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Synthesis and Anticonvulsant activity of Various Mannich and Schiff bases of 1,5-benzodiazepines

Surendra N. Pandeya and Neha Rajput*

Department of Pharmaceutical Sciences, Saroj Institute Of Technology & Management, Sultanpur road, Lucknow(U.P.), India.

ABSTRACT

Benzodiazepines have a various behavioural effects in addition to their anxiolytic action . There is every reason to believe that the BZ/GABA receptor complex is involved in these effects since gabamimetic manipulations modify the effect of BZ in tests of convulsive activity, motor function, and appetitive behavior. 1, 5- benzodiazepines are biologically important molecules and are extensively used clinically as analgesic, hypnotic, sedative and antidepressive agents. Hence 1,5-benzodiazepines were synthesized by condensation of o-phenylenediamine and ketones e.g., cyclohexanone and acetone in presence of Sulfated Zirconia (catalyst).Mannich bases were synthesized with acetophenone, p-nitroacetophenone, p-chloroacetophenone and formaldehyde. Schiff bases were synthesized using Mannich base of 1,5-benzodiazepines with p-chloroaniline and p-chlorophenylsemicarbazide in presence of glacial acetic acid. All the synthesized compounds were characterized by ^{1}H NMR and IR spectral analysis. All the synthesized derivatives were evaluated at the dose of 30mg/kg b.w for anticonvulsant activity by isoniazid induced convulsion model and the compounds NBZD-3 & NBZD-8 were found to be most active among all compounds . Among all the synthesized derivatives, compounds NBZD-13, NBZD-17 were found to be most active among all compounds using thiosemicarbazide induced model. NBZD-8, NBZD-10, NBZD-18 are the compounds which had shown good anticonvulsant activity and have advantage over that, they were not sedative.

Keywords: 1,5-benzodiazepines, Anxiolytics, Antidepressive, Cyclohexanone, Sulfated zirconia.

INTRODUCTION

A benzodiazepine is a psychoactive drug whose core chemical structure is the fusion of a benzene ring and a diazepine ring. The first benzodiazepine, chlordiazepoxide (Librium) , discovered accidentally by Leo Sternbach in 1955, & made available in 1960 by Hoffmann La Roche,which has also marketed diazepam (valium) since 1963[1]. 1, 5- benzodiazepines constitute an important class of psychopharmaca[2], in particular as tranquilizers & also as potent Virucides & non – nucleoside inhibitors of HIV -1 reverse transcriptase [3].

Benzodiazepines has a traditional place in antiepileptic therapy. The clinical use of BZDs can divided into two categories. First in the acute treatment of seizures as drugs of choice in status epilepticus and also in some cases of febrile seizures. Second, the BZDs are utilized in long term therapy of certain seizures types primarily in the paediatrics population[4].
There are some differences between the effects of 1,5- and 1,4- benzodiazepines. A greater therapeutic potential and lower incidence of side effects were described for 1,5-BZDs when compared to 1,4-BZDs .1,5- BZD is used as adjuvant therapy in resistant cases of epilepsies[5]. BZDs exhibit potent anticonvulsant actions in a wide variety of animal seizures models. They are particularly effective against seizures induced by electroshock[6], various chemoconvulsants , in kindled seizures and in absence seizures [7].

Beside this 1,5-benzodiazepines show antifungal , antibacterial [8], antifeedant [9], anti inflammatory analgesic [10] & anticonvulsant activities [11]. The benzodiazepines nucleus is a well studied traditional pharmacophoric scaffold that has emerged as a core structure unit of various biological activity [12].

Although, the first benzodiazepine was introduced as a drug nearly 35 years ago, the research in this area is still very active & is directed towards the synthesis of compounds with enhanced pharmacological activity[13].The chemical structure of the benzodiazepines seems at first sight to be unique among the various types of central depressant drugs[14].1, 5-benzodiazepines derivatives shows a large number of pharmacological properties such as they acted as sedatives[15], Cerebrovasodiatotors [16], neuroleptics[17], antispasmodic [18], anticonvulsant [19], tranquilizing agents[20], antibacterial [21], psoriasis[22] & for treatment of small pox[23].

MATERIALS AND METHODS

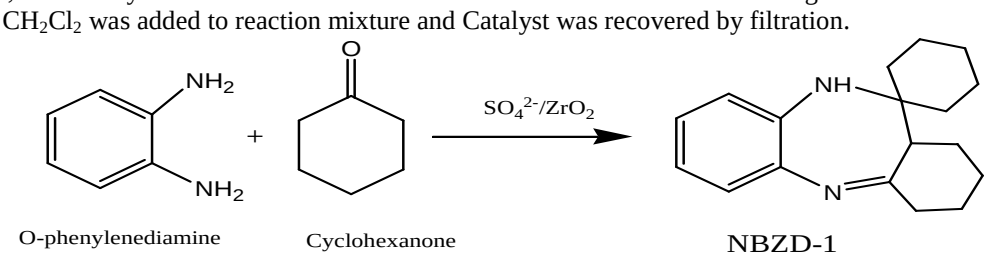
Starting material and reagents were procured from commercial chemical suppliers. All the chemicals and solvents used were of laboratory grade. Melting points were determined in open capillary tubes and are uncorrected. IR spectra (KBr, cm^{-1}) were recorded on Perkin Elmer Spectrometer, 1H NMR(δ, ppm) spectra was recorded on a Brucker 300 MHz NMR spectrometer using TMS as an internal standard. The purity of compounds and progress of the reaction was checked by TLC using silica gel-G as adsorbent.

