# GREEN SYNTHESIS, SPECTRAL ANALYSIS OF SOME NOVEL LAWSONE DERIVATIVES AND ITS ANTI-MYCOBACTERIAL TUBERCULOSIS ACTIVITY

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## Abstract

Lawsone derivatives are synthesized by greener method. The synthesized compounds are analyzed by FT-IR, Mass, Elemental analysis, $^{1}H$ and $^{13}C$ NMR analysis. All the compounds are tested for their anti-mycobacterial tuberculosis activity. The compound **2a** having the Chlorine as a substituent exhibits higher activity with lower minimum inhibition concentration of 30µg/ml.

**Keywords:** Novel lawsone derivatives, Green synthesis, Tuberculosis, Spectral analysis.

## Introduction

Naturally occurring quinones have several different roles in organisms, they are functional constituents of several biochemical systems (e.g. ubiquinones and vitamin K1). 2-Hydroxy-1,4-naphthoquinone (Lawsone) is the principal natural dye in the leaves of henna, Lawsonia inermis. Henna has been used more than 4000 years not only as a hair dye, but also as a body paint and tattoo dye. Today, semi-permanent hair dyes containing Henna as well as its pure dye ingredient are widely used and have become increasingly popular due to their natural origin.[1] Lawsone was first isolated from the leaves of Lawsonia inermis L. in 1959,[2] Lawsone and related compounds have been reported to possess interesting biological activities such as antitumor, antibacterial and antifungal properties.[3-5] It is also used as a hair dye[6] and as an ultra-violet (UV) filter in sunscreen formulation. [7] Naphthoquinones constitute one of the largest and diverse groups of plant secondary metabolites with a broad range of properties[8,9] antifeedent[10] and allelopathic activity[11] which contribute to plant defence. They also possess important pharmacological activities, such as antioxidant, [12] anti-inflammatory,[13] anticancer.[14] With nearly one-third of the global population infected by Mycobacterium tuberculosis (MTB), tuberculosis (TB) is still a major cause of death. Indeed, in 2006 over nine million new cases and 1.7 million deaths occurred due to TB, and there is now a significant concern about the emergence of multi-drug resistant (MDR) strains of TB with an estimated 0.5 million cases worldwide.[15]

However, the nature of many biological properties of lawsone and its derivatives are remains unclear, therefore the investigation of the structure and reactivity of this prolific medicinal agent is important for organic chemists. In the present work, we have summarized a comprehensive study of the novel unexpected lawsone derivatives and its molecular structure

was analyzed by NMR spectroscopy, Mass elemental analysis and also screened for their anti-mycobacterial tuberculosis activity.

**Results and discussion**

The lawsone derivatives **1a-5a** is synthesized with excellent yields by the reaction of substituted lawsone with ethanol amine in different solvent conditions. We have tried this reaction in ten different solvents, we got a higher yield of product in water (water is a greener and eco-friendly solvent) (Scheme 1). Then the compound **1a** is reacted with acetic anhydride gives the unexpected dimer product along with the acetylation **1b** (Scheme 2) also checked the compound **1a** with chloroacetyl chloride, it gives the only unexpected dimer product **1c** without chloroacetylation (Scheme 3). The reaction time, the yields, melting points and the substitutions (–R) of the synthesized products are given in (Table 1). The structures of the all synthesized compounds **1a-5a, 1b, and 1c** are confirmed by FT-IR, $^{1}$H NMR, $^{13}$C NMR, HSQC, HMBC, Mass spectral studies and elemental analysis. FT-IR and elemental analysis data for the all synthesized compounds are given in (Table 2).

In order to determine the structures of the synthesized compounds, compound **1a, 1b, 1c** is taken as the representative compounds.

