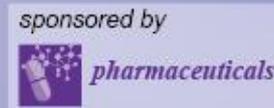




# 3rd International Electronic Conference on Medicinal Chemistry

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chaired by Dr. Jean Jacques Vanden Eynde



## Synthesis and Antimicrobial Activity of Benzofuroxans and Structurally Related N-oxide-Containing Heterocycles

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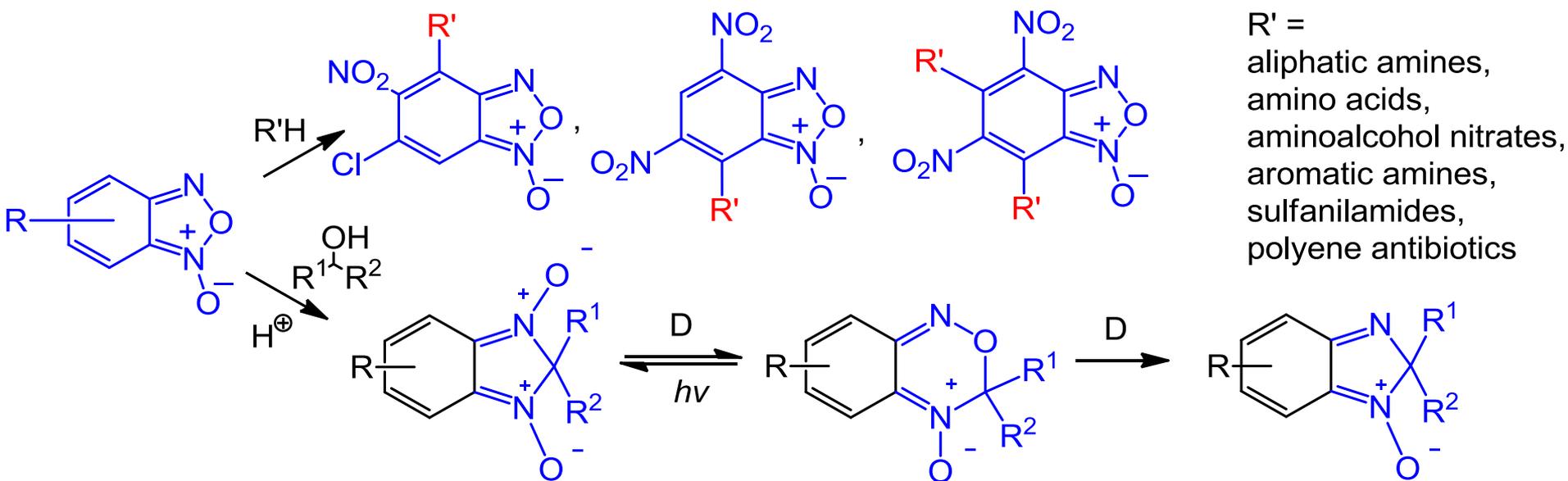
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# Synthesis and Antimicrobial Activity of Benzofuroxans and Structurally Related N-oxide-Containing Heterocycles

## Graphical Abstract



## Abstract:

Synthesis of new organic compounds possessing biological activity is very challenging and is a current trend in medicinal chemistry. In recent studies benzofuroxan derivatives have been described as drugs active against bacteria and fungi, based on the ability of these compounds to induce intracellular release of NO. Consequently, many substances arising from reactions between (di)chloro(di)nitrobenzofuroxans and different aliphatic, aromatic amines, amino acids, aminoalcohol nitrates, sulfanilamides, polyene antibiotics and other nucleophiles have been prepared. Novel 2*H*-benzimidazole 1,3-dioxides were also obtained by interaction of benzofuroxans with alcohols in sulfuric or perchloric acids. We also proposed a new method for the preparation of 2*H*-benzimidazole 1,3-dioxides by reaction of *o*-benzoquinonedioxime with ketones. Further nitration of compounds yielded a wide range of Sepin-1 analogues (separase inhibitors) with various substituents in the 2-position.

Beside their high biological activity, 2*H*-benzimidazole 1,3-dioxides can be involved into thermal reactions. Heating of 2*H*-benzimidazole 1,3-dioxides resulted in the formation of 3*H*-[2,1,4]benzoxadiazine 4-oxides, which are unstable and easily transformed into initial 2*H*-benzimidazole 1,3-dioxides on exposure to sunlight. More prolonged heating of 3*H*-[2,1,4]benzoxadiazine 4-oxides caused sequential elimination of the *N*-oxide oxygen atom to form 2*H*-benzimidazole mono *N*-oxides.

**Keywords:** benzofuroxan, 2*H*-benzimidazole 1,3-dioxide, antimicrobial activity



# Introduction:

Benzofuroxans were first synthesized about a hundred years ago, but they continue to attract attention of researchers due to their synthetic availability, rich chemistry and biological activity of most compounds of this series. In medicinal and biological fields growing interest has been devoted to this organic scaffold owing to its ability to release nitric oxide (NO) molecules under physiological conditions

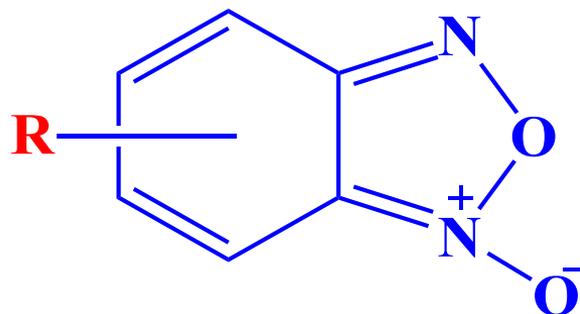
## Antiparasitic agents

Calcium channel modulators

Antibacterial and antifungal agents

Vasodilator agents

Antioxidants



Herbicides

Explosives

Liquid-crystalline materials

Immunosuppressive compounds

Antileukemic compounds

Inhibitors of platelet aggregation



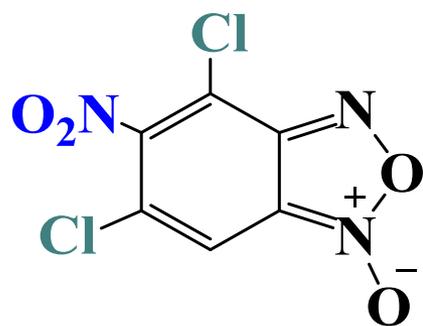
## Purpose of study:

- *the synthesis of new biologically active substances on the base of benzofuroxans;*
- *the study of biological activity and toxicity of obtained benzofuroxan derivatives*