2.1) Synthesis of fused ring benzodiazepine nucleus.

2.1.a). Synthesis of fused ring benzodiazepine nucleus : Synthesis of fused ring benzodiazepines in presence of Sulphated Zirconia involves 2 steps which are as follows[9]:

Preparation of catalyst: 25 gm of Zirconium Oxychloride was dissolved in doubly distilled water (pH-2). Dilute aq. Ammonia was then added drop wise from a burette with vigorous (pH= 8). Precipitate was washed with distilled water several times and dried for 24 h. Sample was ground to fine powder and immersed in an 0.5 M H_2SO_4 solution (30 ml) for 30 min. Excess water was evaporated on water bath and the resulting sample was oven dried.

Synthesis of benzodiazepines: 1:2.5 mole ratio mixture of o-phenylenediamine and ketone **(**Cyclohexanone **(scheme 1)** , with catalytic amount of sulfated zirconia were taken in RBF with stirring at ambient condition for 2-3 h. 10 ml of CH_2Cl_2 was added to reaction mixture and Catalyst was recovered by filtration.

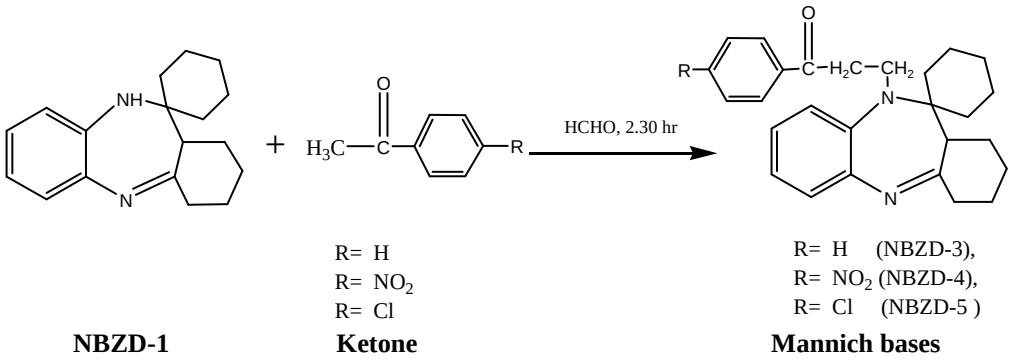


Scheme 1: Synthesis of fused ring benzodiazepine

2.2) Procedure for preparation of Mannich base derivatives:

2.2.a). Synthesis of various Mannich base derivatives of fused ring benzodiazepine (Scheme 2).

Equimolar quantity of fused ring benzodiazepine **(NBZD-1, 0.01M)**, formaldehyde, and various acetophenone (i.e., acetophenone, p-nitroacetophenone, p-chloroacetophenone) were taken in RBF and mixture was refluxed for 2.30 h. Completion of reaction was monitored by TLC analysis for several times. Then reaction mixture was evaporated on water bath and dried. Melting point, Rf value, and % yield were noted. Various Mannich base derivatives are shown in **Scheme 2.**

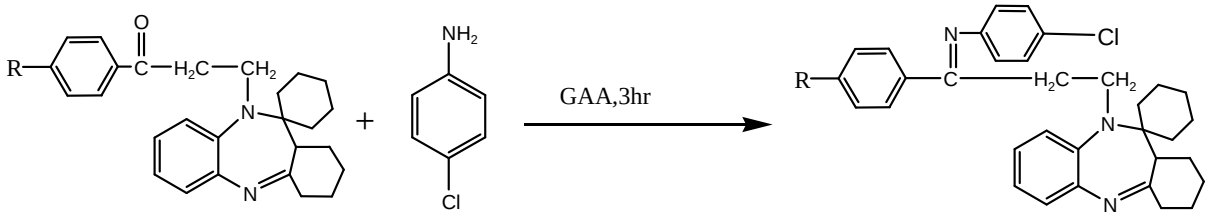


Scheme 2: Synthesis of various Mannich base derivatives of fused ring benzodiazepines.

2.3) Procedure for preparation of Schiff base derivatives:

2.3.a). Synthesis of various Schiff base derivatives of fused ring benzodiazepines

Equimolar quantities of Mannich base derivatives **(0.01M, NBZD-3,NBZD-4, NBZD-5)** in individual reactions , were dissolved in glacial acetic acid and added with p-chloroaniline **(Scheme 3)** or p-chlorophenylsemicarbazide **(Scheme 4)** were taken in RBF and mixture was refluxed for 3 h respectively. Completion of reaction was monitored by TLC analysis for several times in chloroform: ethanol (1:1). Then reaction mixture was evaporated on water bath and dried. Melting point, Rf value, and % yield were noted..

