Article

JOURNAL OF THE CHINESE CHEMICAL SOCIETY

5(4*H*)-Oxazolones as Novel Antitubercular Agents: Synthesis, Characterisation and Structure Activity Study

K. P. Suhasini,[a,b]* P. K. Chintakindi,[c,d] K. Chaguruswamy[a] and Y. L. N. Murthy[a]
[a]*Department of Organic Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India*
[b]*Vignan Institute of Engineering for Women, Visakhapatnam, Andhra Pradesh, India*
[c]*Bio-Organic Chemistry Division, Indian Institute of Integrative Medicine (IIIM), Jammu, India*
[d]*Catalysis and Peptide Research Unit, University of KwaZulu-Natal, Durban, South Africa*

(Received: Mar. 22, 2015; Accepted: Jul. 31, 2015; Published Online: Sept. 18, 2015; DOI: 10.1002/jccs.201500111)

In search of novel anti tubercular agents, a series of twelve 4-(substituted benzylidene)-2-*p*-tolyloxazol-5(4*H*)-ones **(5a-5ℓ)** has been synthesized, characterised and subjected to evaluate their antitubercular activity for the first time against *Mycobacterium tuberculosis* H37Rv (ATCC 27294). The out-put of these studies disclosed that all the synthesized target molecules of the series displayed good to moderate activity with MIC values ranging 2-32 μg/mL in comparison with the standard first line antitubercular drugs Rifampicin and Isoniazid. Compound **5e** with three methoxy groups meta to each other, is the most distinctive compound identified amongst the series, because of its remarkable *in vitro* antitubercular activity and thus may act as a promising lead molecule for further explorations.

Keywords: 5(4*H*)-Oxazolones; Erlenmeyer-Plochl azlactone synthesis, 4A° molecular sieves; Antitubercular; *M. tuberculosis* H37Rv; Rifampicin; Isoniazid.

INTRODUCTION

Tuberculosis (TB) is a highly contagious and airborne disease caused by *Mycobacterium tuberculosis*, emerged with multi-drug resistant strains (MDR-TB) and responsible for more human deaths than any other single infectious disease.[1,2] In spite of the increasing worldwide incidence of TB, no new drugs have been evolved into the market over the past four decades.[3,4] Therefore, there is an imperative need to develop novel antitubercular drugs.

Oxazolones, which are internal anhydrides of acyl amino acids make an important class of five-membered heterocycles and can be easily prepared from *N*-acyl amino acids by dehydration.[5] These are highly versatile intermediates used for the synthesis of several peculiar organic molecules, including amino acids, peptides,[6] heterocyclic precursors,[7] for biosensors coupling, and photosensitive composition devices for proteins.[8] Further, these compounds exhibit an assemblage of biological activities such as antimicrobial,[9] analgesic,[10] anti-inflammatory,[11] muscle relaxant,[12] neuroleptic,[13] anticancer,[14] antagonistic,[15] antiangiogenic,[16] immuno-modulator,[17] cardiotonic,[18] cyclooxygenase-2 inhibitor,[19] and tyrosinase inhibitor[20] etc.

In addition, a well known oxazolone-induced ear edema model, which is based on oxazolone moiety, was reported by Evans *et al.*[21] in mice with 2% oxazolone solution. It was a model of delayed contact hypersensitivity that permits the quantitative evaluation of the topical and systemic anti-inflammatory activity of a target compound following topical administration. In a while, Griswold *et al.*[22] modified this method by applying 3% solution of oxazolones.

Afterwards, the role of MAPKAP kinase 2 (MK2) in skin inflammation was investigated using the same model of oxazolone-induced acute allergic contact dermatitis in mice for the first time[23] and thus helped in some more investigative studies for new drug development.[24,25]

But, to the best of our knowledge, these biologically valuable molecular entities i.e., 5-(4*H*)-oxazolones remained unutilized for developing as new antituberculosis drugs. Thus, in continuation of our investigations on new heterocyclic pharmacophores, a series of twelve 4-(substituted benzylidene)-2-*p*-tolyloxazol-5(4*H*)-ones **(5a-5ℓ)** has been synthesized, characterized and evaluated for their efficacy as antitubercular agents against *M. tuberculosis* H37Rv in comparison to the two most powerful first-line tuberculosis drugs Rifampacin (RIF) and Isoniazid (INH).

