

EFFECTIVENESS OF ANTIEPILEPTIC GABA ANALOGUES FOR THE TREATMENT OF NEUROPATHIC PAIN

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Summary

Enhancement of γ -aminobutyric acid (GABA)ergic neurotransmission and blockade of voltage-gated sodium channels have been reported to possess palliative roles in neuropathic pain conditions. We undertook the present study to assess the peripheral analgesic, antiallodynic and antihyperalgesic activities of the antiepileptic GABA analogues (**SKP-1-16**) in acute tissue-injury model and two animal models of neuropathic pain, namely chronic constriction injury (CCI), and spinal nerve ligation (SNL). Most of the compounds suppressed the acetic acid induced writhing response significantly ($P < 0.01$) in comparison to control. In particular, **SKP-12** (100 mg/kg i.p.) was found to be more active than aspirin (96.0% inhibition of writhing response as against 93.0% for aspirin). Intraperitoneal administration of compounds **SKP-6 and SKP-9** (100 mg/kg) attenuated spontaneous pain up to 150 min of sensory testing in both CCI and SNL rats. Compounds **SKP-2, SKP-3 and SKP-4** (100 mg/kg i.p.) showed activity against dynamic allodynia throughout the 150 min period of observation in both the neuropathic pain models. Overall, from the study, **SKP-4** (100 mg/kg i.p.) emerged as the most active compound being effective in five nociceptive assays (including the mechanical hyperalgesia screen). In conclusion, we have demonstrated the analgesic, antiallodynic and antihyperalgesic activities of N-phthaloyl GABA hydrazones.

Keywords: γ -aminobutyric acid; Allodynia; Hyperalgesia; Writhing.

Introduction

Neuropathic pain is defined as “pain initiated or caused by a primary lesion, injury or dysfunction in the central or peripheral nervous system” and is an area of largely unmet therapeutic need. Consequently, at present, there are very few effective and well-tolerated therapies for neuropathic pain. In particular, tricyclic antidepressants (TCAs) and antiepileptic drugs (AEDs) have been reported to possess antiallodynic and antihyperalgesic activities in animal models of neuropathic pain.^[1-3]

4-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain.^[4] It is well documented that attenuation of GABAergic neurotransmission is involved in the pathophysiology of anxiety, epilepsy and pain.^[5-7] More specifically, the reduced spinal GABAergic tone, which results just after nerve injury is reported to be responsible for the incidence of allodynia and hyperalgesia in neuropathic pain conditions.^[8] Reports on the efficacy of GABA derivatives namely baclofen (a GABA_B receptor agonist),^[9] gabapentin and pregabalin^[10] in various animal models of inflammatory and neuropathic pain, firmly established their therapeutic usefulness. Recently we initiated efforts to prepare newer GABA derivatives with multiple pharmacological actions effective in the treatment of epilepsy and neuropathic pain.^[11-15] In the present work, the GABA derivatives (**SKP 1-16**, N-phthaloyl GABA hydrozones) are expected to exhibit dual action on both the voltage-sensitive sodium channels and GABA receptors,^[11] the present study was undertaken to test the effects of these hybrids in rodent models of neuropathic pain. The antinociceptive activities were established in the chronic constriction injury (CCI) and spinal nerve ligation (SNL) models.

Materials and methods

Animals used for the experiments: The experimental protocols used were approved by the Institutional Animal Ethics Committee of Birla Institute of Technology & Science Pilani (Protocol Nos: IAEC/RES/6/3 and IAEC/RES/6/2). Swiss albino mice (either sex) with weights ranging from 20-25 g were used for the acetic acid induced writhing model. Wistar rats of either sex (200-320 g) were used for both the neuropathic pain models. Animals were housed six (mice) and four (rats) per cage at a constant room temperature of $23 \pm 2^\circ\text{C}$ under a 12-h light/dark cycle (lights on at 8:00 AM), with food and water *ad libitum*.

Acetic acid induced writhing: Mice were divided into groups of six each. Using the method of Siegmund et al.,^[16] writhing was induced by an intraperitoneal injection of 0.1 ml of 3% v/v acetic acid. Test group received acetic acid one hour after drug-treatment. The number of writhings occurring for a 30 min time period was recorded. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition of the writhing response was then calculated.

Surgery-Chronic constriction injury model: Unilateral mononeuropathy was produced in rats using the CCI model as described by Bennett and Xie.^[17] The rats were anesthetized with an intraperitoneal dose of pentobarbital sodium (65 mg/kg) with additional doses of the anesthetic given as needed. Under aseptic conditions, a 3-cm incision was made on the lateral aspect of the left hindlimb (ipsilateral) at the mid-thigh level with the right hindlimb serving as the control (contralateral). The left paraspinal muscles were then separated from the spinous processes and the common left sciatic nerve was exposed just above the trifurcation point. Four loose ligatures were then made with a 4-0 braided silk suture around the sciatic nerve with about 1mm spacing. The wound was then closed by suturing the muscle using chromic catgut with a continuous suture pattern. Finally, the skin was closed using silk thread with horizontal-mattress suture pattern. A sham surgery (n=4) was performed by exposing the sciatic nerve as described above, but not damaging it. Povidone iodine ointment was applied

topically on the wound and gentamicin sulfate (4 mg/kg) was given intramuscularly for five days after surgery. The animals were then transferred to their home-cages and left for recovery.

Surgery- Selective segmental L5 SNL model: A left L5 spinal nerve ligation, as described by Kim and Chung,^[18] was performed. The rats were anesthetized with an intraperitoneal dose of pentobarbital sodium (65 mg/kg) with additional doses of the anesthetic given as needed. Under aseptic conditions, using the transverse processes of L6 as a guide, the left paraspinous muscles were exposed and separated from the spinous processes of L4 to S2 by blunt dissection. The L5 spinal nerve was then exposed at the level of the dorsal root ganglion, and ligated tightly with a 4-0 braided silk suture. Only one tight ligature was made in this model. After confirmation of hemostasis, the wound was then closed by suturing at both muscle and skin levels. A sham surgery (n=4) was performed by exposing the L5 spinal nerve as described above, but not damaging it. Povidone iodine ointment was applied topically on the wound and gentamicin sulfate (4 mg/kg) was given intramuscularly for five days after surgery. The animals were then transferred to their home-cages and left for recovery.