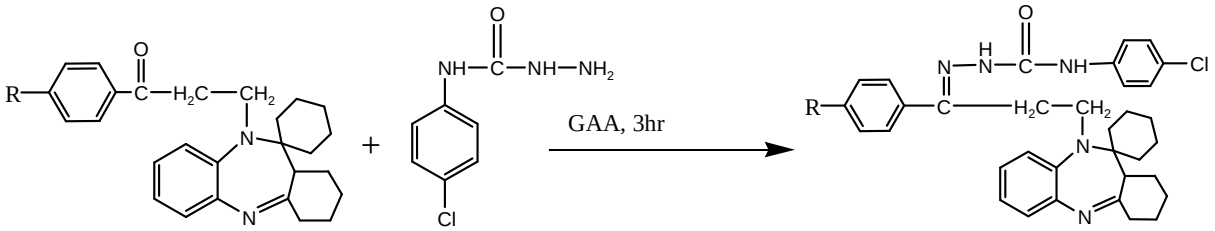


R= H (NBZD-3), R= NO_2 (NBZD-4), R= Cl (NBZD-5) — **Mannich base of 1,5- BZD**

p-chloroaniline

R= H (NBZD-6), R= NO_2 (NBZD-7), R= Cl (NBZD-8) — **Schiff base of 1,5- BZD**

Scheme 3: Synthesis of Schiff base derivative of fused ring benzodiazepine from p-chloroaniline.



R= H (NBZD-3), R= NO_2 (NBZD-4), R= Cl (NBZD-5) — **Mannich base of fused ring BZD**

p-chlorophenylsemicarbazide

R= H (NBZD-9), R= NO_2 (NBZD-10), R= Cl (NBZD-11) — **Schiff base of fused ring BZD**

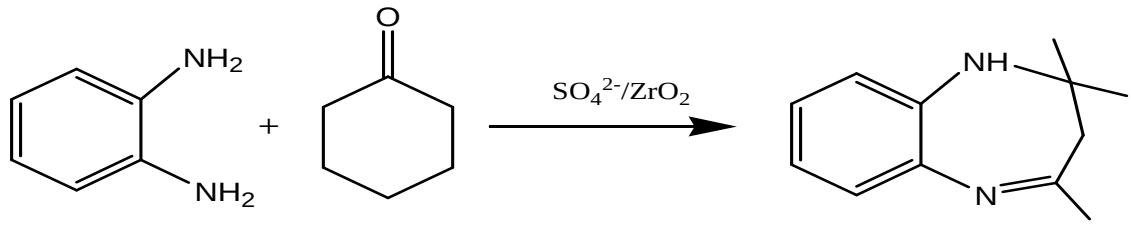
Scheme 4: Synthesis of Schiff base derivative of fused ring benzodiazepine from p-chlorophenylsemicarbazide.

2.4) Synthesis of 1,5- benzodiazepine nucleus.

2.4.a). Synthesis of 1,5- benzodiazepine nucleus : Synthesis of 1,5- benzodiazepines in presence of Sulphated Zirconia involves 2 steps which are as follows[9]:

Preparation of catalyst: 25 gm of Zirconium Oxychloride was dissolved in doubly distilled water (pH-2). Dilute aq. Ammonia was then added drop wise from a burette with vigorous (pH= 8). Precipitate was washed with distilled water several times and dried for 24 h. Sample was ground to fine powder and immersed in an 0.5 M H_2SO_4 solution (30 ml) for 30 min. Excess water was evaporated on water bath and the resulting sample was oven dried.

Synthesis of benzodiazepines: 1:2.5 mole ratio mixture of o-phenylenediamine and ketone **(**Cyclohexanone **(scheme 5)** , with catalytic amount of sulfated zirconia were taken in RBF with stirring at ambient condition for 2-3 h. 10 ml of CH_2Cl_2 was added to reaction mixture and Catalyst was recovered by filtration.



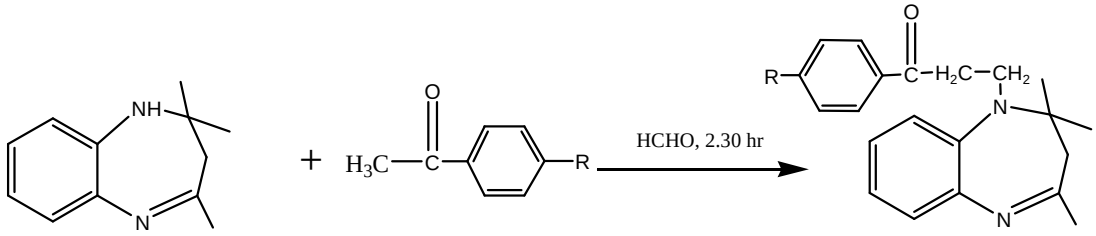
O-phenylenediamine — Cyclohexanone — NBZD-12

Scheme 5: Synthesis of 1,5- benzodiazepine (NBZD-12)

2.5) Procedure for preparation of Mannich base derivatives:

2.5.a). Synthesis of various Mannich base derivatives of 1,5- benzodiazepine (Scheme 6).

Equimolar quantity of fused ring benzodiazepine **(NBZD-12, 0.01M)**, formaldehyde, and various acetophenone (i.e., acetophenone, p-nitroacetophenone, p-chloroacetophenone) were taken in RBF and mixture was refluxed for 2.30 h. Completion of reaction was monitored by TLC analysis for several times. Then reaction mixture was evaporated on water bath and dried. Melting point, Rf value, and % yield were noted. Various Mannich base derivatives are shown in **Scheme 6.**



R= H
R= NO_2
R= Cl

R= H (NBZD-13),
R= NO_2 (NBZD-14),
R= Cl (NBZD-15)

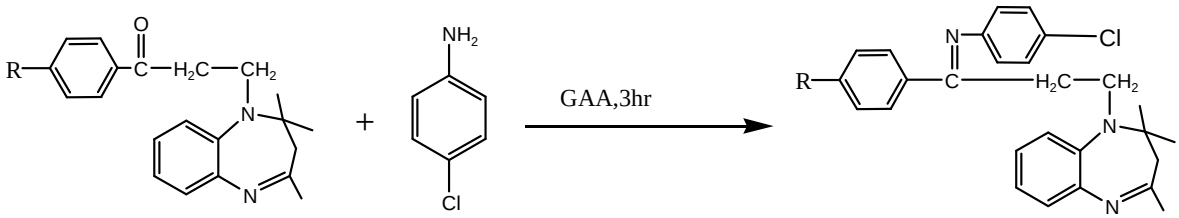
NBZD-1 **Ketone** **Mannich bases**

Scheme 6: Synthesis of various Mannich base derivatives of 1,5- benzodiazepines.

