Med Chem Res
DOI 10.1007/s00044-013-0618-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Efficient synthesis, anticonvulsant and muscle relaxant activities of new 2-((5-amino-1,3,4-thiadiazol-2-yl)methyl)-6-phenyl-4,5-dihydropyridazin-3(*2H*)-one derivatives

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Received: 10 January 2013 / Accepted: 4 May 2013

Abstract A series of 2-(2-(3-(4-chlorophenyl)-6-oxo-5,6-dihydropyridazin-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 derivatives were synthesized, characterized, and evaluated for anticonvulsant activity and muscle relaxant activity. The synthesized compounds **5d** (82.75 %) and **5e** (85.44 %) showed promising anticonvulsant activity by protection against tonic hind limb extensor phase in maximal electroshock model (MES) at (50 mg/kg) compared to standard drug phenytoin and also compounds **5d** (82.75 %), and **5e** (85.44 %) showed significant anticonvulsant activity by protection against pentylenetetrazole-induced generalized convulsions in pentylenetetrazole model (PTZ) at (100 mg/kg) compared to standard drug diazepam. On the other hand, compound **5e** showed significant muscle relaxant activity (84.57 %) by rotarod and traction test model comparing with diazepam as a standard drug.

Keywords Anticonvulsant · Convulsion · Aryl pyridazinone · MES model · PTZ model · Muscle relaxant activity

Introduction

Epilepsy is a major neurological disorder and up to 5 % of the world population develops epilepsy in their lifetime (Sander and Shorvon, 1996; Smith and Bleck, 1991; Samrjn *et al*., 1997), there is a continuing demand for new anticonvulsant agents as it has not been possible to control every kind of seizure with the currently available antiepileptic drugs. About one third of patients do not respond well to current multiple drugs therapy (Schmidt and Löscher, 2005; Kwan and Brodie, 2000), studies reveal that 63 % of patients diagnosed and treated were seizure-free and more than 50 % of epilepsy patients have experienced unwanted side effects (Dichter and Brodie, 1996; Brodie and Dichter, 1996; Kwan and Brodie, 2004); phenobarbital (Hardman *et al*., 1996) and mephobarbital are well-known barbituric acid derivatives which are used for the treatment of epilepsy. These antiepileptic drugs are very effective in controlling the seizure; however, they are associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, substituted heterocyclic/substituted aryl systematic pyridazinone nucleus remarkably increases the antiepileptic activity. Pyridazinone derivatives 3-oxo-5-substituted benzylidene-6-methyl-(*4H*)-2-pyridazinylacetamides and 2-pyridazinylacetyl hydrazides and a considerable number of pyridazin-3(*2H*)-ones are reported for their pronounced anticonvulsant activity (Rubut *et al*., 1990; Asif, 2010; Pooja *et al*., 2009; Siddiqui *et al*., 2006; Oya *et al*., 2004;

Electronic supplementary material The online version of this article (doi:10.1007/s00044-013-0618-0) contains supplementary material, which is available to authorized users.

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Published online: 21 May 2013

Sivakumar *et al*., 2003; Edith *et al*., 2002; Xu *et al*., 1991a, b; Hallot *et al*., 1986; Perio *et al*., 1986), in hope of getting synergistic response of pyridazino methyl 1,3,4-thiadiazole nucleus itself, the present paper reports on the synthesis, anticonvulsant, and muscle relaxant activity of 2-[(5-amino-1,3,4-thiadiazole-2yl)methyl]-6-(4-methoxyphenyl)-4,5-dihydropyridazin-3(*2H*)-one derivatives. The compounds designed so were found to possess much significant anticonvulsant activity with significant reduction in toxicity.

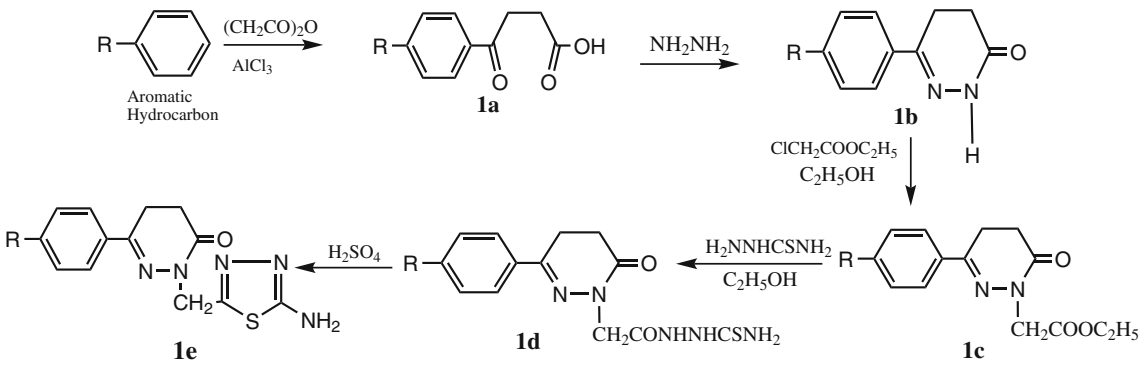
Results and discussion

Chemistry

Synthesis of the target compounds was carried out according to the sequence of reaction outlined in Scheme 1. The key intermediate β-aroyl propionic acid **1a–5a** was synthesized in excellent yield by the friedal craft acylation in the presence of succinic anhydride and aluminum chloride (Khan and Siddiqui, 2000), which on cyclization with hydrazine hydrate form pyridazinone **1b–5b** (Siddiqui and Dogra, 2001; Siddiqui *et al*., 2004), this pyridazinone on treatment with ethyl chloro acetate form 6-oxo-3p-(anisoyl)-5,6-dihydro-*4H*-pyridazine-1yl)-acetic acid ethyl ester **1c**. Then these acetic acid ethyl ester products upon stirring with thiosemicarbazide **1d–5d** were obtained. Then these hydrazinecarbothioamide on dehydrative annulations with conc. sulfuric acid produced 2-[(5-amino-1,3,4-thiadiazole-2yl)methyl]-6-(substituted aroyl)-4,5-dihydropyridazin 3(*2H*)-one derivatives **1e–5e.** The purities of the synthesized compounds were checked using thin layer chromatography (TLC) in three mobile phase system. Each of the synthesized compounds gave isolated spot at different distance from its starting compound. The structures of newly synthesized compounds were identified using infrared (IR), proton nuclear magnetic resonance (^{1}HNMR), and mass spectral data. Elemental analyses of all synthesized compounds for C, H, N, and S were within the theoretical values.

