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SYNTHESIS, CHARACTERIZATION, DOCKING AND BIOLOGICAL ACTIVITIES OF SOME NOVEL SCHIFF BASES OF PYRAZOLINE DERIVATIVES FROM CHALCONE

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ABSTRACT
The substituted aldehydes and ketones were reacted to obtain chalcones using the well known Claisen Schmidt condensation. The chalcone derivatives were further cyclized to pyrazoline carbaldehyde using hydrazine hydrate and formic acid. The cyclized pyrazoline carbaldehyde derivatives were subjected to Schiff base reactions with *p*-amino pyridine. Various *invitro* biological activities were carried out like anti-TB by MABA method, antibacterial by MIC method, anti-inflammatory by BSA method and antioxidant by DPPH and NO radical scavenging methods. 5(a) with hydroxyl and chloro substitutents on the benzene rings showed activity with a MIC value of 25 µg/ml against *Mycobacterium tuberculosis*. Most of the derivatives have shown good activity against *P.aeruginosa* and *S.pneumoniae*. Compounds 5(b) and 5(c) having Br, OH and Cl substitutents respectively exhibited higher percentage inhibition when compared to the standard (89% and 90% at 100 and 200 µg/ml respectively and indomethacin 90% and 91% at the same concentration). However it was observed that the % inhibition exhibited by DPPH method is high as compared to the NO method for both the standard as well as for the test samples. The potential of these molecules as mycobacterial, antibacterial and antiinflammatory agents were predicted using molecular docking studies

KEYWORDS: Pyrazoline carbaldehyde, Schiff base, anti-TB, antibacterial, anti-inflammatory, antioxidant, docking.

INTRODUCTION
Despite significant progress made in the treatment of infectious diseases, caused by bacteria and fungi, it remains a major worldwide health problem due to rapid development of resistance against the existing antimicrobial drugs. Developing novel antimicrobial agents with different mode of action than that of existing drugs is one of the main challenges to overcome the antimicrobial resistance. In view of these facts, it is important to develop more effective antimicrobial agents. Thus, the synthesis and discovery of more efficient antimicrobial agents has been intensively considered during the last decade.

Tuberculosis (TB) is an important disease due to infection by *Mycobacterium tuberculosis* (Mtb) which primarily affects the respiratory tract. According to the World Health Organization (WHO), there are nearly 10 million new cases of TB and 1.8 million deaths each year. The situation is further worsened by the co-infection with HIV due to the devastating effects of Mtb on the vulnerable immune system of the patients.[1] Despite BCG vaccine (Bacillus Calmette–Guerin) and the combined chemotherapy with first-line (isoniazid, ethambutol, rifampicin, pyrazinamide) or second-line (ethionamide) antibiotics (WHO), Mtb is the agent which causes more deaths from infection in the world. The chemotherapy involves the use of these four antibiotics for 6–9 months, resulting in significant lack of patient adherence to long-term therapy. The global emergence of multidrug-resistant (MDR) strains of Mtb is also a serious health problem.[2] For these reasons, there is an urgent need for new safe and effective anti-TB drugs.

Most currently used nonsteroidal anti-inflammatory drugs (NSAIDs) have limitations for therapeutic use since they cause gastrointestinal and renal side effects that are inseparable from their pharmacological activities. Therefore, the synthesis of new compounds devoid of such side effects has become an important goal for medicinal chemists in recent years.

Owing to the strong demand for new antioxidant agents, it becomes very critical to explore novel scaffold for the design and synthesis of new antioxidant agents in order to help in the battle against pathogenic microorganisms.

Pyrazoline and their derivatives are considered to be important for drugs and agricultural chemical.[3] Some substituted pyrazolines and their derivatives have been

reported to possess several interesting biological activities such as antimicrobial,[4-6] anti-inflammatory,[7-8] antitumor,[9] antioxidant,[10] antimalarial,[11] antidepressant,[12] anticonvulsant,[13] and antihistaminic.[14] activities. Some of these compounds have also antiviral, analgesic, antiamoebic, hypotensive, anticancer and molluscicidal properties.[15-20]

Schiff bases are typically formed by the condensation of a primary amine and an aldehyde. These classes of compounds have varied structures and exhibit a great variety of biological activities such as antimicrobial,[21-23] antibacterial,[24-27] antifungal,[28-32] antimalarial,[33] antifeedant,[34] anti-inflammatory,[35-36] anticancer,[37] antitubercular,[38,39] antiviral,[40] anticonvulsant,[41- 44] and analgesic properties.[45 – 48]

Chalcone derivatives have attracted increasing attention due to their numerous pharmacological activities such as antioxidant, anticancer, antitubercular, antimicrobial, anti-inflammatory, analgesic, antiplatelet, antiulcerative, antimalarial, antiviral, antileishmanial, antihyperglycemic, antiproliferative activities.[49-55]

Based upon the above observations it was decided to synthesize some novel pyrazoline derivatives from chalcones and subjected to Schiff base reactions. The final synthesized compounds are subjected to various biological activities.

EXPERIMENTAL

Materials

All the melting points were determined in a Thermonik melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds was recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400 -4000 using KBr pellets and the value of λmax were reported in cm-1. 1HNMR spectra was recorded on Amx - 400 MHz NMR spectrometer using DMSO and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as internal reference. 13C-NMR spectra was recorded on Amx - 400 MHz NMR spectrometer using DMSO and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as an internal reference. A mass spectrum was recorded on Mass spectrophotometer (model Shimadzu) by LC-MS 2010A. The purity of the compounds was checked by thin-layer chromatography on silica gel G plates of 0.5mm thickness as stationary phase and combination of n-hexane: ethyl acetate in different ratios as mobile phase. Elemental analysis were analysed by Thermo Finnigan Flash EA 1112 Series

General procedure for the preparation of 3-(substituted phenyl)-1-(substituted phenyl)-propenone 3(a-j)

Mixture of substituted aromatic benzaldehyde (0.02M) and substituted aromatic ketone (0.02M) was stirred in ethanol (40 ml) and an aqueous solution of KOH (40%, 15 ml) was added to it. The stirring was continued for 6 hrs and the mixture was left overnight at room temperature. The mixture was then poured into crushed ice, acidified with HCl. The solid was filtered and recrystallized from ethanol.