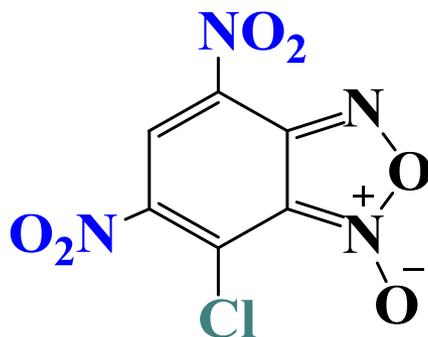


## Results and discussion

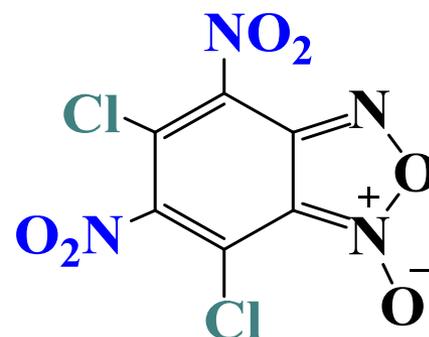
Development of methods of functionalization of benzofuroxans, in particular, of introduction of the nitrogen-containing structural fragments is an actual problem. In this regard the doubtless interest present mono- and dinitrobenzofuroxans containing the halogen atoms in their structure. The presence in molecules of (di)nitrobenzofuroxans (1-3) one or two mobile chlorine atoms enables easy replacement of them by group providing targeted delivery while maintaining the NO-donor properties.



1



2

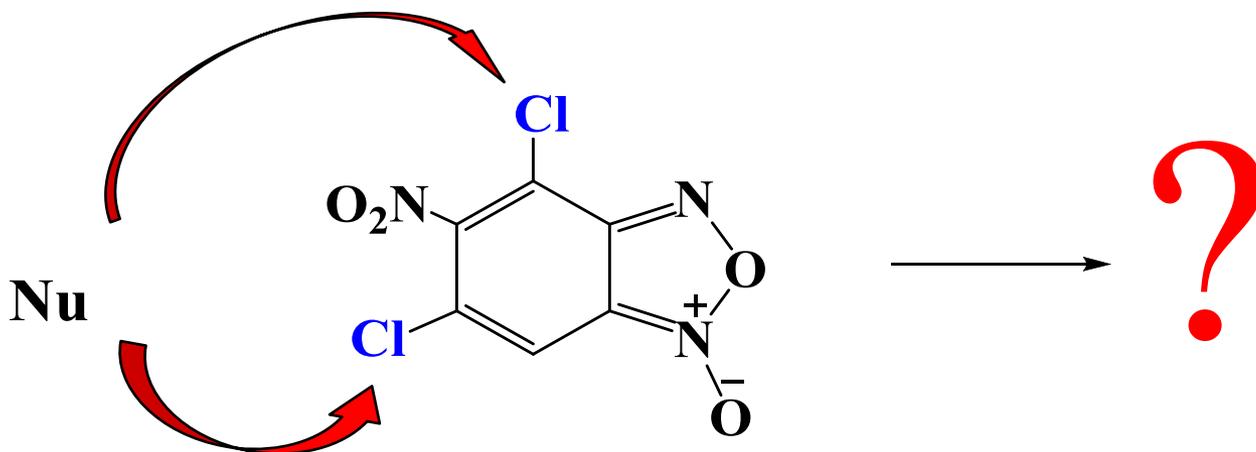


3



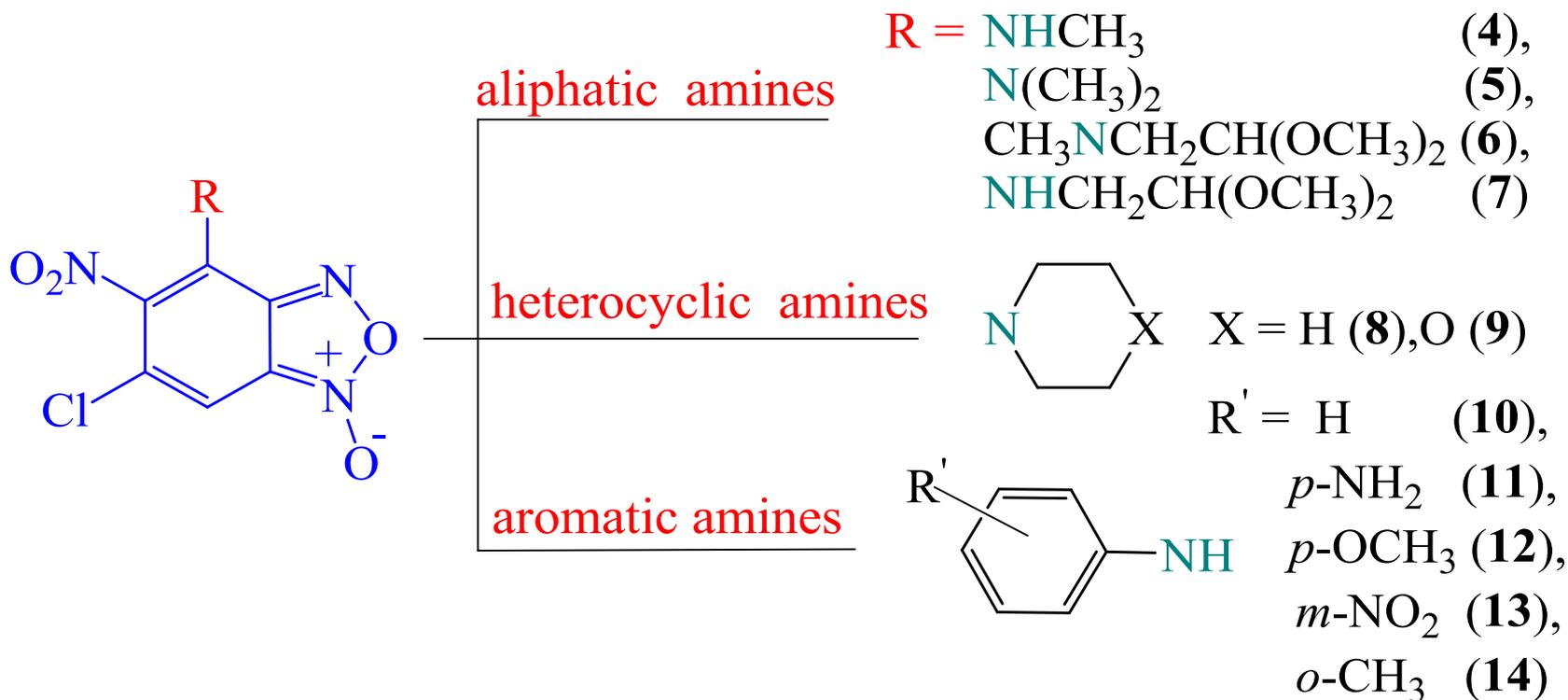
# The interaction of 4,6-dichloro-5-nitrobenzofuroxan with amines

The first task was the study of chlorine atoms reactivity in 4,6-dichloro-5-nitrobenzofuroxan. The interactions between 4,6-dichloro-5-nitrobenzofuroxan and aliphatic, heterocyclic and aromatic amines have been performed in DMSO, isopropyl alcohol, dioxane and chloroform.



