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Diterpene Lipo-Alkaloids with Selective Activities on Cardiac K^+ Channels

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ABSTRACT

Aconitum diterpene alkaloids are known for their remarkable toxicity, which is due to their effect on ion channels. Activation of voltage-gated Na^+ channels is the major cause of their cardiotoxicity, however, influence on K^+ channels may also play a role in the overall effect.

Here we report the synthesis of a series of lipo-alkaloids, including four new compounds, based on the 14-benzoylaconine structure, which is the core of a vast number of diterpene alkaloids naturally occurring in *Aconitum* species. The activities of these compounds were measured *in vitro* on K^+ ion channels using the whole-cell patch-clamp technique. Structure-activity analysis was carried out based on the data of 51 compounds (32 genuine diterpene alkaloids, 5 fatty acids, and 14 lipo-alkaloids). Depending on their substitution, these compounds exert different activities on GIRK (G protein-coupled inwardly-rectifying potassium channel) and hERG (human ether-à-go-go-related gene) channels. Fatty acids and diterpene alkaloids show lower activity on the GIRK channel than lipo-alkaloids. Lipo-alkaloids also have less pronounced hERG inhibitory activity compared to the cardiotoxic aconitine. Considering the GIRK/hERG selectivity as an indicator of perspective therapeutic applicability, lipo-alkaloids are significantly more selective than the genuine diterpene alkaloids. 14-Benzoyl-8-*O*-eicosa-8*Z*,11*Z*,14*Z*-trienoate and 14-benzoyl-8-*O*-eicosa-11*Z*,14*Z*,17*Z*-trienoate are strong and selective inhibitors of GIRK channels, thus, they are promising subjects for further studies to develop diterpene alkaloid-based antiarrhythmic pharmacons.

Introduction

Aconitum species have been widely used in traditional Asian medicinal systems. Tubers and roots are usually used as painkillers and antirheumatic agents after cautious processing in order to reduce their toxicity [1]. Toxicity of unprocessed *Aconitum* drugs is primarily explained by the Na^+ channel activating effect of some of their DAs [e.g., ACON (**18**), hypaconitine (**23**), and mesaconitine (**25**)] [2,3]. Arrhythmogenic alkaloids have a high affinity to the open Na^+ channels at the neurotoxin binding site 2. They activate these voltage-dependent Na^+ channels at their resting potential and inhibit their inactivation, resulting in a final inexcitability of the cells [4]. In case of a fatal *Aconitum* poisoning, the major cause of death is usually arrhythmia or heart arrest [5].

Dzhakhangirov et al. [6] analyzed the structure-cardiac activity relationships of 111 DAs in two animal arrhythmia models. All arrhythmogenic alkaloids possess an aconitane skeleton, but the substituents strongly influence their activity. In particular, a β-OH on C-13, an α-aroyl on C-14, a β-acetate on C-8, and a positively charged nitrogen atom play key roles in the arrhythmogenic activity.

Interestingly, certain DAs have antiarrhythmic effects. This activity is mediated through their interaction with K^+ channels and the inhibition of the voltage-dependent Na^+ channels [4,7]. Na^+ channel blocking DAs are competitive antagonists of the arrhyth-

ABBREVIATIONS

ACON	aconitine
BAE-DI	14-BzA-8-*O*-eicosa-11*Z*,14*Z*-dienoate
BAE-PENT	14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoate
BAE-TETR	14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoate
BAE-8TRI	14-BzA-8-*O*-eicosa-8*Z*,11*Z*,14*Z*-trienoate
BAE-11TRI	14-BzA-8-*O*-eicosa-11*Z*,14*Z*,17*Z*-trienoate
BAL	14-BzA-8-*O*-laurate
g-BALL	14-BzA-8-*O*-γ-linolenate
BAP	14-BzA-8-*O*-palmitate
BAPO	14-BzA-8-*O*-palmitoleate
BAS	14-BzA-8-*O*-stearate
BzA	benzoylaconine
DA	diterpene alkaloid
E-EN	eicosa-11*Z*-enoic acid
E-DI	eicosa-11*Z*,14*Z*-dienoic acid
E-TETR	eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoic acid
E-8TRI	eicosa-8*Z*,11*Z*,14*Z*-trienoic acid
E-11TRI	eicosa-11*Z*,14*Z*,17*Z*-trienoic acid
E-PENT	eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoic acid
FA	fatty acid
GIRK	G protein-coupled inwardly-rectifying potassium channel
hERG	human ether-à-go-go-related gene
LA	lipo-alkaloid
g-LIN	γ-linolenic acid
PALO	palmitoleic acid
SONG	songorine

mogenic, Na^+ channel-activating alkaloids. The most active antiarrhythmic compounds are some C_{18} bisnorditerpene alkaloids. Their common structural features are the presence of an acetylanthranilic or anthranilic acid residue on C-4, methoxy groups on C-1, C-14, and C-16, and an OH on C-8 [6]. Lappaconitine (**24**), a member of this group, irreversibly blocks open human heart Na^+ channels [8] and interacts with K^+ channels [9].

Alkaloids from the C_{19} norditerpene group with antiarrhythmic activity typically have a basic nitrogen and possess an aromatic ester group on either the C-1, C-6, or C-14 position. A representative of this group is 14-benzoyltalatisamine (**22**), which is a potent and selective blocker of the delayed rectifier K^+ channels [10].

Among the C_{20} DAs, members of the Guanfu base series are the most promising antiarrhythmic compounds. Guanfu base A (= acehytisine) (**33**) blocks the fast Na^+ channel, exhibiting a remarkable antiarrhythmic effect in rats [11] and increasing the diastolic period, thereby improving the myocardial blood supply [12]. In a patch-clamp experiment on guinea pig myocytes, it inhibited the delayed rectifier current, which may contribute to the prolongation of cardiac repolarization [7].

