

Eco-friendly synthesis of Biologically active Spiro Heterocycles

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ABSTRACT

In the present synthesis work, some heterocyclic compounds 1,2,3,4, and their derivatives were synthesized by environment benign mechanical grinding assistance or mechanochemical method and as well as conventional methods without assistance of grinding. Structures of synthesized compounds were determined by Elemental analysis, I.R., and NMR spectral data. All synthesized compound were evaluated for their insecticidal and fungicidal activities in which compound 3&4 exhibited better results. It was observed that mechanochemical method gave not only better results but also utilized less quantity of solvent and energy than conventional methods.

Key words: Mechanochemical method, Elemental analysis, Insecticidal activity, Cyclization.

Introduction

Pesticides are required materials not only in the field of agriculture but also in other aspects of human society like as protection of wooden work and fibre from pests, kill vectors of disease and disease causing organisms. Due to their excessive demand, pesticides are synthesised in many agrochemical and pharmaceutical industries (Yuta,2020). Most of the synthesised pesticides are heterocyclic compounds and their synthesis done by old synthetic routes by utilising more solvent and energy (Yadav,2021). In the present work, mechanical grinding assistance were applied with old synthetic routes and found that mechanical grinding assisted reactions were gave better yields with utilisation of less energy and less amount of solvent compared to conventional old routes of synthesis. Synthesised compounds 1,2,3,4, were evaluated for their fungicidal and insecticidal activities against selected species. Compound 5 and derivatives are spiro heterocyclic compounds and have given better biological activity

than others.

Experimental

Materials and Methods

All the chemicals were purchased from Merck, Qualigens and Sigma Aldrich and used without any treatment. Synthesis of compounds were assisted by mechanical grinding at moderate temperature, using Agate Pestle Motor (Zexter lab. solutions Agate Pestle Motor, 220V, 50 Hz, 125 mm dia.)

Scheme: 1, Synthesis was monitored by T.L.C. and determination of melting points done by open capillary tubes and were uncorrected. Compound 1 to 4 and their derivatives were also synthesized by reflux method or by cyclization with H_2SO_4 . Each synthesized compound was characterized on the basis of data obtained from Euro Vector E3000 Elemental Analyser (CHN), Agilent Cary 630 FT IR (4000 – 450 cm^{-1}), H^1 NMR, C^{13} NMR, Bruker Avance 400/Av III HD 300 (FT NMR). Biological activities especially, insecticidal, and fungicidal activities of each synthe-

sized compound were evaluated by appropriate methods.

Synthesis and Characterization

Synthesis of Compound 1 ((E)-2-(pyridine-2ylmethylene) hydrazine-1-carbothioamide) Reaction 1, (Scheme: 1) R=H: Compound 1 was synthesized by a Conventional Known method (G.Doleshall, 1926) and as well as by present, mechanical grinding assistance. In mechanical grinding assistance method, a paste of aqueous solution of semicarbazide hydrochloride (.1M) and sodium acetate (.1M) was prepared and picolinaldehyde (.1M) was added to it and mechanically grinded at 100rpm speed for 1hour. After cooling the reaction mixture white solid was washed and re-crystallized by ethanol, other derivatives were also prepared by same way. Yield: R=H=88%, R=-4CH₃=90%, R=-4Cl=87%, R=-4NO₂=88%. Yield by conventional method: R=H=81%, R=-4CH₃=83%, R=-4Cl=82%, R=-4NO₂=85%.

(i) R=H

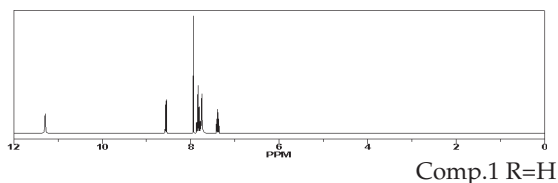


Fig. 1. ¹H NMR: (300 MHz, DMSOd₆, TMS, delta)

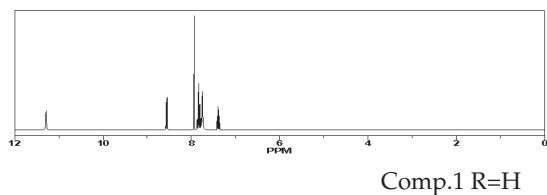


Fig. 2. ¹³C NMR: (62.5 MHz, DMSOd₆, TMS) Characteristic I.R. bands (KBr disc): 3240cm⁻¹, 3000cm⁻¹, 1693cm⁻¹, 1580cm⁻¹, 14590cm⁻¹, 1390cm⁻¹, 1287 cm⁻¹ 890cm⁻¹.

