Bull. Pharm. Sci., Assiut University, Vol. 29, Part 2, December 2006, pp. 253-271.

SYNTHESIS AND PHARMACOLOGICAL ACTIVITIES OF NOVEL 1-ALKYL-4-ARYL-6-HYDROXYPERHYDRO-1,4-DIAZEPINE-2,3-DIONES

A. M. Abdel-Alim[1], M. A. Hussein[*1], A. A. El-Shorbagi[1], A. A. Abu-ElMagd[1], and B. S. El-Menshawi[2]

[1]*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut-71526, Egypt*
[2]*Department of Pharmacognosy, National Research Centre, Dokki, Giza, Egypt*

تشتمل هذه الدراسة على تصميم و تشييد مشتقات جديدة من ١ الكيل ٤ أريل ٦ هيدروكسىبيرهيدرو ٤،١ ثنائي الأزبين ٣،٢ دايون من خلال تفاعل ابيكلوروهيدرين مع أريل الأمين المناسب ثم مع الكيل ، أرالكيل أو سيكلوهكسيل الأمين. ولقد تم تفاعل المركبات الوسيطة (*N,N'* ثنائي مستبدل ثنائىأمينو ٢ بروبانول) مع ثنائي ايثيل أوكسالات للوصول إلى المواد المستهدفة. وقد تم التأكد من التراكيب البنائية للنواتج النهائية اعتمادا على نتائج التحاليل الطيفية المختلفة إلى جانب التحاليل الدقية لعناصرة المكونة.

هذا وقد تم دراسة تأثير خمسة عشر مركبا جديدا كمواد مضاده للتشنجات بالإضافة إلى دراسة تأثير اثنين وعشرين مركبا كمخفضات لضغط الدم. وقد أظهرت أغلب هذه المشتقات قدرتها وسرعتها في الحماية الكاملة من التشنجات المحدثة كيميائيا. وقد وجد أن بعض هذه المركبات تخفض قليلا من ضغط الدم و البعض الآخر له تأثير مساو تقريبا لتأثير عقار البروبرانولول المستخدم علاجيا. ومن جانب اخر فقد تم دراسة التأثيرالمسمم ليرقات الجمبرى لعدد ثمانية وعشرين مركبا جديدا وثبت أن لثلاثة من هذه المركبات تأثير مقبول كذلك تم اختبار درجة السمية الحادة (LD_{50}) لعدد أربعة مركبات على الفئران الصغيرة البيضاء (بالحقن فى الغشاء البريتونى) ووجد أنها تتراوح بين ١٠ ٢٠ مجم / كجم.

The present work involves the synthesis of 1-alkyl-4-aryl-6-hydroxyperhydro-1,4-diazepine-2,3-diones through the reaction of epichlorohydrin with some selected arylamine followed by the reaction of the formed intermediates with the corresponding cyclohexyl, alkyl, or aralkyl amines. The resulting N,N'-disubstituted-1,3-diamino-2-propanols were cyclized with diethyl oxalate to afford the target compounds. The structures of the

Received in 8/4/2006 & Accepted in 19/7/2006

*Corresponding author E-mail address: mostafa1705@yahoo.com

obtained compounds were verified by spectral and elemental methods of microanalysis. Fifteen of the final compounds were subjected to preliminary pharmacological screening as regards their anticonvulsant activity. In addition, evaluation of the hypotensive activity of twenty two compounds was performed. Most of the tested compounds gave 100% protection against pentylenetetrazole-induced convulsions with a faster onset of action (15 min) than diazepam (30 min). On the other hand, most of the tested compounds gave mild to ~ 50-80% reduction in blood pressure in comparison to that of propranolol. Moreover, the cytotoxic activity of twenty eight final compounds was determined and only three of them elicited mild cytotoxic effects. Also, the median lethal dose (LD_{50}) of four target representative compounds was determined and was found to range between 10-20 mg/kg (i.p.).

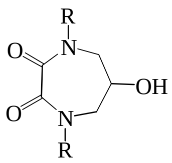
INTRODUCTION

Benzodiazepines[1] represent an important class possessing psycho-therapeutic activities with antianxiety, sedative, and anticonvulsant activities.[2-5] The fused benzene ring is essential for their CNS activity, since it fits to the lipophilic part of their binding sites.[6&7]

Also, it was reported that replacement of the benzene ring of benzodiazepines by a heterocyclic ring retained most of the CNS depressant activities of the parent compounds but with an increased toxicity.[8]

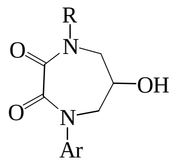
Moreover, other 1,4-diazepines lacking the benzene ring of the parent benzodiazepines were found to be inactive as CNS depressant compounds, however they exihibited anti-inflammatory,[9] neurotropic,[10&11] $5HT_3$ receptor antagonist[12&13] activities. Besides, it has been reported that some 1,4-diazepinones exhibited HIV-1 protease inhibition.[14&15]

In a previous work for this laboratory, the synthesis and biological activities of some symmetrically substituted 1,4-diazepines with the general structure **1,** (Figure 1) was reported.[16] It was found that those compounds exclusively exhibited a 100% protection against pentylenetetrazole-induced convulsions at a dose of 2.8 mmol/kg in comparison to diazepam. On decreasing the dose to 2.1 mmol/kg only four compounds (**1i-l)** exhibited 30-60% of the anticonvulsant activity of diazepam. In addition, most of compounds **1** gave mild to comparable reduction in blood pressure compared to that produced by propranolol.



1

R = C_2H_5 , C_3H_7 , i-C_3H_7 , n-C_4H_9 , i-C_4H_9 , t-C_4H_9 , n-C_5H_{11}, n-C_6H_{13} , cyclo-C_6H_{11} , $C_6H_5CH_2$, $C_6H_5CH_2CH_2$, and $C_6H_5CH(CH_3)$.



(6-9)

Ar = C_6H_5, *p*-$C_6H_4CH_3$, *p*-C_6H_4Br, *p*-C_6H_4Cl.

Figure 1

Taking these findings in consideration, the present work was planned to extend the study by synthesis of the target compounds **[6-9]** which incorporate the R-substituents at N_1 (Table I) and different aryl substituents at N_4 of the diazepindione ring in order to investigate the effect of such aryl substituents on the pharmacological activities previously reported for the symmetrical substituted compounds **(1)**.[16]

EXPERIMENTAL

Chemistry

Materials and equipment

Melting points were determined using an electrothermal melting point apparatus (Stuart Scientific, SMP1, UK) and all are uncorrected. Precoated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck) were used for monitoring the progress of reactions. Visualization of spots was effected by ultraviolet lamp (model CM-10, USA) and/or iodine stain. Silica gel (60-120 mesh, Prolabo) was used for column chromatography (gradient elution) using chloroform/ methanol as a mobile phase unless otherwise specified.

IR spectra (KBr discs or neat samples) were recorded on a Shimadzu 200-91527 spectrophoto-meter and ^{1}H-NMR spectra were scanned on a Varian EM-360 L NMR spectrophotometer (60 MHz) at the Faculty of Pharmacy, Assiut University. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using $CDCl_3$ as a solvent unless otherwise specified, and deuterium oxide was used for assigning of the exchangeable protons. The mass spectra were run on a JEOL JMS 600 mass spectrometer at the units of microanalysis of Assiut and Cairo Universities. Elemental microanalyses were performed on a Perkin Elmer 240 elemental analyzer at unit of microanalysis of Assiut University.

Synthesis of *N,N′*-alkylaryl-1,3-diamino-2-propanols (No. 2-5)[17&18]

A mixture of epichlorohydrin (9.20 mL, 0.03 mol) and the appropriate arylamine (0.031 mol) in ethanol (100 mL) was heated under reflux for 2 h. The selected aliphatic amine (0.032 mol) was then added, and the reaction mixture was refluxed for further 24 h. Ethanol was evaporated under vacuum, the residue was added to 10% aqueous sodium carbonate (70 mL) and then extracted with chloroform (50 mL). The chloroform extract was washed with brine then with water, dried over anhydrous sodium sulfate, filtered and evaporated. The pale yellow liquids obtained were purified by column chromatography using ethyl acetate/hexane as a mobile phase. Data for the compounds prepared by this procedure are listed in Table I.

