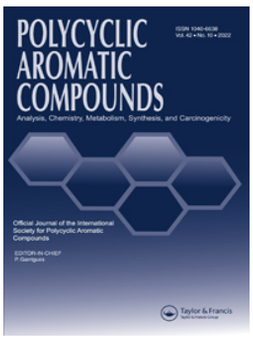

Polycyclic Aromatic Compounds

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[DBUH][OAc]-Catalyzed Domino Synthesis of Novel Benzimidazole Incorporated 3,5-Bis (Arylidene)-4-Piperidones as Potential Antitubercular Agents

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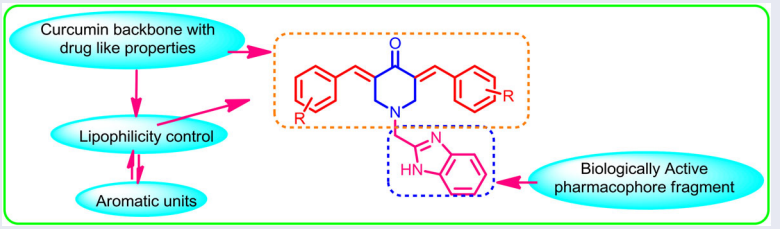
ABSTRACT

A series of new benzimidazole incorporated 3,5-bis (arylidene)-4-piperidones were synthesized by using aryl aldehydes, piperidinone, 2-(chloromethyl)-benzimidazole and DBU acetate [DBUH][OAc] act as a catalyst under solvent free condition in excellent yields. The synthesized compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Ra (*MTB*) and *M. bovis* BCG strains. The compounds **4a**, **4b**, **4e**, **4i**, **4k** and **4l** are highly potent against both the strains. Most of the active compounds are non-cytotoxic against MCF-7, A549, HCT 116 and THP-1 cell lines. Furthermore, a molecular docking study of these compounds was carried out to investigate their binding pattern with the target, active site of mycobacterial enoyl-acyl carrier protein reductase (Inh A). Therefore, these compounds can be subjected for further optimization and drug development which could give promising chemical leads for treatment of TB.



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Introduction

Mycobacterium Tuberculosis (MTB) bacteria causes Tuberculosis (TB), mostly affect the lungs. Nearly 10 million people fell ill with TB worldwide in 2009, in which 1.2 million children, 3.2 million women and 5.6 million men. Health providers ignored child and adolescent TB because it is difficult to diagnose and treat. In 2019, nearly, 87% new TB cases found in 30 countries, among them only eight countries are responsible for the two thirds of the total, with India

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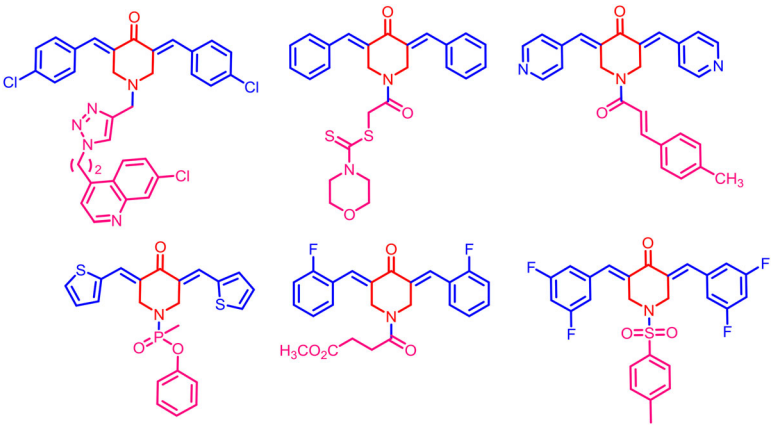


Figure 1. Active monocarbonyl curcumin analogues bearing *N*-substituted piperidone moiety.

leading the count, followed by Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa. Multidrug-resistant TB (MDR-TB) is the biggest public health crisis and a health security threat worldwide. In 2019, globally total 2,06,030 people detected with multidrug- or rifampicin-resistant TB (MDR/RR-TB) which is 10% higher from 186 883 in 2018.[1] Near about 2% TB incidence is falling globally along with 9% cumulative reduction between 2015 and 2019. This was less than half way to the End TB Strategy milestone of 20% reduction between 2015 and 2020. Between 2000 and 2009 it is estimated that 60 million lives were saved through TB diagnosis and treatment. United Nations Sustainable Development Goals (SDGs) health target is to Wind-up the TB epidemic by 2030.[1]

Curcumin [(1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-heptadien-3,5-dion)], a natural component of the rhizome of Curcuma longa, proved to be a powerful chemopreventive.[2] The monocarbonyl curcumin analogues shows wide range of multiple biological activities.[3] The substituents on nitrogen of arylidene-piperidones with various heterocycles systems were reported with different bioactivities such as anticancer,[4] antitubercular,[5] antiinflammatory,[6] antileishmanial,[7] antioxidant[8] and antidiabetic activity.[9] They also exhibits topoisomerase II alpha inhibitors,[10]a proinflammatory cytokines,[10]b murine and human macrophages cell lines[10]c and nitric oxide inhibitors.[10]d The representative structures of biologically active monocarbonyl curcumin analogues/bis arylidene having *N*-substituted piperidinone moieties are given in Figure 1.

