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ORIGINAL RESEARCH

Green synthesis, antitubercular evaluation, and molecular docking studies of ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives

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Abstract

A simple and environment friendly one-pot synthesis of ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives from aryl aldehydes, ethyl cyanoacetate, and ammonium acetate was developed in aqueous medium without using a catalyst. The significant features of this method are easy, inexpensive experimental procedures with short reaction time and high yield. The use of water as the solvent without catalyst makes the reaction meritorious and further fulfilled green chemistry protocols. The compounds were screened for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* 25923) and Gram-negative bacteria (*Escherichia coli* ATCC25922) by disk diffusion method. Compounds **4f**, **4h**, and **4i** showed moderate antibacterial activity *S. aureus*. Intriguingly, compound **4g** exhibited very good antibacterial activity against *E. coli*. Antitubercular activity assay indicates that the compounds **4(a–c)** exhibited activity with varying MICs against *mycobacterium tuberculosis* H37RV control strain and multidrug-resistant tuberculosis (MDR-TB) clinical isolate. Among the three tested compounds, **4c** showed an equipotent antitubercular activity against H37Rv and MDR-TB clinical isolates with MIC 3.13 µg/ml. Further, docking analysis of synthesized piperidinone derivatives with acetate kinase protein reported that these compounds interact effectively with the catalytic residues that are in the vicinity of ATP binding and active sites facilitating inhibition of enzyme function. Thus these derivatives can be promising compounds for antitubercular activity to combat tuberculosis.

Keywords Antitubercular activity · Molecular docking studies · One-pot synthesis · Piperidinones

Introduction

The synthesis of heterocyclic compounds has been an important area of study since long time, as these molecules are usually used in various fields such as agriculture, medicine, pharmacy, and contribute to potent and selective drugs (Varma 1996). The development of an environmentally benign and viable procedure for the synthesis of organic compounds is one of the important goals to be achieved by organic chemists (Ali et al. 2015; Butler and Coyne 2010; Rao et al. 2015). Synthesizing novel bioactive compounds with minimum number of synthetic steps and in a short time is a significant challenge to the scientists (Ali et al. 2012; Kaur et al. 2015; Saleem et al. 2013). One-pot reactions have become popular in organic synthesis and combinatorial chemistry because of their straightforwardness, less reaction time, use of green solvent, fewer byproducts, simple experimentation, and high yield of

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products (Ali et al. 2018a, 2018b, 2019; Tiwari et al. 2016b). If the multi-component reaction could be carried out in water, it would be the most ecofriendly and economical (Konkala and Dubey 2017; Simona and Li 2012; Tiwari et al. 2016a).

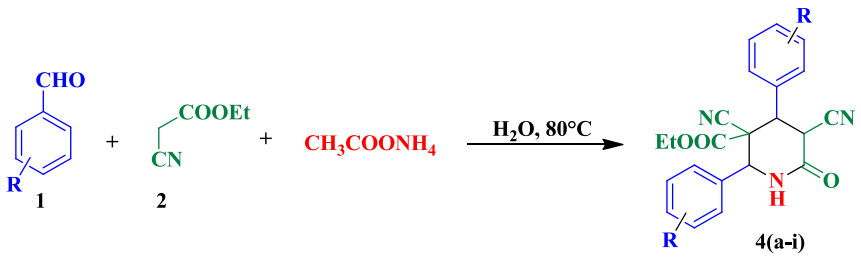
In the context of green chemistry, there are many issues that influence the choice of solvent. Recently, organic synthesis in water medium without using organic solvents has become one of the significant approaches in organic chemistry to fulfill green chemistry needs (Chanda and Fokin 2009; Hayashi 2006; Klijn and Engbers 2005). Water as medium has gained importance in organic synthesis, as it is non-toxic, cost effective, safe, non-flammable, abundant in nature, and above all ecofriendly solvent (Li 2005). In view of the significance of water medium, we have developed a simple and environmentally friendly one-pot synthesis of ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives **4(a–i)** from aryl aldehydes **1**, ethyl cyanoacetate **2**, and ammonium acetate **3** employing aqueous medium without using a catalyst (Scheme 1). The significant features of this method are the simple inexpensive experimental procedure with short reaction time and good yield.

Substituted piperidinones are important class of molecules in the field of synthetic organic, medicinal, and pharmacological chemistry (Blade Front 1980; Marson and Fallah 1994). Literature survey reveals several methods for the preparation of piperidinones such as reaction of ethyl cyanoacetate with aryl aldehydes in ethanolic ammonia (Nagai et al. 1974), reaction of phenyl methanimine with ethyl cyanoacetate in the presence of sodium ethoxide in ethanol (Ajaykumar and Lokanatharai 2006), reaction of ethyl cyanoacetate, aryl aldehyde, and mixture of ammonium acetate, ammonia in methanol as solvent (Chakrabarthy et al. 2007). Although these methods are useful; they suffer from drawbacks like use of toxic organic solvents, longer reaction time, cost of the synthesis, and very low yields. The development of efficient green synthetic methodologies are in demand these days because of the rise in level of pollutants in the atmosphere. Thus catalyst-free and replacement of hazardous solvents by environmentally benign solvents a in chemical process is the core area of research and development. To fulfill green chemistry needs, we therefore report in the present study a convenient simple and environment friendly one-pot synthesis of ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives **4(a–i)**.

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* has been a deadly infectious pathogen causing the death of millions worldwide. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mtb* isolates created havoc and continues to impede the success of current TB control programes (Biecker et al. 2019). There is an urgent need for the design and development of new anti-TB drugs and many researchers across the world have been working for the development of novel anti-TB drugs to combat MDR and XDR TB (Fernandes et al. 2017). Thus, the synthesized piperidinone compounds were screened for antitubercular activity against *mycobacterium tuberculosis* (*Mtb*) H37RV control strain and multidrug-resistant tuberculosis (MDR-TB) clinical isolate. Among the tested ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives **4c** showed excellent antitubercular activity.

