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Design, synthesis and biological evaluation of (E)-5-styryl-1,2,4-oxadiazoles as anti-tubercular agents

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ABSTRACT

Cinnamic acid and its derivatives are known for anti-tubercular activity. The present study reports the synthesis of cinnamic acid derivatives via bioisosteric replacement of terminal carboxylic acid with "oxadiazole". A series of cinnamic acid derivatives (styryl oxadiazoles) were designed and synthesized in good yields by reaction of substituted cinnamic acids (**2**, **15a-15s)** with amidoximes. The synthesized styryl oxadiazoles were evaluated in vitro for anti-tubercular activity against *Mycobacterium tuberculosis* (*Mtb*) *H37*Ra strain. The structure-activity relationship (SAR) study has identified several compounds with mixed anti-tubercular profiles. The compound **32** displayed potent anti-tubercular activity (IC_{50} = 0.045 μg/mL). Molecular docking studies on mycobacterial enoyl-ACP reductase enzyme corroborated well with the experimental findings providing a platform for structure based hit-to-lead development.

1. Introduction

Tuberculosis (TB) is a major global health challenge affecting approximately 10.4 million people worldwide [1]. The current treatment regimen for TB (isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol), has proven successful in efficiently achieving treatment rates of higher than 90%. However, the long treatment duration (6–12 months) and spontaneous gene mutation has increased the cases of multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) in recent years [2,3]. Combinations of new and existing drugs are being evaluated to shorten the span of therapy and to treat multi-drug-resistant tuberculosis. To surmount the difficulties associated with this pandemic, there is a need for new anti-tubercular agents that can shorten and simplify current treatment with potency against both, drug sensitive and drug resistance TB [4]. Nevertheless after 40 years of no new therapies, approval of Bedaquiline and Delamanid for the treatment of tuberculosis is also a cause for optimism [5].

Cinnamic acid and its derivatives were used as antituberculosis agents since many years. When the TB-patients were treated with cinnamic acid **2,** which is prepared from "storax 7" gradual improvement was observed in the activity. Furthermore, in 1920's ethylcinnamate **3**, sodium cinnamate **4** and benzylcinnamate **5** were reported to be efficacious in the treatment of TB [6] (Fig. 1). In the last ten years, the interest of researchers on the cinnamic acid moiety has notably increased. Promising results have been reported by synergistic activity of *trans*-cinnamic acid **2** in drug combinations with isoniazid (INH) **1** and other drugs [7]. Additionally, cinnamyl derivative of rifampicin (RMP) **6** showed improved intracellular and *in-vivo* activities than rifampicin **6a** alone. Interestingly increase in activity was even observed with drug resistant isolates [8,9]. Piplartine **7** (Fig. 1) is another cinnamic-related molecule showing an attractive biological horizon. This cinnamic amide was first-time isolated from the roots of *Piper tuberculatum* and later proved to be a promising anti-cancer scaffold [10]. The compound **8** is dehyrozingerone (DZG) also known as feruloylmethane, having chemical name (*E*)-4-(4-hydroxy-3-methoxyphenyl) but-3-en-2-one has styryl ketone group that resembles the trans cinnamic acid structure. DZG derivative with thiazoleheterocycle **10** showed promising anti-TB activity [11]. In addition it was reported that derivatives

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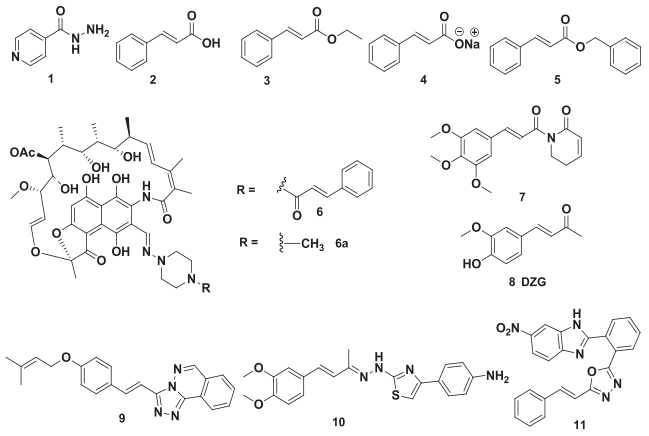


Fig. 1. Structures of substituted cinnamic acid scaffolds reported for anti-mycobacterial agents (**6a**- Rifampicin, RMP).

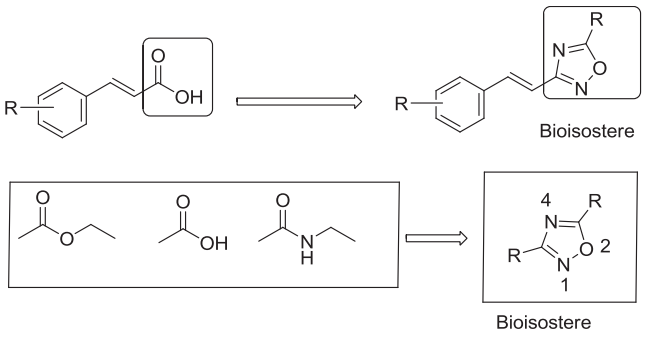


Fig. 2. Cinnamic acid, ester, amide bioisosteric replacement with 1,2,4- oxadiazoles.

resulting from combining cinnamoyl portion with various chemical classes produce promising anti TB active compounds [9,12] (**9**, **10**, and **11**) (Fig. 1). (See Fig. 2.).

