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EVALUATION OF ANTINOCICEPTIVE AND ANTIOXIDANT ACTIVITIES OF MONO AND BIS-MANNICH BASES OF PIPERAZINE DERIVATIVES

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ABSTRACT

Recently mono and bis mannich bases of piperazine derivatives were reported as anticonvulsant agents. Below is an extended study of these derivatives, (4a-4o) for the antinociceptive (formalin induced nociceptive method) and antioxidant activities are described. In formalin induced nociceptive method, the test compounds (**4b-4m**) displayed significant activity in neurogenic phase (early phase). Among these derivatives, the symmetric bis mannich base of piperazine bearing 3-nitrophenyl substitution (**4i**) displayed good antinociceptive activity (71.2%) and other compounds showed moderate activity. Further, the compounds were evaluated for *in vitro* antioxidant activity by DPPH free radical scavenging assay. A correlation between *in vitro* antioxidant and antinociceptive activity was observed for compound **4c**.

Keywords: Formalin induced nociceptive method; DPPH free radical scavenging method; Antinociceptive activity.

1. INTRODUCTION

Neuropathic pain comprises a variety of painful conditions, including post amputation pain, painful neuropathies, post traumatic neuralgia, and others. So far, multiple factors responsible for development of neuropathic pain have been identified: metabolic diseases (e.g., diabetes), neuronal tissue injuries caused by toxicological factors or mechanical damage to the spinal cord, and others (Nickel et al. 2012; Woolf and Mannion 1999). Hence, pharmacotherapy used to relieve neuropathic pain comprises several pharmacological classes, of which anti-epileptic drugs (AEDs), antidepressant drugs, and local anesthetic agents play a pivotal role (Christoph et al. 2011; Davis 2007; Davis 2010; Gilron et al. 2009; Miranda et al. 2012). N-mannich bases and N-aryl piperazine derivatives were reported as anticonvulsant and antinociceptives respectively (Obnisca et al., 2010). In my earlier study, a series of N-mannich bases of piperazine derivatives were screened for anticonvulsant activity (Prasanthi., 2014). Pharmacological and clinical studies have documented the pathophysiological similarities in epilepsy and neuropathic pain models (Baruah et al., 2012).

Therefore, the study was extended for antinociceptive and antioxidant activities for the previously reported derivatives using formalin induced nociceptive assay and DPPH free radical scavenging assay.

2. MATERIALS AND METHODS

Adult male Swiss albino mice (18-25g) were used in the experiments. The animals were kept in groups (6 in each group) at a room temperature 22±3°C, under light/dark (12: 12) cycle and had free access to food and water.

2.1. Chemicals used in pharmacological tests

The synthesis of the investigated compounds, (**1a-1f, 1'a-1'd** and **3a-3f**) was described earlier (Prasanthi., 2014). For the pharmacological experiments, compounds **1a-1f, 1'a-1'd** and **3a-3f** were suspended in 0.5% sodium carboxymethylcellulose and administered orally (50mg/kg body weight; here the compounds were renamed as **4a-4o**). Control animals were given appropriate amount of vehicle (0.5% sodium carboxymethylcellulose), tramadol injection (5 mg/ kg, s.c.) and 2.5% formalin solution (0.92% of formaldehyde, made up in saline solution 137 mM NaCl).

EVALUATION OF ANTINOCICEPTIVE ACTIVITY

Formalin-induced nociception assay

The mice were divided in to eighteen groups each containing six animals. Group 1 was the control group received vehicle; Group 2 received tramadol injection (5 mg/ kg, s.c.), Group 3 -18 received the test compounds (50 mg/kg, p.o) respectively, 1h prior to the formalin injection. Animals were injected subcutaneously in to intraplantar region with 20 µl of 2.5% formalin solution (0.92% of formaldehyde, made up in saline solution 137 mM NaCl). Mice were immediately placed in a glass cylinder 20 cm in diameter and observed from 0 to 30 min following formalin injection. The amount of time spent licking the injected paw was measured with digital timer was considered as indication of nociception. The first phase of the nociceptive response normally peaked 5 min after the formalin injection and the second phase 15 to 30 min after the formalin injection, representing the neurogenic and inflammatory nociceptive responses, respectively (Milanoa *et al.*, 2008).

ANTIOXIDANT STUDIES

Free radical scavenging assay (DPPH Method)

The ability to scavenge 2, 2-diphenyl-1-Picryl-Hydrazyl (DPPH) radical was determined by using DPPH method. In this method, 1 ml of test compound (10, 50, 100, 250, 500 µg/ml) in ethanol was added to 3.9 ml of 0.004% methanol solution of DPPH and incubated in a dark place for 30 min. The absorbance of the samples was read at 517 nm. Ascorbic acid was used as reference standard. Percentage inhibition of DPPH free radical by the test compounds was calculated (Biradhar *et al.*, 2010).

