



Design and synthesis of some new quinoline-3-carbohydrazone derivatives as potential antimycobacterial agents

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ARTICLE INFO

Article history:

Received 10 July 2009

Revised 16 November 2009

Accepted 10 December 2009

Available online 14 December 2009

Keywords:

Quinoline-3-carbohydrazone

TMC 207

Antituberculosis activity

(3R)-3-Amino-N,N-dimethyl-4-

(phenylthio)butanamide

ABSTRACT

A series of 26 new quinoline derivatives carrying active pharmacophores has been synthesized and evaluated for their in vitro antituberculosis activity against *Mycobacterium tuberculosis* H37Rv (MTB), *Mycobacterium smegmatis* (MC²), and *Mycobacterium fortuitum* following the broth micro dilution assay method. Compounds **13e**, **13i**, **13k**, **14a**, **14c**, **14i**, and **14k** exhibited significant minimum inhibition concentrations, when compared with first line drugs isoniazid (**INH**) and rifampicin (**RIF**) and could be ideally suited for further modifications to obtain more efficacious compounds in the fight against multi-drug resistant tuberculosis.

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Tuberculosis (TB) is a worldwide pandemic caused by different species of *mycobacteria*. The latest statistics reveals that around two million people throughout the world die annually from tuberculosis and there are around 8 million new cases each year, out of which developing countries show major share.¹ Among HIV-infected people with weakened immune system, TB is a leading killer epidemic. Every year about 2 million people living with HIV/AIDS die from TB.² Furthermore, in recent times the appearance of multidrug-resistant TB (MDR-TB), a form of TB that does not respond to the standard treatments, is more common. It is a shocking revelation that MDR-TB is present in almost all countries as per the recent survey, made by the World Health Organization (WHO) and its partners. A recent estimation by WHO has revealed that within next 20 years approximately 30 million people will be infected with the *bacillus*.³ Keeping in view of the above statistics, WHO declared TB as a global health emergency and aimed at saving 14 million lives between 2006 and 2015.⁴ All the above facts reveal that there is an urgent need for development of new drugs with divergent and unique structure and with a mechanism of action possibly different from that of existing drugs.

Quinoline moiety is of great importance to chemists as well as biologists as it is found in a large variety of naturally occurring compounds and also chemically useful molecules having diverse

biological activities. Amongst the various activities of their derivatives, antimicrobial activity is noteworthy. Quinoline-based compounds are known to exhibit excellent anti-TB properties.^{5–9} It is

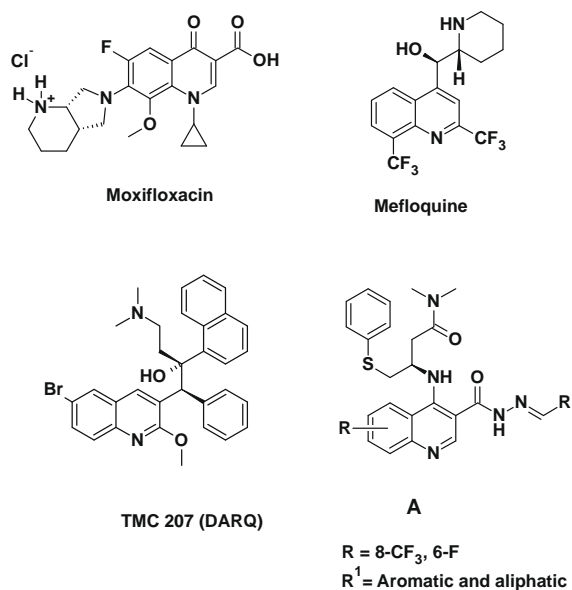


Figure 1. New class of molecules focused on anti-TB therapy and target compounds **A**.

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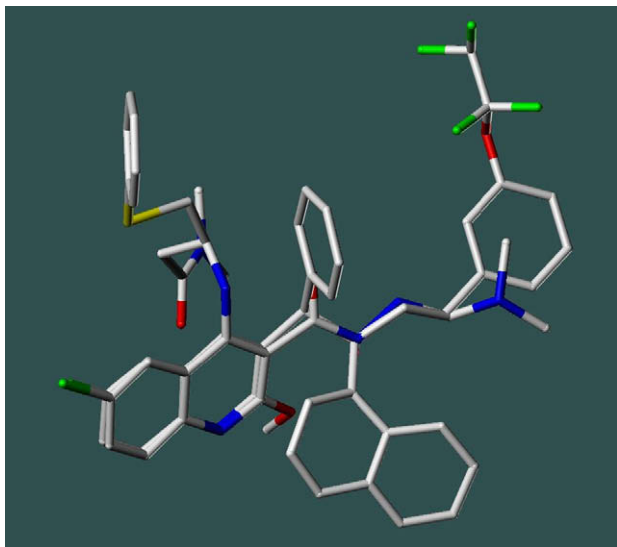