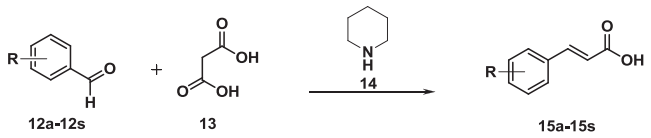
2. Result and discussion

2.1. Chemistry

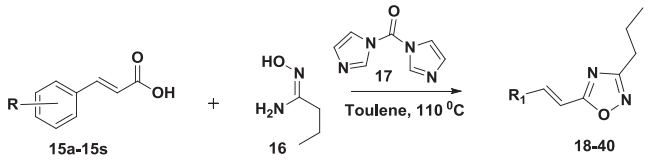
In an attempt to synthesize and evaluate novel compound's activity against TB, herein we report design, synthesis and evaluation of series of cinnamic acid derivatives (styryl oxadiazoles) where carboxyl moiety of cinnamic acid is replaced with its bioisostere 1,2,4-oxadiazole, so as to take care of its metabolism-related liabilities [13] as well as to improve activity. The introduction of such oxadiazole moiety also helps to restrict the rotations around the double bond hence the conformation [14]. Moreover 3,5 di-substituted oxadiazoles are stable while none or monosubstituted are unstable [15–17]. Chemical properties of the 1,2,4-oxadiazoles have been reviewed in the literature [18]. Furthermore, literature report suggest that the oxadiazole motif has the potential to inhibit the enoyl reductase (InhA) a primary molecular target of the frontline ant tubercular drug, which encouraged us in the selection of it as a bioisostere during the design [19]. Further, phenyl ring of cinnamic acid as well as oxadiazole ring substituted with various substituents resulted into novel series of styryl oxadiazoles.

The cinnamic acids **2** and **15a-15s** were synthesized from aldehyde compounds **12** and **12a-12s** were reacted with malonic acid **13** in the presence of catalytic amount of piperidine **14** in pyridine as a solvent (Scheme 1) [20].

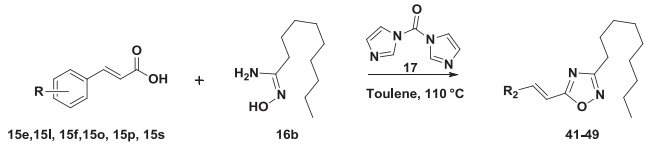
Further, amidoxime compound **16** and **16b** were synthesized from corresponding readily available nitrile compounds and hydroxyl amine hydrochloride using reported method in good yields [21]. Substituted



Scheme 1. [a]Synthesis of cinnamic acid analogues: [a]Reagents and conditions (a) Pyridine, 110 °C for 10–12 h, 70–85%.



Scheme 2. [a]Synthesis of (*E*)-3-propyl-5-styryl-1,2,4-oxadiazole: [a]Reagents and conditions (a): Toluene, CDI, 3–4 h RT, 5–6 h, 100–110 °C, 40–60.0%.



Scheme 3. [a]Synthesis of (*E*)-3-nonyl-5-styryl-1,2,4-oxadiazole. [a]Reagents and conditions: Toluene, CDI, 3–4 h RT, 5–6 h, 100–110 °C, 40–60%.

cinnamic acids (**2** and **15a − 15s**) were reacted with amidoxime (**16** and **16b**) in the presence of CDI **17** to obtained 3,5-disubstituted 1,2,4-oxadiazole derivatives (**18–49**) (Scheme 2 and 3) using reported method [22].

Phenyl ring of cinnamic acid was substituted with various functional groups and diverse set of compounds were synthesized. The compounds **18–40** were having short aliphatic chain, propyl as a substituent in 3rd position of 1,2,4-oxadiazole. Further to explore SAR, amidoxime **16b** with an extended aliphatic chain was selected so as to increase the bulk as well as lipophilicity and compounds **41–49** were synthesized, by using similar method in good yield. Detail synthetic procedure and characterization data is provided in Supplementary data.

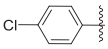
Further, the effect of such structural modification on the anti-tubercular activity of **2** was investigated. New compounds have shown anti-TB activity profile similar to cinnamic acid **2**. Intrigued by these observations, we systematically studied the SAR of compound **18** with various substituents on the aromatic ring as well as at the 3rdposition of 3,5-disubstituted 1,2,4-oxadiazole. A total of two series were designed, synthesized and evaluated for anti-tubercular activity.

2.2. Biological evaluation

The detailed protocol for primary screening of anti-tubercular activity [23] is given in *experimental section.* The title compounds anti-tubercular activity with MIC and IC_{50} values were expressed in μg/mL (Table 1 and 2).

Careful inspection of the preliminary screening data shows the following activity trends. The compound **2** is itself potent compound exhibiting significant anti-tubercular activity (IC_{50} = 0.06 μg/mL) against *Mycobacterium tuberculosis*(*Mtb*) *H37*Ra strain. To begin with compounds synthesized, wherein substitution is made on phenyl ring of cinnamic acid. Compounds **18**, with no substitution over phenyl ring showed ~20 fold decreased in activity as compared to compound **2**. Compounds **19–21** with electron donating substituents on the phenyl ring of cinnamic acid scaffold were design and synthesized and found to be inactive, except compound **34** having hydroxyl group on phenyl ring at *para* position shows moderate activity (IC_{50} = 2.22 μg/mL, Table 1). In case of electron donating (di- substituted) compounds, **35** and **36** shows loss of activity. The compound **37** with heterocyclic aromatic

Table 1
In-vitro anti-tubercular activity of styryl oxadiazoles against *M.tb. H37*Ra (compounds **2, 18–40**).

