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$Mg(ClO_4)_2$ catalyzed eco-benign synthesis of 1,2,4-triazolinone derivatives as anti-tubercular agents

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Abstract: An eco-benign approach towards the synthesis of 1,2,4-triazolinones incorporated with isoindoline-1,3-dione (**4a-j**) and 9*H*-fluoren-9-imine (**5a-j**) was carried out using sydnones (**1a-j**) as synthon under magnesium perchlorate as catalyst**.** The newly synthesized compounds were screened for anti-tubercular activities.

Keywords: Sydnone; isoindoline-1,3-dione; fluroenone; $Mg(ClO_4)_2$; antitubercular activity.

1. Introduction

Sydnones are the most widely studied members of the group of heterocycles *viz.* mesoionic compounds as these compounds act as useful and novel precursors for synthesis of various biologically active heterocycles such as pyrazoles,[1] 1,3,4-oxadiazoles,[2] phenyl indazoles,[3] pyrazolines and tetrazines[4] through 1,3-dipolar cycloaddition and addition-elimination reactions. As of this criterion, we were prompted to work on the ring transformation reactions of sydnone into 1,3,4-oxadiazole which was further converted to 1,2,4-triazolinones incorporated with isoindoline-1,3-dione and 9*H*-fluoren-9-imine.

Tuberculosis (TB), a contagious disease caused by *Mycobacterium tuberculosis* (MTB), still remains a major public health threat worldwide despite ready availability of effective treatment as these pathogens are getting resistance against anti-mycobacterial agents.[5-7] The WHO has estimated that about eight million new cases of tuberculosis occur per annum and up to three million individuals die due to this disease[8] and if this ravage of tuberculosis is left unchecked, there will be about 1 billion more people newly infected with TB till 2020.[9]

Some substituted isoindoline-1,3-dione derivatives appeared to function as pharmacophoric chromophore having diverse biological activities such as tumor necrosis factor-α (TNF-α) regulating activity,[10] hypoglcemic,[11] antiviral,[12] antiandrogenic,[13] antiangiogenic,[14] antiinflammatory,[15] herbicidal,[16]

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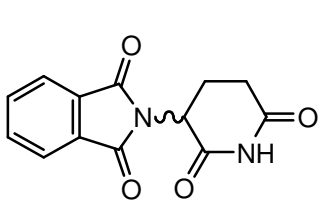
anticonvulsant,[17] hypolipidimic,[18] and antitumour[19]. Thalidomide, (*RS*)-2-(2,6-dioxopiperidin-3-yl)-1*H*-isoindole-1,3(2*H*)-dione (**1**) drug comprises of *N*-substituted isoindoline-1,3-dione derivative which acts as antineoplastic agent[20]. Fluorenone derivatives are associated with DNA intercalation property[21] that has been designed as potential immunomodulatory, cytotoxic and chemotherapeutic agents.[22] Tilorone hydrochloride, 2,7-Bis[2-(diethylamino)ethoxy]fluoren-9-one dihydrochloride (**2**) is a low molecular weight antiviral drug contains fluorenone chromophore, which is effective *in vivo* against several DNA and RNA viruses.[23]



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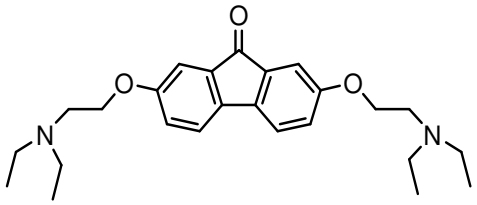

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Figure 1. Pharmacologically active isoindolin-1,3(2*H*)-dione and fluorenone compounds

The demand for green synthesis in organic transformations is increasing popularly day by day. Hence, in the present work, we made an attempt for the eco-benign synthesis of 1,2,4-triazolinones incorporated with phthalimide and fluorenone functions using magnesium perchlorate as Lewis acid catalyst that can interact with the lone pair of electrons present in the functional group required for the synthesis of title compounds through ring insertion reaction. The newly synthesized compounds were characterized by FTIR, ^{1}H NMR, MS and elemental analyses. Further, the title compounds were analyzed for their antitubercular assay.