Sensory testing using nociceptive assays: Four nociceptive assays aimed at determining the severity of behavioral neuropathic responses namely allodynia and hyperalgesia were performed. The assays involved measurement of the degree of spontaneous (ongoing) pain and tests of hind limb withdrawal to cold and mechanical stimuli (dynamic mechanical allodynia, cold allodynia and mechanical hyperalgesia). A minimum of 10 min separated the testing procedures to reduce the influence of prior nociceptive testing. The order of testing was as follows: spontaneous pain, dynamic allodynia, cold allodynia and lastly mechanical hyperalgesia. All of the behavioral responses were timed with a stopwatch.

Spontaneous pain

The paw withdrawal duration (PWDs) due to spontaneous pain was assessed for a total time period of 5 min as described previously by Choi et al.^[19] The operated rat was placed inside an observation cage that was kept 5 cm from the ground level. An initial acclimatization period of 10 min was given to each of the rats. A total number of four rats (n=4) were assigned to this group. The test consisted of noting the cumulative duration that the rat holds its ipsilateral paw off the floor. The paw lifts associated with locomotion or body repositioning were not counted. It's been suggested that those paw lifts in the absence of any overt external stimuli are associated with spontaneous pain, and are correlative of ongoing pain.

Dynamic allodynia

All of the operated rats were assessed for dynamic allodynic response according to the procedure described by Field et al.^[20,21] The operated rat was placed inside an observation cage that was kept 5 cm from the ground level. An initial acclimatization period of 10 min was given to each of the rats. A total number of four rats (n=4) were assigned to this group. A positive dynamic allodynic response consisted of lifting the affected paw for a finite period of time in response to mild stroking on the plantar surface using a cotton-bud. This stimulus is non-noxious to a normal-behaving rat. The latency to paw-withdrawal (PWL)

was then noted down. If no paw withdrawal was shown within 15 s, the test was terminated and animals were assigned this withdrawal time. Hence, 15 s effectively represented no withdrawal.

Cold allodynia

The rats demonstrating unilateral mononeuropathy were assessed for acute cold allodynia sensitivity using the acetone drop application technique as described by Caudle et al.^[22] The operated rat was placed inside an observation cage that was kept 5 cm from the ground level and was allowed to acclimatize for 10 min or until exploratory behaviour ceased. A total number of four rats (n=4) were assigned to this group. Few drops (100-200 μ L) of freshly dispensed acetone were squirted as a fine mist onto the midplantar region of the affected paw. A cold allodynic response was assessed by noting down the duration of paw-withdrawal (PWD) response. For each measurement, the paw was sampled three times and a mean calculated. At least 3 min elapsed between each test.

Mechanical hyperalgesia

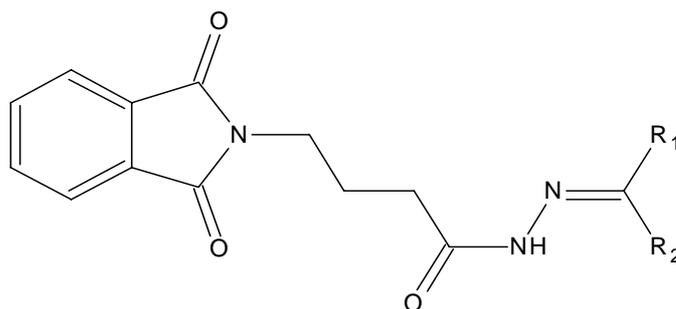
Neuropathic rats were assessed for mechanical hyperalgesia sensitivity according to the procedure described by Gonzalez et al.^[23] The operated rat was placed inside an observation cage that was kept 5 cm from the ground level. An initial acclimatization period of 10 min was given to each of the rats. A total number of four rats (n=4) were assigned to this group. Paw withdrawal duration (PWD) was measured after a mild pin prick stimulus to the midplantar surface of the ipsilateral (left) hindpaw. A withdrawal was defined as being abnormally prolonged if it lasted at least 2 s. The mean withdrawal duration was taken from a set of three responses.

Pharmacological interventions: Compounds **SKP (1-16)** correspond to structures **11-26** whose synthesis and characteristics were reported earlier by our group^[11] (Figure 1)

{**SKP-1:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[(1-phenyl methylene)butanoyl hydrazide; **SKP-2:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[(3-nitrophenyl)methylene]butanoylhydrazide; **SKP-3:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[(4-nitrophenyl)methylene]butanoylhydrazide; **SKP-4:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[(2-hydroxyphenyl)methylene]butanoylhydrazide; **SKP-5:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[(4-hydroxy-3-methoxyphenyl)methylene]butanoylhydrazide; **SKP-6:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[1-phenylethylidene]butanoylhydrazide; **SKP-7:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[1-(4-nitrophenyl)ethylidene]butanoylhydrazide; **SKP-8:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[1-(2-hydroxyphenyl)ethylidene] butanoylhydrazide; **SKP-9:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[1-(4-hydroxyphenyl)ethylidene]butanoylhydrazide; **SKP-10:** *N'*-[1-(3-aminophenyl)ethylidene]-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoylhydrazide; **SKP-11:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[1-(4-methylphenyl)ethylidene]butanoylhydrazide; **SKP-12:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[1-methylpropylidene]butanoylhydrazide; **SKP-13:** 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*-[1-phenylbenzylidene]butanoylhydrazide; **SKP-14:** *N'*-cyclopentylidene-4-(1,3-dioxo-1,3-dihydro-2*H*-

isoindol-2-yl)butanoylhydrazide; **SKP-15**: *N*'-cyclohexylidene-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoylhydrazide; **SKP-16**: 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*'-[2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] butanoylhydrazide} were synthesized according to previously reported procedures.^[11] Lamotrigine and carbamazepine were obtained as gift samples from M/s IPCA Laboratories, India. Gabapentin was obtained as a generous gift sample from M/s Wockhardt Laboratories, India. Aspirin used for the study was commercially available from Central Drug House, India. The solutions of compounds **SKP (1-16)** and standard drugs (lamotrigine, carbamazepine and gabapentin) were prepared with 30% v/v PEG 400 in saline containing 1% Tween 80. Aspirin was suspended in 2% w/v gum acacia.