Cpd No.		*Mtb H37*Ra *(Day 12)*		*Mtb H37*Ra *(Day 8)*	
		MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)
2		0.91	0.011	25.49	0.06
18		7.82	0.02	11.33	1.42
19		> 30	> 30	> 30	> 30
20		> 30	9.82	> 30	> 30
21		> 30	> 30	> 30	> 30
22		6.41	0.018	24.21	4.53
23		2.54	0.014	26.68	9.91
24		7.7	0.087	8.74	2.35
25		5.42	0.028	8.18	1.54
26		2.34	0.087	9.86	0.36
27		> 30	> 30	> 30	> 30
28		> 30	> 30	> 30	> 30
1	INH	0.075	0.0023	0.074	0.0019
6a	RMP	0.043	0.0014	0.048	0.0018

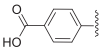
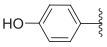
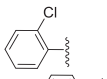
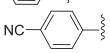
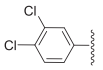
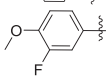
Cpd No.		*Mtb H37*Ra *(Day 12)*		*Mtb H37*Ra *(Day 8)*	
		MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)
29		8.92	0.021	8.95	4.07
30		0.89	0.015	26.49	11.01
31		11.89	0.091	7.69	0.21
32		**8.45**	**0.63**	**4.13**	**0.045**
33		2.78	0.076	21.35	0.56
34		7.61	0.076	16.96	2.22
35		> 30	> 19.04	> 30	> 30
36		> 30	> 30	> 30	> 30
37		> 30	> 30	> 30	> 30
38		> 30	> 30	> 30	> 30
39		> 30	> 30	> 30	> 30
40		> 30	> 30	> 30	> 30
1	INH	0.075	0.0023	0.074	0.0019
6a	RMP	0.043	0.0014	0.048	0.0018

Table 2
In-vitro anti-tubercular activity data of styryl oxadiazoles *M.tb. H37*Ra (compounds **41–49**).

Cpd No.		*Mtb H37*Ra *Day 12*		*Mtb H37*Ra *Day 8*	
		MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)
41		9.16	0.57	8.67	1.83
42		2.88	0.15	7.33	0.17
43		9.87	0.098	19.18	0.56
44		> 30	> 30	> 30	> 30
45		> 30	> 30	> 30	> 30
46		6.95	0.044	10.06	1.54
47		> 30	> 30	> 30	> 30
48		3.1	0.059	2.46	0.1
49		8.75	0.084	9.16	0.36
1	INH	0.075	0.0023	0.074	0.0019
6a	RMP	0.043	0.0014	0.048	0.0018

ring (4-pyridyl) and bicyclic aromatic ring (1-naphthyl, compound **38**) led to completely loss of activity.

The halogen substituted on phenyl ring compound **22**(consisting of 4-chloro substitution on phenyl ring) exhibited moderate activity (IC_{50} = 4.53 μg/mL, Table 1). However change in position of chloro substitution from *para*- to *ortho*-at phenyl ring compound **23** led to decrease in activity. Also, it was observed that 2,3-dichloro phenyl substituted compound **25** is more active than the 3,4-dichloro phenyl compound **24**. The compound **26** with 4-flouro phenyl substitution displayed decent activity (IC_{50} = 0.36 μg/mL, Table 1) (compound **22** versus **26**). In case of bromo substitutions on phenyl ring (compound **27** and **28)** there is complete loss of activity. This is further supported from the results observed for the compound **29** (2-bromo-4-fluoro substitution on phenyl ring) showing moderate activity in-turn suggesting that bromo substituent is not favoured. Above results indicate that presence of fluoro substitution is favored for anti-TB activity.

It was observed that introduction of electron withdrawing group leads to an improvement in anti-TB activity (compound **30–33**, Table 1), except with sulfone and sulfoxide, substitution (compound **39** and **40** respectively, Table1). While 2-nitro and 3-CF_3 phenyl substituted compounds are active (compound **33** IC_{50} = 0.56; compound **31** IC_{50} = 0.21 μg/mL), the compound **32** consisting of polar acid functionality carboxylic acid at para position of phenyl ring displayed strongest anti-TB activity (IC_{50} = 0.045 μg/mL, Table 1) among all and is also greater than the cinnamic acid (**2**) itself. The 4-cyano phenyl substituted compound **30** showed less potent activity (IC_{50} = 11.01 μg/mL, Table 1). Thus the result indicates that the electron withdrawing substituents are favorable for anti-tubercular activity.

Based on the above results few poor to moderately active molecules were specifically chosen and compound **41–49** were synthesized with straight nonyl chain at 3rd position of 1,2,4-oxadiazole (Table 2).

The compound **43** (IC_{50} = 0.56 μg/mL, Table 2) displayed improvement in activity by ~ 4 fold as compared to compound **24** (IC_{50} = 2.35 μg/mL, Table 1). Also compound **23** (IC_{50} = 9.91 μg/mL, Table 1) shows moderate activity whereas with nonyl analogue compound **41** shows increase in activity by ~ 5 fold (IC_{50} = 1.83 μg/mL, Table 2). Similarly, compound **42** shows increase in activity by ~ 60 folds (IC_{50} = 0.17 μg/mL, Table 2) with respect to compound **30** (IC_{50} = 11.01 μg/mL, Table 1). However, poorly active molecules (compound **35** and **36,** Table 1) with electron donating substituents on aromatic ring doesn't show any improvement in their activity with nonyl analogues (compound **44** and **45**, Table 2).