Benzimidazole a privileged pharmacophore, possesses an array of biological activities[11]a,b including tubulin polymerization,[12]a anticancer,[12]b antidiabetic,[12]c antimicrobial,[12]d antimycobacterial,[12]e anti-inflammatory,[12]f anti-HIV,[12]g antiprotozoal,[12]h analgesic,[12]i antimalarial,[12]j antihistamic[12]k and antiviral[12]l activity. Some of the marketed drugs containing benzimidazole nucleus are albendazole (antiprotozoal), nocodazole (anticancer), lerisetron (antihistaminic), andibendeb (phosphodiesterase inhibitor), veliarib (anticancer), maribavir (antiviral) are shown in Figure 2.

Multicomponent reactions (MCRs) permits rapid access to combinatorial libraries of organic molecules[13] for lead structure identification and optimization in drug discovery.[14] The implementation of several transformations in a single manipulation is highly compatible with the goals of sustainable and green chemistry.[15] A variety of ionic liquids have been extensively applied in heterocyclic synthesis as a solvent or catalysts.[16] The [DBUH][OAc] received considerable attention because, it is an inexpensive, nontoxic catalyst as well as solvent for many organic transformations in excellent yields.[17]

We have recently reported the synthesis of 3,5-bis (arylidene)-4-piperidones (Figure 3A) as antitubercular agent (MTB H37Ra and M. bovis BCG strain with MIC values 1.89–26.37 and 2.69–29.14 mg/mL, respectively),[18a] monocarbonyl curcumin analogues bearing propargyl ether

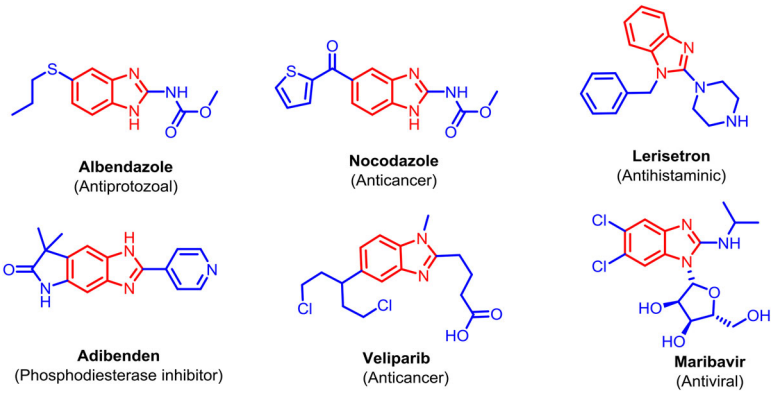


Figure 2. Benzimidazoles containing drugs available in market.

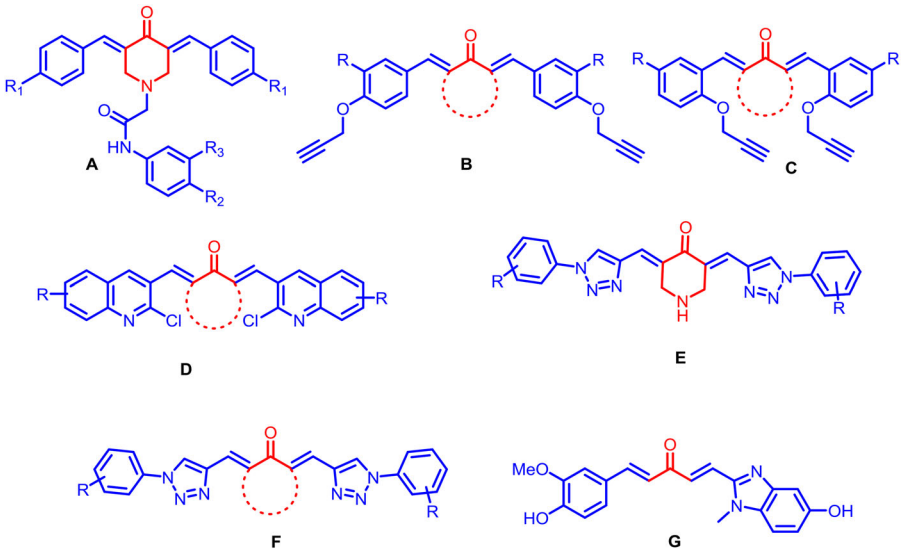


Figure 3. Developed an approach toward the synthesis of curcumin mimics.

(Figure 3B and 3C) (MIC 12.5–175 μg/mL)[18b] and quinoline (Figure 3D)[18c] (MIC 6.25–200 μg/mL) based monocarbonyl curcumin analogues as antifungal agents, 1,2,3-triazole incorporated monocarbonyl curcumin analogues[18d] (Figure 3E) (MIC 6.25–>25 μg/mL) and α,α'-bis(1H-1,2,3-triazol-5-ylmethylene) ketones[18e] (Figure 3F) as potent antitubercular (MIC 3.125–>25 μg/mL) and antioxidant agents (MIC 15.49 ± 0.24–71.09 ± 0.25).

Literature survey reveals that, Lee and coworkers reported synthesis of substituted benzimidazolyl curcumin mimics and their anticancer activity[19] (Figure 3G). However, there is no report on antitubercular activity of benzimidazole incorporated 3,5-bis (arylidene)-4-piperidone derivatives.

In view of the above and continuation of our research programme on the development of new methodology for the synthesis of bioactive heterocyclic compounds,[18,20] herein, we would like to report the synthesis of new benzimidazole incorporated arylidene-piperidone derivatives and their biological evaluation for antibacterial, antitubercular and cytotoxic activity. In addition to this, we have also studied the molecular binding interactions with enoyl-acyl carrier protein reductase enzyme (Inh A) for most active compound.

Table 1. Optimization of solvent and reaction temperature.

