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Synthesis, Structural Analysis & Biological Evaluation of Anticonvulsant Activity of Pyrazole Derivatives Containing Thiourea

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ABSTRACT

Compounds were prepared in two steps. First, the chalcones were synthesized by direct condensation of aromatic aldehydes and the substituted acetophenone, using 20% potassium hydroxide in ethanol to form intermediate. Second, synthesis of compounds VN-1 to VN-5 was carried out by semithiacarbezide and above intermediate. 3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4,5-dihydro-pyrazole-1-carbothioicacid amide, (VN-2) revealed significant activity (75% protection), then 3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid (VN-2) showed 62.5% protection. Compound VN-3 showed mild/intermediate activity. Replacement of the 4-amino phenyl group by 4-bromo-phenyl and 2-bromo-4-methoxy-phenyl moiety as in VN-4 & VN-5 leads to decrease in % of protection of the compounds VN-4 and VN-5 respectively. Moreover, the 3-(4-Amino-phenyl) derivatives **VN-1** and **VN-2** were found to be more active. The order of percentage protection is **VN-2 > VN-1> VN-3> VN-5 >VN-4.** The spectroscopic analysis was performed for synthesized compounds. UV spectra of synthesized compounds were determined on UV shimadzu 1700 spectrophotometer and determined λ_{max} of synthesized compounds. The IR spectra of synthesized compounds were obtained from FTIR, 2000A, ABB spectrophotometer ($ZrCl_2$) in Smriti College of Pharmacy, Indore. The infra red spectra of each of the synthesized compounds shows characterisitic absorption in accordance to their structural functional groups. The ^{1}H-NMR spectrums of synthesized compounds were shows chemical shifts is in good agreement with the structure of the synthesized compounds.

Key word:- Percentage protection, ^{1}H-NMR, UV, Infrared, Chalcones, Acetophenone.

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INTRODUCTION

Hetero aromatic compounds impart considerable attention in the design of biologically active molecules and advanced organic materials[1]. The pyrazole ring is common in a number of biologically active molecules. More recently extensive studies have been focused on pyrazole derivatives for exhibiting analgesic[2], anti-inflammatory[3], anticonvulsant[4], antidepressant[5], antiulcer[6], antidiabetic[7], cytotoxic[8], antitubercular[9] and antibacterial[10]. Antidepressants and anticonvulsants are among the most widely utilized drugs for the treatment of CNS disorders. Considerable interest has been focused on the pyrazole structure, which has been known to possess a broad spectrum of biological activities.

MATERIAL AND METHOD

SYNTHESIS OF NEW COMPOUNDS

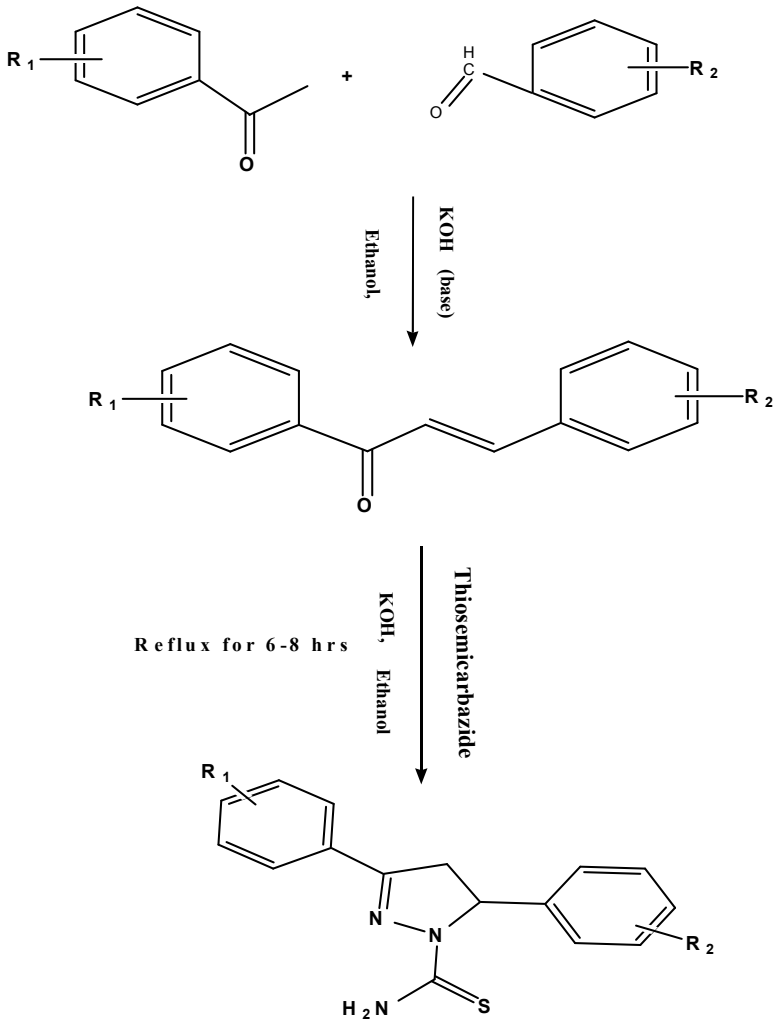
All the chemicals used in these experiments were purchased from ACROS ORGANICS, Bhopal. The synthetic route for the pyrazole derivatives containing thiourea substituted analogues is shown in scheme 1. They were prepared in two steps. First, the chalcones were synthesized by direct condensation of aromatic aldehydes and the substituted acetophenone, using 20% potassium hydroxide in ethanol. Second, synthesis of compounds VN-1 to VN-5 was carried out as given below.

General procedure of synthesis of compounds (VN-1 to VN-5)

The general method of synthesis of pyrazole derivatives is as follows:

Step-1: Synthesis of chalcone: Equimolar quantity of appropriately substituted aromatic aldehydes (0.001mol, 1 equiv) and ketones (0.001 mol, 1 equiv) were dissolved in approximately 15-30 mL of ethanol. The mixture was allowed then to stir for several minutes (15-20minutes). A 10 mL aliquot of a 40% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction flask via a burette. The reaction solution was allowed to stir at room temperature for approximately 1-1.5 hrs. A precipitate formed was then collected by filtration.

Step-2: A mixture of corresponding chalcones (0.001 mol), thiosemicarbazide (0.001 mol), and KOH (0.0025 mol) was refluxed in ethanol (25 -30 mL) for 4-8 hrs. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol.



