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ORIGINAL ARTICLE

Design, in-silico study and biological evaluation of newly synthesized 3-chlorobenzofuran congeners as antitubercular agents

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Benzofuran;
Pyrazole;
Anti-tubercular;
Structure activity relationship;
In-silico study

Abstract Benzofuran is a heterocyclic scaffold present in various natural products and possess excellent pharmacological properties including anti-tubercular activity as well. A novel series 26 compound containing 3-chlorobenzofuran derivatives are designed, synthesized and spectroscopically characterized. In vitro screening of compounds was done against multidrug resistant *Mycobacterium tuberculosis* H37Rv strains. Out of these compounds **3a**, **3b**, **3c**, **4b** and **4c** exhibited excellent inhibitory potency with IC 50 values in the range of 43–104 μM. Compound **3b** was found to be the most potent with IC 50 value of 51.24 μM and IC 90 value of 88.04 μM. The compound may serve as lead for future development of potential and effective anti-tubercular agent.

1. Introduction

Tuberculosis (TB) is a chronic communicable disease caused by *Mycobacterium tuberculosis*, majorly affecting lungs. TB is the 10th leading cause of death globally and every year millions of people get affected with it. According to 2018 global report of WHO, an estimated 1.3 million deaths were caused due to TB. In 2017, 10 million people developed TB globally of which India, China and Indonesia share maximum number of patients. Multi Drug Resistance- Tuberculosis (MDR-TB) continues to be a public health crisis. Over the decades,

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pathogens have become more resistant to the first and second line anti mycobacterial drugs such as isoniazid, rifampicin, ethambutol, streptomycin etc. In 2017, approximately half million people developed drug resistant TB worldwide. Drug resistance can further worsen the situations in future, so it is the critical need of the time to explore more in this field and look for novel, potent anti-TB molecules (Lienhardt et al., 2018; Floyd et al., 2018).

Benzofuran is a heterocyclic fused ring of benzene with furan possessing a number of pharmacologically properties such as antibacterial (Li et al., 2019), antifungal (Liang et al., 2016), anthelmintic (Kenchappa et al., 2017), anticancer (Abbas and El-Karim, 2019; El-Karim et al., 2015; Xie et al., 2015) anti-viral (Gouhar et al., 2018; He et al., 2015; Gao et al., 2019) and anti-tubercular (Zhang et al., 2017; Bodke et al., 2017). Benzofuran has shown important place in the drug development. Currently substituted Benzofurans are gaining interest as anti-mycobacterial agents, linking of pyrazine with benzofuran results into most active compound showing minimum inhibitory concentration (MIC) of 1.21 μg/mL. Exploitation of second and third position of benzofuran has been reported to give compounds with MIC value as low as 3.25 μg/mL (Telvekar et al., 2012; Nevagi et al., 2015).

Pyrazole is nitrogen containing heterocycle having an important role in medicinal chemistry. Pyrazole and its derivatives constitute a number of compounds exhibiting diverse pharmacological activities which includes analgesics, antimicrobial, antibacterial, antifungal, anticonvulsant, anticancer and antitubercular (Harikrishna et al., 2016; Nayak et al., 2016; Domiati et al., 2016; Muhammad et al., 2019; Chougala et al., 2017; El Shehry et al., 2018; Wu et al., 2018; Reddy et al., 2015; Zhang et al., 2019).

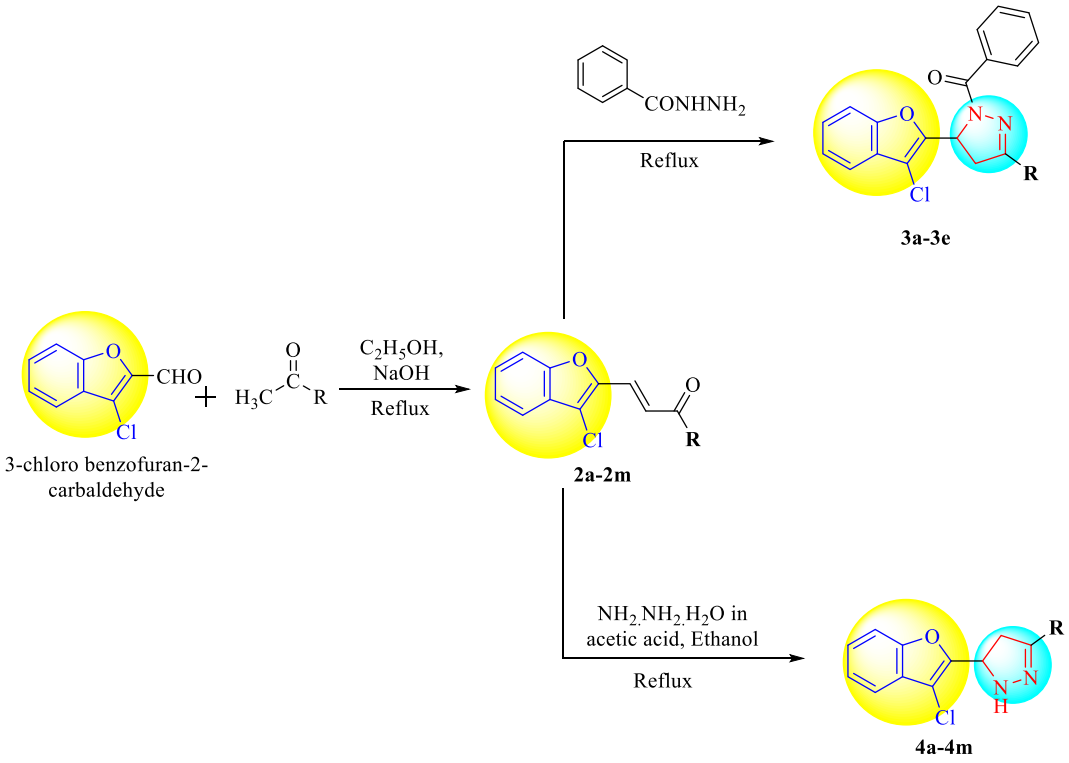
In recent years, a number of compounds having substituted benzofuran bearing pyrazole were reported, they exhibited broad-spectrum antimicrobial activities. (Chu et al., 2019; Manna and Agrawal, 2010). We have designed some novel benzofuran linked pyrazole derivatives to find better therapeutic agents for the treatment of tuberculosis. In present work, a series of 3-chlorobenzofuran 4,5-dihydropyrazole derivatives were synthesized and their antitubercular activity was evaluated against *Mycobacterium tuberculosis* H37RV.

2. Result and discussion

2.1. Chemistry

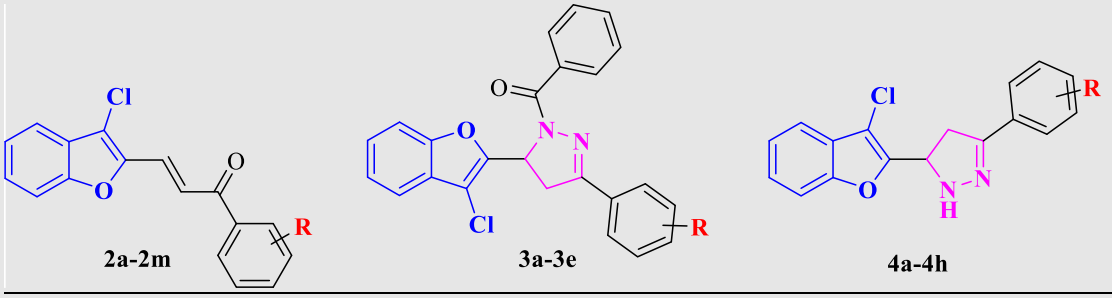
Derivatives of 3-Chlorobenzofuran were synthesized as showed in Scheme 1. 3-chloro benzofuran-2-carbaldehyde was taken as starting material and reacted with different aromatic aldehydes to give intermediates **2a-2n** in excellent yield. Compounds **2a-2n** were refluxed with benzohydrazide and hydrazine hydrate to finally yield compounds **3a-3e** and **4a-4m** respectively.

The yield of the products varied with maximum yield of 98% with anisaldehyde. IR spectra of these chalcones revealed C = O, C = C and C-Cl stretching at 1685, 1605 and 750 cm^{-1}. 1H NMR spectrum further confirmed the structure marked by the disappearance of aldehydic proton and appearance of two characteristic doublets one each for vinylic protons. Selected chalcones (**2b, 2f, 2h, 2l** & **2m**) were reacted with benzoic acid hydrazide in glacial acetic acid to get the respective pyrazoline derivatives (**3a-3e**). All the compounds were purified by column chromatography and their spectral data was found satisfactory for the proposed structures. The



Scheme 1 Synthesis of 3-Chlorobenzofuran derivatives.

Table 1a Physical constant of derivative 2a-4h.