Figure 1: General structure of GABA analogues (SKP 1-16)



Acetic acid induced writhing

Compounds **SKP (1-16)** (100 mg/kg i.p.) were administered 1 h prior to acetic acid administration. The control group mice received normal saline (10 ml/kg). Aspirin at a dose of 100 mg/kg i.p. was used as the standard drug.

Peripheral nerve injury

Baseline sensory response values were measured for each group of animals (n=4) pre-operatively and on 9th day post-operatively. Animals displaying allodynic and hyperalgesic responses in both the models, were then administered the relevant drug according to a pre-determined randomization table and testing was re-performed at 30, 60, 90, 120 and 150 min post drug administration. Each group of animals was used for only one drug administration protocol to ensure no 'carry-over' effects. Compounds **SKP (1-16)** (100 mg/kg i.p.) were administered at t=0. The vehicle control group of rats received only the solvent. Three positive control groups run alongside drug treatment groups using lamotrigine, carbamazepine and gabapentin (100 mg/kg i.p.). The treatment protocol remained the same for the standard drugs. No drug testing was performed for sham-operated rats.

Data analysis: All data are expressed as means \pm S.E.M. The data were analyzed using Student's *t* test only when two means were compared (acute pain assay). In the case of neuropathic pain studies, statistical significance was determined for drug effects by one-way ANOVA, and Bonferroni's post hoc test was used for individual comparisons with postoperative values. In the first assay, significance was assigned to a P value of less than 0.01 and in the chronic pain assay; comparison results with a P value of less than 0.05 were considered statistically significant. The statistical software package PRISM (Graphpad Software Inc, San Diego, CA) was used for the analyses.

Results

Acetic acid induced writhing: Most of the tested compounds suppressed the acetic acid induced writhing response significantly ($P < 0.01$) in comparison to the control. The standard drug, aspirin (100 mg/kg i.p.) exhibited 93.0% inhibition. Compounds **SKP-4**, **SKP-8** and **SKP-12** were the most active compounds with percentage inhibition values more than 80%. Of these compounds, **SKP-12** was observed to be more active than the standard drug aspirin (96.0% inhibition of writhing response as against 93.0% in the case of aspirin) (Table 1).

Table 1: Effects of compounds on writhing induced by acetic acid in mice

Treatment	Number of writhes (per 30 min) ^a	Inhibition (%)
Control	36.00 ± 3.00	-
SKP-1	11.50 ± 1.50	68
SKP-2	20.00 ± 1.00	44
SKP-3	19.50 ± 2.50	46
SKP-4	5.50 ± 3.50	85
SKP-5	27.50 ± 5.50 ^{NS}	24
SKP-6	18.00 ± 10.00	50
SKP-7	30.00 ± 7.00 ^{NS}	17
SKP-8	4.00 ± 2.00	89
SKP-9	24.00 ± 7.00	33
SKP-10	7.50 ± 1.50	79
SKP-11	14.00 ± 2.00	61
SKP-12	1.50 ± 0.50	96
SKP-13	12.50 ± 4.50	65
SKP-14	35.25 ± 1.75 ^{NS}	2
SKP-15	34.25 ± 3.60 ^{NS}	5
SKP-16	24.00 ± 3.00	33
Aspirin	2.65 ± 1.40	93

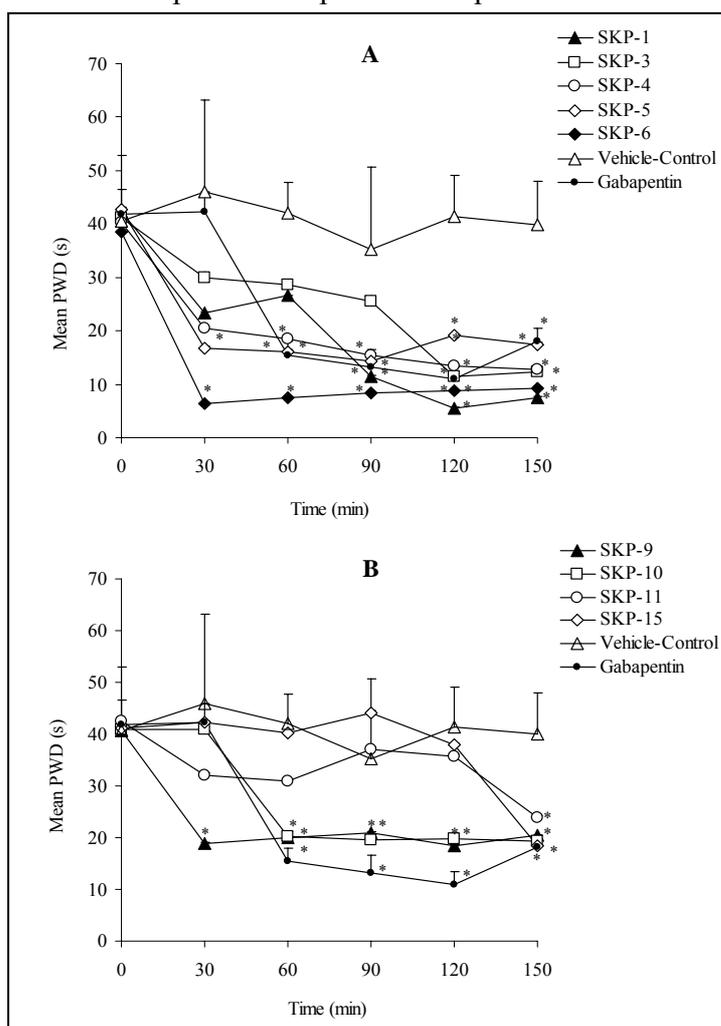
^a Each value represents the mean ± SEM of six mice significantly different from the control at $P < 0.01$ and NS denotes not significant at $P < 0.01$ (Student's t-test).