2. Results and Discussion

2.1 Chemistry

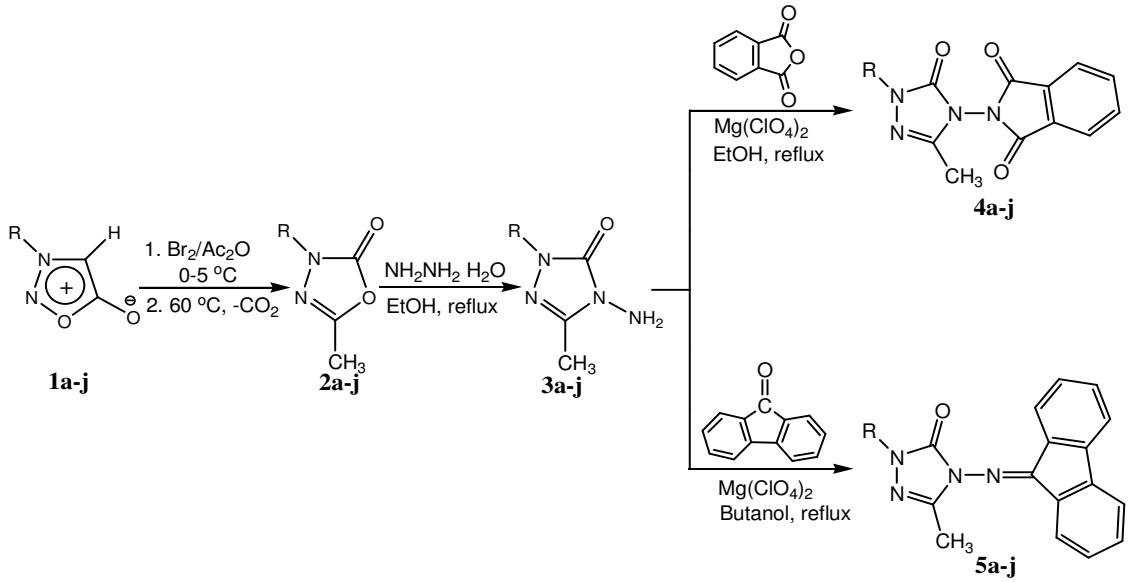
Initially, the preparation of title compounds (**4a-j** and **5a-j**) was attempted in absence of catalyst, but the yield was found to be poor and took prolonged time (8-10 h) for completion of the reaction. The nucleophilic attack by the amine on the carbonyl carbon in the first step is reversible and the rate of formation of product solely depends on removal of water molecule in final step which may be removed under azeotropic distillation, using molecular sieves or dehydrating agents such as tetramethyl orthosilicate and trimethyl orthoformate. But these methods required high reaction temperatures, prolonged time and use of costly reagents.[24] Hence, we underwent to carry out the same reaction in presence of different Lewis acid catalysts *viz.* $ZnCl_2$, $AlCl_3$, $FeCl_3$, $MgBr_2$, $CuSO_4.5H_2O$ and $Mg(ClO_4)_2$ as these can be easily removed from the reaction mixture. The yield of the product (**4a-j**) using these different catalysts was found to be improved and the range of yield observed was 60-65%, 59-61%, 56-58%, 45-50%, 65-68%, 48-51% and 76-82% respectively. As per the earlier reported methods, synthesis of Schiff bases involved the use of few drops of acids *viz*. sulphuric acid[25] or glacial acetic acid[26] to initiate the reaction, but they proved to give lower yields. We made an effort in replacing these acids by Lewis acid catalysts mentioned above and found advantage in respect to mild reaction condition and better yields for the incorporation of 4-amino-1,2,4-triazolinone with flourenone (**5a-j**) using $Mg(ClO_4)_2$. The use of Lewis acid catalyst involves the

formation of "*tight ion pair complex*" between empty *d*-orbitals of transition metal present in the catalyst and non-bonded electrons of carbonyl oxygen which makes the carbonyl carbon more electrophilic towards nucleophilic attack. As a result, the attack of lone pairs of electrons on nitrogen atom of 4-amino-1,2,4-triazolinone (**3a-j**) to carbonyl carbon of both phthalic anhydride and fluorenone (**Scheme 2** and **3**) derivatives was observed with excellent yields (76-82%) within 3-4 hrs with excellent yields. Among all the Lewis acid catalysts we used, $Mg(ClO_4)_2$ catalyzed reaction was found to commence with excellent yields and is considered to be an excellent Lewis acid catalyst for the condensation of less electrophilic carbonyl compounds with poorly nucleophilic amines to afford N-substituted phthalimide (**4a-j**) and imine (**5a-j**) derivatives from 4-amino-1,2,4-triazolinones (**3a-j**). To our satisfaction we found the use of 0.0005mol of $Mg(ClO_4)_2$ resulted in quantitative formation of corresponding products. But we failed to get azetidinone derivatives when Schiff bases (**5a-j**) were treated with chloroacetyl chloride.

