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Synthesis and biological evaluation of some newer 1,5-benzodiazepine derivatives as potential anticonvulsant agents

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ABSTRACT

*Benzodiazepines and their derivatives were reported to have wide biological activities and were synthesized by the reaction between substituted benzaldehydes and substituted ketones in presence of sodium hydroxide to afford chalcones and further reaction between 1,2-diamine under condensation with chalcones in the presence of glacial acetic acid to afford substituted 1,5-benzodiazepines **(1b-10b)** in good yield and further methylation with methyl iodide in basic medium gave 2,4-Disubstituted phenyl-1-methyl-1H-benzodiazepines **(1c-10c)** and on acetylation with acetyl chloride in basic medium yielded 2,4-Disubstituted phenyl-1-acetyl-1H-benzodiazepines **(1d-5d).** The chemical structures of the newly synthesized compounds have been confirmed by IR, ^{1}H-NMR, MASS spectral data and elemental analysis. All the newly synthesized compounds were evaluated for anticonvulsant activities and their neurotoxicity studies by maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (ScPTZ) and rotorod test respectively, of which compounds **1c, 3c, 6c, 8c** and **9c** exhibited potent anticonvulsant results and in the neurotoxicity screening, most of the compounds were devoid of toxicity at the dose of 100 and 300mg/kg.*

Key words: 1, 5- Benzodiazepines, Chalcones, maximal electroshock seizure test, subcutaneous pentylenetetrazole, rotorod.

INTRODUCTION

Benzodiazepines and their derivatives are an important class of bioactive molecules and the medicinal value of benzodiazepines is well documented. Particularly, 1,5-benzodiazepines have received much attention because of their potential structural diversity as a privileged scaffolds in arrays of compounds bioactive towards several major drug target families which include cholecystokinin receptors [1-4], interlukin converting enzymes [5-6] and ion channels [7-8].

Benzodiazepine derivatives have attracted the attention of researchers owing to their interesting pharmacological activities and their low toxicitiy. They are widely used as anti-anxiety [9], analgesic [10], anti-inflammatory [11], DNA binding activity [12-15], anti-cancer [16-22], anti-tumor [23-26], adenosine binding activity [27], anti-sialogogic [28], cutaneous anaphylaxis [29], antimicrobial [30-35], anthelmintic [36-37], anti-neuroinflammatory [38], antiparkinson [39], antileishmanial [40], muscle relexant [41], anticonvulsant [42-43], anti-HIV [44] and sedative and hypnotic [45]. Moreover, 1, 5-benzodiazepines are valuable synthons used for the preparation of other fused ring compounds such as triazolo-, oxadiazolo-, oxazino-, or furano-benzodiazepines [46-50]. Besides this, benzodiazepine derivatives are also used as dyes for acrylic fiber in photography [51-54]. Combinatorial strategy is

used in the design and synthesis of 1,5-benzodiazepine derivatives [55-56]. Benzodiazepine derivatives belong to an important class of heterocyclic compound and have been the subject of extensive study in recent years[57-60].

In the present work, 2,4-Disubstituted-1,5-benzodiazepines **(1b-10b)** were synthesized by reacting substituted chalcones with *o*-phenylenediamine in presence of glacial acetic acid and further methylation with methyl iodide in basic medium gave 2,4-Disubstituted phenyl-1-methyl-1*H*-benzodiazepines **(1c-10c)** and on acetylation with acetyl chloride in basic medium yielded 2,4-Disubstituted phenyl-1-acetyl-1*H*-benzodiazepines **(1d-5d)** and newly synthesized compounds have been confirmed by IR, ^{1}H-NMR, MASS spectral data and elemental analysis. All the newly synthesized compounds were evaluated for anticonvulsant activities and their neurotoxicity studies by MES, ScPTZ and rotorod test respectively.

MATERIALS AND METHODS

Chemistry

The melting points were determined in open capillary tubes and were uncorrected. The homogeneity of all the newly synthesized compounds were checked by TLC on silica gel-protected aluminum sheets (Type 60 F_{254}, Merck) and the spots were detected by exposure to UV-lamp at 254 nm for few seconds. The infrared (IR) spectra were recorded on 470-Shimadzu Infrared Spectrophotometer using KBr disc technique and expressed in cm^{-1}. ^{1}H NMR spectra were recorded on Bruker DRX-300 in DMSO-d_6 as a solvent. The chemical shift was given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet; J: coupling constant. Mass spectrum on Jeol SX 102/DA-6000 mass spectrometer using methanol as solvent. Elemental analysis was carried on Elemental Vario EL III Carlo Erba 1108 and the values were within ±0.4% of the theoretical values.

General procedure for synthesis of substituted chalcones (1a-10a):

A solution of 2.2gm of sodium hydroxide in 20ml of water and 20ml of ethanol was placed in a beaker. Beaker was immersed in a bath of crushed ice, 5.16gm (0.043mol) of acetophenone derivatives was added in the beaker and 4.5gm (0.043mol) of benzaldehyde derivatives was added to it with constant stirring. The temperature of the mixture was kept at about 25ºC and the mixture was vigorously stirred for 2 to 18 hours. The completion of reaction was monitored by TLC. The reaction mixture was kept in the reaction mixture in refrigerator overnight. The reaction mixture was filtered and washed with cold water until the washing was neutral to litmus and recrystallized from ethanol to obtain **1a-10a**.

