RESEARCH ARTICLE

[View Article Online](#)
[View Journal](#) | [View Issue](#) Cite this: *Med. Chem. Commun.*,
2017, 8, 2133

The synthesis, biological evaluation and structure–activity relationship of 2-phenylaminomethylene-cyclohexane-1,3-diones as specific anti-tuberculosis agents†

Muzafar Ahmad Rather,^{‡ab} Ali Mohd Lone,^{‡c} Bisma Teli,^{cd} Zubair Shanib Bhat,^{ad} Paramjeet Singh,^e Mubashir Maqbool,^a Bashir Ahmad Shairgojray,^c Mohd Jamal Dar,^{de} Shajrul Amin,^b Syed Khalid Yousuf,^{*cd} Bilal A. Bhat^{*cd} and Zahoor Ahmad ^{*ad}

The present study utilised whole cell based phenotypic screening of thousands of diverse small molecules against *Mycobacterium tuberculosis* H37Rv (*M. tuberculosis*) and identified the cyclohexane-1,3-dione-based structures **5** and **6** as hits. The selected hit molecules were used for further synthesis and a library of 37 compounds under four families was synthesized for lead generation. Evaluation of the library against *M. tuberculosis* lead to the identification of three lead antituberculosis agents (**37**, **39** and **41**). The most potential compound, 2-(((2-hydroxyphenyl)amino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**39**) showed an MIC of 2.5 $\mu\text{g mL}^{-1}$, which falls in the range of MICs values found for the known anti-tuberculosis drugs ethambutol, streptomycin and levofloxacin. Additionally, this compound proved to be non-toxic (<20% inhibition at 50 μM concentration) against four human cell lines. Like first line anti-tuberculosis drugs (isoniazid, rifampicin and pyrazinamide) this compound lacks activity against general Gram positive and Gram negative bacteria and even against *M. smegmatis*; thereby reflecting its highly specific antituberculosis activity.

Received 12th July 2017,
Accepted 10th October 2017

DOI: 10.1039/c7md00350a

rsc.li/medchemcomm

Tuberculosis (TB) continues to be the most deadly global infectious diseases since prehistoric times.¹ In 2015, 10.4 million people fell ill with TB and 1.8 million died from the disease (including 0.4 million among people with HIV).² It is estimated that one-third of the world's population harbours *Mycobacterium tuberculosis* in its latent state and is at the risk of developing active TB, thereby becoming an infectious reservoir.² The HIV epidemic, one of the major obstacles to achieving global control of the disease, has dramatically increased

the risk of developing active TB and added further complexity to the treatment of TB.³ The increase in the emergence and global spread of drug resistant forms of *M. tuberculosis* isolates is another challenge towards curbing TB.² The above facts and figures of human morbidity and mortality clearly pinpoint the failure of the current strategies used against TB and are the real factors underlining the need for the development of new anti-TB drugs. Furthermore, many of the drug candidates in the TB drug pipeline are either derivatives of existing drugs or they target the same cellular processes as the existing drugs in clinical use.⁴ It is noteworthy that the chances of the successful development of analogues/derivatives of existing drugs is significantly reduced due to cross-resistance, which has been observed with the rifamycins and quinolones.⁵ Thus, there is the pressing need to develop anti-TB agents based on new chemical entities (NCEs) capable of killing drug sensitive, drug resistant and persistent forms of *M. tuberculosis* to counter the TB pandemic. Extensive drug discovery efforts over the past decade started with the screening of millions of small molecules and have identified only a few promising antituberculosis drug candidates that have either received clinical approval or are now in advanced clinical trials.⁶ Diarylquinoline, bedaquiline (TMC207)⁷ (**1**),

^a Clinical Microbiology and PK/PD Division, Clinical Microbiology PK/PD/Laboratory, CSIR-Indian Institute of Integrative Medicine, Sanatnagar, Srinagar, India-190005. E-mail: zahoorap@iiim.ac.in; Fax: +91 1942441331; Tel: +91 194 2431253/55, +91 9906593222

^b Department of Biochemistry, University of Kashmir, Hazratbal Srinagar, India-190006

^c Medicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Sanatnagar, Srinagar, India-190005. E-mail: khalidiim@gmail.com, bilal@iiim.ac.in; Fax: +91 194244133; Tel: +91 1942431253/55

^d Academy of Scientific & Innovative Research (AcSIR), India

^e Cancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu, Jammu & Kashmir, India-180001

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7md00350a

‡ These authors made equal contributions to this manuscript.

nitroimidazole (PA-824, **2**),⁸ the nitroimidazole analogue, delamanid (OPC-67683, **3**)⁹ and the diamine, SQ-109 (**4**)¹⁰ are some representative examples (Fig. 1).