The IR Spectra of all synthesized pyridazinomethyl-1,3,4-thiadiazole derivatives were recorded in terms of wave numbers (cm^{-1}). In the IR spectra broad OH peak is observed in the O–H stretching (1,390) for **1a–5a.** As a consequence of reaction with hydrazine hydrate these bands were not observed for **1b–5b.** Only N–H stretching in the range (3,303.83–3,118), aromatic C–N stretching in the range (1,540–1,680), and absorption band of C=O characteristic of aromatic ketone were observed in the range of (1,600–1,775). The absorption bands of N–H stretching were not observed in compounds **1c–5c,** ester C=O stretching in the range (1750–1740 cm^{-1}) and C–O stretching at (1240) were observed, respectively, in compounds **1c–5c**. As expected on reaction with thiosemicarbazide NH_2 peak in the range (3,400–3,150), C=S absorption band in the range (600–700) and amide absorption band in the range (1,700–1,640) were observed in **1d–5d** derivatives. In the **1e–5e** C–N stretching in the range (1,540–1,680) and C–S–C absorption band were observed in the range of (700–600).

The ^{1}H NMR Spectra of **1a–5a** displayed the OH at 11.00–11.92 ppm; in the **1b–5b** the signal of these protons was not observed in this; N–H peak wase observed at 7.00–8.00; the signal of N–H was not observed in **1c–5c**; methyl and ethyl proton of the ester group showed 3.57–4.16 and 10.10–1.29 in the **1d–5d**; methyl and ethyl proton peak are not observed. NH_2 signal and amide proton peak are observed at 8.00 and 8.56, respectively. In the **1e–5e** NH_2 signal is observed at 6.00–6.99. The other



Scheme	R
1	OCH_3
2	C_2H_5
3	CH_3
4	H
5	Cl

Scheme 1 Synthesis of 2-(5-amino-1,3,4 thiadiazole-2yl)methyl)-6phenyl-4,5-dihydropyridazine-3 (2H)-one derivatives

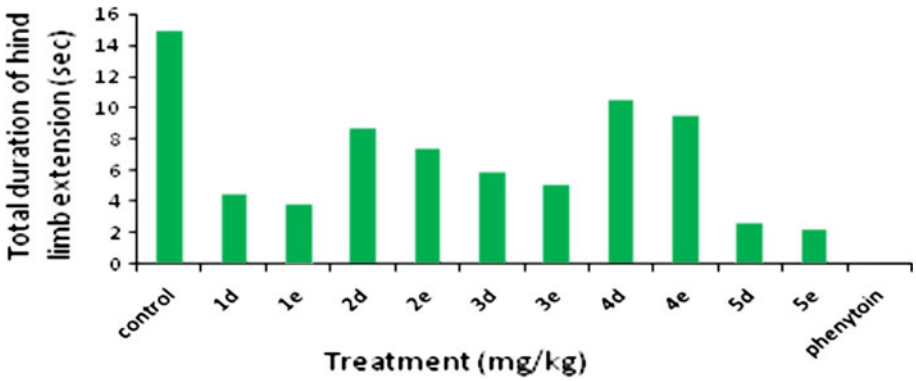


Fig. 1 Comparison of anticonvulsant activity of test compound by MES model

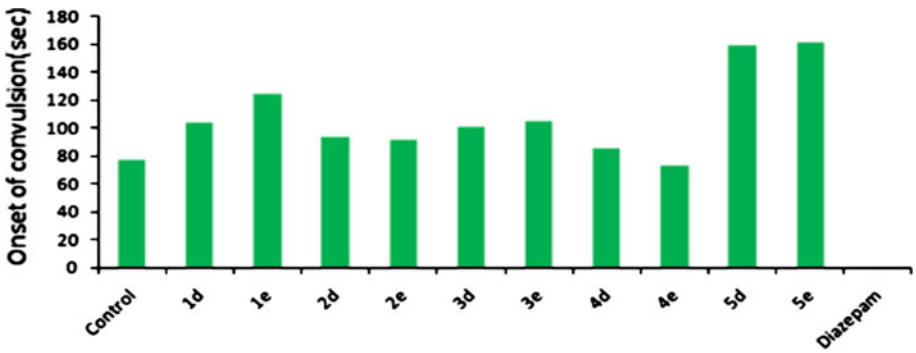


Fig. 2 Comparison of anticonvulsant activity of test compound by PTZ model

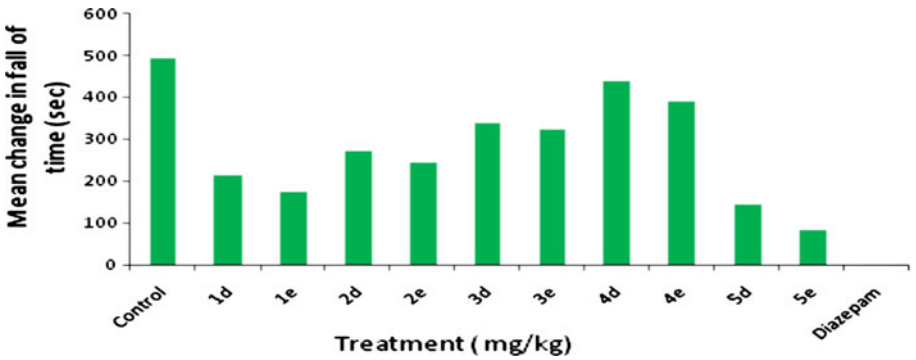


Fig. 3 Comparison of muscle relaxant activity of test compounds using rotarod test model

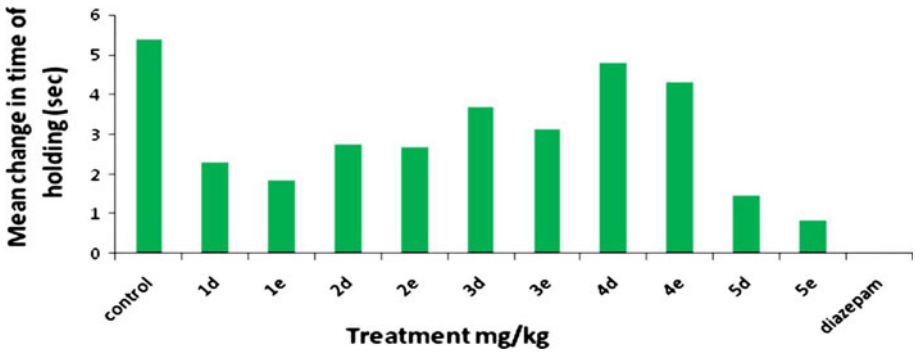


Fig. 4 Comparison of muscle relaxant activity of test compounds using traction test model

protons of the compounds were as expected. The mass spectrum of compounds **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. Standard drug used for MES model was phenytoin (Fig. 1), pentylenetetrazole (PTZ) model was diazepam (Fig. 2), muscle relaxant activity by rotarod test was diazepam (Fig. 3), and traction test (Fig. 4).