Peripheral nerve injury: All animals included in the study showed altered sensory responses in all the four behavioral nociceptive tests, 9 days following CCI and SNL. The sham-operated animals showed no significant difference from the pre-operative baseline sensory response values. In the animals, where only the solvent was administered as a control, there was no significant difference between the pre-drug and post-treatment values, at all time points of observation.

Spontaneous pain in the CCI model

Compounds **SKP-5**, **SKP-6** and **SKP-9** completely reversed the spontaneous pain response, at the dose tested (100 mg/kg) over 150 min (Figure 2). The time to peak-effect (TPEs) of these compounds were 90 min, 30 min and 120 min respectively ($14.25^* \pm 0.17$, $6.44^* \pm 0.24$ and $18.32^* \pm 0.35$ s for **SKP-5**, **SKP-6** and **SKP-9** respectively, versus 42.69 ± 0.54 , 48.43 ± 0.15 and 40.79 ± 0.46 s for pre-drug, $*p < 0.05$). Compound **SKP-1** showed a significant reduction in spontaneous pain response from 90 min through 150 min of sensory testing, while compound **SKP-4** and **SKP-10** were active from 60 min till 150 min. Compound **SKP-3** reversed the ongoing pain response only from 120 min to 150 min. Compounds that showed activity at only one time point include **SKP-11** and **SKP-15** ($23.77^* \pm 0.62$ and $18.44^* \pm 0.21$ s for **SKP-11** and **SKP-15** respectively at 150 min, versus 42.44 ± 0.39 and 41.2 ± 0.19 s for pre-drug, $*p < 0.05$). Compounds **SKP-2**, **SKP-7**, **SKP-8**, **SKP-12**, **SKP-13**, **SKP-14** and **SKP-16** were devoid of any significant effect. Carbamazepine reversed the spontaneous pain response only till 60 min significantly, and lamotrigine was devoid of any activity. On the other hand, gabapentin was found to be effective from 60-150 min ($11.02^* \pm 2.34$ s at 120 min, versus 41.90 ± 4.66 s for pre-drug, $*p < 0.05$).

Figure 2: Effects of compounds in spontaneous pain after CCI in rats (A and B)

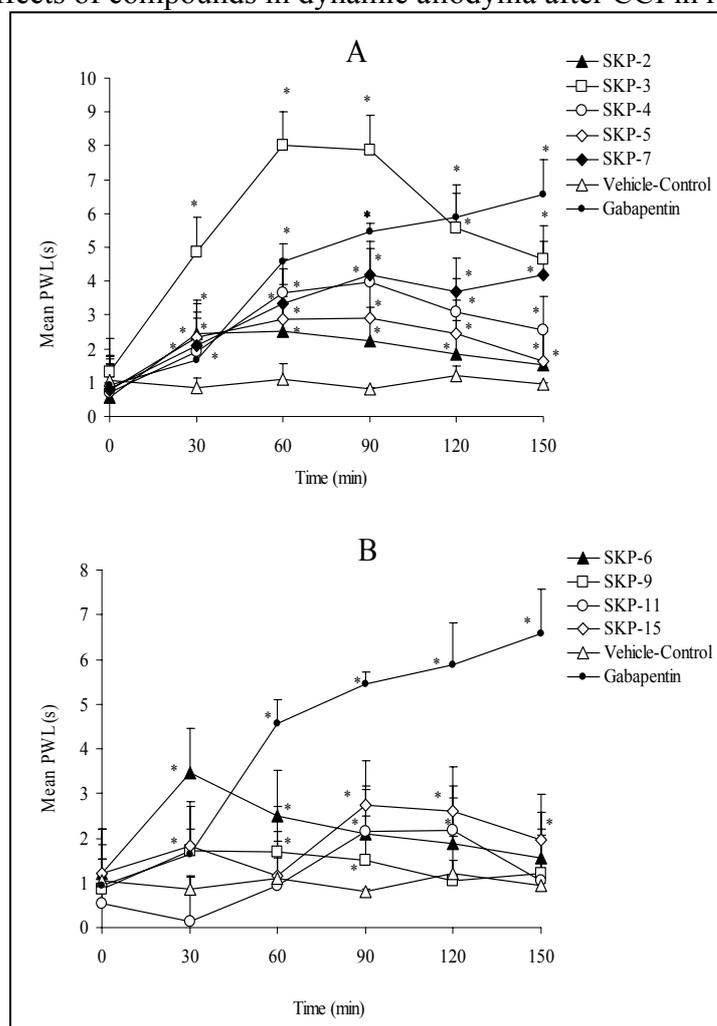


* $P < 0.05$, in comparison with the pre-drug values

Dynamic allodynia in the CCI model

Intraperitoneal administration of compounds **SKP-2**, **SKP-3**, **SKP-4**, **SKP-5** and **SKP-7** showed complete protection against dynamic allodynia throughout the entire 150 min experiment at the dose tested (100 mg/kg). Compounds **SKP-6** (30 min, 60 min), **SKP-9** (30 min, 60 min), **SKP-11** (90 min, 120 min) and **SKP-15** (90 min, 120 min) were found to be active in attenuating the allodynic response only at two time points, while **SKP-1**, **SKP-8**, **SKP-10**, **SKP-12**, **SKP-13**, **SKP-14** and **SKP-16** were completely inactive. Lamotrigine and carbamazepine showed antiallodynic effects only at 30 min and 90 min respectively ($5.66^* \pm 0.39$ and $4.89^* \pm 1.31$ s for lamotrigine and carbamazepine respectively, versus 0.96 ± 0.2 and 1.76 ± 0.67 s for pre-drug, $*p < 0.05$). Gabapentin exhibited antiallodynic activity throughout the entire 150 min period (Figure 3).

