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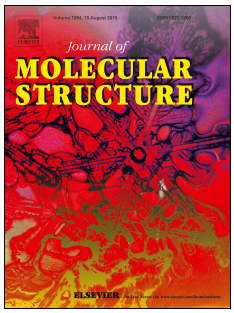

Novel 1,4-dihydropyrano[2,3-c]pyrazole derivatives: Synthesis, characterization, biological evaluation and *in silico* study

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Novel 1,4-dihydropyrano[2,3-c]pyrazole derivatives: Synthesis, characterization, biological evaluation and *in silico* study

Mahesh S. Vasava[a], Manoj N. Bhoi[b], Sanjay K. Rathwa[a], Shilpa S. Shetty[c], Rikin D. Patel[c], Dhanji P. Rajani[d], Smita D. Rajani[d], Alpesh Patel[e], Himanshu A. Pandya[c] and Hitesh D. Patel*[a]

[a]*Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad, Gujarat India.*

E-mail: drhiteshpatel1@gmail.com

[b]*Piramal Enterprise Ltd, Plot No.-18, Pharmez, Matoda Village, Ahmedabad.*

[c]*Department of Botany, Bioinformatics and Climate Change impacts Management, School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.*

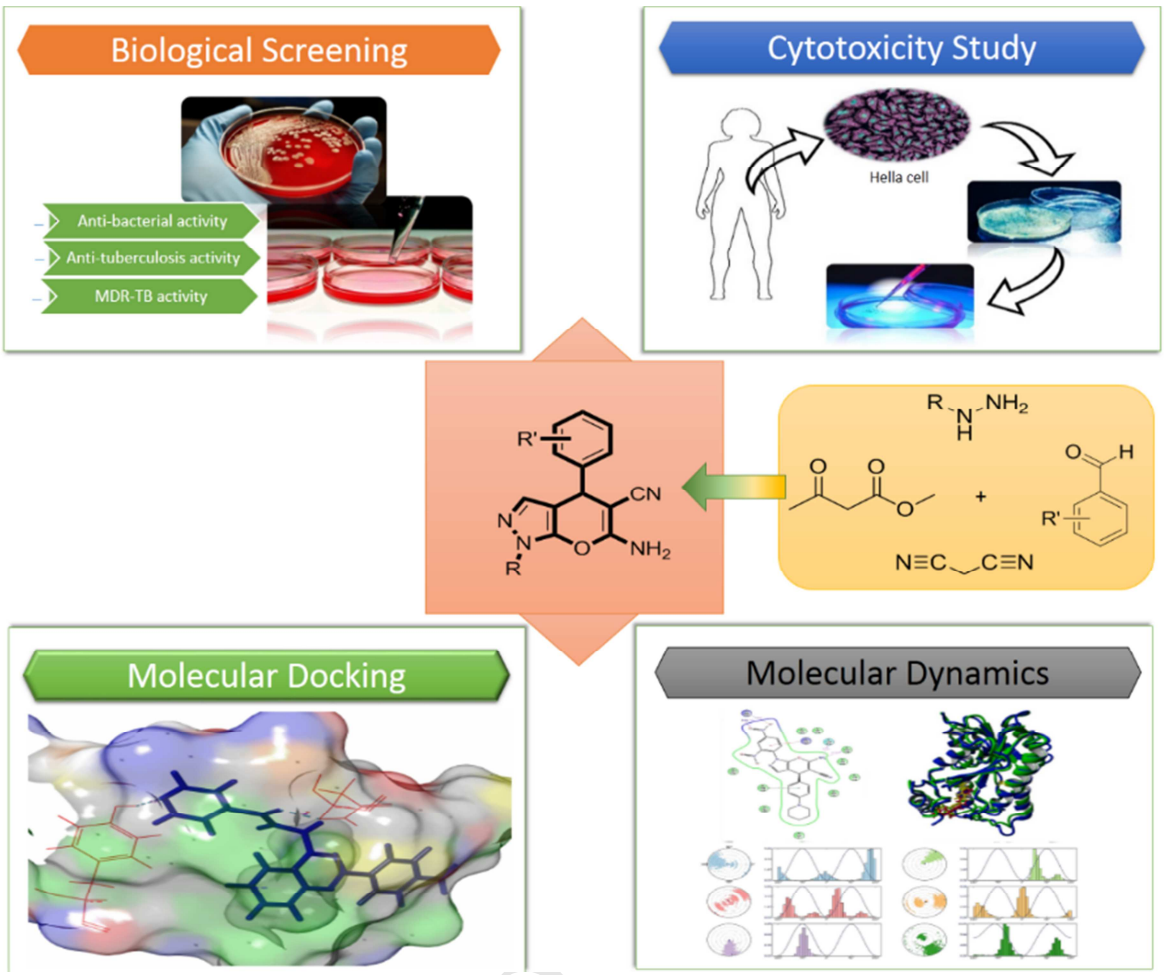
[d]*Microcare Laboratory & Tuberculosis Research Centre, Surat, Gujarat, India,*

[e]*Genxplore Diagnostic and Research Centre Pvt. Ltd., Ahmedabad, Gujarat, India.*

Abstract

In the present study, a series of novel and biologically potent 6-amino-1-(2,4-dinitrophenyl)-4-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile derivatives (**5a-5u)** have been synthesized through multicomponent reaction between various substituted aromatic aldehyde derivative (**4a**-**4u**), 2, 4-dinitrophenyl hydrazine (**1**), ethyl acetoacetate **(2)** and malononitrile **(3)** in the presence of $SnCl_2$ as a prompt catalyst using both microwave irradiation method as well as conventional method. The structure of synthesized compounds were confirmed by various spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR, IR, Mass analysis and elemental analysis. All the synthesized compounds were subjected *in vitro* antibacterial, anti-tuberculosis screening and cytotoxicity MTT assay. *In vitro* biological study revealed that the synthesized compound **5a, 7a** and **8a** are showing good anti-bacterial and anti-tuberculosis activity. The *in silico* study of ADME pharmacokinetic properties were also predicted for synthesized compounds for checking their bioavailability. Furthermore, molecular docking study of synthesized compounds with enoyl-ACP reductase (oxidoreductase) was carry out to find out the binding affinity of compounds. Docking study demonstrated that compound **7b** and **7a** possessed superior binding affinity with target enzyme by strong hydrogen bonding. We have also carried out molecular dynamics simulation to check the stability of docked complex, conformational changes and primary molecular interaction.

Keywords: anti-tuberculosis activity; anti-bacterial activity; molecular docking; molecular dynamics; one-pot synthesis; pyrano[2,3-c]pyrazole;


Biological Screening
Anti-bacterial activity
Anti-tuberculosis activity
MDR-TB activity
Cytotoxicity Study
Hella cell
R'
CN
NH2
R
N
N
O
R
NH2
O
O
O
+
O
H
R'
N≡C
C≡N
Molecular Docking
Molecular Dynamics

1. **Introduction**

The treatment of infectious diseases that are predominantly endemic into the developing countries, it requires simple medications that can be produced in large quantities at low cost. Tuberculosis (TB) is most dangerous infectious disease which causes maximum fatality by subverting the immune system of the human host due to its long and wide association with humans and is often regarded as a successful pathogen [1]. According to the WHO report 2017, one-third of the world population is potentially infected with TB and millions of new cases occur worldwide every year [2].

There are many drugs available for TB in market, among them, Isoniazid (INH), Rifampicin, Pyrazinamide, and Ethambutol are four milestones in the treatment of TB for more than 50 years. INH is pro-drug which activate by the mycobacterial catalyse peroxidase (KatG), which inhibit the enoyl-ACP reductase of the mycobacterial fatty-II type acid. It involved in the biosynthesis of mycolic acid eventually leading to cell death [3-4]. Studies have confirmed that the front-line anti-TB drugs such as INH and ethionamide is primary targeting inhA gene [3]. Freundlich et al. revealed that potent triclosan derivatives that inhibit InhA in the nanomolar range with minimum inhibitory concentrations of 5–10 μg/mL [5]. Additionally, several compounds have been reported, such as arylamides derivatives [6], pyrrolidine carboxamide derivatives [7], pyrazole derivatives and indole-5-amides derivatives [8] which targeting inhA enzyme. However, the increasing prevalence of multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB decrease the effectiveness of these drugs against tuberculosis. Hence, to stop this infection, there is a burning requirement to develop inhibitors targeting InhA directly without prerequisite for activation [9].

Owing to the vast research on anti-tubercular activity, many synthesized heterocyclic compounds have efficiently displayed anti-tubercular activity. Current literature describing that the 4*H*-pyrans and pyran-annulated heterocyclic scaffolds have drawn considerable

interest in medicinal chemistry from the last several years [10-16]. Figure 1 represents some of the bioactive pyran-annulated heterocyclic compounds which are good antibacterial agents. Various studies have indicated 4*H*-pyran derivatives display a potent activity against mycobacterium [17-29] as well as anticancer [20], cytotoxic [21], anti-inflammatory [22], anti-HIV [23-24], antimalarial [25], anti-hyperglycemic, and anti-dyslipidemic [26], anti-neurodegenerative disorders like Alzheimer's, Parkinson disease, Huntington's disease [27], and many more [28-39]. Furthermore, substituted 4*H*-pyran derivatives have encouraged increasing roles in synthetic methodologies to promising compounds in the field of medicinal [30], pigment industries [31], agrochemical and cosmetics [32]. Keeping this knowledge, synthetic chemists develop useful synthetic routes to synthesize these heterocyclic compounds. A lot of synthetic methodologies are already reported.

Nowadays, the most straightforward method for the synthesis of various heterocyclic compounds involves a three-component reaction or multi-component reaction to avoid the setbacks such as time-consuming reaction, low yields, toxicity of the chemicals, harmful organic solvents, dynamic reaction conditions and strenuous work-up. Additionally, Microwave-assisted synthesis is one of the most prominent method increasing its importance in the field of organic chemistry [33]. As compared to conventional heating, microwave irradiation method is much faster and provide higher practical yield in many organic synthesis [34]. Due to this advantage, this technique is widely used in the organic synthetic chemistry. In context to the already existing literature [35-45], we decided to use a simple, rapid, and effective one-pot MCR methodology to synthesize the targeted compounds.

In this current study, our aim was to synthesize new structural moieties under microwave irradiation and conventional heating. The 1,4-dihydropyrano[2,3-c]pyrazole derivatives synthesized by the reaction between substituted aldehyde derivatives, ethyl acetoacetate, malononitrile and 2,4-dinitrophenyl hydrazine using $SnCl_2$ as a rapid catalyst

under solvent-free condition with good practical yields. All the synthesised compounds were screened for their anti-bacterial, anti-tuberculosis activity against various bacterial strain and *$H_{37}Rv$* respectively and cytotoxic activity against HeLa cell line.

In addition, the ADME pharmacokinetic properties of synthesized compounds have studied for gaining preliminary information concerning their possibly drug-like profile. Moreover, based on the favourable *in vitro* antimicrobial results and by considering NADH-dependent enoyl-Acyl Carrier Protein reductase (enoyl-ACP reductase) as the target receptor, Molecular docking and molecular dynamics studies was performed against the active site of the NADH-dependent enoyl-Acyl Carrier Protein reductase (enoyl-ACP reductase) encoded by the Mycobacterium gene inhA *Mycobacterium tuberculosis* oxidoreductase enzyme.

2. Methods and Materials

2.1 Chemistry

All the chemicals and solvents were purchased from commercial source and were used in the reaction without further purification. Synthesis of the desired product was carried out using conventional and microwave irradiation (CEM Discover microwave system, Model No. 908010; Make up CEM Matthews, Inc, USA) methods. Melting points were determined by automated melting point system (MPA- Optimelt) and were uncorrected. The completion of the reactions was checked by thin layer chromatography (TLC). It is silica gel coated aluminium sheets (silica gel G60 F_{254}, Merck) which was visualized by UV radiation and various spray reagent. Elemental analysis were performed on vario MICRO cube, elemental CHNS analyser (Serial Number: 15084053). The IR spectra (in KBr pellets) were recorded on a Perkin-Elmer 377 spectrophotometer with absorption in cm^{-1}. ^{1}H and ^{13}C NMR spectra were run on Bruker 400 and 100 MHz NMR spectrometer respectively, using DMSO-*d6* as solvents. The chemical shifts were expressed in parts per million (δ ppm) with TMS as internal reference. J values are given in hertz (Hz). Mass spectra was recorded on Advion

Expression CMS, USA, with electro-spray ionization (ESI) was used as an ion source and ethanol: formic acid: water was used as a mobile phase. Column Chromatography with 60-120 mesh size of silica gel was used to purify the synthesized compounds.

2.1.1 Synthesis of 4-(piperidin-1-yl) benzaldehyde derivatives (6a-6b):

To a stirred solution of 4-fluoro aldehyde **(5g)** (1.0 eq.) in Methanol was added piperidine (1.0 eq.) and anhydrous Potassium carbonate (10 mol %) at room temperature. The resulting reaction mixture was heated at 130 °C for 20h. The progress of reaction was monitored by TLC plate. The reaction mixture was cooled at room temperature and then poured it into ice cold water and kept for 24h. The solid product was precipitated out, filtered it using vacuum filtration and washed with hot water. The crude material was crystallized with Methanol to get pure compounds *4-(piperidin-1-yl) benzaldehyde derivatives* **6a-6b** with good practical yield.

2.1.2 General procedure for the synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-phenyl-1,4 dihydropyrano[2,3-c]pyrazole-5-carbonitrile derivatives (5a-5u):

2.1.2.1. Microwave irradiation method (Method A)

An equimolar mixture of 2,4-dinitrophenyl hydrazine **(1)** (1.0 eq.), ethyl acetoacetate **(2)** (1.0 eq.), malononitrile **(3)** (1.0 eq.), aldehyde derivatives **(4)** (1.0 eq.) in the presence of $SnCl_2$ (10 mol %) as a catalyst was irradiated in microwave (CEM Discover microwave) at 180 W for 10-20 min. The progress of the reaction was continuously observed by TLC analysis. After the consumption of the starting material, the reaction mixture was cooled at room temperature and poured into ice cold water and extracted with ethyl acetate (2 x 30 mL). The organic layer was dried with anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude material was purified through column chromatography using 20% Ethyl acetate in Hexane as an eluent to get desired compound 6-amino-1-(2,4-

dinitrophenyl)-4-phenyl-1,4dihydropyrano[2,3-c]pyrazole-5-carbonitrile derivatives (**5a-5u**) with good practical yield (61-89%).

