Chem Biol Drug Des 2014; **83:** 498–506

Research Letter

Discovery and Structure Optimization of a Series of Isatin Derivatives as *Mycobacterium tuberculosis* Chorismate Mutase Inhibitors

Variam U. Jeankumar, Reshma Alokam, Jonnalagadda P. Sridevi, Priyanka Suryadevara, Siddharth S. Matikonda, Santosh Peddi, Seedarala Sahithi, Mallika Alvala, Perumal Yogeeswari and Dharmarajan Sriram*

Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Shameerpet, R.R. District, Hyderabad, Andhra Pradesh 500078, India
**Corresponding author: Dharmarajan Sriram, drdsriram@yahoo.com*

In this study, the crystal structure of the *Mycobacterium tuberculosis* (MTB) enzyme chorismate mutase (CM) bound to transition state analogue (PDB: 2FP2) was used as a framework for virtual screening of the BITS-Pilani in-house database (2500 compounds) to identify new scaffold. We identified isatin as novel small molecule MTB CM inhibitors; further twenty-four isatin derivatives were synthesized and evaluated *in vitro* for their ability to inhibit MTB CM, and activity against *M. tuberculosis* as steps towards the derivation of structure–activity relationships (SAR) and lead optimization. Compound 3-(4-nitrobenzylidene)indolin-2-one, 24 emerged as the most promising lead with an IC_{50} of 1.01 ± 0.22 μM for purified CM and MIC of 23.5 μM for M. tuberculosis, with little or no cytotoxicity.

Key words: chorismate mutase, isatin, *Mycobacterium tuberculosis*, tuberculosis

Abbreviations: e-pharmacophore, energy-based pharmacophore; MIC, minimum inhibitory concentration; MTB CM, *Mycobacterium tuberculosis* chorismate mutase.

Received 26 June 2013, revised 25 October 2013 and accepted for publication 4 November 2013

Tuberculosis (TB), an infection caused by *Mycobacterium tuberculosis* (MTB), has become a major global health concern. Presently, MTB kills approximately 1.6 million people annually and has infected one-third of the world population, rendering TB the second most lethal infectious disease after HIV/AIDS.[a] With 400 000 new cases annually, multiple drug-resistant TB is emerging rapidly, calling urgently for new anti-TB therapeutics. No truly new TB drugs have been developed for nearly 40 years since the introduction of rifampicin in 1965. It is thus necessary to search for new and more effective antimycobacterial agents with novel mechanisms of action to combat the emergence of drug resistance and to shorten the duration of therapy (1). Chorismate occupies a central place in the shikimate pathway for the biosynthesis of the aromatic amino acids. Chorismate mutase (CM) (EC 5.4.99.5) catalyses the Claisen rearrangement of chorismate to prephenate *via* an *endo*-oxabicyclic transition state (Figure 1), the first committed step in the biosynthesis of tyrosine and phenylalanine in bacteria (2). *Mycobacterium tuberculosis* possess two chorismate mutases belonging to different subclasses (3), the secreted enzyme (*MtCM, γ-subclass) and the housekeeping enzyme (MtCM, δ-subclass), whereas the former is primarily involved in directing the protein from the cytoplasm to the pseudoperiplasmic space and may have some role in host–pathogen interaction. It is the secreted enzyme (usually referred to as *MtCM) that is likely related to pathogenicity and virulence of MTB (4–8). As vertebrates do not possess the shikimate pathway for the biosynthesis of aromatic compounds and lacks CM activity, this enzyme represents a prime target for the development of antibacterials. In spite of the tremendous opportunity that MTB CM offers as a target for the development of potential antimicrobial agents, very little has been achieved in this regard. A structure-based discovery programme by Agrawal *et al.* has earlier identified four molecules as potential inhibitors of *M. tuberculosis* chorismate mutase (MtCM) with low K_i values (9). The most promising analogue in the study (4-(3,4-dimethoxyphenethylamino)-3-nitro-5-sulfamoylbenzoic acid) exhibited a K_i of 5.7 ± 1.2 μM. But later an *in vitro* (8) and *in vivo* (10) evaluation of a library of molecules designed and synthesized based on Agrawal claims proved to be completely ineffective, with Munack *et al.* (8) recently discrediting Agrawal's work in his study, as the reported lead molecules proved to be inactive. Although the recent research efforts from Pal and his co-workers identifying a series of *N*-aryl substituted thieno[2,3-d]pyrimidin-4(3H)-ones (11), 3–chloroquinoxaline (12), fused triazinone (13) and 2-aminochromenes (14) derivatives as potential *MtCM inhibitors with IC_{50} values in the lower micromolar range are encouraging, MTB CM inhibitors with K_i/IC_{50} values significantly below the μM range still await discovery.

 doi: 10.1111/cbdd.12265

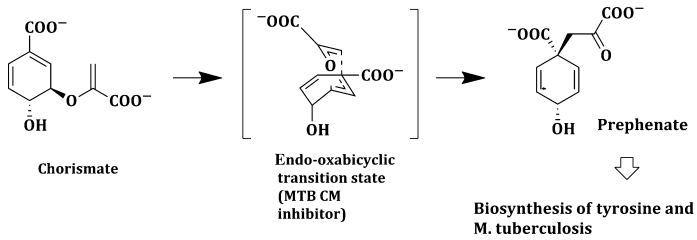
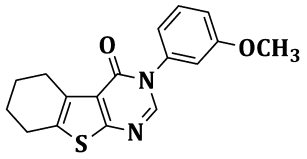


Figure 1: Chorismate mutase reaction scheme.