# The interaction of 4,6-dichloro-5-nitrobenzofuroxan with amines

In spite of the amine excess only the chlorine atom in the position 4 of the benzene ring takes part in the reaction. The research clearly shows that DMSO is the more appropriate solvent to isolate the substitution product in a high yield and with a high purity.

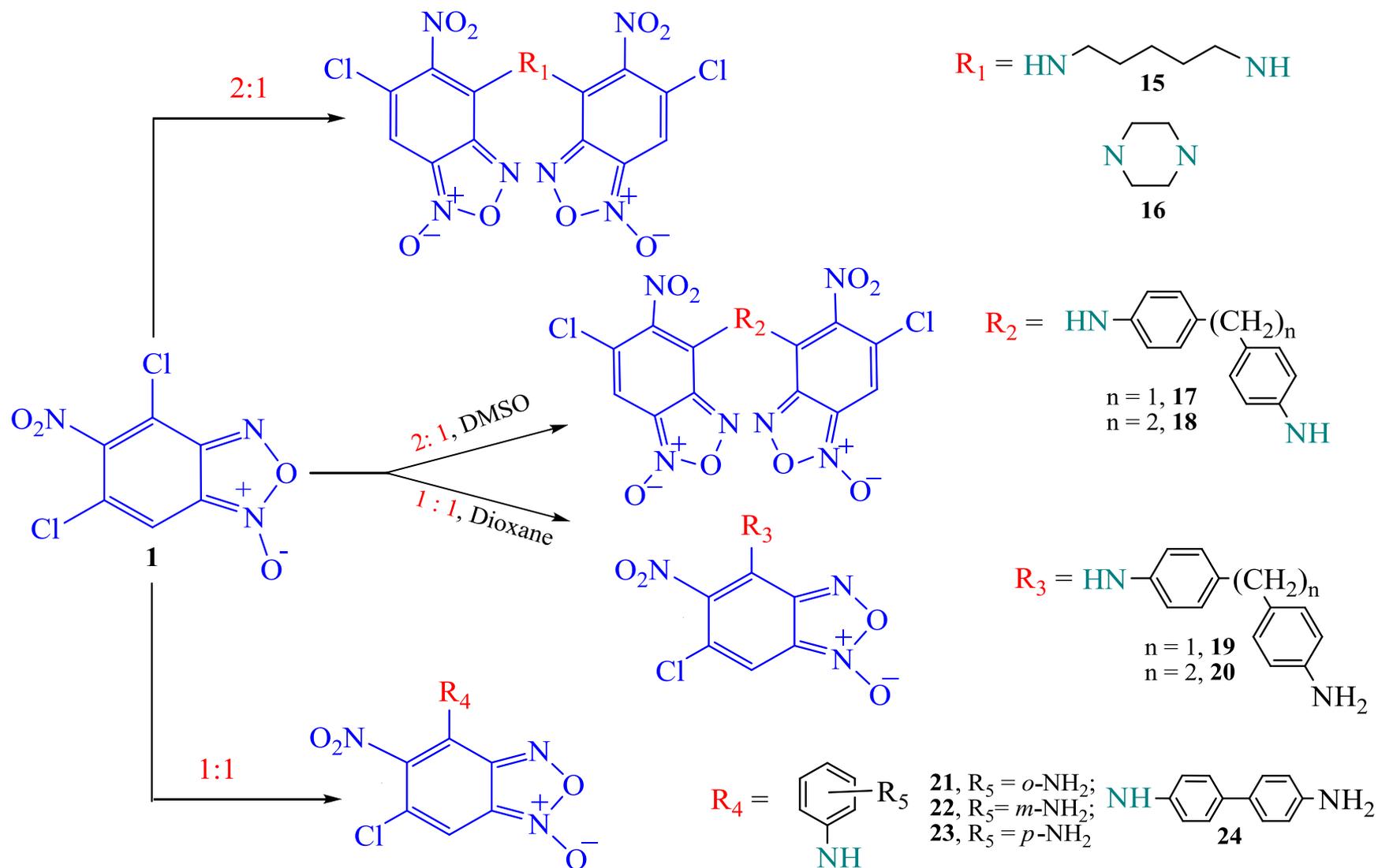


# The interaction of benzofuroxans with diamines

It was found that diamines may be also successfully involved in the above-described reaction. The use of diamines can lead to the formation of two kinds of products: in the ratio of 1 to 1 isomer, when one chlorine atom is replaced and in the ratio of 2 to 1, when two molecules of benzofuroxan are reacting with one molecule of amine. Benzofuroxan **1** reacts with diaminopentane and piperazine in equimolar amount to give the products of double substitution. Interaction of 4,6-dichloro-5-nitrobenzofuroxan with phenylenediamines is going along with the substitution of one chlorine atom and is leading to the formation of compounds 21-24. Reaction of 4,6-dichloro-5-nitrobenzofuroxan with aromatic diamines in different solvents leads to the formation of two kind of products: 1:1 isomer, and 2:1 isomer.



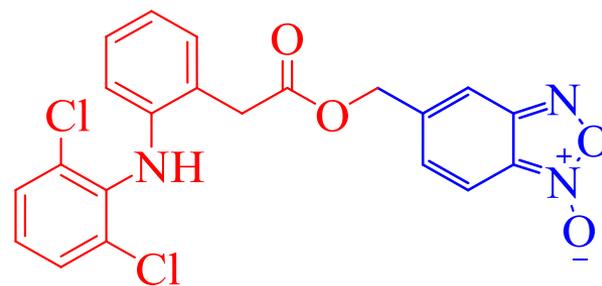
# The interaction of benzofuroxans with diamines



# Creation of «hybrid» compounds on the base of benzofuroxans

Recently, research and development in the medicinal chemistry sphere of benzofuroxan systems have produced hybrid compounds in which benzofuroxanyl moieties together with classical drug moieties are presented in a single molecule. For example, the diclofenac derivative bearing a benzofuroxan moiety in its structure that showed anti-inflammatory activity and with better gastric tolerability with respect to that of native diclofenac, probably related to nitric oxide release ability

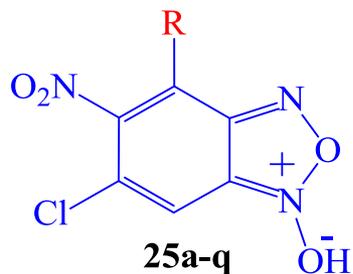
Carvalho P.S, Maróstica M, Gambero A, Pedrazzoli J Jr.,  
Synthesis and pharmacological characterization of a novel  
nitric oxide-releasing diclofenac derivative containing a  
benzofuroxan moiety // Eur. J. Med. Chem. 2010; V. 45, I. 6.  
P. 2489-2493.



