Eur. J. Med. Chem. 36 (2001) 495–506

PII: S0223-5234(01)01246-6/FLA

Original article

3,8-Diazabicyclo-[3.2.1]-octane derivatives as analogues of ambasilide, a Class III antiarrhythmic agent

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Received 17 July 2000; revised 13 March 2001; accepted 8 May 2001

Abstract – Ambasilide, a representative of Class III antiarrhythmics, was reported to prolong the cardiac action potential duration in the dog, with little or no effect on Ca and Na currents. We synthesised a series of ambasilide analogues, having the 3,8-diazabicyclo-[3.2.1]-octane moiety instead of the 3,7-diazabicyclo-[3.3.1]-nonane present in ambasilide. The compounds were tested both in vitro extracellular electrophysiological assays and by the conventional microelectrode technique. Most of them lengthened the effective refractory period (ERP) with no change or slight increase on the impulse conduction time (ICT). Similarly some of the tested compounds lengthened the action potential duration (APD), a typical Class III feature, without exerting any significant effect on the maximal rate of depolarization, therefore apparently lacking Class I antiarrythmic activity. © 2001 Éditions scientifiques et médicales Elsevier SAS

antiarrhythmic activity / ambasilide / diazabicyclo-[3.2.1]-octane

1. Introduction

The results of the cardiac arrhythmia suppression trial (CAST) unexpectedly showed that the sodium channel blocker (Class I) antiarrhythmic drugs flecainide and encainide did not decrease but actually increased sudden cardiac death in postinfarction patients [1]. As a consequence, development of almost all Class I antiarrhythmics was halted and, parallel with this, interest was shifted toward Class III antiarrhythmic agents. Compounds belonging to the latter class, such as D-sotalol, **E-4031** and dofetilide, lengthen repolarization and the effective refractory period (ERP) with no influence on the sodium current and the impulse conduction. The currently available selective Class III antiarrhythmic agents such as D-sotalol, **E-4031** and dofetilide inhibit effectively, the rapid component of the delayed rectifier outward potassium current (I_{Kr}), thereby they prolong the action potential duration (APD) [2]. The APD-lengthening effect is more pronounced at slow heart rate than during tachycardia. This reverse frequency-dependent effect could be dangerous during bradycardia. A recent clinical trial with the selective I_{Kr} blocker D-sotalol (survival with oral D-stall, SWORD) [3] proved that this agent also increased the mortality in patients suffering from myocardial infarction. This negative result has been explained by proarrhythmic potential of the drug as a possible consequence of its reverse frequency dependent repolarization lengthening effect. Recently it was also reported that ambasilide (**1**) (see *figure 1*), a new Class III antiarrhythmic drug, produced less reverse frequency dependent APD prolongation [4] in comparison to the effect of other Class III antiarrhythmic agents [5].

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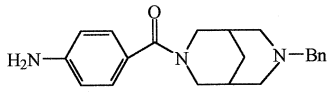


AMBASILIDE (1)

Figure 1. Ambasilide.

Therefore, the goal of the present work was to design and synthesise new ambasilide analogues with a frequency independent APD lengthening effect; such compounds may be expected to exert more advantageous antiarrhythmic activity. Accordingly, compounds **2a** and **3** were prepared, in which the diazabicyclo-[3.3.1]-nonane nucleus of the model was substituted by the lower homologue diazabicyclo-[3.2.1]-octane. In addition, several derivatives (**2b**, **c**, **e**–**g**) which combine the diazabicyclooctane moiety with side chains previously investigated in Class III antiarrhythmics [6–8] were synthesised. Finally, the intermediate nitro derivative **2d** and its open analogue **4** were also considered.

2. Chemistry

As shown in *figure 2*, the 3-benzyl-3,8-diazabicyclo-[3.2.1]-octane [9] was condensed either with the appropriate acyl chloride to give **2a**,**d** or with the required 2-(4-substituted-phenoxy)-1-chloroethane (**5a**,**b**) [10, 11] to provide **2e**,**f**. Treatment of the amino derivative **2a** with methanesulfonyl chloride in pyridine at 0 °C gave **2b**. However, if the reaction was carried out in CH_2Cl_2 at reflux, **2c** was obtained. The reverted isomer 3-(4′-aminobenzoyl)-8-benzyl-3,8-diazabicyclo-[3.2.1]-octane (**3**) was synthesised from 8-Boc-3,8-diazabicyclo-[3.2.1]-octane [12], that was condensed with *p*-amino-benzoyl-chloride in CH_2Cl_2

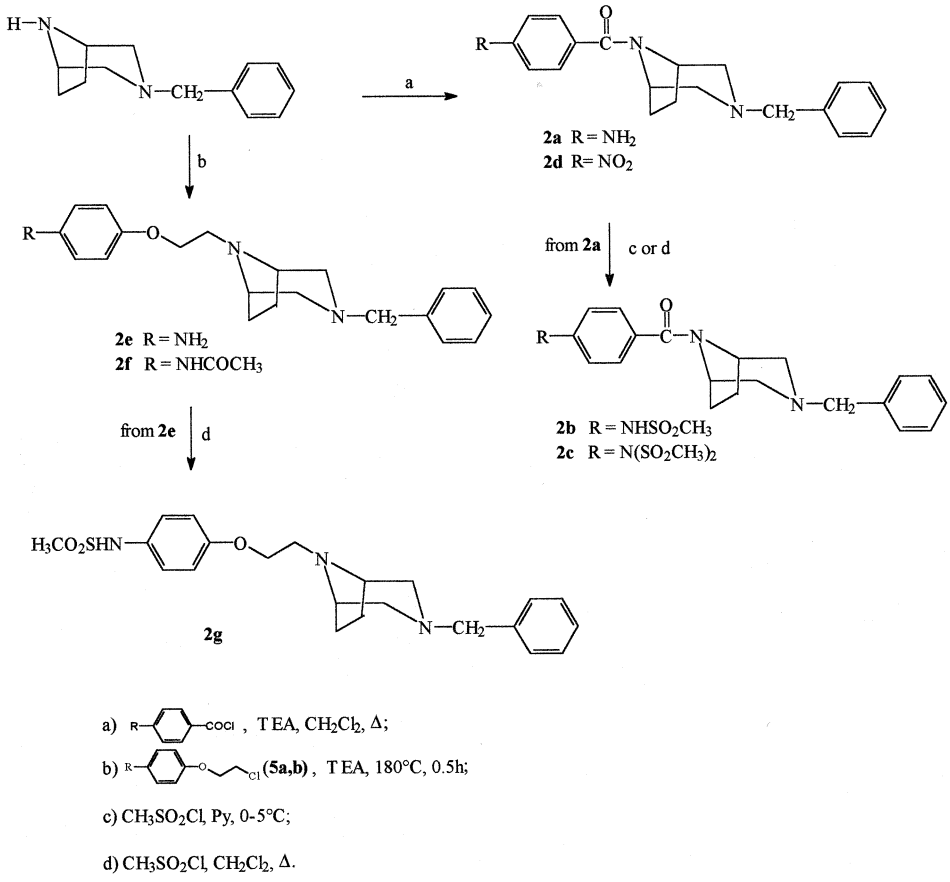


Figure 2. Synthetic route to compounds **2a**–**g**.

