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Synthesis and antituberculostic activity of 5-{3'-oxo-6'-(substituted phenyl)-2',3',4',5'-tetrahydropyridazin-2'-yl}methyl-2-substituted 1,3,4-oxadiazole

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Abstract

*A series of 5-{3'-oxo-6'-(substituted aryl)-2',3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-substituted 1,3,4-oxadiazole has been synthesized. Appropriate aromatic hydrocarbon reacts with succinic anhydride in presence of $AlCl_3$ to yield β-Aroyl propionic acid **(1a)**. The corresponding acid is cyclised with hydrazine hydrate to give 6-(substituted aryl)-2,3,4,5-tetrahydro-3-pyridazinone **(1b)**.This intermediate after reaction with ethyl bromo acetate, hydrazinolysed into 3-oxo-6-(substituted aryl)-2, 3, 4, 5-tetrahydropyridazinyl acetohydrazide **(1c)**.The resulting product was converted into 5-{3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-yl methyl}-2-substituted 1,3,4-oxadiazole. All the final compounds have been structurally elucidated on the basis of IR, ^{1}H-NMR, mass spectral data and elemental analysis and screened for antitubercular activity.*

Key Words: β-Aroyl propionic acid, Acetohydrazide, 1,3,4-oxadiazole and Antitubercular activity

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INTRODUCTION

The 1,3,4-oxadiazoles are known to exhibit diverse pharmacological activities like antimicrobial (1-3), antihistaminics (4), anticancerous (5-6), anti-inflammatory (7-9) and anticonvulsant (11). Hence, some new 1,3,4-oxadiazoles are synthesized as per reaction sequence is outlined in Scheme I. Friedal-crafts acylation of appropriate hydrocarbons with succinic anhydride in presence of anhydrous aluminium chloride yielded β-Aroyl propionic **(1a).**There are hydrazinolysis to get pyridazinones (**1b**) which reacted with ethyl bromo acetate to give ethyl 3-oxo-6-(substituted aryl)-2,3,4,5- tetrahydropyridazinyl acetate **(1c)**.The ester was converted into

3-oxo-6-(substitutedaryl)-2,3,4,5-tetrahydropyridazinyl acetohydrazide **(1d)** with slight excess of 99% hydrazine hydrate in absolute ethanol under reflux. These acetohydrazide acts as starting material for the synthesis of various 2-substituted 1,3,4-oxadiazole:-

i) Equimolar quantities of acetohydrazide **(1d)** and cyanogen bromide in ethanol was refluxed and then neutralized with sodium bicarbonate to yield 5-{3'-oxo-6'-(substituted aryl) 2',3',4',5'-Tetrahydropyridazin-2'-yl methyl}-2-amino 1,3,4-oxadiazole.**(2a-e)**

ii) In an ethanolic solution, equimolar quantities of acetohydrazide (**1d**) and carbon disulphide was refluxed and then acidified with dil. HCl to give 5{3'-oxo-6'-(substituted aryl) 2',3',4',5'-Tetrahydropyridazin-2'-yl methyl}-2-thione 1,3,4-oxadiazole.(**3a-e**)

iii) Thiosemicarbazides **(1g)** were conveniently synthesized by refluxing acid hydrazide **(1d)** with aryl isothiocyanate in ethanol for 3h.The thiosemicarbazides (**1g)** were oxidatively cyclised to 5{3'-oxo-6'-(substituted aryl) 2',3',4',5'-Tetrahydropyridazin-2'-yl methyl}-N-(substituted aryl)-2-amino 1,3,4-oxadiazole **(4a-e)** by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. All the final compounds of each series are structurally confirmed by elemental analysis and spectral data as shown in Table 1.

Table 1.Physicochemical data of 5-{3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-yl methyl}-2-substituted 1,3,4-oxadiazole.