2.1.2.2. Conventional Method (Method B)

All the reaction mixtures were taken same as mention in Method A in 50 ml FBF (Flat Bottomed Flask) in the presence of $SnCl_2$ (10 mol %) as a catalyst. The reaction mixture was stirred at 80 °C for appropriate time mentioned in Table 1. The progress of the reaction was observed by TLC analysis. After the completion of reaction, the reaction mixture allowed cool at room temperature and poured into ice cold water and extracted with ethyl acetate (2 x 30 mL). The organic layer was dried with anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product (**5a-5u**) were then dried and were further purified in to column chromatography using 20 % (v/v) Ethyl acetate: Hexane mixture as an eluent which resulted in 54-81 % yield of the final product.

2.1.2.3. 6-amino-1-(2,4-dinitrophenyl)-4-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5a).

Brownish red solid, m.p. 240-243 °C.; Anal. Calc. for $C_{19}H_{12}N_6O_5$: C, 56.44; H, 2.99; N, 20.78; O, 19.78%. Found- C, 55.85; H, 2.34; N, 20.16%.; IR ν_{max} (KBr) cm^{-1}: 3323 ($N\text{-}H_{str}$), 3106 ($C\text{-}H_{Aromatic,str}$), 1613 ($C{=}C_{str}$), 2249 (-CN), 1023 ($C\text{-}O_{str}$), 1514, 1346 ($N\text{-}O_{str}$).; ^1H NMR: (400 MHz, DMSO): δ; 9.642 (s, 1H), 8.936-8.921 (d, *J* = 6 Hz, 1H), 8.471 (s, 2H), 8.150-8.142 (d, *J* = 3.2 Hz, 1H), 7.900 (s, 1H), 7.572-7.567 (d, *J* = 2 Hz, 2H), 7.358 (s, 1H), 7.317-7.313 (d, *J* = 1.6 Hz, 2H), 4.656 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 174.91. 151.30, 145.86, 142.19, 139.87, 137.22, 135.31, 129.72, 129.46, 128.23, 128.10, 127.35, 125.29, 125.08, 119.78, 117.92, 116.89, 60.18, 28.14; ESI-MS: *m/z* Calculated 404.34, found [*m/z*] $[M+H]^+$405.1.

2.1.2.4. (E)-6-amino-1-(2,4-dinitrophenyl)-4-styryl-1,4-dihydropyrano[2,3-c]pyrazole-5carbonitrile (5b).

Yellow solid, m.p. 248-251 °C.; Anal. Calc. for $C_{21}H_{14}N_6O_5$: C, 58.61; H, 3.28; N, 19.53; O, 18.59%. Found- C, 57.69; H, 3.10; N, 18.83%.; IR ν_{max} (KBr) cm^{-1}: 3417 ($N\text{-}H_{str}$), 2955 ($C\text{-}H_{Aromatic,str}$), 1686 ($C{=}C_{str}$), 2349 (-CN), 1270 ($C\text{-}O_{str}$), 1596, 1349 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ, 9.637 (s, 1H), 8756 - 8.739 (s, *J* = 6.8 Hz1H), 8.644 (s, 2H), 8.573 - 8.562 (d, *J* = 4.4 Hz, 1H), 7.958 (s, 1H), 7.635-7.620 (d, *J* = 6 Hz, 2H), 7.511-7.499 (d, *J* = 4.8 Hz, 1H), 7.480-7.469 (d, *J* = 4.4 Hz, 2H), 6.379-6.361 (s, *J* = 7.2 Hz, 1H), 6.035-6.021 (d, *J* = 5.6 Hz, 1H), 4.357 (s, 1H) .; ^{13}C NMR: (100 MHz, DMSO): δ; 174.82, 152.01, 147.86, 142.97, 140.35, 136.24, 135.87, 131.02, 128.51, 128.48, 128.37, 128.04, 127.35, 127.02, 123.61, 127.02, 120.34, 119.76, 119.07, 118.65, 60.04, 28.17; ESI-MS: *m/z* Calculated 430.37, found [*m/z*] $[M+H]^+$431.3.

2.1.2.5. 6-amino-4-(3-chlorophenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5c).

Light yellow solid, m.p. 260-264 °C.; Anal. Calc. for $C_{19}H_{11}ClN_6O_5$: C, 52.01; H, 2.53; Cl, 8.08; N, 19.15; O, 18.23%. Found- C, 52.34; H, 2.24; N, 19.67%.; IR ν_{max} (KBr) cm^{-1}: 3282 ($N\text{-}H_{str}$), 3093 ($C\text{-}H_{Aromatic,str}$), 1612 ($C{=}C_{str}$), 2192 (-CN), 1059 ($C\text{-}O_{str}$), 1512, 1344 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ, 10.108 (s, 1H), 9.235-9.226 (d, *J* = 3.6 Hz, 1H), 8.982 (s, 2H), 8.834-8.821 (d, *J* = 5.2 Hz, 1H), 7.990 (s, 1H), 7.701-7.692 (d, *J* = 3.6 Hz, 2H), 7.508-7.490 (d, *J* = 7.2 Hz, 2H), 4.887 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 174.62. 151.17, 146.43, 142.30, 140.79, 137.60, 137.21, 134.68, 129.73, 128.23, 127.88, 127.37, 125.62, 124.39, 120.03, 118.40, 117.79, 58.42, 26.54; ESI-MS: *m/z* Calculated 438.78 found, [*m/z*] 438.2 $[M+H]^+$ 439.2.

2.1.2.6. 6-amino-4-(2,5-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5d).

Brownish yellow solid, m.p. 286-289 °C.; Anal. Calc. for $C_{21}H_{16}N_6O_7$: C, 54.31; H, 3.47; N, 18.10; O, 24.12%. Found- C, 54.24; H, 3.31; N, 17.34%.; IR ν_{max} (KBr) cm^{-1}: 3439 ($N\text{-}H_{str}$), 3076 ($C\text{-}H_{Aromatic,str}$), 1626 ($C{=}C_{str}$), 2352 (-CN), 1065 ($C\text{-}O_{str}$), 1525, 1381 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ, 10.929 (s, 1H), 8.914-8.908 (d, *J* = 2.4 Hz, 1H), 8.589 (s, 2H), 8.432-8.420 (s, *J* = 4.8 Hz, 1H), 7.822 (s, 1H), 7.369 (s, 1H), 7.181-7.159 (d, *J* = 8.8 Hz, 1H), 7.066-7.052 (d, *J* = 5.6 Hz, 1H), 5.005 (s, 1H), 3.778 (s, 3H).; ^{13}C NMR: (100 MHz, DMSO): δ; 175.35. 152.08, 151.62, 150.20, 144.78, 142.80, 139.83, 126.71, 124.63, 121.01, 117.90, 116.86, 115.32, 114.13, 113.67, 113.085, 60.64, 55.80, 54.58, 29.01; ESI-MS: *m/z* Calculated 464.39, found [*m/z*] $[M+H]^+$ 465.2.

2.1.2.7. 6-amino-4-(2-chlorophenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5e).

Brown solid, m.p. 260-264 °C.; Anal. Calc. for $C_{19}H_{11}ClN_6O_5$: C, 52.01; H, 2.53; Cl, 8.08; N, 19.15; O, 18.23%. Found- C, 52.42; H, 2.69; N, 19.40%.; IR ν_{max} (KBr) cm^{-1}: 3425 ($N\text{-}H_{str}$), 2979 ($C\text{-}H_{Aromatic,str}$), 1595 ($C{=}C_{str}$), 2197 (-CN), 1060 ($C\text{-}O_{str}$), 1513, 1343 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.978 (s, 1H), 9.540-9.534 (d, *J*=2.4 Hz, 1H), 9.212 (s, 2H), 8.937-8.925 (s, *J* = 4.8 Hz, 1H), 7.962 (s, 1H), 7.802 (s, 1H), 7.404-7.398 (d, *J* = 2.4 Hz, 2H), 7.205-7.192 (d, *J* = 5.2 Hz, 2H), 4.895 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 175.29, 152.00, 146.02, 143.77, 141.90, 39.68, 136.73, 131.90, 129.11, 128.48, 127.95, 126.64, 126.35, 124.51, 120.03, 117.64, 116.84, 58.97, 24.35; ESI-MS: *m/z* Calculated 438.78, found [*m/z*] $[M+H]^+$ 439.3.

2.1.2.8. 6-amino-1-(2,4-dinitrophenyl)-4-(3-nitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5f).

Yellow solid, m.p. 283-286 °C.; Anal. Calc. for $C_{19}H_{11}N_7O_7$: C, 50.79; H, 2.47; N, 21.82; O, 24.92%. Found- C, 51.24; H, 2.19; N, 21.32%.; IR ν_{max} (KBr) cm^{-1}: 3316 ($N\text{-}H_{str}$), 3111 ($C\text{-}H_{Aromatic,str}$), 1619 ($C{=}C_{str}$), 2214 (-CN), 1030 ($C\text{-}O_{str}$), 1513, 1334 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ; 10.124 (s, 1H), 9.582-9.571 (d, *J*=4.4 Hz1H), 8.969 (s, 2H), 8.590-8.578 (d, *J* = 4.8 Hz, 1H), 8.504-8.496 (d, *J* = 3.2 Hz, 2H), 7.946 (s, 1H), 7.877-7.864 (d, *J* = 5.2 Hz, 2H), 4.681 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 175.79. 151.14, 146.02, 145.82, 142.04, 140.60, 137.58, 136.43, 135.29, 133.82, 126.90, 123.73, 121.34, 120.15, 120.06, 119.83, 117.01, 59.42, 26.38; ESI-MS: *m/z* Calculated 449.33, found [*m/z*] $[M+H]^+$ 450.1.

2.1.2.9. 6-amino-1-(2,4-dinitrophenyl)-4-(2-nitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5g).

Yellow solid, m.p. 283-286 °C.; Anal. Calc. for $C_{19}H_{11}N_7O_7$: C, 50.79; H, 2.47; N, 21.82; O, 24.92%. Found- C, 50.19; H, 2.44; N, 21.17%.; IR ν_{max} (KBr) cm^{-1}: 3313 ($N\text{-}H_{str}$), 2978 ($C\text{-}H_{Aromatic,str}$), 1597 ($C{=}C_{str}$), 2349 (-CN), 1088 ($C\text{-}O_{str}$), 1515, 1345 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.628 (s, 1H), 9.064-9.053 (d, *J* = 4.4 Hz, 1H), 8.8669 (s, 2H), 8.179-8.165 (d, *J* = 5.6 Hz, 1H), 8.342-8.312 (d, *J* = 12 Hz, 1H), 7.854-7.833 (d, *J* = 8.4 Hz, 1H), 7.546 (s, 1H), 7.530 (s, 1H), 7.461-7.455(d, *J* = 2.4 Hz, 1H), 4.982 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 176.28. 150.84, 150.05, 146.08, 141.62, 140.32, 137.82, 134.57, 133.34, 128.68, 127.10, 126.34, 123.93, 123.61, 119.83, 117.01, 116.30, 59.63, 25.67; ESI-MS: *m/z* Calculated 449.33, found [*m/z*] $[M+H]^+$ 450.1.

2.1.2.10. 6-amino-1-(2,4-dinitrophenyl)-4-(furan-3-yl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5h).

Dark Red solid, m.p. 263-268 °C.; Anal. Calc. for $C_{17}H_{10}N_6O_6$: C, 51.78; H, 2.56; N, 21.31; O, 24.35%. Found- C, 50.91; H, 2.39; N, 21.67%.; IR ν_{max} (KBr) cm^{-1}: 3330 ($N\text{-}H_{str}$), 3121 ($C\text{-}H_{Aromatic,str}$), 1625 ($C{=}C_{str}$), 2211 (-CN), 1180 ($C\text{-}O_{str}$), 1553, 1349 ($N\text{-}O_{str}$).; ^{1}H NMR:

(400 MHz, DMSO): δ; 9.561 (s, 1H), 8.884-8.875 (d, *J* = 3.6 Hz, 1H), 8.585 (s, 2H), 8.382-8.371 (s, *J* = 4.4 Hz, 1H), 7.676 (s, 1H), 7.340-7.328 (d, *J* = 4.8 Hz, 2H), 6.453-6.442 (d, *J* = 4.4 Hz, 1H), 4.720 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 175.14, 152.24, 148.07, 143.58, 143.29, 141.06, 140.47, 137.01, 127.68, 124.78, 120.09, 119.81, 118.97, 118.00, 109.71, 59.47, 22.59; ESI-MS: *m/z* Calculated 394.30, found [*m/z*] $[M+H]^+$ 395.2.

2.1.2.11. 6-amino-1-(2,4-dinitrophenyl)-4-(pyridin-2-yl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5i).

Brownish Red Solid, m.p. 272-276 °C.; Anal. Calc. for $C_{18}H_{11}N_7O_5$: C, 53.34; H, 2.74; N, 24.19; O, 19.74%. Found- C, 54.25; H, 2.34; N, 23.52%.; IR ν_{max} (KBr) cm^{-1}: 3063 ($N\text{-}H_{str}$), 2956 ($C\text{-}H_{Aromatic,str}$), 1628 ($C{=}C_{str}$), 2798 (-CN), 1123 ($C\text{-}O_{str}$), 1628, 1352 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.534 (s, 1H), 8.970 (s, 1H), 8.813 (d, 2H), 8.452 (s, 1H), 8.180 (s, 1H), 7.821 (s, 1H), 7.590-7.582 (d, *J* = 3.2 Hz, 1H), 7.478-7.462 (d, *J* = 6.4 Hz, 1H), 6.594 (s, 1H), 4.565 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 172.85, 156.40, 150.35, 148.54, 147.03, 141.76, 141.02, 137.81, 136.38, 128.57, 124.78, 123.39, 120.47, 120.02, 118.71, 117.50, 59.05, 27.81; ESI-MS: *m/z* Calculated 405.324, found [*m/z*] $[M+H]^+$ 406.1.

2.1.2.12. 6-amino-1-(2,4-dinitrophenyl)-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5j).

Red Solid, m.p. 272-275 °C.; Anal. Calc. for $C_{18}H_{11}N_7O_5$: C, 53.34; H, 2.74; N, 24.19; O, 19.74%. Found- C, 53.28; H, 2.65; N, 23.75%.; IR ν_{max} (KBr) cm^{-1}: 3669 ($N\text{-}H_{str}$), 2777 ($C\text{-}H_{Aromatic,str}$), 1616 ($C{=}C_{str}$), 2354 (-CN), 1031 ($C\text{-}O_{str}$), 1533, 1359 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.865 (s, 1H), 8.946-8.933 (s, *J* = 5.2 Hz, 1H), 8.265 (s, 2H), 8.255-8.247 (s, *J* = 3.2 Hz, 1H), 7.953-7.943 (d, *J* = 4 Hz, 2H), 7.440-7.426(d, *J* = 5.6 Hz, 2H), 7.281 (s, 2H), 4.968 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 150.35, 149.79, 146.79, 146.12,

142.53, 140.58, 137.14, 127.83, 124.69, 124.27, 123.77, 120.35, 118.94, 118.06, 60.10, 28.90; ESI-MS: *m/z* Calculated 405.32, found [*m/z*] $[M+H]^+$ 406.3.

