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RESEARCH ARTICLE

Synthesis and Biological Evaluation of 3-(2-Benzoyl-4-chlorophenyl)-4H-spiro [indole-3,2-[1,3]thiazolidine]-2,4(1H)-dione Derivatives for Anti-tubercular and Antimicrobial Activities

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Abstract

Objectives: To synthesize a new scaff fold of 3'-(2-Benzoyl-4-chlorophenyl)-4'H-spiro [indole-3,2'-[1,3] thiazolidine]-2,4'(1H)-dione derivatives and to assess the compounds for anti-tubercular and antimicrobial activities. **Methods:** A new series of 3'-(2-Benzoyl-4-chlorophenyl)-4'H-spiro [indole-3,2'- [1,3] thiazolidine]-2,4'(1H)-dione derivatives were prepared by synthesizing schiff's bases followed by condensing schiff base with 2-mercaptopropanoic acid in the presence of zinc chloride. Synthesized compounds were characterized by IR, NMR and Mass spectral data. Their antimicrobial, antitubercular and also docking simulations were evaluated. **Findings:** Compound 3c (floro substituent) showed potent activity against *Mycobacterium tuberculosis* H37Rv with MIC value of 3.125 μg/ml when compared with standard Isoniazid. Compound 3c, 3d and 3f displayed better antimicrobial activity against used bacterial and fungal species with MIC range of 3-20μg/ml comparable to standard drugs ciprofloxacin and ketoconazole respectively. *In silico* studies revealed that compound 3c, 3f and 3d had a better binding affinity with *M. tuberculosis* enoyl-CoA hydratase EchA6 with ΔG values of -9.6kcal/mol, -9.4kcal/mol, and -8.6kcal/mol respectively comparable to isoniazid of -5.4kcal/mol. Compound 3c, 3f and 3d also displayed good binding affinity with DNA gyrase and lanosterol methylase. **Novelty :** Introducing benzoyl moiety to the thiazolidine ring potentiates the antitubercular and antimicrobial activity of spiro indole derivatives.

Keywords: AntiTB; Antimicrobial; Molecular Docking; Spiro indole; Thiazolidine

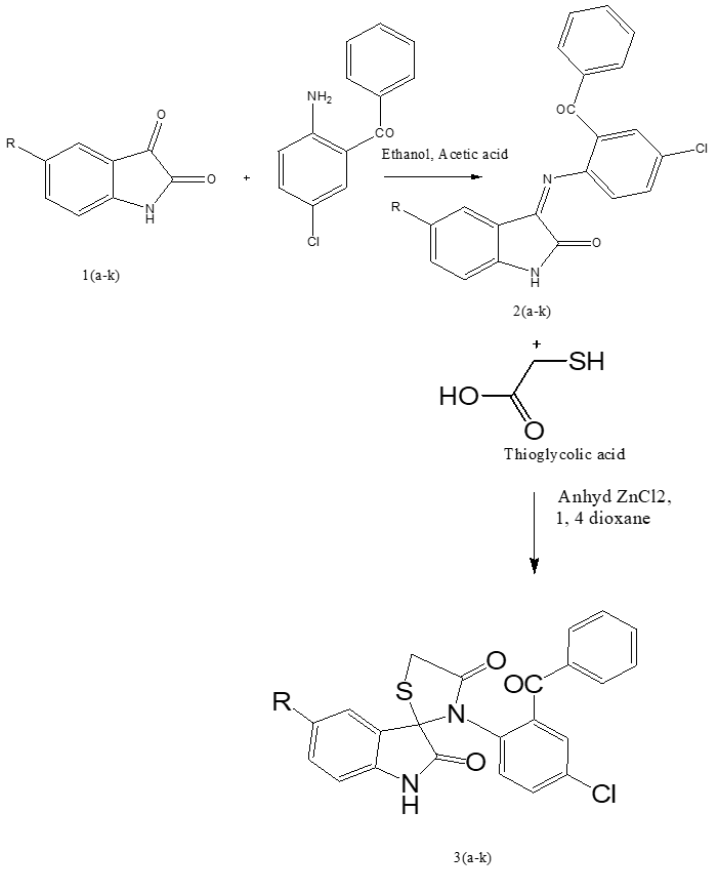
1 Introduction

The emergence of drug-resistant *M. tuberculosis*, drug-resistant bacterial and fungal strains underscore the need for novel therapeutic agents. Hetero cyclic compounds offer a rich source of chemical diversity for designing new drug. Spiro cyclic compounds