(ii) R= -4CH₃

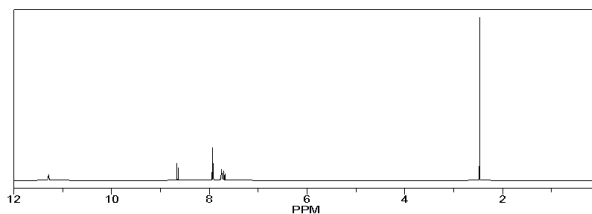


Fig. 3¹H NMR: (300 MHz, DMSOd₆, TMS, del)

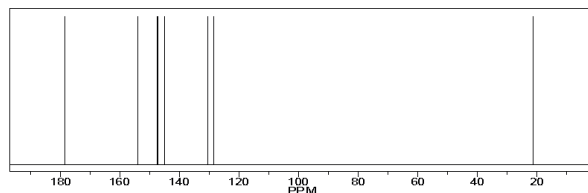


Fig. 4¹³C NMR: (62.5 MHz, DMSOd₆, TMS, del)

Characteristic I.R. bands (KBr disc): 3253cm⁻¹, 3218cm⁻¹, 29750 cm⁻¹, 1673cm⁻¹, 1595cm⁻¹, 1465cm⁻¹, 1355cm⁻¹, 855cm⁻¹.

(iii) R= -4 Cl

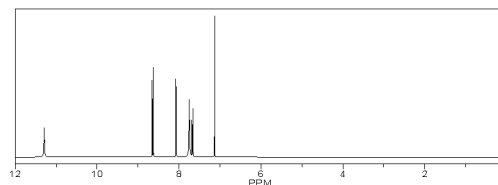


Fig. 5¹H NMR: (300 MHz, DMSOd₆, TMS, delta)

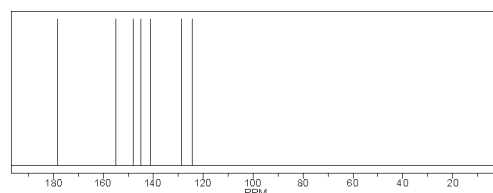
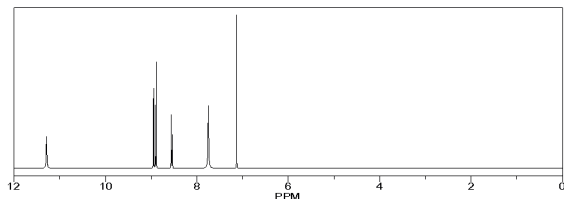
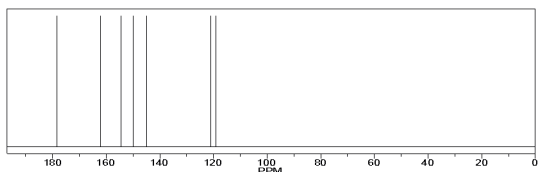


Fig. 6¹³C NMR: (62.5 MHz, DMSOd₆, TMS)

Characteristic I.R. bands (KBr disc): 3375cm⁻¹, 3210cm⁻¹, 1667cm⁻¹, 1585cm⁻¹, 1358cm⁻¹, 1393cm⁻¹, 887cm⁻¹

Table 1. Characterization of Compound 1:

R	M.P. (°C)	Color	Molecular Formula	Analysis					
				C%		H%		N%	
				Found	Calcd.	Found	Calcd.	Found	Calcd.
-H	206	White	C ₇ H ₈ SN ₄	46.64	46.65	4.47	4.47	31.10	31.09
-4CH ₃	204	White	C ₈ H ₁₀ N ₄ S	46.10	46.13	5.57	5.53	30.74	30.74
-4Cl	198	Light Yellow	C ₇ H ₇ ClN ₄ S	39.18	39.16	3.25	3.29	26.10	26.10
-4NO ₂	201	Yellow Brown	C ₇ H ₇ N ₅ O ₂ S	37.30	37.33	3.13	3.13	31.09	31.10

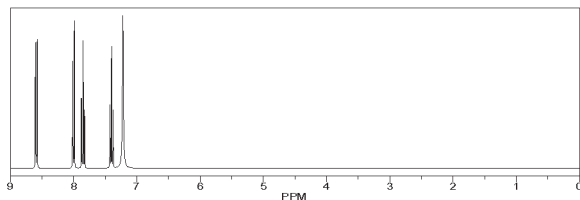
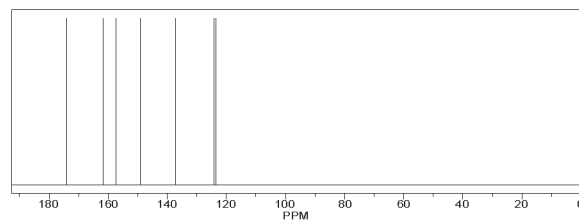
(iv) R= -4 NO₂Fig. 7. H¹ NMR: (300 MHz, DMSOd6, TMS, del.)Fig. 8C¹³ NMR: (62.5 MHz, DMSOd6, TMS)

Characteristic I.R. bands (KBr disc): 3367cm⁻¹, 3224cm⁻¹, 1680cm⁻¹, 1585cm⁻¹, 1478cm⁻¹, 1420cm⁻¹, 875cm⁻¹.

