NITROGEN BASED INDOLE-2-ONE SCHIFF'S BASES CONTAINING PYRIMIDINE-2,3-DIONE MOIETY: DESIGN, SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL EVALUATION AND MOLECULAR DOCKING STUDIES.

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Abstract:
A novel analog of some pyrimidine containing nitrogen based Indole-2-one Schiff's bases II-4(a-j) were synthesized by n-alkylation/benzylation with substituted Istains and followed by Schiff's base mechanism with 5-aminopyrimidine-2,4-dione. These synthesized derivatives gave a good yield between 75-86% and all structures were confirmed by FTIR, ^{1}HNMR and Mass spectroscopy. The antibacterial activity of different synthesized nitrogen based indole-2-one Schiff's bases has been assessed with zone of inhibition by well agar diffusion method, which has shown good activity. Compound **II-4f, II-4h** and **II-4j** showed good antibacterial activity against gram positive (*Staphylococcus aureus, Bacillus subtilis*) and negative bacteria (*Escherichia coli, Salmonella paratyphi*) with comparison of streptomycin as standard drug. The synthesized molecules were subjected to Molecular docking studies with AUTODOCK VINA suite of MGL tools by using Fgb1 receptor with PDB ID: 3K4P and the compound **II-4f (-9.3**), **II-4h (-9.3)** and **II-4j(-9.2)** were reported highest docking ligands.The docking score of the ligands ranged from -7.2 (compound II-4d) to -9.2(Compound II-4f).

Keywords: Substituted Isatins, 5-amino-Pyrimidine-2,4-dione, Molecular Docking, Antimicrobial activity, Streptomycin.

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INTRODUCTION:

Indole, Pyrimidine based heterocyclic compounds are well-known biologically active nitrogen containing heterocyclic compound [1]. In recent years' researchers are much interested in the synthesis of Indole and pyrimidine analogues. Indole derivatives posse fungicidal, herbicidal, antidepressant, and antitumor properties. Synthetically prepared amino pyrimidine derivatives display a wide range of biological activities [2-4] such as antibacterial, antitumor, and antiviral [5]. Therefore, the substituted Indole-2-one structure can be found in diverse clinically approved drugs. Interestingly, a substituted indole-2-one with pyrimidine moiety was also suggested to account for the antibacterial activity.

Recently, the incidence of systemic bacterial infection has become an important complication and a significant cause of disorder and fatality in immune-compromised individuals such as patients going through anticancer chemotherapy or organ transplants. In recent therapeutic chemistry and drug designing, Nitrogen based heterocyclic compounds were becoming the first choice for researchers and scientists because of its potential biological activity [6]. Therefore, it becomes an interesting impression for medicinal chemistry researchers. Most of the types of scaffolds are known for their multiple beneficial uses such as their anti-inflammatory, antibacterial, antifungal, antioxidant, antimalarial, anticancer and antiparastitic[7-9]. The identification of lead compounds showing pharmacological activity against a biological target and the progressive optimization of the pharmacological properties and potency of these compounds are the focal points of early-stage drug discovery. To this end, the pharmaceutical industry has adopted the experimental screening of large libraries of chemicals against a therapeutically-relevant target (highthroughput screening or HTS) as a means to identify new lead compounds.

Indole and its derivatives are an important class of heterocyclic compounds that exhibiting biological and pharmacological properties[10-11]. Also, Schiff's base heterocyclic compounds are active compounds against both bacterial infections. So, according to this survey and in continuation of our heterocyclic synthesis of novel active compounds against some bacterial cultures, we aim to synthesize novel nitrogen based Indole-2-one Schiff's bases with pyrimidine as promising antimicrobial agents towards gram positive and gram negative strains.

MATERIALS AND METHOD:

All the chemicals, reagents and solvents used for the synthesis were obtained from TCI, SD Fine and Hychem Laboratories. The melting point of all synthesized compounds was determined on the Thieles tube apparatus. The synthesized derivatives are physical characterized by TLC methods by using silica gel plates. It is carried out by using mobile phase n-hexane: ethyl acetate (7:3). Then analytical techniques were performed by Spectral analysis like FTIR spectroscopy (Shimadzu), ^{1}HNMR spectroscopy (300MHZ) solvent DMSO-d6, Mass spectrometry (Shimadzu). Finally, Molecular docking studies were carried out by using Auto dock software.

General Procedures:

Step: I. Synthesis of nitrosoacetanilides from substituted anilines: 9 gm of Chloral hydrate was taken into the round bottom flask and dissolved in 120 ml water. To that 13 gm of sodium sulphate, a solution of 5.4 gm of substituted aniline in 30 ml of water containing 5.12 gm of concentrated hydrochloric acid (4.34 ml) to dissolve the amine and solution of 11 gm of hydroxylamine hydrochloride in 50 ml of water were added. Flask was then heated vigorously until the reaction was completed. After it, the solution containing beaker was cooled in running water followed by the filtration of reminder crystallized product with suction pump and air dried

Step: II: Step 2: Synthesis of substituted Isatins (2a-2e) from nitrosoacetanilides: 18.4 gm of concentrated sulphuric acid (10.0 ml) was warmed to 50^{0}C and 2.5 gm of dry nitrosoacetanilide was added in such a rate so as to keep the temperature between 60-70^{0}C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound was finished. The solution was heated to 80^{0}C and kept at this temperature for about 10 min to complete the reaction. Then the reaction mixture was cooled to room temperature and poured it into ten times its volume of cracked ice. After standing for 90 mint, the final product was filtered with suction pump followed by washing with cold water to remove sulphuric acid and dried in air.

Step-III: Synthesis of N-substituted-5-substituted-1,2-3H-indol-dione (3a-3e).

The compound (2a-2e, 0.01mol) was taken in a mixture of 6-amino-pyrimidine-2,3-dione (0.01 mole), glacial acetic acid(2-5ml) and ethanol 30ml in round bottom flask. Then the reaction mixture

was refluxed for 2-3hrs. The progress of the reaction was monitored by TLC (n-hexane: Ethyl acetate) (8:2). The reaction mixture was cooled to room temperature. A solid was obtained, which was filtered off, washed with hexane and recrystallized from methanol to give crystalline solid.

