Bull. Pharm. Sci., Assiut University, Vol. 27, Part 2, December 2004, pp. 193-202.

NEW 1,4-DISUSTITUTED-6-HYDROXYPERHYDRO-1,4-DIAZEPINE-2,3-DIONE DERIVATIVES

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تم في هذه الدراسة تصميم وتشييد بعض مشتقات ٦ هيدروكسي ٤،١ ثنائي مستبدل بيرهيدرو ٤،١ ثنائى الأزبين ٣،٢- دايون من خلال تفاعل ابيكلوروهيدرين مع زيادة من الأمينات الأولية ومفاعلة النواتج الوسيطة مع ثنائى ايثيل أوكسالات للوصول إلى النواتج المستهدفة. وقد تم التأكد من التراكيب البنائية للنواتج النهائية اعتمادا على نتائج طرق التحاليل الطيفية المختلفة إلى جانب التحاليل الدقية لعناصرة المكونة.

وقد تم دراسة ثلاثة عشر مشتقا جديدا من حيث تأثيرها كمثبطات للجهاز العصبي وذلك كمهدئات ومنومات ومواد مانعة للتشنجات ومواد باسطة للعضلات الإرادية بالإضافة لدراسة تأثيرها كمخفضات لضغط الدم.

وقد أظهرت أغلب هذه المشتقات قدرتها على تقليل الحركة التلقائية لفئران التجارب بنسب مئوية متفاوتة مقارنة بعقار الديازيبام. كما أظهرت الدراسة قدرة تلك المركبات على التقليل من فترة بقاء الفئران على جهاز قياس حفظ الاتزان مقارنة بالعقار المرجعى المستخدم. وبالإضافة إلى التأثيرات سالفة الذكر فقد أثبتت هذه المشتقات قدرتها وسرعتها في الحماية الكاملة من التشنجات المحدثة كيميائيا. ومن جانب أخر فقد وجد أن بعض هذه المشتقات يخفض قليلا من ضغط الدم والبعض الاخر له تأثير مساو تقريبا لتأثير عقار البروبرانولول. وقد تمت أيضا دراسة درجة السمية الحادة لأحد هذه المشتقات.

A series of 1,4-disubstituted-6-hydroxyperhydro-1,4-diazepine-2,3-diones were designed and synthesized through the reaction of epichlorohydrin with an excess of the appropriate primary amine. The resulting secondary diamine derivatives were allowed to react with diethyl oxalate to afford the target compounds in good yields. Structures of the target compounds were verified on the basis of spectral and elemental methods of analysis. Twelve new derivatives were subjected to preliminary pharmacological screening regarding their CNS depressant activity such as sedative, hypnotic, anticonvulsant as well as muscle relaxant activities, in addition to evaluation of the hypotensive activity of some representative compounds. Most of the tested compounds gave high percentage reduction in spontaneous locomotor activity (SLA) compared with diazepam. Concerning the rota-rod coordination test, mice cannot remain on the rod more than 20 seconds in comparison with the reference drug used indicating a good muscle relaxant activity of the test compounds. Moreover, these compounds gave 100% protection against pentylenetetrazole-induced convulsions with a rapid onset of action. On the other hand, most of the test compounds gave mild to comparable reduction in blood pressure in comparison to that produced by using propranolol. Moreover, the acute toxicity (LD_{50}) test was carried out for only one representative compound.