Synthesis of 1-alkyl-4-aryl-6-hydroxyperhydro-1,4-diazepine-2,3-diones (No. 6-9)

To a well stirred solution of the appropriate *N*,*N'*-alkylaryl-1,3-diamino-2-propanols (No. 2-5) (0.073 mol) in dry ether (50 mL); diethyl oxalate (9.9 mL, 0.073 mol) was added. The reaction mixture was further stirred at the ambient temperature for 24 h, concentrated, and the separated solid residue was filtered. The products were crystallized from the appropriate solvents. Data for the prepared compounds are listed in Tables II and III.

Pharmacological screening

All screened compounds and reference drugs were tested as solutions or suspensions in 5% w/v aqueous solution of sodium carboxymethylcellulose (NaCMC). Also, NaCMC solution was used as a negative control all over these tests.

I- Anticonvulsant activity

Mice were housed in separate cages, each containing six animals, in temperature-controlled rooms at 25°. Animals were allowed a free access to food, water, and maintained at 12 h light/dark cycle. The work was conducted in accordance with the internationally accepted principles for laboratory animal's use and care as found in the European Community Guidelines.[19]

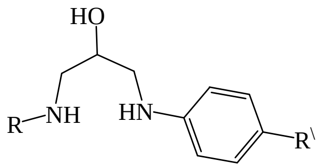
Materials

Male adult albino mice were obtained from the animal house of Faculty of Medicine, Assiut University, Egypt. Diazepam (Valinil® 5 mg Tablets, Nile company, Egypt) pentylenetetrazole (Sigma, USA), other chemicals and solvents were obtained from the local market.

Method

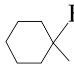
Fifteen new compounds namely **[**No. **6c-f, 7f-i, 8b-e** and **9f-h]** were screened for their anticonvulsant activity by following the anticonvulsant drug development (ADD) program protocol.[20&21] Test compounds or diazepam solutions were injected (*i.p.*) (1.40 mmol/kg) to three groups of mice (each of six animals); fifteen minutes later, pentylenetetrazole 0.3 mL (1.50 mg) of an aqueous solution (0.5%) was administered (*i.p.*). The elapsed time before the onset of clonic convulsions, tonic convulsions, and/or death was recorded, Table IV.

Table I: Yields and ^{1}H-NMR data of *N*,*N*′-alkylaryl-1,3-diamino-2-propanols (No. **2-5**).



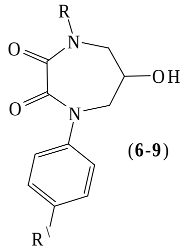
No.	R	R\	Yield %	^{1}H-NMR ($CDCl_3$, δ ppm)*
2a	$CH_3(CH_2)_2$	H	50	1.00 (t, 3H, CH_3); 1.20-2.00(m, 2H, $C\underline{H}_2CH_3$); 3.00-4.20 (m, 6H, $\underline{H}_2CNH$ $C\underline{H}_2CHOHC\underline{H}_2$); 4.26-4.90 (m, 4H, 2N$\underline{H}$, and $C\underline{H}O\underline{H}$); and 7.50 (s, 5H, $C_6\underline{H}_5$).
2b	$(CH_3)_2CH$	H	62	0.95 (d, 6H, 2CH3); 2.00-4.00 (m, 9H, $C\underline{H}N\underline{H}C\underline{H}_2$ $C\underline{H}O\underline{H}C\underline{H}_2N\underline{H}$); and 6.90 (m, 5H, C6H5).
2c	C_6H_{11}	H	70	0.80-2.55 (m, 17H, c-hexyl, and NH $C\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2$); 3.20 (s, 2H, 2NH); and 7.80 (s, 5H, $C_6\underline{H}_5$).
2d	$C_6H_5CH_2$	H	71	3.40 (brs, 6H, $HNC\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2$); 4.50 (d, 2H, $CH_2C_6H_5$); 5.20 (s, 2H, 2NH); and 7.35 (s, 10H, $2C_6\underline{H}_5$).
2e	$C_6H_5(CH_2)_2$	H	70	2.00-4.20 (m, 12H, $C\underline{H}_2C\underline{H}_2N\underline{H}C\underline{H}_2C\underline{H}O\underline{H}$ $C\underline{H}_2N\underline{H}$); and 6.90-7.40 (m, 10H, $2C_6\underline{H}_5$).
2f	$C_6H_5CH(CH_3)$	H	72	1.20 (d, 3H, $CHC\underline{H}_3$); 2.00-4.30 (m, 8H, $N\underline{H}C\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2N\underline{H}$); 6.00 (q, 1H, $C\underline{H}CH_3$); and 6.95-7.80 (m, 10H, $2C_6\underline{H}_5$).
3a	CH_3CH_2	CH_3	60	1.20 (t, 3H, $CH_2C\underline{H}_3$); 2.30 (s, 3H,CH_3); 3.00-4.40 (m, 8H, $CH_3C\underline{H}_2NHC\underline{H}_2C\underline{H}O\underline{H}$ $C\underline{H}_2NH$); 4.70 (s, 2H, 2NH); and 7.00 (dd, 4H, C_6H_4).
3b	$CH_3(CH_2)_2$	CH_3	65	0.83 (t, 3H, $CH_2C\underline{H}_3$); 1.43 (m, 2H, $H_2CC\underline{H}_2CH_3$); 2.20 (s, 3H, CH_3); 2.50-4.43 (m, 8H, $C\underline{H}_2NHC\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2$); 4.90 (s, 2H, 2NH); and 6.86 (dd, 4H, C_6H_4).
3c	$(CH_3)_2CH$	CH_3	62	0.90 (d, 6H, $2CH_3$); 2.10 (s, 3H, CH_3); 2.60-4.63 (m, 6H, $C\underline{H}NHC\underline{H}_2C\underline{H}OH$ $C\underline{H}_2NH$); 5.40 (s_{br}, 3H, 2NH,OH); and 6.36-7.46 (m, 4H, C_6H_4).
3d	$(CH_3)_2CHCH_2$	CH_3	70	0.95 (d, 6H, $2C\underline{H}_3$); 2.30 (s, 3H, CH_3); 2.45-4.20 (m, 9H, $C\underline{H}C\underline{H}_2NHC\underline{H}_2C\underline{H}O\underline{H}$ $C\underline{H}_2NH$); 4.30 (s, 2H, 2NH); and 6.80 (dd, 4H, C_6H_4).
3e	$(CH_3)_3C$	CH_3	65	1.30 (s, 9H, *t*-butyl); 2.30 (s, 3H, CH_3); 2.60-4.60 (m, 6H, $HNC\underline{H}_2C\underline{H}O\underline{H}$ $C\underline{H}_2NH$); 5.30 (s, 2H, 2NH); and 7.00 (dd, 4H, C_6H_4).
3f	C_6H_{11}	CH_3	70	0.73-3.30 (m, 17H, c-hexyl, $C\underline{H}_2C\underline{H}$ $O\underline{H}C\underline{H}_2$); 3.43 (s, 3H, CH_3); 4.95 (s, 2H, 2NH); and 7.00 (dd, 4H, C_6H_4).
3g	$C_6H_5CH_2$	CH_3	75	2.30 (s, 3H, CH_3); 2.50-4.50 (m, 8H, $C\underline{H}_2NHC\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2NH$); 4.65 (s, 2H, 2NH); 7.00 (dd, 4H, C_6H_4); and 7.42 (s, 5H, C_6H_5).

Table I: Continued.