Step: IV. Synthesis of 6-[(Z)-(1-substituted-5-substituted-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione [II-4(a-j)]. In the round bottomed flask take N-substituted-5-substituted-1,2-3H-indol-dione (3a-3e) (3.37mM) and equimolar quantity of benzyl/Methyl chloride (3.7mM), mix with 20ml of DMF and to this mixture add 2gm of K_2CO_3. After gentle mixing of this reaction mixture, reflux for 2 hr, cool and pour to 100 ml of ice cold water. The resultant orange red ppt. collected wash with water and dried and recrystallized from acetonitrile.

Compound-II-4a:6-[(Z)-(1-methyl-2-oxo-1,2-dihydro-3H-indol-3ylidene)amino]pyrimidine-2,4(1H,3H)-dione. M.P. 131-133°C; Mol. Formula: $C_{13}H_{10}N_4O_3$; % Yield: 78%; IR(vcm^{-1}): 3350(-NH *Str*, Pyrimidine-2,3-dione); 3052(CH *Str*, Aromatic), 2920(-CH Str, Alphatic), 1707(-CO *Str*, Indole), 1696(-CO *Str*, Pyrimidine-2,3-dione), 1616(-C=N *Str*), 1512(-C=CH *Str*), 1425(-C=C *Str*),1063(-C-N *Str*). ^{1}H-NMR(DMSO) δ ppm: 11.0532, 10.8632(2H, s, -NH protons in Pyrimidine ring), 8.0654(1H, s, pyrimidine proton), 7.8984-7.8743(2H, d, Indole Ar-H), 7.6003-7.5875(2H, t, Indole Ar-H), 2.2034(3H, s, Ar-CH_3). Mass (LC-MS): m/z 270.08(M), 271.19(M+1, 100%).

Compound.II-4b:6-[(Z)-(1-methyl-4-chloro-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione. . M.P. 166-168°C; Mol.Formula: $C_{13}H_9ClN_4O_3$; % Yield: 76%; IR(vcm^{-1}): 3408(-NH *Str*, Pyrimidine-2,3-dione); 3024(CH *Str*, Aromatic), 2930, 2873(-CH Str, Alphatic), 1717(-CO *Str*, Indole), 1694(-CO *Str*, Pyrimidine-2,3-dione), 1614(-C=N *Str*), 1579(-C=CH *Str*), 1436(-C=C *Str*), 1029(-C-N *Str*), 794(-Cl, *Str*, Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 11.2546, 10.7803(2H, s, -NH protons in Pyrimidine ring), 8.2095(1H, s, pyrimidine proton), 7.8894(1H, s, Indole Ar-H), 7.6987-7.6032(2H, d, Indole Ar-H), 2.0972(3H, s, Ar-CH_3). Mass (LC-MS): m/z 304.04(M), 305.14(M+1, 100%), 306.32(M+2, 30%).

Compound-II-4c:6-[(Z)-(15-dimethyl-2-oxo-1,2-dihydro-3H-indol-3ylidene)amino]pyrimidine-2,4(1H, 3H)-dione. M.P. 206-208°C; Mol.Formula: $C_{14}H_{12}N_4O_3$; % Yield: 84%; IR(vcm^{-1}): 3313(-NH *Str*, Pyrimidine-2,3-dione); 3078(CH *Str*, Aromatic), 2989, 2730(-CH Str, Alphatic), 1716(-CO *Str*, Indole), 1688(-CO *Str*, Pyrimidine-2,3-dione), 1637(-C=N *Str*), 1573(-C=CH *Str*), 1431(-C=C *Str*), 1031(-C-N *Str*). ^{1}H-NMR(DMSO) δ ppm: 12.0654, 11.0142(2H, s, -NH protons in Pyrimidine ring), 8.2898(1H, s, pyrimidine proton), 8.0102(1H, s, Indole Ar-H), 7.8432-7.8143(2H, d, Indole Ar-H), 2.2093(3H, s, N-CH_3), 1.9921(3H, s, Ar-CH_3). Mass (LC-MS): m/z 284.09(M), 285.32(M+1, 100%).

Compound-II-4d:6-[(Z)-(1-methyl-4-fluoro-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione. M.P. 193-195°C; Mol.Formula: $C_{13}H_9FN_4O_3$; % Yield: 80%; IR(vcm^{-1}): 3286(-NH *Str*, Pyrimidine-2,3-dione); 3086(CH *Str*, Aromatic), 2989, 2776(-CH Str, Alphatic), 1712(-CO *Str*, Indole), 1701(-CO *Str*, Pyrimidine-2,3-dione), 1621(-C=N *Str*), 1543(-C=CH *Str*), 1421(-C=C *Str*), 1056(-C-N *Str*). ^{1}H-NMR(DMSO) δ ppm: 11.8530, 11.2732(2H, s, -NH protons in Pyrimidine ring), 8.1652(1H, s, pyrimidine proton), 7.9843(1H, s, Indole Ar-H), 7.6532-7.6021(2H, d, Indole Ar-H), 2.5423(3H, s, N-CH_3). Mass (LC-MS): m/z 288.07(M), 289.21(M+1, 100%), 290.03(M+1, 30%).