* Corresponding author. Email: suhasini.kl@gmail.com

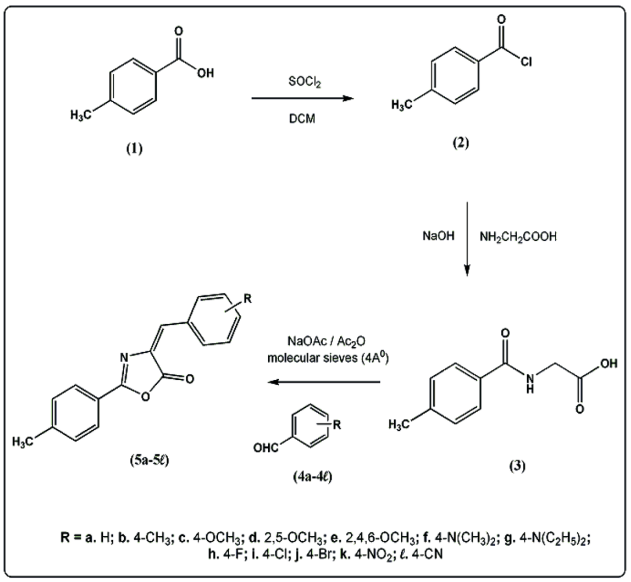
Supporting information for this article is available on the www under http://dx.doi.org/10.1002/jccs.201500111

RESULTS AND DISCUSSION

Chemistry

The present study reports the synthesis, characterization and *in vitro* antitubercular activity of the novel 5(4*H*)-oxazolones. The twelve title compounds have been synthesized from commercially available compound *p*-toulic acid **(1)**. It was converted to *p*-toulic acid chloride **(2)** using thionyl chloride and dichloro methane (DCM). The acid chloride **(2)** obtained was immediately treated *in-situ* with glycine (aminoacetic acid) in sodium hydroxide to yield 2-(*p*-methyl benzamido) acetic acid **(3)**, which on further reaction with various substituted aromatic aldehydes **(4a-4ℓ)** in presence of sodium acetate, acetic anhydride and freshly activated 4A° molecular sieves yielded the target molecules **(5a-5ℓ)** in high yields. The use of 4A° molecular sieves in the reaction, enhanced the reaction rates by the way of affecting cyclodehydration, thus increase in the yield of the products when compared to the classical Erlenmeyer-Plochl azlactone synthesis. The crude 4-(substituted benzylidene)-2-*p*-tolyloxazol-5(4*H*)-ones **(5a-5ℓ)** obtained *via* modified Erlenmeyer-Plochl azlactone synthesis, on recrystallization yielded crystalline solids. The steps involved in the synthesis are presented in Scheme 1. The structures of the target molecules are confirmed on the basis of physical and spectral data viz., FT- IR, FT-^{1}H NMR, FT-^{13}C NMR, ESI-MS and elemental analysis. All the synthesized compounds were in conformity with the structures envisaged. (Spectra are provided in the supplementary material).

Scheme 1 Synthetic route of 4-(substituted benzylidene)-2-*p*-tolyloxazol-5(4*H*)-ones **(5a-5ℓ)**



In general, the IR spectra of these compounds showed bands around ≈1700 cm^{-1}, ≈1600 cm^{-1} and ≈1100 cm^{-1} indicating the characteristic of C=O, C=N and C-O-C stretchings of 5-oxazolone ring respectively. In ^{1}H NMR spectra, the synthesized compounds showed prominent signals for the aromatic protons as doublets and multiplets between δ 7.2-8.8 ppm. The characteristic benzylidene proton showed a prominent singlet around δ 7.1 ppm for all compounds. ^{13}C NMR spectra, showed the characteristic benzylidene carbon around δ 107-115 ppm, whereas signal for characterstic C=O of 5-oxazolone ring was found between δ 170-175 ppm. The results of the elemental analyses were within ±0.4% of the theoretical values.