INTRODUCTION

Benzodiazepines represent an important class of psychotherapeutic agents performing antianxiety, sedative, antipsychotic, and anticonvulsant activities.[1-5] Recently, some examples of heterocyclic rings fused to the seven-membered diazepine ring system have appeared in the literatures.[6,7] Moreover, other derivatives devoid of the aromatic part have been synthesized and tested for their antiinflamatory,[8] neurotropic,[9] serotonin 3-

Received in 23/3/2004 & Accepted in 27/6/2004

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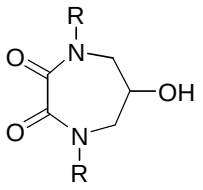
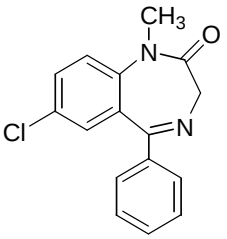
receptor antagonist,[10] and inhibition of spontaneous motor activities.[11] In addition, other 1,4-diazepine derivatives were reported without biological evaluation.[12,13]

Accordingly, the present work aims at the synthesis of 1,4-disubstituted-6-hydroxy-perhydro-1,4-diazepine-2,3-dione derivatives (**I**). The designed compounds (**I**) are, structurally, related to the clinically used 1,4-benzodiazepine (**II**) (Diazepam®), since both (**I**) and (**II**) are 1,4-diazepine derivatives.



I



II

The new designed structural pattern may result in modification of the physicochemical properties of the main skeleton and gives compounds with modified biological activities. Thus, several 1,4-disubstituted-6-hydroxy-perhydro-1,4-diazepine-2,3-diones were synthesized and tested for their CNS depressant activity (sedative, hypnotic, anticonvulsant as well as muscle relaxant properties). In addition, evaluation of the hypotensive activity of some selected derivatives was performed.

EXPERIMENTAL

Melting points were determined on an electrothermal melting point apparatus [Stuart Scientific, UK], and all are uncorrected. Precoated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck) were used for thin layer chromatography. Developing solvent system of chloroform/methanol (3:1) was used and the spots were visualized by ultraviolet light and/or iodine.

IR spectra (KBr disc) were recorded on IR-470 Shimadzu spectrometer, Japan. ^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz) USA. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using $CDCl_3$ or $DMSOd_6$ as solvents. Mass spectra were made on JEOL JMS600 mass spectrometer at Assiut University Central Laboratory, Assiut, Egypt. Elemental analyses were performed at Assiut University Central Laboratory, Assiut University, Assiut, Egypt. Pharmacological screening was performed at the Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Chemistry

General procedure for synthesis of N,N'-dialkyl and diaralkyl-1,3-diamino-2-propanol, (2a-l)[14-16]

A mixture of epichlorohydrin (30 mmol) and the appropriate primary amine (70 mmol) in ethanol (100 ml) was heated under reflux for 24 hr. The reaction mixture was evaporated under reduced pressure and hydrogen chloride gas was bubbled in the methanolic solution of the crude products. The precipitated dihydro-chloride salts were separated by filtration, dissolved in aqueous sodium carbonate solution (10%) and extracted with chloroform. The combined organic extract was washed with brine, water, dried (Na_2SO_4), and evaporated. The products separated out either as oils or solids as mentioned in Table 1.

General procedure for synthesis of 1,4-dialkyl and diaralkyl-6-hydroxy- perhydro-1,4-diazepine-2,3-dione (3a-l)

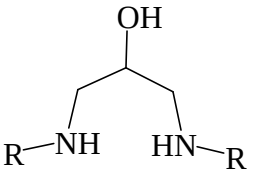
To a stirred solution of the appropriate N,N'-disubstituted-1,3-diaminopropan-2-ol (**2a-l**) (73 mmol) in dry ether (200 ml), diethyl oxalate was added (73 mmol). Few minutes later, crystalline precipitate was formed and the reaction mixture was further stirred at ambient temperature overnight. The crystalline product was separated, washed with ether, dried, and recrystallized from the proper solvent. Physicochemical properties and spectral data are mentioned in Table 2 and Table 3.

Pharmacological screening

i- Measurement of the spontaneous locomotor activity (SLA)

The locomotor activity of compounds (**3a-l**) was determined in mice (25-30 g), three groups each of six animals, using the activity cage method.[17] The cage contains an electromagnetic field, which is sensitive to any motion within it. Movement of animals causes an alteration in the energy of the field that is recorded as a locomotion count. Each animal was placed on the cage five minutes after the

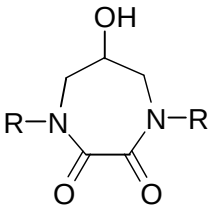
Table 1: Physicochemical and spectral data of compounds **(2a-l)**.