Compound	R	M.P.	Mol.Formula	%yield
2a	-C_6H_5	187	$C_{13}H_{13}N_5O_2$	80
2b	3,5-$(CH_3)_2$-C_6H_5	204	$C_{15}H_{18}N_5O_2$	78
2c	4-CH_3-C_6H_5	184	$C_{14}H_{15}N_5O_2$	69
2d	4-(OC_6H_5)-C_6H_5	198	$C_{19}H_{17}N_5O_3$	71
2e	4-Cl-C_6H_5	186	$C_{13}H_{12}N_5O_2Cl$	63
3a	-C_6H_5	167	$C_{13}H_{12}N_4O_2S$	85
3b	3,5-$(CH_3)_2$-C_6H_5	209	$C_{15}H_{16}N_4O_2S$	75
3c	4-CH_3-C_6H_5	162	$C_{14}H_{14}N_4O_2S$	62
3d	4-(OC_6H_5)-C_6H_5	173	$C_{19}H_{16}N_4O_2S$	76
3e	4-Cl-C_6H_5	179	$C_{13}H_{11}N_4O_2SCl$	82
4a	-C_6H_5	157	$C_{13}H_{17}N_5O_2$	69
4b	3,5-$(CH_3)_2$-C_6H_5	199	$C_{15}H_{21}N_5O_2$	70
4c	4-CH_3-C_6H_5	164	$C_{14}H_{19}N_5O_2$	66
4d	4-(OC_6H_5)-C_6H_5	168	$C_{19}H_{21}N_5O_3$	93
4e	4-Cl-C_6H_5	188	$C_{13}H_{16}N_5O_2Cl$	53

Antitubercular activity (10)

Antitubercular activity was determined using the BACTEC 460 system. Stock solutions of test compounds was prepared in DMSO.MIC of rifampicin is calculated by established procedures. All the synthesized compounds screened at 6.25 μg/ml show the percentage inhibition ranging from 48 to 91%.The compound **(4a)** emerged as highly active analogue in this series with 91%

inhibition against *M.tuberculosis* H37 Rv comparable with that of standard rifampicin and isoniazid shown in Table 2

Table 2.*Invitro* Antitubercular activity of the 5-{3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-yl methyl}-2-substituted 1,3,4-oxadiazole

Compound no.	Concentration	% inhibition
2a	6.25	86
2b	6.25	52
2c	6.25	56
2d	6.25	48
2e	6.25	84
3a	6.25	88
3b	6.25	48
3c	6.25	51
3d	6.25	45
3e	6.25	87
4a	6.25	91
4b	6.25	52
4c	6.25	55
4d	6.25	49
4e	6.25	89
Rifampicin	0.25	98
Isoniazid	0.031	95
Tobramycin[1]	10.0	99
Clarithromycin[1]	26.0	99
Ethionamide[1]	1.17	99
PAS[1]	2.31	99
Ethambutol[1]	1.17	99
Gentamycin[1]	6.0	99
Doxycyclin[1]	12.0	99

[1] The concentration represents their MIC.

MATERIALS AND METHODS

The melting points were determined on a X-4 microscope melting point apparatus and are uncorrected. Elementary analysis was carried out on elementar vario EL analyzer. The NMR spectra were recorded in $CDCl_3$ as solvent (using TMS as an internal standard).The NMR and mass spectra were recorded on Jeol FX-100FT-NMR and Jeol BX 102/DA-6000 mass spectrometer respectively. The infrared spectra in KBr were recorded, on Buck Scientific M-500 Infrared Spectrophotometer. Solvent system used through out the experimental work for running TLC plates Toluene, Ethyl formate, and Formic acid in the ratio of 5:4:1.

The oxadiazole derivatives was synthesized as per Scheme-I. It is illustrated with the synthesis of compound **(2a, 3a, and 4a).**

Synthesis of β-benzoyl propionic acid:(1a)