3-(4-bromo-phenyl)-1-(4-hydroxy-phenyl)-propenone 3a

M.W : 303, yield 51%, M.P: 160^{o}C, Rf value 0.94, solvent ratio: ethyl acetate: n-hexane (7:3).IR (KBr *υ*max cm−1): 3500-3200 (O-H str), 2700 (Ar-H str), 1640 (C=O str), 1600 (CH=CH str), 760 (Ar-Br str).^{1}H-NMR400 MHz, (DMSO-*d6*, *δ*-ppm): 7.97- 7.95 (2H, CH=CH), 7.68-7.59 (8H, Ar-H), 6.84 (1H, O-H).m/z : M^{+} 303, (M+1) 304. (M+2) 305.

3-(4-chloro-phenyl)-1-(4-hydroxy-phenyl)-propenone 3b

M.W: 258, yield 54%, M.P: 120^{o}C, Rf value 0.93, solvent ratio: ethyl acetate: n-hexane (7:3) .IR (KBr *υ*max cm−1): 3240 (O-H str), 2840 (Ar-H str), 1700 (C=O str), 1600 (CH=CH str), 800 (Ar-Cl str).

3-(4-chloro-phenyl)-1-(3,4-dimethoxy-phenyl)-propenone 3e

M.W: 302, yield 52%, M.P: 130^{o}C, Rf value 0.83, solvent ratio: ethyl acetate: n-hexane (7:3).IR (KBr *υ*max cm−1): 2700 (Ar-H str), 1700 (C=O str), 1600 (CH=CH str), 1000 (C-O-C str), 800 (Ar-Cl str).

3-(4-bromo-phenyl)-1-(4-dimethylamino-phenyl)-propenone 3f

M.W: 285, yield 88%, M.P: 190^{o}C, Rf value 0.8, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr *υ*max cm−1): 2840 (ArC-H str), 1640 (C=O str), 1560 (CH=CH str), 1360 (C-N str), 760 (Ar-Br str).

3-(4-bromo-phenyl)-1-(3, 4, 5-trimethoxy-phenyl)-propenone 3g

M.W: 377, yield 56%, M.P: 132^{o}C, Rf value 0.9, solvent ratio: ethyl acetate: n-hexane (7:3). IR (KBr *υ*max cm−1): 2840 (Ar-H str), 1660 (C=O str), 1640 (CH=CH str), 1000 (C-O-C str), 760 (Ar-Br str).

3-(4-chloro-phenyl)-1-(3, 4, 5-trimethoxy-phenyl)-propenone 3h

M.W: 332, yield 79%, M.P: 136^{o}C, Rf value 0.84, solvent ratio: ethyl acetate: n-hexane (7:3).IR (KBr *υ*max cm−1): 2840 (Ar-H str), 1640 (C=O str), 1600 (CH=CH str), 1100 (C-O-C str), 840 (Ar-Br str).

3-(4-chloro-phenyl)-1-phenyl-propenone 3i

M.W: 242, yield 70%, M.P: 120^{o}C, Rf value 0.86, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr *υ*max cm−1): 3140 (ArC-H str), 1720 (C=O str), 1600 (CH=CH str), 760 (Ar-Br str).

3-(4-bromo-phenyl)-1-(4-methoxy-phenyl)-propenone 3j

M.W: 317, yield 54%, M.P: 149^{o}C, Rf value 0.88, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr υmax cm−1): 2940 (ArC-H str), 1660 (C=O str), 1600 (CH=CH str), 1000 (C-O-C str), 720 (Ar-Br str)

General procedure for the preparation of 5-(substituted phenyl)-3-(substituted phenyl)-4, 5-dihydro-pyrazole-1-carbaldehyde 4(a-j)

A solution of 3-(substituted phenyl)-1-(substituted phenyl)-propenone i.e chalcone intermediates **3(a-j)** (0.008M), hydrazine hydrate (0.016M) in 4 ml of formic acid was heated under reflux for 4-6 hrs, then poured into crushed ice. The solid was separated by filtration, washed with water and recrystallized from ethanol.

5-(4-bromo-phenyl)-3(4-hydroxy-phenyl)-4,5-dihydro-pyrazole-1-carbaldehyde 4a

M.W: 345, yield 54%, M.P: 210^{o}C, Rf value 0.86, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr υmax cm−1): 3300 (O-H str), 2880 (Ar C-H str), 1700 (C=O str), 1500 (C=N str), 760 (Ar-Br str).(1H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.84(s, 1H, CHO), 7.69-7.58 (m, 8H, Ar), 5.41 (1H of pyrazole +1H of OH), 2.55 (s, 2H, CH_2 of pyrazoline). M/z: M^+ 345, (M+1) 346. (M+2) 347.

5-(4-bromo-phenyl)-3-phenyl-4, 5-dihydro-pyrazole-1-carbaldehyde 4b

M.W: 329, yield 93%, M.P: 158^{o}C, Rf value 0.72, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr υmax cm−1): 3100 (Ar C-H str), 1660 (C=O str), 1560 (C=N str), 760 (Ar-Br str).

5-(4-chloro-phenyl)-3(4-hydroxy-phenyl)-4, 5-dihydro-pyrazole-1-carbaldehyde 4c

M.W: 300, yield 86%, M.P: 190^{o}C, Rf value 0.78, solvent ratio: ethyl acetate: n-hexane (7:3). IR (KBr υmax cm−1): 3160 (O-H str), 2860 (Ar C-H str), 1700 (C=O str), 1500 (C=N str), 840 (Ar-Cl str).

5-(4-bromo-phenyl)-3(3, 4-dimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbaldehyde 4d

M.W: 389, yield 53%, M.P: 140^{o}C, Rf value 0.6, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr υmax cm−1): 2900 (Ar C-H str), 1680 (C=O str), 1560 (C=N str), 1100 (C-O-C str), 760 (Ar-Br str).

5-(4-bromo-phenyl)-3(3, 4, 5-trimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbaldehyde 4e

M.W: 403, yield 52%, M.P: 176^{o}C, Rf value 0.8, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr υmax cm−1): 2900 (Ar C-H str), 1700 (C=O str), 1500 (C=N str), 1000 (C-O-C str), 760 (Ar-Br str).