General synthesis of substituted 1,5-benzodiazepines (1b-10b):

A mixture of 2gm (0.01mol) of chalcone, 1gm (0.01mol) of *o*-Phenylenediamine and 5 ml of glacial acetic acid in 15ml of DMF were taken in a 250ml beaker and exposed to microwave irradiation at 750 watt for 15 to 20 minutes with intermittent cooling after every minute. The completion of the reaction was monitored by TLC. The reaction was allowed to attain room temperature and treated with cold water, the solid was separated with the help of filtration and washed with water and then recrystallized from methanol to obtain **1b-10b**.

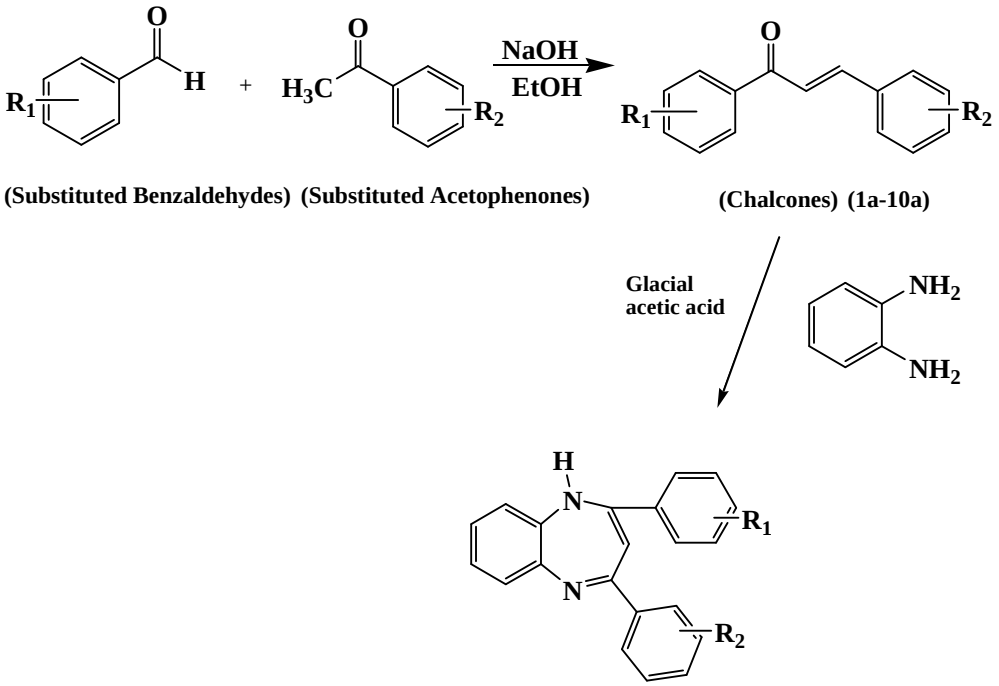
General synthesis of *N*-methylation (1c-10c):

1.36gm (9.60mmol) of methyl iodide was added to the solution of 0.23gm (0.80 mmol) of benzodiazepine in 10ml of 0.5% NaOH ethanolic solution and was stirred at room temperature for 5 to 10 hours. The completion of the reaction was monitored by TLC and then the reaction mixture was diluted with water, the compound was precipitated and washed with water and recrystallized with methanol to obtain **1c-10c.**

General synthesis of *N*-acetylation (1d-5d):

1-*H* Benzodiazepine derivatives 1.44gm (0.01mol), acetyl chloride 0.78gm (0.01mol) and pyridine 0.79gm (0.01mol) were taken in 15 ml DMF and was refluxed for 3 to 6 hours. The completion of the reaction was monitored by TLC. The reaction was allowed to attain room temperature and treated with cold water; the solid was separated with the help of filtration and washed with water, recrystallized from methanol to obtain **1d-5d**.

Fig. 1: Synthesis of 2, 4-disubstituted 1, 5- benzodiazepines (1c-10c and 1d-5d) from substituted chalcones (SCHEME)



(Substituted Benzaldehydes) (Substituted Acetophenones) **(Chalcones) (1a-10a)**

Substituted 1*H*-1, 5-benzodiazepine (1b-10b)

(1c-10c) **(1d-5d)**

***N*-methylated substituted 1*H*-1, 5-benzodiazepine** ***N*-acetylatedsubstituted 1*H*-1, 5-benzodiazepine**

Table 1: Where, R_1- Benzaldehyde derivatives, R_2- Acetophenone derivatives

S. No	Compound no.	R_1	R_2
1.	1c	4-Fluoro	H
2.	1d	4-Fluoro	H
3.	2c	4-Methoxy	H
4.	2d	4-Methoxy	H
5.	3c	4-Chloro	H
6.	3d	4-Chloro	H
7.	4c	4-Nitro	H
8.	4d	4-Nitro	H
9.	5c	3-Nitro	H
10.	5d	3-Nitro	H
11.	6c	3-Nitro	4-Chloro
12.	7c	4-Chloro	4-Chloro
13.	8c	4-Nitro	4-Chloro
14.	9c	2-Chloro	H
15.	10c	4-Nitro	4-Methoxy

