Synthesis, Antimicrobial activity and Multi-Functional Docking Studies of Few Substituted Benzofuranyl Pyrazoles

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Abstract:

Ten compounds that belong to either substituted phenylpropenones (5a-e) or substituted anilines (6a-e) containing benzofuran and pyrazole nucleus were synthesized from 5-chlorosalicylaldehyde using multistep synthetic strategy. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, mass, elemental analysis and further screened for their antibacterial and anti-tubercular activities. The binding affinity of the synthesized compounds to Escherichia coli Topoisomerase IV and Mycobacterium tuberculosis enoyl-ACP reductase was determined using Schrodinger docking simulation. Compounds 5a-e and 6a-e showed notable antibacterial activity at 100 µg dose level when compared with ciprofloxacin standard. In particular, compounds 5d and 6b possessed maximum activity towards all bacterial strains. Compounds 5b, 5d, 6b and 6e exhibited anti-tubercular activity at all concentrations tested. The outcome of present study reveals that these novel substituted benzofuranyl pyrazoles could be used as new hits in lead optimization studies to get better and safer drugs.

Key words: Benzofuranylpyrazoles, Antibacterial, Anti-tubercular activity, Multifunctional docking.

1. Introduction:

Bacterial and fungal infections are common stumbling blocks of viral pneumonia, especially in critically ill patients affected with severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). The pathogens causing secondary infections in SARS patients were diverse: negative bacilli were the most common [1-2]. Despite this appalling predicament, antibiotic resistance is increasing at a dangerous rate. A fattening list of infections like tuberculosis (TB) is becoming stiff and at times impossible to treat while antibiotics are becoming less effective [3-4].

The COVID-19 pandemic threatens to reverse recent progress in reducing the global burden of TB disease. An increase in frequency and distribution of multi-drug resistant TB (MDR-TB) and extensive drug resistant TB (XDR-TB) throughout the world is considered as one of the contributing reasons for TB death. An estimated 9.7% of people with MDR-TB have XDR-TB. To make the conditions even worse, totally drug resistant TB (TDR-TB) has emerged over several countries including India, no effective treatment options exist for those patients as the strain is resistant to all available anti-TB drugs [5-7]. Beside drug repurposing and novel United States Food and Drug Administration (US FDA) approved anti-infective drugs, 10 million deaths are predicted to occur by 2050 as a result of multi drug resistant pathogens [8-9]. Those reports necessitated an increased medicinal chemists' effort in the discovery and development of novel antibacterial and antitubercular agents.

Literature survey revealed the versatile biological activities of benzofurans, pyrazoles and benzofuranyl pyrazoles. It has been established these two heterocyclic structures possess anti-inflammatory, analgesic agents, antibacterial, antifungal, antitubercular, antitumor, and anticancer activities [10-14]. Various reviews also indicate the therapeutic potential of benzofuran, pyrazole and benzofuranyl pyrazole derivatives [15-20]. This inspired numerous researchers to put a lot of effort in search of novel benzofuran and pyrazole based anti-tubercular drugs. In continuation of our interest in the synthesis of nitrogen heterocyclics [21-23], herein, we report the synthesis of five 3-[3-(5-chloro-1-benzofuran-2-yl)-1*H*- pyrazol-4-yl]-1-(substituted phenyl) prop-2-en-1-ones (5a-e) and five *N*- {[3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl] methylene} (substituted) anilines (6a-e) as antibacterial and anti-tubercular agents. All the synthesized compounds were screened for antibacterial and anti-tubercular activities. Docking simulation was done in order to predict the binding affinity of the synthesized benzofuranyl pyrazole derivatives against bacterial Topoisomerase IV and *Mycobacterium tuberculosis* enoyl-ACP reductase.

2. Experimental:

2.1 Materials and Methods:

All chemicals, reagents and solvents were purchased from Sigma Aldrich Co., (St Louis, MO, USA), Merck (Whitehouse Station, NJ, USA), Qualigens Fine Chemicals (Mumbai, India), Loba Chemie Pvt. Ltd (Mumbai, India), and Himedia Laboratories Pvt. Ltd (Mumbai, India). The melting point of the synthesized compounds was determined in an open capillary tube using digital melting point apparatus and uncorrected. The homogeneity and purity of the compounds was verified by thin layer chromatography (TLC) on silica gel G-plates using hexane: ethyl acetate and the spots were visualized in UV chamber. Infrared spectra were recorded on a SHIMADZU FT-IR-8400S using KBr disks and the values expressed in cm^{-1}. CHNO elemental analysis was carried out by a Carlo Erba 1108 elemental analyzer. Mass spectra were obtained either on MSD-1 SPC or API-ES LC-MS. The ^{1}H NMR spectra of the compounds were taken on Mercury Plus 400

MHz NMR spectrophotometer and chemical shifts expressed in delta ppm by using TMS as an internal standard.