2.6) Procedure for preparation of Schiff base derivatives:

2.6.a). Synthesis of various Schiff base derivatives of 1,5- benzodiazepines

Equimolar quantities of Mannich base derivatives **(0.01M, NBZD-13,NBZD-14, NBZD-15)** in individual reactions , were dissolved in glacial acetic acid and added with p-chloroaniline **(Scheme 7)** or p-chlorophenylsemicarbazide **(Scheme 8)** were taken in RBF and mixture was refluxed for 3 h respectively. Completion of reaction was monitored by TLC analysis for several times in chloroform: ethanol (1:1). Then reaction mixture was evaporated on water bath and dried. Melting point, Rf value, and % yield were noted..

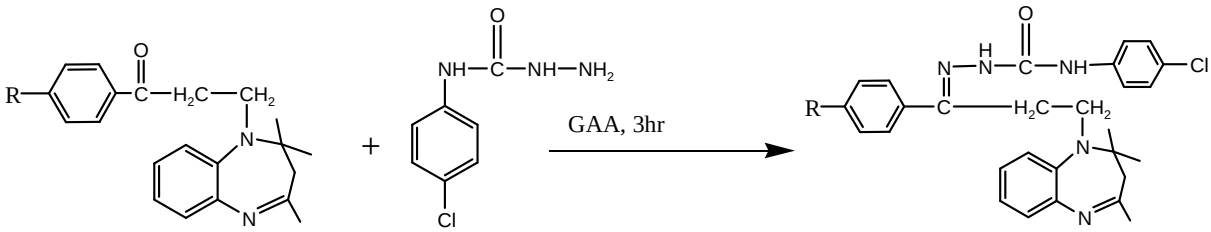


R= H (NBZD-13),
R= NO_2 (NBZD-14),
R= Cl (NBZD-15)

R= H (NBZD-16),
R= NO_2 (NBZD-17),
R= Cl (NBZD-18)

Mannich base of 1,5- BZD **p-chloroaniline** **Schiff base of 1,5- BZD**

Scheme 7: Synthesis of Schiff base derivative of 1,5-benzodiazepine from p-chloroaniline.



R= H (NBZD-13),
R= NO_2 (NBZD-14),
R= Cl (NBZD-15)

R= H (NBZD-19),
R= NO_2 (NBZD-20),
R= Cl (NBZD-21)

Mannich base of 1,5- BZD **p-chlorophenylsemicarbazide** **Schiff base of 1,5- BZD**

Scheme 8: Synthesis of Schiff base derivative of 1,5-benzodiazepine from p-chlorophenylsemicarbazide.

ANTICONVULSANT ACTIVITY

4.1 Chemical induced model :-

➢ Ten mice of either sex with a weight of 22-25g were treated with the test compounds (30 mg/kg b.w) or the standard (e.g. diazepam 10 mg/kg b.w) by i.p administration. Controls received the vehicle only. 30 min after i.p. treatment the animals were injected with a subcutaneous dose of (300 mg/kg, s.c) isoniazid, thiosemicarbazide (20mg/kg, s.c), The occurrence of clonic seizures, tonic seizures and death or recovery were recorded after 0.5hr, 1hr, 2hr, & 4hr respectively for Isoniazid induced convulsion (Table 2) and also Thiosemicarbazide induced convulsion (Table 3).

4.2 NEUROTOXICITY SCREEN

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotates at 20 revolutions per minute. The rod diameter was 3.2 cm. neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of three trials. The dose at which the animals were unable to grasp the rotorod, was determined (Table 2,3).

➢ DOSE : Test drug : 30 mg/Kg bw i.p

4.3 SEDATIVE–HYPNOTIC ACTIVITY

➢ This test was performed with the test substances in a dose of 30 mg/kg by phenobarbitone induced narcosis in rats. The compounds in PEG(Polyethylene gycol) were administered i.p to a group of six rats. After 30 min, rats were then placed on their back and loss of righting reflex was taken as onset of sleep. The time taken by the rats to awake was noted. A control was also performed after pretreatment with test substances vehicle (PEG) and injected phenobarbitone(Table 4) .

