

SHORT COMMUNICATION

Antimycobacterial activity: synthesis of novel 3-(substituted phenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole analogues

Mohamed Ashraf Ali^{1,2}, Rusli Ismail¹, Tan Soo Choon¹, Suresh Pandian², and Mohamed Zaheen Hassan Ansari²

¹Pharmacogenetic and Novel Therapeutic, Institute for Research in Molecular Medicine, Universiti of Sains Malaysia, Pennag-11800, Malaysia, and ²New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan 301030, India

Abstract

In this study, a series of novel 3-(substituted phenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole analogues were synthesized and evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* (MTB) H₃₇Rv and isoniazid resistant *M. tuberculosis* (INHR-MTB). All the newly synthesized compounds were showing moderate to high inhibitory activities. The compound 6,7-dimethoxy-3-(4-chloro phenyl)-4H-indeno[1,2-c]isoxazole (**4b**) was found to be the most promising compound, active against MTB H₃₇Rv and INHR-MTB with minimum inhibitory concentrations of 0.22 and 0.34 μM.

Keywords: Isoxazole, antimycobacterial agents, isoantimycobacterial agents, tuberculosis

Introduction

Many human illnesses are especially caused by infections with microbes such as virus, bacteria, or fungi. Among various illnesses to human beings, certain viral, tubercular, bacterial, and fungal infections are more common and dangerous. This is because of their tendency to develop new strains under any circumstances for developing resistance with the available drugs. This condition has paved the way for scientists to make the efforts to work on several molecules for coming out with novel entities to combat the illnesses.

Tuberculosis (TB) is making a worldwide resurgence. Several factors may be responsible for the increase in the infection rate such as infection with human immunodeficiency virus, change in economic and social circumstances, and decline in TB control programs.¹ In addition, outbreaks of multidrug resistant TB have been identified.² When the AIDS pandemic began, one-third of the world population was infected with *Mycobacterium tuberculosis* (MTB). Each year, eight to 10 million people are

developing active disease and three million people die from TB. Currently, the available first-line anti-TB agents such as rifampicin, ethambutol, streptomycin, and pyrazinamide are highly effective and are generally well tolerated. Problems in the chemotherapy of TB arise when any patients develop resistance to any of these drugs. This is due to the fact that the second-line drugs such as *p*-aminosalicylic acid, amikacin, cycloserine, capreomycin, and ethionamide are less effective and more toxic.³ The global mortality rate for TB is very high and the development of new kinds of TB such as Multi-drug-resistant tuberculosis (MDR-TB) and Extensively drug-resistant tuberculosis (XDR-TB) alarm for the discovery of new drugs to reduce the potential hazards caused by the fatal disease.

In the past decade, most heterocyclic systems have been used as a source to discover new compounds with varied biological potentials. Especially, nitrogen-containing heterocyclic systems such as novel pyrazolines, substituted oxadiazole, and substituted triazole moieties play a vital role in discovering novel candidates having antimicrobial

Address for Correspondence: Mohamed Ashraf Ali, New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan 301030, India. Tel.: +91-99-11-128001; Fax: +91-11-26059666. E-mail: asraf80med@rediffmail.com

(Received 30 March 2010; revised 01 October 2010; accepted 04 October 2010)

potentials.⁴⁻⁹ Recently, we reported that the azole derivatives possess anti-TB activity.¹⁰⁻¹² Also, substituted azole derivatives have been evaluated for their antiproliferative activity against human lung cancer A549.¹³

From the above facts, it was evident that the microbial infections making worldwide resurgence and the whole scientific community have to pave their useful hands in exploring novel entities with good therapeutic response without allowing the microbes to develop their resistance. In view of these findings and in continuation of our previous work on the synthesis of isoxazole derivatives, we report the synthesis and evaluation of antimycobacterial activity against MTB and isoniazid resistant *M. tuberculosis* (INHR-MTB).