2.2 Synthetic Procedure:

All the compounds were synthesized from 5-chloro-2-acetylbenzofuran (2) and conversion of acetyl functionality into hydrazone by reaction with hydrazine hydrate. The resultant 1-(5-chloro-1-benzofuran-2-yl) ethanone hydrazone was converted into pyrazole carbaldehyde by Vilsmeier-Hack reaction. Pyrazole carbaldehyde was allowed to react with various substituted acetophenones and aromatic amines to get the desired compounds 5a-e and 6a-e respectively.

2.2.1 Synthesis of 5-chloro-2-acetylbenzofuran (2):

The synthon 5-chloro-2-acetylbenzofuran was prepared from 5-chlorosalicylaldehyde (1) as shown in scheme 1. Its purity was established by TLC using a mixture of hexane and ethylacetate. The mixture of 5-chlorosalicylaldehyde (1) (7.8 g, 0.05 mol), chloroacetone (4.63 g, 0.05 mol) and anhydrous potassium carbonate (15 g) was gently refluxed in dry acetone (50 mL) for 12 hrs. The reaction product after cooling was filtered and the filtrate on the removal of the solvent under reduced pressure furnished 5-chloro-2-acetylbenzofuran (2) as light brownish solid. The product obtained was recrystallized from ethanol. Yield 75%, m.p. 83-85 ^{o}C. IR cm-1: 1666 (C=O), 1461 (C=C) and 790 (C-Cl), MS (m/z, %): 194(M^{+}) and 196($M+2^{+}$), ^{1}H NMR (δ ppm): 7.2 – 7.8 (4Ar-H), 2.6 (3H, s, CH3).

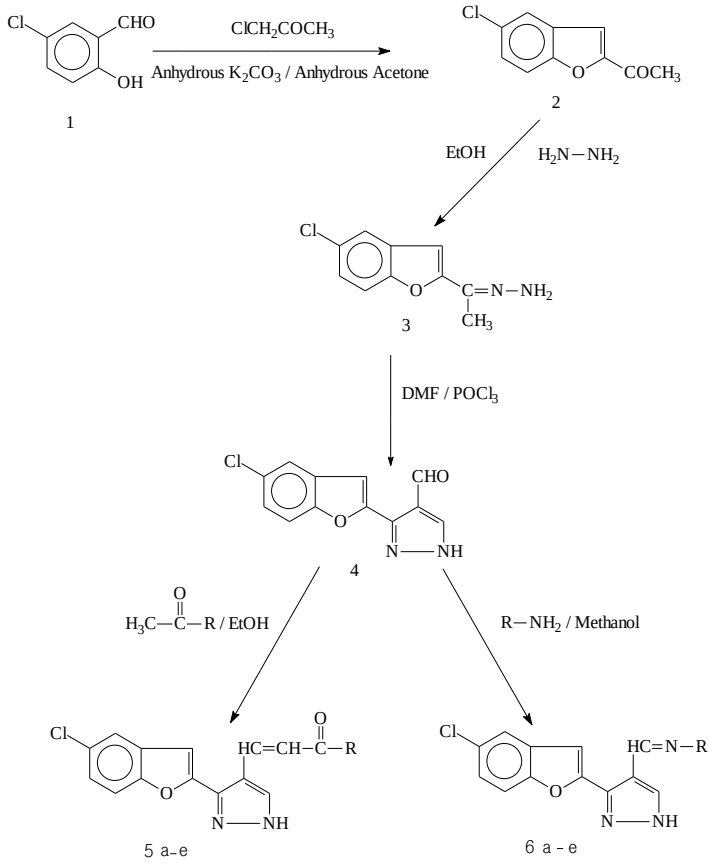
2.2.2 Synthesis of 1-(5-chloro-1-benzofuran-2-yl) ethanone hydrazone (3):

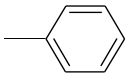
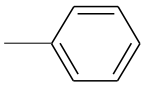
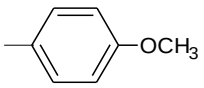
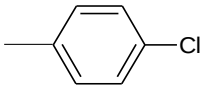
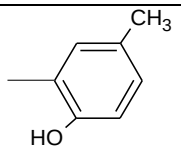
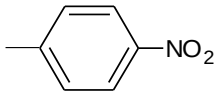
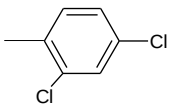
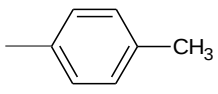
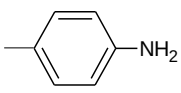
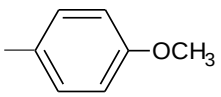
To a solution of 5-chloro-2-acetylbenzofuran 2 (1.94 g, 0.01 mol) in absolute ethanol (30 mL), hydrazine hydrate (0.5 mL, 0.01 mol) and few drops of hydrochloric acid were added. The reaction mixture was heated gently under reflux for 6 hr. The product separated on cooling was collected and crystallized from aqueous ethanol. The purity of the compound was established by TLC using a mixture of hexane and ethyl acetate as mobile phase. Yield 65 %, m.p. 145-147 ^{o}C. IR cm-1: 3361, 3269 (NH_2), 2956, 2932 (CH_3), 1575 (C = N), 1461 (C =C) and 800 (C-Cl), MS (m/z, %): 208(M^{+}) and 210($M+2^{+}$), ^{1}H NMR (δ ppm): 6.9 – 8.1 (4Ar-H), 5.2(2H,s,NH_2), 2.3 (3H, s, CH_3).