Table 1 The effect of compounds on seizure in tonic hind limb extensor phase by MES Model

S. no.	Compound (25 mg/kg)	R	Duration of HLTE ± SEM	% Inhibition
1.	Control	–	14.906 ± 0.67998*	–
2.	Phenytoin	–	00 ± 00	100
3.	**1d**	OCH_3	4.438 ± 0.12487*	70.24
4.	**1e**	OCH_3	3.844 ± 0.04163*	74.2
5.	**2d**	C_2H_5	8.66 ± 0.1144*	43.24
6.	**2e**	C_2H_5	7.396 ± 0.00263*	50.38
7.	**3d**	CH_3	5.788 ± 0.25932*	61.43
8.	**3e**	CH_3	5.024 ± 0.19198*	66.29
9.	**4d**	–	10.496 ± 0.06083*	29.585
10.	**4e**	–	9.42 ± 0.1034*	36.80
11.	**5d**	Cl	2.57 ± 0.17745*	82.75
12.	**5e**	Cl	2.17 ± 0.03245*	85.44

Values are expressed as mean ± SEM; statistical significant test for comparison was done by ANOVA; followed by chi-square test ($n = 5$)

* $P < 0.05$ when compared to control group

Pharmacology

Anticonvulsant activity (MES model)

Maximal electroshock and pentylenetetrazole induced model was used for the evaluation of anticonvulsant study of the test compounds. For the MES model the results are summarized in Table 1. Compound 2-(2-(3-(4-chlorophenyl)-6-oxo-5,6-dihydropyridazin-1(*4H*)yl)acetyl)hydrazine carbothioamide **5d** and 2-((5-amino-1,3,4-thiadiazol-2-yl)methyl)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(*2H*)-one **5e** showed excellent protection against HLTE phase (82.75 and 85.44 % protection, respectively), whereas compounds **4d**, **4e**, **2d**, and **2e** were found to have the least protection against Hind limb tonic extensor phase. Compounds **1d, 1e, 3d**, and **3e** also showed good anticonvulsant activity by protection against HLTE phase with a percentage of 70.24, 74.2, 61.43, and 66.29 %, respectively, in comparison to phenytoin.

Anticonvulsant activity (PTZ model)

Pentylenetetrazole model for evaluation of anticonvulsant activity, according to this seizure latency was defined as the time elapsed from the injection of pentylenetetrazole to the first two myoclonic jerk of the forelimbs. This has been concluded to be the first sign of the beginning of the seizure activity. Animals devoid of generalized convulsion were considered to be protected and results were represented as protection (%). These results of the test compound (100 mg/kg) were compared with that of diazepam (4 mg/Kg) as a standard for pentylenetetrazole-induced seizures. Onset of seizure was assessed by the chi-square test and expressed as mean ± SEM. The results are summarized in Table 2. In the PTZ model again **5d** and **5e** showed maximum protection (81.99 and 84.91, respectively) and rest of the compounds were protective in delaying of the onset of the first myoclonic twitches. So it may be considered that compounds **5d** and **5e** may be considered promising for the development of new anticonvulsant agents.

Muscle relaxant activity

The effect on motor co-ordination was assessed using rotarod apparatus and traction test results are shown in Table 3. The rotarod test has been performed to detect the motor deficit in mice. The time each animal was able to maintain its balance walking on top of the rod was measured. Compounds **1d, 1e, 2e**, and **3e** showed good muscle relaxant activity (57.43, 65.79, 50.92, and 42.0 %, respectively) in comparison to standard diazepam (4 mg/kg), whereas compound **5d** and **5e** showed maximum muscle relaxant activity (73.23, and 84.57 %, respectively). Rest of the compounds showed the least muscle relaxant activity compared with the standard drug diazepam (100 %).

Experimental section

All the solvents and reagents used were of laboratory grade (LR). Melting points of all synthesized compounds were determined using open capillary tube and were uncorrected. The purity of the compounds and completion of the reaction was monitored from time to time by TLC using E-Merk 0.25 mm silica gel plates. Solvent system used for running TLC Plates was toluene, ethyl formate, and formic acid in the ratio of 5:4:1. Visualization was accomplished with UV light (256 nm) and iodine chamber. Synthesized compounds were purified by column chromatography. All the solvents were dried by appropriate drying agents before use. The IR spectra (KBr, in cm^{-1}) were recorded by using potassium bromide (KBr) pellet technique on Schimadzu spectrometer. The ^1H NMR spectra (chemical shift in δ ppm) were recorded in deuterated chloroform using tetramethylsilane (TMS) as an internal reference standard on Bruker Avance II 400 NMR spectrometer.

General procedure for preparing the compounds in Scheme 1 from (**1a–1e**). All the remaining compounds were synthesized by analogous method.