Results and discussion

An efficient catalyst-free green synthetic protocol has been developed for the synthesis of piperidinones **4(a–i)**. According to literature, the formation of knoevenagel condensation intermediate can be accomplished either under solvent-free condition or inorganic solvent medium. Considering the significance of green chemistry protocol, we synthesized piperidinone derivative **4a** through a reaction of benzaldehyde **1**, ethyl cyanoacetate **2**, and ammonium acetate **3** in water without using catalyst at room temperature. It was observed that reaction ensued slowly with the formation of very low **4a** product and high knoevenagel intermediate. The yield of **4a** was very low even after stirring for 10 h at room temperature but with 80 °C temperature and 6 h stirring the yield of **4a** product increased with low intermediate (unreacted intermediate was recovered). There was no further improvement of yield, beyond 80 °C and 6 h time. The reaction was also carried out in different solvents such as dichloromethane, ethanol, acetonitrile, toluene but the reaction was incomplete even after 10 h and results were given in (Table 1).



Scheme 1 Synthesis of ethyl 3, 5-dicyano-6-oxo-2, 4-diarylpiperidine-3-carboxylate derivatives **4(a–i)**

Table 1 Optimization of reaction medium

Entry	Solvent	Temp °C	Time (h)	Yield[a] (%)
1	Dichloromethane	38	10	Trace
2	Acetonitrile	78	10	4
3	Ethanol	80	10	12
4	Toluene	80	10	5
5	Water	80	6	60

[a]Isolated yields

Table 2 Synthesis of ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives[a] **4(a–i)**

Entry	R	Product 4	Time (h)	Yield[b] (%)
1	H	**4a**	6	60
2	4-Fluoro	**4b**	5	70
3	4-Chloro	**4c**	5	65
4	2-Bromo	**4d**	6	34
5	2-Chloro	**4e**	6	45
6	4-Methoxy	**4f**	6	41
7	3-Bromo	**4g**	6	31
8	4-Methyl	**4h**	6	16
9	2,4-Dichloro	**4i**	6	21

[a]Reaction of Ethyl cyanoacetate **2** (10 mmol), Ammonium acetate **3** (15 mmol) with various Aryl aldehydes **1** (10 mmol) in aqueous medium

[b]Isolated yields

The structure of the products **4(a–i)** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectra (HRMS). IR spectra of **4a** showed prominent sharp NH stretching in region 3251 cm^{-1} and showed strong absorption band at 2358 cm^{-1} indicating the presence of CN groups and 1693 (C=O of amide) and 1739 cm^{-1} (C=O of ester) absorption bands indicating the presence of two C=O groups. The ^{1}H NMR spectrum **4a** exhibited 8.94 ppm for NH and a singlet at 5.36 ppm for C_2–H, two doublets at 4.55 and 5.01 ppm for C_4–H and C_5–H of piperidinone. Moreover, **4a** also exhibited quadret at 3.81 ppm for CH_2 and triplet at 0.77 ppm for CH_3 of ester. The ^{13}C spectra of **4a** showed characteristic peaks of C(sp3) at 59.03, 47.32, 39.50, 13.79, and 163.47, 164.50 ppm (C=O) of amide and ester.

After optimization of reaction conditions, various kinds of aryl aldehydes were investigated for synthesis of corresponding piperidinone derivatives. The results are given in (Table 2). Aryl aldehydes having the groups Chlorine (Cl), Fluorine (F) reacted fast and obtained corresponding piperidinone with excellent yield and purity. However, we observed that reaction was slow with aryl aldehyde having electron releasing groups like CH_3, OCH_3, and fewer yields were obtained.

Biological evaluation

Antibacterial screening

The antibacterial activity screening results of the synthesized piperidinone derivative compounds **4(a–i)** are summarized in Table 3. The width of zone of inhibition (ZOI) represents the potency of piperidinone derivatives. The synthesized piperidinone derivatives showed varied antibacterial activity. No antibacterial activity was observed with compounds **4(a–e)** at all the tested concentrations against the *Escherichia coli* and the *Staphylococcus aureus*. However, compound **4f** showed moderate inhibition at 600, 800, 1000, and 1500 µg/ml against *S. aureus*. On the other hand, compounds **4h** and **4i** displayed moderate inhibition at 1500 µg/ml against *S. aureus*. Intriguingly, compound **4g** (Ethyl 2,4-bis(3-bromophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate) exhibited very good antibacterial activity against *E. coli*. Dimethyl sulfoxide (DMSO) served as a negative control with no inhibition zone. Streptomycin and ceftazidime served as a positive control with a wider ZOI against the tested organisms.

Further, the compounds **4(a–c)** were evaluated for antitubercular properties using resazurin microtiter assay (REMA) plate method against *Mtb* H37Rv control strain and MDR-TB clinical isolate and the resulting minimum inhibitory (MIC) values are summarized in Table 4. The results of REMA plate method indicate that the compounds **4(a–c)** exhibited notable antitubercular activity with varying MICs against tested control and MDR-TB clinical isolates. Among the three tested compounds, Compound **4c** (Ethyl 2,4-bis(4-chlorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate) exhibited an excellent antitubercular activity against control and MDR-TB isolates followed by **4b** (Ethyl 3,5-dicyano-2,4-bis(4-fluorophenyl)-6-oxopiperidine-3-carboxylate), and **4a** (ethyl 3,5-dicyano-6-oxo-2,4-diphenylpiperidine-3-carboxylate) (Fig. 1). Further, **4c** showed an equipotent antitubercular activity against control and MDR-TB isolates with MIC 3.13 µg/ml. Isoniazid (INH) MICs for *Mtb* control and MDR-TB clinical isolates were 0.06 and 0.5 µg/ml, respectively. Similarly, Rifampicin (RIF) MICs were 0.06 and 0.25 µg/ml for *Mtb* control and MDR-TB clinical isolate, respectively.