Compound	R	Mol. Wt.	Mol. formulae	% Yield	Melting point (°C)
2a	H	282	$C_{17}H_{11}ClO_2$	78	140–42
2b	4-Chloro	317	$C_{17}H_{10}Cl_2O_2$	87	148–50
2c	4-Methyl	296	$C_{18}H_{13}ClO_2$	76	126–28
2d	4-Nitro	327	$C_{17}H_{10}ClNO_2$	70	180–82
2e	4-Amine	297	$C_{17}H_{12}ClNO_2$	84	184–86
2f	4-Hydroxy	298	$C_{17}H_{11}ClO_3$	76	210–12
2 g	2-Hydroxy	298	$C_{17}H_{11}ClO_3$	76	110–12
2 h	4-Methoxy	312	$C_{18}H_{13}ClO_3$	98	106–08
2i	2,4-Dihydroxy	314	$C_{17}H_{11}ClO_4$	80	100–02
2j	2,4-Dimethoxy	342	$C_{19}H_{15}ClO_4$	84	138–40
2 k	3,4-Dimethoxy	342	$C_{19}H_{15}ClO_4$	89	172–74
2l	4-Hydroxy 3-methyl	312	$C_{18}H_{13}ClO_3$	76	202–04
2m	4-Hydroxy 2-methyl	312	$C_{18}H_{13}ClO_3$	76	160–62
3a	4-Chloro	435	$C_{24}H_{16}Cl_2N_2O_2$	62	150–52
3b	4-Hydroxy	416	$C_{24}H_{17}ClN_2O_2$	56	150–52
3c	4-Methoxy	430	$C_{25}H_{19}ClN_2O_3$	67	100–02
3d	4-Methoxy 3-methyl	430	$C_{25}H_{19}ClN_2O_3$	66	144–46
3e	4-Hyroxy 2-methyl	430	$C_{25}H_{19}Cl_2N_2O_3$	70	148–50
4a	H	296	$C_{17}H_{13}ClN_2O$	78	126–28
4b	4-Chloro	331	$C_{17}H_{12}Cl_2N_2O$	82	66–68
4c	4-Methyl	310	$C_{18}H_{15}ClN_2O$	80	146–48
4d	4-Nitro	341	$C_{17}H_{12}ClN_3O_3$	74	126–28
4e	4-Amino	311	$C_{17}H_{14}ClN_3O$	70	120–22
4f	4-Hydroxy	312	$C_{17}H_{13}ClN_2O_2$	78	268–70
4g	2-Hydroxy	312	$C_{17}H_{13}ClN_2O_2$	82	148–50
4h	4-Methoxy	326	$C_{18}H_{15}ClN_2O_2$,	87	182–84

chalcones **(2a-2n)** were reacted separately in ethanol with hydrazine hydrate by using molecular sieves in presence of glacial acetic acid. In few cases two products were obtained which were isolated by column chromatography, the minor product was identified as acetyl derivative. The pyrazolines **(4a-4m)** thus obtained (major product) were characterized on the basis of elemental analysis, IR, 1H NMR spectra. Physical constant of all the synthesized derivatives are presented in Table 1a.

2.2. Biological activity

2.2.1. Antitubercular

The newly synthesized compounds were evaluated *in vitro* for antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain (ATCC27294) by Microplate Alamar Blue Assay (MABA) (Wahyuningrum et al., 2017; Xu et al., 2016; Bunalema et al., 2015; Nkenfou et al., 2015), at Tuberculosis Antimicrobial Acquisition and Coordination facility (TAACF). Isoniazid (INH) is reported to have MIC at 0.43 µM with selectivity index of > 1,250 by MABA (D Sriram, 2006). For each compound both IC_{50} and IC_{90} were determined, Table 1b represent anti-mycobacterial activity against H37Rv strain of mycobacterium tuberculosis of all synthesized inhibitors.

Selected compounds of the series **(2a-4 m)** were evaluated for *in vitro* antitubercular activity. Eight compounds of the series were found to be weakly active with MICs ranging from 88.04 to 178.58 µM while thirteen were found inactive, particularly chalcones **(2a-2m)**, except *3-(3-Chlorobenzofuran-2-yl)-1-phenyl-2-propen-1-one* **(2a)** that exhibited IC_{50} of 106.83 µM and IC_{90} of 252.06 µM. Derivatives **3a-3e** showed good potency, *5-(3-Chlorobenzofuran-2-yl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-1-pyrazolyl-phenyl methanone* **(3b)** is the most potent in all exhibited IC_{50} of 51.24 µM and IC_{90} of 87.98 µM. Pyrazoline derivatives **(4a-4m)** were active in comparison to parent chalcones **(**Fig. 2**)**.

2.3. Molecular docking and ADME studies

Molecular docking was performed to understand the molecular interactions of the synthesized compounds at the binding site of the target protein. The target protein for anti-mycobacterial activity was obtained from Protein Data Bank (PDB ID: 5OIF). ADME calculation (Table 3) depicts that

Table 1b Antimycobacterial activity of compounds (2a-4 h) against of mycobacterium tuberculosis.

Compound	R	MABA: H37Rv data	
		IC_{50} (μM)	IC_{90} (μM)
2a	H	118.70	252.06
2b	4-Chloro	> 400	> 400
2c	4-Methyl	> 400	> 400
2d	4-Nitro	> 400	> 400
2e	4-Amine	> 400	> 400
2f	4-Hydroxy	113.72	> 400
2g	2-Hydroxy	> 400	> 400
2h	4-Methoxy	> 400	> 400
2i	2,4-Dihydroxy	311.69	> 400
2j	2,4-Dimethoxy	> 400	> 400
2k	3,4-Dimethoxy	> 400	> 400
2l	4-Hydroxy 3-methyl	> 400	> 400
2m	4-Hydroxy 2-methyl	< 0.1950	> 400
3a	4-Chloro	106.84	165.66
3b	4-Hydroxy	51.30	88.04
3c	4-Methoxy	89.36	143.82
3d	4-Methoxy 3-methyl	119.84	> 400
3e	4-Hyroxy 2-methyl	178.37	277.27
4a	H	> 400	> 400
4b	4-Chloro	101.81	158.90
4c	4-Methyl	102.35	163.58
4d	4-Nitro	242.03	> 400
4e	4-Amino	153.80	> 400
4f	4-Hydroxy	316.81	> 400
4g	2-Hydroxy	> 400	> 400
4h	4-Methoxy	92.68	178.64
Isoniazid	–	0.054	0.076

all compounds completely get absorbed when administered orally. Compounds **3b** and **3c** possessed two rotational bonds, **3a**, has one rotational bond, while **4a** and **4c** have no rotational bonds. **3b**, **4a** and **4c**, each has at least one hydrogen bond donor while all compounds have good number of h-bond acceptor. All compounds exhibited drug gable properties as per Lipinski's rule. Molecular docking analysis suggests that all compounds were active as compared to isoniazid and pyrazinamide (Table 2). Compound **3b** displayed maximum docking (−7.061 Kcal/mol) and glide score (−7.063 Kcal/mol) compared to isoniazid which has docking score of −5.696 Kcal/mol and glide score of −5.697 Kcal/mol. Binding site interactions reveal that **3b** forms two strong hydrogen bonds with methionine (MET98- 1.95 Å) and threonine (THR196-1.86 Å) residues while isoniazid and pyrazinamide forms four hydrogen bonds (GLY14, ALA22, SER94 and LYS165) and one hydrogen bond (ILE194) respectively at the binding site. Although the hydrogen bond forming residues are different when compared to isoniazid and pyrazinamide, they all share some common hydrophobic interactions suggesting binding in the similar region of the target. Images depicting two dimensional interactions and three dimensional binding poses of compound **3b** and isoniazid have been shown in Fig. 3.

Table 2 Docking and glide score of potent compounds (PDB ID: 5OIF).

Compound	Docking score (Kcal/mol)	Glide score (Kcal/mol)
3a	−6.051	−6.051
3b	−7.061	−7.063
3c	−6.466	−6.466
4b	−6.324	−6.325
4c	−6.404	−6.405
Isoniazid	−5.696	−5.697
Pyrazinamide	−5.418	−5.418

3. Conclusion

In this work, we have designed, synthesized and evaluated a novel series of chloro benzofuran derivatives for antitubercular activity. Most of the derivatives exhibited potent antimycobacterial property against *Mycobacterium tuberculosis* H37RV strain. Compound **3a**, **3b**, **3c**, **4b** and **4c** showed good inhibitory potential. Structure activity relationship **(**Fig. 1**)** reveals in series **3a- 3e**, compound **3a** and **3c** substituted with chloro and methoxy group at $\mathbf{R_1}$ position respectively have showed excellent inhibitory potency. Whereas compound from **4a to**

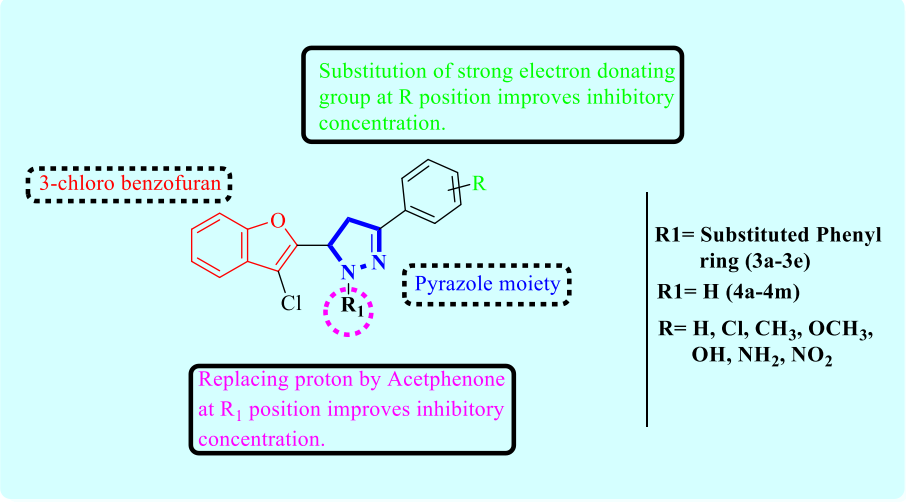


Fig. 1 Structure activity relationship of.