Figure 2. 3D overlay of compound **14i** with **TMC206** showing the superimposition of common pharmacophore.

interesting to note that quinoline is a core pharmacophore in the recently developed anti tuberculosis drugs, viz. TMC207, a diaryl-quinoline (DARQ),¹⁰ mefloquine,¹¹ and moxifloxacin¹² (Fig. 1). The well-known TMC207, during its mechanism of action interacts with the enzyme adenosine triphosphate (ATP) synthase by blocking the main energy source for bacterium.¹³

Further it has been well established that substituted butanamides is an important pharmacophore and are very good antibacterial agents.¹⁴ However, there is no literature report available on antibacterial activity of (3*R*)-3-amino-*N,N*-dimethyl-4-(phenylthio) butanamide, which is structurally similar to reported butanamide derivatives. Since it is an analog of an amino acid, it was expected to show some biological activity. This moiety is predicted to cause a reduction in *clogP* value and there by increasing lipophilicity of the molecule. Also substituted carbohydrazone moiety has been reported to be a well known pharmacophoric group^{15–19} for antituberculosis activity. On the basis of these observations and

as a part of our general program in the continued research for new antibacterials²⁰ and antimycobacterial agents, we have designed some new quinoline derivatives carrying active carbohydrazone moiety and (3*R*)-3-amino-*N,N*-dimethyl-4-(phenylthio) butanamide group at positions 3 and 4 of core quinoline, respectively, with the hope that newly designed molecules would exhibit enhanced activity. Here, the introduction of $-N(CH_3)_2$ group imparts hydrophilic end, carbohydrazone provides hydrogen bonding domain and phenyl moiety carries a hydrophobic part to the active base moiety quinoline carrying chiral centre (*R*) attached to it. Further, indirect type of molecular modeling study of newly designed quinoline derivatives reveals that there exists 3D structural similarity with that of TMC207, as given in Figure 2.

Thus, in this paper we herein report the synthesis of hitherto unknown 26 (*E*)-4-((*R*)-1-(dimethylcarbamoyl)-3-(phenylthio)propan-2-ylamino)-*N'*-(2-fluoro-4-methoxybenzylidene)-6- or 8-(un)substituted quinoline-3-carbohydrazides (**13a–m** and **14a–m**) starting from substituted anilines through multistep reactions. The retrosynthetic approach as shown in Figure 3 has been followed for the synthetic design of target compounds. Also, we report their *in vitro* activity against *Mycobacterium tuberculosis H37Rv* (MTB), *Mycobacterium smegmatis* (MC²), and *Mycobacterium fortuitum*. It has been expected that the target molecules carrying quinoline would inhibit mycobacterium membrane-bound ATP synthase and hence follow the same mechanism, as shown by TMC207.

The total synthesis of the key reactant, that is, (3*R*)-3-amino-*N,N*-dimethyl-4-(phenylthio)butanamide (**6**) was carried out in six steps according to the literature procedure²¹ starting with optically pure *D*(–)-aspartic acid, as shown in Scheme 1. The intermediate benzyl [(3*R*)-5-oxotetrahydrofuran-3-yl]carbamate (**4**) was converted to compound **5** via two steps under microwave condition, wherein the reaction was completed in 20–25 min at a constant power of 210 W with improved yields, whereas in the reported procedure the reaction was completed in 18–24 h with less yield. This is a significant achievement in the synthesis of the compound **6**.

The reaction sequences employed for synthesis of title compounds are shown in Scheme 2. The starting materials **7a** and **b** were conveniently converted to diethyl phenyl amino methyldene propanedioate derivatives **8a** and **b**, by condensing them with