Pharmacological evaluation

The newly synthesised 5(4*H*)-oxazolones **(5a-5ℓ)** have been evaluated for their *in vitro* antitubercular activity against *M. tuberculosis* H37Rv (ATCC 27294) by Resazurin Microtitre Assay (REMA) method[26] and the results are summarised in Table 1. Initially, stock solutions (1 mg/mL) of the test compounds **(5a-5ℓ)** were prepared in dimethyl sulphoxide (DMSO). At the outset, the compounds were tested at 10 and 100 μg/mL concentrations. The growth of the bacteria in the microtitre plate is indicated by the colour change of resazurin (a redox dye) from blue to pink. Further progressive double dilution with DMSO was performed to obtain the required concentrations 64, 32, 16, 8, 4, 2 μg/mL for the determination of Minimum Inhibitory Concentration (MIC) values. MICs'

Table 1. The minimum inhibitory concentrations (MIC)[#] of 4-(substituted benzylidene)-2-*p*-tolyloxazol-5(4*H*)-ones **(5a-5ℓ)** against *M. tuberculosis* H37Rv

Compound	-R group	MIC (μg/ml)
5a	H	16
5b	4-CH_3	16
5c	4-OCH_3	04
5d	2,5-OCH_3	04
5e	2,4,6-OCH_3	02
5f	4-$N(CH_3)_2$	04
5g	4-$N(C_2H_5)_2$	04
5h	4-F	16
5i	4-Cl	16
5j	4-Br	32
5k	4-NO_2	08
5ℓ	4-CN	08
Rifampicin		02
Isoniazide		02

[#] Results are from one experiment of at least two independent repeats.

adopted for the study, were the lowest concentrations of the compounds required for complete inhibition of bacterial growth, with no colour change of the dye resazurin, relative to controls. Each evaluation was performed in duplicate, in two separate set of experiments and the average value is taken. The control received equivalent amount of DMSO. The standard drugs viz., Rifampicin and Isoniazid were used as reference drugs. All the tested 5(4*H*)-oxazolone derivatives exhibited potent to moderate antitubercular activities. The results are tabulated in Table 1 and graphically represented in Figure 1.

Structure activity relationship (SAR)

From Table 1, it has been observed that all the 5(4*H*)-oxazolone derivatives exhibited notable antitubercular activity with MIC values ranging from 2-32 μg/mL against *M.tuberculosis* H37Rv. The activity is considerably affected by substitution at the benzylidene ring attached to C-4 carbon of 5(4*H*)-oxazolone ring (Scheme 1).

For the structure activity relationship (SAR) studies, it is interesting to note that compound **5e** having strong electron donating 2,4,6-trimethoxy group (meta to each other) as a substituent, exhibited equivalent *in vitro* antitubercular activity to the standards Rifampicin and Isoniazid with MIC value of 2 μg/mL, amongst the twelve title compounds studied. Compounds **5c**, **5d**, **5f** and **5g** with 4-methoxy, 2,5-dimethoxy, 4-dimethyl amino and 4-diethyl amino substitutents are equipotent with MIC value 4 μg/mL. Compounds **5b**, **5h**, **5i** with 4-methyl, 4-fluoro and 4-chloro substituents respectively, exhibited equivalent activity without no difference to the unsubstituted parent compound i.e., 4-benzylidene-2-*p*-tolyloxazol-5(4*H*)-one **(5a)** with MIC value 16 μg/mL. Moreover, it is clearly evident that the electron wihtdrawing substituent 4-bromo on the benzene ring in compound **5j** showed moderate activity with MIC value 32 μg/mL against tested organism *M. tuberculosis* H37Rv. Among the compounds **5h-5ℓ** containg electron withdrawing groups, both nitro and cyano substituents **(5k** and **5ℓ)** with MIC values 8 μg/mL are considered as better inhibitors than fluoro, chloro, and bromo substituents in the series. On the whole, compound **5e** with strong electron donating methoxy groups, exhibited prominent *in vitro* antitubercular activity against *M. tuberculosis* H37Rv.