5-(4-bromo-phenyl)-3-(4-dimethylamino-phenyl)-4, 5-dihydro-pyrazole-1-carbaldehyde 4f

M.W: 372, yield 57%, M.P: 186^{o}C, Rf value 0.79, solvent ratio: ethyl acetate: n-hexane (7:3) IR (KBr υmax cm−1): 3100 (Ar C-H str), 1660 (C=O str), 1560 (C=N str), 1380 (C-N str), 760 (Ar-Br str).

General method for the preparation of [5-(substituted phenyl)-3-(substituted phenyl)-4, 5-dihydro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5(a-j)

Mixture of 5-(substituted phenyl)-3-(substituted phenyl)-4, 5-dihydro-pyrazole-1-carbaldehyde 4(a-j) (0.1 M) and *p*-amino pyridine (0.2M) were dissolved in absolute ethanol and were stirred for 12 hours using conc HCl as a catalyst (2drops). The reaction mixture was poured in crushed ice. The solid was filtered and recrystallized from isopropyl alcohol

4-[5-(4-bromo-phenyl)-1-(pyridine-4-yliminomethyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenol 5a

M.W:420, yield 51%, M.P: 250^{o}C, Rf value 0.9, solvent ratio: ethyl acetate: n-hexane (1:1).

IR (KBr υmax cm−1): 3398 (O-H str), 2364 (Ar-CH str), 1587 (C=N str), 1269 (Ar C=N str), 827 (Ar-Br str).[1]H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.85 (2H of pyridine), 7.89-6.64 (10 ArH + 1H of CH=N), 4.03 (1H, O-H), 3.41 (1H of pyrazole), 1.04-1.03 (2H of pyrazole).[13] CNMR (DMSO, ppm): 159.46, 115.07, 133.07, 128.77, 156.17, 41.75, 49.78, 140.59, 131.46, 135.62, 127.88, 163.09, 123.59, and 151.07.m/z : M^+ 420, (M+2) 422. CHN: Found C=59.87%, H= 4.12%, N= 13.38%. Calculated C= 59.87%, H= 4.07%, N= 13.30%

5-(4-bromophenyl)-3-phenyl-4, 5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5b

M.W :404, yield 55%, M.P: 150^{o}C, Rf value 0.89, solvent ratio: ethyl acetate: n-hexane (1:1).IR (KBr υmax cm−1): 2360 (Ar-CH str), 1654 (C=N str), 1323 (Ar C=N str), 761 (Ar-Br str).1H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.83 (2H of pyridine), 7.67-6.99 (11 ArH + 1H of CH=N), 3.34 (1H of pyrazole), 1.97-1.96 (2H of pyrazole).13CNMR (DMSO, ppm): 159.46, 127.51, 128.46, 129.61,159.72, 42.22, 52.67, 141.19, 128.73, 135.10, 125.69, 63.20, 115.50,155.17.m/z : (M-1) 403.CHN: Found C= 62.15%, H= 4.23%, N= 13.82%. Calculated C= 62.23%, H= 4.19%, N= 13.76%.

4-[5-(4-chloro-phenyl)-1-(pyridine-4-yliminomethyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenol 5c

M.W:376, yield 58%, M.P: 210^{o}C, Rf value 0.86, solvent ratio: ethyl acetate: n-hexane (1:1).

IR (KBr υmax cm−1): 3400 (OH str), 2922 (Ar-CH str) 1597 (C=N str), 1361 (Ar C=N str), 829 (Ar-Cl str).[1]H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.91 (2H of pyridine), 8.03-7.07 (10 ArH + 1H of CH=N), 5.50 (1H, O-H), 3.43 (1H of pyrazole), 1.08 (2H of pyrazole).[13]CNMR (DMSO, ppm): 159.60, 127.04, 131.55, 128.42, 156.17, 42.13, 49.73, 135.28, 128.53, 128.89, 134.72, 165.52, 156.74, 127.89, and 155.15.m/z: (M+) 376. CHN: Found C=66.82%, H= 4.78%, N= 14.45%. Calculated C= 66.93%, H= 4.55%, N= 14.87%.

[5-(4-bromophenyl)-3-(3, 4-dimethoxy-phenyl)-4, 5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5d
M.W:465, yield 60%, M.P: 130°C, Rf value 0.84, solvent ratio: ethyl acetate: n-hexane (1:1).
IR (KBr *υ*max cm−1): 2359 (Ar-CH str) 1593 (C=N str), 1259 (Ar C=N str), 1070 (C-O-C str), 640 (Ar-Br str).^{1}H-NMR400 MHz, DMSO-*d6*, *δ*-ppm): 8.83 (2H of pyridine), 7.67-7.16 (9 ArH + 1H of CH=N), 3.67 (1H of pyrazole), 3.28 (6H, OCH_3), 1.98 (2H of pyrazole).^{13}CNMR (DMSO, ppm): 148.81, 148.09, 109.66, 123.86, 159.76, 44.04, 50.52, 141.39, 131.89, 133.69, 128.64, 166.09, 111.85, 151.30, and 60.50.m/z: (M+) 464.CHN: Found C=59.26%, H= 4.51%, N= 12.10%. Calculated C= 59.36%, H= 4.55%, N= 12.04%.

[5-(4-bromophenyl)-3-(3, 4, 5-trimethoxy-phenyl)-4,5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5e
M.W:495, yield 62%, M.P: 180°C, Rf value 0.86, solvent ratio: ethyl acetate: n-hexane (1:1).
IR (KBr *υ*max cm−1): 2937 (Ar-CH str) 1593 (C=N str), 1246 (Ar C=N str), 1122 (C-O-C str), 756 (Ar-Br str).^{1}H-NMR400 MHz, DMSO-*d6*, *δ*-ppm): 8.94 (2H of pyridine), 7.75-6.38 (8 ArH + 1H of CH=N), 3.75 (1H of pyrazole), 3.36 (9H, OCH_3), 1.92 (2H of pyrazole).^{13}CNMR (DMSO, ppm): 147.33, 136.61, 102.73, 128.67, 155.27, 42.26, 52.83, 136.91, 130.02, 131.80, 123.88, 165.55, 159.92, 117.23, 153.05, and 59.88. m/z: (M+) 494. CHN: Found C=58.24%, H= 4.62%, N= 11.26%. Calculated C= 58.19%, H= 4.68%, N= 11.3%.

