Med. Chem. Commun., 2011, 2, 378-384

Synthesis, in silico screening and bioevaluation of dispiro-cycloalkanones as antitubercular and mycobacterial NAD+-dependent DNA ligase inhibitors

Rama P. Tripathi [a]*, Jyoti Pandey[$a], Vandana Kukshal[$c], Arya Ajay[a], Mridul Mishra[a], Divya Dube[c], Deepti Chopra[c], R. Dwivedi[b] Vinita Chaturvedi[b] and Ravishankar Ramachandran[c]*

[a]Medicinal and Process Chemistry Division, [b]Drug Target Discovery and Development Division and [c]Molecular and Structural Biology Division, Central Drug Research Institute Chattar Manzil, P.O. Box 173, Mahatma Gandhi Marg, Lucknow-226001, India.

*Corresponding authors. Tel.: +91 0522 2612411; fax +91 522 2623405/2623938/2629504.
E-mail address: mailto:rpt.cdri@gmail.com (R.P. Tripathi); r_ravishankar@cdri.res.in (R. Ramachandran)
$= authors having equal contribution
ÀElectronic Supplementary Information (ESI) available: Experimental procedures, characterization data and copies of ^{1}H NMR and ^{13}C NMR spectra, protocols of biological assays. See DOI:

Abstract
A series of *dispiro*-cycloalkanones were synthesized using ñCorey Chaykovskyò reaction in Ů,ŮNj *(EE)-bis*(benzylidene)-cycloalkanones/methanone in good yields. The compounds were evaluated for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv and *in silico* screening. Some selected compounds were screened for mycobacterial NAD^+- dependent DNA ligase inhibitory activity. Two of the compounds showed good *in vitro* antitubercular and NAD^+-dependent DNA ligase inhibitory activity along with good correlation to *in silico* results.

Keywords: Trimethylsuphoxonium iodide, *bis*-benzylidene cycloalkanones, dispiro cycloalkanones, *Mycobaceterium tuberculosis*, DNA ligases.

Introduction

Despite current multidrug therapy and ongoing drug development, [1-3] tuberculosis continues to be a major public health concern today. With more than 1.6 million deaths and 9.2 million new cases being reported each year, it is a leading infectious disease claiming millions of death globally [4,5]. *Mycobacterium tuberculosis* has ability to survive for extended periods of time in human host and thus requires prolonged drug treatment (six to nine months) and resulting in low compliance. Moreover, the evolution of multidrug-resistant (MDR), extremely drug resistant (XDR, recent mortality rate >98%) tuberculosis [6-10] and the AIDS epidemic [11-13] further makes the situation more worsening. In *Mycobacterium tuberculosis* drug resistance is not due to a common mechanism for all drugs, but different mechanisms for different class of drugs[14,15]. Almost all the conventional targets and drugs became inadequate to control the resistant TB infection and therefore the discovery of novel, sensitive and selective targets or new chemical entity is needed for development of new generation of antitubercular drugs.

DNA ligases are vital enzymes in replication and repair of DNA. They catalyze the formation of a phosphodiester linkage between adjacent termini in double stranded DNA through similar mechanism.[16] Two types of DNA ligases *viz*. NAD^+- dependent and ATP - dependent ligases are known based on their respective co-factor specificities. [17] NAD^+ ligases, commonly called LigA, occur almost exclusively in bacteria while ATP dependent ligases are more

ubiquitous and occur additionally in viruses, archaea, eukaryotes and higher organisms. [18, 19] although, there is little sequence homology between the eubacterial and eukaryotic enzymes and they exhibit some structural homology in specific domains but the mechanistic steps are broadly conserved. [20, 21] Different steps involved in action of DNA ligases involve large conformational changes as also encircling and partial unwinding of the nicked DNA substrate [22-24]. *M. tuberculosis* codes for at least three different types of ATP- dependent ligases and one LigA. Gene knockout and other studies have shown LigA to be indispensable in several pathogens including *E.coli*, *S. typhimurium*, *S.aureus* and *B.subtilis* in contrast to ATP-dependent ligases which are dispensable in *M. tuberculosis* also [25-30]. To find out new prototypes as specific inhibitors for NAD^+ ligase is one of the approaches for antiTB drug research as no drug is known to act against this enzyme so far. NAD^+ ligase specific inhibitors including aryl amines [31] and pyridochromanones [32] have been reported. We have also shown that glycosyl ureides [33] and glycosyl amines [34] inhibit the Mycobaceterial NAD^+ ligase and have shown broad bactericidal activity (Fig. 1). As a part of our continuing efforts in tuberculosis chemotherapy [35-39] we have recently shown potent antitubercular activities in bis-benzylidene cycloalakanones [40], phenylcyclopropyl methanones [41] and alkylaminoaryl phenyl cyclopropyl methanones [42] (Fig. 2). The compounds were designed as possible inhibitors of FAS-II but the enzyme inhibition by these compounds was not very significant although few of them showed very promising antitubercular activities.