2-(4'-Flourophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine 1(c)

UV (λ_{max}) (DMSO): 394.0nm. IR (KBr, cm^{-1}): 3047.32 (aromatic C-H stretching), 2963.96 (aliphatic C-H stretching), 1667.71 (C=N stretching), 1496.50 (C=C stretching), 1396.37 (C-F stretching), 1290.59 (C-N stretching) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.744 (s, 3H, N-CH_3), δ 5.121 (s, 1H, -C=CH), δ 6.475-6.626 (m, 2H, Ar-H), δ 7.033-7.060 (d, 2H, Ar-H, J=8.10), δ 7.187-7.247 (m, 2H, Ar-H), δ 7.269-7.289 (m, 1H, Ar-H), δ 7.323-7.345 (d, 2H, Ar-H, J=6.61), δ 7.515-7.526 (d, 2H, Ar-H, J=3.30), δ 7.806-7.812 (d, 2H, Ar-H, J=4.81). ESI full mass-MS**:** m/z (%) 328 $[M]^+$, Fragments: 329 $[M+1]^+$, 310, 252, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{17}FN_2$: C; 80.47, H; 5.22, F; 5.79, N; 8.53%. Found: C; 80.45, H; 5.21, F; 5.77, N; 8.51%.

2-(4'-Fluorophenyl)-1-acetyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (1d)

UV (λ_{max}) (Ethanol)**:** 297.0nm. IR (KBr)**:** 3062.32 (aromatic C-H stretching), 2857.95 (aliphatic C-H stretching), 1720.51 (C=O stretching), 1666.38 (C=N stretching), 1528.22 (C=C stretching), 1346.22 (C-F stretching), 1288.36 (C-N stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.821 (s, 3H, N-$COCH_3$), δ 5.124 (s, 1H, -C=CH), δ 6.526-6.629 (m, 2H, Ar-H), δ 6.711-6.821 (m, 3H, Ar-H), δ 6.962-6.985 (d, 1H, Ar-H, J=6.90), δ 7.102-7.123 (d, 1H, Ar-H, J=6.30), δ 7.205-7.228 (d, 2H, Ar-H, J=6.91), δ 7.422-7.449 (d, 2H, Ar-H, J=8.10), δ 7.602-7.624 (d, 2H, Ar-H, J=6.6). ESI full mass-MS**:** m/z (%) 356 $[M]^+$ Fragments: 357 $[M+1]^+$, 358 $[M+2]^+$, 338, 313, 295, 262, 219, 158, 143. Elemental analysis: Calcd for $C_{23}H_{17}FN_2O$: C; 77.51, H; 4.81, F; 5.33, N; 7.86%. Found: C; 77.49, H; 4.78, F; 5.31, N; 7.89%.

2-(4'-Methoxyphenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (2c)

UV (λ_{max}) (Ethanol): 387.0nm. IR (KBr): 3042.32 (aromatic C-H stretching), 2948.93 (aliph. C-H stretching), 1676.74 (C=N stretching), 1574.71 (C=C stretching), 1266.04 (C-N stretching), 1230.50 (C-O-C stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.780 (s, 3H, N-CH_3), δ 3.745 (s, 3H, OCH_3), δ 4.912 (s, 1H, -C=CH), δ 6.658-6.683 (d, 2H, Ar-H, J=7.51), δ 6.718-6.786 (m, 2H, Ar-H), δ 7.040-7.058 (d, 2H, Ar-H, J=5.40), δ 7.180-7.198 (m, 2H, Ar-H), δ 7.282-7.298 (d, 2H, Ar-H, J=4.81), δ 7.312-7.326 (t, 1H, Ar-H, J=4.22), δ 7.614-7.627 (d, 2H, Ar-H, J=3.91). ESI full mass-MS**:** m/z (%) 340 $[M]^+$, Fragments: 341 $[M+1]^+$, 310, 234, 158, 143. Elemental analysis: Calcd for $C_{23}H_{20}N_2O$: C; 81.15, H; 5.92, N; 8.53%. Found: C; 81.13, H; 5.90, N; 8.51%.

2-(4'-Methoxyphenyl)-1-acetyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (2d)

UV (λ_{max}) (Ethanol)**:** 292.0nm. IR (KBr): 3018.39 (aromatic C-H stretching), 2813.95 (aliph. C-H stretching), 1723.11 (C=O stretching), 1663.51 (C=N stretching), 1471.59 (C=C stretching), 1315.36 (C-O-C stretching), 1209.28 (C-N stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.928 (s, 3H, N-$COCH_3$), δ 3.747 (s, 3H, OCH_3), δ 4.916 (s, 1H, -C=CH), δ 6.524-6.542 (d, 2H, Ar-H, J=5.41), δ 6.714-6.768 (m, 2H, Ar-H), δ 6.949-6.967 (d, 2H, Ar-H, J=5.40), δ 7.185-7.204 (d, 1H, Ar-H, J=5.72), δ 7.368-7.392 (d, 1H, Ar-H, J=7.21), δ 7.449-7.498 (m, 3H, Ar-H), δ 7.589-7.604 (d, 2H, Ar-H, J=4.50). ESI full mass-MS**:** m/z (%) 368 $[M]^+$, 369 $[M+1]^+$, 338, 325, 295, 262, 219, 143.. Elemental analysis: Calcd for $C_{24}H_{20}N_2O_2$: C; 78.24, H; 5.47, N; 7.60%. Found: C; 78.26, H; 5.45, N; 7.58%.