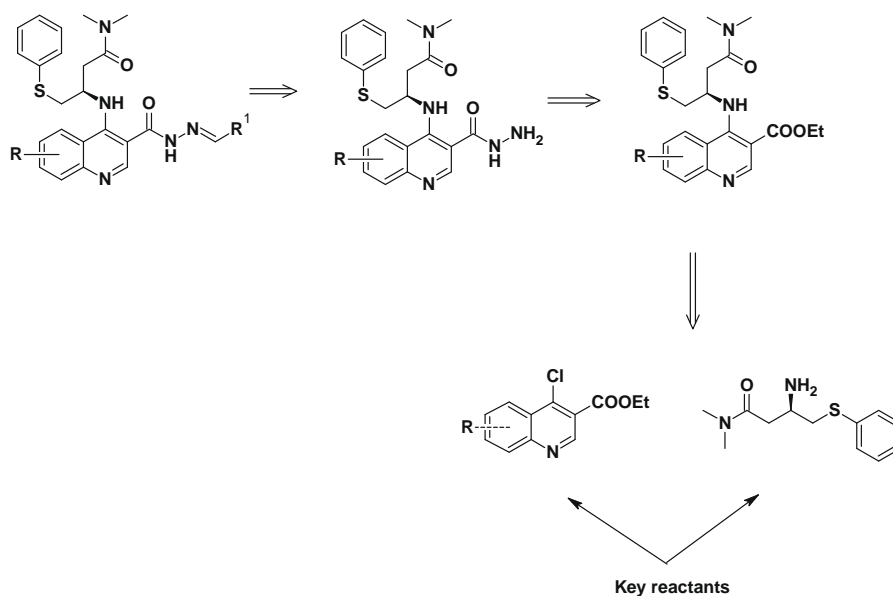
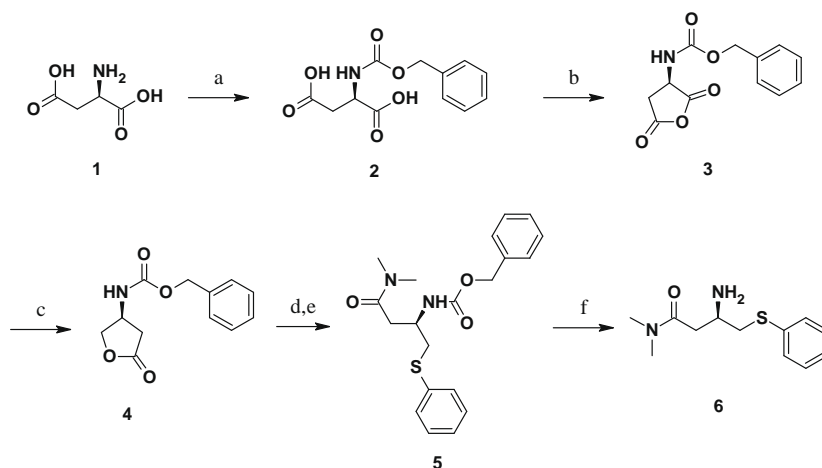
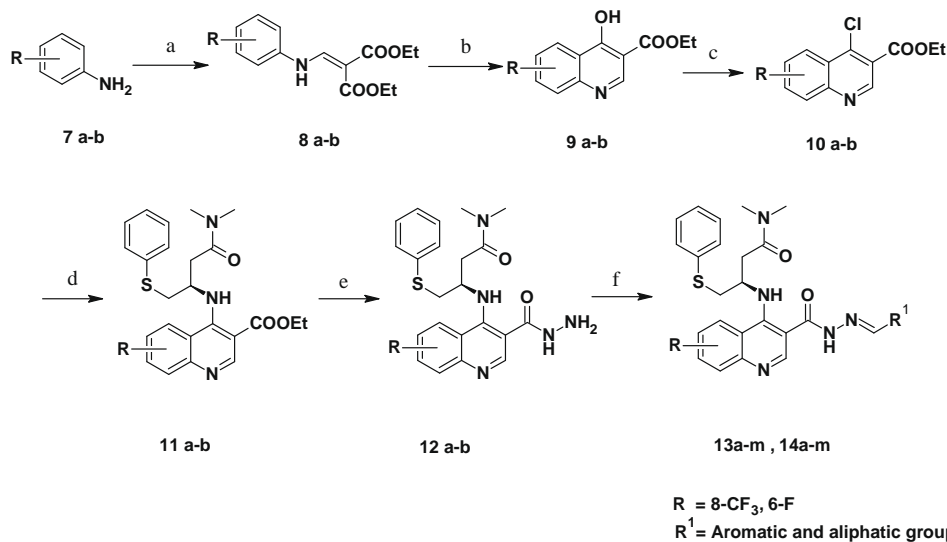


Figure 3. Design and retrosynthetic analysis of quinoline derivatives.



