Discovery of 4-Aminobutyric Acid Derivatives Possessing Anticonvulsant and Antinociceptive Activities: A Hybrid Pharmacophore Approach

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Antiepileptic drugs are often utilized in the treatment of neuropathic pain. The present study aims at the design and synthesis of newer γ-aminobutyric acid (GABA) derivatives with the combination of aryl semicarbazone and the GABA pharmacophores in order to develop a multifunctional drug useful in the treatment of neurological disorders like epilepsy and neuropathic pain. Various GABA semicarbazones were synthesized and screened for anticonvulsant, peripheral analgesic, antiallodynic, and antihyperalgesic activities. The structures of the synthesized compounds were confirmed by the use of their spectral data in addition to elemental analysis. The synthesized derivatives of the inhibitory neurotransmitter GABA produced anticonvulsant and antinociceptive actions in the acetic acid induced writhing test and peripheral nerve injury (chronic constriction injury and L5 spinal nerve ligation) models of neuropathic pain. The underlying mechanisms are expected to be enhancement of peripheral GABAergic neurotransmission owing to their activity in the scPIC screen and due to various reports on the involvement of GABAergic pathway in peripheral models of neuropathic pain.

Introduction

Neuropathic pain responds to many antiepileptic drugs (AEDs), suggesting that it shares with epilepsy underlying neurophysiological mechanisms. Specifically, most of these drugs either stabilize membrane hyperexcitability or enhance inhibitory γ-aminobutyric acid (GABA) and/or glycine neurotransmission.[1,2] Most antiepileptics have multiple potential modes of action that may be beneficial for neuropathic pain. Anticonvulsants, such as carbamazepine or phenytoin, have been traditionally used for the management of neuropathic pain. However, the efficacy of this class of drugs has not been unequivocally established, and their use has often been associated with numerous side effects.[3,4] More recently, some of the newer anticonvulsants, in particular gabapentin and to a lesser extent topiramate and lamotrigine, received increased attention as analgesics for treating neuropathic pain.[4] GABA is the major inhibitory neurotransmitter in the mammalian brain. It has been well documented that the reduction of GABAergic neuronal activity plays an important role in a number of neurological disorders, including epilepsy, anxiety, and pain. Gabapentin, a structural analogue of GABA, has been found to exert significant analgesic effects.[5−7] However, despite the progress made with these compounds, neuropathic pain remains under treated, and in many patients gabapentin does not provide adequate pain relief. In behavioral studies, it has been shown that various chemical analogues of GABA attenuate allodynic and hyperalgesic responses in chronic constriction injury rats.[8−11] Hence there is a current focus on screening the potential of anticonvulsants as therapeutic agents and also in the development of newer lead molecules for neuropathic pain treatment. In the recent literature, various aryl semicarbazones with established pharmacophore requirements have been reported as a novel class of anticonvulsant agents with lesser central nervous system side effects and hepatotoxicity.[12−19] Given the promising biological profile of GABA derivatives and aryl semicarbazones, we initiated a drug discovery program focusing on newer GABA derivatives as the lead. In the present paper, effort has been taken to design GABA semicarbazones by combining the GABA and semicarbazone pharmacophores. Rather than following the old pattern of first completing the development of novel compounds as AEDs and then testing them as analgesics, we proceeded to assess the new pharmacophoric hybrids for anticonvulsant, analgesic, antiallodynic, and antihyperalgesic activity in various rat models.

Chemistry

The synthesis of GABA semicarbazones was achieved as presented in Figure 1. The method was based on an earlier reported procedure.[13,20] The starting material, 4-aminobutyric acid, was treated with phenyl chloroformate in aqueous sodium hydroxide at a range of 0−5 °C to yield 4-(phenoxycarbonylamino)butanoic acid with a yield of 70% after crystallization with 95% ethanol. The carbamate, on condensation with hydrazine hydrate in ethanol, gave the 4-(hydrazinecarbonylamino)butanoic acid. Finally, the required semicarbazone derivatives (**1**−**15**, Table 1) were obtained by reaction of the appropriate aryl/alkyl aldehydes, ketone, or isatin derivatives with the 4-(hydrazinecarbonylamino)butanoic acid in ethanol to give 4-(alkylidene/arylidene hydrazine carbonylamino)-butanoic acids in yields ranging between 47% and 81%. The purity was assessed by TLC using chloroform:methanol in the ratio 9:1 as the eluant system. The structures were characterized by both spectral and elemental analysis, and the data were within ± 0.4% of the theoretical values. In general, the IR spectra showed the C═N peak at 1620−1590 cm^{-1} and the NH stretching vibrations at 3220 cm^{-1}. The synthesized compounds exhibited characteristic amide bonds at 1650−1620 cm^{-1}. The ^{1}H NMR spectrum revealed that the hydrazino proton (═N−NH) showed a singlet at δ 10.0−10.56 and the alkyl NH at

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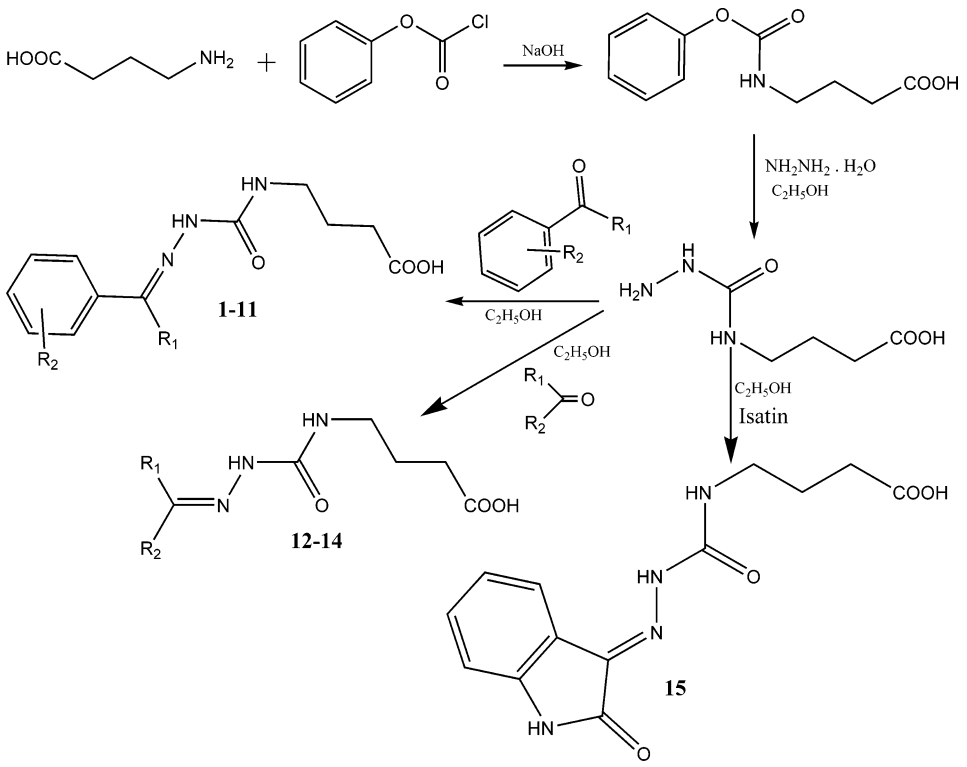
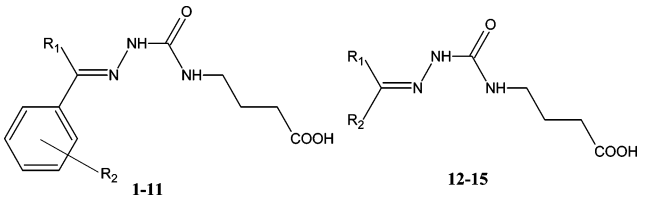


Figure 1. Synthetic protocol of 4-(alkylidene/arylidene hydrazine carbonylamino)butanoic acids.