2-(4'-Chlorophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (3c)
UV (λ_{max}) (Ethanol)**:** 312.0nm. IR (KBr): 3013.48 (aromatic C-H stretching), 2935.46 (aliph. C-H stretching), 1613.46 (C=N stretching), 1450.37 (C=C stretching), 1344.27 (C-N stretching), 1108.99 (C-Cl stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.608 (s, 3H, N-CH_3), δ 5.271 (s, 1H, -C=CH), δ 6.622-6.694 (m, 2H, Ar-H), δ 6.901-6.987 (m, 2H, Ar-H), δ 7.158-7.182 (d, 2H, Ar-H, J=7.21), δ 7.241-7.256 (d, 2H, Ar-H, J=4.50), δ 7.301-7.375 (m, 1H, Ar-H), δ 7.429-7.462 (d, 2H, Ar-H, J=6.90), δ 7.519-7.538 (d, 2H, Ar-H, J=5.71). ESI full mass-MS**:** m/z (%): 344 $[M]^+$, 345 $[M+1]^+$, 346 $[M+2]^+$, 310, 268, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{17}ClN_2$: C; 76.63, H; 4.97, Cl; 10.28, N; 8.12%. Found: C; 76.61, H; 4.95, Cl; 10.26, N; 8.10%.

2-(4'-Chlorophenyl)-1-acetyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine 3(d)
UV (λ_{max}) (Ethanol)**:** 292.0nm. IR (KBr): 3023.39 (aromatic C-H stretching), 2877.60 (Aliph. C-H stretching), 1719.96 (C=O stretching), 1671.59 (C=N stretching), 1573.81 (C=C stretching), 1311.36 (C-N stretching), 1040.63 (C-Cl stretching).^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.828 (s, 3H, N-$COCH_3$), δ 5.289 (s, 1H, -C=CH), δ 6.528-6.682 (m, 2H, Ar-H), δ 6.810-6.892 (m, 3H, Ar-H), 6.942-6.965 (d, 1H, Ar-H, J=6.21), δ 7.101-7.119 (d, 1H, Ar-H, J=5.42), δ 7.324-7.347 (d, 2H, Ar-H, J=6.91), δ 7.456-7.479 (d, 2H, Ar-H, J=6.90), δ 7.553-7.594 (d, 2H, Ar-H, J=6.31). ESI full mass-MS**:** m/z (%): 372 $[M]^+$, Fragments: 373 $[M+1]^+$, 374 $[M+2]^+$, 338, 329, 295, 219, 143. Elemental analysis: Calcd for $C_{23}H_{17}ClN_2O$: C; 74.09, H; 4.60, Cl; 9.51, N; 7.51%. Found: C; 74.07, H; 4.58, Cl; 9.49, N; 7.49%.

2-(4'-Nitrophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (4c)
UV (λ_{max}) (Ethanol)**:** 396.0 nm. IR (KBr): 3049.22 (aromatic C-H stretching), 2928.96 (aliph. C-H stretching), 1666.74 (C=N stretching), 1602.74 (C=C stretching), 1485.50 (N=O stretching), 1270.57 (C-N stretching), 871.86 (C-N-O stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.784 (s, 3H, N-CH_3), δ 5.216 (s, 1H, -C=CH), δ 6.612-6.682 (m, 2H, Ar-H), δ 6.741-6.752 (m, 2H, Ar-H), δ 6.841-6.853 (d, 1H, Ar-H, J=3.61), δ 7.214-7.242 (m, 1H, Ar-H), δ 7.362-7.380 (d, 1H, Ar-H, =5.42), δ 7.483-7.490 (d, 2H, Ar-H, J=2.10), δ 7.521-7.548 (d, 2H, Ar-H, J=8.10), δ 7.941-7.952 (d, 2H, Ar-H, J=3.30). ESI full mass-MS**:** m/z (%): 355 $[M]^+$, Fragments: 356 $[M+1]^+$, 310, 279, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{17}N_3O_2$: C; 74.35, H; 4.82, N; 11.82%. Found: C; 74.33, H; 4.80, N; 11.80%.

