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Research Article

DESIGN, SYNTHESIS AND CHARACTERIZATION OF NOVEL 3, 5- DINITRO PHENYL CLUBBED AZOLES AGAINST ACTIVE AND LATENT TUBERCULOSIS

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ABSTRACT

In the present study a novel series of 3,5-dinitrophenyl clubbed 1,2,4-triazoles and 1,3,4-oxadiazole derivatives have been designed, synthesized and characterized by IR,^{1}H NMR and mass spectral analysis in order to target both active and latent tuberculosis. Synthesized compounds were evaluated for their preliminary anti tubercular activity against mycobacterium tuberculosis $H_{37}Rv$ strain by Microplate Alamar blue assay and cytotoxicity. Compounds with better selectivity index were tested against recombinant M.Tb $H_{37}Rv$ (pFCA-luxAB) strain by low oxygen recovery assay. Docking studies have been designed in order to find out the "best-fit" orientation of a ligand that binds to the given protein of interest. Among all the synthesized compounds 7F, 3F and 4C are effective against both active and latent tuberculosis.

KEY WORDS: Latent tuberculosis, isocitrate lyase, lora assay, dinitro phenyl azoles, tuberculosis

INTRODUCTION

An infection with *Mycobacterium tuberculosis* (M.Tb) can result in engages a balanced "symbiosis" for survival, by which 10% of infected people will develop the active disease i.e., Tuberculosis (TB) while the rest show no symptoms yet carry the long-lived M. Tb commonly, latent tuberculosis infection (LTBI). Even though the introduction of Delamanid and Bedaquiline[1, 2, 3] to the repertoire of TB therapies, the majority of current drugs are ineffective for long-term regimens due to their drug resistance mechanisms.

About one third of the world population is infected with latent tuberculosis acts as a reservoir for disease reactivation and also creates a hindrance for treatment. Unfortunately both latent and active tuberculosis exhibits different metabolic pathways. With this idea, Pharmacophores with diverse mechanism are clubbed together into a single scaffold molecules targeting both active and latent tuberculosis simultaneously.

In the light of literature, Dinitrophenyl moiety was found as a fortunate pharmacophore in encompassing inhibition of crucial enzymes i.e., Isocitrate Lyase (ICL) in glyoxylate shunt responsible for sustaining M.Tb in chronic phase[4,5] and Isocitrate dehydrogenase (IDH) involved in citric acid cycle and also shows significant effect on non-replicating bacilli are established. ICL is a persistent and essential factor in latent phase and absence of ICL orthologs in mammals makes it an attractive target for drug discovery. An evidence of nitro group containing molecules are effective against latent tuberculosis and also influences on M.Tb ICL[6].Azoles are group of molecules which can reach the target by transmembrane diffusion because of its lipophilic property and also inhibits sterol synthesis via sterol demethylase inhibition in a eukaryotic organism[7, 8, 9]. In addition to this, these show an evidence of potential prevention in the formation of latent bacilli in mice which are more effective than rifampicin[10]. Docking is for identifying an optimized orientation between protein and ligand such that the free energy of the overall system is minimized[11].

So far enough efforts have not been made in hyphenation of these pharmacophores as a single scaffold, creates a huge interest in crafting 3,5-Dinitrophenyl clubbed azoles targeting replicating and non-replicating bacilli i.e., active and latent tuberculosis. Rational design of molecules is depicted in figure 1.

The present study illustrates the 3, 5-dinitrophenyl clubbed 1, 3, 4-triazoles and 1, 2, 4-triazoles – synthesis and their biological activity against active and latent tuberculosis.

MATERIALS AND METHODS

Chemistry

The reaction sequences employed for synthesis of target compounds are shown in general scheme of synthesis in figure 2 and their properties are depicted in table 1.

Hydrazinolysis involves the reaction between ester and hydrazine hydrate in appropriate solvent results in the formation of hydrazide (1) (scheme I). Reaction of starting compound (1) with ketone results in the formation of corresponding hydrazones (2 A-G) on further reaction with acetic anhydride afforded 1,3,4-oxadiazole derivatives (3 A-G) in good yield (scheme II a). Compound (1) on reacting with appropriate aromatic acid in presence of phosphorus oxychloride yields substituted Oxadiazoles (4 A-F) (scheme II b). Compound (1) on heating with carbon disulphide under strong basic conditions followed by acidification with dilute hydrochloric acid converted into corresponding potassium dithiocarbazinate, which on cyclization with hydrazine hydrate yields 4-amino-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (5) (scheme III).

Reaction of triazole (5) was condensed with various substituted benzaldehydes in presence of catalytic amount of concentrated sulphuric acid in ethanol to afford a series of 3, 5-dinitrophenyl-4-substituted-1, 2, 4-triazole-3-thiols (6 A-L) (scheme IV a). Reaction of triazole (5) with appropriate hetero aromatic acids in presence of phosphorus oxychloride produced a series of fused triazolo thiadiazoles (7 A-F) (scheme IV b).

EXPERIMENTAL
Chemical Protocols

The melting points of all the synthesized compounds were recorded on a digital melting point apparatus (EZ melt, Stanford Research Systems) and were uncorrected. Preloaded silica gel TLC plates (Silica gel 60 F254, Merck) were used for monitoring the reaction and purity of the compounds. Iodine vapors and Sulphuric acid spray reagent (10% H_2SO_4/MeOH) were used as visualizing agents.IR spectra were recorded on BRUKER FT-IR (OPUS 6.4) spectrometer using KBr disc method and the values were expressed in cm^{-1}.The ^1H NMR spectra of the compounds were recorded on BRUKER AVANCE 400 MHz NMR spectrometer (Topspin) chemical shifts were reported as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal. The elemental analysis was conducted on Thermo Finnigan flash. The mass spectra were recorded on AGILENT QQQ LC-MS (ESI-MS) spectrometer (Mass hunter B.03.01).

Scheme I: General Procedure for Synthesis of 3, 5-dinitrobenzohydrazide

An equimolar ratio of methyl 3,5-dinitrobenzoate with 60% hydrazine hydrate in methanol refluxed for 3 hrs monitored by TLC yields 3,5-dinitrobenzohydrazide (1) which is starting material for the further synthesis. Then formed hydrazide was distilled by using rotavapor and kept aside for overnight. Crystals of hydrazide was obtained.

Scheme II a: General Procedure for Synthesis of 3, 5-dinitrophenyl substituted-1, 3, 4-oxadiazol-3 (2H)-yl ethanone (3 A-G)

Equimolar quantities of hydrazide (1) and different substituted ketones were refluxed in alcohol for about 4-6 hrs in the presence of few drops of glacial acetic acid. The solvent was evaporated and the product was poured onto cold water, filtered and dried. The crude solid was recrystallized in the appropriate solvent systems to give the products (2 A-G). A mixture of compound (2 A-G) (0.003 mol) and acetic anhydride (10 ml) was refluxed for 2-4 hrs. After the reaction mixture attained room temperature, excess of acetic anhydride was decomposed by water and the mixture was stirred for further 30 min. the separated product was filtered, washed with water, dried and recrystallized with methanol (3 A-G).