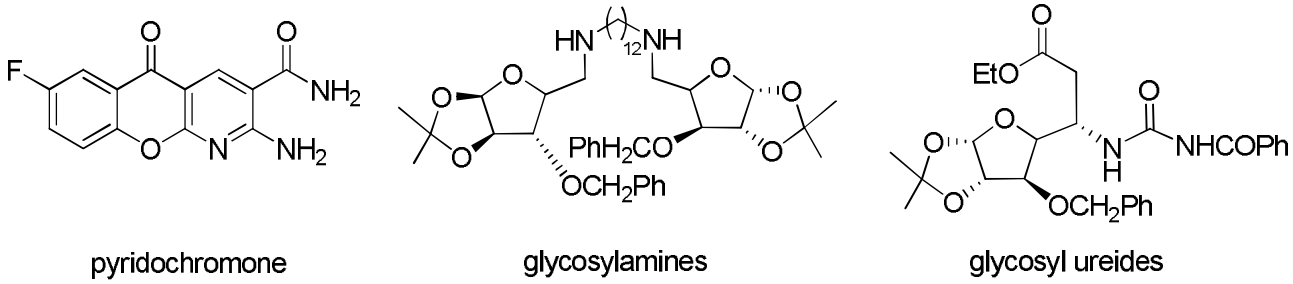


Fig. 1 Potential NAD^+ Ligase inhbitors of different classes

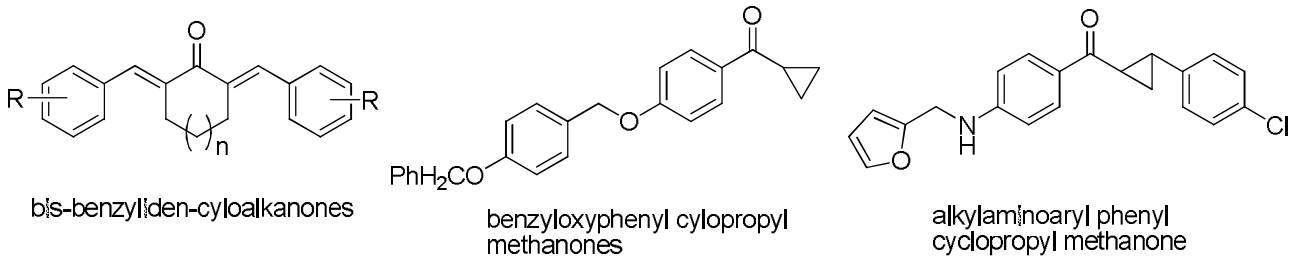


Fig. 2 Potent antitubercular compounds

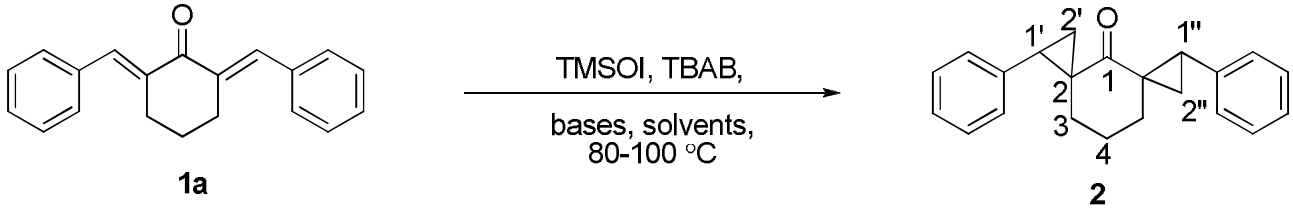
In continuation of this programme we have synthesized a series of *dispiro*-cycloalkanones using TMSOI for methylene insertion, NaOH as base and TBAB as phase transfer catalyst (Corey Chaykovsky reaction) [43] in *bis* benzylidene cyloalkanones. *In silico* screening results indicated

NAD- ligase as targets of these compounds and the compounds were evaluated *in vitro* too against *M. tuberculosis* H37Rv and full length of NAD^+-dependent enzyme from *M. tuberculosis.*

Results and discussion

Chemistry

The starting substartes Ŭ,Ŭ-(*EE*)-*bis*(benzylidene)-cycloalkanones/methanones **(1a-1r)**[40] were prepared by simple condensation of two equivalents of aromatic aldehydes with one equivalent of cycloalkanones/methanone in presence of KOH (5 mol%) in ethanol as earlier reported by us.[40] Cyclopropanation of double bond in Ŭ, Ŭ-(*EE*)-*bis*(benzylidene)-cycloalkanones was carried out with trimethyl sulphoxonium iodide (TMSOI) to give respective disipricycloalkanones. In order to establish a suitable reaction condition a model reaction of Ŭ, Ŭ-(*EE*)-*bis*(benzylidene)-cyclohexanone **(1a)** with TMSOI in presence of tetrabutylammonium bromide (TBAB) was Carried out in different solvent and bases to give 2, 6-bis-(phenyl)-dispiro[2.1.2.3]decan-4-one (**2**) (Scheme 1) and the results are shown in Table 1.



Scheme 1. Optimization of the cyclopropanation reaction using different solvent and base

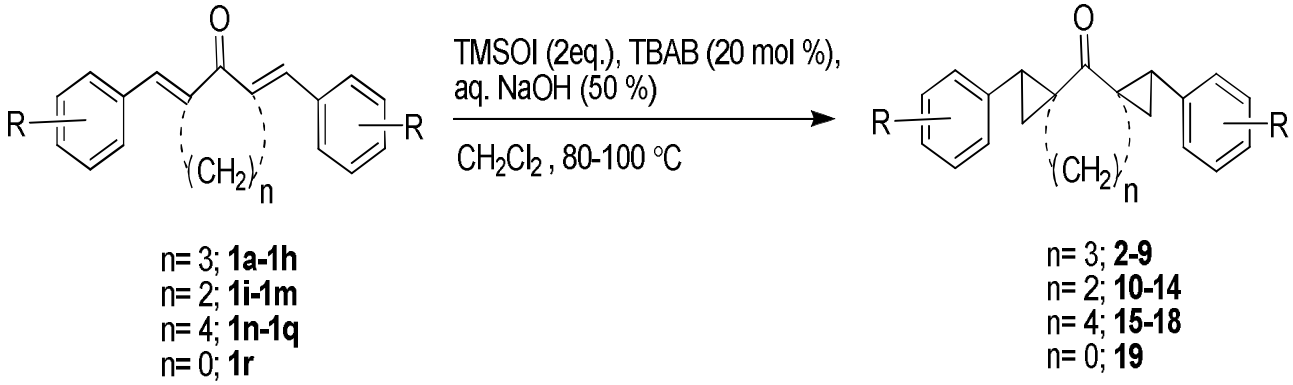
Table 1. Optimization of the cyclopropanation reaction using different solvent and base

Entry	Base (Conc)	Solvent	Time (h)	Temp (°C)	Yield %
1	NaH (2eq.)	DMSO	20	100	30
2	NaH (2eq.)	DMF	24	100	25
3	aq. NaOH (50%)	DMSO	20	100	30
4	aq. NaOH (50%)	DMF	24	100	20
5	aq. NaOH (30%)	CH_2Cl_2	18	80	20
6	aq. NaOH (40%)	CH_2Cl_2	18	80	35
7	aq. NaOH (50%)	CH_2Cl_2	18	80	60