2-(4'-Nitrophenyl)-1-acetyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (4d)
UV (λ_{max}) (Ethanol): 301.0 nm. IR (KBr): 3041.06 (aromatic C-H stretching), 2946.27 (aliph. C-H stretching), 1719.51 (C=O stretching), 1672.17 (C=N stretching), 1630.38 (C=C stretching), 1517.87 (N=O stretching), 1294.07 (C-N stretching), 871.05 (C-N-O stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.248 (s, 3H, N-$COCH_3$), δ 4.952 (s, 1H, -C=CH), δ 6.552-6.692 (m, 2H, Ar-H), δ 6.816-6.893 (m, 3H, Ar-H), δ 6.963-6.981 (d, 1H, Ar-H, J=5.40), δ 7.105-7.129 (d, 1H, Ar-H, J=7.20), δ 7.564-7.581 (d, 2H, Ar-H, J=5.10), δ 7.741-7.767 (d, 2H, Ar-H, J=7.81), δ 7.967-7.989 (d, 2H, Ar-H, J=6.61). ESI full mass-MS**:** m/z (%): 383 $[M]^+$, Fragments: 384 $[M+1]^+$, 338, 340, 295, 219, 143. Elemental analysis: Calcd for $C_{23}H_{17}N_3O_3$: C; 74.09, H; 4.60, N; 7.51%. Found: C; 74.08, H; 4.58, N; 7.49%.

2-(3'-Nitrophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (5c)
UV (λ_{max}) (Ethanol)**:** 397.0 nm. IR (KBr): 3043.22 (aromatic C-H stretching), 2929.11 (aliph. C-H stretching), 1657.44 (C=N stretching), 1612.14 (C=C stretching), 1395.37 (N=O stretching), 1277.57 (C-N stretching), 871.27 (C-N-O stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.682 (s, 3H, N-CH_3), δ 5.425 (s, 1H, -C=CH), δ 6.542-6.559 (m, 2H, Ar-H), δ 6.748-6.754 (m, 3H, Ar-H), δ 6.921-6.948 (d, 1H, Ar-H, J=8.10), δ 7.352-7.380 (d, 1H, Ar-H, J=8.41), δ 7.452-7.476 (d, 2H, Ar-H, J=7.20), δ 7.521-7.528 (m, 1H, Ar-H), δ 7.851-7.878 (d, 1H, Ar-H, J=8.10), δ 7.951-7.962 (d, 2H, Ar-H, J=3.31). ESI full mass-MS**:** m/z (%): 355 $[M]^+$, Fragments: 356 $[M+1]^+$, 310, 279, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{17}N_3O_2$: C; 74.35, H; 4.82, N; 11.82%. Found: C; 74.33, H; 4.80, N; 11.80%

2-(3'-Nitrophenyl)-1-acetyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (5d)
UV (λ_{max}) (Ethanol)**:** 301.0 nm. IR (KBr): 3097.39 (aromatic C-H stretching), 2876.67 (aliph. C-H stretching), 1709.93 (C=O stretching), 1672.29 (C=N stretching), 1575.11 (C=C stretching), 1416.72 (N=O stretching), 1313.56 (C-N stretching), 871.35 (C-N-O stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.793 (s, 3H, N-$COCH_3$), δ 5.259 (s, 1H, -C=CH), δ 6.562-6.697 (m, 2H, Ar-H), δ 6.729-6.749 (m, 3H, Ar-H), δ 6.842-6.867 (d, 1H, Ar-H, J=7.51), δ 7.102-7.119 (d, 1H, Ar-H, J=5.10), δ 7.474-7.492 (d, 2H, Ar-H, J=5.41), δ 7.515-7.597 (m, 1H, Ar-H), δ 7.814-

7.839 (d, 2H, Ar-H, J=7.50), δ 7.957-7.981 (d, 2H, Ar-H, J=7.21). ESI full mass-MS**:** m/z (%): 383 $[M]^+$, Fragments: 384 $[M+1]^+$, 338, 340, 295, 219, 143. Elemental analysis: Calcd for $C_{23}H_{17}N_3O_3$: C; 72.05, H; 4.47, N; 10.96%. Found: C; 72.03, H; 4.45, N; 10.95%.

4-(4''-Chloro)-2-(3'-Nitrophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (6c)

UV (λ_{max}) (Ethanol): 397.0 nm. IR (KBr): 3029.30 (aromatic C-H stretching), 2870.61 (Aliph. C-H stretching), 1671.56 (C=N stretching), 1585.81 (C=C stretching), 1471.59 (N=O stretching), 1269.28 (C-N stretching), 1034.63 (C-Cl stretching), 869.34 (C-N-O stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.780 (s, 3H, N-CH_3), δ 5.216 (s, 1H, -C=CH), δ 6.512-6.583 (m, 2H, Ar-H), δ 6.612-6.624 (d, 1H, Ar-H, J=3.20), δ 6.824-6.848 (d, 1H, Ar-H, J=7.20), δ 7.241-7.267 (d, 2H, Ar-H, J=7.81), δ 7.372-7.485 (m, 1H, Ar-H), δ 7.531-7.578 (m, 2H, Ar-H), δ 7.724-7.742 (d, 1H, Ar-H, J=5.40), δ 7.929-7.952 (d, 2H, Ar-H, J=6.91). ESI full mass-MS**:** m/z (%): 389$[M]^+$, Fragments: 390 $[M+1]^+$, 391 $[M+2]^+$, 344, 310, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{16}ClN_3O_2$: C; 67.78, H; 4.14, Cl; 9.09, N; 11.82%. Found: C; 67.76, H; 4.12, Cl; 9.07, N; 11.79%.