β-Anisoyl-propionic acid (**1a**)

A mixture of anisole (30 ml) and anhydrous aluminum chloride (0.15 mol) was suspended in a two-necked flask and this mixture was refluxed under anhydrous condition

Table 2 The effect of compounds on onset and duration of seizure and protection against pentylenetetrazole-induced generalized convulsion

S. no.	R	Onset of seizure (s)	Duration of clonic seizure (s)	Seizure protection (%)
1d	OCH_3	103.8 ± 2.24*	61 ± 1.702*	69.65
1e	OCH_3	124.4 ± 1.50*	51.2 ± 0.8*	74.2
2d	C_2H_5	93.4 ± 1.32*	112 ± 3.30*	44.27
2e	C_2H_5	91.6 ± 1.74*	99.8 ± 0.8*	50.34
3d	CH_3	101 ± 1.51*	95.4 ± 3.86*	62.48
3e	CH_3	105.8 ± 2.28*	70.2 ± 0.66*	65.07
4d	–	85.8 ± 3.02*	156 ± 4.0*	22.2
4e	–	73 ± 0.70*	125.4 ± 1.6*	38.3
5d	Cl	159 ± 3.67*	38.2 ± 1.06*	81.99
5e	Cl	161.4 ± 1.77*	30.2 ± 0.66*	84.97
Control	–	76.8 ± 1.06*	201 ± 1.06*	00
Diazepam	–	00 ± 00	00 ± 00	100

Values are expressed as mean ± SEM; statistical significant test for comparison was done by ANOVA; followed by chi-square test ($n = 5$)

* $P < 0.05$ when compared to control group

Table 3 The effect of compounds on muscle relaxant activity by rotarod test and traction test

S. no.	Test compounds	R	Rotarod test fall of time in (s)	Decrease in time (%)	Traction test time of holding (s)	Failure to put hind limb (%)
1.	**1d**	OCH_3	105.404 ± 4.54*	57.05	2.296 ± 0.03*	57.43
2.	**1e**	OCH_3	85.99 ± 2.54*	64.96	17.70 ± 16.05*	65.79
3.	**2d**	C_2H_5	135.34 ± 1.78*	44.88	2.954 ± 0.02*	48.88
4.	**2e**	C_2H_5	120.64 ± 3.25*	50.92	2.682 ± 0.05*	50.92
5.	**3d**	CH_3	168.9 ± 4.10*	31.59	3.686 ± 0.10*	31.59
6.	**3e**	CH_3	160.53 ± 3.21*	34.00	3.126 ± 0.03*	42.00
7.	**4d**	–	218.16 ± 2.53*	11.11	4.806 ± 0.12*	11.52
8.	**4e**	–	194.24 ± 1.59*	21.67	4.292 ± 0.07*	20.26
9.	**5d**	Cl	71.65 ± 0.78*	70.80	1.442 ± 0.04*	73.23
10	**5e**	Cl	40.96 ± 1.21*	83.31	0.83 ± 0.18*	84.57
11	Control	–	245.458 ± 2.093*	0.00	5.38 ± 0.16*	0.00
12	Diazepam	–	00.00 ± 00	100	0.00 ± 00	100

Values are expressed as mean ± SEM; statistical significant test for comparison was done by ANOVA; followed by chi-square test ($n = 5$)

* $P < 0.05$ when compared to control group

(anhydrous condition was maintained by guard tube) followed by addition of succinic anhydride (0.10 mol) in small quantities with continuous stirring & heating at 80 °C for 4 h. Contents after leaving overnight at room temperature were poured into ice-cold hydrochloric acid (2.5 % v/v) followed by steam distillation. After completion of the distillation the aqueous solution was concentrated to a small volume by evaporating on a water bath to obtain the crude compound. 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 anisoyl propionic acid, and this was then crystallized from aqueous ethanol (Khan and Siddiqui, 2000).

White crystals (55 %); mp 155 °C; Rf value; 0.66, IR: 750, 1,275, 1,670, 1,027, 1,666–1,698, 1,390 cm^{-1}.^{1}H NMR, δ ppm: 3.26 (2H, t, CH_2), 2.7 (2H, t, CH_2), 7.96 (2H, d, H-2′,6), 6.97 (2H, d, H-3′,5), 11.92 (OH), 3.86 (3H,s); ^{13}C NMR (75 MHz, $CDCl_3$) 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), ms: *m/z* 208.07 (M^+), ms: *m/z* 208.07 (M^+), Anal. Calcd. For $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; o, 30.74. Found: C, 63.52; H, 5.79; O, 30.71.

6-(Anisoyl)2,3,4,5-tetra-hydro-pyridazine-3-ones (**1b**)

The above acid anisoyl propionic acid **1a** (0.1 mol) was refluxed for 6 h with hydrazine hydrate (1 ml) in methanol (25 ml) containing sodium acetate (0.5 g). After completion of the reaction the contents were concentrated and then poured into ice-cold water. A solid was separated, which was filtered and crystallized from ethanol to get compound **1b** (Siddiqui and Dogra, 2001; Siddiqui *et al.*, 2004).

White powder (65 %); mp 175 °C; Rf value; 0.71, IR: 1,275, 3,303.83, 1,540, 1,665, 1,490 cm^{-1}. ^{1}H NMR, 2.6 (2H, t, CH_2), 2.94 (2H, t, CH_2), 7.66 (2H, d, H-2′,6), 6.93 (2H, d, H-3′,5), 3.85 (3H, s), 7.27 (NH); ^{13}C NMR (75 MHz, $CDCl_3$) 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 (1C, Imine). ms: *m/z* 204.09 (M^+), Anal. Calcd. For $C_{11}H_{12}N_2O_2$: C, 64.06; H, 6.84; o, 15.52; N, 13.58. Found: C, 64.02; H, 6.78; O, 15.54; N, 13.51.

6-Oxo-3p-(anisoyl)-5,6-dihydro-*4H*-pyridazine-1yl)-acetic acid ethyl ester (**1c**)

The appropriate pyridazinone **1b** (0.02 mol) was added to an ethanolic solution (50 ml) of sodium (0.46 g, 0.02 g atom). This mixture was first refluxed for 30 min, then ethyl bromoacetate (0.02 mol) was added by drops to the cooled solution, which was then refluxed for 24 h and then the mixture was concentrated to a small volume. The residue was triturated with diisopropyl ether and the solid which was formed was collected by filtration and the dried compound was recrystallized from a mixture of ethanol and water (50:50) to get the compound **1c** (Rubut *et al*., 1990).

Yellow powder (75 %); mp 180 °C; Rf value; 0.58, IR: 760, 1,280, 1,664, 1,490, 1,740 cm^{-1}. ^1H NMR (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 (75 MHz, $CDCl_3$) 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 (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-(2-(3-(4-Methoxyphenyl)-6-oxo-5,6-dihydropyridazine-1(*4H*)-yl)acetyl)hydrazine-1(*4H*)-yl)acetyl) hydrazinecarbothioamide (**1d**)

Take **1c** (0.08 mol) 22 and 7 g (0.08 mol) of thiosemicarbazide in ethanol 50 ml and stir the mixture for 6 h and then reflux on steam bath for 3 h. The excess of solvent was removed under reduced pressure and recrystallized from chloroform/hexane (3;1) v/v to yield yellow crystals of compound **1d** (Namdeo *et al*., 2009).