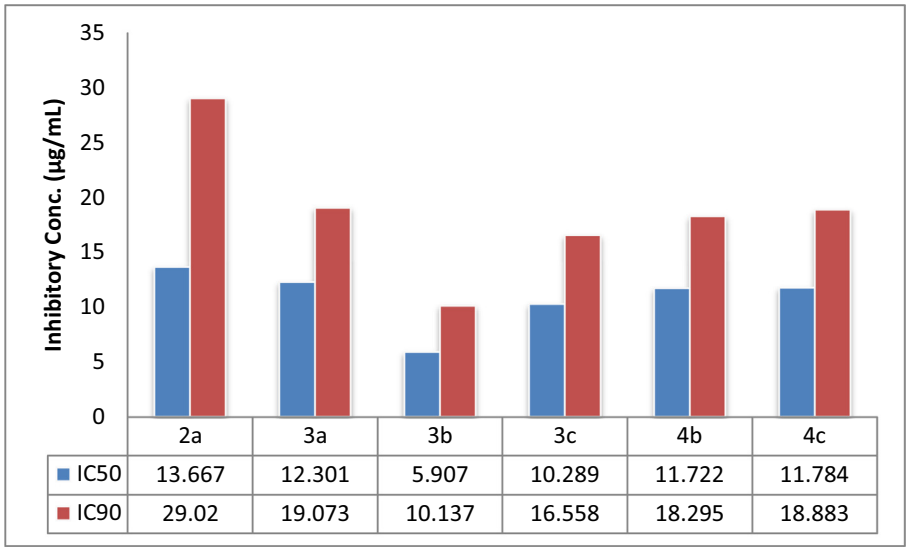


Fig. 2 Graph representing inhibitory constants (IC_{50} & IC_{90}) of potent compounds.

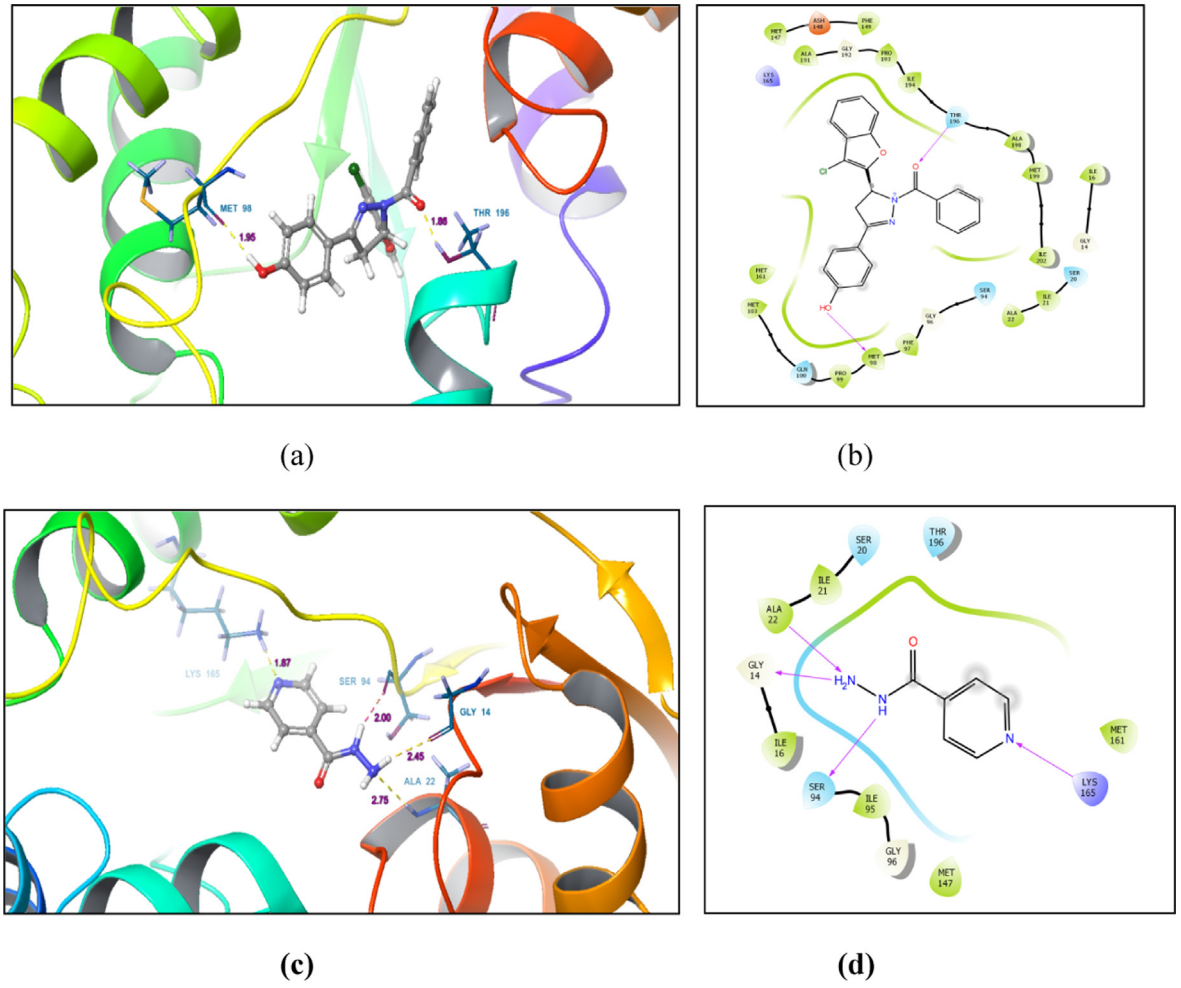


Fig. 3 Molecular docking of compound **3b** and isoniazid **(a)** 3D interaction pose of compound **3b** at the binding site **(b)** 2D interaction of compound **3b** with different residues at the binding site **(c)** 3D interaction pose of isoniazid at the binding site **(d)** 2D interaction of isoniazid with different residues at the binding site.

4m series, **4b** and **4c** substituted with chloro and methyl group at $\mathbf{R_1}$ position has shown good inhibitory potency. Compound **3b** is the representative compound which is substituted with hydroxyl group at $\mathbf{R_1}$ position is reported as most potent compound (IC_{50} = 51.24 and IC_{90} = 87.98 μM). The activity has been further supported by *in silico* study.

4. Experimental

4.1. General

All the chemicals used were of laboratory grade and were supplied by E. Merck, Germany and S.D. Fine Chemicals, India. The Melting points were determined by the open tube capillary method and was not corrected. Thin layer chromatography (TLC) plates prepared with silica gel G were used to monitor the reactions as well as to confirm the purity of the compounds synthesized and to check the purity of the commercial reagents. For this purpose, two distinct solvent systems; a) toluene: ethyl acetate: formic acid (5:4:1) and b) petroleum ether: toluene: acetic acid (5:4:1), have been used to run the TLC. The spots were viewed under iodine vapors/ultra violet light. Infrared spectral response was obtained on a Perkin-Elmer 1720 FT-IR spectrometer using KBr Pellets. $^{1H\ NMR}$ spectra were recorded on Bruker AC 400 MHz in general (whereas in some cases, 300 MHz spectrometer was also used and sited accordingly) using TMS as internal standard in $CDCl_3$ / DMSO d_6. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer.