Bedaquiline has recently received approval by the US Food and Drug Administration (US-FDA) and by the European Medicines Agency (EMA) as part of a combination regimen for treating pulmonary MDR-TB in adults.¹¹ Similarly, delamanid has also received approval by the European Commission against pulmonary MDR-TB in adult patients in whom effective regimens are not available due to resistance or tolerability.¹²

Keeping in view the need of new antituberculosis drugs and some tangible success of whole cell based phenotypic screening as an approach to identifying new antituberculosis agents, the presented study utilizes the same technique on thousands of small molecules to identify a hit compound against *M. tuberculosis*.¹³ A diverse library, comprised of 20 000 compounds, procured from ChemBridge (San Diego, USA) was screened in our Institute to identify the compounds with significant anti-TB potential.¹⁴ Our study identified 2-(((2-hydroxyphenyl)thio)methylene)-cyclohexane-1,3-diones (**5** and **6**, Fig. 2) as two hits. Structural-activity relationship guided synthesis was carried out using these hit compounds to generate a diverse library of molecules that were screened against *M. tuberculosis*. Three molecules were observed to exhibit significant antituberculosis potential (**37**, **39** & **41**, Table 1) and the most potent compound, **39** was studied further.

Initial screening and synthesis of the active compounds

All compounds were assessed for their potential to inhibit *M. tuberculosis* H37Rv growth in Middlebrook 7H9 broth with supplements as previously described.¹⁵ The minimal inhibitory concentrations (at which no visible growth was observed MICs) of the standard antituberculosis drugs rifampicin, isoniazid, ethambutol, streptomycin and levofloxacin are 0.078, 0.313, 1.56, 1.25 and 2.5 $\mu\text{g mL}^{-1}$, respectively. These results

Fig. 2 Two hit molecules from the phenotypic screening process.

are in good agreement with the literature.¹⁶ Our initial phenotypic screening of small synthetic molecules identified two compounds (**5** and **6**, Fig. 2) as potential antituberculosis hits. Though these compounds exhibited some activity against *M. tuberculosis*, this activity was not significant enough to proceed with them for further antituberculosis drug discovery as their MIC values ranged between 40–80 $\mu\text{g mL}^{-1}$, which was significantly higher than the standard drugs. However, this anti-TB activity was a clue to the synthesis of a diverse library of molecules using this scaffold in order to find better lead structures for further study.

Initially, we synthesized few 2-alkyl-aminomethylene-cyclohexane-1,3-dione-based compounds (family A, Table 1, entries 1–5) *via* the condensation reaction of 1,3-cyclohexadiones with various aliphatic amines in the presence of triethylorthoformate (Scheme 1). It is notably that all these compounds were obtained in excellent yield and their structures were confirmed using various spectroscopic techniques. When these compounds were evaluated against *M. tuberculosis*, it was observed that this synthetic approach totally diminished the activity of the parent compounds and the MIC values increased and were found to be $>80 \mu\text{g mL}^{-1}$ (highest experimental concentration). Therefore, a new synthesis was designed to produce 2-phenylaminomethylene-cyclopentane-1,3-dione-based compounds from 1,3-cyclopentadione and substituted-anilines (family B, Table 1, entries 6–8) in the presence of triethylorthoformate. Like family A, the compounds of family-B were also screened against *M. tuberculosis* and this approach also led to a loss of activity (MIC $>80 \mu\text{g mL}^{-1}$). These synthetic approaches indicated that the 1,3-cyclohexanedione moiety in the parental structures was critical

Fig. 1 Representative compounds that are either under clinical trials or have acclaimed clinical use against tuberculosis.