White powder (80 %); mp 125 °C; Rf value; 0.73, IR: cm^{-1} 2,918, 3,099, 1,022, 1,664, 1,612, 3,318, 1,100, ^1H NMR, (δ ppm) 7.6 (d, 1H, Ar–H, $J = 7.5$ Hz), 6.85 (d, 1H, Ar–H, $J = 1.5$ Hz), 3.7 (s, 3H, O–CH_3,), 2.88 (t, 2H, CH_2, $J = 7.1$ Hz), 2.44 (t, 2H, CH_2, $J = 7.1$ Hz), 3.2 (s, 2H, CH_2), 7.86 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 114.4–168 (6C, Ar),162.4 (1C, Pyridazinone and amide), 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-[(5-Amino-1,3,4-thiadiazole-2yl)methyl]-6-(4-methoxyphenyl)-4,5-dihydropyridazin-3(*2H*)-one derivatives (**1e**)

(0.08 mol) of compound **1d** was added in conc. H_2SO_4 (10 ml) in a Petri dish and kept overnight at room temperature, neutralized with ammonia and extracted with ether. The ether was distilled off and the product so obtained was crystallized from 80 % ethanol to get yellowish leaflets of compound **1e** (Namdeo *et al*., 2009).

Brown powder (85 %); mp 70 °C, Rf value; 0.82, IR: 2,918, 3,028, 1,008, 1,698, 1,575, 3,319, 613 cm^{-1}. ^1H NMR, δ ppm: 6.99 (d, 1H, Ar–H, $J = 7.5$ Hz), 7.95 (d, 1H, Ar–H, $J = 1.5$ Hz), 2.59 (2.61 (t, 2H, CH_2, $J = 7.1$ Hz), 3.19 (t, 2H, CH_2, $J = 7.1$ Hz), 4.60 (s, 2H, N–CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) 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; S, 10.10 Found: C, 52.90; H, 4.8; O, 10.05.; N, 22.83; S, 10.18.

β-Ethyl-phenyl-propionic acid (**2a**)

White crystals (55 %); mp 110 °C; Rf value; 0.69, IR: 709.76, 2,781,1,670, 1,309 cm^{-1}. ^1H NMR, δ ppm: 2.83 (2H, t, CH_2), 3.30 (2H, t, CH_2), 7.91 (2H, d, H-2′,6), 7.29 (2H, d, H-3′,5), 2.73 (2H, m, CH_2), 1.25 (3H, t, CH_3), 11.0 (1H, O–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 127.6–148.7 (6C, Aryl), 28.2 (1C, CH_2–CH_3), 14.5 (1C, CH_2–CH_3), 29.1 (1C, CH_2–(=O)–O), 32.9 (1C, CH_2–(=O)–C). ms: *m/z* 205.1 (M^+), Anal. Calcd. For $C_{12}H_{14}O_3$: C, 69.88; H, 6.84; O, 23.27; Found: C, 69.85; H, 6.7; O, 23.25.

6-(Ethyl phenyl)2,3,4,5 tetra-hydro-pyridazine-3-one (**2b**)

White powder (60 %); mp 150 °C, Rf value; 0.72, IR: 700.11, 2,987, 3,303, 1,614, 1,666, 1,483 cm^{-1}. ^1H NMR, δ ppm: 2.29 (2H, t, CH_2), 2.59 (2H, t, CH_2) 7.66 (2H, d, H-2′,6), 7.25 (2H, d, H-3′,5), 1.24 (3H, t, CH_3), 2.68 (2H, m, CH_2), 7.85 (NH,s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 127.6–148.7 (6C, Aryl), 28.2 (1C, CH_2–CH_3), 14.5 (1C, CH_2–CH_3), 35.0 (1C, CH_2–(=O)–N–N), 24.1 (1C, CH_2–C=N), 146.6 (1C, C=N), 167.7 (1C, Pyridazinone and amide), 146.5 (1C, Imine). ms: *m/z* 202.11 (M^+), Anal. Calcd. For $C_{12}H_{14}$ N_2 O: C, 70.56; H, 7.90; O, 7.83; N, 13.17 Found: C, 70.52; H, 7.8; O, 7.83.; N, 13.15.

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

Yellow powder (75 %); mp 116–120 °C, Rf value; 0.68, IR: 731, 2,926.2 C–H, 3,212 N–H, 1,280 C–N, 1,505, 1,723.8 cm^{-1}. ^1H NMR, 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 (75 MHz, $CDCl_3$) 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 (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-(3-(4-Ethylphenyl)-6-oxo-5,6-dihydropyridazin-1(*4H*)-l)acetyl)hydrazinecarbothioamide (**2d**)

White powder (85 %); mp 136–140 °C, Rf value 0.81, IR: 2,928, 3,106, 1,022, 1,666, 1,614, 3,212, 622, 3,323 cm^{-1}. ^1H NMR, δ ppm: 6.9 (d, 1H, Ar–H, $J = 5$ (1.5 Hz), 2 (7.5 Hz), 7.23 (d, 1H, Ar–H, $J = 3$ (7.5 Hz), 6 (1.5 Hz), 2.65 (t, 2H, CH_2, $J = (11)$ 7.1 Hz), 2.51 (q, 2H $J = 15$ (8.0 Hz), 1.25 (t, 3H, $J = 14$ (8.0 Hz), 3.82 (s, 2H, CH_2 $J = (11)$ 7.1 Hz), 7.65 (d, 1H,), 2.56 (s, NH), 7.95 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) 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 (1C, Imines), 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-((5-Amino-1,3,4-thiadiazol-2-yl)methyl)-6-(4-ethylphenyl)-4,5-dihydropyridazin-3(*2H*)-one (**2e**)

Brown powder (80 %); mp 85–88 °C, Rf value; 0.92, IR cm^{-1} 2,915, 3,063, 1,678, 1,573, 613, 3,351, ^1H NMR, δ ppm: 7.3 (d,1H, Ar–H, $J = 6$ (1.5 Hz), 3 (7.5 Hz), 7.9 (d, 1H, Ar–H, $J = 2$ (7.5 Hz), 5 (1.5 Hz), 3.2 (t, 2H, $J = (11)$ 7.1 Hz), 2.6 (t, 2H, $J = (12)$ 7.1 Hz), 2.7 (q, 2H, $J = (22)$ 8.0 Hz), 3.8 (s, 2H), 1.2 (t, 3H $J = (21)$, 8.0 Hz), 6.9 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) 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; S, 10.17; Found: C, 57.09; H, 5.41; O, 5.02; N, 22.20; S, 10.26.