occupy a unique place within heterocyclic compounds due to their rigidity. Among all Spiro cyclic compounds, Spiro indole-containing compounds represent an important branch of this class which usually consists of an indole ring fused with another ring system. Indoles are heterocyclic aromatic structures consisting of a benzene ring fused to a nitrogen-containing five-remembered pyrrole ring. Based on their diverse pharmacological actions, small compounds containing indole have been shown to have medicinal promise[1–3]. Numerous pharmaceutical compounds with an indole ring have been brought to market to address a range of medical ailments. Among these are the anticancer medications apaziquone (marketed as an anti-microbial agent) and vincristine, vinblastine, vinorelbine, vindesine, and mitraphylline), and also anti-hypertensive drugs, such as vincamine, reserpine, and perindopril. Some anti-depressant drugs such asamedalin, pindolol, siramesine, carbinedaline, trandolapril, and indalpine[4]. Because of their rigidity, spirocyclic molecules have a special position among organic chemical compounds. Spiroindole-containing compounds are a significant subclass of all spirocyclic chemicals. This is explained by the broad range of biological features demonstrated by different synthetic and natural analogues, which can come from the C-2 or C-3 indolyl ring and have several heterocyclics that provide different patterns. Indole derivatives, particularly those introducing heterocyclic ring at 3rd position exhibited notable efficacy[4–6]. Considering the importance of Spiro indoles, the current work was aimed to synthesize Spiro indole thiazolidine derivatives and were evaluated for anti-tubercular and antimicrobial activities.

2 Methodology

2.1 Synthesis of 3-(2-Benzoyl-4-chlorophenyl)-4H-spiro [indole-3, 2- [1, 3] thiazolidine]-2,4(1H)-dione derivatives



3a R=H, 3b R=Cl, 3c R=F, 3d R=I, 3eR=Br, 3f R=NO2, 3g R=-OCH3, 3h R=-CH3, 3i R= -CF3, 3j R=NH2, 3k R= OH

Fig 1. Scheme for Synthesis of 3-(2-Benzoyl-4-chlorophenyl)-4H-spiro [indole-3, 2- [1, 3] thiazolidine]-2,4(1H)-dione derivatives

Step-1: Synthesis of shiff bases

A blend of 2-amino-5-chlorobenzophenone and 5-substituted indole-2, 3-dione (istatin) was combined in 20 milliliters of ethanol along with a small amount of acetic acid to form an equimolar (0.03 mole) solution. The mixture was heated while experiencing reflux. The liquid was chilled and left to stand overnight once the reaction was finished. The result was filtered using ethanol, and it was then recrystallized (2a-2k) [7].

Step-2: 3'-(2-Benzoyl-4-chlorophenyl)-4'H-spiro [indole-3,2'- [1,3] thiazolidine]-2,4'(1H)-dione derivatives

The mixture containing compounds (2a-2k) (0.01 mol) and 2-mercaptopropanoic acid was heated over a period of 12 hours while anhydrous zinc chloride and 1,4-dioxane were present. TLC served to track the completion of the reaction. Upon its completion, the reaction mixture was poured into ice-cold water. Following isolation, a water rinse, and recrystallization using ethanol, the resulting product (3a-3k) was obtained this is employed in Figure 1.

2.2 Characterization of synthesized compounds

Synthesized Compounds were characterized by R_f values, IR, NMR and Mass Spectral data.

