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New substituted 2-(pyrazol-1-yl)-dialkylacetanilides with potential local anesthetic and antiarrhythmic action. Part II

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Abstract

Nine substituted 2-(pyrazol-1-yl)-dialkylacetanilides were synthesized by N-alkylation of pyrazole and its derivatives with several 2-iodoacetanilides. The new compounds exhibited local anesthetic and antiarrhythmic actions. They have been characterized by elemental chemical analysis, UV–Vis, IR, ^{1}H NMR, ^{13}C NMR, SM spectra and pharmacology research.

Keywords: Pyrazolyl-acetanilides; Local anesthetics; Antiarrhythmics

1. Introduction

Löfgren [1] investigated a lot of new substituted acetanilides and found out that a local anesthetic should contain both a lipophilic aromatic structure and a hydrophilic one having a tertiary amino-group, such as lidocaine, the drug used even nowadays (Fig. 1). Between the two fragments there is an anesthesiophoric group.

In a previous paper [2] we reported the synthesis and characterization of some new substituted 2-(pyrazol-1-yl)-acetanilides where the benzene ring is substituted by a methyl radical in the *o*-, *m*-, *p*-positions. Some of these compounds were tested. They exhibited infiltration and surface local anesthetic and antiarrhythmic actions, but their potency was lower than that of lidocaine and quinidine, respectively.

The present paper, therefore, reports the synthesis and the characterization of some new compounds analogous to lidocaine, where the amino-group is replaced by less basic pyrazole and the benzene ring is disubstituted by a methyl and ethyl radical in the *o*,*o*′-positions. We were to observe the influence of the substituents on the benzene ring on the chemical, the physical properties and the pharmacological activity of the new compounds.

2. Chemistry

Nine compounds **1**–**9** were synthesized by treatment of substituted 2-iodoacetanilides with pyrazole and its derivatives in DMSO and in presence of sodium carbonate, according to the reaction (Scheme 1).

The new synthesized compounds were characterized by elemental analyses (C, H, N, I), determinations of molecular weights by mass spectrometry, purity determinations and UV–Vis, IR, NMR, SM spectra.

3. Experimental

All the compounds used in the present paper: 2-chloroacetanilides, 2-iodoacetanilides and the substituted pyrazoles were prepared according to literature [3–7].

Carbon, hydrogen, nitrogen and iodine analyses were carried out by microcombustion. Analytical results for C, H, N, I were within ±0.4% of calculated values.

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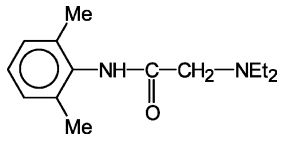
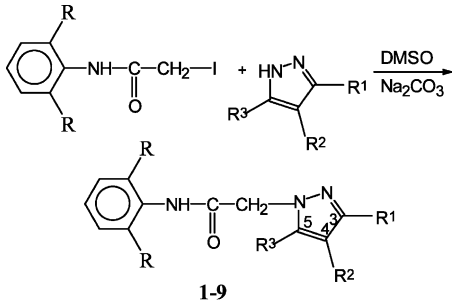


Fig. 1.



1-9

where: R = Me, Et

R^1	R^2	R^3
H	H	H
Me	H	Me
Me	I	Me
Me	NO_2	Me
Ph	H	Me

Scheme 1.

Thin layer chromatography (TLC) was made on silica gel Merck plates, in one dimensional technique; for the development solution of 7.5:1:2:1 petroleum ether:ethyl ether:methylene chloride:ethyl acetate were used. The visualization was made with an UV lamp, $\lambda = 254$ nm.

Molecular weight was obtained with a GC MS 8000 MD 800 Fissions spectrometer at 70 eV, carrier gas He at 2 ml/min.

The melting points (m.p.) were determined with a Boetius apparatus and are not corrected.

Electronic spectra within 200–800 nm range were obtained with Unicam UV–Vis spectrometer in ethanol solution 10^{-5} M.

IR spectra were recorded within 4000–400 per cm range by BIO-RAD FTS-135 spectrometer, in KBr pellets.

NMR spectra were recorded on a Varian Gemini 300 spectrometer operating at 300 MHz (^{1}H NMR), 75 MHz (^{13}C NMR), respectively, in $CDCl_3$ or DMSO-d_6 in 5 mm NOREL-57 PP grade sample tubes. The chemical shifts were referred to TMS as internal standard.