[5-(4-bromophenyl)-3-(4-dimethylamino-phenyl)-4,5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5f
M.W:448, yield 65%, M.P: 208°C, Rf value 0.81, solvent ratio: ethyl acetate: n-hexane (1:1).IR (KBr *υ*max cm−1): 3240 (CH str of CH_3), 2800 (Ar-CH str) 1618 (C=N str), 1350 (Ar C=N str), 756 (Ar-Br str).^{1}H-NMR400 MHz, DMSO-*d6*, *δ*-ppm): 7.69-6.69 (12 ArH + 1H of CH=N), 3.24-3.19 (1H of pyrazole), 2.83 (6H, $N(CH_3)_2$), 1.99-1.98 (2H of pyrazole)^{13}CNMR (DMSO, ppm): 163.20, 158.72, 156.17, 140.19, 134.10, 129.91, 128.98, 128.83, 128.66, 127.51, 126.69, 116.10, 113.21, 59.67, 48.84, and 41.22m/z : (M+1) 449 and (M+2) 451.CHN: Found C=61.15%, H= 4.71%, N= 15.76%. Calculated C= 61.61%, H= 4.95%, N= 15.62%.

[5-(4-chlorophenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5g
M.W:450, yield 69%, M.P: 180°C, Rf value 0.83, solvent ratio: ethyl acetate: n-hexane (1:1).IR (KBr *υ*max cm−1): 2935 (Ar-CH str) 1589 (C=N str), 1242 (Ar C=N str), 1128 (C-O-C str), 825 (Ar-Cl str).^{1}H-NMR400 MHz, DMSO-*d6*, *δ*-ppm): 8.93 (2H of pyridine), 7.75-6.33 (8H ArH + 1H of CH=N), 3.63 (1H of pyrazole), 3.33 (9H of $O(CH_3)_3$), 1.38 (2H of pyrazole)^{13}CNMR (DMSO, ppm): 164.84, 159.26, 155.73, 154.65, 148.88, 136.92, 132.55, 130.02, 127.10, 124.83, 116.47, 103.68, 58.80, and 53.23.m/z : (M+1) 451 and (M+2) 453.CHN: Found C=63.24%, H= 5.62%, N= 12.26%. Calculated C= 63.93%, H= 5.14%, N= 12.43%.

[5-(4-bromophenyl)-3-(4-methoxy-phenyl)-4,5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5h
M.W:435, yield 70%, M.P: 185°C, Rf value 0.79, solvent ratio: ethyl acetate: n-hexane (1:1).IR (KBr *υ*max cm−1): 2833 (Ar-CH str), 1514 (C=N str), 1251 (Ar C=N str), 1182 (C-O-C str), 752 (Ar-Br str). ^{1}H-NMR400 MHz, DMSO-*d6*, *δ*-ppm: 7.89-7.01 (12H ArH + 1H of CH=N), 3.85 (1H of pyrazole), 3.34 (3H of OCH_3), 1.09-1.06 (2H of pyrazole).[13] CNMR (DMSO, ppm): 165.57, 161.59, 159.45, 156.17, 151.57, 140.79, 135.72, 133.77, 131.06, 128.25, 127.67, 123.88, 115.08, 58.83, 49.46, and 41.04m/z : (M+1) 436 and (M+2) 438. CHN: Found C=60.75%, H= 4.21%, N= 12.38%. Calculated C= 60.70%, H= 4.40%, N= 12.87%.

[5-(4-chlorophenyl)-3-(3, 4-dimethoxy-phenyl)-4,5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5i
M.W:420, yield 74%, M.P: 96°C, Rf value 0.86, solvent ratio: ethyl acetate: n-hexane (1:1).IR (KBr *υ*max cm−1): 2935 (Ar-CH str), 1593 (C=N str), 1238 (Ar C=N str), 1139 (C-O-C str), 756 (Ar-Cl str). ^{1}H-NMR400 MHz, DMSO-*d6*, *δ*-ppm: 8.84 (2H of pyridine), 7.77-6.61 (9H ArH + 1H of CH=N), 3.83-3.77 (1H of pyrazole), 3.28 (6H of $(OCH_3)_2$), 1.95-1.94 (2H of pyrazole).^{13}CNMR (DMSO, ppm): 166.87, 159.66, 150.36, 148.83, 148.03, 141.04, 133.50, 130.55, 127.66, 124.45, 116.55, 111.65, 110.25, 60.56, 51.08 and 43.87m/z: (M+1) 421 and (M+2) 423.CHN: Found C=65.26%, H= 5.51%, N= 13.10%. Calculated C= 65.63%, H= 5.03%, N= 13.31%.

[5-(4-chlorophenyl)-3-phenyl)-4,5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine 5j
M.W:360, yield 74%, M.P: 176°C, Rf value 0.86, solvent ratio: ethyl acetate: n-hexane (1:1).IR (KBr *υ*max cm−1): 2891 (Ar-CH str) 1597 (C=N str), 1247 (Ar C=N str), 744 (Ar-Cl str). ^{1}H-NMR400 MHz, DMSO-*d6*, *δ*-ppm: 8.82 (2H of pyridine), 7.69-6.97 (11H ArH + 1H of CH=N), 3.32-3.20 (1H of pyrazole), 1.98-1.97 (2H of pyrazole).^{13}CNMR (DMSO, ppm): 164.90, 158.66, 154.14, 140.10, 134.19, 129.45, 128.93, 128.73, 128.24, 127.03, 124.99, 114.19, 51.08, and 41.87.m/z : (M+) 360 and (M+3) 363.CHN: Found C=69.15%, H= 4.79%, N= 15.53%. Calculated C= 69.90%, H= 4.75%, N= 15.53%.