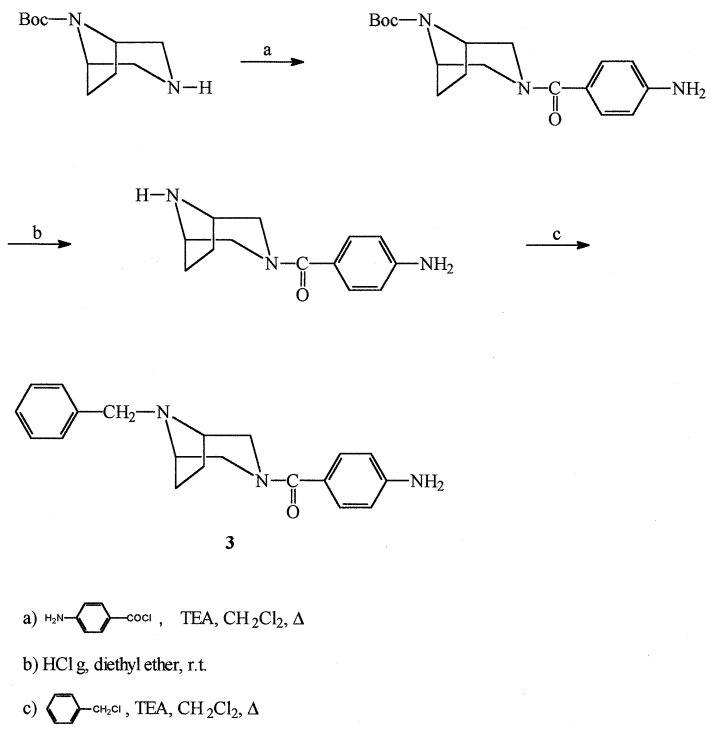


Figure 3. Synthetic route to compound **3**.

in the presence of triethylamine (TEA). Deprotection of the amino group by an ether solution of hydrochloride acid and subsequent condensation with benzyl chloride gave **3** (see *figure 3*). Finally, (*figure 4*) compound **4** was prepared from the 2,6-dimethyl piperazine by condensing first with benzyl chloride in toluene at reflux overnight and subsequently with *p*-nitrobenzoyl chloride (see *table I* and Section 6 for data).

3. Pharmacology

The compounds were tested both by in vitro extracellular electrophysiological assays and by the conventional microelectrode technique (see Section 6 for details).

4. Results and discussion

4.1. Biological data

The compounds were first tested by in vitro extracellular electrophysiological methods measuring the impulse conduction time (ICT) and effective refractory (ERP) period in isolated dog cardiac right ventricular trabecular muscles. As *table II* shows, all the tested compounds with the exception of **2g**, lengthened the ERP with either only slightly or not changing the ICT.

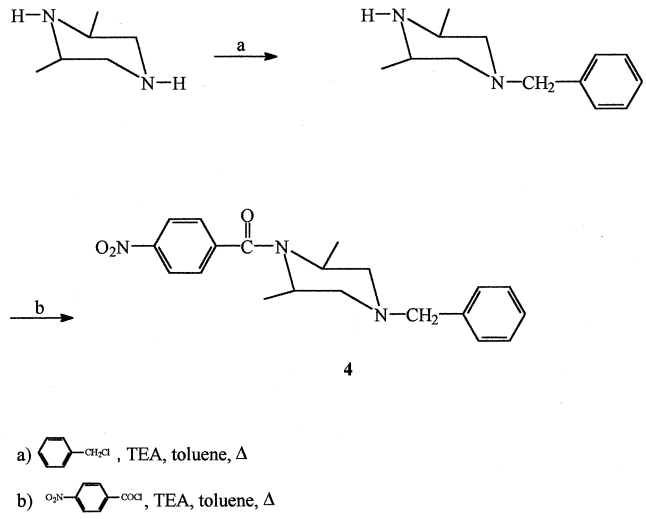
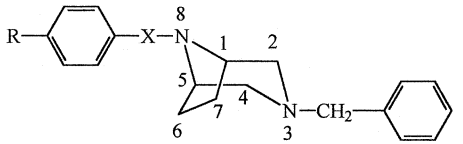


Figure 4. Synthetic route to compound **4**.