Figure 3: Effects of compounds in dynamic allodynia after CCI in rats (A and B)



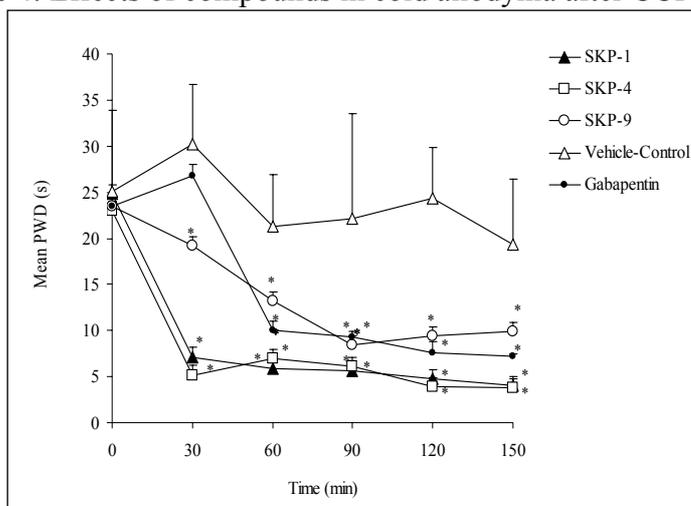
*P<0.05, in comparison with the pre-drug values

Cold allodynia in the CCI model

Paw withdrawal duration (PWD) after cold stimuli in CCI was significantly attenuated by the administration (100 mg/kg i.p.) of compounds **SKP-1**, **SKP-4**, **SKP-6**, **SKP-7**, **SKP-9**, **SKP-11**, **SKP-13** and **SKP-16**. Of these compounds, **SKP-1**, **SKP-4**, **SKP-9** and **SKP-13** were the most active, exhibiting antiallodynic activity over the entire 150 min testing (Peak effects of $4.02^* \pm 0.32$ s at 150 min

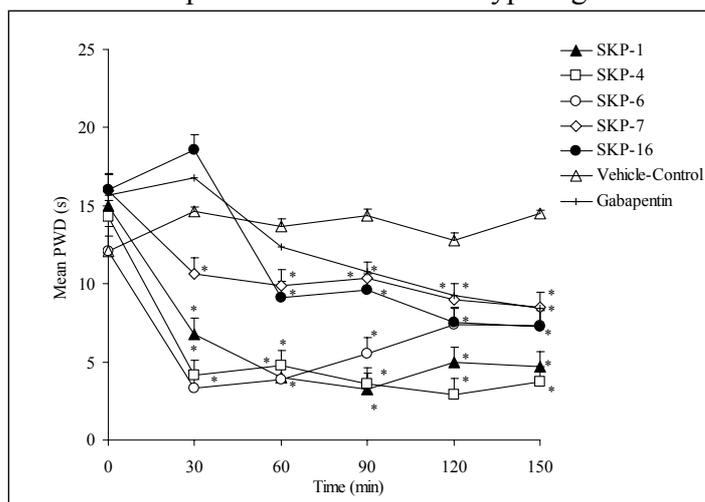
, $3.75^* \pm 0.32$ s at 150 min, $8.44^* \pm 5.30$ s at 90 min and $13.52^* \pm 1.02$ s at 60 min exhibited by **SKP-1**, **SKP-4**, **SKP-9** and **SKP-13** respectively, versus 24.81 ± 1.21 , 23.03 ± 1.39 , 23.46 ± 1.06 and 28.25 ± 0.95 s for pre-drug, $*p < 0.05$). Compounds **SKP-11** and **SKP-16** showed activity only from 60-90 min and 60-150 min respectively while other compounds were completely inactive. So was the standard drug lamotrigine, but carbamazepine was active till 60 min period ($10.56^* \pm 1.55$ s at 60 min, versus 27.74 ± 3.01 s for pre-drug, $*p < 0.05$). In this assay, gabapentin showed antiallodynic activity in a time-dependent manner, with the maximum activity observed at 150 min ($7.25^* \pm 0.25$ s at 150 min, versus 23.45 ± 0.56 s for pre-drug, $*p < 0.05$) (Figure 4).