No	R	Yield %	Physical state	IR (neat) cm^{-1}	^{1}H-NMR ($CDCl_3$, δ ppm)
2a*	C_2H_5	65	Colorless liquid	3455, 2920, 1500, and 513	1.13 (6H, t, $2CH_2C\underline{H}_3$); 2.25-3.00 (8H, m, $C\underline{H}_2NHC\underline{H}_2CH$ (OH)$C\underline{H}_2NHC\underline{H}_2$); and 3.50-4.20 (4H, m, 2NH, and CH OH).
2e	$(CH_3)_2CHCH_2$	57	Colorless liquid	3415, 2965, 1464, and 1436	0.90 (12H, d, $2CH(C\underline{H}_3)_2$); 1.30-2.20 (2H, m ,$2C\underline{H}(CH_3)_2$); 2.40-3.25 (10H, m, $C\underline{H}_2N\underline{H}C\underline{H}_2CH(OH)C\underline{H}_2N\underline{H}$ $C\underline{H}_2$); and 4.00 (2H, m, CHOH).
2i	Cyclo-C_6H_{11}	65	m.p 66-8º	3455, 2925, and 1540**	0.80-2.15 (20H, m, $2C_6H_{11}$); 2.30-3.15 (9H, m, $2C\underline{H}N\underline{H}$ $C\underline{H}_2$, and OH); and 3.50-4.10 (1H, m, $C\underline{H}OH$).
2j	C_6H_5-CH_2	60	Yellow oil	3400, 3050, 1550, 740, and 690	2.85-5.00 (12H, m, $C\underline{H}_2N\underline{H}$ $C\underline{H}_2C\underline{H}(O\underline{H})CH_2NHCH_2$); and 7.70 (10H, s, $2C_6\underline{H}_5$).
2k	$C_6H_5(CH_2)_2$	55	Yellow oil	3480, 3025, 2920, 1570, 739, and 691	2.45-3.25 (15H, m, $C\underline{H}_2C\underline{H}_2$ $N\underline{H}C\underline{H}_2CH(O\underline{H})C\underline{H}_2N\underline{H}C\underline{H}_2$ $C\underline{H}_2$); 3.50-4.00 (1H, m, CH); and 7.25 (10H, s, $2C_6\underline{H}_5$).
2l	C_6H_5-$CH(CH_3)$	80	Yellow oil	3470, 3100, 750, and 700.	1.50 (6H, d, $2CHC\underline{H}_3$); 3.00-3.50 (7H, m, $N\underline{H}C\underline{H}_2C\underline{H}$ (OH) $C\underline{H}_2N\underline{H}$); 4.00 (1H, hump, OH), 6.00 (2H, q, $2CH_3C\underline{H}$); 7.20 (10H, s, $2C_6\underline{H}_5$).

* Compounds **2b**, **2c**, **2d**, **2f**, **2g** and **2h** were reported.[14,15]
** Measured as disc with KBr.

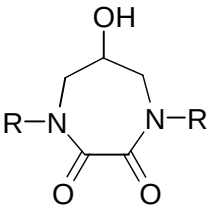
Table 2: Physicochemical data of compounds **(3a-l)**.



