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EFFICACIOUS SYNTHESIS, *IN VIVO* ANTI-INFLAMMATORY AND *IN VITRO* ANTITUBERCULAR ACTIVITY OF NOVEL PYRIDAZINONES AND THEIR EXPECTED ANTI-INFLAMMATORY MECHANISM

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ABSTRACT

A new series of 2-(5-amino-1,3,4 thiadiazol-2-yl)methyl)-6-phenyl-4,5-dihydropyridazin-3(2H)-one derivatives has been synthesized and evaluated for their *In-vitro* antitubercular and *In-vivo* anti-inflammatory activity. Antitubercular activity of synthesized compounds were screened by serial dilution method and disc diffusion method using middlebrook 7H9 medium (broth and agar based) and ATCC 25175 strain of *M.Tuberculosis*. The anti-inflammatory activity of the synthesized compounds was studied using a carageenan-induced hind paw edema model in rat. Test compounds (100 mg/Kg) and indomethacine (10 mg/Kg) dose level were administered orally to the experimental animals. All pyridazinone derivatives bearing acetic acid hydrazide moieties (**1c, 1e, 3c**) showed remarkably potent anti-inflammatory activity, especially after 3 and 4hr the drug was administered, but compounds containing P-chlorophenyl group substitution in aromatic ring in the structure (**5c, 5d** & **5e**) had the most potent anti-inflammatory agents. Derivatives with 4-ethyl phenyl substitution (**2e**) and chloro phenyl substitution (**5d, 5e**) at p-position of pyridazinone nuclei showed good antitubercular activity with MIC value of 1.25 µg/ml then other aryl substitution like 4-methoxy phenyl **1d** &**1e,** 4-methyl phenyl **3d** & **3e** with MIC value of 3.125 & 2.5 µg/ml.

KEYWORDS: Pyridazinone, anti-inflammatory agents, Antitubercular agents, Paw edema model, Aryl-substituted pyridazinones.

1. INTRODUCTION

The causative organism for (TB), Mycobacterium Tuberculosis, claims several human lives annually, [1] Tuberculosis is a serious public unhealthiness in each industrialized and developing countries and is chargeable for omit 2 million deaths every year. Medical aid is complicated because of each demand for prolonged treatment with a combination of drug and also the emergence of drug-resistant strains. 3 major approaches are utilized to regulate TB; the hospital with contemporary air and nutrition diet; vaccination; and therapy. [2]

A numbers of agents, together with para- amino salicylic acid (PAS), isoniazide (INH), rifampicin (RMP), pyrazinamide (PZA) and antibiotic are used against TB. However, the resistant towards available medication is rapidly becoming a significant worldwide downside. Antitubercular medications with new mechanisms of action haven't been developed within the last thirty years. [3] Thus, this is often a requirement to design new antitubercular compounds to subsume this resistant having fewer adverse effects. [4] Pyridazinone is a versatile moiety that exhibit a wide variety of biological activities viz. differently substituted pyridazinones has been found to possess potential activities like antibacterial drug. [5, 6, 7] Antifungal, [8] analgesic and anti-inflammatory.[9, 10, 11, 12]

In addition to the development of recent and effective antitubercular agents against multidrug resistant microorganism, recently attention has targeted on the treatment of inflammation. Most presently used anti-inflammatory drug medications (NASIDs) have limitations for therapeutic use since they cause gastrointestinal and renal side effects that square measure inspirable from their medical specialty activities. Therefore, the synthesis of recent compounds innocent of such facet effects has become a crucial goal for medicinal chemist's chemists in recent years. [13]

The aim of the current research work was to explore and investigate some new categories of compounds with improved potential for treating T.B. and inflammation. During this paper we tend to communicate the synthesis, antitubercular and anti inflammatory activity of varied 2-((5-amino-1,3,4 thiadiazol-2-yl)methyl)-6-phenyl-4,5-dihydropyridazin-3(2H)-one derivatives.

2. MATERIALS AND METHODS

2.1. Chemistry

Melting points, which are uncorrected, were determined using open capillary tubes on an electric melting point apparatus. Synthesized compounds were purified by column chromatography. Required reagents and chemicals were purchased from E Merck (India) Ltd., S.D. Fine (India) and Qualigens (India). All the solvents were dried by appropriate drying agents before use. The progress of the reaction and the purity of the synthesized compounds were verified on ascending thin layer chromatography (TLC) Plates coated with silica gel G (Merck). Solvent system used for running TLC Plates was toluene, ethyl format, & formic acid in the ratio of 5: 4:1. An iodine chamber and UV light were used for the visualization of the TLC spots. The IR Spectra were recorded by using KBr pellet technique on Schimadzu spectrometer (υ_{max} in cm^{-1}). ^1H NMR spectra were recorded in DMSO/$CDCl_3$ on Bruker Avance II 400 MH_z and 125 MH_z spectrometer, using tetramethylsilane (TMS) as the internal reference (chemical shift is measured in δ ppm) . Mass spectra were measured on a JEOL-Accu TOF JMS-T100LS mass spectrometer with a DART (Direct analysis in real time) source.

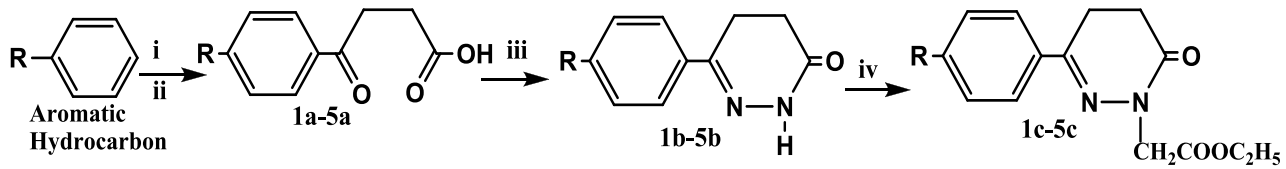
2.1.1. General procedure for the synthesis of β - (substituted aroyl) prop ionic acid (a)

The β-(substituted aroyl) propionic acids (**a**) according to Siddiqui method were obtained by the Friedal craft's acylation of appropriate hydrocarbons. [14]

2.1.2. General procedure for the synthesis of 6 - (substituted aroyl) 2,3,4,5 tetra hydro- pyridazine 3-ones (b). [14]

The Siddiqui method has been followed from β-(substituted aroyl) prop ionic acid (**a**) cyclisation of β-(substituted aroyl) prop ionic acid, with hydrazine hydrate to afford pyridazinones (**b**) in Scheme 1.

2.1.3. 6-oxo-3p-(β-(substituted aroyl) propionic acid)-5, 6-dihydro-4H-pyridazine-1yl)-acetic acid ethyl ester (c). [15] in Scheme 1.



Scheme 1 Synthesis of compounds (**1c-5c).** Reagents and conditions: **i**. succinic anhydride, **ii**.anhydrous alluminium chloride, stirring & heating at 80°C for 4 hr. **iii.** hydrazine hydrate **iv**. ethylchloro acetate, ethanol, refluxed 24hr

	R
1c	OCH3
2c	$CH2CH_3$
3c	CH3
4c	H
5c	Cl

2.1.4. General procedure for the synthesis of 2- (2- (3-(substituted aroyl) – 6 oxo-5, 6-dihydropy ridazine - 1(4H) - yl) acetyl) hydrazine-1(4H) - yl) acetyl) hydrazine carbothioamide (d).