Table 1 Library of 2-alkyl/aryl-aminomethylene cycloalkane-1,3-diones and their MIC values against *Mycobacterium tuberculosis* H37Rv

Entry	Family	Compound ^a	MIC ($\mu\text{g mL}^{-1}$) against <i>M. tuberculosis</i> ^b
1	A 2-alkyl-aminomethylene-cyclohexane-1,3-diones		>80
2		7 (74) ^c	>80
3			>80
4		8 (72)	>80
5			>80
6	B 2-phenylaminomethylene-cyclopentane-1,3-diones	9 (75)	>80
7			>80
8		11 (71)	>80
9			>80
10		12 (78)	>80
11	C 2-phenylaminomethylene-cyclohexane-1,3-diones		>80
12		13 (76)	>80
13			>80
14		14 (78)	80
15			80
16		15 (78)	80
17			40–80
18		16 (77)	>80
19			>80
20		17 (82)	>80
21			>80
22		18 (83)	>80

Table 1 (continued)

Entry	Family	Compound ^a	MIC ($\mu\text{g mL}^{-1}$) against <i>M. tuberculosis</i> ^b
13		19 (74)	>80
14		20 (85)	>80
15		21 (78)	>80
16		22 (84)	>80
17		23 (82)	>80
18		24 (87)	>80
19		25 (79)	>80
20		26 (76)	>80
21		27 (76)	>80
22		28 (80)	>80
23		29 (70)	>80

Table 1 (continued)

Entry	Family	Compound ^a	MIC ($\mu\text{g mL}^{-1}$) against <i>M. tuberculosis</i> ^b
24		30 (74)	>80
25		31 (70)	>80
26		32 (72)	>80
27		33 (79)	>80
28		34 (82)	>80
29		35 (80)	>80
30		36 (80)	>80
31		37 (91)	5–10
32		38 (78)	20
33		39 (90)	2.5
34		40 ^d (78)	10–20

Table 1 (continued)

Entry	Family	Compound ^a	MIC ($\mu\text{g mL}^{-1}$) against <i>M. tuberculosis</i> ^b
35	D 5-aryl-2-phenylaminomethylene-cyclohexane-1,3-diones		5–10
36			10–20
37			20

^a All compounds were synthesized using the route outlined in Scheme 1. ^b The MIC values of isoniazid, rifampicin, ethambutol, streptomycin and levofloxacin were 0.313, 0.078, 1.25, 1.56 and 2.5 $\mu\text{g mL}^{-1}$, respectively. ^c Figures in the parenthesis represent the percentage yields after isolation of the pure compounds. ^d Compound 40 is an inseparable mixture of *E:Z* isomers approximately in 1:1 ratio based NMR spectroscopy.

for activity and therefore, in the next synthetic strategy this was preserved like in the parental compounds 5 and 6. Variations were made only on the right-hand portion of the scaffold with various substituted anilines in order to produce a series of 2-phenylaminomethylene-cyclohexane-1,3-diones (family C, Table 1, entries 9–34). When this family was screened against *M. tuberculosis*, it was observed that most of the compounds also had no activity ($\text{MIC} > 80 \mu\text{g mL}^{-1}$), however, some of the compounds from this family exhibited some enhanced activity, specifically two compounds, 2-(((2-hydroxyphenyl)amino)methylene)-cyclohexane-1,3-dione (37) and 2-(((2-hydroxyphenyl)amino)methylene)-5,5-dimethylcyclohexane-1,3-dione (39), which showed excellent activity with MIC values of 5–10 and 2.5 $\mu\text{g mL}^{-1}$, respectively. Indeed, the MIC of 39 was found in the MIC range found for the standard antituberculosis drugs ethambutol, levofloxacin and streptomycin. It is worth mentioning that these results demonstrate about a 32-fold activity enhancement of the parent compounds due to the above structure activity guided synthesis. Further, by these observations, it is clear that by changing the substituent at the C-5 position of the 1,3-cyclohexanedione ring changes its MIC values considerably. Based on this fact, we next sought to synthesize 5-aryl-2-

phenylaminomethylene-cyclohexane-1,3-dione-based compounds (family D, Table 1, entries 35–37). It was observed that this structure activity guided synthesis also resulted in an improvement of the antituberculosis activity when compared to parent compounds 5 and 6, especially one of the compounds, 2-(((2-hydroxyphenyl)amino)methylene)-5-phenylcyclohexane-1,3-dione (41), which exhibited a significant MIC value of 5–10 $\mu\text{g mL}^{-1}$ (Table 1).