Interestingly the de-methylated hydroxyl analogue (compound **46** and **48**) of compound **45** and **47** (Table 2) showed drastic change in anti-TB activity (from inactive to IC_{50} = 1.54 and 0.1 μg/mL, respectively). In case of mono & dihydroxy analogues of 2,5-dimethoxy compound **47**, mono hydroxy analogue **48** is more potent than its dihydroxy analogue compound **49** (IC_{50} = 0.1 and 0.36 μg/mL, respectively) (Table 2).

2.3. Docking studies

Structure based drug design approach *viz.* molecular docking has emerged as a powerful tool to predict the binding affinity of the candidate molecules towards the biological target and their modes of interaction within, especially in the absence of available resources to carry out the enzymatic studies. Promising antimycobacterial activity demonstrated by the various cinnamic acid derivatives in the cell-based assay motivated us to apply this insilico approach to estimate the binding affinity and mode of interaction of these compounds into the active site of mycobacterial enoyl-ACP reductase enzyme. The NADH-specific enoyl-ACP reductase encoded by the *Mycobacterium* gene InhA has been validated as the primary molecular target of the frontline antitubercular drug isoniazid (INH) [19]. It catalyzes the conversion of Δ2-unsaturated to saturated fatty acids and is involved in the elongation of long-chain fatty acids to mycolic acids that are central constituents of the mycobacterial cell wall (mycobacterial type II fatty acid biosynthesis pathway). Therefore, InhA the enoyl acyl carrier protein reductase is regarded as one of the key enzymes in the type II fatty acid biosynthesis pathway of *Mtb*. Inhibition of InhA will block the mycolic acid biosynthesis, thereby impairing the integrity of the cell wall and eventually leading to mycobacterial cell death [24]. Furthermore, literature reports suggests that the oxadiazole motif has the potential to inhibit the enoyl reductase (InhA) which encouraged the selection of this target to evaluate the binding potential of the title compounds towards this crucial mycobacterial cell target [25,26].

Molecular docking simulation performed on the title compounds showed that all of them could snugly fit into the active site of the enoyl acyl carrier protein reductase (ENR) in co-ordinates that were similar to the native ligand in the crystal structure through a network of close bonded and non-bonded interactions. Their docking scores varied from −9.858 for the most active compound with a glide binding energy of −49.078 kcal/mol to −6.019 (glide energy −28.016 kcal/mol) for least active with an average docking score of −7.845 (glide energy −35.016 kcal/mol)(Supplementary data, Table 1S). A linear correlation was observed as well between the theoretical predictions from molecular docking score and the experimentally observed anti-TB activity with the active compounds showing a higher docking score while those with relatively low inhibition were predicted to have lower docking scores (Supplementary data, Table 1S). Furthermore to gain an insight into residues guiding the anchoring of these ligands into the target and thermodynamics elements involved in this binding event, a detailed per-residue interaction analysis between the InhA enzyme and all the active compounds **2, 25, 31, 32, 33, 42, 43, 48** and **49** has been carried out. For the sake of brevity of text, the details of this analysis have been elucidated for most active compound **32**(Fig. 3) (Supplementary data, Table 1S).

3. Conclusion

The present study attempt the replacement of carboxylic acid functionality of cinnamic acid **2** with its bioisostere (1,2,4-oxadiazole) and subsequent SAR investigations. Some of the compounds showed promising anti-TB activity against *MtbH37*Ra. Based on the observation made during the study we can conclude that electron withdrawing and halogen substituents are most favored whereas electron donating and bulky substituents are least favored at the phenyl ring of cinnamic acid. As well as we can conclude that the increasing chain length i.e. bulk or lipophilicity at 3rd position of 1,2,4-oxadiazole is favored. Compounds **25, 26, 31–33, 42, 43, 48** and **49** (IC_{50} 1.54, 0.36, 0.21, 0.045, 0.56, 0.17, 0.56, 0.1 and 0.36 μg/mL, respectively) exhibited moderate anti-TB activity. The compound **32** shows promising anti-TB activity against *MtbH37*Ra among all. Furthermore molecular docking studies against the mycobacterial cell wall target enoyl acyl carrier protein reductase (InhA) provided well-clustered solutions to the mode of binding for these compounds. These *in-silico* results correlated with the observed experimental values in good agreement and could provide a detailed insight into the various thermodynamic interactions governing the binding of these analogues with InhA, which is essential preliminary information for structure based optimization of this motif. This study provides novel molecules for further exploration in our quest for novel anti-tubercular agents. In our opinion, this is a valuable investigation with significant impact on anti-tubercular drug development field.

4. Experimental

4.1. Organic chemistry

4.1.1. Material and methods

Reagents and solvents were obtained from Indian commercial suppliers, Sigma-Aldrich - USA and were used as received without further purification unless otherwise indicated. The thin layer chromatography was performed on Merck pre-coated silica gel 60 F254 plates, with visualization under UV light. The 1H NMR and 13C NMR spectra were routinely obtained with a Varian Mercury Plus 300 MHz NMR (Bruker) instrument and J values are in Hertz and chemical shifts (δ) are reported in ppm relative to internal tetramethylsilane. Mass spectral (MS) data were recorded on 6110 AA Series Quadrupole LC/MS system (Agilent Technologies, Santa Clara, USA), and Bruker esquires 4000 Quadrupole LC/MS system. HRMS spectrums were recorded on Bruker LCMS QToF, Model: IMPACT HD. The purity of all compounds was determined by HPLC (Agilent 1100 Series with autosampler and PDA detector) system implementing either Method A, Method B for chromatographic separation. Melting points were recorded using a Veego (VMP)-D capillary melting point apparatus (Veego-Instruments Corp. Mumbai, India) and were uncorrected.