No.	R	Yield %	R_f*	m.p ° / cryst. solvent	Molecular formula (M.Wt.)	Microanalysis		
							Calcd. %	Found %
3a	C_2H_5	91	0.794	175-80 Ethanol	$C_9H_{16}N_2O_3$. ¼ H_2O (204.72)	C H N	52.80 8.12 13.68	52.68 8.20 13.54
3b	$CH_3(CH_2)_2$	80	0.690	110-15 Hexane/ Ethanol	$C_{11}H_{20}N_2O_3$ (228.27)	C H N	57.87 8.83 12.26	57.16 8.41 12.12
3c	$(CH_3)_2CH$	83	0.722	200-2 Hexane/ Ethanol	$C_{11}H_{20}N_2O_3$ (228.27)	C H N	57.87 8.83 12.26	57.82 9.53 12.29
3d	$CH_3(CH_2)_3$	86	0.700	104-6 Ethanol	$C_{13}H_{24}N_2O_3$ (256.33)	C H N	60.91 9.44 10.93	60.08 10.00 10.76
3e	$(CH_3)_2CHCH_2$	93	0.861	160-2 Ethanol	$C_{13}H_{24}N_2O_3$ (256.33)	C H N	60.91 9.44 10.93	60.42 10.49 10.83
3f	$(CH_3)_3C$	60	0.769	220-5 Ethanol	$C_{13}H_{24}N_2O_3$ (256.33)	C H N	60.91 9.44 10.93	60.19 9.47 10.81
3g	$CH_3(CH_2)_4$	61	0.789	85-90 Ethanol	$C_{15}H_{28}N_2O_3$. ¼ H_2O (288.88)	C H N	62.36 9.94 9.69	61.93 9.95 9.52
3h	$CH_3(CH_2)_5$	60	0.789	95-100 Ethanol	$C_{17}H_{32}N_2O_3$. ½ H_2O (321.47)	C H N	63.52 10.35 8.71	62.93 10.09 8.68
3i	*Cyclo*-C_6H_{11}	81	0.853	>300 Ethanol	$C_{17}H_{28}N_2O_3$ (308.40)	C H N	66.20 9.15 9.08	65.51 9.85 9.14
3j	$C_6H_5CH_2$	90	0.857	190-2 Ethanol	$C_{19}H_{20}N_2O_3$ (324.36)	C H N	70.35 6.21 8.63	70.31 6.68 8.66
3k	$C_6H_5(CH_2)_2$	70	0.886	195-6 Chloroform/ Ether	$C_{21}H_{24}N_2O_3$. ½ H_2O (361.20)	C H N	69.70 6.97 7.75	69.03 7.22 7.74
3l	$C_6H_5CH(CH_3)$	80	0.760	170-2 Ethanol	$C_{21}H_{24}N_2O_3$. ½ H_2O (361.20)	C H N	69.70 6.97 7.75	69.22 7.15 7.51

*Developing system is chloroform/methanol (3/1).

Table 3: Spectral data of compounds **(3a-l)**.