The spectra of title compounds (**4a-j** and **5a-j**) were in good agreement with the assigned structures. The IR spectra of isoindoline-1,3-diones revealed a sharp band for carbonyl group of 1,2,4-triazolinone ring around 1713-1727 cm^{-1}. The carbonyl stretching of phthalimide was observed around 1724-1766 cm^{-1}. The C=N band was observed around 1611-1628 cm^{-1}. In case of ^1H NMR, a singlet around δ 2.17-2.22 ppm (3H) was attributed to *p-tolyl* methyl protons (**4b**, **4f**, **5b**, **5f**). In all the compounds a singlet in the region δ 2.42-2.56 ppm (3H) was assigned for the C5 methyl protons of 1,2,4-triazolinone ring. A singlet around δ 3.59-3.68 ppm (3H) was assigned for methoxy protons (**4d**, **4h**, **5d**, **5h**). The aromatic protons were resonated as muliplet around δ 7.08-8.91 ppm.

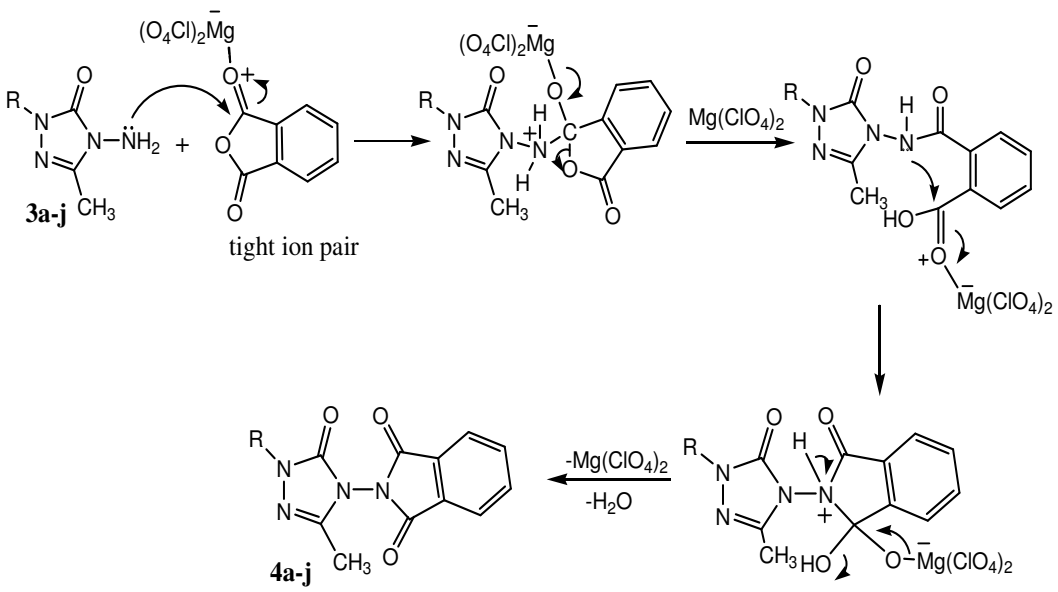
2.2 Antitubercular assay

All the title compounds prepared have been successfully screened for their potential biological activities against standard strain of *Mycobacterium tuberculosis* H_{37} RV (ATCC-27294) by Alamar blue assay method. In case of isoindoline-1,3-diones (**4a-j**), the halogen substituted (**4c**, **4e**, **4g**) and **4a**, **4b**, **4f** derivatives have shown very good activity. The nitro substituted compounds (**4i**, **4j**) have shown moderate activity, whereas methoxy substituted compounds (**4d**, **4h**) have shown least activity. However, the fluoren-9-imine derivatives (**5a-j**) did not show any considerable activity (**Table 1**).