4.2. Chemical synthesis

*4.2.1. Synthesis of 3-chlorobenzofuran-2-carbaldehyde **(1)***

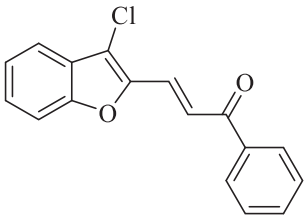
A freshly prepared solution of 2-(2-carboxyphenoxy)acetic acid (1 g) in 5 mL dimethyl formamide (DMF) was added drop wise with constant stirring to 7.8 mL of Vilsmeier reagent maintained at 0 °C. Once the addition was completed the reaction mixture was allowed to attain the room temperature and gradually temperature was increased till it reaches to 90 °C. The reaction was allowed to continue at 90 ± 2 °C for further 6 h. After completion of the reaction, the mixture was cooled and poured on to 250 mL crushed ice with constant stirring. The solid obtained was filtered, washed with plenty of water and recrystallized from ethanol. Percentage yield was found to be 15–20% and melting point recorded to be 74–76 °C.

FT-IR (KBr, cm^{-1}): 2840 (Aldehydic C-H), 1679 (C = O) 758 (C-Cl); 1H NMR ($CDCl_3$, δ, ppm): 7.39–7.43 (1H, m, Ar-H), 7.58 (2H, d, J = 3.6 Hz, Ar-H), 7.74 (1H, d, J = 8.0 Hz, Ar-H), 10.03 (1H, s, –CHO).

*4.2.2. General method for compound synthesis **(2a-2m)***

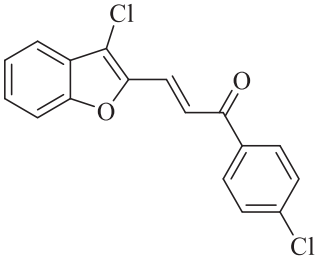
To an ethanolic solution (25 mL) of 3-chlorobenzofuran-2-car baldehyde (0.01 mol), an appropriately substituted acetophenone (0.01 mol) and 5 mL 10% aqueous solution of NaOH were added. The reaction mixture was stirred at 25 °C for 2–3 h. After the reaction was completed, the content was poured onto the crushed ice and neutralized with dilute HCl. The resulting precipitates were filtered, water washed, dried and re-crystallized from ethanol for the desired product **(2a-2m)**.

*4.2.2.1. 3-(3-chlorobenzofuran-2-yl)-1-phenylprop-2-en-1-one (**2a**).*



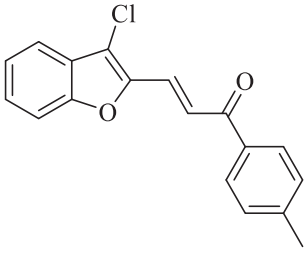
Molecular Formulae: $C_{17}H_{11}ClO_2$, Molecular weight: 282, %Yield: 78, Melting Point: 140–42 °C FT-IR (KBr, cm^{-1}): 1685 (C = O), 1606 (C = C), 755 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 6.80 (1H, d, Hα, J = 8.4 Hz), 7.26–7.66 (9H, m, Ar-H), 7.68 (1H, d, Hβ, J = 8.8 Hz). Anal. Calcd. for $C_{17}H_{11}ClO_2$: C, 72.22; H, 3.92. Found: C, 72.06; H, 3.93%.

*4.2.2.2. 3-(3-Chlorobenzofuran-2-yl)-1-(4-chlorophenyl)-2-propen-1-one (**2b**).*



Molecular Formulae: $C_{17}H_{10}Cl_2O_2$, Molecular weight: 317, %Yield: 87, Melting Point: 148 °C FT-IR (KBr, cm^{-1}):1682 (C = O), 1605 (C = C), 755 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 6.86 (1H, d, Hα, J = 8.0 Hz), 6.90 (2H, d, Ar-H, J = 8 Hz), 7.26 (4H, m, Ar-H), 7.66 (2H, d, Ar-H, J = 8.8 Hz), 7.96 (1H, d, Hβ, J = 8.4 Hz). Anal. Calcd. for $C_{17}H_{10}Cl_2O_2$: C, 64.38; H, 3.18. Found: C, 64.16; H, 3.19%.

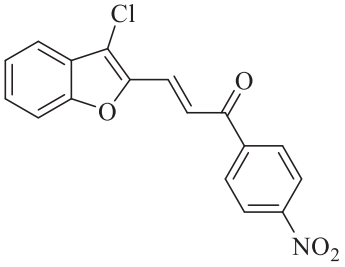
*4.2.2.3. 3-(3-Chlorobenzofuran-2-yl)-1-(4-methylphenyl)-2-propen-1-one (**2c**).*



Molecular Formulae: $C_{18}H_{13}ClO_2$, Molecular weight: 296, %Yield: 76, Melting Point: 126–128 °C FT-IR (KBr, cm^{-1}): 1690 (C = O), 1601 (C = C), 755 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 2.46 (3H, s, -CH_3), 6.72 (1H, d, Hα,

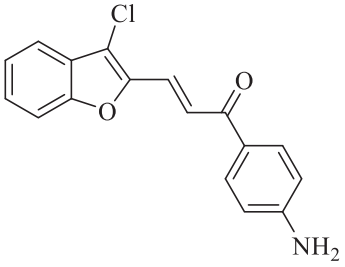
J = 8.0 Hz), 7.25–7.39 (5H, m, Ar-H), 7.48 (1H, d, Ar-H, J = 6.4 Hz), 7.66 (2H, d, Ar-H, J = 8.4 Hz), 7.86 (1H, d, Hβ, J = 8.0 Hz). Anal. Calcd. for $C_{18}H_{13}ClO_2$: C, 72.85; H, 4.42. Found: C, 72.96; H, 4.41%.

*4.2.2.4. 3-(3-Chlorobenzofuran-2-yl)-1-(4-nitrophenyl)-2-propen-1-one (**2d**).*



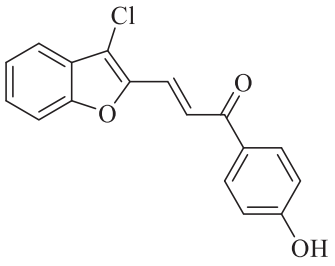
Molecular Formulae: $C_{17}H_{10}ClNO_2$, Molecular weight: 327, %Yield: 70, Melting Point: 182–84 °C FT-IR (KBr, cm^{-1}): 1680 (C = O), 1608 (C = C), 1523 & 1377 (NO_2), 747 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 6.79 (1H, d, Hα, J = 8.8 Hz), 6.95(2H, d, Ar-H, J = 8.4 Hz) 7.32–7.56 (4H, m, Ar-H), 7.60 (2H, d, Ar-H, J = 8.4 Hz), 7.65 (1H, d, Hβ, J = 8.8 Hz). Anal. Calcd. for $C_{17}H_{10}ClNO_4$: C, 62.30; H, 3.08; N, 4.27. Found: C, 62.22; H, 3.08; N, 4.28%.

*4.2.2.5. 3-(3-Chlorobenzofuran-2-yl)- 1-(4-aminophenyl)-2-propen-1-one (**2e**).*



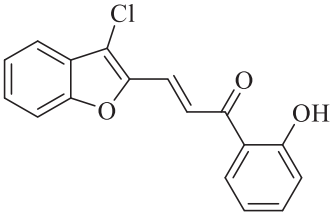
Molecular Formulae: $C_{17}H_{12}ClNO_2$, Molecular weight: 297, %Yield: 84, Melting Point: 184–86 °C. FT-IR (KBr, cm^{-1}): 1685 (C = O), 1606 (C = C), 755 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 3.23 (2H, s, NH_2), 6.86 (1H, d, Hα, J = 8.4 Hz), 7.26–7.48 (4H, m, Ar-H), 7.49 (1H, d, Ar-H, J = 6.4 Hz), 7.51 (1H, d, Ar-H, J = 6.4 Hz), 7.55 (2H, d, Ar-H, J = 8.4 Hz), 7.85 (1H, d, Hβ, J = 8.8 Hz). Anal. Calcd. for $C_{17}H_{12}ClNO_2$: C, 68.58; H, 4.06; N, 4.70. Found: C, 68.46; H, 4.08; N, 4.72%.

*4.2.2.6. 3-(3-Chlorobenzofuran-2-yl)-1-(4-hydroxyphenyl)-2-propen-1-one (**2f**).*



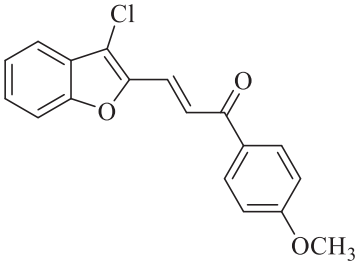
Molecular Formulae: $C_{17}H_{11}ClO_3$, Molecular weight: 298, %Yield: 76, Melting Point: 210–212 °C FT-IR (KBr, cm^{-1}): 3320 (OH), 1685 (C = O), 1606 (C = C), 755 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 6.81 (1H, d, Hα, J = 8.4 Hz), 7.22–7.64 (8H, m, Ar-H), 7.87 (1H, d, Hβ, J = 8.8 Hz), 9.90 (1H, s, OH). Mass m/z: 299 (M^{+1}). Anal. Calcd. for $C_{17}H_{11}ClO_3$: C, 68.35; H, 3.71. Found: C, 68.38; H, 3.72%.

