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5-(4-Chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives as lipophilic cyclic analogues of baclofen: Design, synthesis, and neuropharmacological evaluation

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ABSTRACT

In trials to preserve the pharmacological profile and improve the bioavailability via lipophilicity increment of baclofen **1** and searching for more potent and less toxic muscle relaxants and analgesics, nine substituted cyclic analogues of **1** were designed and synthesized. The target derivatives 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one (**11**–**19**) were obtained through amide formation to the corresponding intermediates (**2**–**10**) followed by cyclization using acetic anhydride. The structures of the target compounds (**11**–**19**) were confirmed by IR, ^{1}H NMR, MS, and elemental analyses. The neuropharmacological activities of these lipophilic cyclic analogues (**11**–**19**) were assessed for their effects on motor activity, muscle relaxation, pain relief and impaired cognition, by intraperitoneal administration at a dose of 3 mg/kg with reference to those of baclofen **1**. Our results showed that compounds **11**–**14** are devoid of all of the tested pharmacological effects associated with **1**. In all paradigms tested, undecyl, tridecyl, heptdec-8-enyl and benzyl substituted analogue derivatives (**15**, **16**, **18**, and **19**) revealed a significant neurological activity being vividly favorable comparable with baclofen **1**. 2-Benzyl-5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivative **19** is the most active candidate with high significant neurological potencies, while 5-(4-chlorophenyl)-2-(dec-8-enyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivative **17** displayed activity at relatively higher time interval. These results probe a new structurally distinct class incorporating 1,3-oxazepine nucleus as promising candidates as $GABA_B$ agonists for further investigations.

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1. Introduction

γ-Amino butyric acid (GABA), the most abundant inhibitory neurotransmitter in the central nervous system, is implicated in the regulation of several physiological and psychological processes.[1] Ionotropic GABA receptors, including $GABA_A$ and $GABA_C$ receptors, and metabotropic $GABA_B$ receptors are the two subtypes of GABA receptors.[2] The $GABA_B$ receptor is a heterodimer made up of two subunits, $GABA_{B(1)}$ and $GABA_{B(2)}$, both necessary for $GABA_B$ receptors to be functionally active.[3] The former contains the GABA-binding domain, whereas $GABA_{B(2)}$ provides the G-protein-coupling mechanism and also incorporates an allosteric modulatory site within its heptahelical structure.[4] The receptors are located in the brain both pre- and post-synaptically where they are coupled to Ca^{2+} and K^+ channels. Therefore, when receptor activation occurs, a variety of effects might be expected to occur as a consequence of inhibition of transmitter release and/or neuronal hyperpolarization.[5] Having a selective agonist for the receptor, together with information derived from 'knockout' mice, has provided the basis for much of the speculation about the potential therapeutic benefits of both agonists and antagonists for the $GABA_B$ receptor. Pharmacological studies with 4-amino-3-(4-chlorophenyl)butyric acid (baclofen **1**), the highly selective $GABA_B$ receptor agonist, have pointed to a role for $GABA_B$ receptors in a number of central nervous system disorders such as epilepsy, cognition, pain, muscle relaxation gastroesophageal reflux, and addiction.[6] The most predominant pharmacological effects of baclofen are the muscle relaxant, anti-nociceptive and anti-drug craving effects, as well as the reduction in cognitive behavior.[7] Baclofen **1** has been in clinical use for the treatment of spasticity associated with brain and spinal cord injuries, as well as dystonia for over 30 years.[8] The centrally mediated muscle-relaxant properties of baclofen **1** are well established clinically, making it the drug of choice in spasticity associated with cerebral palsy, multiple sclerosis, stiff-man syndrome, and tetanus. However, the side effects produced by this drug, including drowsiness, insomnia, dizziness, weakness, ataxia, orthostatic hypotension, and impairment of cognitive functions are frequently not tolerated by patients.[9–13] Various clinical studies demonstrated that baclofen **1** does not penetrate the blood–brain

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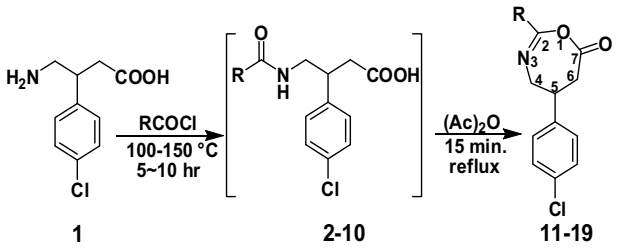
barrier (BBB) in a sufficient extent. This has been largely overcome by the introduction of intrathecal administration using an indwelling pump.[14,15] But there are several potential complications of chronic intrathecal baclofen infusion, including surgical side effects and malfunction of the system.[16] Serious surgical complications include epidural hematoma and abscess formation of the surgical site or in the epidural space. Seroma formation at the pump pocket is fairly common and can be minimized by careful homeostasis and a small size of subcutaneous pocket. Severe problems associated with persistent cerebrospinal fluid leak, including dural puncture headache, cranial nerve palsy, and intracranial subdural hematoma, have also been reported.[17] In an effort to improve the bioavailability of baclofen **1**, in the present study we design and synthesize a series of 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**) as lipophilic cyclic analogues of baclofen **1** in a trial to enhance the permeation of **1** through blood–brain barrier (BBB) to improve its therapeutic potency, and to reduce the possible adverse effects. The central neuropharmacological properties of target compounds (**11**–**19**) were studied in mice and compared with the neuropharmacological effects and structure of the reference drug baclofen **1**.

2. Results and discussion

2.1. Chemistry

Scheme 1 illustrates the procedures used for the synthesis of 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**). Acylation of the amino group of baclofen **1** with acyl chloride afforded the intermediates *N*-acylamino-3-(4-chlorophenyl)butyric acid derivatives (**2**–**10**). Intramolecular cyclization through dehydration of **2**–**10** afforded the lactone type structures (**11**–**19**). Acetic anhydride as mild and rapid dehydrating agent was used successfully for the synthesis of the target lactones **11**–**19**. The structures of baclofen derivatives (**11**–**19**) were confirmed by spectroscopic means including IR, ^{1}H NMR, and EI-MS (Tables 1 and 2). Evidence of lactone formation in **11**–**19** emerged from the presence of absorption bands in their IR spectra at 1758–1770 cm^{-1}, assigned to ester C=O. ^{1}H NMR spectra of compounds (**11**–**19**) exhibited a multiplet signal at δ 3.37–3.47 assigned to the methine proton (H-5). A characteristic pair of doublets at δ 2.62–2.74, 2.77–2.87, 3.47–3.56, and 3.71–3.84 recognized to be H-6 and H-4, respectively. Two doublets appeared at δ 7.12–7.18 and 7.30–7.36 reflected the *p*-substituted pattern of aromatic protons of all derivatives (**11**–**19**) except compound **19** showed a multiplet signal at δ 7.16–8.12 assigned for nine aromatic protons. The presence of ^{1}H NMR spectral data for residues in position 2 is also assigned. The EI-MS spectra of the target derivatives (**11**–**19**) revealed molecular ion peaks consistent with their respective molecular formulas. Based on the above findings, with the aid of elemental analyses that showed satisfactory results within the accepted experimental errors, the structures of **11**–**19** were established.