After suspending anhydrous aluminum chloride (0.15 mol) in dry benzene (50ml) under anhydrous conditions, the contents was refluxed on a water bath. Succinic anhydride (0.10 mol) was then added to the reaction mixture in small portions with continuous stirring. Stirring and heating was continued for 6 h and the contents after leaving overnight at room temperature, ice cold solution of concentrated hydrochloric acid (2.5% v/v) was then added to the reaction mixture and the contents was concentrated to a small volume by heating on a water bath. The solid compound which separated out, was filtered. It was purified by dissolving in 5% w/v sodium bicarbonate solution, followed by extraction with ether. The aqueous layer on acidification with dilute hydrochloric acid gave benzoyl propionic acid, crystallized from aqueous ethanol to give a colorless compound. m.p.125^{o}C; R_f 0.25; %yield 73; ^{1}H-NMR(δ): 2.59 (t, 2H, CH_2), 3.23(t, 2H, CH_2), 7.53-7.62(m, 3H, H-3'-H-5'), 7.97 (d, 2H, H-2', H-6'), 12.17(s, 1H, COOH).

All the remaining acids were synthesized by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as solvent.

Synthesis of 6-phenyl-2, 3, 4, 5-tetrahydro pyridazin-3-one:(1b)

To a solution of β-benzoyl propionic acid **(1a)** (0.1 mol) in methanol (30ml), hydrazine hydrate (1ml) and sodium acetate (0.5g) was added and the contents refluxed for 6 h. After completion of the reaction, methanol was distilled off and the contents were poured into cold water. The solid that separated out, was filtered and crystallized from methanol, m.p.250^{o}C; R_f 0.45; % yield 72; IR (cm^{-1}) 3306(NH), 1678(C=O); ^{1}H-NMR (δ)2.45 (t, 2H, CH_2), 2.93(t, 2H, CH_2), 7.41(m, 3H, H-3'-H-5'), 7.74(d, 2H, H-2', H-6'), 10.94(s, 1H, CONH); Ms (m/z) 174, 159, 147, 130, 115, 109.

Synthesis of ethyl-3-oxo-6-phenyl-2, 3, 4, 5-tetrahydropyridazinyl acetate:(1c)

The above compound (**1b)** was added to an ethanolic solution (50 ml) of sodium (0.46g). Then the reaction mixture was refluxed for 30 min, and then cooled down. To this reaction mixture ethyl bromoacetate (3.34 gm, 0.02 mole) was added by drops to the cooled solution, which was refluxed for 24 h. The solvent was evaporated off and the resulting residue, triturated with di-isopropyl ether. The solid that was formed, was collected by filtration and then dried. The compound was recrystalised from a mixture of ethanol- water (50:50); m.p 189^{o}C; R_f 0.69; % yield 50; IR (cm^{-1}) 3204(OH), 1680 (C=O), 1599 (C=C), 1345, 1207, 751; ^{1}H-NMR (δ)1.2 (t, 3H, CH_3), 2.60(s, 2H, N-CH_2CO), 2.64(t, 2H, CH_2), 3.00(t, 2H, CH_2), 4.2(q, 2H, $COCH_2$) 7.41-7.72(m, 5H, Ar-H); MS: m/z 260(M^+), 247, 186.8, 173.1.

Synthesis of 3-oxo-6-phenyl-2, 3, 4, 5-tetrahydropyridazinyl acetohydrazide:(1d)

To a solution of compound **(1c)** (0.01 mol) in methanol (30ml), hydrazine hydrate (1ml) was added and the contents was refluxed for 8 h. The contents was concentrated, cooled and filtered to get the solid compound. It was recrystallized with alcohol to get TLC pure compound, m.p.179^{o}C; R_f 0.64; % yield 50; IR (cm^{-1}) 1701(CONH), 1686(CO), 1652(C=C); ^{1}H-NMR (δ)2.6 (t, 2H, CH_2), 2.8(m, 2H, NH_2), 3.00(t, 2H, CH_2), 3.2(s, 2H, N-CH_2CO), 7.37-7.71(m, 5H, Ar-H), 8.85(s, 1H, CONH).