Materials

The entire chemicals were supplied by Merck Limited, Worli, Mumbai, Maharashtra, India and S. D. Fine Chemicals, Mumbai, Maharashtra, India. Melting points were determined by open-tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene-ethyl formate-formic acid (5:4:1) and benzene-methanol (8:2), the spots were located under iodine vapors or UV light. Infra-red (IR) spectrums were obtained on a Perkin-Elmer 1720 Fourier transform infrared spectroscopy (FT-IR) (KBr pellets). Proton nuclear magnetic resonance spectroscopy (¹H-NMR) spectra were recorded on a Bruker AC 300 MHz spectrometer using Tetramethylsilane (TMS) as internal standard in Dimethyl sulfoxide (DMSO)/CDCl₃. The mass spectra under Electron impact-Mass spectroscopy (EI-MS) were recorded at 70 eV ionizing voltage with a VG ProSpec instrument and are presented as *m/z*.

Methods

Chemistry

General method for the preparation of 2-[(*E*)-1-(substituted aryl)methylidene]-5,6-dimethoxy-1-indanone (**3a-3o**)

5,6-Dimethoxy-1-indanone (1.92 g, 0.01 mol) and appropriate aldehyde (1.02 g, 0.01 mol) were dissolved in ethanol and sodium hydroxide (30%, 5 ml) with 10 ml of petroleum ether. The reaction mixture was stirred at room temperature for 4 h. The resulting solution was allowed to stand overnight then poured into ice-cold water followed by neutralization with HCl. The solid separated was filtered, dried, and purified from ethanol.^{5,6}

General method for the preparation of 3-(3-substituted aryl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-*c*]isoxazole (**4a-4o**)

To 2-[(*E*)-1-(substituted phenyl)methylidene]-5,6-dimethoxy-1-indanone (**3a-3o**) (0.001 mol) in 15 ml of glacial acetic acid, 0.002 mol hydroxylamine hydrochloride was added and the reaction mixture was refluxed for 15 h and cooled. Excess of solvent was removed under reduced pressure and the reaction mixture was cooled and poured

on to crushed ice (20 g). The product obtained was filtered, washed with water, and purified from ethanol by recrystallization.

3-(4-Methoxyphenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-*c*]isoxazole (**4a**)

IR: (KBr) cm⁻¹: 3042 (CH), 1320 (C-N); ¹H-NMR (DMSO-*d*₆) ppm: 3.0-3.2 (2H, m, CH₂), 3.82 (6H, s, OCH₃), 3.97-4.0 (1H, m, CH), 5.17 (d, *J* = 6.4 Hz, 1H, CH), 6.6-7.3 (6H, m, aromatic); ¹³C-NMR (75 MHz, CDCl₃): 28.4, 35.1, 56.1, 56.1, 56.1, 91.3, 104.0, 110.4, 112.5, 120.3, 131.3, 131.9, 147.1, 149.9, 147.2, 147.8, 148.4, 152.2, 157.2; EI-MS (*m/z*): 326 (M⁺); C₁₉H₁₉NO₄; calculated: C = 70.14, H = 5.89, N = 4.30; found: C = 70.12, H = 5.87, N = 4.32%.

3-(4-Chlorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-*c*]isoxazole (**4b**)

IR: (KBr) cm⁻¹: 3042 (CH), 1320 (C-N), 786 (C-Cl); ¹H-NMR (DMSO-*d*₆) ppm: 3.0-3.2 (2H, m, CH₂), 3.82 (6H, s, OCH₃), 3.97-4.0 (1H, m, CH), 5.17 (d, *J* = 6.4 Hz, 1H, CH), 6.6-7.4 (6H, m, aromatic); ¹³C-NMR (75 MHz, CDCl₃): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 127.9, 128.7, 128.9, 128.9, 130.6, 131.0, 147.2, 147.8, 152.2, 157.2; EI-MS (*m/z*): 330 (M⁺); C₁₈H₁₆ClNO₃; calculated: C = 65.56, H = 4.89, N = 4.25; found: C = 65.58, H = 4.87, N = 4.26%.