6-oxo-3p-(β-(substituted aroyl) propionic acid)-5, 6-dihydro-4H-pyridazine-1yl)-acetic acid ethyl ester (**c**) (0.08 mol, 22gm) and (0.08 mol, 7gm) of thiosemicarbazide in ethanol 50 ml were mixed, and the reaction mixture was stirred for 6 hours and then refluxed on steam bath for 3 hours. The excess of solvent was removed under reduced pressure and recrystallized from chloroform- hexane (3: 1) v/v to obtained yellow crystals of compound (**d**) (Table 1).

Table 1 Synthesis of derivatives (1c-1e, 2c-2e, 3c-3e, 4c-4e, and **5c-5e)**

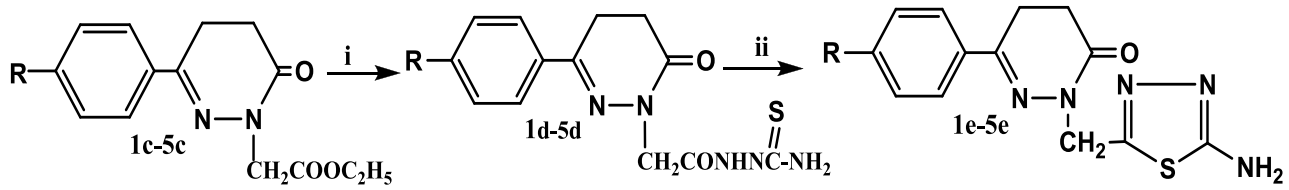
Entry	Compounds	R	Yield[a] (%)
1	1c	OCH_3	75
2	1d	OCH_3	80
3	1e	OCH_3	81
4	2c	CH_2CH_3	75
5	2d	CH_2CH_3	82
6	2e	CH_2CH_3	80
7	3c	CH_3	72
8	3d	CH_3	80
9	3e	CH_3	65
10	4c	H	73

11	4d	H	58
12	4e	H	78
13	5c	Cl	74
14	5d	Cl	74
15	5e	Cl	80

a. Yields are indicated in isolated compounds

2.1.5. General procedure for the synthesis of 2-[(5-amino-1, 3, 4-thiadiazole-2yl) methyl] -6 - (4-substituted aroyl) - 4, 5-dihydropyridazin 3(2H)-one derivatives (e)

2- (2- (3- (substituted aroyl) – 6 oxo-5, 6-dihydropyridazine - 1(4H) - yl) acetyl) hydrazine-1(4H) - yl) acetyl) hydrazine carbothioamide (**d**) (0. 08 mol) was added in conc. H_2SO_4 (10ml) in a Petridis and kept it overnight at room temperature, neutralized with ammonia and extracted with ether. The ether was distilled off and the product so obtained was recrystallized from 80% ethanol to obtain yellowish leaflets product of 2-[(5-amino-1, 3, 4-thiadiazole-2yl) methyl] -6 - (4-substituted aroyl) - 4, 5-dihydropyridazin 3(2H)-one derivatives (**e**) (Table 1) and Scheme 2.



Scheme 2 Synthesis of compounds (**1d-5d** and **1e-5e**). Reagents and conditions: **i.** (1c-5c) individually stirred 6hr with thiosemicarbazide in ethanol **ii.** (1d-5d) individually add conc. H_2 SO4 overnight at r.t.

	R
1d, 1e	**OCH_3**
2d, 2e	**CH2CH3**
3d, 3e	**CH_3**
4d, 4e	**H**
5d, 5e	**Cl**

2.1.6. Characterization and spectral analysis of synthesized derivatives (1c-1e, 2c-2e, 3c-3e, 4c-4e, 5c-5e)

2.1.6.1 6-oxo-3p-(β-(anisoyl) propionic acid)-5, 6-dihydro-4H-pyridazine-1yl)-acetic acid ethyl ester (1c)

Yellow power; mp 180°C; Rf- 0.58; IR (KBr) Cm^{-1}: 2966 **(**ali-CH_3),3099(Ar-C-H),1023(C-O-C Symm in Anisol), 1251 (C-O-C Asymm in Anisol) (1664 (C=N), 1463 (Ar C=C), 2835 (C-H str in Anisol), 1751 (C=O str in ester), 1301 (C-O str in ester), 3008 (C-H str in ester); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 2.59 (2H,t,CH_2), 2.95 (2H,t,CH_2), 7.69 (2H,d,H-2',6), 6.94 (2H,d,H-3',5), 3.94 (2H,s), 3.84 (2H,m), 1.28 (3H,t); ^{13}C NMR ($CDCl_3$): δ 114.4-168.0 (6C, Aryl), 55.8 (1C,O-CH_3), 29.1 (1C, CH_2-(=O)-O),35.0 (1C, CH_2-(=O)-C), 167.7(1C, Pyridazinone and amide),146.5 146.5 (1C, imine), 52.3 (1C, CH_2-C(=O)-O), 61.0 (1C, CH_2-O-C=O), 14.5(aliphatic-C), 169.5 (1C, Carboxyl). ms: m/z 295.2 (M^+), Anal. Calcd. For $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25; o, 22.04; N, 9.65. Found: C, 62.02; H, 6.22; o, 22.00; N, 9.60.

2.1.6.2. 2- (2- (3- (anisoyl) – 6 oxo-5, 6-dihydropyridazine - 1(4H) - yl) acetyl) hydrazine-1(4H) - yl) acetyl) hydrazine carbothioamide (1d)

White powder; mp 125°C; Rf - 0.73; IR (KBr) Cm^{-1}: 2918 (ali-CH_2),3007(Aro-CH),1022 (C-O-C symm in anisol),1251 (C-O-C Asymm in anisol), (1664 (Ar C=O), 1612 (Ar C=N), 3318 (Pri NH_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 7.6 (d, 1H, Ar-H, J=7.5Hz), 6.85 (d, 1H, Ar-H, J=1.5Hz), 3.7 (s, 3H, O-CH_3,), 2.88 (t, 2H, CH_2, J=7.1Hz), 2.44 (t, 2H, CH_2, J=7.1Hz), 3.2 (s, 2H, CH_2), 7.86 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 114.4-168 (6C, Ar),162.4 (1C, Pyridazinone and Amide),146.5 146.5 146.5 (1C,imine), 32.5 (2C, -(C=O)-N-N), 24.4 (2C, -C=N), 182.5 (1C, Thioamide), 170.3 (1C, amide), 55.8 (1C, O-CH_3), 57.6(1C, -(C=O)-N-N). ms: m/z 335.7 (M^+), Anal. Calcd. For $C_{14}H_{17}N_5O_3S$: C, 50.41; H, 5.11; o, 14.31; N, 20.88; Found: C, 50.38; H, 5.10; o, 14.29; N, 20.85

2.1.6.3. 2-[(5- amino-1, 3, 4- thiadiazole-2yl) methyl] -6 - (4- anisoyl) - 4, 5- dihydropyridazin 3 (2H)-one derivatives (1e)

Yellowish leaflets; mp 70°C; Rf - 0.82; IR (KBr) Cm^{-1}: 2918(Ali C-H), 3028(Aro C-H), 1698 (Aro C=O), 1575 (Ar C=C), 3319(Pri NH_2), 613 (C-S-C stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 6.99 (d, 1H, Ar-H,

J=7.5Hz), 7.95 (d, 1H, Ar-H, J=1.5Hz), 2.59 (2.61 (t, 2H, CH_2, J=7.1Hz), 3.19 (t, 2H, CH_2, J=7.1Hz), 4.60 (s, 2H, N-CH_2), 6.9 (s,2H,NH_2); ^{13}C NMR ($CDCl_3$): δ 114.4-168 (6C, Ar), 162.4 (1C, Pyridazinone and Amide), 146.5 (1C,imine), 32.8 (2C, -(C=O)-N-N), 24.4 (2C, -C=N), 55.8 (1C, O-CH_3), 51.0 (2C,CH_2-N),161.4(1C,Thiadiazole C-NH_2),168.0 (1C, Thiadiazole –C-S). ms: m/z 335.7 (M^+), Anal. Calcd. For $C_{15}H_{14}N_5O_2S$: C, 52.98; H, 4.76; o, 10.08; N, 22.07; Found: C, 52.90; H, 4.8; o, 10.05; N, 22.83.