Entry	Solvent	Time (h)	Temp (°C)	Yield[a] (%)
1	DMSO	9	rt	63
2	EtOH	7.5	rt	55
3	MeOH	7	rt	59
4	DMF	6.5	rt	74
5	CH_3CN	8	rt	61
6	Toulene	7.5	rt	75
7	Solvent-free	4	rt	85

Reaction conditions: **1a** (2 mmol), **2** (1 mmol), **3** (1 mmol) and 20 mol% [DBUH][OAc].
[a]Isolated yield.

Results and discussion

Chemistry

To optimize the best reaction condition for condensation-alkylation, the benzaldehyde **1a**, piperidinone **2**, and 2-(chloromethyl)-benzimidazole **3** in the presence of [DBUH][OAc] as a medium/catalyst was considered for model reaction (Scheme 1). Firstly, we have screened different solvents for model reaction *viz*. DMSO, EtOH, MeOH, DMF, CH_3CN and Toluene at room temperature provides low yield of product (Table 1, **Entries 1–6**). Under the solvent-free condition, the desired product **4a** was obtained in 85% yield (Table 1, Entry 7).

The amount of catalyst plays a vital role to carry the reaction in forward direction. Further investigation was carried out to determine the quantity of [DBUH][OAc]. An increase in the amount of [DBUH][OAc] from 5 to 20 mol% led to an increase in the yield (Table 2, entries 2–5). The use of 25 mol% of [DBUH][OAc] did not improve the yield (Table 2, entry 6). However, when reaction was carried out in the absence of [DBUH][OAc], no product was obtained (Table 2, entry 1). It proves that a [DBUH][OAc] may be essential for better catalytic activity.

Because [DBUH][OAc] can play a role of acid or a base or both simultaneously, as a nucleophile. Thus, the optimum conditions required the use of [DBUH][OAc] as 20 mol % at room temperature.

To check the eco friendliness of [DBUH][OAc], we recycled the ionic liquid [DBUH][OAc] for five times, Table 3. The reaction proceeded cleanly with good yields (85, 83, 80, 80 and 75%); although a weight loss of ∼5% of [DBUH][OAc] was observed from cycle to cycle due to mechanical loss (Table 3, entries 1–5).

The structure of **4a** has been established on the basis of IR, ^1H, ^{13}C NMR and HRMS. In the IR spectrum of **4a** displayed characteristic signal for C = O at 1647 cm^{-1}. In the ^1H NMR spectrum for **4a** exhibited a sharp singlet resonating at δ 4.95 ppm for two protons, has been assigned to methylene protons attached to benzimidazole. The peak observed at δ 4.56 ppm for the four protons of the piperidone ring. ^{13}C NMR spectrum was also in good agreement with the proposed structure displaying characteristic signals for C = O and two CH_2 group at δ 188.4, 56.8 and 51.4 ppm, respectively.

Using the above optimized reaction conditions, the scope and efficiency of this approach was explored for the synthesis of other new benzimidazole based 3,5-bis(arylidene)-4-piperidones with electron donating and withdrawing groups on phenyl ring in excellent yields (Scheme 2). The structures of all the derivatives are confirmed by physical data and spectral analysis.

A plausible mechanistic pathway (Scheme 3) is proposed to illustrate the synthesis of monocarbonyl curcumin analogues catalyzed by [DBUH][OAc]. The initial step is believed to be the protonation of the aldehyde **I** by ionic liquid [DBUH][OAc] to form intermediate **II**, which facilitates the nucleophilic attack of piperidinone to promote the formation of C-C bond to yield intermediate **IV**. The subsequent elimination of H_2O molecule by the reaction of intermediate **IV** to yield compound **V**. The final step involves deprotonation of intermediate **V** followed by 2-

Table 2. The effect of [DBUH][OAc] loading on model reaction **4a**[a].

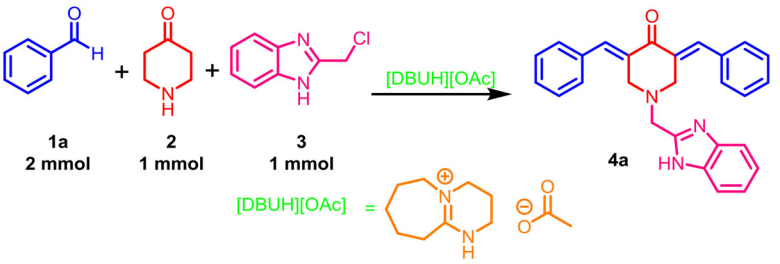
Entry	Catalyst (mol%)	Yield (%)[b]
1	No Catalyst	Trace
2	5	50
3	10	65
4	15	73
5	20	85
6	25	85

[a]Reaction conditions: **1a** (2 mmol), **2** (1 mmol), **3** (1 mmol), rt, 4 h.
[b]Isolated yield.

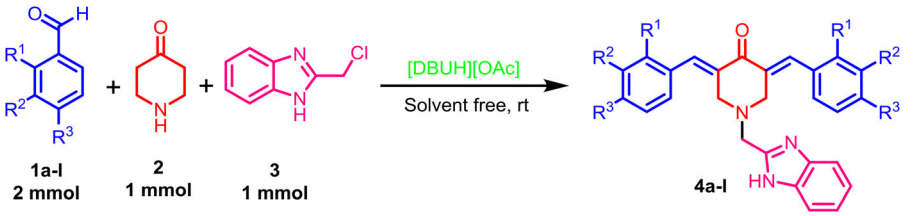
Table 3. Reusability of [DBUH][OAc] in the synthesis of **4a**[a].