Scheme 1: General Scheme of synthesis of designed compounds

Synthesis of 3-(4-Amino-phenyl)-5-(4-chlro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide (VN-1)

Equimolar portions of the appropriately 4-chloro Benzaldehyde 140 mg (0.001 mol,1 equiv) and 4-amino acetophenone 135 mg (0.001 mol, 1 equiv) were dissolved in approximately 15 mL of ethanol. The mixture was allowed to stir for several minutes. A 10 mL aliquot of a 40% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction flask via a burette. The reaction solution was allowed to stir at room temperature for approximately 1 hr. Precipitate was formed and collected by filtration. A mixture of chalcone 257 mg (0.001 mol), thiosemicarbazide 91 mg (0.001 mol), and KOH 14 mg (0.0025 mol) was then refluxed in ethanol (25 mL) for 8 hrs. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol. Finally we obtained

the 3-(4-Amino-phenyl)-5-(4-chlro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide with 24% yield.

3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide (VN-2)

Equimolar portions of the appropriately 3-nitrobenzaldehyde 151 mg (0.001mol, 1 equiv) and 4-amino acetophenone 135mg (0.001mol, 1 equiv) were dissolved in approximately 15 mL of ethanol. The mixture was allowed to stir for several minutes. A 10 mL aliquot of a 40% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction flask via a burette. The reaction solution was then allowed to stir at room temperature for approximately 1 hrs. Precipitate was formed and collected by filtration. A mixture of chalcone 268 mg (0.001 mol), thiosemicarbazide 91 mg (0.001 mol), and KOH 14 mg (0.0025 mol) was refluxed in ethanol (25 mL) for 8 hrs. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol. Finally we obtained 3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide with 36% yield.

3-(4-Amino-phenyl)-5-(3, 4-dimethoxy-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide (VN-3)

Equimolar portions of the appropriately 3, 4-dimethoxybenzaldehyde 166 mg (0.001mol, 1 equiv) and 4-amino acetophenone 135 mg (0.001mol, 1 equiv) were dissolved in approximately 15 mL of ethanol. The mixture was allowed to stir for several minutes. A 10 mL aliquot of a 40% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction flask via a burette. The reaction solution was allowed to stir at room temperature for approximately 1.5 hrs. Precipitate was formed and collected by filtration. A mixture of chalcone 283 mg (0.001 mol), thiosemicarbazide 91mg (0.001 mol), and KOH 14 mg (0.0025 mol) was refluxed in ethanol (25 mL) for 6 hrs. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol. Finally we obtained 3-(4-Amino-phenyl)-5-(3, 4-dimethoxy-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide with 48% yield.

3-(2-bromo-4-methoxy-phenyl)-5-(3, 4-dimethoxy-phenyl)-4, 5-dihydro-pyrazole-1 carbothioic acid amide (VN-4)

Equimolar portions of the appropriately 3, 4- dimethoxybenzaldehyde 166 mg (0.001mol, 1 equiv) and 2-bromo, 4-methoxyacetophenone 228 mg (0.001mol, 1 equiv) were dissolved in approximately 30 mL of ethanol. The mixture was allowed to stir for several minutes. A 10 mL aliquot of a 40% aqueous

potassium hydroxide solution was then slowly added dropwise to the reaction flask via a burette. The reaction solution was allowed to stir at room temperature for approximately 1.5 hrs. Precipitate was formed and collected by filtration. A mixture of chalcone 337 mg (0.001 mol), thiosemicarbazide 91 mg (0.001 mol), and KOH 14 mg (0.0025 mol) was refluxed in ethanol (25 mL) for 7 hrs. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol. Finally we obtained 3-(2-bromo-4-methoxy-phenyl)-5-(3, 4-dimethoxy-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide with 38% yield.

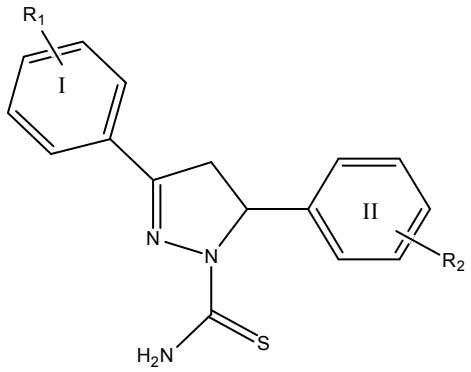


Table 1: List and structures of synthesized compounds

S.No.	Compounds	R_1	R_2	Molecular formula	% Yield	Molecular Weight
1	VN-1	4-amino	4-chloro	$C_{16}H_{15}Cl\ N_4S$	24%	330.843
2	VN-2	4-amino	3-nitro	$C_{16}H_{15}N_5O_2S$	36%	341.396
3	VN-3	4-amino	3,4-dimethoxy	$C_{18}H_{20}N_4O_2S$	48%	356.451
4	VN-4	2-bromo,4-methoxy	3,4-dimethoxy	$C_{19}H_{20}BrN_3O_3S$	38%	450.359
5	VN-5	4-bromo	4-chloro	$C_{16}H_{13}BrCl\ N_3S$	40%	394.724

3-(4-bromo-phenyl)-5-(4-chloro-phenyl)-4,5-dihydro-pyrazole-1-carbothioic acid amide (VN-5)

Equimolar portions of the appropriately 4- chlorobenzaldehyde 140 mg (0.001mol, 1 equiv) and 4-bromoacetophenone 199 mg (0.001mol, 1 equiv) were dissolved in approximately 15 mL of ethanol. The mixture was allowed to stir for several minutes. A 10 mL aliquot of a 40% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction flask via a burette. The reaction solution was allowed to stir at room temperature for approximately 4 hrs. Precipitate was formed and

collected by suction filtration. A mixture of chalcone 321 mg (0.001 mol), thiosemicarbazide 91 mg (0.001 mol), and KOH 14 mg (0.0025 mol) was refluxed in ethanol (25 mL) for 8 h. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol. Finally we obtained 3-(4-bromo-phenyl)-5-(4-chloro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide with 40% yield.

PHYSIOCHEMICAL CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

Physiochemical characterizations of synthesized compounds were performed by using of various parameters such as melting point, solubilities, R_f value etc.

Melting point determination

VEEGO apparatus was used for the determination of melting point of synthesized compounds. Practically obtained melting points were approximately sharp which indicates the purity of the synthesized compounds after crystallization. Table 2 shows the melting point of synthesized compounds.