RESULTS

3.1 Physicochemical Characterization

Table 1 : Physicochemical data of synthesized compounds

Compound Code	Molecular Formula	Molecular weight	Melting Point	% yield	Rf Value	Log P Value
NBZD-1	$C_{18}H_{24}N$	268.19	110	76.48	0.689	4.25
NBZD-3	$C_{27}H_{32}N_2O$	400.25	92	85.7	0.78	6.25
NBZD-4	$C_{33}H_{36}N_3Cl$	510.12	132	83.3	0.73	6.15
NBZD-5	$C_{34}H_{28}ClN_5O$	568.15	140	72	0.83	6.81
NBZD-6	$C_{27}H_{31}N_3O_3$	445.55	98	84.3	0.63	8.93
NBZD-7	$C_{27}H_{31}ClN_2O$	435.00	98	79.2	0.82	6.70
NBZD-8	$C_{33}H_{35}ClN_4O_2$	554.24	130	67.2	0.57	9.49
NBZD-9	$C_{34}H_{37}ClN_6O_3$	612.26	126	56.98	0.52	8.07
NBZD-10	$C_{33}H_{35}Cl_2N_3$	543.22	136	65.8	0.54	9.56
NBZD-11	$C_{34}H_{37}Cl_2N_5O$	601.24	130	66	0.66	8.62
NBZD-12	$C_{12}H_{16}N_2$	188.60	96	72	0.68	2.22
NBZD-13	$C_{21}H_{24}N_2O$	320.3	98	72.32	0.72	4.22
NBZD-14	$C_{21}H_{23}N_3O_3$	365.43	102	92.1	0.72	4.83
NBZD-15	$C_{21}H_{24}N_2O$	320.43	110	57.6	0.75	4.78
NBZD-16	$C_{21}H_{23}ClN_2O$	354.87	108	82.9	0.73	6.90
NBZD-17	$C_{27}H_{28}ClN_3$	429.98	140	90.4	0.78	7.20
NBZD-18	$C_{27}H_{27}ClN_4O_2$	474.98	136	64.06	0.75	7.46
NBZD-19	$C_{27}H_{27}Cl_2N_3$	464.43	142	85.6	0.63	6.04
NBZD-20	$C_{27}H_{27}ClN_4O_2$	474.18	138	67.9	0.55	-
NBZD-21	$C_{28}H_{29}ClN_6O_3$	532.20	142	65.2	0.76	6.59

Rf value : Solvent system ; chloroform: methanol 1:1.

3.2 REPRESENTATIVE SPECTRAL ANALYSIS:

1. 10-Spirocyclohexane-1,2,3,9,10,10a hexahydro benzo[b cyclohexane [e] [1,4] diazepine (NBZD-1) :

^{1}H NMR (300 MHz , δ) : CH (m, 6.4-7.0, 4H, phenyl), NH(s, 4.1, 1H), CH_2(m, 1.2-1.6, 18H, Cyclohexane), CH(s, 2.7, 1H, Diazepine ring). **IR (KBr) :** NH (Ar, 3030 cm^{-1}, str), CH (Ar, 3180cm^{-1}, str), CH(Ar, 800cm^{-1}, bend) ,C=N (1618cm^{-1}, Str)CH_2 (1490 cm^{-1}, str), C-C (Ar, 1600cm^{-1}), C=C (Ar,1410,1500,1580cm^{-1}) .

2. 1-Phenyl-3-(10-Spirocyclohexane-1,2,3,9,10,10a-hexahydrobenzo[b]cyclohexane [e][1,4]diazepine-1-yl) propan-1-one (NBZD-3) :

^{1}H NMR (300 MHz , δ) : CH(m, 7.3-7.9, 5H, Acetophenone), CH_2(s, 2.8, 2H, -$COCH_2$), CH_2 (s, 3.5, 2H, -$NHCH_2$), CH (m, 6.4-7.0, 4H, phenyl), CH_2(m, 1.2-1.5, 18H, Cyclohexane), CH(s, 2.5, 1H, Diazepine ring).

IR (KBr) : C=O (1700cm^{-1}, str), CH (Ar, 3180cm^{-1}, str), CH(Ar, 810cm^{-1}, bend) , C=N (1618cm^{-1}, Str) , CH_2 (1490 cm^{-1}, str), C-C (Ar, 1600cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1})

3. 1-(4-Nitrophenyl)-3-(10-Spirocyclohexane-1,2,3,9,10,10a hexahydro benzo[b]cyclohexa[e][1,4]diazepine-1-yl) propan-1-one (NBZD-4).
^{1}H NMR (300 MHz , δ) : CH(m, 8.1-8.2 , 4H, p-nitroacetophenone), CH_2(s, 2.7, 2H, -$COCH_2$), CH_2 (s, 3.5, 2H, -$NHCH_2$), CH (m, 6.4-7.0, 4H, phenyl), NH(s, 4.0, 1H,), CH_2(m, 1.22-1.59, 18H, Cyclohexane), CH(s, 2.7, 1H, Diazepine ring). **IR (KBr) :** C=O (1710cm^{-1}, str), CH (Ar, 3150cm^{-1}, str), CH(Ar, 800cm^{-1}, bend) , , C=N (1658cm^{-1}, Str), CH_2 (1490 cm^{-1}, str), C-C (Ar, 1610cm^{-1}), C=C (Ar, 1410,1560,1580cm^{-1}) , N-O (1350cm^{-1}, str) .