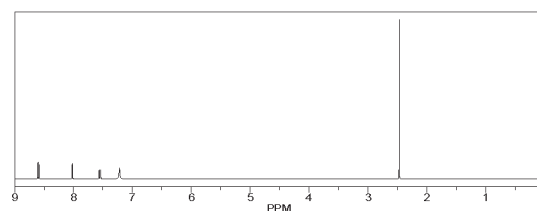
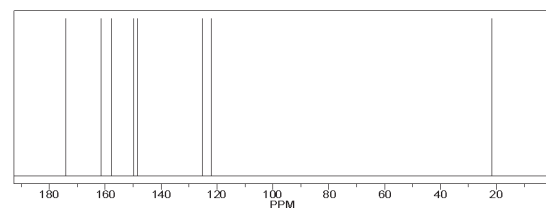
Synthesis of Compound 2(5-(pyridine-2-yl)-1,3,4-thiadiazole-2-amine), Reaction: 2, (Scheme: 1) R=H: It was prepared by mechanical assisted M.S. Gibson method and by Gibson method(M.S. Gibson, 1963). In mechanically assisted Gibson method slurry of compound 1 is grinded at 100 rpm with 1 M bromine in small quantity glacial acetic acid and 1M sodium acetate was added. After some time red coloured solution obtained, grinding was stopped and added distilled water, thus obtained solid was washed and re-crystallized. Similarly other derivatives were also synthesized. Yield: R=H=76%, R=-4CH₃=77%, R=-4Cl=75%, R=-4NO₂=73%. Yield without grinding method: R=H=73%, R=-4CH₃=73%, R=-4Cl=72%, R=-4NO₂=73%.

Characterization of Compound 2: Table 2

(i) R=H

Fig. 9. H¹ NMR: (300 MHz, DMSOd6, TMS, delta).Fig. 10. C¹³ NMR: (62.5 MHz, DMSOd6, TMS)

Characteristic I.R. bands (KBr disc): 3267cm⁻¹, 3213cm⁻¹, 1670cm⁻¹, 1565cm⁻¹, 1458cm⁻¹, 1460cm⁻¹, 1234 cm⁻¹, 875cm⁻¹.

(ii) R= -4CH₃Fig. 11. H¹ NMR: (300 MHz, DMSOd6, TMS, delta).Fig. 12C¹³ NMR: (62.5 MHz, DMSOd6, TMS)

Characteristic I.R. bands (KBr disc): 3270cm⁻¹, 3213cm⁻¹, 1670cm⁻¹, 1559cm⁻¹, 1414cm⁻¹,

(iii) R= -4 Cl

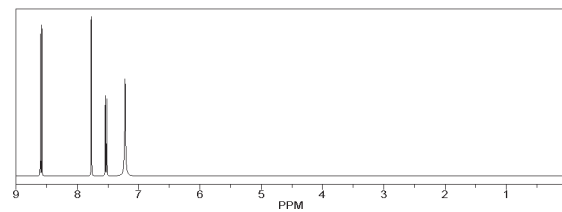
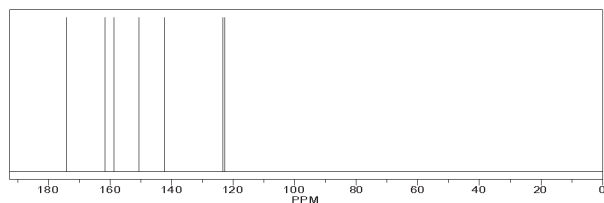
Fig.13 H¹ NMR: (300 MHz, DMSOd6, TMS, delta).

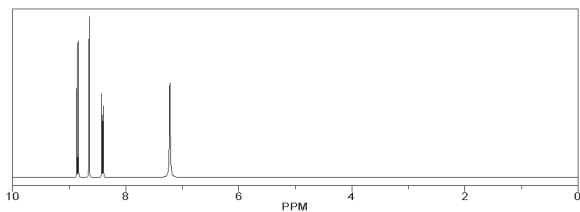
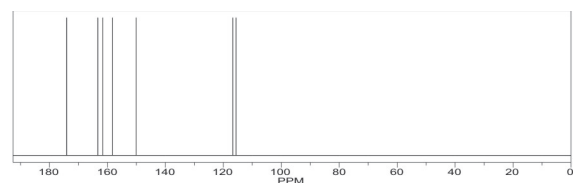
Table 2. 1470cm⁻¹, 1244 cm⁻¹,873cm⁻¹.