Synthesis of 5-{3'-oxo-6'-phenyl 2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-amino- 1, 3, 4-oxadiazole:(2a)

Equimolar quantities of compound **(1d)** (0.001 mole) and cyanogen bromide (0.001 mole) in ethanol was refluxed for 14 h. The resulting solution was cooled and neutralized with sodium bicarbonate. The solid thus separated out was filtered, washed with water, dried and recrystallized from methanol. m.p. 187^{o}C; R_f 0.74; % yield 80; IR (cm^{-1}) 3210(NH), 3050(CH), 1680(C=O), 1630(C=N); ^{1}H-NMR (δ)2.48 (t, 2H, CH_2), 2.84(t, 2H, CH_2), 3.05(s, 2H, NCH_2), 7.42(s, 2H, NH_2), 8.02-8.68(m, 5H, Ar-H).

Synthesis of 5-{3'-oxo-6'-phenyl 2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-mercapto-1, 3, 4-oxadiazole:(3a)

To an ethanolic solution of compound (**1d)** (0.001 mole), KOH (0.001 mole) and carbon disulphide (5 ml) was refluxed on water bath for 24 h.The solution was then concentrated, cooled and acidified with dilute HCl.The solid mass that separated out was filtered, washed with ethanol, dried and crystallized from ethanol. m.p. 167^{o}C; R_f 0.74; % yield 78; IR (cm^{-1}) 1267 (C=S), 1593(C=N), 1680(C=O), 3046(CH), 3128(NH); ^{1}H-NMR (δ)2.32 (t, 2H, CH_2), 2.61(t, 2H, CH_2), 3.02(s, 2H, CH_2), 8.00-8.62(m, 5H, C=S NH).

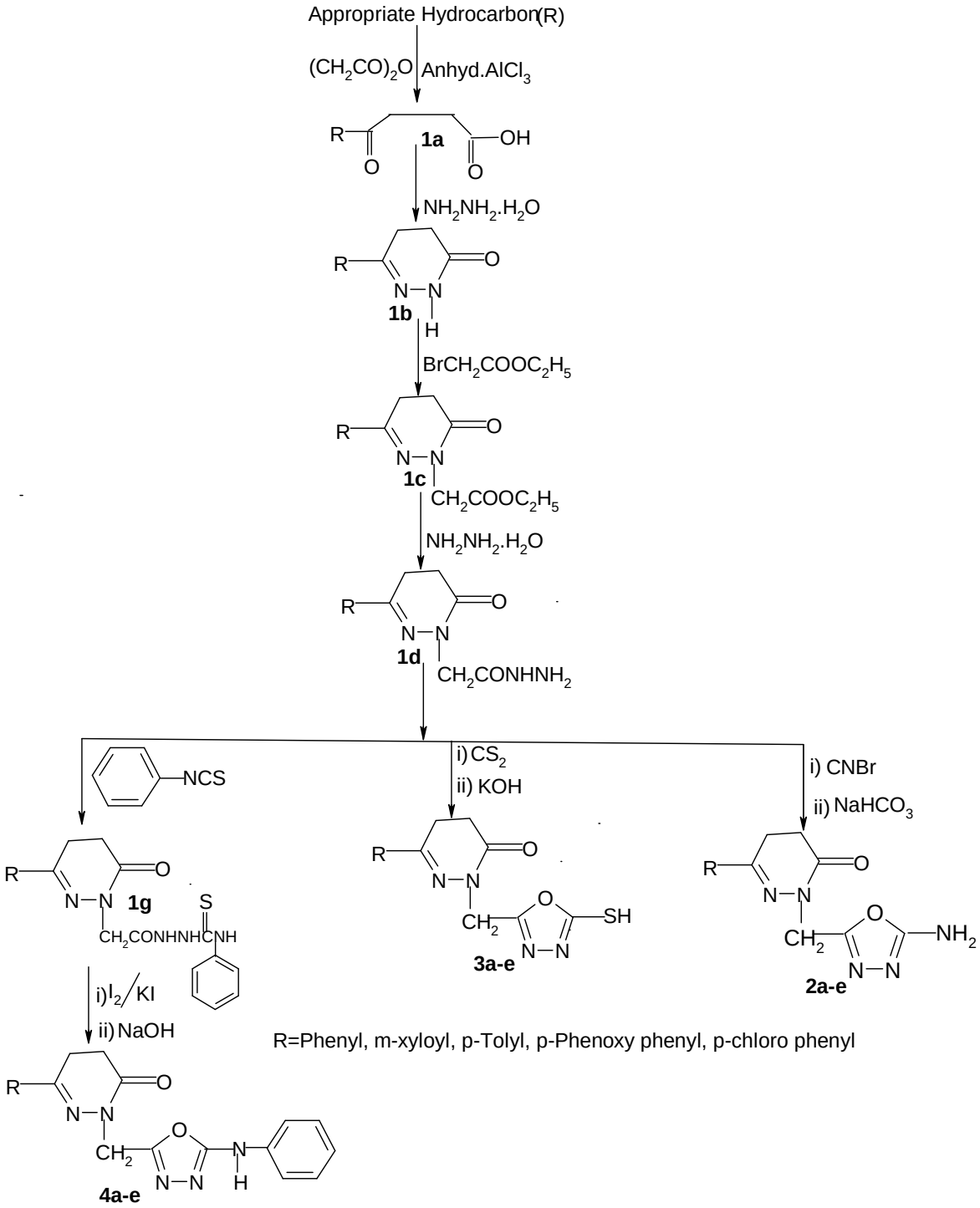
Synthesis of 5-{3'-oxo-6'-phenyl 2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-phenyl amino-1, 3, 4-oxadiazole:(4a)