No.	R	IR (KBr) Cm^{-1}	^{1}H-NMR ($CDCl_3$, δ ppm)
3a	C_2H_5	3455, 2935, 1636, 1657 1331, 779, 513 cm^{-1}	1.20 (6H, t, $2CH_3$), 3.10-3.90 (9H, m, $CH_2NCH_2CH(OH)CH_2NCH_2$), 4.45 (1H, hump, OH).
3b	$CH_3(CH_2)_2$	3450, 2940, 648, 1658, 375,434 714 cm^{-1}	1.00 (6H, t, $2CH_3$), 1.25-2.00 (4H, m, $2CH_2CH_3$),3.1-4.00 (8H,m, $CH_2NCH_2CH(OH)CH_2NCH_2$), 4.21-5.00 (2H, m, $CH_2CH(OH)CH_2$).
3c	$(CH_3)_2CH$	3370 1639,1650 cm^{-1}	1.20 (12H, d, $2CH_3CHCH_3$), 3.51 (4H, s_{br}, $CH_2CH(OH)CH_2$), 4.10-4.65 (2H, m, CH(OH)) 4.70-5.20 (2H, m, $2CH(CH_3)_2$).
3d	$CH_3(CH_2)_3$	3450, 1636,1671, 1332, 774, 513 cm^{-1}	1.00-2.00 (14H, m, $2CH_2CH_2CH_3$), 3.00-4.00(8H,m, $CH_2NCH_2CH(OH)$ CH_2NCH_2),4.20-5.00 (2H, m, CH(OH)).
3e	$(CH_3)_2CHCH_2$	3415, 2965, 1633, 1654, 1174, 1286, 1436,1464 cm^{-1}	1.00 (12H, d, $2CH_3CHCH_3$),1.65-2.35 (2H, m, $2CH_3CHCH_3$), 3.00-3.85 (m, $CH_2NCH_2CH(OH)NCH_2$), 4.15-4.85 (2H, m, CH(OH)).
3f	$(CH_3)_3C$	3420, 2935, 1650,1662, and 727 cm^{-1}	1.50 (18H, s, *t*-butyl),3.50 (5H, s_{br}, $NCH_2CH(OH)CH_2N$),4.16 (1H, hump, OH).
3g	$CH_3(CH_2)_4$	3410, 2960, 1647,1650 and 1332 cm^{-1}	0.90 (6H, t, 2 $CH_2(CH_2)_3CH_3$), 1.00-2.00 (12H, m, 2 $CH_2(CH_2)_3CH_3$),3.00-4.00 (8H, d, $CH_2NCH_2CH(OH)CH_2NCH_2$),4.10-4.75 (2H, m, CH(OH)).
3h	$CH_3(CH_2)_5$	3425, 2900, 1643, 1375, 720, and 524 cm^{-1}	1.00 (6H, t, 2 CH_3), 1.50 (16H, s_{br}, 2 $CH_2(CH_2)_4CH_3$), 3.10-4.00 (8H, m, 2 $CH_2(CH_2)_4CH_3$),$CH_2CH(OH)CH_2N$), and 4.10-4.90 (2H, m, CH(OH)).
3i	*Cyclo*-C_6H_{11}	3445, 2925, 1641, and 1650 cm^{-1}.	1.10-2.25 (22H, m, 2 *c*-hexyls), 3.60 (4H, s_{br}, $NCH_2CH(OH)CH_2N$),4.10-4.83 (2H, m, CH(OH)).
3j	$C_6H_5CH_2$	3285, 2965, 1651, 691 and 714 cm^{-1}.	3.10-3.90 (6H, m, $CH_2CH(OH)CH_2$) 4.66 (4H, dd, J = 6 Hz, $2CH_2Ph$),7.50 (10H, s, Ar-H).
3k	$C_6H_5(CH_2)_2$	3450, 2965, 1621, 1650, 691 and 739 cm^{-1}	3.00 (4H, t, $2CH_2CH_2Ph$),3.23-4.26 (10H, m, $NCH_2CH(OH)CH_2N$ $2CH_2CH_2Ph$),7.50 (10H, s,2 Ar-H).
3l	$C_6H_5CH(CH_3)$	3355, 2965, 1621, 1651, 696, and 725 cm^{-1}	1.60 (6H, d, 2 $CHCH_3$),3.00-3.50 (5H, m, $CH_2CH(OH)CH_2$),4.10 (1H, hump, OH), 6.10 (2H, q, $2CH_3CH$),7.50 (10H, s, 2 Ar-H).

i.p. injection of the test compounds as well as the reference drug (diazepam) at a dose level of 2.8 mmol/kg in NaCMC. The total number of steps in 10 minutes over one hour was recorded. The results are given in Table 4.

ii- The rota-rod motor coordination test

The ability of mice, three groups each of six animals, to remain on a rotating rod for 15 seconds without falling was determined at 10 min intervals over a period of 2 hr following the i.p. injection of the test compounds and the reference drug (diazepam) at a dose level of 2.8 mmol/kg in NaCMC.[17]

iii- Anticonvulsant activity

All compounds (**3a-l**) were screened for their anticonvulsant properties and the activity was established by following anticonvulsant drug development program protocol.[18,19] Pentylenetetrazole 125 mg/kg in NaCMC was administered i.p. to the mice (three groups each of six animals) 15 minutes after injection of the test compound and the reference drug (diazepam) at a dose level of 2.8 mmol/kg in NaCMC. The elapsed time before onset of clonic convulsions, tonic convulsions or death was recorded. The mice were considered to be survived, if they lived for longer than 20 min after pentylenetetrazole administration and the results are given in Table 5.