Spectral data of Compounds

3'-(2-chlorophenyl-4-benzoyl)-4'H-spiro [thiazolidine-3,2'-indole-3,2']-2,4'(1H)-dione(3a)

IR(KBr) cm^{-1}: 3285 N-H str, 2958 C-H Ar str, 1749(thiazole C=O srt of CON), 1054 C-N str, 1H NMR DMSO, 400 MH)3.72 s, 2H, CH), 6.83-6.85 d,1H of Ar-H, 7.3(d, Ar-H, 7.48 m, 5H Ar-H, 11.06(s, 1H of NH are the values of δ (ppm). MS m/z:434[M-H] + Elemental composition, Found: C, 63.62; H, 3.38; Cl, 8.25; N, 6.34; O, 11.24; S, 7.17; Calculated: C, 63.52; H, 3.38; Cl, 8.14; N, 6.43; O, 11.02; S, 7.36, Rf:0.66 (Ethyl acetate 7:3 chloroform); 2-chlorophenyl-4-benzoyl-3'-1.

[1,3]Indole-3,2'-thiazolidine-5-chloro-4'H-spiro-2,4'(1H)-dione (3 b)

IR(KBr) cm^{-1}: 3448 N-H str of, 3038, 2960(C-H Ar str, 1750 C=O of thiazolodinone ring), 1596 C=O srt of CONH, 1481 C-H, Str, 1264 C-O, str, 1H NMRDMSO, 400 MHzδ (ppm):3.76 (s, 2H, CH2), 6.84-6.86 (d, 1H of Ar-H), 7.32 (d, Ar-H), 7.48 (m, 5H Ar-H), 11.07 (s, 1H of NH) Elemental composition calculated using MS m/z:468[M-H]+Found: C, 63.62; H, 3.38; Cl, 8.25; N, 6.34; O, 11.24; S, 7.17; -C, 63.52; H, 3.48; Cl, 8.15; N, 6.44; O, 11.04; S, 7.37; Rf:0.68 (Chloroform: Ethyl acetate 7:3).

3'-(2-chlorophenyl-4-benzoyl)1-[1,3]thiazolidine-5-fluro-4'H-spiro[indole-3,2'-]-2,4'(1H)-dione 3C

IR(KBr) cm^{-1}: 3448 N-H str of benzimidazol), 3038, 2960 C-H Ar str, 1750 C=O of thiazolodinone rin), 1596 C=O srt of CONH, 1481(C-H, Str,1264 C-O, str, 1H NMR DMSO, 400 MHzδ (ppm):3.7(s, 2H, CH2, 6.84-6.86 d, 1H of Ar-H, 7.32(d, Ar-H, 7.48 m, 5H Ar-H, 11.07s, 1H of NH : MS m/z:468[M-H]+, Calculated Elemental compositionN, 6.44; O, 11.04; S, 7.37; Found- C, 63.62; H, 3.38; Cl, 8.25; N, 6.34; O, 11.24; S, 7.1, Rf:0.68 (Chloroform: Ethyl acetate 7:3).

3'-(2-chlorophenyl-4-benzoyl)Indole-3,2'-[1,3]thiazolidine-5-iodo-4'H-spiro[-2,4'(1H)-dione (3d)

FT-IR(KBr) cm^{-1}: 3432 N-H str, 2972 C-H Ar st, 1761 thiazolodinone ring's C=O, 1611C=O srt of CON, 1478C-H, Str, 1259 C-O, str, 1H NMRDMSO, 400 MHzδ (ppm): 3.7 s, 2H, CH), 6.7-7.2d, 1H of Ar-H, 7.47m, 5H Ar-H, 11.1 s, 1H of NH : MS m/z:559 [M-H]+, Elemental composition; The following values were found: C, 49.35; H, 2.12; Cl, 6.23; I, 22.72; N, 5.11; O, 8.35; S, 5.71; Calculated: C, 49.26; H, 2.52; Cl, 6.32; I, 22.63; N, 5.00; O, 8.56; S, 5.72, Rf: 0.72 (Ethyl acetate: Chloroform 7:3).