RESULTS AND DISCUSSION

In formalin induced nociceptive method, the test compounds (**4b-4m**) displayed significant activity in neurogenic phase (early phase). Among these derivatives, the symmetric bis mannich base of piperazine bearing 3-nitrophenyl substitution (**4i**) displayed good antinociceptive activity (71.2%) and other compounds showed moderate activity (**Table-I**). The second phase of the formalin induced nociceptive method indicates inflammatory responses. Here, symmetric bis mannich bases of piperazine possessing 3-nitrophenyl substitution and asymmetric bis mannich bases of piperazine bearing 3-nitrophenyl and phenyl group exhibited good activity in inflammatory phase. However, the activities of these compounds are less than tramadol. Baruah *et al.,* reported the pathophysiological similarities in epilepsy and neuropathic pain models and the potentiality of antiepileptic agents to manage neuropathic pain (Baruah *et al.,* 2012). In accordance to these reports, it was observed that compounds **4f** and **4i** which displayed good anti-MES protection also exhibited significant activity in formalin induced method.

Further, the compounds were evaluated for *in vitro* antioxidant activity by DPPH free radical scavenging assay. IC_{50} values were calculated and illustrated in **Table-II**. Compound **4a** possessing phenyl substitution on the 4^{th} position of piperazinyl mannich base and free-NH group displayed low IC_{50} values and showed good antioxidant activity, comparable to standard ascorbic acid. Other derivatives **4c, 4d, 4g, 4j, 4k** and **4o** exhibited moderate antioxidant activity. A correlation between *in vitro* antioxidant and antinociceptive activity was observed for compound **4c**. Symmetric and asymmetric bis mannich bases displayed higher activities than mono mannich bases. There is no significant influence of symmetric or asymmetric substitution of mannich bases on biological activities. However, the anticonvulsant and antinociceptive activities of the compounds **4f, 4i, 4k** and **4l** were not correlated to antioxidant activity.

CONCLUSION

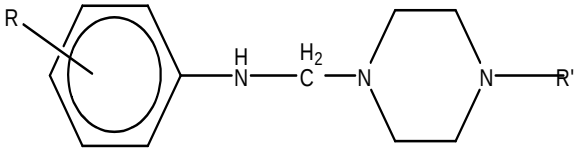
The present study revealed that compounds **4f** and **4i** showed promising anticonvulsant and antinociceptive activities. The presence of 3-nitro group on phenyl and (phenylamino) methyl substitution at the 4^{th} position of the nitrogen of piperazinyl mannich bases are probably the desirable features for good anticonvulsant and antinociceptive activities. Further, compound 4c showed good correlation between *in vitro* antioxidant and antinociceptive activity.

Table 1: Antinociceptive activity of N-[(substitutedpiperazin-1-yl) methyl] benzenamine derivatives (4a-4m) in formalin induced nociceptive model.

Compound	Number of Paw licking during 0-5 min. (Mean±SEM)	Percentage Protection during 0 -5 min (%)	Number of Paw licking during 15-30 min. (Mean±SEM)	Percentage Protection during 15-30 min (%)
Control	22±1.764	-----	9±0.8563	----
Tramadol	2.75±0.6922***	87.5	0.75±0.2500***	91.6
4a	17.5±0.9916^{ns}	20.4	7±0.8563^{ns}	22.2
4b	10.6±2.044***	51.8	3.5±0.4282***	61
4c	9.5±1.147***	56.8	2.5±0.339***	72.2
4d	11.8±3.631***	46.3	5.5±0.4282**	38.8
4e	----------	----------	----------	----------
4f	7±0.8563***	68	1.5±0.8466***	83.3
4g	12±1.983***	45.4	2.66±0.8819***	70.4
4h	7.667±1.022***	65	1.5±0.4282***	83.3
4i	6.33±0.6280***	71.2	1±0.50***	88.8
4j	14.17±0.8333***	35.5	2±1.183***	77.7
4k	9.333±0.6667***	57.5	1.5±0.4282***	83.3
4l	8.167±0.8333***	62.8	1.083±0.2713***	87.9
4m	15.67±1.116**	28.7	1.5±0.4282***	83.3
4n	----------	----------	----------	----------
4o	------	----------	----------	----------

The test compounds were administered orally (at the dose of 50mg/kg) 1h before the injection of formalin (2.5%, s.c in intraplantar region), Values were expressed as mean±SEM, n=6. One-way analysis of variance (ANOVA) followed by Dunnet's.*** P<0.0001 vs control, **P<0.05 Vs control, ^{ns}P>0.05 vs control.

Table 2: Antioxidant activity of N-[(substitutedpiperazin-1-yl) methyl] benzene amine derivatives (4a-4o) in DPPH free radical scavenging assay



Compound	R	R^1	IC_{50} (µg/ml)
4a	H	H	29.25
4b	H	H	91.6
4c	H	H	163.0
4d	H	H	123.5
4e	C_6H_5	C_6H_5	190.0
4f	C_6H_5	C_6H_5	227.3
4g	C_6H_5	C_6H_5	105
4h	O-NO_2	-CH_2-NH-C_6H_4-2-$N0_2$	392
4i	m-NO_2	-CH_2-NH-C_6H_4-3-$N0_2$	360
4j	p-NO_2	-CH_2-NH-C_6H_4-4-$N0_2$	172.3
4k	2-NO_2	-CH_2-NH-C_6H_5	132
4l	3-NO_2	-CH_2-NH-C_6H_5	217.3
4m	4-NO_2	-CH_2-NH-C_6H_5	410
4n	2-NO_2	4-F-C_6H_4-	588
4o	2-NO_2	4-Cl-C_6H_4-	190.4
Ascorbic acid	----	-----	26.1

Reduction of DPPH free radical by the test compounds at various concentrations was expressed as IC_{50} value, which was estimated in ethanol solution, absorbance was measured at 517nm.

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