Scheme 1. Synthesis of enantiomerically pure (3R)-3-amino-N,N-dimethyl-4-(phenylthio) butanamide (**6**). Reagents and conditions: (a) D(-)-Aspartic acid, NaHCO₃, Cbz-Cl·H₂O/THF, 0 °C to rt, 8 h; (b) EtOAc, SOCl₂, rt, 12 h; (c) NaBH₄, THF, 0 °C to rt, 4 h; (d) N(CH₃)₂, THF, MW, 100 °C, 20 min; (e) Ph₂S₂, (Bu)₃P, toluene, MW, 105 °C, 25 min; (f) 30% HBr in acetic acid, rt, 4 h.



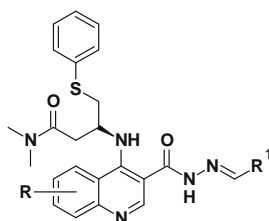
Scheme 2. Synthetic protocol of title compounds **13a-m**, **14a-m**. Reagents and conditions: (a) substituted-aniline, (COOEt)₂C=CH-OEt, 110 °C, 4 h; (b) diphenylether, 260 °C, 4 h; (c) POCl₃, 80 °C, 3 h; (d) (3R)-3-amino-N,N-dimethyl-4-(phenylthio) butanamide, K₂CO₃, CH₃CN, 80 °C, 1 h; (e) NH₂NH₂, 80 °C, 2 h; (f) substituted aldehyde, EtOH, AcOH, rt, 10 min.

diethyl ethoxymethylene malonate. The diesters **8a** and **b**, on heating at 260 °C in Dowtherm medium, were readily cyclized to ethyl 4-hydroxy-substituted quinoline-3-carboxylate (**9a** and **b**), which on refluxing with phosphorus oxychloride yielded the corresponding 4-chloro quinoline derivatives **10a** and **b** in very good yield. The conversion of chloro derivatives **10a** and **b** to the respective amines **11a** and **b** was carried out by treating them with (3R)-3-amino-N,N-dimethyl-4-(phenylthio) butanamide at 80 °C in presence of potassium carbonate in acetonitrile. These amines, on condensation with hydrazine hydrate in alcoholic medium gave the key intermediates quinoline-3-carbohydrazide derivatives **12a** and **b** in good yield. Finally, the target compounds, viz. (E)-4-((R)-1-(dimethylcarbamoyl)-3-(phenylthio)propan-2-ylamino)-N'-(benzylidene)-substituted quinoline-3-carbohydrazide (**13a-m**, **14a-m**) were synthesized from their precursors **12a** and **b** by condensing them with different bio-active aldehydes in ethanol/acetic acid medium.

The compounds were screened for their in vitro antimycobacterial activity against *M. tuberculosis* H37Rv ATCC 27294, and non-

tubercular mycobacterial (NTM) species like *M. smegmatis* (MC²) ATCC 19420, and *M. fortuitum* ATCC 19542 by Resazurin Assay method^{22,23} and their MIC values were determined. The standard drugs, viz. isoniazid (**INH**) and rifampicin (**RIF**) were used for comparison. The screening results of the title compounds and the standard drugs are reported in Table 1.

The compounds **13e**, **13i**, **13k**, **14a**, **14c**, **14i**, and **14k** exhibited moderate to good activity at 1 and 10 µg/mL, against all tested *Mycobacterium* strains. The SAR study revealed that the presence of -CF₃ group at position-8 enhanced the activity while the introduction of -F at position-6 partially lowered the activity. Amongst the tested title compounds, -F and -OCH₃ substituents (**13a**, **13c**, **13d**, **13f**, **13g**, **13i**, **14a**, **14c**, **14d**, **14g**, and **14i**) brought about increased activity whereas presence of thiophene and -OH groups (**13h**, **13f**, **13i**, **13m**, **14f**, **14h**, **14i**, and **14m**) caused decrease in activity when compared to standards. It is interesting to note that eight compounds, viz. **13a**, **13e**, **13i**, **13k**, **14a**, **14c**, **14i**, and **14k** were found to be more potent than **INH** (MIC: 50) against *M. smegmatis*, while 13 compounds, viz. **13c**, **13d**, **13e**, **13g**, **13i**, **13k**,

Table 1Physical constants, preliminary results of antimycobacterial activities and cytotoxicity of the title compounds **13a–m**, **14a–m**