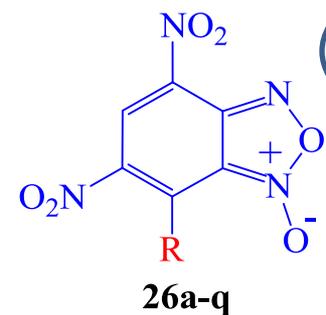
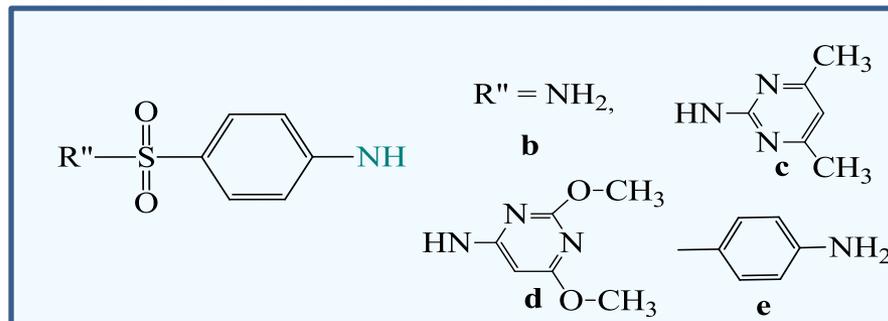
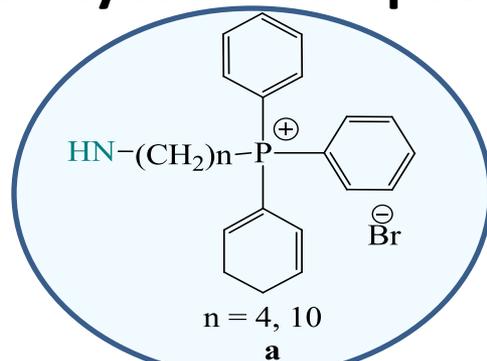
“Hybrid” compounds were prepared in the reaction of benzofuroxans with *N*-containing phosphonium bromides (a), antibacterial sulfonamides such as sulfanilamide (b), sulfadimezin (c) and sulfadimethoxine (d), aminoalcohol nitrates (f-g), amino acids (h-l), *N*-substituted naphthalimides (m-o), and antifungal polyene antibiotics such as Amphotericin B (p) and Nystatin (q)



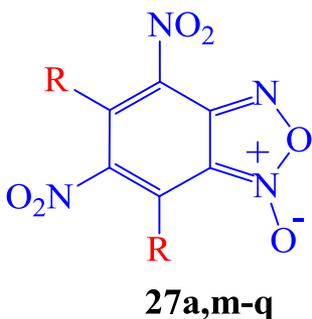
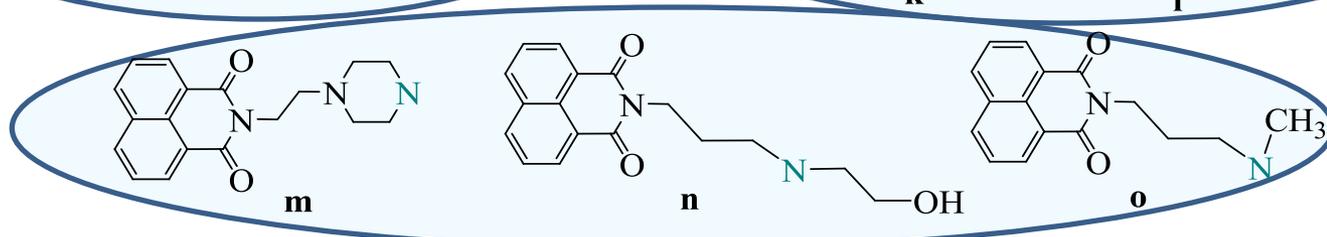
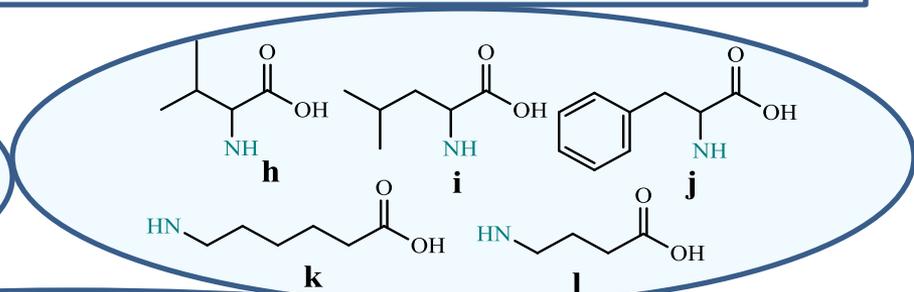
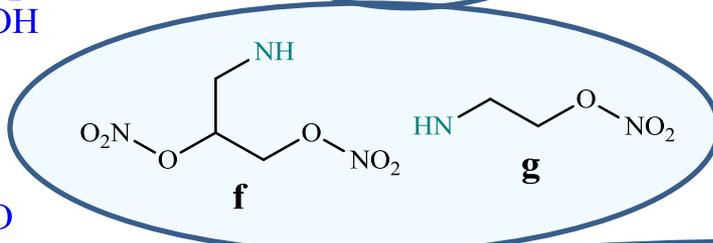
# Preparation of "hybrid" compounds based on benzofuroxans