No.	R	R\	Yield %	^{1}H-NMR ($CDCl_3$, δ ppm)*
3h	$C_6H_5(CH_2)_2$	CH_3	73	2.10 (s, 3H, CH_3); 2.40-3.30 (m, 10H, $CH_2CH_2NHCH_2CHOHCH_2NH$), 4.60 (s, 2H, 2NH); 6.80 (dd, 4H, C_6H_4); and 7.40 (s, 5H, C_6H_5).
3i	$C_6H_5CH(CH_3)$	CH_3	70	1.43 (d, 3H, $CHCH_3$); 2.20 (s, 3H, CH_3); 2.50-4.40 (m, 7H, $CHNHCH_2CHOH$ CH_2NH); 4.80 (s, 2H, 2NH); 7.00 (dd, 4H, C_6H_4); and 7.40 (s, 5H, C_6H_5).
4a	CH_3CH_2	Br	60	1.40 (t, 3H, CH_2CH_3); 3.00-4.80 (m, 10H, $CH_2NHCH_2CHOHCH_2NH$); and 7.80 (dd, 4H, C_6H_4).
4b	C_6H_{11}	Br	70	0.73-2.20 (m, 10H, $(CH_2)_5$); 2.30-4.23 (m, 7H, $CH_aNHCH_2CHOHCH_2NH$); 4.90 (s, 2H, 2NH); and 7.00 (dd, 4H, C_6H_4).
4c	$C_6H_5CH_2$	Br	75	3.00-4.00 (m, 8H, $NHCH_2CHOHCH_2NH$); 4.80 (dd, 2H, $CH_2C_6H_5$); and 7.45 (s_{br}, 9H, C_6H_5, C_6H_4).
4d	$C_6H_5(CH_2)_2$	Br	70	2.80-4.45(m, 8H, $CH_2CH_2NHCH_2CHOHCH_2$ NH); 4.80 (s, 4H, 2NH, CHOH); 6.76 (dd, 4H, C_6H_4); and 7.50 (s_{br}, 5H, C_6H_5).
4e	C_6H_5-$CH(CH_3)$	Br	70	1.53 (d, 3H, $CHCH_3$); 2.10-4.55 (m, 7H, $CHNHCH_2CHOHCH_2NH$); 5.50 (s, 2H, 2NH); and 6.40-7.45 (m, 9H, C_6H_5, C_6H_4).
5a	CH_3CH_2	Cl	55	1.40 (t, 3H, CH_2CH_3); 3.00-4.00 (m, 7H, $CH_2NHCH_2CHOHCH_2$); 4.50 (m, 1H, CHOH); 5.20 (s, 2H, 2NH); and 7.50 (dd, 4H, C_6H_4).
5b	$CH_3(CH_2)_2$	Cl	56	0.80 (t, 3H, CH_3); 1.46 (m, 2H, CH_2CH_3); 2.00-4.30 (m, 6H, CH_2NH $CH_2CHOHCH_2$); 4.80 (s, 4H, 2NH, CHOH); and 7.00 (dd, 4H, C_6H_4).
5c	$(CH_3)_2CH$	Cl	60	0.90 (d, 6H, $2CH_3$); 2.00-4.00 (m, 7H, $CHNHCH_2CHOHCH_2NH$); 4.30 (s, 2H, 2NH); and 6.80 (dd, 4H, C_6H_4).
5d	$(CH_3)_2CHCH_2$	Cl	50	1.96 (d, 6H, $2CH_3$); 2.20-3.90 (m, 9H, CH CH_2 $NHCH_2CHOHCH_2NH$); 4.10 (s, 2H, 2NH); and 7.00 (dd, 4H, C_6H_4).
5e	$(CH_3)_3C$	Cl	52	1.40 (s, 9H, *t*-butyl); 2.70-5.30 (m, 8H, $NHCH_2CHOHCH_2NH$); and 7.00 (dd, 4H, C_6H_4).
5f	 H_a	Cl	65	1.50 (m, 10H, $(CH_2)_5$); 2.20-4.40 (m, 6H, $CH_aNHCH_2CHOHCH_2NH$); 5.20 (s_{br}, 3H, 2NH,OH); and 7.00 (dd, 4H,C_6H_4).
5g	$C_6H_5CH_2$	Cl	60	2.40-4.00 (m, 10H, CH_2NHCH_2CH $OHCH_2NH$); and 7.35 (s, 9H, C_6H_5,C_6H_4).
5h	$C_6H_5(CH_2)_2$	Cl	68	2.35-4.85 (m, 12H, $CH_2CH_2NHCH_2CH$ $OHCH_2NH$); 6.60 (dd, 4H, C_6H_4); and 7.35 (s, 5H, C_6H_5).
5i	C_6H_5-$CH(CH_3)$	Cl	70	1.30 (d, 3H, $CHCH_3$); 2.20-4.90 (m, 5H, CH $NHCH_2CHOHCH_2$ NH); 5.49 (s_{br}, 4H, 2NH, CHOH); and 6.70-7.50 (m, 9H, C_6H_5, C_6H_4).

* Protons of OH & NH groups are exchangeable by D_2O.

Table II: Physical and microanalytical data of 1-alkyl-4-phenyl-6-hydroxyperhydro-1,4-diazepine-2,3-diones (**6-9**).



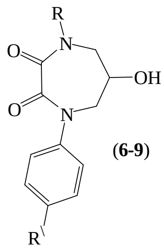
(**6-9**)

No.	R	R\	Yield %	M.p°*	Molecular formula (M.wt.)	Microanalyses			ClogP[26]
							Calcd. %	Found %	
6a	$CH_3(CH_2)_2$	H	50	208-10	$C_{14}H_{18}N_2O_3$ ½ H_2O (271.33)	C H N	61.90 7.06 10.32	62.14 6.83 10.39	0.82
6b	$(CH_3)_2CH$	H	70	198-200	$C_{14}H_{18}N_2O_3$ ½ H_2O (271.33)	C H N	61.90 7.06 10.32	62.14 6.83 10.39	0.65
6c	C_6H_{11}	H	80	210-12	$C_{17}H_{22}N_2O_3$ (302.37)	C H N	67.53 7.33 9.26	68.33 7.80 8.85	1.54
6d	C_6H_5-CH_2	H	60	>300	$C_{18}H_{18}N_2O_3$ $2H_2O$ (346.38)	C H N	62.42 6.40 8.08	61.73 6.93 8.78	1.73
6e	C_6H_5-$(CH_2)_2$	H	70	194-6	$C_{19}H_{20}N_2O_3$ (324.37)	C H N	70.35 6.21 8.64	69.83 6.42 7.90	2.01
6f	C_6H_5-CH-(CH_3)	H	75	190-2	$C_{19}H_{20}N_2O_3$ (324.39)	C H N	70.35 6.21 8.64	69.68 7.03 7.92	2.41
7a	CH_3CH_2	CH_3	56	170-5	$C_{14}H_{18}N_2O_3$ ¼ H_2O (266.80)	C H N	63.02 6.98 10.49	63.09 6.62 10.55	0.82
7b	CH_3-$(CH_2)_2$	CH_3	60	188-90	$C_{15}H_{20}N_2O_3$ ¼ H_2O (280.35)	C H N	64.26 7.37 9.99	63.86 6.90 9.94	1.31
7c	$(CH_3)_2$-CH	CH_3	55	190-2	$C_{15}H_{20}N_2O_3$ ¼ H_2O (280.35)	C H N	64.26 7.37 9.99	63.75 7.03 9.64	1.14
7d	$(CH_3)_2$-$CHCH_2$	CH_3	70	195-7	$C_{16}H_{22}N_2O_3$ ¼ H_2O (294.86)	C H N	65.17 7.96 9.50	65.25 7.29 9.47	1.71
7e	$(CH_3)_3C$	CH_3	78	200-2	$C_{16}H_{22}N_2O_3$ (290.36)	C H N	66.18 7.64 9.65	65.50 7.27 9.51	1.36
7f	C_6H_{11}	CH_3	60	210-2	$C_{18}H_{24}N_2O_3$ ½ H_2O (325.39)	C H N	66.44 7.74 8.61	66.21 7.21 8.58	2.03
7g	C_6H_5-CH_2	CH_3	60	217-9	$C_{19}H_{20}N_2O_3$ ¼ H_2O (328.87)	C H N	69.39 6.28 8.50	69.21 6.05 8.47	2.22

Table II: Continued.

