dataset ligand were showed in Table.4 along with the interaction amino acids. Among the docked compounds **I-4a, I-4f, I-4h** & and **I-4i** reported the lowest binding energy between **-8.1 to -9.1 Kcal/mol**. Compounds **I-4f** & and **I-4h** possess two hydrogen bonds each. Compounds **I-4a, & I-4i** possess one hydrogen bond each. **I-4b** had no hydrogen bond interactions.

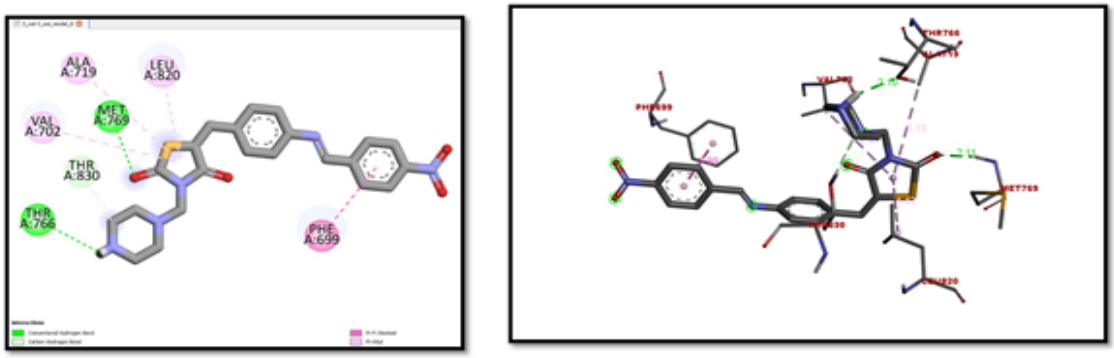
Table.4. Insilco EGFR inhibition of nitrogen based new Azole compounds (I-4(a-l))-Glide dock sores of the dataset ligands

Compound No	Binding Energy (Kcal/mol)	No of H-bonds	Interacting amino acids	H-bond lengths (Å)
I-4a	-8.8	1	VAL:702, LYS:721, ASP: 813, ASP:831, PRO: 853, LEU:834	2.14
I-4b	-7.8	Nil	VAL:702, ALA:719, GLU: 734, GLU: 738, ASP: 813, LEU:820, ASP:831	Nil
I-4c	-8.2	1	ALA:719, GLU: 734, ASP: 813, LEU:820, ASP:831	2.5
I-4d	-8.1	Nil	GLU: 734, GLU: 738, ASP: 813, LEU:820, ASP:831	Nil
I-4e	-8.2	1	VAL:702, ALA:719, GLU: 738, ASP: 813, LEU:820	2.32

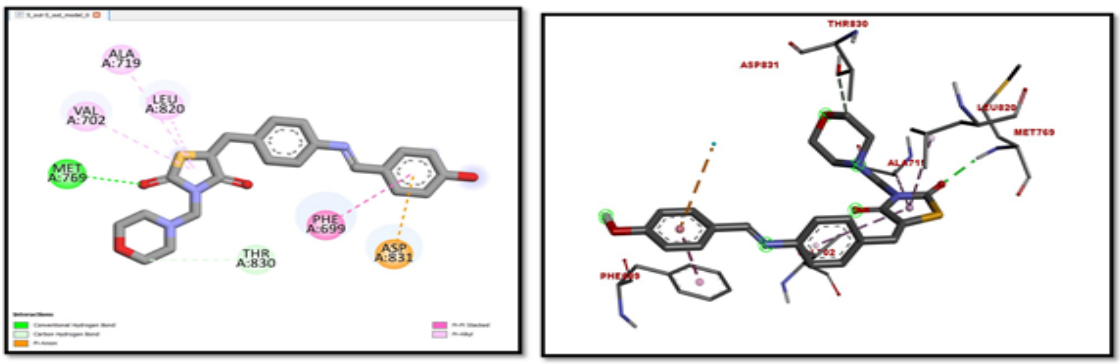
I-4f	-9.1	2	PHE: 699, VAL:702, ALA:719, THR:766, MET:769, THR:830, LEU:820	2.10, 2.11
I-4g	-7.9	Nil	VAL:702, ALA:719, THR:766, MET:769, LEU:820	2.31
I-4h	-8.1	2	PHE: 699, VAL:702, ALA:719, LYS: 721, MET:742, THR:766, ASP:831	2.34, 2.47
I-4i	-8.1	1	VAL:702, ALA:719, THR:766, MET:769, THR:830	2.32
I-4j	-8.6	Nil	LYS:721, ASP: 813, ASP:831, PRO: 853, LEU:834	2.1
I-4k	-8.0	1	GLU: 734, GLU: 738, ASP: 813, LEU:820	2.12
I-4l	-8.9	1	PHE: 699, VAL:702, ALA:719, ASP: 831, MET:769, THR:830, LEU:820	2.14



Compound-4a-2D and 3D picture



Compound:I-4f-2D and 3D picture



Compound:I-4l-2D and 3D picture

Figure.6. Molecular docking poses between ligand with targe-2D and 3D picture

CONCLUSION:

In this study, we reported synthesis, structural and biological activity of a series of nitrogen based new azole derivatives. All the derivatives were characterized by physical and spectral data and the % yield was to be between 78-86% (Scheme-I)**.** All the compounds revealed significant antibacterial, anticancer activities. Most of the Docking score of the synthesized nitrogen based new Azole derivatives were ranged between -8.1 to -9.1 Kcal/mol. Compounds I-4f & and I-4h possess two hydrogen bonds each. Compounds I-4a, & I-4i possess one hydrogen bond each. I-4b had no hydrogen bond interactions.

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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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