1 2-10 11-19

Compound No.	R
2, 11	-CH_3
3, 12	-C_7H_{15}
4, 13	-C_8H_{17}
5, 14	-$C_{10}H_{21}$
6, 15	-$C_{11}H_{23}$
7, 16	-$C_{13}H_{27}$
8, 17	—$(CH_2)_8$—CH=CH_2
9, 18	—$(CH_2)_7$—CH=CH—$(CH_2)_7CH_3$
10, 19	H_2C—

Scheme 1. Synthesis of the target derivatives (**11**–**19**).

2.2. Neuropharmacological activity

The aim of the present studies was to broadly characterize the behavioral neuropharmacological effects of 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**) as lipophilic cyclic analogues of baclofen **1**. Loss of righting reflex, locomotor activity, rotarod test, traction test, hot-plate test, and effect on passive avoidance behavior are paradigms employed. Baclofen **1**, as a positive control, and the tested derivatives (**11**–**19**) were administered in mice intraperitoneally at a dose of 3 mg/kg; 30, 45, 60, and 90 min before experimental testing. Our results demonstrated that the methyl, heptyl, octyl, and decyl substituted analogues (**11**–**14**) are devoid of all the tested neuropharmacological effects associated with baclofen **1**. While undecyl, tridecyl, dec-9-enyl, heptadec-8-enyl, and benzyl substituted derivatives (**15**–**19**) showed a comparable activity in the selected neuropharmacological effects tested (Tables 3–5).

Results obtained from the righting reflex test (Fig. 1) showed a highly significant increase ($p < 0.01$) in the onset of the loss of righting reflex after administration of **15**, **18**, and **19** derivatives compared with baclofen **1** (Fig. 1A). While the tridecyl and dec-8-enyl substituted analogues (**16** and **17**) displayed a significant increase ($p < 0.05$) in the onset of the loss of righting reflex compared with baclofen **1** (Fig. 1A). Also there was significant increase ($p < 0.05$) in the duration of loss of righting reflex after administration of compounds **15** and **17**, and highly significant increase ($p < 0.01$) after administration of compounds **16**, **18**, and **19** relative to baclofen-treated group (Fig. 1B). Measurement of locomotor activity of mice showed significant ($p < 0.05$) decrease in locomotor activity record after administration of undecyl derivative (**15**) compared with baclofen-treated group after 30 and 45 min, which became highly significant ($p < 0.01$) after 60 and 90 min. On the other hand, the tridecyl, heptadec-8-enyl, and benzyl substituted analogues (**16**, **18**, and **19**) showed highly significant ($p < 0.01$) decrease in locomotor activity after all time intervals tested (Fig. 2A–D) relative to that of baclofen **1**. While administration of compound **17** resulted in significant decrease ($p < 0.05$) in locomotor activity only after 60 min and highly significant decrease ($p < 0.01$) after 90 min of its administration (Fig. 2C and D).

Table 1
Physical and IR data of substituted 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**)

Compound[a]	Clog*P*[b]	Mp (°C)	Yield (%)	Solvent[c]	Molecular formula[d] (Molecular weight)	IR KBr (cm^{-1})
11	4.58	184–186	74	A	$C_{12}H_{12}ClNO_2$ (237.68)	1768 (C=O, ester), 1580, 1090
12	7.76	154–155	79	B	$C_{18}H_{24}ClNO_2$ (321.84)	1765 (C=O, ester), 1575, 1085
13	8.29	116–118	69	B	$C_{19}H_{26}ClNO_2$ (335.87)	1765 (C=O, ester), 1575, 1085
14	9.35	101–102	71	B	$C_{21}H_{30}ClNO_2$ (363.92)	1762 (C=O, ester), 1570, 1085
15	9.87	112–114	81	B	$C_{22}H_{32}ClNO_2$ (377.95)	1760 (C=O, ester), 1570, 1085
16	10.93	94–95	73	B	$C_{24}H_{36}ClNO_2$ (406.00)	1760 (C=O, ester), 1570, 1085
17	8.86	107–109	64	B	$C_{21}H_{28}ClNO_2$ (361.91)	1762 (C=O, ester), 1575, 1085
18	12.56	77–78	72	B	$C_{28}H_{42}ClNO_2$ (460.09)	1758 (C=O, ester), 1570, 1085
19	6.15	219–220	84	A	$C_{18}H_{16}ClNO_2$ (313.78)	1770 (C=O, ester), 1580, 1090

[a] The mass spectrum revealed a proper molecular ion $[M]^+$ for all compounds.
[b] Calculated partition coefficient and Clog*P* for baclofen = −1.332.[18]
[c] Solvent of crystallization: A, ethanol; B, ethanol–$CHCl_3$.
[d] Satisfactory microanalysis was obtained (±0.04%) for C, H and N.

Table 2
^{1}H NMR data of substituted 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**)