R	M.P. (°C)	Color	Molecular Formula	Analysis					
				C%		H%		N%	
				Found	Calcd.	Found	Calcd.	Found	Calcd.
-H	198	reddish	C7H6SN4	47.18	47.18	3.40	3.39	31.44	31.44
-4CH3	200	brownish	C8H9N4S	49.98	49.98	4.19	4.19	29.16	29.15
-4Cl	212	Light brown	C7H5ClN4S	39.53	39.54	2.35	2.37	26.33	26.35
-4NO2	190	deep Brown	C7H5N5O2S	37.65	37.67	2.26	2.26	31.38	31.38

**Fig. 14.** C¹³NMR: (62.5 MHz, DMSO_d6, TMS)

Characteristic I.R. bands (KBr disc): 3277cm⁻¹, 3242cm⁻¹, 2900cm⁻¹, 1677cm⁻¹, 1555cm⁻¹, 1448cm⁻¹, 1460cm⁻¹, 1214 cm⁻¹, 865cm⁻¹.

(iv) R= -4 NO₂

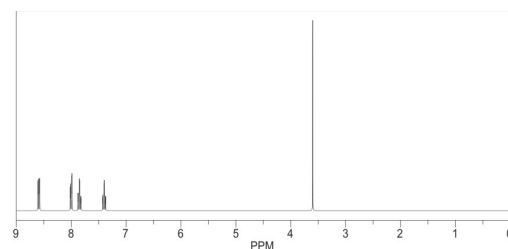
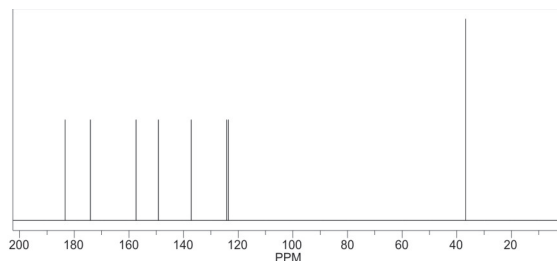
**Fig. 15.** H¹NMR: (300 MHz, DMSO_d6, TMS, delta)**Fig. 16**C¹³NMR: (62.5 MHz, DMSO_d6, TMS)

Characteristic I.R. bands (KBr disc): 3263cm⁻¹, 3212cm⁻¹, 1725 cm⁻¹, 1680cm⁻¹, 1529cm⁻¹, 1400cm⁻¹, 1435cm⁻¹, 1234 cm⁻¹, 888cm⁻¹.

Synthesis of Compound 3 via Intermediate compound A (sodium(5-(pyridine-2-yl)-1,3,4-thiadiazol-2-yl) carbonimidodithioate), Reaction: 3&4 (Scheme:1) R=H: This compound was prepared by grinding slurry in methanol of compound 2 (.1M), sodium hydroxide (.1M) and CS₂ (.1M) at 100 rpm speed for one hour, compound A thus prepared (Scheme:1) was ground again for one hours

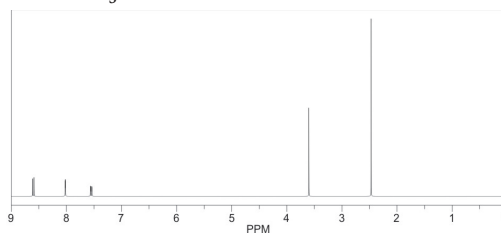
with 1M DCM. Resultant reaction mixture was transferred in a beaker containing water, thus obtained solid compound was filtered, washed and recrystallized by hot ethanol. Similarly derivatives were also synthesized. Yield R=H=70%, R=-4CH₃=69%, R=-4Cl=65%, R=-4NO₂=64%. Yield without grinding method: R=H=64%, R=-4CH₃=63%, R=-4Cl=65%, R=-4NO₂=62%. Characterization of Compound 3: Table 3

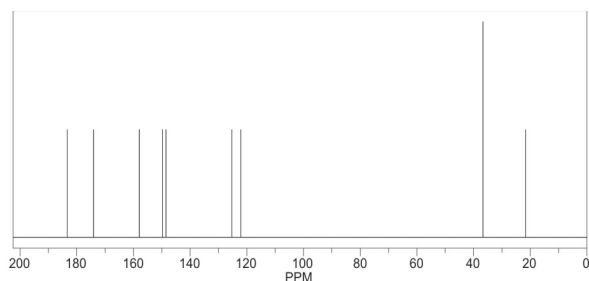
(i) R=H

**Fig. 17** H¹NMR: (300 MHz, DMSO_d6, TMS, delta).**Fig. 18**C¹³NMR: (62.5 MHz, DMSO_d6, TMS)

Characteristic I.R. bands (KBr disc): ,2920cm⁻¹, 2850cm⁻¹, 1710, 1677cm⁻¹, 1555cm⁻¹, 1448cm⁻¹, 1460cm⁻¹, 1214 cm⁻¹, 1140, 865cm⁻¹.