1-(5-(3,5-dinitrophenyl)-2,2-dimethyl-1,3,4-oxadiazol-3 (2H)-yl) ethanone (3A) : M.P (°C) :166 ; IR (KBr,Cm^{-1}): 1302,1503 (Nitro,NO_2), 1441 (C=C, Ar),1631 (C=N),1713 (C=O);^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 2.36 (s, 3H), 1.40 (s, 6H). MS (m/z, M+):309.00 (M+1); Yield: 81%

1-(5-(3,5-dinitrophenyl)-2-methyl-2-phenyl-1,3,4-oxadiazol-3 (2H)-yl) ethanone (3B): M.P (°C) :156; IR (KBr,Cm^{-1}): 1323,1520 (Nitro,NO_2), 1491 (C=C, Ar),1637 (C=N),1718 (C=O) ;^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.08 – 6.66 (m, 5H), 2.36 (s, 3H), 1.53 (s, 3H). MS (m/z, M+):371.90 (M+1); Yield: 76%

1-(2-(4-chlorophenyl)-5-(3,5-dinitrophenyl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl) ethanone
(3C): M.P (°C) :160; IR (KBr,Cm^{-1}): 1350,1519 (Nitro,NO_2), 1498 (C=C, Ar),1656 (C=N),1707 (C=O), 693 (Ar-Cl);^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.23(d, *J=8.0*, 2H), 7.12 (d, *J=8.0*, 2H), 2.36 (s, 3H), 1.72 (s, 3H). MS (m/z, M+):405.90 (M+1); Yield: 87%

1-(5-(3,5-dinitrophenyl)-2-methyl-2-(4-nitrophenyl)-1,3,4-oxadiazol-3 (2H)-yl) ethanone (3D): M.P (°C) :168; IR (KBr,Cm^{-1}): 1314,1517 (Nitro,NO_2), 1511 (C=C, Ar),1625 (C=N),1717 (C=O) ;^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.92(d, *J= 7.8*, 2H), 7.69 (d, *J= 7.8*, 2H), 2.36 (s, 3H), 1.96 (s, 3H). MS (m/z, M+):416.50 (M+1); Yield: 84%

1-(2-(4-bromophenyl)-5-(3,5-dinitrophenyl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl) ethanone (3E): M.P (°C) :176; IR (KBr,Cm^{-1}): 1375,1508 (Nitro,NO_2), 1515 (C=C, Ar),1608 (C=N),1735 (C=O), 785 (Ar-Br);^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.21(d, *J=8.0*, 2H), 7.03 (d, *J=8.0*, 2H), 2.36 (s, 3H), 1.72 (s, 3H). MS (m/z, M+):450.10 (M+1); Yield: 71%

1-(5-(3,5-dinitrophenyl)-2-methyl-2-p-tolyl-1,3,4-oxadiazol-3(2H)-yl) ethanone (3F) : M.P (°C) :156; IR (KBr,Cm^{-1}): 1377,1517 (Nitro,NO_2), 1510 (C=C, Ar),1608 (C=N),1717 (C=O) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.95(d, *J=7.8*, 2H), 6.77 (d, *J=8.0*, 2H), 2.36 (s, 3H),1.82 (s,3H), 1.51 (s, 3H). MS (m/z, M+):385.90 (M+1); Yield: 74%

1-(5-(3,5-dinitrophenyl)-2-(4-hydroxyphenyl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl)ethanone(3G): M.P (°C) :164; IR (KBr,Cm^{-1}): 1377,1508 (Nitro,NO_2), 1517 (C=C, Ar),1621 (C=N),1718 (C=O), 3482 (Ar-OH); ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.69(d, *J=7.8*, 2H), 6.55 (d, *J=8.0*, 2H), 5.09 (s, 1H), 2.36 (s, 3H), 1.53 (s, 3H) MS (m/z, M+):387.80 (M+1); Yield: 70%

Scheme II b: General Procedure for Synthesis of 2-(3, 5-dinitrophenyl) Substituted-1,3,4-oxadiazoles (4 A-F)

An equimolar ratio of compound (1) and the appropriate aromatic acid in phosphorus oxychloride (10 ml) was refluxed for 3-5 hrs. Then the reaction mixture was slowly poured over crushed ice and kept overnight aside. The solid thus separated out was filtered, treated with dilute sodium hydroxide, washed with water and recrystallized with ethyl acetate: hexane in 2:4 (4 A-F)**.**

2-(3,5-dinitrophenyl)-5-phenyl-1,3,4-oxadiazole (4A): M.P (°C) :271 ; IR (KBr,Cm^{-1}): 1324,1524 (Nitro,NO_2), 1460 (C=C, Ar),1664 (C=N),3061 (C-H, Ar) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.08 – 6.66 (m, 5H). MS (m/z, M+):313.0 (M+1); Yield: 80%

2-(4-chlorophenyl)-5-(3,5-dinitrophenyl)-1,3,4-oxadiazole (4B): M.P (°C) :305; IR (KBr,Cm^{-1}): 1322,1501 (Nitro,NO_2), 1530 (C=C, Ar),1614 (C=N),3080 (C-H, Ar), 666 (Ar-Cl) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.21 (d, *J = 8.0*, 2H), 7.18 (d, *J = 8.8*, 2H) MS (m/z, M+):347.5 (M+1); Yield: 87%

2-(3,5-dinitrophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (4C): M.P (ºC): 316; IR (KBr,Cm^{-1}): 1306,1515 (Nitro,NO_2), 1533 (C=C, Ar),1691 (C=N),3060 (C-H, Ar) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.30 (d, *J = 7.8*, 2H), 7.26 (d, *J = 7.8*, 2H). MS (m/z, M+):358.9 (M+1); Yield: 91%

2-(3,5-dinitrophenyl)-5-P-tolyl-1,3,4-oxadiazole (4D): M.P (ºC) :285; IR (KBr,Cm^{-1}): 1314,1530 (Nitro,NO_2), 1566 (C=C, Ar),1690 (C=N),3090 (C-H, Ar) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.92 (d, *J = 8.0*, 2H), 6.66 (d, *J = 7.8*, 2H),1.53 (s, J=2.2, 3H) MS (m/z, M^-):325.4 (M-1); Yield: 92%

4-(5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl) phenol (4E): M.P (ºC) :287; IR (KBr,Cm^{-1}): 1311,1532 (Nitro,NO_2), 1612 (C=C, Ar),1691 (C=N),3055 (C-H, Ar), 3316 (Ar-OH) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.01 (d, *J = 8.8*, 2H), 6.84 (d, *J = 8.0*, 2H),5.43(s, 1H). MS (m/z, M^-):327.7 (M-1); Yield: 85%

2-(3,5-dinitrophenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (4F) : M.P (ºC) :300; IR (KBr,Cm^{-1}): 1310,1464 (Nitro,NO_2), 1583 (C=C, Ar),1664 (C=N),3099 (C-H, Ar), 1727 (Ar-OCH_3) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.71 (d, *J = 7.8*, 2H), 6.52 (d, *J = 7.8*, 2H),4.2 (s, J=2, 3H). MS (m/z, M^+):343.0 (M+1); Yield: 88%

Scheme III: General Procedure for Synthesis of 4-amino-5-(3, 5-dinitrophenyl)-4H-1, 2, 4-triazole-3-thiol (5)

Compound (**1**) (0.003 mol) and carbon disulphide (0.006 mol) were added in a solution of potassium hydroxide (0.0067 mol) in absolute ethanol (30 ml) refluxed for about 16 hrs. To the resulting solution anhydrous ether was added and the precipitate of potassium dithiocarbazinate was collected by filtration, washed with ether and dried under vacuum. A suspension of the potassium salt, hydrazine hydrate (1.5 ml) and water (10 ml) was refluxed for 5 hrs gave a precipitate. Then filtered, washed with water and recrystallized with methanol.

4-amino-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (5): M.P (ºC) :231; IR (KBr,Cm^{-1}): 1369,1541 (Nitro,NO_2), 1427 (C=C, Ar),1641 (C=N),3034 (C-H, Ar), 2561 (C-SH), 3460 (NH, str) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 4.42 (s, 1H), 4.02 (s,2H). MS (m/z, M^-):281.9(M-1); Yield: 86%

Scheme IVa: General Procedure for Synthesis of 3, 5-dinitrophenyl-4-substituted-1, 2, 4-triazole-3-thiols (6 A-L)

An equimolar ratio of compound (5) and substituted benzaldehydes with 4-5 drops of concentrated sulphuric acid in ethanol was refluxed for 3-4 hrs. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol.