Compound[a]	–CH_2–CO–	Methine H	–CH_2–N=	Ar-H	Others
11	2.72 (1H, dd, *J* = 16.2, 6.9 Hz, H-6b), 2.84 (1H, dd, *J* = 16.2, 7.2 Hz, H-6a)	3.43 (1H, m, H-5)	3.52 (1H, dd, *J* = 16.1, 6.8 Hz, H-4b), 3.79 (1H, dd, *J* = 16.1, 7.2 Hz, H-44a)	7.18 (2H, d, *J* = 8.6, H-3′, 5′), 7.36 (2H, d, *J* = 8.6, H-2′, 6′)	1.76 (3H, s, CH_3)
12	2.69 (1H, dd, *J* = 16.1, 6.8 Hz, H-6b), 2.80 (1H, dd, *J* = 16.1, 7.0 Hz, H-6a)	3.41 (1H, m, H-5)	3.49 (1H, dd, *J* = 16.1, 6.9 Hz, H-4b), 3.73 (1H, dd, *J* = 16.1, 7.3 Hz, H-4a)	7.13 (2H, d, *J* = 8.5, H-3′, 5′), 7.31 (2H, d, *J* = 8.5, H-2′, 6′)	2.12 (2H, t, –CH_2–), 1.34 (2H, p, –CH_2–), 1.21 (8H, m, 4× –CH_2–), 0.80 (3H, t, –CH_3)
13	2.67 (1H, dd, *J* = 16.0, 6.8 Hz, H-6b), 2.79 (1H, dd, *J* = 16.1, 6.9 Hz, H-6a)	3.39 (1H, m, H-5)	3.50 (1H, dd, *J* = 16.2, 6.8 Hz, H-4b), 3.75 (1H, dd, *J* = 16.2, 7.3 Hz, H-4a)	7.12 (2H, d, *J* = 8.5, H-3′, 5′), 7.32 (2H, d, *J* = 8.5, H-2′, 6′)	2.16 (2H, t, –CH_2–), 1.36 (2H, p, –CH_2–), 1.22 (10H, m, 5× –CH_2–), 0.81 (3H, t, –CH_3)
14	2.66 (1H, dd, *J* = 16.1, 6.9 Hz, H-6b), 2.78 (1H, dd, *J* = 16.1, 7.1 Hz, H-6a)	3.37 (1H, m, H-5)	3.48 (1H, dd, *J* = 16.2, 6.9 Hz, H-4b), 3.72 (1H, dd, *J* = 16.2, 7.0 Hz, H-4a)	7.13 (2H, d, *J* = 8.5, H-3′, 5′), 7.31 (2H, d, *J* = 8.5, H-2′, 6′)	2.18 (2H, t, –CH_2–), 1.37 (2H, p, –CH_2–), 1.24 (14H, m, 7× –CH_2–), 0.82 (3H, t, –CH_3)
15	2.64 (1H, dd, *J* = 16.1, 6.9 Hz, H-6b), 2.80 (1H, dd, *J* = 16.0, 7.1 Hz, H-6a)	3.38 (1H, m, H-5)	3.47 (1H, dd, *J* = 16.1, 6.8 Hz, H-4b), 3.71 (1H, dd, *J* = 16.1, 7.0 Hz, H-4a)	7.14 (2H, d, *J* = 8.5, H-3′, 5′), 7.31 (2H, d, *J* = 8.5, H-2′, 6′)	2.23 (2H, t, –CH_2–), 1.40 (2H, p, –CH_2–), 1.29 (16H, m, 8× –CH_2–), 0.84 (3H, t, –CH_3)
16	2.62 (1H, dd, *J* = 16.1, 7.0 Hz, H-6b), 2.77 (1H, dd, *J* = 16.0, 7.1 Hz, H-6a)	3.39 (1H, m, H-5)	3.48 (1H, dd, *J* = 16.1, 6.9 Hz, H-4b), 3.73 (1H, dd, *J* = 16.1, 7.1 Hz, H-4a)	7.14 (2H, d, *J* = 8.6, H-3′, 5′), 7.30 (2H, d, *J* = 8.6, H-2′, 6′)	2.24 (2H, t, –CH_2–), 1.40 (2H, p, –CH_2–), 1.30 (20H, m, 10× –CH_2–), 0.85 (3H, t, –CH_3)
17	2.69 (1H, dd, *J* = 16.0, 6.9 Hz, H-6b), 2.81 (1H, dd, *J* = 16.2, 7.2 Hz, H-6a)	3.42 (1H, m, H-5)	3.51 (1H, dd, *J* = 16.2, 6.8 Hz, H-4b), 3.80 (1H, dd, *J* = 16.1, 7.2 Hz, H-4a)	7.17 (2H, d, *J* = 8.6, H-3′, 5′), 7.33 (2H, d, *J* = 8.6, H-2′, 6′)	5.27 (1H, m, –C*H*=CH_2–), 4.23 (2H, m, –CH=CH_2–), 2.14 (2H, q, –CH_2–), 1.74 (2H, t, –CH_2–), 1.35 (2H, p, –CH_2–), 1.01 (10H, m, 5× –CH_2–)
18	2.70 (1H, dd, *J* = 16.1, 6.9 Hz, H-6b), 2.80 (1H, dd, *J* = 16.1, 7.1 Hz, H-6a)	3.41 (1H, m, H-5)	3.50 (1H, dd, *J* = 16.0, 6.8 Hz, H-4b), 3.79 (1H, dd, *J* = 16.0, 7.2 Hz, H-4a)	7.16 (2H, d, *J* = 8.5, H-3′, 5′), 7.32 (2H, d, *J* = 8.5, H-2′, 6′)	5.11 (2H, m, 2× –C*H*–), 2.13(4H, m, 2× –CH_2–), 1.81 (2H, t, –CH_2–), 1.70 (2H, p, –CH_2–), 1.11 (20H, m, 10× –CH_2–), 0.83 (3H, t, –CH_3)
19	2.74 (1H, dd, *J* = 16.2, 6.9 Hz, H-6b), 2.87 (1H, dd, *J* = 16.2, 7.2 Hz, H-6a)	3.47 (1H, m, H-5)	3.56 (1H, dd, *J* = 16.1, 7.1 Hz, H-4b), 3.84 (1H, dd, *J* = 16.1, 6.9 Hz, H-4a)	7.16–8.12 (9H, m)	3.52 (2H, s, –CH_2–)

[a] All compounds measured in DMSO.

Table 3
Effect of baclofen **1** and 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**) in dose 3 mg/kg, ip on the righting reflex and locomotor activity in mice

Compound	Righting reflex		Locomotor activity (m)			
	Onset (min)	Duration (min)	After 30 min	After 45 min	After 60 min	After 90 min
Control	–	–	45.24 ± 5.46	43.46 ± 6.71	42.79 ± 5.63	40.13 ± 5.18
Baclofen	20.24 ± 5.46**	35.23 ± 4.36**	30. 21 ± 4.32*	28.48 ± 6.47**	30.76 ± 4.15*	35.64 ± 4.64
11	–	–	45.24 ± 5.46	43.46 ± 6.71	42.79 ± 5.63	40.13 ± 5.18
12	–	–	45.24 ± 5.46	43.46 ± 6.71	42.79 ± 5.63	40.13 ± 5.18
13	–	–	45.24 ± 5.46	43.46 ± 6.71	42.79 ± 5.63	40.13 ± 5.18
14	–	–	45.24 ± 5.46	43.46 ± 6.71	42.79 ± 5.63	40.13 ± 5.18
15	38.23 ± 6.38**	60.25 ± 5.67**	20.12 ± 4.73**	16.21 ± 4.22**	14.11 ± 4.47**	15.66 ± 4.63**
16	32.16 ± 6.84**	75.93 ± 6.38**	15.24 ± 3.42**	13.73 ± 3.56**	14.76 ± 3.22**	13.11 ± 3.54**
17	36.63 ± 4.23**	55. 33 ± 7.39**	25.36 ± 5.62*	20. 56 ± 5.94**	19.43 ± 5.97**	18.56 ± 5.73**
18	38.65 ± 7.72**	93.37 ± 5.32**	11.45 ± 3.54**	9.33 ± 3.14**	10.61 ± 3.46**	9. 25 ± 3.44**
19	42.12 ± 5.32**	112.36 ± 5.61**	0.00 ± 0.0**	0.00 ± 0.0**	0.00 ± 0.0**	0.00 ± 0.0**

Results represent the mean ($n = 5$) ± SEM.
* $p < 0.05$ vs control group.
** $p < 0.01$ vs control group.