Table 1. Physical Data of the Synthesized Compounds



no.	R_1	R_2	molecular formula	molecular weight	yield (%)	mp (°C)	R_f [a]
1	H	2-OH	$C_{12}H_{15}N_3O_4$	264	55.0	212	0.71
2	H	4-NO_2	$C_{12}H_{14}N_4O_5$	294	56.0	216[b]	0.65
3	H	4-Cl	$C_{12}H_{14}N_3O_3Cl$	283	69.0	203	0.63
4	H	3-NO_2	$C_{12}H_{14}N_4O_5$	294	47.0	182	0.71
5	H	4-$N(CH_3)_2$	$C_{14}H_{20}N_4O_3$	292	65.0	259	0.73
6	CH_3	H	$C_{13}H_{17}N_3O_3$	263	59.0	119	0.77
7	CH_3	4-CH_3	$C_{14}H_{19}N_3O_3$	277	49.0	120	0.78
8	CH_3	3-NH_2	$C_{13}H_{18}N_4O_3$	278	61.0	260	0.81
9	CH_3	4-NO_2	$C_{13}H_{16}N_4O_5$	308	59.0	197	0.72
10	C_6H_5	H	$C_{18}H_{19}N_3O_3$	325	66.0	47	0.82
11	C_6H_5	4-Br	$C_{18}H_{18}N_3O_3Br$	404	79.0	73	0.86
12	CH_2-C_6H_5	CH_2-C_6H_5	$C_{20}H_{23}N_3O_3$	353	55.0	89	0.87
13	cyclohexylene		$C_{11}H_{19}N_3O_3$	241	55.0	122	0.71
14	cyclopentylene		$C_{10}H_{17}N_3O_3$	227	58.0	121	0.65
15	isatinyl		$C_{13}H_{14}N_4O_4$	290	81.0	114[b]	0.77

[a] The solvent system used was chloroform:methanol (9:1). [b] Melting with decomposition.

6.0−6.03, both of which were D_2O exchangeable. All compounds showed a characteristic D_2O exchangeable singlet due to OH proton of the acid function at δ 12.0−12.2. The aryl ring protons resonated at $\delta \sim$ 7.2−7.66 ppm.

Results and Discussion

The aim of this drug discovery program was to prepare newer GABA derivatives with multiple pharmacological actions effective in the treatment of epilepsy and neuropathic pain. The synthesized compounds (**1**−**15**) were evaluated at dose levels of 30, 100, and 300 mg/kg intraperitoneally in mice for anticonvulsant activity by following the standard anticonvulsant drug development (ADD) program protocols.[21−22] Table 2 lists the results obtained from the initial anticonvulsant evaluation of the synthesized compounds compared to the clinically proven antiepileptics such as phenytoin, and ethosuximide also tested at the same dose levels. The tests included one electrical and three chemoshock tests, i.e., maximal electroshock seizure test (MES), subcutaneous pentylenetetrazole (scPTZ) seizure threshold test, subcutaneous strychnine (scSTY) pattern test, and subcutaneous picrotoxin (scPIC) seizure threshold test. The acute neurological toxicity was determined by the rotorod test. Five

Table 2. Analgesic and Anticonvulsant Activity of the Synthesized Compounds

	intraperitoneal administration to mice[a]										
	MES		scPTZ	scSTY		scPIC		neurotoxicity		acetic acid induced writhing[b]	
compd	0.5 h	4 h	0.5 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	number of writhes (per 30 min)	% inhibition
control	−	−	−	−	−	−	−	−	−	183.3 ± 3.8	−
1	−	−	−	30	30	30	30	−	300	19.0 ± 2.1	89
2	−	−	−	−	−	−	−	300	−	18.7 ± 0.9	90
3	−	−	−	300	300	100	100	−	−	59.3 ± 4.6	68
4	−	−	300	−	−	300	300	−	−	17.7 ± 5.5	90
5	−	−	−	−	−	30	100	−	−	13.0 ± 2.6	93
6	−	−	−	100	300	300	300	−	−	33.7 ± 1.4	82
7	−	−	300	−	−	300	−	300	−	32.5 ± 2.4	82
8	300	300	−	−	−	−	−	300	300	19.7 ± 0.9	89
9	−	−	−	−	−	−	−	−	−	61.0 ± 0.9	67
10	300	−	−	300	300	300	300	300	−	13.3 ± 1.2	93
11	300	−	−	300	300	−	−	−	−	21.0 ± 2.1	89
12	−	−	−	300	300	100	100	−	−	10.3 ± 2.6	94
13	−	−	300	−	−	300	−	−	−	32.0 ± 2.5	82
14	−	300	−	−	−	−	−	300	300	18.0 ± 1.5	90
15	300	300	300	−	−	300	300	100	300	47.3 ± 6.2	74
phenytoin	30	30	−	−	−	−	−	100	100	−	−
ethosuximide	−	−	300[c]	−	−	−	−	−	−	−	−
aspirin	−	−	−	−	−	−	−	−	−	5.7 ± 3.5	97

[a] Doses of 30, 100, and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice (three in each group). The animals were examined at 0.5 and 4 h. The line (−) indicates an absence of anticonvulsant activity or neurotoxicity at the maximum dose tested. [b] The compounds were administered intraperitoneally at a dose level of 100 mg/kg. Control animals were administered 30% v/v PEG 400 in water. Each value represents the mean ± SEM of six mice significantly different from the control at $P < 0.01$ (Student's t test). [c] Ethosuximide at 100 mg/kg exhibited protection in one out of three mice.

compounds (**8**, **10**, **11**, **14**, and **15**) showed activity in the MES screen indicative of their ability to prevent seizure spread. Compounds **8** and **15** exhibited longer duration of action (i.e., activity until 4 h interval) whereas compounds **10** and **11** showed shorter duration of action (0.5 h). In the scPTZ screen, a test used to identify compounds that elevate seizure threshold, four compounds (**4**, **7**, **13**, and **15**) showed marginal protection. These compounds showed protection at 300 mg/kg at 0.5 h time period only. Six compounds (**1**, **3**, **6**, and **10**−**12**) showed protection in the subcutaneous strychnine-induced seizure model. Compound **1** was the most effective in this model exhibiting protection at 30 mg/kg for a longer duration (until 4 h). Compound **6** showed activity at 100 mg/kg, and other compounds showed activity at 300 mg/kg. All of the active compounds exhibited a longer duration (0.5 and 4 h) of action. In the scPIC-induced seizure threshold test, most of the compounds exhibited activity indicative of the possible involvement of GABA-mediation in the anticonvulsant action. All of the compounds except **2**, **8**, **9**, **11**, and **14** showed protection in the scPIC test. Compounds **1** and **5** showed pronounced activity at 30 mg/kg and compounds **3** and **12** showed activity at 100 mg/kg. In general, it appears that except compounds **2**, **5**, **8**, **9**, and **14**, all other compounds were found to be effective in at least two models of seizure with compounds **10** and **15** effective in three animal models that include MES, scSTY or scPTZ, and scPIC screens. Compounds **8** and **14** showed activity only in the MES model, whereas compound **5** showed activity in the scPIC model, and compounds **2** and **9** were completely inactive in the anticonvulsant screening.