***In vitro* Biological Activities**

***In vitro* Anti –TB activity using Alamar Blue Dye**

The anti mycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of ster ile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C

for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink[56]

Organism: *Mycobacterium tuberculosis H37RV* strain/ *ATCC no. 2729*

***In vitro* anti-bacterial activity study**

Nine dilutions of each drug have to be done with BHI for MIC. In the initial tube 20microliter of drug was added into the 380microliter of BHI broth. For dilutions 200microliter of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity.[57]

Brain heart infusion broth	500g
Ingredients	*Gms/litre*
Calf brain, infusion from	200.00
Beef heart, infusion from	250.00
Proteose peptone	10.00
Dextrose	2.00
Sodium chloride	5.00
Disodium phosphate	2.50

Final pH (at 25ºC) 7.4+/-0.2
Organism: *Staphylococcus aureus*
Streptococcus pneumonia
Escherichia coli
Pseudomonas aeruginosa

In vitro anti-inflammatory activity

Bovine serum albumin assay

Procedure

A solution of 0.2% w/v of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 1000µg/ml of all test samples were prepared by using methanol as a solvent, from these stock solutions two different concentrations of 100µg/ml and 200µg/ml were prepared by using methanol as a solvent. 100µl (0.1ml) of each test sample was transferred to volumetric flask (10 ml) using 1ml micropipette; 5ml of 0.2% BSA was added to all the above flasks. The control consists of 5ml 0.2%w/v BSA solution with 0.1ml methanol. The 0.1ml standard consist 100µg/ml of indomethacin in methanol with 5ml 0.2%w/v BSA solution. The volumetric flasks were heated at 72˚C for five minutes and then cooled for 10 min. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. The % inhibition of precipitation (denaturation of the protein) was determined on a percentage basis relative to the control using the following formula.[58]

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

***In vitro* antioxidant activity**

(a). Screening of antioxidant activity by DPPH method

Procedure

Preparation of Control (DPPH) Solution

10 mg of DPPH was dissolved in 10 ml of methanol. From this stock solution dilutions were made to obtain concentrations of 10 to 40 µg/ ml. The absorbance was recorded for these dilutions at 516 nm.

Preparation of standard solution (Ascorbic acid)

10 g of ascorbic acid was dissolved in 10 ml of methanol. From this stock solution dilutions were made to obtain concentrations of 10 to 40 µg/ ml. 1 ml from each of these solutions was taken in different volumetric flasks to which 1 ml of DPPH solution (300 µg/ ml concentration) was added and volume was made up to 10 ml. The absorbance was recorded for these dilutions at 516 nm after duration of 30 min.

Preparation of test or sample solutions

The test solution were prepared in similar manner as that of standard ascorbic acid and the absorbance were recorded at 516 nm after duration of 30 min.

% inhibition was calculated by following formula[59]

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

(b). Nitric oxide free radical scavenging activity

Procedure

Sodium nitroprusside solution

Weighed accurately 0.2998 g of sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM).

Naphthyl ethylene diaminedihydrochloride (NEDD, 0.1%)

Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 50% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water.

Preparation of test or sample solutions

The reaction mixture (6 ml) containing 10 mM sodium nitroprusside in phosphate buffer solution and the test or reference compound (ascorbic acid) at different concentrations (10-100 µg/ml) were incubated at 250 C

for 150 min. About 0.5 ml aliquot of the incubated sample was removed at 30 min. interval and 0.5 ml Griess reagent was added. The absorbance of the chromophore formed was measured at 546 nm. Inhibition of the nitricoxide generated was measured by comparing the absorbance values of control, test samples and ascorbic acid.

% inhibition was calculated by following formula.[60]

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Docking Studies

Molecular docking studies to the target proteins are performed in an effort to understand the results obtained through *invitro* studies. Molecular docking has been frequently used to predict the prominent and acknowledged geometry of a protein-ligand complex and to understand the interaction studies of the target with specific ligands. Docking is often used with scoring functions to predict binding affinities of ligands in virtual screening experiments. It is also important in studying the structure activity relationship of the newly synthesized compounds. The function of docking is to define the energetics of the system and the efficiency of the ligand molecule to bind to its target, as it forms the basis of the docking algorithms attempt. AutoDock Vina is a new open source program for drug discovery, molecular docking and virtual screening, offering multi-core capability, high performance and enhanced accuracy and ease of use. AutoDock Vina significantly improves the average accuracy of the binding mode predictions.

Protein preparation: The proteins were extracted from PDB files and all the water molecules were removed. The missing atoms and hydrogen were added, polar hydrogen atoms and charges of the Gasteiger-type were assigned by using PMV and saved in pdbqt format.

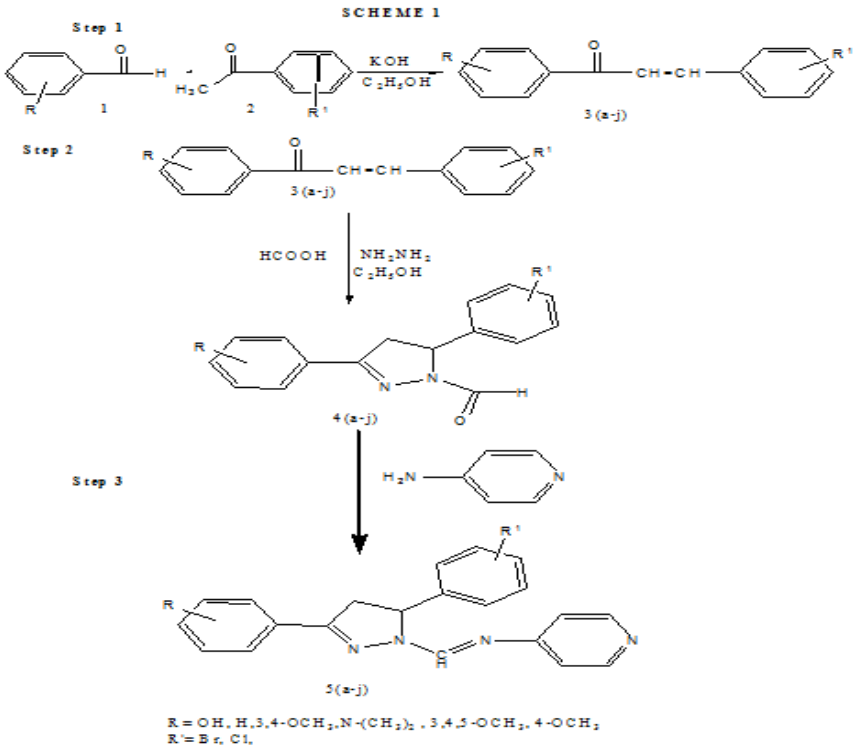
Ligand preparation: The molecules were drawn in JME editor and then optimized by using prodrg server. The pdb formats of the ligand were opened in ADT, polar hydrogens and charges of the Gasteiger-type were assigned and the non polar hydrogens were merged with the carbons and saved in pdbqt format.