Results of rotarod test (Fig. 3) reflected that no significant change in rotarod endurance after 30 min of administration of compounds **15**–**19** and after 45 min of administration of compounds **15** and **17** relative to baclofen **1** (Fig. 3A and B, respectively). Also, administration of compounds **16** and **18** caused significant ($p < 0.05$) reduction in the rotarod endurance relative

Table 4
Effect of baclofen **1** and 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**) in a dose 3 mg/kg, ip on the rotarod and traction tests in mice

Compound	After 30 min		After 45 min		After 60 min		After 90 min	
	Rotarod endurance	Traction test % successful	Rotarod endurance	Traction test % successful	Rotarod endurance	Traction test % successful	Rotarod endurance	Traction test % successful
Control	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0
Baclofen	214 ± 16**	50 ± 3.1**	225 ± 16*	52 ± 5.3**	234 ± 16*	53 ± 5.4**	254 ± 13*	53 ± 4.5**
11	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0
12	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0
13	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0
14	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0	300 ± 0	100 ± 0
15	232 ± 14*	45 ± 4.3**	188 ± 14**	43 ± 3.2**	178 ± 14**	40 ± 3.8**	145 ± 14*	40 ± 3.6**
16	219 ± 9**	40 ± 3.5**	174 ± 9**	40 ± 3.1**	169 ± 9**	35 ± 2.4**	133 ± 9**	35 ± 5.7**
17	222 ± 15*	55 ± 3.2**	198 ± 15**	50 ± 2.1**	185 ± 15**	50 ± 4.3**	155 ± 13**	53 ± 4.4**
18	198 ± 12**	35 ± 4.1**	175 ± 12**	35 ± 4.2**	155 ± 12**	33 ± 3.3**	128 ± 12**	31 ± 2.4**
19	234 ± 16*	27 ± 4.3**	185 ± 16**	25 ± 3.7**	0.00**	25 ± 2.5**	0.00**	22 ± 2.8**

Results represent the mean ($n = 5$) ± SEM.
* $p < 0.05$ vs control group.
** $p < 0.01$ vs control group.

Table 5
Effect of baclofen **1** and 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**) in a dose 3 mg/kg (ip) on the hot plate and passive avoidance reflex (PAR) tests in mice

Compound	Retention latency (s)							
	After 30 min		After 45 min		After 60 min		After 90 min	
	Hot-plate test	PAR test	Hot-plate test	PAR test	Hot-plate test	PAR test	Hot-plate test	PAR test
Control	8.12 ± 2.14	163.23 ± 6.26	8.22 ± 2.57	163.23 ± 6.24	9.23 ± 2.35	160.43 ± 8.46	9.33 ± 2.13	164.56 ± 8.66
Baclofen	13.42 ± 2.46*	125.34 ± 4.54*	15.35 ± 2.23*	121.46 ± 4.83*	14.36 ± 2.13*	121.44 ± 7.52*	14.22 ± 2.25*	128.56 ± 7.64*
11	8.12 ± 2.24	163.23 ± 6.36	8.22 ± 2.58	163.23 ± 6.24	9.22 ± 2.37	160.43 ± 7.43	9.33 ± 2.13	164.56 ± 8.66
12	8.12 ± 2.13	163.23 ± 6.26	8.22 ± 2.57	163.23 ± 6.44	9.23 ± 2.25	160.43 ± 8.46	9.33 ± 2.13	164.56 ± 8.66
13	8.12 ± 2.14	163.33 ± 6.47	8.22 ± 2.67	163.23 ± 6.34	9.23 ± 2.35	160.43 ± 8.26	9.33 ± 2.13	164.56 ± 8.66
14	8.12 ± 2.34	163.53 ± 6.22	8.22 ± 2.55	163.23 ± 6.27	9.23 ± 2.45	160.43 ± 8.38	9.33 ± 2.13	164.56 ± 8.66
15	7.82 ± 3.51	152.34 ± 7.32	13.21 ± 3.11*	114.73 ± 7.33**	18.56 ± 1.52*	88.14 ± 13.4**	24.43 ± 1.52**	72.45 ± 13.66**
16	8.33 ± 2.21	147.24 ± 7.63	15.32 ± 2.36*	109.34 ± 7.54**	18.45 ± 2.35*	82.36 ± 12.23**	26.13 ± 2.47**	67.56 ± 12.56**
17	8.32 ± 3.22	148.82 ± 5.23	14.34 ± 3.41*	118.32 ± 6.85**	19.73 ± 3.11*	95.35 ± 9.32*	21.42 ± 3.21**	95.45 ± 11.23**
18	9.34 ± 2.88	146.23 ± 5.52*	15.77 ± 2.47*	110.36 ± 6.54**	23.61 ± 2.17**	75.44 ± 12.76**	28.43 ± 2.51**	68.44 ± 12.38**
19	9.53 ± 2.36	125.64 ± 6.57*	15.38 ± 3.32*	106.56 ± 6.77**	25.63 ± 3.44**	70.34 ± 9.55**	28.73 ± 3.25**	65.34 ± 9.27**

Results represent the mean ($n = 5$) ± SEM.
* $p < 0.05$ vs control group.
** $p < 0.01$ vs control group.

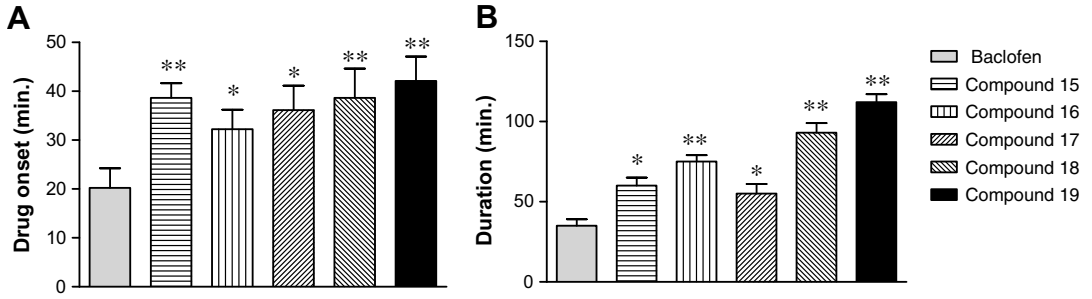


Figure 1. Effect of baclofen **1** and compounds (**15**–**19**) in dose 3 mg/kg, ip on the righting reflex test in mice. (A) onset and (B) duration of the loss of righting reflex. Results represent mean ($n = 5$) ± SEM. * $p < 0.05$ significant increase vs baclofen-treated group. ** $p < 0.01$ significant increase vs baclofen-treated group.

to baclofen **1** after 45 min (Fig. 3B) and compounds **15**–**18** after 60 and 90 min (Fig. 3C and D). While compound **19** showed a significant decrease ($p < 0.05$) in rotarod endurance after 45 min (Fig. 3B) and highly significant ($p < 0.01$) reduction in the rotarod endurance relative to baclofen **1** after 60 and 90 min of its administration (Fig. 3C and D). In traction test, the dec-8-enyl substituted derivative (**17**) revealed no significant decrease in the percent of success of the traction test in measurements carried out at all time intervals relative to baclofen-treated group (Fig. 4A–D). While compound **15**, showed significant ($p < 0.05$) decrease in the percent of success of the traction test only at 90 min after its administration (Fig. 4D). Also significant activity ($p < 0.05$) showed by compounds **16** and **18** at 30 and 45 min time intervals compared with baclofen-treated group (Fig. 4A and B). Highly significant ($p < 0.01$) decrease in the percent of success of the traction test at all time intervals relative to that of baclofen was displayed by the benzyl substituted derivative **19** (Fig. 4A–D). Many studies indicated the role of $GABA_B$ receptors in sedation and muscle-relaxant effect, suggesting that activation of $GABA_B$ receptors may decrease anxiety.[25] Baclofen **1** has demonstrated anxiolytic-like effects in a number of tests and reversed the anxiogenic response induced by withdrawal from chronic diazepam or alcohol treatment.[26–29] Clinically, baclofen **1** reversed the anxiety associated with alcohol withdrawal,[30] post-traumatic stress,[31] and panic disorder.[32]