2.2.3 Synthesis of 3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazole-4-carbaldehyde (4):

Vilsmeier-Hack reagent, prepared from dimethylformamide (10 mL) and phosphorousoxychloride (1.1 mL) was added in small portions to 1-(5-chloro-1-benzofuran-2-yl) ethanonehydrazone (2.08 g, 0.01 mol) at 0-5^{o}C and the reaction mixture heated at 60^{o}C for about 4 hr and poured into crushed ice. The mixture was then neutralized with dilute sodium hydroxide solution, heated at 50-60^{0}C cooled and acidified; the separated solid was filtered and recrystallized from methanol. The purity of the compound was established by TLC using ethyl acetate and hexane mixture (30:70) as mobile phase. Yield 60 %, m.p. 119-122^{o}C. IR cm-1: 3330 (NH), 1666 (C =O), 1581 (C = N), 1438 (C =C) and 798 (C-Cl), MS (m/z, %): 246(M^{+}) and 248($M+2^{+}$), ^{1}H NMR (δ ppm): 6.0 – 7.1 (4Ar-H + 1CH of pyrazole ring), 8.2(1H,s,NH), 9.5 (1H, s, CHO).

Scheme 1:



Compound Number	R	Compound Number	R
5a		6a	
5b		6b	
5c		6c	
5d		6d	
5e		6e	

2.2.4 General synthesis of 2-[2-(5-chloro-1-benzofuran-2-yl)-1H-pyrazol-4-yl]-(substituted phenyl) prop-2-en-1-ones (5a-e)

To a mixture of 3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazole-4-carbaldehyde 4 (0.246 g, 0.001 mol) and various substituted acetophenones (0.001 mol) in ethanol (50 mL) cooled at 5-10^{o}C, sodium hydroxide (70 %, 5 mL) was added drop wise with constant stirring. The reaction mixture was stirred for 2 hr, left overnight and neutralized with concentrated hydrochloric acid. The solid then separated was filtered and recrystallized from ethanol. The purity of the compounds was established by TLC using a mixture of hexane and ethyl acetate as mobile phase.

2.2.5 General synthesis of N-{[3-(5-chloro-1-benzofuran-2-yl)-1H-pyrazol-4-yl] methylene (substituted) anilines (6a-e)

A mixture of 3-(5-chloro-1-benzofuran-2-yl)-1 *H*-pyrazole-4-carbaldehyde 4 (0.246 g, 0.001 mol), various substituted anilines (0.001 mol) and acetic acid (0.5 mL) was added in methanol (50 mL), refluxed for 4 hr, then the product was separated by filtration, washed with water and recrystallized from benzene. The purity of the compound was established by TLC using a mixture of hexane and ethyl acetate as a mobile phase.

2.3 The IUPAC and spectra details of the synthesized compounds 5a-e and 6a-e:

3-[3-(5-Chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]-1-phenylprop-2-en-1-one (5a)

Yield 65%, m.p. 160-162 ^{o}C. IR cm-1: 3320 (NH), 1660 (C=O), 1550 (C=N), 1450 (C=C), 789 (C-Cl), ^{1}H NMR (δ ppm): 6.7 (1H, d, -CO-CH=), 7.2 (1H, d, =CH-R), 7.5–8.0 (9H, m, Ar-H), 8.1(1H, s, NH).

3-[3-(5-Chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]-1-(4-methoxyphenyl)prop-2-en-1-one(5b)

Yield 68%, m.p. 167-169^{o}C. IR cm-1: 3310 (NH), 1670 (C=O), 1550 (C=N), 1450 (C=C), 780 (C-Cl).

3-[3-(5-Chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]-1-(2-hydroxy-5-methylphenyl) prop-2-en-1-one (5c)

Yield 75 %, m.p. 145-147^{o}C. IR cm-1: 3628 (OH), 3307 (NH), 1639 (C=O), 1582(C=N), 1438 (C=C), 800 (C-Cl), MS (m/z, %): 378 $[M]^{+}$, 380 $[M+2]^{+}$, ^{1}H NMR (δ ppm): 2.6 (3H, s, CH_3), 6.6 (1H, d, -CO-CH=), 7.6 (1H, d, =CH-R), 8.1- 8.9 (7H, m, Ar-H) 9.2 (1H, s, NH), 12.2 (1H, s, OH).