Table 2: Melting points of different synthesized compounds determined.

S.No.	Compounds	appearance	Melting point by VEEGO (^oC)
1	VN-1	Dull brown	280-282
2	VN-2	Brownish yellow	320
3	VN-3	Brownish red	334-336
4	VN-4	Brownish yellow	338-340
5	VN-5	Brownish red	330-332

Solubility studies

The solubility of synthesized compounds was determined in various polar and nonpolar solvents. There solubilities are summarized here under in table 3.

Table 3: Solubility of synthesized compounds in polar and non polar solvents

Solvent Comp. → / ↓	Cold Water	Methanol	Acetone	Ethyl acetate	Chloroform	Dioxane	Benzene	n-hexane
VN-1	-	++	++	+	++	-	++	-
VN-2	-	++	++	+	++	-	++	-
VN-3	-	++	++	+	++	-	++	-
VN-9	-	++	++	+	++	-	++	-
VN-10	-	++	++	+	++	-	++	-

++ Soluble; + Sparingly soluble; - Insoluble

Thin layer chromatograaphy (TLC)

The thin layer chromatography was performed of the synthesized compounds. Silica gel G was used as stationary phase. In TLC, we found single spot some time with tailing with different R_f values for each compound .This single spot indicates the purity of synthesized compounds. The R_f values of synthesized compounds is given in table 4.

Table 4: R_f values of synthesized compounds

S.No.	Compounds	R_f value of synthesized compounds
1	VN-1	0.64
2	VN-2	0.74
3	VN-3	0.58
4	VN-4	0.72
5	VN-5	0.65

Solvent system- *Chloroform: ethyl acetate: formic acid (5:4:1)*

STRUCTURAL DETERMINATION OF SYNTHESIZED COMPOUNDS

The structure of our synthesized compounds was determined by using various spectroscopic methods.

UV spectroscopy

The wavelength of light in the ultra violet range at which the compounds shows the maximum absorbance called as $\lambda_{max.}$ Benzene is used as the solvent because all synthesized compounds are soluble in the benzene. The λ_{max} of compounds are shown in the table 5.

Table 5: λ_{max} data's of synthesized compounds

S.No.	Compounds	λ_{max} (nm)
1	VN-1	340
2	VN-2	344
3	VN-3	335
4	VN-4	320
5	VN-5	330

IR Spectroscopy

IR spectra were recorded on FTIR, 2000A, ABB spectrophotometer (ZrCl2) in Smriti College of Pharmacy, Indore. IR spectra of VN-1 to VN-5 compounds are shown in figures 4.1 to 4.5 respectively. The interpretation along with characteristics peaks of compounds VN-1 to VN-5 is shown in table 4.6 to 4.10 respectively.

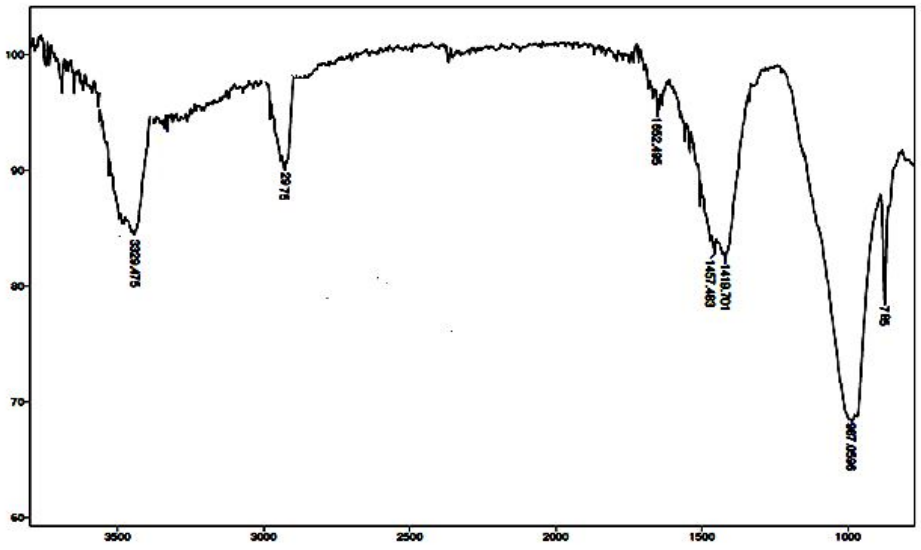


Figure 1: IR Spectrum of VN-1

Table 6: Interpretation of IR Spectrum of VN-1

S. No.	Wave number (cm^{-1})	Interpretation
1	3329	N-H stretching
2	2975	C-H stretching
3	1652 and 1457	C=C stretching (Aromatic)
4	1419	C-N stretching
5	1216	C=S stretching
6	785	C-Cl stretching
7	1590	C=N stretching

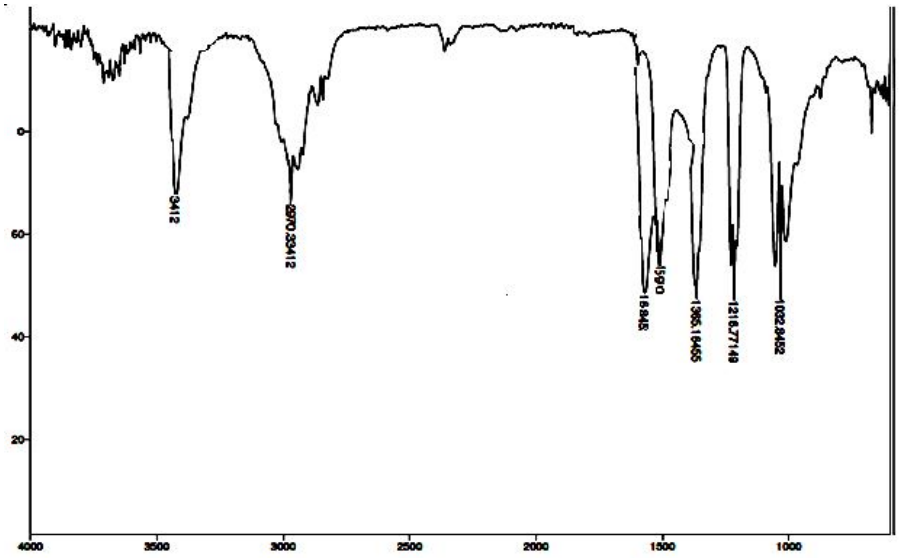


Figure 2: IR Spectrum of VN-2

Table 7: Interpretation of IR Spectrum of VN-2

S. No.	Wave number (cm^{-1})	Interpretation
1	3420	N-H stretching
2	2970	C-H stretching
2	1606 and 1480	C=C stretching (Aromatic)
3	1590	C=N stretching
4	1550 and 1350	NO_2 stretching
5	1337	C-N stretching
6	1216	C=S stretching