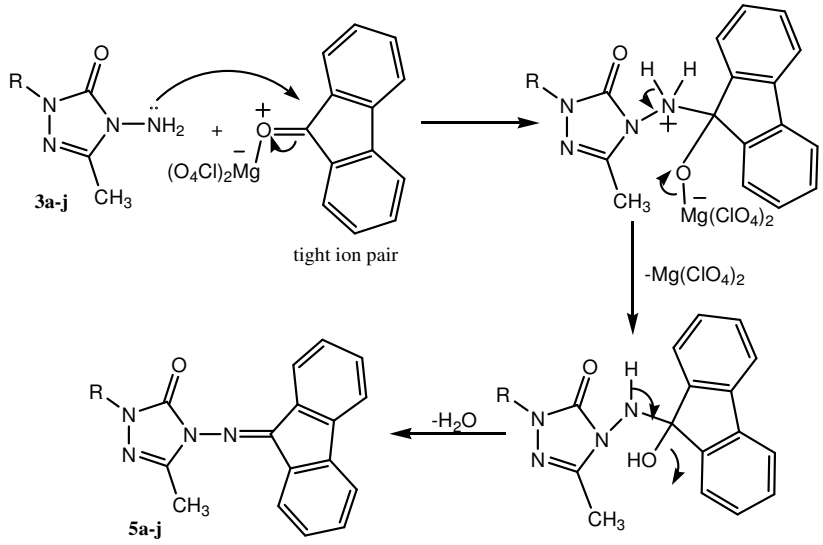


a; R= C_6H_5, **b**; R= *p*-$CH_3C_6H_4$, **c**; R= *p*-ClC_6H_4, **d**; R= *p*-$OCH_3C_6H_4$, **e**; R= *p*-BrC_6H_4, **f**; R= *m*-$CH_3C_6H_4$, **g**; R= *m*-ClC_6H_4, **h**; R= *m*-$OCH_3C_6H_4$, **i**; R= *p*-$NO_2C_6H_4$, **j**; R= *m*-$NO_2C_6H_4$

Scheme 1. Synthetic route of title compounds **4a-j** and **5a-j**



Scheme 2. Proposed mechanism for the formation of compound **4a-j**



Scheme 3. Proposed mechanism for the formation of compound **5a-j**

Table 1. Antitubercular activity of synthesized compounds (**4a-j** and **5a-j**)

Compound	R	MIC (µg/mL)
4a	C_6H_5	06.6
4b	*p*- $CH_3C_6H_4$	07.3
4c	*p*-ClC_6H_4	05.7
4d	*p*-$OCH_3C_6H_4$	21.0
4e	*p*-BrC_6H_4	05.3
4f	*m*- $CH_3C_6H_4$	07.2
4g	*m*-ClC_6H_4	05.2
4h	*m*-$OCH_3C_6H_4$	23.0
4i	*p*-$NO_2C_6H_4$	15.0
4j	*m*-$NO_2C_6H_4$	18.0
5a	C_6H_5	10.7
5b	*p*-$CH_3C_6H_4$	08.2
5c	*p*-ClC_6H_4	08.8
5d	*p*-$OCH_3C_6H_4$	25.0
5e	*p*-BrC_6H_4	09.9
5f	*m*- $CH_3C_6H_4$	08.2
5g	*m*-ClC_6H_4	11.6
5h	*m*-$OCH_3C_6H_4$	28.0
5i	*p*-$NO_2C_6H_4$	21.0
5j	*m*-$NO_2C_6H_4$	25.0
Standard	Streptomycin	06.5

All compounds were tested at 5, 10, 15, 20, 25, 30 µg/mL concentrations by serial dilution.

3. Conclusion

From the present work, it can be concluded that the methodology followed provides simple, mild and facile route for the condensation of 4-amino-1,2,4-triazolinones (**3a-j**) with phthalic anhydride and fluorenone functions to yield N-substituted phthalimide (**4a-j**) and fluoren-9-imine (**5a-j**) derivatives using magnesium perchlorate as catalyst with excellent yields. The formed bisheterocycles were found to exhibit good antitubercular activities.