4.1.2. General procedure for synthesis of cinnamic acids (2, 15a to 15 s)

To a solution of pyridine (3 vol), was added aldehyde (**1** mmol) malonic acid (2 mmol) followed by catalytic amount of piperidine (0.1 mmol). The reaction mass was slowly heated to 110 °C and maintained for 10 to 12 hr. at 110 °C. Reaction was monitored by TLC. Reaction mass was cooled to room temperature and quenched into 10 vol of water of pyridine. To the quenched mass was added, NaOH (2 mmol). Reaction mixture was stirred to obtain clear solution. Then reaction mixture was washed with ethyl acetate (20 vol X 2). Aqueous layer was then acidified with 50% sulfuric acid till pH 2. The precipitated solid was filtered and washed with water (5 vol X 2) followed by pet ether wash 2 vol The product was suck dried on buchner funnel for 15 min to 60 min. The solid product was dried in oven at 50 to 60 °C overnight. Cinnamic acids were obtained in 80 to 90% yield.

4.1.2.1. Synthesis of cinnamic acid (2). To a solution of pyridine (30 ml), added benzaldehyde (**12**) (10 g, 94 mmol) malonic acid (**13**)

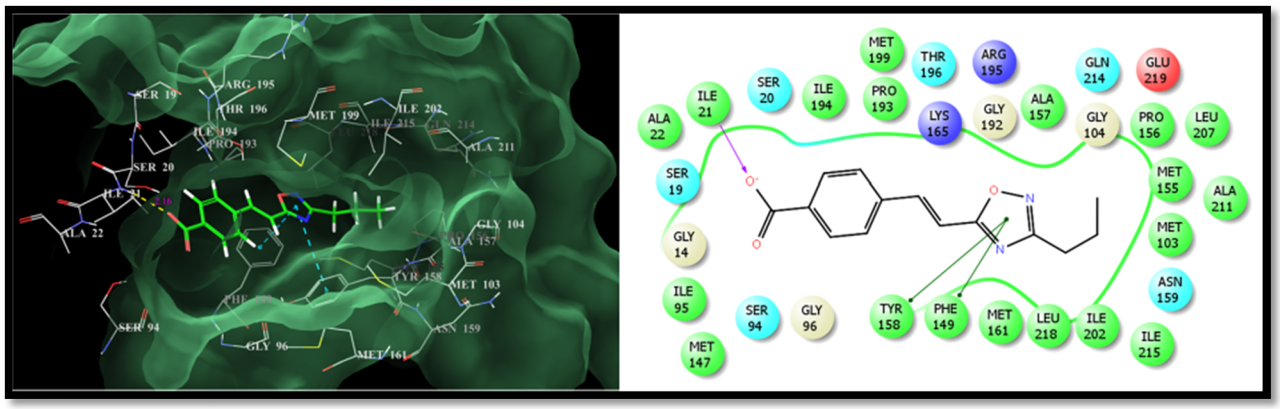


Fig. 3. Binding mode of compound **32** into the active site of mycobacterial enoyl reductase (InhA). Hydrogen bonding interactions are represented the yellow dotted lines while blue dotted lines signify the π-π stacking interactions.

(19.61 g, 188 mmol) followed by catalytic amount of piperidine (**14**) (0.933 ml, 9.42 mmol). The reaction mass was slowly heated to 110 °C and maintained for 10 to 12 h. at 110 °C. The reaction was monitored by TLC. The reaction mass was cooled to room temperature and quenched into 300 ml of water. To the quenched mass was added, NaOH (7.54 g, 188 mmol). Reaction mixture was stirred to obtain clear solution. The reaction mixture was washed with ethyl acetate (200 ml X 2). Aqueous layer was then acidified with 50% sulfuric acid till pH 2.The precipitated solid was filtered and washed with water (50 ml X 2) followed by pet ether wash. The product was suck dried on buchner funnel for 15 to 60 min. The solid product was dried in oven at 50 to 60 °C overnight, Cinnamic acid (**2**) (11 g).

Nature: Pale yellow crystalline solid; Yield: 79%; m.p. 133–134 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 12.42 (s, 1H, COOH), 7.70–7.67 (m, 2H, Ar), 7.62–7.57 (d, J = 16.2 Hz, 1H, trans), 7.42–7.40 (t, J = 3.6 Hz, 3H, Ar), 6.56–6.51(d, J = 15.9 Hz, 1H, trans), LC-MS (ESI -ve) m/z 147.1 $[M-H]^+$; HPLC: 99.97% .

Similarly compounds from **15a** to **15 s** were synthesized using general procedure as demonstrated for compound**2**.