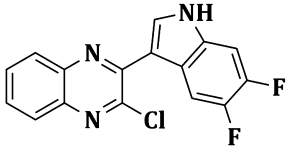
This study describes the *in silico* discovery of isatin as novel small molecule inhibitor of MTB chorismate mutase, and further, a library of its derivatives was synthesized and was evaluated *in vitro* for their ability to inhibit MTB CM enzyme and whole-cell MTB as steps towards the derivation of structure–activity relationships.

Rational inhibitor design relies both on mechanistic and structural information about the target enzyme. The mechanism of the uncatalysed MTB CM reaction is known to involve a transition state with a chair-like conformation, Figure 1. In fact, the *endo*-oxabicyclic dicarboxylic acid (15), a good geometric mimic of this transition state, is currently the best inhibitor of a broad range of chorismate mutases consistent with the hypothesis that the enzymatic process proceeds through a corresponding bicyclic transition state (16). In this study, an energy-based pharmacophore model (e-pharmacophore), developed using chorismate mutase-transition state analogue (CM-TSA) complex as a template, was explored to carry out a high-throughput virtual screening of our in-house (BITS-Pilani) small molecule database (2500 compounds). Glide XP (extra precision) module of Schrödinger 9.2 was utilized for docking of the molecules on to the active site pocket of MTB CM, and isatin was identified as a potential lead (Figure 2). The methodology employed in e-pharmacophore generation and molecular docking approach has been described thoroughly in the experimental section of supporting information (Appendix S1). Important interactions as evidenced from docking studies for isatin were associated with Arg 134 and Lys 60. Such interactions were predicted to be crucial for enzyme activity, diagrammatic representation of which in comparison with that of transition state analogue is given in Figure 3. In the enzyme inhibition studies, isatin showed excellent MTB CM inhibition with IC_{50} of 1.08 ± 0.1 μM. To investigate the potential of this lead, a series of substituted isatin derivatives were synthesized and evaluated for their ability to inhibit MTB CM enzyme, to explore the structure-activity relationship (SAR) as well as to evaluate their antimycobacterial potency.

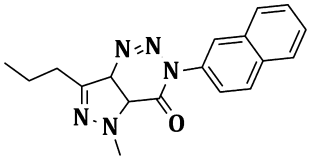
A library was designed with the goal of obtaining a lead series with tractable SAR and potencies better than the previously identified virtual screening hit, isatin **1**. The isatin derivatives were synthesized from isatin by a simple and straightforward route as delineated in Schemes 1, 2 and 3. It was decided to first explore the *N*-1 position of isatin by introducing various alkyl, aryl and amide derivatives to understand its importance in activity determination. Alkylation of isatin with corresponding alkyl iodides using Cs_2CO_3 as base in acetonitrile gave the desired



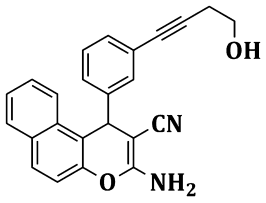
3-(3-Methoxyphenyl)-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one
IC_{50} = 19.80 ± 1.02 μM



2-chloro-3-(5,6-difluoro-1*H*-indol-3-yl)quinoxaline
IC_{50} = 19.74 μM



5-Naphthyl-7-propyl-3H-pyrazolo [4,3-d][1,2,3]triazin-4(5h)-one
IC_{50} = 15.13 ± 0.95 μM



3-amino-1-(3-(4-hydroxybut-1-yn-1-yl)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile
IC_{50} = 15.63 μM

Figure 2: Reported *Mycobacterium tuberculosis* chorismate mutase inhibitors.