3'-(2-chlorophenyl-4-benzoyl)Indole-3,2'-[1,3]thiazolidine-5-bromo-4'H-spiro-4'(1H)dione(3e)

IR(KBr) cm^{-1}: 3442 N-H str, 2982 C-H Ar st, 1764, thiazolodinone ring's C=O, 1668 C=O srt of CONH, 1421 C-H, Str, 1270(C-O, str, 1H NMRDMSO, 400 MHzδ (ppm): 3.64 s, 2H, CH2, 6.6-7.0 d, 1H of Ar-H, 7.4m, 5H A r-H, 11.0s, 1H of NH : MS m/z: 512[M-H]+, analyses of elements N, 5.46; O, 9.33; S, 6.23; Calculated: C, 53.76; H, 2.76; Br, 15.56; Cl, 6.91; Found: C, 52.78; H, 3.74; Br, 15.44; Cl, 6.91; N, 5.43; O, 9.36; S, 6.23.Rf:0.78 (Ethyl acetate: Chloroform 7:3).

3'-(2-Benzoyl-4-chlorophenyl)-5-nitro-4'H-spiro[indole-3,2'-[1,3]thiazolidine]-2,4'(1H)-dione (3f);

IR N-H str 3444, C-H Ar str 2985, C=O srt of CONH 1758, C=O str of 1613, C-H, Str 1486, C-O, str 1274, 1H NMR DMSO, 400 MHz δ (ppm):6.8-7.3 d, 1H of Ar-H, 7.7 m, 5H Ar-H, 11.06(s, 1H of NH, and 3.6 s, 2H, CH2, MS m/z478[M-H]+:: Calculated elemental composition for A total of C, 57.54; H, 2.93; Cl, 7.38; N, 8.76; O, 16.66; S, 6.69; and C, 57.65; H, 2.83; Cl, 7.48; N, 8.85; O, 16.56; S, 6.77 .Rf:0.69 (Ethyl acetate: chloroform 7:3).

2.3 *In silico* Studies

2.3.1 Molecular properties & ADME prediction:

ADME Properties: Evaluation of drug likeliness and molecular properties of synthesized compounds had been conducted using SWISS ADME web server.

2.3.2 Docking studies

To understand the structural processes behind the compounds' *in vitro* anti-tubercular activities, They were carried out by utilizing the Schrodinger suite's inbuilt Glide module[5,6] to find molecules with high binding affinities Molecular interactions of 3(a-k) compounds were done with M. tuberculosis enoyl-CoA hydratase, EchA6 (PDB ID: 5DU4) and M. tuberculosis Decaprenyl-phosphoryl-ribose 2′-epimerase, DprE1 (PDB ID: 6G83) and both these chosen proteins had the co-crystallized inhibitors, DNA gyrase 6RKS and lanosterol demethylase 5QEB.

2.4 *In vitro* studies

2.4.1 Anti-tubercular activity

Micro plate Alamar blue assay

Mycobacterium tuberculosis H37Rv (ATTCC 27294) frozen stock culture suspension from Lowenstein-Jensen slants in full 7H9 broth was vortexed and adjusted to turbidity equal to a 1 McFarland standard (3x108 CFU/ml). It was utilised as an inoculum in the MABA assay[8] after being further diluted with media-I to a concentration of 2x105 CFU/ml**.**

Preparation of the standard and test sample stock/ working solutions

Test samples 3(a-k) and to generate stock solutions with a concentration of 20,000 μg/ml, standard compounds (Isoniazid) were diluted in DMSO and filtered via syringe-driven filters (0.22 μm, 13 mm, Himedia). Using media-IIssay, the stock solutions were serially diluted to afford working solutions of 4X.[9]

2.4.2 Antibacterial activity

The antibacterial activity of the synthesized compounds was assessed using the dilution method. Four distinct bacterial microorganisms were chosen for this study: *Staphylococcus aureus* (NCIM 5345) and *Bacillus cereus* (NCIM 5346), both belonging to the Gram-positive bacteria category, and *Escherichia coli* (NCIM 5346) and *Pseudomonas aeruginosa* (NCIM 5514), both falling under the Gram-negative bacteria category.[10–12]