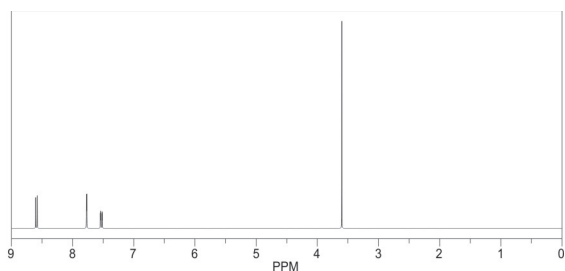
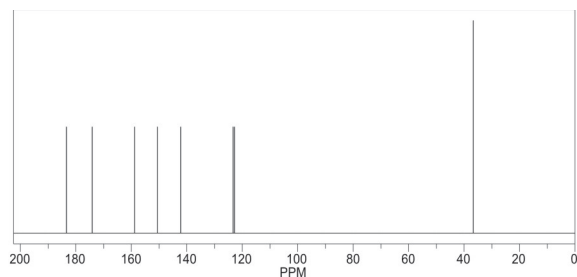
(ii) R= -4CH₃

**Fig. 19**H¹NMR: (300 MHz, DMSO_d6, TMS, delta).

Fig. 20 C^{13} NMR: (62.5 MHz, DMSO d_6 , TMS)

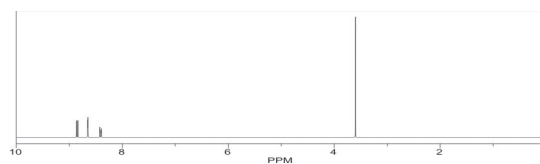
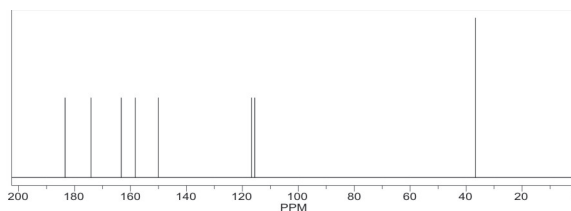
Characteristic I.R. bands (KBr disc): ,2980 cm^{-1} ,
 ,2750 cm^{-1} ,
 1700,1623 cm^{-1} , 1505 cm^{-1} , 1408 cm^{-1} , 1410 cm^{-1} , 1224
 cm^{-1} ,1100,875 cm^{-1} .

(iii) R= -4 Cl

Fig. 21. H^1 NMR: (300 MHz, DMSO d_6 , TMS, delta).Fig. 22 C^{13} NMR: (62.5 MHz, DMSO d_6 , TMS)

Characteristic I.R. bands (KBr disc): ,2970 cm^{-1} ,
 2780 cm^{-1} ,
 1690,1643 cm^{-1} , 1515 cm^{-1} , 1410 cm^{-1} , 1390 cm^{-1} , 1212
 cm^{-1} ,1050,895 cm^{-1} .

(iv) R= -4 NO $_2$

Fig. 23. H^1 NMR: (300 MHz, DMSO d_6 , TMS, delta).Fig. 24. C^{13} NMR: (62.5 MHz, DMSO d_6 , TMS)

Characteristic I.R. bands (KBr disc): ,2960 cm^{-1} ,
 ,2770 cm^{-1} ,1670,1633 cm^{-1} , 1515 cm^{-1} , 1410 cm^{-1} ,1390 cm^{-1} ,
 1212 cm^{-1} ,1050,875 cm^{-1} .

Synthesis of Compound 4 (3- chloro-1-(5-(pyridine-2-yl)-1,3,4-thiadiazol-2-yl)-5,8-dithia-1-azaspiro[3,4]octan-2-one), Reaction 5,(Scheme:1)

R=H: This compound was synthesized by grinding compound 3(.01M) + triethylamine (.02M) in 10 ml dioxin in cold then chloroacetylchloride (.02M) added drop wise with constant grinding for 6 hours then grinding was stopped and leaved for 8 hours then reaction mixture was poured in water and compound obtained as precipitate, filtered ,washed and re-crystallized from cyclohexane. Derivatives of this compound were prepared by following above procedure. All the compounds were also synthesized without use of mechanical grinding. Yield: R=H=64%,R=-4CH $_3$ =63%, R=-4Cl=64%, R=-4NO $_2$ =63%.Yield without grinding method: R=H=61%, R=-4CH $_3$ =61%, R=-4Cl=62%, R=-4NO $_2$ =63%.