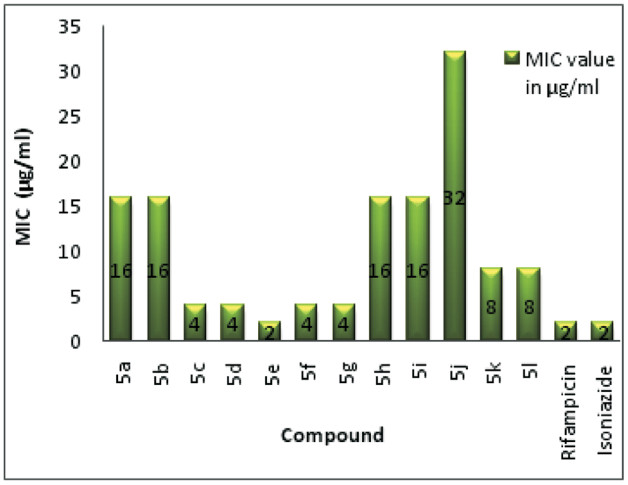


Fig. 1. MIC values of 4-(substituted benzylidene)-2-*p*-tolyloxazol-5(4*H*)-ones **(5a-5ℓ)** against M. tuberculosis H37Rv.

EXPERIMENTAL

Materials and methods: All the chemicals used were of synthetic grade and commercially procured from Qualigens. All the organic solvents adopted in the study were commercially available and dried using standard procedures. Melting points were taken in open capillaries and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1330 infrared spectrophoto- meter using KBr disc method. ^{1}H NMR and ^{13}C NMR spectra were recorded by a "Mercury-400" spectrometer using either deuteriated dimethyl sulphoxide (DMSO) or deuteriated chloroform ($CDCl_3$) as solvents. The chemical shifts δ are expressed in ppm. Electrospray Ionization (ESI) mass spectra were recorded on an Ionspec QFT FT-ICR mass spectrometer. Elemental analyses were performed on an Elementar Vario EL elemental analyzer. To assess the progress of reaction thin layer chromatography (TLC) was performed by using pre coated silica gel plates (G 350, Merck) and the compounds were visualized with aqueous $KMnO_4$. The title compounds **(5a-5ℓ)** were recrystallized twice from hot benzene to enhance the degree of purity for the pharmacological studies.

General synthesis of p-toulic acid chloride (2): A mixture of *p*-toulic acid **(1)** (0.1 mol) in DCM (30 mL) was added slowly to thionyl chloride (0.3 mol) and the reaction mixture was heated to re?ux for 4 h under anhydrous conditions. The excess thionyl chloride was distilled off and the resultant acid chloride **(2)** was used *in situ* for further reaction with glycine (aminoacetic acid).

General synthesis of 2-(p-methylbenzamido) acetic acid (3): Glycine (0.1 mol) was dissolved in NaOH (30 mL, 2 N) and stirred vigorously with mechanical stirrer, until it is totally dissolved. This was added drop wise to the crude acid chloride **(2)** (0.1 mol) and stirred vigorously for 1 h. Later it was poured into crushed ice and acidified with concentrated hydrochloric acid

with stirring. The obtained crude compound **(3)** was washed with cold water, dried and recrystallized from boiling water. White powder, Yield: 75%, m.p.: 245 °C, IR (KBr, ν_{max}, cm^{-1}): 1191, 1431, 1543, 1675, 3072, 3272. ^{1}H NMR (400 MHz, DMSO, δ, ppm): 2.75 (s, 3H, CH_3), 3.72 (s, 2H, CH_2), 7.33 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.45 (d, 2H, *J* = 8.8 Hz, Ar-H), 8.15 (s, 1H, NH), 12.66 (br s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO) δ: 22.8, 41.1, 127.6, 128.6, 132.4, 134.8, 170.5, 171.2; ESI-MS (*m/z*): 193 (M^+), Anal. Cald. for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25%; Found C, 62.15; H, 5.76; N, 7.21%.