4. (4-Chloro-phenyl)-[1-Chlorophenyl-3-10-Spirocyclo hexane -1,2,3,9,10,10a-hexahydrobenzo[b]cyclo hexane [e][1,4]diazepine-1yl)-propylidene] –amine (NBZD-8)
^{1}H NMR (300 MHz , δ) : CH(m, 7.30-7.5, 4H, p-chloroacetophenone), CH(m, 7.2-7.3, 4H, p-chloroaniline), CH_2(s, 1.6, 2H, -$COCH_2$), CH_2 (s, 3.4, 2H, -$NHCH_2$), CH (m, 6.6-7.1, 4H, phenyl), CH_2(m, 1.3-1.5, 18H, Cyclohexane), CH(s, 2.7, 1H, Diazepine ring). **IR (KBr) :** C=N (1569 cm^{-1}, str), C-Cl (727cm^{-1}, str),C-Cl (760cm^{-1}),C-H (2975cm^{-1}, str assym), CH(1383.9cm^{-1}, def sym.), C-H (Ar, 3072cm^{-1}, str), CH (Ar, 3150cm^{-1}, str), CH(Ar, 860cm^{-1}, bend) , , C=N (1678cm^{-1}, Str)CH_2 (1490 cm^{-1}, str), C-C (Ar, 1600cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1}) .

5. (4-Chlorophenylhyrazinecarboxamide)[1-Nitrophenyl-3-(10-Spirocyclo hexane-1,2,3,9,10,10a-hexahydro benzo [b] cyclohexane [e][1,4] diazepine-1-yl)-propylidine]-amine (NBZD-10)
^{1}H NMR (300 MHz , δ) : CH(m,7.9-8.2 , 4H, p-nitroacetophenone), NH (s, 7.0,1H, =NNH, p-chlorophenylsemicarbazide), NH (s, 6.0, 1H, -NHC_6H_4Cl, p-chlorophenylsemicarbazide), CH(m, 7.2-7.6,4H, p-chlorophenylsemicarbazide), CH (m, 6.6-7.1, 4H, phenyl), CH_2(m, 1.3-1.5, 18H, Cyclohexane), CH(s, 2.3, 1H, Diazepine ring), CH_2(s, 1.6, 2H, -$COCH_2$), CH_2 (s, 3.4, 2H, -$NHCH_2$). **IR (KBr) :** C=N (1569 cm^{-1}, str), C-Cl (728cm^{-1}, str), C-H (2970cm^{-1}, str assym), CH(1353.9cm^{-1}, def sym.), C-H (Ar, 3062cm^{-1}, str), CH (Ar, 3180cm^{-1}, str), CH(Ar, 810cm^{-1}, bend) , , C=N (1638cm^{-1}, Str)CH_2 (1490 cm^{-1}, str), C-C (Ar, 1680cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1}) , NO (1380cm^{-1}, str)

6. (4-Chlorophenylhyrazinecarboxamide)[1-Nitrophenyl-3-(10-Spirocyclo hexane-1,2,3,9,10,10a-hexahydro benzo [b] cyclohexane [e][1,4] diazepine-1-yl)-propylidine]-amine (NBZD-11) :
^{1}H NMR (300 MHz , δ) : CH(m,7.6-7.7 , 4H, p-chloroacetophenone), NH (s, 9.0,1H, =NNH, p-chlorophenyl semicarbazide), NH (s, 6.0, 1H, -NHC_6H_4Cl, p-chlorophenyl semicarbazide), CH(m, 7.2-7.6,4H, p-chlorophenylsemicarbazide), CH (m, 6.6-7.1, 4H, phenyl), CH_2(m, 1.3-1.6, 18H, Cyclohexane), CH(s, 2.7, 1H, Diazepine ring), CH_2(s, 1.6, 2H, -$COCH_2$), CH_2 (s, 3.4, 2H, -$NHCH_2$). **IR (KBr) :** C=N (1559 cm^{-1}, str), C-Cl (787cm^{-1}, str),C-Cl (769cm^{-1}),C-H (2975cm^{-1}, str assym), CH(1353.9cm^{-1}, def sym.), C-H (Ar, 3062cm^{-1}, str), CH (Ar, 3180cm^{-1}, str), CH(Ar, 880cm^{-1}, bend) , , C=N (1638cm^{-1}, Str)CH_2 (1490 cm^{-1}, str), C-C (Ar, 1600cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1}) .

7. (1-Phenyl-3-(2,2,4-trimethyl-2,3-dihydro-benzo [b][1,4] diazepin-1-yl)-propan-1-one) (NBZD-13).
^{1}H NMR (300 MHz , δ) : CH(m, 7.3-7.8, 5H, Acetophenone), CH_2(s, 2.7, 2H, -$COCH_2$), CH_2 (s, 3.5, 2H, -$NHCH_2$), CH (m, 6.6-7.1, 4H, phenyl), 2xCH_3 (s, 1.28, 6H) , CH_3 (s,0.9, 3H) , CH_2 (s, 2.5, 2H, Diazepine ring).
IR (KBr) : C=O (1700cm^{-1}, str), NH (Ar, 3230 cm^{-1}, str), CH (Ar, 3180cm^{-1}, str), CH(Ar, 800cm^{-1}, bend) , , C=N (1618cm^{-1}, Str), CH_3 (2980cm^{-1}, str), C-C (Ar, 1610cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1}) .