3-(4-Dimethyl aminophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-*c*]isoxazole (**4c**)

IR: (KBr) cm⁻¹: 3042 (CH), 1320 (C-N); ¹H-NMR (DMSO-*d*₆) ppm: 2.82 (6H, s, NCH₃), 3.0-3.2 (2H, m, CH₂), 3.82 (6H, s, OCH₃), 3.97-4.0 (1H, m, CH), 5.17 (d, *J* = 6.4 Hz, 1H, CH), 6.6-7.0 (6H, m, aromatic); ¹³C-NMR (75 MHz, CDCl₃): 28.4, 35.1, 41.3, 41.3, 56.1, 56.1, 91.3, 104.0, 112.0, 113.0, 113.0, 127.2, 128.0, 127.2, 131.2, 147.2, 147.8, 148.4, 152.2, 157.2; EI-MS (*m/z*): 339 (M⁺); C₂₀H₂₂N₂O₃; calculated: C = 70.99, H = 6.55, N = 8.28; found: C = 70.98, H = 6.53, N = 8.26%.

6,7-Dimethoxy-3-phenyl-3a,4-dihydro-3H-indeno[1,2-*c*]isoxazole (**4d**)

IR: (KBr) cm⁻¹: 3042 (CH), 1320 (C-N); ¹H-NMR (DMSO-*d*₆) ppm: 3.0-3.2 (2H, m, CH₂), 3.82 (6H, s, OCH₃), 3.97-4.0 (1H, m, CH), 5.17 (d, *J* = 6.4 Hz, 1H, CH), 6.6-7.6 (7H, m, aromatic); ¹³C-NMR (75 MHz, CDCl₃): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 126.0, 128.0, 128.1, 128.8, 128.8, 130.5, 131.2, 147.2, 147.8, 152.2, 157.2; EI-MS (*m/z*): 296 (M⁺); C₁₈H₁₇NO₃; calculated: C = 73.20, H = 5.80, N = 4.74; found: C = 73.22, H = 5.78, N = 4.76%.

3-(3,4-Dimethoxyphenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-*c*]isoxazole (**4e**)

IR: (KBr) cm⁻¹: 3042 (CH), 1320 (C-N); ¹H-NMR (DMSO-*d*₆) ppm: 3.0-3.2 (2H, m, CH₂), 3.82, 3.85 (12H, s, OCH₃), 3.97-4.0 (1H, m, CH), 5.17 (d, *J* = 6.4 Hz, 1H, CH), 6.6-7.0 (5H, m, aromatic); ¹³C-NMR (75 MHz, CDCl₃): 28.4, 35.1, 56.1, 56.1, 56.1, 56.1, 91.3, 104.0, 110.4, 112.5, 120.3, 131.3, 131.9, 147.1, 149.9, 147.2, 147.8, 148.4, 152.2, 157.2; EI-MS (*m/z*): 356 (M⁺); C₂₀H₂₁NO₅; calculated: C = 65.59, H = 5.96, N = 3.94; found: C = 67.57, H = 5.97, N = 3.96%.

3-(3,4,5-Trimethoxyphenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4f)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82, 3.86 (15H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.0 (4H, m, aromatic); EI-MS (m/z): 386 (M^+); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 56.1, 56.1, 56.1, 91.3, 104.0, 110.4, 112.5, 120.3, 131.3, 131.9, 147.1, 149.9, 147.2, 147.8, 148.4, 152.2, 157.2; $\text{C}_{21}\text{H}_{23}\text{NO}_6$; calculated: C=65.46, H=6.01, N=3.59; found: C=65.42, H=6.00, N=3.61%.

3-(4-Fluorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4g)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N), 776 (C-F); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.2 (6H, m, aromatic); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 115.6, 115.6, 127.9, 127.9, 131.2, 134.1, 147.2, 147.8, 152.2, 157.2, 160.2; EI-MS (m/z): 314 (M^+); $\text{C}_{18}\text{H}_{16}\text{FNO}_3$; calculated: C=69.00, H=5.15, N=4.47; found: C=69.02, H=5.13, N=4.46%.

3-(2-Chlorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4h)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N), 786 (C-Cl); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.4 (6H, m, aromatic); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 126.9, 127.4, 128.9, 133.4, 128.9, 135.2, 131.0, 147.2, 147.8, 152.2, 157.2; EI-MS (m/z): 330 (M^+); $\text{C}_{18}\text{H}_{16}\text{ClNO}_3$; calculated: C=65.56, H=4.89, N=4.25; found: C=65.58, H=4.87, N=4.26%.

