Antiarrhythmic agents based on diterpenoid alkaloids*

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Diterpenoid alkaloids comprise a large group of natural compounds produced by the plants of the genera Aconitum and Delphinium. Some of these compounds were found to manifest valuable pharmacological properties, including the unique antiarrhythmic activity. Allapinine (derived from the alkaloid lappaconitine) was approved as a drug. The investigation of the structure—activity relationship revealed structural elements responsible for the arrhythmogenic and antiarrhythmic properties.

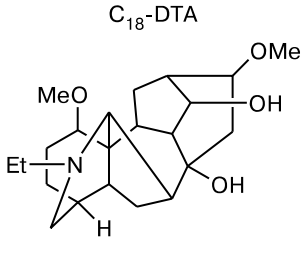
Key words: diterpenoid alkaloids, antiarrhythmic agents, arrhythmogenic agents, structure—activity relationships.

In spite of advances in the development and use of new antiarrhythmic agents, the pharmacotherapy of cardiac arrhythmia remains a challenge. The present review summarizes some aspects related to the design of antiarrhythmic agents based on diterpenoid alkaloids.

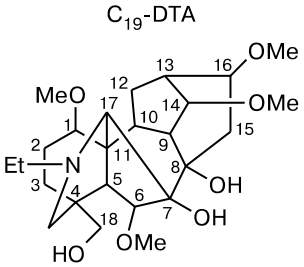
Diterpenoid alkaloids (DTA) comprise one of the most widespread groups of alkaloids. Plants producing these substances have long since been used in folk medicine and have attracted attention of chemists and pharmacologists for a long time.[1]

The structures of DTA were for the most part determined in the 1950s. They can be divided into the following main types: C_{19}- and C_{20}-DTA belonging to the most abundant group; C_{18}-DTA in which one carbon atom of the skeleton is absent; heteratisine-type alkaloids containing the lactone ring (only nine compounds of this group are currently known). The structures of representatives of these groups are given below.

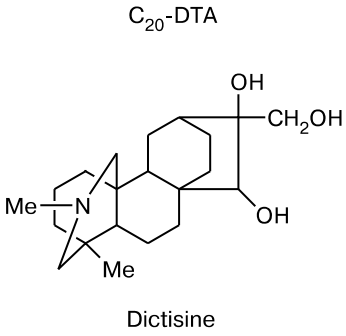
Plants of the genera Aconitum and Delphinium, which are widespread in the Northern hemisphere, including



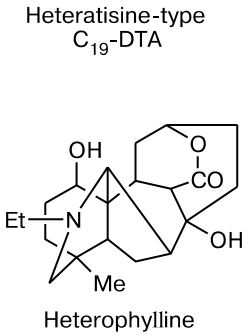
Aconosine



Licoctonine



Dictisine



Heterophylline

Russia, where apparently the largest stocks of these plants are localizes, are the main sources of DTA.[2]

Plants of the genus Aconitum are highly toxic. Many C_{19}-DTA are arrhythmogenic agents (cause arrhythmia). The diterpenoid alkaloid aconitine (**1**) is used by pharmacologists for the development of a cardiac arrhythmia model (the so-called aconitine model) and is one of the strongest plant toxins (comparable with the cobra venom).[1]

Diterpenoid alkaloids were found to possess various pharmacological properties, including antiarrhythmic, arrhythmogenic, local anaesthetic, anti-inflammatory, psychostimulant, antidepressant, spasmolytic, ganglionic blocking, curare-like, antidote (against aconitine), antiulcer, and antitumor activities.[1,3,4]

Prior to our studies, only one drug based on the diterpenoid alkaloid methyllycaconitine (**2**) (methyllycaconitine hydroiodide, the trade name Mellictine) belonging to the muscle relaxants (agents with curare-like activity) has been approved in medicine, but it has found little use. Nevertheless, this drug has attracted attention because it proved to be relatively effective when administered orally.[5]