General synthesis of 4-(substituted benzy-lidene)-2-p-tolyloxazol-5(4H)-ones (5a-5ℓ): A mixture of dry 2-(*p*-methyl benzamido) acetic acid **(3)** (0.01 mol), substituted aromatic aldehyde **(4a-4ℓ)** (0.01 mol), powdered anhydrous sodium acetate (0.01 mol) and high-grade acetic anhydride (0.03 mol) was heated at 110 °C with constant shaking. Then 4A° molecular sieves (2 g) were added to the reaction mixture and heated on a water bath for 1 h with occasional stirring. After completion of the reaction, the molecular sieves were removed by filtration and ethanol (10 mL) was added slowly to the reaction mixture and left overnight at room temperature. The crystalline product thus obtained was ?ltered, washed successively with cold alcohol, boiling water and dried. The obtained 5-oxazolones **(5a-5ℓ)** were recrystallized from hot benzene. **4-Benzylidene-2-p-tolyloxazol-5(4H)-one (5a):** Yellow crystals, Yield: 78%, m.p.: 167 °C, IR (KBr, ν_{max}, cm^{-1}): 964, 1134, 1476, 1657, 1756, 3028. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.32 (s, 3H, CH_3), 7.11 (s, 1H, =CH-), 7.28 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.39 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.53 (m, 3H, Ar-H), 7.61 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.1, 110.6, 121.4, 121.6, 122.4, 122.9, 127.2, 128.7, 129.1, 133.4, 134.9, 141.6, 166.0, 174.9; ESI-MS (*m/z*): 263 (M^+), Anal. Cald. for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32%; Found: C, 77.50; H, 4.59; N, 5.30%. **4-(4-Methyl benzylidene)-2-p-tolyloxazol-5(4H)-one (5b):** Pale yellow crystals, Yield: 80%, m.p.: 173 °C, IR (KBr, ν_{max}, cm^{-1}): 960, 1158, 1455, 1562, 1666, 1789, 3014. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.39 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.07 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.09 (s [merged with d], 1H, =CH-), 7.27-7.38 (m, 4H, Ar-H), 7.54 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 21.9, 23.7, 112.3, 128.0, 129.5, 131.2, 132.5, 135.8, 143.2, 166.7, 174.1. ESI-MS (*m/z*): 277 (M^+), Anal. Cald. for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05%; Found C, 77.90; H, 5.43; N, 5.01%. **4-(4-Methoxy benzylidene)-2-p-tolyloxazol-5(4H)-one (5c):** Yellow crystals, Yield: 82%, m.p.: 181 °C, IR (KBr, ν_{max}, cm^{-1}): 959, 1156, 1263, 1476, 1591, 1668, 1788, 2879. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.36 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.78 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.08 (s, 1H, =CH-), 7.21 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.49 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.62 (d, 2H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.0, 55.5, 110.5, 126.4, 128.0, 131.1, 132.1, 134.6, 142.2, 158.1, 166.8, 173.8. ESI-MS (*m/z*): 293 (M^+), Anal. Cald. for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78%; Found C, 73.73; H, 5.18; N, 4.75%. **4-(2,5-Dimethoxy benzylidene)-2-p-tolyl oxazol-5(4H)-one (5d):** Bright yellow crystals, Yield: 79%, m.p.: 179 °C, IR (KBr, ν_{max}, cm^{-1}): 919, 1146, 1256, 1288, 1443, 1665, 2854. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.38 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 7.06 (s, 1H, =CH-), 7.22 (s, 1H, Ar-H), 7.28-7.33 (m, 2H, Ar-H), 7.38 (d, 2H, *J* = 9.2 Hz, Ar-H), 7.41 (d, 2H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.1, 54.5, 58.2, 109.7, 120.9, 122.3, 124.4, 127.9, 132.1, 136.5, 143.1, 154.5, 158.2, 166.4, 172.6. ESI-MS (*m/z*): 323 (M^+), Anal. Cald. for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33%; Found C, 70.54; H, 5.28; N, 4.30%. **4-(2,4,6-Trimethoxy benzylidene)-2-p-tolyloxazol-5(4H)-one (5e):** Deep yellow crystals, Yield: 84%, m.p.: 174 °C, IR (KBr, ν_{max}, cm^{-1}): 940, 1097, 1155, 1287, 1452, 1581, 1667, 1775, 2987. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.36 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.48 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.15 (s, 1H, =CH-), 7.38 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.49 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.72 (d, 1H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.7, 56.2, 60.9, 62.0, 107.6, 120.6, 126.2, 127.9, 129.6, 130.9, 135.0, 141.0, 154.7, 156.8, 158.0, 166.8, 173.8. ESI-MS (*m/z*): 353 (M^+), Anal. Cald. for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96%; Found C, 67.99; H, 5.40; N, 3.97%. **4-(4-Dimethylamino benzylidene)-2-p-tolyl-oxazol-5(4H)-one (5f):** Deep red crystals, Yield: 72%, m.p.: 178 °C, IR (KBr, ν_{max}, cm^{-1}): 957, 1159, 1243, 1376, 1428, 1586, 1660, 3017. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.40 (s, 3H, CH_3), 3.09 (s, 6H, 2xCH_3), 6.53 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.07 (s, 1H, =CH-), 7.43-7.51 (m, 4H, Ar-H), 7.59 (d, 2H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.6, 40.1, 111.4, 121.5, 126.4, 128.2, 130.8, 133.6, 134.8, 135.9, 145.9, 167.4, 173.0. ESI-MS (*m/z*): 306 (M^+), Anal. Cald. for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14%; Found C, 74.45; H, 5.55; N, 9.12%. **4-(4-Diethylamino benzylidene)-2-p-tolyloxazol-5(4H)-one (5g):** Orange red crystals, Yield: 70%, m.p.: 197 °C, IR (KBr, ν_{max}, cm^{-1}): 956, 1196, 1274, 1407, 1577, 1664, 1753, 2957. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.22-1.26 (t, 6H, *J* = 6.8 Hz, 2xCH_3), 2.38 (s, 3H, CH_3), 3.42-3.48 (q, 4H, *J* = 7.2 Hz, 2xCH_2), 6.51 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.05 (s, 1H, =CH-), 7.38 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.49 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.57 (d, 2H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 12.7, 23.6, 44.8, 111.1, 120.9, 126.0, 127.9, 130.7, 133.6, 135.2, 135.9, 150.1, 167.5, 172.8. ESI-MS (*m/z*): 334 (M^+), Anal. Cald. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38%; Found C, 75.40; H, 6.60; N, 8.29%.