Table I. Physicochemical properties of compounds **2a–g.**



2a-g

Comp.	X	R	%Yield	Mp/°C[a]	Formula[b]	^{1}H-NMR (DMSO), δ (ppm)[c]
2a	CO	NH_2	37	186–189	$C_{20}H_{23}N_3O$ HCl	1.9–2.4 (m, 4H H-6,7); 3.3–3.7 (m, 4H H-2,4); 4.2–4.3 (m, 1H H-1); 4.4 (s, 2H CH_2–Ph); 4.69 (bs., 2H NH_2 exch. with D_2O); 4.9–5.0 (m, 1H H-5); 6.6 (d, 2H H-3′,5′); 6.7 (d, 2H H-2′,6′); 7.5–7.7 (m, 5H H_{Ar}·–Ph).
2b	CO	$NHSO_2CH_3$	35	157–159	$C_{21}H_{25}N_3O_3S$ HCl	1.9–2.4 (m, 4H H-6,7); 3.2 (s, 3H SO_2CH_3); 3.3–3.7 (m, 4H H-2,4); 4.2–4.3 (m, 1H H-1); 4.4 (s, 2H CH_2–Ph); 4.3–4.6 (m, 1H H-5); 4.9–5.1 (m, 1H exch. with D_2O NH); 7.5–7.7 (m, 7H H_{Ar}·–Ph H-3′,5′); 7.4 (d, 2H H-2′,6′).
2c	CO	$N(SO_2CH_3)_2$	50	210–212	$C_{22}H_{27}N_3O_5S_2$ HCl	1.9–2.4 (m, 4H H-6,7); 3.3–3.5 (m, 4H H-2,4); 3.6 (s, 6H $(SO_2CH_3)_2$); 4.3–4.5 (m, 1H H-1); 4.4 (s, 2H CH_2–Ph); 4.9–5.1 (m, 1H H-5); 7.5–7.6 (m, 5H H_{Ar}·–Ph); 7.6–7.8 (m, 4H H-2′,3′,5′,6′).
2d	CO	NO_2	45	190–192	$C_{20}H_{21}N_3O_3$ HCl	1.9–2.4 (m, 4H H-6,7); 3.2–3.8 (m, 4H H-2,4); 4.3–4.4 (m, 1H H-1); 4.5 (s, 2H CH_2–Ph); 4.9–5.1 (m, 1H H-5); 7.5–7.7 (m, 5H H_{Ar}·–Ph); 7.7 (d, 2H H-2′,6′); 8.4 (d, 2H H-3′,5′).
2e	OCH_2CH_2	NH_2	35	180–181	$C_{21}H_{27}N_3O$ HCl	1.8–2.0 (m, 4H H-6,7); 2.4 (m, 2H CH_2-N); 2.5–2.7 (m, 2H H-2,4); 2.7–2.9 (m, 2H H-2,4); 3.2-3.3 (m, 2H CH_2-O); 3.3 (s all., 2H exch. with D_2O NH_2); 3.5 (s, 2H CH_2–Ph); 4.0–4.1 (m, 2H H-1,5); 6.6 (d, 2H H-3′,5′); 6.7 (d, 2H H-2′,6′); 7.5–7.7 (m, 5H H_{Ar}·–Ph).
2f	OCH_2CH_2	$NHCOCH_3$	12	95–97[c]	$C_{23}H_{29}N_3O_2$	1.8–2.0 (m, 4H H-6,7); 2.1 (s, 3H $COCH_3$); 2.3 (d, 2H CH_2-N); 2.5–2.7 (m, 2H H-2,4); 2.7–2.9 (m, 2H H-2,4); 3.1–3.3 (m, 2H CH_2-O); 3.5 (s, 2H CH_2–Ph); 4.0–4.2 (m, 2H H-1,5); 6.8 (d, 2H H-2′,6′); 7.1–7.3 (m, 5H H_{Ar}·–Ph); 7.4 (d, 2H H-3′,5′); 8.1–8.4 (m, 1H exch. with D_2O)[d].
2g	OCH_2CH_2	$NHSO_2CH_3$	28	198–199	$C_{22}H_{29}N_3O_3S$ HCl	1.8–2.0 (m, 4H H-6,7); 2.3–2.7 (m, 4H H-2,4); 2.7–2.9 (m, 2H CH_2-N); 2.9 (s, 3H SO_2CH_3); 3.2–3.4 (m, 2H CH_2-O); 3.5 (s , 2H CH_2–Ph); 4.0–4.2 (m, 2H H-1,5); 5.8–6.4 (m, 1H exch. with D_2O NH); 6.8 (d, 2H H-3′,5′); 7.2 (d, 2H H-2′,6′); 7.2–7.4 (m, 5H H_{Ar}·–Ph);

[a] As the hydrochloride.
[b] Analyses were within $\pm$ 0.4% of the theoretical values.
[c] As the free base.
[d] $CDCl_3$ as the solvent.

Table II. Effects of **2a**–**g**, **3**, **4** and reference compounds on the impulse conduction time and effective refractory period in isolated dog right ventricular trabecular muscle[a].

Compounds (10 μM)	*n* [b]	ICT [c] (ms)	ERP [d] (ms)
Control	8	15.6 ± 1.7	238.7 ± 3.5
2a	8	17.5 ± 1.6 [e]	264.4 ± 5.5 [e]
Control	8	15.6 ± 1.2	251.2 ± 4.8
2b	8	18.0 ± 1.2 [e]	275.0 ± 5.9 [e]
Control	12	14.7 ± 1.3	250.8 ± 3.8
2c	12	14.8 ± 1.2	279.6 ± 5.2 [e]
Control	12	13.3 ± 0.5	246.7 ± 5.7
2d	12	14.2 ± 0.5 [e]	261.2 ± 6.0 [e]
Control	8	14,5 ± 1.3	253.7 ± 5.0
2e	8	17.0 ± 1.2 [e]	267.5 ± 4.1 [e]
Control	12	17.4 ± 0.9	240.0 ± 2.1
2f	12	19.3 ± 1.1 [e]	257.5 ± 2.8 [e]
Control	8	13.1 ± 0.8	238.7 ± 4.1
2g	8	15.0 ± 1.1 [e]	243.7 ± 4.2
Control	8	17.5 ± 1.4	251.2 ± 4.8
3	8	20.9 ± 1.5 [e]	268.7 ± 4.5 [e]
Control	12	11.8 ± 0.6	230.8 ± 4.2
4	12	12.7 ± 0.6 [e]	247.1 ± 4.2 [e]
Control	12	14.5 ± 1.3	249.2 ± 3.1
Ambasilide (5 μM)	12	18.7 ± 2.2 [e]	275.0 ± 3.8 [e]
Control	12	13.2 ± 1.2	245.0 ± 5.1
E-4031 (5 μM)	12	14.8 ± 0.9 [e]	266.2 ± 3.9 [e]

[a] Stimulation frequency 1 Hz.
[b] *n* = number of preparation.
[c] ICT = impulse conduction time.
[d] ERP = effective refractory period.
[e] $P < 0.05$.