*Infra-red spectral analysis of representative compounds **1a, 1b, 1c***

The FT-IR spectrum of compound **1a** shows characteristic absorption frequencies appeared at 3352cm$^{-1}$ due to aromatic CH stretching vibrations. The absorption bands around 2923-2843cm$^{-1}$ due to the aliphatic CH stretching vibrations. The absorption band at 1674cm$^{-1}$ is assigned to carbonyl stretching vibration. The absorption band at 3428cm$^{-1}$ is assigned to NH stretching vibrations. Compound **1b** shows characteristic absorption frequencies around 3101cm$^{-1}$, 3038-2849 cm$^{-1}$, 1761 cm$^{-1}$ respectively as compound **1a** and it doesn't show the NH stretching

frequency. The absorption bands of compound **1c** 3073cm$^{-1}$, 2924-2849 cm$^{-1}$, 1678cm$^{-1}$ and 3174 cm$^{-1}$ are also similar to the compound **1a.**.

*$^{1}$H NMR spectral analysis of **1a***

In the $^{1}$H NMR spectrum of compound **1a**, the H2 protons attached to the oxygen observed as a triplet at 3.59ppm. A triplet observed at 2.88ppm is due to the H3 protons attached to the nitrogen. The NH proton H4 absorbed as a singlet at 5.44ppm. The aromatic protons are absorbed in the range of 7.52-7.78ppm.

*$^{1}$H NMR spectral analysis of **1b***

In the $^{1}$H NMR spectrum of compound **1b**, the singlet observed at 7.04ppm is due to the H3 proton attached to the nitrogen. The acetyl methyl protons are absorbed as a singlet at 2.37ppm. The aromatic protons are absorbed in the range of 7.89-8.04ppm.

*$^{1}$H NMR spectral analysis of **1c***

In the $^{1}$H NMR spectrum of compound **1c**, the singlet observed at 6.17ppm is due to the H3 proton is attached to the nitrogen. The NH proton H4 absorbed as a singlet at 11.72ppm. Aromatic protons are absorbed in the range of 7.79-8.01ppm.

*$^{13}$C NMR spectral analysis of **1a***

In the $^{13}$C NMR spectrum of compound of **1a**, 13C resonate at 57.63ppm is due to the C2 carbon attached to the oxygen and the 41.26ppm is due to the C3 carbon attached to the nitrogen. The $^{13}$C resonate at 171.81ppm is due to the ipso carbon C4a attached to the nitrogen and the $^{13}$C resonate at 106.83ppm is due to the C10a carbon which is attached to the oxygen. The two signals observed at 186.94, 180.94ppm are due to the two carbonyl carbons C5 and C10 respectively. The aromatic carbons resonated at in the range of 124.75-135.42ppm.

*$^{13}$C NMR spectral analysis of **1b***

In the $^{13}$C NMR spectrum of compound **1b**, $^{13}$C resonate at 133.13ppm is due to the C2 carbon attached to the oxygen and the 125.31ppm is due to the C3 carbon attached to the nitrogen. The $^{13}$C resonate at 153.78ppm is due to the ipso carbon C4a attached to the nitrogen and the signal at 134.77ppm is due to the C10a carbon attached to the oxygen. The two signals observed at 178.32, 184.30ppm are due to the two carbonyl carbons C5 and C10 respectively. The acetyl carbonyl carbon observed at 167.78ppm and acetyl methyl carbon observed at 20.95ppm. The aromatic carbons resonated in the range of 125.96-134.36ppm.

*$^{13}$C NMR spectral analysis of **1c***

In the $^{13}$C NMR spectrum of compound **1c**, $^{13}$C resonate at 133.14ppm is due to the C2 carbon attached to the oxygen and the 110.91ppm is due to the C3 carbon attached to the nitrogen. The $^{13}$C resonate at 159.39ppm is due to the ipso carbon C4a attached to the nitrogen and the signal at 134.39ppm is due to the C10a carbon attached to the oxygen. The two signals observed at 181.16, 184.61ppm are due to the two carbonyl carbons C5 and C10 respectively. The aromatic carbons resonated in the range of 125.31-134.80ppm.