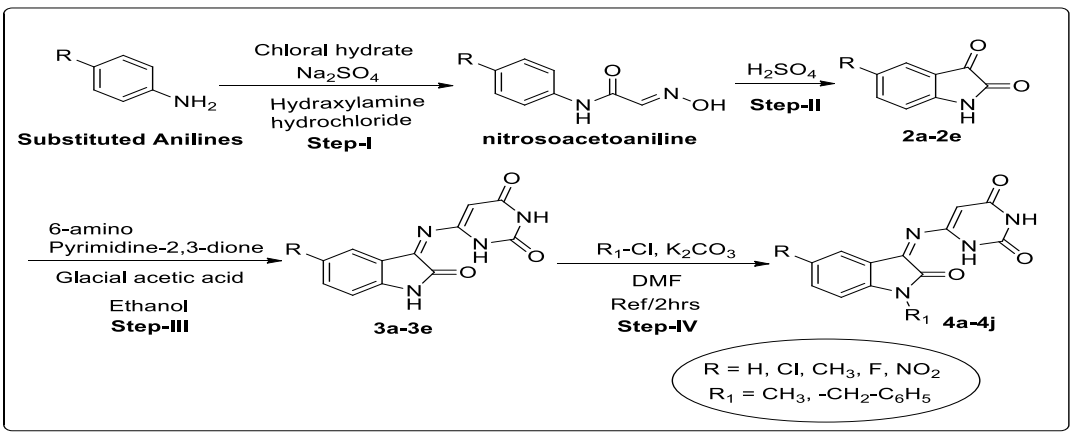


Fig.1. Scheme

Compound-II-4e:6-[(Z)-(1-methyl-4-niitro-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione. M.P. 223-225°C; Mol.Formula: $C_{13}H_9N_5O_5$; % Yield: 83%; IR(νcm^{-1}): 3388(-NH *Str*, Pyrimidine-2,3-dione); 3012(CH *Str*, Aromatic), 2933, 2830(-CH Str, Alphatic), 1712(-CO *Str*, Indole), 1692(-CO *Str*, Pyrimidine-2,3-dione), 1623(-Ar-NO_2 *Str*), 1580(-C=CH *Str*), 1417(-C=C *Str*), 1079(-C-N *Str*). ^{1}H-NMR(DMSO) δ ppm: 12.1209, 11.1254(2H, s, -NH protons in Pyrimidine ring), 8.2092(1H, s, pyrimidine proton), 8.0988(1H, s, Indole Ar-H), 7.9109-7.9035(2H, d, Indole Ar-H), 2.2083(3H, s, N-CH_3). Mass (LC-MS): m/z 315.06(M), 316.03(M+1, 100%).

Compound-II-4f:6-[(Z)-(1-benzyl-4-methyl-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione. M.P. 179-181°C; Mol.Formula: $C_{20}H_{16}N_4O_3$; % Yield: 79%; IR(νcm^{-1}): 3290(-NH *Str*, Pyrimidine-2,3-dione); 3062(CH *Str*, Aromatic), 2981, 2827(-CH Str, Alphatic), 1711(-CO *Str*, Indole), 1698(-CO *Str*, Pyrimidine-2,3-dione), 1558(-C=CH *Str*), 1436(-C=C *Str*), 1066(-C-N *Str*). ^{1}H-NMR(DMSO) δ ppm: 11.6532, 10.9874(2H, s, -NH protons in Pyrimidine ring), 8.3109(1H, s, pyrimidine proton), 8.1043(1H, s, Indole Ar-H), 7.9876-7.9034(2H, d, Indole Ar-H), 7.6998-7.6898(2H, d, Ar-H), 7.5276-7.5190(3H, t, Ar-H), 4.6533-4.6001(1H, s, -N-CH_2-), 2.0982(3H, s, Ar-CH_3). Mass (LC-MS): m/z 360.12(M), 361.21(M+1, 100%).

Compound-II-4g:6-[(Z)-(1-benzyl-4-chloro-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione. M.P. 201-203°C; Mol.Formula: $C_{19}H_{13}ClN_4O_3$; % Yield: 83%; IR(νcm^{-1}): 3398(-NH *Str*, Pyrimidine-2,3-dione); 3089(CH *Str*, Aromatic), 2989, 2878(-CH Str, Alphatic), 1715(-CO *Str*, Indole), 1703(-CO *Str*, Pyrimidine-2,3-dione), 1602(-C=CH *Str*), 1489(-C=C *Str*), 1099(-C-N *Str*), 801(-Cl, *Str*, Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 12.2973, 11.5432(2H, s, -NH protons in Pyrimidine ring), 8.2983(1H, s, pyrimidine proton), 8.0973(1H, s, Indole Ar-H), 7.8963-7.7763(2H, d, Indole Ar-H), 7.5843-7.4874(2H, d, Ar-H), 7.3845-7.2932(3H, t, Ar-H), 4.5384-4.5002(1H, s, -N-CH_2-). Mass (LC-MS): m/z 380.07(M), 381.23(M+1, 100%), 382.12(M+2, 30%).

Compound-II-4h:6-[(Z)-(1-benzyl-4-nitro-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione. M.P. 209-211°C; Mol.Formula: $C_{19}H_{13}N_5O_5$; % Yield: 78%; IR(νcm^{-1}): 3401(-NH *Str*, Pyrimidine-2,3-dione); 3036(CH *Str*, Aromatic), 2978, 2876(-CH Str, Alphatic), 1720(-CO *Str*, Indole), 1705(-CO *Str*, Pyrimidine-2,3-dione), 1643(-NO *Str*, Ar-NO_2), 1598(-C=CH *Str*), 1480(-C=C *Str*), 1120(-C-N *Str*). ^{1}H-NMR(DMSO) δ ppm: 11.8976, 11.0344(2H, s, -NH protons in Pyrimidine ring), 8.3092(1H, s, pyrimidine proton), 8.2012(1H, s, Indole Ar-H), 7.9873-7.8653(2H, d, Indole Ar-H), 7.6693-7.5632(2H, d, Ar-H), 7.4775-7.3982(3H, t, Ar-H), 4.8032-4.7832(2H, s, -N-CH_2-). Mass (LC-MS): m/z 391.09(M), 392.32(M+1, 100%).

