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Non-natural 2*H*-azirine-2-carboxylic acids: an expedient synthesis and antimicrobial activity†

Pavel A. Sakharov,[a] Alexander N. Koronatov,[a] Alexander F. Khlebnikov,[a] Mikhail S. Novikov,[a] Artem G. Glukharev,[a] Elizaveta V. Rogacheva,[ab] Liudmila A. Kraeva,[b] Vladimir V. Sharoyko,[a] Tatiana B. Tennikova[a] and Nikolai V. Rostovskii*[a]

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Non-natural 2*H*-azirine-2-carboxylic acids were obtained in high yields by $FeCl_2$-catalyzed isomerization of 5-chloroisoxazoles to azirine-2-carbonyl chlorides followed by their hydrolysis. The 3-aryl- and 3-heteroaryl-substituted acids are stable during prolonged storage, exhibit antibacterial activity against ESKAPE pathogens and show a low level of cytotoxicity.

Introduction

Infectious diseases still remain among the five main causes of death in the World and cause 13 million deaths per year.[1] At the same time, the effectiveness of existing antibacterial drugs has been increasingly reduced during recent decades due to the constant growth in the number of microorganisms that are resistant to the drugs.[2] Resistant microorganisms are becoming clinically widespread and they pose a serious threat to world health. Therefore, development of new compounds with antibacterial activity is one of the most significant challenges in modern medicinal and pharmaceutical chemistry.[3]

In 1971, azirinomycin (3-methyl-2*H*-azirine-2-carboxylic acid), the first example of an azirine-2-carboxylic acid, was isolated from the soil bacterial strain *Streptomyces aureus* (Fig. 1).[4] It was found that azirinomycin exhibited a wide spectrum of antibacterial activity *in vitro* against both Gram-positive and Gram-negative bacteria.[4a] *In vivo* studies in mice have shown rather high toxicity of azirinomycin of 50% purity isolated from natural raw materials.[4a] As far as azirinomycin was very unstable compound, it was not definitely established whether the observed toxicity was related to azirinomycin, its decomposition products or impurities present in the sample.

Motualevic acid F, isolated from the marine sponge *Siliquariaspongia* (Fig. 1), was the first reported example of long chain 2*H*-azirine-2-carboxylic acids.[5] Motualevic acid F showed inhibitory activity against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* at low acid loadings.[5] According to observations, the presence of a carboxyl group in the structure of azirine derivative was important for displaying antimicrobial activity that was confirmed by the fact that esters of azirinomycin[4a] and motualevic acid F ((4*E*)-(*R*)-antazirine)[5] showed much lower antimicrobial activity.

This data shows that azirine-2-carboxylic acid derivatives are perspective candidates in antibiotics development. Although the compounds with a free carboxyl group showed high antibacterial activity, further studies in this direction slowed down. This was primarily due to the lack of methods for the synthesis of azirine-2-carboxylic acids. The hydrolysis of motualevic acid F methyl ester has long been the only example of the synthesis of this class of carboxylic acids.[6] Very recently another synthetic acid, 3-phenyl-2*H*-azirine-2-carboxylic acid,[7] was prepared by the hydrolysis of its methyl ester. The poor availability of some azirine-2-carboxylic esters and the strongly basic conditions of the hydrolysis motivate the search for new approaches to azirine-2-carboxylic acids as potential antibiotics.

In this work, a new effective method for the synthesis of azirine-2-carboxylic acids has been developed involving $FeCl_2$-

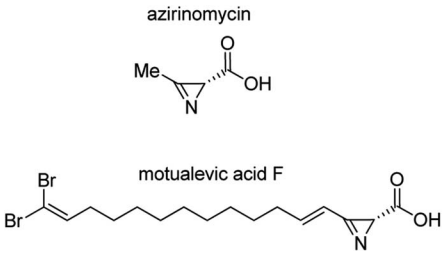

Fig. 1 2*H*-Azirine-2-carboxylic acids found in nature.

[a]*Institute of Chemistry, Saint Petersburg State University, 7/9 Universitetskaya nab., Saint Petersburg, 199034, Russia. E-mail: n.rostovskiy@spbu.ru*

[b]*Pasteur Institute of Epidemiology and Microbiology, 14 Mira Street, Saint Petersburg, 197101, Russia*

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catalyzed isomerization of 5-chloroisoxazoles to azirine-2-carbonyl chlorides followed by their hydrolysis (Scheme 1).