These changes suggested that these compounds exert repolarisation lengthening (action potential duration lengthening) effect in the dog ventricle. This possibility was further tested by the conventional microelectrode technique (see later). The possible effect of compounds on the ATP-sensitive potassium current was also tested with extracellular in vitro electrophysiological technique measuring the ERP before and after the application of 2 μM Cromakalim. Cromakalim [13] is a known opener of the ATP-sensitive potassium channels and thereby shortens cardiac repolarisation and consequently the ERP. The attenuation or prevention of the Cromakalim induced ERP shortening was considered as inhibition of the ATP sensitive potassium current. As *table III* shows **2a**, **2e** and **2f** significantly attenuated the Cromakalim evoked ERP shortening which suggests that these compounds may inhibit the ATP sensitive potassium current in some degree. The effects of the **2a**,**b**,**d**–**g**, **4** and reference compounds on the action potential parameters were studied in dog right ventricular papillary muscles by the conventional microelectrode technique. The results are summarised in *table IV*. Moreover, the most significant results are illustrated in the *figure 5*. Compounds **2b**, **2d** and **4** but not **2a**, **2e**, **2f**, **2g** lengthened the action potential duration (APD) without apparently influencing the action potential amplitude (APA) and maximal rate of depolarisation (V_{max}). The repolarisation lengthening — so called Class III antiarrhythmic effect — observed with most of the available Class III antiarrhythmic drugs strongly depends on the heart rate or stimulation frequency. The slower the rate the more pronounced APD lengthening was observed, which phenomenon was called reverse use or rate dependency [14]. This is considered as a disadvantageous feature since the excessive APD prolongation at slow rate may induce early after depolarisation (EAD) and consequently torsade de pointes tachycardia. In addition, at high rate, i.e. at tachycardia, where possible APD lengthening would be especially important to prevent re-entry type tachycardia, the less pronounced APD prolongation may diminish antiarrhythmic effectiveness. Therefore we

Table III. Effects of **2a**–**g** and **4** on the 2 μM Cromakalim (CR) evoked ERP shortening in dog cardiac right ventricular trabecular muscle[a].

Compounds (10 μM)	*n* [b]	ERP [c] (ms)		Change (%)
		Before CR [d]	After CR	
Control	12	233.3 ± 4.8	176.2 ± 3.8	−24.3 ± 1.5
2a	12	249.6 ± 3.8	212.5 ± 5.3	−14.9 ± 1.6 [e]
Control	12	230.8 ± 7.5	160.4 ± 6.5	−30.6 ± 0.9
2b	12	256.2 ± 8.0	166.7 ± 6.7	−35.1 ± 1.0
Control	12	253.3 ± 4.1	190.0 ± 4.1	−25.0 ± 1.0
2c	12	252.9 ± 4.0	185.0 ± 2.3	−26.7 ± 1.2
Control	12	236.7 ± 2.6	147.5 ± 7.3	−37.8 ± 2.8
2d	12	242.5 ± 3.1	157.5 ± 9.4	−34.9 ± 4.1
Control	12	255.0 ± 3.8	160.0 ± 6.1	−37.2 ± 2.4
2e	12	270.0 ± 4.4	229.2 ± 6.0	−14.7 ± 2.9 [e]
Control	12	249.2 ± 3.1	172.5 ± 3.7	−30.7 ± 1.7
2f	12	267.1 ± 3.4	233.3 ± 4.5	−12.5 ± 1.7 [e]
Control	12	246.7 ± 4.7	165.0 ± 5.4	−32.6 ± 2.5
2g	12	261.7 ± 3.7	190.0 ± 4.1	−27.3 ± 1.8
Control	12	241.7 ± 3.2	161.7 ± 5.9	−33.3 ± 1.7
4	12	260.4 ± 5.2	179.6 ± 5.2	−31.0 ± 2.0

[a] Stimulation frequency 1 Hz.
[b] *n* = number of preparation.
[c] ERP = effective refractory period.
[d] CR = cromakalin (2 μM).
[e] $P < 0.05$.

Table IV. Effects of **2a**,**b**,**d**–**g**, **4** and reference compounds on the action potential parameters in isolated dog right papillary muscle.

Compounds (10 μM)	*N*	APA [a] (mV)	APD_{90} [b] (ms)	V_{max} [c] (V/s)
Control	3	107.0 ± 2.1	263.7 ± 25.3	198.3 ± 26.9
2a	3	109 ± 3.2	261.0 ± 28.7	203.7 ± 22.4
Control	4	107.2 ± 1.2	235.5 ± 2.0	208.5 ± 22.4
2b	4	111.2 ± 2.6	280.2 ± 8.2 [d]	206.7 ± 15.37
Control	4	109.0 ± 1.3	237.5 ± 10.4	245.7 ± 21.1
2d	4	108.7 ± 1.9	260.7 ± 11.5 [d]	240.2 ± 23.2
Control	3	104.3 ± 1.5	245.3 ± 4.9	196.7 ± 30.1
2e	3	105.2 ± 2.0	245.3 ± 16.5	176.3 ± 22.1
Control	3	109.7 ± 0.9	242.0 ± 14.5	285.3 ± 21.7
2f	3	111.7 ± 0.3	229.0 ± 17.4	283.0 ± 20.6
Control	3	110.0 ± 2.9	257.7 ± 5.5	186.7 ± 15.6
2g	3	110.3 ± 2.0	257.0 ± 7.0	187.0 ± 18.0
Control	3	106.0 ± 3.0	241.7 ± 6.7	199.3 ± 9.3
4	3	106.0 ± 3.1	254.7 ± 3.7 [d]	202.0 ± 10.2
Control	4	111.0 ± 1.7	247.7 ± 13.7	220.5 ± 12.9
Ambasilide (10 μM)	4	111.7 ± 2.6	299.7 ± 17.5 [d]	210.0 ± 5.0
Control	4	113.0 ± 0.7	258.5 ± 10.4	174.5 ± 7.5
E-4031 (1 μM)	4	113.5 ± 0.6	301.2 ± 4.2 [d]	179.7 ± 1.7

Stimulation frequency 1 Hz.
[a] APA = action potential amplitude.
[b] APD_{90} = 90% repolarisation.
[c] V_{max} = maximal rate of depolarisation.
[d] $P < 0.05$.

investigated the frequency dependent effect of **2b**, **2d**, **E-4031** and ambasilide on the APD in dog right papillary muscle. As *figure 5* shows, **E-4031** exerted the unfavourable reverse rate dependent APD prolongation while after **2b**, **2d** and ambasilide application the APD lengthening effect was not apparently dependent on the stimulation frequency.

4.2. Modeling

4.2.1. Conformation analysis

Results are summarised in *table V*, where minimal and maximal conformational energy values for each compound are listed. All generated structures were used in DISCO computations.