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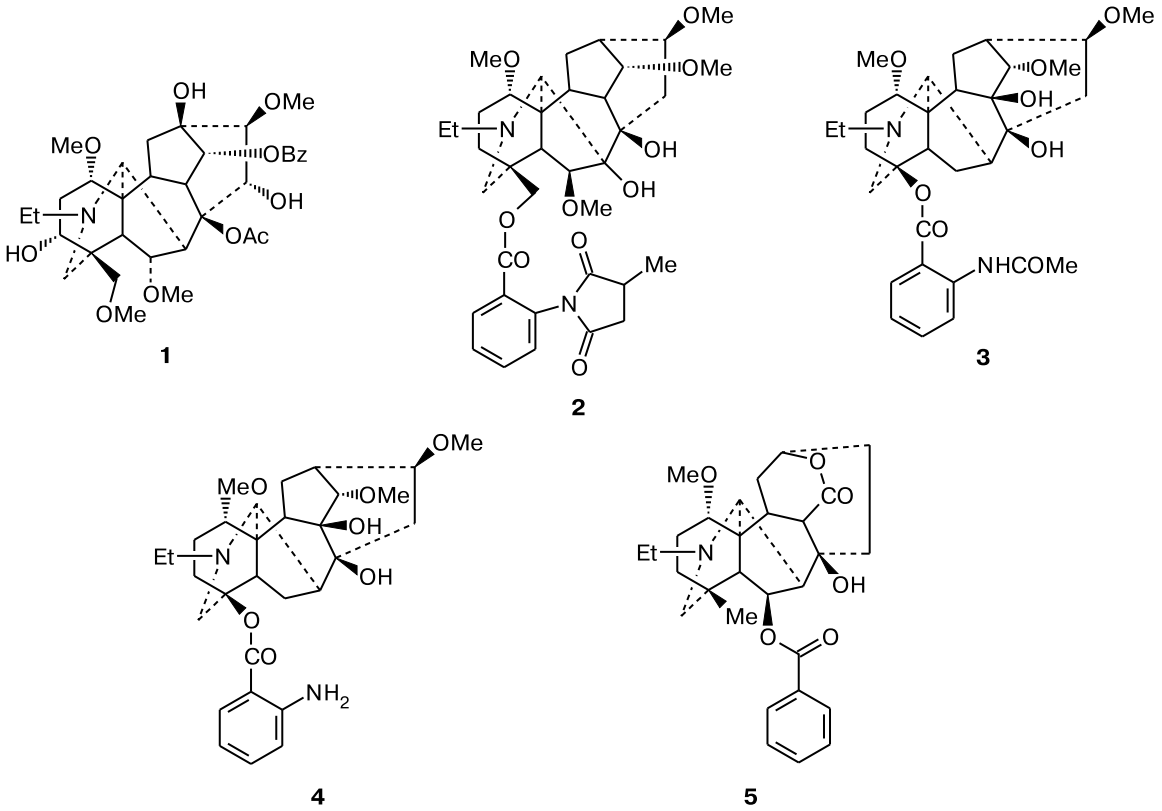


Collaborative works of botanists, chemists, and pharmacologists showed that certain DTA and their derivatives are unique antiarrhythmic agents. The following four agents were subjected to comprehensive trials: lappaconitine (**3**), *N*-deacetyllappaconitine (**4**), *O*-benzoylheteratisine (**5**), and azopelline (**6**) (1-benzoylnapelline (**7**)) (Scheme 1). Each agent has its own specificity (Table 1).

Allapinine (lappaconitine hydrobromide) has passed clinical trials and was commercially marketed and used in medical practice since 1987.[6] Cardiologists believe that Allapinine can be considered as the first-line drug for the treatment of certain types of cardiac arrhythmia due to its unique properties.[4,7] It is constantly included in the list of vitally important drugs issued by the Ministry