2.1.3.13. 6-amino-4-(4-(dimethylamino)phenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5k).

Brownish green solid, m.p. 275-279 °C.; Anal. Calc. for $C_{21}H_{17}N_7O_5$: C, 56.38; H, 3.83; N, 21.91; O, 17.88%. Found- C, 56.15; H, 3.11; N, 20.57%.; IR ν_{max} (KBr) cm^{-1}: 3324 ($N\text{-}H_{str}$), 2986 ($C\text{-}H_{Aromatic,str}$), 1692 ($C{=}C_{str}$), 2208 (-CN), 1187 ($C\text{-}O_{str}$), 1522, 1356 ($N\text{-}O_{str}$). ^1H NMR: (400 MHz, DMSO): δ; 9.513 (s, 1H), 8.834-8.824 (s, *J* = 4 Hz, 1H), 8.413 (s, 2H), 8.285-8.278 (d, *J* = 2.8 Hz, 1H), 7.880 (s, 1H), 7.385-7.374 (d, *J* = 4.4 Hz, 2H), 6.794-6.782 (d, *J* = 4.8 Hz, 2H), 4.571 (s, 1H) 2.9335 (s, 6H).; ^{13}C NMR: (100 MHz, DMSO): δ; 175.69. 151.12, 150.06, 145.67, 142.20, 139.92, 138.29, 129.11, 128.76, 127.08, 124.81, 124.51, 120.34, 117.70, 116.64, 112.83, 112.09, 60.49, 42.76, 42.7, 29.38; ESI-MS: *m/z* Calculated 447.40, found [*m/z*] $[M+H]^+$ 448.3.

2.1.2.14. 6-amino-1-(2,4-dinitrophenyl)-4-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5l).

Light orange solid, m.p. 296-298 °C.; Anal. Calc. for $C_{22}H_{18}N_6O_8$: C, 53.44; H, 3.67; N, 17.00; O, 25.89. Found- C, 53.20; H, 3.27; N, 17.13%.; IR ν_{max} (KBr) cm^{-1}: 3312 ($N\text{-}H_{str}$), 3098 ($C\text{-}H_{Aromatic,str}$), 1621 ($C{=}C_{str}$), 2312 (-CN), 1135 ($C\text{-}O_{str}$), 1512, 1335 ($N\text{-}O_{str}$).; ^1H NMR: (400 MHz, DMSO): δ; 9.328 (s, 1H), 8.871-8.846 (d, *J* = 10 Hz, 1H), 8.364 (s, 2H), 7.952 (s, 1H), 6.748-6.720 (d, *J* = 11.2 Hz, 1H), 6.671-6.654 (d, *J* = 6.8 Hz, 1H), 4.483 (s, 1H), 3.589 (s, 9H).; ^{13}C NMR: (100 MHz, DMSO): δ; 175.19. 151.75, 151.50, 150.18, 143.93, 142.76, 138.92, 125.68, 123.53, 121.13, 117.86, 116.37, 115.40, 114.34, 112.59, 112.21, 60.37, 54.76, 53.87, 29.21; ESI-MS: *m/z* Calculated 494.41, found [*m/z*] $[M+H]^+$ 495.2.

2.1.2.15. 6-amino-1-(2,4-dinitrophenyl)-4-(4-fluorophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5m).

Dark Brown solid, m.p. 254-257 °C.; Anal. Calc. for $C_{19}H_{11}N_6O_5F$: C, 54.03; H, 2.63; F, 4.50; N, 19.90; O, 18.94 %; found C, 54.32; H, 2.14; N, 19.02%; IR ν_{max} (KBr) cm^{-1}: 3325 (N-H_{str}), 3036-2028 (C-$H_{Aromatic}$), 1615 (C=C_{str}), 2230 (-CN), 1163 (C-O $_{str}$), 1511, 1377 (N-O_{str}); ^{1}H NMR: (400 MHz, DMSO): δ 10.034 (s, 1H), 9.358 - 9.341 (d, *J* = 6.8 Hz, 1H), 9.039 (s, 2H), 8.649 - 8.635 (d, *J* = 5.6 Hz, 1H), 7.967 (s, 1H), 7.161 - 7.140 (d, *J* = 8.4 Hz, 2H), 7.051 - 7.036 (d, *J* = 6 Hz, 2H), 4.562 (s, 1H); ^{13}C NMR: (100 MHz, DMSO): δ 169.95, 158.82, 150.74, 145.90, 142.33, 140.39, 138.86, 130.84, 130.31, 129.38, 128.07, 124.19, 121.32, 118.81, 118.04, 116.41, 115.80, 59.95. 27.93; ESI-MS: *m/z* Calculated 422.33, found [*m/z*] $[M+H]^+$ 423.5.

2.1.2.16. 6-amino-4-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5n).

Brown solid, m.p. 260-265 °C.; Anal. Calc. for $C_{19}H_{11}ClN_6O_5$: C, 52.01; H, 2.53; Cl, 8.08; N, 19.15; O, 18.23%. Found- C, 52.38; H, 2.26; N, 19.55%.; IR ν_{max} (KBr) cm^{-1}: 3317 (N-H_{str}), 2921 (C-$H_{Aromatic,str}$), 1615 (C=C_{str}), 2209 (-CN), 1180 (C-O $_{str}$), 1521, 1331 (N-O_{str}).; ^{1}H NMR: (400 MHz, DMSO): δ, 10.089 (s, 1H), 8.986-8.978 (s, *J* = 3.2 Hz, 1H), 8.692 (s, 2H), 8.495-8.482 (s, *J* = 5.2 Hz, 1H), 7.989 (s, 1H), 7.692-7.654 (d, *J* = 15.2 Hz, 2H), 7.492-7.480 (d, *J* = 4.8 Hz, 2H), 4.892 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 170.31, 150.88, 145.89, 142.67, 140.39, 138.30, 132.35, 131.27, 130.65, 130.51, 127.55, 125.81, 125.32, 125.06, 120.32, 118.21, 117.73, 59.48, 28.38; ESI-MS: *m/z* Calculated 438.78, found [*m/z*] $[M+H]^+$ 439.4.

2.1.2.17. 6-amino-4-(4-cyanophenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5o).

Light yellow solid, m.p. 266-268 °C.; Anal. Calc. for $C_{20}H_{11}N_7O_5$: C, 55.95; H, 2.58; N, 22.84; O, 18.63%. Found- C, 53.89; H, 2.68; N, 21.38%.; IR ν_{max} (KBr) cm^{-1}: 3443 ($N\text{-}H_{str}$), 3028 ($C\text{-}H_{Aromatic,str}$), 1696 ($C{=}C_{str}$), 2324 (-CN), 1124 ($C\text{-}O_{str}$), 1498, 1365 ($N\text{-}O_{str}$).; ^1H NMR: (400 MHz, DMSO): δ; 9.724 (s, 1H), 8.954 - 8.942 (d, 1H), 8.571 (s, 2H), 8.336 - 8.321 (d, *J* = 6 Hz, 1H), 7.898 (s, 1H), 7.620-7.612 (d, *J* = 3.2 Hz, 2H), 7.589-7.560 (d, *J* = 11.6 Hz, 2H), 4.632 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 173.02, 152.02, 147.41, 142.30, 140.67, 137.39, 130.11, 129.76, 127.20, 124.17, 120.53, 118.95, 118.84, 117.91, 109.42, 109.13, 108.86, 59.40, 28.71; ESI-MS: *m/z* Calculated, 429.35 found [*m/z*] $[M+H]^+$ 430.3.

2.1.2.18. 6-amino-1-(2,4-dinitrophenyl)-4-(3-hydroxyphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5p).

Dark brown solid, m.p. 258-260 °C.; Anal. Calc. For $C_{19}H_{12}N_6O_6$: C, 54.29; H, 2.88; N, 19.99; O, 22.84%. Found- C, 54.75; H, 2.16; N, 19.34%.; IR ν_{max} (KBr) cm^{-1}: 3313 ($N\text{-}H_{str}$), 3103 ($C\text{-}H_{Aromatic,str}$), 1620 ($C{=}C_{str}$), 2313 (-CN), 1085 ($C\text{-}O_{str}$), 1514, 1368 ($N\text{-}O_{str}$).; ^1H NMR: (400 MHz, DMSO): δ_H *(ppm)* 9.859 (s, 1H), 9.158-9.135 (d, *J* = 9.2 Hz, 1H), 8.859 (s, 2H), 8.372-8.356 (d, *J* = 6.4 Hz, 1H), 7.759 (s, 1H), 7.251-7.236 (d, *J* = 6 Hz, 2H), 7.047 (s, 1H), 6.939-6.921 (d, *J* = 7.2 Hz, 1H), 5.051 (s, 1H), 4.667 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 171.83, 155.74, 150.62, 146.71, 142.10, 139.90, 137.52, 136.71, 129.87, 127.35, 124.17, 122.05, 120.32, 117.58, 113.43, 112.18, 59.52, 28.82; ESI-MS: *m/z* Calculated 420.34, found [*m/z*] $[M+H]^+$ 421.1.

2.1.2.19. 6-amino-4-(4-bromophenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5q).

Brown solid, m.p. 273-276 °C.; Anal. Calc. for $C_{19}H_{11}BrN_6O_5$: C, 47.22; H, 2.29; Br, 16.54; N, 17.39; O, 16.55%. Found- C, 48.42; H, 2.64; N, 17.11%.; IR ν_{max} (KBr) cm^{-1}: 3314 (N-

H_{str}), 2938 (C-$H_{Aromatic,str}$), 1596 (C=C_{str}), 2350 (-CN), 1126 (C-O $_{str}$), 1506, 1346 (N-O_{str}).; ^{1}H NMR: (400 MHz, DMSO): δ_H *(ppm)* 9.694 (s, 1H), 8.869-8.836 (d, *J* = 13.2 Hz, 1H), 8.574 (s, 2H), 8.352-8.330 (d, *J* = 8.8 Hz, 1H), 7.895 (s, 1H), 7.621-7.594 (d, *J* 10.8 = , 2H), 7.047 (s, 1H), 7.320-7.304 (d, *J* = 6.4 Hz, 2H), 4.835 (s, 1H).^{13}C NMR: (100 MHz, DMSO): δ; 170.56. 151.67, 146.38, 143.16, 140.32, 138.04, 134.09, 132.70, 131.61, 131.27, 130.74, 127.36, 124.15, 120.48, 120.28, 118.43, 117.59, 59.87, 29.09; ESI-MS: *m/z* Calculated 483.23, found [*m/z*] $[M+H]^+$ 483.5.

2.1.2.20. 6-amino-4-(5-bromo-2-hydroxyphenyl)-1-(2,4-dinitrophenyl)-1,4dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5r).

Orange solid, m.p. 288-293 °C.; Anal. Calc. for $C_{19}H_{11}BrN_6O_6$: C, 45.71; H, 2.22; Br, 16.01; N, 16.83; O, 19.23%. Found- C, 45.22; H, 2.68; N, 16.20%.; IR ν_{max} (KBr) cm^{-1}: 3387 (N-H_{str}), 2803 (C-$H_{Aromatic,str}$), 1598 (C=C_{str}), 2214 (-CN), 1127 (C-O $_{str}$), 1513, 1345 (N-O_{str}).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.361 (s, 1H), 8.670-8.651 (s, *J* = 7.6 Hz, 1H), 8.576 (s, 2H), 8.281-8.270 (s, *J* = 4.4 Hz, 1H), 8.148 (s, 1H), 7.873-7.861 (d, *J* = 4.8 Hz, 1H), 7.472-7.460 (d, *J* = 4.8 Hz, 2H), 6.363 (s, 1H), 5.827 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 172.24, 156.88, 151.23, 146.64, 142.21, 139.85, 137.22, 136.00, 126.94, 124.61, 122.45, 120.38, 119.55, 118.20, 117.58, 115.68, 114.79, 59.68, 26.76; ESI-MS: *m/z* Calculated 499.23, found [*m/z*] $[M+H]^+$ 500.6.

2.1.2.21. 6-amino-1-(2,4-dinitrophenyl)-4-(4-nitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5s).

Dark orange solid, m.p. 283-285 °C.; Anal. Calc. for $C_{19}H_{11}N_7O_7$: C, 50.79; H, 2.47; N, 21.82; O, 24.92%. Found- C, 50.33; H, 2.29; N, 21.08%.; IR ν_{max} (KBr) cm^{-1}: 3281 (N-H_{str}), 3110 (C-$H_{Aromatic,str}$), 1612 (C=C_{str}), 2314 (-CN), 1137 (C-O $_{str}$), 1514, 1346 (N-O_{str}).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.584 (s, 1H), 8.727-8.703 (d, *J* = 9.6 Hz, 1H), 8.538 (d, 2H),

8.371-8.359 (d, *J* = 4.8 Hz, 1H), 8.247 (s, 1H), 7.773-7.762 (d, *J* = 4.4 Hz, 2H), 7.658-7.629 (d, *J* = 11.6 Hz, 2H), 4.594 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δc (*ppm*) 27.87, 59.62, 116.87, 118.03, 120.06, 123.79, 124.04, 124.91, 127.08, 130.22, 130.90, 136.83, 139.13, 140.60, 142.11, 145.95, 146.29, 151.37, 173.20.; ESI-MS: *m/z* Calculated 449.33, found [*m/z*] $[M+H]^+$ 450.1.

2.1.2.22. 6-amino-4-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-1,4-dihydropyrano[2,3c]pyrazole-5-carbonitrile (5t).

Black solid, m.p. 286-289 °C.; Anal. Calc. for $C_{21}H_{16}N_6O_7$: C, 54.31; H, 3.47; N, 18.10; O, 24.12%. Found- C, 54.62; H, 3.27; N, 17.86%.; IR ν_{max} (KBr) cm^{-1}: 3315 ($N\text{-}H_{str}$), 2975 ($C\text{-}H_{Aromatic,str}$), 1615 ($C{=}C_{str}$), 2215 (-CN), 1085 ($C\text{-}O_{str}$), 1511, 1379 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.580 (s, 1H), 8.784-8.772 (s, *J* = 4.8 Hz, 1H), 8.264 (s, 2H), 7.97-7.963 (s, *J* = 2.8 Hz, 1H), 7.579 (s, 1H), 7.242-7.231 (s, *J* = 4.4 Hz, 1H), 6.817-6.790 (d, *J* = 10.8 Hz, 2H), 4.861 (s, 1H), 3.871 (s, 6H).; ^{13}C NMR: (100 MHz, DMSO): δ; 172.22, 151.12, 150.67, 146.24, 145.92, 142.29, 140.40, 136.87, 128.24, 128.07, 124.20, 122.31, 120.42, 117.92, 117.31, 113.12, 112.83, 60.09, 56.67, 55.82, 28.71; ESI-MS: *m/z* Calculated 464.39, found [*m/z*] $[M+H]^+$ 465.2.