4.2.2. DISCO analysis

The calculation forced to find at least one donor atom, one acceptor atom and two hydrophobic centres resulted in 119 models. The user must constrain the search to those solutions whose numbers of pharmacophore points are as high as possible (because more common pharmacophore points will present a better overall fit) and whose superposition is still acceptable. On the other hand, consideration based on X-ray crystallographic evidence suggests that a rigid superposition of pharmacophore points is unnecessary. Therefore one generally considers a 1 Å tolerance between pharmacophoric elements to be very good and tolerance of 2 Å to be acceptable. Therefore 1.5 Å tolerance, with the maximum number of pharmacophoric elements seems to

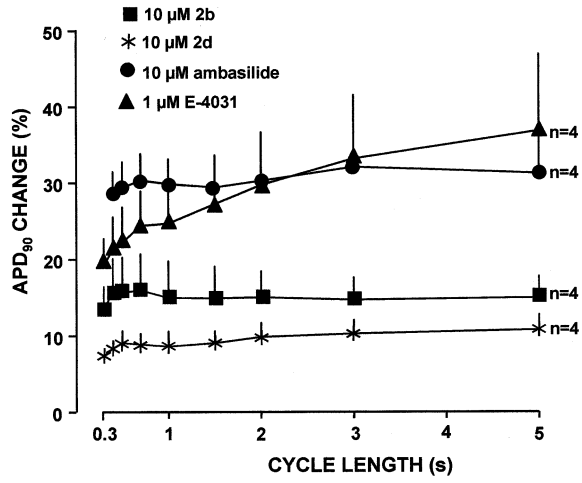


Figure 5. The frequency dependent effect of **2b**, **2d**, ambasilide (**1**) and **E-4031** on the action potential duration at 90% of repolarization (APD90) in dog right ventricular papillary muscle is represented. The abscissa shows the stimulation frequency expressed in cycle lengths, the ordinate indicates the normalized APD lengthening expressed in percentage. Values represent the mean of four successful experiments, bars indicate ±standard error.

Table V. Minimal and maximal conformational energy values (Kcal/mol) of compounds **2a**–**g** and ambasilide.

Compound	E_{min}	E_{max}
Ambasilide	66.08	70.41
2a	19.48	27.72
2b	19.63	28.58
2c	19.20	28.68
2d	21.67	29.90
2e	14.64	25.87
2f	17.73	30.21
2g	13.58	27.04

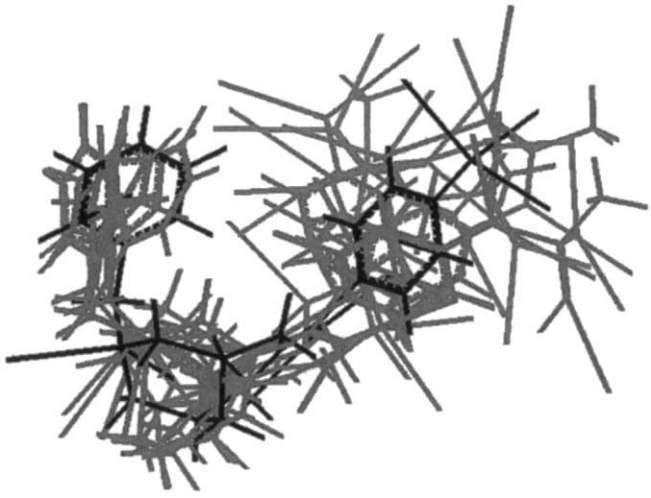


Figure 6. The compounds as aligned by DISCO. The reference molecule, ambasilide, is black.

Table VI. Scores of the final model for compounds **2a**–**g** and ambasilide.

Compound	FIT [a]	Overlap [b]	ΔE [c]	Tolerance [d]
Ambasilide	0.00	273.12	0.00	0.00
2a	0.82	170.37	0.42	1.19
2b	0.83	167.75	0.80	1.19
2c	1.74	144.00	0.72	1.19
2d	1.73	144.00	0.40	1.19
2e	1.28	157.25	2.15	1.00
2f	1.09	177.75	4.08	1.46
2g	1.55	156.37	7.90	1.12

[a] FIT, RMS deviation of feature atom coordinates from those of the corresponding reference features (Å).
[b] OVERLAP, intersection of the van der Waals volumes of the reference and the superimposed molecule (Å^3).
[c] ΔE, the increase in conformational energy of the conformer over the minimal energy conformation of the compound.
[d] Tolerance, maximal distances between the corresponding faetures of the reference and the superimposed molecule.

be a good compromise. The final model has been selected from the set of models mentioned above by the energy values of the reference conformers, since the bioactive conformation probably is one of the low-energy conformers. Moreover a visual inspection of the alignment of this model, also revealed a favorable overlap of molecular parts other than those identified for the pharmacophore. The final model is displayed in *figure 6*. It contains one donor atom, (protonated N), one acceptor oxygen atom and four hydrophobic centers. Scores (fit, overlap, relative energy of the selected conformer) of the final model are displayed in *table VI*. The distances of the most important pharmacophoric elements are shown in *table VII and VIII*. Relative energy (E_{rel}) is the increase in conformational energy of a conformer over the lowest energy conformer of the compound. Reference structure ambasilide with pharmacophoric elements found by DISCO is displayed by *figure 7*. The key features of this pharmacophore (DA, HYD5 and HYD6) are in appropriate agreement with the most important elements of the model defined in a previous study [15]. The pharmacophoric model defined could be useful for designing novel compounds with Class III antiarrhythmic activity.

5. Conclusions

A new series of ambasilide analogues were synthesised which have the 3,8-diazabicyclo-[3.2.1]-octane

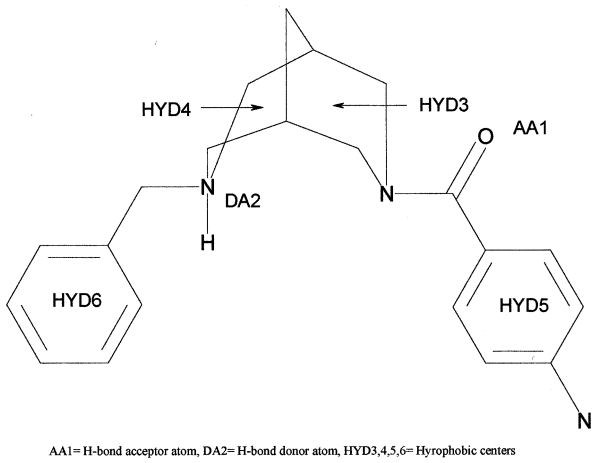
Table VII. Distances (in Å) between pharmacophoric elements of the final model.