Table 4: Spontaneous locomotor activity (SLA) of compounds **(3a-l)**.

Compd.	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	Diazepam	Control*
% Mean decrease in SLA	49	61	68	51	78	86	51	51	86	88	86	82	70	0.0

*****Sodium carboxymethylcellulose (NaCMC).

Table 5: Anticonvulsant activity of the test compounds **(3a-l)**.

Compd. No.	Time* of clonic convulsion	Time of tonic convulsion	Protected animals	Dead animals	% Protection
3a	-**	-	8	none	100
3b	-	-	8	none	100
3c	-	-	8	none	100
3d	-	-	8	none	100
3e	-	-	8	none	100
3f	-	-	8	none	100
3g	-	-	8	none	100
3h	-	-	8	none	100
3i	-	-	8	none	100
3j	-	-	8	none	100
3k	-	-	8	none	100
3l	-	-	8	none	100
Diazepam	-	-	8	none	100
Control	2	5	none	8	0***

* Time in minutes.
**No clonic or tonic convulsions.
*** Animals died after 10 minutes.

Hypotensive activity

Three rabbits were anaesthetized with an i.p. injection of urethane in a dose of 1.60 g/kg in water (6 ml).[20] Arterial blood pressure was recorded via the carotid artery, the latter was cannulated to Burden blood pressure transducer. Heparin sodium (0.25 units) was placed in the tip of a cannula to prevent clotting. Blood pressure was recorded by using an oscillograph (400 MD 2 C Bioscience, Kent, U.K.). The transducer was calibrated and the test compounds were injected i.p. (28.9 mmol/kg in NaCMC). Blood pressure was recorded before and after administration of the test compounds (**3c, 3e, 3f, 3j, 3k, and 3l**) over a period of 4 hrs and the results are given in Table 6.

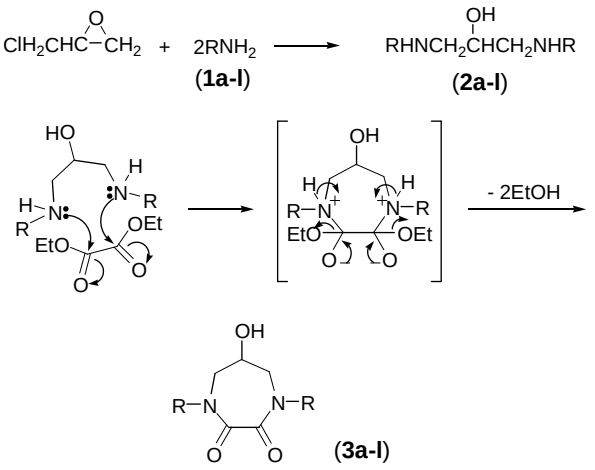
Acute toxicity (LD_{50})

Medium lethal dose (LD_{50}) of compound **3e** was determined in mice. Groups of male adult albino mice of DDY type each of six animals (25-30 g) were injected i.p. with graded doses of the tested compound. The percentage of mortality in each group of the animals was determined 48 hrs after injection. Computation of LD_{50} was processed by graphical method.[21]

RESULTS AND DISCUSSION

Chemistry

The target compounds were synthesized by reaction of epichlorohydrin with excess of the appropriate amine (**1a-l**).[14-16] The resulting secondary diamines (**2a-l**) were subjected to cyclization with diethyl oxalate to provide the desired derivatives (**3a-l**), Scheme 1.