2.4.3 Antifungal activity

The *in vitro* antifungal efficacy of the three synthesized compound series was examined against two distinct classes of fungi, each consisting of three sub types. The yeast fungus *Candida* species were included: *C. albicans* (*Candida albicans*), *C. uti.* (*Candida utilis*), and *C. kru.* (*Candida krusei*). Additionally, the filamentous fungi *Aspergillus* species were incorporated: *A. fum.* (*Aspergillus fumigatus*), *A. nig.* (*Aspergillus niger*), and *R. bat.* (*Rhizoctonia bataticola*). This assessment was carried out through the tube dilution method. As points of reference in the study, Itraconazole and Miconazole were employed.[13–15]

3 Results & Discussion

3.1 Molecular Properties

Molecular Properties predictions revealed that most of the compounds has poor and moderate solubility, while compound 3c (-F) had a better solubility profile among them. All of the compounds exhibited high GI absorption. In general, the predicted ADME values are all the compounds had a favourable ADME predictions and this predicted that compounds have favorable Pharmacokinetic and safety profiles[16]. Compounds investigated in this study all of them are in compliance with the Lipinski's rule of five (Table 1).

Table 1. Molecular properties and drug-likeness prediction of compounds

S.No	Log P o/w	No. of. H-bond Donors	No.of H-bond Acceptors	Solubility	TPSA (Å^2)	GI absorption	BBB permeation	P-gp substrate	Drug likeness	CYP450 isoforms inhibition
3a	4.50	2	3	moderate	100.20	High	No	No	Yes	No
3b	4.48	3	3	moderate	102.45	High	No	No	yes	2C19/2C9/3A4
3c	3.10	1	2	good	91.78	High	No	No	yes	No
3d	4.51	1	3	soluble	92.46	High	No	No	Yes	No
3e	4.45	3	3	soluble	91.96	High	No	No	yes	No
3f	3.95	1	4	moderate	91.85	High	No	No	yes	NO
3g	4.26	2	2	moderate	94.59	High	No	No	Yes	NO

Continued on next page

Table 1 continued

3h	3.85	2	3	poor	104.86	High	No	No	yes	2C19/2C9/3A4
3i	3.20	1	2	poor	91.86	High	No	No	yes	NO
3j	4.43	2	2	moderate	100.56	High	No	No	yes	2C19/2C9/3A4
3k	3.55	1	3	moderate	103.54	High	NO	No	yes	No

3.2 Docking Studies

3.2.1 ***Antitubercular activity***

Molecular Docking studies were conducted with EchA6, DprE1 protein and these results were employed in (Table 2). Molecular docking studies showed compound 3c, with a 5-fluoro substitution, had strong binding (-9.6 kcal/mol) with EchA6 (Figure 2) and DprE1 proteins (Figure 3). It formed a hydrogen bond with PRO72 and pi-pi stacking interactions with TRP220 and PHE216 in EchA6. In DprE1, it had halogen bond interaction with THR118 and hydrogen bond interaction with -C=O carbonyl group of the indole ring. Compound 3e, with 5-bromo substitution, displayed similar interactions with EchA6 and DprE1. Compound 3f, with a 5-nitro substitution, showed comparable interactions with EchA6 and DprE1, including hydrogen bond and pi-pi stacking interactions.

Table 2. Docking score synthesized compounds in the binding pocket of *M. tuberculosis*enoyl-CoA hydratase, EchA6 and Decaprenyl-phosphoryl-ribose 2′-epimerase, DprE1

Sr. no	Compounds	Docking ScoreK cal/mole 5DU4	Docking ScoreKcal/mole 6G83
1	3a	-9.1	-7.8
2	3b	-8.9	-7.6
3	3c	-9.6	-8.8
4	3d	-8.7	-7.7
5	3e	-9.3	-8.6
6	3f	-9.4	-8.5
7	3g	-8.5	-7.8
8	3h	-8.4	-7.5
9	3i	-8.3	-7.4
10	3j	-8.2	-8.3
11	3k	-8.1	-7.2
12	Isoniazid	-5.4	-4.8