Entry	Reaction cycle	**Isolated yield** (%)[b]
1	1st (fresh run)	85
2	2nd cycle	83
3	3rd cycle	80
4	4th cycle	80
5	5th cycle	75

[a]Reaction conditions: **1a** (2 mmol), **2** (1 mmol), **3** (1 mmol), [DBUH][OAc] (20 mol%), rt, 4 h.
[b]Isolated yield.



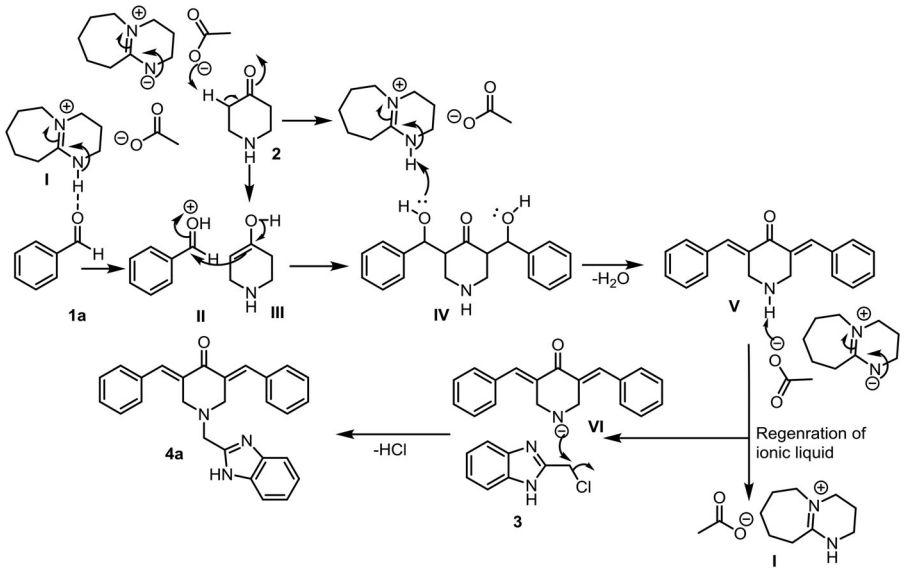
Scheme 1. Model reaction.



4a: R^1=H, R^2=H, R^3=H, **4b**: R^1=H, R^2=H, R^3=Me
4c: R^1=H, R^2=H, R^3=-OMe, **4d**: R^1=-Cl, R^2=H, R^3=H
4e: R^1=H, R^2=-Cl, R^3=H, **4f**: R^1=H, R^2=H, R^3=-Cl
4g: R^1=-OH, R^2=H, R^3=H, **4h**: R^1=H, R^2=H, R^3=-OH
4i: R^1=H, R^2=H, R^3=-F, **4j**: R^1=H, R^2=H, R^3=-Br
4k: R^1=H, R^2=-NO_2, R^3=H, **4l**: R^1=H, R^2=H, R^3=-NO_2

Scheme 2. Synthesis of benzimidazole incorporated 3,5-bis (arylidene)-4-piperidones **4a-l**.

(chloromethyl)-1*H*-benzo[*d*]imidazole (**3**) expedites the formation of C-N bond to yield compound **4a** by the elimination of HCl molecule by the nucleophilic attack of nitrogen to the carbon group to promote C-N bond formation, accelerated by ionic liquid [DBUH][OAc] eventually leads to the formation of final product **4a**.



Scheme 3. Plausible mechanistic catalytic cycle for the synthesis of compound **4a**.

Biological evaluation

Antitubercular activity

In a standard primary screening, all the newly synthesized compounds **4a-l** were evaluated for their *in vitro* antitubercular activity against *MTB* H37Ra and *M. bovis* BCG strains at concentrations of 30, 10 and 3 µg/mL using an established XTT Reduction Menadione assay (XRMA) and NR (Nitrate reductase) assay, respectively.[21] Compounds showing 90% inhibition of bacilli at or lower than 30 µg/mL were selected for further dose response curve (Tables S1–S13, supplementary material). Rifampicin was used as standard drug and the obtained results are presented in Table 4.

The compounds **4a**, **4b**, **4e**, **4i**, **4k** and **4l** (MIC = 1.37, 0.64, 2.46, 1.3, 1.38 and 2.19 µg/mL, respectively) were found to be highly active against *MTB* H37Ra strain. Similarly, compounds **4a**, **4b**, **4e**, **4i**, **4k** and **4l** (MIC = 1.36, 3.15, 2.5, 1.33, 2.3 and 2.52 µg/mL, respectively) were found to be active against *M. bovis* BCG. Remaining all the compounds (MIC = >30 µg/mL) were found to be less active against both the strains. According to the data, the activity depends on the substituents present on phenyl rings.

For *MTB* H37Ra and *M. bovis* BCG strains, the compound **4a** showed promising antitubercular activity with MIC values 1.37 and 1.36 µg/mL, respectively. The compound **4b** (R^3 = Me) showed very promising antitubercular activity as compared to remaining with MIC value of 0.64 and 3.15 µg/mL, against the *MTB* H37Ra and *M. bovis* BCG strains, respectively. The compound **4e** (R^2 = Cl) showed significant antimycobacterial activity with MIC value of 2.46 and 2.5 µg/mL, against both the strains. Remaining chloro containing compounds does not displayed significant change in antitubercular activity. The position of *chloro-* group is considered to be important on the phenyl ring for activity. For compound **4i** (R^3 = OH), the MIC values 1.3 and 1.33 µg/mL, showed excellent antitubercular activity. The presence of nitro group in compounds **4k** and **4l** displays prominent antitubercular activity with MIC values 1.38 and 2.19 µg/mL, against the *MTB* H37Ra strain and 2.3 and 2.52 µg/mL, against the *M. bovis* BCG strain respectively. The similar type of trend was occurred for active compounds **4a**, **4b**, **4c**, **4i**, **4k** and **4l** with lower IC_{50} values against both the strains. However, all the synthesized compounds exhibited poor activity compared to the standard antitubercular drug Rifampicin.