*4.1.3. Synthesis of (E)-3-propyl-5-styryl-1,2,4-oxadiazole (**18**)*

To a solution of Cinnamic acid (**2**) (1.48 g, 9.99 mmol) in Toluene (21.28 ml, 200 mmol), was added CDI (**17**) (1.944 g, 11.99 mmol), Stirred the reaction mass at room temperature for 10 to 15 min till effervescence of CO_2 ceases. Add (Z)-N'-hydroxybutyrimidamide (**16**) (1.785 g, 17.48 mmol) and stirred the mass at room temperature for 3 to 4 h. till completion of reaction by TLC. This is the formation of an intermediate. Further the reaction mass was heated to 110 °C and maintained for 5 to 6 h. till completion of reaction by TLC. The reaction mass was cooled to room temperature and quenched into 20 to 30 ml of water. Separate the organic i.e. toluene layer. The aqueous layer was extracted with toluene approx. 10 to 20 ml. The combined Toluene (organic) layer was washed with water about 10 to 20 ml, followed by washed with dil. HCl (1 N) about 10 to 15 ml. Again the toluene i.e. organic layer was washed with dil. sodium bicarbonate (5% solution) about 10 to15 ml. The organic layer was washed with brine. The toluene was distilled off on rota-evaporator under vacuum at 50 to 60°Cto obtain the crude product. The crude product was purified from IPA. The solid product was filtered at 15 to 20 °C, suck dried on buchner funnel for 15 min to 60 min. The solid product was dried in oven at 40 to 60 °C overnight. (E)-3-propyl-5-styryl-1,2,4-oxadiazole (**18**) (1.28 g).

Nature: light pale yellow semi solid; Yield: 60.1%; m.p. low melting semi solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.86–7.80 (d, J = 18 Hz, 1H, trans), 7.80 (m, 2H, Ar), 7.452–7.44 (t, J = 3 Hz, 3H, Ar), 7.37–7.31 (d, J = 18 Hz, 1H, trans), 2.71–2.66 (t, J = 6 Hz, 2H, attached to hetrocyclic ring), 1.77–1.65 (m, 2H, attached to CH_3), 0.95–0.90 (t, J = 6 Hz, 3H, -CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 175.03 (oxadiazole *N*—C═O, -1C), 170.74 (oxadiazole N—C═N, -1C), 142.59 (trans, -1C), 134.61(Ar, -1C), 130.83 (Ar, -1C), 129.28 (Ar, -2C), 128.60 (Ar, -2C), 110.66 (trans, -1C), 27.43 (CH_2 attached to oxadiazole, -1C), 20.16 (CH_2 attached to CH_3, -1C), 13.73 (terminal CH_3, -1C); LC-MS (ESI +ve) m/z 215.1 $[M+H]^+$; HPLC Purity: 94.18%.

*4.1.4. Synthesis of Synthesis of (E)-5-(2-chlorostyryl)-3-nonyl-1,2,4-oxadiazole (**41**)*

To a solution of (E)-3-(2-chlorophenyl)acrylic acid (**15e**) (1.83 g, 10.0 mmol) in toluene (50.5 g, 548 mmol) was added CDI (**17**) (1.94 g, 12.0 mmol).Stirred the reaction mass at room temperature for 10 to 15 min till effervescence of CO_2 ceases. Add (Z)-N'-hydroxydecanimidamide (**16b**) (3.26 g, 17.5 mmol) and stirred the mass at room temperature for 3 to 4 h. till completion of reaction by TLC. This is the formation of an intermediate. Further the reaction mass was heated to 110 °C and maintained for 5 to 6 h. till completion of reaction by TLC. The reaction mass was cooled to room temperature and quenched into 20 to 30 ml of water. Separate the organic i.e. toluene layer. The aqueous layer was extracted with toluene approx. 10 to 20 ml. The combined Toluene (organic) layer was washed with water about 10 to 20 ml, followed by washed with dil. HCl (1 N) about 10 to 15 ml. Again the toluene i.e. organic layer was washed with dil. sodium bicarbonate (5% solution) about 10 to 15 ml. The organic layer was washed with brine. The toluene was distilled off on rota-evaporator under vacuum at 50 to 60 °C to obtain the crude product. The crude product was purified by column chromatography (silica gel Chloroform – ethyl acetate) to yield the title compound. The solid product was dried in oven at 40 to 60°Covernight. (E)-5-(2-chlorostyryl)-3-nonyl-1,2,4-oxadiazole (**41**) (1.9 g).

Nature: white semi solid; Yield: 60.0%; m.p. low meting semi solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.105–8.080 (dd, J = 7.5 Hz, J = 1.8 Hz, 1H, Ar), 8.080–8.025 (d, J = 16.5 Hz, 1H, trans), 7.603–7.572 (m, 1H, Ar), 7.505–7.444 (m, 2H, Ar), 7.484–7.430 (d, J = 16.2 Hz, 1H, trans), 3.808 (s, 3H, -OCH_3), 2.75–2.704 (t, J = 7.2 Hz, 2H, attached to hetrocyclic ring), 1.713–1.667 (m, 2H, CH_2 - attached to CH_3), 1.295–1.244 (m, 12H, -CH_2), 0.869–0.825 (t, J = 7.2 Hz, 3H, terminal CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 174.298 (oxadiazole N—C═O, -1C), 170.724 (oxadiazole N—C═N, -1C), 136.672 (trans, -1C), 133.683 (Ar, -1C), 131.965 (Ar substituted Cl, -1C), 131.795 (Ar, -1C), 130.045 (Ar, -1C), 128.121 (Ar, -1C), 127.822 (Ar, -1C), 113.296 (trans, -1C), 31.295 (CH_2 attached to oxadiazole, -1C), 28.866 (aliphatic CH_2, -1C), 28.673 (aliphatic CH_2, -2C), 28.393 (aliphatic CH_2, -1C), 26.395 (aliphatic CH_2, -1C), 25.753 (aliphatic CH_2, -1C), 22.120 (CH_2 attached to CH_3, -1C), 13.945 (terminal CH_3, -1C); LC-MS (ESI +ve) m/z 333.1 $[M+H]^+$; HPLC Purity: 97.95%.