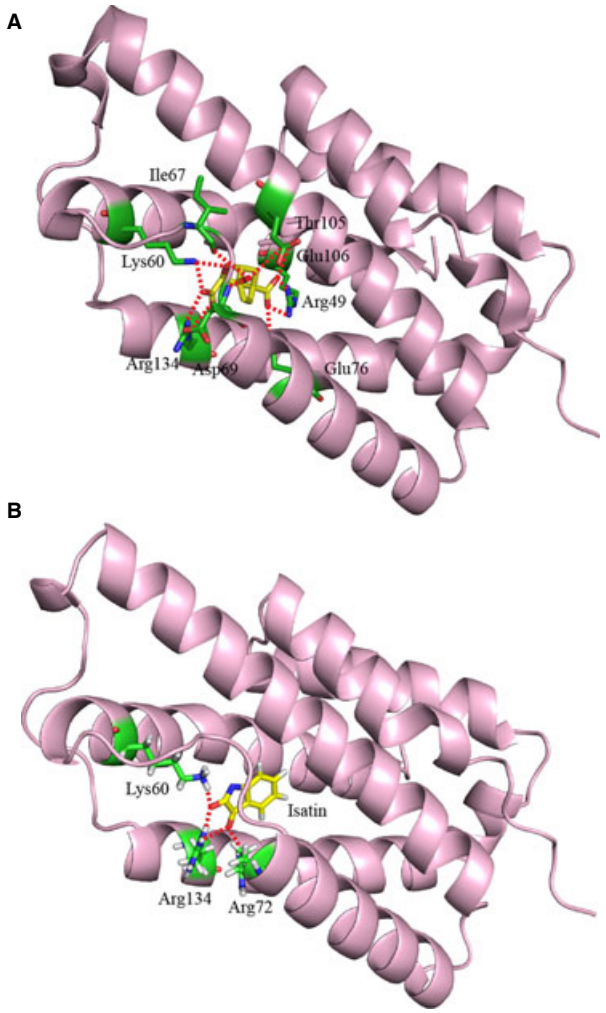
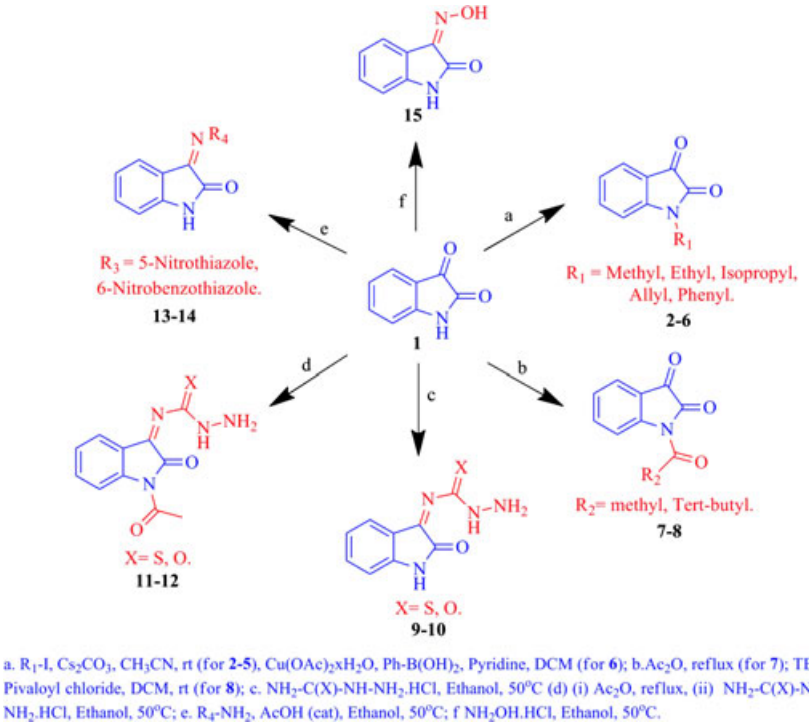


Figure 3: View of key interactions among the residues in the active site of MTB CM. Amino acids are represented in green (C), blue (N), red (O) and hydrogen (H); ligand molecules are represented with yellow. (C) coloured atoms. Polar contacts are in blue dashes. (A) Interaction of TSA, (B) interaction of isatin.

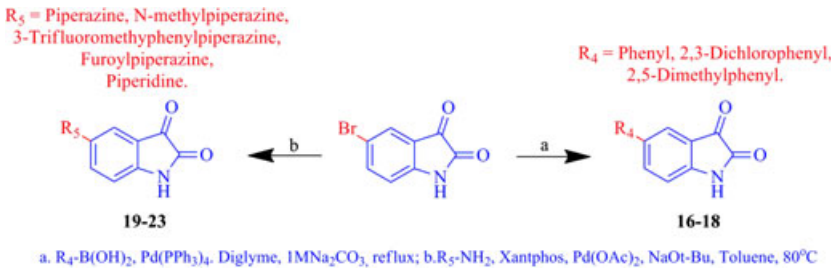
alkylated derivatives, compounds **2**–**5** in good yield (17,18). The Chan–Lam methodology (19,20) was successful in achieving the *N*-phenyl derivative **6**. *N*-Acetyl derivative **7** was prepared by acetylating with acetic anhydride under refluxing conditions (21), and pivaloyl group **8** was introduced by treatment with pivaloyl choride using triethylamine as base in tetrahydrofuran. We then focused on the carbonyl group at C-3 position, which was further modified into their corresponding semicarbazone, thiosemicarbazone and oxime derivatives (22–24) **9**–**10** & **15**, by refluxing with semicarbazide, thiosemicarbazide and hydroxylamine hydrochloride, respectively, in ethanol. A similar strategy was also employed in the case of the *N*-acetyl isatin as well to give the *N*-acetyl analogue **11**–**12**. Schiff bases **13**–**14** were prepared by refluxing isatin with the corresponding amines in ethanol in the presence of catalytic amount of acetic acid (25). All the reactions went smoothly giving the desired products in good yield. To understand the role of various substituents in phenyl core of isatin, various aryl and heteroaryl/amino groups were introduced at the 5th position of isatin core. The introduction of the aryl functionality was brought about by palladium catalysed cross-coupling reaction as described by Martinez *et al.* (26). Buchwald–Hartwing cross coupling was utilized to synthesize the *N*-aryl derivatives (27–30). Two condensation products **24**–**25** were also synthesized via Knoevenagel condensation of the corresponding aldehydes (31,32) with oxindole, which in turn was prepared by the one-pot Wolff–Kishner like reduction in isatin with hydrazine hydrate (33,34). All the synthesized compounds (Table 1) were characterized by ^{1}H & ^{13}C NMR, LC-MS and purity checked by elemental analysis.