4-(4-bromobenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (6A): M.P (ºC) :160; IR (KBr,Cm^{-1}): 1338,1544 (NO_2,Nitro), 1116 (C-N), 1617 (C=N),1452 (C=C, Ar), 2575 (C-SH),520 (Ar-Br); ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.41 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.26 (d, *J = 8.0*, 2H), 7.20 (d, *J = 8.8*, 2H), 4.48(s, 1H, N=C-SH). MS (m/z, M^+):449.0 (M+1); Yield: 69%

5-(3,5-dinitrophenyl)-4-(3,4,5-trimethoxybenzylideneamino)-4H-1,2,4-triazole-3-thiol (6B) : M.P (ºC) :190; IR (KBr,Cm^{-1}): 1360,1510 (NO_2,Nitro), 1105 (C-N), 1625 (C=N),1450 (C=C, Ar), 2560 (C-SH) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.73 (s, 1H, N=CH), 10.04 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.02 (s, 2H), 4.48(s, 1H, N=C-SH), 3.83 (s, 3H), 3.67(s, 6H). MS (m/z, M^+):461.1(M+1); Yield: 86%

4-(4-aminobenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (6C): M.P (ºC) :198; IR (KBr,Cm^{-1}): 1382,1524 (NO_2,Nitro), 1117 (C-N), 1664 (C=N),1460 (C=C, Ar), 2552 (C-SH),3122 (NH, Stretch) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.38 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.46 (d, *J = 8.0*, 2H), 7.35 (d, *J = 8.0*, 2H), 4.48(s, 1H, N=C-SH), 3.32 (s, 2H, NH_2). MS (m/z, M^-):384.9 (M-1); Yield: 80%

4-(3,4-dimethoxybenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (6D): M.P (ºC) :206; IR (KBr,Cm^{-1}): 1324,1525 (NO_2,Nitro), 1134 (C-N), 1679 (C=N),1441 (C=C, Ar), 2565 (C-SH),1148 (Ar-OCH_3) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.91 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.63 – 8.01(m, 3H), 4.48(s, 1H, N=C-SH), 3.82 (s, 3H), 3.61(s, 3H). MS (m/z, M^+):431.1 (M+1); Yield: 75%

5-(3,5-dinitrophenyl)-4-(4-methoxybenzylideneamino)-4H-1,2,4-triazole-3-thiol(6E): M.P (ºC) :214; IR (KBr,Cm^{-1}): 1312,1508 (NO_2,Nitro), 1120 (C-N), 1684 (C=N),1445 (C=C, Ar), 2579 (C-SH),1142 (Ar-OCH_3) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.17 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.64 (d, *J = 8.0*, 2H), 7.53 (d, *J = 8.0*, 2H), 4.48(s, 1H, N=C-SH), 3.52 (s, 3H). MS (m/z, M^+):401.8 (M+1); Yield: 82%

2-((3-(3,5-dinitrophenyl)-5-mercapto-4H-1,2,4-triazol-4-ylimino)methyl phenol (6F): M.P (ºC) :240; IR (KBr,Cm^{-1}): 1320,1516 (NO_2,Nitro), 1672 (C=N),1447 (C=C, Ar), 2573 (C-SH),3350 (Ar-OH) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.68 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.58 – 7.81(m, 4H), 4.59 (s, *1*H), 4.48(s, 1H, N=C-SH). MS (m/z, M^+):387.7 (M+1); Yield: 81%

4-(2-chlorobenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (6G): M.P (ºC) :170; IR (KBr,Cm^{-1}): 1313,1558 (NO_2,Nitro), 1611 (C=N),1439 (C=C, Ar), 2559 (C-SH),619 (Ar-Cl) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.76 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.63 – 7.98(m, 4H), 4.48(s, 1H, N=C-SH). MS (m/z, M^+):405.5 (M+1); Yield: 86%

4-(benzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (6H): M.P (ºC) :156; IR (KBr,Cm^{-1}): 1370,1560 (NO_2,Nitro), 1690 (C=N),1465 (C=C, Ar), 2580 (C-SH) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.38 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.55 – 7.61(m, 5H), 4.48(s, 1H, N=C-SH). MS (m/z, M^-):369.9 (M-1); Yield: 79%

5-(3,5-dinitrophenyl)-4-(4-methylbenzylideneamino)-4H-1,2,4-triazole-3-thiol (6I): M.P (ºC) :186; IR (KBr,Cm^{-1}): 1355,1540 (NO_2,Nitro), 1685 (C=N),1460 (C=C, Ar), 2575 (C-SH),2878 (Ar-CH_3) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.65 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.80 (d, *J = 7.8*, 2H), 8.03 (d, *J = 7.8*, 2H), 4.48(s, 1H, N=C-SH), 2.14 (s, 3H). MS (m/z, M^+):385.1 (M+1); Yield: 76%

4-((3-(3,5-dinitrophenyl)-5-mercapto-4H-1,2,4-triazol-4-ylimino)methyl)-2-methoxy phenol (6J): M.P (ºC) :240; IR (KBr,Cm^{-1}): 1371,1519 (NO_2,Nitro), 1697 (C=N),1487 (C=C, Ar), 2558 (C-SH),1194 (Ar-OCH_3),3304 (Ar-OH) ; ^{1}HNMR

(□,400 MHz,DMSO-d_6,PPm): 9.76 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.63 – 7.75(m, 3H), 5.04 (s, 1H), 4.48(s, 1H, N=C-SH), 3.59(s, 3H).MS (m/z, M^+):417.5 (M+1); Yield: 88%

4-((1H-pyrrol-2-yl)methyleneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol (6K): M.P (ºC) :232; IR (KBr,Cm^{-1}): 1380,1538 (NO_2,Nitro), 1697 (C=N),1464 (C=C, Ar), 2555 (C-SH),3120 (NH- Stretch) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 12.16 (s, 1H, Pyr-NH), 9.23 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.12 – 7.25(m, Pyr-3H), 4.48(s, 1H, N=C-SH). MS (m/z, M^-):358.9 (M-1); Yield: 83%

5-(3,5-dinitrophenyl)-4-(3-phenylallylideneamino)-4H-1,2,4-triazole-3-thiol (6L): M.P (ºC) :204; IR (KBr,Cm^{-1}): 1340,1521 (NO_2,Nitro), 1685 (C=N),1513 (C=C, Ar), 2610 (C-SH),3034 (C-H, Ar) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.18 (s, 1H, N=CH), 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.65 – 7.93 (m, 5H), 6.52 (d, 1H, Allyl-CH), 5.89 (d, 1H, Allyl-CH), 4.48(s, 1H, N=C-SH). MS (m/z, M^+):397.0 (M+1); Yield: 89%

Scheme IV b: General Procedure for Synthesis of 3, 5-dinitrophenyl fused triazolo thiadiazoles (7 A-F)

An equimolar mixture of compound (**5**) and appropriate aromatic acids in phosphorus oxychloride (10 ml) was refluxed for 4-5 hrs. Then the reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, treated with dil. Sodium hydroxide solution and washed thoroughly with cold water. The compound so obtained was dried and recrystallized with Ethanol: DMF in 2:1.