The nine new compounds were synthesized according to the following example. Several changes of the reaction conditions were made as a function of the substituents nature in the heterocycle.

3.1. 2-(Pyrazol-1-yl)-2′,6′-diethylacetanilide

A total of 1.16 g (3.6 mmol) 2′,6′-diethyl-2-iodoacetanilide, 0.25 g (3.6 mol) pyrazole were dissolved in 4 ml of DMSO to which a sodium carbonate was then added. The mixture was heated at 60 °C for 5 h. The solid raw product was recrystallized from ethanol.

4. Pharmacology

4.1. Acute toxicity (LD_{50})

White mice weighing 20 ± 2 g each were used in batches of six; one batch was the control one. The new compounds were administered in doses ranging between 250 and 750 mg/kg of body weight, per os. The graphic method [8,9] was used.

The strain of the experimental animals has been maintained at a minimum all through the testing.

4.1.1. Infiltration local anesthetic action

Infiltration local anesthetic action was determined by Bianchi's method [10,11]. The mice were injected subcutaneously about 1 cm from the tail's bases with 1% suspension or 0.05 ml solution of the compounds to be tested and 1% lidocaine hydrochloride, respectively. Every 15 min after the mice were injected, the pain reflex of all the injected animals was tested applying a small artery clip to the zone where the compound was injected. The time in seconds of enduring pain without the reflex movement of the tail was registered. The mean enduring time versus the effect of lidocaine considered to be equal to 100 was expressed. Lidocaine hydrochloride solutions were used for comparison. The results were interpreted using the 't' Student's test [8,9].

4.1.2. Antiarrhythmic action

Eleven batches, each of five white male mice weighing 20 ± 2 g were used as follows: to eight batches were administered the studied compounds per os in an equal dose of 1/10 LD_{50}, and to two batches were given 50 mg/kg of body weight lidocaine and 75 mg/kg of body weight quinidine sulfate. The remaining batch was the control one. Heart fibrillation artificially induced by chloroform atmosphere appeared after 30 min. The latent time of the appearance of heart fibrillation was measured using Hackenberger's technique [12] for the new compounds comparatively with lidocaine and quinidine sulfate.

5. Results and discussion

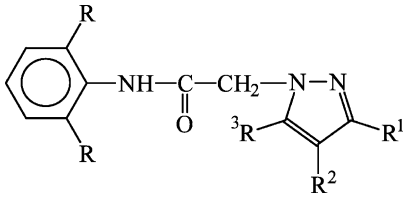
5.1. Chemical and spectral results

Some properties are given in Table 1. The purity of the new compounds was checked trough TLC using silica gel G chromatoplates; R_F are given in Table 1.

The electronic spectra of the compounds recorded in ethanolic solution show the λ_{max} values exist in the characteristic ranges (231–274 and 261–280 nm) of the chromophores present in the molecule (>C=O, >C=C<, >C=N–). These bands are assigned to the π–π* transitions.

IR spectra recorded in the 4000–400 per cm range in KBr pellets reflect the molecular structure of the new compounds and showed the bands characteristic of the secondary amides [13]. The strong band due to the stretching frequency, ν_{NH} appears within the 3240–3267 per cm range. The very strong amide band I, ν_{CO} appears within the 1656–1668 per cm range; the strong

Table 1
Some properties of the prepared compounds **1**–**9**



Number	Comp.				Formula	Molecular mass		Base peak *m/e* 100%	M.p. (°C)	Yield (%)	R_F
	R	R^1	R^2	R^3		Calc.	Exp. (SM)				
1	Me	H	H	H	$C_{13}H_{15}N_3O$	229.28	229	81	174–175	65.5	0.10
2	Me	Me	H	Me	$C_{15}H_{19}N_3O$	257.34	257	109	185–186	50.1	0.11
3	Me	Me	I	Me	$C_{15}H_{18}N_3OI$	383.23	383	235	234–236	13.9	0.16
4	Me	Me	NO_2	Me	$C_{15}H_{18}N_4O_3$	302.33	302	154	254–256	39.4	0.10
5	Et	H	H	H	$C_{15}H_{19}N_3O$	257.34	257	81	178–179	78.7	0.17
6	Et	Me	H	Me	$C_{17}H_{23}N_3O$	285.39	285	109	149–150	79.9	0.16
7	Et	Me	I	Me	$C_{17}H_{22}N_3OI$	411.29	411	235	212–213	60.9	0.29
8	Et	Me	NO_2	Me	$C_{17}H_{22}N_4O_3$	330.39	330	154	234–235	57.8	0.14
9	Et	Ph	H	Me	$C_{22}H_{25}N_3O$	347.46	347	171	155–158	63.9	0.30