2-(4'-Chloro)-4-(4''-chlorophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (7c)

UV (λ_{max}) (Ethanol): 397.0 nm. IR (KBr): 3018.48 (aromatic C-H stretching), 2891.81 (aliph. C-H stretching), 1646.66 (C=N stretching), 1492.37 (C=C stretching), 1344.29 (C-N stretching), 1100.39 (C-Cl stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.684 (s, 3H, N-CH_3), δ 4.624 (s, 1H, -C=CH), δ 6.512-6.584 (m, 2H, Ar-H), δ 6.787-6.802 (d, 1H, Ar-H, J=4.50), δ 6.982-7.104 (d, 1H, Ar-H, J=6.61), δ 7.297-7.310 (d, 2H, Ar-H, J=3.91), δ 7.539-7.557 (d, 4H, Ar-H, J=5.40), δ 7.598-7.612 (d, 2H, Ar-H, J=4.21). ESI full mass-MS**:** m/z (%): 378 $[M]^+$, 379 $[M+1]^+$, 380 $[M+2]^+$, 344, 310, 268, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{13}Cl_2N_2$: C; 69.67, H; 4.25, Cl; 18.69, N; 7.31%. Found: C; 69.65, H; 4.23, Cl; 18.67, N; 7.29%.

4-(4''-Chloro)-2-(4'-nitrophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (8c)

UV (λ_{max}) (Ethanol): 394.0 nm. IR (KBr): 3082.04 (aromatic C-H stretching), 2896.88 (aliph. C-H stretching), 1656.74 (C=N stretching), 1484.58 (C=C stretching), 1372.76 (N=O stretching), 1305.72 (C-N stretching), 1016.42 (C-Cl stretching), 871.62 (C-N-O stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.781 (s, 3H, N-CH_3), δ 5.148 (s, 1H, -C=CH), δ 6.624-6.629 (m, 2H, Ar-H), δ 6.871-6.894 (d, 1H, Ar-H, J=6.91), δ 7.162-7.189 (d, 1H, Ar-H, J=8.10), δ 7.372-7.389 (d, 2H, Ar-H, J=5.11), δ 7.577-7.598 (d, 4H, Ar-H, J=6.30), δ 7.972-7.998 (d, 2H, Ar-H, J=7.81). ESI full mass-MS**:** m/z (%): 389 $[M]^+$, Fragments: 390 $[M+1]^+$, 391 $[M+2]^+$, 355, 344, 310, 268, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{16}ClN_3O_2$: C; 67.78, H; 4.14, Cl; 9.09, N; 11.82%. Found: C; 67.76, H; 4.12, Cl; 9.07, N; 11.80%.

2-(2'-Chlorophenyl)-1-methyl-4-phenyl-1*H*-benzo[*b*][1,5]diazepine (9c)

UV (λ_{max}) (Ethanol): 311.0 nm. IR (KBr): 3090.33 (aromatic C-H stretching), 2896.88 (aliph. C-H stretching), 1664.24 (C=N stretching), 1585.81 (C=C stretching), 1265.22 (C-N stretching), 1104.21 (C-Cl stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.604 (s, 3H, N-CH_3), δ 5.262 (s, 1H, -C=CH), δ 6.512-6.589 (m, 2H, Ar-H), δ 6.724-6.892 (m, 3H, Ar-H), δ 7.189-7.208 (d, 1H, Ar-H, J=5.70), δ 7.397-7.425 (d, 1H, Ar-H, J=8.40), δ 7.567-7.624 (m, 2H, Ar-H), δ 7.691-7.702 (d, 2H, Ar-H, J=3.31), δ 7.876-7.895 (d, 2H, Ar-H, J=5.71). ESI full mass-MS: m/z (%):344 $[M]^+$, Fragments: 345 $[M+1]^+$, 310, 268, 234, 158, 143. Elemental analysis: Calcd for $C_{22}H_{17}ClN_2$: C; 76.63, H; 4.97, Cl; 10.28, N; 8.12%. Found: C; 76.61, H; 4.95, Cl; 10.26, N; 8.10%.

4-(4''-Methoxyphenyl)-2-(4'-nitrophenyl)-1-methyl-1*H*-benzo[*b*][1,5]diazepine (10c)

UV (λ_{max}) (Ethanol): 394.0 nm. IR (KBr): 3048.14 (aromatic C-H stretching), 2893.08 (aliph.C-H stretching), 1643.84 (C=N stretching), 1495.18 (C=C stretching), 1369.71 (N=O stretching), 1271.62 (C-N stretching), 1230.29 (C-O-C stretching), 870.02 (C-N-O stretching). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.781 (s, 3H, N-CH_3), δ 3.734 (s, 3H, -OCH_3), δ 4.945 (s, 1H, -C=CH), δ 6.569-6.584 (d, 2H, Ar-H, J=4.51), δ 6.612-6.691 (m, 2H, Ar-H), δ 6.815-6.839 (d, 1H, Ar-H, J=7.20), δ 6.912-6.943 (d, 1H, Ar-H, J=9.31), δ 7.462-7.489 (d, 2H, Ar-H, J=8.11), δ 7.652-7.678 (d, 2H, Ar-H, J=7.80), δ 7.970-7.988 (d, 2H, Ar-H, J=5.40). ESI full mass-MS: m/z (%): 385 $[M]^+$, Fragments: 340, 310, 234, 158, 143. Elemental analysis: Calcd for $C_{23}H_{19}N_3O_3$: C; 71.67, H; 4.97, N; 10.90%. Found: C; 71.65, H; 4.95, N; 10.88%.