3-(3,5-dinitrophenyl)-6-phenyl-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole (7A): M.P (ºC) :225; IR (KBr,Cm^{-1}): 1384,1551 (NO_2,Nitro), 1691 (C=N),1484 (C=C, Ar), 3031 (C-H, Ar) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.12-7,03 (m, Ar-5H) MS (m/z, M^+):369.10 (M+1); Yield: 85%

6-(4-chlorophenyl)-3-(3,5-dinitrophenyl)-[1,2,4] triazolo [3,4-b][1,3,4]thiadiazole (7B): M.P (ºC) :201; IR (KBr,Cm^{-1}): 1385,1562 (NO_2,Nitro), 1691 (C=N),1465 (C=C, Ar), 3031 (C-H, Ar),748 (Ar-Cl) ; ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.42 (d, *J = 8.8*, 2H), 7.63 (d, *J = 8.8*, 2H). MS (m/z, M^+):403.02 (M+1); Yield: 77%

3-(3,5-dinitrophenyl)-6-(4-nitrophenyl)-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole (7C): M.P (ºC) :215; IR (KBr,Cm^{-1}): 1306,1566 (NO_2,Nitro), 1685 (C=N),1530 (C=C, Ar),3051 (C-H, Ar); ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 7.82 (d, *J = 7.8*, 2H), 7.79 (d, *J =8.0*, 2H). MS (m/z, M^+):414.32 (M+1); Yield: 69%

3-(3,5-dinitrophenyl)-6-p-tolyl-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole (7D): M.P (ºC) :224; IR (KBr,Cm^{-1}): 1321,1529 (NO_2,Nitro), 1685 (C=N),1510 (C=C, Ar),3030 (C-H, Ar),2854 (Ar-CH_3); ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.98 (d, *J = 8.0*, 2H), 6.74 (d, *J = 7.8*, 2H),1.94 (s, 3H) MS (m/z, M^+):383.10 (M+1); Yield: 71%

3-(3,5-dinitrophenyl)-6-(4-methoxyphenyl)-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole (7E): M.P (ºC) :201; IR (KBr,Cm^{-1}): 1370,1540 (NO_2,Nitro), 1693 (C=N),1510 (C=C, Ar),1155 (Ar-OCH_3); ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.80 (d, *J = 8.0*, 2H), 6.73 (d, *J = 8.0*, 2H), 3.93(s, 1H). MS (m/z, M^+):399.10 (M+1); Yield: 73%

4-(3-(3,5-dinitrophenyl)- [1,2,4] triazolo [3,4-b][1,3,4] thiadiazol-6-yl phenol (7F): M.P (ºC) :223; IR (KBr,Cm^{-1}): 1379,1525 (NO_2,Nitro), 1696 (C=N),1528 (C=C, Ar), 3195 (Ar-OH); ^{1}HNMR (□,400 MHz,DMSO-d_6,PPm): 9.02 (d, *J = 1.6*, 1H), 8.94 (d, *J = 2.0*, 2H), 6.96 (d, *J = 7.8*, 2H), 6.85 (d, *J = 7.8*, 2H), 5.03 (s, 1H),4.02 (s,3H). MS (m/z, M^+):385.10 (M+1); Yield: 62%

BIOLOGICAL PROTOCOL

Anti Tubercular Activity

Anti tubercular activity for synthesized compounds against $H_{37}Rv$ middle brook 7H9 agar with OADC medium using Microplate Alamar Blue Assay[12]. 96 well plate received 100 µl of the inoculum and serial dilution of compounds were made directly on the plate at concentration of 0.01-100 µg/ml incubated at 37ºC for five days. After this time, 1:1 mixture of Alamar blue reagent and 10% tween 80 incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth compared with against positive control, negative control and with standard isoniazid. MIC (µg/ml) of each compound was determined and is defined as the lowest concentration of compound which inhibits ≤ 99% of bacterial population presents at the beginning of the assay.

MTT Assay for Cell Viability

Cytotoxicity for synthesized compounds against A_{549} cell lines in the presence of 10% and 0.2% PBS respectively using 3-(4,5-dimethyl thizole-2-yl)-2,5-diphenyl tetrazolium bromide reduction assay [13]. The compounds were dissolved in DMSO at 10mM concentration and stored at -20ºC. The dilutions were made in culture medium before treatment. Selectivity index for each compound was determined.

LORA for Non-replicating M.Tb

Compounds with better selectivity indexes were tested against latent tuberculosis i.e., Non-replicating M.Tb using Low Oxygen Recovery Assay. A recombinant M.Tb H37R*v* (pFCA-luxAB) [14] expressing luciferase and provides results of drug activity against non-replicating M.Tb surviving under hypoxic conditions. MIC's was determined in micro molar concentration.

INSILICO PROTOCOL

Docking Studies

Chemsktech is used to draw the ligand compounds. Auto dock 4.0 is the preliminary docking program used in this work for the semi-flexible protein-ligand docking studies [11].

RESULTS AND DISCUSSIONS

The IR spectra of compound (1) revealed broad stretch band at 1711 cm^{-1} accounting for C=O group of hydrazide moiety, a band at 3250 cm^{-1} accounting for NH group of hydrazide and a band absorption at 1525 and 1360 cm^{-1} two bands accounting for nitro groups. The HNMR signals at □ 1.98 and □ 8.82 accounts for the formation of hydrazide which is confirmed by the molecular ion peak at 225.9 m/z of compound (**1**) found to be in conformity with its molecular formula of the assigned structure.

The IR spectra of compound (3F) revealed stretching band 1717 cm^{-1} accounting for C=O amide carbonyl group. Lack of resonance of NH and NH_2 and appearance of sharp singlet at □ 1.51 for CH_3 group in the HNMR spectra of compound (3F) accounted for the formation of oxadiazole ring. Further the

assigned structure is confirmed by its molecular ion peak at 385.9 m/z confirmed the structure of compound (3F). The anti tubercular activity of the synthesized 3,5-dinitrophenyl substituted 1,3,4-oxadiazol-3(2H)-yl ethanone derivatives (3 A-G) among them compounds 3C, 3D and 3F are active against M.Tb $H_{37}Rv$ strain at MIC 6.25 µg/ml.

HNMR spectra of compound (4D) has showing 5 signals out of none of the signals corresponds to NH and NH2, further the appearance of peaks at 6.92,6.66 and 1.53 confirms the formation of oxadiazoles ring with tolyl group. The assigned structure is confirmed further by the molecular ion peak at m/z 325.40 confirmed the structure of compound (4D) Compounds 4A, 4C, 4F are active against M.Tb $H_{37}Rv$ at 6.25 µg/ml and 4D 12.5 µg/ml.

Lack of resonance at 1.98 and 8.82 and appearance of singlet at 4.42 for SH and two singlet at 4.30 for NH_2 confirm the formation of compound (5). Further confirm by its molecular ion peak at m/z 281.9 in mass spectra.

The appearance of C=N at 1664 cm^{-1} in the IR spectra of compound 6C serves as a diagnostic feature for the formation of Schiff base. One proton singlet corresponds to N=CH at 9.38 and one proton singlet corresponds to N=C-SH at 4.48 from HNMR spectra. Further the assigned structure of 6C is confirmed by the molecular ion peak at m/z 384.9. Compounds 6C, 6K are active against M.Tb $H_{37}Rv$ at12.5 µg/ml.

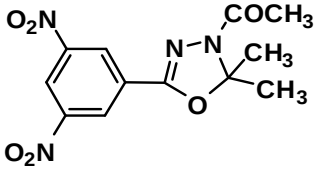
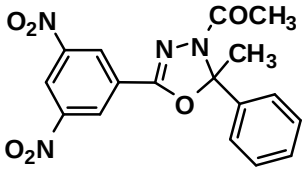
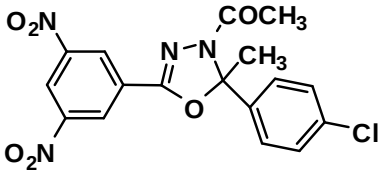
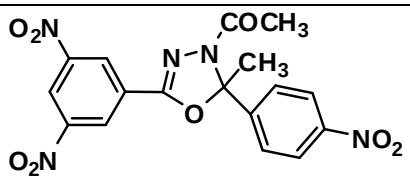
The absence of peaks at 4.42 and 4.30 from the HNMR spectra and molecular ion peak at m/z 385.1 confirms the formation of 7F. Among this series of compounds, 7C and 7D are active against M.Tb $H_{37}Rv$ at12.5 µg/ml where as 7F is active against M.Tb $H_{37}Rv$ at6.25 µg/ml.