An ethanolic solution of compound (**1d)** (0.001 mole) and phenyl isothiocyanate (0.001 mole) was refluxed for 3 h. The contents was concentrated and poured into crushed ice, filtered and dried to get crude thiosemicarbazide (**1g**).To the crude thiosemicarbazide (**1g**) (0.003 mole) in ethanol (60 ml), aq NaOH (6N, 5 ml) was added. 10% solution of I_2 in KI was then added drop wise and the reaction mixture was kept at 10^{0}C. The addition of I_2 was continued till the colour of I_2 persisted and the reaction mixture was then refluxed for 4 h. On cooling, the separated solid was washed thoroughly with water and recrystallized from ethanol; m.p. 157 ^{o}C; R_f 0.84; % yield 80; IR (cm^{-1}), 1030-1020(C-O-C), 1680-1600(CN), 1678(C=O), 3140-3100(NH); ^{1}H-NMR, (δ)2.59 (t, 2H, CH_2), 2.94(t, 2H, CH_2), 3.7(m, 2H, NCH_2), 7.01-7.68(m, 10H, Ar-H), 9.86(s, 1H, Ar-NH).

The remaining compounds were synthesized with analogue procedure

5-{3'-Oxo-6'-(2'', 4''-dimethylphenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl-methyl}-2-amino-1, 3, 4-oxadiazole (2b, R=m-xyloyl)

m.p. 204^{o}C, R_f 0.81, %yield 98; IR(cm^{-1}) 3210(NH), 3060(CH), 1682(C=O), 1635(C=N), 1032-1025(C-O-C); ^{1}H-NMR(δ) 2.31(s, 6H, 2xCH_3), 2.58(s, 2H, CH_2), 2.92(t, 2H, CH_2), 2.72(s, 2H, NCH_2), 7.31(s, 2H, NH_2), 7.9-8.3(m, 3H, Ar-H).



Scheme I. Synthesis of 5-{3'-oxo-6'-(substituted phenyl)-2', 3', 4', 5'- tetrahydropyridazin-2'-yl} methyl-2-substituted 1, 3, 4-oxadiazole

5-{3'-Oxo-6'-(4''-toloyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-amino-1, 3, 4-oxadiazole (2c, R=Toloyl)
m.p. 189^{o}C, R_f 0.71, % yield 90; IR(cm^{-1}) 3205(NH), 3051(CH), 1680(C=O), 1630(C=N); ^{1}H-NMR(δ) 2.06(s, 3H, CH_3), 2.46(t, 2H, CH_2), 2.89(t, 2H, CH_2), 3.09(s, 2H, NCH_2), 7.40(s,2H, NH_2), 8.0-8.5(m, 4H, Ar-H).