4-(4-Fluoro benzylidene)-2-p-tolyloxazol-5(4H)-one (5h): Pale yellow crystals, Yield: 68%, m.p.: 182 °C, IR (KBr, ν_{max}, cm^{-1}): 958, 1168, 1216, 1418, 1675, 1764, 3033. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.39 (s, 3H, CH_3), 7.13 (s, 1H, =CH-), 7.21 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.36 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.30 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.42 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.5, 112.1, 125.7, 126.5, 128.1, 129.6, 131.1, 131.9, 132.7, 142.5, 164.6, 174.8. ESI-MS (*m/z*): 281 (M^+), Anal. Cald. for $C_{17}H_{12}FNO_2$: C, 72.59; H, 4.30; N, 4.98%; Found C, 72.55; H, 4.32; N, 4.96%. **4-(4-Chloro benzylidene)-2-p-tolyloxazol-5(4H)-one (5i):** Yellow crystals, Yield: 78%, m.p.: 179 °C, IR (KBr, ν_{max}, cm^{-1}): 784, 960, 1163, 1424, 1667, 1791, 3014. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.37 (s, 3H, CH_3), 7.14 (s, 1H, =CH-), 7.22 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.32 (d, 2H, *J* = 9.2 Hz, Ar-H), 7.51 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.56 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.8, 114.5, 128.0, 128.9, 130.2, 131.2, 131.7, 133.4, 135.8, 137.3, 143.2, 166.2, 174.5. ESI-MS (*m/z*): 297 (M^+), 299 (M+2), Anal. Cald. for $C_{17}H_{12}ClNO_2$: C, 68.58; H, 4.06; N, 4.70%; Found C, 68.55; H, 4.02; N, 4.68%. **4-(4-Bromo benzylidene)-2-p-tolyloxazol-5(4H)-one (5j):** Pale yellow crystals, Yield: 70%, m.p.: 188 °C, IR (KBr, ν_{max}, cm^{-1}): 956, 1159, 1486, 1661, 1785, 2956. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.39 (s, 3H, CH_3), 7.18 (s, 1H, =CH-), 7.28 (d, 2H, Ar-H), 7.35 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.43-7.55 (m, 4H, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.6, 113.8, 125.5, 127.0, 128.8, 129.8, 131.9, 132.1, 132.5, 133.1, 146.7, 165.8, 175.4. ESI-MS (*m/z*): 341 (M^+), 343 (M+2), Anal. Cald. for $C_{17}H_{12}BrNO_2$: C, 59.67; H, 3.53; N, 4.09%; Found C, 59.66; H, 3.55; N, 4.04%. **4-(4-Nitro benzylidene)-2-p-tolyloxazol-5(4H)-one (5k):** Bright yellow crystals, Yield: 72%, m.p.: 193 °C, IR (KBr, ν_{max}, cm^{-1}): 962, 1167, 1340, 1498, 1511, 1668, 1794, 3045. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.41 (s, 3H, CH_3), 7.10 (s, 1H, =CH-), 7.48 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.59 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.77 (d, 2H, *J* = 8.8 Hz, Ar-H), 8.08 (d, 2H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.4, 115.3, 123.6, 128.1, 131.4, 132.6, 134.5, 135.8, 139.1, 148.1, 165.5, 175.3. ESI-MS (*m/z*): 308 (M^+), Anal. Cald. for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09%; Found C, 66.20; H, 3. 90; N, 9.04%. **4-(4-Cyano benzylidene)-2-p-tolyloxazol-5(4H)-one (5*ℓ*):** Yellow crystals, Yield: 65%, m.p.: 202 °C, IR (KBr, ν_{max}, cm^{-1}): 985, 1136, 1478, 1678, 1758, 2215, 2958. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.32 (s, 3H, CH_3), 7.15 (s, 1H, =CH-), 7.22 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.42 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.31 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.38 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (75 MHz, DMSO) δ: 23.4, 113.4, 121.4, 124.0, 126.5, 128.5, 132.1, 132.9, 133.4, 134.2, 138.3, 141.6, 164.4, 171.7. ESI-MS (*m/z*): 288 (M^+), Anal. Cald. for $C_{18}H_{12}N_2O_2$: C, 74.99; H, 4.20; N, 9.72%; Found C, 74.95; H, 4.26; N, 9.70%.