Grid box generation: A grid box was generated using spacing of 0.375 A˚ with 70 x 70 x 70 points was set. The grid was placed around the catalytic clef of the protein for docking.

Docking: Lamarckian genetic algorithm was used for the conformational sampling. For each docking simulation, 10 independent runs were carried out with a population size of 150 and $25x10^5$ energy evaluations. The lowest binding energy with maximum cluster size was considered for all further interaction studies.

RESULTS AND DISCUSSION

Chemistry



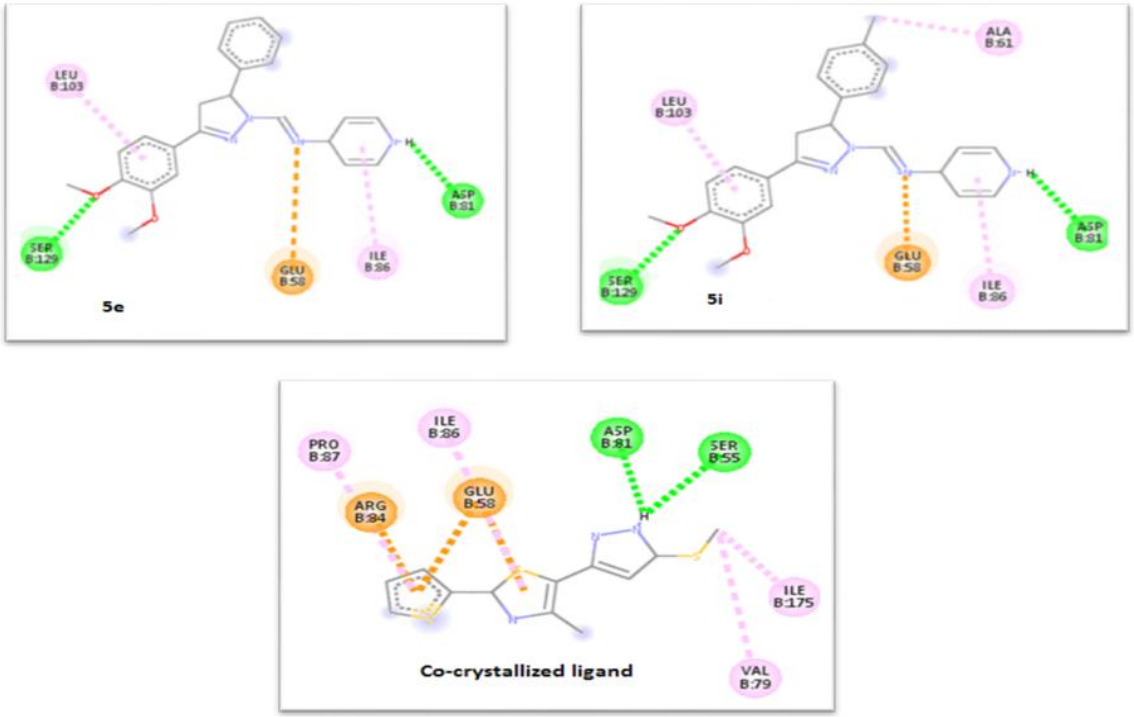


Fig 1: Intearction diagrams for compounds 5e, 5i and the co-crystallized ligand into the active site of G75. Green lines indicate H-bonds formed between the compounds and the enzyme active site residues.

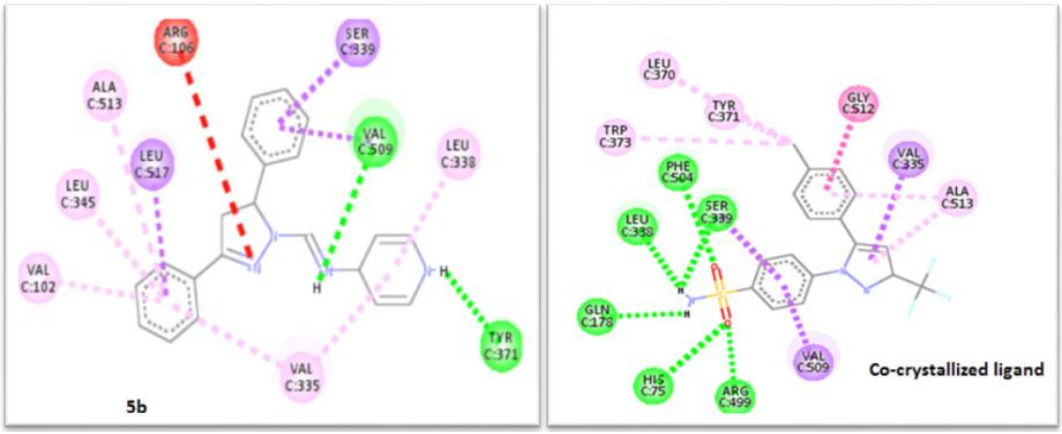


Fig 2: Intearction diagrams for compounds 5b and the co-crystallized ligand into the active site of 3LN1.Green lines indicate H-bonds formed between the compounds and the enzyme active.

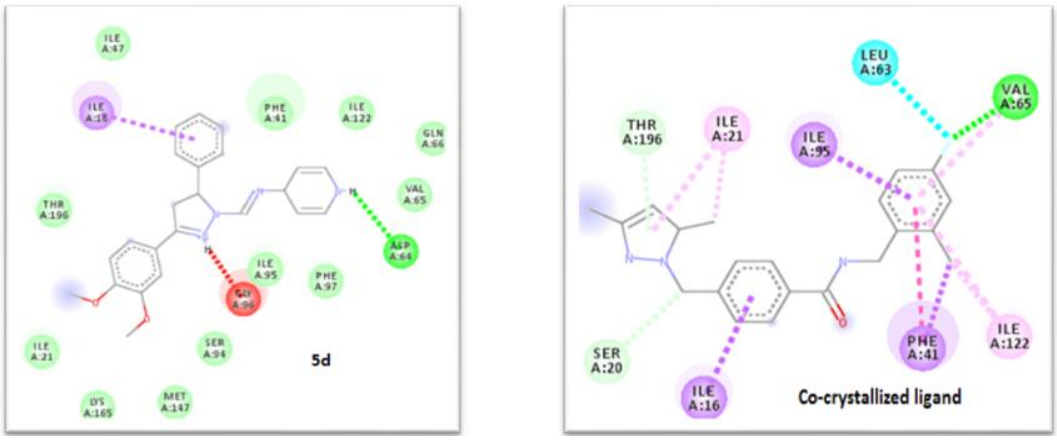


Fig 3: Intearction diagrams for compounds 5d and the co-crystallized ligand into the active site of 4QXM.Green lines indicate H-bonds formed between the compounds and the enzyme active.