2.1.6.4. 6-oxo-3- (ethyl phenyl) - 5, 6-dihydro-4H-pyridazine-1yl) -acetic acid ethyl ester (2c)

Yellow power; mp 116-120°C; Rf -0.68; IR (KBr) Cm^{-1}: 3099, (Aro C-H), 2967, 29279 (Ali C-H), 1511 (Ar C=C), 1613 (Ar C=N),1666 (Ar C=O),1740 (Ester C=O), 1251 (Ester C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 2.98 (2H,t,CH_2), 2.6 (2H,t,CH_2), 7.64 (2H,d,H-2',6), 7.26 (2H,d,H-3',5), 2.69 (2H,m,CH_2), 1.25 (3H,t,CH_3), 3.94 (2H,s,CH_2),4.17 (2H,m,CH_2), 1.4 (3H,t,CH_3); ^{13}C NMR ($CDCl_3$): δ 127.6-146.7 (6C, Aryl), 28.2 (1C, CH_2-CH_3), 14.5 (1C, CH_2-CH_3), 32.5 (1C, CH_2-(=O)-N-N),24.4 (1C, CH_2-C=N),146.5 (1C, C=N), 162.4(1C, Pyridazinone and amide),146.5 146.5 (1C, imine), 52.3 (1C, CH_2-C(=O)-O),61.0 (1C, CH_2-O-C=O), 14.5 (aliphatic-C),169.5 (1C, Carboxyl). ms: m/z 288.15 (M^+), Anal. Calcd. For $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; o, 16.65; N, 9.72 Found: C, 66.62; H, 6.90; o, 16.61; N, 9.71.

2.1.6.5. 2- (3-(4-ethyl phenyl) -6-oxo-5, 6-dihydropyridazin-1(4H)-l) acetyl) hydrazine carbo thioamide (2d)

White powder**;** mp 136-140°C; Rf-0.81; IR (KBr) Cm^{-1}: 2928 (ali-CH_2), 3106 (Aro-CH), 1022(C-O), 1666 (C=O), 1614(C=N), 3212(NH_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 6.9 (d, 1H, Ar-H, J=5(1.5 Hz), 2(7.5Hz), 7.23 (d, 1H, Ar-H, J=3(7.5Hz), 6(1.5 Hz), 2.65(t, 2H, CH_2, J=(11)7.1Hz), 2.51(q,2H J=15 (8.0Hz), 1.25 (t, 3H, J=14(8.0Hz), 3.82 (s, 2H, CH_2 J=(11)7.1Hz), 7.65(d,1H,), 2.56 (s,NH), 7.95(s,2H, NH_2); ^{13}C NMR ($CDCl_3$): δ 127.6-146.7 (6C, Aryl), 28.2 (1C, CH_2-CH_3), 14.5 (1C, CH_2-CH_3), 32.5 (1C, CH_2-(=O)-N-N),24.4 (1C, CH_2-C=N),146.5 (1C, C=N), 162.4(1C, Pyridazinone and amide),146.5 146.5 (1C, imine), 57.6 (1C, CH_2-C(=O)-N-N), 170.3 (1C, amide), 182.5 (1C, Thioamide). ms: m/z 333.13 (M^+), *Anal*. Calcd. For $C_{15}H_{19}N_5O_2S$: C, 54.15; H, 5.36; o, 9.18; N, 21.10 Found: C, 54.11; H, 5.32; o, 9.16; N, 21.08.

2.1.6.6. 2- (5-amino-1, 3, 4-thiadiazol-2-yl) methyl) - 6- (4-ethylphenyl) -4, 5-dihydropyridazin-3(2H)-one (2e)

Brown powder; mp 85-88°C; Rf -0.92; IR (KBr) Cm^{-1}: 2915 (Ali C-H), 3063(Aro C-H), 1678 (Aro C=O), 1573 (Ar C=C), 3351 (Pri NH_2), 613 (C-S-C stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 7.3 (d, 1H, Ar-H, J=6 (1.5 Hz), 3(7.5Hz), 7.9 (d, 1H, Ar-H, J=2(7.5Hz), 5 (1.5 Hz), 3.2(t,2H, J=(11)7.1Hz), 2.6 (t,2H,J=(12)7.1Hz), 2.7 (q,2H,J=(22) 8.0Hz), 3.8(s, 2H),1.2(t,3H J=(21), 8. 0Hz),), 6.9 (s,2H,NH_2); ^{13}C NMR ($CDCl_3$): δ 127.6-146.7 (6C, Aryl), 28.2 (1C, CH_2-CH_3), 14.5 (1C, CH_2-CH_3), 32.5 (1C, CH_2-(=O)-N-N),24.4 (1C, CH_2-C=N),146.5 (1C, C=N), 162.4(1C, Pyridazinone and amide), 51.0 (1C, CH_2-N), 168.0 (1C, Thiadiazole, C-S), 161.6 (1C, Thiadiazole, C-NH_2). ms: m/z 315.12 (M^+), Anal. Calcd. For $C_{15}H_{17}N_5OS$: C, 57.12; H, 5.43; o, 5.07; N, 22.21 Found: C, 57.09; H, 5.41; o, 5.02; N, 22.20.

2.1.6.7. 6-oxo-3p-(toloyl)-5, 6-dihydro-4H-pyridazine-1yl) -acetic acid ethyl ester (3c)

Yellow power; mp 148-152°C; Rf -0.77; IR (KBr) cm^{-1}: 2920, 2973 **(**ali-CH_3),3036, 3090 (Aro-CH), 1660(C=O aromatic ketone),1754(ester ketone), 1231(ester C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 2.57(2H,t,CH_2), 2.95(2H,t,CH_2), 7.6(2H,d,H-2',6, j=2(7.5), 7.2(2H,d,H-3',5,J=3(7.5)), 3.9 (2H,s), 3.7 (2H,m), 1.23 (3H,t); ^{13}C NMR ($CDCl_3$): δ 127.0-140.7 (6C, Aryl), 21.3 (1C, CH_2-CH_3), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 162.4(1C, Pyridazinone and amide),146.5 146.5 (1C, imine), 52.3 (1C, CH_2-C(=O)-O),61.0 (1C, CH_2-O-C=O), 14.1 (aliphatic-C),169.5 (1C, Carboxyl). ms: m/z 273.2 (M^+), Anal. Calcd. For $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; o, 17.50; N, 10.21 Found: C, 65.66; H, 6.59; o, 17.48; N, 10.20.