The characterized isatin derivatives were then subjected to MTB CM enzyme inhibition study by adapting a previously reported protocol (5) to a 96-well plate format, as detailed in the experimental section of supporting information (Appendix S1) and the IC_{50} values reported in Table 1. The original hit compound isatin **1** showed IC_{50} of 1.08 ± 0.1 μM. Of twenty-four isatin derivatives synthesized, three molecules exhibited promising potency comparable with that of the initial lead isatin with IC_{50} of 0.99 ± 0.14 μM for 1-acetyl isatin, **7**, 1.01 ± 0.22 μM for 3-(4-nitrobenzylidene)indolin-2-one, **24**, and 0.93 ± 0.1 μM for 3-((5-nitrothiophen-2-yl)methylene)indolin-2-one, **25**. Dose–response curves were plotted for the more potent inhibitors **1**, **7**, **24**, **25** and the positive control carvacrol, using graphpad prism software by taking log (inhibitor concentration) on *X*-axis and response (% inhibition) on *Y*-axis as shown in Figure 4. To analyse the interaction pattern of the active molecule reported from assay in the active site of protein, molecular docking approach was adopted. The compounds were docked to the protein active site using extra precision mode (XP) of Glide module and their docking score, and interaction pattern was compared with that of their *in vitro* activity, as steps towards establishing a structure–activity relationship.

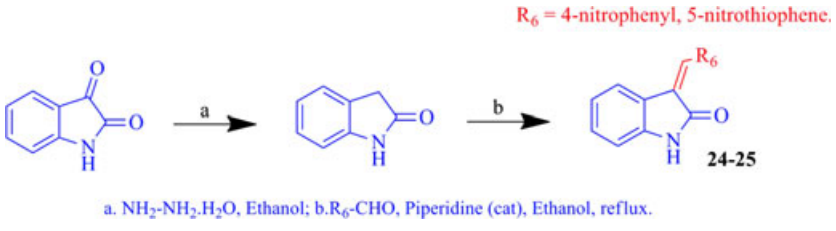
The lead compound isatin, which exhibited an *in vitro* activity of about 1.08 ± 0.10 μM, was found to be associated in five prominent hydrogen bonds with the active site residues. The oxygen atom at R^1 position was found to be bonded with Arg 72 and Arg 134. The hydrogen on nitrogen atom (N-1) was found to be in polar contact with the carboxyl oxygen of Glu 109, and the carbonyl oxygen next to nitrogen atom was found associated with Lys 60 and Arg 134. Various substitutions on isatin nucleus gave compounds with a wide range of activity. With respect to structure–activity relationship, introduction of alkyl group at N-1 position of isatin made the compounds **2**–**5** less active (IC_{50} ranging from 1.96 ± 0.43 to 96.56 ± 5.2 μM) and increasing chain length decreased the activity drastically (*N*-methyl > *N*-ethyl > *N*-*iso*propyl > *N*-allyl). This decline in activity may be attributed to



Scheme 1: Synthetic protocol adopted to attain compounds **2–15**; modifications explored at N-1 and C-3 positions.



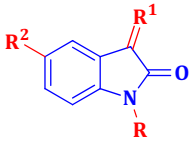
Scheme 2: Synthetic protocol adopted to attain compounds **16–23**; modifications explored at C-5 position.



Scheme 3: Synthetic protocol adopted to attain compounds **24–25**; modifications explored at C-3 position.

the loss of polar contact of compounds with Glu 109. *N*-Phenyl isatin showed a moderate IC_{50} of 23.3 ± 2.0 μM. Introduction of simple acetyl group made the compound (**7**) equally active (0.99 ± 0.14 μM) or slightly more potent than isatin, Figure 5, whereas bulky acyl derivative like pivaloyl derivative **8** reduced the activity many fold (41.0 ± 3.6 μM). Replacement of oxygen on isatin at R^1 position with semicarbazones and thiosemicarbazones **9–10** and its *N*-acyl derivatives **11–12** brought about a decline in activity, indicating the importance of third ketone group, which forms the hydrogen bond with Arg 72 and Arg134 of MTB CM. Schiff bases of isatin derivatives **13–14** with 2-amino-5-nitrothiazole and 2-amino-6-nitrobenzothiazole made the compounds completely inactive (>100 μM), suggesting that the substitution of aromatic bulkier groups at R^1

