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SYNTHESIS AND EVALUATION OF DEPROTECTED N-BOC PIPERAZINE DERIVED MONO-MANNICH BASES

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ABSTRACT

A series of deprotected N-boc piperazine derived mannich bases were synthesized and evaluated for anticonvulsant activity using subcutaneous pentylenetetrazole (PTZ) induced and maximal electroshock (MES) induced methods. Of all the derivatives, compound **1a** displayed good protection against PTZ induced seizures where as other derivatives were exhibited significant protection. In case of MES induced method, compound 1c showed good protection against shock induced seizures and all are less active than phenytoin as reference standard.

Keywords: Mannich bases, Pentylenetetrazole induced method, MES induced method.

1. INTRODUCTION

Mannich bases exhibit diverse pharmacological activities like antiinflammatory, antihelminthic, antibacterial, antineoplastic, analgesic, anticonvulsant, antinociceptive etc. Therefore, these compounds have gained the importance in drug discovery. N-aryl piperazines were also reported as key pharmacophoric moieties for antidepressant, anticonvulsant, antinociceptive and anti-inflammatory activities (Orjales *et al.,* 1996; Bayrak *et al.,* 2009; Wang *et al.,* 2010). A series of new N-mannich bases of [7,8-f]benzo-2-aza-spiro[7,8f]-benzo-1,3-aza-spiro[4,5] decane-1,3-diones and [7,8-f]benzo-2-aza-spiro[7,8f]-benzo-1,3-aza-spiro[4,5] decane-2,4-diones were reported and screened the derivatives for anticonvulsant activity using maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure test by Obniska et al (Obniska et al., 2010). Based on the literature, synthesis and evaluation of N-boc piperazine derived mono mannich bases were reported in this paper.

2. EXPERIMENTAL METHODOLOGY

2.1 Materials and Methods:

Aldehydes and esters were procured from Sigma-Aldrich and Merck chemicals. All other chemicals are of AR grade. Purity of the samples was monitored by TLC analysis using precoated aluminium plates (Merck), coated with silica gel (Kieselgel 60) with F_{254} indicator. Melting points were determined in open capillaries using Analab melting point apparatus and were uncorrected. IR spectra were recorded as KBr diluted pellets on a Jasco FTIR (FTIR-4100) Spectrophotometer. ^1H NMR spectra were carried out on Jeol-400 MHz NMR Spectrophotometer (JNM-400) using TMS as internal reference. Chemical shifts (δ) values are given in parts per million (ppm) using $CDCl_3$ as solvent and coupling constants (J) in Hz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectral data was obtained on LCMS (schimadzu) APCI model LC-2010 EV. Elemental analyses were performed on Perkin Elmer 2400 C, H and N elemental analyser.

General method for the synthesis of mono mannich bases of piperazines (1a-1d) (Abuo-Rahama *et al.,* 2009)

An equimolar concentration of boc-piperazine and the substituted aniline dissolved in suitable quantity of ethanol, 1ml of formalin (37%). The reaction mixture was refluxed for 5-hours, completion of the reaction was monitored by TLC (n-hexane: methanol 8:2). After the completion of the reaction, poured the contents in ice water and filtered it off to obtain

boc-protected 1a-1d**.** Compound 1a-1d were obtained by dissolving the intermediate boc-protected 1a-1d in 6N HCl (60 ml) and washed with ether (3×50 ml). The aqueous phase was basified with solid KOH to adjust the pH 11. It was then extracted with ethyl acetate (3×100 ml). The combined organic phases were dried over Na_2SO_4 and concentrated to give the compounds **1a-1d** (Repine *et al*., 2007).

Synthesis of the N-((piperazin-1-yl) methyl) benzenamine (1a, R=H):

0.01 mol (1.86 g) of boc-piperazine and 0.01 mol of aniline (1 ml) dissolved in suitable quantity of ethanol. To the stirred reaction mixture 1ml of formalin (37 %) was added and refluxed for 5 h, completion of the reaction was monitored by TLC. After the completion of the reaction, poured the contents in ice water and filtered it off. The resulting piperazine was deprotected by dissolving in 6N HCl (60 ml) and washed with ether (3×50 ml). The aqueous phase was basified with solid KOH to adjust pH 11. It was then extracted with ethyl acetate each quantity of 100ml for 3-4times. The combined organic phases were dried over Na_2SO_4 and concentrated to obtain the compound **1a**. The product was obtained as pale yellow solid.