Molecular docking studies

Acetate kinase (AckA) is an essential protein in pathogenic bacteria including *Mycobacterium tuberculosis*. It catalyzes the formation of acetate phosphate from acetate and ATP in the presence of divalent cation. During chronic infection, host-derived lipid components act as the major carbon source at the site of infection. Previous studies reported that fatty acid consumption by *Mycobacterium tuberculosis*

Table 3 Antibacterial activity of synthesized piperidinone derivatives **4(a–i)**

S. No.	Compound	*E. coli*						*S. aureus*					
		A	B	C	D	E	F	A	B	C	D	E	F
1	**4a**	–	–	–	–	–		–	–	–	–	–	–
2	**4b**	–	–	–	–	–							
3	**4c**	–	–	–	–	–		–	–	–	–	–	–
4	**4d**	–	–	–	–	–		–	–	–	–	–	–
5	**4e**	–	–	–	–	–		–	–	–	–	–	–
6	**4f**	–	–	–	–	–	+	–	–	++	++	++	++
7	**4g**	–	–	–	–	–	+++	–	–	–	–	–	+
8	**4h**	–	–	–	–	–		–	–	–	–	–	++
9	**4i**	–	–	–	–	–		–	–	–	–	–	++
11	+ve control (30 µg /ml) streptomycin	+++ +						+++ +					
12	+ve control (30 µg/ml) ceftazidime	+++						+++					
13	−ve (DMSO)	–						–					
14	−ve (Water)	–						–					

– no antibacterial activity, + inhibition zone 0–5 mm, mild sensitivity, = inhibition zone 5–10 mm, ++ moderate sensitivity, +++ inhibition zone 10–15 mm, highly sensitive, Inhibition zone15–20mm, A 100 µg/ml, B 400 µg/ml, C 600 µg/ml, D 800 µg/ml, E 1000 µg/ml, F 1500 µg/ml

Table 4 In vitro evaluation of anti-TB activity of piperidinone derivatives **4(a–c)** against H37Rv control isolate and MDR-MTB clinical isolate by REMA plate method

S. No.	Compound	Compound name	Anti-TB activity—MIC (µg/mL)	
			H37RV control isolate	MDR-TB clinical isolate
1	INH	Isoniazid	0.06	0.5
2	RIF	Rifampicin	0.06	0.25
3	**4a**	Ethyl 2,4-diphenyl-3,5-dicyano-6-oxopiperidine-3-carboxylate	25	100
4	**4b**	Ethyl 3,5-dicyano-2,4-bis(4-fluorophenyl)-6-oxopiperidine-3-carboxylate	6.25	25
5	**4c**	Ethyl 2,4-bis(4-chlorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate	3.13	3.13

activates metabolic pathway, causing the pathogen to release acetate as carbon intermediates (Rucker et al. 2015). AckA is one of the key enzymes of acetate utilization pathway, regulate flux of metabolites in glycolysis, gluconeogenesis, TCA cycle, and fatty acid metabolism resulting in energy generation, survival, and growth of pathogen (Chittori et al. 2012). Earlier reports suggested that piperidinone derivatives inhibit the growth of pathogenic *Mtb* by targeting AckA with equal or higher efficacy than the clinically proven antibiotics (Tiwari et al. 2018). Therefore AckA protein of *Mycobacterium marinum* bearing PDBID:4DQ8 is chosen as a suitable target for docking ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives. It is a homodimer protein consisting of Chain A and Chain B. Each subunit is further divided into N-terminal and C-terminal domains. Crystal structure of AckA enzyme of *Mycobacterium marinum* reveals the following binding sites. ATP binding site residues include 21, 197–201, 271–273, and 319–323. The potential substrate binding site residue is 80 and active site residue is 137. Metal binding site residues are 14 and 373.

Analysis of ligand poses demonstrated the binding affinity of the molecules to catalytic subunit of chain A of AckA protein. All the compounds **4a**, **4b**, and **4c** showed significant Moldock scores revealing good interaction with the protein target as shown in Table 5. The MolDock score, Rerank score, and interacting residues for **4a**, **4b**, and **4c** are listed in Table 6. 3D and 2D images after docking along with hydrogen and other bond interactions are depicted in Figs 2–4. The compounds **4b** and **4c** reported higher moldock scores of −135.65 and −135.06, respectively. The compound **4b** shows hydrogen bond interaction with