8	aq. NaOH (60%)	CH_2Cl_2	18	80	60
9	aq. NaOH (70%)	CH_2Cl_2	18	80	60

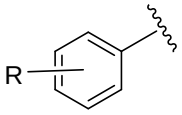
According to results shown in Table-1, 50% aq. NaOH in CH_2Cl_2 (entry 7) is the most suitable protocol for the reaction. The success of this method is based on the solvation of the ionic species formed during reaction. Solvation favors the reaction rate. Since solvation of ionic species is better in aqueous NaOH/CH_2Cl_2 combination than DMSO or DMF as solvent, the reaction yield is accordingly enhanced in aqueous NaOH/CH_2Cl_2 than NaOH/DMSO or DMF combination [44]. The structure of compound **2** was established on the basis of its spectroscopic data and microanalysis (see supplementary information). The *trans* geometry of the cylopropyl rings in compound **2** was established on the basis of literature precedents where cyclopropanation of *trans*-propenones with TMSOI under basic conditions (Corey-Chaykovsky Reaction) is always reported to result in *trans* products. [45-47]

After establishing the standard reaction condition, we explored the scope of different substrates in the cyclopropanation. Thus, we carried out the cyclopropanation of different Ŭ, Ŭ-(*EE)-bis*(benzylidene)-cyclohexanones (**1a-1h**) with TMSOI (trimethylsilyl sulfoxonium iodide) to get the desired products **2-9** in moderate to good yields (Table 2, Scheme 2). To see the effect of ring size of cycloalkanone moiety on this cyclopropanation reaction, the study was extended with Ŭ, Ŭ-(*EE)-bis*(benzylidene)-cyclopentanones (**1i-1m**) and Ŭ, Ŭ-(*EE)-bis*(benzylidene)-cycloheptanones (**1n-1q**). The reaction of different Ŭ, Ŭ-(*EE)-bis*(benzylidene)-cyclopentanones/cycloheptanones with TMSOI under similar reaction condition yielded compounds **10-18** in good yields (Scheme 2) and results are shown in Table 2. All the synthesized prototypes were well characterized by their spectroscopic data and microanalysis (see supplementary data).



Scheme 2. Synthesis of dispiro compounds **2-19** from different Ŭ, Ŭ-(*EE)-bis*(benzylidene)-cycloalkanones/methanone

Table 2. Synthesized *dispiro*-cycloalcanones **(2-19)** and their *in vitro* antitubercular activity

Compd. No.	n		*C* log*P*[a]	MIC[b] (µM) *M. tuberculosis* H37 Rv
2	3	Phenyl	5.20	>41.39
3	3	4-Fluorophenyl	5.32	36.98
4	3	4-Chlorophenyl	6.43	16.84
5	3	4-Bromophenyl	6.60	13.58
6	3	4-Methoxyphenyl	4.99	>34.53
7	3	3,4-Dimethoxyphenyl	4.78	29.62
8	3	3, 4, 5-Trimethoxyphenyl	4.57	25.93
9	3	4-Benzyloxyphenyl	7.73	>24.31
10	2	4-Fluorophenyl	5.00	38.58
11	2	4-Bromophenyl	6.28	>28.02
12	2	4-Methoxyphenyl	4.68	35.91
13	2	3,4-Dimethoxyphenyl	4.47	>30.63
14	2	4-Benzyloxyphenyl	7.41	>25.00
15	4	4-Chlorophenyl	6.75	>32.29
16	4	4-Methoxyphenyl	5.31	>33.24
17	4	3,4-Dimethoxyphenyl	5.10	>28.66
18	4	3, 4, 5-Trimethoxyphenyl	4.89	>25.20
19	0	4-Benzyloxyphenyl	6.81	26.37

C log*P*[a] was determined by OSIRIS Property Explorer Programme which is available at http://www.organic-chemistry.org/prog/peo/.

[b]MIC= Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of mycobacterium >90%; MIC of the drugs used as control, INH 4.74 and ethambutol 15.90 ε M against *M. tuberculosis H37 Rv.*

Biological activities

The *in vitro* antitubercular activity against *M. tuberculosis* H37Rv was determined using agar microdilution method [48]. The *in silico* docking studies were carried out in above synthesized *dispiro*-cycloalkanones using autodock tool [49, 50] and some of the active hits were screened for their mycobacterial NAD^+-dependent DNA ligase inhibitory activity against the full length of NAD^+-dependent enzyme from *M. tuberculosis*, the major human DNA ligase I and bacteriophage T4 DNA ligase. The compounds were assayed for their antibacterial activity via *in vivo* assay against *S. typhimurium* LT2 strain as per earlier reported protocols [51, 52].