4. Experimental

4.1 Methods and materials

Melting points were determined in open capillaries. FTIR spectra (KBr) were recorded on Nicolet Impact-410 FT-IR spectrometer. ^{1}H NMR spectra were recorded on Bruker-300 MHz FT-NMR spectrometer with TMS as an internal standard. Mass spectra were recorded using Finnegan MAT (Model MAT 8200) spectrometer and elemental analyses were carried out using Heraus CHN rapid analyzer. Purity of the compounds was monitored by TLC on silica gel plate using n-hexane and ethyl acetate as eluent. 4-Amino-2-aryl-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**3a-j**) were prepared from 3-arylsydnones (**1a-j**) according to the reported method *via* 5-methyl-3-aryl-oxadizolin-2-one (**2a-j**). [2, 27-28]

4.1.1. Procedure for the preparation of isoindoline-1,3-diones (4):

4-Amino-2-aryl-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (0.001mol), phthalic anhydride (0.001 mol) and magnesium perchlorate (0.0005 mol) were taken in dry butanol (20 mL) and refluxed for 5 h. Completion of the reaction was monitored by TLC using hexane and ethyl acetate (7:3) as eluent. After completion of reaction, the reaction mixture was poured into water and partitioned between water and dichloromethane. The organic layer was washed with brine solution, dried over anhydrous sodium sulphate and solvent was evaporated to dryness to get solid compound **4a-j**.

2-(3-Methyl-5-oxo-1-phenyl-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4a**). Yield 85%; M.W. 320.3; m.p. 114-116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.52 (s, 3H, C5-CH_3), 7.19-8.02 (m, 9H, Ar-H); IR (KBr) cm^{-1}: 1762, 1724 (C=O, phth), 1717 (C=O), 1605 (C=N); MS m/z: 320 (M^+, 35), 306 (20), 278 (20), 250 (15), 235 (10), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{17}H_{12}N_4O_3$: C, 63.75%; H, 3.78%; N, 17.49%. Found: C, 63.72%; H, 3.77%; N, 17.50%.

2-(3-Methyl-5-oxo-1-*p*-tolyl-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4b**). Yield 79%; M.W. 334.33; m.p. 123-125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.17 (s, 3H, Ar-CH_3), 2.45 (s, 3H, C5-CH_3), 7.22-8.11 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1758, 1725 (C=O, phth), 1719 (C=O), 1615 (C=N); MS m/z: 334 (M^+, 20), 320 (25), 292 (20), 264 (15), 250 (15), 235 (10), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{18}H_{14}N_4O_3$: C, 64.66%; H, 4.22%; N, 16.76%. Found: C, 64.67%; H, 4.23%; N, 16.74%.

2-(1-(*p*-Chlorophenyl)-3-methyl-5-oxo-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4c**). Yield 76%; M.W. 354.75; m.p. 106-108 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.53 (s, 3H, C5-CH_3), 7.22-8.23 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1759, 1732 (C=O, phth), 1723 (C=O), 1615 (C=N); MS m/z: 356 $(M+2)^+$, 30), 354 (M^+, 10), 328 (20), 326 (10), 314 (20), 312 (10), 286 (30), 250 (15), 235 (10), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{17}H_{11}ClN_4O_3$: C, 57.56%; H, 3.13%; N, 15.79%. Found: C, 57.55%; H, 3.12%; N, 15.77%.

2-(1-(*p*-Methoxyphenyl)-3-methyl-5-oxo-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (4d). Yield 78%; M.W. 350.33; m.p. 107-109 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.51 (s, 3H, C5-CH_3), 3.65 (s, 3H, OCH_3), 7.31-8.15 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1760, 1732 (C=O, phth), 1713 (C=O), 1616 (C=N); MS m/z: 350 (M^+, 10), 336 (10), 308 (25), 280 (15), 252 (20), 237 (25), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{18}H_{14}N_4O_4$: C, 61.71%; H, 4.03%; N, 15.99%. Found: C, 61.72%; H, 4.05%; N, 15.98%.

2-(1-(*p*-Bromophenyl)-3-methyl-5-oxo-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4e**). Yield 76%; M.W. 399.2; m.p. 135-137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.47 (s, 3H, C5-CH_3), 7.26-8.29 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1763, 1728 (C=O, phth), 1718 (C=O), 1618 (C=N); MS m/z: 401 $(M+2)^+$, 20), 399 (M^+, 20), 373 (10), 371 (10), 343 (25), 341 (25), 263 (35), 249 (30), 234 (25), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{17}H_{11}BrN_4O_3$: C, 51.15%; H, 2.78%; N, 14.03%. Found: C, 51.17%; H, 2.79%; N, 14.01%.