PHYSICAL AND SPECTRAL DATA OF COMPOUNDS (1a-1e)

1. N-[(piperazin-1-yl) methyl] benzenamine (1a, R=H, R^1=H): R_f : (n-hexane : methanol, 8:2**)** 0.43; **λmax** 400nm. **IR (KBr) ν_{max}, cm^{-1}:** 3352 (2°amine, N-H str), 3041 & 3021 (Ar-C-H str), 2975, 2932 (alkyl, C-H str). **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm):** 2.40-2.55 (br d, 4H, $2CH_2$ of piperazine), 2.85 (br s, 1H, NH of piperazine), 3.4-3.5 (br d, 4H, $2CH_2$N of piperazine), 4.0 (br s, 1H, $NHCH_2$), 6.9-7.3 (m. 5H, aromatic). **APCI-MS:** m/z = 190.9 $(M)^+$. Anal. Calc. for $C_{11}H_{17}N_3$: C, 69.07; H, 8.96; N, 21.97. Found: C, 68.90; H, 8.91; N, 21.81.

2. 2-Nitro-N-[(piperazin-1-yl) methyl] benzenamine (1b, R=2-NO_2): 0.01mol (1.86g) of boc-piperazine and 0.01 mol of 2-nitroaniline (1.38g) dissolved in suitable quantity of ethanol and proceeded as in **1a** and obtained as yellow powder. **R_f: (**n-hexane : methanol, 8:2**)** 0.56; **λmax** 403nm. **IR (KBr) ν_{max} , cm^{-1} :** 3458, 3450 & 3446 (2°amine, N-H str), 3081 & 3021 (Ar, C-H str), 2975 & 2932 (alkyl, C-H str), 1302 & 1276 (Ar, C-N str). **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm):** 3.05-3.12 (t, 4H, $2CH_2$ of piperazine), 3.65-3.84 (br d, 4H, 2CH2 of piperazine)**,** 4.25 (s, 2H, CH_2), 4.75 (br s, 1H, NH), 6.04 (br s, 1H, NH), 6.69-8.13 (m, 4H, Ar-H of nitrophenyl). **APCI-MS:** m/z = 236.8 $(M+H)^+$.

3. 3-Nitro-N-[(piperazin-1-yl) methyl] benzenamine (1c, R=2-NO_2): 0.01mol (1.86g) of boc-piperazine and 0.01 mol of 3-nitroaniline (1.38g) dissolved in suitable quantity of ethanol and proceeded as in **1a** and obtained as yellow crystals. **R_f: (**n-hexane : methanol, 8:2**)** 0.54; **λmax** 395nm. **IR(KBr) ν_{max} , cm^{-1} :** 3452 , 3414 (2°amine,N-H str), 3093, 3041 & 3022 (Ar, C-H str), 2995 & 2962 (alkyl, C-H str), 1470 (N-O asym N-O Str), 1366 (N-O sym N-O str). **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm):** 2.43-2.49 (d, 4H, $2CH_2$ of piperazine), 2.82 (br s, 1H, NH), 3.40-3.55 (dt, 4H, 2CH2 of piperazine)**,** 4.01 (br s, 1H, NH), 5.21 (s, 2H, CH_2), 6.93-7.58 (m, 4H, Ar-H of nitrophenyl). **^{13}C NMR (100MHz, $CDCl_3$) δ (ppm):** 47.9 (**C**H_2-NH), 53.7 (N-**C**H_2), 72.4 (-HN-**C**H_2-N), 109.0, 116.8, 121.0, 135.0, 147.8 (CH_{ar}, C_{ar}), 150.6 (C-NO_2, C_{ar}). **APCI-MS:** m/z = 235.9 $(M)^+$. Anal. Calc. for $C_{11}H_{16}N_4O_2$: C, 55.92; H, 6.83; N, 23.71. Found: C, 55.85; H, 6.78; N, 22.67.

4. 4-Nitro-N-[(piperazin-1-yl) methyl] benzenamine (1d, R=4-NO_2): 0.01mol (1.86g) of Boc-piperazine and 0.01 mol of 4-nitroaniline (1.38g) dissolved in suitable quantity of ethanol and proceeded as in **1a** and obtained as yellow powder. **R_f: (**n-hexane : methanol, 8:2**)** 0.48; **λ max** 401nm.

2.3. PHARMACOLOGICAL STUDIES

2.3.1. Experimental animals

Male Swiss albino mice (18-22g) and male Wistar rats (150-200g) were used as experimental animals. They were obtained from King Institute of Preventive Medicine, Chennai. The animals were acclimatized for a week under standard husbandary conditions, room temperature of 24±1°C, relative humidity 45-55% and 12: 12 h light/dark cycle. The animals had free access to rodent pellet diet (Pranav Agro Industry, Bangalore) and water under strict hygienic conditions. All animal experiment protocols were approved by the

Institutional Animal Ethical Committee (IAEC) of Annamacharya college of Pharmacy, Rajampet, India (1220/a/08/CPCSEA/ANCP/06).