Table 4. *In vitro* antitubercular activity of **4a-l** (μg/mL).

Cpd	*MTB* H37Ra		*M. bovis* BCG		Glide score	Glide energy (Kcal/mol)	H-bonding (Å)	π-π/cation-π stacking (Å)
	MIC	IC_{50}	MIC	IC_{50}				
4a	1.37	0.11	1.36	0.11	−9.760	−58.003	Thr196 (2.085)	Tyr158(5.096), Phe149(4.349)
4b	0.64	0.18	3.15	1.3	−8.956	−49.971	Ile194 (2.158)	Tyr158(5.350), Trp222(4.825)
4c	>30	>30	>30	>30	−7.853	−41.635	–	Tyr158(5.363)
4d	>30	>30	>30	>30	−9.375	−46.883	–	Phe149(4.331)
4e	2.46	0.25	2.5	0.21	−8.135	−55.35	Thr196 (1.866)	Tyr158(5.161), Phe149(4.331)
4f	>30	>30	>30	>30	−7.802	−41.562	–	Tyr158(5.276)
4g	>30	>30	>30	>30	−7.842	−41.521	–	Tyr158(5.003)
4h	>30	>30	>30	>30	−7.347	−41.117	–	Tyr158(5.062)
4i	1.3	0.13	1.33	0.11	−9.442	−54.663	–	Tyr158(5.096), Phe149(4.296)
4j	>30	>30	>30	>30	−7.685	−41.863	–	Tyr158(5.309)
4k	1.38	0.09	2.3	0.09	−9.957	−59.653	Thr196 (2.034)	Tyr158(5.230), Phe149(4.378)/Phe149(5.573)
4l	2.19	0.24	2.52	0.13	−8.799	−46.538	Ile194 (2.074)	Tyr158(5.224), Trp222(5.018)/ Tyr158(5.572)
RP	0.045	0.0017	0.017	0.0015	–	–	–	–

Cpd: Compound; RP: Rifampicin.

Cytotoxicity

After identifying a good number of active antitubercular leads, the compounds were tested against human cell lines, MCF-7, A549, HCT-116 and THP-1 using MTT assay[22] with paclitaxel as a positive control. The cytotoxicity results are expressed in terms of GI_{50} indicating the 50% growth inhibition concentration (Table 5). None of the active compounds exhibited any significant cytotoxic effects against all the cell lines, suggesting a great potential for their *in vivo* use as antimycobacterial agents.

Antibacterial activity

The most active antitubercular compounds **4a**, **4b**, **4e**, **4i**, **4k** and **4l** were further confirmed from their dose dependent effect against four bacteria strains Gram-negative and Gram-positive bacteria.[23] The most promising compounds **4a**, **4b**, **4i**, **4k** and **4l** showed strong specificity against *MTB* and BCG as compared to **4e** (Table 6). The results clearly indicates that, compounds **4a**, **4b**, **4i**, **4k** and **4l** are mycobacteria specific and that can be explored further for potential antitubercular drug.

Molecular docking study

All compounds were successfully docked into the active site of mycobacterial InhA. The docking score of most active compounds **4a**, **4b**, **4e**, **4i**, **4k** and **4l** was found to be −9.760, −8.956, −8.135, −9.442, −9.957 and −8.799 respectively, which were comparable with respect to *in vitro* antitubercular activity. We have discussed only the docking study of most active compound **4k**. The interactions of **4k** with the active site of mycobacterial InhA is shown in Figure 4. The lowest energy docking pose of **4k** revealed the presence of hydrogen bonding interactions between nitrogen of imidazole ring and Thr196 with a distance of 2.034 Å. Also, highly hydrophobic π-π stacking interactions were observed between phenyl ring and Tyr158 and Phe149 and also π-π stacking interaction between nitro group and Phe149. These hydrogen binding and π stacking interactions helps in predilection of these ligands within the active site which increases the steric and electrostatic interactions of ligands with the amino acid residues present within the active site of mycobacterial InhA.

The compound **4k** was stabilized within the active site through favorable van der Waals interactions observed with Met199 (−3.783 kcal/mol), Ala198 (−0.773 kcal/mol), Thr196 (−2.165 kcal/

Table 5. *In vitro* cytotoxicity of active compounds (GI_{50} in μg/mL).

Compound	MCF-7	A549	HCT-116	THP-1
4a	>100	>100	>100	>100
4b	>100	>100	>100	>100
4e	>100	>100	>100	>100
4i	>100	>100	>100	>100
4k	>100	>100	>100	>100
4l	>100	>100	>100	>100
Rifampicin	>100	>100	>100	>100
Paclitaxel	0.0048	0.0035	0.0260	0.1374

GI_{50} indicates concentration to inhibit 50% growth of cells.

Table 6. *In vitro* antibacterial activity (MIC in μg/mL).