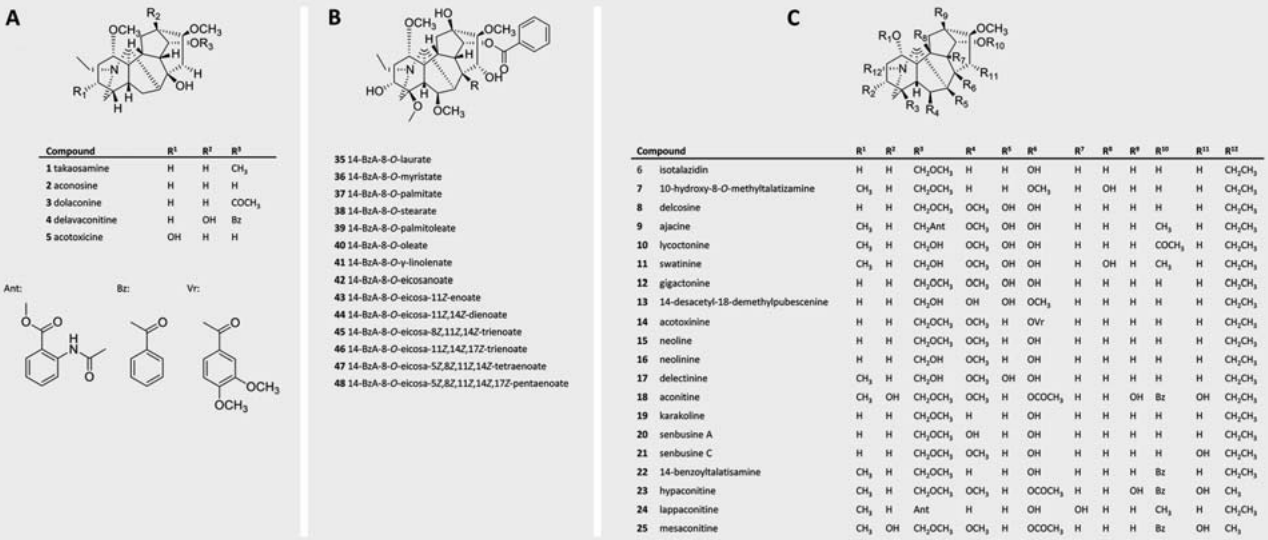
A comprehensive investigation of cardioactive *Aconitum* alkaloids led to the development of a new group of antiarrhythmic drugs [13]. Lappaconitine hydrobromide, produced from the roots of *Aconitum leucostomum* Vorosch. and *Aconitum septentrionale* Koelle, was the first diterpenoid alkaloid-based drug on the market, approved in the 1980s as a class 1C antiarrhythmic drug. Acehytisine (**33**), an alkaloid of *Aconitum koreanum* R. Raymond, was approved for the treatment of paroxysmal supraventricular tachycardia in 2005 [14].

The hERG encodes a voltage-gated potassium channel that provides the major repolarizing current (rapidly activating the delayed rectifier potassium current, I_{Kr}) in phase 3 of the cardiac action potential. Blockade of this channel may lead to the prolongation of the QT interval and thereby could enhance the risk of arrhythmia and sudden cardiac death [15]. The increased expression of GIRK channels in the atria is associated with chronic atrial fibrillation [16], thus, it is assumed that selective antagonists of these channels might be useful in the treatment of atrial fibrillation [17]. Therefore, selective blockers of GIRK channels without any inhibitory activity on hERG channels may be candidates for further studies focusing on their potential application as antiarrhythmics.

Interestingly, systematic studies on the effects of DAs on K^+ channels are scarce. A previous study reported the hERG channel inhibitory effect of ACON (**18**) [18], which may also contribute to the toxicity of *Aconitum* plants. Later, an animal experiment confirmed similar activity for hypaconitine (**23**), reassuring the QT prolonging potential of this compound [19]. For acehytisine (**33**), relatively weak hERG channel inhibitory activity was observed compared to the structurally related Guanfu base G (**34**), which justifies its superiority in the case of human therapeutic application [20]. In one of our previous papers we reported that ACON (**18**), BAP (**47**), gigactonine (**12**), neolinine (**16**), and songoramine (**31**) demonstrated significant hERG channel inhibition using the whole-cell patch-clamp technique [21]. In a subsequent study, 15 DAs [aconosine (**2**), dolaconine (**3**), delavaconitine (**4**), acotoxicine (**5**), 14-deacetyl-18-demethyl-pubescenine (**13**), acotoxinine (**14**), neoline (**15**), neolinine (**16**), delectinine (**17**), ACON (**18**), karakoline (**19**), senbusine A (**20**), senbusine C (**21**), SONG (**30**), and napelline (**32**)] were tested for their GIRK inhibitory activities, but none of them exerted remarkable activity. In this study, no hERG activity was recorded for neoline (**15**), karakoline (**19**), senbusine A (**20**), senbusine C (**21**), and napelline (**32**) [22].

Processing of aconite roots results in the transesterification of diester alkaloids with FAs at the C-8 carbon. The amount of the so-called LAs increases during processing, and in properly processed roots, the major native alkaloids [ACON (**18**), hypaconitine (**23**), and mesaconitine (**25**)] can be found only in traces. Contrary to ACON-type alkaloids, LAs are substantially less toxic; the intravenous lethal single doses in animal experiments were 3 magnitudes higher for the latter compounds [23].

The aim of the current study was the synthesis of a series of ACON-derived LAs to study their effects on cardiac K^+-channels. The investigation of compounds that are present in traditionally applied herbal medicines may result in the better understanding of the effects of *Aconitum*-based products and lead to the discovery of new therapeutically useful derivatives.



Compound	R^1	R^2	R^3
1 takaosamine	H	H	CH_3
2 aconosine	H	H	H
3 dolaconine	H	H	$COCH_3$
4 delavaconitine	H	OH	Bz
5 acotoxicine	OH	H	H

35 14-BzA-8-*O*-laurate
36 14-BzA-8-*O*-myristate
37 14-BzA-8-*O*-palmitate
38 14-BzA-8-*O*-stearate
39 14-BzA-8-*O*-palmitoleate
40 14-BzA-8-*O*-oleate
41 14-BzA-8-*O*-γ-linolenate
42 14-BzA-8-*O*-eicosanoate
43 14-BzA-8-*O*-eicosa-11*Z*-enoate
44 14-BzA-8-*O*-eicosa-11*Z*,14*Z*-dienoate
45 14-BzA-8-*O*-eicosa-8*Z*,11*Z*,14*Z*-trienoate
46 14-BzA-8-*O*-eicosa-11*Z*,14*Z*,17*Z*-trienoate
47 14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoate
48 14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoate

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
6 isotalazidin	H	H	CH_2OCH_3	H	H	OH	H	H	H	H	H	CH_2CH_3
7 10-hydroxy-8-*O*-methyltalatizamine	CH_3	H	CH_2OCH_3	H	H	OCH_3	H	OH	H	H	H	CH_2CH_3
8 delcosine	H	H	CH_2OCH_3	OCH_3	OH	OH	H	H	H	H	H	CH_2CH_3
9 ajacine	CH_3	H	CH_2Ant	OCH_3	OH	OH	H	H	H	CH_3	H	CH_2CH_3
10 lycoctonine	CH_3	H	CH_2OH	OCH_3	OH	OH	H	H	H	$COCH_3$	H	CH_2CH_3
11 swatinine	CH_3	H	CH_2OH	OCH_3	OH	OH	H	OH	H	CH_3	H	CH_2CH_3
12 gigactonine	H	H	CH_2OCH_3	OCH_3	OH	OH	H	H	H	H	H	CH_2CH_3
13 14-desacetyl-18-demethylpubescenine	H	H	CH_2OH	OH	OH	OCH_3	H	H	H	H	H	CH_2CH_3
14 acotoxinine	H	H	CH_2OCH_3	OCH_3	H	OVr	H	H	H	H	H	CH_2CH_3
15 neoline	H	H	CH_2OCH_3	OCH_3	H	OH	H	H	H	H	H	CH_2CH_3
16 neolinine	H	H	CH_2OH	OCH_3	H	OH	H	H	H	H	H	CH_2CH_3
17 delectinine	CH_3	H	CH_2OH	OCH_3	OH	OH	H	H	H	H	H	CH_2CH_3
18 aconitine	CH_3	OH	CH_2OCH_3	OCH_3	H	$OCOCH_3$	H	H	OH	Bz	OH	CH_2CH_3
19 karakoline	H	H	CH_2OCH_3	H	H	OH	H	H	H	H	H	CH_2CH_3
20 senbusine A	H	H	CH_2OCH_3	OH	H	OH	H	H	H	H	H	CH_2CH_3
21 senbusine C	H	H	CH_2OCH_3	OCH_3	H	OH	H	H	H	H	OH	CH_2CH_3
22 14-benzoyltalatisamine	CH_3	H	CH_2OCH_3	H	H	OH	H	H	H	Bz	H	CH_2CH_3
23 hypaconitine	CH_3	H	CH_2OCH_3	OCH_3	H	$OCOCH_3$	H	H	OH	Bz	OH	CH_3
24 lappaconitine	CH_3	H	Ant	H	H	OH	OH	H	H	CH_3	H	CH_2CH_3
25 mesaconitine	CH_3	OH	CH_2OCH_3	OCH_3	H	$OCOCH_3$	H	H	H	Bz	OH	CH_3

▶ **Fig. 1** Structures of bisnorditerpene alkaloids (**A**), lipo-alkaloids (**B**), and norditerpene alkaloids (**C**). Ant: Antranoyl, BzA: Benzoyl aconine, Vr: Veratroyl.

Results and Discussion

The transesterification of ACON (**18**) in the C-8 position with unsaturated FAs (E-EN, E-DI, E-8TRI, E-11TRI, and E-TETR) resulted in five LAs (**43–47**) (▶ **Fig. 1**). These LAs have not yet been identified from plants or processed drugs [24, 25] and four of these compounds (**43–46**) have been synthetized for the first time. The ^{1}H NMR spectroscopic data of compounds **43–46** are reported in the Materials and Methods section.

The inhibitory activity of alkaloids and a series of FAs was examined on cardiac potassium (GIRK and hERG) channels. The structure-activity relationships were analyzed together with the previously published activities of LAs (**35–42**), DAs (**1–27**), and FAs (PALO, g-LIN, E-DI, E-TERT, and E-PENT) (▶ **Table 1**) [21, 22, 26]. The GIRK inhibitory activity and the hERG activity of LAs **43–48** are reported here for the first time.

The analysis of the semisynthetic LA series provided information about their possible beneficial cardiac effects and leads to the conclusion of some structure-activity relationships concerning the esterifying FAs. All semisynthetic LAs exerted inhibitory activity on the GIRK channel at 1 and 10 µM concentrations. The GIRK inhibitory activity of saturated and unsaturated FAs was also measured. Free FAs were significantly less active than LAs (**35–48**) ($p < 0.05$). This indicates that the 14-BzA part of the molecule is necessary for the ion channel inhibition (▶ **Fig. 2**). LAs exerted significantly higher activity on GIRK channels than DAs ($p < 0.01$) (▶ **Fig. 3**). At the 10 µM concentration, the inhibitory activities of compounds **35, 39, 41**, and **45–48** were higher than 50 %. The unsaturation of the esterifying FAs seems to be a crucial factor for GIRK inhibitory activity. 14-BzA-*O*-γ-linolenate (**41**), BAE-TETR (**47**), and BAE-PENT (**48**) exerted remarkable activity even at the 1 µM concentration.

Eicosaenoic acid esters **43–48** were the most effective. Generally, compounds with a higher unsaturation degree exerted more potent inhibitory activity (▶ **Fig. 4**), except for compounds having one or two double bonds. The inhibitory potency increased with the number of double bonds in the order 2 < 1 < 3 < 4 < 5.

Compounds that are active on hERG channels are considered cardiotoxic, thus for safety reasons, are not suitable for medical application. The LAs were less cardiotoxic, in general, than ACON (▶ **Fig. 5**), however, compounds **41** and **48** are exceptions since their activity is similar to that of ACON. The GIRK/hERG selectivity of compounds belonging to different structural groups is shown on ▶ **Fig. 6 A**. The selectivity of LAs is significantly higher than that of DAs ($p < 0.001$) (▶ **Fig. 6 B**).

The compounds with the highest GIRK inhibitory activity are g-BALL (**41**) and BAE-PENT (**47**), however, they are also active on hERG channels. Although these compounds are highly selective to GIRK channels, their hERG activity is raising safety concerns, thus making them unsuitable for cardiac application. Two compounds, BAE-8TRI (**45**) and BAE-11TRI (**46**), possess low inhibitory activity on hERG and at the same time they are potent GIRK inhibitors, which renders them worthy of consideration for further pharmacological studies.

In summary, the most characteristic compound for the *Aconitum* genus, ACON (**18**), acts both on the hERG and GIRK potassium channels. However, transesterified ACON-based LAs are selective inhibitors of K^+ channels, with higher activity on GIRK and lower activity on hERG channels. FAs and DAs exert much lower activity on the GIRK channel. This demonstrates that the DA skeleton and the aliphatic FA substituent are both prerequisites for GIRK channel inhibition. The most potent compounds are those with polyunsaturated acyl groups. The most selective and thus particularly promising compounds with the lowest hERG activity are the newly synthesized BAE-8TRI (**45**) and BAE-11TRI (**46**).

▶ **Table 1** GIRK and hERG activity of the compounds (mean ± SD, n = 3).