3-[3-(5-Chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]-1-(2,4-dichlorophenyl)prop-2-en-1-one (5d)

Yield 72%, m.p.172-174^{o}C. IR cm-1: 3320 (NH), 1650 (C=O), 1585(C=N), 1448 (C= C), 800 (C-Cl).

1-(4-Aminophenyl)-3-[3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]prop-2-en-1-one (5e)

Yield 60%, m.p. 152-154 ^{o}C. IR cm-1: 3444, 3423 (NH_2/NH), 1663 (C=O), 1541 (C=N), 1448 (C=C), 806 (C-Cl), ^{1}H NMR (δ ppm): 4.0 (2H, s, NH_2),6.6 (1H, d, -CO-CH=), 7.2 (1H, d, =CH-R),7.4-8.0 (8H, m, Ar-H) 8.3 (1H, s, NH).

N-{[3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl] methylene}aniline (6a)

Yield 72%, m.p. 168-170 ^{o}C. IR cm-1: 3344 (NH), 1568 (C=N), 1436 (C=C), 780 (C-Cl). ^{1}H NMR (δ ppm): 7.0–8.1 (10H, m, Ar-H), 8.3(1H, s, NH).

N-{[3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]methylene}-4-chloroaniline (6b)

Yield 78%, m.p.110-112^{o}C. IR cm-1: 3320 (NH), 1590 (C=N), 1460 (C=C), 790 (C-Cl).

N-{[3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]methylene}-4-nitroaniline (6c)

Yield 60%, m.p. 158-160 ^{o}C. IR cm-1: 3340 (NH), 1580(C=N), 1456 (C=C), 800 (C-Cl).^{1}H NMR (δ ppm): 6.9–7.8 (9H, m, Ar-H), 8.2(1H, s, NH).

N-{[3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]methylene}-4-methylaniline (6d)

Yield 58 %, m.p. 176-178^{o}C. IR cm-1: 3320 (NH), 1560 (C=N), 1438 (C= C), 790 (C-Cl), MS (m/z, %): 335 $[M]^{+}$, 337 $[M+2]^{+}$, ^{1}H NMR (δ ppm): 2.5 (3H, s, CH_3),7.2 – 8.1 (8H, m, Ar-H), 8.3 (1H, s, NH),10.4 (1H, s, N=CH).

N-{[3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazol-4-yl]methylene}-4-methoxyaniline (6e)

Yield 65%, m.p.147-149^{o}C. IR cm-1: 3340 (NH), 1570 (C=N), 1448 (C=C), 790 (C-Cl).

2.4 Antimicrobial activity:

2.4.1 Antibacterial activity:

The antibacterial activity of the synthesized benzofuranylpyrazoles (5a-e and 6a-e) was evaluated using agar cup plate method. Accordingly, the compounds were screened against Gram-negative organisms, namely, *Escherichia coli and Pseudomonas aeruginosa* and Gram-positive organisms *Staphylococcus epidermatitis and Bacillus subtilis* using the minimum inhibitory concentration (MIC) method. Ciprofloxacin was employed as a reference standard to compare the results.

Brain heart infusion agar was used at room temperature. The required colonies were transferred to the plates and the turbidity was adjusted visually with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. The entire surface of agar plate was swabbed three times, rotating plates ∼60° between streaking to ensure even distribution. The inoculated plate was allowed to stand for at least 5 minutes before applying disks.

A 5 mm hollow tube was heated, pressed on the inoculated agar plate, and removed immediately five times by making five wells in the plate. Subsequently, 75, 50, 25, 10, and 5 μL of the synthesized compounds were added into the respective wells on each plate. The plates were incubated within 15 minutes, after compounds application, for 24 hours at 37°C in incubator. The diameter of inhibition zone was measured to the nearest whole millimeter by holding the measuring device. According to the MIC procedure, the serial dilution was repeated up to 10^{-9} dilution for each synthesized compound [24, 25].

2.4.2 Antitubercular activity:

The synthesized benzofuranylpyrazoles (5a-e and 6a-e) were screened for antitubercular activity using the microplate Alamar blue assay method. Accordingly, each compound

was screened against *M. tuberculosis* H37 RV strain in the Middlebrook 7H9 (MB 7H9) broth using isonicotinic acid hydrazide (INH) as standard drug.

An amount of 200 µL of sterile deionized water was added to all outer perimeter wells of a sterile 96-well plate to minimize evaporation of the medium in the test wells during incubation. The 96-well plate received 100 µL of the MB 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. Plates were covered and sealed with Parafilm and incubated at 37°C for 5 days. After this time, 25 µL of freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween 80 was added to the plate and incubated for 24 hours. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC, which is the required concentration to inhibit 90% of the standardized bacterial inoculums, was defined as the lowest drug concentration which prevented the color change from blue to pink [26- 28].