Characterization of Compound 4 and its derivatives: Table 4

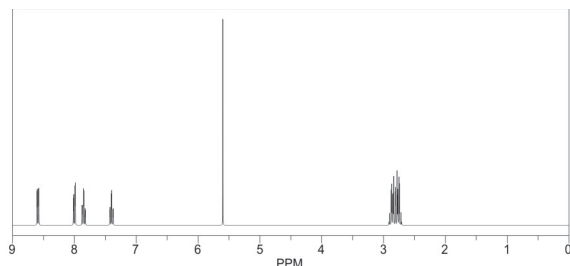
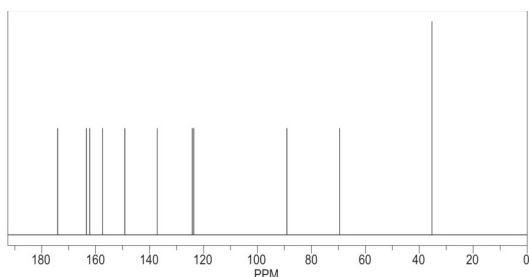
Table 3. 1690, 1643 cm^{-1} , 1515 cm^{-1} , 1410 cm^{-1} , 1390 cm^{-1} , 1212 cm^{-1} ,1050,895 cm^{-1} .

R	M.P. ($^{\circ}C$)	Color	Molecular Formula	Analysis					
				C%		H%		N%	
				Found	Calcd.	Found	Calcd.	Found	Calcd.
-H	152	Light brown	C $_{10}$ H $_8$ S $_3$ N $_4$	42.81	42.84	2.86	2.88	34.30	34.30
-4CH $_3$	145	brown	C $_{11}$ H $_{10}$ N $_4$ S $_3$	44.88	44.88	3.42	3.42	19.03	19.03
-4Cl	150	Yellow brown	C $_{10}$ H $_7$ ClN $_4$ S $_3$	38.13	38.15	2.24	2.24	17.73	17.83
-4NO $_2$	155	Dark Brown	C $_{10}$ H $_7$ N $_5$ O $_2$ S $_3$	36.90	36.91	2.17	2.17	21.52	21.52

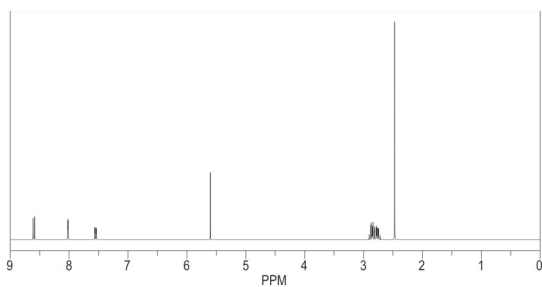
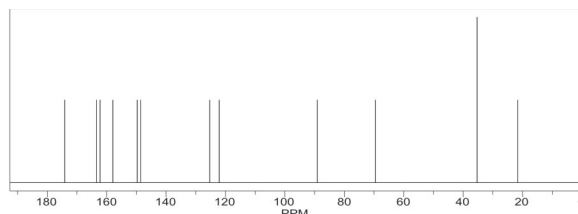
Table 4.

R	M.P. (°C)	Color	Molecular Formula	Analysis					
				C%		H%		N%	
				Found	Calcd.	Found	Calcd.	Found	Calcd.
-H	189	reddish	$C_{12}H_{10}S_3N_4OCl$	40.27	40.25	2.82	2.82	15.60	15.66
-4CH ₃	180	pinkish	$C_{13}H_{12}N_4S_3OCl$	41.98	41.98	3.25	3.25	15.07	15.03
-4Cl	195	brown	$C_{12}H_9Cl_2ON_4S_3$	36.71	36.74	2.31	2.31	14.28	14.28
-4NO ₂	201	Brick Colour	$C_{12}H_9N_5O_3S_3Cl$	35.78	35.78	2.25	2.25	17.38	17.38