In the acute neurological toxicity screen, the compounds **1**, **3**−**6**, and **11**−**14** emerged as promising anticonvulsants with less or no neurotoxicity. There was no separation between the anticonvulsant dose and the neurotoxic dose (300 mg/kg) for compounds **7**, **8**, **10**, and **15**, in which **15** showed greater neurotoxicity at 0.5 h at 100 mg/kg. Compound **2** which was ineffective in the seizure model showed neurotoxicity at 300 mg/kg at 0.5 h.

To examine the potential therapeutic value of the newer GABA derivatives (**1**−**15**) in the treatment 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. All of the tested compounds suppressed the acetic acid induced writhing response significantly ($P < 0.01$) in comparison to the control (Table 2). The standard drug, aspirin, exhibited the highest percentage inhibition (97.0%). Compounds **2**, **4**, **5**, **10**, **12**, and **14** were the most active compounds with percentage inhibition 90% or above. Of these compounds, **12** was observed to be most active with 94% inhibition.

In the next phase of screening two well-known peripheral neuropathic pain models were used that included the chronic constriction injury (CCI) and L5 spinal nerve ligation (SNL) models in rats. In the CCI model, the left sciatic nerve proximal to the trifurcation point was constricted with four loose ligatures using 3−0 braided silk thread, while in the SNL model, a tight ligation was tied around the L5 spinal nerve using 3−0 braided silk thread. 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. Baseline sensory response values were measured for each group of animals ($n = 4$) preoperatively and 9 days postoperatively. Animals displaying allodynic and hyperalgesic responses in both the models were then administered the relevant drug according to a predetermined randomization table, and testing was reperformed at 0.5, 1, 1.5, 2.0, and 2.5 h postdrug administration. All of the behavioral responses were timed with a stopwatch. All of the compounds were tested at a single dose of 100 mg/

Table 3. Effects of Compounds on Spontaneous Pain in the CCI Model

study group	spontaneous pain (PWD) (s)[a]					
	predrug	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h
control	40.6 ± 12.3	45.9 ± 17.3^{NS}	42.1 ± 5.6^{NS}	35.3 ± 15.3^{NS}	41.4 ± 7.7^{NS}	39.9 ± 8.1^{NS}
1	31.3 ± 3.4	10.5 ± 2.0	19.3 ± 2.7^{NS}	23.7 ± 1.3^{NS}	11.2 ± 1.7	7.3 ± 1.4
2	21.2 ± 2.3	10.2 ± 1.1	9.2 ± 2.1	13.6 ± 1.9^{NS}	11.3 ± 0.6^{NS}	17.2 ± 2.3^{NS}
5	52.7 ± 8.7	47.2 ± 1.2^{NS}	48.8 ± 3.5^{NS}	38.2 ± 1.5^{NS}	11.9 ± 5.2	5.2 ± 2.1
6	42.9 ± 3.7	13.2 ± 0.8	18.8 ± 3.0	18.3 ± 1.6	12.9 ± 2.6	10.6 ± 1.1
7	27.7 ± 2.7	23.9 ± 2.4^{NS}	21.8 ± 2.7^{NS}	18.3 ± 2.1^{NS}	10.9 ± 1.4	10.7 ± 0.9
8	12.7 ± 1.4	1.7 ± 0.1	1.2 ± 1.0	0.9 ± 0.2	1.4 ± 0.1	2.8 ± 1.0
12	22.8 ± 1.3	9.2 ± 1.2	9.7 ± 1.8	1.3 ± 0.6	3.2 ± 0.9	8.5 ± 0.2
15	48.6 ± 4.3	38.6 ± 5.2^{NS}	34.9 ± 1.3^{NS}	21.6 ± 2.3	20.6 ± 1.9	20.2 ± 2.7
lamotrigine	69.6 ± 4.7	69.2 ± 3.2^{NS}	79.0 ± 2.6^{NS}	76.9 ± 1.0^{NS}	71.2 ± 2.3^{NS}	67.8 ± 2.7^{NS}
carbamazepine	66.8 ± 7.9	24.6 ± 2.0	17.8 ± 2.9	32.8 ± 2.7^{NS}	67.4 ± 5.8^{NS}	76.9 ± 6.9^{NS}
gabapentin	74.9 ± 4.6	62.2 ± 3.8^{NS}	15.5 ± 2.5	13.1 ± 3.4	11.0 ± 2.3	18.1 ± 2.3

[a] All compounds were administered intraperitoneally at a dose level of 100 mg/kg. Control animals were administered 30% v/v PEG 400 in water. Each value represents the mean ± SEM of four rats, significantly different from predrug at $P < 0.05$; NS denotes not significant at $P < 0.05$ (one-way ANOVA, followed by post-hoc Bonferroni test).

Table 4. Antihyperalgesic Effects of Compounds in the CCI Model

study group	mechanical hyperalgesia (PWD) (s)[a]					
	predrug	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h
control	12.1 ± 3.5	14.7 ± 5.9^{NS}	13.6 ± 2.4^{NS}	14.3 ± 4.1^{NS}	12.8 ± 5.6^{NS}	14.5 ± 4.1^{NS}
1	25.7 ± 2.4	7.5 ± 2.7	7.7 ± 2.0	4.4 ± 2.0	4.1 ± 1.0	7.3 ± 1.9
3	14.8 ± 2.5	7.9 ± 1.8^{NS}	7.8 ± 1.7^{NS}	4.4 ± 1.1	4.2 ± 1.1	7.2 ± 1.4
5	41.9 ± 29.0	17.4 ± 12.8	13.1 ± 10.9	9.7 ± 8.2	5.8 ± 2.6	4.8 ± 1.2
7	28.8 ± 1.3	27.3 ± 1.8^{NS}	13.2 ± 0.8	9.6 ± 0.6	5.3 ± 0.2	4.2 ± 1.1
8	24.7 ± 0.1	9.3 ± 0.1	9.6 ± 0.1	9.9 ± 0.1	6.6 ± 1.9	4.8 ± 0.6
11	23.6 ± 1.1	20.5 ± 2.3^{NS}	8.5 ± 2.0	5.1 ± 1.0	7.3 ± 1.2	5.6 ± 1.5
13	21.2 ± 1.3	20.3 ± 1.1^{NS}	24.8 ± 1.0^{NS}	4.8 ± 1.0	5.2 ± 1.2	7.0 ± 1.1
14	72.1 ± 21.6	32.1 ± 8.2	30.7 ± 0.9	17.7 ± 7.9	22.8 ± 8.1	25.3 ± 8.2
15	11.3 ± 0.3	10.8 ± 3.9^{NS}	4.9 ± 0.5	8.8 ± 3.4^{NS}	5.0 ± 0.1	27.8 ± 9.3*
lamotrigine	34.8 ± 6.0	36.2 ± 5.0^{NS}	36.7 ± 0.9^{NS}	35.7 ± 1.0^{NS}	33.7 ± 1.9^{NS}	29.8 ± 2.9^{NS}
carbamazepine	21.8 ± 1.9	13.3 ± 3.0^{NS}	15.7 ± 2.3^{NS}	22.9 ± 0.7^{NS}	21.7 ± 1.7^{NS}	24.0 ± 2.2 ^{NS}
gabapentin	45.6 ± 1.5	36.8 ± 0.2^{NS}	12.3 ± 0.5	10.7 ± 0.4	9.2 ± 0.4	8.4 ± 0.1

[a] All compounds were administered intraperitoneally at a dose level of 100 mg/kg. Control animals were administered 30% v/v PEG 400 in water. Each value represents the mean ± SEM of four rats, significantly different from predrug at $P < 0.05$; asterisk (*) represents the mean ± SEM of a pharmacologically inactive response significantly different from predrug at $P < 0.05$, and NS denotes not significant at $P < 0.05$ (one-way ANOVA, followed by post-hoc Bonferroni test).