2.5 Molecular Docking studies:

2.5.1 Preparation of Target molecules:

The molecular docking study was carried out on GLIDE (Schrodinger 2020-1) docking program [29]. All the synthesized compounds (5a-e and 6a-e) were docked in the active site of the crystal structure of *Escherichia coli* Topoisomerase IV ParE 24kDa subunit (PDB code: 1S14) and *Mycobacterium tuberculosis* enoyl-ACP reductase (PDB code: 2PR2).

The Ramachandran plot of all the three prepared target molecules was obtained using RAMPAGE [30].The protein structures were verified, validated and evaluated by ERRAT, Verify 3D, Structural Analysis and Verification Server [31-33]. Obtained results suggest that all protein models are acceptable and of good quality.

2.5.2 Preparation of ligand molecules:

The 2D chemical structures of the ligands were drawn and saved in binary format using Chem Draw Ultra Version 8.0.3 [34]. These binary files were converted to SDF format using the Open Babel GUI version 2.4.1 virtual screening tool for windows was availed to get spatial data file of ligands [35, 36]. Energy was minimized employing OPLS3e force field by keeping default settings in Ligprep like ionization (possible states at target pH 7.0±2.0 using Epik), desalt, retain specified chiralities and etc tools [37]. Binding affinity of compounds was compared using ATP as docking control. The results were investigated by comparing the binding interactions and docking score obtained from GLIDE_SP ligand docking.

3. Results and Discussion:

3.1 Synthesis:

The schematic representation for the synthesis of benzofuranylpyrazoles (5a-e and 6a-e) is provided in Scheme 1. First, the key synthon 5-chloro-2-acetylbenzofuran (2) is synthesized by the condensation of 5-chlorosalicylaldehyde (1) and chloroacetone. Heating 5-chloro-2-acetylbenzofuran (2) with hydrazine hydrate yielded the corresponding hydrazone derivative (3). Reaction of 1-(5-chloro-1-benzofuran-2-yl) ethanonehydrazone (3) with Vilsmeier-Hack reagent resulted in the formation of basic unified structure with benzofuran and pyrazole scaffolds, 3-(5-chloro-1-benzofuran-2-yl)-1*H*-pyrazole-4-carbaldehyde (4).

Constant stirring of a mixture containing benzofuranylpyrazole carbaldehyde (4) and substituted acetophenones at 0-5^0 C under basic conditions eventually rendered 2-[2-(5-chloro-1-benzofuran-2-yl)-1H-pyrazol-4-yl]-(substituted phenyl) prop-2-en-1-ones (5a-e).

Schiff's base reaction of benzofuranylpyrazole carbaldehyde (4) with various substituted aromatic primary amines produced N-{[3-(5-chloro-1-benzofuran-2-yl)-1H-pyrazol-4-yl] methylene (substituted) anilines (6a-e).

The purity of all the synthesized compounds (2, 3, 4, 5a-e and 6a-e) is established by TLC using a mixture of hexane and ethyl acetate as mobile phase. Their identity was confirmed by single spot on TLC, sharp melting point and by spectral characteristics.

In the Infra Red (IR) spectra of the synthesized compounds, 5a−5e, a characteristic band observed in the region between 1640 and 1685 cm−1 confirms α, β-unsaturated ketone system. In the proton nuclear magnetic resonance (^{1}HNMR) spectra of synthesized compounds, 5a, 5c and 5e, the signals for aromatic protons resonate in the region between 7.4 and 8.9 ppm, whereas signals for =CH-CO- resonate around 6.6 ppm. In 5c, one-proton singlet results at 12.2 ppm corresponding to proton of the hydroxy group located at ortho position of the phenyl ring attached with α, β-unsaturated ketone system. Furthermore, the signal for pyrazole's -NH resonates at 8.1, 9.2 and 8.3ppm in 5a, 5c and 5e.

Similarly for compounds 6a-6e, an IR absorption band observed in the region between 1550 and 1600 cm−1 confirms Schiff's base C=N, whereas bands around 3340 cm−1 and 790 cm−1 corresponds to –NH and –Cl respectively. In the ^{1}HNMR spectra of 6a, 6c and 6d, the signals for aromatic protons resonate in the region between 6.9 and 8.1 ppm.

3.2 Antimicrobial activity:

All the synthesized benzofuranylpyrazoles (5a-e and 6a-e) were screened for their antibacterial and antitubercular activities.

All the synthesized compounds have been evaluated for their antibacterial activity using agar cup-plate method. The results of this evaluation have been viewed by taking ciprofloxacin as reference standard. Compounds (5a-e and 6a-e) showed notable antibacterial activity at 100 µg dose level when compared with standard drug (Figure 1). In particular, compounds 5d and 6b possessed maximum activity towards all bacterial strains which may be due to the presence of *p*-chlorophenyl at third position of the pyrazole ring. Compounds 5b, 5c, 5e, 6c, 6d and 6e also possessed good antibacterial activity against all bacterial strains which may be due to the presence of methoxy, hydroxyl, amino, nitro, methyl and methoxy groups at third position of the pyrazole ring respectively, besides the favorable effect of the benzofuran moiety.