3-(2,6-Dichlorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4i)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N), 786 (C-Cl); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.5 (5H, m, aromatic); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 127.8, 128.9, 130.0, 130.0, 133.8, 134.8, 131.2, 147.2, 147.8, 152.2, 157.2; EI-MS (m/z): 364 (M^+); $\text{C}_{18}\text{H}_{15}\text{Cl}_2\text{NO}_3$; calculated: C=59.36, H=4.15, N=3.85; found: C=59.34, H=4.13, N=3.83%.

3-(4-Nitrophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4j)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.7 (6H, m, aromatic); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 124.0, 124.0, 129.0, 129.0, 131.2, 144.6, 145.2, 147.2, 147.8, 152.2, 157.2; EI-MS (m/z): 341 (M^+); $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_5$; calculated: C=63.53, H=4.74, N=8.23; found: C=63.51, H=4.76, N=8.26%.

3-(2-Furyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4k)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0

(1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.4 (2H, m, aromatic), 7.2–7.8 (3H, m, furyl); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 110.2, 110.2, 112.0, 131.2, 141.5, 147.2, 147.8, 152.2, 153.5, 157.2; EI-MS (m/z): 286 (M^+); $\text{C}_{16}\text{H}_{15}\text{NO}_4$; calculated: C=67.36, H=5.30, N=4.91; found: C=67.35, H=5.28, N=4.90%.

6,7-Dimethoxy-3-(2-thienyl)-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4l)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.0 (2H, m, aromatic), 7.2–7.5 (3H, m, thiophenyl); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 124.9, 125.1, 126.4, 131.2, 142.9, 147.2, 147.8, 152.2, 157.2; EI-MS (m/z): 302 (M^+); $\text{C}_{16}\text{H}_{15}\text{NO}_3\text{S}$; calculated: C=63.77, H=5.02, N=4.65; found: C=63.75, H=5.00, N=4.66%.

3-(4-Bromophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole (4m)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.2 (6H, m, aromatic); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 112.0, 120.4, 128.2, 128.2, 131.2, 131.7, 137.5, 147.2, 147.8, 152.2, 157.2, 160.2; EI-MS (m/z): 374 (M^+); $\text{C}_{18}\text{H}_{16}\text{BrNO}_3$; calculated: C=57.77, H=4.31, N=3.74; found: C=57.74, H=4.29, N=3.76%.

4-(6,7-Dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazol-3-yl)-2-benzonitrile (4n)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (6H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 6.6–7.2 (6H, m, aromatic); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 91.3, 104.0, 109.9, 112.0, 118.6, 127.3, 127.3, 131.2, 132.3, 132.3, 142.8, 147.2, 147.8, 152.2, 157.2; EI-MS (m/z): 321 (M^+); $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_3$; calculated: C=71.24, H=5.03, N=8.74; found: C=71.22, H=5.01, N=8.72%.

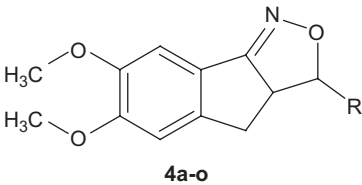
4-(6,7-Dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazol-3-yl)-2-methoxyphenol (4o)

IR: (KBr) cm^{-1} : 3042 (CH), 1320 (C-N); $^1\text{H-NMR}$ (DMSO- d_6) ppm: 3.0–3.2 (2H, m, CH_2), 3.82 (9H, s, OCH_3), 3.97–4.0 (1H, m, CH), 5.17 (d, $J=6.4$ Hz, 1H, CH), 5.6 (1H, s, OH), 6.6–7.3 (5H, m, aromatic); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): 28.4, 35.1, 56.1, 56.1, 56.1, 91.3, 104.0, 109.9, 110.8, 112.0, 120.7, 132.1, 142.8, 146.0, 147.6, 147.2, 147.8, 152.2, 157.2; EI-MS (m/z): 342 (M^+); $\text{C}_{19}\text{H}_{19}\text{NO}_5$; calculated: C=66.85, H=5.61, N=4.10; found: C=66.83, H=5.59, N=4.08%.