Table 2: Physicochemical properties of synthesized compounds

Compound No.	Molecular formula	Molecular weight	m.p.(°C)	R_f* value	Yield (%)	Log P[a]
1c	$C_{22}H_{17}FN_2$	328.38	65-66	0.52	78.20	1.98
1d	$C_{23}H_{17}FN_2O$	356.39	86-87	0.51	61.79	2.50
2c	$C_{23}H_{20}N_2O$	340.42	62-63	0.57	72.83	3.50
2d	$C_{24}H_{20}N_2O_2$	368.43	82-83	0.62	68.05	3.34
3c	$C_{22}H_{17}ClN_2$	344.84	64-65	0.53	77.50	1.64
3d	$C_{23}H_{17}ClN_2O$	372.85	85-86	0.61	65.94	2.81
4c	$C_{22}H_{17}N_3O_2$	355.39	70-71	0.63	77.38	2.33
4d	$C_{23}H_{17}N_3O_3$	383.40	94-95	0.61	65.26	2.21
5c	$C_{22}H_{17}N_3O_2$	355.39	72-73	0.61	79.76	2.80
5d	$C_{23}H_{17}N_3O_3$	383.40	100-101	0.56	66.84	3.12
6c	$C_{22}H_{16}ClN_3O_2$	389.83	76-77	0.59	65.59	1.87
7c	$C_{22}H_{13}Cl_2N_2$	379.28	71-72	0.59	65.56	3.87
8c	$C_{22}H_{16}ClN_3O_2$	389.83	73-74	0.58	63.44	1.86
9c	$C_{22}H_{17}ClN_2$	344.84	63-64	0.53	73.17	2.01
10c	$C_{23}H_{19}N_3O_3$	385.42	79-80	0.58	66.30	3.02

** Solvent system used: Toluene: methanol (8:2, v/v).*
[a] Log P was calculated using ACD lab version 8.0

Pharmacology

All the synthesized compounds were evaluated for their antiepileptic effects using albino rats of either sex (150-200 g). The primary qualitative evaluations were performed in rats involved two epilepsy tests (MES: Maximal Electroshock Seizure test and ScPTZ: Subcutaneous pentylenetetrazole). Phenytoin sodium (30 mg/kg) was used as the standard drug for the comparison. Acute neurological toxicity induced by the compounds in rats was assessed through standardized rotorod test. In the initial screening, test compounds were screened for their antiepilepsy potential through MES and ScPTZ models in rats at a dose level of 30, 100, 300 mg/kg by intraperitoneal (*i.p*) route and the groups of rats are tested at different time points (i.e., 0.5 and 4 h) post administration of the test compounds. All the experimental protocols were carried out with the permission from Institutional Animal Ethics committee (IAEC), form no. 10/11; registration no. is 882-ac/05/CPCSEA Animals were obtained from Animal House Facility, Rajiv Academy for Pharmacy, Mathura, U.P.

Anticonvulsant activity:

Maximal electroshock seizure test (MES)

Each compound was administered through *i.p* route at dose level of 30, 100, 300 mg/kg body weight. The rats were subjected to maximal electroshocks by delivering 60 Hz, 150 mA electrical stimuli for 0.2 s via corneal electrodes primed with an electrolyte solution containing an anaesthetic agent. This procedure caused immediate hindlimb tonic extension. After 0.5 h and 4.0 h of drug administration, electroshocks were via corneal electrodes. Absence of tonic extension suggests that the tested compounds were considered as positive criteria. [61-63].

ScPTZ-induced seizures test

This screen utilizes a dose of pentylenetetrazole (70 mg/kg in rats) that produces clonic seizures lasting for a period of at least 5 s in 97% (CD_{97}) of animals tested. At the anticipated time of testing the convulsant is administered subcutaneously. The time needed for the development of clonic seizure activity involving limbs and duration of seizure was carefully noted. Seizure free period of 1 h was considered as protection. The number of animals protected in each group was recorded and percentage of protection was calculated [64, 65].

Neurotoxic studies (NT)

The acute neurological toxicity (NT) induced by a compound was detected in rats using the standardized minimal motor impairment- rotorod test. The albino rats (100-250 g) were trained to stay on an accelerating rotorod (diameter 3.2 cm) that rotated at 10 rpm. Only those rats were taken for the test, which can stay on the revolving rod

for at least one minute. Trained animals were injected *i.p*. with the test compounds at doses of 30, 100, 300mg/kg. The acute motor impairment (Neurotoxicity) can be demonstrated by the inability of the animal to maintain equilibration on the rod for at least one minute [66].