*Mass spectral analyses*

The mass spectrum of compound **1a** gives M-2 peak m/z value 212.7 by losing two protons which are consistent with the proposed molecular mass of compound **1a**. Compound 1b gives m/z value 238.9 by identical cleavage of dimer along with the removal of methyl group which is consistent with the proposed molecular mass of compound **1b**. Compound 1c gives m/z value 212.1by identical cleavage of dimer which is consistent with the proposed molecular mass of compound **1c.**

*Anti-Mycobacterial activity*

All the synthesized compounds **1a-5a**, **1b** and **1c** are tested for the Anti-Mycobacterial activity the compound **2a** having the Chlorine as a substituent higher shows activity with lower minimum inhibition concentration of 30µg/ml than that of all other compounds and the compound **1c** is having the lower activity with high concentration of 500 µg/ml.The minimum inhibition concentration of the all compounds are given in (Table 3).

**Experimental:**

*Spectroscopy*

The infrared spectra are recorded on a Thermo Nicolet-Avatar-330 FT-IR spectrophotometer using KBr (pellets) and noteworthy absorption values ($cm^{-1}$) are obtained. $^{1}H$ and $^{13}C$ NMR spectra are recorded at 293K on BRUKER AMX-400 Spectrometer operating with the frequencies of 400 MHz and 100 MHz respectively using DMSO-$d_6$ as solvent. Samples are prepared by dissolving about 5 mg of sample in 0.5ml of DMSO-$d_6$. All the chemical shift values are referenced to TMS. Mass spectrum was recorded on APPLIED BIO-SYSTEM Mass Spectrometer using Electron Spray Ionization technique. The sample was prepared by dissolving about 2mg of compound in 5mL of HPLC grade methanol.

*Mycobacterial activity minimum inhibitory concentration (MIC) method*

Compounds demonstrating at least 90% inhibition were tested against M. Tuberculosis H37Rv at a lower concentration to determine the actual minimum inhibitory concentration (MIC) in the MicroplateAlamar Blue Assay (MABA). The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. 10-500 µg concentrations tested for antimicrobial study. Rifampin (Sigma Chemical Company, St. Louis, MO) was included as a positive drug control.

*Experimental procedure for the synthesis of compounds **1a-5a***

With a mixture of appropriate substituted lawsone (0.001M), ethanol amine (0.001 M) and 25 ml of water was added in a 50 ml borosil round bottom flask was added. The reaction mixture was stirred for 30 min at room temperature (monitored by TLC). The reaction mixture was then concentrated in vacuum to afford the products **1a-5a** (Scheme 1).

*Experimental procedure for the synthesis of compound of **1b***

The 0.001 M of 3,4-dihydro-2H-naphtho[2,3-b][1,4]oxazine-5,10-dione was dissolved in 0.004 m of acetic anhydride and it was refluxed for two hours. The reaction mixture was poured into crushed ice and filtered under vacuum to afford the product 1b (Scheme 2).

*Experimental procedure for the synthesis compound of **1c***

The 0.001 M of 3,4-dihydro-2H-naphtho[2,3-b][1,4]oxazine-5,10-dione was dissolved in 25ml of acetonitrile and 0.001 M of chloroacetylchloride was added drop wise at $0^{o}$C. The mixture was refluxed for two hours. The reaction mixture was poured into crushed ice and filtered under vacuum to afford the product **1c** (Scheme 3).

**NMR Spectral data of all compounds:**

*Compound **1a**:*

$^{1}$H NMR (δ ppm):3.59 (H2, t), 2.88 (H3, t), 5.44 (H4, s), 7.52-7.78 (Ar-H, m). $^{13}$C NMR (δ ppm): 57.63 (C2), 41.26 (C3), 171.81 (C4a), 106.83 (C10a), 186.94 (C5), 180.94 (C10). 124.75-135.42 (Ar-C).

*Compound **2a**:*

$^{1}$H NMR (δ ppm):3.42 (H2, t), 3.16 (H3, t), 5.42 (H4, s), 7.54-7.99 (Ar-H, m). $^{13}$C NMR (δ ppm): 59.17 (C2), 41.53 (C3), 161.81 (C4a), 106.55 (C10a), 168.12 (C5), 165.41 (C10). 128.79-144.18 (Ar-C).