	GIRK inhibitory activity (%)		hERG inhibitory activity (%)		
	1 µM	10 µM	1 µM	10 µM	30 µM
Takaosamine (**1**)	3 ± 26[c]	20 ± 5[c]		12.5 ± 2.9[a]	
Aconosine (**2**)	15 ± 6	24 ± 9		15.3 ± 3.5[a]	
Dolaconine (**3**)	4 ± 21	10 ± 16		8.3 ± 1.4[a]	
Delavaconitine (**4**)	28 ± 10	44 ± 3			
Acotoxicine (**5**)	15 ± 1	31 ± 7		17.3 ± 3.3[a]	
Isotalazidine (**6**)	21 ± 0[c]	30 ± 1[c]		19.1 ± 3.2[a]	
10-Hydroxy-8-*O*-methyltalatizamine (**7**)	− 3 ± 6[c]	− 5 ± 58[c]		15.8 ± 1.1[a]	
Delcosine (**8**)	23 ± 18[c]	45 ± 1[c]		17.9 ± 2.4[a]	
Ajacine (**9**)	25 ± 8[c]	33 ± 3[c]		13.0 ± 1.7[a]	
Lycoctonine (**10**)	0 ± 4[c]	11 ± 26[c]		13.7 ± 3.3[a]	
Swatinine (**11**)	28 ± 5[c]	37 ± 1[c]		8.9 ± 1.6[a]	
Gigactonine (**12**)	13 ± 3[c]	27 ± 4[c]		38.0 ± 7.4[a]	
14-Desacetyl-18-demethylpubescenine (**13**)	16 ± 6	33 ± 9		6.5 ± 1.9[a]	
Acotoxinine (**14**)	11 ± 8	35 ± 2		6.5 ± 2.2[a]	
Neoline (**15**)	17 ± 1	32 ± 18	3 ± 4	19 ± 5[b] (14.4 ± 3.7[a])	
Neolinine (**16**)	17 ± 4	35 ± 12		35.8 ± 4.7[a]	
Delectinine (**17**)	30 ± 1	40 ± 7		7.7 ± 2.3[a]	
Aconitine (**18**)	15 ± 9[b]	45 ± 9[b]		44.9 ± 7.4[b]	
Karakoline (**19**)	10 ± 2	17 ± 1	3 ± 7[b]	16 ± 10[b]	
Senbusine A (**20**)	19 ± 1	26 ± 8	4 ± 1[b]	11 ± 0[b]	
Senbusine C (**21**)	17 ± 5	20 ± 9	− 1 ± 10	3 ± 19	
Acovulparine (**26**)	12 ± 9[c]	26 ± 6[c]		10.8 ± 2.3[a]	
Septentriodine (**27**)	23 ± 10[c]	37 ± 13[c]		20.9 ± 1.0[a]	
Finetiadine (**28**)	9 ± 11[c]	0 ± 8[c]			
Hetisinone (**29**)	11 ± 8[c]	25 ± 12[c]		14.3 ± 3.9[a]	
Songorine (**30**)	10 ± 10	47 ± 9		13.2 ± 1.8[a]	
Songoramine (**31**)				36.4 ± 5.4[a]	
Napelline (**32**)	14 ± 5	21 ± 8	0 ± 6	10 ± 8	
14-BzA-8-*O*-laurate (**35**)	17 ± 13[c]	65 ± 16[c]		20 ± 18[c]	52 ± 25[c]
14-BzA-8-*O*-myristate (**36**)	12 ± 5[c]	25 ± 9[c]			
14-BzA-8-*O*-palmitate (**37**)	11 ± 9[c]	22 ± 14[c]		39.6 ± 5.6[a]	
14-BzA-8-*O*-stearate (**38**)	31 ± 12[c]	57 ± 13[c]		24 ± 2	43 ± 13
14-BzA-8-*O*-palmitoleate (**39**)	32 ± 6[c]	76 ± 4[c]		20 ± 16[c]	60 ± 6[c]
14-BzA-8-*O*-oleate (**40**)	14 ± 14[c]	35 ± 3[c]			
14-BzA-8-*O*-γ-linolenate (**41**)	42 ± 19[c]	85 ± 8[c]		45 ± 5[c]	92 ± 3[c]
14-BzA-8-*O*-eicosanoate (**42**)	21 ± 4[c]	47 ± 6[c]			
14-BzA-8-*O*-eicosa-11*Z*-enoate (**43**)	8 ± 0	17 ± 9			
14-BzA-8-*O*-eicosa-11*Z*,14*Z*-dienoate (**44**)	18 ± 8	59 ± 13		8 ± 12	33 ± 2
14-BzA-8-*O*-eicosa-8*Z*,11*Z*,14*Z*-trienoate (**45**)	36 ± 14	84 ± 1		22 ± 17	46 ± 19
14-BzA-8-*O*-eicosa-11*Z*,14*Z*,17*Z*-trienoate (**46**)	22 ± 21	78 ± 15		18 ± 4	50 ± 4
14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoate (**47**)	41 ± 4	88 ± 1		37 ± 12	75 ± 8
14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoate (**48**)	42 ± 8	91 ± 1		42 ± 5	82 ± 3
Palmitoleic acid	11 ± 7[c]	36 ± 7[c]			
γ-Linolenic acid	15 ± 5[c]	22 ± 5[c]			

continued

▶ **Table 1** *Continued*

	GIRK inhibitory activity (%)		hERG inhibitory activity (%)		
	1 µM	10 µM	1 µM	10 µM	30 µM
Eicosa-11*Z*,14*Z*-dienoic acid	23 ± 1[c]	31 ± 7[c]			
Eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoic acid	20 ± 6[c]	17 ± 16[c]			
Eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoic acid	20 ± 1[c]	13 ± 19[c]			

BzA: Benzoyl aconine. Data are published in references [a] [21], [b] [22], and [c] [26].

Materials and Methods

Diterpene alkaloids

The examined bisnorditerpene (C_{18}) (**1–5**), norditerpene (C_{19}) (**6–28**), and DAs (C_{20}) (**29–32**) were previously isolated from *Aconitum anthora* L. (**6, 7, 29**) [21], *Aconitum moldavicum* Hacq. (**9, 11, 12**) [27], *Aconitum toxicum* Rchb. (**2–5, 12, 14–16, 18**) [28, 29], *Aconitum vulparia* Rchb. (**8, 10, 17, 26–28**) [30], *Aconitum napellus* L. subsp. *firmum* (**19–21, 32**) [22], and *Consolida orientalis* Gay. (**1, 13**) [31], all belonging to the Ranunculaceae family. The purity (≥ 95 %) of the isolated compounds was confirmed by HPLC and ^{1}H NMR spectroscopy.

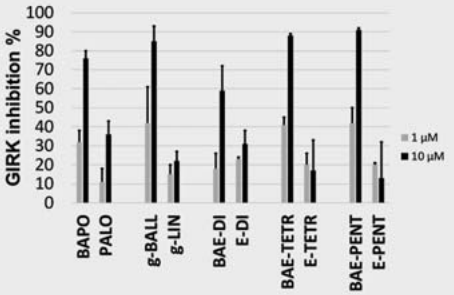
Lipo-alkaloids

The semisynthesis and purification of the compounds was carried out according to the method reported previously by our research group [23]. The identification and structure elucidation of the compounds were performed by means of NMR spectroscopy. The LAs (**35–42, 48**) were obtained by semisynthesis, as reported in our previous work [25]. The synthesis of new LAs **43–48** was carried out according to the modified method of Bai et al. [25, 32]. In the reaction, 20 mg ACON (**18**) was transesterified by 40 mg E-EN, E-DI, E-8TRI, or E-11TRI. The products were separated and purified by a multistep chromatographic method, including centrifugal planar chromatography (Chromatotron 8924, Harrison Research; stationary phase: Al_2O_3 60G neutral type E, Merck, 1 mm; 5 mL/min, cyclohexane/CH_2Cl_2/MeOH 1–5 fraction: 70 : 30 : 1, 6–30 fraction 70 : 30 : 3), gel filtration chromatography (Lipophilic Sephadex LH20100, Sigma-Aldrich; $CHCl_3$/MeOH 1 : 1), and preparative layer chromatography (stationary phase: Al_2O_3 $60F_{254}$ neutral, Merck; eluent: toluene/acetone/EtOH/*cc.* NH_3 70 : 40 : 10 : 3). All chemical substances, including ACON (**18**), were highly purified test reagents and were purchased from Sigma-Aldrich Ltd.