2.1.2.23. 6-amino-1-(2,4-dinitrophenyl)-4-(4-hydroxy-3-methoxyphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5u).

Reddish brown solid, m.p. 278-283 °C.; Anal. Calc. for $C_{20}H_{14}N_6O_7$: C, 53.34; H, 3.13; N, 18.66; O, 24.87% Found- C, 52.74; H, 3.42; N, 18.17%.; IR ν_{max} (KBr) cm^{-1}: 3313 ($N\text{-}H_{str}$), 3075 ($C\text{-}H_{Aromatic,str}$), 1622 ($C{=}C_{str}$), 2314 (-CN), 1134 ($C\text{-}O_{str}$), 1514, 1366 ($N\text{-}O_{str}$).; ^{1}H NMR: (400 MHz, DMSO): δ; 9.428 (s, 1H), 8.965-8.952 (d, *J* = 5.2 Hz, 1H), 8.689 (s, 2H), 8.224-8.204 (d, *J* = 8 Hz, 1H), 7.847 (s, 1H), 7.042-7.037 (d, *J* = 2 Hz, 1H), 6.748-6.725 (d, *J* = 9.2 Hz, 2H), 4.865 (s, 1H), 4.834 (s, 1H), 3.674 (s, 3H).; ^{13}C NMR: (100 MHz, DMSO): δ;

171.22, 152.14, 148.05, 146.18, 145.23, 142.20, 140.00, 136.71, 129.04, 127.10, 124.26, 122.19, 120.21, 118.09, 117.66, 114.79, 112.82, 59.48, 55.91, 28.59; ESI-MS: *m/z* Calculated 450.36, found [*m/z*] $[M+H]^+$ 451.6.

2.1.3. Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-(4-(piperidin-1-yl)phenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile derivatives (7a-7b):

An equimolar mixture of 2, 4-dinitrophenyl hydrazine **(1)** (1.0 eq.), ethyl acetoacetate **(2)** (1.0 eq.), malononitrile **(3)** (1.0 eq.) and aldehyde derivatives (**6a-6b**) (1.0 eq.) were taken into 50 ml FBF (Flat Bottomed Flask) in the presence of $SnCl_2$ (10 mol %) as a catalyst. The reaction mixture was stirred at 80 °C for appropriate time mentioned in **Table 1** and also heated at 180 W for 10-20 min under microwave irradiation. The isolation of yield performed same as **5a-5u** to get compound **7a-7b**.

2.1.3.1. 6-amino-1-(2,4-dinitrophenyl)-4-(4-morpholinophenyl)-1,4-dihydropyrano[2,3-c] pyrazole-5-carbonitrile (7a).

Dark brown solid, m.p. 302-304 °C.; Anal. Calc. for $C_{23}H_{19}N_7O_6$: C, 56.44; H, 3.91; N, 20.03; O, 19.61%. Found- C, 55.76; H, 3.82; N, 20.34%.; IR ν_{max} (KBr) cm^{-1}: 3284 ($N\text{-}H_{str}$), 2804 ($C\text{-}H_{Aromatic,str}$), 1599 ($C{=}C_{str}$), 2216 (-CN), 1060 ($C\text{-}O_{str}$), 1469, 1381 ($N\text{-}O_{str}$).; ^1H NMR: (400 MHz, DMSO): δ, 9.921 (s, 1H), 8.854 (s, 2H), 8.630 (s, 1H), 7.834-7.811 (d, *J* = 9.2 Hz, 1H), 7.756-7.738 (d, *J* = 7.2 Hz, 1H), 7.223-7.201 (d, *J* = 8.8 Hz, 2H), 7.051-7.031 (d, *J* = 8 Hz, 2H), 4.131 (s, 1H), 3.818-3.806 (t, *J* = 4.8 Hz, 4H), 3.402-3.364 (t, *J* = 15.2 Hz, 4H).; ^{13}C NMR: (100 MHz, DMSO): δ; 169.08. 151.80, 149.47, 144.49, 144.41, 141.54, 137.11, 129.41, 129.07, 128.11, 124.27, 123.80, 121.92, 116.63, 115.90, 112.19, 111.94, 65.87, 64.90, 60.64, 54.34, 53.20, 29.01; ESI-MS: *m/z* Calculated 489.44, found [*m/z*] $[M+H]^+$ 490.3.

2.1.3.2. 6-amino-1-(2,4-dinitrophenyl)-4-(4-(piperidin-1-yl)phenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (7b).

Brownish orange solid, m.p. 298-301 °C.; Anal. Calc. for $C_{24}H_{21}N_7O_5$: C, 59.13; H, 4.34; N, 20.11; O, 16.41%. Found- C, 59.31; H, 4.08; N, 20.65%.; IR ν_{max} (KBr) cm^{-1}: 3140 ($N\text{-}H_{str}$), 2989 ($C\text{-}H_{Aromatic,str}$), 1641 ($C{=}C_{str}$), 2316 (-CN), 1174 ($C\text{-}O_{str}$), 1544, 1383 ($N\text{-}O_{str}$).; ^1H NMR: (400 MHz, DMSO): δ, 9.454 (s, 1H), 8.821-8.794 (d, *J* = 10.8 Hz, 1H), 8.554 (s, 2H), 8.046-8.022 (d, *J* = 9.6 Hz, 1H), 7.590 (s, 1H), 7.069-7.046 (d, *J* = 9.2 Hz, 2H), 7.000-6.978 (d, *J* = 8.8 Hz, 2H), 4.153 (s, 1H), 3.321-3.308 (t, *J* = 5.2 Hz, 4H), 1.494-1.395(m, 6H).; ^{13}C NMR: (100 MHz, DMSO): δ; 174.59, 153.09, 151.64, 148.05, 143.29, 138.75, 135.83, 130.16, 129.85, 128.07, 124.15, 123.81, 121.01, 119.13, 116.89, 113.00, 112.87, 60.24, 54.06, 53.64, 25.07, 24.91, 23.90; ESI-MS: *m/z* Calculated 487.47, found [*m/z*] $[M+H]^+$ 488.4.

2.1.4. Synthesis of 6-amino-1-isonicotinoyl-4-phenyl-1, 4-dihydropyrano [2, 3-c] pyrazole-5-carbonitrile derivative (8a):

An equimolar mixture of isonicotinohydrazide **(1a)** (1.0 eq.), ethyl acetoacetate **(2)** (1.0 eq.), malononitrile **(3)** (1.0 eq.) and aldehyde derivatives **(4a)** (1.0 eq.) were taken in 50 ml FBF (Flat Bottomed Flask) in the presence of SnCl2 (10 mol %) as a catalyst. The isolation method of the product yield is same as **5a-5u** to obtain compound 6-amino-1-isonicotinoyl-4-phenyl-1, 4-dihydropyrano [2, 3-c] pyrazole-5-carbonitrile **8a**.

2.1.4.1. 6-amino-1-isonicotinoyl-4-(4-nitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (8a).

Light yellow solid, m.p. 268-270 °C.; Anal. Calc. for $C_{19}H_{12}N_6O_4$: C, 58.76; H, 3.11; N, 21.64; O, 16.48%. Found- C, 58.25; H, 3.07; N, 21.38%.; IR ν_{max} (KBr) cm^{-1}: 3186 ($N\text{-}H_{str}$), 3001 ($C\text{-}H_{Aromatic,str}$), 1685 ($C{=}C_{str}$), 2205 (-CN), 1142 ($C\text{-}O_{str}$), 1560 ($N\text{-}O_{str}$).; ^1H NMR:

(400 MHz, DMSO): δ; 8.815-8.797 (d, 2H), 7.957 (s, 2H), 7.650-7.638 (d, *J* = 4.8 Hz, 2H), 7.479-7.458 (d, *J* = 8.4 Hz, 2H), 6.534-6.512 (d, *J* = 8.8 Hz, 2H), 6.492 (s, 1H), 4.526 (s, 1H).; ^{13}C NMR: (100 MHz, DMSO): δ; 174.82. 167.02, 157.34, 152.01, 150.67, 142.25, 141.03, 131.68, 130.09, 129.57, 124.02, 123.83, 123.04, 122.34, 117.86, 117.02, 60.62, 29.18; ESI-MS: *m/z* Calculated 388.34, found [*m/z*] $[M+H]^+$ 389.2.

2.2 Biological assay

2.2.1 *In vitro* anti-bacterial activity

The in vitro anti-bacterial activity of synthesized compounds were performed against four different bacterial strains (*E. coli* MTCC 443, *P. aeruginosa* MTCC 1688, *S. aureus* MTCC 96, *S. pyogenus* MTCC 442) and compered with standard drugs Ampicillin, Chloramphenicol and Ciprofloxacin. This activity was performed in accordance with method in Patel et al. and Mungra et al. The highest dilution showing at least 99% inhibition has taken as MIC value [53-62].

2.2.2 *In vitro* anti-tuberculosis activity and MDR-TB study

The anti-tuberculosis screening of the synthesized compounds was carried out using method describe by Patel et al. and Mungra et al. [53-62]. The MIC of test compound was taken as < 20 colonies or no development of colonies occurred at the concentration. The *M. tuberculosis* $H_{37}Rv$ strain was tested with standard drug isoniazid for the comparison intense. The multi-drug resistance tuberculosis activity of the synthesized compounds against *M. tuberculosis* $H_{37}Rv$. (MDR-TB strain was collected from National Institute for Research in Tuberculosis, Chennai, Tamil Nadu, and India). (The strain was identified by phenotype and genotype).

2.2.3 Cytotoxicity assay protocol

Human cervical cancer cell line (HeLa) were used for in vitro cytotoxicity study of the synthesized compounds **7a, 8a, 5a** and **5r**. The cytotoxic activity protocol was used according method used in Desai et al. [63].

2.3 Computational Study

2.3.1 ADMET property prediction

A set of ADMET-related properties of the synthesized compounds were predicted by using Qikprop programs (Schrödinger, LLC, New York, NY, 2015) [64, 65]. *LigPrep* module were used to prepare the compounds and utilized for the calculation of pharmacokinetic parameters by *QikProp* module. The program *QikProp* generates physically relevant descriptors, and uses them to perform ADMET predictions and utilizes the method of Jorgensen [67] to calculate pharmacokinetic properties and descriptors.

2.3.2 Molecular Docking

The potential binding mode and the binding interaction of the ligand with enoyl-ACP reductase (oxidoreductase) have been investigated by using Maestro 10.7 Schrödinger, LLC, New York, NY, 2012. The 3D structures of all the compounds were obtained using Marvin suites and saved as SDF file. The 3D crystal structures of enoyl-ACP reductase (oxidoreductase) from *Mycobacterium tuberculosis* (PDB ID: 5MTQ; resolution 2.6cÅ) were retrieved from the protein data bank (www.rcsb.org). The protein (5MTQ) was choose based on structure similarity of ligands, resolution and expression system. Morevere, The protein organism is *Mycobacterium Tubercolisis* ($H_{37}Rv$ Strain) which is encourage us to do the docking in the active site of this protein. The ligand with the lowest energy, correct Lewis structure, tautomers and ionization states (pH 7.0±2.0) for each of the ligands were generated and optimized with default settings were prepared for molecular docking using *Ligprep*

module of Schrödinger. The OPLS_2005 [65] force field were used for computing partial atomic charges. The proteins were prepared for docking using Protein Preparation Wizard module in Maestro. Bond order and formal charges were assigned and hydrogen atoms were added to the crystal structure. Further to refine the structure OPLS-2005 force field parameter was used to alleviate steric clashes. The receptor grid, which was generated using Glide 5.8 (Maestro, Schrödinger, LLC, New York, NY, 2012) with default settings for all parameters. An approximately large grid size was preferred to include all active site residues involved in substrate binding. The Glide extra-standard precision (XP) mode was used for docking of the generated receptor grid file along with all prepared ligand conformers. Default settings were retained for the scoring and refinement.

2.3.4 Molecular Dynamics

The natural dynamics on different timescales of docked complex of compound **7b** and protein, thermal average of the molecular properties of complex is carried with the help of molecular dynamics (MD) stimulation. MD simulations have been conducted with Desmond [65-67] program, as implemented Schrödinger Materials Science Suite 2015-4 [68]. OPLS_2005 [65] force field with NPT ensemble class have been used, while pressure and temperature were set to 1.0325 bar and 300 K, respectively. The system was modelled by placing one NATPB (5-[(4-nitrophenyl)acetamido]-2-(4-tert-butylphenyl)benzoxazole) molecule into the cubic box with around 3000 water molecules and the simulation time was set to 10 ns cut off radius was set to 12 Å, pressure to 1.0325 bar, while temperature was set to 300 K. In all cases when Desmond was used, input and output files were manipulated by Maestro graphical user interface application of Schrödinger Materials Science Suite 2015-4.

3. Results and Discussion

3.1 Chemistry

In the present study. We have synthesized a series of novel compounds based on pyrano[2,3-c]pyrazole (**5a-5u, 7a-7b** and **8a**) which was illustrated in Scheme 1-3. The synthesis of the series of heterocyclic scaffold performed using MCR methodology following direct addition of the substituted aldehyde derivative (**4a-4u**) with the corresponding 2, 4-dinitrophenyl hydrazine (**1**), ethyl acetoacetate **(2)** and malononitrile **(3)** in the presence of $SnCl_2$ using Methanol as solvent under microwave irradiation and conventional method (**Scheme 2-3**). Targeted compound 6-amino-1-isonicotinoyl-4-phenyl-1, 4-dihydropyrano [2, 3-c] pyrazole-5-carbonitrile (**8a**) (**Scheme 3)** was synthesised using substituted aldehyde derivative (**4a-4u**), isonicotinohydrazide (**1a**), ethyl acetoacetate (**2**) and malononitrile (**3**) in the same condition as a **5a-5u**. Whereas, to synthesise compound **7a-7b**, a 4- substituted aldehyde derivatives were initially synthesized **(6a-6b**) (**Scheme 1**) via condensation of 4-fluoro aldehyde, piperidine and morpholine in the presence of anhydrous potassium carbonate using Methanol as solvent.