Figure 4: Effects of compounds in cold allodynia after CCI in rats



*P<0.05, in comparison with the pre-drug values

Figure 5: Effects of compounds in mechanical hyperalgesia after CCI in rats



*P<0.05, in comparison with the pre-drug values

Mechanical hyperalgesia in the CCI model

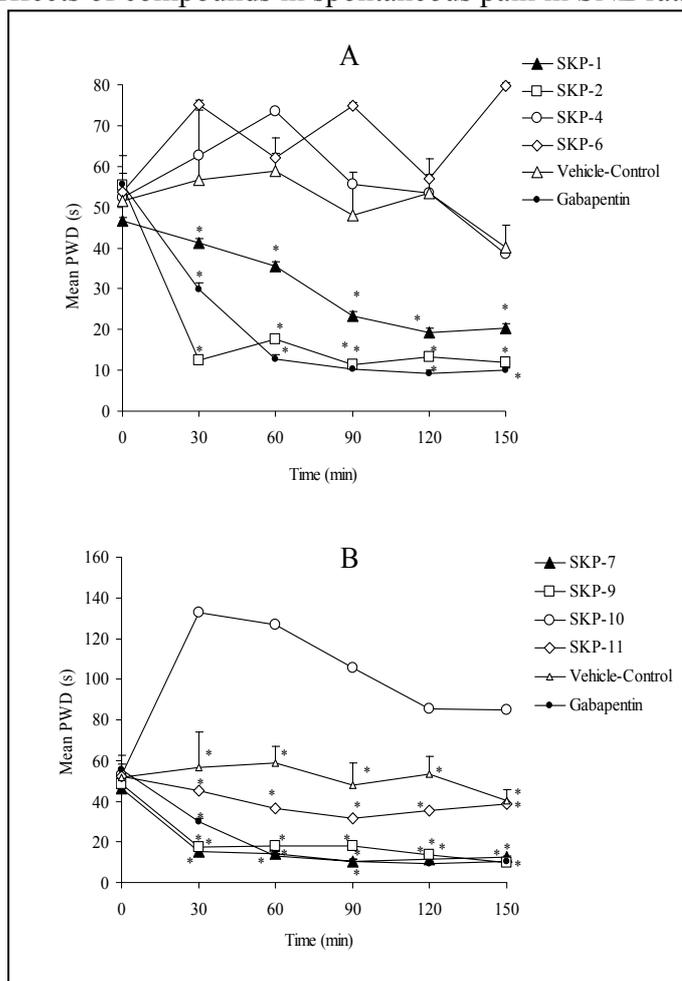
Hyperalgesia evoked by a mechanical pin-prick stimulus was effectively attenuated at all time-points of study by compound **SKP-1**, **SKP-4**, **SKP-6**, **SKP-7** (100 mg/kg i.p.) Compound **SKP-16** was observed to be active in reversing the hyperalgesic response at three time-points of study. Compounds **SKP-2**, **SKP-3**,

SKP-5, SKP-8, SKP-10, SKP-11, SKP-12, SKP-13, SKP-14 and SKP-15 possessed no antihyperalgesic activity in any of the time-points. The standard drug lamotrigine (100 mg/kg i.p.) showed significant activity only at 30 min, whereas carbamazepine was devoid of any antihyperalgesic effect. Gabapentin exhibited time-dependent antihyperalgesic effect ($8.41^* \pm 0.15$ s at 150 min, versus 15.65 ± 1.55 s for pre-drug, $*p < 0.05$) (Figure 5).

Spontaneous pain in the SNL model

Spontaneous pain was significantly reduced at all time-points by compounds **SKP-2, SKP-7, SKP-9 and SKP-11** (100 mg/kg i.p.). Compound **SKP-5**, which was active in reversing the pain response in the CCI model, was observed to be completely inactive in the SNL model. Compounds **SKP-3, SKP-4, SKP-6, SKP-8, SKP-10, SKP-12, SKP-13, SKP-14, SKP-15 and SKP-16** were totally inactive. Compound **SKP-1** showed activity from 60-150 min period of testing. Carbamazepine showed activity till 90 min (peak effect of $7.85^* \pm 0.99$ s at 60 min, versus 45.81 ± 6.78 s for pre-drug, $*p < 0.05$), while lamotrigine was active only at 90 min ($2.56^* \pm 0.65$ s versus 43.07 ± 2.34 s for pre-drug, $*p < 0.05$). Gabapentin was found to be active from 60-150 min in reversing the spontaneous pain ($9.12^* \pm 0.98$ s at 120 min, versus 55.46 ± 2.89 s for Pre-drug, $*p < 0.05$) (Figure 6).

Figure 6: Effects of compounds in spontaneous pain in SNL rats (A and B).