The IR spectra of pyrazoline carbaldehyde series have shown the presence of carbonyl stretch at a higher range of 1700 cm^{-1} which is absent in the Schiff bases 5(a-j). The imine formations have been confirmed by the presence of a prominent peak at 1587 cm^{-1} (5a). All the derivatives have shown a prominent peak at 1269 cm^{-1} indicating the presence of aromatic C=N group.

The ^{1}HNMR spectra of the titled compounds revealed the presence of imine proton along with the multiplet of aromatic protons in the range of 7.0192-7.8932 ppm. The pyridyl protons are distinctly seen in the range of 8.858 ppm.

The formation of imine carbon is confirmed by the presence of a single peak at δ value of 165 ppm confirming the formation of titled Schiff bases. The δ values of pyridyl carbon (150-155 ppm) and the aromatic carbon (120-130 ppm) clearly indicate their presence in the titled compounds. The δ values of carbon in the range of 40-50 ppm indicate the presence of pyrazole moiety.

Mass spectra of the compounds are in agreement with their molecular formula

The structures are also confirmed from CHN analysis wherein the calculated and experimental values of the elements present in the compounds do not differ by ±0.5.

Biological Activities

Antitubercular activity: All the synthesized compounds were screened for antitubercular activity by MABA method. All the compounds showed mild activity with a MIC value of 100 µg/ml as compared to standard Pyrazinamide- 3.125µg/ml and Streptomycin- 6.25µg/ml. **5a** with hydroxyl and chloro substitutents on the benzene rings showed activity with a MIC value of 25 µg/ml.

Antibacterial activity: In case of antibacterial, some interesting results have been highlighted. Most of the derivatives have shown good activity against *P.aeruginosa* and *S.pneumoniae*. Some of the derivatives 5b, 5c, 5e and 5i with Br, OH and Cl, 1, 2, 3-OCH_3 and Br, 3, 4-OCH_3 and Cl substitutents respectively have shown MIC value in the range of 3.12 µg/ml as compared to the standard having MIC value of 3.125 µg/ml whereas one derivative 5j has shown MIC value more than the standard against *P.aeruginosa*. Derivatives 5a, 5d, 5f, 5h with OH and Br, 3,4-OCH_3 and Br, N-$(CH_3)_2$ and Br,4-OCH_3 and Br substitutents respectively having MIC value of 6.25 µg/ml have shown moderate antibaceterial activity.

Except for 5g with 3,4,5-OCH_3 and Cl substitutents , all the derivatives have shown MIC value more than that of standard indicating that they can be good candidate for antibacterial action against *S.pneumoniae*.

The activity may be contributed by the presence of three different moiety in the titled Schiff's bases namely benzene, pyrazole, pyridine and imine N. But all the derivatives have shown poor activity against *E.coli* and *S.aureus* which indicates our synthesized compounds have narrow spectrum of activity.

Anti-inflammatory Activity: It is interesting to note that that most of the derivatives have shown good activity when compared to the standard indomethacin. Compounds 5b and 5c having Br, OH and Cl substitutents respectively exhibited higher percentage inhibition when compared to the standard (89% and 90% at 100 and 200 µg/ml respectively and indomethacin 90% and 91% at the same concentration). Within the series 5f, 5g and 5j with N-$(CH_3)_2$ and Br, 1, 2, 3-OCH_3 and Cl, Cl substitutents respectively have shown mild activity. The activity can be attributed due to the presence of pyrazole moiety in the ring as some of the standard antiinflammtory agents like phenylbutazone, oxyphenbutazone and celecoxib contain pyrazole as the prominent moiety.[61]

Antioxidant Activity: All the synthesized compounds were screened for antioxidant activity by DPPH and NO radical scavenging methods. However it was observed that the % inhibition exhibited by DPPH method is high as compared to the NO method for both the standard as well as for the test samples. This may be attributed due to low generation of nitric ions by reaction with sodium nitroprusside at physiological pH in phosphate buffer. By comparing both the methods it was observed that compounds 5a, 5d, 5i having OH and Br, 3,4-OCH_3 and Br, 3,4-OCH_3 and Cl substitutents respectively have shown good % inhibition (86, 87, 91 by DPPH and 37, 39, 33 by NO) as compared to the standard (97 by DPPH and 40 by NO) at the same concentration i.e 10 µg/ml. Within the series 5b, 5c, 5e, 5f, 5h, 5i having Br, OH and Cl, 3, 4, 5-OCH_3 and Br, N-$(CH_3)_2$ and Br, 4-OCH_3 and Br, Cl substitutents have shown mild antioxidant activity as compared to the standard. But none of the compounds have shown activity greater than the standard.

Docking Results

Molecular docking studies of the synthesized compounds were performed in order to rationalize the obtained biological results. Molecular docking studies further help in understanding the various interactions between the ligand and enzyme active site in detail. The Pdb codes 3G75,[62] 3LN[63] and 4QXM[64] were downloaded from the protein data bank www.pdb.org then docking was carried out for the structural proteins.

Antibacterial: As evident from the invitro studies all the derivatives showed a good docking score more than the reference ligand against 3G75 (co-crystallized ligand as pyrazothiazole). These derivatives formed hydrogen bonding with Ser B129, Asp B81, Asn B54. Some of these amino acids were also present in the binding interactions of the co-crystallized ligand. It was a clear indication that the pyridine ring and the imine N had an enough role in binding of drug with the receptor. It was

also observed that among all the substituent in the series, the methoxy group which was present in 5a, 5d, 5e, 5h and 5i formed good binding interactions which correlated well with the obtained MIC values (shown in table 2).