Scheme 1

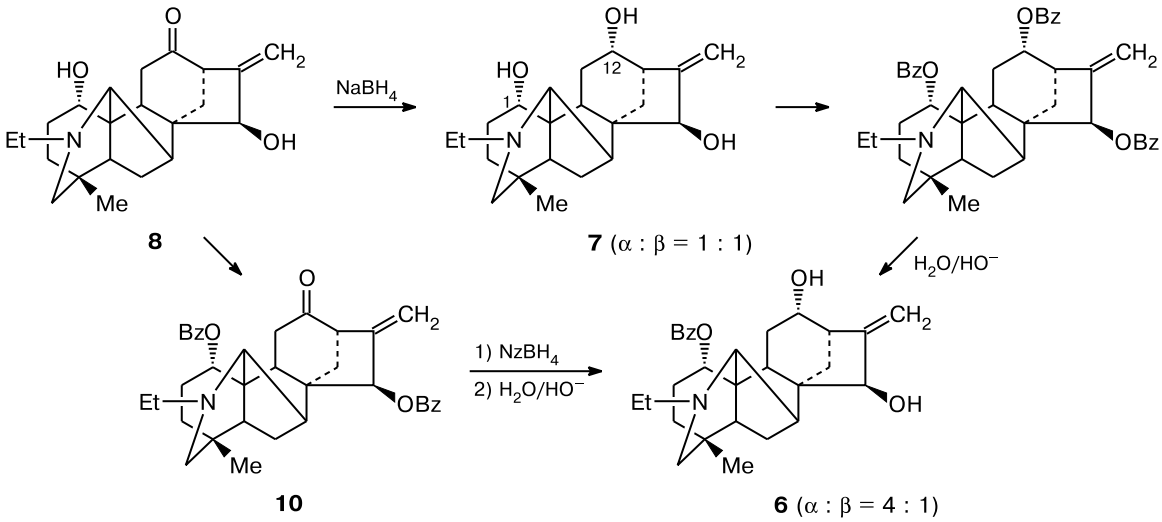


Table 1. Comparative efficacy of diterpenoid alkaloids and known antiarrhythmic agents for aconitine-induced arrhythmia in rats

Agents	ED_{50}	LD_{50}	Antiarrhythmic index, LD_{50}/ED_{50}
	mg kg^{-1}		
Allapinine*	0.05	5.9	118.0
Azopelline (**6**)*	0.24	32.0	133.3
Napelline (**7**)*	10.0	87.5	8.7
Heteratisine (**11**)*	12.6	180.0	14.4
Benzoylheteratisine (**5**)*	0.035	4.0	114.3
Dihydroatisine (**14**)*	1.0	38.0	38.0
Quinidine	20.0	66.9	3.3
Novocainamide	40.0	138.0	3.4
Aimaline	5.0	33.0	6.6
Rytmilen	4.0	42.0	10.5
Mexiletine	7.0	35.0	5.0
Lidocaine	15.0	39.0	2.6
Obsidane	5.7	28.0	4.9

* Diterpenoid alkaloids.

of Healthcare and Social Development of the Russian Federation.

Allapinine plays an important role in the prevention of attacks of paroxysmal ventricular fibrillation.[7,8] The antiarrhythmic activity of Allapinine is associated with suppression of the fast incoming sodium current. An important characteristic feature of the interaction of Allapinine with sodium channels is the long-term binding.[7]

The major metabolite of Allapinine, *viz.*, *N*-deacetyllappaconitine (**4**), is as efficient as Allapinine on most of arrhythmia models but it is less toxic, has the wider therapeutic index, and is characterized by the more rapid development of the antiarrhythmic effect compared to Allapinine. However, the metabolite has a shorter-term effect compared to Allapinine.[7] Other metabolites (minor) of Allapinine (eight metabolites were characterized)[9,10] also have pronounced antiarrhythmic activity and are similar in the spectrum and strength of pharmacological activities to Allapinine. The biotransformation of Allapinine occurs quite rapidly as evidenced by the appearance of metabolites in urine and blood within 2 h after its single intragastric administration. The main biotransformation pathways of Allapinine are deacetylation, hydroxylation, demethylation, and demethoxylation. The pharmacological action observed after the administration of Allapinine into the organism should be considered as the sum of the effects of Allapinine and its metabolites.

The availability is an important problem for Allapinine, like for other phytoagents. In the Russian Federation, northern wolfsbane (*Aconitum septentrionale* Koelle) serving as a source for the preparation of this drug occupies large areas ranging from the Kola Peninsula to the Moscow, Chelyabinsk, Kemerovo, Magadan, Syktyvkar, Arkhangelsk, and other regions.[3] However, the conditions of harvesting of wolfsbane are quite difficult.