The antitubercular activity of the synthesized benzofuranylpyrazoles (5a-e and 6a-e) was screened against *Mycobacterium tuberculosis* H37Rv in the middle brook 7H9 broth media (MB 7H9 broth) by using INH as standard drug (Table 1). The results of antitubercular activity revealed that compounds 5b and 6e having *p*-methoxyphenyl and compounds 5d and 6b having *p*-chlorophenyl at the third position of the pyrazole ring exhibited activity at all concentrations and the remaining compounds showed no much activity.

Figure 1. Antibacterial activity of Benzofuranyl pyrazole derivatives (5a-e and 6a-e)

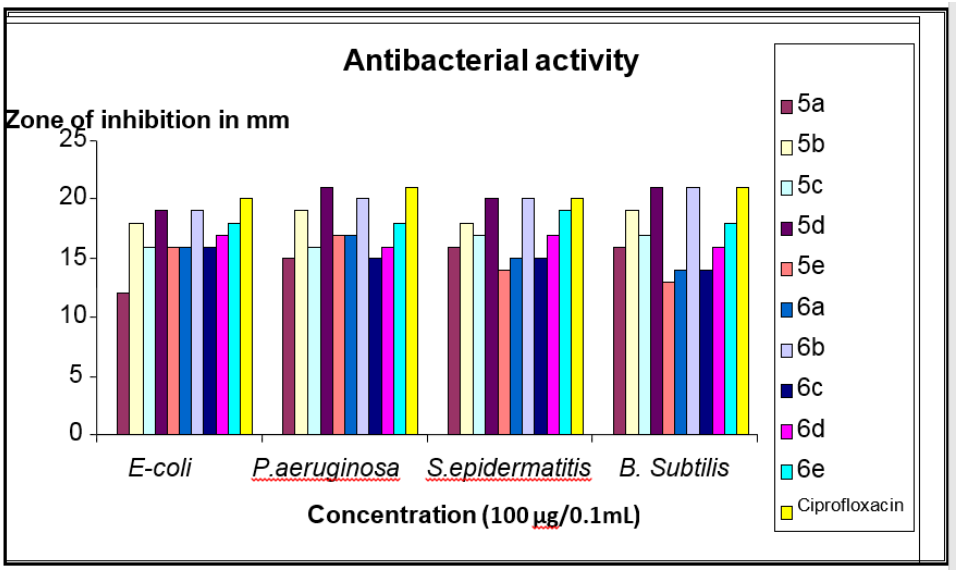


Table 1. Antitubercular activity of Benzofuranylpyrazole derivatives (5a-e and 6a-e)

Minimum inhibitory concentration (MIC)			
Comp. No.	**Concentration (µg/mL)**		
	25 µg/mL	**50 µg/mL**	**100 µg/Ml**
INH (std)	S	S	S
5a	R	R	S
5b	S	S	S
5c	R	R	S
5d	S	S	S
5e	R	S	S
6a	R	R	S
6b	S	S	S
6c	S	S	S
6d	R	R	S
6e	S	S	S

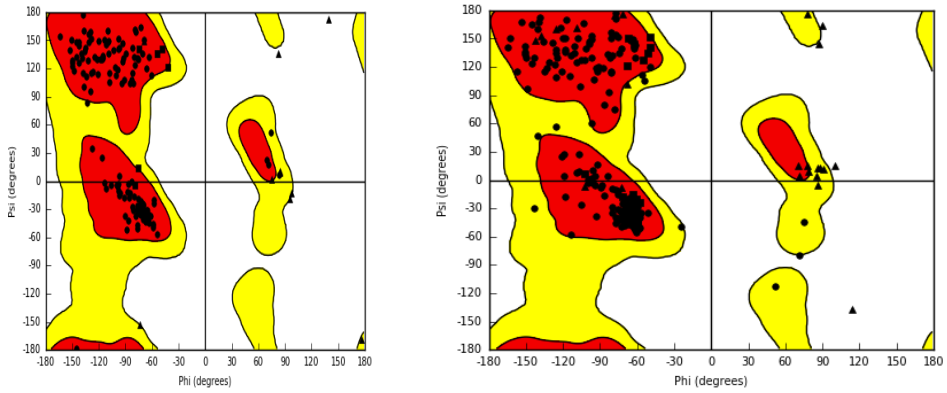
Note: R =Resistance S = Sensitive

3.3 Molecular Docking:

The quality of 3D target molecule models was evaluated with Ramachandran plot calculations using RAMPAGE. The percentage of residues in the favoured region, allowed region and outlier region is 94.6%, 5.4%, 0% (1S14), 89.8%, 9.8%, 0.4% (4WMZ), 89.8%, 8.9% and 0.9% (2PR2) respectively. Generally a score close to 100% indicates a good quality of the model. Therefore, these results suggest that the predicted model was of good quality (Figure 2)

Figure 2. Ramachandran plots generated via RAMPAGE for (A) 1S14 and (B) 2PR2. Residues in favoured (red), allowed (yellow) and outlier regions (white).