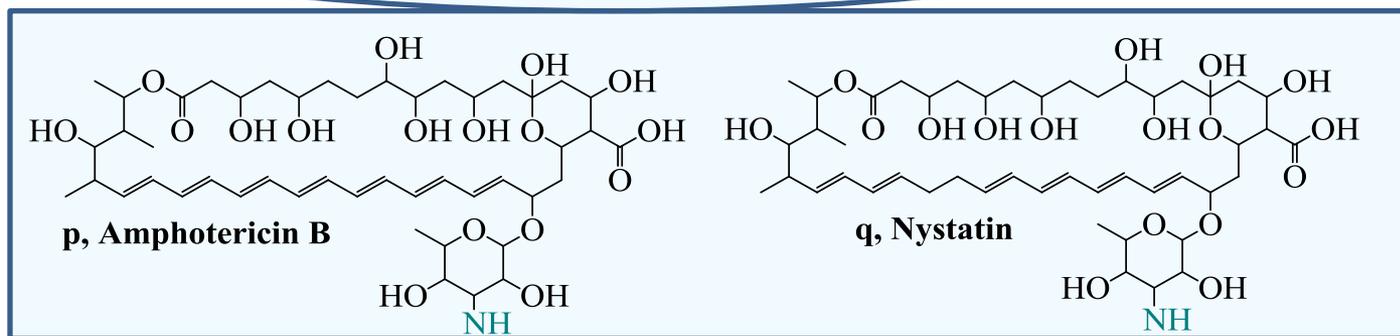
R=



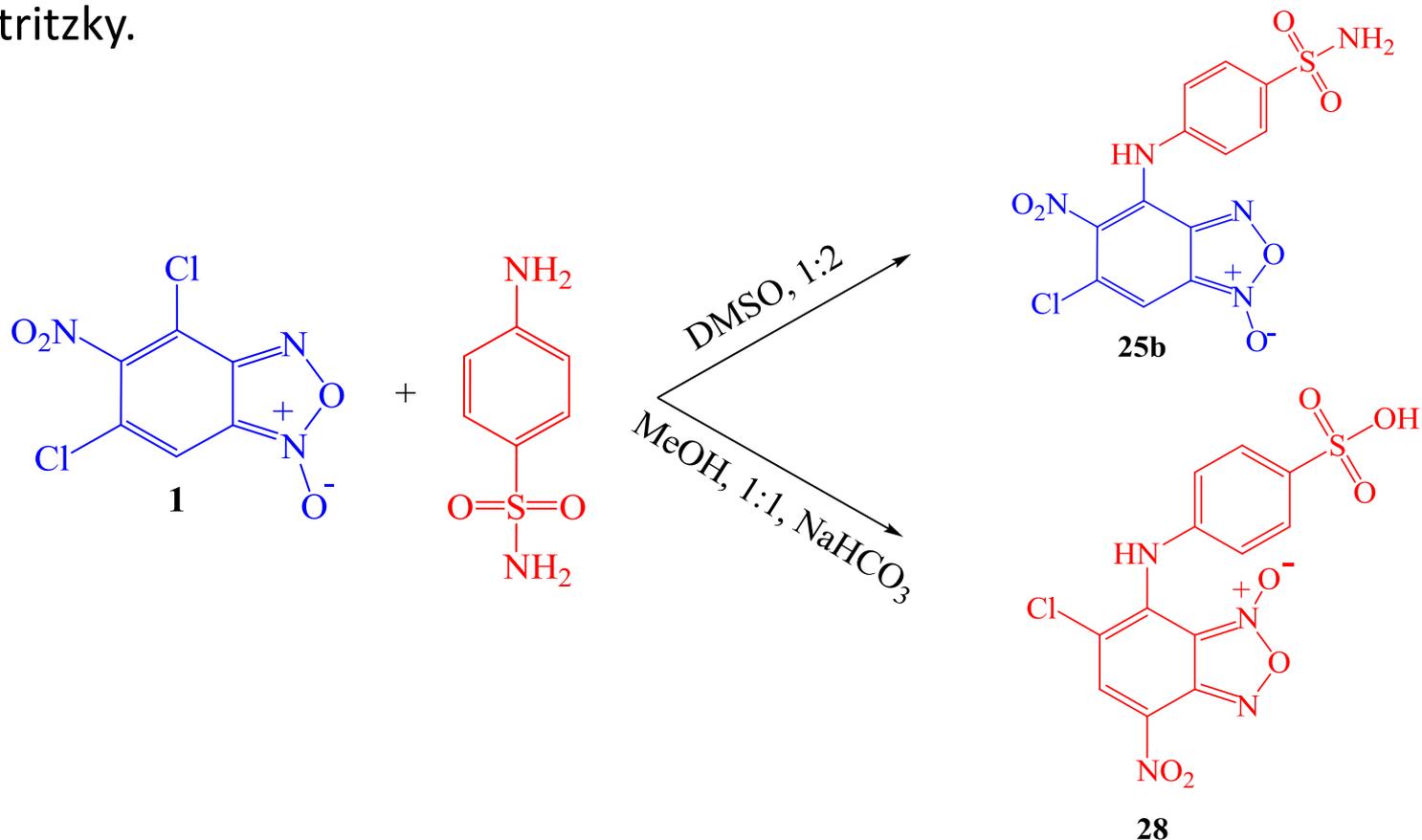
**26a-q**



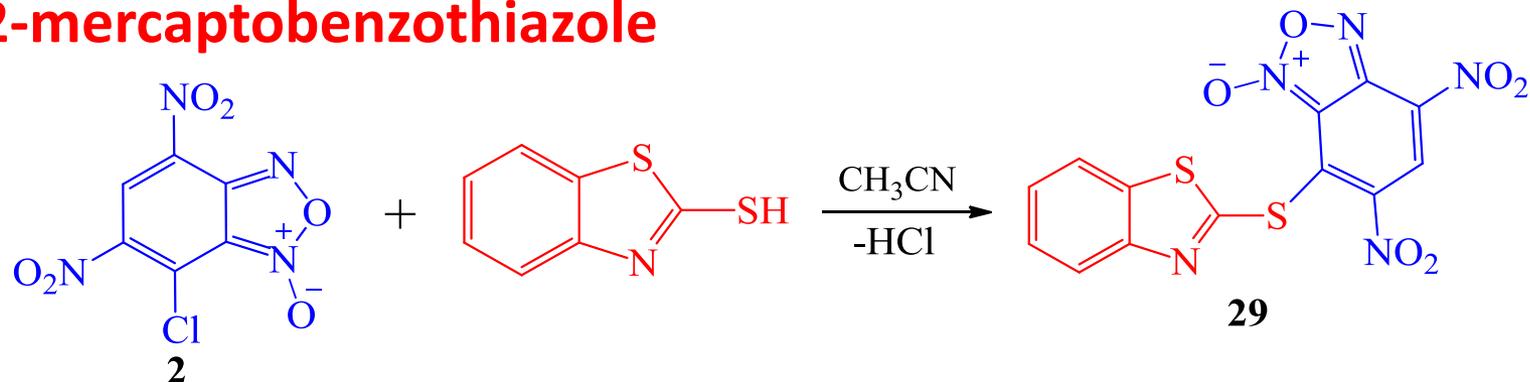
**27a,m-q**



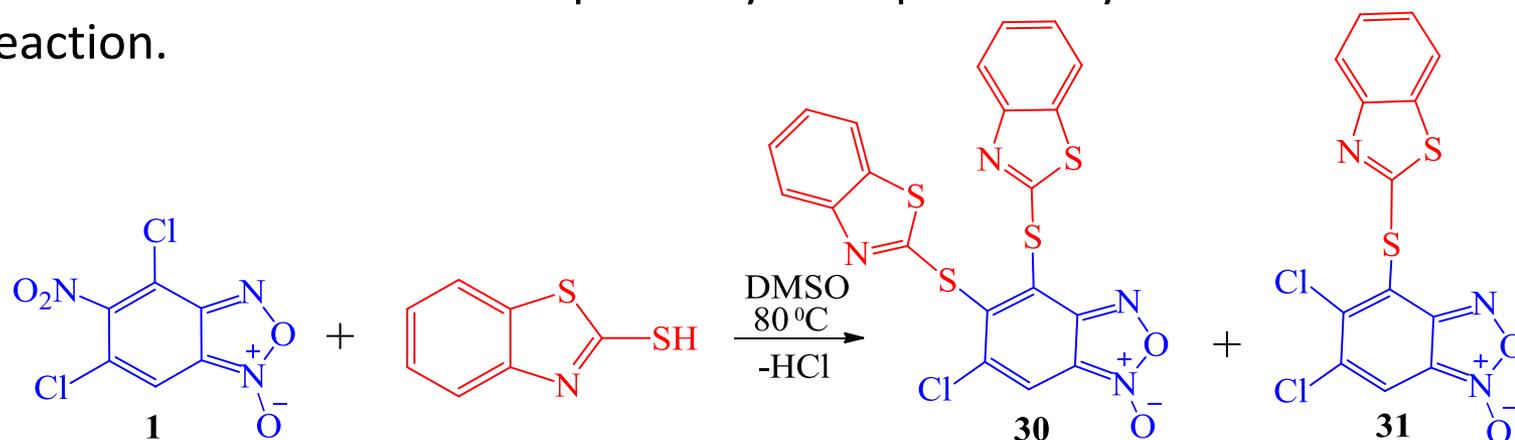
Importantly, by reacting of 4,6-dichloro-5-nitrobenzofuroxan with sulfanilamide at various ratios of reagents and conditions, we have a variety of products: in a ratio of 1:2, we obtained compound **25b** and by performing the reaction in the presence of sodium bicarbonate reactant ratio of 1:1 was formed compound **28**. Probably, this phenomenon can be explained by the rearrangement of Boulton-Katritzky.