Antitubercular activity: *M. tuberculosis* strains were grown in Middlebrook 7H9 broth supplemented with 10% OADC. The culture was diluted to McFarland 2 standard with the same medium. From this, 50 μL of this culture was added to 150 μL of fresh medium in 96-well microtitre plates. The 96-well test plates were incubated at 37 °C for 7 days in CO_2 incubator. At the end of incubation period, 20 μL of 0.01% sterile resazurin solution was added to each tube and plates were again incubated for 48 h. After completion of 2nd day incubation, MIC was determined by visual inspection of the dye colour. Resazurin, a redox dye, is blue in the oxidized state and turns pink when reduced by the growth of viable cells. Compounds which prevented the change of color of the dye were considered to be inhibitory to *M. tuberculosis* H37Rv species.

CONCLUSIONS

A new series of twelve 5(4*H*)-oxazolones were obtained by a modified Erlenmeyer-Plochl azlactone synthesis, fully characterized and evaluated for their efficacy as antitubercular agents for the first time. The newly synthesized compounds exhibited promising activity against *M. tuberculosis* H37Rv and could be regarded as new hits for further development as a novel class of Anti-Mycobacterium tuberculosis agents. In particular, compound **5e** possessing strong electron donating methoxy groups was found to be prominent *in vitro* antitubercular agent among the series studied. The results were promising and worth further investigations.