β-Toloyl-propionic acid (**3a**)

White crystals (59 %); mp 125–130 °C, Rf value; 0.79, IR: 2,750, 1,600, 1,250, 1,460 cm^{-1}, ^1H NMR, δ ppm: 2.4 (2H, t, CH_2, $J = 10$ (7.1), 2.8 (2H, t, CH_2, $J = 9$ (7.1), 7.8 (CH_2, d, H-2′,6), 7.2 (CH_2, d, H-3′,5), 2.4 (3H, s), 11 (1H, O–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 128.7–142.8 (6C, Aryl), 21.3 (1C, CH_2–CH_3), 29.1 (1C, CH_2–(=O)–O), 32.9 (1C, CH_2–(=O)–C), 198.3 (1C, Carbonyl). ms: *m/z* 192.08 (M^+), Anal. Calcd. For $C_{11}H_{12}O_3$: C, 68.74; H, 6.29; O, 24.97; Found: C, 68.71; H, 6.25; O, 24.94.

6-(Toloyl)2,3,4,5-tetra-hydro-pyridazine-3-ones (**3b**)

White powder (66 %); mp 140–144 °C, Rf value −0.65, IR: 2,384.64, 3,400, 1,623, 1,490, 720 cm^{-1}. ^1H NMR, δ ppm: 2.9(2H, t, CH_2 $J = 7.1$), 2.6(2H, t, CH_2 $J = 7.1$), 7.6 (2H, d, H-2′,6 $J = 7.5$), 7.21 (2H, d, H-3′,5 $J = 7.5$), 2.34 (3H, s), 7.269 (NH, s). ^{13}C NMR (75 MHz, $CDCl_3$) 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), 167.7 (1C, Pyridazinone and amide), 146.5 (1C, Imine). ms: *m/z* 188.09 (M^+), Anal. Calcd. For $C_{11}H_{12}N_2O$: C, 69.45; H, 7.42; O, 8.41; N, 14.73 Found: C, 69.42; H, 7.40; O, 8.39; N, 14.71.

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

Yellow powder (72 %); mp 148–152 °C, Rf value; 0.77, IR: 720, 2,389.64, 1,250, 1,490 cm^{-1}. ^1H NMR, 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 (75 MHz, $CDCl_3$) 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), 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-(2-(6-Oxo-3-p-tolyl-5,6-dihydropyridazin-1(*4H*)yl)acetyl)hydrazinecarbothioamide (**3d**)

White powder (80 %); mp 135–140 °C, Rf value; 0.82, IR: 730, 2,385, 1,185, 1,490, 1,545, 3,257 cm^{-1}. ^1H NMR, 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 (75 MHz, $CDCl_3$) 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-((5-Amino-1,3,4-thiadiazol-2-yl)methyl)-6-p-tolyl-4,5-dihydropyridazin-3(*2H*)-one (**3e**)

Brown powder (75 %); mp 90 °C, Rf value; 0.94, IR: 2,925, 3,029,1,682, 1,607, 3,352, 613 cm^{-1}. ^1H NMR, δ ppm: 7.85 (d, 1H, Ar–H, $J = 5$ (1.5 Hz), 2 (7.5 Hz), 7.30 (d, 1H, Ar–H, $J = 3$ (7.5 Hz), 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 (75 MHz, $CDCl_3$) 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; S, 10.64 Found: C, 55.75; H, 5.00; O, 5.28; N, 23.20; S, 10.70.

β-Aroyl-propionic acid (**4a**)

White crystals (72 %); mp 129 °C, Rf value; 0.80, IR: 2,925, 1,684, 1,250, 1,460 cm^{-1}. ^1H NMR, δ ppm: 3.25 (2H, t, CH_2, $J = 9$ (7.1), 2.65 (2H, t, CH_2, $J = 10$ (7.1), 7.96 (CH_2, d, H-2′,6, $J = 6$ (7.6), 7.53 (CH_2, d, H-3′,5, $J = 5$ (7.5), 7.62 (H, d), 11 (1H, O–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 128.6–136.4 (6C, Aryl), 32.9 (1C, CH_2–(=O)–C), 198.3 (1C, Carbonyl), 29.1 (1C, CH_2–(=O)–O), 177.3 (1C, Carboxyl). ms: *m/z* 179.0 (M^+), Anal. Calcd. For $C_{10}H_{10}O_3$: C, 67.41; H, 5.66; O, 26.94; Found: C, 67.38; H, 5.62; O, 26.91.

6-(Aroyl)2,3,4,5-tetra-hydro-pyridazine-3-ones (**4b**)

White powder (68 %); mp 140 °C, Rf value; 0.62, IR: 2,384.64, 3,400, 1,665, 1,490, 720 cm^{-1}. ^1H NMR (δ ppm) 2.92 (2H, t, CH_2 $J = 11$ (7.1), 2.43 (2H, t, CH_2 $J = 12$ (7.1), 7.70 (2H, d, H-2′,6, $J = 6$ (7.5), 7.35 (2H, d, H-3′,5 $J = 6$ (7.5), 8.1 (NH,s). ^{13}C NMR (75 MHz, $CDCl_3$) 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 (1C, Imine) ms: *m/z* 175.1 (M^+), Anal. Calcd. For $C_{10}H_{10}N_2O_3$: C, 68.16; H, 6.86; O, 9.08; N, 15.90 Found: C, 68.14; H, 6.83;, O9.00; N, 15.88.