From the current literature survey, we concluded that many researcher have reported desired product formation with lower product yields and longer hours reaction time by using different solvent and catalyst for the synthesis of 1,4-dihydropyrano[2,3-c]pyrazole derivatives [35-45, 69]. Despite this challenge, we have optimized the reaction conditions using conventional and microwave irradiation to find out the superior method for the synthesis of targeted compound **5a** with higher yield and shorter reaction time. In both the conditions, we first performed a trial reaction with equivalent mole of benzaldehyde, malononitrile and 2,4-dinitrophenyl hydrazine in the presence of $SnCl_2$ using water, Methanol, ethanol as solvent and without solvent at various temperature (**Table 1**). In the conventional method, Compound **5a** with yield of 67% in 4.2 hrs was obtained when the reaction was performed without catalyst at 80 ºC temperature. While, when the reaction was performed in Methanol at 80 °C temperature by using $SnCl_2$ as a catalyst the yield and time

of product **5a** could be improved to 80 % in 1.4 hours. However, the targeted product **5a** was synthesized in 25 min with 88 % of yield in microwave irradiation method, where Methanol was used as a solvent in the presence of $SnCl_2$ as a catalyst at 180 W (140 °C) temperature. Most of the compounds were synthesized using neat condition in the microwave irradiation method. However, we have also used neat condition while synthesizing compounds via conventional method but the yield of the reaction was quite low as compared to in Methanol. The synthesized compounds are enantiomeric mixture there is no synthetic selectivity. The yield of compound **5a** (80 % by conventional method and 88 % by microwave method) synthesized by our method was significantly higher, than the previously reported protocols [35-45, 69].

All the synthesized compounds **5a-5u**, **7a-7b**, **8a** were characterized by various spectroscopic methods such as ^{1}H NMR, ^{13}C NMR, IR spectra, Mass analysis and elemental analysis. We have confirmed the synthesised compound **5a** from IR analysis. IR band at 3323 and 3106 cm^{-1} which confirmed the presence of $N\text{-}H_{stretch}$ and aromatic character ($C\text{-}H_{stretch}$) in the moiety respectively. When -CN group showed vibration peak at 2249 cm^{-1}, while $C\text{-}O_{stretch}$ showed peak at band at 1023 cm^{-1} confirm the formation of pyran ring in molecule. From the ^{1}H NMR data of compound **5a, The** aromatic protons were resonated at 9.642, 8.936-8.921 δ ppm (Nitro containing aromatic ring) and other benzene (Aromatic) ring showed peak at 7.572-7.567, 7.358 and 7.317-7.313 δ ppm. The $-NH_2$ group resonated at 8.471 δ ppm. The chiral carbon containing proton resonated at 4.656 δ ppm which also supported to synthesised compound **5a.** Also ^{13}C-NMR gave full structural conformation of synthesised compound. The peaks at 27.93, 60.18 and 174.91 δ ppm correspond to cyclised chiral carbon, –C-CN containing carbon and $-NH_2$ containing carbon respectively which gave full confirmation of **5a**.The mass spectra of compound **5a** showed molecular ion peak at *m/z* = 404.2 (M+H) which support to the structure of compound **5a**.

3.2 Biology

3.2.1 *In vitro* anti-bacterial activity

A series of synthesized compounds were evaluated for their anti-bacterial activity against different bacterial stain such as *E. coli*, MTCC 443, *P. aeruginosa* MTCC 1688, *S. aureus* MTCC 96, *S. pyogenus* MTCC 442 as compared to the standard drug. The activity data were summarized in Table 2. From the bioassay, it can be stated that the compound **8a** which was hybridised molecule of isoniazid with a pyran ring showed significant antibacterial activity against *E. coli* and *P. aeruginosa* at MIC value 12.5 µg/mL. Analogue **5a** with high lipophilicity (C log P = 4.1319) exhibited excellent antibacterial activity against *E. coli* and *P. aeruginosa* at MIC value 12.5 µg/mL and 6.25 µg/mL respectively. In addition, Compound **5l** which contained two methoxy groups showed good activity against *E. coli* and *P. Aeruginosa* at MIC value 12.5 µg/mL and 25 µg/mL correspondingly. Compound **5n** (C log P = 4.7019) and **7a** (C log P =3.4449) with higher lipophilicity displayed higher MIC value 12.5 µg/mL, 12.5 µg/mL, respectively amongst all the active compounds when tested against *S. pyogenus*. Moreover, analogue **5b** and **7a** (MIC value 25 µg/mL) shows better inhibitory effect against *S. aureus.* Based on the results, it can be stated that in case on inhibition of the bacterial strain, higher inhibiting efficiency of compounds comes with higher lipophilicity than with lower lipophilicity. Remaining compounds exhibited comparatively superior antibacterial activity against all the tested bacterial strains in comparison to the standard drugs Ampicillin, Chloramphenicol and Ciprofloxacin. The C log P values (P is the partition coefficient) of all the synthesised compounds as well as standard drugs are mentioned in Table 2. Overall, all the results showed that the MIC value less than that of standard drugs were considered as promising anti-bacterial agent.

3.2.3 *In vitro* anti-tuberculosis activity and MDR-TB study

The promising results obtained from the antibacterial activity encouraged us to go for further biological screening such as *in vitro* anti-tubercular activity. *In vitro* anti-tuberculosis activity of all the newly synthesized compounds was investigated against *Mycobacterium tuberculosis* $H_{37}Rv$ strain by Lowenstein-Jensen method and the corresponding results are shown in Table 2. The result showed that analogue **8a** enhance good activity (25 µg/mL) due to the presence of isoniazid moiety instead of 2, 4-dinitrophenyl hydrazine presented in rest molecules. In addition, compound **7a** and **5r** showed 25 µg/mL of MIC against mycobacterial strain.

Moreover, Compound **5u** showed moderate activity against $H_{37}Rv$ strain with MIC value 50 µg/mL. All the remaining compounds were found to display reasonable activity at MIC ranging from 50 to 500 µg/mL. Further, the compounds were screened against MDR-TB (First-line anti-TB drug resistance) in clinical strains. From the data (Table 2) of activity against MDR strain it was observed that only compound **7a** demonstrate moderate activity at MIC value 50 µg/mL. The remaining result of tested compounds against MDR-TB strain were comparatively poor. The lower effectiveness of synthesized compounds against strain not being adapted to laboratory environments and different media, which may affect their apparent susceptibility to certain inhibitors and they exhibit poor effect against strain in vitro resulted in the poor activity.

3.2.4 Cytotoxicity assay

In vitro cytotoxic activity of synthesized compounds **5a, 5r, 7a** and **8a** was evaluated against HeLa cell line (human cervical cancer) by the MTT colorimetric assay [70]. The result are summarized in table 3. Percentage cell viability of HeLa cell line at various concentrations for these compounds are shown in figure 2. It was observed that there is no any remarkable cytotoxic effect on HeLa cell line, Perticulery the tested compound **7a**. This

activity suggest that these compounds have good proficiency for their future *in vivo* activity for antimicrobial agents.

3.3 Computational Study

3.3.1 ADMET property prediction

In silico, pharmacokinetic properties leading to drug-likeness of the synthesized compounds were predicted using Qikprop module (Schrodinger, LLC, New York, NY, 2015). The predicted important pharmacokinetic properties of synthesized compounds along with their acceptable limits are showed in Table 4 as compared with the reference compounds. Most of the compounds were found to have good bioavailability except **5s**. One of the important factor to be studied in concern with the absorption of the drug molecule is intestinal absorption, which was ascertained by Caco-2 cell permeability (QPPCaco) prediction. The synthesized compound shows moderate results predicting intestinal absorption in Caco-2 cell permeability. Further, The Qikprop descriptor for blood/brain partition coefficient QPlogBB showed reliable prediction for the synthesized compounds and reference drugs.

The predicted results for QPlogkhsa descriptor of Qikprop indicating the predicted values of human serum albumin binding indicated that molecules were found to in the permissible range (-1.5-1.5). The active molecules were accessed for IC_{50} value of HERG K^+ channel blockage prediction, which indicated that the predicted values are in the acceptable range (<-5) as compared to the standard reference entities. The aqueous solubility parameter (QPlog S) of the synthesized compounds were assessed and the compounds were found to be falling out of the acceptable range (-6.5-0.5) signifying incomplete solubility of the synthesized compounds which can be further improved using solubility enhancers.

3.3.2 Molecular docking

Due to lack of enzymatic study, we decided to perform *in silico* computational study of the synthesized compounds which provided more information that could be applied to design new molecules with more potent biological activity. Therefore, Molecular docking analysis was performed to obtain the detailed molecular interactions and to estimate the binding affinity of the synthesized compounds with enoyl-ACP reductase (oxidoreductase) (PDB ID: 5MTQ). Synthesized pyrano [2,3-c]-pyrazole derivatives and standard ligand Isoniazid was docked into the active site of enoyl-ACP reductase (oxidoreductase). The docking results of the ligands with enoyl-ACP reductase (oxidoreductase) as per the Glide calculations are given in Table 5. The results of docking simulations demonstrated high binding affinity with good interactions for critical residues present in enoyl-ACP reductase (oxidoreductase). Synthesized compound **7b**, standard compound isoniazid and isoniazid with co-factor (NAD^+) showed the catalytic site with binding energies of -10.19, -6.34 and -6.23 kcal/mol, respectively. The purpose of docking with INH-NAD^+ is to compare the docking result with INH because most of known INH inhibitors bind next to the NAD^+ co-factor. The docking pose of INH without co-factor and with co-factor is presented in figure 4. As per the docking simulations, **7b** was docked into enoyl-ACP reductase (oxidoreductase) active site with -10.19 kcal/mol of binding energy higher than Isoniazid was seen to be forming conventional hydrogen bond with Gln214 and Arg225, along with carbon hydrogen bond with Pro156 which also show Pi-Cation interaction and Vai203, Met199, Phe149, Leu218, Met155 shows alkyl and Pi-Alkyl interaction with amino acids of enoyl-ACP reductase (oxidoreductase) in Figure 3a. When the docking snapshot of isoniazid in complex with enoyl-ACP reductase (oxidoreductase) from *Mycobacterium tuberculosis* was analysed, it was seen that it docked into the active site of enoyl-ACP reductase (oxidoreductase) with -6.34 kcal/mol of energy and found to be forming direct hydrogen bonds with Gly239; Lys377, and Heme, along with hydrophobic interactions with Ile232; Leu384; Leu230;

Leu234; Phe163; Phe226; Val350; Ala264; Ile354; Ala260; Phe291; and Pro241 of enzyme in figure 3b.

Among 25 compounds analysed, all compounds were successfully docked with a binding energy range of -10.19 to -2.39 kcal/mol. The compound **7b** showed the highest binding affinity of -10.19 kcal/mol of binding energy. The compound with highest binding affinity, **7b** was further analysed using molecular dynamic simulation to check the stability of docked complex.

3.3.3 Molecular Dynamics

Molecular Dynamics (MD) simulations were performed to analyse the stability, conformational changes and underlying molecular interactions at atomic level of enoyl-ACP reductase (oxidoreductase) with compound **7b** at 10 ns. The MD simulations were carried out within the given temperature, pressure and volume defined by simulation quality parameters. The studies were performed in different standard simulation parameters: (i) Root mean square deviation (RMSD), (ii) Root mean square fluctuations (RMSF), (iii) The Ligand Root Mean Square Fluctuation, (iv) Protein-Ligand contacts; (v) total energy, and (vi) Ligand Torsion Profile and properties. The main objective of MD simulation was to understand the dynamics and stability of enoyl-ACP reductase (oxidoreductase) from *Mycobacterium tuberculosis* protein and its interaction with compound **7b**. The overall statistical data after the analysis of the MD trajectories are explained with figure 5.

RMSF of the enoyl-ACP reductase (oxidoreductase) protein in complex with **7b** pattern of residue fluctuations were found to be similar from docked structure of protein-ligand shown in Figure 3 and 5 (a). Protein depicted quite stable structure without lacking any major interaction throughout the simulation period. Intra molecular hydrogen bonds were analysed to understand the underlying forces better in relation to protein structure stability. Protein rigidity is represented by increased intra molecular hydrogen bonds. This observation

clearly shows that the proposed compound **7b** have the potential to inhibit the protein, with no alterations in enoyl-ACP reductase (oxidoreductase) rigidity. The overall energy involved for stabilization of enoyl-ACP reductase (oxidoreductase) state; in complex with **7b** was studied with many other parameters shown in the figures. This data suggests that protein in complex with **7b** has better inhibiting potential as compared to standard drug. A timeline representation of the interactions and contacts shows the total number of specific contacts in which residues interact with the ligand in each trajectory frame shown in Figure 5 (d). Molecular interactions between protein complex and **7b** during MD simulations shown in Figure 5 (a).

The detailed inter-molecular interactions were studied with simulation interactions diagram using Desmond module of Schrödinger. There were about 0-6 contact found in between protein-compound with 4 hydrogen bonds, with residues Arg225, Arg153, Gln214 and Pro156. Compound also formed pi-pi stacking with PHE149 and various hydrophobic contacts with residues Trp222, Leu218, Ile202, Tyr158, Pro193, Ile21, Met199 and Met155. On the other hand, residues Arg225 was found to be actively participating in forming water-bridging bonds.

The torsional degrees of freedom in the ligand given by rotational bonds were studied to understand the dynamics related with it. Each rotatable bond torsion is accompanied by a dial plot and bar plots of the same colour. A total of six rotatable bonds have been observed in **7b**, which are present between ligand atoms 27 and 33; 2 and 24; 25 and 30; 9 and 36; 7 and 12 and finally between 15-21.

From the dial panels, the above-mentioned rotatable bonds are rotating around, some of them rotated in parts around the clock-wise with gap -130° to -180° / 50˚ to -60˚ / 110˚ to 180˚; 10° to 160°; 180° to 180°; −70° to −180° / 70˚ to -50˚ / 130˚ to 180˚; -10˚to -130˚ and 40° to −180° / 50˚to 180˚, respectively in figure 7.

Finally, the post docking analysis to check for the similarities between the docked structure and after 10ns simulation structure of *Mycobacterium tuberculosis* enoyl-ACP reductase (oxidoreductase) with **7b** compound is done via superimposing the structures. The proposed compound's mode of inhibition doesn't show any vast difference after simulation structure specifically, same prominent alignments were observed with the residues shown in Figure 6 (b) indicating protein (blue) with ligand (yellow) structure after molecular dynamic simulation on docked structure protein (green) with ligand (red).

The discoveries of compounds that can inhibit the activation of *Mycobacterium tuberculosis* oxidoreductase protein. This suggest their capability to act as anti-tuberculosis agents. Compound **7b** was found to be strong inhibitors for protein as compared with standard compound Isoniazid. All the compounds discovered here bind the same active binding site of *Mycobacterium tuberculosis* oxidoreductase, by creating hydrogen bonds and various non-covalent interactions. The computational analysis also supports good binding energy being involved in present compounds being investigated here with the protein combining their complex's thermodynamic stability.

To verify it further, molecular dynamic simulations for 10 ns suggest that the residues of *Mycobacterium tuberculosis* oxidoreductase and ligand interactions, could be useful for its inhibitory activity. The present study gives us vision of the valuable ligand molecule with strong binding affinity towards *Mycobacterium tuberculosis* oxidoreductase protein for plausible anti-tuberculosis activity.