*4.2.2.7. 3-(3-Chlorobenzofuran-2-yl)-1-(2-hydroxyphenyl)-2-propen-1-one (**2g**).*



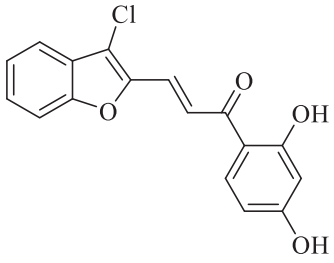
Molecular Formulae: $C_{17}H_{11}ClO_3$, Molecular weight: 298, %Yield: 76, Melting Point: 110–12 °C FT-IR (KBr, cm^{-1}): 3528 (OH), 1682 (C = O), 1614 (C = C), 743 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 6.94 (1H, d, Hα, J = 8.0 Hz), 7.23–7.81 (8H, m, Ar-H), 7.92 (1H, d, Hβ, J = 8.0 Hz), 10.11 (1H, s, OH). Anal. Calcd. for $C_{17}H_{11}ClO_3$: C, 68.35; H, 3.71. Found: C, 68.28; H, 3.70%.

*4.2.2.8. 3-(3-Chlorobenzofuran-2-yl)-1-(4-methoxyphenyl)-2-propen-1-one (**2h**).*



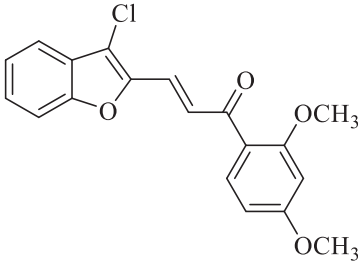
Molecular Formulae: $C_{18}H_{13}ClO_3$, Molecular weight: 312, %Yield: 98, Melting Point: 106–08 °C FT-IR (KBr, cm^{-1}): 1685 (C = O), 1645 (C = C), 750 (C–Cl).; 1H NMR (DMSO d_6, δ, ppm): 3.52 (3H, s, OCH_3), 6.82 (1H, d, Hα, J = 8.4 Hz), 6.91 (2H, d, Ar-H, J = 8 Hz), 6.99–7.67 (5H, m, Ar-H), 7.69 (1H, d, Ar-H, J = 6.8 Hz), 7.78 (1H, d, Hβ, J = 8.8 Hz). Anal. Calcd. for $C_{18}H_{13}ClO_3$: C, 69.13; H, 4.19. Found: C, 69.26; H, 4.20%.

*4.2.2.9. 3-(3-Chlorobenzofuran-2-yl)-1-(2,4-dihydroxyphenyl)-2-propen-1-one (**2i**).*



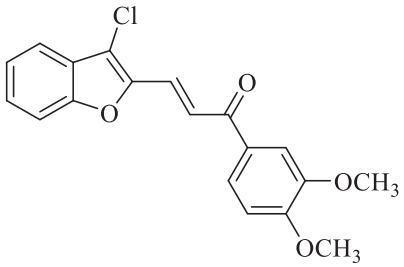
Molecular Formulae: $C_{17}H_{11}ClO_4$, Molecular weight: 314, %Yield: 80, Melting Point: 98–100 °C FT-IR (KBr, cm^{-1}): 3530 (OH), 1682 (C = O), 1606 (C = C), 755 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 6.83 (1H, d, Hα, J = 8.8 Hz), 7.26–7.67 (7H, m, Ar-H), 7.72 (1H, d, Hβ, J = 8.8 Hz) 10.23 (2H, s, 2 × OH). Anal. Calcd. for $C_{17}H_{11}ClO_4$: C, 64.88; H, 3.52. Found: C, 64.90; H, 3.53%.

*4.2.2.10. (3-Chlorobenzofuran-2-yl)-1-(2,4-dimethoxyphenyl)-2-propen-1-one (**2j**).*



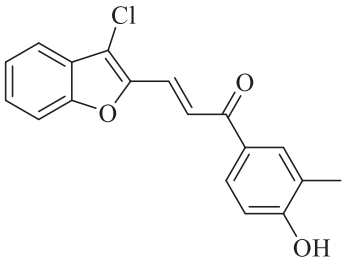
Molecular Formulae: $C_{19}H_{15}ClO_4$, Molecular weight: 342, %Yield: 84, Melting Point: 138–40 °C FT-IR (KBr, cm^{-1}): 1685 (C = O), 1653 (C = C), 735 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 3.66 (6H, s, 2 × OCH_3), 6.92 (1H, d, Hα, J = 8.0 Hz), 7.24–7.84 (7H, m, Ar-H), 7.89 (1H, d, Hβ, J = 8.4 Hz). Anal. Calcd. for $C_{19}H_{15}ClO_4$: C, 66.58; H, 4.41. Found: C, 66.66; H, 4.40%.

*4.2.2.11. 3-(3-Chlorobenzofuran-2-yl)-1-(3,4-dimethoxyphenyl)-2-propen-1-one (**2k**).*



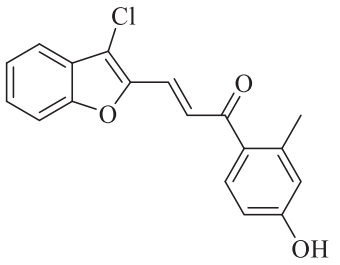
Molecular Formulae: $C_{19}H_{15}ClO_4$, Molecular weight: 342, %Yield: 89, Melting Point: 172–74 °C FT-IR (KBr, cm^{-1}): 1685 (C = O), 1602 (C = C), 765 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 3.68 (6H, s, 2 × OCH_3), 6.82 (1H, d, Hα, J = 8.4 Hz), 6.89–7.68 (7H, m, Ar-H), 7.29 (1H, d, Hβ, J = 8.0 Hz). Anal. Calcd. for $C_{19}H_{15}ClO_4$: C, 66.58; H, 4.41. Found: C, 66.48; H, 4.42%.

*4.2.2.12. 3-(3-Chlorobenzofuran-2-yl)-1-(4-hydroxy-3-methylphenyl)-2-propen-1-one (**2l**).*



Molecular Formulae: $C_{18}H_{13}ClO_3$, Molecular weight: 312, %Yield: 76, Melting Point: 202–04 °C FT-IR (KBr, cm^{-1}): 3530 (OH), 1685 (C = O), 1606 (C = C), 756 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 2.26 (3H, s, CH_3), 6.76 (1H, d, Hα, J = 8.8 Hz), 6.95–7.62 (7H, m, Ar-H), 7.68 (1H, d, Hβ, J = 8.8 Hz) 8.83 (1H, s, OH). Anal. Calcd. for $C_{18}H_{13}ClO_3$: C, 69.13; H, 4.19. Found: C, 69.26; H, 4.22%.

*4.2.2.13. 3-(3-Chlorobenzofuran-2-yl)-1-(4-hydroxy-2-methylphenyl)-2-propen-1-one (**2m**).*

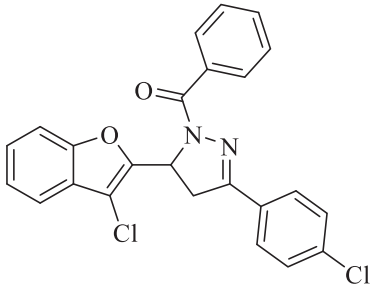


Molecular Formulae: $C_{18}H_{13}ClO_3$, Molecular weight: 312, %Yield: 76, Melting Point: 160–62 °C FT-IR (KBr, cm^{-1}): 3530 (OH), 1686 (C = O), 1606 (C = C), 754 (C-Cl).; 1H NMR (DMSO d_6, δ, ppm): 2.42 (3H, s, CH_3), 6.83 (1H, d, Hα, J = 8 Hz), 7.27–7.67 (7H, m, Ar-H), 7.68 (1H, d, Hβ, J = 8.4 Hz), 8.93 (1H, s, OH). Anal. Calcd. for $C_{18}H_{13}ClO_3$: C, 69.13; H, 4.19. Found: C, 69.12; H, 4.22%.

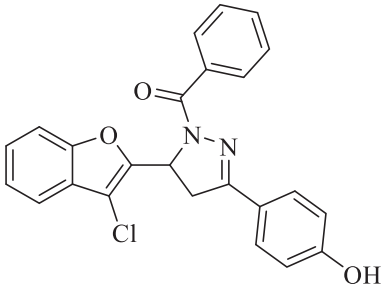
*4.2.3. General procedure for synthesis of compounds (**3a-3e**)*

An equimolar mixture of benzoic acid hydrazide (1-benzenecarbohydrazide) and appropriate chalcone (**2b, 2f, 2h, 2l** & **2m**) were refluxed in glacial acetic acid for 10–12 h. The excess solvent removed under reduced pressure after completion of reaction and the viscous contents were poured on crushed ice and neutralized with diluted ammonia solution. The products (**3a-3e**) thus precipitated was filtered, washed with cold water and purified by column chromatography using (Petroleum: Hexane, 4:1).