No.	R	R\	Yield %	M.p°*	Molecular formula (M.wt.)	Microanalyses	Calcd. %	Found %	ClogP[26]
7h	C_6H_5-$(CH_2)_2$	CH_3	80	≥ 300	$C_{20}H_{22}N_2O_3$ ¼ H_2O (342.90)	C H N	70.05 6.60 8.16	70.18 6.49 7.81	2.50
7i	C_6H_5CH-(CH_3)	CH_3	80	225-30	$C_{20}H_{22}N_2O_3$ (338.40)	C H N	70.99 6.55 8.28	71.49 6.35 8.27	2.54
8a	CH_3CH_2	Br	60	140-2	$C_{13}H_{15}BrN_2O_3$ (327.17)	C H N	47.72 4.62 8.56	47.35 4.82 8.68	1.16
8b	C_6H_{11}	Br	82	180-5	$C_{17}H_{21}BrN_2O_3$ (381.26)	C H N	53.55 5.55 7.35	52.51 5.28 7.42	2.37
8c	C_6H_5-CH_2	Br	73	190-2	$C_{18}H_{17}BrN_2O_3$ (389.24)	C H N	55.54 4.40 7.20	55.86 4.83 7.52	2.56
8d	C_6H_5-$(CH_2)_2$	Br	65	>300	$C_{19}H_{19}BrN_2O_3$ (403.06)	N	6.95	7.01	2.84
8e	C_6H_5CH-(CH_3)	Br	72	198-200	$C_{19}H_{19}BrN_2O_3$ (403.27)	C H N	56.59 4.75 6.95	56.85 4.22 7.34	2.88
9a	CH_3CH_2	Cl	65	139-40	$C_{13}H_{15}ClN_2O_3$ (282.72)	C H N	55.23 5.35 9.91	55.85 5.00 9.76	0.89
9b	CH_3-$(CH_2)_2$	Cl	60	164-6	$C_{14}H_{17}ClN_2O_3$ (296.75)	C H N	56.66 5.77 9.44	56.06 5.99 9.34	1.38
9c	$(CH_3)_2$-CH	Cl	65	188-90	$C_{14}H_{17}ClN_2O_3$ ½ H_2O (305.77)	C H N	54.99 5.93 9.16	54.41 5.50 9.10	1.21
9d	$(CH_3)_3C$	Cl	67	194-6	$C_{15}H_{19}ClN_2O_3$ ½ H_2O (319.78)	C H N	56.34 6.30 8.76	56.50 5.85 8.83	1.43
9e	C_6H_{11}	Cl	80	126-8	$C_{17}H_{21}ClN_2O_3$ ½ H_2O (345.81)	C H N	59.04 6.41 8.10	58.18 5.98 8.72	2.10
9f	C_6H_5-CH_2	Cl	80	189-92	$C_{18}H_{17}ClN_2O_3$ (344.79)	C H N	62.78 4.97 8.12	62.67 4.95 8.48	2.29
9g	C_6H_5-$(CH_2)_2$	Cl	90	Decomp. with charring	$C_{19}H_{19}ClN_2O_3$ (358.82)	C H N	63.60 5.34 7.81	64.25 4.58 7.93	2.57
9h	C_6H_5CH-(CH_3)	Cl	90	228-30	$C_{19}H_{19}ClN_2O_3$ (358.82)	N	7.81	8.21	2.61

Table III: ^{1}H-NMR data of compounds **(6-9)**.



(**6-9**)

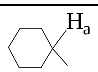
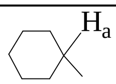
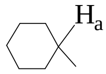
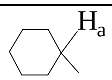
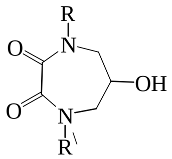
No.	R	R\	^{1}H-NMR ($CDCl_3$, δ ppm)*
6a	$CH_3(CH_2)_2$	H	0.90 (t, 3H, CH_3); 1.20-2.00 (m, 2H, $H_2CC\underline{H}_2CH_3$); 3.00-4.20 (m, 6H, $C\underline{H}_2NC\underline{H}_2CHOHC\underline{H}_2N$); 4.53 ($s_{br}$, 2H, $C\underline{H}\,O\underline{H}$); and 7.50 (5H, s_{br}, $C_6\underline{H}_5$).
6b	$(CH_3)_2CH$	H	1.20 (d, 6H, $2C\underline{H}_3$); 3.00-4.10 (m, 5H, $C\underline{H}NC\underline{H}_2CH\ OHC\underline{H}_2N$); 4.15-5.13 (m, 2H, $C\underline{H}O\underline{H}$); and 7.34-8.10 (m, 5H, $C_6\underline{H}_5$).
6c		H	0.86-2.26 (m, 10H, $(CH_2)_5$); 2.50-4.33 (m, 5H, $C\underline{H}_aN\ C\underline{H}_2CHOHC\underline{H}_2$); 4.86 ($s_{br}$, 2H, $C\underline{H}O\underline{H}$); and 6.60-7.73 (m, 5H, $C_6\underline{H}_5$).
6d	$C_6H_5CH_2$	H	3.40 (s, 6H, $NC\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2N$); 4.50 (d, 2H, $C\underline{H}_2C_6H_5$); and 7.35 (s, 10H, $2C_6\underline{H}_5$).
6e	$C_6H_5(CH_2)_2$	H	2.40-4.20 (m, 9H, $C\underline{H}_2C\underline{H}_2NC\underline{H}_2C\underline{H}OHC\underline{H}_2$); 5.45 (hump, 1H, $O\underline{H}$); and 7.50 (s, 10H, $2C_6H_5$).
6f	C_6H_5-$CH(CH_3)$	H	1.50 (d, 3H, $CHC\underline{H}_3$); 2.32-4.46 (m, 6H, $NC\underline{H}_2C\underline{H}\ O\underline{H}C\underline{H}_2N$); 5.80 (q, 1H, $C\underline{H}CH_3$); and 7.60 (s, 10H, $2C_6H_5$).
7a	CH_3CH_2	CH_3	1.15 (t, 3H, $CH_2C\underline{H}_3$); 2.35 (s, 3H, $C_6H_4C\underline{H}_3$); 3.10-4.10 (m, 6H, $CH_3C\underline{H}_2NC\underline{H}_2CHOHC\underline{H}_2N$); 4.20-4.90 (m, 2H, $C\underline{H}O\underline{H}$); and 7.33 (s, 4H, $C_6\underline{H}_4$).
7b	$CH_3(CH_2)_2$	CH_3	0.93 (t, 3H, $CH_2C\underline{H}_3$); 1.16-2.00 (m, 2H, $C\underline{H}_2CH_3$); 2.45 (s, 3H, $C_6H_5C\underline{H}_3$); 3.10-4.00 (m, 6H, $C\underline{H}_2NC\underline{H}_2C\underline{H}_2$); 4.23-4.76 (m, 2H, $C\underline{H}O\underline{H}$); 7.33 (s, 4H, $C_6\underline{H}_4$).
7c	$(CH_3)_2CH$	CH_3	1.10 (d, 6H, $(C\underline{H}_3)_2CH$); 2.26 (s, 3H, $C_6H_5C\underline{H}_3$); 3.00-5.00 (m, 7H, $C\underline{H}NC\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2N$); and 7.26 (dd, 4H, $C_6\underline{H}_4$).
7d	$(CH_3)_2CHCH_2$	CH_3	0.90 (d, 6H, $CH(C\underline{H}_3)_2$); 2.33 (s, 3H, $C_6H_5C\underline{H}_3$), 3.00-5.00 (m, 9H, $C\underline{H}C\underline{H}_2NC\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2N$); and 7.30 (s, 4H, $C_6\underline{H}_4$).
7e	$(CH_3)_3C$	CH_3	1.50 (s, 9H, *t*-butyl); 2.40 (s, 3H, $C_6H_4C\underline{H}_3$); 3.20-4.00 (m, 5H, $NC\underline{H}_2CHO\underline{H}C\underline{H}_2N$); 4.10-4.55 (m, 1H, $C\underline{H}OH$); and 7.50 (s, 4H, $C_6\underline{H}_4$).
7f		CH_3	0.73-2.10 (m, 10H, $(CH_2)_5$); 2.40 (s, 3H, $C_6H_4C\underline{H}_3$); 2.93-4.66 (m, 6H, $C\underline{H}_aNC\underline{H}_2C\underline{H}OHC\underline{H}_2$); 5.63 ($s_{br}$, 1H, $O\underline{H}$); and 7.44 (s, 4H, $C_6\underline{H}_4$).
7g	$C_6H_5CH_2$	CH_3	2.50 (s, 3H, $C_6H_4C\underline{H}_3$); 3.00-4.75 (m, 6H, $NC\underline{H}_2C\underline{H}O\underline{H}\ C\underline{H}_2N$); 5.20 (d, 2H, $C\underline{H}_2C_6H_5$); and 7.45 (s_{br}, 9H, C_6H_5, C_6H_4).
7h	$C_6H_5(CH_2)_2$	CH_3	2.70-4.40 (m, 13H, $C\underline{H}_2C\underline{H}_2NC\underline{H}_2C\underline{H}O\underline{H}C\underline{H}_2NC_6H_4\ C\underline{H}_3$); and 7.50 ($s_{br}$, 9H, C_6H_5, C_6H_4).