*Compound **3a**:*

$^{1}$H NMR (δ ppm):3.19 (H2, t), 3.09 (H3, t), 5.48 (H4, s), 7.58-7.99 (Ar-H, m). $^{13}$C NMR (δ ppm): 59.15(C2), 41.60 (C3), 159.98 (C4a), 106.54 (C10a), 164.92 (C5), 161.24 (C10). 119.64-143.51 (Ar-C).

*Compound **4a**:*

$^{1}$H NMR (δ ppm):3.40 (H2, t), 3.18 (H3, t), 5.43 (H4, s), 7.26-8.00 (Ar-H, m), 2.35 (Methyl proton, s). $^{13}$C NMR (δ ppm): 59.64 (C2), 41.23 (C3), 161.25 (C4a), 106.87 (C10a), 167.76 (C5), 164.91 (C10). 128.94-142.46 (Ar-C), 21.05 (Methyl Carbon).

*Compound **5a**:*

$^{1}$H NMR (δ ppm):3.22 (H2, t), 3.12 (H3, t), 5.45 (H4, s), 7.87-8.07(Ar-H, m), 3.78 (Methoxy proton, s). $^{13}$C NMR (δ ppm): 59.60 (C2), 41.59 (C3), 159.99 (C4a), 106.99 (C10a), 164.96 (C5), 161.30 (C10). 129.06-142.58 (Ar-C), 57.68 (Methoxy carbon).

*Compound **1b**:*

$^{1}$H NMR (δ ppm): 7.04 (H3, s), 2.37 (Acetyl methyl proton, s), 7.89-8.04 (Ar-H, m). $^{13}$C NMR (δ ppm): 133.13 (C2), 125.31 (C3), 153.78 (C4a), 134.77 (C10a), 178.32 (C5), 184.30 (C10). 125.96-134.36 (Ar-C).

*Compound **1c**:*

$^{1}$H NMR (δ ppm):6.17 (H3, s), 11.72 (H4, s), 7.79-8.01 (Ar-H, m). $^{13}$C NMR (δ ppm): 133.14 (C2), 110.91 (C3), 159.39 (C4a), 134.39 (C10a), 181.16 (C5), 184.61 (C10), 125.31-134.80 (Ar-C).

## Conclusion

Lawsone derivative compounds **1a-5a**, **1b** and **1c**are synthesized by using ten different solvents, among these water is a better solvent for this reaction and it gives high yield. The synthesized compounds are analyzed by FT-IR, Mass, Elemental analysis, $^{1}$H and $^{13}$C spectral

studies. All the compounds are tested for their anti-mycobacterial tuberculosis activity. The compound **2a** having the Chlorine as a substituent shows higher activity with lower minimum inhibition concentration of 30µg/ml than that of all other compounds and the compound **1c** is having the lower activity with high concentration of 500 µg/ml.

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**Figure Captions:**

**Scheme. 1 Synthetic route for compounds 1a-5a.**

**Scheme. 2 Synthetic route for compound 1b.**

**Scheme. 3 Synthetic route for compound 1c.**

**Tables Captions:**

**Table-1 Solvent and reaction condition of the compounds 1a-5a.**

**Table-2 Substitutions –R, IR Stretching frequencies data ($cm^{-1}$) and physical properties of compounds 1a-5a, 1b and1c.**