2.1.6.8. 2-(2-(6-oxo-3-p-tolyl-5,6-dihydropyridazin-1(4H) yl) acetyl) hydrazine carbothioamide (3d)

White powder; 135-140°C; Rf -0.82; IR (KBr) cm^{-1}: 2920 (ali-CH_2), 3090 (Aro-CH), 1660 (Ar C=O), 1622 (Ar C=N), 3312 (Pri NH_2),1100, 1100, 3465 (Sec amide N-H Str); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 2.58 (2H,t,CH_2, j=11(7.1), 2.91(2H,t,CH_2, j=12(7.1), 7.6 (2H,d,H-2',6, j=2(7.5), 7.2 (2H,d,H-3',5, j=3(7.5), 2.4 (3H,s), 3.95 (2H, s), 8.09 (NH), 2.2 (NH_2); ^{13}C NMR ($CDCl_3$): δ 127.0-140.7 (6C, Aryl), 21.3 (1C, CH_2-CH_3), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 162.4(1C, Pyridazinone and amide), 146.5 (1C, imine), 57.6 (1C, CH_2-C(=O)-N-N), 170.3 (1C, amide), 182.5 (1C, Thioamide). ms: m/z 319.11 (M^+), *Anal*. Calcd. For $C_{14}H_{17}N_5O_2S$: C, 52.65; H, 5.38; o, 10.02; N, 21.93 Found: C, 52.58; H, 5.32; o, 10.00; N, 21.91.

2.1.6.9. 2 - (5-amino-1, 3, 4-thiadiazol – 2 - yl) methyl) – 6 – p – tolyl - 4, 5-dihydropyridazin-3(2H)-one (3e)

Brown powder; mp 90°C; Rf -0.94; IR (KBr) Cm^{-1}: 2925 (Ali C-H), 3029(Aro C-H), 1682 (Aro C=O), 1573 (Ar C=C), 3352 (Pri NH_2), 613 (C-S-C stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 7.85 (d, 1H, Ar-H, J=5(1.5 Hz), 2(7.5Hz), 7.30 (d, 1H, Ar-H, J=3(7.5Hz), 6(1.5 Hz), 2.41(s,3H,CH_3), 2.64(t,2H,CH_2 J=12(7.1 Hz), 3.21(t,2H,CH_2 J=11 (7.1 Hz), 2.57 (s,2H,CH_2),7.98 (s,2H,NH_2); ^{13}C NMR ($CDCl_3$): δ 127.0-140.7 (6C, Aryl), 21.3 (1C, CH_2-CH_3), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 162.4(1C, Pyridazinone and amide), 146.5 (1C, imine), 51.0 (1C, CH_2-N), 168.0 (1C, Thiadiazole, C-S), 161.6 (1C, Thiadiazole, C-NH_2). ms: m/z 301.10 (M^+), Anal. Calcd. For $C_{14}H_{17}N_5OS$: C,

55.80; H, 5.02; o, 5.31; N, 23.24 Found: C, 55.75; H, 5.00; o, 5.28; N, 23.20.

2.1.6.10. 6-oxo-3p-(Aroyl)-5, 6-dihydro-4H-pyridazine-1yl)-acetic acid ethyl ester (4c)

Yellow power; mp 155°C; Rf -0.65; IR (KBr) cm^{-1}: 2929 (Ali C-H), 3066 (Aro C-H), 1595(Aro C-N), 1665 (Aro C=O),1205 (ester C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 2.44 (2H,t,CH_2, J=12(7.1), 2.92 (2H,t,CH_2, J=11(7.1), 7.75 (2H,d,H-2',6, J=2(7.5), 7.40 (2H,d,H-3',5, J=3(7.5), 3.5 (2H,m), 1.9 (3H,t); ^{13}C NMR ($CDCl_3$): δ 128.2-136.4 (6C, Aryl), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 167.7(1C, Pyridazinone and amide), 146.5 146.5 (1C, imine), 52.3 (1C, CH_2-C(=O)-O), 61.0 (1C, CH_2-O-C=O), 14.1 (aliphatic-C),169.5 (1C, Carboxyl). ms: m/z 263.1 (M^+), Anal. Calcd. For $C_{14}H_{16}$ N_2O_3: C, 64.60; H, 6.20; o, 18.44; N, 10.76 Found: C, 64.60; H, 6.20; o, 18.44; N, 10.76.

2.1.6.11. 2-(2-(6-oxo-3-phenyl-5, 6-dihydropyridazine-1(4H) yl) acetyl) hydrazine carbothioamide (4d)

White powder; mp 115-122°C; Rf -0.74; IR (KBr) cm^{-1}: 2944 (ali-CH_2),3098(Aro-CH), 1676 (Ar C=O), 1618 (Ar C=N), 3456 (Pri NH_2), 3303 (Sec amide N-H Str), 1676 1676 (C=O stre in amide), 1492 amide), 1492 (N-H bending in the amide); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 7.36 (t, 1H, Ar-H-2'6 J=5(7.5 Hz), 1(1.5Hz, 2 (1.5 Hz), 7.70 (d,1H, Ar-H-3'5, J=2(7.5Hz), 5 (7.5Hz,1(1.5Hz), 2.43 (t,2H,CH_2J=12(7.1Hz), 2.92 (t,2H,CH_2J=11(7.1Hz), 3.5 (s,2H,CH_2), 8.15(s,2H,NH_2), 3.38 (s,2H,CH_2), 2.50 (s,NH); ^{13}C NMR ($CDCl_3$): δ 128.2-136.4 (6C, Aryl), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 167.7 (1C, Pyridazinone and amide), 146.5 146.5 (1C, imine), 57.6 (1C, CH_2-C(=O)-N-N), 170.3 (1C, amide), 182.5 (1C, Thioamide). ms: m/z 305.1 (M^+), Anal. Calcd. For $C_{13}H_{15}$ N_5O_2S: C, 51.13; H, 4.95; o, 10.50; N, 12.94 Found: C, 51.10; H, 4.92; o, 10.49; N, 12.93.

2.1.6.12. 2-(5-amino-1, 3, 4-thiadiazol-2-yl) methyl)-6-phenyl-4,5-dihydropyridazin-3(2H)-one (4e)

Brown powder; mp 98-100°C; Rf -0.89; IR (KBr) Cm^{-1}: 2924 (Ali C-H), 3028 (Aro C-H), 1683 (Aro C=O), 1594 (Ar C=C), 3350 (Pri NH_2), 614 (C-S-C stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 7.98 (t, 1H, Ar-H-3'5 J=6(7.5 Hz),3(1.5Hz, 1 (1.5 Hz),),7.52(t,1H, Ar-H-4 J=2(7.5 Hz),5(1.5Hz, 1(1.5 Hz),),7.65(d,1H, Ar-H-2'6) 3.24(t, 2H, CH2 J=12(7.1 Hz),2.63(t, 2H, CH2 J=11(7.1 Hz),3.63(2H,s,CH_2),2.5(2H,s,NH_2); ^{13}C NMR ($CDCl_3$): δ 128.2-136.4 (6C, Aryl), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 167.7(1C, Pyridazinone and amide),146.5 146.5 (1C, imine), 51.0 (1C, CH_2-N), 168.0 (1C, Thiadiazole, C-S), 161.8 (1C, Thiadiazole, C-NH_2). ms: m/z 289.0 (M^+), Anal. Calcd. For $C_{13}H_{15}$ N_5OS: C, 54.34; H, 4.56; o, 5.57; N, 24.37 Found: C, 54.31; H, 4.54; o, 5.54; N, 24.35.