Table III: Continued.

No.	R	R\	^{1}H-NMR ($CDCl_3$, δ ppm)*
7i	$C_6H_5CH(CH_3)$	CH_3	1.80 (d, 3H, CH_3); 2.40 (s, 3H, $C_6H_4CH_3$); 2.26-5.00 (m, 7H, $CHNCH_2CHOHCH_2N$); 6.76 (d, 2H, Ar-H); and 7.13-8.10 (m, 7H, Ar-H).
8a	CH_3CH_2	Br	1.40 (t, 3H, CH_2CH_3); 3.00-4.80 (m, 8H, $CH_3CH_2NCH_2CH\ OHCH_2N$); and 7.80 (s, 4H, C_6H_4).
8b		Br	1.00-2.32 (m, 10H, $(CH_2)_5$); 3.00-4.83 (m, 7H, $CH_aNCH_2\ CHOHCH_2N$); 6.50-7.76 (dd, 4H, C_6H_4).
8c	$C_6H_5CH_2$	Br	3.00-4.75 (m, 6H, $NCH_2CHOHCH_2N$); 5.20 (d, 2H, $CH_2C_6H_5$); and 7.45 (s_{br}, 9H, C_6H_5, C_6H_4).
8d	$C_6H_5(CH_2)_2$	Br	2.76-4.60 (m, 10H, $CH_2CH_2NCH_2CHOHCH_2N$); and 7.35 (s_{br}, 9H, C_6H_5, C_6H_4).
8e	$C_6H_5CH(CH_3)$	Br	1.80 (d, 3H, CH_3); 2.26-5.00 (m, 7H, $CHNCH_2\ CHOHCH_2N$); 6.76 (d, 2H, Ar-H); and 7.13-8.10 (m, 7H, Ar-H).
9a	CH_3CH_2	Cl	1.40 (t, 3H, CH_2CH_3); 3.00-4.65 (m, 8H, $H_3CCH_2NCH_2CH\ OHCH_2N$); and 7.33-8.00 (dd, 4H, C_6H_4).
9b	$CH_3(CH_2)_2$	Cl	1.00 (3H, t, CH_2CH_3); 1.20-2.00 (2H, m, CH_2CH_3); 2.10-3.83 (8H, m, $CH_3CH_2CH_2NCH_2CHOHCH_2$); and 7.96 (4H, dd, C_6H_4).
9c	$(CH_3)_2CH$	Cl	1.20 (d, 6H, $2CH_3$); 3.10-3.96 (m, 4H, $NCH_2CHOH\ CH_2N$); 4.10-4.50 (m, 1H, $CHOH$); 4.60-5.10 (m, 1H, $CH(CH_3)_2$); 5.33 (s_{br}, 1H, $CHOH$); and 7.56 (dd, 4H, C_6H_4).
9d	$(CH_3)_3C$	Cl	1.50 (s, 9H, *t*-butyl); 3.33-4.00 (m, 4H, $NCH_2CHOHCH_2N$); 4.55 (s_{br}, 1H, $CHOH$); 5.75 (hump, 1H, OH); and 7.66 (s, 4H, C_6H_4).
9e		Cl	0.73-2.53 (m, 10H, $(CH_2)_5$); 2.80-4.36 (m, 6H, $CH_aNCH_2CH\ OHCH_2$); 5.00 (hump, 1H, OH); and 6.90-7.30 (m, 4H, C_6H_4).
9f	$C_6H_5CH_2$	Cl	3.33 (s_{br}, 6H, $NCH_2CHOHCH_2N$); 4.40 (d, 2H, $CH_2C_6H_5$); and 7.26 (s_{br}, 9H, C_6H_5, C_6H_4).
9g	$C_6H_5(CH_2)_2$	Cl	2.66-4.76 (m, 10H, $CH_2CH_2NCH_2CHOHCH_2N$); and 7.40 (s_{br}, 9H, C_6H_5, C_6H_4).
9h	$C_6H_5CH(CH_3)$	Cl	1.50 (d, 3H, $CHCH_3$); 3.35 (s, 6H, $NCH_2CHOHCH_2N$); 5.16 (q, 1H, $CHCH_3$); 7.66 (s_{br}, 9H, C_6H_5, C_6H_4).

Compounds **6b, 6d, 6e, 7c, 7g, 7h, 7i, 8c, 8d,** and **8e** were measured in DMSO-d_6 and proton of OH is exchangeable with D_2O.

Table IV: %Protection against pentylenetetrazole-induced convulsions of compounds (**6-9**) in comparison to the corresponding symmetrical compounds (**1**)[16] at 1.4 mmol/kg.



No.	R	R\	C logP	% protection
1i	*c*-hexyl	*c*-hexyl	1.43	0%
6c	*c*-hexyl	C_6H_5	1.54	50%
7f	*c*-hexyl	*p*-$C_6H_5CH_3$	2.03	100%
8b	*c*-hexyl	*p*-C_6H_5Br	2.37	75%
9e	*c*-hexyl	*p*-C_6H_5Cl	2.10	75%
1j	benzyl	benzyl	1.80	0%
6d	benzyl	C_6H_5	1.73	75%
7g	benzyl	*p*-$C_6H_5CH_3$	2.22	100%
8c	benzyl	*p*-C_6H_5Br	2.56	75%
9f	benzyl	*p*-C_6H_5Cl	2.29	75%
1k	β-phenethyl	β-phenethyl	2.36	30%
6e	β-phenethyl	C_6H_5	2.01	100%
7h	β-phenethyl	*p*-$C_6H_5CH_3$	2.50	100%
8d	β-phenethyl	*p*-C_6H_5Br	2.84	100%
9g	β-phenethyl	*p*-C_6H_5Cl	2.57	100%
1l	α-phenethyl	α-phenethyl	2.44	30%
6f	α-phenethyl	C_6H_5	2.41	100%
7i	α-phenethyl	*p*-$C_6H_5CH_3$	2.54	100%
8e	α-phenethyl	*p*-C_6H_5Br	2.88	100%
9h	α-phenethyl	*p*-C_6H_5Cl	2.61	100%
Diazepam			2.98	100%

II- Hypotensive activity

Twenty two of the newly synthesized 1,4-perhydrodiazepines namely (**6b-f, 7c-i, 8b-e,** and **9c-h**) were tested for their effect on the blood pressure of anaesthetized normotensive rabbits at 28.9 mmol/kg dose level as solutions or suspensions in 5% NaCMC (w/v) in comparison to propranolol as a reference drug.