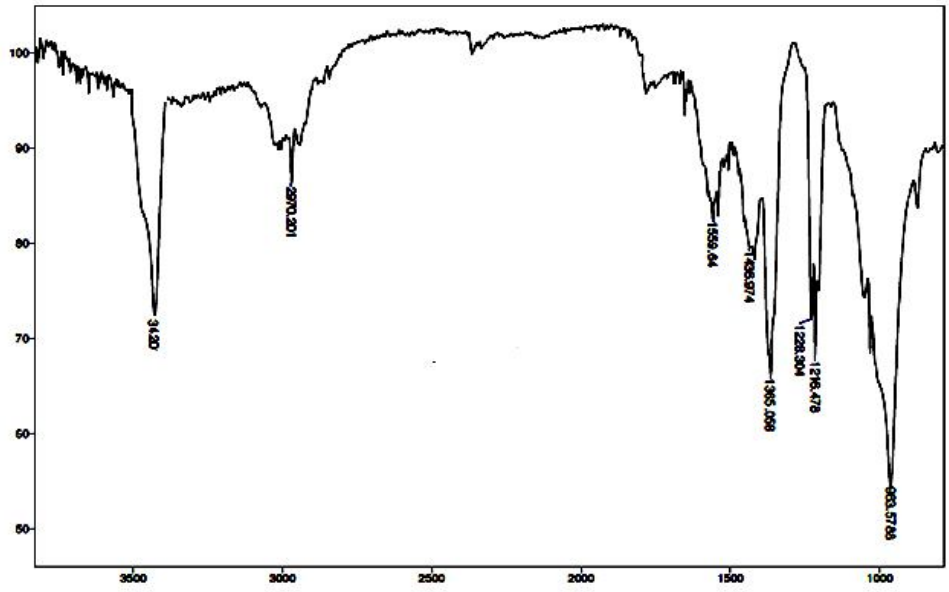


Figure 3: IR Spectrum of VN-3

Table 8: Interpretation of IR Spectrum of VN-3

S. No.	Wave number (cm^{-1})	Interpretation
1	3420	N-H stretching
2	2970	C-H stretching
3	1559 and 1436	C=C stretching (Aromatic)
4	1365	C-N stretching
5	1228	C-O stretching
6	1216	C=S stretching
7	1590	C=N stretching

Table 9: Interpretation of IR Spectrum of VN-4

S. No.	Wave number (cm^{-1})	Interpretation
1	3369	N-H stretching
2	2964	C-H stretching
3	1647 and 1458	C=C stretching (Aromatic)
4	1558	C=N stretching
5	1265	C=S stretching
6	Less than 667	C-Br stretching