Distance (Å)	TOL	1	2
5.53	1.50	AA1	DA2
3.59	1.50	AA1	HYD3
4.87	1.50	AA1	HYD4
3.63	1.50	AA1	HYD5
6.44	1.50	AA1	HYD6
2.87	1.50	DA2	HYD3
1.52	1.50	DA2	HYD4
6.94	1.50	DA2	HYD5
3.81	1.50	DA2	HYD6
1.58	1.50	HYD3	HYD4
5.02	1.50	HYD3	HYD5
5.23	1.50	HYD3	HYD6
6.28	1.50	HYD4	HYD5
4.72	1.50	HYD4	HYD6
6.31	1.50	HYD5	HYD6

TOL = tolerance of the distances. AA1 = H-bond acceptor atom, DA2 = H-bond donor atom, HYD3,4,5,6 = Hydrophobic centers.

Table VIII. Atomic coordinates of the final model.

Atom	*X*	*Y*	*Z*
AA1	−5.764856	−1.590058	−3.191282
DA2	−9.541248	−4.035973	0.026391
HYD3	−7.804212	−4.352076	−2.264429
HYD4	−9.055249	−4.621854	−1.237577
HYD5	−2.912106	−3.460097	−1.956332
HYD6	−7.024588	−3.495673	2.834229



AA1= H-bond acceptor atom, DA2= H-bond donor atom, HYD3,4,5,6= Hyrophobic centers

Figure 7. Pharmacophoric elements of ambasilide found by DISCO.

moiety instead of the 3,7-diazabicyclo-[3.3.1]-nonane present in the reference compound. The compounds were tested to verify their capability to exert Class III antiarrhythmic effects, both in vitro extracellular electrophysiological assays and by the conventional microelectrode technique. Most of them lengthened the effective refractory period (ERP) with significant increase on the impulse conduction time (ICT). Moreover, they lengthened the action potential duration (APD), a typical Class III feature, without exerting any significant Class I antiarrythmic activity.

Compounds reported in this paper are expected to possess significant Class III type antiarrhythmic activity. Further work is now in progress to investigate the in vivo effects of these substances.

6. Experimental

6.1. Chemistry

Melting points were determined with a Büchi 510 capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Brucker AC200 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as internal standard; dimethyl sulphoxide-d_6 was used as the solvent, unless otherwise noted. TLC on silica-gel plates was used to check product purity. Silica gel 60 (Merck 70–23 mesh) was used for column chromatography while Silica gel 60 (Merck 230–400 mesh) for column flash chromatography. Elemental analyses were within ±0.4 of the theoretical values. The structures of all compounds were consistent with their analytical and spectroscopic data.

*6.1.1. 3-Benzyl-8-substituted benzoyl-3,8-diazabicyclo-[3.2.1]-octanes (**2a**,**d**)*

A mixture of the appropriate benzoyl chloride (0.01 mol), 3-benzyl-3,8-diazabicyclo-[3.2.1]-octane [9] (0.01 mol) and triethylamine (0.01 mol) in dichloromethane (20 mL) was refluxed overnight. After cooling, the inorganic salts were filtered off, the solvent evaporated and the crude product purified by silica-gel chromatography, eluting with dichloromethane–ethyl acetate (9:1) (see *table I* for data).

*6.1.2. 3-Benzyl-8-(4′-methanesulfonanilide)-carbonyl-3,8-diazabicyclo-[3.2.1]-octane (**2b**)*

To a vigorously stirred solution of **2a** (1 g, 3.1 mmol) in pyridine (20 mL) cooled at 0–5 °C, methanesulfonyl chloride (0.25 mL, 3.1 mmol) was added dropwise. The reaction mixture was poured into water (80 mL) and extracted with ethyl acetate (3×30mL). The organic layers were collected and dried (Na_2SO_4) and the solvent evaporated to give **2b**, which was purified by silica gel chromatography, eluting with dichloromethane–methanol (49:1) (see *table I* for data).

*6.1.3. 3-Benzyl-8-(4′-bis-methansulfonanilide)-carbonyl-3,8-diazabicyclo-[3.2.1]-octane (**2c**)*

To a vigorously stirred solution of **2a** (1 g, 3.1 mmol) in dichloromethane (20 mL), cooled at 0–5 °C, methanesulfonyl chloride (0.5 mL, 6.2 mmol) was added dropwise and the reaction refluxed for 1 h. After cooling, the reaction mixture was poured into water (80 mL) and extracted with ethyl acetate (3×30 mL). The organic layers were collected and dried (Na_2SO_4) and the solvent evaporated to give **2c**, which was purified by silica gel chromatography, eluting with dichloromethane–methanol (19:1) (see *table I* for data).

*6.1.4. 3-Benzyl-8-aryloxy-ethylen-3,8-diazabicyclo-[3.2.1]-octane (**2e**,**f**)*

A mixture of the appropriate chloride (**5a**,**b**) (0.01 mol), 3-benzyl-3,8-diazabicyclo-[3.2.1]-octane [9] (0.01 mol) and triethylamine (0.01 mol) was heated at 180 °C for 0.5 h. After cooling the crude product was purified by silica gel chromatography, eluting with dichloromethane–methanol (19:1) (see *table I* for data).

*6.1.5. 3-Benzyl-8-(4′-methanesulfonanilide)-ethylen-3,8-diazabicyclo-[3.2.1]-octane (**2g**)*

Compound **2g** was prepared as above reported for **2b** starting from **2e** (see *table I* for data).

*6.1.6. 3-(4′-Aminobenzoyl)-8-benzyl-3,8-diazabicyclo-[3.2.1]-octane (**3**)*

The 8-Boc-3,8-diazabicyclo-[3.2.1]-octane [12] was condensed with *p*-amino-benzoyl chloride as above reported for **2a** to give the 3-(4′-aminobenzoyl)-8-Boc-3,8-diazabicyclo-[3.2.1]-octane. Yield 60%, oil.