2.3.2. Acute toxicity studies

The study was conducted as per OECD-425 guide lines for testing of chemicals acute oral toxicity. The test was used to fix the safe dose for the compounds **1a-1d**. Swiss albino mice were divided into six groups each containing 10 animals and repeated for all the test compounds and were administered by oral route in different concentrations (2000, 1000, 500, 250, 100 and 50mg/kg body weight). The animals were observed for their death over a period of 7days. The LD_{50} values were calculated by up and down method and dose was fixed as 50mg/kg body weight.

2.3.3. METHODS

Evaluation of Anticonvulsant Activity subcutaneous Pentylenetetrazole Seizure test (Sc PTZ)

This method utilizes a dose of pentylenetetrazole (PTZ) 80mg/kg subcutaneously, in rats that produces clonic seizures. The rats were divided into several groups (six rats each). Group 1 animals were kept as control and were received vehicle; Group 2 received Diazepam (5 mg/kg, intraperitoneally), other groups received the test compounds **1a-1d** respectively (50mg/kg, oral), which were prepared by suspending in 0.5% sodiumcarboxymethylcellulose. 1 h after administration of vehicle, diazepam and test compounds **1a-1d**. The time of onset of clonic convulsions and the protection against mortality were observed (Subudhi *et al.,* 2009). The percentage protection against mortality was calculated using the formula: [number of animals used − number of animals died/ number of animals used] 100.

Maximal Electric Shock test (MES)

Anticonvulsant property of the test compounds in this model was assessed by its ability to protect against Maximal Electric Shock induced convulsions. Male Wistar albino rats were divided into several groups (six rats each). Group 1 was the control group which received vehicle (0.5% sodium carboxy methyl cellulose, oral); Group 2 received Phenytoin (30 mg/kg, oral), Other groups received each of test compounds **1a-1d** respectively (50 mg/kg, oral), which were prepared by suspending in 0.5% sodium carboxy methyl cellulose. 1 h after the administration of vehicle, Phenytoin, test compounds, Maximal Electric Shock of 150 mA current for 0.2 sec was applied through corneal electrodes to induce convulsions using an Electroconvulsiometer (INCO, Ambala, India) in the control, standard and test compounds treated animals. Duration of hind limb tonic extensor was noted. Abolition or reduction in the duration of tonic extension was considered as the index for antiepileptic activity (Obniska. J. *et al.,* 2010). The percentage protection against shock induced seizures was calculated using the formula: $[C_{\text{hind limb extension}} - T_{\text{hind limb extension}} / C_{\text{hind limb extension}}] 100$.

3. RESULTS AND DISCUSSION:

In the present study, the synthesis of the substituted N-[(piperazine-1-yl) methyl] benzenamine derivatives **(1a-1d)** was carried out via the mannich reaction (Abuo-Rahama *et al.,* 2009). The condensation of commercially available piperazine or substituted piperazines and various substituted anilines with formaldehyde in equimolar or 1:2 molar ratios resulted in the compounds **(1a-1d)** in good yields (52-80%). In the synthesis of **1a-1d,** Boc-protected piperazine was used in the above method and the reaction was further continued for the deprotection of piperazinyl moiety by acidification followed by basification (Repine *et al.,* 2007).

The IR spectra of the title compounds showed a broad band at 3500-3400cm^{-1} assignable to the secondary amine group. A band at 1450-1350 cm^{-1} indicated symmetric and asymmetric stretching of nitro functional group and at 1050-1020 cm^{-1} showed C-N stretching. The ^{1}H NMR spectrum of the compounds supported the structures of title compounds. These compounds showed a broad singlet at 2.3-2.8 ppm which indicated the NH proton of piperazinyl moiety and a triplet or doublet in the region of 2.4-3.8 ppm representing alkyl protons of piperazine ring. A singlet was observed in the region of 4.2-5.2 ppm assignable to the methylene protons flanked by two nitrogens and multiplets in the region of 6.4-8.2 ppm due to the presence of aryl protons. The mass spectra of the

compounds **(1a-1d)** showed the molecular ion peaks at their respective molecular weights as M^+ and $(M+H)^+$ and the elemental analysis for the compounds were within the limits of ±0.4% of theoretical value.