2.1.6.13. 6-oxo-3p-(chloro)-5, 6-dihydro-4H-pyridazine-1yl)-acetic acid ethyl ester (5c)

Yellow power; mp 170°C; Rf -0.71; IR (KBr) cm^{-1}: 2935 (ali-CH_3), 3105 (Aro-CH), 1677(C=O aromatic ketone), 1749 (ester ketone), 1305, 1279 (ester C-O), 2979, 3106 (Ali ester stretch), 708,742 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 2.43 (2H,t,CH_2), 2.92 (2H,t,CH_2), 7.98 (2H,d,H-2',6), 7.52 (2H,d,H-3',5), 3.9 (2H,s), 3.7 (2H,m), 1.23 (3H,t); ^{13}C NMR ($CDCl_3$): δ 128.7-138.7 (6C, Cl-aryl), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 167.7(1C, Pyridazinone and amide), 146.5 (1C, imine), 52.3 (1C, CH_2-C(=O)-O), 61.0 (1C, CH_2-O-C=O), 14.1 (aliphatic-C),169.5 (1C, Carboxyl). ms: m/z 293.3 (M^+), Anal. Calcd. For $C_{14}H_{15}Cl$ N_2O_3: C, 57.05; H, 5.13; o, 16.29; N, 9.50 Found: C, 57.00; H, 5.11; o, 16.20; N, 9.49.

2.1.6.14. 2 - (2- (3- (4 - chlorophenyl) – 6 – oxo -5, 6-dihydropyridazine-1(4H) yl) acetyl) hydrazine carbothioamide (5d)

White powder; mp 170°C; Rf -0.71; IR (KBr) cm^{-1}: 2935 (ali-CH_2), 3109(Aro-C-H), 1492 (Ar C=C) 1679(aromatic C=O), 1612 (C=N), 1748 (amide C=O), 3204 (Amide N-H), 3420(C-NH_2), 708,743 (aromatic C-Cl), 1215, 1279 (C-N-H Strech); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 7.40 (d, 1H, Ar-H, J=3(7.5 Hz), 6(1.5Hz), 7.74 (d, 1H, Ar-H, J=2(7.5Hz), 5 (1.5Hz); ^{13}C NMR ($CDCl_3$): δ 128.7-138.7 (6C, Cl-aryl), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 167.7(1C, Pyridazinone and amide), 146.5 (1C, imine), 57.6 (1C, CH_2-C(=O)-N-N), 170.3 (1C, amide), 182.5 (1C, Thioamide). ms: m/z 339.06 (M^+), Anal. Calcd. For $C_{13}H_{14}Cl$ N_5O_2S: C, 45.94; H, 4.15; o, 9.42; N, 20.61 Found: C, 45.90; H, 4.09; o, 9.30; N, 20.59.

2.1.6.15. 2-(5-amino-1, 3, 4-thiadiazol-2-yl) methyl)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2H)-one (5e)

Brown powder; mp 102-106°C; Rf -0.90; IR (KBr) Cm^{-1}: 2927 (Ali C-H), 3060 (Aro C-H), 1680 (Aro C=O), 1590 (Ar C=C), 3348 (Pri NH_2), 658 (C-S-C stretching); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 7.51 (d, 1H, Ar-H, J=3(7.5 Hz), 6(1.5Hz), 7.98(d,1H, Ar-H, J=2(7.5Hz), 5(1.5 Hz), 3.25(t,2H,CH_2 J=12(7.1 Hz), 2.6(t,2H,CH_2 J=13(7.1 Hz), 2.5(s,2H,CH_2); ^{13}C NMR ($CDCl_3$): δ 128.7-138.7 (6C, Cl-aryl), 35.0 (1C, CH_2-(=O)-N-N), 24.1 (1C, CH_2-C=N), 167.7(1C, Pyridazinone and amide), 146.5 (1C, imine), 51.0 (1C, CH_2-N), 168.0 (1C, Thiadiazole, C-S), 161.8 (1C, Thiadiazole, C-NH_2). ms: *m/z* 321.05 (M^+), *Anal*. Calcd. For $C_{13}H_{14}$ ClN_5OS: C, 48.52; H, 3.76; o, 4.97; N, 21.76 Found: C, 48.50; H, 3.72; o, 4.95; N, 21.72.

2.2. ANTITUBERCULAR ACTIVITY

All the synthesized compounds **(1c-1e, 2c-2e, 3c-3e, 4c-4e, 5c-5e)** were evaluated for antitubercular activity by Serial dilution method and disc Diffusion Method using Middle brook 7H9 medium (broth and agar based) and ATCC 25175 strain of mycobacterium Tuberculosis. [16] Activity of test compounds was determined from the zone of inhibition surrounding the well. The sensitivity

of microorganism to the sample was determined by measuring the zones of inhibition surrounding the well using a millimeter scale. The actual diameter of zone of inhibition was measured including diameter of the well. Compounds effecting <90% inhibition in the primary screen were not evaluated further. The derivatives were initially screened against *Mycobacterium tuberculosis* strain at single concentration of 6.25 µg/ml.[17,18] Compounds demonstrating growth inhibition ≥ 90% in the primary screening were considered active. The antimycobacterial activity data were compared with standard drug Rifampcin at 0.25 µg/ml and INH (MIC= 0.025- 0.05 µg ml^{-1}) concentration which showed 98% inhibition. The active compounds were re-tested by serial dilution at initial concentration of 6.25 µg/ml against *M. tuberculosis* to determine the actual minimum inhibitory concentration (MIC) in Middle brook 7H9 medium. The MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation INH (MIC= 0.025- 0.05 µg ml^{-1}) and RMP (0.025- 0.125 µg ml^{-1}) was used as positive control drugs, results are given in Table 2 and (Fig 1 and 2).

Table 2 Antitubercular activity expressed as MIC (µg/ml) and Zone of inhibition (mm) of derivatives 1c-1e, 2c-2e, 3c-3e, 4c-4e, and 5c-5e.

Diameter of zone of inhibition (mm)		
C Compounds	**MIC[c] (µg/ml)**	**Mean[a] ± S.D[b]**
1c	6.25	16.04±0.52
1d	3.125	18.94±0.51
1e	2.5	20.028±0.86
2c	3.125	15.89±0.55
2d	2.5	19.98±0.66
2e	1.25	21.04±0.86
3c	6.125	12.57±0.56
3d	3.125	13.52±0.59
3e	2.5	14.22±0.31
4c	6.25	4.98 ±0.130
4d	6.25	4.52±0.192
4e	3.125	5.20±0.02
5c	2.5	21.30±1.00
5d	1.25	23.46±0.30
5e	1.25	25.40±0.37
Rifampcine	0.125	28.37±0.16
Isoniazide	0.05	29.15±0.06

a. Mean value of measured diameter of zone of inhibition, b. S.D. denotes the standard deviation, c. MIC denotes minimum inhibitory concentration

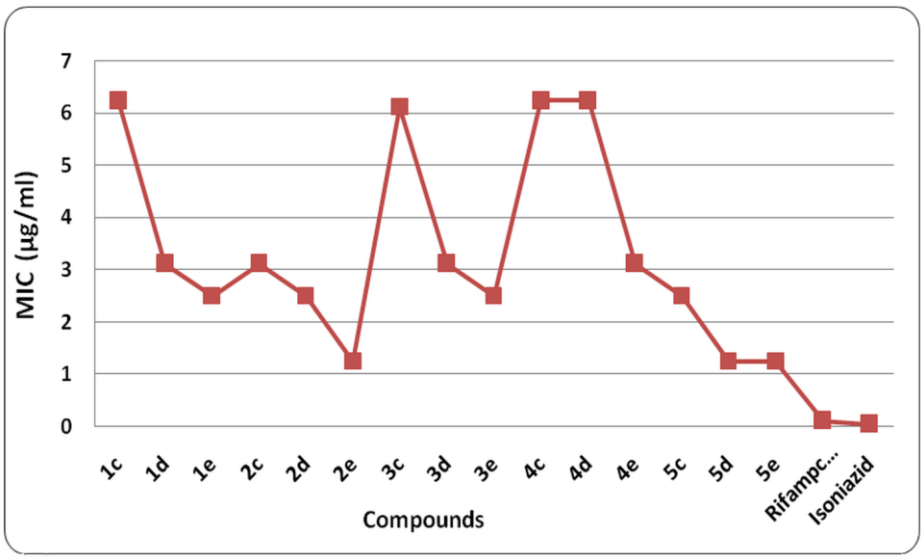


Fig.1 Antitubercular activity minimum inhibitory concentration of derivatives (µg/ml)