^{1}H NMR: ($CDCl_3$) 1.5 (s, 9H $3\times CH_3$); 1.7–2.0 (m, 4H H-6,7); 3.0–3.4 (m, 4H H-2,4); 3.9 (bs, 2H exch. with D_2O NH_2); 4.1–4.4 (m, 2H H-1,5); 6.7 (d, 2H H-3′,5′); 7.2 (d, 2H H-2′,6′).

To a cooled solution of 3-(4′-aminobenzoyl)-8-Boc-3,8-diazabicyclo-[3.2.1]-octane (0.01 mol) in diethyl ether (10 mL) a solution of HCl in diethyl ether was added until the pH was about 1 and the mixture stirred overnight at room temperature (r.t.). Sodium hydroxide (6 N) was then added until pH 9, the layers separated and the aq phase extracted twice by diethyl ether (2×20 mL) The reunited organic layers were dried over sodium sulfate and the solvent evaporated to give 3-(4′-amino-benzoyl)-3,8-diazabicyclo-[3.2.1]-octane (90%) which was directly condensed with equimolar benzyl chloride in CH_2Cl_2 at reflux in the presence of TEA to give **3**. Yield 65%, oil.

^{1}H NMR: ($CDCl_3$) 1.4–2.0 (m, 4H H-6,7); 2.2–2.5 (m, 1H H-2); 2.6–2.8 (m, 1H H-4); 3.0-3.4 (m, 2H exch. with D_2O NH_2); 3.4–3.5 (m, 2H H-2,4); 3.8–4.0 (m, 1H H-1); 4.3 (s, 2H CH_2–Ph); 4.6–4.8 (m, 1H H-5); 6.5–6.7 (m, 2H H-3′,5′); 7.2–7.5 (m, 7H $5H_{Ar}$–Ph, H-2′,6′).

*6.1.7. 4-Benzyl-1-(4′-nitro-phenyl)-carbonyl-2,6-dimethyl-piperazine (**4**)*

A mixture of 2,6-dimethyl-piperazine (1 g, 8.75 mmol), benzyl chloride (1.1 g, 8.75 mmol) and triethylamine (0.85 mL, 8.75 mmol) in toluene (10 mL) was refluxed overnight. After cooling, the inorganic salts were filtered off and the solvent evaporated to give 4-benzyl-2,6-dimethyl-piperazine which was used without further purification.

A mixture of 4-benzyl-2,6-dimethyl-piperazine (0.74 g, 3.6 mmol), *p*-nitro-benzoyl chloride (0.67 g, 3.6 mmol) and triethylamine (0.35 mL, 3.6 mmol) in toluene (10 mL) was refluxed overnight. After cooling, the inorganic salts were filtered off and the solvent evaporated to give a crude product (0.12 g), which was purified by silica gel chromatography, eluting with dichloromethane–ethyl acetate (19:1).

Yield 10% oil. ^{1}H NMR: ($CDCl_3$) 1.3 (s, 3H CH_3); 1.4 (s, 3H CH_3); 2.1–2.3 (m, 2H H-3,5); 2.7 (m, 2H H-3,5); 3.5 (s, 2H CH_2–Ph); 3.8–4.4 (m, 2H H-2,6); 7.2–7.4 (m, 5H H_{Ar}–Ph); 7.5 (d, 2H H-2′,6′); 8.2 (d, 2H H-3′,4′).

*6.1.8. 2-(4-acetamidophenoxy)-1-ethanol (**5a**) and 2-(4-aminophenoxy)-1-chloroethane (**5b**)*

To a well stirred solution of 4-acetamidophenol (33.75 g, 0.222 mol) and sodium hydroxide (9.38 g, 0.235 mol) in water (118 mL), ethylene chlorohydrine (18.75 g, 0.232 mol) was added, and the reaction mixture heated at 60–70 °C for 8 h. After cooling, the solution was kept in the refrigerator overnight. The so formed crystals were filtered, washed with water (2×50 mL) and dried to give 2-(4-acetamidophenoxy)-1-ethanol (**5a**) [10] (36.68 g; 84.6%). mp 124–125 °C ^{1}H NMR ($CDCl_3$): 2.1 (s, 3H CH_3); 2.2 (bs, 1H exch. with D_2O OH); 3.8 (m, 2H CH_2–N); 4.2 (t,2H CH_2–O); 6.8 (d, 2H H-2′,6′); 7.2 (bs, 1H exch. with D_2O NH); 7.4 (d, 2H H-3′,5′).

To a well stirred solution of 2-(4-acetamidophenoxy)-1-ethanol (13.6 g, 0.07 mol) in chloroform (26 mL) and *N*,*N*-dimethyl-formamide, at 0–5 °C, a cold (0–5 °C) solution of thionyl chloride (6 mL) in chloroform (9 mL) was added dropwise during 30 min. The mixture was refluxed for 2.5 h, then evaporated to dryness under vacuum to give an orange oil, which was added of water (70 mL) and conc. HCl (68 mL), refluxed for 0.5 h and filtered while still hot. After cooling, the so formed crystals were separated to give 2-(4-aminophenoxy)-1-chloroethane hydrochloride [10], which was dissolved in water (74 mL) at 60 °C and the pH brought to 7 by dropwise addition of conc. ammonium hydroxide (5.2 mL). The mixture was cooled and filtered, the crystals washed with water (2×50 mL) and dried to give **5b** [11] (10.1 g, 85%), m.p.: 88–90 °C. ^{1}H NMR ($CDCl_3$): 3.8 (t, 2H CH_2–Cl); 4.2 (t, 2H CH_2–O); 6.8 (d, 2H H-3′,5′); 7.2 (bs, 1H exch. with D_2O); 7.4 (d, 2H H-2′,6′).

6.2. Biological section

6.2.1. Extracellular electrophysiological recordings [16]

Adult mongrel dogs (5–12 kg) were anaesthetized by 30 mg/kg intravenous pentobarbital. Their hearts were removed and small pieces of right ventricular papillary and trabecular muscle muscles were prepared and placed into the tissue bath (50 mL) which contained modified Tyrode's solution (Na^+ 140 mM, K^+ 4 mM, Ca 1.8 mM, Mg^{2+} 1.0 mM, Cl^- 129.6 mM, HCO_3^- 20 mM and

glucose 11 mM) and was oxygenated with 95% O_2 and 5% CO_2. The temperature was kept constant at 36± 0.5 °C. The pH was 7.4±0.05.