REFERENCES

1. Johnson, R.; Streicher, E. M.; Louw, G. E.; Warren, R. M.; Van Helden, P. D.; Victor, T. C. *Curr. Issues Mol. Biol.* **2006**, *8*, 97.
2. Brooks, J. T.; Kaplan, J. E.; Holmes, K. K.; Benson, C.; Pau, A.; Masur, H. *Clin. Infect. Dis.* **2009**, *48*, 609.
3. Raviglione, M. C.; Snider, Jr., D. E.; Kochi, A. *JAMA* **1995**, *273*, 220.
4. Farmer, P.; Bayona, J.; Beccera, M.; Henry, J.; Furin, C.; Hiarr, H.; Kim, J. Y.; Mimic, C.; Nardell, E.; Shin, S. *Int. J. Tuberc. Lung Dis.* **1998**, *2*, 869.
5. Khan, K. M.; Zia-Ullah, Mughal, U. R.; Perveen, S.; Khan, M. T. H.; Choudhary, M. I. *Bioorg. Med. Chem.* **2006**, *14*, 6027.
6. Gottwald, K.; Seebach, D. *Tetrahedron* **1999**, *55*, 723.
7. Cannella, R.; Clerici, F.; Gelmi, M. L.; Penso, M.; Pocar, D. *J. Org. Chem*. **1996**, *61*, 1854.
8. Kojima, S.; Ohkawa, H.; Hirano, T.; Maki, S.; Niwa, H.; Ohashi, M.; Inouye, S.; Tsuji, F. I. *Tetrahedron Lett.* **1998**,

39, 5239.
9. Gill, C. H.; Argade, N. D.; Kalrale, B. K. *E-J. Chem*. **2008**, *5*, 120.
10. Mariappan, G.; Dipankar, S.; Rashmi, K.; Plipika, P.; Deepak, K. *J. Global Pharma Tech*. **2010**, *2*, 28.
11. Goksen, U. S.; Kelekci, N. G.; Goktas, O.; Koysal, Y.; Kilic, E.; Isik, S.; Aktay, G.; Ozalp, M. *Bioorg. Med. Chem*. **2007**, *15*, 5738.
12. Chatpalliwar, V. A.; Jadhav, J. S.; Khandse, S. C.; Patil, R. R. *Indian J. Heterocycl. Chem*. **2008**, *17*, 343.
13. Cascio, G.; Manghisi, E.; Fregnan, G. *J. Med. Chem.* **1989**, *32*, 2241.
14. Jat, L. R.; Mishra, R.; Pathak, D. *Int. J. Pharm. Pharm. Sci*. **2012**, *4*, 378.
15. Tsukumo, Y.; Harada, D.; Manabe, H. *J. Pharmacol. Sci.* **2010**, *113*, 255.
16. Sierra, F. M. P.; Pierre, A.; Burbridge, M.; Guilbaud, N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1463.
17. Mesaik, M. A.; Choudhary, M. I.; Rahat, S.; Murad, S.; Khan, A. A. K. M.; Ismail, Z. *Bioorg. Med. Chem.* **2004**, *12*, 2049.
18. George, P. C.; Winton, Jr.; D. J.; Richard, A. S. United States Patent, Patent No: US 4698353 A, 1987.
19. Puig, C.; Crespo, M. I.; Godessart, N.; Feixas, J.; Ibarzo, J.; Jimenez, J. M.; Soca, L.; Cardelus, I.; Heredia, A.; Miralpeix, M.; Puig, J.; Beleta, J.; Huerta, J. M.; Lopez, M.; Segarra, V.; Ryder, H.; Palacios, J. M. *J. Med. Chem.* **2000**, *43*, 214.
20. Hamidian, H.; Tagizadeh, R.; Fozooni, S.; Abbasalipour, V.; Taheri, A.; Namjou, M. *Bioorg. Med. Chem.* **2013**, *21*, 2088.
21. Evans, P. D.; Hossack, M.; Thomson, D. S. *Br. J. Pharmacol*. **1971**, *43*, 403.
22. Griswold, D. E.; Dilorenzo, J. A.; Calaresi, P. *Cell Immunol*. **1974**, *11*, 198.
23. Anne, T. F.; Claus, J.; Matthias, G.; Bom, B.; Louise, L. L.; Kragballe, K.; Iversen, L. *J. Invest. Derm.* **2009**, *129*, 891.
24. Mohamed, E.; Debprasad, C.; Naoufel, A. Z. *J. Evid. Based Complementary Altern. Med.* **2012**, *2012*, Article ID 142087.
25. Shivraj, H. N.; Se Won, P. *Mini. Rev. Med. Chem*. **2013**, *13*, 95.
26. Tyagi, S. T.; Neetu, K. T. *J. Antimicrob. Chemother.* **2007**, *60*, 288.