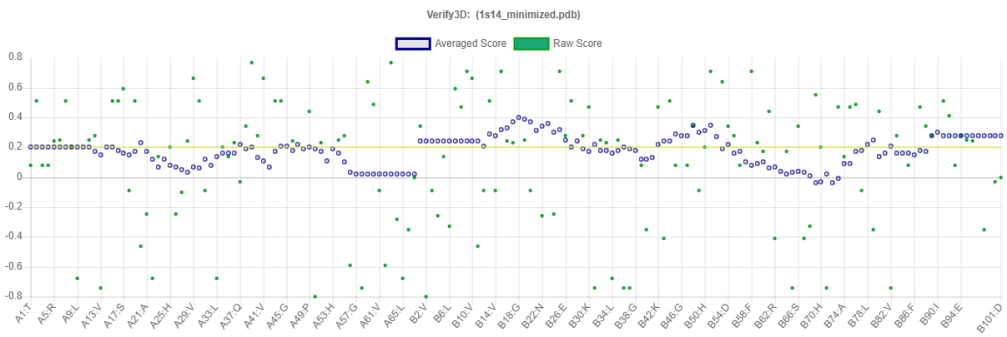
(A) (B)



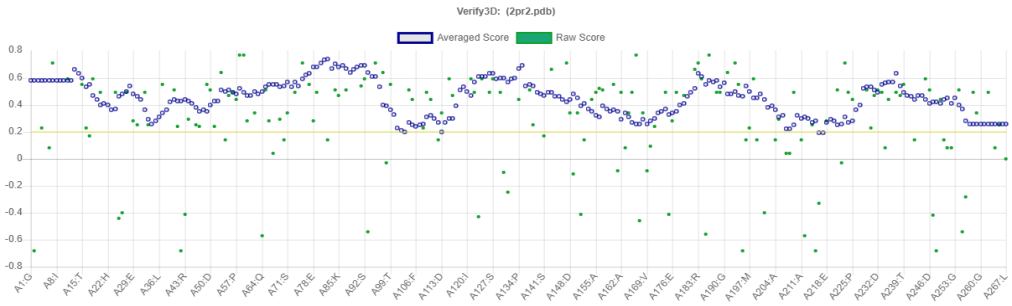
These structures were also validated by other servers such as ERRAT and Verify 3D. The overall quality factor of target molecules obtained in ERRAT analysis is in the order of 94.21% (2PR2) < 95.83% (1S14). All these values are either higher or closer to the 95% rejection limit. This reinstates the quality of target protein models. In the Verify 3D analysis, it was found that none of the amino acids of 2PR2 had a negative score, but very few residues in 1S14 exhibited marginal negative score. The percentage of aminoacid residues with 3D-1D score greater than or equal to 0.2 is 99.25% (2PR2) and 46.43% (1S14). It should be noted that compatibility scores above zero correspond to an acceptable structural environment (Figure 3).

Figure 3. Verify 3D results of (A) 1S14 and (B) 2PR2.

(A)



(B)



The docking score of each ligand against target proteins was predicted using Glide, which is one of the most commonly used Schrodinger's docking software. Docking of compounds 5d and 6b with two target protein models are shown in figures 4 and 5. In the docking procedure, ten binding poses were obtained, and the binding pose with the highest docking score was selected. Amongst all synthesized compounds, 6c showed highest docking score (−9.269). All compounds exhibited interesting docking scores comparable with respective standards.

Figure 4. Docking of compounds 5d and 6b with 1S14 Protein model:

5d 6b

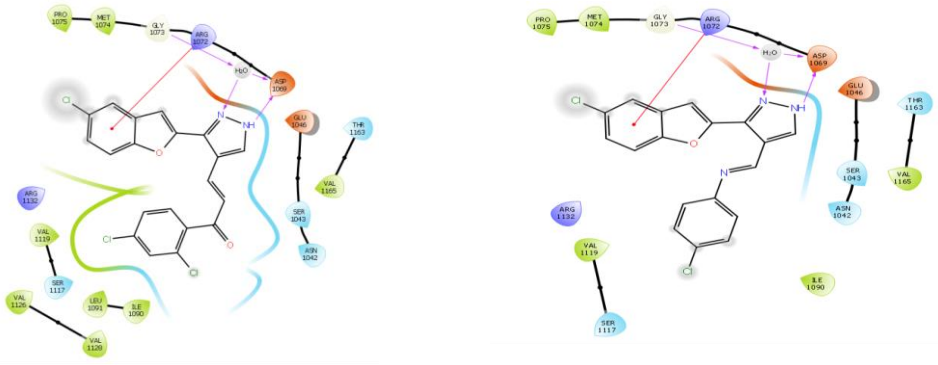
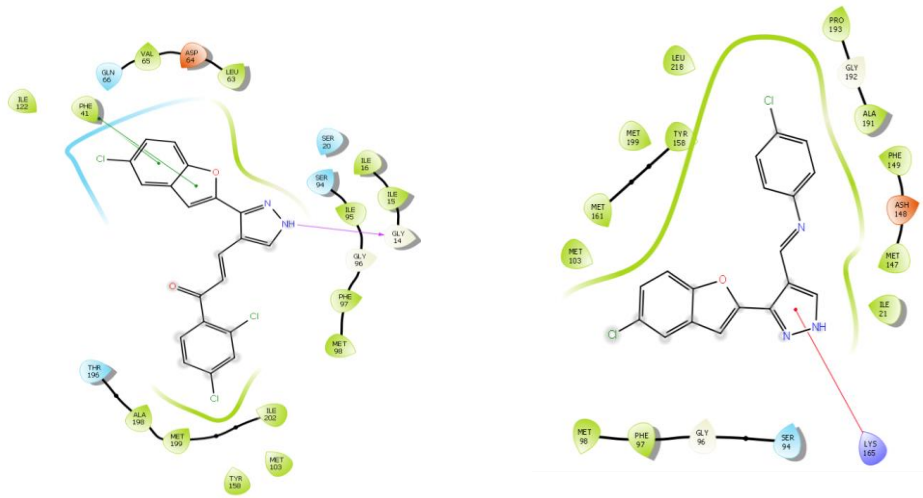


Figure 5. Docking of compounds 5d and 6b with 2PR2 Protein model:

5d 6b



The summary of interactions observed between synthesized benzofuranylpyrazoles and amino acid residues of target proteins are given in Tables 2 and 3. With the exception of compounds 5e and 6c, all synthesized compounds showed hydrophobic interactions with 1S14.

Except 5a, 5b and 5e, rest of all synthesized compounds were found to mimicking the standard drug ciprofloxacilin regarding hydrogen bond interaction sites. Compound 6d exhibited neither hydrogen bonding nor hydrophobic interaction with target 2PR2. Compounds 5c and 5d were the only molecules that interacted with both hydrophobic and hydrogen binding sites.

Table 2. Interactions between synthesized compounds and residues of 1S14.

Compound Code	Docking Score	H- Bond Interactions	Hydrophobic interactions (Pi-Pi stacking or Pi-cation)
5a	-8.429	ASP 1069	ARG 1072
5b	-7.482	ASP 1069	ARG 1072
5c	-7.723	ASP 1069, GLY 1073	ARG 1072
5d	-8.264	ASP 1069, GLY 1073	ARG 1072
5e	-7.442	ASP 1069	---
6a	-7.116	ASP 1069, GLY 1073	ARG 1072
6b	-6.893	ASP 1069, GLY 1073	ARG 1072
6c	-6.702	ASP 1069, GLY 1073	---
6d	-7.021	ASP 1069, GLY 1073	ARG 1072
6e	-7.136	ASP 1069, GLY 1073	ARG 1072
Ciprofloxacin	-7.066	ASP 1069, GLY 1073	VAL 1118

Table 3. Interactions between synthesized compounds and residues of 2PR2.

Compound Code	Docking Score	H- Bond Interactions	Hydrophobic interactions (Pi-Pi stacking or Pi-cation)
5a	-7.422	---	LYS 165
5b	-7.462	---	LYS 165
5c	-7.289	GLY 96	PHE 97
5d	-7.361	GLY 14	PHE 41
5e	-7.518	SER 94, MET 98	---
6a	-7.26	---	LYS 165
6b	-7.604	---	LYS 165
6c	-7.654	---	TRP 222
6d	-7.645	---	---
6e	-7.341	---	LYS 165
Isoniazid	-7.244	VAL 95, GLY 96	PHE 41

Remaining compounds interacted either through hydrophobic pockets or hydrogen bonding residues.

4. Conclusion:

The procedures employed in the synthesis of title compounds are classical, well-established, and have been in practice for many years. The spectral and analytical data from Fourier transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (1H NMR), and mass spectrometry (MS) were analysed to confirm the structure of selected compounds. In all cases, the products were retrieved in their purest form. Furthermore, they were purified by recrystallization from ethanol (5a-e) and benzene (6a-e).

Compounds having electron donating groups on the aromatic ring bonded to the 3rd position of the pyrazole nucleus had the strongest antibacterial activity among the benzofuranylpyrazoles synthesised. Molecular docking investigations revealed that all of the synthesised compounds had a high dock score and hence may be exploited as lead structures in rationally designing antibacterial molecules. The findings indicate that these heterocyclic structures might serve as significant biological agents and/or be employed as

competent intermediates to establish substantial biologically active molecules, specifically 5a-e.

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Conflict of interest:
The authors report no conflicts of interest in this work.

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