Table 1: Biological activities of isatin derivatives



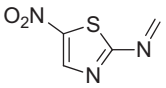
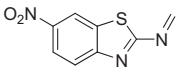
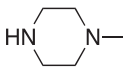
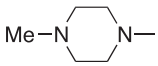
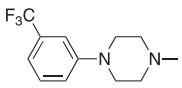
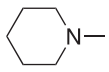
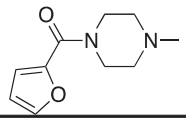
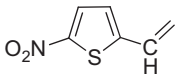
Compound ID	*R*	R^1	R^2	M.P (°C)	IC_{50} (μM)[a]	MIC (μM)[b]	Cytotoxicity[c] % inhibition at 50 μM	Cytotoxicity[c] % inhibition at 100 μM
1	H	O	H	199–200	1.08 ± 0.10	340.13	39.53 ± 0.04	47.10 ± 0.03
2	CH_3–	O	H	133–134	1.96 ± 0.43	310.60	28.1 ± 0.12	38.6 ± 0.06
3	C_2H_5–	O	H	86–88	22.70 ± 3.12	285.71	14.06 ± 0.10	31.6 ± 0.06
4	$(CH_3)_2CH$–	O	H	76–77	75.12 ± 5.20	132.28	13.93 ± 0.02	25.80 ± 0.08
5	CH_2=$CHCH_2$–	O	H	89–90	96.56 ± 5.20	267.38	39.89 ± 0.15	64.90 ± 0.24
6	C_6H_5–	O	H	139–141	23.30 ± 2.00	28.02	9.77 ± 0.04	29.09 ± 0.13
7	CH_3CO–	O	H	141–142	0.99 ± 0.14	228.30	17.31 ± 0.05	24.62 ± 0.02
8	$(CH_3)_3CCO$–	O	H	157–158	41.00 ± 3.60	125.63	27.81 ± 0.02	38.57 ± 0.06
9	H	$NH_2NHC(S)N$=	H	232–234	66.90 ± 5.80	60.96	29.97 ± 0.01	31.71 ± 0.05
10	H	$NH_2NHC(O)N$=	H	266–268	54.09 ± 2.40	132.28	30.07 ± 0.14	40.36 ± 0.21
11	CH_3CO–	$NH_2NHC(S)N$=	H	247–249	30.50 ± 2.60	50.59	25.91 ± 0.02	38.6 ± 0.01
12	CH_3CO–	$NH_2NHC(O)N$=	H	235–237	25.00 ± 3.40	108.24	22.99 ± 0.10	32.31 ± 0.04
13	H		H	191–193	>100.00	91.24	0.05 ± 0.15	23.27 ± 0.20
14	H		H	124–126	>100.00	77.16	29.53 ± 0.07	36.58 ± 0.07
15	H	HON=	H	227–229	98.14 ± 6.20	77.16	25.22 ± 0.06	42.62 ± 0.08
16	H	O	C_6H_5–	208–211	37.10 ± 2.50	112.10	0.46 ± 0.18	32.76 ± 0.02
17	H	O	2,3-$(Cl)_2C_6H_3$–	223–225	95.70	85.64	25.10 ± 0.14	32.43 ± 0.10
18	H	O	2,5-$(CH_3)_2C_6H_3$–	156–159	36.50	119.20	26.74 ± 0.08	31.95 ± 0.09
19	H	O		109–111	39.90 ± 2.80	108.22	31.61 ± 0.01	42.56 ± 0.03
20	H	O		133–135	31.90 ± 2.60	102.04	23.32 ± 0.01	31.18 ± 0.02
21	H	O		131–133	55.70 ± 1.80	66.50	60.58 ± 0.04	87.10 ± 0.01
22	H	O		154–156	25.60 ± 1.50	108.69	29.97 ± 0.07	40.98 ± 0.04
23	H	O		146–149	28.80 ± 1.20	38.46	21.22 ± 0.01	33.27 ± 0.07

Table 1: continued

Compound ID	*R*	R^1	R^2	M.P (°C)	IC_{50} (μM)[a]	MIC (μM)[b]	Cytotoxicity[c] % inhibition at 50 μM	Cytotoxicity[c] % inhibition at 100 μM
24	H	4-NO_2-C_6H_4CH=	H	223–225	1.01 ± 0.22	23.50	19.97 ± 0.05	24.17 ± 0.02
25	H		H	234–236	0.93 ± 0.10	183.83	18.03 ± 0.01	25.57 ± 0.05
26		Isoniazid		nd	nd	0.66	nd	nd
27		Rifampicin		nd	nd	0.23	nd	nd
28		Ethambutol		nd	nd	15.31	nd	nd

nd, not determined.
[a]MTB chorismate mutase.
[b]Minimum inhibitory concentration against *Mycobacterium tuberculosis* H37Rv.
[c]RAW 264.7 cell line.