*4.2.3.1. Spectral Data of 5-(3-Chlorobenzofuran-2-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-1-pyrazolyl-phenyl methanone (**3a**)*. Molecular Formulae: $C_{24}H_{16}Cl_2N_2O_2$, Molecular weight: 435, %Yield: 62, Melting Point: 150–52 °C, FT-IR (KBr, cm^{-1}): 1654 (C = O), 1583 (C = N). 1H NMR ($CDCl_3$, δ, ppm): 3.21 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.84 (1H, dd, Hb, J = 12.4, 13.2 Hz), 5.29 (1H, dd, Hx, J = 5.6, 6.0 Hz), 7.35–7.82 (13H, m, Ar-H). Anal. Calcd. for $C_{24}H_{16}Cl_2N_2O_2$: C, 66.22; H, 3.70; N, 6.44. Found: C, 66.35; H, 3.71; N, 6.46%.

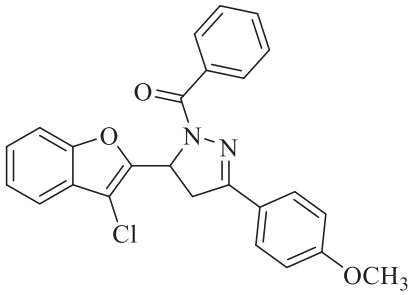


*4.2.3.2. 5-(3-Chlorobenzofuran-2-yl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-1-pyrazolyl-phenyl methanone (**3b**)*.



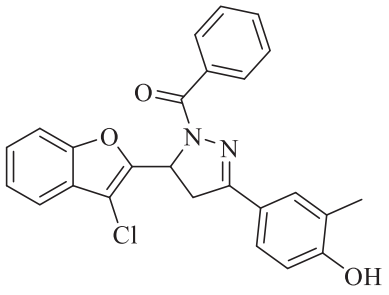
Molecular Formulae: $C_{24}H_{17}ClN_2O_2$, Molecular weight: 416, %Yield: 56, Melting Point: 150–52 °C FT-IR (KBr, cm^{-1}): 3433 (OH), 1665 (C = O), 1592 (C = N). 1H NMR ($CDCl_3$, δ, ppm): 3.34 (1H, dd, Ha, J = 5.2, 5.6 Hz), 3.64 (1H, dd, Hb, J = 12.4, 12.4 Hz), 5.29 (1H, dd, Hx, J = 5.6, 5.6 Hz), 7.11 (2H, d, Ar-H, J = 8 Hz), 7.15–7.99 (13H, m, Ar-H). Anal. Calcd. for $C_{24}H_{17}ClN_2O_3$: C, 69.15; H, 4.11; N, 6.72. Found: C, 68.97; H, 4.10; N, 6.71%.

*4.2.3.3. 5-(3-Chlorobenzofuran-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-1-pyrazolyl-phenyl methanone (**3c**)*.



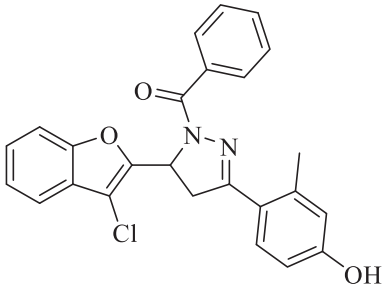
Molecular Formulae: $C_{25}H_{19}ClN_2O_3$, Molecular weight: 430, %Yield: 67, Melting Point: 100–02 °C, FT-IR (KBr, cm^{-1}): 1655 (C = O), 1585 (C = N). 1H NMR ($CDCl_3$, δ, ppm): 3.34 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.58 (1H, dd, Hb, J = 12.4, 12.4 Hz), 3.68 (3H, s, –OCH_3), 5.38 (1H, dd, Hx, J = 6.0, 5.6 Hz), 7.14–7.78 (13H, m, Ar-H). Anal. Calcd. for $C_{25}H_{19}ClN_2O_3$: C, 69.69; H, 4.44; N, 6.50. Found: C, 69.81; H, 4.43; N, 6.49%.

*4.2.3.4. 5-(3-Chlorobenzofuran-2-yl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-phenylmethanone (**3d**)*.



Molecular Formulae: $C_{25}H_{19}ClN_2O_3$, Molecular weight: 430, %Yield: 66, Melting Point: 144–46 °C, FT-IR (KBr, cm^{-1}): 1650 (C = O), 1598 (C = N). 1H NMR ($CDCl_3$, δ, ppm): 2.45 (3H, s, -CH_3), 3.34 (1H, dd, Ha, J = 6.0, 5.6 Hz), 3.46 (1H, dd, Hb, J = 12.0, 12.4 Hz), 5.57 (1H, dd, Hx, J = 6.0, 6.0 Hz), 7.13–7.56 (12H, m, Ar-H), 10.16 (1H, s, OH). Anal. Calcd. for $C_{25}H_{19}ClN_2O_3$: C, 69.69; H, 4.44; N, 6.50. Found: C, 69.74; H, 4.42; N, 6.52%.

*4.2.3.5. 5-(3-Chlorobenzofuran-2-yl)-3-(4-hydroxy-2-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-phenlmethanone (**3e**).*

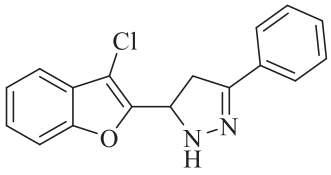


Molecular Formulae: $C_{25}H_{19}Cl_2N_2O_3$, Molecular weight: 430, %Yield: 66, Melting Point: 144–46 °C, FT-IR (KBr, cm^{-1}): 1655 (C = O), 1589 (C = N). 1H NMR ($CDCl_3$, δ, ppm): 2.44 (3H, s, $-CH_3$), 3.38 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.66 (1H, dd, Hb, J = 12.4, 12.4 Hz), 5.77 (1H, dd, Hx, J = 6.0, 6.0 Hz), 7.19 (1H, d, Ar-H, J = 8.4), 7.39–7.51 (2H, m, Ar-H), 7.57–7.61 (8H, m, Ar-H), 10.05 (1H, s, OH). Anal. Calcd. for $C_{25}H_{19}ClN_2O_3$: C, 69.69; H, 4.44; N, 6.50. Found: C, 69.56; H, 4.43; N, 6.48%.

*4.2.4. General method for synthesis of compounds (**4a-4m**)*

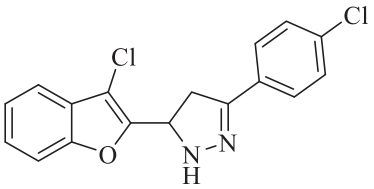
To an ethanolic solution of (**2a-2m**) (0.01 mol; 20 mL), a solution of 0.015 mol of hydrazine hydrate in 5 mL glacial acetic acid was added, this reaction mixture was allowed to reflux for 3–6 h in the presence of 100 mg of 5A × 1.5 mm molecular sieves. The contents were concentrated and poured onto 100 g of crushed ice after completion of the reaction, and neutralized with ammonia. To get the pure product, solid mass was filtered, washed with enough cold water and recrystallized with hydrated ethanol (**4a-4m**).

*4.2.4.1. 2-(3-Phenyl-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride (**4a**).*



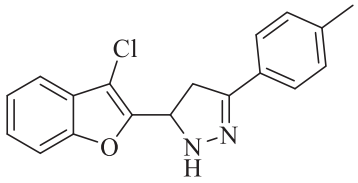
Molecular Formulae: $C_{17}H_{13}ClN_2O$, Molecular weight: 296, %Yield: 78, Melting Point: 126–28 °C, FT-IR (KBr, cm^{-1}): 3580 (N-H), 1596 (C = N), 1324 (C-N), 757 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 3.49 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.73 (1H, dd, Hb, J = 12.0, 12.4 Hz), 5.87 (1H, dd, Hx, J = 5.6, 5.6 Hz), 7.2–7.8 (9H, m, Ar-H). 8.5 (1H, s, NH). Anal. Calcd. for $C_{17}H_{13}ClN_2O$: C, 68.81; H, 4.42; N, 9.44. Found: C, 68.72; H, 4.41; N, 9.45%.

*4.2.4.2. 2-[3-(4-Chlorophenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride (**4b**).*



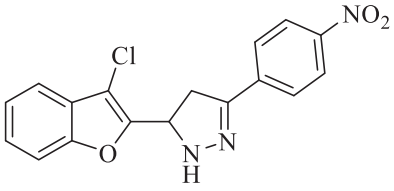
Molecular Formulae: $C_{17}H_{12}Cl_2N_2O$, Molecular weight: 331, %Yield: 82, Melting Point: 66–68 °C, FT-IR (KBr, cm^{-1}): 3569 (N-H), 1596 (C = N), 1322 (C-N), 747 (C-Cl). 1H NMR ($CDCl_3$, δ, ppm): 3.53 (1H, dd, Ha, J = 5.2, 5.6 Hz), 4.24 (1H, dd, Hb, J = 12, 12 Hz), 5.72 (1H, dd, Hx, J = 5.6, 5.6 Hz), 6.96 (1H, d, Ar-H, J = 6.4 Hz), 7.34 (2H, m, Ar-H and NH pyrazoline), 7.52 (1H, d, Ar-H, J = 6.4 Hz), 7.58 (2H, d, Ar-H, J = 7.6 Hz), 7.80 (2H, d, Ar-H, J = 8 Hz). Anal. Calcd. for $C_{17}H_{12}Cl_2N_2O$: C, 61.65; H, 3.65; N, 8.46. Found: C, 61.54; H, 3.64; N, 8.45%.