**Table-3 *Mycobacterial activity* of compounds 1a-5a, 1b and 1c.**

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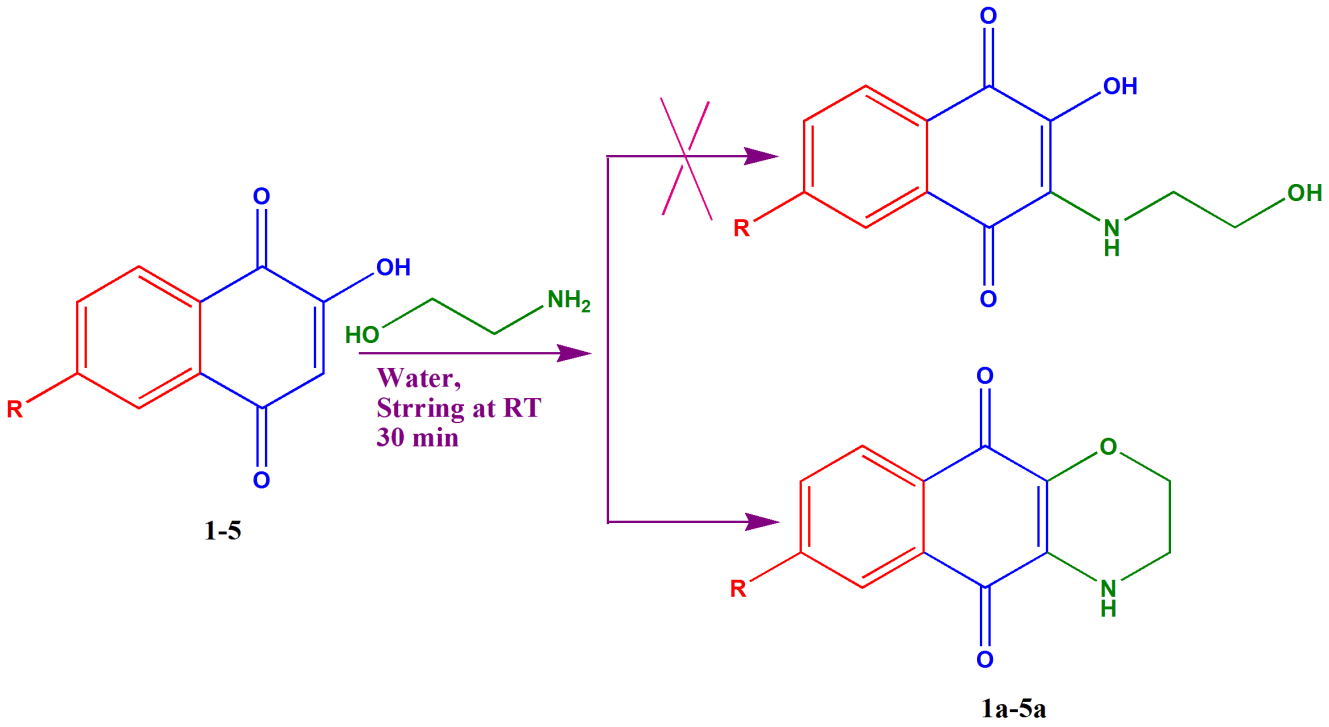
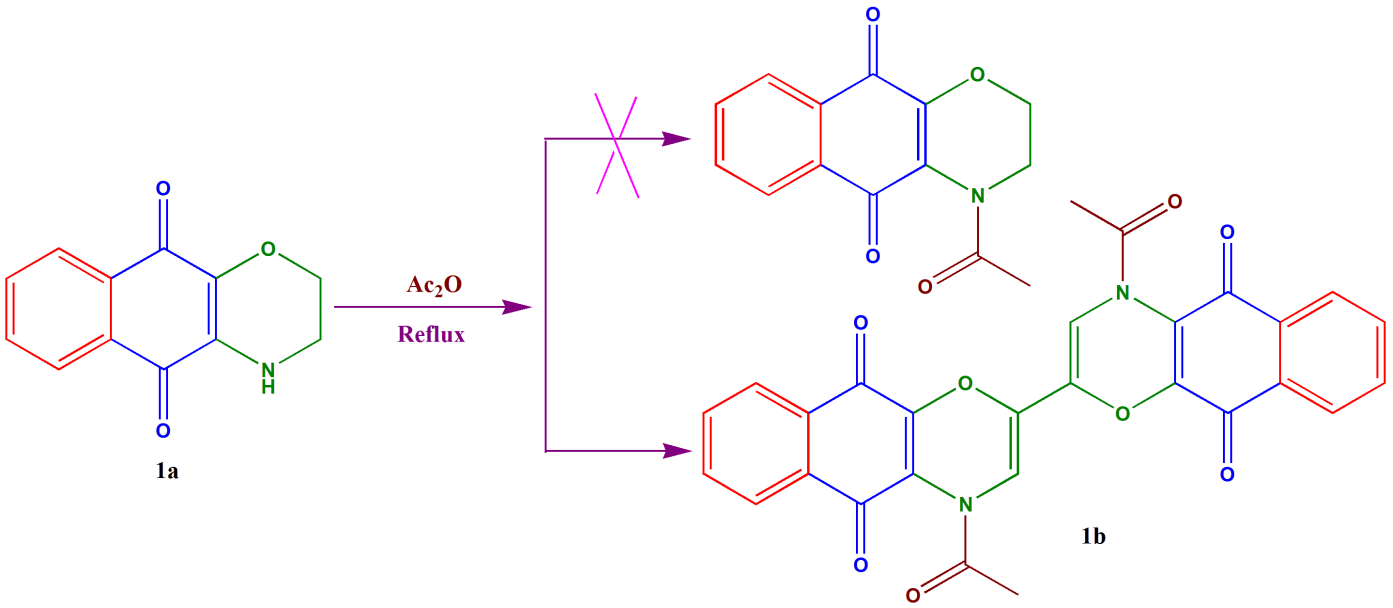