# Synthesis of novel structural hybrids of benzofuroxan and 2-mercaptobenzothiazole

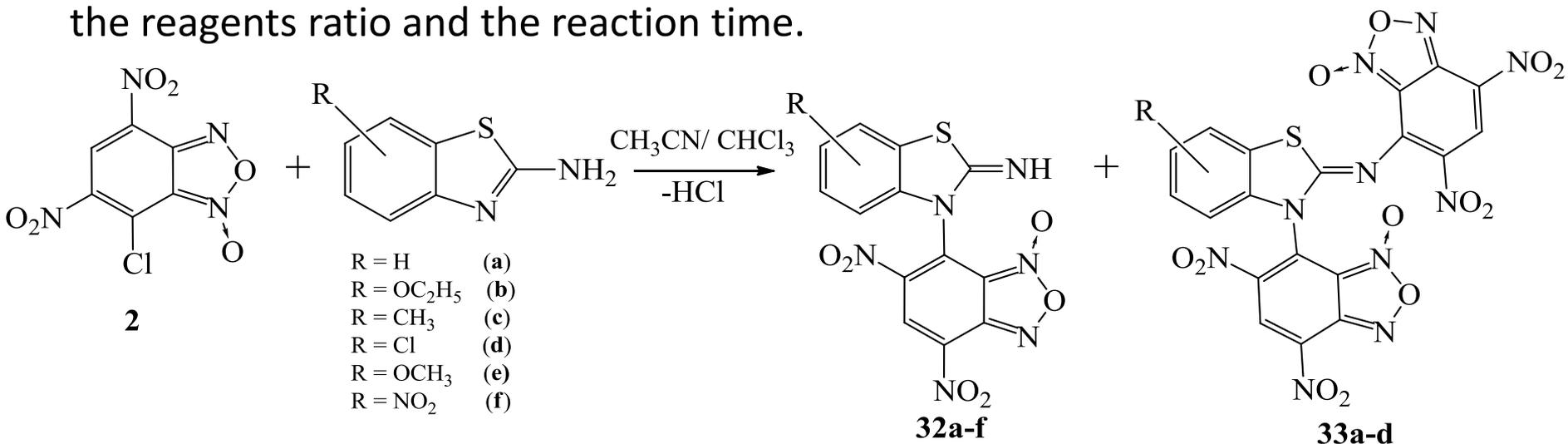


In contrast to 7-chloro-4,6-dinitrobenzofuroxan, the reaction of 4,6-dichloro-5-nitrobenzofuroxan with 2-mercaptobenzothiazole takes place only in polar DMSO at 80-90 °C, and unexpectedly leads to the formation of a mixture of two products. We assume that the substitution of a nitro group for a chlorine atom or mercaptothiazole in this case can probably be explained by the radical mechanism of the reaction.