Samples of the plant collected from approximately 100 areals were investigated. The lappaconitine content varies from 0.3 to 2.5% of the dry root weigh (on the average, 0.6—0.7%) and depends only slightly on the vegetation period. Currently, the raw material is procured primarily in the Altai territory. The substance is produced at the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic Uzbekistan (Tashkent), and the drug formulation is produced at the Plant of the All-Russian Institute of Medicinal Plants (Moscow Region). The production of the substance has increased by approximately a factor of 30 since 1987 and amounted up to 250 kg in 2010. However, all attempts to culture northern wolfsbane (*Aconitum septentrionale* Koelle, Russia) and *Aconitum leucostomum* Worosch (Kirgiziya, Kazakhstan), including the use of bioengineering techniques (cell cultures, tissue cultures, clonal reproduction), failed.

The problem of an increase in the availability of antiarrhythmic agents based on DTA can be solved in another way by designing new drug formulations with the aim of increasing the activity of the agents. For example, the synthesis of complexes of lappaconitine (**3**) (the active substance of the drug Allapinine) with glycyrrhizic acid showed promise. The new drug is much cheaper, 3—4 times more active, approximately 10 times less toxic, and, consequently, more available; it is devoid of undesirable adverse side effects.[11] Synthetic aza(diaza)bicyclononane compounds, which can be considered as fragments of diterpenoid alkaloids,[12] also exhibit high activity.

Azopelline (**6**), benzoylheteratisine (**5**), and *N*-deacetyllappaconitine (**4**) are other antiarrhythmic agents based on diterpenoid alkaloids, which have been studied in detail.

Azopelline is particularly efficient against ventricular fibrillation and is a good antidote for aconitine poisoning.[4]

Azopelline was designed based on the alkaloid napelline (**7**), it is its 1-*O*-benzoyl derivative (**6**).[13] Unfortunately, there are no good plant sources of napelline. Meanwhile, azopelline (**6**) can be synthesized from songorine (**8**).

We found that *Aconitum monticola* Steinb is a good source of songorine (**8**) (see Scheme 1).[14] The remarkable feature of this plant is that it does not contain the highly toxic alkaloids aconitine (**2**) and mesaconitine (**9**) (see Scheme 1), which generally accompany songorine.

Our research group developed a facile method for the synthesis of azopelline (**6**) from songorine (**8**) (see Scheme 1). The problem was to develop the stereospecific method for the reduction of the carbonyl group of songorine to the α-hydroxy group (napelline). The direct reduction of songorine with $NaBH_4$ in ethanol afforded a mixture of napelline and isonapelline (C(12)—β-OH) in a ratio of 1 : 1. It was difficult to isolate napelline from this mixture, and the yield of napelline was low. The reduction of the dibenzoyl

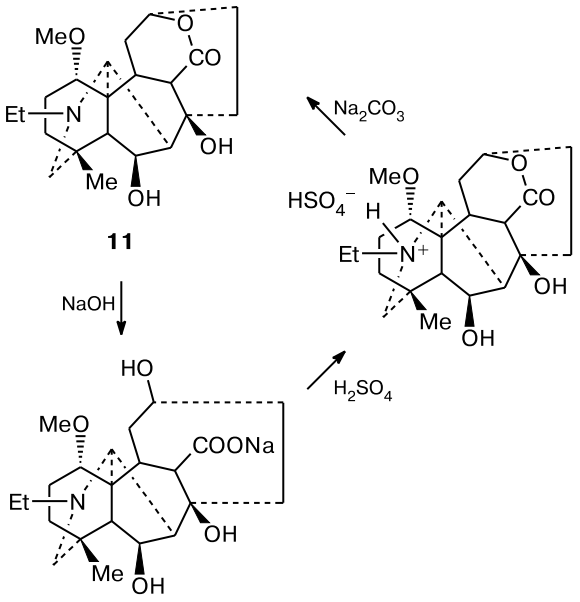
derivative of songorine (**10**) appeared to be a good idea. In this case, dibenzoylnapelline and dibenzoylisonapelline formed in a ratio of 4 : 1, and the former was easily separated in the pure form by recrystallization.[13]

Earlier, our research group has shown that saponification of the acetyl group in the position C(1) of C_{19}-DTA occurs with difficulty due to which 1-*O*-acetyl derivatives can be synthesized in good yields.[15]

This observation was used for the preparation of the 1-*O*-benzoylnapelline, *viz.*, azopelline. It appeared that the benzoyl group in the position C(1) is more difficult to remove than the 1-*O*-acetyl group, which made it possible to perform saponification of dibenzoylnapelline and obtain 1-*O*-benzoylnapelline in quantitative yield.[16]