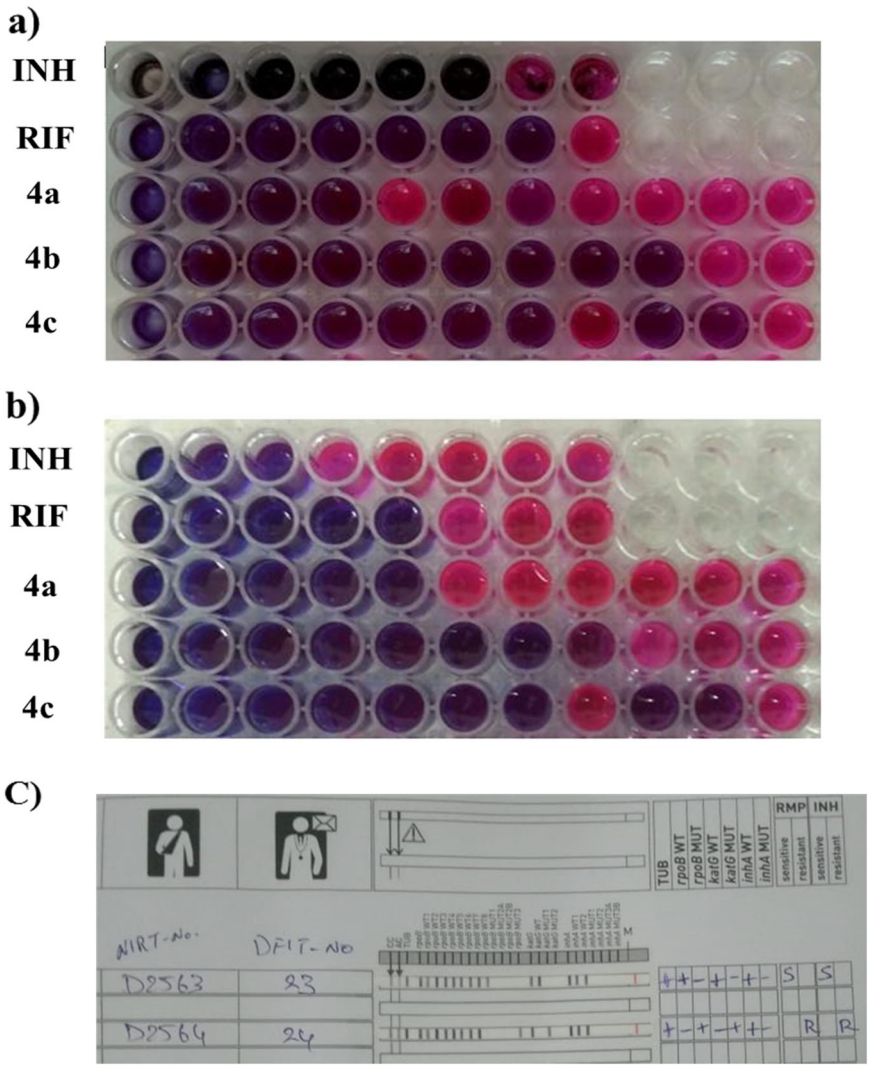


Fig. 1 Screening of 2-piperidinone derivatives for antitubercular properties against (**a**) H37Rv control isolate and (**b**) MDR-TB clinical isolate (D2564) by resazurin-based 96-well plate microdilution method. Serial twofold dilutions of compounds were tested at doses of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 0.0 µg/ml. RIF at doses of 2.0–0.0156 µg/ml and INH of 1.0–0.063 µg/ml doses were used. First and last columns are sterility and growth controls, respectively. **c** DNA strip patterns of genotype MTBDR-plus strip of MDR-TB clinical isolate (D2564)

Table 5 Molecular docking result of compounds on acetate kinase chain a protein of *Mycobacterium marinum* (PDBID:4DQ8)

Ligand	MolDock score	Rerank score	HBond
4a	−126.93	−95.57	−4.75
4b	−135.65	−100.12	−2.91
4c	−135.06	−97.351	−4.20

Asn14, Glu373, Arg80 residues along with pi interactions with residues like Val82, Pro221, and Met217. A halogen (fluorine) interaction with Glu373 and His197 is also present which may contribute to enhanced antibacterial activity. In the case of **4c** hydrogen bond interactions were seen with Asn14, Arg80 in addition to carbon hydrogen bonds with Arg80 and His169. Pi interactions were displayed with Asp137 (active site residue), Val82, Phe168, His169, His197, Met217, Pro221, Ala318, and Glu373 residues. Furthermore, halogen interaction of chlorine substituent with Met217 may contribute to the enhanced inhibition of AckA that resulted in good antitubercular activity. In compound **4a** hydrogen bonds were identified between the ligand molecule and residues Asn14, Arg80, Asp137, and Glu373 of protein. In addition to these Val81 and Pro221 formed Pi-alkyl bond, while Met217 displayed Pi–Sulfur bond interaction. All the compounds displayed optimum binding with active site residues. Thus these compounds may serve as potential AckA inhibitors contributing to antitubercular activity.

Discussion

In the present study, ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives were synthesized in aqueous medium using an efficient environment friendly one-pot synthesis method and bio assayed for their antibacterial activity. The tested analogs were showing meager

Table 6 Docking score results of top compounds with interacting amino acid residues in the active site of acetate kinase (PDBID:4DQ8)

Ligand	Moldock score	Rerank score	Interacting residues
4a	−126.93	−95.57	Val82, Pro221, Met217, arg80, Asp137, Glu373, Asn14
4b	−135.65	−100.12	Val82, Pro221, Met217, Asn14, Glu373, His197, Arg80
4c	−135.06	−97.35	Asn14, Arg80, Val82, Asp137, Phe168, His169, His197, Met217, Pro221, Ala318, Glu373.