Table 5. Effects of Compounds on Spontaneous Pain in the SNL Model

study group	spontaneous pain (PWD) (s)[a]					
	predrug	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h
control	51.6 ± 10.9	56.8 ± 17.0 ^{NS}	58.9 ± 8.1^{NS}	48.1 ± 10.5^{NS}	53.5 ± 8.4^{NS}	40.3 ± 5.4^{NS}
3	37.5 ± 2.2	33.1 ± 1.3^{NS}	14.0 ± 2.9	12.7 ± 2.1	11.2 ± 1.2	15.9 ± 1.1
5	76.4 ± 4.6	12.4 ± 1.5	21.2 ± 9.5	16.4 ± 3.9	29.2 ± 5.8	43.0 ± 4.7^{NS}
7	26.3 ± 1.3	22.2 ± 1.3^{NS}	11.1 ± 1.0	6.4 ± 2.9	2.9 ± 1.0	3.1 ± 0.1
10	26.3 ± 2.1	15.1 ± 1.1^{NS}	18.5 ± 1.2^{NS}	13.4 ± 1.8^{NS}	10.9 ± 1.1	8.8 ± 1.2
11	29.2 ± 2.0	25.1 ± 1.1^{NS}	28.5 ± 2.0^{NS}	23.1 ± 1.1^{NS}	10.1 ± 1.0	8.7 ± 0.1
12	25.9 ± 2.6	22.2 ± 2.^{NS}	7.9 ± 1.0	8.9 ± 2.2	11.8 ± 1.1	7.4 ± 1.1
13	30.8 ± 1.3	21.2 ± 0.2^{NS}	20.2 ± 1.2^{NS}	10.8 ± 1.2	10.6 ± 1.2	10.8 ± 1.7
15	10.8 ± 2.3	1.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	9.8 ± 1.8^{NS}
lamotrigine	23.1 ± 2.3	15.6 ± 3.4^{NS}	13.3 ± 2.7^{NS}	2.6 ± 0.6	22.9 ± 3.4^{NS}	24.4 ± 3.6^{NS}
carbamazepine	85.8 ± 6.8	14.6 ± 1.0	7.9 ± 0.9	22.3 ± 3.7	85.4 ± 8.9^{NS}	94.9 ± 7.6^{NS}
gabapentin	35.5 ± 2.9	29.7 ± 1.8^{NS}	12.9 ± 1.0	10.2 ± 1.0	9.1 ± 1.0	10.1 ± 0.6

[a] All compounds were administered intraperitoneally at a dose level of 100 mg/kg. Control animals were administered 30% v/v PEG 400 in water. Each value represents the mean ± SEM of four rats, significantly different from predrug at $P < 0.05$; NS denotes not significant at $P < 0.05$ (one-way ANOVA, followed by post-hoc Bonferroni test).

kg. The results along with data on standard drugs lamotrigine, carbamazepine, and gabapentin are represented in Tables 3−6.

In the CCI model, compounds **6**, **8**, and **12** completely reversed the spontaneous pain response throughout the time period of testing (0.5−2.5 h), more effective when compared to carbamazepine (Table 3). The times to peak effect of these compounds were 1.5 h for **8** and **12** and 2.5 h for **6**. Lamotrigine was ineffective in this test, while gabapentin showed activity from 1 h until 2.5 h of testing. Compound **2** showed activity up to 1 h. The onset of action of compounds **5** and **7** was 2 h and for **15** 1.5 h. Compound **1** showed an irregular activity profile as presented in Table 3. Compounds **3**, **4**, **9**, **10**−**11**, and **13**−**14** were ineffective in this test.

Six compounds (**1**, **5**, **6**, **11**, **12**, and **15**) were active in attenuating the dynamic allodynic response (results given as Supporting Information) throughout the entire 2.5 h experiment similar to gabapentin. Compound **3** showed an irregular response, and all other compounds were inactive. In the cold

Table 6. Antihyperalgesic Effects of Compounds in the SNL Model

study group	mechanical hyperalgesia (PWD) (s)[a]					
	predrug	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h
control	15.7 ± 3.7	13.7 ± 2.5^{NS}	15.0 ± 2.1^{NS}	14.7 ± 2.6^{NS}	15.5 ± 3.7^{NS}	12.1 ± 4.5^{NS}
1	23.9 ± 1.2	17.6 ± 1.5^{NS}	17.3 ± 2.8^{NS}	10.6 ± 1.9	11.3 ± 2.1^{NS}	17.3 ± 1.1^{NS}
3	34.9 ± 5.8	24.6 ± 3.8^{NS}	17.3 ± 2.8^{NS}	12.3 ± 1.8	5.6 ± 1.2	17.9 ± 1.2^{NS}
5	19.1 ± 3.6	4.7 ± 0.7	5.8 ± 2.3	5.5 ± 2.0	4.4 ± 1.4	5.8 ± 1.4
6	34.3 ± 6.8	23.2 ± 8.4^{NS}	27.6 ± 3.3^{NS}	18.7 ± 2.9^{NS}	16.7 ± 1.3	17.3 ± 1.7^{NS}
8	17.1 ± 3.0	2.4 ± 0.7	6.5 ± 0.8	7.3 ± 0.8	19.5 ± 3.3^{NS}	17.3 ± 2.2^{NS}
10	32.1 ± 7.2	18.4 ± 1.6^{NS}	19.9 ± 3.8^{NS}	18.5 ± 0.1^{NS}	13.7 ± 1.2	15.0 ± 1.9
13	29.0 ± 3.2	14.7 ± 2.2^{NS}	19.7 ± 3.0^{NS}	15.6 ± 2.9^{NS}	11.3 ± 2.3	12.8 ± 2.6
14	36.6 ± 3.1	5.3 ± 0.9	9.9 ± 1.2	5.1 ± 1.9	9.6 ± 1.3	8.6 ± 1.2
15	39.5 ± 10.1	4.7 ± 0.2	9.6 ± 3.2	45.7 ± 10.6^{NS}	19.7 ± 1.2^{NS}	18.7 ± 1.3
lamotrigine	17.9 ± 3.4	22.5 ± 1.9^{NS}	8.2 ± 1.1	10.1 ± 2.3^{NS}	6.4 ± 0.4	17.3 ± 1.8^{NS}
carbamazepine	19.1 ± 2.6	11.1 ± 3.1^{NS}	5.2 ± 2.3	6.6 ± 0.7	5.5 ± 1.6	5.8 ± 1.2
gabapentin	24.4 ± 0.5	20.1 ± 0.2^{NS}	10.2 ± 0.2	10.0 ± 0.3	9.9 ± 0.2	8.8 ± 0.3