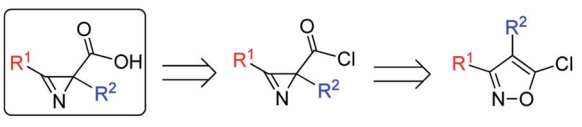
Results and discussion

The catalytic isomerization of isoxazoles, bearing a heteroatom substituent at the C5 position, to 2*H*-azirine-2-carboxylic acid derivatives has been intensively studied and widely used in recent years.[8,9] In particular, it has been demonstrated that 5-chloroisoxazoles can be transformed to 2*H*-azirine-2-carbonyl chlorides under treatment with anhydrous $FeCl_2$ in acetonitrile.[10]

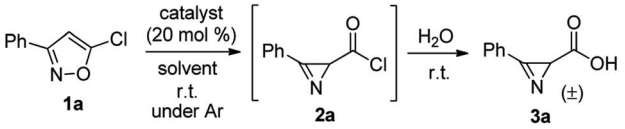
Keeping this fact in mind we started searching for a catalyst suitable for the implementation of the two step reaction sequence "isoxazole isomerization – azirinecarbonyl chloride hydrolysis" in one synthetic operation. At first we tested $FeCl_2 \cdot 4H_2O$ in acetonitrile using isoxazole **1a** as a model compound (Scheme 2, Table 1, entry 1). However, only traces of azirinecarboxylic acid **3a** were detected even after addition of water. The use of anhydrous $FeCl_2$ in acetonitrile for isomerization of **1a** and addition of water after full conversion of the isoxazole into chloride **2a** allowed to obtain azirine-2-carboxylic acid **3a** in almost quantitative yield (entry 2). $FeCl_2$ catalysis of **2a** isomerization in other solvents (acetone, tetrahydrofuran, toluene) turned out to be less effective (entries 3–5). We also tested several other salts as catalysts for isomerization of chloroisoxazoles. Among $Fe(NTf_2)_2$,[9d,i] CuI and $Rh_2(Piv)_4$ (ref. 9*a*) only the latter allowed to synthesize acid **3a** in moderate yield (entry 8).

Next, we examined the scope of acids that could be prepared by the method using anhydrous $FeCl_2$ (Scheme 3). The most starting materials, 5-chloroisoxazoles, were obtained from the corresponding isoxazol-5-ones and $POCl_3$ (see ESI†). Azirinecarboxylic acids **3** bearing halogen, alkyl, and methoxy groups in the aryl ring were synthesized under these conditions in good to excellent yields (compounds **3a–k**). Moreover, azirinecarboxylic acids containing heterocyclic substituent (furan, thiophene, pyrrole) at the C3 atom were also obtained but in slightly lower yields (compounds **3l–n**). This method was applied to the synthesis of racemic form of natural azirinomycin (compound **3o**). It was synthesized in high yield under the standard reaction conditions. Finally, 4-aryl- and 4-alkyl-substituted isoxazoles gave acids with quaternary azirine C2 atom in good yields (compounds **3p–t**). It is important that all reactions were carried out at room temperature and in most cases there was no need to perform chromatographic purification in order to obtain pure products.

The structures of acids were established on the basis of ^1H, ^{13}C NMR and IR spectroscopy as well as the data of HRMS. The



Scheme 1 Retrosynthetic scheme to 2*H*-azirine-2-carboxylic acids.



Scheme 2 Synthesis of acid **3a**.

strong band of azirine C=N bond in IR spectra lies in 1763–1781 cm^{-1} range which is characteristic of C3-substituted azirines.[11]

rac-Azirinomycin was found to be unstable in pure form without solvent: it underwent spontaneous explosive decomposition. On the contrary, other synthesized acids containing an aryl or heteroaryl substituent at the C3 atom are quite stable and can be stored at −20 °C for months without notable decomposition. It was noticed that the solid acids melted with vigorous decomposition. We studied this process in detail for acid **3a** using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). According to the TGA data, weight loss of the sample of acid **3a** under heating is 27.1% that corresponds to the loss of CO_2 (27.3%) (ESI†). The DSC analysis showed that the acid melted first (a small endothermic region) and afterwards the exothermic decomposition of the sample occurred (ESI†).

2*H*-Azirine-2-carboxylic acids **3a–t** are soluble in water and common organic solvents except aliphatic hydrocarbons. The measured solubility of acid **3a** in water is *ca*. 1.5 mg mL^{-1}. The acidity of compound **3a** ($pK_a \approx 3.2$) was also estimated using the potentiometric method (ESI†).

Acids **3** can be easily converted to the corresponding potassium salts **4** in high yield (95% for compound **3a**) using potassium *tert*-butoxide in methanol. The salt is much more soluble in water (*ca*. 35 mg mL^{-1}) and more thermally stable than the acid. Thus, the preparation of the salts is especially useful as a solution to the problem of long-term storage of thermally unstable azirine-2-carboxylic acids.