Structural activity relationship (SAR)

Based on the above observations, we arrived at the SAR depicted in Fig. 3: (a) the presence of the 1,3-cyclohexadione motif and *o*-hydroxyl aryl ring in the scaffold are crucial for anti-TB activity (compounds 5, 6, 37–43). By replacing the 1,3-cyclohexadione motif with 1,3-cyclopentadione, a drastic reduction in activity was observed (compound 12); (b) the presence of 5,5-*gem*-dimethyl substitution at C-5 in the 1,3-cyclohexadione moiety seems to be optimal for significant MIC values (compound 39). Compounds with no substitution at the C-5 position (37) or bearing other substituents (compounds 38, 41–43) are comparatively less active than compound 39. By changing the position of the *gem*-dimethyl group to the C-4 position in the 1,3-cyclohexadione motif also substantially diminishes the anti-TB potential (40); (c) it was interesting to note that the compounds containing a S-linkage (5, 6) exhibit lower activity than the compounds containing a –NH– linkage (37 and 39) even if the other framework was preserved. This was possibly due to the hydrogen bonding between –NH– and one of the carbonyl groups in the 1,3-dione moiety that imparts rigidity in the structure; (d) finally, it was also observed that the compounds containing an

Scheme 1 Synthesis of 2-alkyl and aryl-aminomethylene-cycloalkane-1,3-diones.

Fig. 3 The SAR of 2-alkyl/aryl-aminomethylene cycloalkane-1,3-diones against *M. tuberculosis*.

aliphatic moiety are not active (compounds 7–11), while as those containing an aromatic motif with appropriate substitution are generally active (compounds 33–39). Among these compounds, the compound containing a 5,5-*gem*-dimethyl substitution on the 1,2-cyclohexanedione moiety connected through a –NH– linkage to the *o*-hydroxyl-aryl moiety (compound 39) was the most active compound found in this study. Compounds containing C-5 aryl substitution (compound 41) also look promising for future exploration. Based on this study and keeping in consideration the significant MIC value of compound 39 ($2.5 \mu\text{g mL}^{-1}$), it was further studied towards the advancement of tuberculosis drug discovery.

The antibacterial activity spectrum of compound 39

Historically, *M. tuberculosis* has been resistant to antibiotics, which is the reason why antibiotic development against tu-

berculosis has been so challenging and indeed bacterial diseases that have appeared much later than TB do not exist anymore due to antibiotic developments. Surprisingly, antibiotics that are active against TB and together constitute the standard first line antituberculosis regimen include INH, RIF and PZA are either totally inactive or poorly active against other bacteria. Indeed RIF and INH have been shown to exhibit almost no activity even against a fast growing non-virulent mycobacterial strain, *Mycobacterium smegmatis* *i.e.* these drugs exhibit 500- to 1000-fold higher MIC values against this strain when compared to *M. tuberculosis*.¹⁷ Keeping in view this fact we evaluated the test compound against a panel of Gram positive and Gram negative bacteria as well as against *M. smegmatis* to evaluate its spectrum of activity (Table 2) as previously described.¹⁸ Briefly, all the bacterial strains were challenged with the test compound at concentrations ranging between 0.25 to $128 \mu\text{g mL}^{-1}$. The standard drug, levofloxacin (LVX) was used as the control in a

Table 2 The antibacterial activity of 2-((2-hydroxyphenyl)amino)methylene)-5,5-dimethylcyclohexane-1,3-dione (compound 39) against Gram positive bacteria, Gram negative bacteria and non-pathogenic mycobacteria

Type of bacteria	Bacterial strain	MIC ($\mu\text{g mL}^{-1}$)	
		Test compound	LVX ^a
Gram positive	<i>Staphylococcus aureus</i> (ATCC 25923)	>128	0.078–0.156
	<i>Staphylococcus epidermidis</i> (ATCC 12228)	>128	0.039–0.078
	<i>Bacillus subtilis</i> (ATCC 11774)	>128	0.39
	<i>Micrococcus luteus</i> (ATCC 10240)	128	0.078
	<i>Enterococcus faecalis</i> (ATCC 51299)	>128	0.156
	<i>Escherichia coli</i> (10536)	32–64	0.078
Gram negative	<i>Klebsiella pneumoniae</i> (ATCC BAA-2146)	>128	0.625–1.25
	<i>Pseudomonas aeruginosa</i> (ATCC 10145)	>128	0.312–0.625
	<i>M. smegmatis</i> (ATCC 607)	>80	0.156
Mycobacteria	<i>M. tuberculosis</i> H37Rv (ATCC 25177)	2.5	2.5

RIF and INH have been reported to show 500- and 1000-fold higher MIC values against *M. smegmatis* (100 and $25 \mu\text{g mL}^{-1}$) when compared to those of *M. tuberculosis* (0.2 and $0.02 \mu\text{g mL}^{-1}$), respectively.¹⁷ ^a LVX = levofloxacin, which was used as a reference antibiotic. The data are obtained from triplicate sets of three independent experiments.