[a] All compounds were administered intraperitoneally at a dose level of 100 mg/kg. Control animals were administered 30% v/v PEG 400 in water. Each value represents the mean ± SEM of four rats, significantly different from predrug at $P < 0.05$; NS denotes not significant at $P < 0.05$ (one-way ANOVA, followed by post-hoc Bonferroni test).

allodynia produced in CCI rats, the paw withdrawal durations (PWDs) were significantly reduced by the administration of compounds **1**, **5**, and **12**, whose onset of action was at 1 h (results given as Supporting Information). Lamotrigine did not show any protection in this test, while carbamazepine was effective only until 1 h. All other compounds were found to be ineffective in this test. Hyperalgesia evoked by a mechanical pin-prick stimulus was effectively attenuated at all time-points of study by compounds **1**, **5**, **8**, and **14**. Compounds **7**, **11**, and **15** showed significant reductions in paw withdrawal duration with an onset of action at 1 h similar to gabapentin, whereas compounds **3** and **13** had shown onset of action from 1.5 h. Compounds which were inactive in this test included **2**, **4**, **6**, **9**, **10**, **12**, and the standard drugs lamotrigine and carbamazepine (Table 4). Overall, it appears that, in the CCI model of neuropathic pain, compounds that showed promising results include **1** and **5** effective in all the four tests, **12** and **15** in three out of four tests, and **6**, **7**, **8**, **11**, and **15** in two tests. Compounds that showed moderate activity include **3** in two tests.

In the SNL model, the paw withdrawal durations due to spontaneous ongoing pain were significantly reduced by compounds **3**, **7**, and **12** from 1 h until 2.5 h similar to gabapentin, while compounds **5** and **15** showed activity from 0.5 to 2 h. Carbamazepine showed significant activity until 1.5 h only. Compounds **10** and **11** showed onset of action at 2 h, while that for compound **13** occurred at 1.5 h. All other compounds including lamotrigine were ineffective in this test. Compounds **6** and **8**, which were active in completely reversing the pain response in the CCI model, were inactive in the SNL model (Table 5).

The dynamic mechanical allodynia produced by SNL was effectively reversed by all compounds except **8**, **9**, **10**, **12**, and **15**. In fact, all of the compounds tested in the dynamic allodynia assay showed a reversal effect at least in two time-points. Five compounds (**1**, **6**, **7**, **11**, and **14**) were found to be completely effective until 2.5 h, hence more effective than carbamazepine which showed activity until 1 h and lamotrigine which was ineffective. Compounds **3** and **5** were effective until 1.5 h and compounds effective until 1 h were **2** and **13**. Gabapentin reversed the dynamic allodynia from 1 to 2.5 h (results given as Supporting Information).

Cold allodynia produced by SNL model was completely reversed by compound **1** as seen with CCI model. Administration of compounds **2**−**5**, **9**, **10**, **12**, and **13** to animals with an allodynic response to cold stimuli showed no antiallodynic activity. Compounds **5** and **12** were active in CCI rats. Compounds **8** and **15** were found to be effective but only until 1 and 2 h, respectively. Other active compounds had different times of onset as follows: **7** and **14** (1.5 h), **6** (1 h), **11** (2 h), and gabapentin (1 h). Lamotrigine and carbamazepine which were effective in CCI were inactive in the SNL model (results given as Supporting Information).

Mechanical hyperalgesia produced by SNL was completely reversed significantly at all time-points by compounds **5** and **14**. Compound **8**, which showed complete reversal until 2.5 h in the CCI model, showed activity until 1.5 h only in the SNL model. Gabapentin was effective after 1 h of observation. Compounds that showed moderate activity included **3**, **10**, **13**, and **15**. Carbamazepine showed a significant antihyperalgesic effect with an onset of action at 1 h while lamotrigine showed an irregular activity profile. Compounds **1** and **6** were active at only one time-point of observation (Table 6).

Overall, it appears that, in the SNL model, compounds that showed promising results included **3**, **5**, **6**, **7**, **11**, **13**, **14**, and **15** in three out of four tests, and **8** and **10** in two tests while compounds **2** and **12** exhibited moderate activity. The most active compounds that had been found to be effective in both neuropathic pain models would be compounds **1** and **5**.

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. It is well-established that GABAergic mechanisms are involved in antinociceptive processes. As evidence to this, peripheral administration of GABAergic agents increases the antinociceptive effect of morphine, but central administration inhibits this effect, suggesting that multiple interactions might occur.[23] Second, 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]

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. In 1999, Eaton et al.[25] provided evidence for the involvement of GABA in spinal antinociception, when a single intrathecal injection of GABA permanently reversed neuropathic pain after nerve injury. Moreover, a reduced spinal GABAergic tone has been suggested just after nerve injury.[26] Intrathecal administration of GABA receptor antagonists dose-dependently produced tactile allodynia,[27] suggesting that inhibiting endogenous GABA can lead to an excited sensory state.

In conclusion, we have shown that the synthesized derivatives of the inhibitory neurotransmitter GABA produce anticonvulsant and antinociceptive actions 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 enhancement of peripheral GABAergic neurotransmission owing to their activity in the scPIC screen and due to various reports on the involvement of GABAergic pathway in peripheral models of neuropathic pain. This study presents the first report on the antiallodynic and antihyperalgesic activities of GABA semicarbazones. Further research is required to confirm the hypothesized molecular mechanisms of action of the reported compounds.

Structure−Activity Relationship

The bioevaluation led to an understanding of the importance of the size of the group at the carbimino carbon atom. In the preliminary anticonvulsant screening, replacement of the proton on the carbimino carbon atom by methyl did not improve the anticonvulsant activity profile when compared to compounds with carbimino hydrogen. The benzylidene compounds were less neurotoxic than their phenylethylidene derivatives. When replaced with bulkier groups like phenyl (**10**), the spectrum of activity improved with no separation from neurotoxicity, while benzyl compounds (**12**) showed activity only in scPIC and scSTY screens with no neurotoxicity. Generally at the 2- and 4-positions of the aryl ring, the electron donating group is favored as seen with compounds **1**, **3**, **5**, and **7** in which compound **7** was found to exhibit neurotoxicity at the anticonvulsant dose. The electron withdrawing group at the 4-position (**2** and **9**) was found to result in complete loss of activity. This is in agreement with our earlier report on aryl semicarbazones.[15] At the carbimino terminal, introduction of cycloalkylidene group (**13**, **14**) showed decreased activity and introduction of isatinimino function showed improved spectrum of activity in MES, scPTZ, and scPIC but elevated the neurotoxicity. Analysis of this data reveals that compounds **7**, **8**, **10**, and **13**−**15** are poor anticonvulsants as the neurotoxic dose is equal to the anticonvulsant dose. Overall it appears that compounds **1** and **5** emerged as promising candidates with significant activity and not neurotoxic at the anticonvulsive dose.