The structure of acid **3a** was also studied by X-ray diffraction analysis (Fig. 2). In crystal phase, the acid **3a** does not exist in form of zwitter-ion unlike amino acids: according to the carbon–oxygen bond distances (1.21 and 1.33 Å), there is

Table 1 Optimization of acid **3a** synthesis[a]

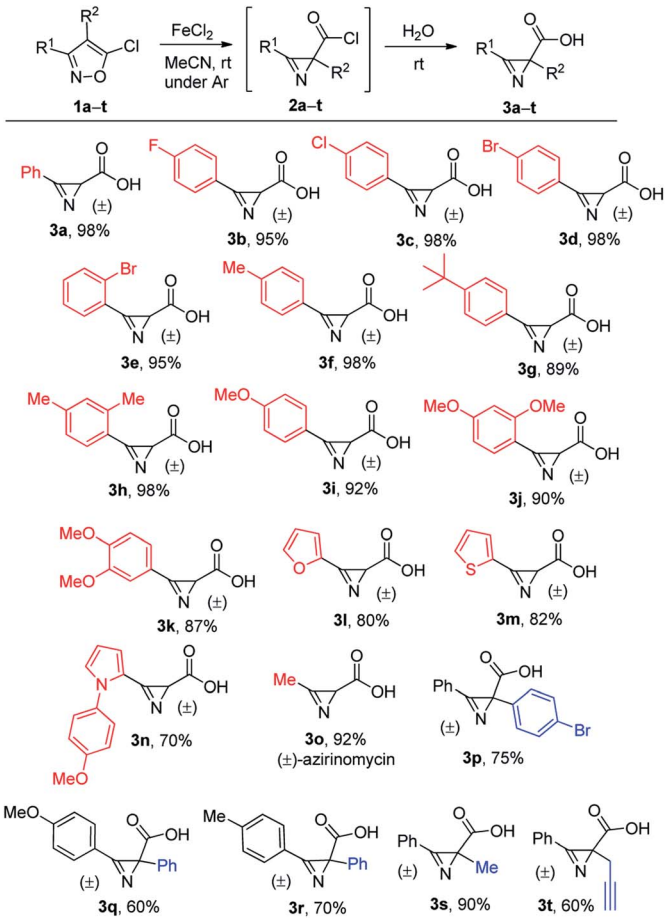
Entry	Solvent	Catalyst (20 mol%)	Time, h	Yield of **3a**[b] (%)
1	MeCN	$FeCl_2 \cdot 4H_2O$	100	Trace
2	MeCN	$FeCl_2$	2	98
3	Acetone	$FeCl_2$	24	60
4	THF	$FeCl_2$	48	35
5	PhMe	$FeCl_2$	100	10
6	MeCN	$Fe(NTf_2)_2$	100	0
7	MeCN	CuI	100	Trace
8	PhMe	$Rh_2(Piv)_4$	2	51[c]

[a] NTf_2 = bis(trifluoromethylsulfonyl)imide, Piv = trimethylacetate. [b] Isolated yields. [c] Isomerization was carried out at 110 °C with 5 mol% of $Rh_2(Piv)_4$.

Table 2 Antimicrobial activity of compounds **3a,b,d,e,m** against ESKAPE pathogens evaluated by determining the minimum inhibitory concentration (MIC)[a]

Acid	*E. faecium*	*S. aureus*	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	*E. aerogenes*
3a	56	56	225	450	56	56
3b	50	50	200	600	600	600
3d	304	19	76	608	608	304
3e	152	38	76	608	608	608
3m	>864	864	864	216	54	>864
Sulfamethoxazole	16	64	64	128	32	32

[a] MIC values are reported as μM.



Scheme 3 Scope of 2*H*-azirine-2-carboxylic acids.

a carboxylic group in the structure. However, chains which are formed by intermolecular hydrogen bonding between azirine nitrogen atom of one molecule and the hydrogen atom of the carboxylic group of another azirine molecule are observed in the crystal structure.

We also obtained primary data on antibacterial activity of the non-natural 2*H*-azirine-2-carboxylic acids. A screening of the antibacterial activity of synthesized acids against of the so-called ESKAPE pathogens (*i.e.* the ones most prone to developing resistance to drugs)[12] was carried out initially by the disk diffusion method (DDM; see ESI† for details). Subsequently, for the lead compounds **3a,b,d,e,m** antibacterial activities were evaluated by determining the minimum inhibitory concentration (MIC) and compared to Sulfamethoxazole used as a positive control (Table 2). In many cases, MICs of 2*H*-azirine-2-carboxylic acids toward ESKAPE pathogens were similar to that of Sulfamethoxazole. Moreover, compounds **3a,b,d,e** inhibited growth of *Staphylococcus aureus* at concentrations even lower than Sulfamethoxazole. Notably, methyl ester of acid **3a** (compound **3a′**) displayed activity only against *Klebsiella pneumoniae* (DDM; see ESI† for details). This fact indicates that a free carboxylic group is essential for displaying antibacterial activity of azirine-2-carboxylic acids.