Compound-II-4i:6-[(Z)-(1-benzyl-2-oxo-1,2-dihydro-3H-indol-3ylidene)amino] pyrimidine-2,4(1H,3H)-dione. M.P. 243-245°C; Mol. Formula: $C_{19}H_{14}N_4O_3$; % Yield: 84%; IR(νcm^{-1}): 3340(-NH *Str*, Pyrimidine-2,3-dione); 3087(CH *Str*, Aromatic), 2999, 2890(-CH Str, Alphatic), 1716(-CO *Str*, Indole), 1698(-CO *Str*, Pyrimidine-2,3-dione), 1601(-C=CH *Str*), 1493(-C=C *Str*), 1098(-C-N *Str*). ^{1}H-NMR(DMSO) δ ppm: 11.7863, 10.8973(2H, s, -NH protons in Pyrimidine ring), 8.2093(1H, s, pyrimidine proton), 8.1023-8.0022(2H, d, Indole Ar-H), 7.7874-7.7323(2H, t, Indole Ar-H), 7.5643-7.5032(2H, d, Ar-H), 7.3764-7.3453(3H, t, Ar-H), 4.6543-4.5102(2H, s, -N-CH_2-). Mass (LC-MS): m/z 346.11(M), 347.21(M+1, 100%).

Compound-II-4j:6-[(Z)-(1-benzyl-4-fluoro-2-oxo-1,2-dihydro-3H-indol-3ylidene) amino] pyrimidine-2,4(1H,3H)-dione. M.P. 249-251°C; Mol.Formula: $C_{19}H_{13}FN_4O_3$; % Yield: 79%; IR(νcm^{-1}): 3372(-NH *Str*, Pyrimidine-2,3-dione); 3054(CH *Str*, Aromatic), 2976, 2882(-CH Str, Alphatic), 1709(-CO *Str*, Indole), 1702(-CO *Str*, Pyrimidine-2,3-dione), 1612(-C=CH *Str*), 1486(-C=C *Str*), 1103(-C-N *Str*), 815(-F *Str*, Ar-F).. ^{1}H-NMR(DMSO) δ ppm: 12.2763, 11.5643(2H, s, -NH protons in Pyrimidine ring), 8.1982(1H, s, pyrimidine proton), 8.0943(1H, s, Indole Ar-H), 7.9843-7.8964(2H, d, Indole Ar-H), 7.6754-7.6102(2H, d, Ar-H), 7.2035-7.1872(3H, t, Ar-H), 4.7833-4.7102(2H, s, -N-CH_2-). Mass (LC-MS): m/z 364.10(M), 365.22(M+1, 100%), 366.34(M+2, 30%).

Physical properties II(4a-4j)

Compounds	Molecular Formula	R	R_1	Molecular weight(gm)	M.P(^{0}C)	%Yield
II-4a	$C_{13}H_{10}N_4O_3$	H	CH_3	270.08	131-133	78
II-4b	$C_{13}H_9ClN_4O_3$	Cl	CH_3	304.04	166-168	76
II-4c	$C_{14}H_{12}N_4O_3$	CH_3	CH_3	284.09	206-208	84
II-4d	$C_{13}H_9FN_4O_3$	F	CH_3	288.07	193-195	80
II-4e	$C_{13}H_9N_5O_5$	NO_2	CH_3	315.06	223-225	83
II-4f	$C_{20}H_{16}N_4O_3$	CH_3	$-CH_2-C_6H_5$	360.12	179-181	79
II-4g	$C_{19}H_{13}ClN_4O_3$	Cl	$-CH_2-C_6H_5$	380.07	201-203	83
II-4h	$C_{19}H_{13}N_5O_5$	NO_2	$-CH_2-C_6H_5$	391.09	209-211	77
II-4i	$C_{19}H_{14}N_4O_3$	H	$-CH_2-C_6H_5$	346.11	243-245	84
II-4j	$C_{19}H_{13}FN_4O_3$	F	$-CH_2-C_6H_5$	364.10	249-251	79

Pharmacological activity

Antibacterial activity:

The antibacterial activity studies against Bacterial Strains *Staphylococcus aureus, Bacillus subtilis* (Gram positive) and *Escherichia coli, Salmonella paratyphi* (Gram negative) were selected based on pharmacological and clinical significance [12-13]. The bacterial microorganisms were cultured on nutrient agar/YEPD by making use of spread plate (cup-plate/disk plate) methodology, following refrigeration storage at 4°C. Bacterial strains were developed in Mueller-Hinton agar (MHA) plates at temperature 37°C (bacteria developed in nutrient broth and retained as for nutrient agar slants at 4°C). The typical stock cultures were kept at 4°C.

The antibacterial activity *in vitro* were examined for sample by the Zone of inhibition method. The activity of samples against two gram-positive, two-gram negative bacteria were investigated by the method of agar disk diffusion. Each compound was dissolved in DMSO (dimethyl sulfoxide), ethanol, and after sterilization, it was filtered by making use of sintered glass filter, which was kept at 4°C. As for the calculation of zone of inhibition, Gram-negative, Gram-positive strains were considered a standard antibiotic for collation of results.

All the compounds were tested for their respective antibacterial activity against gram-positive and gram negative strains. The compound dilutions (100µg/ml) and respective standard drugs (Streptomycin, 50 µg/ml) were provided in double-distilled water by utilizing nutrient agar tubes. The plates of Mueller-Hinton sterile agar were seeded by standard bacterial strains and which were allowed to continue at 37°C for 3 hours with controlled experiments and under a similar environment using Streptomycin as standard drug. From the disks zone of developed inhibition were evaluate succeeding of 18 to 24 hours of incubation at temperature 37°C bacteria. The sensitiveness of microorganism to synthesized drug were calculated by using dimensions of inhibitory zones (counting diameter of a disk) of the agar surface region of the disk and value < 8 mm were measured as not active. All the results were recorded in **Table 2**.

Molecular Docking Studies.

The molecular modelling studies has constantly been proven to be a robust tool for justifying and ranking the conformations using a scoring function and also helps in finding the interactions for making this information available to virtual screening techniques [14-16]. In addition, it also helps us to propose structural hypotheses of how the ligand inhibits the target. The molecular docking models was applied to investigate the binding mode of target molecules via selected proteins with PDB ID (3K4P) for the protein active pocket of the modelled Fgb1. I have docked 10 ligands like novel nitrogen based Indole-2-one Schiff's bases containing pyrimidine-2,4-dione II-4(a-j) in to active site of the Fgb1 protein using AUTODOCK suite of MGL Tools. The protein-ligand interactions of the dataset ligands were observed by using structurally optimized protein shape with Glide Xp docking protocol. Primarily, a 3D grid used to be set up to the binding active site of the Fgb1 protein into all the dataset ligands had been docked. It is a combination of hydrophilic, hydrophobic, metal binding groups, Van der Waals energy, Freezing rotatable bonds and polar interactions with receptor. Highest docked pose with lowest glide score was recorded for each ligand and extra precision was performed by using Auto dock suite. Finally, the binding interactions and it was calculated in phrases of Glide score was recorded in **Table 3**.