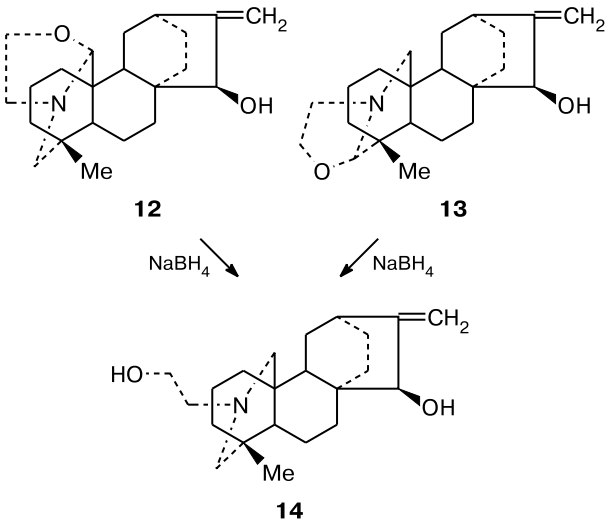
Heteratisine (**11**), atisine (**12**), and isoatisine (**13**) (Schemes 2 and 3) are components of the plant *Aconitum zeravshanicum* Steinb. endemic to Tajikistan.[17] It is difficult to separate atisine and isoatisine from heteratisine; besides, atisine is easily transformed into isoatisine.

Scheme 2



Our research group developed a facile method for the quantitative separation of heteratisine from atisine and isoatisine (see Scheme 2). The lactone ring opening in heteratisine upon heating in ethane alkali easily afforded the salt of the corresponding hydroxy acid. Other alkaloids were extracted with chloroform and precipitated as a mixture of hydrochlorides. The reduction of a mixture of atisine and isoatisine (with $NaBH_4$ in EtOH) gave the only product, *viz.*, dihydroatisine (**14**) (see Scheme 3), which proved to be an efficient antiarrhythmic agent.[18] The alkaline solution was neutralized. The pure heteratisine was extracted with chloroform and was used for the synthesis of benzoylheteratisine (**5**).

Scheme 3



The effect of benzoylheteratisine (**5**) is achieved very rapidly, at the moment of the administration, but the duration of its action is 1.5—2 h.[14,19,20]

It should be noted that all antiarrhythmic agents based on DTA are good antidotes for poisoning with lethal doses of aconitine and related alkaloids. This is an important problem because the poisoning in humans and animals with plants containing aconitine and related alkaloids still happens.

Figure 1 schematically presents the relative efficiency of the known antiarrhythmic agents. Is can be seen that agents based on the diterpenoid alkaloids, particularly azopelline (**6**) and benzoylheteratisine (**5**), and much more efficient than other antidotes in the case of administration of the 1/10 dose of LD_{50}.

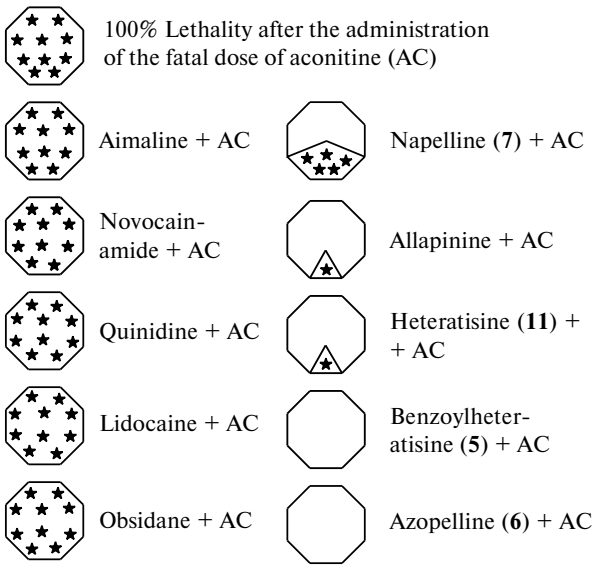
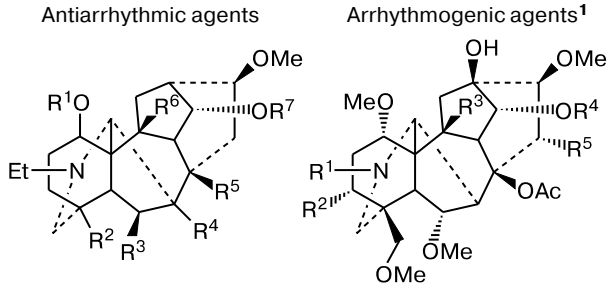


Fig. 1. Antidote action of agents in a dose equal to 1/10 of LD_{50} for aconitine poisoning.