Compd	R	R ¹	Mp range (°C)	Yield ^a (%)	MIC (μg/mL)			% Inhibition	IC ₅₀ ^e (μg/mL)
					MTB ^b	MS ^c	MF ^d		
13a	8-CF ₃	2-Fluoro phenyl	98–100	82	10	10	>100 ^f	90	>62.5
13b	8-CF ₃	4-Methoxy phenyl	160–161	86	>100 ^f	>100 ^f	>100 ^f	0	ND
13c	8-CF ₃	4-Fluoro phenyl	152	91	10	>100 ^f	10	90	>62.5
13d	8-CF ₃	4-Trifluoro methoxy phenyl	121–123	80	10	>100 ^f	10	90	>62.5
13e	8-CF ₃	3-Pyridyl	141–143	73	1	10	1	>95	>62.5
13f	8-CF ₃	5-Bromo thiophene	173–175	84	10	>100 ^f	>100 ^f	90	>62.5
13g	8-CF ₃	3-Hydroxy-4-methoxy phenyl	191	83	10	>100 ^f	10	90	>62.5
13h	8-CF ₃	5-Fluoro-2-hydroxy phenyl	139–141	86	10	>100 ^f	>100 ^f	0	ND
13i	8-CF ₃	3-(1,1,2,2-Tetrafluoro ethoxy) phenyl	130–132	82	1	10	10	>95	>62.5
13j	8-CF ₃	2-fluoro-4-methoxy phenyl	183–185	86	10	>100 ^f	>100 ^f	90	ND
13k	8-CF ₃	Octyl	—	81	1	10	10	>95	>62.5
13l	8-CF ₃	2-(Methylthio) phenyl	136–138	84	>100 ^f	>100 ^f	>100 ^f	0	ND
13m	8-CF ₃	3-(Methylthio) propyl	—	80	10	>100 ^f	>100 ^f	90	ND
14a	6-F	2-Fluoro phenyl	144–146	88	1	10	10	>95	>62.5
14b	6-F	4-Methoxy phenyl	130–132	90	10	>100 ^f	>100 ^f	0	ND
14c	6-F	4-Fluoro phenyl	124–127	92	1	10	10	95	>62.5
14d	6-F	4-Trifluoro methoxy phenyl	130–133	80	10	>100 ^f	10	90	>62.5
14e	6-F	3-Pyridyl	151–153	86	10	>100 ^f	10	90	>62.5
14f	6-F	5-Bromo thiophene	171–172	90	>100 ^f	>100 ^f	>100 ^f	0	ND
14g	6-F	3-Hydroxy-4-methoxy phenyl	196–198	78	10	>100 ^f	10	90	>62.5
14h	6-F	5-Fluoro-2-hydroxy phenyl	146–148	82	10	>100 ^f	>100 ^f	0	ND
14i	6-F	3-(1,1,2,2-Tetrafluoro ethoxy) phenyl	143–144	92	1	10	1	>95	>62.5
14j	6-F	2-Fluoro-4-methoxy phenyl	179–180	90	10	>100 ^f	>100 ^f	0	ND
14k	6-F	Octyl	81–82	88	1	10	10	>95	>62.5
14l	6-F	2-(Methylthio) phenyl	140–141	82	>100 ^f	>100 ^f	>100 ^f	0	ND
14m	6-F	3-(Methylthio) propyl	—	76	>100 ^f	>100 ^f	>100 ^f	0	ND
		Isoniazid (INH)			1	50	12.5	>95	>62.5
		Rifampicin (RIF)			0.5	1.5	1.5	>95	>62.5

—: Thick liquid.

ND: not determined.

^a After column purification.^b *Mycobacterium tuberculosis* H37Rv.^c *Mycobacterium smegmatis* (ATCC 19420).^d *Mycobacterium fortuitum* (ATCC 19542).^e Cytotoxicity in mammalian Vero cell lines.^f MIC > 100 μg/mL indicates that the strain is resistant to tested substance.

14a, **14c**, **14d**, **14e**, **14g**, **14i**, and **14k** were found to be more potent than **INH** (MIC:12.5) against *M. fortuitum*. In **13k**, the enhanced activity may be due to the presence of hydrophobic linear octyl group which may mimic the micolate α -chain or mycolic acid β -branch of protein, during its mode of action. Moreover, the linear octyl group exerts electron releasing property in **13k**. In conclusion, compounds **13e** and **14i** showed exceptionally good activity against both *M. fortuitum* and *M. tuberculosis* H37Rv and were more potent than **INH** and they also showed better activity than **RIF**.