RESULTS AND DISCUSION.

Chemistry: A series of nitrogen based novel Indole-2-one Schiff's bases contains pyrimidine [6-[(1-methyl-2-oxo-1,2-dihydro-3H-indol-3ylidene)amino]pyrimidine-2,4(1H,3H)-dione] was synthesized as depicted in Scheme 1. The structures assigned to the synthesized compounds **II-4(a-j)** on the basis of IR, ^{1}HNMR and Mass spectroscopic analysis. In the IR (ν cm^{-1}) spectra

appearance of nitrogen based novel Indole-2-one Schiff's bases contains pyrimidine II-(a-j) at 3320-3450cm^{-1} shows that –NH stretching bands in pyrimmidne-2,4-dione. All the compounds having carbonyl stretching(>C=O) were observed at between 1685-1730cm^{-1} and compounds contain aromatic and aliphatic C-H stretching were observed around observed at around 3010-3099cm^{-1} and 2998-2822cm^{-1}. Some of the derivatives containing Ar-Cl/F group showed strong absorption peak around in the region of 785-825cm^{-1}. In the ^{1}HNMR spectrum of all the derivatives showed that singlet protons at δ 12.5674-10.0342 due to -NH protons of Pyrimidine rings. All the synthesized compounds were showed singlets, doublets and triplets at δ 6.9322-8.3501 due to aromatic protons. In the ^{13}C NMR of novel novel Schiff's bases of Indole derivatives showed that peak appeared around at δ 176-168 ppm were confirmed by carbonyl carbon (C=O) and imine carbons(C=N) were conformed at δ 158-143ppm. Most of compounds were showed signal at δ 28-36ppm were confirmed by methyl carbon(-CH_3). The Mass spectrum of all derivatives II-4(a-j) are confirmed by their molecular ion peak and molecular weight given in the spectrum.

Antibacterial activity:

The results of antibacterial activity of the synthesized novel indole-2-one Schiff's bases containing pyrimidine moiety and standard drug using gram positive (*Staphylococcus aureus, Bacillus subtilis*), and Gram negative (*Escherichia coli, Salmonella paratyphi*) are presented in Table.2. respectively. Al the synthesized Indole-2-one Schiff's bases were potent antibacterial agents in comparison to streptomycin taken as standard drug respectively. Among the synthesized derivatives, compound **II-4a [(25*** against ***Staphylococcus aureus***), **26*** against ***Bacillus subtilis***), **26*** against ***Salmonella paratyphi***)]; compound **II-4a [(24*** against ***Staphylococcus aureus***), **23*** against ***Salmonella paratyphi***)] and compound **II-4a [(23*** against ***Staphylococcus aureus***), **25*** against Bacillus subtilis), 24* against ***Escherichia coli***)] are showed better activity and reaming compounds are showing moderate activity.

Table.2. Antibacterial activity of novel nitrogen based pyrimidine contained Indole-2-one Schiff's bases II-4(a-j)

Compounds	Zone of Inhibition (in mm)			
	Staphylococcus aureus	***Bacillus subtilis***	***E.Coli***	***Salmonella paratyphi***
II-4a	09	14	17	10
II-4b	18	10	17	12
II-4c	12	17	15	17
II-4d	15	10	10	18
II-4e	19	12	16	14
II-4f	**25***	**26***	10	**26***
II-4g	15	12	16	12
II-4h	10	**24***	15	**23***
II-4i	13	15	10	12
II-4j	**23***	**25***	**24***	15
Streptomycin	**28**	**31**	**33**	**32**

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

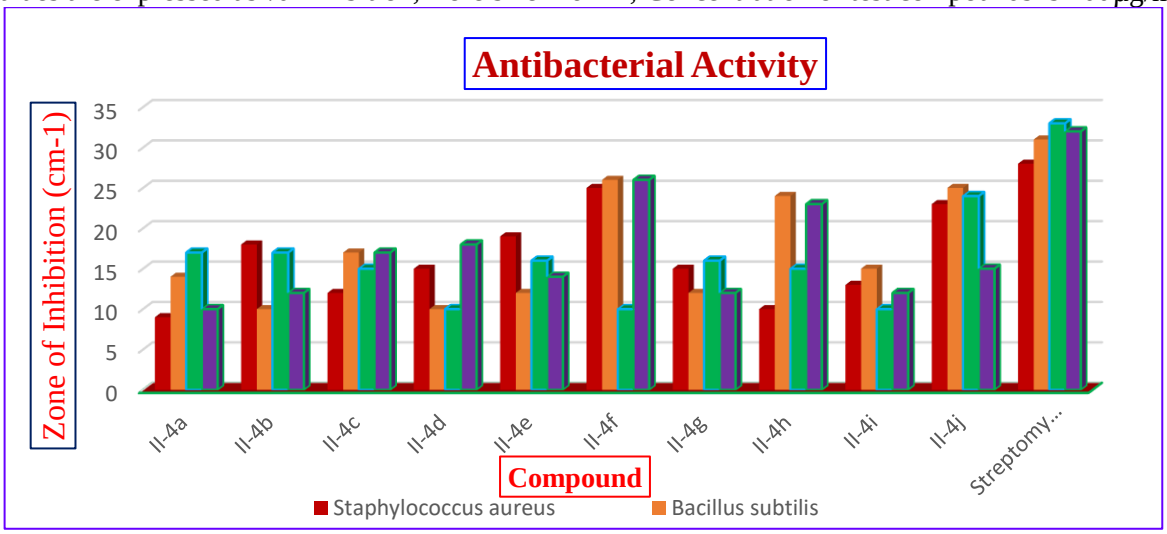


Fig.2.Graphical representation of novel nitrogen based pyrimidine contained

Indole-2-one Schiff's bases II-4(a-j)