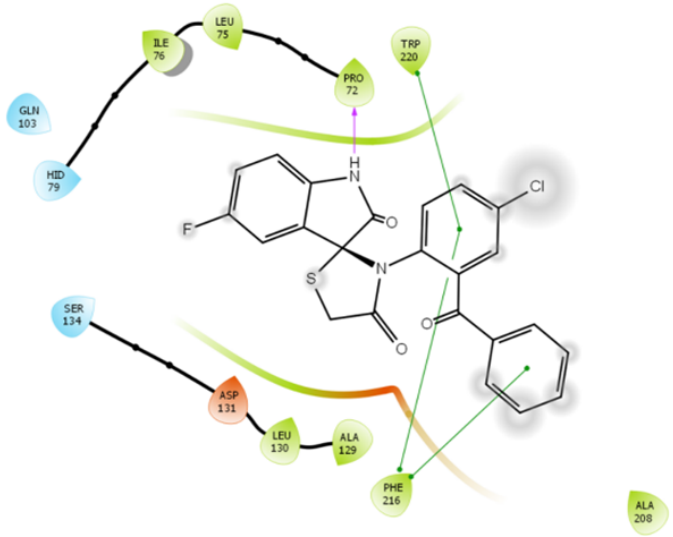


Fig 2. Molecular interactions 3c and with *Mycobacterium tuberculosis* enoyl-CoA hydratase , EchA6

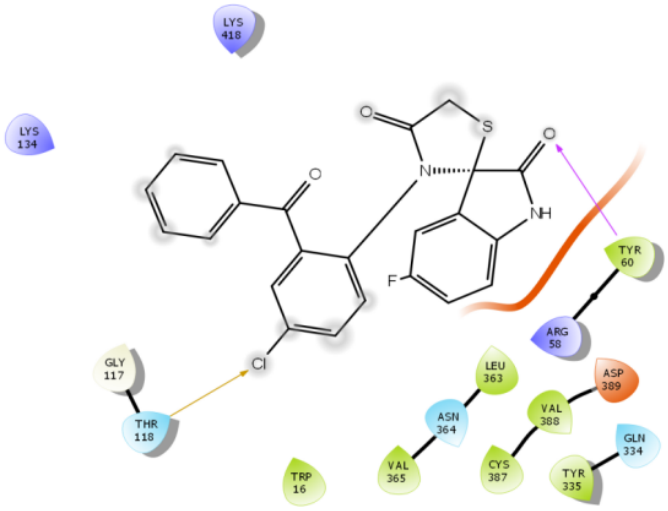


Fig 3. Molecular interactions of 3c with *Mycobacterium tuberculosis* Decaprenyl-phosphoryl-ribose 2′-epimerase, DprE1

3.2.2 ***Antibacterial activity***

Compound 3c (fluoro) showed marked binding affinity with ΔG value of -8.9 kcal/mol. All the compounds showed better binding affinity with DNA gyrase 6RKS. 3c participated aromatic ring pi interaction with GLU-145, hydrogen bond with ASP-136, Compound 3f(-NO_2) showed good binding affinity with ΔG value of -8.2 kcal/mol, Compound 3f (nitro) participated in aromatic ring pi interaction with GLU-145, Lue-96, Hydrogen bond with Asp-136. Other Compounds 3e (bromo) with 8.1 Kcal.mole, showed moderate affinity. Standard drug ciprofloxacin participated in Pi interaction with GLU-145, hydrogen bond interaction with ASP-136, LEU-96, VAL-91. Compounds bearing electron with drawing groups 3c (fluoro), 3f (nitro) showed potent binding affinity with the target. Compounds bearing electron donating groups 3h (-CH_3), 3j (-NH_2) groups showed moderate affinity with the targets. Based on binding it can be predicted that GLU-145 ASP-136, LEU-96 are participated in the interactions with the ligands. Electron with drawing groups on aromatic ring is important for Antibacterial activity.