4.2. Biology

4.2.1. Material

All the chemicals such as sodium salt XTT, DMSO, sulfanilic acid, sodium nitrate, HCl, NEED and rifampicin were purchased from Sigma-Aldrich, USA. Dubos medium was purchased from DIFCO, USA. Synthesized compounds were dissolved in DMSO and it was used as stock solution (10 mg/ml) for further biological testing.

4.2.2. Anti-mycobacterial activity

Cultivation of mycobacteria: Microbial strains such as Mycobacterium tuberculosis *H37*Ra (ATCC 25177) and M. bovis BCG (ATCC 35734) were obtained from AstraZeneca, India. The stock culture was maintained at −80 °C and subculture once in a liquid medium before inoculation into an experimental culture. Cultures were grown in Dubos media (enrichment media). For antimycobacterial assay, M. phalli medium (minimal essential medium) was used. It contains 0.5 g KH_2PO_4, 0.25 g trisodium citrate, 60 mg $MgSO_4$, 0.5 g asparagine and 2 ml glycerol in distilled water (100 ml) followed by pH adjustment to 6.6. All bacterial stock cultures were first grown in Dubos media at 37 °C at 150 RPM. It takes at least 8–10 days for OD 1 at 620 nm.

4.2.3. Anti-mycobacterial assay

All the synthetic compounds were screened for their in vitro activity against M. tuberculosis *H37*Ra (MTB) (ATCC 25177) and M. bovis BCG (ATCC 35743) at two different time point (day 8 and 12) by established XTT Reduction Menadione Assay (XRMA) and Nitrate Reductase (NR) method, respectively, both of the method were developed earlier in our lab. Briefly, 0.1 OD620 cultures of MTB/ BCG was treated with synthesized compound at three different concentrations (30, 10 and 3 μg/mL) and incubated for 8 and 12 days at 37 °C. The XRMA and NR was then carried out to estimate viable cells present in different wells of the assay plate (33, 34). The optical density was read on a micro plate reader (Spectramax plus 384 plate reader, Molecular Devices Inc.) at 470 nm filter for XTT and at 540 nm filter for NR against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as 100% cell growth. Initially primary screening was done at 30, 10 and 3 μg/ml. Compounds showing 90% inhibition of bacilli at 30 μg/ml which were selected for further dose response curve. MIC and IC50 values of selected compound were calculated from their dose response curves by using Origin 6 software.

Percent inhibition was calculated by using following formula:

% Inhibition = [(absorbance of Control − absorbance of Test)

/(absorbance of Control−−absorbance of Blank)] X 100

where control is the medium with bacilli along with vehicle and blank is cell free medium.

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Appendix A. Supplementary material