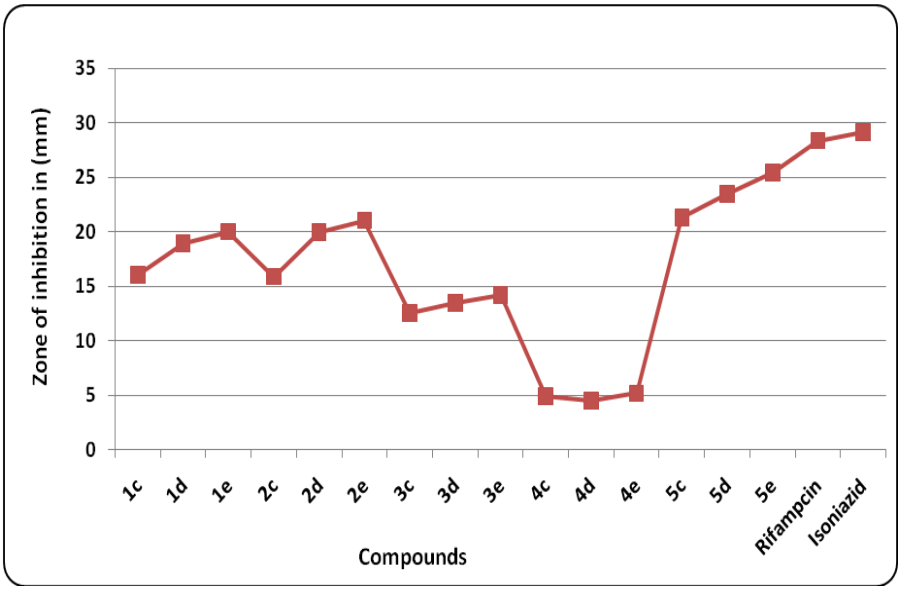


Fig.2 Antitubercular activity diameter of zone of inhibition of the derivatives (mm)

2.3. ANTI-INFLAMMATORY ACTIVITY

2.3.1. Animals

Wistar albino rat of (150-200g) were used for the assessment of anti-inflammatory activity. Ethical clearance and animals for performing the experimental procedure and protocols used in this study were reviewed by the institutional animal ethical committee (IAEC) of the institute with reference no. BU/Pharm/IAEC/11/031 (approved by CPCSEA Regd No. 716/02/a/CPCSEA). Before initiation of the experiment the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature (25±2^{o}C), relative humidity (55-65%) and 12hr dark/light cycle were maintained in the quarantine.

2.3.2. Acute toxicity study (LD50)

Acute toxicity studies [19] were carried on albino rats as per the guidelines (OECD NO: 423) given by the organization for economic co-operation and development. Overnight-fasted wistar albino rats (180 ± 20g) of either sex were used for the study. The animals were divided into five groups of three animals each. The synthesized compounds were administered separately to the all the three animals in each group at starting single dose of 5mg/kg. Animals were observed for the period of 1h, the occasionally for three hours for severity of any toxic sign and mortality. If no mortality is observed at this dose, the same procedure will be repeated for dose level of 50, 400, 2000 mg/kg of synthesized compounds separate newer groups. The LD50 was thus determine, which was selected for the anti inflammatory animals study, the animals were observed up to 7 days after drug administration to find out any delayed mortality.

2.3.3. Carageenan-induced paw edema Model

For the determination of the effect on acute inflammation, the carageenan induced paw edema model. [20] was selected, this model is based on the principle of release of various inflammatory mediators by carageenan subcutaneous injection of carageenan produces inflammation resulting from plasma extravasations, increased tissue water and plasma protein exudation along with neutrophile extravasation, all due to the metabolism of arachidonic acid.[21]

Rats were divided in to 12 groups (n=5). The test compound and indomethacine used as a reference were administered orally respectively at doses of 100 mg/kg and 10 mg/kg as a suspension in 0.2 ml of 0.2 ml of 0.5% CMC Na in carageenan induced paw edema in rats. The control group received only saline water. The dose of 0.1 ml of 1% w/v carageenan was administered in sub plantar region in rats hind paw for causing paw edema. The rats were pre-treated with vehicle, test compounds and indomethacine 1hr before carageenan administration.

2.3.4. Data Analysis

The paw size was measured in mm using plethysmometer at an interval of 1Hr, 2hr, 3hr and 4r after carageenan administration. The percentage inhibition was calculated by following formula **[21]**. Percent edema inhibition = {Vc _Vt/Vc} x100. Where Vc is the mean increase in paw volume in the absence of test compound (control) and Vt is the mean increase of paw volume after treatment with the test compound and standard drug. Results are presented as mean ± S.E.M. (standard error of mean) of five rats and the percentage inhibition in paw edema expressed in Table 3 and 4. Results were expressed as means ± S.E.M. Statistical significance was analyzed by using the one-way analysis of variance followed by Turkey's Multiple Comparison Test where $p < 0.05$ was accepted to be a significant difference (Fig 3 and 4).

Table 3 Anti-inflammatory activity of derivatives 1c-1e, 2c-2e,3c-3e, 4c-4e and 5c-5e				
Compounds	**Change in mean paw volume[a] (ml)**			
	1hr	**2hr**	**3hr**	**4hr**
1c	0.51±0.012	0.55±0.017	0.60±0.014	0.47±0.016
1d	0.53±0.016	0.6±0.014	0.63±0.017	0.54±0.024
1e	0.48±0.016	0.54±0.014	0.55±0.021	0.44±0.021
2c	0.54±0.021	0.54±0.015	0.62±0.016	0.51±0.018
2d	0.53±0.019	0.62±0.014	0.62±0.023	0.57±0.016
2e	0.57±0.020	0.64±0.019	0.70±0.014	0.62±0.023
3c	0.37±0.016	0.45±0.013	0.54±0.020	0.44±0.021
3d	0.50±0.012	0.54±0.021	0.62±0.017	0.52±0.023
3e	0.43±0.016	0.51±0.013	0.52±0.017	0.51±0.021
4c	0.54±0.010	0.64±0.023	0.73±0.014	0.63±0.023
4d	0.56±0.012	0.64±0.013	0.71±0.021	0.65±0.021
4e	0.56±0.029	0.63±0.016	0.71±0.021	0.66±0.022
5c	0.30±0.014	0.37±0.016	0.44±0.02	0.36±0.018
5d	0.33±0.018	0.37±0.010	0.43±0.011	0.33±0.021
5e	0.31±0.018	0.36±0.010	0.37±0.016	0.32±0.020
Indomethacene	0.26±0.016	0.34±0.010	0.37±0.010	0.32±0.32
Solvent control	0.65±0.007	0.74±0.027	0.82±0.005	0.78±0.006
a. Change in mean paw volume (ml) ± Standard deviation, P < 0.05				