# Synthesis of novel structural hybrids of benzofuroxan and benzothiazole derivatives

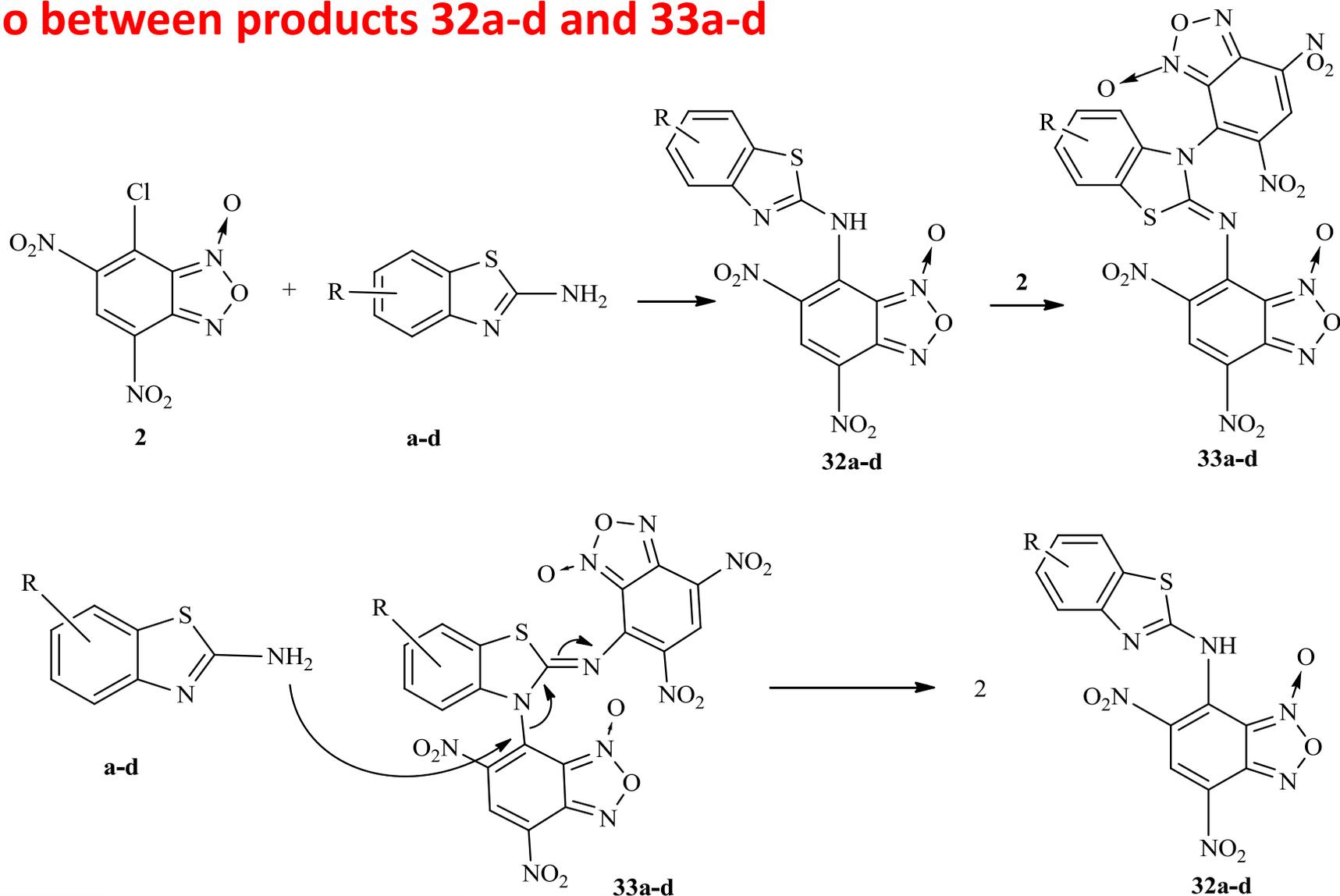
The reaction between 7-chloro-4,6-dinitrobenzofuroxan and 2-aminobenzothiazole derivatives gave two products, one bearing the benzofuroxan moiety linked to the exocyclic amino nitrogen, and the second derived from the attack of two molecules of electrophile to both the nitrogen atoms of the benzothiazole reagent. Their relative ratio is modifiable by tuning the reagents ratio and the reaction time.



Synthesis and antimicrobial activity of novel structural hybrids of benzofuroxan and benzothiazole derivatives // Elena Chugunova, Carla Boga, Ivan Sazykin, Silvia Cino, Gabriele Micheletti, Andrea Mazzanti, Marina Sazykina, Alexander Burilov, Ludmila Khmelevtsova, Natalia Kostina // European Journal of Medicinal Chemistry. 2015. Vol. 93. P. 349-359

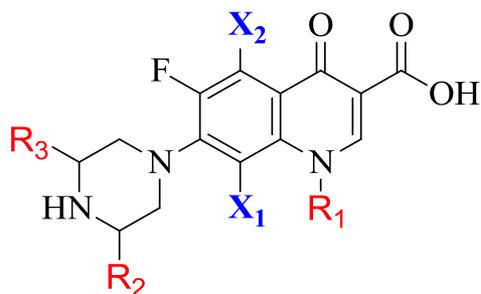


# Proposed pathway to explain the observed time-dependence of the ratio between products 32a-d and 33a-d



# Preparation of "hybrid" compounds based on benzofuroxans and fluoroquinolones

Unexpectedly for us we have salt-like products are formed during hydrolysis of benzofuroxans by water present in the solvent as a result of reactions of benzofuroxans with fluoroquinolones instead of the expected replacement products.



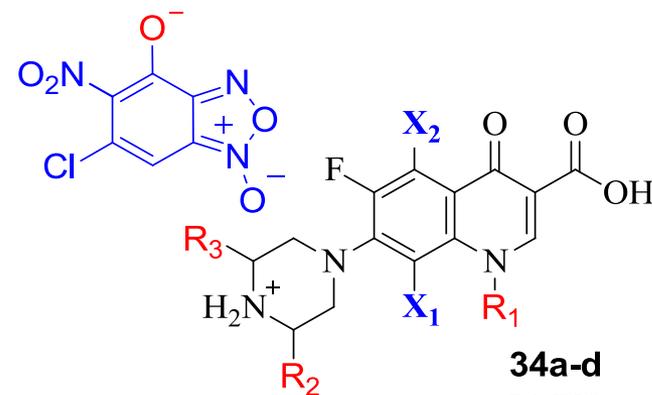
- a) R<sub>1</sub> = Cyclopropyl, X<sub>1</sub> = F, X<sub>2</sub> = NH<sub>2</sub>, R<sub>2</sub> = R<sub>3</sub> = CH<sub>3</sub>;
- b) R<sub>1</sub> = Cyclopropyl, X<sub>1</sub> = X<sub>2</sub> = R<sub>2</sub> = R<sub>3</sub> = H;
- c) R<sub>1</sub> = Ethyl, X<sub>1</sub> = X<sub>2</sub> = R<sub>2</sub> = R<sub>3</sub> = H;
- d) R<sub>1</sub> = Ethyl, X<sub>1</sub> = F, X<sub>2</sub> = R<sub>3</sub> = H, R<sub>2</sub> = CH<sub>3</sub>