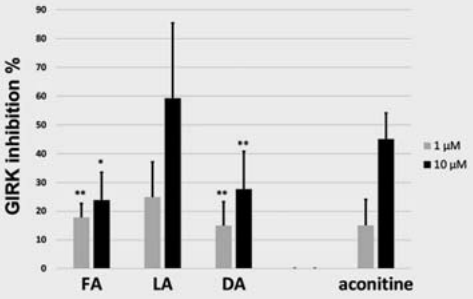
Identification of compounds and structure elucidation

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX 500 spectrometer at 500 MHz. The signals of the deuterated solvent were taken as the reference.

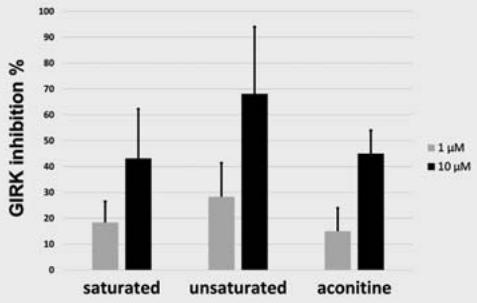
*14-Benzoyl-aconine-8-*O*-eicosa-11Z-enoate* (**43**): colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.03 (2H, d, J = 7.4 Hz, H-2′, H-6′), 7.56 (1H, t, J = 7.3 Hz, H-4′), 7.45 t (2H, J = 7.6 Hz, H-3′, H-5′), 5.36 (2H, m, H-11″, H-12″), 4.86 (1H, d, J = 4.9 Hz, H-14), 4.49 (1H, d, J = 2.3 Hz, 15-OH), 4.44 (1H, m, H-15), 4.03 (1H, d, J = 6.4 Hz, H-6), 3.96 (1H, s, 13-OH), 3.78 (1H, m, H-3), 3.76 (3H, s, 16-OMe), 3.61 (1H, d, J = 8.9 Hz, H-18a), 3.46 (1H, d, J = 8.9 Hz, H-18b), 3.34 (1H, d, J = 5.3 Hz, H-16), 3.30 (3H, s, 18-OMe), 3.26 (3H, s, 1-OMe), 3.16 (3H, s, 6-OMe), 3.13 (1H, t, J = 6.7 Hz, H-1), 3.11 (1H, s, H-17), 2.91 (1H, t, J = 5.8 Hz, H-9), 2.88 (1H, d, J = 11.3 Hz, H-19), 2.84 (1H, s, H-7), 2.73 (1H, m, H-20a), 2.70



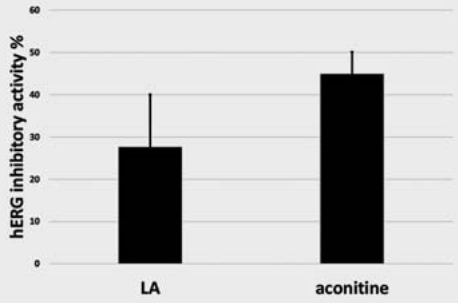
▶ **Fig. 2** GIRK activities of lipo-alkaloids and their corresponding fatty acids. Values are the mean ± SD. BAPO: 14-BzA-8-*O*-palmitoleate, PALO: palmitoleic acid, g-BALL: 14-BzA-8-*O*-γ-linolenate, g-LIN: γ-linolenic acid, BAE-DI: 14-BzA-8-*O*-eicosa-11*Z*,14*Z*-dienoate, E-DI: eicosa-11*Z*,14*Z*-dienoic acid, BAE-TETR: 14-BzA-8-O-eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoate, E-TETR: eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoic acid, BAE-PENT: 14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoate, E-PENT: eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoic acid.



▶ **Fig. 3** GIRK inhibitory activities of lipo-alkaloids (LA, n = 14) compared to fatty acids (FA, n = 5), diterpene alkaloids (DA, n = 27), and aconitine (n = 3). Values are the mean ± S. E. M.; $^*p < 0.05$, $^{**}p < 0.01$.



▶ **Fig. 4** GIRK inhibitory activity of lipo-alkaloids containing saturated (n = 5) and unsaturated (n = 9) acyl groups. Values are the mean ± S. E. M.



▶ **Fig. 5** hERG inhibitory activity of lipo-alkaloids (LA, mean ± S. E. M., n = 10) compared with aconitine (mean ± S. E. M., n = 3) at a 10 µM concentration.

(1H, m, H-12a), 2.34–2.41 (3H, m, H-20b, H-2a, H-19b), 2.14 (3H, m, H-5, H-10, H-12b), 2.01 (5H, m, H-2b, H-10″, H-13″), 1.80 (1H, m, H-2″a), 1.45 (1H, m, H-2″b), 1.32–1.14 (26H, m, 13 × CH_2), 1.10 (3H, t, *J* = 7.0, H-21), 0.89 (3H, t, *J* = 6.5, H-20″).

*14-Benzoyl-aconine-8-*O*-eicosa-11Z,14Z-dienoate* (**44**): colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.02 (2H, d, *J* = 7.4 Hz, H-2′, H-6′), 7.55 (1H, t, *J* = 7.3 Hz, H-4′), 7.45 t (2H, *J* = 7.6 Hz, H-3′, H-5′), 5.37 (4H, m, H-11″, H-12″, H-14″, H-15″), 4.86 (1H, d, *J* = 4.9 Hz, H-14), 4.49 (1H, d, *J* = 2.2 Hz, 15-OH), 4.45 (1H, m, H-15), 4.03 (1H, d, *J* = 6.5 Hz, H-6), 3.96 (1H, s, 13-OH), 3.78 (1H, m, H-3), 3.76 (3H, s, 16-OMe), 3.61 (1H, d, *J* = 8.9 Hz, H-18a), 3.46 (1H, d, *J* = 8.9 Hz, H-18b), 3.34 (1H, d, *J* = 5.4 Hz, H-16), 3.30 (3H, s, 18-OMe), 3.26 (3H, s, 1-OMe), 3.16 (3H, s, 6-OMe), 3.13 (1H, t, *J* = 6.4 Hz, H-1), 3.09 (1H, s, H-17), 2.90 (2H, m, H-9, H-19), 2.84 (1H, s, H-7), 2.79 (2H, m, H-13″), 2.73 (1H, m, H-20a), 2.70 (1H, m, H-12a), 2.37 (3H, m, H-20b, H-2a, H-19b), 2.14 (3H, m, H-5, H-10, H-12b), 2.05 (4H, m, H-10″, H-16″), 1.96 (1H, m, H-2b), 1.79 (1H, m, H-2″a), 1.45 (1H, m, H-2″b), 1.32–1.05 (22H, m, 11 × CH_2), 1.09 (3H, t, *J* = 7.1, H-21), 0.89 (3H, t, *J* = 6.5, H-20″).