Antiinflammatory: The derivatives had docking score very near to the reference ligand (Celecoxib as the co-crystalized ligand). Among the series 5b (having H and Br substitutent) had the highest docking score of -10.4 which is in agreement with the obtained percentage inhibition (89 % and 90% at 100 and 200 µg/ml respectively**)** result obtained through invitro BSA method. Some of the amino acid residues which are involved in H-bonding interactions were Val C509, Tyr C371, Phe C504 and Ser C457.

Antitubercular: Although the binding interactions and the docking scores obtained were quite satisfactory but the obtained MIC value through MABA method did not validate with each other indicating that pyrazole moiety may not be an active pharmacophore for inhibiting *Mycobacterium tuberculosis***.**

Table 1: Antitubercular activity by MABA method.

Sl. No	Compound Code	MIC (µg/ml)
1	5a	50
2	5b	100
3	5c	100
4	5d	100
5	5e	100
6	5f	100
7	5g	100
8	5h	100
9	5i	100
10	5j	100

Pyrazinamide- 3.125µg/ml Streptomycin- 6.25µg/ml

Table 2: MIC value of the synthesized compounds against various strains of bacteria.

Sl No.	Comp code	*E.coli*	*P.aeruginosa*	*S. aureus*	*S.pneumoniae*
		MIC in µg/ml			
1	5 a	100	6.25	50	0.4
2	5b	50	3.12	100	0.8
3	5c	100	3.12	50	0.8
4	5d	100	6.25	100	0.4
5	5e	25	3.12	100	0.4
6	5f	25	6.25	100	0.4
7	5g	50	25	100	25
8	5h	50	6.25	100	0.4
9	5i	50	3.12	100	0.8
10	5j	50	0.4	100	0.8

Ciprofloxacin-3.125µg/ml

Table 3: *In-vitro* anti-inflammatory activity.

Sl No.	Comp Code	% Inhibition	
		100 µg/ml	200µg/ml
1	5a	88	89
2	5b	89	90
3	5c	89	90
4	5d	86	90
5	5e	85	87
6	5f	77	88
7	5g	56	60
8	5h	87	89
9	5i	84	85
10	5j	66	72
Standard	Indomethacin	90	91

Table 4: DPPH free radical scavenging activity.

Sl No.	Comp Code	% Inhibition			
		10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
1	5a	86	91	92	93
2	5b	48	49	49	52
3	5c	40	42	43	50
4	5d	87	88	89	90
5	5e	40	43	49	54
6	5f	50	56	59	63

7	5g	83	84	85	87
8	5h	65	67	68	72
9	5i	91	92	92	94
10	5j	70	71	72	73
11	Ascorbic acid	97	98	98	99

Table 5: Nitric oxide free radical scavenging activity.

Sl No.	Comp Code	% Inhibition		
		10 µg/ml	50 µg/ml	100 µg/ml
1	5a	37	40	41
2	5b	20	23	28
3	5c	18	26	31
4	5d	39	42	48
5	5e	19	23	32
6	5f	22	25	40
7	5g	25	28	32
8	5h	30	36	41
9	5i	33	40	45
10	5j	24	25	28
11	Ascorbic acid	40	55	62

Table 6: The data representing binding energies of synthesized compounds to various protein targets.

Compound Code	Binding energy score (Kcal/mol)					
	3G75	Hydrogen bond	3LN1	Hydrogen bond	4QXM	Hydrogen bond
5a	-8.3	SerB129, Asp B81	-7.8	-	-8.9	PheA41
5b	-8	Asn B54	-10.4	ValC509, TyrC371	-9.3	GlyA14
5c	-8.1	Asn B54	-9.7	PheC504	-9.2	AspA64
5d	-8.1	SerB129	-8.1	SerC457	-10.4	AspA64
5e	-8.4	SerB129, Asp B81	-7.9	-	-8.7	ThrA196
5f	-7.7	Asn B54	-8.2	-	-9.4	SerA94
5g	-7.7	Asp B81	-7.7	-	-9	IleA21
5h	-8.3	SerB129, Asp B81	-7.8	-	-8.9	PheA41
5i	-8.4	SerB129, Asp B81	-7.8	-	-9.3	GlyA96, LeuA63
5j	-8	Asn B54	-7.8	ValC509	-9.5	GlyA14
Reference Ligand	-6.5	AspB81, SerB55	-13.1	PheC504,ArgC499, HisC75,LeuC338, SerC339, GlnC178	-9.3	ValA65

Table 7: Calculation of molecular properties of [5-(4-substituted-phenyl)-3-(substituted-phenyl)-4, 5-dihyro-pyrazol-1-ylmethylene]-pyridin-4-yl-amine MKS2(a-j).

Comp Code	miLog P	TPSA	Natoms	MW	nON	nOHNH	nviolations	nrotb	volume
5a	4.15	61.09	27	421.30	5	1	0	4	330.70
5b	4.63	40.86	26	405.30	4	0	0	4	322.68
5c	4.02	61.09	27	376.85	5	1	0	4	326.35
5d	4.28	59.33	30	465.35	6	0	0	6	373.77
5e	4.26	68.56	32	495.38	7	0	0	7	399.32
5f	4.73	44.10	29	448.37	5	0	0	5	368.59
5g	4.13	68.56	32	450.93	7	0	0	7	394.97
5h	4.69	50.09	28	435.32	5	0	0	5	348.23
5i	4.15	59.33	30	420.90	6	0	0	6	369.42
5j	4.50	40.86	26	360.85	4	0	0	4	318.33

CONCLUSION

The titled Schiff's bases were obtained by reaction of substituted aldehydes with ketones to obtain chalcones. These chalcones were cyclized with formic acid and hydrazine hydrate to form pyrazole carbaldehyde which undergone reaction with p-amino pyridine to form the corresponding Schiff's bases. The compounds were characterized by IR, NMR (1H & C-13), Mass and CHN analysis. All the compounds were obtained from moderate to good yields. Various invitro biological activities were carried out like anti-TB, antibacterial, anti-inflammatory and antioxidant. All the derivatives were subjected to drug likeness profile , in which all the derivatives fall within the limits criteria of Lipinski Rule.Some of the derivatives have shown good activity which are in agreement with the obtained *insilico* studies

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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