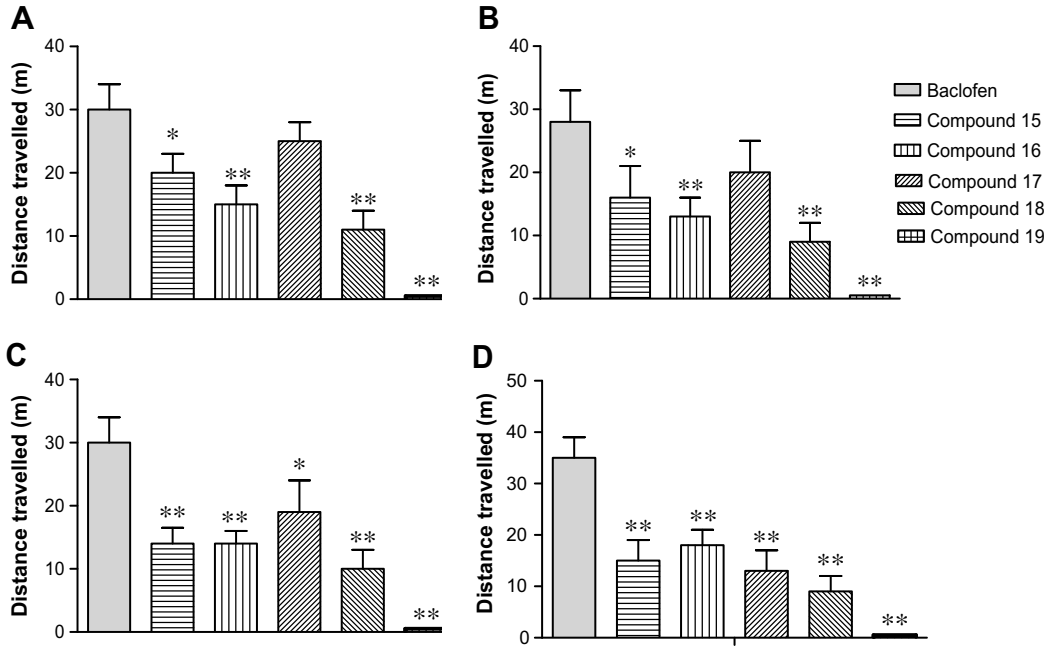


Figure 2. Effect of baclofen **1** and compounds (**15**–**19**) in dose 3 mg/kg, ip on the locomotor activity of mice after (A) 30 (B) 45 (C) 60, and (D) 90 min of administration. Results represent mean ($n = 5$) ± SEM. $^{*}p < 0.05$ significant decrease vs baclofen-treated group. $^{**}p < 0.01$ significant decrease vs baclofen-treated group.

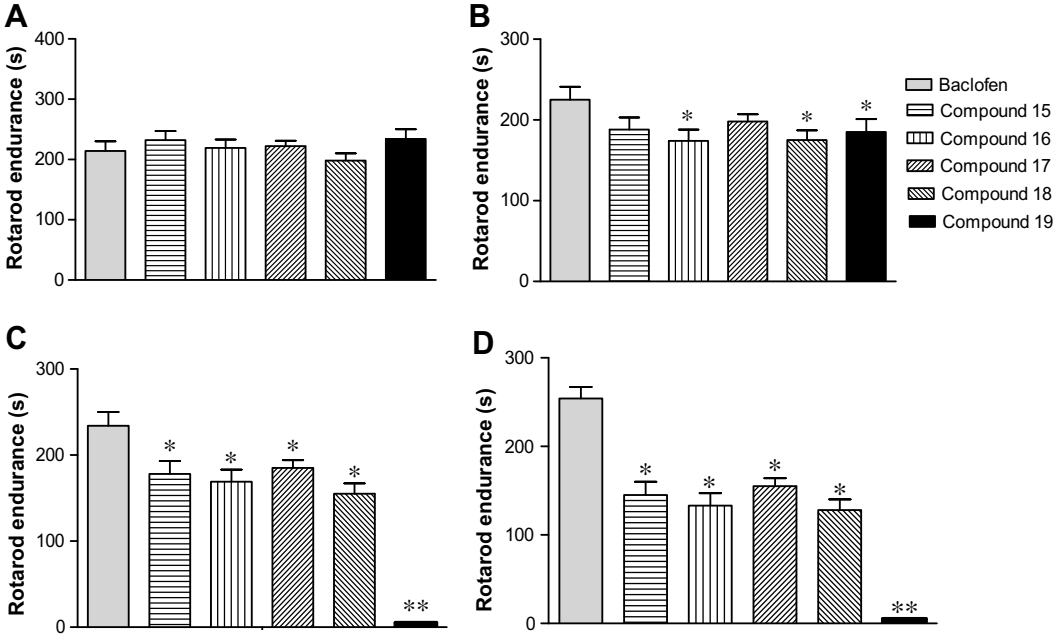


Figure 3. Effect of baclofen **1** and compounds (**15**–**19**) in dose 3 mg/kg, ip on the rotarod test endurance in mice after (A) 30 (B) 45 (C) 60, and (D) 90 min of drug administration. Results represent mean ($n = 5$) ± SEM. $^{*}p < 0.05$ significant decrease vs baclofen-treated group. $^{**}p < 0.01$ significant decrease vs baclofen-treated group.

Results obtained from measurement of hot-plate retention latency shown in Table 5 indicted that baclofen **1** showed significant increase ($p < 0.05$) in hot-plate retention latency at all time intervals compared with control group. While administration of undecyl, tridecyl, and dec-9-enyl, hepatdec-8-enyl, and benzyl substituted analogues **15**–**19** showed no significant change in hot-plate latency compared with baclofen (Fig. 5A and B). Administration of compounds **15** and **16** resulted in significant increase ($p < 0.05$) in hot-plate retention latency measured at 60 min, and highly significant increase in hot-plate latency at 90 min following administration of these compounds (Fig. 5C and D, respectively). On the other hand, relative to baclofen-treated group, the heptadec-8-enyl and benzyl substituted derivatives **18** and **19** revealed highly significant ($p < 0.01$) increase in hot-plate retention latency after 60 and 90 min, and compound **17** showed significant increase ($p < 0.05$) in hot-plate latency measured 60 and 90 min after its administration (Fig. 5C and D). Our results indicated that the cyclic baclofen analogues **15**–**19** exert analgesic activity, but with relatively delayed onset and longer duration than baclofen **1**, which is known to exert analgesic activity.[33] These results are in accordance with the study shown by Cutting and Jordan who reported that baclofen **1** prolonged the time to foot withdrawal in the mouse hot-plate test.[34] Also, study of Balerio and Rubio demonstrated that baclo-