*14-Benzoyl-aconine-8-*O*-eicosa-8Z,11Z,14Z-trienoate* (**45**): colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.03 (2H, d, *J* = 7.4 Hz, H-2′, H-6′), 7.56 (1H, t, *J* = 7.3 Hz, H-4′), 7.45 t (2H, *J* = 7.6 Hz, H-3′, H-5′), 5.37 (6H, m, H-8″, H-9″, H-11″, H-12″, H-14″, H-15″), 4.86 (1H, d, *J* = 5.0 Hz, H-14), 4.49 (1H, d, *J* = 2.3 Hz, 15-OH), 4.44 (1H, m, H-15), 4.03 (1H, d, *J* = 6.5 Hz, H-6), 3.95 (1H, s, 13-OH), 3.79 (1H, m, H-3), 3.76 (3H, s, 16-OMe), 3.61 (1H, d, *J* = 8.9 Hz, H-18a), 3.47 (1H, d, *J* = 8.9 Hz, H-18b), 3.34 (1H, d, *J* = 5.4 Hz, H-16), 3.30 (3H, s, 18-OMe), 3.27 (3H, s, 1-OMe), 3.16 (3H, s, 6-OMe), 3.14 (1H, t, *J* = 6.4 Hz, H-1), 3.09 (1H, s, H-17), 2.89 (2H, m, H-9, H-19), 2.84 (4H, m, H-9″, H-13″), 2.83 (1H, s, H-7), 2.73 (1H, m, H-20a), 2.68 (1H, m, H-12a), 2.37 (3H, m, H-20b, H-2a, H-19b), 2.14 (3H, m, H-5, H-10, H-12b), 2.06 (4H, m, H-7″, H-16″), 1.98 (1H, m, H-2b), 1.80 (1H, m, H-2″a), 1.45 (1H, m, H-2″b), 1.30–1.00 (18H, m, 9 × CH_2), 1.10 (3H, t, *J* = 7.0, H-21), 0.89 (3H, t, *J* = 6.5, H-20″).

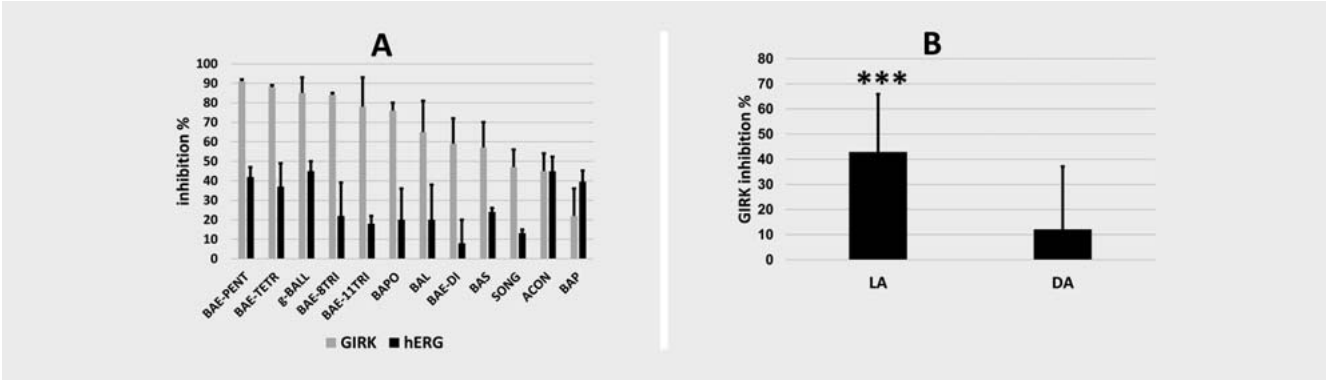
*14-Benzoyl-aconine-8-*O*-eicosa-11Z,14Z,17Z-trienoate* (**46**): colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.04 (2H, d, *J* = 7.3 Hz, H-2′, H-6′), 7.56 (1H, t, *J* = 7.2 Hz, H-4′), 7.46 t (2H, *J* = 7.5 Hz, H-3′, H-5′), 5.37 (6H, m, H-11″, H-12″, H-14″, H-15″, H-17″, H-18″), 4.86 (1H, d, *J* = 5.0 Hz, H-14), 4.49 (1H, d, *J* = 2.2 Hz, 15-OH), 4.44 (1H, m, H-15), 4.03 (1H, d, *J* = 6.5 Hz, H-6), 3.95 (1H, s, 13-OH), 3.79 (1H, m, H-3), 3.76 (3H, s, 16-OMe), 3.61 (1H, d, *J* = 8.9 Hz, H-18a), 3.46 (1H, d, *J* = 8.9 Hz, H-18b), 3.34 (1H, d, *J* = 5.4 Hz, H-16), 3.30 (3H, s, 18-OMe), 3.26 (3H, s, 1-OMe), 3.16 (3H, s, 6-OMe), 3.12 (1H, t, *J* = 6.5 Hz, H-1), 3.09 (1H, s, H-17), 2.90 (2H, m, H-9, H-19), 2.83 (1H, s, H-7), 2.81 (4H, m, H-12″, H-15″), 2.72 (1H, m, H-20a), 2.69 (1H, m, H-12a), 2.37 (3H, m, H-20b, H-2a, H-19b), 2.12 (3H, m, H-5, H-10, H-12b), 2.07 (4H, m, H-10″, H-18″), 1.96 (1H, m, H-2b), 1.78 (1H, m, H-2″a), 1.43 (1H, m, H-2″b), 1.32–1.10 (18H, m, 9 × CH_2), 1.10 (3H, t, *J* = 7.0, H-21), 0.98 (3H, t, *J* = 7.5, H-20″).

Electrophysiological investigations

The automated patch-clamp experiments were executed at room temperature by using planar patch-clamp technology in the whole-cell configuration with a four-channel medium throughput fully automated patch-clamp platform (Patchliner Quattro, Nanion). The pipetting protocols were controlled by PatchControlHT software (Nanion). Data acquisition and online analysis were performed with an EPC-10 Quadro patch-clamp amplifier (HEKA), using PatchMaster software (HEKA).

Automated patch-clamp experiments were carried out on suspension of stable transfected cell lines. Suspension of cells for measurements was derived from the running cell culture. Cells were maintained in an incubator at 37 °C in 5 % CO_2. Before experiments, cells were washed twice with PBS (Life Technologies) and then detached with trypsin-EDTA (PAA) for 30–180 s depending on the cell line. Trypsin was blocked with serum containing complete culture medium. The cell suspension was centrifuged (2 min, 100 *g*) next, resuspended in serum-free base medium at a final density of 1 × 10^6-5 × 10^6 cells/mL, and kept in the cell hotel of the Patchliner. Cells were recovered after 15–30 min and remained suitable for automated patch-clamp recordings for up to 4 h.