Considering initially documented toxicity of azirinomycin,[4a] the *in vitro* data on cytotoxicity of synthetic azirine-2-carboxylic acids **3** is of obvious interest. Compounds **3a,b,d,e,m** were tested at 1–100 μM concentrations for their ability to affect the cell culture viability of non-cancerous human retinal pigment epithelial cell line ARPE-19 (ref. 13) and human epithelial kidney cell line HEK293.[14] As followed from the MTT assay data (Fig. 3; see ESI† for details), the studied compounds displayed no appreciable cytotoxicity within the concentration range tested on both the cell lines. The pattern of cell viability in the presence of tested compounds was practically the similar in ARPE-19 and HEK293 cell lines (Fig. 3). Overall HEK293 cell line was less sensitive to cytotoxic effect of the tested compounds compared to ARPE-19 cell line. The lowest cell viability was detected for compounds **3b,d** at 100 μM concentrations in ARPE-19 cell line.

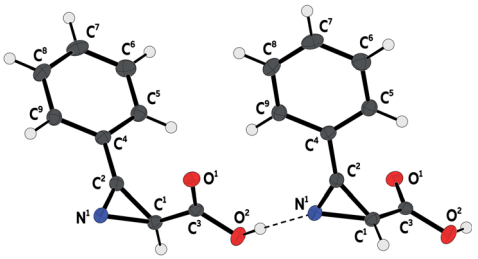


Fig. 2 X-ray structure of azirine-2-carboxylic acid **3a** indicating hydrogen bond.

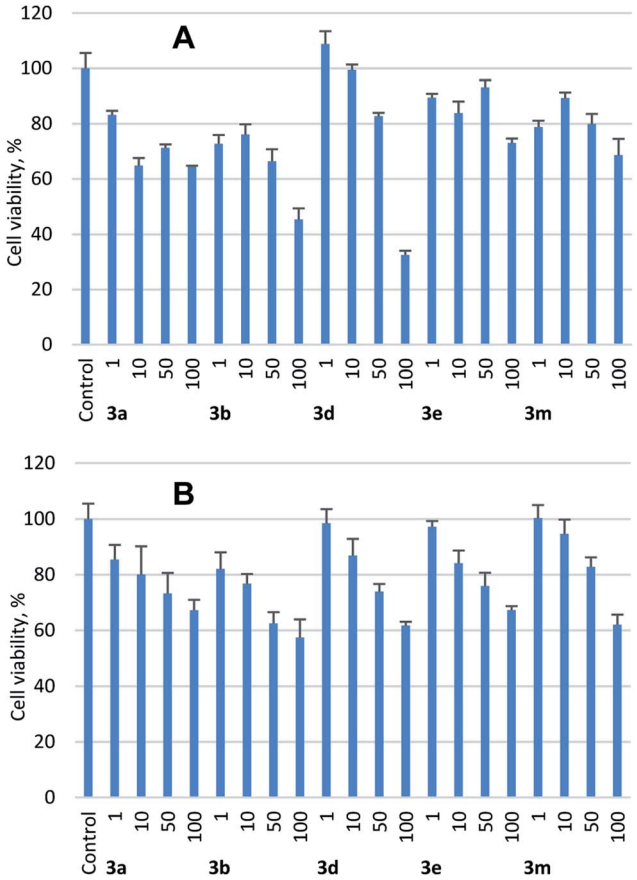


Fig. 3 Cell viability MTT assay results for compounds **3a,b,d,e,m** (1 μM, 10 μM, 50 μM and 100 μM) against (A) APRE-19 cell line and (B) HEK293 cell line (values are expressed as the mean ± SEM of three experiments): (*) $P < 0.05$ and (**) $P < 0.01$ in comparison to control.

Conclusions

In conclusion, a high-yield synthesis of 3-mono- and 2,3-disubstituted 2*H*-azirine-2-carboxylic acids from readily accessible 5-chloroisoxazoles has been developed. The method is based on $FeCl_2$-catalyzed isomerization of 5-chloroisoxazoles followed by hydrolysis of formed azirine-2-carbonyl chlorides. 3-Aryl-2*H*-azirine-2-carboxylic acids exist as OH form in crystal, are stable during prolonged storage at −20 °C, but undergo decarboxylation after melting. These compounds exhibit antibacterial activity against ESKAPE pathogens comparable to that of Sulfamethoxazole and show low-level cytotoxicity.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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