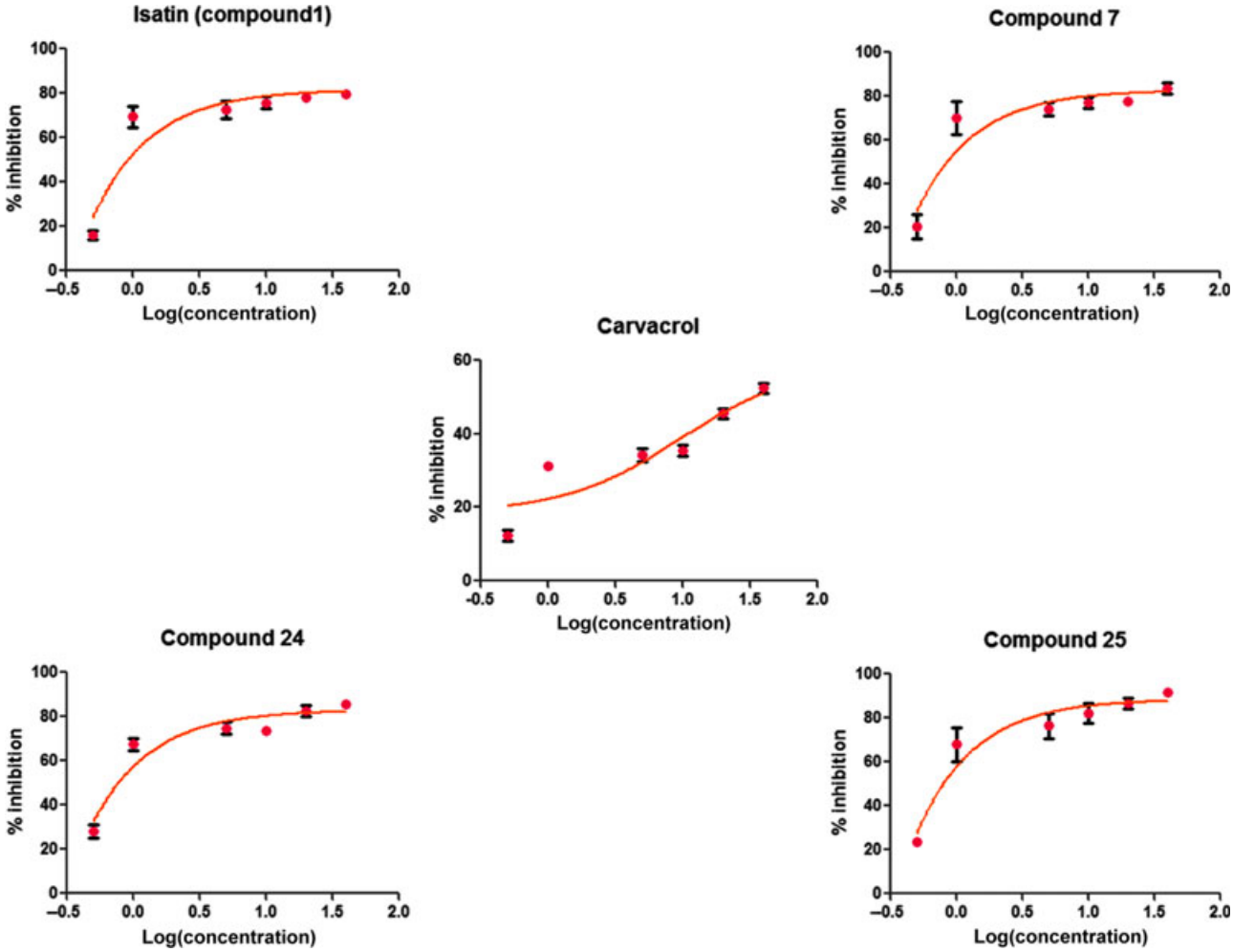


Figure 4: Dose–response curve with error bars (black colour) for the positive control carvacrol and potent inhibitors compound **1**, **7**, **24** and **25**.

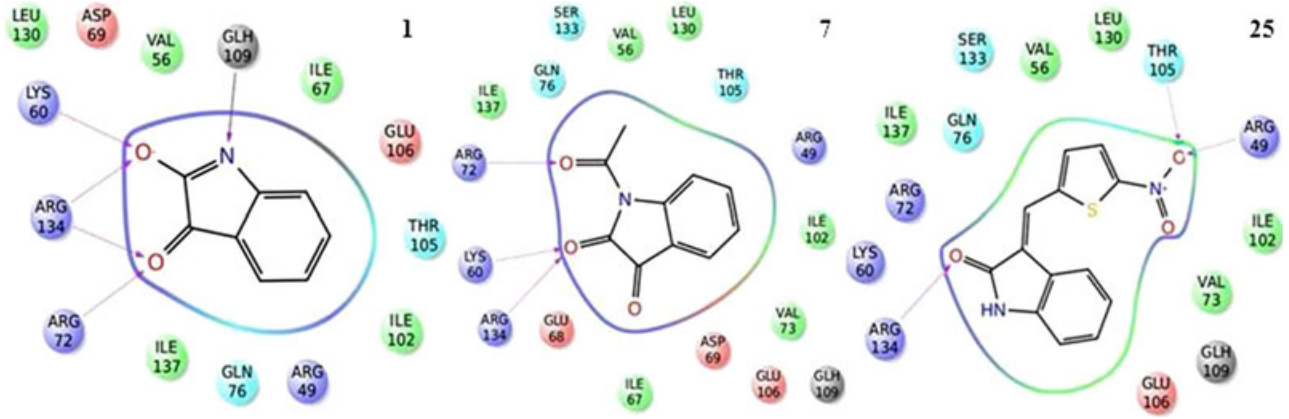


Figure 5: 2D interaction profile of compounds **1**, **7** and **25** with the active site residues. GLH 109 indicates the protonated glutamate residue. The pink dashed lines representing hydrogen bonding with the side chains of the residues. Amino acids are represented in green (hydrophobic), blue (polar), red (negatively charged) and purple (positively charged).

position depletes the activity. These substitutions on isatin ring completely changed the orientation of the compound at its active site, moving it slightly out of the pocket, facing towards the solvent. Similarly, 3-(hydroxyimino)indolin-2-one **15** also showed poor activity. We modified the phenyl ring of isatin by introducing various aryl groups at the C-5 position via Suzuki coupling and amino group via Buchwald–Hartwig cross-coupling reactions. Two of the 5-phenyl-substituted isatins **16** and **18** and two of 5-amino-substituted isatins **22** and **23** showed moderate activity. Compounds with substitution at R^2 position of isatin with piperazine derivatives **19**–**21** were found to be less active, with activity ranging between 31 and 55 μM, suggesting the unsuitability of piperazine substitution. This may be probably attributed to the orientation of these compounds towards the solvent as observed earlier. Surprisingly, two of the 3-substituted isatin derivatives like 3-(4-nitrobenzylidene)indolin-2-one **24** and 3-((5-nitrothiophen-2-yl)methylene)indolin-2-one **25** showed very potent activity with compound **25** in lower micromolar range. These substitutions were found to be suitable as the nitro group was involved in key interaction with Arg 49 and Thr 105 (Figure 5). In conclusion, only few substitutions such as acetyl group on N-1 nitrogen **7** and small arylidenes at R^1 position **24** and **25** were found to be favourable, and their activity supports the same.