During the equilibration period (60 min) the right ventricular trabecular muscle preparations were driven with stimuli of 2 ms duration and twice the diastolic threshold strength at 1000 ms of cycle length through bipolar platinum electrodes. To measure conduction time, bipolar extracellular platinum electrodes (diameter = 0.1 mm) were placed on the surface of the right ventricular wall along the direction of the trabecular muscle fibers, 10–12 mm away from the stimulating electrodes, and the propagated biphasic action potentials were recorded extracellularly. The propagated extracellular action potentials appeared at the recording electrode, and the time difference between the sign of the stimulus artifact and the first inflexion point of the extracellular potential provided a measure of conduction time. The extracellular action potentials were amplified with an amplifier (Eltron GMK) and were displayed on the screen of an oscilloscope (Medicor VM 62 A).

The effective refractory period (ERP) was determined at three times the threshold strength using twin impulses with gradually increasing the coupling intervals.

The stimulation frequency was varied between 3 and 0.5 Hz. Measurements were taken after sufficient adaptation of the fibers to the new cycle length.

Drugs were diluted directly in the tissue bath from 10 mM stock solution dissolved in DMSO to reach the appropriate final concentrations. The solvent at 0.1% did not evoke significant changes in the measured parameters.

The drug effects were determined after 30–45 min equilibration period after which steady-state drug effects were achieved. Student's *t*-test for paired data was used to determine statistical significance of the results. The results were considered significant when P was <0.05.

6.2.2. Conventional microelectrode technique [17]

Adult mongrel dogs (5–12 kg) of both sexes were used. After animals were anaesthetized with sodium pentobarbital 30 mg/kg intravenously, their hearts were rapidly removed through right lateral thoracotomy and immediately rinsed in oxygenated Tyrode's solution containing (in mM): Na^+ 140, K^+ 4, Ca^{2+} 1.8, Mg^{2+} 1, Cl^- 129.6, HCO_3^- 20, and glucose 11. The pH of this solution was 7.35–7.45 when gassed with 95% O_2/5% CO_2 at 37 °C. Right ventricular papillary muscles were individually mounted in a tissue chamber (~ 50 mL vol). Each preparation was initially stimulated (HSE stimulator type 215/II.) at a basic cycle length (BSL) of 1000 ms (frequency 1 Hz), using 2 ms rectangular constant current pulses isolated from the ground across bipolar platinum electrodes in contact with the preparation. At least 1 h was allowed for each preparation to equilibrate while it was continuously superfused with Tyrode's solution. The temperature was kept constant at 37 °C. Transmembrane potentials were recorded by conventional microelectrode techniques. Microelectrodes filled with 3 M KCl with tip resistances of 5–20 MΩ were connected to the input of a high-impedance electrometer (HSE microelelctrode amplifier type 309) referenced to the ground. The first derivative of transmembrane voltage with respect to time (V_{max}) was electronically derived by (HSE differentiator type 309) with linear response in the range of 20–1000 V/s. Voltage outputs from all amplifiers were displayed on a dual-beam memory oscilloscope (Tektronix 2230 100 MHz digital storage oscilloscope).

Resting membrane potential (RP), action potential amplitude (APA), and action potential duration at 90% of repolarization (APD90) were obtained with software (developed in our institution) with an 386 IBM-compatible computer connected to the digital output of the oscilloscope. When different steady-state stimulation cycle lengths were applied, action potential parameters were measured at each cycle length after adaptation to the new pacing cycle length (usually after 2 min).

The preparations were superfused for 30 min with the tested compounds before measurements started. If possible, the same impalement was maintained throughout the experiment. When impalement was lost during measurement, readjustment was attempted. If the readjusted parameters deviated more than 10% from the previous parameters experiments were terminated.

6.3. Molecular modeling methods

Mapping the conformational spaces of ligands was carried out with an energy window high enough to include the putative bioactive conformation. Each conformer set was next provided for a DISCO analysis. All calculations were performed on a Silicon Graphics INDY R4400 workstation by utilizing Tripos' SYBYL molecular modeling software.

6.3.1. Molecular conformations

Starting structures were drawn by Sketch Molecule option, and geometry optimizations were carried out with Tripos force field.

Sets of conformations of all compounds were generated by Multisearch option in DISCO interface. This conformer generator, like Randomsearch, searches various energy minima available to a molecule by randomly perturbing torsions, followed by minimisation and, finally eliminating duplicates. In Multisearch method, torsions are limited to those which can change the internal geometry of atoms that determine DISCO features. Therefore, in connection with a DISCO conformation is not necessarily identical with the lowest energy conformation, energy cutoff was set relatively high value (15 kcal/mol). Searches were done with the following parameters:

Analysis, the Multisearch method seems to be superior over Randomsearch that does not check distances of features.

In our cases, computations were always stopped after 100 conformations have been found, since DISCO was limited to study less than 100 conformations of each compound. Since bioactive conformation is not necessarily identical with the lowest energy conformation, energy cutoff was set relatively high. Searches were done with the following parameters:

PRESCREENING:

Minimizer: Maxmin

Cutoff: 15 kcal/mol
Maxmin RMS Gradient: 3.0

TERMINATION:

Max. Cycles: 3000

Max. Conformers: 100

Maxmin RMS Gradient: 0.05

CONFORMER COMPARISON:

Method: Fit

Criteria: Max. Distance

Deviation Limit: 0.40.

6.3.2. DISCO analysis

Using scan rows for reference option of DISCO, Ambasilide was offered as reference with the summary of possible model components: one donor atom (DA), one acceptor site (AS), three donor sites (DS), two acceptor atoms (AA) and four hydrophobe rings (Hyd). (DISCO as a rule, selects the reference molecule with the least number of possible points for superposition and least number of conformations.) These class features were found common in all structures.

The analysis was done with the following parameters:

STRUCTURE REQUIREMENTS:

Match_All

FEATURE REQUIREMENTS:

Do 3 To 8

DISTANCE TOLERANCE

Coarse Fit (range of tolerance was varied between 0.5 A- 5.0 A)

Acknowledgements

This work was supported by grants obtained from the Hungarian National Research Foundation (OTKA T 032558 and T031910), Hungarian Ministry of Health (ETT 536/2000) and from the Hungarian Ministry of Education FKFP (1025/1997). Financial support from CNR and Ministero dell'Universita' e della Ricerca Scientifica is also acknowledged.

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