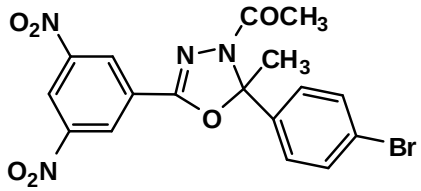
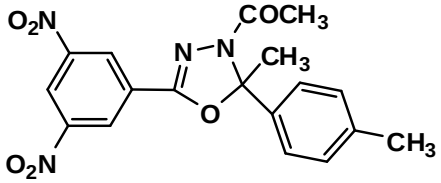
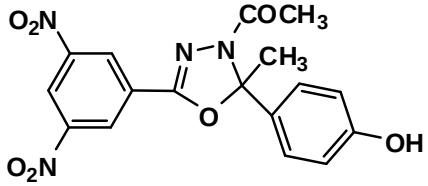
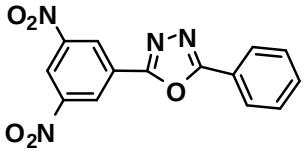
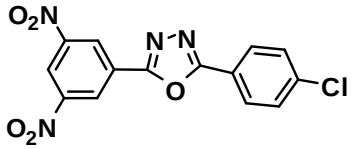
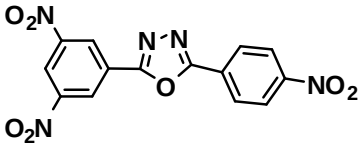
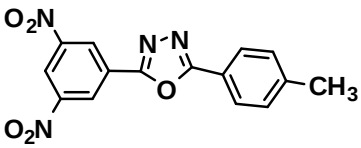
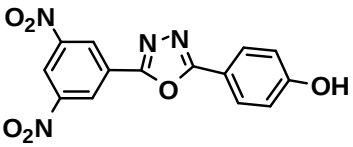
The results of anti tubercular activity against M.Tb $H_{37}Rv$ are illustrated in table 2. Compounds with MIC's of ≤12.5 µg/ml were tested against A_{549} to determine invitro cytotoxicity (IC_{50}) and selective index (SI) are illustrated in table 3. From the result, it is evident that the compounds 3C,3D,3F,4A,4C,4F and 7F have shown better selective indices-16.82,16.97,19.48,16.90, 18.42,17.99 and 19.00 respectively.

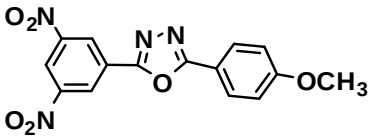
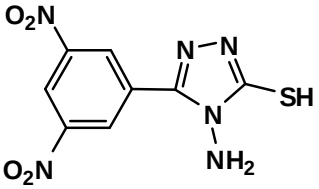
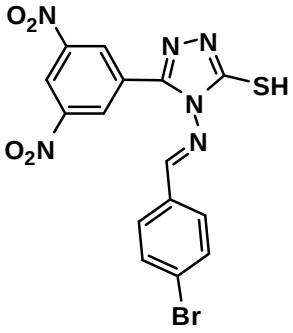
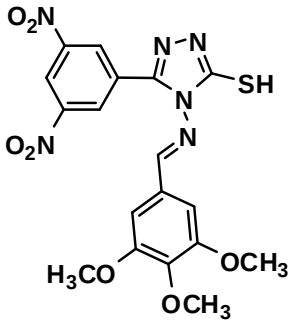
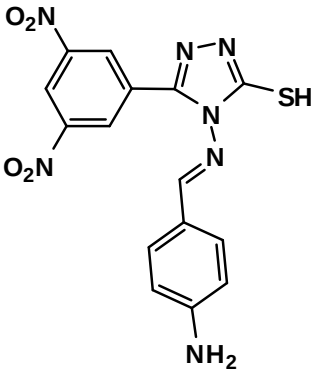
Compounds with better selectivity index were tested against non-replicating M.Tb using LORA. Among them compounds 7F, 3F and 4C shows its MIC's (µm) as 1.25, 1.8 and 2 respectively are despite in table 4.

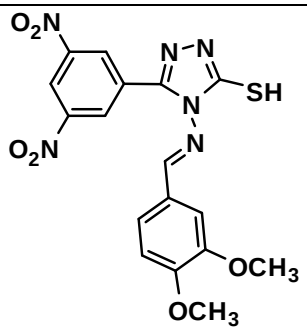
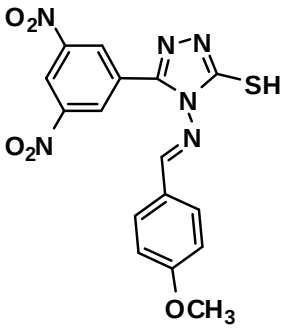
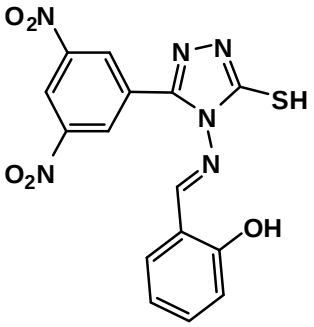
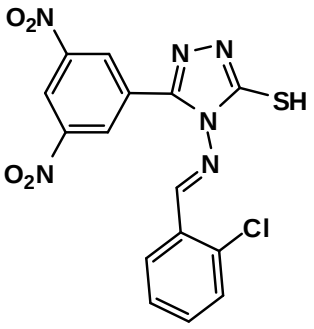
Docking results are illustrated in table 5. All the molecules have shown to be successfully docking inside the active site of protein (isocitrate lyase) with a binding energy ranging from -4.6 to -8.9 Kcal /mol. As per the docking results, it was revealed that Compound 3F has the best estimated -8.9 Kcal/mol of binding energy with protein.

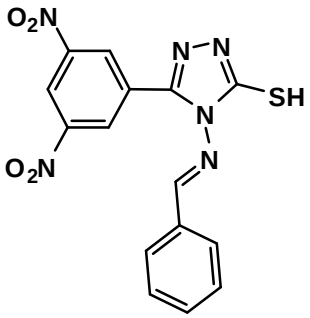
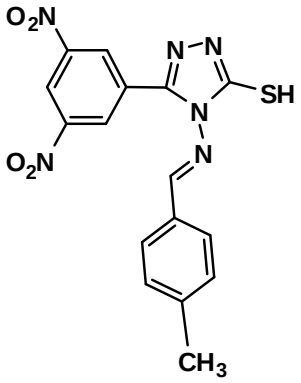
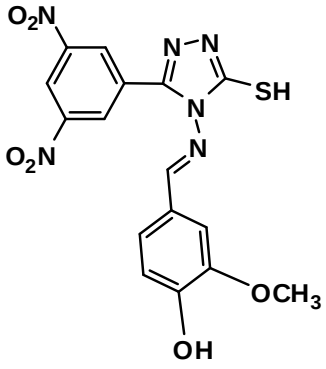
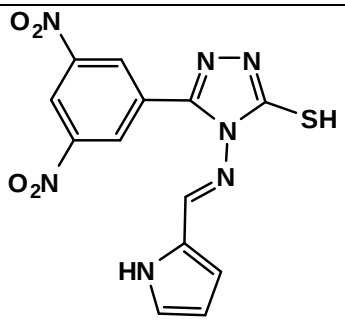
TABLE 1: ANALYTICAL AND PHYSICO-CHEMICAL DATA OF SYNTHESIZED COMPOUNDS

