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-tuberculcar 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 (Mtbg) H37R strain. The structure-activity relationship (SAR) study has identified several compounds with mixed anti-tubercular profiles. The compound 32 displayed potent anti-tubercular activity (IC50 = 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 multidrug-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 antitubercular 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]. Piplarine 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...
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 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 3rd position 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 IC50 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 (IC50 = 0.06 µg/mL) against Mycobacterium tuberculosis (Mtb) H37Ra 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 (IC50 = 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
The halogen substituted on phenyl ring compound 22 (consisting of 4-chloro substitution on phenyl ring) exhibited moderate activity (IC₅₀ = 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 (IC₅₀ = 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, Table 1). While 2-nitro and 3-CF₃ phenyl substituted compounds are active (compound 33 IC₅₀ = 0.56; compound 31 IC₅₀ = 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 (IC₅₀ = 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 (IC₅₀ = 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 (IC50 = 0.56 µg/mL, Table 2) displayed improvement in activity by ∼4 fold as compared to compound 24 (IC50 = 2.35 µg/mL, Table 1). Also compound 23 (IC50 = 9.91 µg/mL, Table 1) shows moderate activity whereas with monoy analogue compound 41 shows increase in activity by ∼5 fold (IC50 = 1.83 µg/mL, Table 2). Similarly, compound 42 shows increase in activity by ∼60 folds (IC50 = 0.17 µg/mL, Table 2) with respect to compound 30 (IC50 = 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 IC50 = 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 (IC50 = 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 in-silico 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 anti-tubercular 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 MtbH37Rv. 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 (IC50 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 MtbH37Rv 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 15s)

To a solution of pyridine (3 vol), was added aldehyde (2mmol) followed by catalytic amount of piperidine (0.1mmol). 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 (2mmol). 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) (10g, 94 mmol) malonic acid (13)

510
(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 acetone (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;
1H NMR (300 MHz, DMSO-d6) 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 Purity: 94.18%.

Similarly compounds from 15a to 15s were synthesized using general procedure as demonstrated for compound2.

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 ef-
fervescence of CO2 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 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 °C.

Nature: white semi solid; Yield: 60.0%; m.p. low melting semi solid;
1H NMR (300 MHz, DMSO-d6) 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), 3.757–3.704 (t,
J = 3.6 Hz, 3H, Ar), 2.75–2.704 (t, J = 15.9 Hz, 1H, trans), LC-MS (ESI
+ve) m/z 175.03 [M+H]+; HPLC Purity: 97.95%.

4.1.4. 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 CO2 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
describe the title compound. The solid product was dried in oven at 40 to
60 °C.

(E)-5-(2-chlorostyryl)-3-nonyl-1,2,4-oxadiazole (41) (1.9 g).

Nature: white semi solid; Yield: 60.0%; m.p. low melting semi solid;
1H NMR (300 MHz, DMSO-d6) δ (ppm): 7.86–7.80 (d, J = 18 Hz, 1H, trans), 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 heterocyclic ring), 1.77–1.65 (m, 2H, attached to CH3), 0.95–0.90 (t, J = 6 Hz, 3H, -CH3); 13C NMR (75 MHz, DMSO-d6) 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 (CH3 attached to oxadiazole, -1C), 20.16 (CH3 attached to CH2, -1C), 13.73 (terminal CH3, -1C); LC-MS (ESI +ve) m/z 215.1 [M + H]+; HPLC Purity: 97.95%.
4.2. Biology

4.2.1. Material

All the chemicals such as sodium salt XTT, DMSO, sulfuric 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 H37Ra (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 KH₂PO₄, 0.25 g trisodium citrate, 60 mg MgSO₄, 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 to 620 nm.

4.2.3. Anti-mycobacterial assay

All the synthesized compounds were screened for their in vitro activity against M. tuberculosis H37Ra (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 were 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 primary 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:

\[
\text{Percent inhibition} = \left(1 - \frac{\text{absorbance of Control}}{\text{absorbance of Test}}\right) \times 100
\]

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.054.

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