(A) *In vitro* antitubercular evaluation

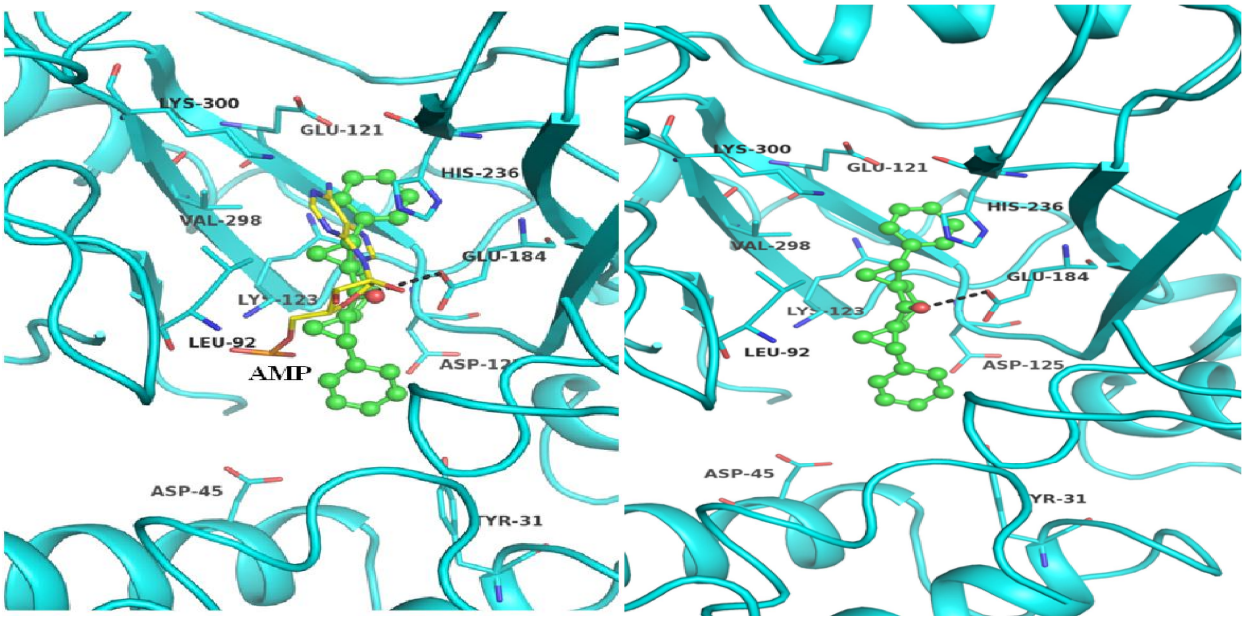
The above synthesized *dispiro* derivatives **2-19** were evaluated against virulent strain *M. tuberculosis H37Rv*. The MIC values were determined using the agar microdilution method [48]. As evident from Table 2, among all the compounds screened compounds **4** and **5** were found to possess good activity with MIC 6.25 µg/mL against virulent strain. However, compounds **3**, **7**, **8**, **10**, **12** and **19** displayed a moderate antitubercular activity with MIC of 12.5 µg/mL against virulent strain, while other compounds possess MIC values >12.5 µg/mL.

The activity results suggest that the conformation as well as substitution pattern in aromatic ring both govern the biological activity in synthesized molecules. The conformational changes in central alicyclic ring system have more impact over the substitution pattern in aromatic ring system. All these antiTB results are strongly correlated with the *in silico* as well as *in vitro* enzymatic assays.

(B) *In silico* screening

Molecular Interactions of LigA with the compounds

An analysis of the AutoDock predicted docking poses of all the compounds suggests that these inhibitors interact with several essential residues lining the AMP binding site with one of the aromatic ring of the inhibitor overlapping the adenine base of AMP and rest of the aromatic/aliphatic moieties projecting down the NAD^+ binding tunnel (Fig. 3a). All the compounds are making polar interactions with important active site residues like E184, R211, G126, and D125. The compounds are also making hydrophobic interactions particularly stacking with the H236 (Fig. 3a and 3b). Thus the stacking interaction seems to be the characteristic hallmark of the ligand recognition in the *Mtu*LigA inhibition and catalysis. Among all the compounds used in AutoDock study only four compounds **2**, **3**, **4** and **5** proved to be the best scored (Table 3).



(A) **(B)**

Fig 3a. Compound **2** (ball and stick) occupying same cavity as that occupied by AMP in the LigA binding pocket. The AMP is shown in yellow stick in panel A. In panel B, Compound I is shown as docked in the ligA binding cavity. The hydrogen bonding interactions are marked by dotted line.

(C) *In vitro* enzymatic assays

To identify the drug target, compounds **2**, **3**, **4** and **5** which were sorted out based on the scoring function and fitness scores (minimum docking energy) as implemented in the AUTODOCK program, were assayed against the full length NAD^+-dependent enzyme from *M. tuberculosis*, the major human DNA ligase I and bacteriophage T4 DNA ligase, respectively for the determination of *in vitro* inhibitory potency. Two compounds **3** and **5** showed selective inhibition of *M. tuberculosis* ligase were further evaluated for *in vivo* antibacterial activities.

***In vitro* inhibition of nick joining activity**

DNA ligase nick joining activity was done as described earlier in presence of varying concentration of inhibitors. To quick screening of inhibitors we carried out DNA ligation assay at high concentrations 100µM against both Mtu-LigA and T4Lig. This served as a sieve for selecting compounds with the potential to distinguish between NAD^+ and ATP-dependent ligases for detailed experiments. Based on the obtained results, subsequent efforts were focused on four compounds which we assayed for its *in vitro* inhibitory potency against the full length NAD^+-dependent enzyme from *M. tuberculosis*, the major human DNA ligase I and bacteriophage T4 DNA ligase, respectively. *In vitro* inhibition data IC_{50} (Table 3) shows that these compounds are inhibiting *Mtu*LigA in low micromolar range.

Table 3. In vitro inhibition of *M. tuberculosis* NAD^+-dependent DNA ligase (*Mtu*ligA), Human DNA ligase (HuligI) and T4 DNA ligase (T4 lig)

Compounds	IC_{50} (ε M) *Mtu*ligA	T4 lig	HuligI	Docking energy (Kcal/mol)
2	180±5	210±8.3	130±10.5	-6.30
3	8.6±0.3	33.4±3.1	45.4±2.2	-6.02
4	12.0±0.7	20.2±1.1	18.5±0.7	-6.85
5	7.3±0.5	70.2±3.6	58.6±3.2	-7.95

Out of four compounds, compound **2**, bound to the Human DNA ligase (HuligI) and T4 DNA ligase (T4 lig) with low affinities there is no selectivity of this compound for NAD^+ and ATP DNA ligases. On the other hand, compound **3** distinguishes between the ATP dependent DNA ligase and NAD^+ -dependent DNA ligase of *M. tuberculosis* by a factor of four and between human and *M. tuberculosis* enzyme by a factor of five and has high affinity for *M. tuberculosis* enzyme with IC_{50} of 8.6 µM. Compound **4** also showed greater affinity for *M. tuberculosis* enzyme while compound **5** showed highest affinity among all the four compounds. For *M. tuberculosis* enzyme the IC_{50} value for this compound is 7.3 µM and it can distinguish between NAD^+ dependent DNA ligase and ATP dependent DNA ligase by a factor of 8-10 (Fig. 4). A good correlation have been observed between the MIC of *in vitro* antitubercular activity and IC_{50} of *in vitro* inhibition of nick joining activity in compound 4, compound 5 is also have in good agreement.