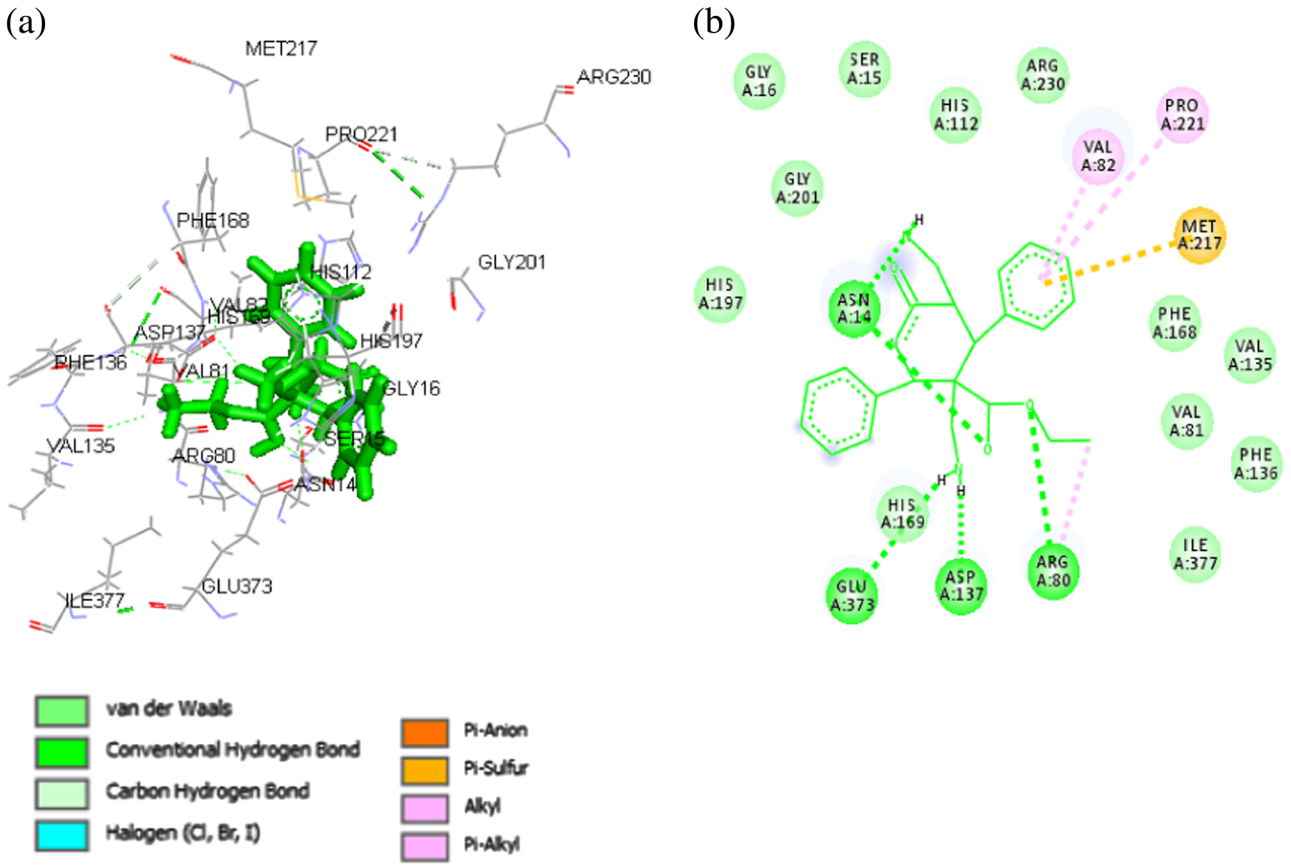


Fig. 2 **a** 3D structure view of molecular docking of **4a** (green sticks) with *Mycobacterium* AckA protein (gray lines). **b** Ligand interaction diagram of **4a** showing hydrogen bond (green dotted lines) interaction with AckA residues Asn14, Arg80, Asp137, Glu373, and Pi interaction (pink- and yellow-dotted lines) with Val82, Met217, and Pro221 (2D structure view)

antibacterial activity when tested against Gram-negative bacteria. Intriguingly, piperidinone analogs, in particular, ethyl 2,4-bis(4-chlorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (**4c**) exhibited excellent antitubercular property against the MTB control strain as well as MDR-TB clinical isolate. MIC values observed in our study were tested for the first line of drugs using REMA plate method and were in accordance with the previous report by (Palomino et al. 2002). Meticulous observation of in vitro antitubercular activity studies indicates that the presence of fluorine and chlorine at the fourth position of the phenyl ring in **4b** and **4c** is considered to be effective. Thus, the compounds screened in this study, ethyl 2,4-bis (4-chlorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (**4c**) and ethyl 2,4-bis(2-fluorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (**4b**) were very effective against TB causing *Mycobacterium tuberculosis*. Significant Moldock scores of molecular docking analysis demonstrate that all the compounds displayed optimum binding with active site residues. Affecting AckA might alter the Pta-AckA pathway which in turn alters the acetate metabolism, an important pathway of *Mycobacterium tuberculosis* for its survival inside the host (Rucker et al. 2015). Thus these compounds may serve as potential AckA inhibitors contributing to antitubercular activity. Further, in-depth studies are warranted to know the mechanism of action of compounds and its synergistic activity with the existing antitubercular drugs against MDR and XDR-TB. In addition, these compounds can be used as a source compound to develop novel antitubercular drugs.

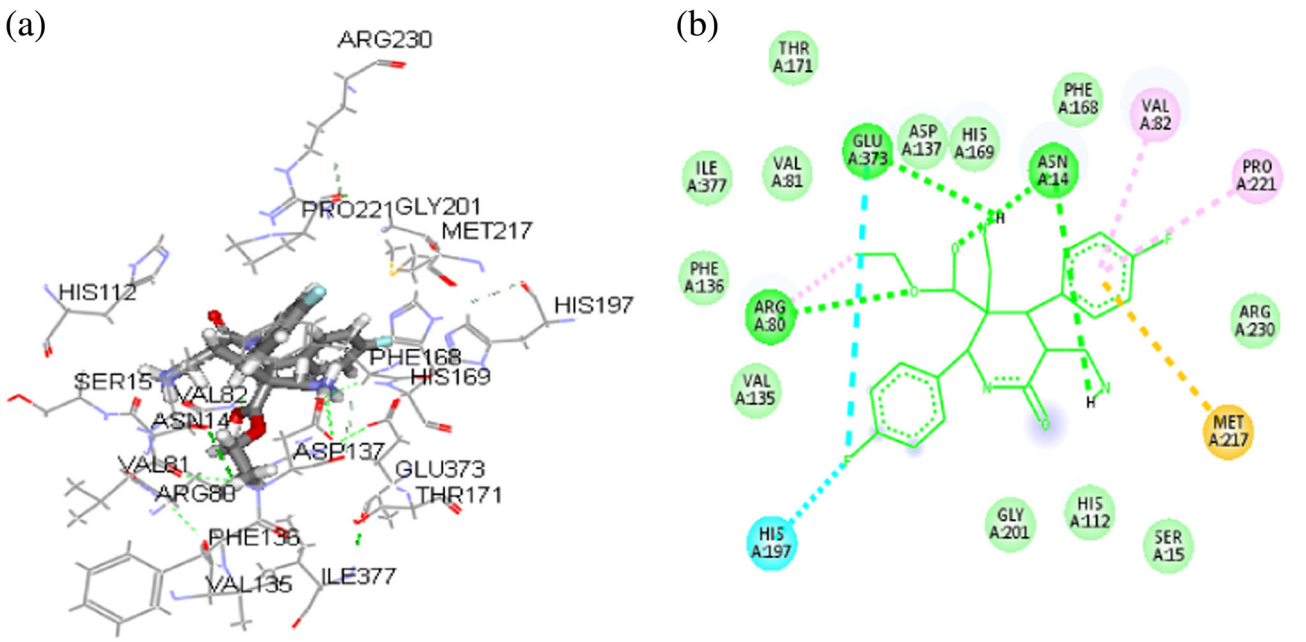


Fig. 3 **a** 3D representation of molecular docking of compound **4b** (gray sticks) with *Mycobacterium* AckA protein (gray lines). **b** 2D diagram of **4b** showing hydrogen bond (green-dotted lines) interaction with AckA residues Asn14, Arg80, halogen bond (cyan dotted lines) interaction with His197, and Glu373