8. 1-(4-Chloro-phenyl)-3-(2,2,4-trimethyl-2,3-dihydrobenzo[b] [1,4]diazepin-1-yl)-propan-1-one (NBZD-15).
^{1}H NMR (300 MHz , δ) : CH(m, 7.3-7.8, 4H, p-chloracetophenone), CH_2(s, 2.78, 2H, -$COCH_2$), CH_2 (s, 3.5, 2H, -$NHCH_2$), CH (m, 6.6-7.1, 4H, phenyl), 2xCH_3 (s, 1.2, 6H) , CH_3 (s,0.9, 3H) , CH_2 (s, 2.4, 2H, Diazepine ring).
IR (KBr) : C=O (1710cm^{-1}, str), NH (Ar, 3030 cm^{-1}, str), CH (Ar, 3280cm^{-1}, str), CH(Ar, 800cm^{-1}, bend) , , C=N (1618cm^{-1}, Str), CH_3 (2990cm^{-1}, str), C-C (Ar, 1600cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1}) , C-Cl (760cm^{-1})

9. (4-Chloro-phenyl)-[1-(4-nitrophenyl)-3-(2,2,4-trimethyl- 2,3-di hydro - benzo [b] [1,4] dizepine-1-yl) -propylidene (NBZD-17).
^{1}H NMR (300 MHz , δ) : CH(m, 7.8-8.2, 4H, p-nitrocetophenone),CH(m, 7.2-7.3, 4H, p-chloroaniline), CH_2(s, 1.6, 2H, -N=C-CH_2), CH_2 (s, 3.4, 2H, -$NHCH_2$), CH (m, 6.6-7.1, 4H, phenyl), 2xCH_3 (s, 1.2, 6H) , CH_3 (s,0.9, 3H) , CH_2 (s, 2.5, 2H, Diazepine ring). **IR (KBr) :**C=N (1599 cm^{-1}, str), C-Cl (728cm^{-1}, str), C-H (2985cm^{-1}, str assym), CH(1353.9cm^{-1}, def sym.), C-H (Ar, 3062cm^{-1}, str), CH (Ar, 3180cm^{-1}, str), CH(Ar, 800cm^{-1}, bend) , , C=N (1618cm^{-1}, Str), CH_3 (2990cm^{-1}, str), C-C (Ar, 1600cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1}) , NO (1350cm^{-1}, str)

3.3 Anticonvulsant activity using chemical induced method

TABLE 2: Anticonvulsant activity using Isoniazid induced convulsion model

Compound Code	Isoniazid induced model			Neurotoxicity study	
	0.5 hr	1hr	2hr	1hr	4hr
				Dose : 30mg/kg	
NBZD-1	30mg	30mg	Not protected	NN	NN
NBZD-3	30mg	30mg	30mg	NN	NN
NBZD-4	30mg	Not protected	Not protected	NN	NN
NBZD-5	30mg	Not protected	Not protected	NN	NN
NBZD-6	30mg	30mg	Not protected	NN	NN
NBZD-7	30mg	Not protected	Not protected	NN	NN
NBZD-8	30mg	30mg	30mg	NN	NN
NBZD-9	30mg	Not protected	Not protected	NN	NN
NBZD-10	30mg	30mg	Not protected	NN	NN
NBZD-11	30mg	30mg	Not protected	NN	NN
CONTROL	--------	-------	-------		

Note: Symbol (NN) indicates no neurotoxicity at 30 mg /kg B.W

TABLE 3: Anticonvulsant activity using Thiosemicarbazide induced convulsion model

Compound Code	Thiosemicarbazide induced model			Neurotoxicity study	
	0.5 hr	1hr	2hr	1hr	4hr
				Dose : 30mg/kg	
NBZD-12	30mg	30mg	Not protected	NN	NN
NBZD-13	30mg	30mg	30mg	NN	NN
NBZD-14	30mg	Not protected	Not protected	NN	NN
NBZD-15	30mg	Not protected	Not protected	NN	NN
NBZD-16	30mg	30mg	Not protected	NN	NN
NBZD-17	30mg	Not protected	Not protected	NN	NN
NBZD-18	30mg	30mg	30mg	NN	NN
NBZD-19	30mg	Not protected	Not protected	NN	NN
NBZD-20	30mg	Not protected	Not protected	NN	NN
NBZD-21	30mg	30mg	Not protected	NN	NN
CONTROL	--------	-------	-------		

Note: Symbol (NN) indicates no neurotoxicity 30 mg/kg of B.W

3.4 SEDATIVE ACTIVITY

Table 13: Sedative activity of synthesized compound

COMPOUNDS CODE	SLEEPING TIME(MEAN ±SEM) (Min.)
NBZD-1	120±9.00**
NBZD-3	138±10.53**
NBZD-4	140±11.92**
NBZD-7	141±11.21**
NBZD-8	68±12.6 NS
NBZD-10	63±9.05 NS
NBZD-11	148±12.15**
NBZD-12	124±10.12**
NBZD-14	110±11.41 **
NBZD-15	100±10.98**
NBZD-17	148±11.54**
NBZD-18	76 ±10.26 NS
NBZD-20	112±9.62**
NBZD-21	157±12.09**
Phenobarbitone (control)	56±11.47

Values represent the mean ± SEM of six animals for each group.
**significant at p<0.05, **significant at p<0.01 (Dunnett's test)*
Test drug (30mg/kg), Phenobarbitone (40mg/kg)
NS denotes not significant at p<0.01(student's t-test)

10. 4-Chlorophenylhyrazine carboxamide) [1-nitrophenyl-3-(2,2,4trimethyl-2,3-dihydrobenzo[b][1,4] diazepine-1-yl)-propylidine]-amine (NBZD-20).