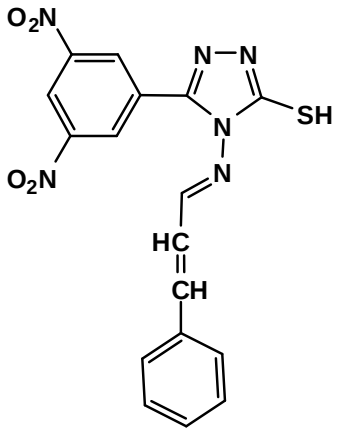
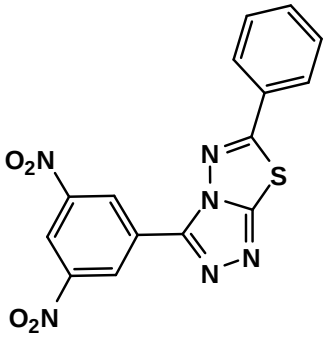
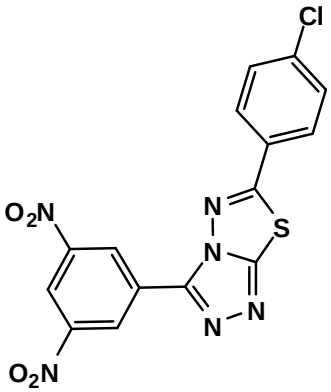
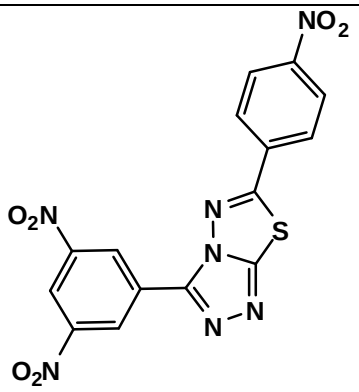
Compound code	Structure and IUPAC nomenclature	Molecular formula	Elemental analysis		
				Required	Found
3A	 1-(5-(3,5-dinitrophenyl)-2,2-dimethyl-1,3,4-oxadiazol-3 (2H)-yl) ethanone	$C_{12}H_{12}N_4O_6$	C H N	46.76 3.92 18.18	45.92 3.86 18.12
3B	 1-(5-(3,5-dinitrophenyl)-2-methyl-2-phenyl-1,3,4-oxadiazol-3 (2H)-yl) ethanone	$C_{17}H_{14}N_4O_6$	C H N	55.14 3.81 15.13	55.05 3.74 15.02
3C	 1-(2-(4-chlorophenyl)-5-(3,5-dinitrophenyl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl) ethanone	$C_{17}H_{13}ClN_4O_6$	C H N	50.45 3.24 13.84	50.4 3.19 13.58
3D	 1-(5-(3,5-dinitrophenyl)-2-methyl-2-(4-nitrophenyl)-1,3,4-oxadiazol-3 (2H)-yl) ethenone	$C_{17}H_{13}N_5O_8$	C H N	49.16 3.16 16.86	49.12 3.12 16.80

3E	 1-(2-(4-bromophenyl)-5-(3,5-dinitrophenyl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl) ethanone	$C_{17}H_{13}BrN_4O_6$	C H N	45.45 2.92 12.47	45.41 2.94 12.41
3F	 1-(5-(3,5-dinitrophenyl)-2-methyl-2-p-tolyl-1,3,4-oxadiazol-3(2H)-yl) ethanone	$C_{18}H_{16}N_4O_6$	C H N	56.25 4.20 14.58	56.21 4.16 14.45
3G	 1-(5-(3,5-dinitrophenyl)-2-(4-hydroxyphenyl)-2-methyl-1,3,4-oxadiazol-3(2H)-yl) ethanone	$C_{17}H_{14}N_4O_7$	C H N	52.85 3.65 14.50	52.81 3.60 14.45
4A	 2-(3,5-dinitrophenyl)-5-phenyl-1,3,4-oxadiazole	$C_{14}H_8N_4O_5$	C H N	53.85 2.58 17.94	53.08 2.52 17.1
4B	 2-(4-chlorophenyl)-5-(3,5-dinitrophenyl)-1,3,4-oxadiazole	$C_{14}H_7ClN_4O_5$	C H N	48.50 2.04 16.16	48.46 2.01 16.12
4C	 2-(3,5-dinitrophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole	$C_{14}H_7N_5O_7$	C H N	47.07 1.98 19.6	47.01 1.92 19.2
4D	 2-(3,5-dinitrophenyl)-5-P-tolyl-1,3,4-oxadiazole	$C_{15}H_{10}N_4O_5$	C H N	55.22 3.09 17.17	55.12 3.02 17.11
4E	 4-(5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl) phenol	$C_{14}H_8N_4O_6$	C H N	51.23 2.46 17.07	51.20 2.41 17.04

4F	 2-(3,5-dinitrophenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole	$C_{15}H_{10}N_4O_6$	C H N	52.64 2.94 16.37	52.61 2.94 16.3
5	 4-amino-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol	$C_8H_6N_6O_4S$	C H N	34.04 2.14 29.78	33.8 2.08 29.71
6A	 (E)-4-(4-bromobenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol	$C_{15}H_9BrN_6O_4S$	C H N	40.1 2.02 18.71	39.8 1.96 18.5
6B	 (E)-5-(3,5-dinitrophenyl)-4-(3,4,5-trimethoxybenzylideneamino)-4H-1,2,4-triazole-3-thiol	$C_{18}H_{16}N_6O_7S$	C H N	46.96 3.5 18.25	46.85 3.42 18.19
6C	 (E)-4-(4-aminobenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol	$C_{15}H_{11}N_7O_4S$	C H N	46.75 2.88 25.44	46.71 2.82 25.4

6D	 (E)-4-(3,4-dimethoxybenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol	$C_{17}H_{14}N_6O_6S$	C H N	47.44 3.28 19.53	47.41 3.22 19.49
6E	 (E)-5-(3,5-dinitrophenyl)-4-(4-methoxybenzylideneamino)-4H-1,2,4-triazole-3-thiol	$C_{16}H_{12}N_6O_5S$	C H N	48.00 3.02 20.99	47.85 3.00 20.71
6F	 (E)-2-((3-(3,5-dinitrophenyl)-5-mercapto-4H-1,2,4-triazol-4-ylimino)methyl phenol	$C_{15}H_{10}N_6O_5S$	C H N	46.63 2.61 21.75	46.51 2.53 21.71
6G	 (E)-4-(2-chlorobenzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol	$C_{15}H_9ClN_6O_4S$	C H N	44.51 2.24 20.7	44.42 2.2 20.1

6H	 (E)-4-(benzylideneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol	$C_{15}H_{10}N_6O_4S$	C H N	48.65 2.72 22.69	48.58 2.68 22.61
6I	 (E)-5-(3,5-dinitrophenyl)-4-(4-methylbenzylideneamino)-4H-1,2,4-triazole-3-thiol	$C_{16}H_{12}N_6O_4S$	C H N	50.0 3.15 21.86	49.8 3.1 21.81
6J	 (E)-4-((3-(3,5-dinitrophenyl)-5-mercapto-4H-1,2,4-triazol-4-ylimino)methyl)-2-methoxy phenol	$C_{16}H_{12}N_6O_6S$	C H N	46.15 2.9 20.18	45.94 2.87 20.12
6K	 (E)-4-((1H-pyrrol-2-yl)methyleneamino)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazole-3-thiol	$C_{13}H_9N_7O_4S$	C H N	43.45 2.52 27.29	43.38 2.35 27.12

6L	 (E)-5-(3,5-dinitrophenyl)-4-(3-phenylallylideneamino)-4H-1,2,4-triazole-3-thiol	$C_{17}H_{12}N_6O_4S$	C H N	51.5 3.05 21.2	51.1 3.01 20.8
7A	 3-(3,5-dinitrophenyl)-6-phenyl-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole	$C_{15}H_8N_6O_4S$	C H N	48.91 2.19 22.82	48.81 2.08 22.82
7B	 6-(4-chlorophenyl)-3-(3,5-dinitrophenyl)-[1,2,4] triazolo [3,4-b][1,3,4]thiadiazole	$C_{15}H_7ClN_6O_4S$	C H N	44.73 1.75 20.87	44.69 1.74 20.77
7C	 3-(3,5-dinitrophenyl)-6-(4-nitrophenyl)-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole	$C_{15}H_7N_7O_6S$	C H N	43.59 1.71 23.72	43.51 1.65 23.7