In the antiallodynic and antihyperalgesic assays in CCI and SNL rats, among the benzylidene derivatives (**1**−**5**), compounds **1** and **5** with 2-hydroxy and 4-*N*,*N*-(dimethylamino) substituent, respectively, showed more effectiveness in CCI rats in alleviating dynamic and cold allodynia and mechanical hyperalgesia. While in SNL rats, compounds with an electron rich group at 4-position (especially **3** and **5**) showed activity in three behavioral assays compared to compounds with substitution at the 2- (**1**) or 3-positions (**4**, **8**) which were less active or totally inactive. Among the phenylethylidene derivatives (**6**−**9**), compounds with unsubstitution and substitution with electron donating groups were active, while compound **9** with 4-nitro group was found to be ineffective in both CCI and SNL rats. Replacement of carbimino hydrogen with phenyl (**10**) showed lesser effectiveness than dibenzyl (**12**) or 4-bromophenyl (**11**) compound in CCI rats, while in the SNL rats, dibenzyl compound (**12**) was less effective than **10** and **11**. Replacement of benzylidene group with cycloalkylidene group (**13**, **14**) showed moderate activity against spontaneous pain and mechanical hyperalgesia in CCI rats and pronounced activity in SNL rats. Lastly, isatinimino derivative (**15**) showed similar effectiveness in CCI and SNL rats.

In conclusion, the present study revealed that some of the newer GABA derivatives possessed a broad spectrum of anticonvulsant activity with less neurotoxicity. These derivatives were also observed to be useful in the treatment of acute and chronic pain conditions.

Experimental Section

Chemistry. Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Mass spectra were measured with a Shimadzu GC-MS-QP5000 spectrophotometer. Only molecular ions (M^+) and base peaks are given. Elemental analyses (C, H, and N) were undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within ±0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silicagel-G (Merck) coated aluminum plates, visualized by iodine vapor and UV light. Developing solvents were chloroform−methanol (9:1).

Synthesis of 4-[(Phenoxycarbonyl)amino]butanoic Acid. 4-Aminobutanoic acid (0.1 mol, 10.32 g) was dissolved in 10 mL of water and was stirred at 0−5 °C vigorously. **A**n equimolar amount of phenyl chloroformate (0.1 mol, 15.65 mL) and 0.1 N sodium hydroxide (10 mL) were added dropwise simultaneously. The reaction mixture was maintained alkaline throughout the course of the reaction. Stirring was continued for 2 h. The resulting precipitate was filtered, dried, and recrystallized using ethanol. Mp 98 °C; IR (KBr) ν_{max} 3260, 3040, 1710, 1650−1590, 1240 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 1.88 (m, 2H, CH_2 β to COOH), 2.20 (t, 2H, CH_2 α to COOH), 2.99 (t, 2H, CH_2 γ to COOH), 6.77 (s, 1H, NH, D_2O exchangeable), 7.2−7.34 (m, 5H, Ar−H), 12.32 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 223 (M^+, 100). Anal. ($C_{11}H_{13}NO_4$) C, H, N.

Synthesis of 4-(Hydrazinecarbonylamino)butanoic Acid. 4-[(Phenoxycarbonyl)amino]butanoic acid (0.05mol, 11.16 g) was dissolved in 25 mL of ethanol. To it an equimolar amount of hydrazine hydrate (99%) (0.05 mol, 2.425 mL) was added and refluxed for 6 h after which the solvent was stripped off by distillation in vacuo. The resultant product was dried and recrystallized in 95% ethanol. Mp 202 °C; IR (KBr) ν_{max} 3450, 3300, 1650−1620, 1590−1540, 1310, 1210 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 1.82 (m, 2H, CH_2 β to COOH), 2.20 (t, 2H, CH_2 α to COOH), 3.16 (t, 2H, CH_2 γ to COOH), 5.34 (s, 2H, NH_2^-, D_2O exchangeable), 6.03 (s, 2H, NHCONH, D_2O exchangeable), 12.24 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 161 (M^+, 100). Anal. ($C_5H_{11}N_3O_3$) C, H, N.

General Procedure for the Synthesis of 4-(Alkylidene/Arylidene Hydrazinecarbonylamino)butanoic Acids (1−15). To a solution of 4-(hydrazinecarbonylamino)butanoic acid (0.005 mol, 0.806 g) in ethanol (20 mL) was added an equimolar quantity of appropriate alkyl/aryl aldehydes or ketones (including isatin) in ethanol (5-6 mL), and the mixture stirred for 1−3 h until the completion of the reaction. The resultant precipitate was then filtered, dried, and recrystallized from 95% ethanol. The physical data of the compounds are presented in Table 1. The IR spectra of the compounds were identical in the following aspects: 3320−3200, 1650−1620, 1620−1590, 1350, 840 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) spectra and *m*/*z* of some of the representative compounds are as follows.

4-({[2-(2-Hydroxybenzylidene)hydrazino]carbonyl}amino)-butanoic Acid (1). 1.80 (m, 2H, CH_2 β to COOH), 2.20 (t, 2H, CH_2 α to COOH), 3.0 (t, 2H, CH_2 γ to COOH), 6.01 (s, 1H, NHCO, D_2O exchangeable), 7.2−7.4 (m, 4H, Ar−H), 8.0 (s, 1H, carbimino H), 9.9 (s, 1H, Ar−OH, D_2O exchangeable), 10.56 (s, 1H, NHN═, D_2O exchangeable), 12.0 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 265 (M^+, 100).

4-({[2-(4-Chlorobenzylidene)hydrazino]carbonyl}amino)-butanoic Acid (3). 1.80 (m, 2H, CH_2 β to COOH), 2.22 (t, 2H,

CH_2 α to COOH), 3.12 (t, 2H, CH_2 γ to COOH), 6.0 (s, 1H, NHCO, D_2O exchangeable), 7.4−7.6 (m, 4H, Ar−H), 8.0 (s, 1H, carbimino H), 10.36 (s, 1H, NHN═, D_2O exchangeable), 12.0 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 283 (M^+, 100).

4-[({2-[4-(Dimethylamino)benzylidene]hydrazino}carbonyl)-amino]butanoic Acid (5). 1.80 (m, 2H, CH_2 β to COOH), 2.22 (t, 2H, CH_2 α to COOH), 3.0 (s, 6H, $N(CH_3)_2$), 3.12 (t, 2H, CH_2 γ to COOH), 6.0 (s, 1H, NHCO, D_2O exchangeable), 7.2−7.4 (m, 4H, Ar−H), 8.0 (s, 1H, carbimino H), 10.56 (s, 1H, NHN═, D_2O exchangeable), 12.0 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 292 (M^+, 100).

4-({[2-(1-Phenylethylidene)hydrazino]carbonyl}amino)-butanoic Acid (6). 1.0 (s, 3H, carbimino CH_3), 1.80 (m, 2H, CH_2 β to COOH), 2.22 (t, 2H, CH_2 α to COOH), 3.12 (t, 2H, CH_2 γ to COOH), 6.0 (s, 1H, NHCO, D_2O exchangeable), 7.4−7.65 (m, 5H, Ar−H), 10.0 (s, 1H, NHN═, D_2O exchangeable), 12.22 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 263 (M^+, 100).

4-[({2-[1-(3-Aminophenyl)ethylidene]hydrazino}carbonyl)-amino]butanoic Acid (8). 0.98 (s, 3H, carbimino CH_3), 1.80 (m, 2H, CH_2 β to COOH), 2.22 (t, 2H, CH_2 α to COOH), 3.12 (t, 2H, CH_2 γ to COOH), 5.86 (s, 2H, Ar−NH, D_2O exchangeable), 6.0 (s, 1H, NHCO, D_2O exchangeable), 7.0−7.22 (m, 4H, Ar−H), 10.56 (s, 1H, NHN═, D_2O exchangeable), 12.0 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 278 (M^+, 100).