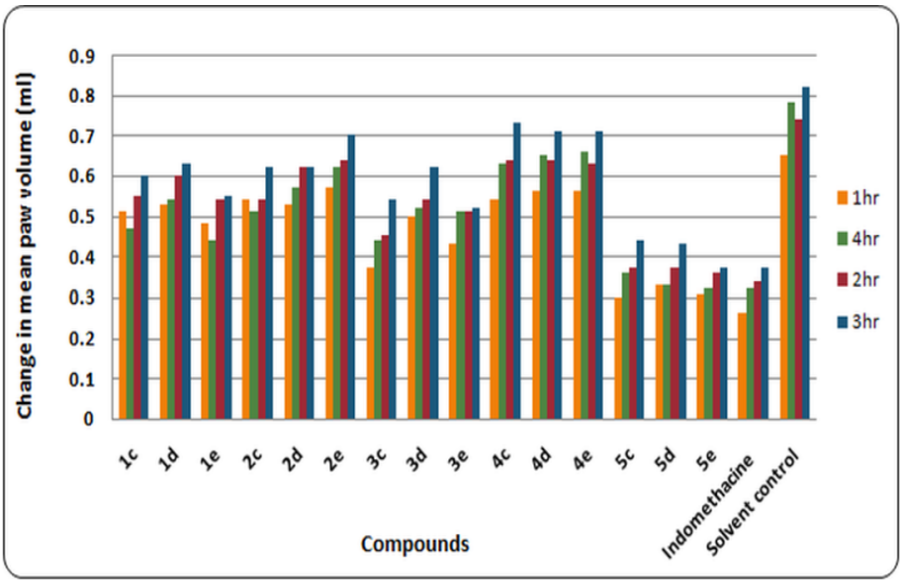


Fig.3 Anti-inflammatory activity change in mean paw volume of the derivatives (ml)

Table 4 Anti-inflammatory activity of derivatives 1c-1e, 2c-2e, 3c-3e, 4c-4e, and 5c-5e				
Compounds	**Percentage inhibition of carrageenan-induced paw edema[a] (%)**			
	1hr	**2hr**	**3hr**	**4hr**
1c	21.53	25.67	26.82	39.74
1d	18.46	18.91	23.17	30.76
1e	26.15	27.02	32.92	43.58
2c	16.92	27.02	24.39	34.61
2d	18.40	16.21	24.39	26.92
2e	12.30	13.51	14.63	20.51
3c	43.07	39.18	34.14	43.58
3d	23.07	2 7.02	24.39	33..33
3e	33.85	31.08	36.58	34.61
4c	16.92	13.51	10.97	19.23
4d	13.84	13.51	13.97	16.66
4e	13.84	14.86	13.97	15.3

5c	53.84	50.00	46.34	53.84
5d	49.23	50.00	47.56	57.64
5e	52.30	51.00	54.87	58.97
Indomethacene	60	54.05	54.87	58.97

a Percentage inhibition of carageenan induced paw edema (%)

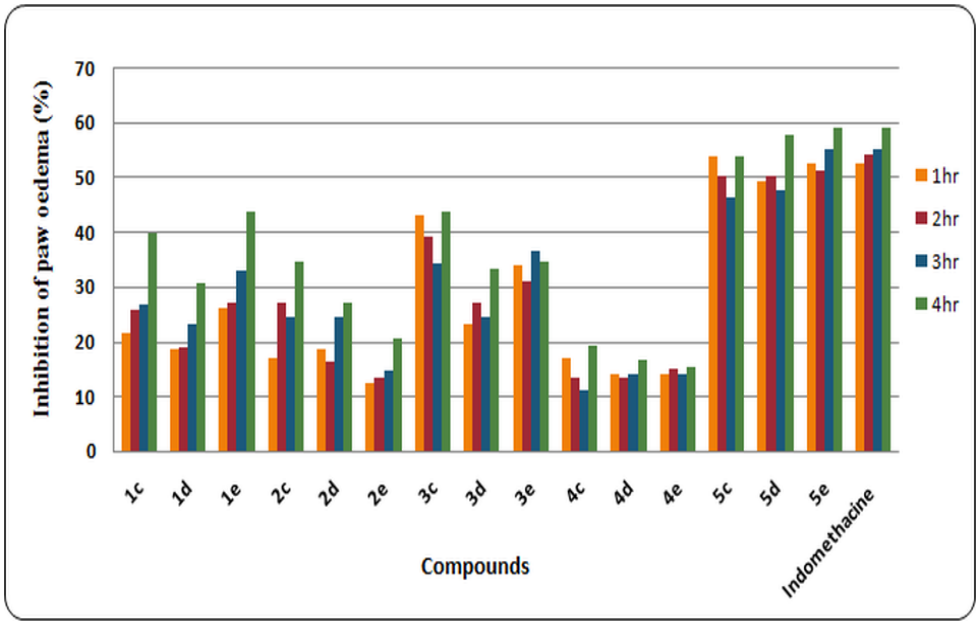


Fig.4 Anti-inflammatory activity percentage inhibition of Carrageenan induced paw edema (%)

3. RESULTS AND DISCUSSION

3.1. Chemistry

The target compounds 4a–e and 6a–g, and their intermediates (**1a–5a, 1b-5b & 1c-5c**) and (**1d-5d, 1e-5e**) is depicted in Schemes 1 and 2. β-aroyl prop ionic acid **1a-5a** was synthesized in excellent yield by the friedal craft acylation in presence of succinic anhydride and alluminium chloride by literature methods. [22] The 6 - (substituted aroyl) 2,3,4,5 tetra hydro- pyridazine **(1b-5b)** were synthesized by cyclisation of β-aroyl prop ionic acid with hydrazine hydrate. [15] The pyridazinone on treatment with ethyl chloro acetate form 6-oxo-3p-(anisoyl)-5,6-dihydro-4H-pyridazine-1yl)-acetic acid ethyl ester (**1c-5c)**. The acetic acid ethyl ester derivatives upon stirring with Hydrazine hydrate produced **1d-4d**. Dehydrative annulations of these hydrazine carbothioamide with conc. Sulphuric acid produced the final compounds (**1e-5e)**. The purities of the synthesized compounds were checked using thin layer chromatography in three mobile phase system. All structures were analyzed by using IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. Elemental analysis of all derivatives was also in complete agreement with the proposed structures. The IR Spectra of all synthesized derivatives were recorded in terms of wave numbers (cm^{-1}).

In the IR spectra broad OH peak is observed in the O-H stretching (1390) for β-aroyl propionic acid**.** These bands were not observed for **1b-5b** only N-H stretching in the range (3303.83-3118), aromatic C-N stretching in the range (1540-1680), and absorption band of C=O characteristic of aromatic ketone were observed in the range of (1600-1775). Which was further confirmed by the appearance of singlet at δ =7.9 (NH, 1H) in the ^{1}HNMR spectra of this compounds.

In the third step (**1c-5c**) appearance of >C=O (Ester) stretching in the range (1750-1735) and doublet for CH_3 and CH_2 at (3099) cm^{-1} indicate the attachment of ester group at position N^1 of pyridazinone. This was further confirmed by the appearance of singlet at δ 4.46 in the ^{1}HNMR Spectra of this compound due to CH_2 in the (**1c-5c**).

The synthesis of (**1d-5d**) have been confirmed by the shifting of C=O (Ester) stretching to the >C=O (amide) stretching range (1700-1640) cm^{-1} and emergence of C=S Stretching at (622 and 722) cm^{-1} and N-H stretching for primary amine in the range (3300-3370) cm^{-1} and sec. amide N-H stretching in the range (3500-3400) cm^{-1}. Further confirmation have been done by the appearance of singlet for two protons at δ= 4.09 for N-CH_2 in their ^{1}HNMR Spectra.