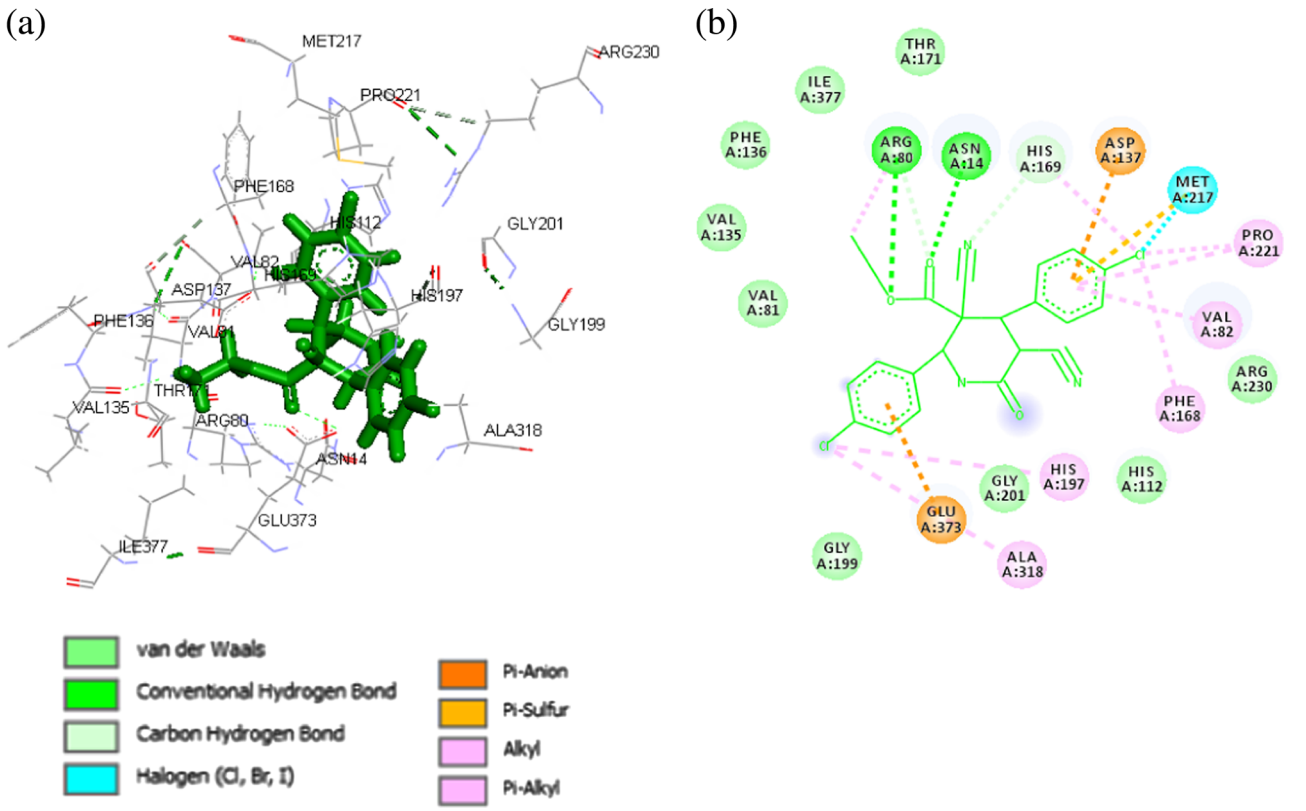


Fig. 4 **a** 3D structure view of molecular docking of compound **4c** (green sticks) with *Mycobacterium* AckA protein (gray lines). **b** 2D representation of **4c** interactions at the active site of AckA

Experimental

General consideration

All chemicals were obtained from commercial suppliers and were used without purification. Infrared spectra were recorded on a Bruker spectrophotometer with KBr pellets ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on 500 MHz Broker spectrometer with tetra methyl silane as the internal standard and resonance (δ) are given in ppm. Melting points were determined in open capillaries and were uncorrected. HRMS were recorded using a Bruker spectrometer. Thin-layer chromatography (TLC) was performed using MERK precoated silica gel and the components were visualized under a UV or an iodine chamber.

General procedure for the synthesis of ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives (4)

In a 50 ml RBF mixture of aryl aldehyde **1** (10 mmol), ethyl cyanoacetate **2** (10 mmol) and ammonium acetate **3** (15 mmol) were taken in 10 ml water. The reaction mixture was allowed to stir magnetically at 80 °C. The progress of the reaction was monitored by TLC. After completion of the reaction, mass was cooled to room temperature and solid was filtered. Filtered solid was purified by column chromatography by using mobile phase (ethyl acetate and hexane).

Bacterial cultures and antibiotics

Antibacterial activity of the synthesized piperidinone derivatives was carried out using the disk diffusion method against Gram-positive bacteria (*S. aureus* 25923) and Gram-negative bacteria (*E. coli* ATCC25922). Further, antitubercular activity was tested against *Mycobacterium tuberculosis* (MTB) H37Rv and MDR MTB clinical isolate which is resistant to two key first line of anti-TB drugs (RIF and INH). The bacterial culture media Luria Bertani (LB) broth, Mueller–Hinton (MH) Agar, antibiotics streptomycin sulfate, and ceftazidime were purchased from HiMedia, Mumbai. The compound screening for antitubercular activity was carried out at Damien Foundation Urban Leprosy and TB Research Centre, Nellore, Andhra Pradesh, India. The research center is a TB culture and drug susceptibility testing (C and DST) referral laboratory for Revised National Tuberculosis Control Program and accredited by the National Mycobacteriology Accreditation System of Central TB Division Ministry of Health, Government of India.

Antibacterial activity assay

DMSO was used as solvent to dissolve the test compounds. A stock concentration of 20 mg/ml was prepared with each of the test compounds. Sterile filter paper disks of about 6 mm are impregnated with six different working concentrations of the test compounds at 100, 400, 600, 800, 1000, and 1500 µg/ml. A single colony was inoculated into 5 ml of the LB broth and incubated at 37 °C for 8–10 h. After adjusting the turbidity to 0.5 Mac Farland standard (1.5×10^8 CFU/ml), the culture was swabbed onto the MH agar media. The plates were left for 5–10 min for drying the excess swabbed culture. The dried filter paper disks impregnated with test compounds were kept onto the culture plate not less than 24 mm from center to center. After gentle pressing of the disks onto the culture plate, the plates were incubated for about 24 h at 37 °C. Streptomycin sulfate and ceftazidime (30 µg/ml) served as a positive controls and DMSO and water (10 µl) as negative controls included in the study. Each of the test compounds was tested in duplicates.