The compounds were further screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by microplate alamar blue assay method (35). The lead compound isatin **1**, which showed excellent enzyme inhibition activity in the *in vitro* enzyme assay, did not show promising activity in MTB cell line, exhibiting an MIC of 340.13 μM. The lack of translation of strong enzyme inhibition potency to antitubercular activity can be probably attributed to the inability of these molecules to penetrate the mycobacterial cell wall as evident from a lower LogP value of 0.34. All the twenty-four isatin derivatives synthesized were found to be more potent than isatin as antitubercular compounds with MIC's range from 23.5 to 310.60 μM. Seven compounds **6**, **9**, **11**, **17**, **21**, **23** and **24** inhibited MTB with an MIC <100 μM, and the compound **24** was found to be most potent derivative against MTB with an MIC of 23.5 μM. The LogP of compound **24** was also very high (2.68) when compared to the lead molecule isatin. When compared to first line antitubercular drugs such as isoniazid (MIC: 0.66 μM), rifampicin (MIC: 0.23 μM) and ethambutol (MIC: 15.31 μM), compound **24** was found to be less active.

The safety profile of all the compounds were also accessed by testing *in vitro* cytotoxicity against RAW 264.7 cells at 50 and 100 μM concentrations by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (36). Mouse macrophage RAW 264.7 cells were selected for this study, as MTB generally reside in macrophage cells of humans. Percentage inhibitions of cells are reported in Table 1. The entire tested compound demonstrated good safety profile with very low toxicity towards the macrophage and showed good selectivity index (CC_{50}/MIC), indicating the suitability of these compounds for further drug development. The most promising anti-TB compound **24** showed 24.17% inhibition at 100 μM.

In this study, a structure-based e-pharmacophore modelling has been employed to identify small molecule inhibitors of *Mycobacterium tuberculosis* (MTB) chorismate mutase (CM) enzyme. Isatin **1** was identified as a potential lead with an IC_{50} of 1.08 ± 0.10 μM. Hit expansion of compound **1** by chemical synthesis leads to a more potent inhibitor, compound 3-(4-nitrobenzylidene)indolin-2-one **24** with promising *in vitro* activity against MTB CM enzyme and whole-cell MTB. Structural elements were identified that provide the basis for further optimization of the new inhibitors.

Acknowledgment

This work was supported by a fund from the Aditya Birla Science & Technology Company Limited Mumbai, India.

References

1. Duncan K. (2004) Identification and validation of novel drug targets in *tuberculosis*. Curr Pharm Des;10:3185–3194.
2. Okvist M., Dey R., Sasso S., Grahn E., Kast P., Krengel U. (2006) 1.6 A crystal structure of the secreted chorismate mutase from *Mycobacterium tuberculosis*: novel fold topology revealed. J Mol Biol;357:1483–1499.
3. Roderer K., Kast P. (2009) Evolutionary cycles for pericyclic reactions – or why we keep mutating mutases. Chimia;63:313–317.
4. Sasso S., Ramakrishnan C., Gamper M., Hilvert D., Kast P. (2005) Characterization of the secreted chorismate mutase from the pathogen *Mycobacterium tuberculosis*. FEBS J;272:375–389.
5. Kim S.K., Reddy S.K., Nelson B.C., Vasquez G.B., Davis A., Howard A.J., Patterson S., Gilliland G.L., Ladner J.E., Reddy P.T. (2006) Biochemical and structural characterization of the secreted chorismate mutase (Rv1885c) from *Mycobacterium tuberculosis* H37Rv: an *AroQ enzyme not regulated by the aromatic amino acids. J Bacteriol;24:8638–8648.
6. Sasso S., Okvist M., Roderer K., Gamper M., Codoni G., Krengel U., Kast P. (2009) Structure and function of a complex between chorismate mutase and DAHP synthase: efficiency boost for the junior partner. EMBO J;28:2128–2142.
7. Djamei A., Schipper K., Rabe F., Ghosh A., Vincon V., Kahnt J., Osorio S. *et al.* (2011) Metabolic priming by a secreted fungal effector. Nature;478:395.
8. Munack S., Leroux V., Roderer K., Okvist M., van Eerde A., Gundersen L.L., Krengel U., Kast P. (2012) When inhibitors do not inhibit: critical evaluation of rational drug design targeting chorismate mutase from *Mycobacterium tuberculosis*. Chem Biodivers;11:2507–2527.
9. Agrawal H., Kumar A., Bal N.C., Siddiqi M.I., Arora A. (2007) Ligand based virtual screening and biological evaluation of inhibitors of chorismate mutase (Rv1885c) from *Mycobacterium tuberculosis* H37Rv. Bioorg Med Chem Lett;17:3053–3058.
10. Sikdar S.. Ph.D. thesis, Aston University, UK, 2010.
11. Adepu R., Kumar K.S., Sandra S., Rambabu D., Rama Krishna G., Reddy C.M., Kandale A., Misra P., Pal M. (2012) C–N bond formation under Cu-catalysis: Synthesis and in vitro evaluation of N-aryl substituted thieno[2,3-d]pyrimidin-4(3H)-ones against chorismate mutase. Bioorg Med Chem;20:5127–5138.
12. Kumar K.S., Rambabu D., Sandra S., Kapavarapua R., Krishna G.R., Basaveswara Rao M.V., Chatti K., Reddy C.M., Misra P., Pal M. (2012) $AlCl_3$ induced (hetero)arylation of 2,3-dichloroquinoxaline: a one-pot synthesis of mono/disubstituted quinoxalines as potential antitubercular agents. Bioorg Med Chem;20:1711–1722.
13. Shiva Kumar K., Adepu R., Sandra S., Rambabu D., Rama Krishna G., Malla Reddy C., Misra P., Pal M. (2012) Cu-mediated N-arylation of 1,2,3-triazin-4-ones: synthesis of fused triazinone derivatives as potential inhibitors of chorismate mutase. Bioorg Med Chem Lett;22:1146–1150.
14. Ram Reddy T., Srinivasula Reddy L., Rajeshwar Reddy G., Nuthalapatia V.S., Lingappa Y., Sandra S., Kapavarapua R., Misra P., Pal M. (2011) A Pd-mediated new strategy to functionalized 2-aminochromenes: their in vitro evaluation as potential anti tuberculosis agents. Bioorg Med Chem Lett;21:6433–6439.
15. Bartlett P.A., Johnson C.R. (1985) An inhibitor of chorismate mutase resembling the transition-state conformation. J Am Chem Soc;107:7792–7793.
16. Sogo S.G., Widlanski T.S., Hoare J.H., Grimshaw C.E., Berchtold G.A., Knowles J.R. (1984) Stereochemistry of the rearrangement of chorismate to prephenate: chorismate mutase involves a chair transition state. J Am Chem Soc;106:2701–2703.
17. Garden S.J., Torres J.C., da Silva L.E., Pinto A.C. (1998) A convenient methodology for the N-Alkylation of isatin compounds. Syn Comm;28:1679–1689.
18. Silviya D.F., Suhaib S., Srinivasaraghava K., Wolfgang S., Manfred J. (2012) Rhodanine carboxylic acids as novel inhibitors of histone acetyltransferases. Med Chem Commun;3:305–311.
19. Chan D.M.T., Monaco K.L., Wang R.P., Winter M.P. (1998) New N- and O-arylations with phenylboronic acids and cupric acetate. Tetrahedron Lett;39:2933–2936.
20. Lam P.Y.S., Clark C.G., Saubern S., Adams J., Winters M.P., Chan D.M.T., Combs A. (1998) New aryl/Heteroaryl C–N bond cross-coupling reactions via arylboronic acid/cupric acetate arylation. Tetrahedron Lett;39:2941–2944.
21. Jacobs T.L., Winstein S., Linden G.B., Robson J.H., Levy F.E., Seymour D. (1955) 2-Hydroxycinchoninic acid. Org Synth;3:456.
22. Chiyanzu I., Hansell E., Gut J., Rosenthal P.J., McKerrow J.H., Chibale K. (2003) Synthesis and evaluation of isatins and thiosemicarbazone derivatives against cruzain, falcipain-2 and rhodesain. Bioorg Med Chem Lett;13:3527–3530.
23. Bal T.R., Balasubramani A., Yogeeswari P., Sriram D. (2005) Synthesis and evaluation of anti-HIV activity of isatin beta-thiosemicarbazone derivatives. Bioorg Med Chem Lett;15:4451–4455.
24. Frank W., Elsebet O.N., Jergen D., Jensen L.H. (1993) Isatin oximes - A novel series of bioavailable non-NMDA antagonists. Bioorg Med Chem Lett;3:105–106.