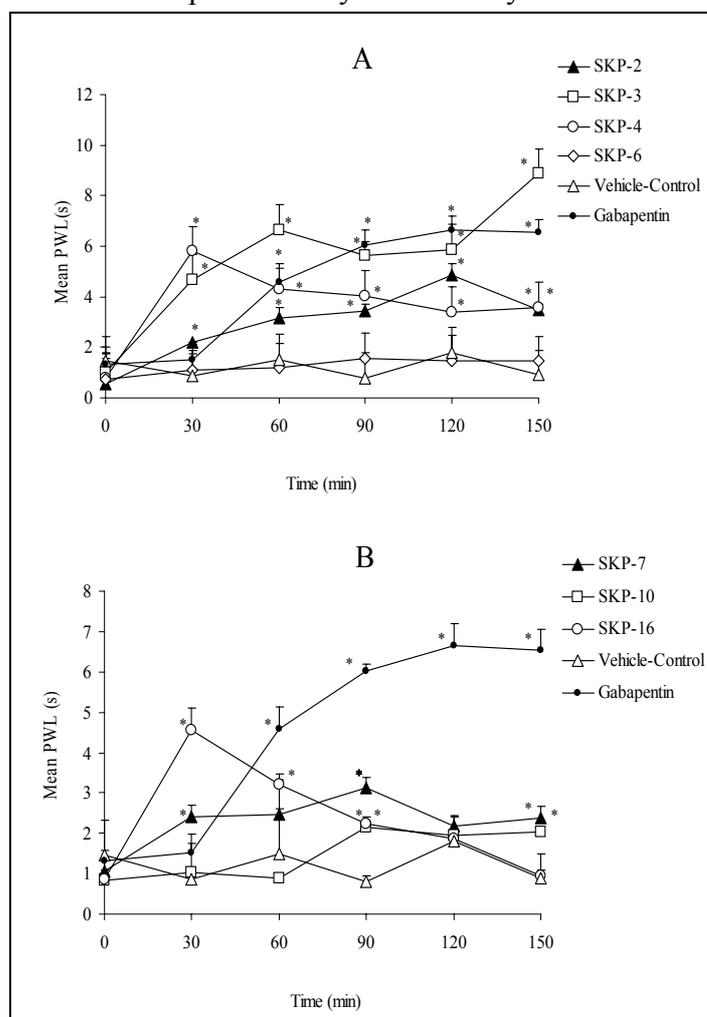


*P<0.05, in comparison with the pre-drug values

Dynamic allodynia in the SNL model

The dynamic mechanical allodynia produced by SNL was reversed at all time-points of observation by intraperitoneal administration of compounds **SKP-2**, **SKP-3** and **SKP-4** (100 mg/kg) (Peak effects of $4.85^* \pm 0.45$, $8.87^* \pm 1.95$ and $5.8^* \pm 0.54$ s for **SKP-2**, **SKP-3** and **SKP-4** respectively, versus 0.54 ± 0.12 , 1.01 ± 0.24 and 0.8 ± 0.12 s for Pre-drug, $*p < 0.05$). Here again, compound **SKP-5**, which was active in the CCI model, was found to be completely inactive in the SNL model. The other compounds that showed no antiallodynic activity include **SKP-1**, **SKP-8**, **SKP-9**, **SKP-11**, **SKP-12**, **SKP-13**, **SKP-14** and **SKP-15**. Compound **SKP-16** was active from 30-120 min period of testing. Carbamazepine was active at 30 min and 60 min time-points ($14.67^* \pm 1.01$ s at 30 min, versus 2.76 ± 0.78 s for pre-drug, $*p < 0.05$), while lamotrigine showed no antiallodynic effect. Unlike in CCI rats, gabapentin was active in attenuating the allodynic response only from 60-150 min ($6.65^* \pm 0.55$ s at 120 min, versus 1.32 ± 0.25 s for Pre-drug, $*p < 0.05$) (Figure 7).

Figure 7: Effects of compounds in dynamic allodynia in SNL rats (A and B)



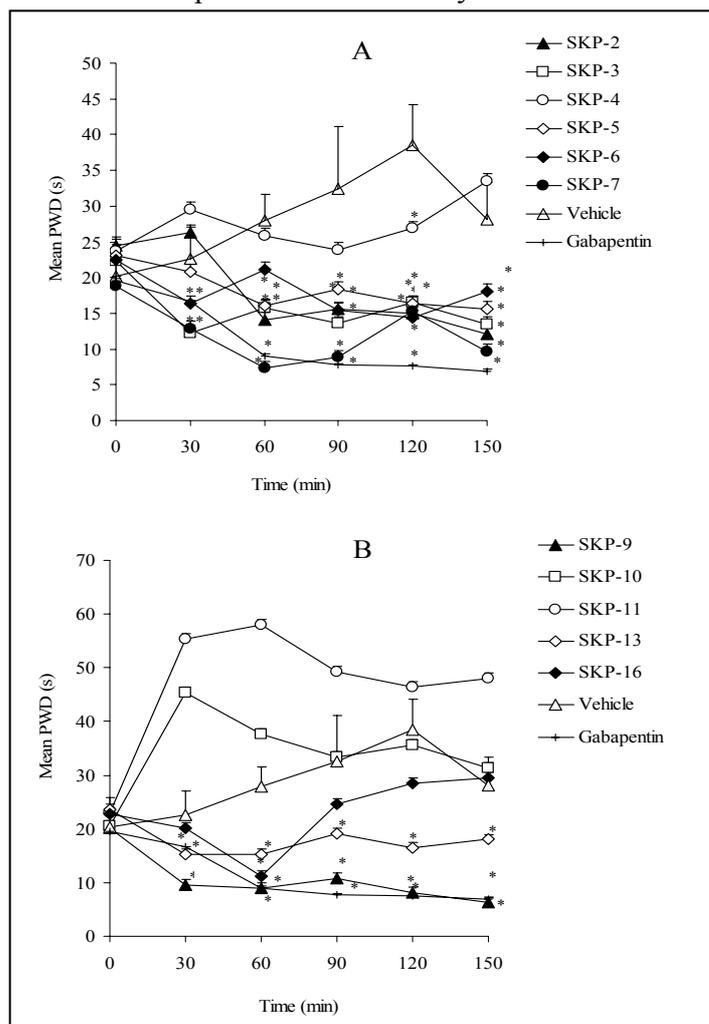
*P<0.05, in comparison with the pre-drug values

Cold allodynia in the SNL model

Intraperitoneal administration of compounds **SKP-1**, **SKP-8**, **SKP-12**, **SKP-14** and **SKP-15** (100 mg/kg) to animals with an allodynic response to cold stimuli,

showed no antiallodynic activity. Compounds **SKP-8**, **SKP-12**, **SKP-14** and **SKP-15** were inactive in CCI rats as well. Compounds that showed complete protection (30-150 min) include **SKP-3**, **SKP-6**, **SKP-9** and **SKP-13**. Compound **SKP-16**, which showed prolonged activity from 60-150 min in CCI rats was observed to be active only at the 60 min time-point in SNL rats. Both lamotrigine and carbamazepine showed no antiallodynic effects in SNL rats, whereas gabapentin was active from 60-150 min ($6.95^* \pm 0.25$ s at 150 min, versus 19.58 ± 0.58 s for pre-drug, $*p < 0.05$) (Figure 8).

Figure 8: Effects of compounds in cold allodynia in the SNL rats (A and B)