Table 3 The cytotoxicity of compounds (37–43) at a concentration of 50 μ M expressed as percentage growth inhibition in four human cell lines

Compound	HEK-293	MCF-7	HCT-116	PC-3
37	28.27	14.52	10.57	29.51
38	6.08	16.6	35.70	8.20
39	9.03	15.06	20.22	10.13
40	64.12	n.d. ^a	60.70	41.22
41	52.79	16.52	n.d.	20.36
42	46.44	2.92	42.75	26.06
43	44.04	23.37	28.65	18.55

^a n.d. = not determined.

concentration range between 0.019 to 10 μ g mL⁻¹. The MIC was determined in Muller Hinton Broth and the results were read after 24 h of incubation at 37 °C. The test compound (39) was not active at all against most of the Gram negative bacterial strains tested (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*) as an MIC was not observed even at the highest tested concentration (128 μ g mL⁻¹). However, the test compound exhibited poor activity against *E. coli* with a MIC value of 32–64 μ g mL⁻¹. Similarly, the test compound did not exhibit any activity against the Gram positive bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Micrococcus luteus* and *Enterococcus faecalis*) up to the highest tested concentration (128 μ g mL⁻¹). Indeed when the test compound was evaluated against a non-virulent mycobacterial strain, *M. smegmatis*, it did not exhibit any activity (MIC >80 μ g mL⁻¹) (Table 2). Thus, compound 39, like the standard antituberculosis drugs INH, RIF and PZA, was specifically active against *M. tuberculosis*. This finding was also of crucial significance as TB treatment at the best is usually for six months and therefore, highly specific TB drugs are expected to affect least normal flora otherwise *vice versa* observations are expected.

Cytotoxicity evaluation of lead compounds 37–43

One of the critical most considerations for any molecule to proceed successfully during the drug discovery process is its ability to differentiate between bacterial and mammalian cells as its by this virtue its activity and toxicity are separated.¹⁹ We therefore studied the cytotoxic potential of the lead compounds (37–43) against a human embryonic kidney cell line 293 (HEK-293) and three cancer cell lines; breast (MCF-7), human colon (HCT-116) and human prostate (PC-3) cell lines using an MTT cell viability assay at 1–50 μ M.²⁰ The MCF-7, HCT-116 and PC3 cells were obtained from the National Cancer Institute (NCI), USA and the HEK-293 cells were purchased from the National Centre For Cell Science (NCCS), Pune, India. It was observed that overall all the compounds had little effect on all four cell lines from 1–40 μ M (<20% growth inhibition approximately). The results at 50 μ M obtained for all the compounds against the four cell lines are presented in Table 3. All the compounds showed <50%

growth inhibition in the tested cell lines at this concentration. With the exception of compound 40, which inhibited the growth of HEK-293 and HCT-116 by 64.12% and 60.70%, respectively, and compound 41, which inhibited 52.79% growth of HEK-293 cells. Compound 39 (best lead) was observed to be non-toxic against all the tested cell lines with <20% inhibition at a concentration of 50 μ M. This compound was further tested up to 80 μ M against HEK-293 (normal human cell line) and <20% inhibition was observed. Considering the fact that its MIC was 2.5 μ g mL⁻¹ against *M. tuberculosis* and it does not affect human cells even up to 50 μ M, this compound has a significantly suitable therapeutic index and thus, merits *in vivo* evaluation.

In conclusion, the present study describes the synthesis and evaluation of a library of 2-phenylaminomethylene-cyclohexane-1,3-diones against *M. tuberculosis*. This study identified “2-(((2-hydroxyphenyl)amino)methylene)-5,5-dimethylcyclohexane-1,3-dione” as a potential lead molecule whose MIC matches those of the standard anti-TB drugs ethambutol, levofloxacin and streptomycin. Further, its activity is highly selective against *M. tuberculosis* when compared to other microbes and it proved to be non-toxic to human cell lines. The structure–activity relationship data is also presented for future study. To the best of our knowledge, this study has introduced a new chemical entity with potential that is worth exploring as a future drug candidate for TB.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

The authors are highly thankful to Director IIIM, Ram A. Vishwakarma for support and encouragement. SKY is thankful to DST for INSPIRE faculty award (IF-CH-18) and research grant under budget head GAP-1179. BT. BAB wishes to thank SERB for the research support under budget head SR/WOS-A/CS-54/2016. This work was supported by funding from the Council of Scientific and Industrial Research Govt. of India (MLP-6010 and BSC-203) and the Ramalingaswami Fellowship grant from the Department of Biotechnology Govt. of India (GAP-1160) to ZA. IIIM publication No: IIIM/2170/2017.