Anticonvulsant activity

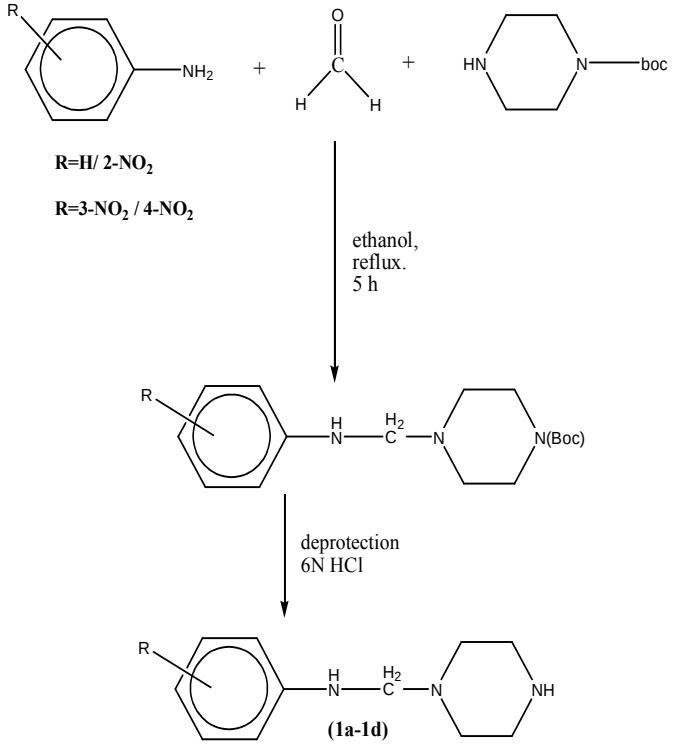
The profile of anticonvulsant activity of compounds **1a-1d** was evaluated by subcutaneous pentylenetetrazole (scPTZ) and maximal electroshock (MES) induced seizure methods after oral administration of the drug candidate to male Wistar albino rats at the dose of 50 mg/kg body mass. The dose was fixed by up and down method as per OECD-425 guidelines. Seizure inducing pentylenetetrazole (80mg/kg) or maximal electroshock (150 mAmp, 0.2 sec) was applied 1 hour after the administration of drug candidate. Compounds **1a-1d** showed significant anti-PTZ activity (P<0.05 Vs control). Of these compounds, **1a** exhibited good anticonvulsant activity and delayed the onset of convulsions, which may be due to the presence of mannich bases containing phenylaminobenzyl substitution on piperazine (P>0.0001). Other derivatives displayed moderate activity and are not comparable to diazepam as reference standard (**Table 1**). The protection against PTZ induced mortality is very less and other compounds did not exhibit significant protection.

In **MES model**, compounds **1a-1c** showed significant decrease in the duration of hind limb extension and percentage protection against electro convulsions except compound 1d (P<0.05vs control; **Table 1**). Among these derivatives, piperazine possessing 3-nitrophenyl substitutions on the nitrogen of piperazine (**1c)** displayed good anti-MES protection and other derivatives showed moderate protection. However, the activities of the compounds **1a-1c** are less than Phenytoin.

4. CONCLUSION

The present study revealed that, compounds, **1a** exhibited good anticonvulsant activity which may be due to the presence of mannich bases containing phenylaminobenzyl substitution on piperazine. Compound **1c** bearing 3-nitrophenyl substitutions on the nitrogen of piperazine displayed good anti-MES protection and less active than phenytoin as reference standard.

Scheme 1: Synthetic protocol of the piperazine derived mono mannich bases (1a-1f)



compound	R
1a	H
1b	2-NO_2
1c	3-NO_2
1d	4-NO_2

Table 1: Anticonvulsant activity of N-[(piperazin-1-yl) methyl] benzenamine derivatives **(1a-1d)** in pentylenetetrazole induced seizure model and maximal electroshock method.

Compound	Latency Period mean ± SEM	Percentage mortality (%)	Duration of Limb Extension mean ± SEM	Percentage protection (%)
Control	66.67±2.789	000	21±1.317	-------
Diazepam	300±0.0***	100	------	-----
Phenytoin	-----	-----	6.333±0.5578***	69.8
1a	231.7±23.12***	16.6	15.33±1.116**	27
1b	200±33.47***	16.6	14±0.6325***	33.3
1c	193.3±29.51***	33.3	11.67±0.5578***	44.4
1d	213.3±27.65***	33.3	18.67±1.174 ^{ns}	11.0

The test compounds were administered orally (at the dose of 50mg/kg) 1h before the injection of pentylenetetrzole (80mg/kg, i.p) or application of maximal electroshock (150mAmp, 0.2 sec). Values were expressed as mean±SEM, n=6. One-way analysis of variance (ANOVA) followed by Dunnet's multiple comparision test. *** P<0.0001 vs control, **P<0.05 vs control, ^{ns}P>0.05 vs control.

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