Stock of extra- and intracellular solutions were made for automated patch-clamp recordings on stable transfected cell lines. Chemicals were purchased from Sigma-Aldrich Ltd. All solutions were sterile filtered. Aliquots were stored at − 20 °C and warmed up to room temperature before use.



▶ **Fig. 6** **A** Selectivity of the most potent compounds on the GIRK and hERG channels (n = 3, values are the mean ± SD.). **B** Inhibitory activities of lipo-alkaloids (LA, mean ± S. E. M., n = 10) and diterpene alkaloids (DA, mean ± S. E. M., n = 25) on GIRK channels at 10 µM. BAE-PENT: 14-BzA-8-*O*-eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoate, BAE-TETR: 14-BzA-8-O-eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoate, g-BALL: 14-BzA-8-*O*-γ-linolenate, BAE-8TRI: 14-BzA-8-*O*-eicosa-8*Z*,11*Z*,14*Z*-trienoate, BAE-11TRI: 14-BzA-8-*O*-eicosa-11*Z*,14*Z*,17*Z*-trienoate, BAPO: 14-BzA-8-*O*-palmitoleate, BAL: 14-BzA-8-*O*-laurate, BAE-DI: 14-BzA-8-*O*-eicosa-11*Z*,14*Z*-dienoate, BAS: 14-BzA-8-*O*-stearate, SONG: songorine, ACON: aconitine, BAP: 14-BzA-8-*O*-palmitate. Selectivity was taken as a difference of GIRK and hERG activity (**A**); ***p < 0.001 (**B**).

GIRK channel inhibitory assay

Experiments were executed on HEK293 cells stably expressing the GIRK1/4 (Kir3.1/3.4) K^+ channels. The GIRK channel inhibitory assay was developed in our laboratory as the manual patch-clamp method of Hashimoto et al. [33] was adapted to the automated planar patch-clamp technology. The cell line was purchased from UCL Business PLC. Cells were maintained in MEM medium (PAA) supplemented with 10% FBS (PAA) and 182 µg/mL zeocin (Life Technologies).

The undermentioned solutions were used during patch-clamp recordings (compositions in mM): external solution: NaCl 140, KCl 4, glucose-monohydrate 5, $MgCl_2$ 1, $CaCl_2$ 3, and HEPES 10 (pH 7.4, NaOH); high K^+ external solution: NaCl 135, KCl 25, $MgCl_2$ 1, $CaCl_2$ 3, and HEPES 10 (pH 7.4, NaOH); K^+-free external solution: NaCl 160, $MgCl_2$ 1, $CaCl_2$ 3, and HEPES 10 (pH 7.4, NaOH); internal solution: K-gluconate 40, NaCl 20, KF 60, EGTA 20, and HEPES 10 (pH 7.2, KOH), supplemented with 0.9 mM GTPγS before the experiments to induce channel activation.

The voltage protocol for GIRK ion channel assays started with a depolarizing voltage step to 60 mV for 100 ms before a 500-ms hyperpolarizing ramp to − 140 mV was applied. The membrane potential then remained at − 140 mV for 100 ms before returning to the holding potential of − 40 mV. The inward currents were calculated from the − 140 mV segment. The pulse frequency was 0.1 Hz. Recording started in the normal external solution (4 mM K^+). High K^+ external solution was then applied to increase the current amplitude. After a 3-min control period, the test compounds were added to the cells in two concentrations, each for approximately 3 min. Propafenone (1 µM, purity > 98%; Sigma-Aldrich Ltd.) was used as a reference compound, and then K^+-free external solution was applied. The data were corrected with the current values measured in the K^+-free external solution, which served as the baseline.

hERG channel inhibitory assay

A modified assay by Polonchuk [34] was used for measurements of the hERG channel effect of the investigated compounds. Measurements were performed on HEK293 cells stably expressing the hERG (K_v 11.1) K^+ channel. The cell line was purchased from Cell Culture Service. Cells were maintained in IMDM (PAA) medium supplemented with 10% FBS (PAA), 2 mM L-glutamine (Life Technologies), 1 mM Na-piruvate (PAA), and 500 µg/mL G418 (PAA).

The following solutions were used during patch-clamp experiments (compositions in mM): external solution: NaCl 140, KCl 4, glucose-monohydrate 5, $MgCl_2$ 1, $CaCl_2$ 3, and HEPES 10 (pH 7.4, NaOH); internal solution: KCl 50, NaCl 10, KF 60, EGTA 20, and HEPES 10 (pH 7.2, KOH).

The voltage protocol for the hERG ion channel started with a short (100 ms) − 40 mV step as a reference. A 20 mV depolarizing step was applied for 3 s, and then the test potential was − 40 mV for 1 s to evoke an outward tail current. Holding potential was − 80 mV; pulse frequency was 0.1 Hz. The peak tail current was corrected and the leak current defined during the first period to − 40 mV. The experiment started in external solution. After the control period, increasing concentrations of the test compound were applied, each for approximately 5 min. Ten µM amitriptyline (purity > 98%; Sigma-Aldrich Ltd.) was applied as a reference inhibitor, then a wash-out step terminated the pipetting protocol.

Statistical analysis

The distribution of data was checked for normality by the Shapiro-Wilk test. In cases of normal distribution, two sample Student's test, one-way ANOVA, and a post hoc Bonferroni test ($p < 0.05$) were used. When a variable was not normally distributed, the Kruskal-Wallis test was used for evaluation. In cases of significance ($p < 0.05$), the data were tested using the Mann-Whitney test to show which groups were significantly different from each other. The experimental data are expressed as the mean ± SD or mean ± SEM. All statistical analyses were carried out in R (version

3.3.2; The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org).