Table 2. Compounds having antiarrhythmic and arrhythmogenic properties

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7
			Antiarrhythmic agents				
14-Benzoyltalatisamine[21]	Me	CH_2OMe	H	H	OH	H	Bz
1-Benzoylkaracoline[22]	Bz	Me	H	H	OH	H	H
6-Benzoyleldelidine[23]	Me	Me	Bz	—OCH_2O—		OH	Me
6-Benzoyldelcorine[4]	Me	CH_2OMe	Bz	—OCH_2O—		H	Me
			Arrhythmogenic agents				
Aconitine (**2**)	Et	OH	H	Bz	OH		
Mesaconitine (**9**)	Me	OH	H	Bz	OH		
Bikaconitine	Et	H	H	$COC_6H_3(OMe)_2$-3,4	H		
Pseudoaconitine	Et	OH	H	$COC_6H_3(OMe)_2$-3,4	H		
Aconifine	Et	OH	OH	Bz	OH		
Jesaconitine	Et	OH	H	COC_6H_4OMe-4	OH		



It should be noted that DTA containing the same skeleton and similar functionalization can exhibit both arrhythmogenic and antiarrhythmic properties (Table 2).

It was of interest to reveal which functional groups are responsible for particular properties.

Our research group studied approximately 200 DTA and their derivatives and revealed structure—antiarrhythmic (or arrhythmogenic) activity relationships. Thus the modeling of the structure of aconitine (Table 3) by selective acetylation of hydroxy groups showed that the blocking of the hydroxy groups at positions 3 and 15 (**15**, **16**) does not lead to the disappearance of the aconitine-like toxicity. However, the acylation of all hydroxy groups, including position 13 (**17**), leads to the disappearance of toxicity. The removal of all ester groups from the aconitine molecule also results in the formation of the nontoxic compound, *viz.*, aconine (**18**). Based on the experimental data and the analysis of the literature, it can be concluded that the hydroxy group at C(13) and the benzoyl group or another aromatic acid group at O(14) are required for the arrhythmogenic properties of C_{19}-DTA. The presence of the acetoxy group at the C(8) atom is responsible for toxic properties.

Table 3. Toxicity of aconitine and its derivatives

Compound	LD_{50}*
Aconitine (**2**)	0.125
3-Acetylaconitine (**15**)	0.27
3,15-Diacetylaconitine (**16**)	3.5
3,15,13-Triacetylaconitine (**17**)	150.0
Aconine (**18**)	200.0
14-Benzoylaconine	16.0

* Studies were carried out in mice, the agent was administered intravenously.

The absence of the hydroxy group at C(13) and the presence of the aromatic (heteroaromatic) acid or benzyl group at the position O(1), O(4), O(6), or O(14) are prerequisites for the manifestation of antiarrhythmic properties. The *N*-(β-hydroxyethyl) group and the aromatic acid group at C(1) play an important role in the antiarrhythmic properties of C_{20}-DTA. It should be noted (see Table 1) that certain diterpenoid alkaloids, such as heteratisine (**11**) (C_{19}-DTA), songorine (**8**), napelline (**7**), and dihydroatisine (**14**) (C_{20}-DTA), which do not contain ester groups, also exhibit pronounced antiarrhythmic properties.[4] However, although the antiarrhythmic index of these alkaloids is quite high, the activity of phytoagents is insufficient for their use in medicine because of their limited availability.

The revealed relationship allows one to perform the rational synthesis of compounds with antiarrhythmic activity from inactive DTA.

Antiarrhythmic agents based on diterpenoid alkaloids are efficient and often unique. Taking into account the structure—activity relationship, it is reasonable to design efficient antiarrhythmic agents based on available alkaloids isolated from plants of the genus Delphinium because these plants are easy to culture.

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