Table 3: Anticonvulsant activity and neurotoxicity of compounds 1c-10c and 1d-5d administered intraperitoneally to rats

Compound	MES[a] screening		scPTZ[b] screening		NT[c] screening	
	0.5h[d]	4.0h[d]	0.5h[d]	4.0h[d]	0.5h[d]	4.0h[d]
1c	100	_	300	_	300	300
1d	300	_	_	300	_	_
2c	300	300	_	300	_	_
2d	_	300	300	_	_	_
3c	30	30	100	300	300	300
3d	300	300	300	_	_	_
4c	300	300	300	_	_	_
4d	300	_	_	_	_	_
5c	300	_	_	300	_	_
5d	300	300	300	_	_	_
6c	100	_	300	_	_	_
7c	_	300	300	_	100	100
8c	30	30	100	_	_	_
9c	30	100	100	300	300	300
10c	_	300	_	_	_	_
Phenytoin sodium	30	30	_	_	100	100

The sign – (dash) represents an absence of activity at maximum dose administered (300mg/kg).
[a] Maximal electroshock test (administered intraperitoneally to rats at doses ranging from 30 to 300 mg/ kg).
[b] Subcutaneous pentylenetetrazole test (administered intraperitoneally to rats at doses ranging from 30 to 300 mg/ kg).
[c] Neurotoxicity (administered intraperitoneally to rats at doses ranging from 30 to 300 mg/ kg).
[d] Time of test after drug administration

RESULTS AND DISCUSSION

2,4-Disubstituted-1,5-benzodiazepines **(1b-10b)** were synthesized by reacting substituted chalcones with *o*-phenylenediamine in presence of glacial acetic acid and further methylation with methyl iodide in basic medium gave 2,4-Disubstituted phenyl-1-methyl-1*H*-benzodiazepines **(1c-10c)**, on acetylation with acetyl chloride in basic medium yielded 2,4-Disubstituted phenyl-1-acetyl-1*H*-benzodiazepines **(1d-5d).** The reactions were monitored by TLC on silica gel G plates, their R_f values were noted and the final compounds were purified by recrystallization from various solvents and their melting point range noted. The novel benzodiazepines were synthesized successfully in moderate to good yield.

The newly synthesized compounds were identified on the basis of R_f value, melting point range, solubility analysis, FTIR, ^{1}HNMR, MASS Spectral data and elemental analysis. The FTIR spectra of the newly synthesized compounds showed the presence of characteristic absorption bands in the region 3078-2927 cm^{-1} for aromatic C-H str., 2948-2867 cm^{-1} for aliphatic C-H str., 1689-1619 cm^{-1} for C=C str., 1628-1539 cm^{-1} for C=N str. and 1382-1327 cm^{-1} for C-N str. of benzodiazepine ring.

^{1}H NMR spectra of the synthesized compounds showed the characteristic peaks of N-CH_3 group, -C=C-H and aromatic protons between δ 2.604-2.812 ppm, 4.912-5.425 ppm and δ 6.501-7.985 ppm respectively **(1c-10c)**, the peaks for protons of the acetyl group of the *N*-acetylated compounds (**1d-5d)** fall within the range of δ 2.793-3.248 ppm.

The compounds (**1c-10c** and **1d-5d**) were administrated intraperitoneally (*i.p*) into the rats using dose 30, 100 and 300 mg/kg, the observations were taken at two different time intervals (0.5 and 4.0 h). Neurotoxicity was measured by the rotorodtest. The results are shown in Table 3.

In the present series of compounds, substituted benzodiazepine derivatives were designed and synthesized to meet structural requirements essential for anticonvulsant activity. In the MES test, compounds **3c**, **8c** and **9c** were active against MES test at dose of 30 mg/kg at 0.5 h and afforded 100% protection at dose of 30 mg/kg at 0.5 h. interestingly, compounds **3c** and **8c** continued to protect from the seizures at a dose of 30 mg/kg at 4.0 h also, which

indicates that **3c** and **8c** have rapid and long duration of anticonvulsant activity at lower dose. Compound **9c** was active at 4.0 h but at the higher dose i.e 100 mg/kg.

Compounds that were active at a dose of 100 mg/kg only at 0.5 h in MES screen included **1c** and **6c**, indicating that they have rapid onset and short duration of anticonvulsant activity. The characteristic feature of these compounds is substitution with electronegative groups that makes the compounds highly lipophilic.

In the ScPTZ screen, compounds that showed protection at 0.5h were **1c, 2d, 3c, 3d, 4c, 5d, 6c, 7c, 8c** and **9c**. Among these compounds **3c, 8c** and **9c** showed anti-ScPTZ activity at the dose of 100mg/kg at time periods 0.5 h. Compounds **1d, 2c** and **5c** were active only at 4.0 h at dose of 300 mg/kg indicative of the long duration of action of these compounds.

In the neurotoxicity screening, compounds **1d, 2c, 2d, 3d, 4c, 4d, 5c, 5d, 6c, 8c, 10c** were devoid of minimal motor impairment at the highest dose (300 mg/kg). Compounds **1c, 3c** and **9c** were less neurotoxic and exhibited motor impairment at the dose of 300 mg/kg at 0.5 h and also at 4.0 h. Compound **7(c)** revealed neurotoxicity at a dose of 100 mg/kg at 0.5h and also at 4.0 h.

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