Materials

Two rabbits were housed per cage in temperature-controlled rooms (25°). Animals were allowed a free access to food, water and maintained at 12 h light/dark cycle. The work was conducted in accordance with the internationally accepted principles for laboratory animal's use and care as found in the European Community Guidelines.[19]

1) Adult healthy male rabbits (6 months old, 1.25-1.50 Kg) were available from the animal house of Assiut University.
2) Urethane (Aldrich chemical company, USA) 25% w/v aqueous solution was used as an anaesthetic agent, Heparin (Amoun, Egypt) 12000 I.U./ 0.5 mL (*i.v.*) was used as an anticoagulant.
3) Pure standard propranolol hydrochloride inderal® (Astra pharmaceuticals, Astra and Zeneca, UK and Sweden) 0.1% *i.v.* injection, was used as a reference hypotensive agent. The same molar concentration of the tested compounds (28.9 mmol/kg) was used.

Method

A group of three rabbits was used for testing each compound. Animals were prepared for the experiment by being first anaesthetized with an *i.p.* injection of urethane in a dose of 1.6 g/kg (6 mL).[22] Arterial blood pressure was recorded via the carotid artery which was cannulated to Burden blood pressure transducer. Heparin (0.5 mL) was placed in the tip of the cannula to prevent blood clotting. Blood pressure was recorded by using an oscillograph 400 MD 2C Bioscience (Kent, U.K.). The transducer was calibrated and the tested compounds were injected *i.p.* Blood pressure was recorded before and after administration of the tested compounds over a period of 4 h and results are recorded in Tables V&VI.

III- Cytotoxic activity

Cytotoxicity of twenty eight final compounds was done using the Brine Shrimp Lethality Bioassay method.[23] Stock solutions of the tested compounds (10 mg in 2 mL DMSO) representing a 5000 ppm/mL was prepared. In 10 mL disposable glass scintillation Wheaton vials containing 1 mL saline and 10 napulii; 25, 50, and 100 μL of the stock solution were added. Saline was added to complete the volume of each vial to 5 mL. The vials were put in a thermostatically controlled water bath at 24° in a constant cold light source for 24 h. The number of surviving larvae per vial was counted, each test was done in triplicate, and results obtained, LC_{50}, and LC_{90} were calculated using the probit analysis.[24]

IV- Acute toxicity (LD_{50})

The median lethal doses (LD_{50}) of compounds **6f, 7g, 8e**, and **9h** were determined in mice by the graphical Litchfield method[25]. Groups of adult albino mice each of six animals (25-30g) were injected *i.p.* with graded doses of the tested compounds. The percentage of mortality in each group of the animals was determined 48 h after injection. Computation of LD_{50} was processed by the graphical method.[25]

Table V: Hypotensive activity of compounds **(6-9)** and propranolol.

Time (min.)	% Mean decrease in blood pressure[a]						
	6b	**6c**	**6d**	**6e**	**6f**	Propranolol	5%NaCMC
D[b]	5±0.63**	5±0.71**	3±0.45**	3.0±0.42**	2.4±0.46**	13.5±1.30	2±0.81
1	7±1.13*	4±0.52**	3±0.46**	0.9±0.10**	2±0.39**	19.0±1.82	0.5±0.09
3	10±1.25**	4±0.54**	2±0.27**	0.9±0.12**	2±0.29**	23.0±3.23	1±0.39
5	12±1.52**	5±0.97**	1±0.21**	0.9±0.11**	2.5±0.52**	27.4±2.77	1.2±1.34
10	15±1.86**	5±0.63**	2±0.29**	1±0.18**	2.7±0.29**	32.5±4.85	1.5±0.42
15	15±1.70**	7±0.84**	6±0.65**	1±0.12**	3±0.42**	41.0±6.11	2.5±0.16
30	22±2.53**	12±1.58**	7±0.89**	3±0.86*	6.7±0.98**	47.0±6.21	2±0.79
45	25±2.96**	18±2.31**	8±2.58*	6±0.84**	9.3±1.28**	52.3±6.53	3±0.57
60	26±3.21**	20±2.34**	10±3.89*	8±1.23**	11±1.54**	45.0±5.53	5±0.87
90	27±3.22**	13±1.42**	11±1.23**	10±1.58**	11±1.46**	48.0±5.56	7±0.87
120	30±3.32**	9±1.27**	7±0.97**	12±1.56**	12±1.56**	42.3±5.24	5±0.91
150	28±4.32	5±0.67**	7±0.89**	12±1.97**	11±1.35**	34.9±4.31	3±1.10
180	26±2.58**	4±1.24**	4±0.87**	7±1.28**	6.9±0.98**	20.0±2.38	2±0.78
240	10±1.87*	2±2.71	3±0.68**	4±1.21*	2±1.31	6.3±0.53	1±0.37

Table V: Continued**.**

Time (min.)	% Mean decrease in blood pressure[a]						
	7c	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**
D[b]	29±3.21**	24±3.45**	20±2.32**	30±4.87**	40±6.54**	32±4.52**	35±4.25**
1	2.3±2.65**	5±0.97**	2±0.45**	3±0.64**	0±0.00**	2±0.65**	2±0.45**
3	4±0.68**	5±0.67**	3±0.57**	5±0.68**	2±0.37**	3±0.69**	3±0.58**
5	4.5±0.57**	7±0.97**	4±1.10*	8±2.10*	4.8±0.67**	6±0.84**	5±0.69**
10	5.7±0.83**	8±1.57**	5±0.86**	8±0.97**	4.6±1.11**	7±0.92**	9±1.67**
15	8.7±1.56**	7±1.32**	6±1.21**	8±1.28**	6.6±1.12**	7.5±0.98**	10±1.56**
30	13±1.97**	10±1.37**	6±0.94**	9±1.38**	4.3±0.67**	8±1.25**	9±0.99**
45	15±1.85**	15±1.94**	10±1.26**	7±0.67**	4±1.89*	7±1.27**	8±1.47**
60	32±5.56*	18±2.87**	20±3.42**	6±1.28**	4±1.23**	5±0.54**	6±1.23**
90	39±2.56**	20±3.24**	25±3.27**	7±1.50**	3±0.56**	4±1.57*	5±0.87**
120	20±2.86**	30±2.74**	20±2.49**	6±1.17**	2±0.48**	3±0.98*	4±0.47**
150	3±1.10**	20±2.58**	10±1.57**	5±0.60**	1±0.34*	2±0.41**	2±0.31**
180	3±1.11**	10±1.29**	5±0.80**	3±0.54**	0±0.00**	1±0.24**	2±0.84*
240	3±1.12*	6±0.87**	3±0.64**	1±0.27***	0±0.00**	0±0.00**	0±0.00**

Table V: Continued.

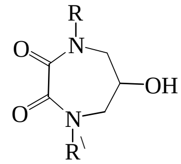
Time (min.)	% Mean decrease in blood pressure[a]			
	8b	**8c**	**8d**	**8e**
D[b]	24±2.58**	32±1.23**	20±2.68**	25±1.29**
1	5±0.24**	2±0.14**	5±0.34**	3±0.79*
3	6±1.68*	7±0.89**	7±0.97**	5±0.27**
5	10±1.47**	22±1.97**	10±1.25**	10±0.95**
10	13±2.40**	33±4.67**	12±3.24*	15±4.21*
15	15±2.13**	25±2.84**	14±1.28**	22±3.29**
30	20±2.13**	15±1.87**	20±2.45**	24±3.21**
45	25±3.64**	10±1.78**	18±2.34**	17±2.21**
60	20±3.54**	5±2.31*	15±2.57**	16±2.89**
90	20±3.58**	5±2.74*	15±2.80**	13±2.31**
120	16±3.11**	3±1.21*	10±2.32**	12±2.25**
150	15±2.14**	2±0.78*	5±1.47*	10±1.91**
180	10±2.14**	1±0.48*	5±0.98**	7±1.47**
240	5±2.14*	1±0.54*	2±0.47**	5±0.87**

Table V: Continued.