Compound	*E. coli*	*P. fluorescens*	*S. aureus*	*B. subtilis*
4a	>30	>30	>30	>30
4b	>30	>30	>30	>30
4e	7.86	8.66	9.46	10.26
4i	>30	>30	>30	>30
4k	>30	>30	>30	>30
4l	>30	>30	>30	>30
Ampicillin	4.17 ± 1.04	12.47 ± 1.28	2.86 ± 0.78	29.53 ± 1.88
Kanamycin	3.34 ± 0.41	1.01 ± 0.09	> 61.92	2.78 ± 0.85

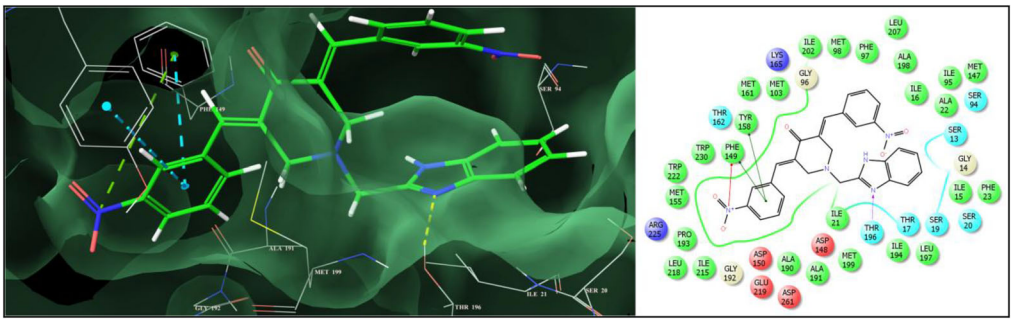


Figure 4. 3D and 2D view of binding of **4k** with the active site of mycobacterial InhA.

mol), Ile194 (−2.334 kcal/mol), Pro193 (−3.911 kcal/mol), Gly192 (−1.982 kcal/mol), Ala191 (−1.793 kcal/mol), Lys165 (−1.254 kcal/mol), Met161 (−2.452 kcal/mol), Tyr158 (−2.711 kcal/mol), Phe149 (−5.313 kcal/mol), Met147 (−1.241 kcal/mol), Phe97 (−0.931 kcal/mol), Gly96 (−1.218 kcal/mol), Ile95 (−1.561 kcal/mol), Ser94 (−1.603 kcal/mol) and Ile21 (−1.484 kcal/mol) residues. Also, several strong electrostatic interactions were observed with Met199 (−4.544 kcal/mol), Ala198 (−0.811 kcal/mol), Thr196 (−1.842 kcal/mol), Ile194 (−3.533 kcal/mol), Pro193 (−4.558 kcal/mol), Gly192 (−2.501 kcal/mol), Ala191 (−2.531 kcal/mol), Lys165 (−1.155 kcal/mol), Met161 (−2.831 kcal/mol), Tyr158 (−3.306 kcal/mol), Phe149 (−4.791 kcal/mol), Met147 (−0.926 kcal/mol), Phe97 (−0.994 kcal/mol), Gly96 (−1.272 kcal/mol), Ile95 (−2.193 kcal/mol), Ser94 (−4.620 kcal/mol) and Ile21 (−0.908 kcal/mol) which fits the compound **4k** in to the cavity of InhA and leads to its firm binding with docking score of −9.957 and was found to be most potent among the series. From, the docking studies it is clear that these compounds have significant binding with the active site of mycobacterial InhA.

Conclusion

A series of new benzimidazole incorporated 3,5-bis (arylidene)-4-piperidones were synthesized by using aryl aldehydes, piperidinone, 2-(chloromethyl)-benzimidazole and [DBUH][OAc] act as a

catalyst under solvent free condition in excellent yields. The synthesized compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Ra (*MTB*) and *M. bovis* BCG strains. The compounds **4a**, **4b**, **4e**, **4i**, **4k** and **4l** are highly potent against both the strains. Most of the active compounds are non-cytotoxic against MCF-7, A549, HCT 116 and THP-1 cell lines. Furthermore, a molecular docking study of these compounds was carried out to investigate their binding pattern with the target, active site of mycobacterial enoyl-acyl carrier protein reductase (Inh A) and can be developed as oral drug candidate.

Experimental

All the solvents and reagents were purchased from commercial suppliers, Spectrochem Pvt. Ltd., Rankem India Ltd. and Sigma Aldrich, and were used without further purification. The completion of the reactions was monitored by thin-layer chromatography (TLC) on aluminum plates coated with silica gel 60 F_{254}, 0.25 mm thickness (Merck). The detection of the components was made by exposure to iodine vapors or UV light. Melting points were determined by open capillary methods and are uncorrected. ^{1}H NMR & ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker DRX-400 MHz spectrometer. IR spectra were recorded using a Bruker ALPHA ECO-ATR FTIR spectrometer. High-resolution mass spectra (HRMS) were recorded on Agilent 6520 (QTOF) mass spectrometer.

General procedure for (3E,5E)-1-((1H-benzo[d]imidazol-2-yl)methyl)-3,5-dibenzylidenepiperidin-4-one derivatives (4a-l)

In a 50 mL round bottom flask, aromatic aldehydes (1 mmol), piperidin-4-one (1 mmol) and 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (1 mmol) and 20 mol% [DBUH][OAc] was stirred at room temperature under solvent-free condition for 4 h. The progress of the reaction was monitored using thin layer chromatography (TLC) (Petroleum ether: Ethyl acetate 1:1). After completion of reaction water was added the reaction mixture was further stirred for 5 min. The solid obtained was removed by filtration, washed with water and then recrystallized from ethanol. The water was removed from filtrate under reduced pressure to recover [DBUH][OAc], which was then reused in subsequent cycles. The identity of the products was confirmed by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectra.