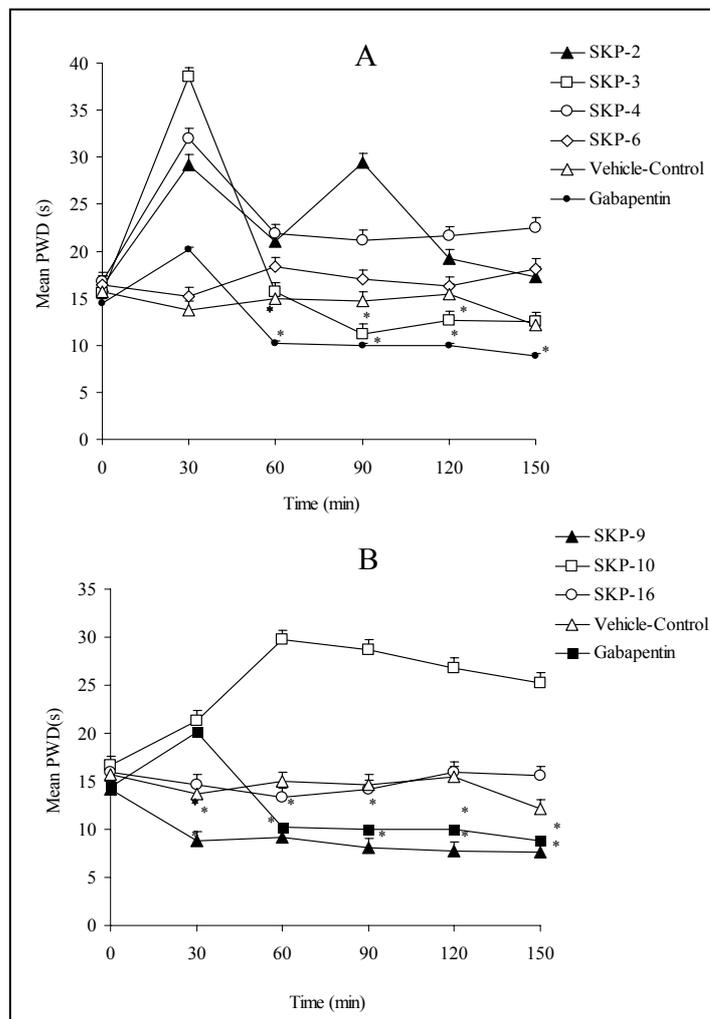
* $P < 0.05$, in comparison with the pre-drug values

Mechanical hyperalgesia in the SNL model

Hyperalgesia produced by mechanical stimulation in SNL rats was reversed significantly at all time-points by compound **SKP-9** (100 mg/kg i.p.) (Peak effects of $7.58^* \pm 0.26$, versus and $14.13^* \pm 0.43$ for pre-drug, $*p < 0.05$). Compound **SKP-3** was significantly active, 60 min onwards. Compounds **SKP-1**, **SKP-4**, **SKP-6** and **SKP-7** that were active in the CCI model were observed to be completely inactive in the SNL model. The other compounds with no antihyperalgesic effect include **SKP-5**, **SKP-8**, **SKP-10**, **SKP-11**, **SKP-12**, **SKP-13**, **SKP-14** and **SKP-15**. Carbamazepine showed a significant antihyperalgesic effect from 60-150 min (peak effect of $5.18^* \pm 2.27$ s at 60 min, versus $19.11 \pm$

2.65 s for Pre-drug, *P<0.05, n=4). So was gabapentin, which was active in reversing the hyperalgesic response from 60-150 min period of testing (peak effect of 8.85 ± 0.27 s at 150 min, versus 14.45 ± 0.55 s for pre-drug, *p<0.05) (Figure 9).

Figure 9: Effects of compounds in mechanical hyperalgesia in SNL rats (A and B)



*P<0.05, in comparison with the pre-drug values

Discussion

This study examined the potential therapeutic value of N-phthaloyl GABA hydrazones [SKP (1-16)] in the treatment of neuropathic pain using the CCI and SNL models of neuropathic pain. The first part of this study examined the ability of the synthesized derivatives of GABA to inhibit writhing responses in the acetic acid induced writhing test, a chemical pain test used to evaluate acute antinociceptive function.

The results of this study indicated that the newer GABA derivatives, apart from exhibiting significant analgesic activity in the acetic acid induced writhing model, also possessed antiallodynic and antihyperalgesic actions in both the CCI and SNL models of neuropathic pain. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors.^[24] Hence, taking

into consideration both of the above-mentioned findings, it could be concluded that the analgesic activity of **SKP (1-16)** observed in the tissue-injury model is primarily mediated by the peripheral GABAergic pathway. Of the compounds tested, the results presented here indicate that **SKP-12** emerged as the most active compound with percent inhibition greater than that of the standard drug aspirin (96.0% inhibition of writhing response as against 93.0% shown by aspirin).

As in the case of acute nociceptive pain states, there is considerable evidence supporting a palliative role for GABAergic neurotransmission in neuropathic pain conditions as well. Hence, in the present work, we tested the hypothesis that administration of structural analogues of GABA relieves the pain associated with neuropathy. The present data demonstrated that most of the compounds exhibited antiallodynic or antihyperalgesic activities in one or more of the sensory nociceptive assays. In CCI rats, compounds **SKP-4**, **SKP-5** and **SKP-6** were the most active compounds, each being active up to 150 min of observation in at least one nociceptive behavioral assay. In the case of SNL rats, intraperitoneal administration of compound **SKP-2** afforded the maximum protection against spontaneous pain, dynamic allodynia and cold hyperalgesia. Compounds with activity in two nociceptive assays include **SKP-7** and **SKP-9** wherein they were observed to be active against spontaneous pain and cold allodynia.

Overall, from the study compound **SKP-4** (4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N'*[(2-hydroxyphenyl)methylene]butanoylhydrazide) emerged as the most active compound with activities against spontaneous pain, dynamic allodynia, cold allodynia and mechanical hyperalgesia in the CCI model, and against dynamic allodynia in SNL rats. Chronic pain syndromes consist of a wide range of symptoms likely to be mediated by multiple mechanisms. Therefore, blockade of just one of these mechanisms may not provide full pain relief in every patient. A better approach may be, as in this case designing and developing novel compounds with more than just one mechanism of action.

In conclusion, we have shown that the synthesized derivatives of the inhibitory neurotransmitter GABA produce antinociception in the acetic acid induced writhing test and peripheral nerve injury (CCI and SNL) models of neuropathic pain. The underlying mechanisms are expected to be neuronal voltage-sensitive sodium channel blockade and enhancement of peripheral GABAergic neurotransmission owing to the presence of both the phthalimido and GABA pharmacophores. Further research is required to confirm the hypothesized molecular mechanisms of action of the reported compounds.

Acknowledgements

The authors wish to thank the University Grants Commission (UGC), New Delhi (India) for funding the project (Ref. No: 32-124/2006(SR)).

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