(i) R=H

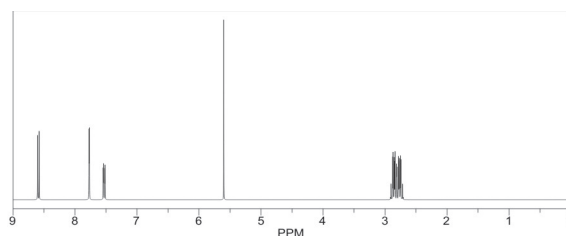
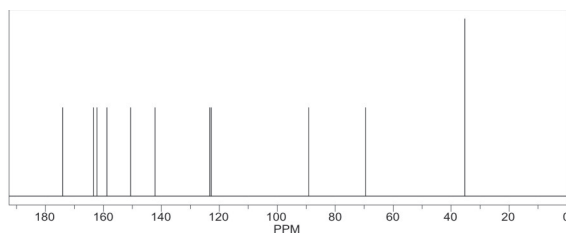
Fig. 25. ¹H NMR: (300 MHz, DMSOd₆, TMS, delta)Fig. 26. ¹³C NMR: (62.5 MHz, DMSOd₆, TMS)

Characteristic I.R. bands (KBr disc): ,2980cm⁻¹, 2680cm⁻¹, 1695, 1645cm⁻¹, 1525cm⁻¹, 1415cm⁻¹, 1395cm⁻¹, 1202 cm⁻¹, 1040, 900, 895cm⁻¹.

(ii) R= -4CH₃Fig. 27 ¹H NMR: (300 MHz, DMSOd₆, TMS, delta).Fig. 28. ¹³C NMR: (62.5 MHz, DMSOd₆, TMS)

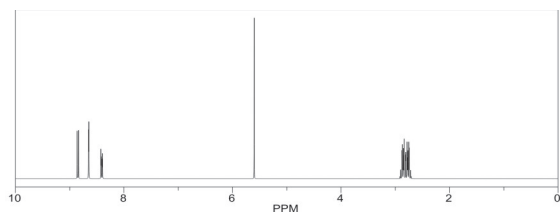
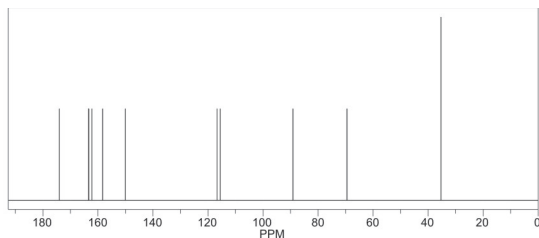
Characteristic I.R. bands (KBr disc): ,2970cm⁻¹, 2687cm⁻¹, 1685, 1635cm⁻¹, 1505cm⁻¹, 1405cm⁻¹, 1365cm⁻¹, 1102 cm⁻¹, 1000, 900, 895cm⁻¹.

(iii) R= -4 Cl

Fig. 29 ¹H NMR: (300 MHz, DMSOd₆, TMS, delta).Fig. 30. ¹³C NMR: (62.5 MHz, DMSOd₆, TMS)

Characteristic I.R. bands (KBr disc): ,2960cm⁻¹, 2677cm⁻¹,

1675, -1, 1605cm⁻¹, 1500cm⁻¹, 1421cm⁻¹, 1365cm⁻¹, 1112 cm⁻¹, 1050, 900, 895cm⁻¹.

(iv) R= -4 NO₂Fig.31: ¹H NMR: (300 MHz, DMSO_{d6}, TMS, delta).Fig. 32 ¹³C NMR: (62.5 MHz, DMSO_{d6}, TMS)

Characteristic I.R. bands (KBr disc): ,2900cm⁻¹, 2777cm⁻¹, 1605, ⁻¹, 1585cm⁻¹, 1508cm⁻¹, 1480cm⁻¹, 1367cm⁻¹, 1124cm⁻¹, 1060, 910, 885cm⁻¹.

Evaluation of Insecticidal Activities: Insecticidal activity of compound 1,2,3,4 and their derivatives were evaluated for common cutworm :

1-Common Cutworm (*Spodoptera litura*)

1-Common Cutworm (*Spodoptera litura*): Each compound to be investigated was applied to third instar larvae with artificial diet. Freezed dry food for common cutworm was put in 10 ml of the 500 ppm aqueous suspension (contained 1500 ppm of emulsifying agent and 10 ppm of wetting agent) of each compound. After the dry food absorbed, suspension to be wet food. 10 third instar larvae were infested on the wet food. After the third instar larvae grew up to be the sixth instar larvae in an incubator at 25°C insect growth regulatory activities were estimate G.I. and P.I. were calculated by following formula and results are given in Table 5.

Growth Inhibition

$$\left(1 - \frac{\text{active 6}^{\text{th}} \text{ instar larvae}}{10}\right) \times 100$$

Population Inhibition :

$$\left(1 - \frac{\text{No. of treated alive pupae}}{\text{No. of treated alive larvae}}\right) \times 100$$

Evaluation of Fungicidal Activity: The assessment of fungicidal activity of each synthesized compounds were done against *Pyricularia oryzae* by filter paper disc method (Nizamuddin, 1999) at 100 ppm and 500 ppm concentration. Carbendazim was taken as standard fungicide. Percentage inhibition calculated by following formula and results in Table 6.

$$\text{Percentage Inhibition} = \frac{(C-T)}{C} \times 100$$

C= diameter of the mycelia colony (in mm) on control plate

T= diameter of the mycelia colony on treated plate.