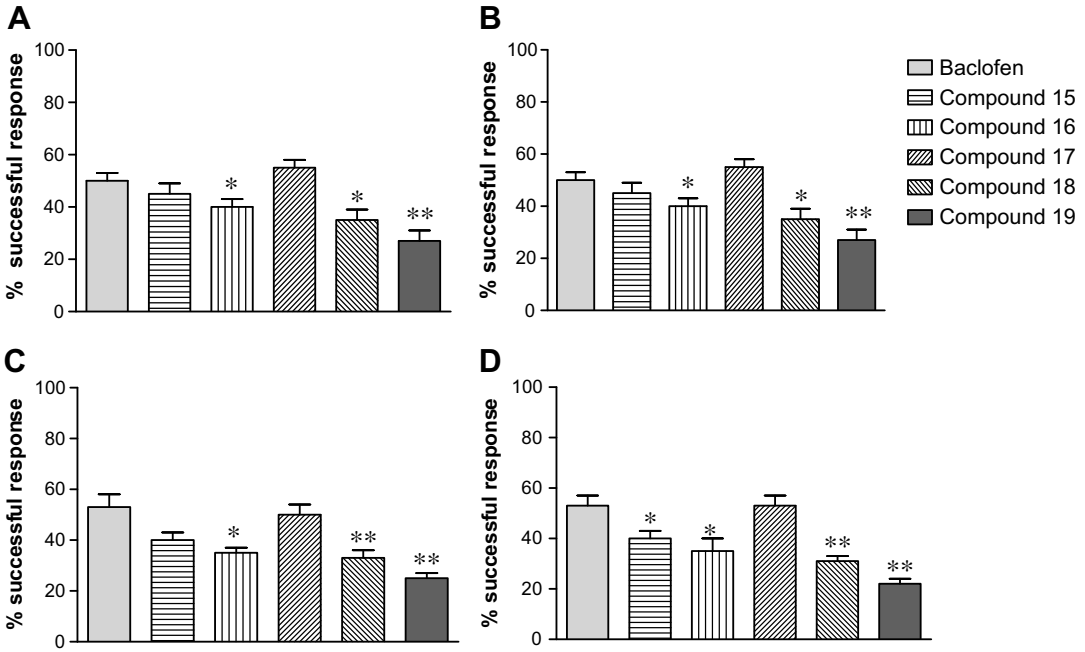


Figure 4. Effect of baclofen **1** and compounds (**15**–**19**) in dose 3 mg/kg, ip on the %. Success of traction in mice after (A) 30 (B) 45 (C) 60, and (D) 90 min of drug administration. Results represent mean ($n = 5$) ± SEM. $^{*}p < 0.05$ significant decrease vs baclofen-treated group. $^{**}p < 0.01$ significant decrease vs baclofen-treated group.

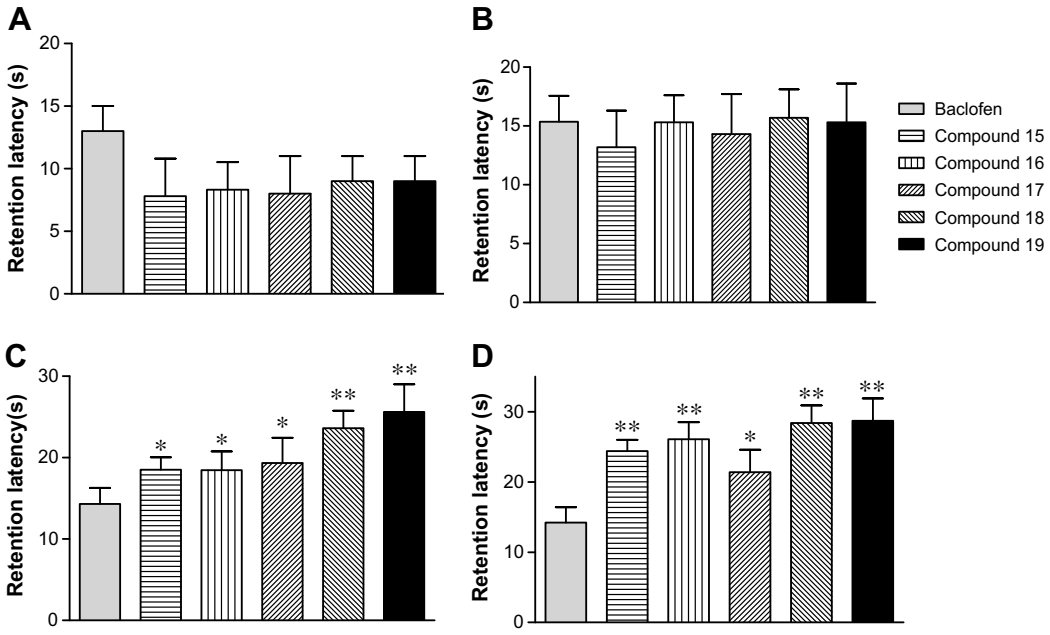


Figure 5. Effect of baclofen **1** and compounds (**15**–**19**) in dose 3 mg/kg, ip on the retention latency of hot-plate test in mice after (A) 30 (B) 45 (C) 60, and (D) 90 min of administration. Results represent mean ($n = 5$) ± SEM. $^{*}p < 0.05$ significant increase vs baclofen-treated group. $^{**}p < 0.01$ significant increase vs baclofen-treated group.

fen **1** elicits a dose-dependent anti-nociceptive effect in mice using the hot-plate test.[35]

As shown in Table 5, compounds (**11**–**19**) also differed in their influence on cognitive function compared with the control group. Baclofen **1** and its derivatives (**15**–**19**) showed significant decrease in the retention latency of passive avoidance reflex (PAR) test compared with control (Table 5). While compared with baclofen **1**, results showed that there was no significant change in the retention latency of PAR test after 30 min of their administration of these compounds **15**–**19** (Fig. 6A). Compounds **16**, **18**, and **19** showed significant decrease in the retention latency of the PAR test after 45 min of their administration (Fig. 6B). Compound **16** showed significant decrease in the retention latency of the PAR test at level $p < 0.05$ after 60 min of its administration (Fig. 6C) that became highly significant at level $p < 0.01$ after 90 min of its administration (Fig. 6D). Compounds **18** and **19** showed highly significant ($p < 0.01$) decrease in the retention latency of the PAR test after 60 and 90 min of its administration (Fig. 6C and D). Compound **15** showed no significant change in PAR test latency compared with baclofen after 30 and 45 min of its administration, while it started to show significant decrease ($p < 0.05$) in PAR latency after 60 min and highly significant decrease ($p < 0.01$) after 90 min of its