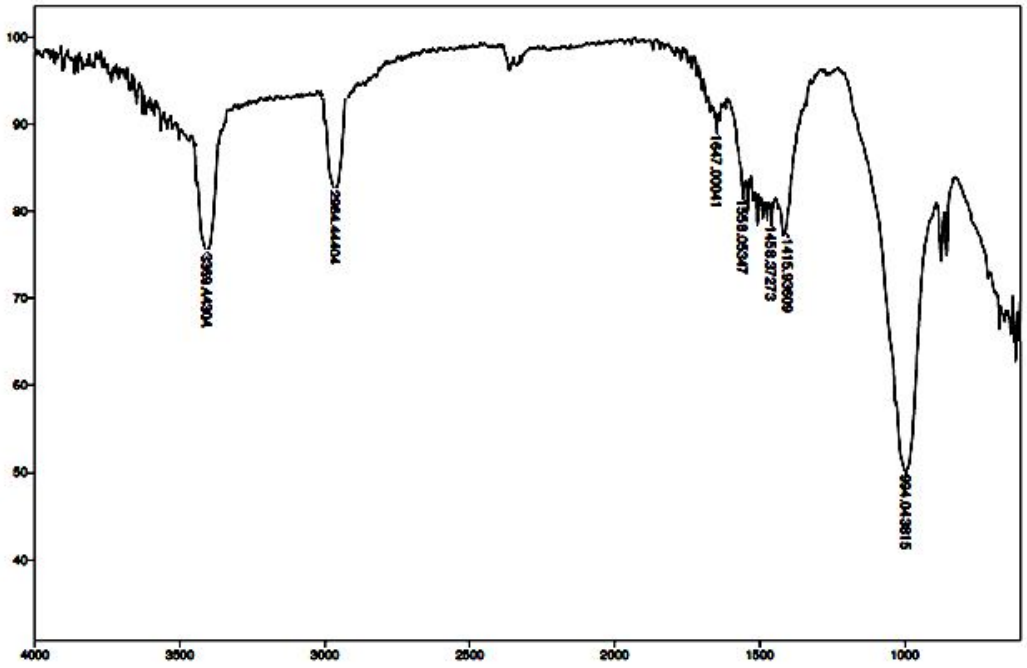


Figure 4: IR Spectrum of VN-4

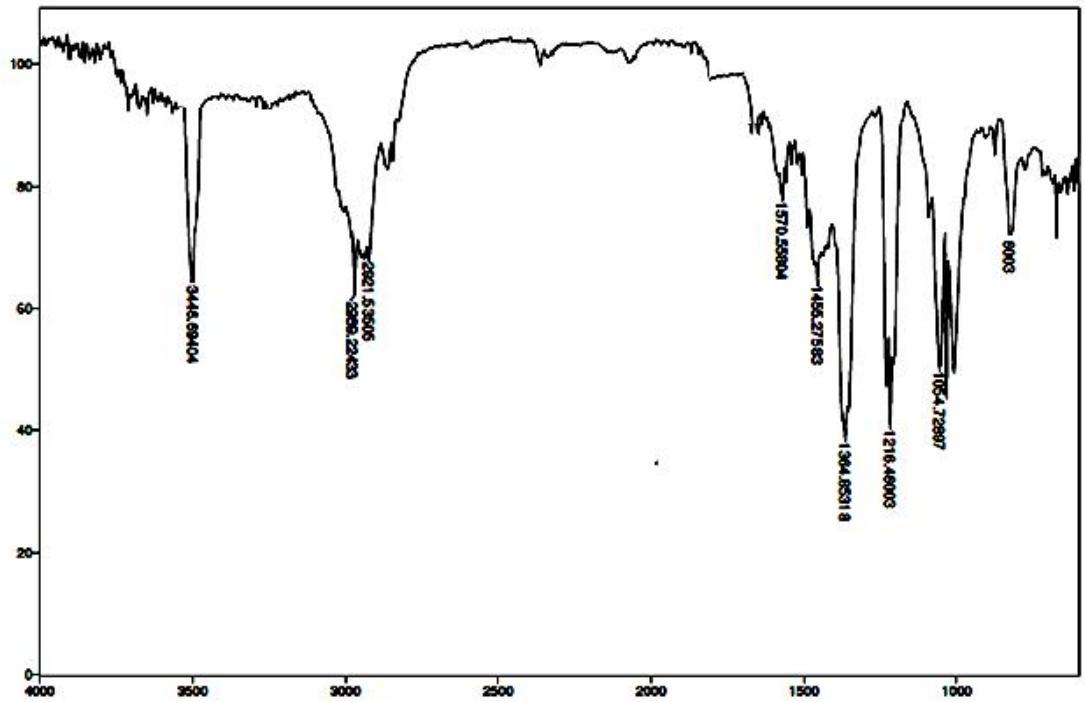


Figure 5: IR Spectrum of VN-5

Table 10: Interpretation of IR Spectrum of VN-5

S. No.	Wave number (cm^{-1})	Interpretation
1	3446	N-H stretching
2	2969, 2921	C-H stretching
3	1570 and 1455	C=C stretching (Aromatic)
4	1364	C-N stretching
5	1216	C=S stretching
6	785	C-Cl stretching
7	Less than 667	C-Br stretching

^{1}HNMR Spectroscopy

^{1}HNMR spectra were recorded at Indian Institute of Education and Science Research (IISER), Bhopal (M.P.), on a mercury plus 300 MH_Z NMR spectrometer in suitable solvent (CDCl3), with tetramethylsilane as internal standard. ^{1}H NMR spectra of VN-1 to VN-5 compounds are shown in figure 4.6 to 4.10 and their interpretation is reported in table 4.11 to 4.15.

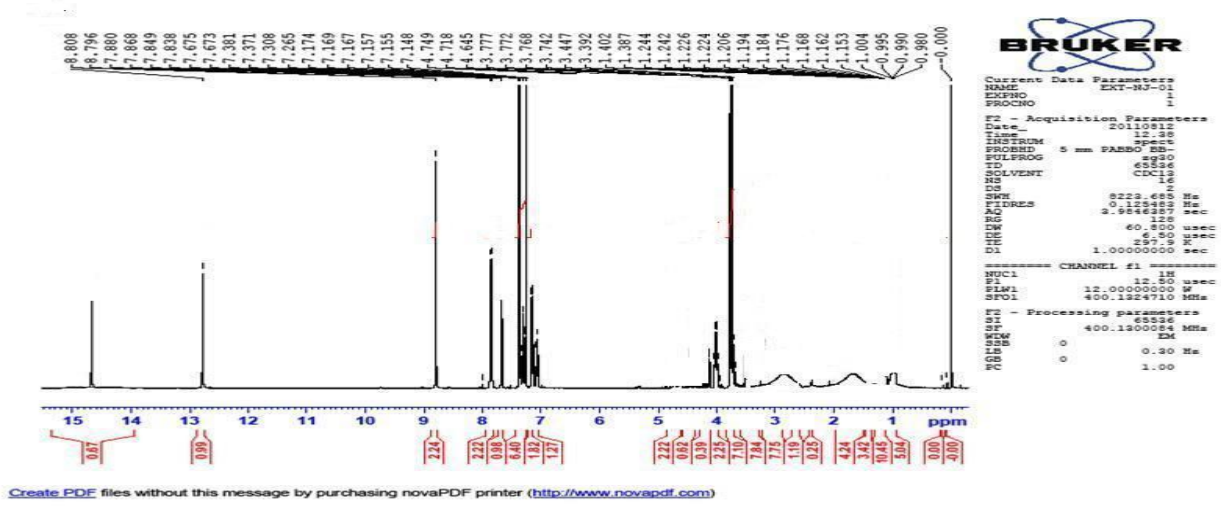


Figure 6: ^{1}H-NMR- spectrum of VN-1

Table 11: Interpretation of ^{1}H-NMR- spectrum of VN-1

^{1}H-NMR δ ppm (300 MHz)	Interpretation
2.0	s, 2H, S=C-N<u>H</u>$_2$
4.0	t, 2H, H_4C_6-N<u>H</u>$_2$
1.9	d, 2H, -C<u>H</u>$_2$- (4,5-dihydropyrazole)
3.9	m, 1H, -C<u>H</u>-(4,5-dihydropyrazole)
6.5-7.4	m, 4H, =C<u>H</u>- (benzylidinimin)
7.06-7.22	m, 4H, =C<u>H</u>- (benzene)

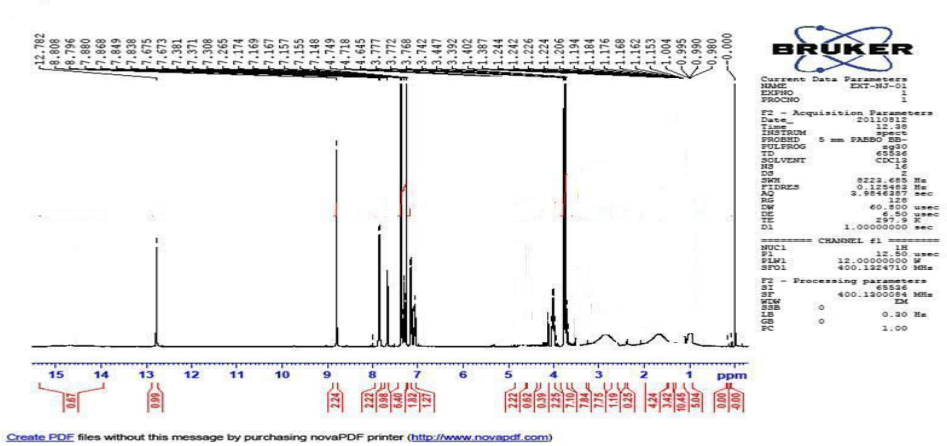


Figure 7: ^{1}H-NMR- spectrum of VN-2

Table 12: Interpretation of ^{1}H-NMR- spectrum of VN-2

^{1}H-NMR δ ppm (300 MHz)	Interpretation
2.0	s, 2H, S=C-N<u>H</u>$_2$
4.0	t, 2H, H_4C_6-N<u>H</u>$_2$
1.9	d, 2H, -C<u>H</u>$_2$- (4,5-dihydropyrazole)
3.9	m, 1H, -C<u>H</u>-(4,5-dihydropyrazole)
6.5-7.4	m, 4H, =C<u>H</u>- (benzylidinimin)
7.51-8.05	m, 4H, =C<u>H</u>- (benzene)