3.2.3 ***Antifungal activity***

Compound 3c (fluoro) showed strong binding (-8.9 kcal/mol) with 5QEB, engaging in pi-pi stacking with TRP220 and PHE216, and a hydrogen bond with Leu-135. Similarly, 3f (-NO2) and 3d (iodo) displayed notable affinities (-8.2 kcal/mol and -7.8 kcal/mol respectively), while 3i (CF3) exhibited moderate affinity (-7.7 kcal/mol). Compounds with electron-withdrawing groups like fluoro, nitro, and iodo showed potent binding, whereas those with electron-donating groups displayed moderate affinities. Enhanced electron-withdrawing capabilities of substituents were key to improving binding affinities at the target site.

3.3 *In vitro* activities

3.3.1 ***Antitubercular Activity***

The Alamar Blue assay was used to evaluate anti tubercular activity of the synthesized compounds (3a-3k). MIC values of synthesized compounds were determined. Compound 3c having fluro substituent, showed potent anti-*Mycobacterium tuberculosis* H37Rv activity**.** Compound 3f (-NO2) showed modest activity against *Mycobacterium tuberculosis* H37Rv, with a MIC value of 3.125μg. Compound 3e (-Br) exhibited a MIC value of 12.5μg, and so forth. Comparing all other synthesized compounds to standard isoniazid, they showed mild to moderate activity against *Mycobacterium* TB H37Rv. These findings are consistent with previous research [17]. Compounds containing iodo, bromo, or nitro groups exhibited strong activity when compared to the standard. A robust correlation was observed between *in vivo* and *in vitro* investigations.

3.3.2 ***Antibacterial activity***

Synthesised compounds were evaluated for antibacterial activity against range of pathogens, including *Escherichia coli, Bacillus cereus*, and *Staphylococcus aureus*. Tube dilution method was used. Results are employed in (Figure 4). The screening revealed mild to strong antimicrobial properties across the bacterial spectrum. Compound 3C(florogroup) showed notable activity when compared with ciprofloxacin, with MIC values of 3.21 μg/ml against S. aureus, 2.65 μg/ml against *B. cereus*, 7.24 μg/ml against

E. coli, and 3.54 μg/ml against *P. aeruginosa*. When it came to Gram-positive strains, compounds 3e (-Br) and 3b (-Cl) showed moderate to good activity, but when it came to Gram-negative strains, they showed moderate to poor potency. The experimental outcomes closely correlated with molecular docking simulations, identifying compound 3f as a promising candidate for further investigation. These results align closely with conclusions drawn in previous studies[18,19].

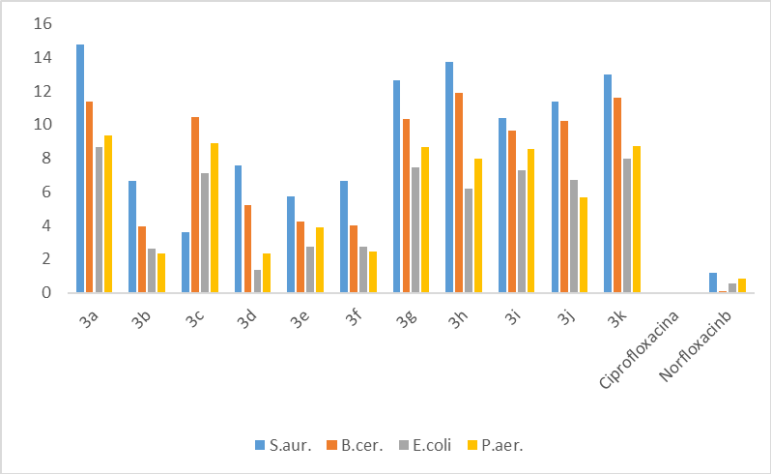


Fig 4. Anti-bacterial activity of title compounds *S. aur.*: *Staphylococcus aureus*; *B.cer.*: *Bacillus cereus*; *E. coli.*: *Escherichia coli*; *P.aer* . : *Pseudomonas aeruginosa*