Time (min.)	% Mean decrease in blood pressure[a]					
	9c	**9d**[c]	**9e**	**9f**	**9g**	**9h**
D[b]	10±1.57*	7.4±0.97**	2±0.81*	30±0.78*	4±0.28**	5±0.59**
1	5±1.27**	21±2.87**	0.5±0.09**	0.9±0.10**	1±0.18**	1±0.24**
3	9±1.21**	21±1.84**	1±0.39*	2±0.27**	2.5±0.32**	3±0.21**
5	13±1.65**	25±3.24**	1.2±1.34	2.5±2.98	4±0.57**	5±0.42**
10	15±1.23**	27±2.41**	1.5±0.42*	3±0.21**	6±0.67**	8±1.11**
15	20±2.31**	28±2.15**	2.5±0.16**	5±0.72**	7±0.87**	10±0.86**
30	27±2.21**	30±3.84**	2±0.79*	7±0.98**	9±1.54**	10±1.32**
45	28±3.24**	35±2.57**	3±0.57**	9±1.21**	12±0.98**	8±0.75**
60	30±4.57*	41±3.57**	5±0.87**	11±1.54**	15±1.60**	6±0.70**
90	30±4.52*	25±3.47**	7±0.87**	8±0.46**	10±1.83**	4±0.67**
120	25±3.57**	31±4.13**	5±0.91**	6±0.74**	5±0.74**	3±0.47**
150	23±3.10*	36±4.65**	3±1.10*	4±0.84**	3±0.84*	2±0.64*
180	15±1.87*	36±4.13**	2±0.78*	2±0.34**	2±0.37**	2±0.31**
240	10±1.25**	39±4.58**	1±0.37*	1±0.21**	1±0.24**	1±0.27*

a- Mean value of three observations ± standard deviation.
b- Direct after injection.
c- This compound exhibited delayed effect, since at 5 hr (20%) and at 6 hr (10%)
*- Significant at $P<0.05$ vs. Propranolol value (student's-t-test).
**- Significant at $P<0.01$ vs. Propranolol value (student's-t-test).

Table VI: Comparison of maximum hypotensive activity of 3 symmetrical dialkyl derivatives with the corresponding unsymmetrical 1-alkyl-4-aryl analogues.



R=R\	Activity	R	R\	Activity	Propranolol
i-Propyl	7.45	*i*-Propyl	C_6H_5	18.4	32.3
		i-Butyl	C_6H_5	-	
		t-Butyl	C_6H_5	-	
i-Butyl	12.9	*i*-Propyl	*p*-tol.	13.01	
		i-Butyl	*p*-tol.	13.2	
		t-Butyl	*p*-tol.	9.9	
t-Butyl	22.5	*i*-Propyl	*p*-ClC_6H_4	18.5	
		i-Butyl	*p*-ClC_6H_4	22.5	
		t-Butyl	*p*-ClC_6H_4	28.7	

RESULTS AND DISCUSSION

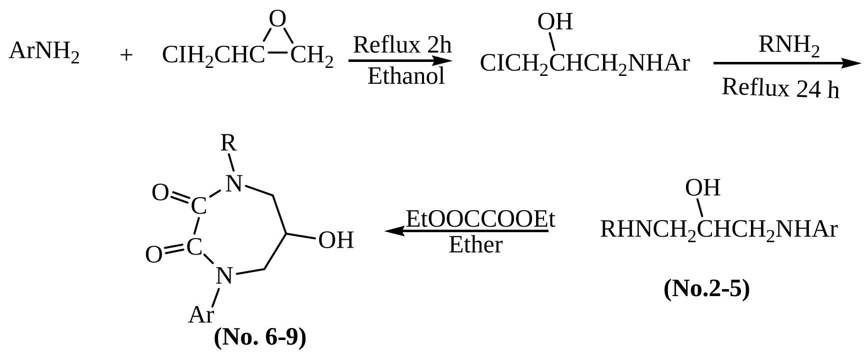
Chemistry

The unsymmetrical disubstituted 1,3-diamino-2-propanol derivatives (*No*. **2-5**) were prepared by interaction of the selected aromatic amines with epichlorohydrin in a 1:1 molar ratio by reflux in ethanol for 2 h then another mole of the appropriate alkyl or aralkylamine was added and reflux was continued for 24 h[17] (Scheme 1). These compounds are liquids and were purified by extraction and column chromatography using ethyl acetate/hexane as a mobile phase. These intermediates were cyclized with diethyl oxalate[18] in ether at room temperature to yield the corresponding perhydro-1,4-diazepine -2,3-diones (*No*. **6-9**), Scheme 1.

The 1-arylamino-3-chloro-2-propanols can't be separated,[27] however, only the *p*-tolyl derivative was separated, crystallized (ethanol) and its m.p was determined (80-2ºC) which is identical to that reported.[28]

Attempts to prepare compounds (*No*. **2-5**) by a reversed technique, i.e interaction first of epichlorohydrin with alkyl (aralkyl) amines followed by the aromatic ones, were unsuccessful. This may be attributed to the higher basicity and nucleophilicity of the alkyl and aralkyl amines.[29]

The structures of the pure intermediates were elucidated by IR and ^{1}H-NMR spectrophotometry. IR spectra of compounds (*No*. **2-5**) are characterized by the presence of broad bands at 3410-3380 cm^{-1} (υ OH stretching) and at 3320-3200 cm^{-1} (υ NH stretching).



Ar = C_6H_5, *p*-$C_6H_4CH_3$, *p*-C_6H_4Br, *p*-C_6H_4Cl
R = C_2H_5, $CH_3(CH_2)_2$, $(CH_3)_2CH$, $(CH_3)_2CHCH_2$, $(CH_3)_3C$, cyclo-C_6H_{11}, $C_6H_5CH_2$, $C_6H_5SCH_2CH_2$, $C_6H_5CH(CH_3)$

Scheme 1

^{1}H-NMR spectra of compounds (*No*. **2a-f, 3a-i, 4a-e, 5a-i**) showed a common pattern for the $HNCH_2CHOHCH_2NH$ moiety as a multiplet in the range of δ 2.25-5.30 and the OH was exchangeable with D_2O. The spectral differences were attributed to the two substituents on the *N* and *N*$^\backslash$ of the intermediates.

IR spectra of the final compounds (*No*. **6-9**) revealed the disappearance of the broad bands corresponding to the 2NH groups of the intermediates and characterized by the presence of two strong bands at 1671-1621 cm^{-1} (υ C=O stretching) in addition to a broad band at 3480-3285 cm^{-1} (υ OH stretching).

^{1}H-NMR spectra revealed a common pattern for the $CH_2CH(OH)CH_2$ part of the ring with some characteristic differences attributed to the 1,4-substituents.

The mass spectrum of compound (**8e**) revealed the molecular ion peak M^+ at *m/z* 403 (10.4%) corresponding to $C_{19}H_{19}{}^{79}BrN_2O_3$, the M-1 at *m/z* 402 (1.2%), the (M-1)+2 at *m/z* $C_{19}H_{19}{}^{81}BrN_2O_3$ 404 (8.6%) and this confirms the presence of bromine. The base peak at *m/z* 105 (100%) corresponds to the α-phenethyl radical cation. The mass spectrum of compound (**9d**) showed the molecular ion peak M^+ at *m/z* 311 (9.2%) corresponding to $C_{15}H_{19}{}^{35}ClN_2O_3$, the M+2 at *m/z* 313 (3.3%) corresponding to $C_{15}H_{19}{}^{37}ClN_2O_3$, and this confirms the presence of chlorine. The base peak at *m/z* 140 (100%) corresponding to *N*-methyl-*p*-chloroaniline radical cation.