6-Oxo-3p-(aroyl)-5,6-dihydro-*4H*-pyridazine-1yl)-acetic acid ethyl ester (**4c**)

Yellow powder (70 %); mp 155 °C, Rf value; 0.65, IR: 2,929, 3,066, 1,595, 1,665.1,756,1,205 cm^{-1}. ^1H NMR, 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 (75 MHz, $CDCl_3$) 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 (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-(2-(6-Oxo-3-phenyl-5,6-dihydropyridazine-1(*4H*)yl)acetyl) hydrazinecarbothioamide (**4d**)

White powder (80 %); mp 115–122 °C, Rf value; 0.74, IR (KBr)- 2,944, 3,098 1,676, 1,676 1,618 (C=N), 3,206, 685, 3,456 cm^{-1}. ^1H NMR, δ ppm: 7.36 (t, 1H, Ar–H-2′6 $J = 5$ (7.5 Hz), 1 (1.5 Hz, 2 (1.5 Hz), 7.70 (d, 1H, Ar–H-3′5, $J = 2$ (7.5 Hz), 5 (7.5 Hz, 1 (1.5 Hz), 2.43 (t, 2H, CH_2 $J = 12$ (7.1 Hz), 2.92 (t, 2H, CH_2 $J = 11$ (7.1 Hz), 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 (75 MHz, $CDCl_3$) 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 (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-((5-Amino-1,3,4-thiadiazol-2-yl)methyl)-6-phenyl-4,5-dihydropyridazin-3(*2H*)-one (**4e**)

Brown powder (78 %); mp 98–100 °C, Rf value; 0.89, IR: 2,924, 3,028,1,683, 1,594, 614, 3,350 cm^{-1}. 1H NMR, δ ppm: 7.98 (t,1H, Ar–H-3′5 $J = 6$ (7.5 Hz), 3 (1.5 Hz, 1 (1.5 Hz), 7.52 (t,1H, Ar–H-4 $J = 2$ (7.5 Hz), 5 (1.5 Hz, 1 (1.5 Hz), 7.65 (d, 1H, Ar–H-2′6) 3.24 (t, 2H, CH_2 $J = 12$ (7.1 Hz), 2.63 (t, 2H, CH_2 $J = 11$ (7.1 Hz), 3.63 (2H, s, CH_2), 2.5 (2H, s, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) 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 (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; S, 11.16 Found: C, 54.31; H, 4.54; O, 5.54; N, 24.35; S, 11.20.

β-Chloro propionic acid (**5a**)

White crystals (70 %); mp 120–125 °C, Rf value; 0.81, IR: 2,586, 1,680, 1,250, 3,060, 1,460 cm^{-1}, ^1H NMR, δ ppm: 3.25 (2H, t, CH_2, $J = 9$ (7.1 Hz), 2.65 (2H, t, CH_2, $J = 10$ (7.1 Hz), 7.9 (CH_2, d, H-2′,6, $J = 2$ (7.5 Hz)), 7.4 (CH_2, d, H-3′,5, $J = 3$ (7.5 Hz)), δ ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 128.7–138.7 (6C, Cl-aryl), 32.9 (1C, CH_2–(=O)–C), 29.1 (1C, CH_2–(=O)–O), 198.3 (1C, Carbonyl), 29.1 (1C, CH_2–(=O)–O), 177.3 (1C, Carboxyl). ms: *m/z* 212.02 (M^+), Anal. Calcd. For $C_{10}H_9ClO_3$: C, 56.49; H, 4.27; O, 22.57; Found: C, 56.28; H, 4.20; O, 22.5.

6-(Chloro)2,3,4,5-tetra-hydro-pyridazine-3-ones (**5b**)

White powder (70 %); mp 178–182 °C, Rf value; 0.79, IR; 2,384.64, 3,400, 1,674 1,250, 1,490, 720 cm^{-1}. ^1H NMR, δ ppm: 2.92 (2H, t, CH_2 $J = 12$ (7.1 Hz), 2.45 (2H, t, CH_2 $J = 13$ (7.1 Hz), 7.6 (2H, d, H-2′,6 $J = (37.5$ Hz), 7.21 (2H, d, H-3′,5 $J = 2$ (7.5 Hz), 2.34 7.269 (NH, s). ^{13}C NMR (75 MHz, $CDCl_3$) 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). ms: *m/z* 209.1 (M^+), Anal. Calcd. For $C_{10}H_9ClN_2O_3$: C, 57.57; H, 4.35; O, 7.67; N, 13.43 Found: C, 57.51; H, 4.30; O, 7.62; N, 13.44.

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

Yellow powder (74 %); mp 170 °C, Rf value; 0.71, IR: 2,935, 3,105, 1,677, 1,611, 1,224 cm^{-1}; ^1H NMR, 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 (75 MHz, $CDCl_3$) 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-(2-(3-(4-Chlorophenyl)-6-oxo-5,6-dihydropyridazine-1(*4H*)yl)acetyl)hydrazinecarbothioamide (**5d**)

White powder (74 %); mp 170 °C, Rf value; 0.71, Yield –74 %., IR: 2,935, 3,109, 1,679, 1,611, 1,612, 3,332, 624, 3,322 cm^{-1}. ^1H NMR, δ ppm: 7.40 (d, 1H, Ar–H, $J = 3$ (7.5 Hz), 6 (1.5 Hz), 7.74 (d, 1H, Ar–H, $J = 2$ (7.5 Hz), 5 (1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) 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-((5-Amino-1,3,4-thiadiazol-2-yl)methyl)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(*2H*)-one (**5e**)

Brown powder (80 %); mp 102–106 °C, Rf value; 0.90, Yield –80 %., IR: 2,927, 3,060, 1,680, 1,590, 658, 3,348 cm^{-1}. ^1H NMR (δ ppm), 7.51 (d, 1H, Ar–H, $J = 3$ (7.5 Hz), 6 (1.5 Hz), 7.98 (d, 1H, Ar–H, $J = 2$ (7.5 Hz), 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 (75 MHz, $CDCl_3$) 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; S, 9.96 Found: C, 48.50; H, 3.72; O, 4.95; N, 21.72; S, 9.89.

Pharmacology

Experimental animals

Anticonvulsant activity of the synthesized compounds was determined by their ability to provide protection from convulsions in albino mice. Swiss Male albino mice of weight (25–30 g) ($n = 5$) were used in the experiments of MES-induced seizures. Female animals were excluded because of the fact that estrus cycle influence the seizure threshold. Animals were housed in polypropylene cage with dust-free rice husk as bedding material under laboratory condition with controlled environment of temperature 25 ± 2 °C and 12 h light/dark schedule, humidity (60 ± 10 %) and before subjecting them to experimentation, the animals were given a week of time to get acclimatized with laboratory condition. They had free access to standard mouse diet and tap water except during the experiment. On the day of the experiment, animals were transferred to individual cases randomly and allowed to acclimatize for 30 min before injection of drug or vehicle. All 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).