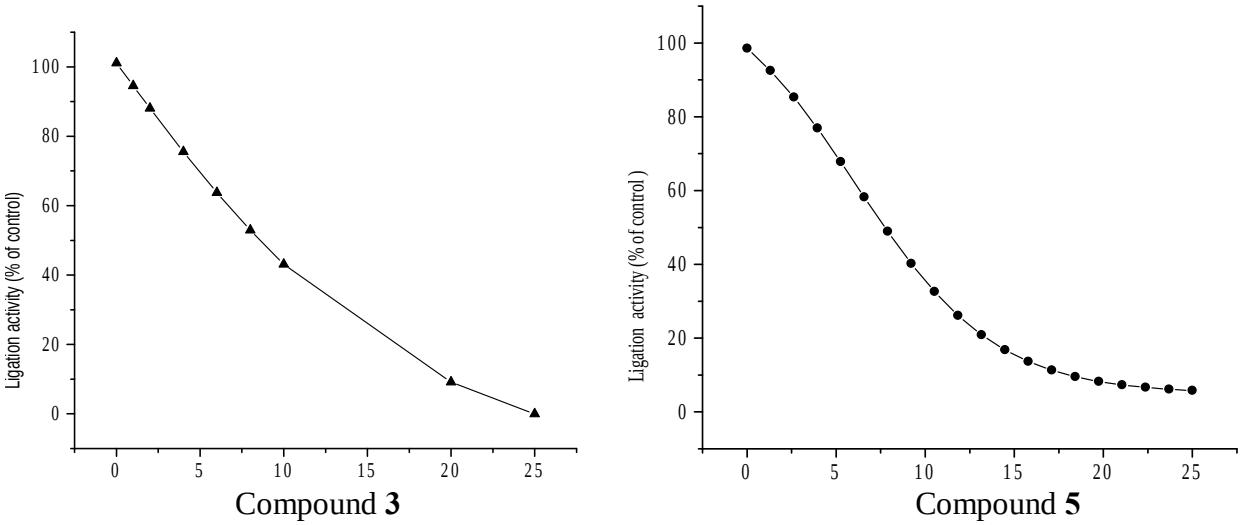
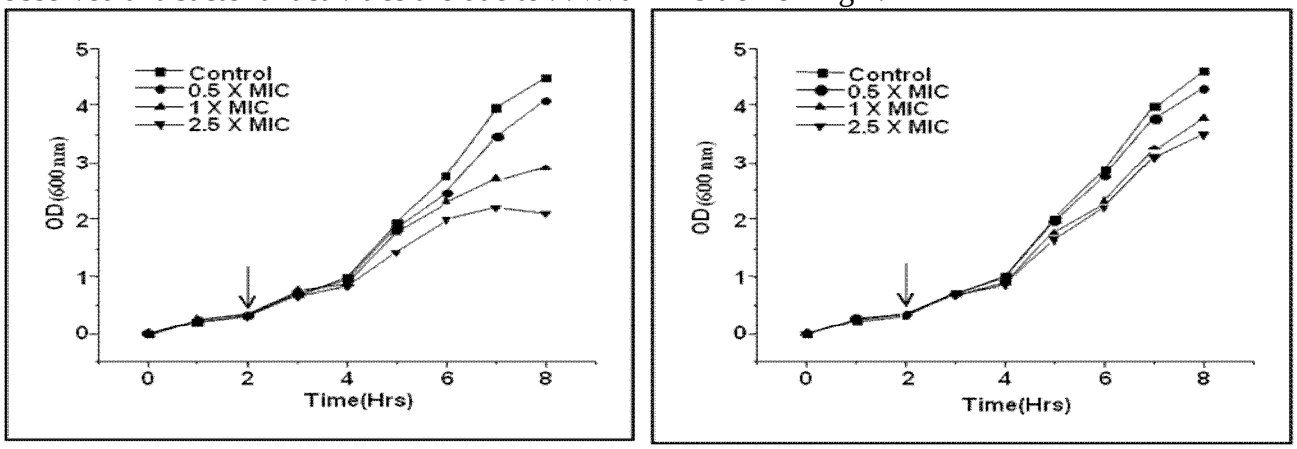


Fig 4. Inhibition of growth of *M. tuberculosis* (ligand affinity)

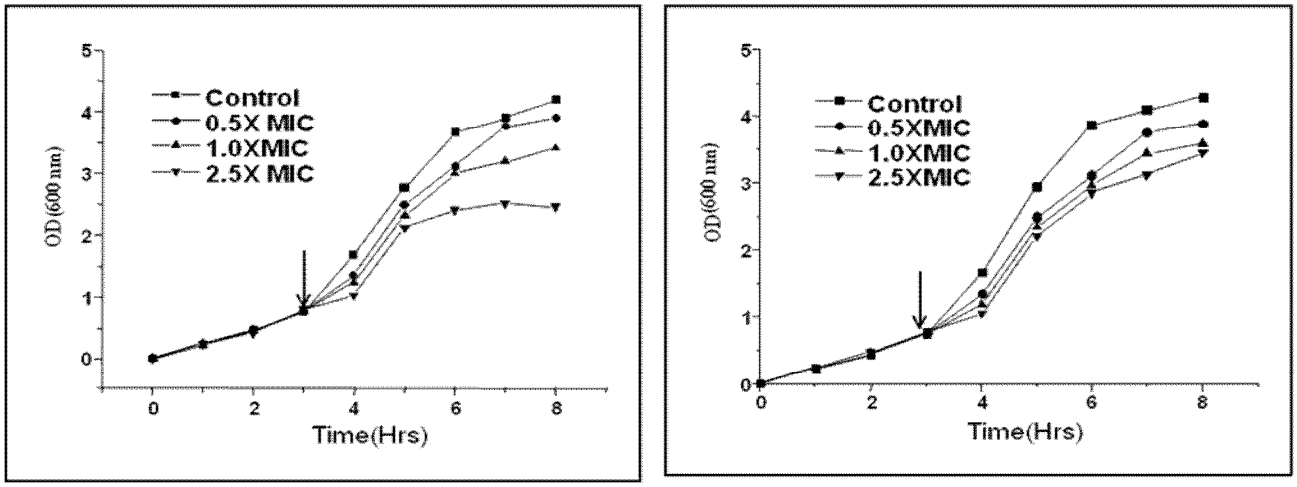
(D) Antibacterial/*in vivo* assay

To evaluate the *in vivo* inhibition of NAD^+ ligases, two bacterial systems were used and result depicted in Table 5 clearly shows that the compounds are more specifically inhibits NAD^+ dependent DNA ligase in compare to ATP dependent DNA ligases. Cell viability which was tested using the compound again shows that the wild type *S. typhimurium* LT2 strain is less viable at same compound concentrations compared to the viability of the ligase deficient variant rescued with T4Lig (Fig 5a and 5b, Table 4). The *In vivo* assay results demonstrate that compound has higher specificity for NAD^+-dependent ligases and strongly suggest that the observed antibacterial activities are due to *in vivo* inhibition of LigA.