Table 2
^{1}H resonance data for representative compounds, δ (ppm), *J* (Hz)

Comp.	Pyrazole protons					CH_2	NH
	R^1 H-3	R^2 H-4	R^3 H-5	R^1 Me (3)	R^3 Me (5)		
1	7.67 d 1.9	6.40 dd 2.3 1.9	7.58 d 2.3			5.00 s	7.70 bs
4 [a]				2.58 s	2.75 s	5.20 s	8.72 bs
5	7.68 d 2.2	6.41 dd 2.0 2.2	7.59 d 2.0			5.01 s	7.70 bs
6		5.93 s		2.24 s	2.32 s	4.84 s	7.50 bs
9		6.47 s		7.31–7.79 [b]	2.38 s	4.93 s	7.68 bs

s, singlet; bs, broad singlet; d, doublet; dd, double doublet.
[a] Solvent: DMSO-d_6.
[b] Ph protons.

amide band II, due to the $\delta_{NH}+\nu_{CN}$ coupling is present within the 1530–1541 per cm range. The bands due to the stretching of the pyrazole ring can be found within the 1367–1476 per cm range [12].

^{1}H NMR and ^{13}C NMR spectra recorded at 300 MHz in the $CDCl_3$ and DMSO-d_6 solution support the structure formulas assigned to these compounds, deduced from the equation of the synthesis reaction. Tables 2 and 3 give the chemical shifts, δ (ppm) and the coupling constants of representative compounds. The positions of the protons and of the substituents in the ring can be found out using HETCOR and COSY. The tautomeric 3(5)-phenyl-methylpyrazole by reaction with 2-iodoacetanilde could lead to a mixture of two isomers but in fact only the formation of one isomer (**9**) was observed by ^{1}H NMR spectroscopy (Scheme 2) [2]. The structure was ascertained by using the Overhauser effect (NOE).

Table 1 gives the *m/e* values (%) of the base peak in the mass spectra of the new compounds. Fragmentation processes (Scheme 3) can support the structure formulas assigned to the compounds.

Scheme 3 shows the fragmentation process specific to the aromatic amides occurring by the cleavage of the bond between the N and C atoms of the carbonyl group followed by CO elimination. The base peak thus appears [14,15].

5.2. Pharmacological results

The acute toxicity (LD_{50}) of the compounds ranges within 469–629 mg/kg body weight, per os. Compounds **8** and **1** are the least toxic (Table 4).

The anesthetic and antiarrhythmic actions were tested using 1/10 of the LD_{50} value of the compounds as a working dose.

5.2.1. Local anesthetic action

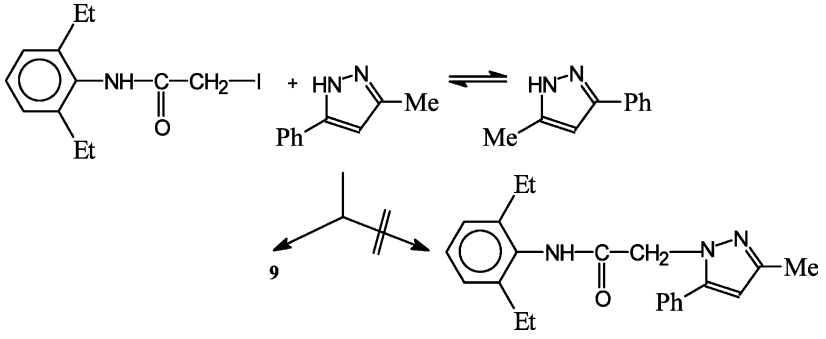
The anesthetic action was tested using Bianchi's [10,11] experiment on mice (Table 5) using lidocaine hydrochloride as reference substance. At first, the animals responded to the mechanical stimuli after 1.5–2 s.