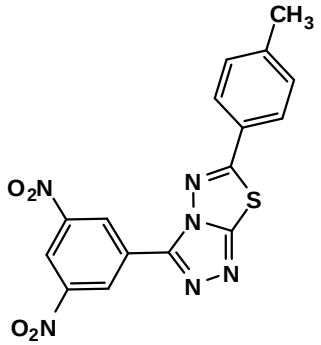
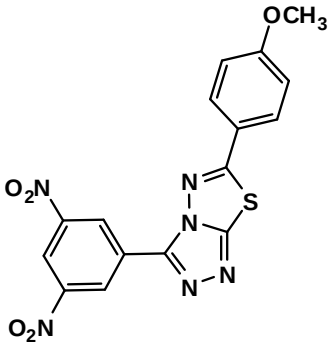
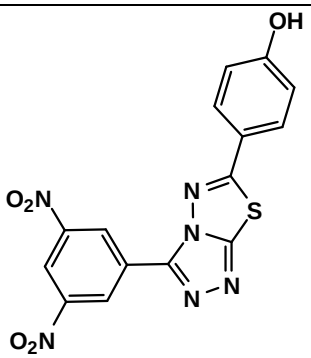
7D	 3-(3,5-dinitrophenyl)-6-p-tolyl-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole	$C_{16}H_{10}N_6O_4S$	C H N	50.26 2.64 21.98	50.21 2.6 21.89
7E	 3-(3,5-dinitrophenyl)-6-(4-methoxyphenyl)-[1,2,4] triazolo [3,4-b][1,3,4] thiadiazole	$C_{16}H_{10}N_6O_5S$	C H N	48.24 2.53 21.1	48.2 2.49 20.9
7F	 4-(3-(3,5-dinitrophenyl)- [1,2,4] triazolo [3,4-b][1,3,4] thiadiazol-6-yl phenol	$C_{15}H_8N_6O_5S$	C H N	46.88 2.1 21.87	46.65 2.0 21.82

TABLE 2: PRELIMINARY ANTI TUBERCULAR ACTIVITY EXPRESSED IN MIC (µg/ml)

Compound	MIC (µg/ml)	Compound	MIC (µg/ml)
3A	50	**6E**	50
3B	25	**6F**	25
3C	6.25	**6G**	50
3D	6.25	**6H**	100
3E	25	**6I**	25
3F	6.25	**6J**	50
3G	50	**6K**	12.5
4A	6.25	**6L**	50
4B	25	**7A**	25
4C	6.25	**7B**	25
4D	12.5	**7C**	12.5
4E	25	**7D**	12.5
4F	6.25	**7E**	50
5	12.5	**7F**	6.25
6A	100	**Rifampicin**	1.6
6B	25	**Isoniazid**	1.6
6C	12.5	**Streptomycin**	6.25
6D	50	**3-Nitro Propionate**	50

TABLE 3: IC_{50} AND SELECTIVE INDEX FOR SELECTED COMPOUNDS

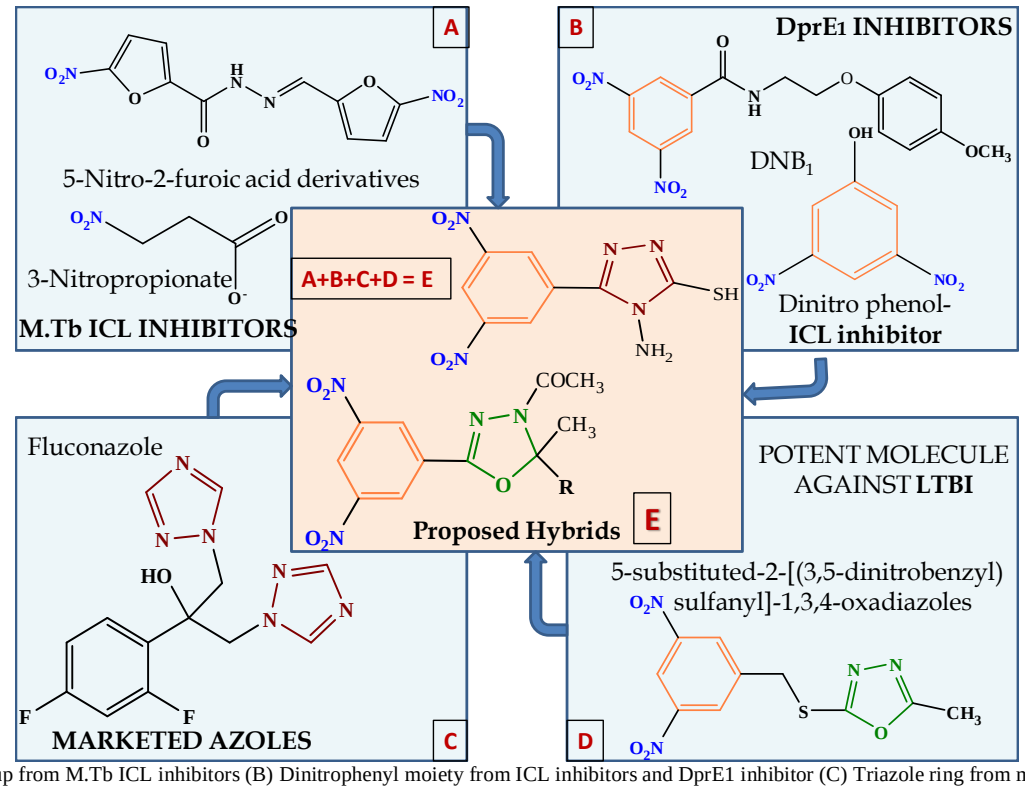
Compound	A 549 cell lines IC_{50} (µg/ml)	*Mtb* $H_{37}Rv$ MIC (µg/ml)	Selectivity index (IC_{50} / MIC)
3C	105.10	6.25	16.81
3D	106.10	6.25	16.97
3F	**121.81**	**6.25**	**19.48**
4A	105.67	6.25	16.90
4C	**115.17**	**6.25**	**18.42**
4D	108.36	12.5	8.66
4F	112.44	6.25	17.99
5	95.21	12.5	7.61
6C	119.57	12.5	9.56
6K	122.19	12.5	9.77
7C	79.05	12.5	6.32
7D	83.47	12.5	6.67
7F	**118.76**	**6.25**	**19.00**

TABLE 4: PRIMARY ACTIVITY AGAINST LATENT TUBERCULOSIS EXPRESSED IN MIC (µm)

Compound	Solvent	MIC (µm)
7F	DMSO	1.25
3F	DMSO	1.8
4C	DMSO	2
Rifampicin	DMSO	0.5
Isoniazid	H_2O	1.024
Streptomycin	H_2O	0.3

TABLE 5: LIGAND BINDING ENERGIES WITH PROTEIN (ICL)