2-(3-Methyl-5-oxo-1-*m*-tolyl-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4f**). Yield 83%; M.W. 334.33; m.p. 118-120 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.18 (s, 3H, Ar-CH_3), 2.52 (s, 3H, C5-CH_3), 7.09-8.18 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1758, 1724 (C=O, phth), 1721 (C=O), 1617 (C=N); MS m/z: 334 (M^+, 20), 320 (25), 292 (20), 264 (15), 250 (15), 235 (10), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{18}H_{14}N_4O_3$: C, 64.66%; H, 4.22%; N, 16.76%. Found: C, 64.64%; H, 4.22%; N, 16.74%.

2-(1-(*m*-Chlorophenyl)-3-methyl-5-oxo-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4g**). Yield 78%; M.W. 354.75; m.p. 104-106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.45 (s, 3H, C5-CH_3), 7.08-8.33 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1761, 1732 (C=O, phth), 1721 (C=O), 1614 (C=N); MS m/z: 356 $(M+2)^+$, 45), 354 (M^+, 15), 328 (20), 326 (10), 314 (20), 312 (10), 286 (30), 250 (15), 235 (10), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{17}H_{11}ClN_4O_3$: C, 57.56%; H, 3.13%; N, 15.79%. Found: C, 57.57%; H, 3.14%; N, 15.77%.

2-(1-(*m*-Methoxyphenyl)-3-methyl-5-oxo-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4h**). Yield 81%; M.W. 350.33; m.p. 125-127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.51(s, 3H, C5-CH_3), 3.59 (s, 3H, OCH_3), 7.23-8.25 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1752, 1726 (C=O, phth), 1718 (C=O), 1611 (C=N); MS m/z: 350 (M^+, 10), 336 (10), 308 (25), 280 (15), 252 (20), 237 (25), 207 (15), 179 (25), 165 (20), 158 (30), 149 (45), 130 (40), 118 (15), 105 (80), 91 (100), 77 (30), 65 (50), 40 (5). Anal. Calcd. for $C_{18}H_{14}N_4O_4$: C, 61.71%; H, 4.03%; N, 15.99%. Found: C, 61.72%; H, 4.02%; N, 15.97%.

2-(3-Methyl-1-(*p*-nitrophenyl)-5-oxo-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4i**). Yield 75%; M.W. 365.3; m.p. 142-144 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.50 (s, 3H, C5-CH_3), 7.53-8.91 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1766, 1726 (C=O, phth), 1721 (C=O), 1628 (C=N), 1560 (NO_2, asym.), 1353 (NO_2, sym.); MS m/z: 365 (M^+, 15), 351 (10), 323 (25), 295 (10), 281 (25), 267 (30), 252 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{17}H_{11}N_5O_5$: C, 55.89%; H, 3.04%; N, 19.17%. Found: C, 55.88%; H, 3.02%; N, 19.19%.

2-(3-Methyl-1-(*m*-nitrophenyl)-5-oxo-1*H*-1,2,4-triazol-4(5*H*)-yl)isoindoline-1,3-dione (**4j**). Yield 84%; M.W. 365.3; m.p. 136-138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.48 (s, 3H, C5-CH_3), 7.47-8.87 (m, 8H, Ar-H); IR (KBr) cm^{-1}: 1759, 1725 (C=O, phth), 1720 (C=O), 1627 (C=N), 1563 (NO_2, asym.), 1353 (NO_2, sym.); MS m/z: 365 (M^+, 15), 351 (10), 323 (25), 295 (10), 281 (25), 267 (30), 252 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{17}H_{11}N_5O_5$: C, 55.89%; H, 3.04%; N, 19.17%. Found: C, 55.90%; H, 3.02%; N, 19.15%.

4.1.2. Method 1: Procedure for preparation of fluoren-9-imine derivatives using acid (5):

4-Amino-2-aryl-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (0.001mol) and fluorenone (0.001mol) were taken in dry ethanol (15mL). Glacial acetic acid (2-3 drops) was added and refluxed for 8-10 h. Completion of reaction was monitored by TLC using hexane and ethyl acetate (8:2) as eluent. After

completion of reaction, the reaction mixture was cooled, concentrated and poured to ice cold water to get crude product which is recrystalized from petroleum ether to get yellow needles. Yield observed was **55-58%** for all the compounds (**5a-j**).