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-dibenzylidenepiperidin-4-one (4a)

The compound **4a** was obtained from **1a**, **2** and **3** as yellow solid; Mp: 214–216 °C; Yield: 85%; IR (cm^{-1}): 1647, 1564, 1486 and 1427; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 9.55 (s, 1H, NH), 7.85 (s, 2H, -C = CH), 7.77–7.66 (m, 6H, Ar-H), 7.56–7.53 (m, 6H, Ar-H), 7.41–7.40 (d, 2H, J = 4 Hz, -Ar-H), 4.95 (s, 2H, -CH_2) and 4.56 (s, 4H, -CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 188.4, 156.2, 137.0, 135.7, 132.6, 129.0, 126.0, 122.4, 121.8, 120.1, 119.6, 117.6, 105.7, 56.8 and 51.4; HRMS (ESI-qTOF): Calcd for $C_{27}H_{23}N_3OKa$ $[M + K]^+$, 444.1388, found: 444.1367.

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(4-methylbenzylidene) piperidin-4-one (4b)

The compound **4b** was obtained from **1b**, **2** and **3** as yellow solid; Mp: 212–214 °C; Yield: 80%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 7.83 (s, 2H, -C = CH), 7.54–7.47 (m, 6H, Ar-H), 7.27–7.23 (m, 6H, Ar-H), 4.94 (s, 2H, -CH_2), 4.49 (s, 4H, -CH_2) and 2.35 (s, 6H, CH_3). Calcd for $C_{29}H_{28}N_3O$ $[M + H]^+$, 434.2232, found: 434.0875.

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(4-methoxybenzylidene)piperidin-4-one (4c)

The compound **4c** was obtained from **1c**, **2** and **3** as yellow solid; Mp: 245–247 °C; Yield: 79%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 7.93 (s, 2H, -C = CH), 7.59–7.55 (m, 6H, Ar-H), 7.27–7.25 (m, 2H, Ar-H), 7.12–7.11 (m, 4H, Ar-H), 5.01 (s, 2H, -CH_2), 4.35 (s, 4H, -CH_2) and 3.80 (s, 6H, OCH_3). Calcd for $C_{29}H_{28}N_3O_3$ $[M+H]^+$, 467.2164, found: 467.2631.

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(2-chlorobenzylidene) piperidin-4-one (4d)

The compound **4d** was obtained from **1d**, **2** and **3** as yellow solid; Mp: 220–222 °C; Yield: 80%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 8.06 (s, 2H, -C = CH), 7.64–7.57 (m, 4H, Ar-H), 7.42–7.25 (m, 8H, Ar-H), 5.10 (s, 2H, -CH_2) and 4.58 (s, 4H, -CH_2).

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(3-chlorobenzylidene) piperidin-4-one (4e)

The compound **4e** was obtained from **1e**, **2** and **3** as yellow solid; Mp: 178–180 °C; Yield: 78%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 7.75 (s, 2H, -C = CH), 7.70–7.65 (m, 4H, Ar-H), 7.49–7.40 (m, 6H, Ar-H), 7.34–7.32 (m, 2H, Ar-H), 4.66 (s, 2H, -CH_2) and 4.09 (s, 4H, -CH_2).

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(4-chlorobenzylidene) piperidin-4-one (4f)

The compound **4f** was obtained from **1f**, **2** and **3** as yellow solid; Mp: 232–234 °C; Yield: 82%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 8.11 (s, 2H, -C = CH), 7.89–7.87 (m, 2H, Ar-H), 7.74–7.58 (m, 10H, Ar-H), 4.64 (s, 2H, -CH_2) and 4.00 (s, 4H, -CH_2).

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(2-hydroxybenzylidene)piperidin-4-one (4g)

The compound **4g** was obtained from **1g**, **2** and **3** as yellow solid; Mp: 242–244 °C; Yield: 81%. Calcd for $C_{27}H_{23}N_3O_3$ $[M+H]^+$, 438.1773, found: 438.0804.

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(4-hydroxybenzylidene)piperidin-4-one (4h)

The compound **4h** was obtained from **1h**, **2** and **3** as yellow solid; Mp: 210–212 °C; Yield: 83%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 8.34 (s, 2H, -C = CH), 7.33–7.32 (m, 2H, Ar-H), 7.89–7.82 (m, 6H, Ar-H), 7.71–7.67 (m, 2H, Ar-H), 7.40–7.38 (m, 2H, Ar-H), 4.83 (s, 2H, -CH_2) and 4.39 (s, 4H, -CH_2).

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(4-fluorobenzylidene) piperidin-4-one (4i)

The compound **4i** was obtained from **1i**, **2** and **3** as yellow solid; Mp: 195–197 °C; Yield: 72%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 7.87 (s, 2H, -C = CH), 7.66–7.64 (m, 2H, Ar-H),

7.58–7.33 (m, 6H, Ar-H), 6.92–6.91 (m, 4H, Ar-H), 4.79 (s, 2H, -CH_2) and 4.26 (s, 4H, -CH_2). Calcd for $C_{27}H_{22}F_2N_3O$ $[M+H]^+$, 442.1686, found: 442.0526.

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(4-bromobenzylidene)piperidin-4-one (4j)

The compound **4j** was obtained from **1j**, **2** and **3** as yellow solid; Mp: 253–255 °C; Yield: 80%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 7.89 (s, 2H, -C = CH), 7.65–7.63 (m, 6H, Ar-H), 7.48–7.47 (m, 4H, Ar-H), 7.33–7.31 (m, 2H, Ar-H), 4.68 (s, 2H, -CH_2) and 4.32 (s, 4H, -CH_2).