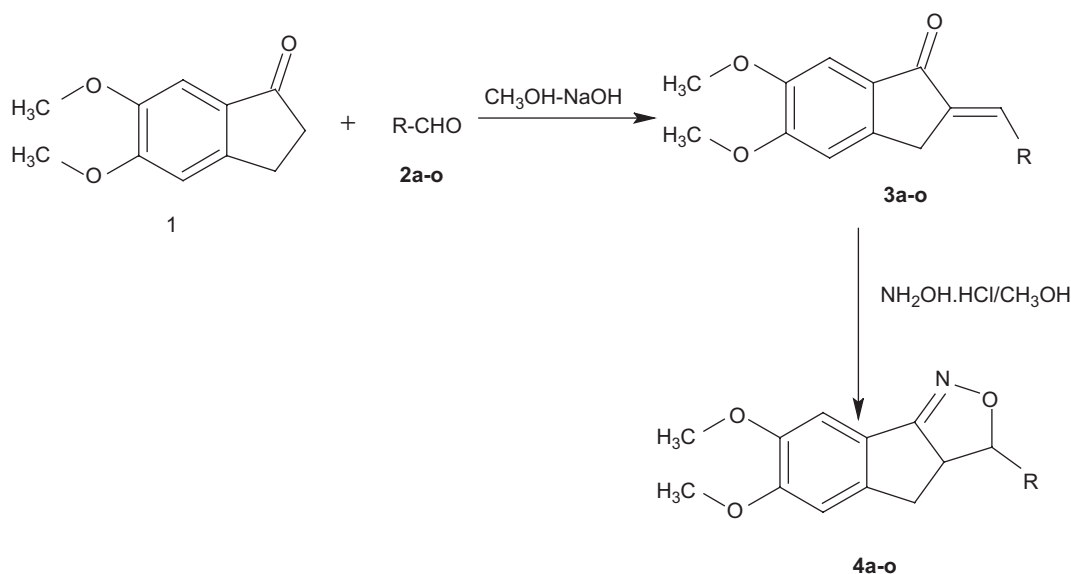
Results and discussion

3-(Substituted aryl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]isoxazole analogues (**4a–o**) described in this study are shown in Table 1 and a reaction sequence for the preparation is outlined in Scheme 1. In the initial step, 5,6-dimethoxy-2-[(*E*)-1-arylmethylidene]-1-indanone was

Table 1. Physical constants and antimycobacterial activity of the synthesized compounds.



Comp.	R	Yield (%)	MP (°C)	MIC (μM)		Cytotoxicity
				MTB ^a	MTB ^b	
4a	4-Methoxy phenyl-	84	144	>6.25	>6.25	>62.5
4b	4-Chloro phenyl-	60	162	0.22	0.34	>62.5
4c	4-Dimethylamino phenyl-	82	143	4.94	24.78	>62.5
4d	Phenyl-	70	141	>6.25	>6.25	>62.5
4e	3,4-Dimethoxy phenyl-	62	128	>6.25	>6.25	>62.5
4f	3,4,5-Trimethoxy phenyl-	75	103	>6.25	>6.25	>62.5
4g	4-Fluoro phenyl-	82	176	1.22	2.10	>62.5
4h	2-Chloro phenyl-	65	145	0.78	1.72	>62.5
4i	2,6-Dichloro phenyl-	67	164	1.98	3.96	>62.5
4j	3-Nitro phenyl-	72	184	3.46	>6.25	>62.5
4k	Furyl-	80	198	4.59	>6.25	>62.5
4l	Thiophenyl	62	168	5.68	>6.25	>62.5
4m	4-Bromo phenyl-	71	154	>6.25	>6.25	>62.5
4n	4-Cyano phenyl-	66	164	>6.25	>6.25	>62.5
4o	4-Hydroxy, 3-methoxy phenyl-	72	192	>6.25	>6.25	>62.5
INH	-	-	-	0.73	11.37	

^a*Mycobacterium tuberculosis* H₃₇R_v.^bINH resistant *Mycobacterium tuberculosis*.