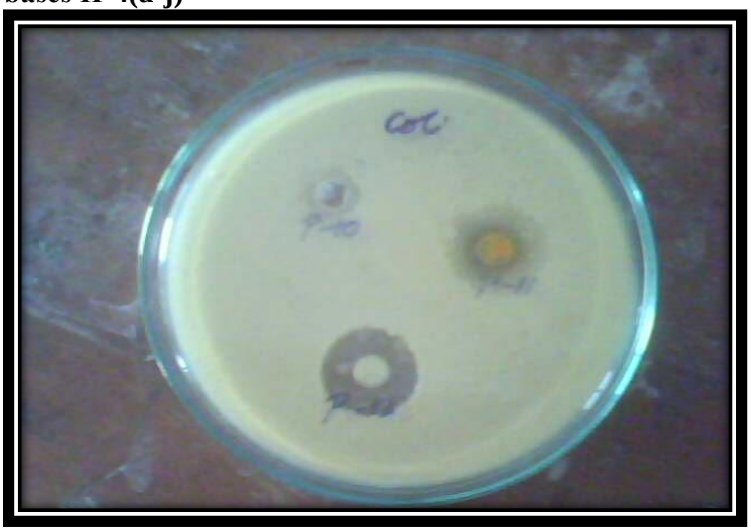


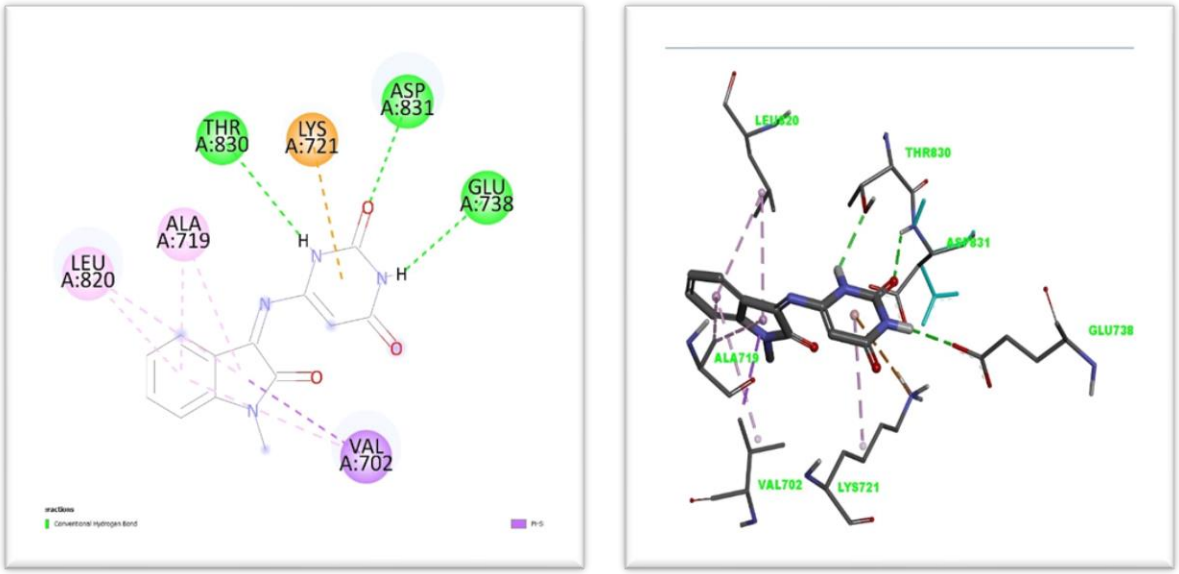
Fig.3: Photographs of Antibacterial activity- novel nitrogen based pyrimidine contained Indole-2-one Schiff's bases II-4(a-j)

Molecular Docking Studies:

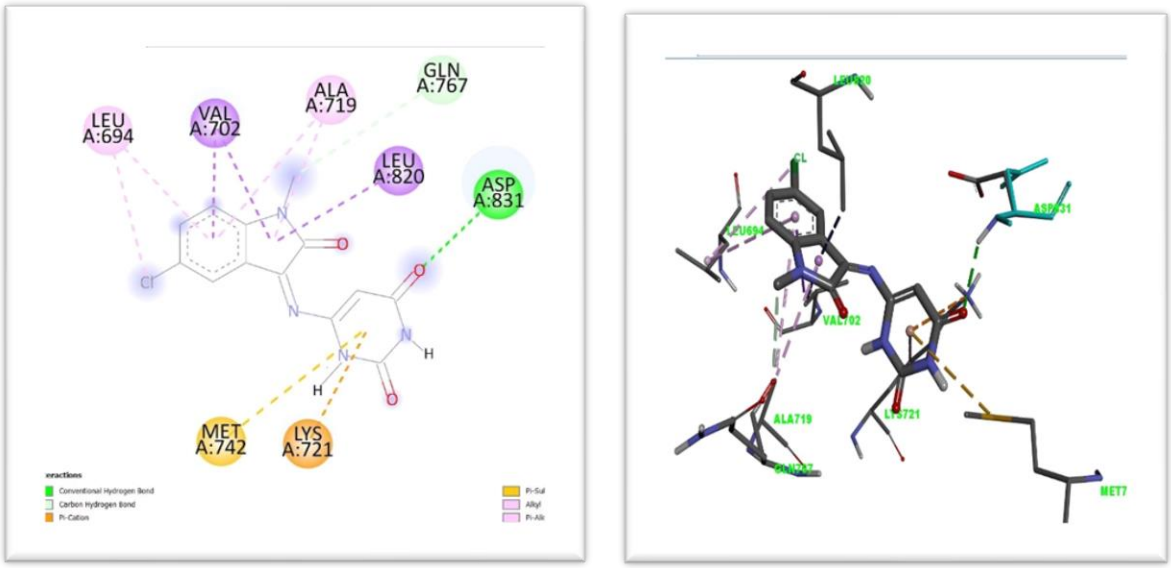
The obtained binding energies(Kcal/mol) and hydrogen bonding of compounds **II-4(a-j)** from
The molecular socking simulations are detailed in Table 3, were the docked conformation of ligand with receptor interactions are shown in Figure 4 respectively. I have docked 10 ligands like novel nitrogen based pyrimidine contained Indole-2-one Schiff's bases II-4(a-j) in to active site of the Fgb1 protein using AUTODOCK suite of MGL Tools. All the ligands are interacted different amino acids like VAL:702, LYS:721, ALA:719, LEU:820, ASP:831, THR:830, GLU:738, LEU:694, MET:749, MET:742, GLN:767, MET:769. The compound **II-4f (-9.3**), **II-4h (-9.3)** and **II-4j(-9.2)** were reported highest docking ligands.The docking score of the ligands ranged from **-7.2 (compound II-4d)** to **-9.2(Compound II-4f)**.