Synthesis of (**1e-5e**) have been confirmed by the disappearance of peak for C=O (either Ester or amide) in the IR spectra of the compound and appearance of C-S-C stretching in the range (600-700) cm^{-1}. Further the synthesis was confirmed by the ^{1}HNMR spectra showed two triplet at 2.92 and 2.43 respectively for two aromatic- CH_2 stretching and two doublet at 7.28 and 7.00 for 4 aromatic protons and a singlet of 2 proton for N-CH_2 only, while no other signal for proton peak were

observed. For ^{13}C NMR, the thioamide carbon of (**1d-5d)** was elucidated at 182–183 ppm while the thiadiazole, (C-NH_2) of (**1e-5e)** was elucidated at 161.8-162 ppm. ^{13}C NMR spectrum indicated the appearance of signals for the N-CH_2, aliphatic-C, carboxyl at 52.3 (1C, CH_2-C (=O)-O), 14.5, 169.5 respectively.
The mass spectrum of compound **5d** and **5e** at m/z 339.06 (M^+), m/z 321.05 (M^+), respectively, and mass spectrum of all other derivatives were given in the experimental section as expected.

3.2. Antitubercular activity

All the synthesized compounds (**1c-1e, 2c-2e, 3c-3e, 4c-4e,** and **5c-5e**) tested against disc diffusion method using middle brook 7H9 medium (broth and agar based) and ATCC 25175 strain of *Mycobacterium Tuberculosis*. Compound (**2e, 5d** & **5e)** showed maximum anti-TB activity with a MIC of 1.25 µg/ml. Compounds (**4c-4e)** found very weak anti-TB agents when compared with the standard drug INH (MIC= 0.025- 0.05 µg ml^{-1}) and RMP (0.025- 0.125 µg ml^{-1}).

Literature survey indicates that the pyridazinone derivatives represent a great important biologically active compounds have acquired more concernment in recent decades for its wide variety of biological activity. The present work describe the anti-inflammatory and anti-TB activity of 4,5-dihydropyridazin-3(2H)-on. In antitubercular activity, compounds with 4-ethyl phenyl substitution (**2e**) and chloro phenyl substitution (**5d, 5e**) at p-position of pyridazinone nuclei showed good antitubercular activity with MIC value of 1.25 µg/ml then other aryl substitution like 4-methoxy phenyl **1d** &**1e,** 4-methyl phenyl **3d** & **3e** with MIC value of 3.125 & 2.5 µg/ml. Therefore, it can be concluded that compound 2-(2-(3-(4-chlorophenyl)-6-oxo-5,6-dihydropyridazine-1(4H)yl)acetyl)hydrazine carbothioamide and 2-(5-amino-1,3,4-thiadiazol-2-yl) methyl)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2H)-one would constitute a useful model for further investigation in the development of new class of antitubercular agents.

3.3. Anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds was studied using a carageenan-induced hind paw edema model in rat. Test compound and indomethacine used as a reference were administered orally at the doses of 100 mg/Kg and 10 mg/Kg respectively. Paw volume was measured immediately after injection and after every one hour. Normal saline was used as a solvent control; results of anti-inflammatory activity are shown in Table 3 and 4.

Carrageenan induced paw edema is a useful model in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. Edema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin, and bradykinin affecting vascular permeability. The inflammatory edema reached its maximum level at the third hour and after that it started declining.

In our study, test drug and indomethacin showed anti-inflammatory effects in carrageenan-induced rat paw edema. All pyridazinone derivatives bearing acetic acid hydrazide moieties (**1c, 1e, 3c**) showed remarkably potent anti-inflammatory activity, especially after 3 and 4hr the drug was administered, but compounds containing P-chlorophenyl group substitution in aromatic ring in the structure (**5c, 5d** & **5e**) had the most potent anti-inflammatory agents.

3.4. Possible Mechanisms

In addition to the development of new and effective antitubercular agents against multidrug resistant bacteria, recently attention has focused on the treatment of inflammation. Most currently used nonsteroidal anti-inflammatory drugs (NASIDs) have limitations for therapeutic use since they cause gastrointestinal and renal side effects that are inspirable from their pharmacological activities. Therefore, the synthesis of new compounds devoid of such side effects has become an important goal for medicinal chemists in recent years.[23] Pyridazinones have been a subject of intensive research owing to their wide spectrum of pharmacological activities.

Carrageenan induced paw edema model that are routinely used by most anti-inflammatory drugs discovery programs. In the present study all compounds were showed anti-inflammatory activities against carrageenan induced paw edema model.

Vinegar et al. claimed that the inhibitory effects of agents that act on the first stage of carrageenan-induced hind paw inflammation are attributable to inhibition of the release of chemical mediators such as histamine and serotonin. They also claimed that the second stage of the hind paw edema may be related to arachidonic acid metabolites since it is inhibited by aspirin and other arachidonate cyclooxygenase inhibitors. [24]

All pyridazinone derivatives bearing hydrazide moiety (**1c, 1e, 3c**) showed remarkable anti-inflammatory activity especially 3-4 hr after the drug was administered, which suggested that compounds **1c, 1e, 3c & 3e** may inhibit arachidonic acid metabolites. Compounds **5c, 5d** and **5e** bearing chloro group at the Para position of the aromatic ring in the structure had the most potent anti-inflammatory activity throughout the study. These derivatives are most effective in the early hyperemia, 0-2 hrs as well as in the second stage 3-4 hr. which suggested that compounds **5c-5e** might have effect in both stages of inflammation.

A further investigation is needed to evaluate the effects of the anti-inflammatory activity in several other animal models for the estimation of neurotransmitters to speculate about the exact possible mechanism of these anti-inflammatory compounds.

3.5. CONCLUSION

Synthesis of the target compounds were carried out according to the sequence of reactions outlined in scheme. Derivatives are synthesized starting with the friedal craft acylation of hydrocarbon to get appropriate β-(substituted aroyl)-propionic acid. Cyclization of the acids with hydrazine hydrate to form respective pyridazinone. pyridazinone on nucleophillic substitution with ethyl chloro acetate (by SN^2 Mechanism) produces ethyl N^1- pyridazino acetate, which was stirred with thiosemicarbazide in absolute ethanol to obtain N^1-pyridazinoactyl thiosemicarbazide **(d)**. Compound 1d on dehydrative annulations with conc. H_2SO_4 Gave 2-(5-amino-1,3,4-thiadiazol-2-yl)methyl)-6-(4-methoxyphenyl)-4,5-dihydropyridazin-3(2H)-one (**e**). All the newly synthesized compounds were screened for antitubercular activity. Compounds tested against disc diffusion method using middle brook 7H9 medium (broth and agar based) and ATCC 25175 strain of *Mycobacterium Tuberculosis*. Compound (**2e, 5d** & **5e)** showed maximum anti-TB activity with a MIC of 1.25 µg/ml. Compounds (**4a**-**4e)** found very weak anti-TB agents when compared with the standard drug INH (MIC= 0.025- 0.05 µg ml^{-1}) and RMP (0.025- 0.125 µg ml^{-1}). Compounds **5c, 5d** and **5e** bearing chloro group at the Para position of the aromatic ring in the structure had the most potent anti-inflammatory activity throughout the study. These derivatives are most effective in the early hyperemia, 0-2 hrs as well as in the second stage 3-4 hr. which suggested that compounds **5c**-**5e** might have effect in both stages of inflammation.

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