Scheme 1. Protocol for synthesis.

synthesized by condensing 5,6-dimethoxy-1-indanone with appropriate aromatic aldehyde in dilute methanolic sodium hydroxide solution at room temperature. The product 5,6-dimethoxy-2-[(*E*)-1-phenylmethylidene]-1-indanone obtained in the first step is treated with hydroxylamine hydrochloride in the presence of glacial acetic acid to get titled compounds in 62–84% yield after recrystallization with ethanol. The purity of the compounds was checked by TLC and elemental analyses.

Both analytical and spectral data (¹H-NMR, IR) of all the synthesized compounds were in full agreement with the proposed structures. The elemental analysis results were within ±0.4% of the theoretical values.

The synthesized compounds (**4a–o**) were tested for their antimycobacterial activity *in vitro* against MTB and INHR-MTB by agar dilution method using double-dilution technique similar to that recommended by the National Committee for Clinical Laboratory Standards.¹⁴

The INHR-MTB clinical isolate was obtained from Tuberculosis Research Center, Alwar, India. The MIC was defined as the minimum concentration of compound required to inhibit 90% of bacterial growth and MICs of the compounds were reported in Table 1 with standard drug INH for comparison.

Among the 15 compounds synthesized eight compounds were found to be most active compounds with minimum inhibitory concentration of $<6 \mu\text{M}$ and were more active than INH against MTB. Compounds with electron withdrawing group substituted on the aryl ring were showing better activity. Among the 15 newly synthesized compounds, compound 6,7-dimethoxy-3-(4-chlorophenyl)-4*H*-indeno[1,2-*c*]isoxazole (**4b**) was found to be the most active agent against MTB H37_{Rv} and INHR-MTB with minimum inhibitory concentration of $<0.30 \mu\text{M}$. When compared with INH, compound (**4b**) was found to be 3.32- and 33.4-fold more active against MTB and INHR-MTB, respectively. Following these compounds, 2-chlorophenyl (**4h**) and 2,6-dichlorophenyl (**4i**) substituents were found to be more active than INH against MTB H_{37Rv} and INHR-MTB with MIC of 0.78 μM , 1.72 μM , and 1.28 μM , 3.96 μM , respectively. However, the electron withdrawing group such as 4-chlorophenyl, 2-chlorophenyl, 2,6-dichlorophenyl, thiophenyl, furyl, and 4-nitrophenyl substituted analogues showed good to excellent inhibitory activity against MTB H_{37Rv} and moderate to good activity against INHR-MTB. On the other hand, the electron donating group containing analogues, such as (OCH₃) group substituted 4-methoxy phenyl (**4a**), 3,4 dimethoxy phenyl (**4e**), 3,4,5 trimethoxy phenyl (**4f**), and 3-hydroxyl, 4-methoxy substituted compound (**4o**), and electron withdrawing group substituted 4-dimethylaminophenyl (**4c**) and 4-fluorophenyl (**4g**) showed moderate anti-tubercular activity. These reports clearly show that the substitution of electron withdrawing group such as 4-chloro makes remarkable improvement in antimycobacterial activity.

All the compounds were tested for cytotoxicity (IC₅₀) in VERO cells at concentrations of 62.5 $\mu\text{g/ml}$ or 10 \times . After 72h of exposure, viability was assessed on the basis of cellular conversion of 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation method.¹⁵ Most of the active compounds were found to be nontoxic up to the concentration of 62.5 $\mu\text{g/ml}$.

Conclusion

The screening of all the isoxazole derivatives identified novel compounds that are endowed with antimycobacterial activity. It is conceivable that derivatives showing more potency, selectivity, and low toxicity make them excellent leads for synthesizing novel derivatives for antimycobacterial activity against MTB and INHR-MTB. Also, these derivatives can be further modified to exhibit better potency than the standard drugs. Further studies are ongoing in our laboratory to acquire more information about quantitative structure-activity relationships

and MDR. The isoxazole derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of anti-tubercular diseases.