4.1.3. Method 2: Procedure for the preparation of fluoren-9-imine derivatives using catalyst (5):

4-Amino-2-aryl-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (0.001mol), fluorenone (0.001mol) and magnesium perchlorate (0.0005mol) were taken in dichloromethane (20 mL) and refluxed for 3h at 60-70 °C on water bath. Completion of reaction was monitored by TLC using hexane and ethyl acetate (8:2) as eluent. After completion of reaction, the contents were poured to water and partitioned between water and dichloromethane. The organic layer was washed with brine solution, dried over anhydrous sodium sulphate and solvent was evaporated to dryness to get analytical pure compound. Yield found to be **75-83%** for all the compounds (**5a-j**).

4-(9*H*-Fluoren-9-ylideneamino)-5-methyl-2-phenyl-2*H*-1,2,4-triazol-3(4*H*)-one (5a). Yield 82%; M.W. 352.39; m.p. 125-127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.47 (s, 3H, C5-CH_3), 7.28-7.87 (m, 13H, Ar-H); IR (KBr) cm^{-1}: 1714 (C=O), 1611 (C=N); MS m/z: 352 (M^+, 35), 337 (30), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{22}H_{16}N_4O$: C, 74.98%; H, 4.58%; N, 15.90%. Found: C, 74.97%; H, 4.43%; N, 15.89%.

4-(9*H*-Fluoren-9-ylideneamino)-5-methyl-2-*p*-tolyl-2*H*-1,2,4-triazol-3(4*H*)-one (**5b**).Yield 78%; M.W. 366.42; m.p. 58-60 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.19 (s, 3H, Ar-CH_3), 2.49 (s, 3H, C5-CH_3), 7.26-7.67 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1718 (C=O), 1623 (C=N); MS m/z: 366 (M^+, 40), 351 (35), 337 (30), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{23}H_{18}N_4O$: C, 75.39%; H, 4.95%; N, 15.29%. Found: C, 75.37%; H, 4.96%; N, 15.30%.

4-(9*H*-Fluoren-9-ylideneamino)-2-(*p*-chlorophenyl)-5-methyl-2*H*-1,2,4-triazol-3(4*H*)-one (**5c**). Yield 79%; M.W. 386.83; m.p. 102-104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.56 (s, 3H, C5-CH_3), 7.27-8.23 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1721 (C=O), 1623 (C=N); MS m/z: 388 $(M+2)^+$, 30), 386 (M^+, 10), 373 (25), 371 (8), 336 (30), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{22}H_{15}N_4ClO$: C, 68.31%; H, 3.91%; N, 14.48%. Found: C, 68.30%; H, 3.92%; N, 14.47%.

4-(9*H*-Fluoren-9-ylideneamino)-2-(*p*-methoxyphenyl)-5-methyl-2*H*-1,2,4-triazol-3(4*H*)-one (**5d**).Yield 83%; M.W. 382.41; m.p. 114-116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.42 (s, 3H, C5-CH_3), 3.64 (s, 3H, OCH_3), 7.21-7.79 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1710 (C=O), 1613 (C=N), 1148 (C-O); MS m/z: 382 (M^+, 25), 367 (30), 353 (35), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{23}H_{18}N_4O_2$: C, 72.24%; H, 4.74%; N, 14.65%. Found: C, 72.22%; H, 4.74%; N, 14.66%.

4-(9*H*-Fluoren-9-ylideneamino)-2-(*p*-bromophenyl)-5-methyl-2*H*-1,2,4-triazol-3(4*H*)-one (**5e**). Yield 76%; M.W. 431.28; m.p. 134-136 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.55 (s, 3H, C5-CH_3), 7.35-8.27 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1723 (C=O), 1626 (C=N); MS m/z: 433 $(M+2)^+$, 40), 431 (M^+, 38), 418 (30), 416 (28), 336 (30), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{22}H_{15}N_4BrO$: C, 61.27%; H, 3.51%; N, 12.99%. Found: C, 61.28%; H, 3.52%; N, 12.97%.