(A) (B)

Fig 5a. Bactericidal activity of compound **3**. (**A**) *S. typhimurium* LT2 and (**B**) its DNA ligase minus (null) derivative TT15151 on their respective exposure to compound **3**



(A) (B)

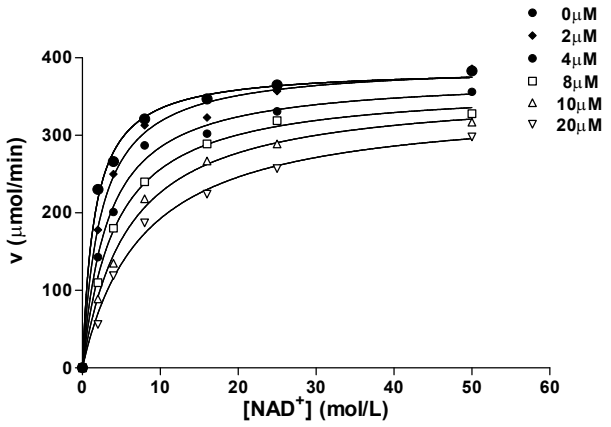
Fig 5b Bactericidal activity of compound **5**. (**A**) *S. typhimurium* LT2 and (**B**) its DNA ligase minus (null) derivative TT15151 on their respective exposure to compound **5**

. **Table 4.** Antibacterial Activity of compounds **3** and **5**

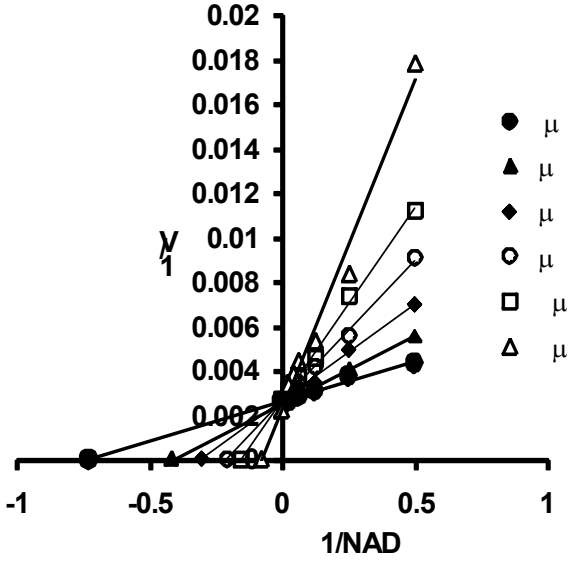
Compd. No.	MIC (ε g/ml)				
	S. typhimurium LT2	*S. typhimurium TT15151*	*E. coliGR501 +pTRC99A*	*E. coliGR501 +MtuNAD+ligase*	*E. coliGR501 T4 DNA ligase*
3	10	50	2	15	60
5	20	35	4	24	30

(E) Mode of inhibition

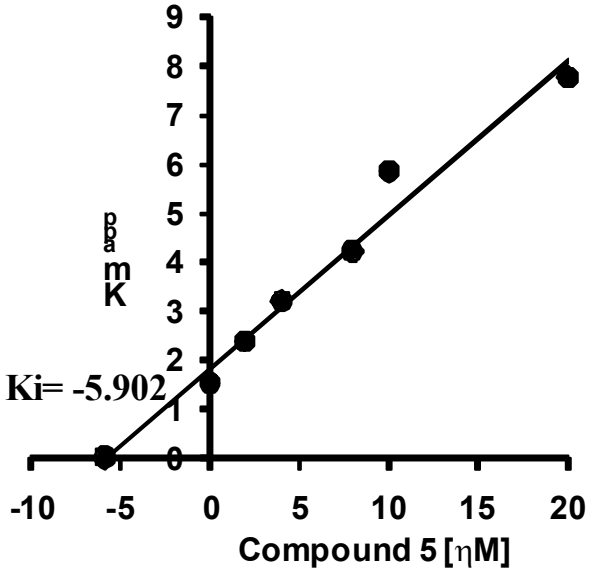
We chose compound **5** to evaluate the mode of inhibition standard kinetics was done with NAD^+ in over all nick sealing reaction *in vitro*. When nick joining activity was measured in presence of different concentration of Inhibitor (0-50µM) with increasing concentration of NAD^+, the kinetics clearly indicates a competitive inhibition of NAD^+ by compound **5** (Fig 6). The linear regration using the apparent Km value leads to Ki value of 5.902µM.