Compound	Common Cutworm	
	% G.I.	% P.I.
Compound 1		
R=-H	39	46
R=- 4CH ₃	40	49
R=-4Cl	43	55
R=NO ₂	49	62
Compound 2		
R=-H	41	45
R=- 4CH ₃	48	50
R=-4Cl	56	66
R=NO ₂	60	67
Compound 4		
R=-H	55	56
R=- 4CH ₃	64	66
R=-4Cl	69	70
R=NO ₂	69	71
Compound 5		
R=-H	64	63
R=- 4CH ₃	68	71
R=-4Cl	72	74
R=NO ₂	74	77

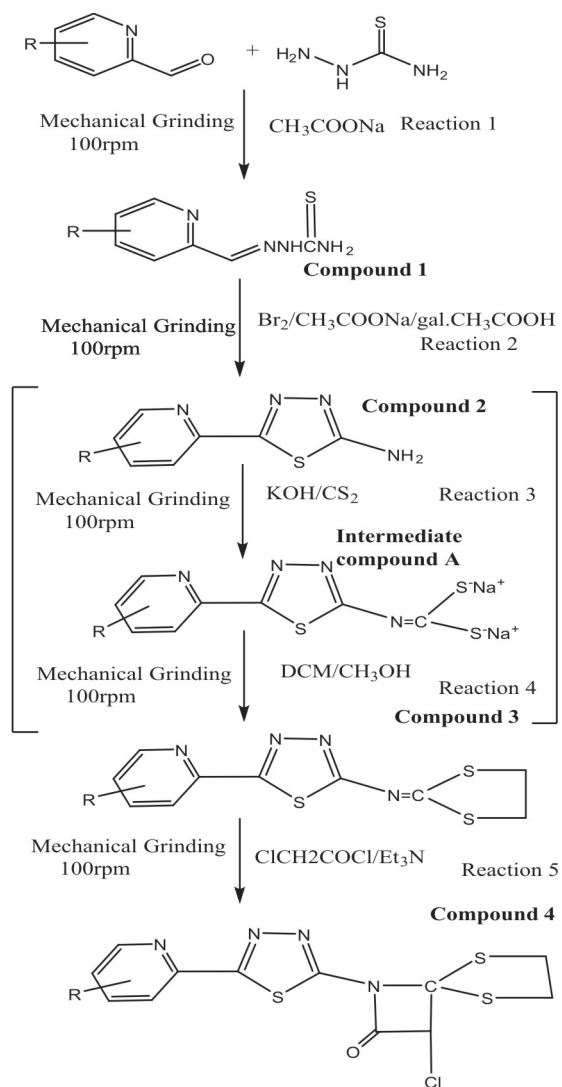
Table 5. (Insecticidal Activity)

Results and Discussion

Observed percentage yields of Compounds 1,2,3,4 and their derivatives which were synthesized by mechanical grinding assistance, exhibited superiority over conventional reflux method. Mechanical grinding assisted reaction not only reasonable but also it is environment benign. Structures of all the synthesized compounds and their derivatives have been proposed by elemental analysis (Table 1, 2, 3, 4) and I.R., NMR spectral data obtained from analysis. All the synthesized compounds have showed appreciable insecticidal and fungicidal activity (Table 5 & 6) against the selected species.

Conclusion

From the results and discussion it is clear that heterocyclic pesticides can also be synthesized by environment benign mechanical grinding assistance method. Conventional reflux method or cyclization reaction in which mechanical grinding was not applied, utilized more solvent and energy thus they are not cost effective.



Scheme 1.

Table 6. (Fungicidal Activity)

Scheme: 1		<i>P. oryzae</i>	
Compounds	Concentrations	500 ppm	100 ppm
Compound 1	R=H	73	65
	R= 4CH ₃	74	66
	R = 4-Cl	75	66
	R = 4-NO ₂	84	71
Compound 2	R = H	61	50
	R= 4CH ₃	69	54
	R = 4-Cl	80	69
	R = 4-NO ₂	83	74
Compound 3	R = H	69	63
	R= 4CH ₃	76	68
	R = 4-Cl	79	64
	R = 4-NO ₂	81	72
Compound 4	R = H	77	69
	R= 4CH ₃	78	70
	R = 4-Cl	85	79
	R = 4-NO ₂	98	83
Carbendazim (Standard)	-	100	88

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Conflict of Interest: The authors declare that they have no conflict of interest.

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