Acute toxicity study of the test compounds

Acute toxicity studies were performed on Swiss albino mice according to Organization for Economic Co-operation and Development (OECD)—425 guidelines (OECD, 2001; Ghosh, 1984; Hardman *et al*., 1996), the animals

were kept fasted for 3 h with free access to water. The test compounds were administered orally at a dose of 50 mg/kg. The dose at which mortality was observed in two out of three mice, was considered as toxic dose (OECD, 2001). However, if no mortality was observed, the procedure was repeated with higher dose such as 100, 300, 500, 2,000 mg/kg body weight. Toxic manifestations like abnormal motor activity, alteration in water or food intake, respiration, sedation, and moribund stages were observed for 6 h and mortality for 24 h. There was no mortality among the graded dose groups of mice up to a dose of 2,000 mg/kg for duration of 72 h. This finding probably suggests that the test compounds are relatively safe or non-toxic in mice at the doses used for this study.

Anticonvulsant activity (MES model)

The anticonvulsant activities of the compounds were determined against MES models (Kulkarni, 1999; Tandon and Gupta, 2005), (Model: Techno Electro Convulsometer). Phenytoin was considered as positive control drug with anticonvulsant effect in MES models. Test compounds and phenytoin were given intraperitoneally (IP) as a freshly prepared solution in 5 % gum acacia to give a concentration of 1 % (w/v), and 50 % sterile normal saline. Control animals were injected with vehicle only. The vehicle had no effect on the test system. Test compounds injected IP at dose level 50 mg/kg body weight to different groups of five mice each (Asif *et al*., 2011; Samanta *et al*., 2011). Phenytoin was given at the dose of (25 mg/kg). Thirty minutes after administration of the test compound, mice were administered the convulsion stimulus (MES, 60 Hz, 37.2 mA for 0.25 s); the abolition of the hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity (Krall *et al*., 1978; Shaharyar and Akhter, 2009); mice were considered protected according to occurrence of HLTE (hind limb tonic extension) in MES model. The results are reported in Table 1.

Anticonvulsant activity (PTZ model)

Animals were divided into three groups each comprising 5 animals. One group was used for studying the effect of PTZ alone (control) (dose 80 mg/kg, IP; a stock solution containing 8 mg/ml of the drug was prepared and 1 ml/100 g of body weight of mouse was injected) and second group for studying the protective effect of diazepam. The test compound (dose 100 mg/kg) and diazepam (dose 4 mg/Kg, IP; prepare a suspension of diazepam in 1 % (w/v) gum acacia containing 0.4 mg/ml of the drug and inject 1 ml/100 g of body weight of mouse). After 30 min of PTZ administration the onset and severity of convulsion were noted. The delay onset of convulsion was referred to be anticonvulsant response the results of test compound were compared with control and standard drug diazepam (Jatav *et al*., 2008, 2010; Tandon and Gupta, 2005; Upmanyu *et al*., 2009). The results are reported in Table 2.

Muscle relaxant activity

Rotarod test

The effect on motor co-ordination was assessed using Rotarod apparatus (Biocraft Scientific System Pvt. Ltd., Agra, India). The Rotarod test has been performed to detect the motor deficit in mice. Mice were placed on a horizontal metal-coated rod with rubber (3 cm diameter) rotating at an initial speed of 10 rpm/min. Terminal speed of the rod was 20 rpm in accelerated studies and rotational velocity of the rod was linearly increased from 10 to 20 rpm within 20 s. The time at which each animal was able to maintain its balance walking on top of the rod was measured. Mice were given two trials with a maximum time of 300 s and a 30–60 min intertribal rest interval (McIlwain *et al*., 2001), before the beginning of all experiments, the riding ability of the animals in the rotarod was checked. Thus, the mice were initially put on a rotating rod, and mice that immediately dropped off (within 30 s) were removed from the experiment. Diazepam (4 mg/kg), test compounds, and normal saline (10 ml/kg) were injected 30, 60, and 60 min, respectively, before the test. The results are reported in Table 3.

Traction test

Forepaws of a mouse were placed on a 15-cm-long twisted wire rigidly supported and 20 cm above the table top. Normal mice grasped the wire with forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 s. Inability to put up at least one hind foot was considered failure to the traction (Villar *et al*., 1992). The test was conducted on five groups of previously screened mice, 1 h after given saline (10 ml/kg) or test compound (mg/kg) and 30 min after the injection of diazepam (4 mg/kg). The results are reported in Table 3.

Statistical analysis of the data

The mean value of the readings (the time duration of the tonic extensor phase of the seizures) was taken and standard error of mean was calculated. The percent protection of the standard drug and the test samples were calculated with respect to the control. Differences between means were considered to be significant at $P < 0.05$ compared to control. Chi-square test was applied for the data expressed in percentage using graph Pd prism. In this case the "*P*"

value less than 0.05 was considered significant when compared to control.

$$\text{Percentage inhibition} = [(\text{mean of control} - \text{mean of treatment})/(\text{mean control})] \times 100$$

Conclusions

These 6(chloro)2,3,4,5 tetra hydro-pyridazine-3-ones derivatives of pyridazinone have shown significant anticonvulsant activity against MES and PTZ induced seizure in albino mice after IP administration of 50 and 100 mg/kg body weight dose, respectively. The potency order of the test compound on the extensor phase and pentylenetetrazole-induced seizures **5e** > **5d** > **1e** > **1d** > **3e** > **3d**. The compound **5d** and **5e** have shown maximum recovery in practice so, the compound **5d and 5e** may be regarded as true anticonvulsant because a substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase in MES convulsions and elapsed from the injection of pentylenetetrazole to the first two myoclonic jerk of the forelimbs and devoid of generalized convulsion were considered to be protected in PTZ Model.

The title compounds thus may have immense potential for contribution to human benefit. Scientific exploration of further studies of more derivatives as well as on more parameters is needed to elucidate the defined role of pyridazinones at the anticonvulsive levels.

Acknowledgments The authors are thankful to the Director, SAIF, Punjab University, Chandigarh for IR, ^{1}H NMR, ^{13}C NMR, and Mass spectroscopy, Authors are also thankful to the Director and Head of the department, Institute of pharmacy, Bundelkhand University, Jhansi for providing facilities to conduct the research work and Mrs. Shalini pathak, department of pharmacology, for her support during the pharmacological screening.

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