25. Verma M., Pandeya S.N.Singh K.N., Stables J.P. (2004) Anticonvulsant activity of Schiff bases of isatin derivatives. Acta Pharm;54:49.
26. Martinez F., Naarmann H. (1990) New isatin derivatives: synthesis and reactions. Synth Met;39:195–203.
27. Hartwig J.F. (1997) Palladium-catalyzed amination of aryl halides: mechanism and rational catalyst design. Synlett;4:329–340.
28. Huang X., Anderson K.W., Zim D., Jiang L., Klapars A., Buchwald S.L. (2003) Expanding Pd-catalyzed C-N bond-forming processes: the first amidation of aryl sulfonates, aqueous amination, and complementarity with Cu-catalyzed reactions. J Am Chem Soc;125:6653–6655.
29. Strieter E.R., Blackmond D.G., Buchwald S.L. (2003) Insights into the origin of high activity and stability of catalysts derived from bulky, electron-rich monophosphinobiaryl ligands in the Pd-catalyzed C-N bond formation. J Am Chem Soc;125:13978–13980.
30. Yin J., Buchwald S.L. (2002) Pd-catalyzed intermolecular amidation of aryl halides: the discovery that xantphos can be trans-chelating in a palladium complex. J Am Chem Soc;124:6043–6048.
31. Fukuyamaa T., Liu G. (1997) Stereocontrolled total synthesis of (±)-gelsemine. Pure Appl Chem;69:501–505.
32. Sumpter W.C. (1945) The chemistry of oxindole. Chem Rev;37:443–479.
33. Crestini C., Saladino R. (1994) A new efficient and mild synthesis of 2-oxindoles by one-pot Wolff-Kishner like reduction of isatin derivatives. Synth Commun; 24:2835–2841.
34. Olgen S., Akaho E., Nebioglu D. (2005) Synthesis and anti-tyrosine kinase activity of 3-(substituted-benzylidene)-1, 3-dihydro-indolin derivatives: investigation of their role against p60c-Src receptor tyrosine kinase with the application of receptor docking studies. Farmaco;60:497–506.
35. Franzbalu G.S., Witzig S.R., McLaughlin C.J., Torres P., Madico G., Hernandez A., Degnan T.M., Cook B.M., Quenzer K.V., Ferguson M.R., Gilman H.R. (1998) Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. J Clin Microbiol;36:362–366.
36. Hansen J., Bross P. (2010) A cellular viability assay to monitor drug toxicity. Methods Mol Biol;648: 303–311.

Note

[a]Tuberculosis, Fact Sheet No. 104. World Health Organization. http://www.who.int/media centre/factsheets/fs104/en/ (reviewed in February 2013).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Methodology used for docking, experimental and spectral details of all the synthesized compounds and the protocol used for biological evaluation.