5-{3'-Oxo-6'-(4''-phenoxy phenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-amino-1, 3, 4-oxadiazole (2d, R=p-phenoxy phenyl)
m.p. 256^{o}C, R_f 0.74, % yield 98; IR (cm^{-1}) 3210(NH), 3050(CH), 1681(C=O), 1630(C=N), 1035 (C-O-C); ^{1}H-NMR(δ) 2.37(t, 2H, CH_2), 2.68(t, 2H, CH_2), 3.00(s, 2H, NCH_2), 7.38(s, 2H, NH_2), 8.01-8.43(m, 9H, Ar-H).

5-{3'-Oxo-6'-(4''-chlorophenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-amino-1, 3, 4-oxadiazole (2e, R=p-chloro phenyl)
m.p.186^{o}C, R_f 0.80; % yield 90; IR(cm^{-1}) 3205(NH), 3051(CH), 1680(C=O), 1630(C=N), 710(C-Cl); ^{1}H-NMR(δ) 2.46(t, 2H, CH_2), 2.82(t, 2H, CH_2), 3.1(s, 2H, NCH_2), 7.40(s, 2H, NH_2), 8.01-8.68(m, 4H, Ar-H).

5-{3'-Oxo-6'-(2'', 4''-dimethylphenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-mercapto-1, 3, 4-oxadiazole (3b, R=m-xyloyl)
m.p. 209^{o}C, R_f 0.73, % yield 90; IR (cm^{-1}) 3205(NH), 3044(CH), 1683(C=O), 1602(C=N), 1165(C=S); ^{1}H-NMR(δ) 2.35(s, 6H, 2xCH_3), 2.54(s, 2H, CH_2), 2.86(t, 2H, CH_2), 3.06(s, 2H, CH_2), 7.19-7.50(m, 3H, Ar-H), 7.96-8.52(s, 1H, 1CSNH).

5-{3'-Oxo-6'-(4''-toloyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-mercapto-1, 3, 4-oxadiazole (3c, R=p-Toloyl)
m.p.162^{o}C, R_f 0.78, % yield 88; IR(cm^{-1}) 3120(NH), 3043(CH), 1683(C=O), 1590(C=N), 1165(C=S); ^{1}H-NMR(δ) 2.02(s, 2H, CH_3), 2.30(τ, 2H, CH_2), 2.59(t, 2H, CH_2), 3.01(s, 2H, CH_2), 7.18-7.52(m, 4H, Ar-H), 8.01-8.60(s, 1H, 1CSNH);

5-{3'-Oxo-6'-(4''-phenoxy phenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-mercapto-1, 3, 4-oxadiazole (3d, R=p-Phenoxy phenyl)
m.p. 219^{o}C, R_f 0.93, % yield 90; IR(cm^{-1}) 3110(NH), 3060(CH), 1680(C=O), 1590 (C=N), 1168 (C=S); ^{1}H-NMR(δ) 2.30(t, 6H, CH_2), 2.60(s, 2H, CH_2), 3.0(s, 2H, CH_2), 7.09-7.45(m, 9H, Ar-H), 8.01-8.58(s, 1H, 1NHC=S).

5-{3'-Oxo-6'-(4''-chlorophenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-mercapto- 1, 3, 4-oxadiazole (3e, R=p-chloro)
m.p.179 ^{o}C, R_f 0.79 , % yield 89; IR(cm^{-1}) 3120(NH), 3043(CH), 1683(C=O), 1590(C=N), 1167(C=S), 715(C-Cl); ^{1}H-NMR(δ) 2.30(t, 2H, CH_2), 2.60(t, 2H, CH_2), 3.10(s, 2H, CH_2), 7.17-7.50(m, 4H, Ar-H), 7.89-8.59(s, 1H, 1CSNH).