Table.3. Docking scores of novel nitrogen based pyrimidine contained Indole-2-one Schiff's bases II-4(a-j)-Glide dock score of the dataset ligands.

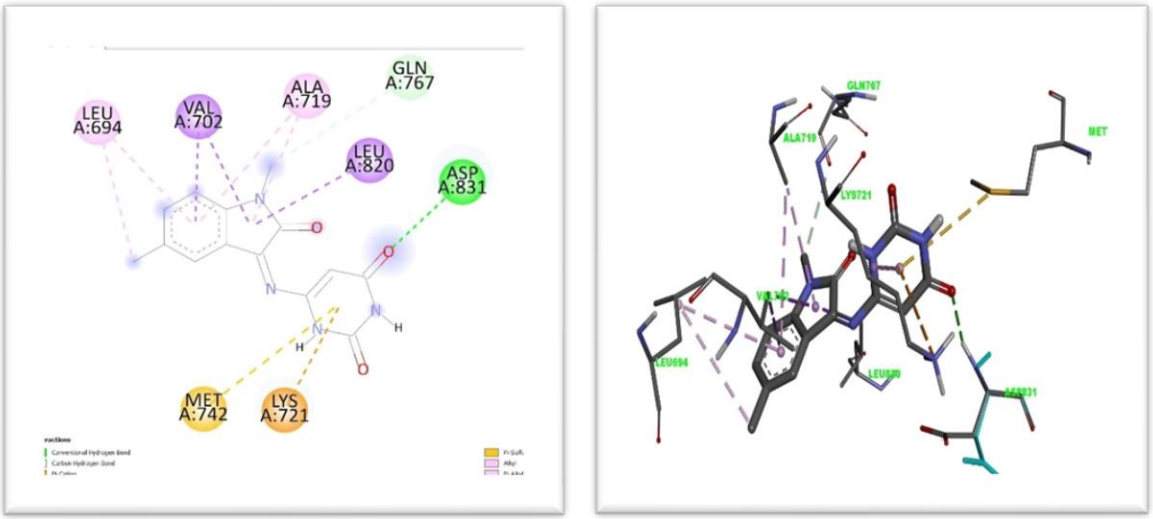
Compound No	Binding Energy (Kcal/mol)	No of H-bonds	Interacting amino acids	H-bond lengths (Å)
II-4a	-8.9	3	VAL:702, LYS:721, ALA:719, LEU:820, ASP:831, THR:830, GLU:738	2.31, 2.48, 2.45
II-4b	-8.9	1	LEU:694, MET:749, MET:742, GLN:767, VAL:702, LYS:721, MET:769, ASP:831, LEU:820, ALA:719	2.54
II-4c	-9.1	1	LEU:694, MET:742, VAL:702, GLN:767, LYS:721, GLU:738, LEU:768, MET:769, ASP:831, LEU:820	2.52
II-4d	-7.2	1	LEU:694, MET:742, ASP:831, LEU:820, ALA:719	2.59
II-4e	-8.2	0	LEU:694, ASP:831, LEU:820, ALA:719MET:742,	0
II-4f	-9.3	4	LEU:694, PHE:699, VAL:702, LYS:721, ALA:719, MET:769, LEU:820, ASP:831	2.29, 2.60, 2.67, 2.99
II-4g	-8.5	1	MET:742, VAL:702, GLN:767, LYS:721, GLU:738	2.88
II-4h	-9.2	1	LEU:694, MET:749, VAL:702, LYS:721, GLU:738, GLY:772, MET:769, ASP:831, LEU:820, THR:830	2.21, 2.58, 2.77, 2.84, 2.98, 2.97
II-4i	-8.6	0	PHE:699, VAL:702, LYS:721, ALA:719, MET:769, LEU:820	0
II-4j	-9.3	3	VAL:702, LYS:721, GLU:738, GLY:772, MET:769, ASP:831, LEU:820, THR:830	2.34, 2.65, 2.72