The new compounds increased the response time to the mechanical stimuli applied on injected area with values ranging between 10.62 ± 0.9 and 21.66 ± 1.6. In the same conditions lidocaine had an effect equal with 25.33 ± 1.7 (Table 5). The analysis of the results for the first three test times (15, 30, 45 min) showed that the most active compounds which produced an effect versus lidocaine hydrochloride were **5** with 85.51%, **8** with 80.54%, **7** with 75.29%. It is interesting that after the first 15 min, the following compounds came very close to the response time of lidocaine 29.8 corresponding to the same test time: **8** with 29.6; **5** with 26.0; **7** with 22.8.

Table 3
^{13}C resonance data for representative compounds, δ (ppm)

Comp.	Pyrazole carbons					CO	CH_2
	R^1 C-3	R^2 C-4	R^3 C-5	R^1 Me (3)	R^3 Me (5)		
1	141.47	106.99	131.15			165.70	55.16
4 [a]	148.16	131.34	143.54	13.44	11.65	164.02	51.56
5	141.59	107.05	131.12			166.25	55.26
6	149.96	106.47	140.72	13.44	10.98	166.87	52.23
9	152.50	103.91	141.24	125.56–132.79 [b]	11.27	166.60	52.59

[a] Solvent: DMSO-d_6.
[b] Ph carbons.



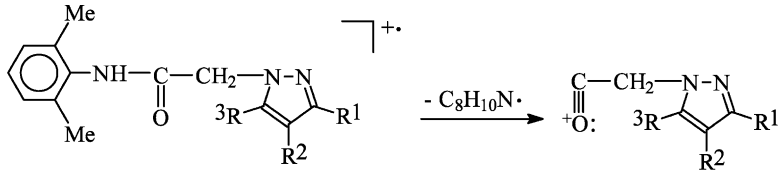
Scheme 2.

After 30 min, the response times versus lidocaine 27.2 were: **5** with 20.4; **7** with 18.2; **8** with 17.0. After 45 min the response times versus lidocaine 19.0 were: **5** with 18.6; **7** with 16.2; **8** with 14.6, respectively.

Structurally, the most active compounds were characterized by the presence of the diethyl group in the *o*,*o*′-position on the benzene ring: compounds **5**, **8** and **7** had 85.51, 80.54 and 75.29, respectively, of the lidocaine action. Compound **1** had 74.22 of the lidocaine action. These compounds have in position four of the pyrazole ring either the hydrogen and iodine atom or a nitro group.

5.2.2. Antiarrhythmic action

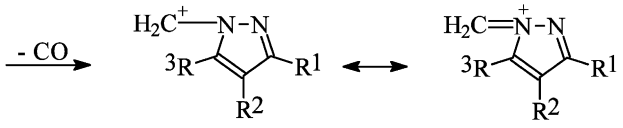
The influence of the studied compounds was determined on the experimental fibrillation induced in mouse by an atmosphere of chloroform [12,16]. The reference substances were quinidine sulfate and lidocaine hydrochloride.



Molecular ion

(1) $R^1 = R^2 = R^3 = H$ m/e = 229 (1) m/e = 109

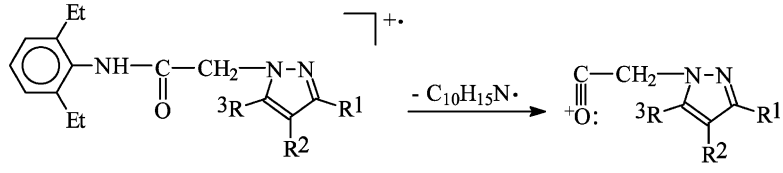
(2) $R^1 = R^3 = CH_3$; $R^2 = H$ m/e = 257 (2) m/e = 137



Base peak

(1) m/e = 81

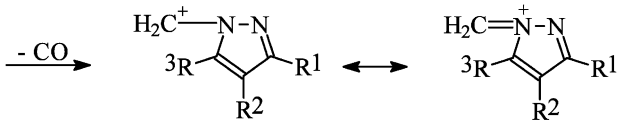
(2) m/e = 109



Molecular ion

(3) $R^1 = R^2 = R^3 = H$ m/e = 257 (3) m/e = 109

(4) $R^1 = R^3 = CH_3$; $R^2 = H$ m/e = 285 (4) m/e = 137



Base peak

(3) m/e = 81

(4) m/e = 109

Scheme 3.