s= singlet, d= doublet, t= triplet, m= multiplet

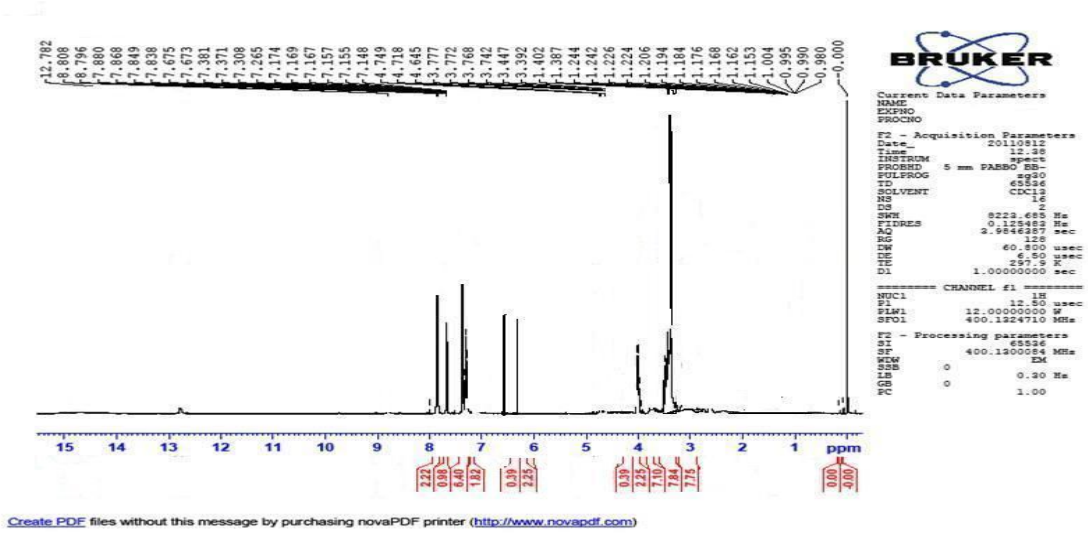


Figure 8: ^{1}H-NMR- spectrum of VN-3

Table 13: Interpretation of ^{1}H-NMR- spectrum of VN-3

^{1}H-NMR δ ppm (300 MHz)	**Interpretation**
2.0	s, 2H, S=C-N$\underline{H}_2$
4.0	t, 2H, H_4C_6-N$\underline{H}_2$
1.9	d, 2H, -C$\underline{H}_2$- (4,5-dihydropyrazole)
3.9	m, 1H, -C$\underline{H}$-(4,5-dihydropyrazole)
6.5-7.4	m, 4H, =C$\underline{H}$- (benzylidinimin)
6.52-6.61	m, 4H, =C$\underline{H}$- (benzene)
3.73	s, 6H, -(O-C$\underline{H}_3$)$_2$

s= singlet, d= doublet, t= triplet, m= multiplet

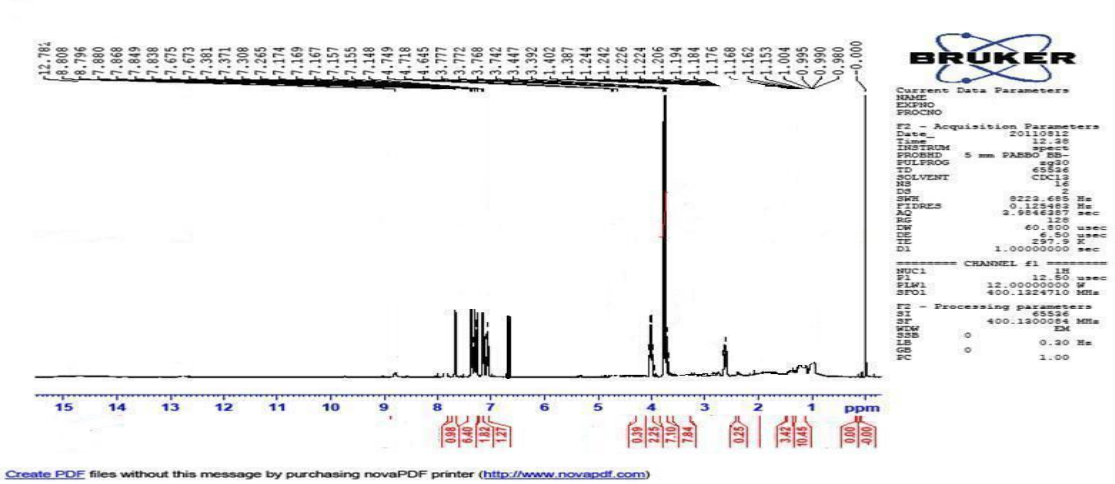


Figure 9: ^{1}H-NMR- spectrum of VN-4

Table 14: Interpretation of ^{1}H-NMR- spectrum of VN-4

^{1}H-NMR δ ppm (300 MHz)	**Interpretation**
2.0	s, 2H, S=C-N$\underline{H}_2$
3.73	s, 9H, -O-C$\underline{H}_3$(benzylidinimin); -O-(C$\underline{H}_3$)$_2$(benzene)
1.9	d, 2H, -C$\underline{H}_2$- (4,5-dihydropyrazole)
3.9	m, 1H, -C$\underline{H}$-(4,5-dihydropyrazole)
6.7-7.4	m, 4H, =C$\underline{H}$- (benzylidinimin)
6.52-6.61	m, 4H, =C$\underline{H}$- (benzene)