Resazurin microtiter assay (REMA) plate method

A low cost, simple, and rapid calorimetric REMA plate method was used to study the antitubercular properties of the synthesized piperidinone derivatives against the *Mycobacterium tuberculosis* H37Rv control strain and multidrug-resistant MTB clinical isolate following the protocol described by (Palomino et al. 2002). Briefly, REMA was performed in 7H9-S medium containing Middlebrook broth, 0.1% Casitone, and 0.5% glycerol and supplemented with oleic acid, albumin, dextrose, and catalase. Antitubercular first line of drugs, INH and RIF were used as positive controls. Stock solutions of RIF and INH and were prepared at concentrations of 10 mg/ml in methanol and 1 mg/ml in distilled water, respectively. Resazurin sodium salt powder was prepared at 0.02% (wt/vol) in distilled water and filter sterilized and frozen until used.

Serial twofold dilutions of test compounds and first line of drugs in 100 µl of 7H9-S medium were prepared directly in 96-well plates at concentrations of 800–3.125, 0 µg/ml for test compounds, 2.0–0.063 µg/ml for RIF, and 1.0–0.063 µg/ml for INH. Only solvents without any first lines of drugs and test compounds were included as negative controls. *Mycobacterium tuberculosis* H37Rv control strain and multidrug-resistant MTB clinical isolate was used as reference strain to test the antitubercular activity of the test compounds. The inoculum prepared in 7H9-S medium was adjusted to a McFarland tube no. 1 and then 100 µl of 1:20 diluted culture was used as an inoculum. The 96-well plates prepared as per the above protocol were covered and incubated at 37 °C in the normal atmosphere. After 7–8 days of incubation, 30 µl of 0.02% resazurin solution prepared in MQ water was added to each well, incubated at 37 °C for overnight and observed for color change. Irreversible conversion of blue-colored resazurin to

pink-colored resorufin indicates bacterial growth. The MIC was defined as the lowest concentration of the test compounds or drugs that prevented color change from blue to pink.

Molecular docking

The interaction of AckA protein with different piperidinone derivatives was studied using Molegro Virtual Docker (MVD 2012.5.5) (CLCbio 2012). Docking was performed with potential active site detected on AckA protein with three piperidinone derivatives **4(a–c)**. The structures of all the compounds were generated using Marvin Sketch 5.6.0.2. (1998–2011, Copyright © ChemAxon Ltd), cleaned in 3D and saved in.pdb format for docking studies. The PDB file of the target protein downloaded from RCSB PDB (www.rcsb.org), AckA (PDBID:4DQ8). During docking first the protein and ligands were prepared by assigning bonds, bond orders, charges, explicit hydrogens, flexible torsions in ligands if they were missing. The search algorithm is taken as MolDock SE and numbers of runs are taken as ten and max iterations were 2000 with population size 50 and with an energy threshold of 100). Further investigation of the binding interactions of the most active (**4a**, **4b**, and **4c**) docked compounds were performed using Biovia discovery studio 2017 R2 Client. To obtain more potent compounds as inhibitors the optimal binding mode of a molecule (compound) to the active site of macromolecule is interpreted.

Spectral characterization data for 4(a–i)

ethyl 3,5-dicyano-6-oxo-2,4-diphenylpiperidine-3-carboxylate (4a) White solid; Yield: 60%; m.p.: 172–174 °C; IR (KBr) cm^{-1}: 3251 (NH), 2358 (CN), 1739 (C=O), 1693 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 8.94 (*s*, 1H, NH), 7.29–7.46 (m, 10H, Ar–H), 5.36 (*s*, 1H, CH), 5.01 (*d*, $J = 13.0$ Hz, 1H, CH), 4.55 (*d*, $J = 13.0$ Hz, 1H, CH), 3.81 (q, 2H, CH_2), and 0.77 (t, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 164.50, 163.47, 135.06, 134.47, 130.01, 129.68, 129.38, 129.06, 128.95, 128.04, 116.98, 114.63, 63.44, 60.95, 59.03, 47.32, 39.50, 13.79; MS (*m/z*): 374.14 $[M + H]^+$.

ethyl 3,5-dicyano-2,4-bis(4-fluorophenyl)-6-oxopiperidine-3-carboxylate (4b) White solid; Yield: 70%; m.p.: 164–165 °C; IR (KBr) cm^{-1}: 3272 (NH), 2377 (CN), 1747 (C=O), 1703 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.43–7.44 (m, 4H, Ar–H), 7.12–7.14 (m, 4H, Ar–H), 6.50 (*s*, 1H, NH), 5.26 (*s*, 1H, CH), 4.35 (*d*, $J = 12.6$ Hz, 1H, CH), 4.08 (*d*, $J = 12.9$ Hz, 1H, CH), 3.84 (q, 2H, CH_2), and 0.79 (t, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 164.84, 164.51, 163.85, 162.84, 162.52, 162.19, 130.16, 130.09, 129.51 (d, $J = 8.6$ Hz), 129.45, 129.11, 128.22, 116.76, 116.65, 116.59 (dd, $J = 21.9$, 14.7 Hz), 116.47, 114.28, 113.70, 63.91, 61.43, 57.99, 48.27, 39.22, and 13.46; MS (*m/z*): 410.13 $[M + H]^+$.

ethyl 2,4-bis(4-chlorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (4c) Half white solid; Yield: 65%; m.p.: 191–193 °C; IR (KBr) cm^{-1}: 3259 (NH), 2365 (CN), 1743 (C=O), 1702 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.26–7.41 (m, 8H, Ar–H), 6.59 (s, 1H, NH), 5.26 (d, $J =$ 4.2 Hz, 1H), 4.34 (dd, $J = 13.3$, 4.2 Hz, 1H), 4.07 (dd, $J =$ 13.7, 4.5 Hz, 1H). 3.87 (q, 2H, CH_2), 0.81 (t, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 165.57, 163.73, 162.22, 136.81, 136.35, 131.74, 130.75, 129.83, 129.68, 129.56, 129.28, 129.19, 129.12, 128.87, 114.27, 113.59, 64.06, 61.46, 57.66, 48.30, 39.00, and 13.46; MS (*m/z*): 442.07/ 444.069 $[M + H]^+$.