**Scheme-1 synthetic route for compounds 1a-5a**




**Scheme-2 synthetic route for compound 1b**

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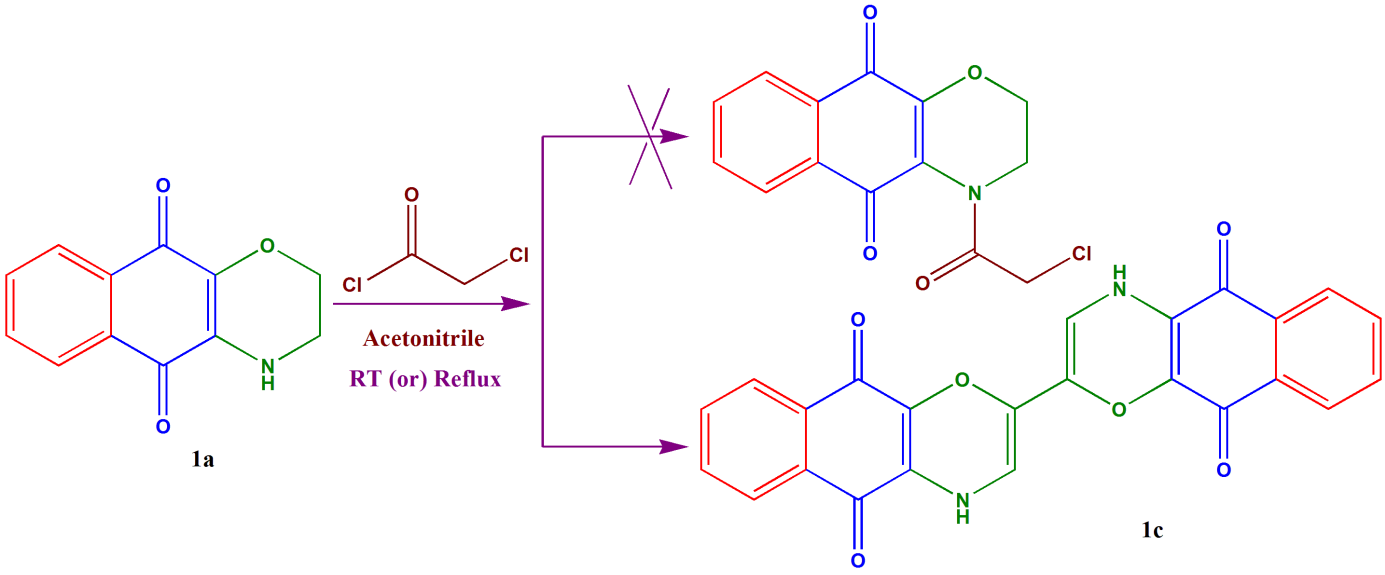