(a)



(b)



(c)

Fig 6: Mode of Inhibition of MtuLigA with respect to NAD^+ by Compound **5**

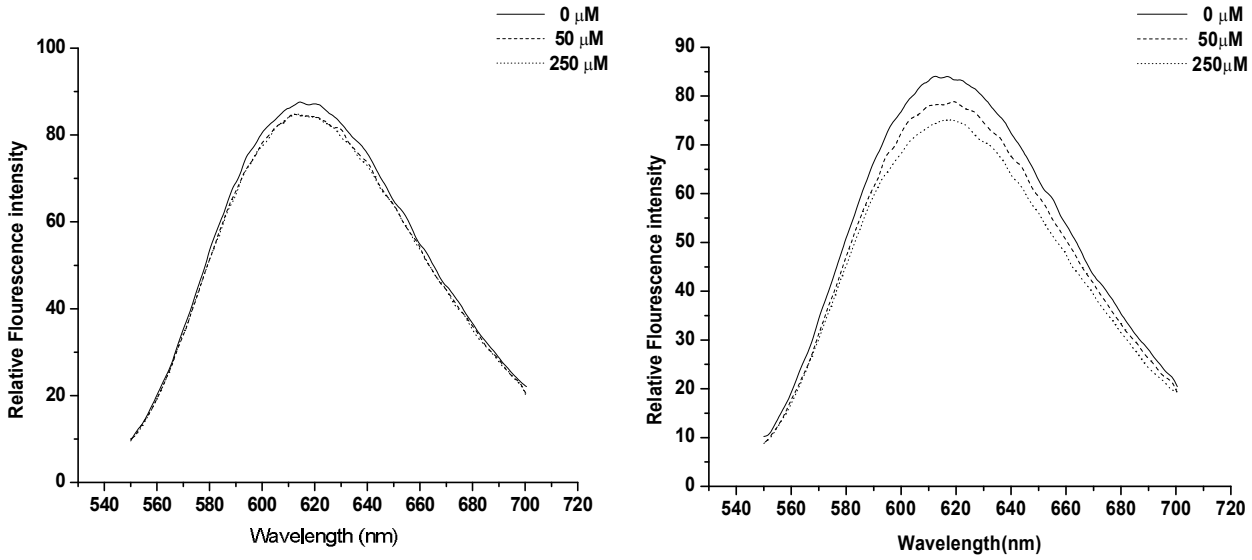
(a) Activity of MtuLigA measured in the presence of rising concentrations of NAD^+ (0 ï 50 µM) and compound 2 (0 ï 20 µM).

(b) A double reciprocal plot of the data clearly indicates competitive binding between NAD^+ and Compound **5.**

(c) Linear regression plot of the inhibitor concentration *versus* the Km_{app}. The K_i value is marked with an arrow

(F) DNA binding assay

In order to check whether the compounds are generally interact with DNA and thereby influence the inhibitory behavior, we carried out ethidium bromide displacement assays. Compounds were added to a maximum concentration of 250 ε M. Even at this high concentration, representing a 50-fold excess over ethidium bromide (5 ε M) no loss in fluorescence was observed (Fig.7). We also carried out gel shift assays where the electrophoretic mobility of plasmid DNA was checked in the presence of increasing inhibitor concentrations. The experiments did not support any general interaction of compounds **3** and **5** with DNA.



Compound **3** Compound **5**

Fig 7. Ethidium bromide displacement assay.

Conclusion

In conclusion, a series of *dispiro*-cycloalkanones with conformationally different cycloalkyl ring systems is synthesized and evaluated against *M. tuberculosis* H37Rv *in vitro* and full length of mycobacterial NAD^+- dependent DNA ligase. Few of the compounds showed moderate to significant antitubercular and DNA ligase inhibitory activities. The possible mode of actions of these compounds was also evaluated by using *in silico* screening, *in vitro* and *in vivo* enzymatic assays.

Acknowledgment

This is a CDRI communication no.8017. The authors thank UGC and CSIR New Delhi for the fellowship. We sincerely acknowledge the financial assistance from DRDO and DBT New Delhi.

SAIF CDRI is also acknowledged for providing the spectral and microanalytical data of the compounds.

References

1. M. Teresa, G. Lugo and C. A. Bewley, *J. Med. Chem.,* 2008, **51***,* 2606ï 2612.
2. Y. Zhang, K. P. Martens and S. Denkin, *Drug Dicov. Today,* 2006, **11**, 21-27.
3. Y. L. Janin, *Bioorg. Med. Chem.,* 2007, **15***,* 2479ï 2513.
4. K. Duncan and C. E. Barry, *Curr. Opin. Microbiol.,* 2004, **7**, 460ï 465.
5. World Health Organization. *Global Tuberculosis Control: Surveillance, Planning, and Financing*; WHO Report 2008; WHO Press: Geneva, Switzerland.
6. M. Jassal and W. R. Bishai, *Lancet Infect.,* 2009, **155**, 19-30.
7. R. P. Tripathi, N. Tewari, N. Dwivedi and V. K. Tiwari, *Med. Res. Rev.,* 2005, **25**, 93-131.
8. S. H. Gillespie, *Antimicrob. Agents Chemother*., 2002, **46**, 267-274.
9. G. J. Ebrahim, *J. Tropic. Pediat.,* 2007, **53**, 147-149.
10. E. Huitric, P. Verhasselt, A. Koul, K. Andries, S. Hoffner and D. I. Andersson, *Antimicrob. Agents Chemother.,* 2010, **54**, 1022-1028.
11. D. Jones, *Nat. Rev. Drug Discovery,* 2005, **4**, 103-103.
12. N. R. Gandhi, A. Moll and A. W. Sturm, *Lancet,* 2006, **4***,* 1575-1580.
13. M. C. Raviglione, *N. Engl. J. Med*., 2008, **7**, 636-638.
14. Y. Zhang, C. Vilcheze and W. R. Jacobs, in *Tuberculosis and the tubercle bacillus,* (ed.) S. T. Cole, K. D. Eisenach, D. N. McMurray and W. R. Jacobs, Jr., ASM Press, Washington, DC. 2005, pp. 115-142.
15. M. K. Machala, E. Rychta, A. Brzostek, H. R. Sayer, A. R. Galewicz, R. P. Bowater and J. Dziadek, *Antimicrob. Agents Chemother*., 2007, **51**, 2888-2897.
16. I. R. Lehman, *Science* 1974, **186**, 790-797.
17. M. J. Engler and C. C. Richardson, in *The Enzymes,* ed. P. D. Boyer, Academic Press, New York., 1982, vol. **15**, pp. 3-29,
18. A. Wilkinson, J. Day, and R. Bowater, *Mol. Microbiol.* 2001*,* **40**, 1241-1248.
19. V. Sriskanda, R. W. Moyer and S. J. Shuman, *Biol. Chem.* 2001, **276**, 36100-36109.
20. D. J. Timson, M. R. Singleton and D. B. Wigley, *Mutat. Res.,* 2000, **460**, 301-318.
21. A. J. Doherty and S. W. Suh, *Nucleic Acids Res.* **2000**, 28, 4051-4058.
22. J. M. Pascal, P. J. OÕBrien, A. E. Tomkinson and T. Ellenberger, *Nature* 2004, **432**, 473-478.
23. K. C. Gajiwala, and C. Pinko, *Structure (Camb*)., 2004, **12**, 1449-1459.
24. J. Y. Lee, C. Chang, H. K. Song, J. Moon, J. K. Yang, H. K. Kim, S. T. Kwon and S. W. Suh, *EMBO J.,* 2000, **19**, 1119-1129.
25. C. Gong, A. Martins, P. Bongiorno, M. Glickman and S. Shuman, *J. Biol. Chem.* 2004, **279**, 20594-20606
26. A. Wilkinson, H. Sayer, D. Bullard, A. Smith, J. Day, T. Kieser and R. P. Bowater, *Proteins Struct. Funct. Genet.,* 2003, **51**, 321-326.
27. J. J. Dermody, G. T. Robinson and R. Sternglanz, *J. Bacteriol.,* 1979, **139**, 701-704.
28. F. S. Kaczmarek, R. P. Zaniewski, T. D. Gootz, D. E. Danley, M. N. Mansour, M. Griffor, A. V. Kamath, M. Cronan, J. Mueller, D. Sun, P. K. Martin, B. Benton, L. McDowell, D. Biek and M. B. Schmid, *J. Bacteriol.,* 2001, **183**, 3016-3024.