Table 4
Acute toxicity (LD_{50}) in mice

Comp.	LD_{50}	
	Body weight per os (mg/kg)	Body weight (mmol/kg)
1	565(515–587)	2.46
2	485(428–530)	1.88
3	487(448–510)	1.27
4	515(480–537)	1.70
5	495(466–520)	1.92
6	535(447–550)	1.87
7	469(443–486)	1.14
8	629(583–638)	1.90
Lidocaine hydrochloride	292 [a]	1.08

[a] See ref. [15].

All tested compounds delayed the appearance of the toxic fibrillating effect of chloroform. Compounds **6** and **8** were the most active, 76.91% in relation to lidocaine and 49.20% in relation to quinidine (**6**), 60.93 and 38.98% (**8**), respectively (Table 6).

6. Conclusions

We have obtained nine new substituted 2-(pyrazol-1-yl)-dialkylacetanilides and characterized them by several methods: elemental chemical analysis, TLC, UV–Vis, ^{1}H NMR, ^{13}C NMR, SM spectra and pharmacological tests.

Table 5
Infiltration local anesthetic action

Comp.	Response time (s) ($x \pm SE$) [a]			Mean response time (s) ($x \pm SE$) [a]	Effect versus lidocaine 25.33 = 100
	15 min	30 min	45 min		
Control mice	2±0.02	3.5±0.04	4.8±0.06	3.43±0.04	
1	20.8±1.6	19.6±1.7	16.0±1.2	18.80±1.5	74.22
2	12.2±1.2	10.4±1.1	9.26±0.5	10.62±0.9	41.93
3	16.8±1.4	19.6±1.5	12.2±1.1	16.2±1.3	63.96
4	19.6±1.3	17.8±1.3	15.6±1.2	17.66±1.2	69.72
5	26±2	20.4±1.6	18.6±1.4	21.66±1.6	85.51
6	13.8±1.2	11.78±1.3	11.2±1.0	12.26±1.2	48.40
7	22.8±2	18.2±1.4	16.2±1.3	19.07±1.5	75.29
8	29.6±2.4	17.0±1.4	14.6±1.1	20.40±1.6	80.54
Lidocaine hydrochloride	29.8±2.3	27.2±2.2	19.0±0.7	25.33±1.7	100

[a] ($x \pm SE$): mean value ± standard error.

Table 6
The action of new compounds on the experimental fibrillation in mice

Comp.	Dose body weight per os mg/kg	Time of fibrillation appearance (s) ($x \pm SE$) [a]	Latency in percentage 60 s = 100	Protective effect versus $CHCl_3$ (s)	Activity of compounds with the standard	
					Lidocaine 45.00 = 100	Quinidine 70.34 = 100
Control mice		5.80±0.09	9.66±0.15			
1	55	12.50±1.02	20.83±1.7	11.17±1.55	24.82	15.88
2	50	19.13±0.96	31.88±1.6	22.22±1.45	49.38	31.59
3	48	12.00±1.50	20.00±2.5	10.34±2.35	22.98	14.70
4	50	19.25±1.58	32.08±2.6	22.42±2.45	49.82	31.87
5	50	12.25±1.03	20.41±1.7	10.75±1.56	23.89	15.28
6	53	26.56±1.60	44.27±2.6	34.61±2.45	76.91	49.20
7	47	18.75±1.60	31.25±2.6	21.59±2.45	47.98	30.69
8	63	22.25±1.60	37.08±2.6	27.42±2.45	60.93	38.98
Lidocaine hydrochloride	50	32.80±2.50	54.66±4.1	45.00±3.95	100	63.98
Quinidine sulfate	75	48.00±3.70	80.00±6.1	70.34±5.95	156.31	100

[a] $x \pm SE$: mean value ± standard error.

The studied compounds have infiltration local anesthetic actions and an antiarrhythmic action, but their potency is lower than that of lidocaine and quinidine, respectively. Anyhow, taking into account their lower toxicity versus lidocaine, the new compounds may deserve further consideration as potential local anesthetic and antiarrhythmic agents.

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