4. Conclusion

In the present study, a series of novel hybrid *4H*–pyran containing pyrazole derivatives were synthesized and characterized by IR, ^{1}H-NMR, ^{13}C NMR and mass spectrometry techniques. We have screened biological activities such as *in vitro* antimicrobial, anti-tuberculosis and cytotoxic MTT assay. From the anti-bacterial activity, it

was observed that the compounds **5a, 5h, 5m** and **8a** were showed good activity against gram-negative bacterial strain, while other compounds **5b, 5p** and **7a** gave significant activity against gram-positive bacteria. We have subjected anti-tubercular activity against *mycobacterium tuberculosis* ($H_{37}RV$ strain). The compounds **5e, 5t, 7a** and **8a** showed potent activity as well as compound **7a** enhanced the moderate activity against MDR-TB. These compounds were further evaluated for their cytotoxic effect in HeLa cell line and was accompanied by relatively low level of cytotoxicity. Compound **7a** were found to be good against biological evolution and cytotoxic assay.

The molecular docking investigation of all the synthesised compounds was carried out in the active site of enoyl-ACP reductase (oxidoreductase). Few compounds (**7b, 7a, 5c, 5r, 5f, 5m**) displayed good binding affinity in comparison to isoniazid as standard drug and to isoniazid with co-factor. From the docking simulations, it can be concluded that these molecules have an excellent affinity for the *Mycobacterium tuberculosis* enoyl-ACP reductase enzyme making them relevant starting points for structure-based drug design. Furthermore, the MD simulation was carried out to understand the dynamics and stability of enoyl-ACP reductase (oxidoreductase) protein with its interaction with compound **7b** and based on MD simulation analysis. It can be stated that the docked complex is thermodynamically stable and inhibit the activation of *Mycobacterium tuberculosis* oxidoreductase protein. This suggests their capability to act as strong anti-tuberculosis agents in comparison to standard drug isoniazid. Additionally, the predicted ADMET properties of the synthesized compounds recognized the drug-likeness of the molecules. Hence, promising in vitro anti-tubercular activity, docking pattern into the active site of enoyl-ACP reductase (oxidoreductase) and ADMET properties shows that few of these compounds effective lead for further development as potential anti-tubercular agents.

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Captions

Table 1: Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-phenyl-1,4 dihydropyrano[2,3-c]pyrazole-5-carbonitrile derivatives (**5a-5u,7a-7b** and **8a**) using SnCl2 as catalyst by Conventional and microwave irradiation method.

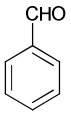
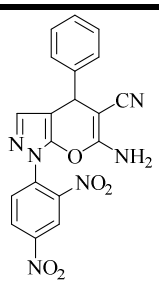
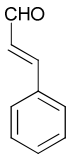
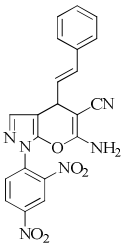
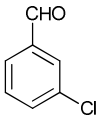
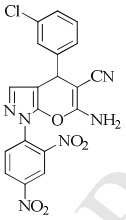
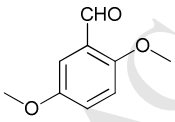
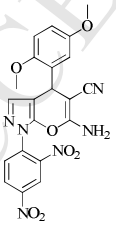
Table 2: *In vitro* anti-bacterial, anti-tuberculosis and MDR-TB screening result of synthesised compounds.

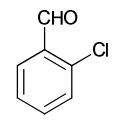
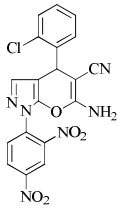
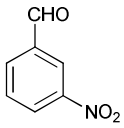
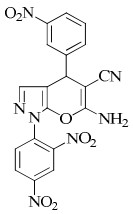
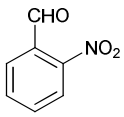
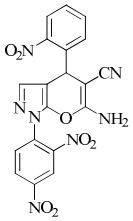
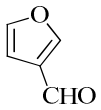
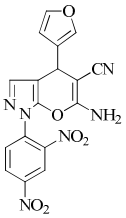
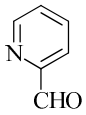
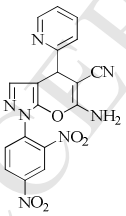
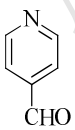
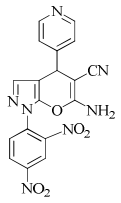
Table 3: *In vitro* % viability of compound **5e, 5r, 7a** and **8a** by MTT assy.

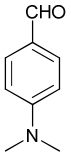
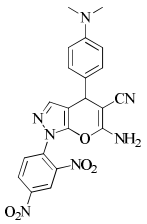
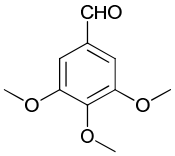
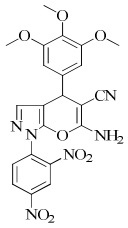
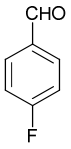
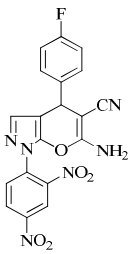
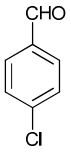
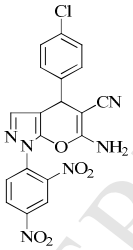
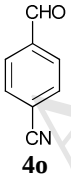
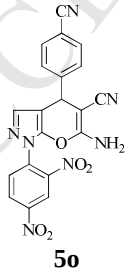
Table 4: Predicted ADME parameters for synthesized compounds.

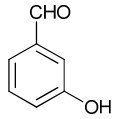
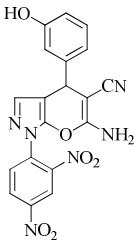
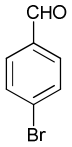
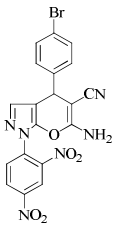
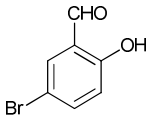
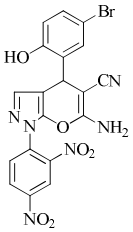
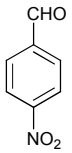
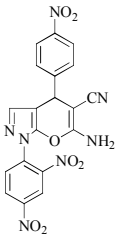
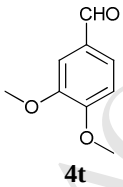
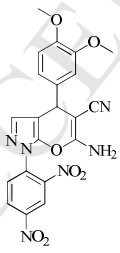
Table 5: Binding energy of Compounds with target protein (kcal/mol)

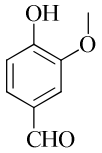
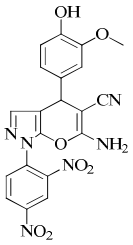
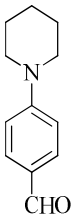
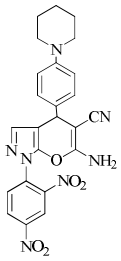
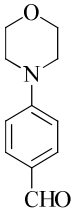
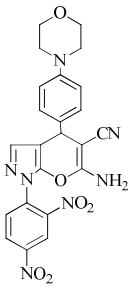
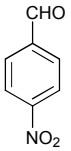
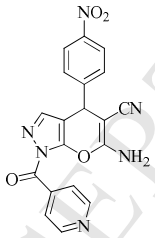
Table 1. Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-phenyl-1,4 dihydropyrano[2,3-c]pyrazole-5-carbonitrile derivatives (**5a-5u**,**7a-7b** and **8a**) using $SnCl_2$ as catalyst by Conventional and microwave irradiation method.

Entry	Aromatic Aldehyde	Product	Conventional Method				Microwave Method			
			Solvent	Temp. °C	Time Min.	Yield %	Solvent	Temp. W	Time Min.	Yield %
1	 **4a**	 **5a**	MeOH	80	100	80	MeOH/Neat	180	25	88
2	 **4b**	 **5b**	MeOH	90	90	78	MeOH/Neat	180	20	82
3	 **4c**	 **5c**	MeOH	80	95	68	Neat	180	15	72
4	 **4d**	 **5d**	MeOH	80	110	74	Neat	180	15	77

5	 **4e**	 **5e**	MeOH	80	95	58	Neat	180	15	67
6	 **4f**	 **5f**	MeOH	80	100	78	Neat	180	15	81
7	 **4g**	 **5g**	MeOH	80	100	69	Neat	180	15	72
8	 **4h**	 **5h**	MeOH	80	90	55	Neat	180	15	64
9	 **4i**	 **5i**	MeOH	80	100	52	Neat	180	15	60
10	 **4j**	 **5j**	MeOH	80	95	54	Neat	180	15	61

11	 **4k**	 **5k**	MeOH	80	120	67	Neat	180	15	71
12	 **4l**	 **5l**	MeOH	80	110	73	Neat	180	15	81
13	 **4m**	 **5m**	MeOH	80	115	74	Neat	180	15	79
14	 **4n**	 **5n**	MeOH	80	100	75	Neat	180	15	78
15	 **4o**	 **5o**	MeOH	80	95	63	Neat	180	15	69

16	 **4p**	 **5p**	MeOH	80	105	60	Neat	180	15	63
17	 **4q**	 **5q**	MeOH	80	100	72	Neat	180	15	76
18	 **4r**	 **5r**	MeOH	80	100	57	Neat	180	15	62
19	 **4s**	 **5s**	MeOH	80	110	81	Neat	180	15	86
20	 **4t**	 **5t**	MeOH	80	100	71	Neat	180	15	76

21	 **4u**	 **5u**	MeOH	80	105	66	Neat	180	15	69
22	 **6a**	 **7a**	MeOH	80	110	60	Neat	180	18	64
23	 **6b**	 **7b**	MeOH	80	120	62	MeOH	180	30	65
24	 **5s**	 **8a**	MeOH	80	100	67	Neat	180	15	70

[a] *Isolated Yield.*

Table 2. *In vitro* anti-bacterial, anti-tuberculosis and MDR-TB screening result of synthesized compounds.

Entry	Compound	C log *P*[a]	Anti-Bacterial				Anti-Tuberculosis	MDR-TB[d] MIC µg/mL
			Gram-negative[b]		Gram-positive[c]			
			E.C. MIC µg/mL	P.A. MIC µg/mL	S.A. MIC µg/mL	S.P. MIC µg/mL	$H_{37}RV$[d] MIC µg/mL	
1	**5a**	4.1319	**12.5**	**6.25**	100	125	125	250
2	**5b**	3.4220	100	50	**25**	125	250	500
3	**5c**	4.3629	250	250	200	250	250	500
4	**5d**	2.4919	62.5	25	100	250	500	100
5	**5e**	4.1539	125	125	250	100	**25**	100
6	**5f**	4.2817	100	125	125	62.5	125	250
7	**5g**	3.1649	125	250	250	100	250	1000
8	**5h**	4.7019	125	**12.5**	100	62.5	200	>1000
9	**5i**	3.9890	200	250	125	100	125	500
10	**5j**	2.4919	200	250	500	250	100	250
11	**5k**	3.6520	250	100	250	500	62.5	100
12	**5l**	3.5969	100	62.5	500	250	100	125
13	**5m**	3.6469	**12.5**	**25**	50	100	250	500
14	**5n**	4.7019	100	125	250	125	125	250
15	**5o**	4.7019	125	250	125	250	100	250
16	**5p**	3.7320	250	125	100	**12.5**	250	500
17	**5q**	3.3220	50	100	125	250	500	1000
18	**5r**	3.1712	100	500	250	200	500	>1000
19	**5s**	3.7320	250	200	125	100	250	1000
20	**5t**	4.8519	125	500	100	50	**25**	100
21	**5u**	3.2891	100	125	125	250	500	1000
22	**7a**	3.4449	100	250	**25**	**12.5**	**25**	**50**
23	**7b**	4.8269	100	50	250	250	125	250
24	**8a**	3.1260	**6.25**	**12.5**	100	100	50	125
25	**Ampicillin**	-1.2045	100		250	100	-	-
26	**Chloramphenicol**	1.2830	50	50	50	50	-	-
27	**Ciprofloxacin**	-0.7252	25	25	50	50	-	-
38	**Isoniazid**	-0.6680	-	-	-	-	0.20	-

[a] C log P calculated using the ChemBioDraw Ultra, version 12.0, software by Cambridge Soft.

[b] Ec: *Escherichia coli* (MTCC-443); Pa: *Pseudomonas aeruginosa* (MTCC-1688).

[c] Sa: *Staphylococcus aureus* (MTCC-96), Sp: *Streptococcus pyogenes* (MTCC-442).

[d] Minimum inhibitory concentration against $H_{37}Rv$ strain of M. Tuberculosis and Multi Drug-Resistance Tuberculosis (μg/mL).

Table 3. *In vitro* % viability of compound **5e, 5r, 7a** and **8a** by MTT assy.

Entry	Compound	Inhibition concentration (μg/mL)	% viability (HeLa Cell-line)
1	5e	5	10.31
		10	22.59
		20	28.21
		25	30.82
		50	48.23
		100	69.53
2	5r	5	19.51
		10	24.45
		20	27.32
		25	30.47
		50	38.27
		100	48.83
		200	49.91
3	7a	5	36.84
		10	49.16
		20	59.47
		25	61.37
4	8a	5	5.94
		10	11.70
		20	22.19
		25	24.88
		50	33.62
		100	38.60
		200	58.75

Table 4. Predicted ADME parameters for synthesized compounds.