4-[({2-[1-(4-Nitrophenyl)ethylidene]hydrazino}carbonyl)amino]-butanoic Acid (9). 0.98 (s, 3H, carbimino CH_3), 1.80 (m, 2H, CH_2 β to COOH), 2.22 (t, 2H, CH_2 α to COOH), 3.12 (t, 2H, CH_2 γ to COOH), 6.02 (s, 1H, NHCO, D_2O exchangeable), 7.6−8.2 (m, 4H, Ar−H), 10.56 (s, 1H, NHN═, D_2O exchangeable), 12.0 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 308 (M^+, 100).

4-({[2-(Diphenylmethylene)hydrazino]carbonyl}amino)-butanoic Acid (10). 1.80 (m, 2H, CH_2 β to COOH), 2.22 (t, 2H, CH_2 α to COOH), 3.12 (t, 2H, CH_2 γ to COOH), 6.0 (s, 1H, NHCO, D_2O exchangeable), 7.2−7.8 (m, 10H, Ar−H), 10.56 (s, 1H, NHN═, D_2O exchangeable), 12.0 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 325 (M^+, 100).

4-{[(2-Cyclohexylidenehydrazino)carbonyl]amino}butanoic Acid (13). 1.32 (m, 4H, *o*-position of cyclohexane ring), 1.66 (m, 6H, *m*- and *p*-positions of cyclohexane ring), 1.80 (m, 2H, CH_2 β to COOH), 2.22 (t, 2H, CH_2 α to COOH), 3.12 (t, 2H, CH_2 γ to COOH), 6.0 (s, 1H, NHCO, D_2O exchangeable), 10.56 (s, 1H, NHN═, D_2O exchangeable), 12.0 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 241 (M^+, 100).

4-({[2-(2-Oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]-carbonyl}amino)butanoic Acid (15). 1.82 (m, 2H, CH_2 β to COOH), 2.24 (t, 2H, CH_2 α to COOH), 3.16 (t, 2H, CH_2 γ to COOH), 6.03 (s, 1H, NHCO, D_2O exchangeable), 7.0−7.66 (m, 4H, Ar−H), 10.54 (s, 1H, NHN═, D_2O exchangeable), 11.0 (s, 1H, NH of isatinyl, D_2O exchangeable), 12.2 (s, 1H, OH of COOH, D_2O exchangeable); MS *m*/*z* 290 (M^+, 100).

Pharmacological Methods. Albino mice (Swiss strain, 20−25 g) and albino rats (Sprague−Dawley, 200−320 g) of either sex were used as experimental animals. All experiments were approved by the Institutional Animal Ethics Committee. Animals were housed six (mice) and four (rats) per cage at constant temperature under a 12 h light/dark cycle (lights on at 7:00 AM), with food and water ad libitum. The synthesized compounds **1**−**15** were suspended in 30% v/v polyethylene glycol (PEG) 400 or 0.5% methyl cellulose/water mixture.

1. Anticonvulsant Screening. The anticonvulsant evaluations were undertaken partly by the National Institutes of Health, using their reported procedures.[21,28] Initially all compounds were administered i.p. at doses of 30, 100, and 300 mg/kg to one to four mice. Activity was established using the MES, scPTZ, scSTY,[29] and scPIC[30] tests.

2. Neurotoxicity Screening. Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals were given an i.p. injection of the test compounds in doses of 30, 100, and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

3. Analgesic Activity. Acetic Acid Induced Writhing. Mice were divided into groups of six each. Using the method of Siegmund et al.,[31] writhing was induced by an intraperitoneal injection of 0.1 mL of 3% v/v acetic acid. Test group mice received acetic acid 1 h 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.

4. Induction of Peripheral Mononeuropathy—CCI Model. Unilateral mononeuropathy was produced in rats using the CCI model performed essentially as described by Bennett and Xie.[32] 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 1 mm 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 antibiotic (4 mg/kg) was given intramuscularly for 5 days after surgery. The animals were then transferred to their home cages and left for recovery.

5. Induction of Peripheral Mononeuropathy—Selective Segmental L5 SNL Model. A left L5 spinal nerve ligation, as described by Kim and Chung,[33] 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 paraspinal 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 antibiotic (4 mg/kg) was given intramuscularly for 5 days after surgery. The animals were then transferred to their home cages and left for recovery.

6. Sensory Testing Using Nociceptive Assays. Spontaneous Pain. Spontaneous pain was assessed for a total time period of 5 min as described previously by Choi et al..[34] 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 has 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.[35,36] 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 was then noted. 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 (results given as Supporting Information).

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.[37] 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 behavior ceased. A total number of four rats ($n = 4$) were assigned to this group. Few drops (100−200 μL) 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 response. For each measurement, the paw was sampled three times and a mean calculated. At least 3 min elapsed between each test (results given as Supporting Information).

Mechanical Hyperalgesia. Mononeuropathic rats were assessed for mechanical hyperalgesia sensitivity according to the procedure described by Gonzalez et al.[38] 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. Hindpaw withdrawal duration 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.

Statistical Analysis. All data are expressed as means ± standard error of mean (SEM). 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 (predrug) 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.