^{1}H NMR (300 MHz , δ) : CH(m, 7.9-8.2, 4H, p-nitrocetophenone), NH (s, 9.0,1H, =NNH, p-chlorophenylsemicarbazide), NH (s, 6.0, 1H, -NHC$_6$H$_4$Cl, p-chlorophenylsemicarbazide), CH(m, 7.2-7.5,4H, p-chlorophenylsemicarbazide), CH$_2$(s, 1.6, 2H, -N=C-CH$_2$), CH$_2$ (s, 3.4, 2H, -NHCH$_2$), CH (m, 6.6-7.1, 4H, phenyl), 2xCH$_3$ (s, 1.28, 6H) , CH$_3$ (s,0.9, 3H) , CH$_2$ (s, 2.5, 2H, Diazepine ring). **IR (KBr) :** C=N (1570 cm^{-1}, str), C-Cl (730cm^{-1}, str), C-H (2975cm^{-1}, str assym), CH(1353.9cm^{-1}, def sym.), C-H (Ar, 3062cm^{-1}, str), CH (Ar, 3180cm^{-}

[1], str), CH(Ar, 800cm^{-1}, bend) , , C=N (1618cm^{-1} , Str), CH_3 (2990cm^{-1}, str), C-C (Ar, 1600cm^{-1}), C=C (Ar, 1410,1500,1580cm^{-1}) , NO (1350cm^{-1}, str)

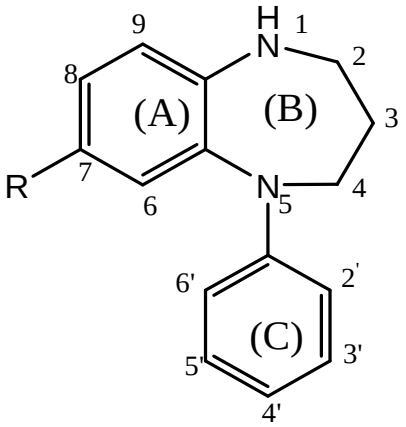
CONCLUSION

All the synthesized derivatives were evaluated at the dose of 30mg/kg b.w for anticonvulsant activity by isoniazid induced convulsion model and the compounds NBZD-3 & NBZD-8 were found to be most active among all compounds . Among all the synthesized derivatives , compounds NBZD-13, NBZD-17 were found to be most active among all compounds using thiosemicarbazide induced model. Activity of the drugs interfering with motor coordination was checked by the rotorod test. None of the synthesized compounds were found to be neurotoxic at a dose of 30mg/kg b.w. among all the tested compounds. The compounds NBZD-1, NBZD-3, NBZD-4, NBZD-7, NBZD-11, NBZD-12, NBZD-14, NBZD-15, NBZD-17, NBZD-20, NBZD-21 were found to cause sedation. Rather NBZD-8, NBZD-10, NBZD-18 are the compounds which had shown good anticonvulsant activity and have advantage over that, they were not sedative.

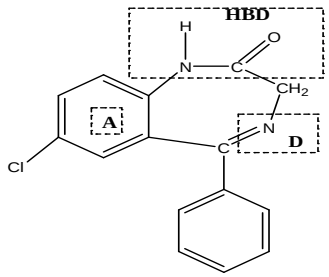
STRUCTURE ACTIVITY RELATIONSHIP

Anticonvulsant:-

I. **HIGHLY ACTIVE**: NBZD- 3, NBZD-8, NBZD-13, NBZD-18.
II. **MODERATELY ACTIVE**: NBZD-1, NBZD-6, NBZD-10, NBZD-11, NBZD-12, NBZD-16, NBZD-21.
III. **LESS ACTIVE**: NBZD-4, NBZD-7, NBZD-9, NBZD-14, NBZD-15, NBZD-17, NBZD-19, NBZD- 20.
IV.



General structure of 1,5-Benzodiazepine



N-desmethyl diazepam

A= Hydrophobic unit, HBD= hydrogen bonding domain, D= electron donor

Most functional subtypes of the $GABA_A$ receptor contain α,β, subunits, with the different Benzodiazepine binding site ligands.BZ-binding site ligands act through mechanisms which modulate the inhibiting effects of GABA.

1. In the basic structure of Benzodiazepine ,early SAR studies indicated that the seven membered imino ring B was essential for its affinity towards the BZ- binding site.
2. 4-5 carbimino double bond has also been shown to substantially contribute to the binding affinity of compound. Saturation leads to complete less of activity. It acts as a two electron donor site.
3. The primary chemical moieties of the compounds which contribute to high receptor binding affinity, are restricted to position 7 , 2, 1.
   - Position 2 – It is most effective place. Presence of an electrophilic & bulky substituent at position 2 results in strong increase in receptor binding affinity of the corresponding compounds.
   - Compounds NBZD- 3 & NBZD-8 had shown good anticonvulsant Activity as they have cyclohexane ring at position 2.
4. Molar refractivity is the most important parameter at position 1, Suggesting that the Molecule size of the substituent needs to be restricted at position 1 for effective ligand binding. Compound NBZD-1& 8 have less substituent as compared to other & hence more active.
5. Compounds NBZD-6, NBZD-10, NBZD-11 were found to be moderately active anticonvulsant action , hence this shows that chloro substituted derivatives are rather good anticonvulsant agent as compared to Nitro substituted derivatives.
6. Among these synthesized compounds which have methyl groups at 2nd & 4th position , NBZD- 13, NBZD-18 were found to be most active. Hence shows that for good activity compounds preferred with less substitution at 1st position.

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