R = C_2H_5 (**3a**), C_3H_7 (**3b**), *i*-C_3H_7 (**3c**), *n*-C_4H_9 (**3d**), *i*-C_4H_9 (**3e**), *t*-C_4H_9 (**3f**), *n*-C_5H_{11} (**3g**), *n*-C_6H_{13} (**3h**), *cyclo*-C_6H_{11} (**3i**), $C_6H_5CH_2$ (**3j**), $C_6H_5CH_2CH_2$ (**3k**), and $C_6H_5CH(CH_3)$ (**3l**).

Scheme 1

Table 6: Hypotensive activity of compounds **3c, 3e, 3f, 3j, 3k and 3l**.

Time (min.)	% Mean decrease in blood pressure*						
	3c	**3e**	**3f**	**3j**	**3k**	**3l**	Propranolol
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D**	5.5	15.0	18.3	24.0	13.6	38.5	13.5
1	2.8	3.1	18.9	19.0	0.8	5.0	19.0
3	3.4	5.6	13.3	21.0	0.9	7.0	23.0
5	1.8	7.3	9.7	23.0	1.1	9.0	27.4
10	1.6	3.9	16.5	24.0	2.0	10.0	32.5
15	3.3	7.8	18.1	26.0	5.7	13.0	41.0
30	4.9	10.5	21.2	37.0	6.0	13.0	47.0
45	13.9	13.8	26.6	44.0	6.0	15.0	52.3
60	13.9	22.0	28.8	46.0	5.0	23.6	45.0
90	13.9	27.7	35.1	41.0	4.2	17.5	48.0
120	17.3	40.8	42.9	43.0	3.3	15.0	42.3
150	10.4	13.9	32.0	40.0	3.3	12.0	34.9
180	8.0	8.2	28.0	26.0	2.0	10.0	20.0
240	4.0	1.0	5.0	9.0	2.0	5.0	6.3

*Average of three results.
**Direct after injection.

Structures of the intermediate compounds (**2a-l**) were verified on the basis of their spectral methods of analysis, the data are in accordance with the proposed structures, Table 1. However, compounds **2b**, **2c**, **2d**, **2f**, **2g**, and **2h** are reported.[14,15] Structures of the designed compounds (**3a-l**) were verified on the basis of spectral and elemental analyses. Physico-chemical constants of the synthesized compounds (**3a-l**) are cited in Table 2.

All the spectral data of compounds (**3a-l**) are in accordance with the assumed structures. IR spectra showed a broad band in the range of 3480-3285 cm^{-1} (OH strech) and strong two absorption bands or some times strong broad one at 1671-1621 cm^{-1} (C=O, stretch of the two carbonyl groups), Table 3.

In ^{1}HNMR spectra, a common pattern for the $CH_2CH(OH)CH_2$ part of the ring was observed, The differences in sets and patterns were only attributed to the 1,4-substituents, where they give results in accordance with the structure of the target compounds, Table 3.

Pharmacological screening

CNS depressant activity

Twelve new derivatives were subjected to preliminary pharmacological screening regarding their CNS depressant activity such as sedative hypnotic, anticonvulsant as well as muscle relaxant activity, in addition to the evaluation of their hypotensive activity.

i- Measurement of the spontaneous locomotor activity (SLA)

The synthesized compounds (**3a-l**) were tested at a dose level of 2.8 mmol/kg, this is the effective dose determined from a dose response curve, dissolved in sodium carboxymethyl-cellulose (NaCMC) intraperitoneally (i.p.) in mice for measurement of SLA in comparison to diazepam as a reference drug. The test was done according to the protocol described in experimental part.[17] The locomotor activity of mice was registered within an activity cage. Each animal was placed on the cage five minutes after the i.p. injection and the total number of steps in 10 minutes over one hour were recorded. The results given in Table 4 indicate that, there are three main factors governing the activity of such compounds. Chain length of the N-substituents, where compounds having straight chain C_2-C_6 (**3a**, **3b**, **3d**, **3g** and **3h**) except the *n*-propyl one (**3b**), exhibit almost the same effect. Branching dramatically increases the activity; thus, data of the constitutional isomers 3d, 3l and 3f clearly show this result, where R = R = *n*-butyl, *i*-butyl, and *t*-butyl respectively. On the other hand, increasing lipophilicity of the compounds,[22] as represented by derivatives **3j**, **3k**, and **3l**, enhances the activity. This indicates that branching and hence the increase of lipophilicity of the compounds is the limiting factor in determining such activity (Table 4).This order of activity might be attributed to the relation between the degree of penetration of the blood brain barrier and the lipophilicity of the administered compounds.[23]