Compound	Percent human oral absorption (>80% – high & <25% – poor)	QPlog BB (-3.0 –1.5)	QPlog HERG (below -5)	QPPCaco (<25 poor, >500 great)	QPlog Khsa (-1.5 –1.5)	PSA (70 –200 Å)	QPlog S (-6.5 –5)
5a	46.28	-2.73	-5.86	10.10	0.49	160.71	-6.70
5b	28.40	-3.75	-6.08	2.10	0.31	186.50	-6.36
5c	49.18	-3.22	-6.77	10.12	0.64	160.70	-7.24
5d	41.41	-2.86	-5.94	9.22	0.26	171.58	-5.99
5e	47.13	-3.09	-6.01	9.76	0.61	164.14	-7.19
5f	37.37	-3.21	-5.84	4.15	0.40	181.55	-6.82
5g	40.65	-2.64	-5.35	10.01	0.19	169.62	-5.40
5h	46.92	-2.73	-5.90	9.95	0.54	160.70	-6.86
7a	45.90	-3.01	-5.95	9.83	0.54	173.97	-7.21
7b	50.96	-3.08	-6.03	9.59	0.95	164.32	-8.14
5j	34.90	-3.07	-5.77	5.49	0.16	173.59	-5.76
5k	28.86	-3.68	-5.89	2.00	0.39	203.10	-6.34
5l	46.63	-3.09	-5.85	10.42	0.49	175.74	-6.84
8a	52.87	-2.53	-5.56	13.59	-0.11	158.08	-4.99
5i	44.90	-2.82	-5.98	10.09	0.45	160.71	-6.34
5n	47.76	-2.71	-5.90	10.11	0.56	160.71	-7.07
5o	47.68	-2.74	-5.97	10.07	0.56	160.71	-7.11
5p	24.38	-3.97	-5.90	1.21	0.42	205.60	-6.51
5q	31.24	-3.46	-5.85	3.06	0.28	183.25	-6.06
5r	35.77	-3.42	-5.84	4.58	0.30	187.44	-6.27
5s	24.36	-3.98	-5.91	1.21	0.42	205.61	-6.52
5t	48.21	-2.72	-5.93	10.11	0.59	160.71	-7.18
5m	46.48	-3.14	-5.94	10.10	0.50	173.91	-6.93
5u	47.11	-3.25	-5.83	10.09	0.51	180.14	-7.07
Ciprofloxacin	49.25	-0.61	-3.20	14.27	-0.007	96.22	-3.79
INH	66.83	-0.84	-3.54	275.98	-0.75	81.40	-0.04
Chloramphenicol	65.44	-1.50	-3.25	60.36	-0.81	121.93	-2.00
Ampicillin	20.18	-1.09	-0.28	1.87	-0.94	135.18	-1.51

Table 5. Binding energy of Compounds with target protein (kcal/mol)

Code	DS*	GGS*	Gevdw*	GEC*	GE*	XP LEvdW*
7b	-10.19	-10.21	-51.02	-3.44	-54.47	-6.49
7a	-9.76	-9.76	-47.55	-2.75	-50.30	-6.07
5c	-8.38	-8.38	-39.16	-2.69	-41.85	-5.21
5r	-7.00	-7.00	-45.02	-0.98	-45.99	-5.49
5f	-6.45	-6.47	-49.95	-1.49	-51.45	-5.69
5m	-6.40	-6.40	-51.73	1.12	-50.60	-5.82
INH	-6.34	-6.34	-16.97	-4.63	-21.60	-1.86
INH with CO-Factor	-6.23	-6.34	-18.45	-4.10	-22.557	-2.09
5h	-6.30	-6.30	-41.72	-3.04	-44.76	-5.20
5q	-6.21	-6.21	-38.72	-2.73	-41.45	-4.77
5p	-6.19	-6.19	-54.19	-1.51	-55.70	-5.72
5t	-6.16	-6.16	-46.18	-2.80	-48.98	-5.16
5l	-6.07	-6.07	-39.39	0.20	-39.19	-5.32
5a	-6.04	-6.04	-43.13	-0.43	-43.56	-5.26
5k	-5.94	-5.94	-48.54	-1.00	-49.54	-5.44
5o	-5.93	-5.93	-45.50	0.10	-45.39	-5.50
5g	-5.68	-5.68	-41.03	-3.65	-44.68	-4.52
5j	-5.61	-5.62	-42.34	-0.14	-42.48	-5.06
5u	-5.50	-5.50	-52.26	-1.41	-53.67	-5.64
5e	-5.38	-5.38	-39.58	-5.54	-45.12	-4.99
5i	-5.36	-5.36	-34.97	0.52	-34.45	-4.86
5n	-5.32	-5.32	-46.26	-2.54	-48.80	-5.28
5d	-5.19	-5.19	-36.62	-0.20	-36.82	-4.29
5s	-5.17	-5.17	-30.73	-1.59	-32.32	-4.49
8a	-4.56	-4.56	-44.94	-0.40	-45.34	-4.72

5b	-10.19	-10.21	-51.02	-3.44	-54.47	-6.49

*Where, DS: Docking Score, GGS: Glide g Score, Gevdw: Glide evdw (Van der Waals energy), GEC: Glide ecoul (Coulomb energy), GE: Glide Energy (Modified Coulomb-Van der Waals energy), XP LEvdW: Lipophilic EvdW.

Captions:

Figure legends

Figure 1: previously reported biologically active pyran-annulated heterocyclic compounds.

Figure 2: *In vitro* % viability of compound 5e, 5r, 7a and 8a by MTT assy.

Figure 3: (a) Interacting amino acids with compound **7b** in 3d (b) 3d diagram of compound **7b** with surface (c) Interacting amino acids with compound **7b** in 2d (d) Hydrophobic interaction of compound **7b** with protein.

Figure 4: Docked pose of INH without co-factor and INH with co-factor NAD. (a) 3d pose of INH without co-factor, (b) 2d pose of INH without co-factor, (c) 2d pose of INH with co-factor, (d) 3d pose of INH with co-factor ribbon view, (e) 3d pose of INH with co-factor surface view.

Figure 5: (a) RMSD of protein-ligand (**7b**) over a period of 10ns, **(e)** RMSF of protein-ligand (**7b**) over a period of 10ns, **(b)** RMSD ligand (**7b**) over a period of 10ns, **(c)** Protein (amino acids)-ligand (**7b**) contact over a period of 10ns, **(d)** Timeline representation of Protein (amino acids)-ligand (**7b**) contact over a period of 10ns.

Figure 6: (a) Protein (amino acids)-ligand (**7b**) interaction over a period of 10ns **(b)** Superimposition of after 10ns simulation structure on docked structure of protein-ligand.

Figure 7: Plot and radial representation of ligand (**7b**) torsion showing rotatable bonds over a period of 10ns.

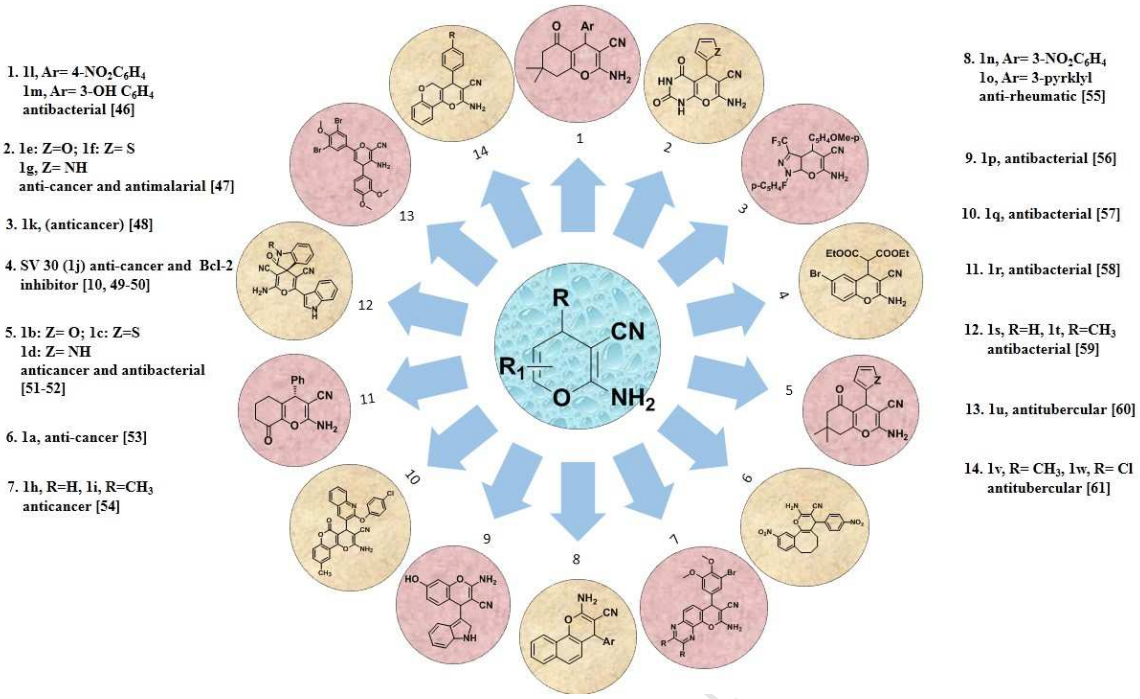


Figure.1

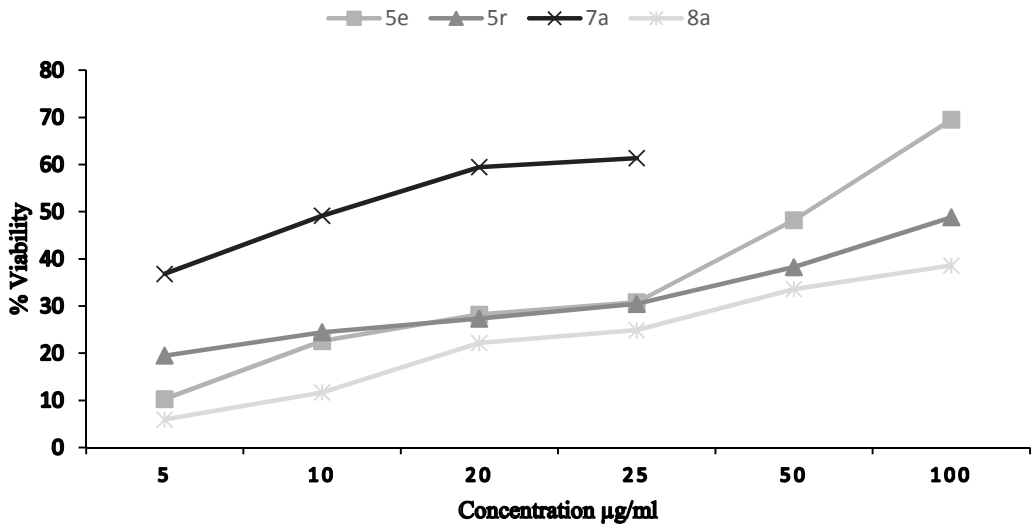
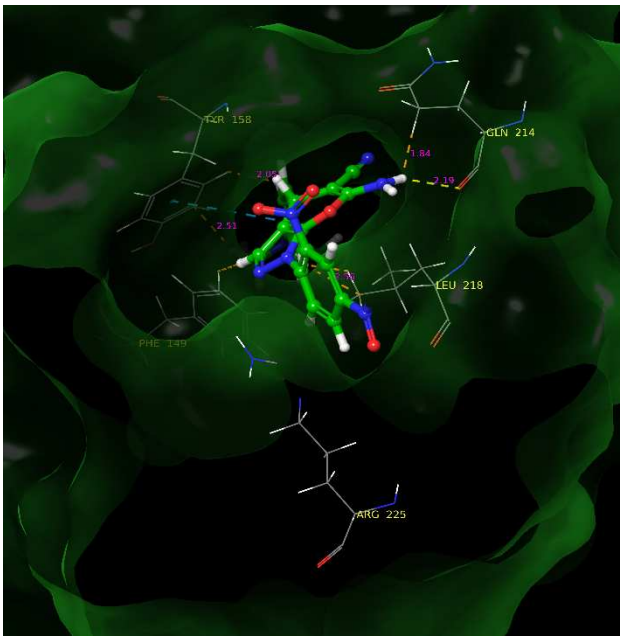
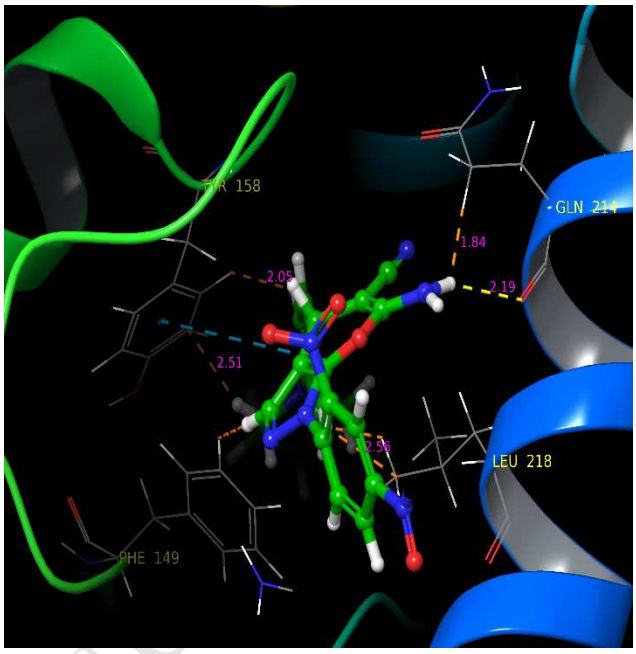
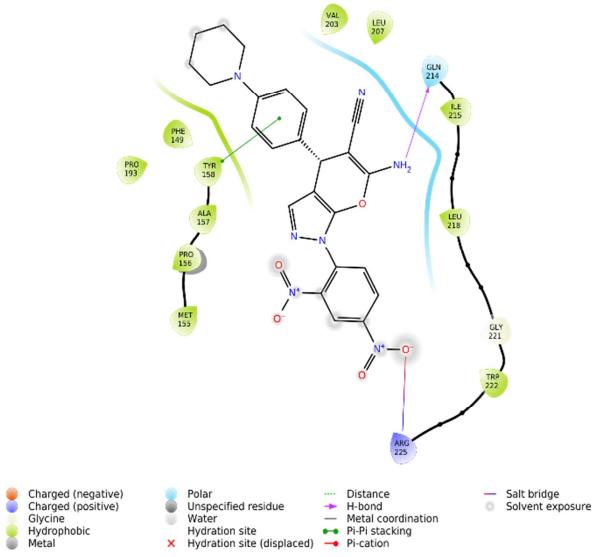
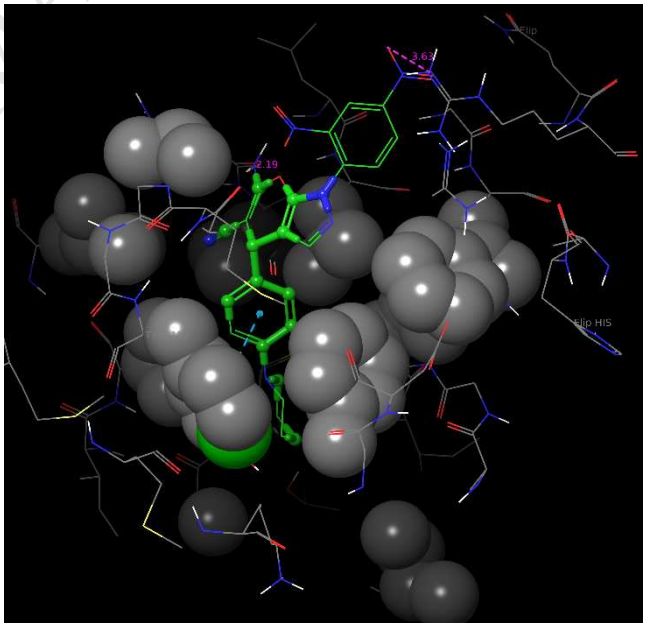


Figure 2.