4-(9*H*-Fluoren-9-ylideneamino)-5-methyl-2-*m*-tolyl-2*H*-1,2,4-triazol-3(4*H*)-one (**5f**). Yield 75%; M.W. 366.42; m.p. 136-138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.22 (s, 3H, Ar-CH_3), 2.46 (s, 3H, C5-CH_3), 7.26-7.89 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1713 (C=O), 1613 (C=N); MS m/z: 366 (M^+, 35), 351 (25), 337 (30), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{23}H_{18}N_4O$: C, 75.39%; H, 4.95%; N, 15.29%. Found: C, 75.38%; H, 4.96%; N, 15.27%.

4-(9*H*-Fluoren-9-ylideneamino)-2-(*m*-chlorophenyl)-5-methyl-2*H*-1,2,4-triazol-3(4*H*)-one (**5g**). Yield 79%; M.W. 386.83; m.p. 112-114 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.58 (s, 3H, C5-CH_3), 7.26-8.27 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1721 (C=O), 1623 (C=N); MS m/z: 389 $(M+2)^+$, 40), 387 (M^+, 14), 359 (35), 357 (17), 345 (25), 343 (12), 330 (20), 328 (10), 337 (30), 293 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{22}H_{15}N_4ClO$: C, 68.31%; H, 3.91%; N, 14.48%. Found: C, 68.33%; H, 3.92%; N, 14.45%.

4-(9*H*-Fluoren-9-ylideneamino)-2-(*m*-methoxyphenyl)-5-methyl-2*H*-1,2,4-triazol-3(4*H*)-one (**5h**). Yield 83%; M.W. 382.41; m.p. 121-123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.45 (s, 3H, C5-CH_3), 3.68 (s, 3H, OCH_3), 7.32-7.81 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1709 (C=O), 1620 (C=N), 1150 (C-O); MS m/z: 382 (M^+, 25), 354 (20), 340 (20), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{23}H_{18}N_4O_2$: C, 72.24%; H, 4.74%; N, 14.65%. Found: C, 72.26%; H, 4.75%; N, 14.65%.

4-(9*H*-Fluoren-9-ylideneamino)-5-methyl-2-(*p*-nitrophenyl)-2*H*-1,2,4-triazol-3(4*H*)-one (**5i**). Yield 75%; M.W. 397.39; m.p. 128-130 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.51 (s, 3H, C5-CH_3), 7.35-8.89 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1724 (C=O), 1628 (C=N), 1565 (NO_2, asym.), 1355 (NO_2, sym.); MS m/z: 397 (M^+, 25), 369 (15), 354 (30), 340 (10), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{22}H_{15}N_5O_3$: C, 66.49%; H, 3.80%; N, 17.62%. Found: C, 66.48%; H, 3.81%; N, 17.60%.

4-(9*H*-Fluoren-9-ylideneamino)-5-methyl-2-(*m*-nitrophenyl)-2*H*-1,2,4-triazol-3(4*H*)-one (**5j**). Yield 81%; M.W. 397.39; m.p. 143-145 °C; ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 2.53 (s, 3H, C5-CH_3), 7.27-8.10 (m, 12H, Ar-H); IR (KBr) cm^{-1}: 1727 (C=O), 1619 (C=N), 1561 (NO_2, asym.), 1351 (NO_2, sym.); MS m/z: 397 (M^+, 25), 369 (15), 354 (30), 340 (10), 322 (15), 305 (20), 291 (25), 278 (15), 251 (15), 237 (10), 211 (25), 198 (30), 183 (35), 180 (45), 163 (50), 151 (55), 125 (35), 105 (40), 85 (60), 57 (80), 44 (100), 40 (10). Anal. Calcd. for $C_{22}H_{15}N_5O_3$: C, 66.49%; H, 3.80%; N, 17.62%. Found: C, 66.50%; H, 3.81%; N, 17.61%.

4.2 Antitubercular assay

The anti-tubercular activity of the test compounds were evaluated against standard strain of *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC-27294) in BACTEC 12B medium using the microplate Alamar blue assay (MABA).[29-30]Antibiotic standard used was streptomycin at 6.5 µg/mL concentration. The compounds were tested at 5, 10, 15, 20, 25, 30 µg/mL concentrations by serial dilution against the *M. tuberculosis* $H_{37}Rv$ to determine minimum inhibition concentration (MIC) using MABA.

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