Some of the compounds listed in **Table 1** were further examined for toxicity (IC₅₀) in a mammalian Vero cell line till 62.5 μg/mL concentrations. After 72 h of exposure, viability was assessed on the basis of cellular conversion of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into a formazan product using the Promega Cell Titer 96® non-radioactive cell proliferation assay.²⁴ None of them were found to be toxic. Hence the activities of the newly synthesized compounds are not due to cytotoxicity of compounds. The physical constants, results of antimycobacterial activities and cytotoxicity of the newly synthesized title compounds **13a–m**, **14a–m** are summarized in **Table 1**. While the sec-

ond level anti tuberculosis screening of the title compound have been incorporate in **Table 2**.

The structures of all the newly synthesized compounds were confirmed by ¹H & ¹³C NMR, LC–MS (ESI) studies, and elemental analysis. The detailed experimental procedures and spectral data for all the synthesized compounds are given in **Supplementary data**. The chiral purity of the title compounds were >97% (ee) which was determined using Agilent 1100 HPLC system (column: chiralcel AD-H 4.6 × 250 mm, 5 μm, flow; 1.0 mL/min, wavelength: 230 nm, mobile phase: *n*-hexane: IPA (80:20)).

In conclusion, the present study described the design, synthesis, and evaluation of antitubercular activities of new quinoline derivatives against sensitive strains of *M. tuberculosis* namely *M. tuberculosis* H37Rv (MTB), *M. smegmatis* (MC²), and *M. fortuitum*. All tested compounds were found to be nontoxic and some of the compounds displayed significant and promising anti-tuberculosis activity. The results of biological activity clearly indicate that the presence of substituted hydrazones and potent (3*R*)-3-amino-*N,N*-dimethyl-4-(phenylthio)butanamide (**6**) respectively at positions-3 and -4 of quinoline skeleton has tremendously enhanced the

Table 2
Second level antituberculosis screening of the title compounds

Compd	MIC ($\mu\text{g}/\text{mL}$)		
	MTB	MS	MF
13a	2.5	5	—
13b	—	—	—
13c	5	—	10
13d	5	—	5
13e	0.625	2.5	1.25
13f	10	—	—
13g	5	—	10
13h	10	—	—
13i	1.25	5	2.5
13j	10	—	—
13k	1.25	10	5
13l	—	—	—
13m	10	—	—
14a	1.25	10	5
14b	10	—	—
14c	1.25	10	5
14d	2.5	—	5
14e	5	—	10
14f	—	—	—
14g	5	—	5
14h	10	—	—
14i	0.625	2.5	1.25
14j	5	—	—
14k	1.25	10	5
14l	—	—	—
14m	—	—	—

— indicates that MIC > 10 $\mu\text{g}/\text{mL}$.

pharmacological property. Thus, the compounds **13e** and **14i** are ideally suited for further modifications to obtain more efficacious and potent antituberculosis drugs. The evaluation of in vivo antituberculosis activity of most promising analogues is currently underway in our laboratory and results will be reported in due course of time.

Acknowledgment

Authors are thankful to Dr. Ganesh Sambhasivam, CEO, Anthem biosciences, Bangalore, India, for his invaluable support and allocation of resources for this work. They are also grateful to the Head, Chemistry Department, NITK for providing necessary laboratory facilities for the research work and valuable support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.045.

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- M. tuberculosis* strains were grown in Middlebrook 7H9 broth (Difco BBL, Sparks, MD, USA) supplemented with 10% OADC Becton Dickinson, Sparks, MD, USA). The culture was diluted to McFarland 2 standard with the same medium. From this, 50 μL of this culture was added to 150 μL of fresh medium in 96 well microtitre plates. Stock solutions (2 mg/mL) of the test compounds were prepared in dimethyl formamide (DMF). The compounds were tested at 1, 10 and 100 $\mu\text{g}/\text{mL}$ concentrations. Further the second level testing was carried out at concentrations 0.3125, 0.625, 1.25, 2.5, and 5 $\mu\text{g}/\text{mL}$. Control tubes had the same volumes of DMF without any substrate. Rifampicin (RIF) and isoniazid (INH) was used as the reference compounds. After incubation at 37 °C for 7 days, 20 μL of 0.01% Resazurin (Sigma, St. Louis, MO, USA) in water was added to each tube. Resazurin, a redox dye, is blue in the oxidized state and turns pink when reduced by the growth of viable cells. The control tubes showed a colour change from blue to pink after 1 h at 37 °C. Compounds which prevented the change of colour of the dye were considered to be inhibitory to *M. tuberculosis*.
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