Acknowledgements

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] Pharmacopoeia of the People's Republic of China. Vol. I. 10th ed. Beijing: People's Medical Publishing House; 2010

[2] Friese J, Gleitz J, Gutser UT, Heubach JF, Matthiesen T, Wilffert B, Selve N. *Aconitum* sp. alkaloids: the modulation of voltage-dependent Na+ channels, toxicity and antinociceptive properties. Eur J Pharmacol 1997; 337: 165–174

[3] Chan TYK, Kelly SP. Aconite poisoning. Med J Aust 1990; 153: 499

[4] Ameri A. The effects of *Aconitum* alkaloids on the central nervous system. Prog Neurobiol 1998; 56: 211–235

[5] Liu Q, Zhuo L, Liu L, Zhu S, Sunnassee A, Liang M, Zhou L, Liu Y. Seven cases of fatal aconite poisoning: forensic experience in China. Forensic Sci Int 2011; 212: e5–e9

[6] Dzhakhangirov FN, Sultankhodzhaev MN, Tashkhodzhaev B, Salimov BT. Diterpenoid alkaloids as a new class of antiarrhythmic agents. Structure-activity relationship. Chem Nat Compd 1997; 33: 190–202

[7] Wang YP, Chen WZ, Wang XL, Hua Z. Inhibition of guan-fu base A on delayed rectifier current (Ik) in guinea pig ventricular myocytes. Yao Xue Xue Bao 1996; 31: 581–584

[8] Wright SN. Irreversible block of human heart (hH1) sodium channels by the plant alkaloid lappaconitine. Mol Pharmacol 2001; 59: 183–192

[9] Vakhitova YV, Farafontova EI, Khisamutdinova RY, Yunusov VM, Tsypysheva IP, Yunusov MS. A study of the mechanism of the antiarrhythmic action of allapinin. Russ J Bioorganic Chem 2013; 39: 92–101

[10] Song MK, Liu H, Jiang HL, Yue JM, Hu GY. Electrophysiological characterization of 14-benzoyltalatisamine, a selective blocker of the delayed rectifier K+ channel found in virtual screening. Eur J Pharmacol 2006; 531: 47–53

[11] Chen WZ, Dong YL, Zhang YF, Ding GS. Anti-arrhythmia effects of guan-fu base A. Zhongguo Yao Li Xue Bao 1983; 4: 247–250

[12] Wang YP, Chen WZ, Sun WK, Wu JX. Hemodynamic actions of guan-fu base A in anesthetized rats. Zhongguo Yao Li Xue Bao 1992; 13: 231–234

[13] Tai CJ, El-Shazly M, Wu TY, Lee KT, Csupor D, Hohmann J, Chang FR, Wu YC. Clinical aspects of *Aconitum* preparations. Planta Med 2015; 81: 1017–1028

[14] Huang X, Yang Y, Zhu J, Dai Y, Pu J. The effects of a novel anti-arrhythmic drug, acehytisine hydrochloride, on the human ether-a-go-go related gene K+ channel and its trafficking. Basic Clin Pharmacol Toxicol 2009; 104: 145–154

[15] Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. Nature 2006; 440: 463–469

[16] Dobrev D, Friedrich A, Voigt N, Jost N, Wettwer E, Christ T, Knaut M, Ravens U. The G protein-gated potassium current I(K,ACh) is constitutively active in patients with chronic atrial fibrillation. Circulation 2005; 112: 3697–3706

[17] Hashimoto N, Yamashita T, Tsuruzoe N. Tertiapin, a selective IKACh blocker, terminates atrial fibrillation with selective atrial effective refractory period prolongation. Pharmacol Res 2006; 54: 136–141

[18] Li Y, Tu D, Xiao H, Du Y, Zou A, Liao Y, Dong S. Aconitine blocks HERG and Kv1.5 potassium channels. J Ethnopharmacol 2010; 131: 187–195

[19] Xie S, Jia Y, Liu A, Dai R, Huang L. Hypaconitine-induced QT prolongation mediated through inhibition of KCNH2 (hERG) potassium channels in conscious dogs. J Ethnopharmacol 2015; 166: 375–379

[20] Huang X, Yang Y, Zhu J, Xu D, Peng J, Liu J. Comparative effects of guanfu base A and guanfu base G on hERG K+ channel. J Cardiovasc Pharmacol 2012; 59: 77–83

[21] Forgo P, Borcsa B, Csupor D, Fodor L, Berkecz R, Molnár VA, Hohmann J. Diterpene alkaloids from *Aconitum anthora* and assessment of the hERG-inhibiting ability of *Aconitum* alkaloids. Planta Med 2011; 77: 368–373

[22] Kiss T, Orvos P, Bánsághi S, Forgo P, Jedlinszki N, Tálosi L, Hohmann J, Csupor D. Identification of diterpene alkaloids from *Aconitum napellus* subsp. *firmum* and GIRK channel activities of some *Aconitum* alkaloids. Fitoterapia 2013; 90: 85–93

[23] Borcsa B, Csupor D, Forgo P, Widowitz U, Bauer R, Hohmann J. *Aconitum* lipo-alkaloids-semisynthetic products of the traditional medicine. Nat Prod Commun 2011; 6: 527–536

[24] Csupor D, Wenzig EM, Zupkó I, Wölkart K, Hohmann J, Bauer R. Qualitative and quantitative analysis of aconitine-type and lipo-alkaloids of *Aconitum carmichaelii* roots. J Chromatogr A 2009; 1216: 2079–2086

[25] Borcsa B, Widowitz U, Csupor D, Forgo P, Bauer R, Hohmann J. Semisynthesis and pharmacological investigation of lipo-alkaloids prepared from aconitine. Fitoterapia 2011; 82: 365–368

[26] Borcsa B, Kiss T, Csupor D, Orvos P, Tálosi L, Hohmann J. The effect of diterpene alkaloids on the GIRK channels. Planta Med 2015; 81: 1546–1547

[27] Borcsa B, Fodor L, Csupor D, Forgo P, Molnár A, Hohmann J. Diterpene alkaloids from the roots of *Aconitum moldavicum* and assessment of Nav 1.2 sodium channel activity of *Aconitum* alkaloids. Planta Med 2014; 80: 231–236

[28] Csupor D, Forgo P, Csedő K, Hohmann J. C19 and C20 diterpene alkaloids from *Aconitum toxicum* Rchb. Helv Chim Acta 2006; 89: 2981–2986

[29] Csupor D, Forgo P, Wenzig EM, Bauer R, Hohmann J. Bisnorditerpene, norditerpene, and lipo-alkaloids from *Aconitum toxicum*. J Nat Prod 2008; 71: 1779–1782

[30] Csupor D, Forgo P, Zupkó I, Szabó P, Hohmann J. Anthranoyl-substituted norditerpene alkaloids from *Aconitum vulparia* Rchb. and their cytotoxic activities. Z Naturforsch 2007; 62 b: 135–141

[31] Hohmann J, Forgo P, Hajdú Z, Varga E, Máthé I. Norditerpenoid alkaloids from *Consolida orientalis* and complete 1H and 13C NMR signal assignments of some lycoctonine-type alkaloids. J Nat Prod 2002; 65: 1069–1072

[32] Bai Y, Desai HK, Pelletier SW. Long-chain fatty acid esters of some norditerpenoid alkaloids. J Nat Prod 1994; 57: 963–970

[33] Hashimoto N, Yamashita T, Tsuruzoe N. Characterization of *in vivo* and *in vitro* electrophysiological and antiarrhythmic effects of a novel IKACh blocker, NIP-151: a comparison with an IKr-blocker dofetilide. J Cardiovasc Pharmacol 2008; 51: 162–169

[34] Polonchuk L. Toward a new gold standard for early safety: automated temperature-controlled hERG test on the PatchLiner®. Front Pharmacol 2012; 3: 3