Ligand	Target	Binding energy (kcal/mol)
3f_mmff94_E=148.36	1F61_A_prep	-8.9
7b_mmff94_E=134.64	1F61_A_prep	-8.9
3c_mmff94_E=145.90	1F61_A_prep	-8.8
3e_mmff94_E=146.27	1F61_A_prep	-8.8
3d_mmff94_E=185.62	1F61_A_prep	-8.7
3b_mmff94_E=135.17	1F61_A_prep	-8.6
7f_mmff94_E=126.20	1F61_A_prep	-8.5
4d_mmff94_E=105.97	1F61_A_prep	-8.3
6l_mmff94_E=145.16	1F61_A_prep	-8.2
4f_mmff94_E=110.14	1F61_A_prep	-8.1
7a_mmff94_E=133.21	1F61_A_prep	-8.1
7c_mmff94_E=173.79	1F61_A_prep	-8.1
7d_mmff94_E=137.12	1F61_A_prep	-8.1
Rifampicin_mmff94_E=311.44	1F61_A_prep	-8.1
4a_mmff94_E=102.24	1F61_A_prep	-8
4b_mmff94_E=103.02	1F61_A_prep	-8
4c_mmff94_E=141.08	1F61_A_prep	-8
4e_mmff94_E=94.96	1F61_A_prep	-8
6i_mmff94_E=158.37	1F61_A_prep	-8
3g_mmff94_E=135.24	1F61_A_prep	-7.9
3a_mmff94_E=96.49	1F61_A_prep	-7.7
6a_mmff94_E=156.36	1F61_A_prep	-7.7
6g_mmff94_E=159.46	1F61_A_prep	-7.7
7e_mmff94_E=138.83	1F61_A_prep	-7.6
6e_mmff94_E=159.69	1F61_A_prep	-7.5
6c_mmff94_E=146.25	1F61_A_prep	-7.4
6f_mmff94_E=157.77	1F61_A_prep	-7.4
6h_mmff94_E=156.59	1F61_A_prep	-7.4
6k_mmff94_E=126.73	1F61_A_prep	-7.4
6j_mmff94_E=163.74	1F61_A_prep	-7.1
6d_mmff94_E=175.76	1F61_A_prep	-7
5_mmff94_E=98.92	1F61_A_prep	-6.8
6b_mmff94_E=190.17	1F61_A_prep	-6.8
Isoniazid_mmff94_E=64.28	1F61_A_prep	-5.9
3-Nitropropinate mmff94_E=-50.27	1F61_A_prep	-4.6



(A) Nitro group from M.Tb ICL inhibitors (B) Dinitrophenyl moiety from ICL inhibitors and DprE1 inhibitor (C) Triazole ring from marketed azoles i.e., Fluconazole (D) Oxadiazole ring and Dinitrophenyl from potent molecule against latent M.Tb clubbed to arise proposed hybrids.

Figure 1: Rational in Design of Proposed Hybrids

CONCLUSION

In this work, anti mycobacterial activity of clubbed 1,3,4-oxadiazole shows effective than clubbed 1,2,4-triazoles. Among them 1-(5-(3,5-dinitrophenyl)-2-methyl-2-p-tolyl-1,3,4-oxadiazol-3(2H)-yl) ethenone (**3F**), 2-(3,5-dinitrophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**4C**) and 4-(3-(3,5-dinitrophenyl)- [1,2,4] triazolo [3,4-b][1,3,4] thiadiazol-6-yl phenol (**7F**) exhibits excellent activity against $H_{37}R_V$ strain. Furthermore, these compound also posses' activity against non replicating M.Tb H37R*v* (pFCA-luxAB).

The main flaw of these synthesized compounds is for its low aqueous solubility. This fastens the metabolic degradation, will be considered in further structural optimization. Moreover, several mutagencity and gene toxicity assay reveals that nitro group containing compounds have low mutagencity.

Docking studies suggests that all the compounds shows good binding energies with M.Tb ICL (isocitrate lyase) enzyme compared with standard isoniazid, rifampicin and 3-nitropropionate, cell free assay did not confirm this assumption. In the light of literature azoles inhibits specific cytochrome system in mycobacterium.

Interestingly, the investigated compounds had low toxicity against A_{549} cell lines and better selectivity index towards mycobacterium. These findings, together indicates that the 3F, 4C and 7F proved to be promising activity against active and latent tuberculosis with least side effects and selectivity, warranting further structural optimization, mechanism and assessment both invitro and in vivo. Therefore, these derivatives are of particular interest need for further studies.

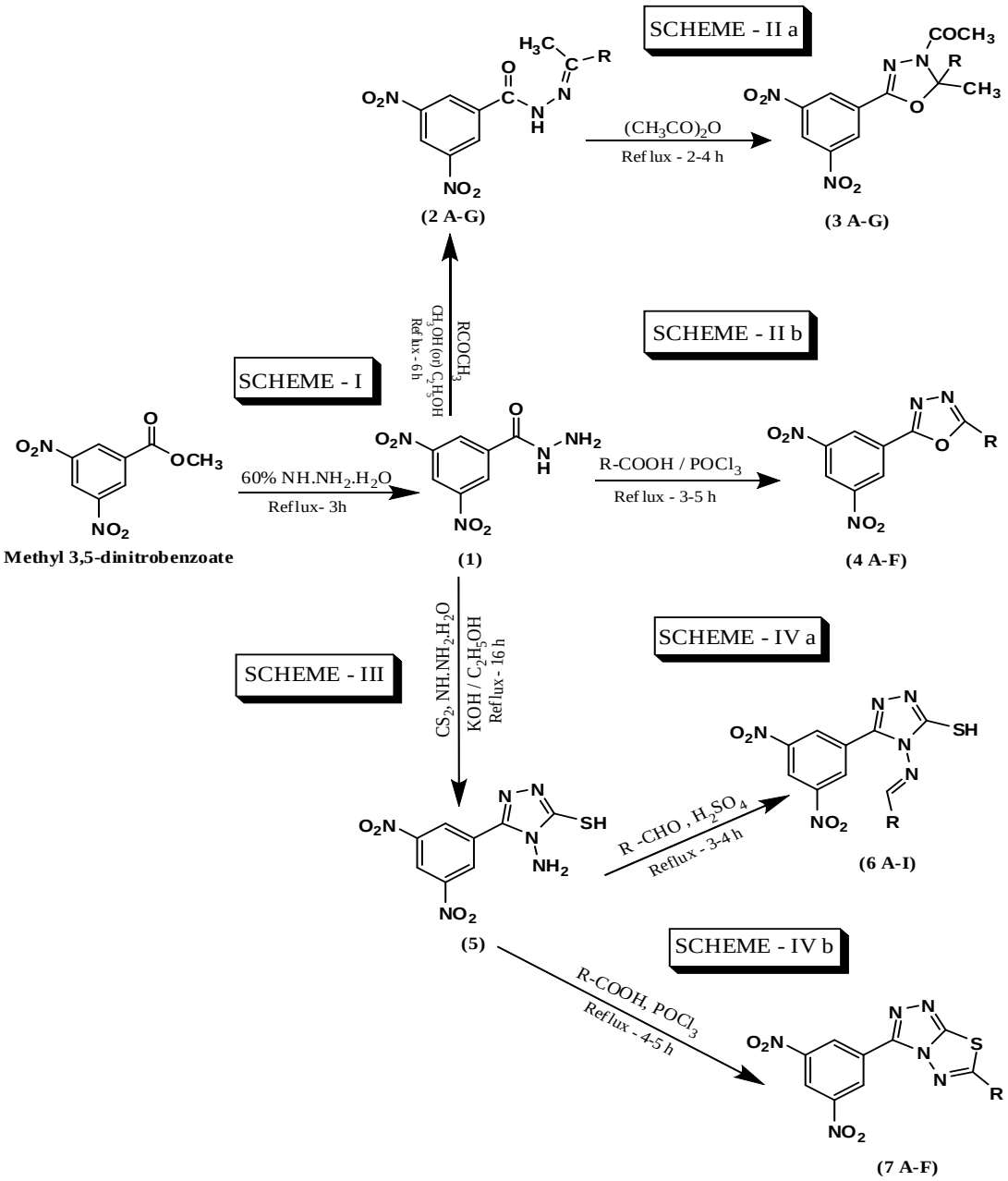


Figure 2 - General Scheme of Synthesis

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