Pharmacological screening

Anticonvulsant activity

Study of results listed in Table IV indicates that substitution of a cyclohexyl, benzyl, α-phenethyl and β-phenethyl **1(i-l)** at N^4 by an aryl group dramatically increases the anticonvulsant activity. This finding more or less, correlates with the C logP values of the corresponding compounds.[25] Also, data listed in Table IV show that, although the C logP values of compounds having *p*-bromo substituents (**8b** and **8c**) are higher than those of the *p*-tolyl derivatives (**7f** and **7g**), yet the latter compounds elicited more protection against seizures. This may be attributed to a better fitting of the tolyl derivatives than the bromine-containing compounds on the specific receptors.

Hypotensive activity

Hypotensive activity of 1-alkyl (aralkyl)-4-aryl-1,4-diazepines (**6-9**) was also carried out and the results are shown in Table V. These data show that maximum activity was reached after 2 h of administration of compounds (**6b-f**). Comparison of the maximum hypotensive activity of the tested compounds indicates that substitution of one *i*-propyl group by an aryl moiety almost doubles the hypotensive effect (Table VI). Substitution of the phenyl moiety by (*p*-chlorophenyl, *p*-bromophenyl or *p*-tolyl) either reserved or increased the activity, but no consistent correlation could be obtained between the activity and the corresponding σ & π constants of these substituents[30] (Tables V &VI).

Cytotoxic activity

Cytotoxicity of twenty eight of the target compounds was determined *in vitro* using the brine shrimp bioassay method.[23&24] Results of Table VII indicate that only compounds **8a**, **8e,** and **9e,** elicited mild cytotoxic activity.

Acute toxicity

LD_{50} of some selected representative compounds **6f, 7g, 8e,** and **9g** was determined using the graphical Litchfield method.[25] Results showed that their LD_{50} equals 10, 20, 17, and 15 mg/kg (*i.p.*) respectively, while that of diazepam was 710 mg/kg[31] showing that these compounds are far more toxic than the reference drug.

Table VII: Results of brine shrimp lethality bioassay.

Compound No.	% Inhibition at tested concentrations (ppm)			LC_{50}* ppm	LC_{90}* ppm
	100	50	25		
8a	97	82.4	18.8	39	67.9
8e	100	86.1	36	31.5	58.1
9f	100	73.5	42	31	69.3

* The LC_{50} and LC_{90} were calculated using probit analysis[24] by the use of the results of 3 concentrations between 1% and 99%.

Acknowledgement

The authors are greatly indepted to Dr. M. M. Hamdy, Department of Pharmacology, Faculty of Medicine, Assiut University for carrying out the CNS depressant and hypotensive activities. Also, they are grateful to Assistant Researcher May El-Manawaty, M.Sc.Pharm., for determining the cytotoxicity using the methods and facilities provided by the project "Use of biotechnological methods for drug discovery", Department of Pharmacognosy, National Research Centre, Dokki, Giza, Egypt.

REFERENCES

1- F. D. Popp, and A. C. Noble, in "Advances in Heterocyclic chemistry" (Ed.: A. R. Katritzky, A. J. Boulton), Vol. 8, Academic Press, New York, 1967, pp. 51-60.

2- E. I. Isaacson, in "Text book of Organic Med. and Pharm. Chemistry" (Ed. By J. N. Delgado and W. A. Remers) 10^{th} Ed., Lippincott-Raven, New York, 1998, pp. 435-443

3- Y. Edoute, J. Giris, S. A. Ben-Haim, A. Lochner, A. Weizman, G. Hayam, Y. Katz and M. Gavish, Pharmacology, 46, 224 (1993).

4- U. Breyer-Pfaff, in"Psychotropic Agents" Part 1:Antipsychotics and Antidepressants (Ed. By F. Hoffmaster, G. Stille), Springer-Verlag Berlin, Heidelberg, New York, 1980, pp. 295-296.

5- J. G. Ochoa, and W. Riche, Antiepileptic drugs, through http://www.emedicine.com/neuro , 2, 1-32 (2002).

6- H. Diaz-Arauzo, K. Koehler, T. J. Hagen and J. M. Cook, Life Sci.; 1991, 49, 207. Through J. Med. Chem., 37, 745 (1994).

7- E. D. Cox, H. Diaz-Arauza, Q. Huang, M. S. Reddy, C. Ma, B. Harris, R. Mckernan, P. Skolnick and J. M. Cook, J. Med. Chem., 41, 2537 (1998).

8- H. A. DeWald, S. Lobbestael, and B. P. H. Poschel, ibid., 24, 982 (1981).

9- B. Bobranski, J. Przytocka-Balik, and M. Wilimowski, Farmaco, Ed. Sci., 36, 135 (1981), (Eng), Chem. Abst., 94, 167625K (1981).

10- K. Stankiewicz, B. Bobranski, E. Tatarczynska and E. Chojnacka – Wojcik, ibid., 39, 1038 (1984), Chem. Abst., 102, 1060675 (1985).

11- S. T. Richard, S. M. Joyce, W. S. Ann, W. M. John and M. C. David, Int. Appl. WO 00 39,105 (CI. C07D243\08), Chem. Abst., 133, 74043c (2000).

12- N. Yoshida, H. Omoya, S. Kato and T. Ito, Eur. J. Pharmacol, 216, 435 (1992).

13- M. Roychoudhury and S. K. Kulkarni, Methods Find. Exp. Clin. Pharmacol., 19, 107 (1997).

14- M. Medou, G. Priem, G. Quélever, M. Camplo, and J. L. Kraus, Tetrahydron Letters, 39, 4021 (1998).

15- P. K. Jadhav and H. W. Man, ibid., 37, 1153 (1996).
16- A. A. Abuel-Magd, A. A. El-Shorbagi, M. A. Hussein, M. M. Hamdy and A. M. Abdel-Alim, Bull. Pharm. Sci., Assiut Univ., 27, 193 (2004).
17- B. J. Ludwig, W. A. West and D. W. Farnsworth, J. Am. Chem. Soc., 76, 2891 (1954).
18- A. Nefzi, J. M. Ostresh, and R. A. Houghten, Tetrahydron Letters, 38, 4943 (1997).
19- Confidential (Study Report) Prefa/Preclinical Pharmacology, Research unit, University of Turku, Homos Medical Ltd.Tykistokatu 6A, Fin-20520 Turku, Finland.
20- Ü. Sayan, S. Cengiz and T. Altug, Pharmacol. Research, 28, 325 (1993).
21- J. F. Reinhard, and J. Jr. F. Reinhard, in “Anticonvulsants”, (Ed. By Vida, J. A.), Academic Press, London, 1977, pp. 86-93.
22- L. L. Setescak, F. W. Dekow, J. M. Kitazen and L. L. Martin, J. Med. Chem., 27, 401 (1984).
23- B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols and J. L. Mclaughlin, J. Med. Plant Research, 45, 31 (1982).
24- M. J. Norusis, “SPSS for Windows, Base System User’s Guide”. Release 11, SPSS, Inc.
25- F. Sztaricskai, I. E. Takàcs, F. Pusztai, G. Szabó and I. Csipõ, Arch. Pharm. Med. Chem., 332, 321 (1999).
26- A. Leo, P. Y. C. Jow, C. Silipo and C. Hansch, J. Med. Chem., 18, 865 (1975).
27- R. F. Homer, J. Chem. Soc., 3690 (1950).
28- D. L. Heywood and B. Phillips, J. Am. Chem. Soc., 80, 1257 (1957).
29- H. K. Hall, J. Org. Chem., 29, 3539 (1964).
30- Y. C. Martin and J. J. Hackbarth, J. Med. Chem., 19, 1033 (1976).
31- A. MacDonald, A. F. Michaelis and B. Z. Senkowski, in “Analytical Profiles Of Drug Substances”; (Ed. By K. Florey), Vol. 1; Academic press, New York, 1972, pp. 80-99.