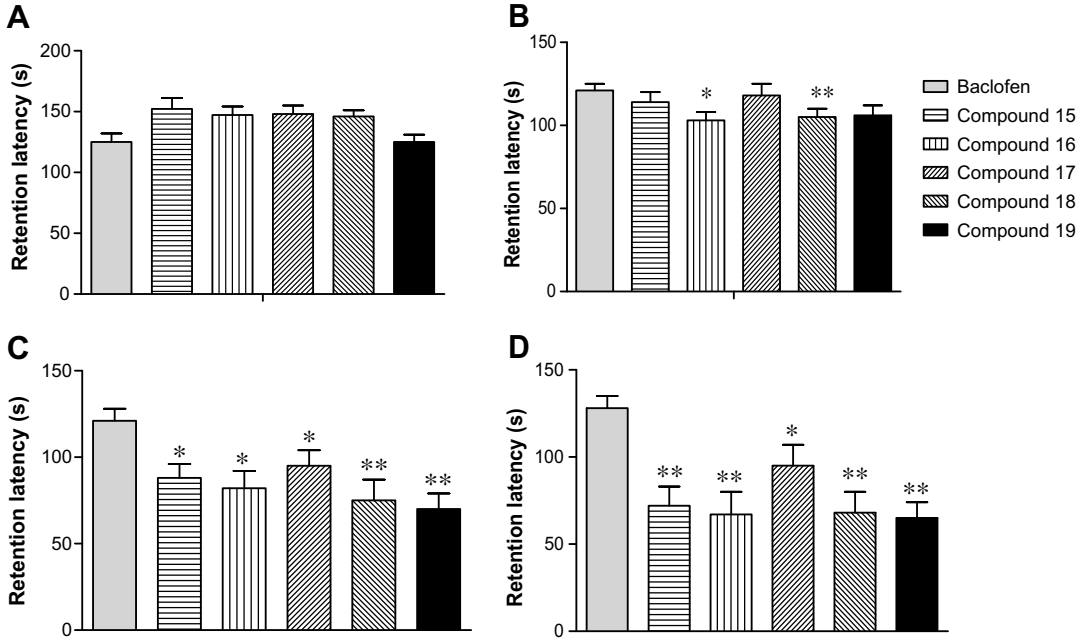


Figure 6. Effect of baclofen **1** and compounds (**15**–**19**) in dose 3 mg/kg, ip on retention latency of the passive avoidance reflex (PAR) test in mice after (A) 30 (B) 45 (C) 60, and (D) 90 min of drug administration. Results represent mean ($n = 5$) ± SEM. $^{*}p < 0.05$ significant decrease vs baclofen-treated group. $^{**}p < 0.01$ significant decrease vs baclofen-treated group.

administration (Fig. 6C and D). Compound **17** showed significant decrease ($p < 0.05$) in PAR test latency only after 60 and 90 min of its administration (Fig. 6C and D). These data are consistent with previous reports demonstrating cognitive impairing effects of baclofen **1** on passive avoidance behavior.[36–38] Baclofen **1**, an agonist of $GABA_B$ receptors, impaired cognition and passive avoidance reflex in a dose-related manner.[39–42] Zarrindast et al. showed that low doses of baclofen (0.125 mg/kg and 0.25 mg/kg) showed only a tendency (without significance) to impair acquisition of passive avoidance in mice but diminished the improvement produced by physostigmine.[43] This is in accordance with our results, where a significant impairment in cognition observed after administration of baclofen **1** and its cyclic derivatives **15**–**19**, but these compounds showed delayed impairment in cognitive function but with more prolonged duration than that of baclofen **1**.

During the last decade, however, innumerable attempts to develop specific agonists for the $GABA_B$ receptors site, baclofen **1** remains the clinical drug of choice in the treatment of spasticity.[44–46] Up to now, baclofen **1** is the only clinically useful selective $GABA_B$ receptors agonist. Therefore, additional efficient $GABA_B$ receptor agonists are eagerly awaited. In ongoing efforts to search for more potent and less toxic $GABA_B$ receptor agonist, nine new lipophilic cyclic analogues of baclofen (**11**–**19**) are designed and synthesized. The main target of our design of 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (**11**–**19**) is to preserve the pharmacological profile and improve the bioavailability through lipophilicity increment of baclofen **1**. Structural modifications of baclofen **1** are designed in a manner where baclofen back-formation via metabolic hydrolysis from the target molecules (**11**–**19**) can be achieved.

From the results obtained, a significant neuropharmacological activity was displayed by undecyl, tridecyl, heptdec-8-enyl, and benzyl substituted analogues derivatives (**15**, **16**, **18**, and **19**) in all paradigms tested. In terms of potency, the neuropharmacological activity of these derivatives (**15**, **16**, **18** and **19**) is vividly favorable comparable with baclofen **1**. On the other hand, vulnerability to metabolic activation of these compounds (**15**, **16**, **18**, and **19**) to baclofen **1** may gives rise to longer duration of action. 2-Benzyl-5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivative **19** is the most active candidate highly affecting the potencies at all time intervals tested in addition to its 10-times reduction in locomotor activity, 6-times reduction in the rotarod endurance, 3-times decrease in the retention latency of PAR, and 2-times increase in hot-plate retention latency and decrease in the percent of success of retention test compared with baclofen **1** after 90 min of its administration. The neurological activity of 5-(4-chlorophenyl)-2-(dec-8-enyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivative **17** which appeared at relatively higher time interval in most terms may prove that metabolic activation of **17** to baclofen **1** is a necessary after crossing BBB to reach the site of action at a higher concentration.

Although no clear correlation between the structure and the neuropharmacological activity emerged from the present results, it is obvious that the R residue at C-2 of baclofen cyclic analogues (**15**–**19**) effectively enhances the neuropharmacological potency. On the other hand, the number of carbon atoms and their nature (saturated, unsaturated, or aromatic) of the R residue at C-2 possibly affected the neuropharmacological activity of these compounds.

Partition coefficient, log*P* values, is an important parameter for determining the biological properties of compounds affecting the central nervous system (CNS). Since the cross of BBB by CNS agents is highly affected by lipophilicity, calculated lipophilicity (Clog*P*) of the target derivatives (**11**–**19**) was estimated by using the Clog*P* program based on the fragment method developed by Leo.[18] As it is clear from our results that it is difficult to correlate all log*P* values with the neuropharmacological potency of the target derivatives (**11**–**19**), which may reflect that the intrinsic activity in addition to the bioavailability plays an important role in their activities.

The most interesting data from this study are that simple cyclization of baclofen **1** to 1,3-oxazepinone with addition of lipophilic moieties may lead to a dramatic increase in the neuropharmacological potency, which may reflect a new era of research on $GABA_B$ agonists. Generally, it can be concluded that 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives represent a new

distinct class of structures with therapeutic strategy for the treatment of various neurological diseases. Although initial evidence obtained from the present experiments about the neuropharmacological potencies of the synthesized derivatives (**11**–**19**) is very encouraging, further experiments are being actively investigated in our laboratory and will be published in due course.

3. Materials and methods

3.1. Chemistry

Melting points were determined on a micromelting point apparatus (L-272, Yanaco, Kyoto, Japan) and are uncorrected. IR spectra were performed with a JASCO FT/IR-230 infrared spectrophotometer. Thin layer chromatography was carried out on pre-coated Silica gel 60 F_{254} plates (0.25 mm thickness, Merck, Darmastat, Germany) and spots were detected under UV light. Silica gel 60 (60–230 mesh, Merck, Darmastat, Germany) was used for column chromatography. ^{1}H NMR spectra were measured with a JEOL-JNM-GX 400 (400 MHz) spectrophotometer and chemical shifts were given in δ ppm relative to tetramethylsilane (TMS). Electron impact (EI) mass spectra were measured with a JEOL GC-mate spectrometer at an ionization voltage of 70 eV. Elemental analyses were performed at the microanalytical center of Toyama Medical and Pharmaceutical University, Toyama, Japan. All chemicals used were of analytical grade.