s= singlet, d= doublet, t= triplet, m= multiplet

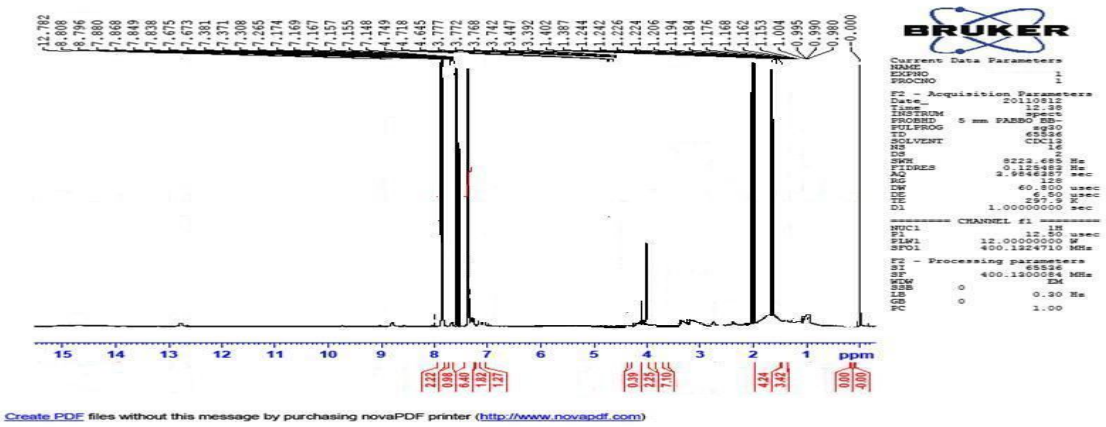


Figure 10: ^{1}H-NMR- spectrum of VN-5

Table 15: Interpretation of ^{1}H-NMR- spectrum of VN-5

^{1}H-NMR δ ppm (300 MHz)	**Interpretation**
2.0	s, 2H, S=C-N$\underline{H}_2$
1.9	d, 2H, -C$\underline{H}_2$- (4,5-dihydropyrazole)
3.9	m, 1H, -C$\underline{H}$-(4,5-dihydropyrazole)
7.5	m, 4H, =C$\underline{H}$- (benzylidinimin)
7.06-7.22	m, 4H, =C$\underline{H}$- (benzene)

s= singlet, d= doublet, t= triplet, m= multiplet

RESULT & DISCUSSION

Synthesis of planned compounds have been performed as shows in scheme 1. After synthesis, characterization of synthesized compounds has been carried out.

Melting points obtained were sharp which indicates the purity of the synthesized compounds.

The results of solubility study shows that all synthesized compounds are soluble in organic solvent. Compounds shows more solubility in polar solvents . This confirms lipophilic nature of the synthesized compounds.

The R_f values of synthesized compounds was determined by thin layer chromatography (TLC) using silica gel- G as stationary phase. A single and clear spot of compounds and values of R_f indicate that synthesized compounds are pure and contain little or no impurities.

The spectroscopic analysis was performed for synthesized compounds. UV spectra of synthesized compounds were determined on UV shimadzu 1700 spectrophotometer and determined λ_{max} of synthesized compounds.

The IR spectra of synthesized compounds were obtained from FTIR, 2000A, ABB spectrophotometer ($ZrCl_2$) in Smriti College of Pharmacy, Indore. The infra red spectra of each of the synthesized compounds shows characterisitic absorption in accordance to their structural functional groups.

The ^{1}H-NMR spectrums of synthesized compounds were obtained from IISER (Indian Institute of Science and Education Research), Bhopal. ^{1}H-NMR shows chemical shifts is in good agreement with the structure of the synthesized compounds.

All the above results comfirmed the synthesis of compounds.

ANTICONVULSANT ACTIVITY

Different type of epilepsies that is grandmal, petitmal or psychomotor type can be studied in laboratory animals. The models we used for Anti-convulsion test is given bellow.

Pentylenetetrazole-Induced Convulsions

Pentylenetetrazole (PTZ) is a central nervous system stimulant. It produces jerky type of clonic convulsions in rats and mice. The convulsive effect of this drug is considered to be analogus to petit mal type of convulsions in man. Recently PTZ has been reported to act through GABA-

benzodiazepine receptor mechanisms in the brain. It is widely used as a tool in experimental pharmacology to study convulsion and anticonvulsant action of drugs **(kulkarni, 2009).**

Procedure

Animals used: Swiss Albino Rats

No. of animals used: 8 animals in each group.

Dose of compound: 10, 20, 30, 40 & 50mg/kg

Dose of standard drug: 5mg/kg (diazepam)

Dose of convulsant (PTZ) drug: 90mg/kg.

Route of administration: intraperitoneal (i.p.)

1. Mice of both sexes of weight 25–30 g were selected and divided in groups. Each containing 8 mice.
2. Firstly, convulsant effect of Pentylenetetrazole on controlled group was studied by injected PTZ by i.p route.
3. Diazepam was given by i.p. route to second group, then after 30 mins PTZ was given and mice were observed for clonic convulsion (indicated by straub's tail, jerky movements of whole body & convulsions) for 60 mins.
4. Procedure third was repeated for compounds VN-1 to VN-5 with dosing 10, 20, 30, 40 & 50mg/kg till they started showing protection against convulsion.
5. Then, number of mice protected (i.e. not showing convulsion) were reported and percentage of protection was calculated by following formula.

$$\% \text{ of protection} = \frac{\text{number of convulsions of control} - \text{number of convulsions of control}}{\text{number of convulsions of control}} \text{x}100$$

Synthesized compounds were screened for their anticonvulsant activity by the Pentylenetetrazole (PTZ) induced seizures method.

Resultant data of *in vivo* study is given in Table 16 and the % of protection is illustrated in figure 11.