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Supporting Information Available: Behavioral assays of dynamic and cold allodynia in CCI and SNL rats. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jensen, S. T. Anticonvulsants in neuropathic pain: rationale and clinical evidence. *Eur. J. Pain* **2002**, *6*, 61−68.
(2) Rogawski, M. A.; Loscher, W. The neurobiology of antiepileptic drugs. *Neuroscience* **2004**, *5*, 553−562.
(3) Sindrup, S. H.; Jensen, T. S. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain* **1999**, *83*, 389−400.
(4) Jensen, T. S. Anticonvulsants in neuropathic pain: rationale and clinical evidence. *Eur. J. Pain* **2002**, *6*, 61−68.
(5) Rice, A. S.; Maton, S. Gabapentin in postherpetic neuralgia: a randomized double-blind placebo-controlled study. *Pain* **2001**, *94*, 215−224.
(6) Serpell, M. G. Gabapentin in neuropathic pain syndromes: a randomized, double-blind, placebo-controlled trial. *Pain* **2002**, *99*, 557−566.
(7) Backonja, M.; Glanzman, R. L. Gabapentin dosing for neuropathic pain: evidence from randomized, placebo-controlled clinical trials. *Clin. Ther.* **2003**, *25*, 81−104.
(8) Franek, M.; Vaculin, S.; Rokyta, R. $GABA_B$ receptor agonist baclofen has non-specific antinociceptive effect in the model of peripheral neuropathy in the rat. *Physiol. Res.* **2004**, *53*, 351−355.
(9) Smith, G. D.; Harrison, S. M.; Birch, P. J.; Elliott, P. J.; Malcangio, M.; Bowery, N. G. Increased sensitivity to the antinociceptive activity of (+/−)-baclofen in an animal model of chronic neuropathic, but not chronic inflammatory hyperalgesia. *Neuropharmacology* **1994**, *33*, 1103−1108.
(10) Hwang, J. H.; Yaksh, T. L. The effect of spinal GABA receptor agonists on tactile allodynia in a surgically-induced neuropathic pain model in the rat. *Pain* **1997**, *70*, 15−22.
(11) Alves, N. D.; De Castro, C. C. M.; De Carvalho, A. M.; Santos, F. J. C.; Silveira, D. G. Possible analgesic effect of vigabatrin in animal experimental chronic neuropathic pain. *Arq. Neuropsiquiatr.* **1999**, *57*, 916−920.
(12) Dimmock, J. R.; Puthucode, R. N.; Smith, J. M.; Hetherington, M.; Quail, J. W.; Pugazhenthi, U.; Lechler, T.; Stables, J. P. (Aryloxy)-aryl semicarbazones and related compounds: a novel class of anticonvulsant agents possessing high activity in the maximal electroshock screen. *J. Med. Chem.* **1996**, *39*, 3984−3997.
(13) Dimmock, J. R.; Vashishtha, S. C.; Stables, J. P. Anticonvulsant properties of various acetyl hydrazones, oxamoyl hydrazones and semicarbazones derived from aromatic and unsaturated carbonyl compounds. *Eur. J. Med. Chem.* **2000**, *35*, 241−248.
(14) Yogeeswari, P.; Sriram, D.; Veena, V.; Kavya, R.; Rakhra, K.; Mehta, S.; Ragavendran, J. V.; Thirumurugan, R.; Stables, J. P. Synthesis of novel aryl semicarbazones as potential anticonvulsant agents. *Biomed. Pharmacother.* **2005**, *59*, 51−55.
(15) Yogeeswari, P.; Sriram, D.; Thirumurugan, R.; Ragavendran, J. V.; Sudhan, K.; Kumar, R. P.; Stables, J. Discovery of *N*-(2,6-dimethylphenyl) substituted semicarbazones as anticonvulsants: hybrid pharmacophore based design. *J. Med. Chem.* **2005**, *48*, 6202−6211.
(16) Yogeeswari, P.; Sriram, D.; Raghavendran, J. V.; Thirumurugan, R.; Induja, S.; Stables, J. P. Synthesis and structure−activity relationship on anticonvulsant aryl semicarbazones. *Med. Chem.* **2006**, *2*, 55−62.
(17) Yogeeswari, P.; Sriram, D.; Saxena, A.; Thirumurugan, R.; Stables, J. 2,4-Dimethoxyphenyl semicarbazones with anticonvulsant activity against three animal models of seizure: Synthesis and pharmacological evaluation. *Bioorg. Med. Chem.* **2006**, *14*, 3106−3112.
(18) Yogeeswari, P.; Thirumurugan, R.; Sriram, D.; Suniljit, L. R. J.; Ragavendran, J. V.; Kavya, R.; Rakhra, K.; Stables, J. P. Synthesis of some new semicarbazone derivatives as anticonvulsants with GABA- mediated mechanism. *Acta Pharm.* **2006**, *56*, 259−272.
(19) Mehta, S.; Yogeeswari, P.; Sriram, D.; Kudyar, P.; Kumar, P. R.; Induja, S.; Stables, J. P. Quantum mechanical modeling of N^4-(2,5-disubstituted phenyl) semicarbazones: synthesis and anticonvulsant activity of N^4-(2,5-dimethylphenyl/-2-fluoro-5-methyl phenyl) semicarbazones. *Med. Chem.* **2006**, *2*, 617−626.
(20) Beukers, M. W.; Wanner, M. J.; Künzel, J. K. V. F. D.; Klaasse, E. C.; Ijzerman, A. P.; Koomen, G. J. N^6-Cyclopentyl-2-(3-phenylaminocarbonyltriazene-1-yl) adenosine (TCPA), a very selective agonist with high affinity for the human adenosine A_1 receptor. *J. Med. Chem.* **2003**, *46*, 1492−1503.
(21) Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. Antiepileptic drug development program. *Cleveland Clin. Quart.* **1984**, *51*, 293−305.
(22) Kupferberg, H. J. Antiepileptic drug development program: a cooperative effort of government and industry. *Epilepsia* **1989**, *30*, S51−56.
(23) Sawynok, J. GABAergic mechanisms in antinociception. *Prog. Neuropsychopharmacol. Biol. Psychiat.* **1984**, *8*, 581−586.
(24) Chakraborty, A; Devi, R. K. B.; Rita, S.; Sharatchandra, K. H.; Singh, T. H. I. Preliminary studies on anti-inflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models. *Indian J. Pharmacol.* **2004**, *36*, 148−150.
(25) Eaton, M. J.; Martinez, M. A.; Karmally, S. A single intrathecal injection of GABA permanently reverses neuropathic pain after nerve injury. *Brain Res.* **1999**, *835*, 334−339.
(26) Ibuki, T.; Hama, A. T.; Wang, X. T.; Pappas, G. D.; Sagen, J. Loss of GABA immunoreactivity in the spinal dorsal horn of rats with peripheral nerve injury and promotion of recovery by adrenal medullary grafts. *Neuroscience* **1997**, *76*, 845−858.
(27) Yaksh, T. L. Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effects of modulatory receptor systems and excitatory amino acid antagonist. *Pain* **1989**, *37*, 111−123.
(28) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferbeng, H. J.; Swinyard, E. A. Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia* **1978**, *19*, 409−428.

(29) Lapin, I. P. Antagonism of L-glycine to seizures induced by L-kynurenine, quinolinic acid and strychnine in mice. *Eur. J. Pharmacol.* **1981**, *17*, 495−502.
(30) Barrada, O.; Oftedal, S. I. The effect of diazepam (valium) and nitrazepam (mogadon) on picrotoxin induced seizures in rabbits. *Electroencephalogr. Clin. Neurophysiol.* **1970**, *29*, 220−221.
(31) Siegmund, E. A.; Cadmus, R. A. A method for evaluating both nonnarcotic and narcotic analgesics. *Proc. Soc. Exp. Biol.* **1957**, *95*, 729−731.
(32) Bennett, G. J.; Xie, Y. K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **1988**, *33*, 87−107.
(33) Kim, S.; Chung, J. M. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* **1992**, *50*, 355−363.
(34) Choi, Y.; Yoon, Y. W.; Na, H. S.; Kim, S. H.; Chung, J. M. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. *Pain* **1994**, *59*, 369−376.
(35) Field, M. J.; Bramwell, S.; Hughes, J.; Singh, L. Detection of static and dynamic components of mechanical allodynia in rat models of neuropathic pain: Are they signalled by distinct primary sensory neurones? *Pain* **1999**, *83*, 303−311.
(36) Field, M. J.; McCleary, S.; Hughes, J.; Singh, L. Gabapentin and pregabalin, but not morphine and amitriptyline, block both static and dynamic components of mechanical allodynia induced by streptozocin in the rat. *Pain* **1999**, *80*, 391−398.
(37) Caudle, R. M.; Mannes, A. J.; Benoliel, R.; Eliav, E.; Iadarola, M. J. Intrathecally administered cholera toxin blocks allodynia and hyperalgesia in persistent pain models. *J. Pain* **2001**, *2*, 118−127.
(38) Gonzalez, M. I.; Field, M. J.; Hughes, J.; Singh, L. Evaluation of selective NK1 receptor antagonist CI-1021 in animal models of inflammatory and neuropathic pain. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 444−450.

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