Notes and references

- 1 Z. Ahmad, H. N. Makaya and J. H. Grosset, History of TB Drug Discovery: Early Evaluation Studies and Lessons Learnt from Them. Antituberculosis Chemotherapy, *Prog. Respir. Res.*, 2011, **40**, 1–8.
- 2 WHO Global tuberculosis report, 2016, http://reliefweb.int/sites/reliefweb.int/files/resources/gtbr2016_main_text.pdf.
- 3 U. G. Laloo, S. Pillay and R. Mngqibisa, *et al.*, HIV and COPD: a conspiracy of risks, *Respirology*, 2016, **21**, 1166–1172.
- 4 B. Lechartier, J. Rybníček and A. Zumla, *et al.*, Tuberculosis drug discovery in the post genomic era, *EMBO Mol. Med.*, 2014, **6**, 158–168.

- 5 B. P. Goldstein, Resistance to rifampicin: a review, *J. Antibiot.*, 2014, **67**, 625–630.
- 6 G. Gualano, S. Capone and A. Matteelli, *et al.*, New Antituberculosis Drugs: From Clinical Trial to Programmatic Use, *Infect. Dis. Rep.*, 2016, **8**, 6569, DOI: 10.4081/idr.2016.6569. eCollection 2016 Jun 24.
- 7 K. Andries, P. Verhasselt and J. Guillemont, *et al.*, A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*, *Science*, 2005, **307**, 223–227.
- 8 C. K. Stover, P. Warren and D. R. VanDevanter, *et al.*, A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis, *Nature*, 2000, **405**, 962–966.
- 9 M. Matsumoto, H. Hashizume and T. Tomishige, *et al.*, OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice, *PLoS Med.*, 2006, **3**, e466.
- 10 L. Jia, J. E. Tomaszewski and C. Hanrahan, *et al.*, Pharmacodynamics and pharmacokinetics of SQ109, a new diamine-based antitubercular drug, *Br. J. Pharmacol.*, 2005, **144**, 80–87.
- 11 S. K. Field, Bedaquiline for the treatment of multidrug-resistant tuberculosis: great promise or disappointment?, *Ther. Adv. Chronic Dis.*, 2015, **6**, 170–184.
- 12 R. Rustomjee and A. Zumla, Delamanid expanded access novel treatment of drug resistant tuberculosis, *Infect. Drug Resist.*, 2015, **8**, 359.
- 13 U. H. Manjunatha and P. W. Smith, Perspective: Challenges and opportunities in TB drug discovery from phenotypic screening, *Bioorg. Med. Chem. Lett.*, 2015, **23**, 5087–5097.
- 14 C. Rani, R. Mehra, R. Sharma, R. Chib, P. Wazir, A. Nargotra and I. A. Khan, High-throughput screen identifies small molecule inhibitors targeting acetyltransferase activity of *Mycobacterium tuberculosis* GlmU, *Tuberculosis*, 2015, **95**(6), 664–677.
- 15 Z. Ahmad, S. Tyagi and A. Minkowski, *et al.*, Antimicrobial activity of 5-Cl-Pyrazinamide in mice infected with *Mycobacterium tuberculosis* or *Mycobacterium bovis*, *Indian J. Med. Res.*, 2012, **136**, 808–814.
- 16 K. Bartmann, *Antituberculosis Drugs*, Springer Berlin Heidelberg, 1988.
- 17 V. Chaturvedi, N. Dwivedi and R. P. Tripathi, *et al.*, Evaluation of *Mycobacterium smegmatis* as a possible surrogate screen for selecting molecules active against multi-drug resistant *Mycobacterium tuberculosis*, *J. Gen. Appl. Microbiol.*, 2007, **53**, 333–337.
- 18 A. Shah, Rather MA, Qazi PH, *et al.* Discovery of antimicrobial and antitubercular molecules from *Fusarium solani*: an endophyte of *Glycyrrhiza glabra*, *J. Appl. Microbiol.*, 2017, **122**, 1168–1176.
- 19 A. Ebbensgaard, H. Mordhorst and M. T. Overgaard, *et al.*, Comparative evaluation of the antimicrobial activity of different antimicrobial peptides against a range of pathogenic bacteria, *PLoS One*, 2015, **10**, e0144611.
- 20 J. Carmichael, W. G. Degraff and A. F. Gazdar, *et al.*, Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing, *Cancer Res.*, 1987, **47**, 936–942.