3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4,5-dihydro-pyrazole-1-carbothioicacid amide, (VN-2) revealed significant activity (75% protection), then 3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid (VN-2) showed 62.5% protection. Compound VN-3 showed mild/intermediate activity. Replacement of the 4-amino phenyl group by 4-bromo-phenyl and 2-bromo-4-methoxy-phenyl moiety as in VN-4 & VN-5 leads to decrease in % of protection of the compounds

VN-4 and VN-5 respectively. Moreover, the 3-(4-Amino-phenyl) derivatives **VN-1** and **VN-2** were found to be more active. The order of percentage protection is **VN-2 > VN-1> VN-3> VN-5 >VN-4.**

Table 16: data of *in vivo* study

S. No.	compounds		Response (number of protected mice)	Percentage of protection (%)
1	controlled		-	0
2	Diazepam (10 mg/kg)		7	87.5
3	VN-1	10mg/kg	-	62.5
		20mg/kg	-	
		30mg/kg	5	
4	VN-2	10mg/kg	-	75
		20mg/kg	-	
		30mg/kg	6	
5	VN-3	10mg/kg	-	50
		20mg/kg	-	
		30mg/kg	4	
6	VN-4	10mg/kg	-	25
		20mg/kg	-	
		30mg/kg	2	
7	VN-5	10mg/kg	-	37.5
		20mg/kg	-	
		30mg/kg	3	

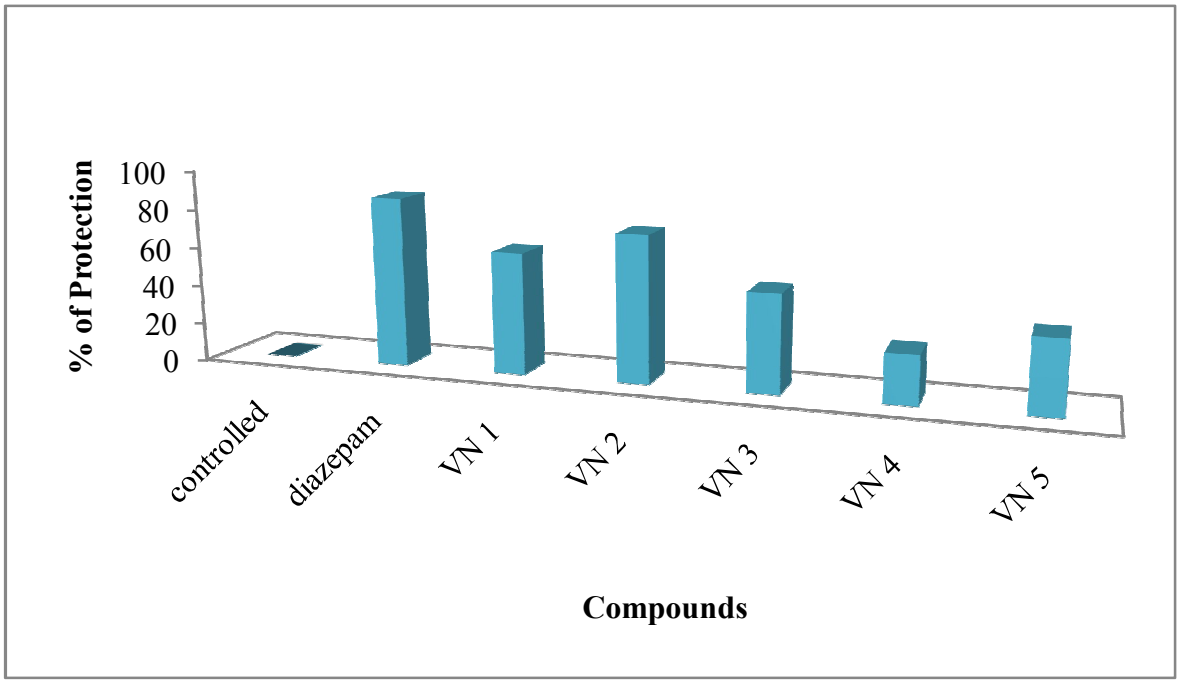


Figure 11: percentage of protection

Epilepsy is a disorder of brain electrical activity that results in recurrent seizures. The type of seizure depends on the portion of the brain affected. While there are many different causes of seizures, including brain tumor, head injury, stroke, and alcohol withdrawal, the discussion in this article is limited mainly to cases in which the cause is idiopathic (primary epilepsy). Conventional treatment of epilepsy consists primarily of anticonvulsant medications. Although these drugs often control or reduce the frequency of seizures, some patients show little or no improvement. A number of dietary modifications, nutritional supplements, and hormones have been found to be beneficial for some patients with epilepsy. Potentially useful dietary interventions include measures to stabilize blood glucose levels, identification and avoidance of allergenic foods, and avoidance of potential inciting agents (such as ethanol and aspartame). The ketogenic diet has been successful for many patients, but because of its highly restrictive nature and potential to cause significant adverse effects, its use is restricted to severe cases that fail to respond to other treatments. A less restrictive version of the ketogenic diet, the Atkins diet, has shown promise and deserves further study. Several different nutrients (and two hormones) may also be beneficial in selected patients with epilepsy. The fact that nutritional factors are involved in the regulation of electrical activity in the brain is indicated by the fact that severe deficiency of thiamine, magnesium, or vitamin B6 can cause seizures. A subnormal concentration of each of these nutrients has been found to be common in patients with epilepsy. While

the severity of these deficiencies is probably not great enough in most cases to cause seizures in otherwise healthy people, marginal status with respect to any of these nutrients could conceivably exacerbate a seizure disorder due to another cause. In addition, some patients with epilepsy might have a higher-than-normal requirement for one or more nutrients that play a role in brain electrical activity. That phenomenon has been clearly demonstrated in the case of vitamin B6-dependent epilepsy, a condition in which intractable seizures can be completely controlled by administration of large doses of vitamin B6. The existence of this relatively rare syndrome raises the possibility that more subtle forms of nutrient dependency occur more commonly. While mildly or moderately increased requirements for vitamin B6 or other nutrients may not by themselves be sufficient to cause seizures, a failure to meet these increased requirements could aggravate an existing seizure disorder. Some studies have found that supplementation with individual nutrients reduced seizure frequency or improved other aspects of health in patients with epilepsy, but other studies have failed to confirm those findings.

The newly synthesized compounds were screened for their anticonvulsant activity by the Pentylenetetrazole (PTZ) induced seizures method. Resultant data of in vivo study is given in Table 5.1 and the % of protection from convulsion is illustrated in figure 11.

3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid amide, (VN-2) revealed significant activity (75% protection), then 3-(4-Amino-phenyl)-5-(3-nitro-phenyl)-4, 5-dihydro-pyrazole-1-carbothioic acid (VN-2) showed 62.5% protection. Compound VN-3 showed mild/intermediate activity. Replacement of the 4-amino phenyl group by 4-bromo-phenyl and 2-bromo-4-methoxy-phenyl moiety as in VN-4 & VN-5 leads to decrease in % of protection of the compounds VN-4 and VN-5 respectively. Moreover, the 3-(4-Amino-phenyl) derivatives **VN-1** and **VN-2** were found to be more active. The order of percentage protection is **VN-2 > VN-1> VN-3> VN-5 >VN-4.**

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