*4.2.4.3. 2-[3-(4-Methylphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride (**4c**).*



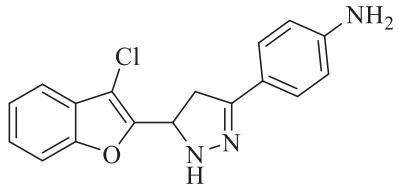
Molecular Formulae: $C_{18}H_{15}ClN_2O$, Molecular weight: 310, %Yield: 80, Melting Point: 146–48 °C, FT-IR (KBr, cm^{-1}): 3560 (N-H), 1590 (C = N), 1326 (C-N), 747 (C-Cl). 1H NMR ($CDCl_3$, δ, ppm): 2.40 (3H, s, $-CH_3$), 3.41 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.65 (1H, dd, Hb, J = 12, 12.4 Hz), 5.82 (1H, dd, Hx, J = 5.6, 5.6 Hz), 7.25 (2H, d, Ar-H, J = 8 Hz), 7.34 (1H, d, Ar-H, J = 7.6 Hz) 7.48 (2H, m, Ar-H and NH pyrazoline), 7.52 (1H, d, Ar-H, J = 6.8 Hz), 7.65 (2H, d, Ar-H, J = 8 Hz). Anal. Calcd. for $C_{18}H_{15}ClN_2O$: C, 69.57; H, 4.86; N, 9.01. Found: C, 69.68; H, 4.85; N, 9.00%.

*4.2.4.4. 2-[3-(4-Nitrophenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride (**4d**).*



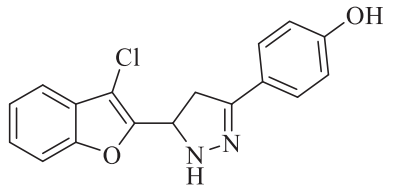
Molecular Formulae: $C_{17}H_{12}ClN_3O_3$, Molecular weight: 341, %Yield: 74, Melting Point: 126–28 °C, FT-IR (KBr, cm^{-1}): 3569 (N-H), 1596 (C = N), 1322 (C-N), 747 (C-Cl).; 1H NMR ($CDCl_3$, δ, ppm): 3.22 (1H, dd, Ha, J = 5.6, 5.6 Hz), 4.10 (1H, dd, Hb, J = 12.0, 12.0 Hz), 5.52 (1H, dd, Hx, J = 5.6, 5.2 Hz), 6.95(2H, d, Ar-H, J = 8.4 Hz), 7.32–7.43 (4H, m, Ar-H and pyrazoline), 7.90 (2H, d, Ar-H, J = 8.4 Hz). Anal. Calcd. for $C_{17}H_{12}ClN_3O_3$: C, 59.75; H, 3.54; N, 12.30. Found: C, 59.64; H, 3.56; N, 12.28%.

*4.2.4.5. 2-[3-(4-Aminophenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride (**4e**).*



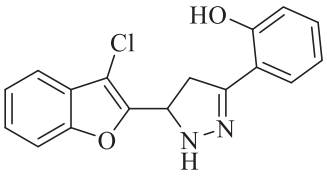
Molecular Formulae: $C_{17}H_{14}ClN_3O$, Molecular weight: 311, %Yield: 70, Melting Point: 120–22 °C, FT-IR (KBr, cm^{-1}): 3280 (N-H), 1596 (C = N), 1324 (C-N), 757 (C-Cl). 1H NMR ($CDCl_3$, δ, ppm): 2.38 (2H, s, NH_2), 3.40 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.64 (1H, dd, Hb, J = 12.0, 12.8 Hz), 5.82 (1H, dd, Hx, J = 5.6, 5.6 Hz), 7.26 (2H, m, Ar-H), 7.34 (1H, d, Ar-H, J = 6.8 Hz), 7.43 (1H, s, NH pyrazoline), 7.51 (1H, d, Ar-H, J = 6.8 Hz), 7.58 (2H, d, Ar-H, J = 7.6 Hz), 7.60 (2H, d, Ar-H, J = 8 Hz). Anal. Calcd. for $C_{17}H_{14}ClN_3O$: C, 65.49; H, 4.53; N, 13.48. Found: C, 65.48; H, 4.52; N, 13.50%.

*4.2.4.6. 2-[3-(4-Hydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride (**4f**).*



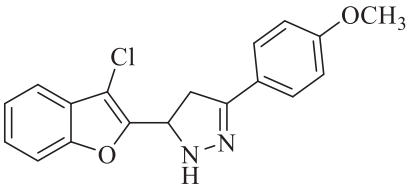
Molecular Formulae: $C_{17}H_{13}ClN_2O_2$, Molecular weight: 312, %Yield: 78, Melting Point: 268–70 °C, FT-IR (KBr, cm^{-1}): 3532 (OH), 3285 (N-H), 1594 (C = N), 1324 (C-N), 756 (C-Cl).; 1H NMR (DMSO d_6, δ, ppm): 3.79 (1H, dd, Ha, J = 5.2, 4.8 Hz), 4.07 (1H, dd, Hb, J = 12.4, 12.8 Hz), 6.07 (1H, dd, Hx, J = 5.6, 5.6 Hz), 7.27(2H, d, Ar-H, J = 8 Hz), 7.64–7.71 (3H, m, Ar-H and NH pyrazoline), 7.76(1H, d, Ar-H, J = 7.2 Hz), 8.00 (2H, d, Ar-H, J = 8 Hz), 9.91 (1H, s, OH). Mass m/z: 312 (M^+). Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; N, 8.96. Found: C, 65.30; H, 4.20; N, 8.97%.

*4.2.4.7. 2-[3-(2-Hydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride (**4g**).*



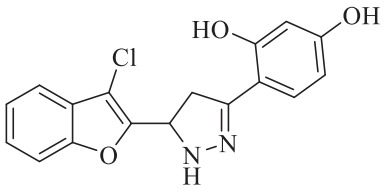
Molecular Formulae: $C_{17}H_{13}ClN_2O_2$, Molecular weight: 312, %Yield: 82, Melting Point: 148–50 °C FT-IR (KBr, cm^{-1}): 3538 (OH), 3295 (N-H), 1596 (C = N), 1324 (C-N), 754 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 3.62 (1H, dd, Ha, J = 5.6, 5.6 Hz), 4.68 (1H, dd, Hb, J = 12.4, 12.4 Hz), 6.01 (1H, dd, Hx, J = 5.6, 5.2 Hz), 7.26–7.73 (6H, m, Ar-H and NH pyrazoline), 7.74 (1H, d, Ar-H, J = 6.8 Hz), 7.78 (1H, d, Ar-H, J = 6.8 Hz), 8.92 (1H, s, OH). Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; N, 8.96. Found: C, 65.38; H, 4.20; N, 8.95%.

*4.2.4.8. 2-[3-(4-Methoxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride (**4h**).*



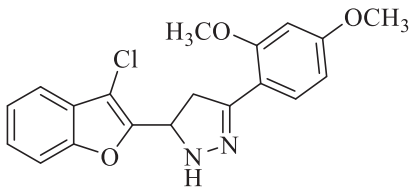
Molecular Formulae: $C_{18}H_{15}ClN_2O_2$, Molecular weight: 326, %Yield: 87, Melting Point: 182–84 °C FT-IR (KBr, cm^{-1}): 3560 (N-H), 1590 (C = N), 1326 (C-N), 750 (C–Cl). 1H NMR (DMSO d_6, δ, ppm): 3.39 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.63 (1H, dd, Hb, J = 12.4, 12.0 Hz), 3.85 (3H, s, -OCH_3), 5.81 (1H, dd, Hx, J = 5.6, 5.6 Hz), 6.94 (2H, d, Ar-H, J = 8.4 Hz), 7.26–7.32 (2H, m, Ar-H and NH pyrazoline), 7.34 (1H, d, Ar-H, J = 8.0 Hz), 7.51 (1H, d, Ar-H, J = 6.4 Hz), 7.70 (2H, d, Ar-H, J = 8.8 Hz). Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57. Found: C, 66.20; H, 4.64; N, 8.55%.