5-{3'-Oxo-6'-(2'', 4''-dimethylphenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-p-tolyl amino-1, 3, 4-oxadiazole (4b, R=m-xyloyl)
m.p.199^{o}C, R_f 0.68, % yield 93; IR(cm^{-1}) 3145-3100(NH), 1680(C=O), 1650-1600(C=N), 1035-1021(C-O-C); ^{1}H-NMR(δ) 2.3(s, 9H, 3xCH_3), 2.59(t, 2H, CH_2), 2.94(t, 2H, CH_2), 3.7(m, 2H, N-CH_2), 4.87(s, 1H, NH), 7.03-7.39(m, 7H, Ar-H).

5-{3'-Oxo-6'-(4''-toloyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-phenyl amino-1, 3, 4-oxadiazole (4c, R=p-Toloyl)
m.p.164^{o}C, R_f 0.80, % yield 76; IR(cm^{-1}) 3125(NH), 1686(C=O), 1676(CN), 1026(C-O-C); ^{1}H-NMR(δ) 2.02(s, 3H, CH_3), 2.42(t, 2H, CH_2), 2.84(t, 2H, CH_2), 3.62(m, 2H, NCH_2), 7.01-7.60(m, 9H, Ar-H), 9.72(s, 1H, Ar-NH).

5-{3'-Oxo-6'-(4''-phenoxy phenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-phenyl amino-1, 3, 4-oxadiazole (4d, R=p-Phenoxy phenyl)
m.p. 204^{o}C, R_f 0.61, % yield 95; IR (cm^{-1}) 3135-3120(NH), 1681(C=O), 1667-1672(C=N), 1035-1025(C-O-C); ^{1}H-NMR(δ) 2.37(t, 2H, CH_2), 2.68(t, 2H, CH_2), 3.00(s, 2H, N-CH_2), 7.38(s, 2H, NH_2), 8.01-8.43(m, 14H, Ar-H).

5-{3'-Oxo-6'-(4''-chlorophenyl)-2', 3', 4', 5'-tetrahydropyridazin-2'-yl methyl}-2-phenyl amino-1, 3, 4-oxadiazole (4e, R=p-chloro phenyl)
m.p. 188^{o}C, R_f 0.67, % yield 99; IR(cm^{-1}) 3150(NH), 1686(C=O), 1670(C=N), 1022(C-O-C), 722(C-Cl); ^{1}H-NMR(δ) 2.54(t, 2H, CH_2), 2.89(t, 2H, CH_2), 3.67(m, 2H, NCH_2), 7.01-7.78(m, 9H, Ar-H), 9.78(s, 1H, Ar-NH).

RESULTS AND DISCUSSION

The antitubercular activity of synthesized compounds was performed by adopting Alamar blue susceptibility test (MABA).Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water, and subsequent two fold dilutions were performed in 0.1 ml of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 ml was added to wells. Subsequent determination of bacterial titers yielded 1 X106 CFU/ml in plate wells for *M. tuberculosis* H37Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by a 1:50 dilution in 7H9GC. Addition of 1/10 ml to wells resulted in final bacterial titers of 2.0 X105 CFU/ml for *M. tuberculosis* H37Rv. Wells containing drug only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of incubation, 20 µL of 10X alamar Blue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 ml of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37°C. Wells were observed at 12 and 24 hours for a color change from blue to pink and for a reading of 50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with

excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 hrs, reagent was added to the entire plate. If the well remained blue or 50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37°C, and results were recorded at 24 hrs post-reagent addition. Visual minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as 1 - (test well FU/mean FU of triplicate B wells) X100. The lowest drug concentration effecting an inhibition of >90% was considered the MIC.

All the final compounds was tested for antitubercular activity at 6.25 µg/ml, showed percentage of inhibition ranging from 45 to 90%.

CONCLUSION

The antitubercular activity of synthesized compounds was performed by adopting Alamar blue susceptibility test (MABA).All the final compounds was tested for antitubercular activity at 6.25 µg/ml, showed percentage of inhibition ranging from 45 to 90%.The compound **(4a)** emerged as highly active analogue of the series with 91% inhibition against *M.tuberculosis* H37 Rv. The order of activity was found to be H>Cl.>O-toluidine>m-xyloyl>Di-phenyl ether.From the above result, it concluded that **(4a)** are highly active against *M.tuberculosis* H37 Rv.

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