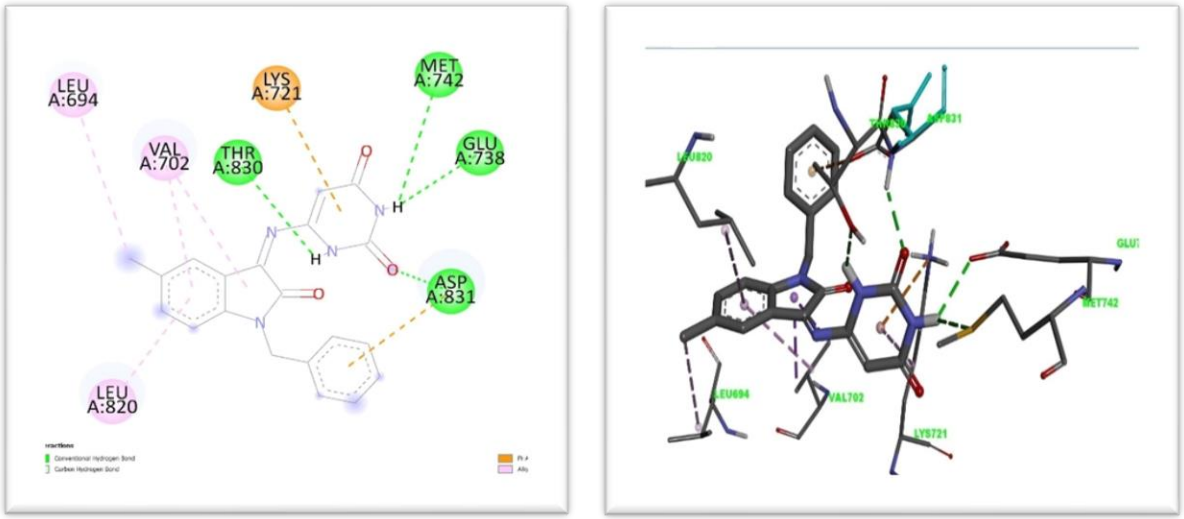
Compound.II-4a.Dock1 and 3d structures



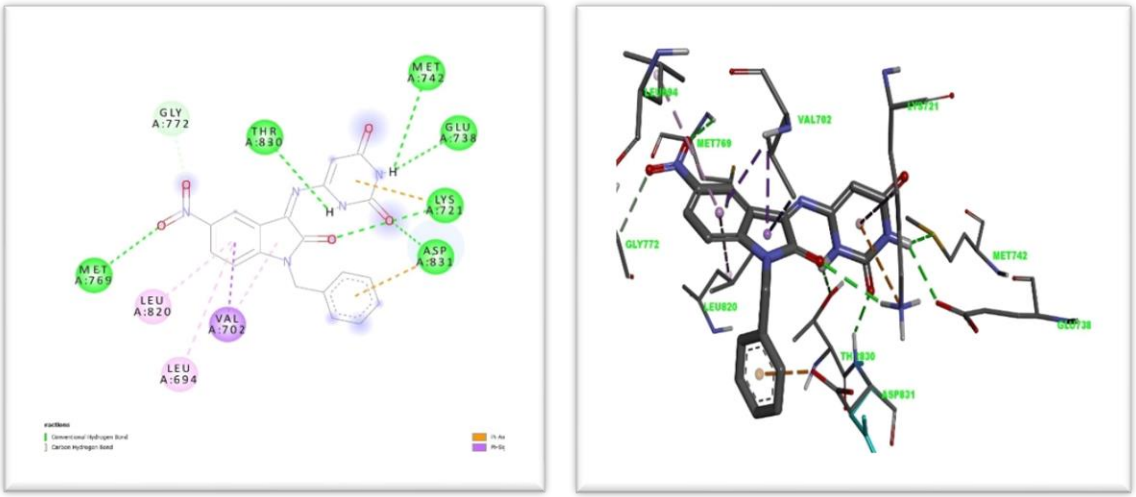
Compound.II-4b.Dock1 and 3d structures



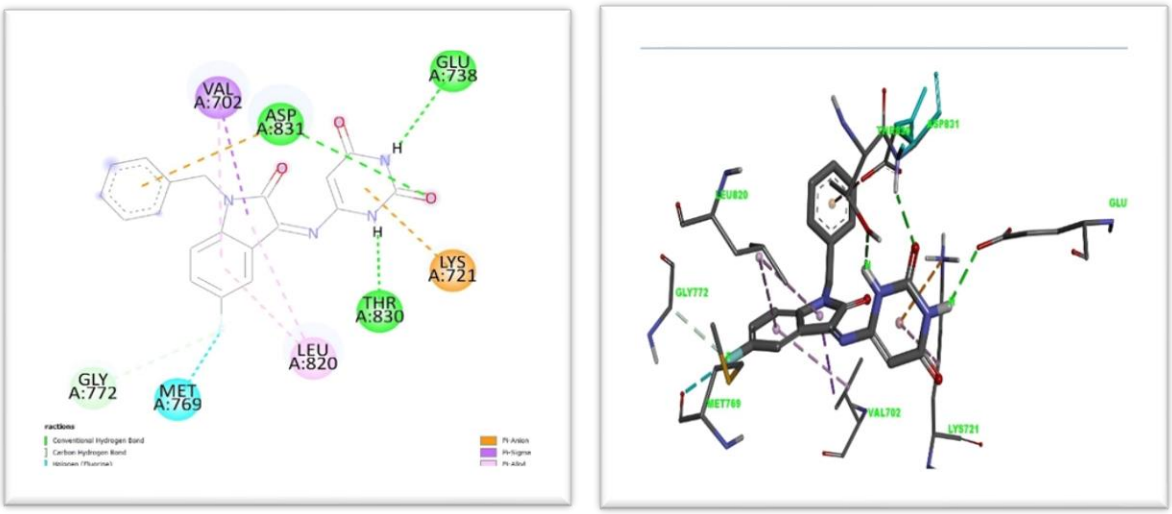
Compound.II-4c.Dock1 and 3d structures



Compound.II-4f.Dock1 and 3d structures



Compound II-4h. Dock1 and 3d structures



Compound II-4j. Dock1 and 3d structures

Fig.4: Graphical illustration of predicted binding mode in the active site of 3K4P for compounds II-4a, II-4b, II-4c, II-4f, II-4h and II-4j. [Key residues involved in the interactions are labelled and the compounds are represented as lines. The hydrogen bond interactions are represented by magenta arrow].

CONCLUSION:

In summary, we have carried out the synthesis of novel nitrogen based pyrimidine contained Indole-2-one Schiff's bases II-4(a-j), carried out their docking studies and screened for in vitro antibacterial activity. All the synthesized structures were confirmed by IR, ^{1}H-NMR and Mass spectrometry. From the antibacterial results indicated, compound II-4a (25*, 26*, 26*) against *Staphylococcus aureus, Bacillus subtilis and Salmonella paratyphi;* compound II-4a (24*, 23*) against *Staphylococcus aureus, Salmonella paratyphi* and compound II-4a (23*, 25* and 23*) against *Staphylococcus aureus,* Bacillus subtilis and *Escherichia coli are* showed good activity. The docking score of the ligands ranged from -7.2 (compound II-4d) to -9.2(Compound II-4f).

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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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