Acknowledgements

The authors thank Institute for Research in Molecular Medicine, Universiti of Sains Malaysia, Pennag, Malaysia and Alwar Pharmacy College, Alwar, Rajasthan, India, for providing research facilities.

Declaration of interest

The authors declare no conflicts of interest.

References

1. Kamr AG, Abdel-Latif NA, Abdalla, MM. Synthesis and antiandrogenic activity of some new 3-substituted androstano[17,16-*c*]-5'-aryl-pyrazoline and their derivatives. *Bioorg Med Chem* 2007;15:373-384.
2. Ruhoglu O, Ozdemir Z, Calis U, Gümüşel B, Bilgin AA. Synthesis of and pharmacological studies on the antidepressant and anticonvulsant activities of some 1,3,5-trisubstituted pyrazolines. *Arzneimittelforschung* 2005;55:431-436.
3. ShafieeA, BagheriM, ShekarchiM, AbdollahiM. The antinociceptive activities of 1-(4-aryl-2-thiazolyl)-3,5-disubstituted-2 pyrazolines in mouse writhing test. *J Pharm Pharm Sci* 2003;6:360-362.
4. Berghot MA, Moawad EB. Convergent synthesis and antibacterial activity of pyrazole and pyrazoline derivatives of diazepam. *Eur J Pharm Sci* 2003;20:173-179.
5. Palaska E, Aytemir M, Uzbay IT, Erol D. Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines. *Eur J Med Chem* 2001;36:539-543.
6. Turan-Zitouni G, Ozdemir A, Güven K. Synthesis of some 1-[(N, N-disubstituted thiocarbamoylthio)acetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives and investigation of their antibacterial and antifungal activities. *Arch Pharm (Weinheim)* 2005;338:96-104.
7. Jeong TS, Kim KS, An S, An SJ, Cho KH, Lee S et al. Novel 3,5-diaryl pyrazolines as human acyl-CoA:cholesterol acyltransferase inhibitors. *Bioorg Med Chem Lett* 2004;14:2715-2717.
8. Foks H, Pancechowska-Ksepko D, Janowiec M, Zwolska Z, Augustynowicz-Kopec E. Synthesis and tuberculostatic activity of some (4-phenylpiperazin-1-ylmethyl)-1,3,4-oxadiazole and (4-phenylpiperazin-1-ylmethyl)-1,2,4-triazole derivatives. *Acta Pol Pharm* 2004;61:473-476.
9. Bethge K, Pertz HH, Rehse K. New oxadiazole derivatives showing partly antiplatelet, antithrombotic and serotonin antagonistic properties. *Arch Pharm (Weinheim)* 2005;338:78-86.
10. Ali MA, Shaharyar M, Siddiqui AA. Synthesis, structural activity relationship and anti-tubercular activity of novel pyrazoline derivatives. *Eur J Med Chem* 2007;42:268-275.
11. Ali MA, Shaharyar M. Oxadiazole mannich bases: synthesis and antimycobacterial activity. *Bioorg Med Chem Lett* 2007;17:3314-3316.
12. Yar MS, Ali MA, Bakht MA, Velmurugan S. Synthesis, antimycobacterial activity of 4-[5-(substituted phenyl)-4, 5-dihydro-3-isoxazolyl]-2-methylphenol. *J Enzyme Inhib Med Chem* 2008;23:432-436.
13. Yar MS, Siddiqui AA, Ali MA, Murugan V, Chandrasekhar R. Synthesis and cytotoxic activity of novel pyrazoline derivatives against human lung tumor cell line (A549). *J Chin Chem Soc* 2007;54:81-86.
14. Heifets LB, Flory MA, Lindholm-Levy PJ. Does pyrazinoic acid as an active moiety of pyrazinamide have specific activity against *Mycobacterium tuberculosis*? *Antimicrob Agents Chemother* 1989;33:1252-1254.
15. Gundersen LL, Nissen-Meyer J, Spilsberg B. Synthesis and antimycobacterial activity of 6-arylpurines: the requirements for the N-9 substituent in active antimycobacterial purines. *J Med Chem* 2002;45:1383-1386.