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References

[1] World Health organization, Global Tuberculosis Report, 2018: http://www.who.int/tb/publications/global_report/en/.
[2] S.K. Parida, R.A. Robertson, M.V. Rao, N. Singh, I. Master, A. Lutckii, S. Keshavjee, J. Andersson, A. Zumla, M. Maeurer, Totally drug-resistant tuberculosis and adjunct therapies, J. Intern. Med. 277 (2017) 388–405.
[3] M. Berry, O.M. Kon, Multidrug and extensively drug-resistant tuberculosis: an emerging threat, Eur. Respir. Rev. 18 (2009) 195–197.
[4] A. Zumla, P. Nahid, S.T. Cole, Advances in the development of new tuberculosis drugs and treatment regimen, Nat. Rev. Drug Discov. 12 (2013) 388–404.
[5] K. Mdluli, T. Kaneko, A. Upton, The Tuberculosis Drug Discovery and Development Pipeline and Emerging Drug Targets, Cold Spring Harbor Perspect. Med, 2015, pp. 1–25.
[6] P. De, D. Veau, F.B. Belval, S. Chassaing, M. Baltas, Cinnamic Derivatives in Tuberculosis, Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance, Dr. Pere-Joan Cardona (Ed.), (2012). ISBN: 978-953-307-948-6, In Tech.
[7] N. Rastogi, K.S. Goh, L. Horgena, W.W. Barrow, Synergistic activities of anti-tuberculous drugs with cerulenin and trans-cinnamic acid against Mycobacterium tuberculosis, Fems Immunol. Med. Mic. 21 (1998) 149–157.
[8] M.D. Kakwani, P. Suryavanshi, M. Ray, M.G.R. Rajan, S. Majee, A. Samad, P. Devarajan, M.S. Degani, Design, synthesis and antimycobacterial activity of cinnamide derivatives: a molecular hybridization approach, Bioorg. Med. Chem. Lett. 21 (2011) 1997–1999.
[9] S.A. Carvalho, E.F. da Silva, M.V.N. de Souza, M.C.S. Lourenço, F.R. Vicente, Synthesis and antimycobacterial evaluation of new trans-cinnamic acid hydrazide derivatives, Bioorg. Med. Chem. Lett. 18 (2008) 538–541.
[10] P. De, G.K. Yoya, P. Constant, F.B. Belval, H. Duran, N. Saffon, M. Daff, M. Baltas, Synthesis design and biological evaluation of new cinnamic derivatives as anti-tuberculosis agents, J. Med. Chem. 54 (5) (2011) 1449–1461.
[11] G.A. Hampannavar, R.V. Karpoormath, M.B. Palkar, M.S. Shaikh, B. Chandrasekaran, Dehydrozingerone inspired styryl hydrazine thiazole hybrids as promising class of anti-mycobacterial agents, ACS Med. Chem. Lett. 7 (2016) 686–691.
[12] R.V. Shingalapur, K.M. Hosamani, R.S. Keri, Synthesis and evaluation of in vitro anti-microbial and anti-tubercular activity of 2-styryl benzimidazoles, Eur. J. Med. Chem. 44 (2009) 4244–4248.
[13] J. Saunders, A.M. MacLeod, K. Merchant, G.A. Shadwell, R.J.B. Snow, L.J. Street, R. Baker, Ester bio-isosteres: synthesis of oxadiazolyl-1-azabicyclo[2.2.1]heptane as muscarinic agonists, J. Chem. Soci Chem. Commun. (1988) 1618–1619.
[14] P. Kaur, G.L. Khatik, Identification of novel 5-Styryl-1,2,4-Oxadiazole/Triazole derivatives as the potential anti-androgens through molecular docking study, Int. J. Pharm. Pharm. Sci. 8 (10) (2016) 72–77.
[15] S. Borg, K. Luthman, F. Nyberg, L. Terenius, U. Hacksell, 1,2,4-Oxadiazole derivatives of phenylalanine: potential inhibitors of substance P endopeptidase, Eur. J. Med. Chem. 28 (1993) 801–810.
[16] M.L. Boys, L.A. Schretzman, N.S. Chandrakumar, M.B. Tollefson, S.B. Mohler, V.L. Downs, T.D. Penning, M.A. Russell, J.A. Wendt, B.B. Chen, H.G. Stenmark, H. Wu, D.P. Spangler, M. Clare, B.N. Desai, I.K. Khanna, M.N. Nguyen, T. Duffin, V.W. Engleman, M.B. Finn, S.K. Freeman, M.L. Hanneke, J.L. Keene, J.A. Klover, G.A. Nickols, M.A. Nickols, C.N. Steininger, M. Westlin, W. Westlin, Y.X. Yu, Y. Wang, C.R. Dalton, S.A. Norring, Convergent, parallel synthesis of a series of bi-substituted 1,2,4-oxadiazole butanoic acids as potent and selective αvβ3 receptor antagonists, Bioorg. Med. Chem. Lett. 16 (2006) 839–844.
[17] L.B. Clapp, 1,2,4-Oxadiazoles, Adv. Heterocycl. Chem. 20 (1976) 65–116.
[18] A. Tolmachev, A.V. Bogolubsky, S.E. Pipko, A.V. Grishchenko, D.V. Ushakov, A.V. Zhemera, O.O. Viniychuk, A.I. Konovets, O.A. Zaporozhets, P.K. Mykhailiuk, Y.S. Moroz, Expanding synthesizable space of disubstituted 1,2,4-oxadiazoles, ACS Comb. Sci. 18 (2016) 616–624.
[19] A. Banerjee, E. Dubnau, A. Quemard, V. Balasubramanian, K.S. Um, T. Wilson, D. Collins, G.L. de, W.R. Jacobs, InhA a gene encoding a target for isoniazide and ethionamide in Mycobacterium tuberculosis, Science 263 (1994) 227–230.
[20] J.P.D. Veldhoven, Structure-activity relationships of trans substituted- propenoic acid derivatives on the nicotinic acid receptor HCA2 (GPR109A) for acid synthesis from aldehydes, Bioorg. Med. Chem. Lett. 21 (2011) 2736–2739.
[21] F. Eloy, R. Lenaers, The chemistry of amidoximes and related compounds, Chem. Rev. 62 (1961) 155–183.
[22] T.L. Deegan, T.J. Nitz, D. Cebzanov, D.E. Pufko, J.A. Porco Jr., Parallel synthesis of 1,2,4-oxadiazoles using CDI activation, Bioorg. Med. Chem. Lett. 9 (1999) 209–212.
[23] A. Khan, D.A. Sarkar, Simple whole cell based high throughput screening protocol using, Mycobacterium bovis BCG for inhibitors against dormant and active tubercle bacilli, J. Micro. Method. 73 (2008) 62–68.
[24] C. Vilchèze, H.R. Morbidoni, T.R. Weisbrod, H. Iwamoto, M. Kuo, J.C. Sacchettini, W.R. Jacobs, Inactivation of the InhA-encoded fatty acid synthase II (FASII) enoyl-acyl carrier protein reductase induces accumulation of the FASI end products and cell lysis of Mycobacterium smegmatis, J. Bacteriol. 14 (2000) 4059–4067.
[25] K.D. Asgaonkar, G.D. Mote, T.S. Chitre, QSAR and molecular docking studies of oxadiazole-ligated pyrrole derivatives as enoyl-ACP (CoA) reductase inhibitors, Sci. Pharm. 82 (2014) 71–85.
[26] S.G. Kini, A.R. Bhat, B. Bryant, J.S. Williamson, F.E. Dayan, Synthesis, anti-tubercular activity and docking study of novel cyclic azole substituted diphenyl ether derivatives, Eur. J. Med. Chem. 44 (2009) 492–500.