(a)

(b)

(c)

(d)

Figure. 3

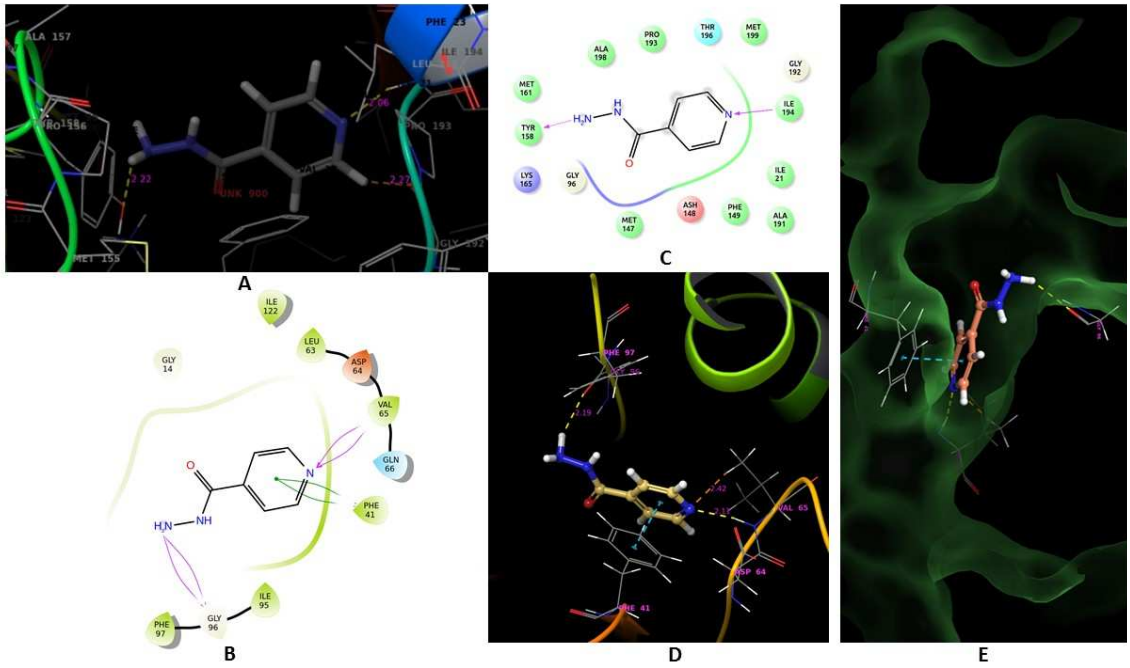


Figure. 4

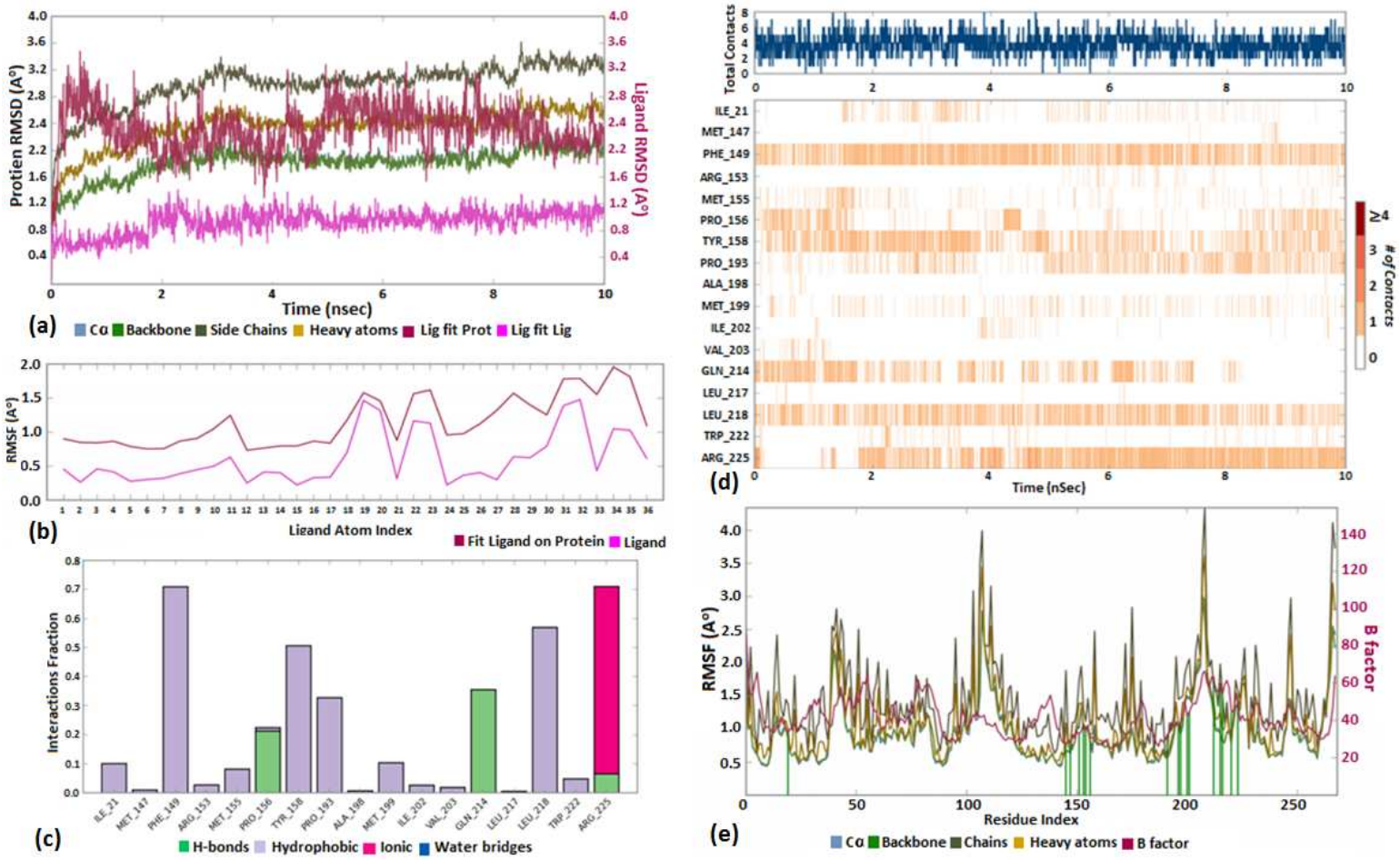
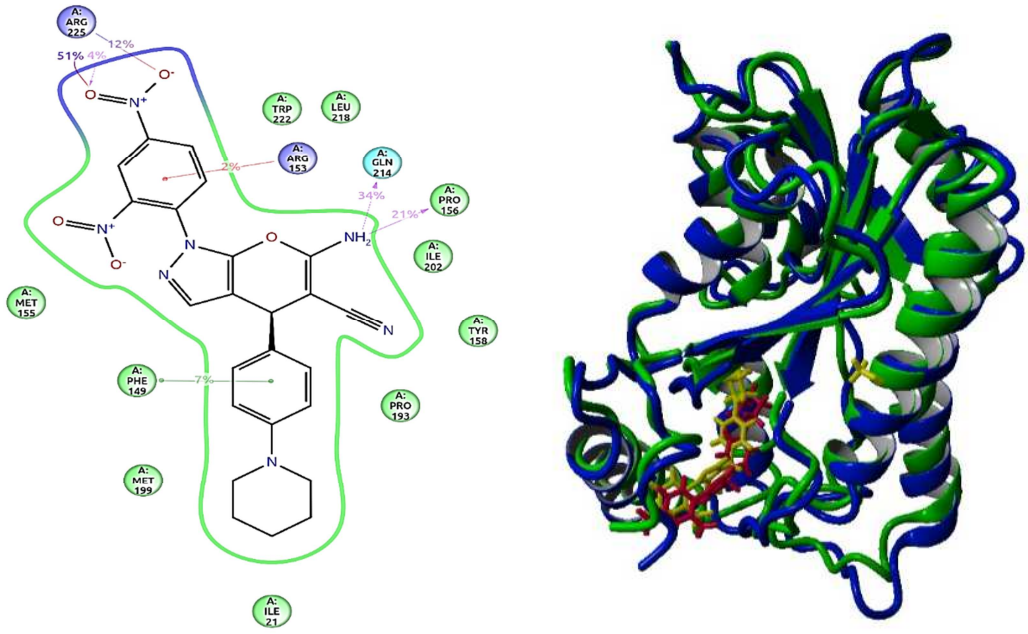


Figure 5.



(a) **(b)**

Figure. 6

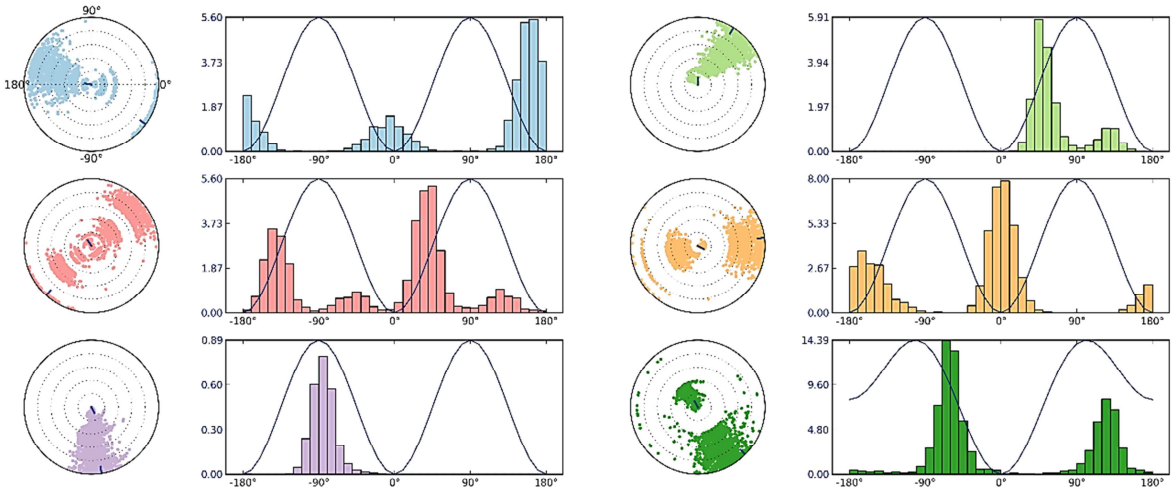
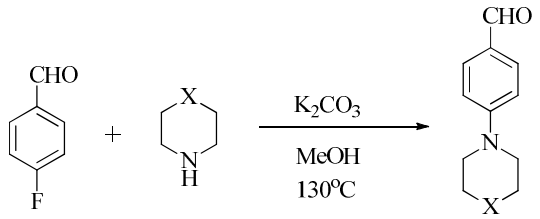


Figure. 7

Reaction schemes

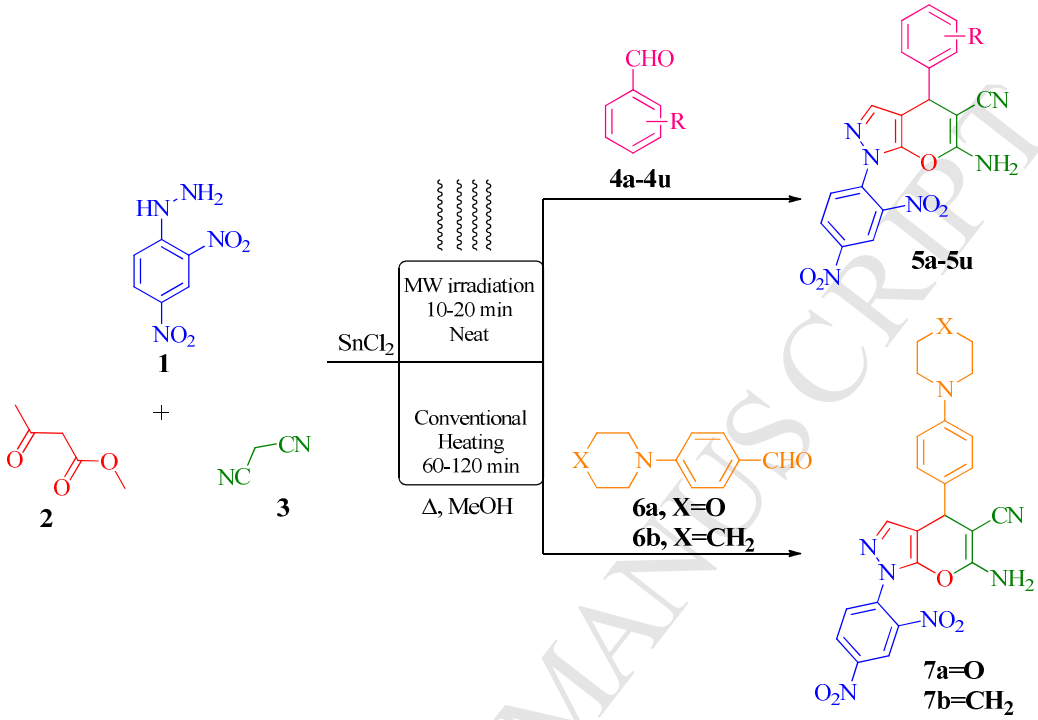
Scheme 1: Synthesis of 4-(piperidin-1-yl) benzaldehyde derivatives (**6a-6b**)



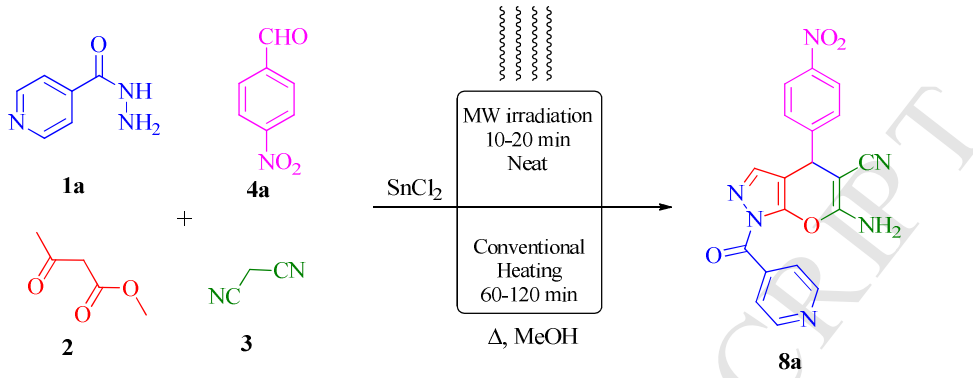
X= O, CH_2

6a, X=O
6b, X=CH_2

Scheme 2: Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-phenyl-1,4 dihydropyrano[2,3-c] pyrazole-5-carbonitrile derivatives (**5a-5u**) and (**7a, 7b**)



Scheme 3. Synthesis of 6-amino-1-isonicotinoyl-4-phenyl-1, 4-dihydropyrano [2, 3-c] pyrazole-5-carbonitrile derivative (**8a**)



Highlights

- A good biological activity against various pathogens is observed in the various derivative of 1,4-dihydropyrano[2,3-c]pyrazole.
- The final motif **5e, 5t, 7a** and **8a** showed potent activity against *Mycobacterium tuberculosis* (H_{37}RV strain) while compound **7a** also enhanced the moderate activity against MDR-TB.
- Compound **7a** was found to be good against biological evolution and cytotoxic assay.
- The molecular docking investigation of all the synthesised compounds was carried out in the active site of enoyl-ACP reductase (oxidoreductase) and compound **7b** shows good binding affinity.