29. M. A. Petit and S. D. Ehrlich, *Nucleic Acids Res.,* 2000, **28**, 4642-4648.
30. C. M. Sassetti, D. H. Boyd and E. J. Rubin, *Mol. Microbiol.,* 2003, **48**, 77-84.
31. G. Ciarrocchi, D. G. MacPhee, L. W. Deady and L. Tilley, *Antimicrob. Agents Chemother.,* 1999, **43**, 2766-2772.
32. H. Brotz-Oesterhelt, I. Knezevic, S. Bartel, T. Lampe, U. Warnecke-Eberz, K. Ziegelbauer, D. Habich and H. Labischiinski, *J. Biol. Chem*., 2003, **278**, 39435-39442.
33. S. K. Srivastava, R. P. Tripathi and R. Ramachandran, *J. Biol. Chem.,* 2005, **280**, 30273-30281.
34. S. K. Srivastava, D. Dube, N. Tewari, N. Dwivedi, R. P. Tripathi and R. Ramachandran *Nucleic Acids Research*, 2005, **33**, 7090ï 7101.
35. D. Katiyar, V. K. Tiwari, R. P. Tripathi, A. Srivastava, V. Chaturvedi, R. Srivastava and B. S. Srivastava, *Bioorg. Med. Chem.,* 2003, **11**, 4369-4375.
36. N. Tewari, V. K. Tiwari, R. P. Tripathi, V. Chaturvedi, A. Srivastava, R. Srivastava, P. K. Shukla, A. K. Chaturvedi, A. Gaikwad, S. Sinha and B. S. Srivastava, *Bioorg. Med. Chem. Lett.,* 2004, **14***,* 329-332.
37. R. C. Mishra, R. Tripathi, D. Katiyar, N. Tewari, D. Singh and R. P. Tripathi, *Bioorg. Med. Chem.,* 2003, **11***,* 5363-5374.
38. N. Tewari, V. K. Tiwari, R. C. Mishra, R. P. Tripathi, A. K. Srivastava, R. Ahmad, R. Srivastava and B. S. Srivastava, *Bioorg. Med. Chem.,* 2003, **11***,* 2911-2922.
39. N. Tewari, V. K. Tiwari, D. Katiyar, N. Saxena, S. Sinha, A. Gaikwad, A. Srivastava, V. Chaturvedi, Y. K. Manju, R. Srivastava and B. S. Srivastava, *Bioorg. Med. Chem*., 2005, **13**, 5668-5679.
40. N. Singh, J. Pandey, A. Yadav, V. Chaturvedi, S. Bhatnagar, A. Gaikwad, S. Sinha, A. Kumar, P. K. Shukla and R. P. Tripathi, *Eur. J. Med. Chem.,* 2009, **44***,* 1705-1709.
41. N. Dwivedi, N. Tewari, V. K. Tiwari, V. Chaturvedi, Y. K. Manju, A. Srivastava, A. Giakwad, S. Sinha and R. P. Tripathi, *Bioorg. Med. Chem. Lett.,* 2005, **15**, 4526-4530.
42. A. Ajay, V. Singh, S. Singh, S. Pandey, S. Gunjan, D. Dubey, S. K. Sinha, B. N. Singh, V. Chaturvedi, R. Tripathi, R. Ramchandran and R. P. Tripathi, *Bioorg. Med. Chem.,* 2010, **18**, 8289-8301.
43. E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, 1965, **87**, 1353-1364.
44. M. H. Abraham and Y. H. Zhao, *J. Org. Chem.* 2004, **69**, 4677-4685.
45. S. Chandrasekhar, C. Narasihmulu, V. Jagadeshwar and K. V. Reddy, *Tetrahedron Lett.*, 2003, **44**, 3629-3630.
46. R. J. Paxton and R. J. K. Taylor, *Synlett*, 2007, **4**, 633-637.
47. A. Hartikka and P. I. Arvidsson, *J. Org. Chem.,* 2007, **72**, 5874-877.
48. H. Saito, H. Tomioka, K. Sato, M. Emori, T. Yamane, K. Yamashita, K. Hosol and T. Hidaka, *Antimicrob. Agents Chemother.,* 1991, **35**, 542-547.
49. ACCELRYS ver., 11. San Diego, *CA, Accelrys Inc*., 2000, 92121-92152.
50. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey and W. E. Hart, *J. Comput. Chem.*, 1998, **19**, 1639-1662.
51. J. B. Le-Pecq and C. Paoletti, *J. Mol. Biol.,* 1967, **27**, 87.
52. S. K. Srivastava, R. P. Tripathi and R. Ravishankar, *J. Biol. Chem.,* 2005, **280**, 30273-30281.