**Scheme-3 synthetic route for compound 1c**

**Table-1** Solvent and reaction condition of the compounds 1a-5a

| Entry | Yield percentage (%) | | | | | Temperature (ºC) | Time (min) |
|---|---|---|---|---|---|---|---|
| | 1a R=H | 2a R=Cl | 3a R=Br | 4a R=$CH_3$ | 5a R=$OCH_3$ | | |
| **Water** | 97 | 92 | 94 | 90 | 91 | RT | 30 |
| **Ethanol** | 89 | 86 | 81 | 80 | 82 | RT | 30 |
| **Methanol** | 85 | 81 | 80 | 76 | 78 | RT | 30 |
| **Acetonitrile** | 71 | 70 | 67 | 63 | 68 | RT | 30 |
| **Tetrahydro furan** | 76 | 74 | 72 | 78 | 71 | RT | 30 |
| **Dioxane** | 78 | 76 | 79 | 74 | 73 | RT | 30 |
| **Ethyl acetate** | 74 | 70 | 73 | 71 | 69 | RT | 30 |
| **Chloroform** | 60 | 65 | 67 | 62 | 65 | RT | 30 |
| **DMF** | 64 | 62 | 68 | 65 | 63 | RT | 30 |
| **Toluene** | 65 | 61 | 66 | 60 | 64 | RT | 30 |

RT=Room temperature

**Table-2** Substitutions –R, IR Stretching frequencies data ($cm^{-1}$) and physical properties of compounds **1a-5a, 1b** and **1c**

| Entry | Aromatic -CH | Aliphatic -CH | >C=O | -NH | Melting point ($^oC$) | Elemental Analysis | | | | | | Molecular formula |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $C_{(cal)}$ | $H_{(cal)}$ | $N_{(cal)}$ | $C_{(Exp)}$ | $H_{(Exp)}$ | $N_{(Exp)}$ | |
| **1a** | 3052 | 2923 -2843 | 1674 | 3428 | 130 | 66.97 | 4.22 | 6.51 | 66.95 | 4.19 | 6.49 | $C_{12}H_9NO_3$ |
| **2a** | 3061 | 2926 -2860 | 1663 | 3386 | 136 | 57.73 | 3.23 | 5.61 | 57.71 | 3.20 | 5.57 | $C_{12}H_8ClNO_3$ |
| **3a** | 3035 | 2923 -2849 | 1713 | 3284 | 132 | 49.01 | 2.74 | 4.76 | 48.98 | 2.72 | 4.73 | $C_{12}H_8BrNO_3$ |
| **4a** | 3013 | 2922 -2853 | 1667 | 3221 | 116 | 68.11 | 4.84 | 6.11 | 68.07 | 4.80 | 6.08 | $C_{13}H_{11}NO3$ |
| **5a** | 3030 | 2922 -2853 | 1668 | 3182 | 110 | 63.67 | 4.52 | 5.71 | 63.65 | 4.48 | 5.69 | $C_{13}H_{11}NO_4$ |
| **1b** | 3101 | 3038 -2849 | 1761 | - | 118 | 66.14 | 3.17 | 5.51 | 66.10 | 3.15 | 5.50 | $C_{28}H_{16}N_2O_8$ |
| **1c** | 3073 | 2924 -2849 | 1678 | 3174 | 122 | 67.93 | 2.85 | 6.60 | 67.91 | 2.82 | 6.57 | $C_{24}H_{12}N_2O_6$ |

**Table-3** *Mycobacterial activity* of compounds **1a-5a**, **1b** and **1c**

| **Tested sample** | **Concentration (MIC) in µg/ml** |
|---|---|
| 1a | 50 |
| 2a | 30 |
| 3a | 40 |
| 4a | 60 |
| 5a | 50 |
| 1b | 100 |
| 1c | >500 |
| Rifampicin | 30 |

Rifampicin=positive drug control