(3E,5E)-1-((1H-Benzo[d]imidazol-2-yl)methyl)-3,5-bis(3-nitrobenzylidene) piperidin-4-one (4k)

The compound **4k** was obtained from **1k**, **2** and **3** as yellow solid; Mp: 209–211 °C; Yield: 75%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 8.45 (s, 2H, -C = CH), 8.26 (s, 2H, Ar-H), 7.88–7.56 (m, 8H, Ar-H), 7.30–7.26 (m, 2H, Ar-H), 4.81 (s, 2H, -CH_2) and 4.38 (s, 4H, -CH_2). Calcd for $C_{27}H_{21}N_5O_5$ $[M+H]^+$, 495.4950, found: 495.3476.

(3E,5E)-1-((1H-benzo[d]imidazol-2-yl)methyl)-3,5-bis(4-nitrobenzylidene)piperidin-4-one (4l)

The compound **4l** was obtained from **1l**, **2** and **3** as yellow solid; Mp: 228–230 °C; Yield: 80%; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 7.90 (s, 2H, -C = CH), 7.60–7.58 (m, 6H, Ar-H), 7.31–7.20 (m, 6H, Ar-H), 4.69 (s, 2H, -CH_2) and 4.10 (s, 4H, -CH_2). Calcd for $C_{27}H_{21}N_5O_5$ $[M+H]^+$, 495.1543, found: 495.1063.

Experimental protocol for biological activity

Antitubercular testing using the XRMA protocol

All the synthesized compounds were screened for their *in vitro* activity against *MTB* H37Ra (ATCC 25177) and *M. bovis* BCG (ATCC 35743) using two-fold dilution technique, in order to determine the actual minimum inhibitory concentration (MIC). Activity against MTB was determined through the XTT reduction menadione assay (XRMA) reading absorbance at 470 nm as per the protocol developed earlier.[21] The nitrate reductase (NR) assay was performed to estimate inhibition of *M. bovis* BCG by compounds. Absorbance for the NR assay was measured at 540 nm. *In vitro* activity against *MTB* and *M. bovis* BCG at active (8 days) and dormant (12 days) stages was performed using the XRMA and NR assay, respectively, as described above. Percentage inhibition was calculated using the following formula:

$$\%\ \text{inhibition} = \left[(\text{control} - \text{CMP})/(\text{control} - \text{blank})\right] \times 100$$

where 'control' is the activity of mycobacteria without compounds, 'CMP' is the activity of mycobacteria in the presence of compounds and 'blank' is the activity of the culture medium without mycobacteria.

Cytotoxicity assay

To check the selectivity, active derivatives **4a**, **4b**, **4e**, **4i**, **4k** and **4l** were assayed for their cytotoxic effects in three different cell lines MCF-7, A549 and HCT 116 using MTT assay[22] (Table 5). The cell lines were maintained under standard cell culture conditions under 5% CO_2 at 37 °C in

95% air humidified environment. Each concentration was tested in duplicates in a single experiment. GI_{50}/GI_{90} values were calculated using Origin Pro Software.

Antibacterial activity

All bacterial cultures were first grown in Lysogeny Broth (LB) media at 37 °C at 180 RPM. Once the culture reaches 1 O.D, it is used for antibacterial assay. Bacterial strains *E. coli* (NCIM 2688), *Pseudomonas fluorescens* (NCIM 2036) as gram-negative and *B. subtilis* (NCIM 2079), *S. aureus* (NCIM 2010) as gram-positive were obtained from NCIM (NCL, Pune) and were grown in Luria Burtony medium from Himedia, India. The assay was performed in 96 well plates after 8 and 12 h. for gram negative and gram-positive bacteria respectively. 0.1% of 1 OD culture at 620 nm was used for screening.[23] 0.1% inoculated culture was added in to each well of 96 well plates containing the compounds to be tested. Optical density for each plate was measured at 620 nm after 8 h for gram negative bacteria and after 12 h for gram positive bacteria.

Molecular docking study

Docking studies were carried out to predict the probable mechanism of action of antitubercular activity of our synthesized imidazole incorporated curcumin conjugates. Docking studies were performed using crystal structure of mycobacterium tuberculosis enoyl-acyl carrier protein reductase (InhA) (PDB ID: 1ZID) using Glide module (Grid-Based Ligand Docking with Energetics Program) of Schrodinger molecular modeling package.[24] All the ligand structure were drawn in Maestro 9.3[25] and were prepared using *Ligand Preparation* tool [26] which gives the low-energy conformers, 3D structures with correct chirality's for each successfully processed input structure. The imported protein was further purified. After, careful examination, the water molecules were deleted. Ionization and tautomeric state of amino acid residues were rectified and H-atoms atoms were added wherever necessary. Missing residues of the side chain were added using Prime.[27] Initially, during protein refinement orientation of polar hydrogens, flip terminal amides and histidine's and protein protonation states were optimized. Further, existing steric clashes present within the protein were relaxed using the OPLS-2005 force field present in the impact refinement module. Minimization was terminated when the energy converged or the root mean square deviation reached a maximum cut off of 0.30 Å.[28]

Further, the active site of receptor was identified by generating grid around the native ligand ZID. Native ligand was selected and a grid box of size of around 20 Å was encompassed around it, so that there will be maximum inclusion of active site of mycobacterial InhA.

Before subjecting the benzimidazole incorporated 3,5-bis (arylidene)-4-piperidones (4a-4l) for molecular docking, the protocol was validated by extracting the co-crystallized ligand and re-docking it into the active site of InhA using the above discussed setup. The best docked conformation of the native ligand was re-produced with a rmsd of less than 1 Å compared to the experimentally observed conformation (Figure 12S) which validates the molecular docking protocol adopted for the molecules being investigated herein.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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