3.3.3 ***Anti-fungal activity***

The synthesized compound series (3a-3k) was subjected to *in vitro* testing for antifungal efficacy against two distinct classes of fungi, each comprising three subtypes. *Candida* species, including *C. albicans, C. uti.*, and *C. kru*, as well as *Aspergillus* species, such as *A. fum., A. nig.*, and *R. bat.*, were included in the assessment. The tube dilution method was employed, and the results are depicted in (Figure 5). Itraconazole and Miconazole served as reference points in the study. Compounds 3c (fluoro), 3f (nitro), and 3d (iodo) exhibited promising antifungal activity against the screened fungal species when compared to standard Itraconazole and Miconazole. They displayed modest to negligible minimum inhibitory concentrations (MICs) against filamentous fungi and moderate to high efficacy against yeast fungi. Compounds with electron-withdrawing groups demonstrated potent activity, while those with electron-donating groups showed poor activity. These findings are consistent with previous reports[16,17]. Compounds containing iodo, bromo, or nitro groups exhibited potent activity compared to the standard. A strong correlation was observed between *in vitro* and *in vivo* studies, as well as between the experimental outcomes and molecular docking simulations. Compound 3d emerged as a promising candidate for further exploration based on these findings.

4 Conclusion

A series of compounds 3-(2-Benzoyl-4-chlorophenyl)-4H-spiro [indole-3,2- [1,3] thiazolidine]-2,4(1H)-dione derivatives(3a-3k) were synthesized. Spectral data revealed that title compounds were pure. Molecular Properties predictions revealed that most of the compounds has poor and moderate solubility, while compound 3c (-F) had a better solubility profile among them. All of the compounds exhibited high gastrointestinal (GI) absorption. None of the compounds are predicted to permeate the blood-brain barrier (BBB) or act as substrates for P-glycoprotein (P-gp). Compound 3c (fluoro) showed potent activity against *Mycobacterium tuberculosis* H37Rv, DNA gyrase 6RKS and lanosterol demethylase 5QEB with MIC value of 3.125μg/ml, 3.21 μg/ml, 8.35μg/ml respectively. All the compounds showed better binding affinity with *Mycobacterium tuberculosis* H37Rv, DNA gyrase 6RKS and lanosterol demethylase 5QEB when compared with standards. *In vitro* studies revealed that compounds 3c(fluoro) 3e (bromo) 3f (nitro) having electron with drawing substituents exhibited potent anti tubercular and antimicrobial activities when compared with electron donating groups. The results closely consistent with the conclusions drawn in previous reports[20]. The commendable correlation was observed between the *in vitro* experimental outcomes and molecular docking simulations in which electron with drawing substituents as a promising candidate for anti-tubercular and antimicrobial activities.

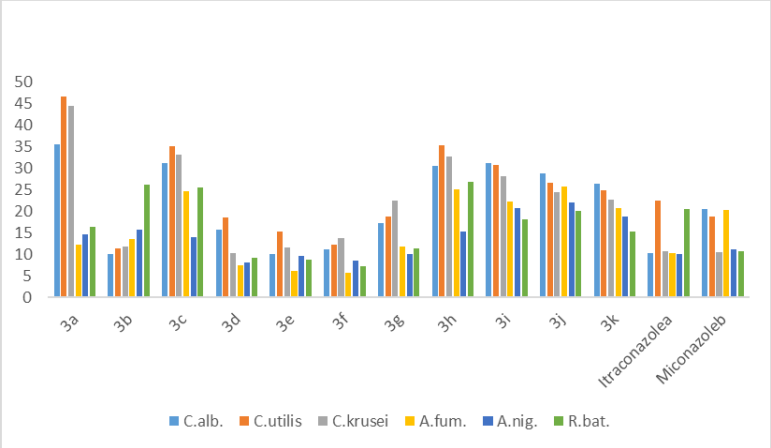


Fig 5. Anti fungal activity of title compounds ***C. albic.*: *Candida albicans*; *C. uti.*: *Candida utilis*; *C. kru.*: *Candida krusei*; *A. fum.*: *Aspergillus fumigatus*; *A.nig.* : *Aspergillus niger* ; *R. bat.*: *Rhizoctonia bataticola***

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