ii- The rota-rod motor coordination test

Compounds (**3a-l**) were tested at a dose level of 2.8 mmol/kg in NaCMC following i.p. injection in mice for rota-rod motor coordination test according to the procedure described in the experimental part.[17] The ability of animals to remain on a rotating rod for 15 seconds without falling was determined at 10 minutes intervals over a period of 2 hr following the i.p. injection of the test compounds. Results of the test showed that the mice couldn't remain on the rod more than 15 seconds in comparison with the reference drug (diazepam) indicating good muscle relaxant activity of the test compounds.

iii- Anticonvulsant activity

Blockade of pentylenetetrazole-induced convulsions in mice is a characteristic effect of some CNS depressant drugs. All compounds (**3a-l**) were investigated for their anti-convulsant activity by following the anti-convulsant drug developments (ADD) program.[18,19] at a dose level of 2.8 mmol/kg in NaCMC. The compounds were administered i.p. in mice using the procedure described in the experimental part. The elapsed time before onset of clonic convulsions, tonic convulsions and death were recorded. Mice have considered to be survived if they lived for longer than 20 min. after pentylenetetrazole administration and the results are given in Table 5.

Neither clonic, nor tonic convulsions have been observed for animals treated with either the test compounds (**3a-l**) or diazepam. It has been observed that, an interval of 30 min

between injection of diazepam and pentylenetetrazole is required for prevention of convulsions and, any decrease in this interval results in convulsions and death of the animals. Also, it is noteworthy to mention that the test compounds elicited a 100% protection against pentylenetetrazole at only 15 min time interval. The rapid onset of action of the test compounds might be attributed to their good lipophilicity and high absorbability.

Hypotensive activity

Six of the synthesized derivatives [**3c** (R = *i*-C_3H_7), **3e** (R = *i*-C_4H_9), and **3f** (R = *t*-C_4H_9), **3j** (R = $C_6H_5CH_2$), **3k** (R = $C_6H_5CH_2CH_2$), and **3l** (R = $C_6H_5CH(CH_3)$)] carrying different alkyl and aralkyl side chains were screened for their hypotensive activities in comparison to propranolol at a dose level of 28.9 mmol/kg in rabbits using the procedure described in the experimental part.[20] The observed change in blood pressure was recorded using an oscillograph. The transducer was calibrated and the test compounds were injected. Blood pressure was recorded before and after administration of the tested compounds (**3c**, **3e**, **3f**, **3j**, **3k**, and **3l**) over a period of 4 hr at intervals indicated in Table 6 and the percentage mean decrease in blood pressure are recorded in Table 6.

After injection, most of the tested compounds as well as the reference drug (propranolol) showed a significant fall in blood pressure of the anaethetized animals. The decrease in blood pressure observed for most of the tested compounds increases by time and the maximum reduction in blood pressure was reached after two hours for only three compounds (**3e, 3f** and **3j**, Table 6) in comparison to that of propranolol. This may be attributed to the good fitting of these compounds (N-substituents are *t*-butyl, *t*-butyl and benzyl) to the receptors than other analogues.

Acute toxicity (LD_{50})

LD_{50} of one of the investigated derivatives (compound **3e**) was determined using graphical method according to the protocol described in the experimental part.[21] LD_{50} of compound **3e** was found to be 4.5 mg/kg while that of the reference drug (diazepam) is 710 mg/kg.[24]

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