ethyl 2,4-bis(2-bromophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (4d) Pale yellow solid; Yield: 34%; m.p.: 205–207 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.63–7.70 (m, 2H, Ar–H), 7.52–7.58 (m, 2H, Ar-H), 7.27–7.44 (m, 4H, Ar–H), 6.10 (*s*, 1H, NH), 5.86 (*s*, 1H, CH), 5.04 (*d*, $J = 12.5$ Hz, 1H, CH), 4.27 (d, $J = 13.0$ Hz, 1H, CH), 3.91 (q, 2H, CH_2), and 0.89 (t, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 162.14, 161.76, 134.27, 133.85, 132.93, 132.48, 132.11, 131.32, 131.11, 129.51, 128.92, 128.78, 127.09, 124.10, 114.93, 113.53, 64.32, 60.33, 56.02, 45.64, 40.40, and 13.32; MS (*m/z*): 529.97/ 531.97 $[M + H]^+$.

ethyl 2,4-bis(2-chlorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (4e) White solid; Yield: 45%; m.p.: 187–188 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.28–7.39 (m, 8H, Ar–H), 6.76 (*s*, 1H, NH), 4.71 (*d*, $J =$ 2.8 Hz, 1H, CH), 4.64 (*d*, $J = 4.05$ Hz, 1H, CH), 4.11 (*s*, 1H, CH), 3.78 (q, 2H, CH_2), and 0.90 (t, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 162.21, 161.84, 135.56, 133.80, 131.78, 131.22, 130.95, 130.83, 130.79, 130.35, 129.27, 128.26, 128.15, 126.99, 114.78, 113.51, 64.23, 57.78, 56.07, 42.85, 40.26, and 13.22; MS (*m/z*) = 442 $[M + H]^+$.

ethyl 3,5-dicyano-2,4-bis(4-methoxyphenyl)-6-oxopiperidine-3-carboxylate (4f) White solid; Yield: 41%; m.p.: 143–144 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.28–7.35 (m, 4H, Ar–H), 6.93–6.95 (m, 4H, Ar–H), 6.92 (s, 1H, NH), 5.21 (*s*, 1H, CH), 4.03 (m, 1H, CH), 4.00 (*d*, $J = 13.2$ Hz, 1H, CH), 3.83 (q, 2H, CH_2), and 1.26–1.27 (6H, *s*, $2 \times CH_3$); 0.81 (t, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 167.32, 163.21, 159.43, 158.00, 137.86, 137.72, 137.65, 131.15, 130.22, 128.53, 128.42, 128.40, 128.15, 127.98, 127.90, 127.73, 73.48,

73.15, 72.31, 70.28, 67.88, 35.67, 29.5, and 14.20; MS (m/z) = 434 $[M + H]^+$.

ethyl 2,4-bis(3-bromophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (4g) Half white solid; Yield: 31%; m.p.: 197–199 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.57–7.50 (m, 4H, Ar–H), 7.30–7.46 (m, 4H, Ar–H), 6.69 (*s*, 1H, NH), 5.22 (*d*, $J = 27.7$ Hz, 1H, CH), 4.15 (*d*, $J = 12.9$ Hz, 1H, CH), 4.05 (*d*, $J = 13.2$ Hz, 1H, CH), 3.86 (q, 2H, CH_2), and 0.87 (t, 3H, CH_3);^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 162.22, 161.92, 154.01, 153.33, 144.38, 130.98, 130.96, 130.10, 130.05, 129.96, 129.01, 128.90, 128.11, 127.99, 127.77, 117.31, 63.54, 61.91, 48.87, 39.20, 29.14, and 13.5; MS (m/z) = 529 $[M + H]^+$.

ethyl 3,5-dicyano-6-oxo-2,4-dip-tolylpiperidine-3-carboxylate (4h) White solid; Yield: 16%; m.p.: 180–183 °C; ^{1}HNMR (500 MHz, $CDCl_3$) 6.98–7.02 (m, 8H, Ar–H), 6.4 (*s*, 1H, NH), 5.5 (*s*, 1H, CH), 4.12 (*d*, $J = 12.8$ Hz, 1H, CH), 3.82 (*d*, $J = 11.3$ Hz, 1H, CH), 3.6 (q, 2H, CH_2), and 0.85 (t, 3H, CH_3); MS (m/z) = 402 $[M + H]^+$.

ethyl 2,4-bis(2,4-dichlorophenyl)-3,5-dicyano-6-oxopiperidine-3-carboxylate (4i) White solid; Yield: 21%; m.p.: 215–217 °C; ^{1}HNMR (500 MHz, $CDCl_3$) δ 7.41–7.21 (m, 5H, Ar–H), 6.4 (m, 2H, Ar–H, NH), 5.26 (*s*, 1H, CH), 4.30 (d, $J = 12.9$ Hz, 1H, CH), 4.17 (*d*, $J = 11.4$ Hz, 1H, CH), 3.84 (q, 2H, CH_2), and 0.82 (t, 3H, CH_3); MS (m/z) = 509 $[M + H]^+$.

Conclusion

In conclusion, we synthesized piperidinone derivatives from reaction of ethyl cyanoacetate, ammonium acetate with various aryl aldehydes in aqueous medium using environment friendly one-pot synthesis. Among the synthesized ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives, compound **4c** substituted with Cl moiety exhibited notable antitubercular activity against *Mtb* H37RV control strain and MDR-TB clinical isolate. Molecular docking study shows that the synthesized piperidinone derivatives are binding efficiently with AckA protein of *Mycobacterium*. Thus, the results of the present study suggest that ethyl 3,5-dicyano-6-oxo-2,4-diarylpiperidine-3-carboxylate derivatives could be promising compounds for antitubercular activity to combat TB.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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