3.1.1. Synthesis of 4-acylamino-3-(4-chlorophenyl)butanoic acid derivatives (2–10)

To a solution of **1** (1 equiv mol) in dioxane, a solution of the appropriate acid chloride (1 equiv mol) in dioxane was added. The reaction mixture was heated at 100 ~ 150 °C for 5 ~ 10 h. The residue after evaporation of dioxane was purified by SiO_2 gel column chromatography using $CHCl_3$/MeOH as eluent, and the residues after solvent evaporation were used for further reaction.

3.1.2. Synthesis of 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives (11–19)

A solution of the appropriate derivative (**2**–**10**) (0.3 g) in acetic anhydride (5 mL) was refluxed for 15 min. After cooling to room temperature, the product precipitated was filtered and crystallized from the appropriate solvent. Yields and physical constants are listed in Table 1, and ^{1}H NMR data are presented in Table 2.

3.2. Calculation of log*P* values

The log*P* values of the synthesized derivatives (**11**–**19**) were computed with a routine method called calculated log*P* (Clog*P*) contained in a PC-software package (Mac log P 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where hydrogens are omitted, or suppressed (SMILES notation), is entered into the program, which computes the log*P* based on the fragment method developed by Leo.[18] Results are given in Table 1.

3.3. Neuropharmacological evaluation

3.3.1. Chemicals

L-Baclofen is purchased from (Sigma co., USA) through the office of International co. of chemicals, Jeddah, Saudi Arabia.

3.3.2. Animals

Male albino mice (Laboratory Animal Breeding Facility, Jazan, KSA) weighing 20–25 g were housed under standard conditions (21–25 °C, 12 h light–dark cycle) with unlimited access to food and water. All experimental procedures were carried out in the morning and procedures for the tested drugs were carried out parallel to those carried on control group. Animals were divided into groups five mice for each. Each group is placed in a separate cage.

3.3.3. Drug treatment

L-Baclofen and tested compounds (**11**–**19**) were dissolved directly before experiments in DMSO to prepare solutions for giving all drugs in a dose 3 mg/kg intrapretoneally, 30, 45, 60, and 90 min before experimental testing. Control group was treated with DMSO free from drugs.

3.3.4. In vivo experiments

3.3.4.1. Loss of righting reflex. The test was carried out as described before by Boehm et al.[19] Animals were injected with baclofen or tested compounds (3 mg/kg, ip), and the length of loss of righting reflex (sleep-time) was measured. Upon loss of the righting reflex, mice were placed on their backs in a sleep trough (90° angle), and the time to regain the righting reflex was measured. Loss of righting reflex was defined as the inability of a mouse to right itself within 30 s. Return of the righting response was defined as the ability of a mouse to right itself twice in 1 min sleep-time, or duration of loss of righting reflex was defined as the time between loss and return of the righting response.

3.3.4.2. Locomotor activity

3.3.4.2.1. Recording device. Horizontal locomotor activity was assessed in transparent Plexiglas boxes (dimensions: 19 × 31 × 16 cm), and activity was detected and registered using the TSE Moti system (TSE, Bad Homburg, Germany), which is based on the registration of infrared light beam interruptions along the *x*-, *y*-, and *z*-axes, as caused by an animal's movements data were directly stored into the computer.[20]

3.3.4.2.2. Experimental procedures. Mice received injections with L-baclofen **1** and the tested derivatives (**11**–**19**) (3 mg/kg ip), or vehicle (DMSO) and also were immediately returned to their home cages for 30, 45, 60, and 90 min. Subsequently, they were individually placed in Plexiglas boxes, and their spontaneous locomotor activity was registered.

3.3.4.3. Rotarod test. The rotarod apparatus consisted of a cylinder subdivided into five available mice positions, each 6 cm in diameter, which was positioned 30 cm above the table and rotated at a speed of 12 rpm.[21] The mice were placed singly on the cylinder. On the day before the start of the experiment, animals were trained to stay on the rotarod for 300 s. Mice that failed to learn the test or did not reach the criterion (300-s endurance) were excluded from the study. During the test day, the length of time each mouse remained on the cylinder ('endurance time,' maximal score 300 s) was measured 30, 45, 60, and 90 min after application of a test compound or vehicle.

3.3.4.4. Traction test. The effect of drugs on muscle strength was examined in the traction test. The animal was suspended by the front paws from a horizontal wire. The untreated mice grasped the stick with both forepaws and, when allowed to hang free, placed at least one hind foot on the stick. The test was successfully completed when the animal was able to touch the wire with at least one hind paw within 5 s. Inability to perform was scored as a failure of traction.[22] The animals were tested 30, 45, 60, and 90 min after application immediately prior to the rotarod test.

3.3.4.5. Hot-plate test. The test was carried out as described by Dambrova et al.[23] Mice were placed into a 19 cm wide glass cylinder on a heated (54 °C) metal plate (Model DS35, Ugo Basile, Italy). The latency to lick one of the hind paws or to jump off the plate was determined. Mice were removed from the hot plate immediately after the response. The cut-off time was set to 30 s

to avoid tissue damage. The animals were tested 30, 45, 60, and 90 min after injection of the tested drugs or vehicle.

3.3.4.6. Effect on passive avoidance behavior. Starting on the day before the training trial and continuing throughout the experiment, they were housed singly in the experimental room. The apparatus and procedure were as previously described in detail.[24] Briefly, for the training trial, mice were gently placed into the light side of the two-compartment trough-shaped apparatus. The door to the dark compartment was opened and a button pressed to initiate timing by the computer. When the mouse broke a photocell beam located 10.5 cm into the dark compartment, the latency from opening the door to the animal breaking the beam (step-through latency) was automatically recorded, and a Campden Instruments 521 C Shock Source (Campden Instruments Ltd, Leicester, UK) was automatically activated. This resulted in the application of a foot shock (0.5-mA rectangular current waves) between the stainless steel plates, which comprised the dark compartment. This ended when the mouse escaped back to the light compartment or after 5 s elapsed, whichever came first. If an animal did not enter the dark compartment within 150 s on the training trial, it was removed from the apparatus without receiving any shock and was excluded from the retention test. The retention test was carried out on the following day, and the same procedure was followed except that the shock generator was switched off.

3.3.4.6.1. Drugs. L-Baclofen **1** and the tested derivatives (**11**–**19**) were administered ip at a dose of 3 mg/kg at 30, 45, 60, and 90 min before the training trial.

3.3.5. Statistical analysis

All results are expressed as means ± SEM. For testing the significance of the differences between groups Student's *t*-test was used. *p*-values less than 0.05 were considered to be significant and less than 0.01 to be highly significant. All statistical analyses of data were carried out with the help of computer system using the commercially available software (Graph pad Prism V5, USA).

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