*4.2.4.9. 2-[3-(2,4-Dihydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride (**4i**).*



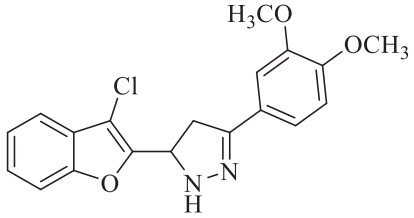
Molecular Formulae: $C_{17}H_{13}ClN_2O_3$, Molecular weight: 328, %Yield: 74, Melting Point: 140–42 °C FT-IR (KBr, cm^{-1}): 3530 (OH), 3280 (N-H), 1596 (C = N), 1324 (C-N), 757 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 3.45 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.98 (1H, dd, Hb, J = 12, 12 Hz), 6.11 (1H, dd, Hx, J = 5.6, 5.6 Hz), 6.98–7.42 (3H, m, Ar-H), 7.45 (1H, d, Ar-H, J = 6.8 Hz), 7.61–7.72 (3H, m, Ar-H and NH pyrazoline), 10.2 (2H, s, 2 X OH). Anal. Calcd. for $C_{17}H_{13}ClN_2O_3$: C, 62.11; H, 3.99; N, 8.52. Found: C, 62.10; H, 4.00; N, 8.51%.

*4.2.4.10. 2-[3-(2,4-Dimethoxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride (**4j**).*



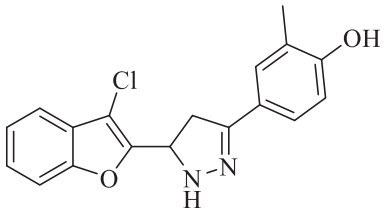
Molecular Formulae: $C_{17}H_{13}ClN_2O_3$, Molecular weight: 328, %Yield: 74, Melting Point: 140–42 °C FT-IR (KBr, cm^{-1}): 3280 (N-H), 1590 (C = N), 1324 (C-N), 755 (C-Cl). 1H NMR ($CDCl_3$, δ, ppm): 3.36 (1H, dd, Ha, J = 5.6, 5.6 Hz), 3.62 (1H, dd, Hb, J = 12, 12 Hz), 3.86 (6H, s, 2 X OCH_3), 5.82 (1H, dd, Hx, J = 5.6, 5.6 Hz), 7.26–7.83 (7H, m, Ar-H and NH pyrazoline). Anal. Calcd. for $C_{19}H_{17}ClN_2O_3$: C, 63.96; H, 4.80; N, 7.85. Found: C, 64.05; H, 4.81; N, 7.84%.

*4.2.4.11. 2-[3-(3,4-Dimethoxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride (**4k**).*



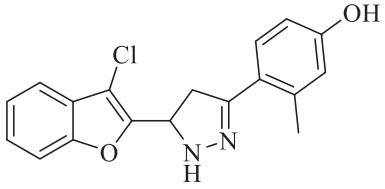
Molecular Formulae: $C_{19}H_{17}ClN_2O_3$, Molecular weight: 326, %Yield: 87, Melting Point: 268–70 °C FT-IR (KBr, cm^{-1}): 3280 (N-H), 1596 (C = N), 1324 (C-N), 757 (C-Cl). 1H NMR ($CDCl_3$, δ, ppm): 3.38 (1H, dd, Ha, J = 5.2, 5.2 Hz), 3.59 (1H, dd, Hb, J = 12.4, 12.4 Hz), 3.89 (6H, s, 2 X OCH_3), 6.17 (1H, dd, Hx, J = 5.2, 5.2 Hz), 6.98 (1H, s, Ar-H), 7.06–7.13 (3H, m, Ar-H), 7.17 (1H, bs, NH), 7.50–7.58 (3H, m, Ar-H). Anal. Calcd. for $C_{19}H_{17}ClN_2O_3$: C, 63.96; H, 4.80; N, 7.85. Found: C, 64.08; H, 4.80; N, 7.84%.

*4.2.4.12. 2-[3-(4-Hydroxy-3-methylphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride (**4l**).*



Molecular Formulae: $C_{18}H_{15}ClN_2O_2$, Molecular weight: 326, %Yield: 76, Melting Point: 150–70 °C FT-IR (KBr, cm^{-1}): 3536 (OH), 3279 (N-H), 1590 (C = N), 1324 (C-N), 755 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 2.38 (2H, d, CH_2 pyrazoline), 2.43 (3H, s, CH_3), 4.52 (1H, t, CH), 7.23–7.72 (7H, m, Ar-H and NH), 9.92 (1H, s, OH). Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57. Found: C, 66.15; H, 4.62; N, 8.58%.

*4.2.4.13. 2-[3-(4-Hydroxy-2-methylphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride (**4m**).*



Molecular Formulae: $C_{18}H_{15}ClN_2O_2$, Molecular weight: 326, %Yield: 77, Melting Point: 232–34 °C FT-IR (KBr, cm^{-1}): 3535 (OH), 3285(N-H), 1594 (C = N), 1327 (C-N), 754 (C-Cl). 1H NMR (DMSO d_6, δ, ppm): 2.36 (2H, d, CH_2 pyrazoline), 2.69 (3H, s, CH_3), 4.52 (1H, t, CH), 7.32–7.82 (7H, m, Ar-H and NH), 10.21 (1H, s, OH). Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57. Found: C, 66.29; H, 4.64; N, 8.56%.

4.3. Antitubercular activity

The newly synthesized compounds were screened *in vitro* for activity against *Mycobacterium tuberculosis* H37Rv strain (ATCC27294) by Microplate Alamar Blue Assay (MABA) (Wahyuningrum et al., 2017; Xu et al., 2016; Bunalema et al., 2015; Nkenfou et al., 2015), at Tuberculosis Antimicrobial Acquisition and Coordination facility (TAACF).

4.4. In silico studies

Synthesized compounds were subjected to different *in silico* studies to predict their ADME properties, understand their

Table 3 ADME properties of potent compounds.

Compound	Nrb	HBD	HBA	QPlogPo/w	%Abs	Rule of five	Rule of three
3a	1	0	4	6.257	100	1	1
3b	2	1	4.75	5.112	100	1	1
3c	2	0	4.75	5.795	100	1	1
4b	0	1	2.5	4.875	100	0	1
4c	0	1	2.5	4.688	100	0	1

Nrb = Number of Rotational Bond, HBD = Hydrogen Bond Donor, HBA = Hydrogen Bond Acceptor, QPlog Po/w = Predicted Octanol/water partition coefficient, %Abs = Percentage of Human Oral Absorption.

Table 4 H-bond and hydrophobic interactions of compounds with the binding site.

Compound	No. of H-bond	H-bond forming residue	H-bond length (Å)	Other residues with hydrophobic interaction
3a	1	SER20	2.4	ILE21, ALA22, MET147, ILE95, PHE97, MET199, ALA168,
3b	2	MET98	1.95	ILE16, ILE21, ALA22, PHE97, PRO99, MET103, ALA191, PRO193, ILE194,
		THR196	1.86	ALA198, MET199, ILE202
3c	1	THR196	1.88	ILE16, ILE21, ALA22, PHE97, MET98, PRO99, MET103, TYR158, PHE149, MET147, ALA191, PRO193, ILE194, ALA198, MET199, ILE202
4b	1	GLY14	2.15	ILE15, ILE16, ILE21, MET147, ILE95, PHE97
4c	2	GLY14	1.98	1LE16, ILE21, PHE41, PHE97, ILE95, ALA191, ILE194
		GLY96	2.34	
Isoniazid	4	GLY14	2.45	ILE16, ILE21, ILE95, MET147, MET161
		ALA22	2.75	
		SER94	2.00	
		LYS165	1.87	
Pyrazinamide	1	ILE194	1.97	ILE21, MET147, PHE149, ALA191, PRO193, MET199

interaction with target protein and their affinity towards at the binding site of the target. Schrodinger Maestro 11.4, on a dell desktop with i5 intel core processor, assembled with 16 GB RAM and 4 GB nividia graphics padwas used to carry out all *in silico* studies.

4.4.1. ADME properties

Chemical structures of all synthesized compounds were drawn on Chemdraw 12.0 as mol files and subjected to energy minimization using Ligprep module of Maestro. The conformers generated after ligand preparation were analysed for ADME properties using Quikprop. Some of the predicted properties include QP log Po/w, QP log BB, overall CNS activity, cell permeability, logKhsa for human serum albumin binding, the percentage of human oral absorption. The results obtained have been indicated in Table 3.

4.4.2. Molecular docking

All compounds which exhibited potent anti-tubercular activity were further studied for their binding with target protein. The co crystallized protein of *Mycobacterium tuberculosis* InhA for molecular docking study was obtained from Protein Data Bank (PDB ID- 5OIF) and prepared using protein preparation wizard module of Maestro. The protein was pre-processed to identify different chains, ligands as hetero atoms and water molecules. Relevant chain containing ligand for the study was retained and other parts were omitted. The protein was then optimised for hydrogen bond and finally subjected to energy minimization. The prepared protein was then treated for receptor grid generation. Once the grid was generated, docking of the prepared ligands along with established standard inhibitors like Isoniazid and Pyrazinamide was performed. The result obtained has been presented in Tables 2 and 4.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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