DMSO

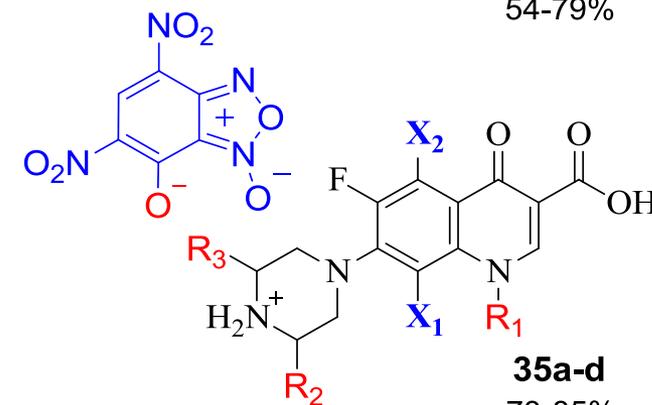
1

2

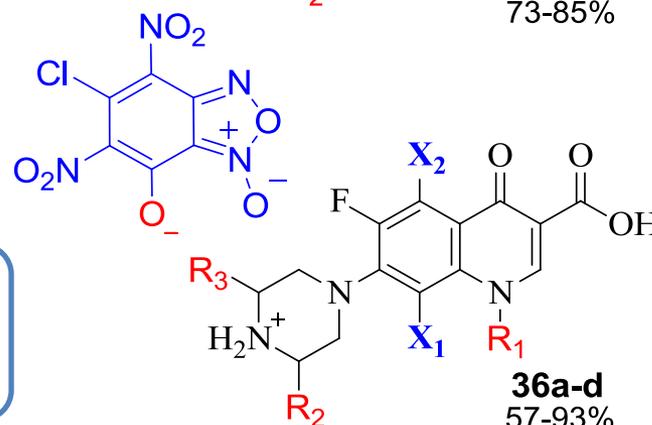
3



**34a-d**  
54-79%



**35a-d**  
73-85%



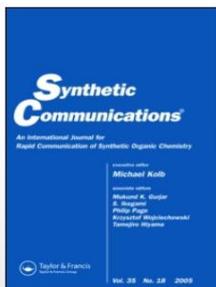
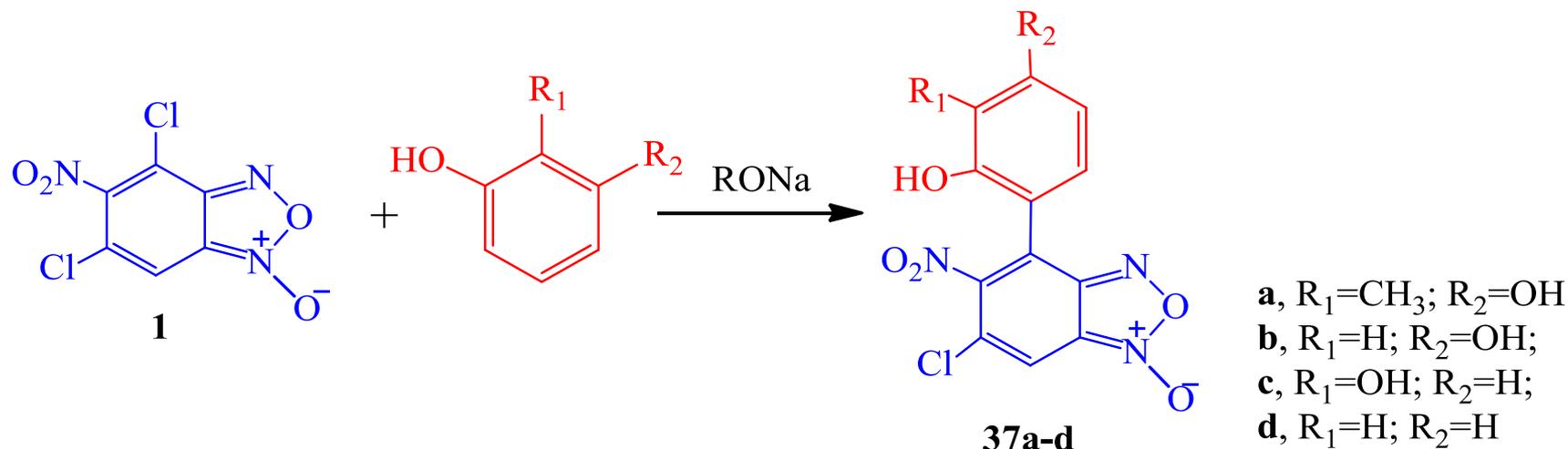
**36a-d**  
57-93%

E. Chugunova, N. Akylbekov, A. Bulatova, N. Gavrilov, A. Voloshina, N. Kulik, V. Zobov, A. Dobrynin, V. Syakaev, A. Burilov, European Journal of Medicinal Chemistry 2016. Vol. 116. P. 165-172.



# Interaction of benzofuroxans with phenols

As a result of reactions with the phenolic derivatives it was found that 4,6-dichloro-5-nitrobenzofuroxan forms substitution products involving carbon atoms with phenolates in isopropyl alcohol medium.



Synthesis and Investigation of Antimicrobial Activity of Compounds Derived from Benzo[c][1,2,5]oxadiazole-1-oxides and Phenolates / E. A. Chugunova, N. I. Akylbekov, A. D. Voloshina, N. V. Kulik, V. V. Zobov, V. M. Babaev, N. V. Gavrilov, A R. Burilov // Synthetic Communications, 2016, Vol. 46, Iss. 18, P. 1560-1565

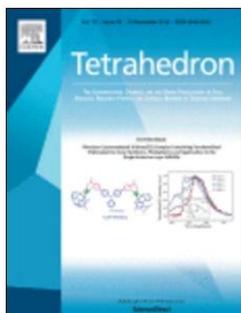
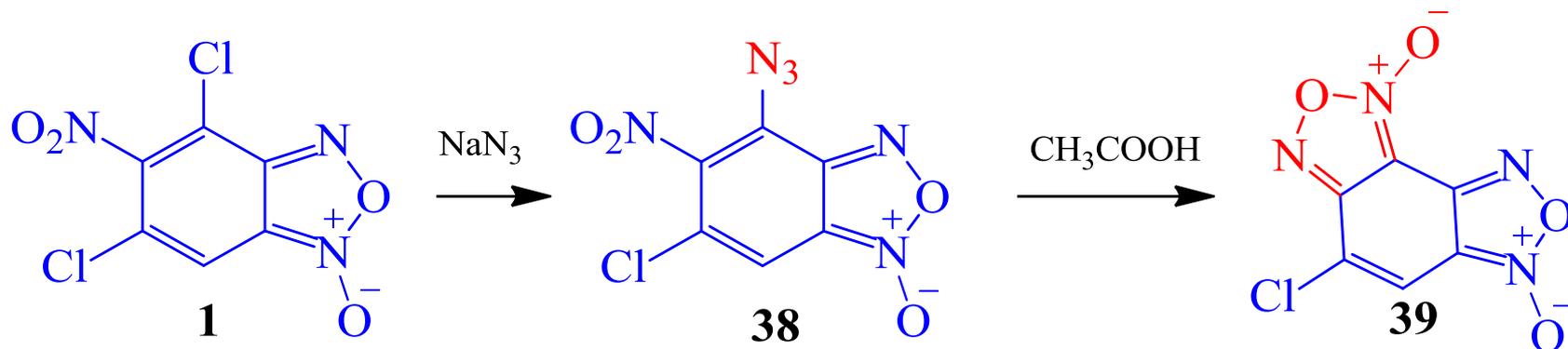


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# Synthesis of benzodifuroxan

It has been found in literature that benzodifurazan derivatives exhibited bacteriostatic, fungistatic, and acaricide properties. The 4-chlorobenzodifuroxan has been synthesized for the first time in two steps from 4,6-dichloro-5-nitrobenzofuroxan **1**.

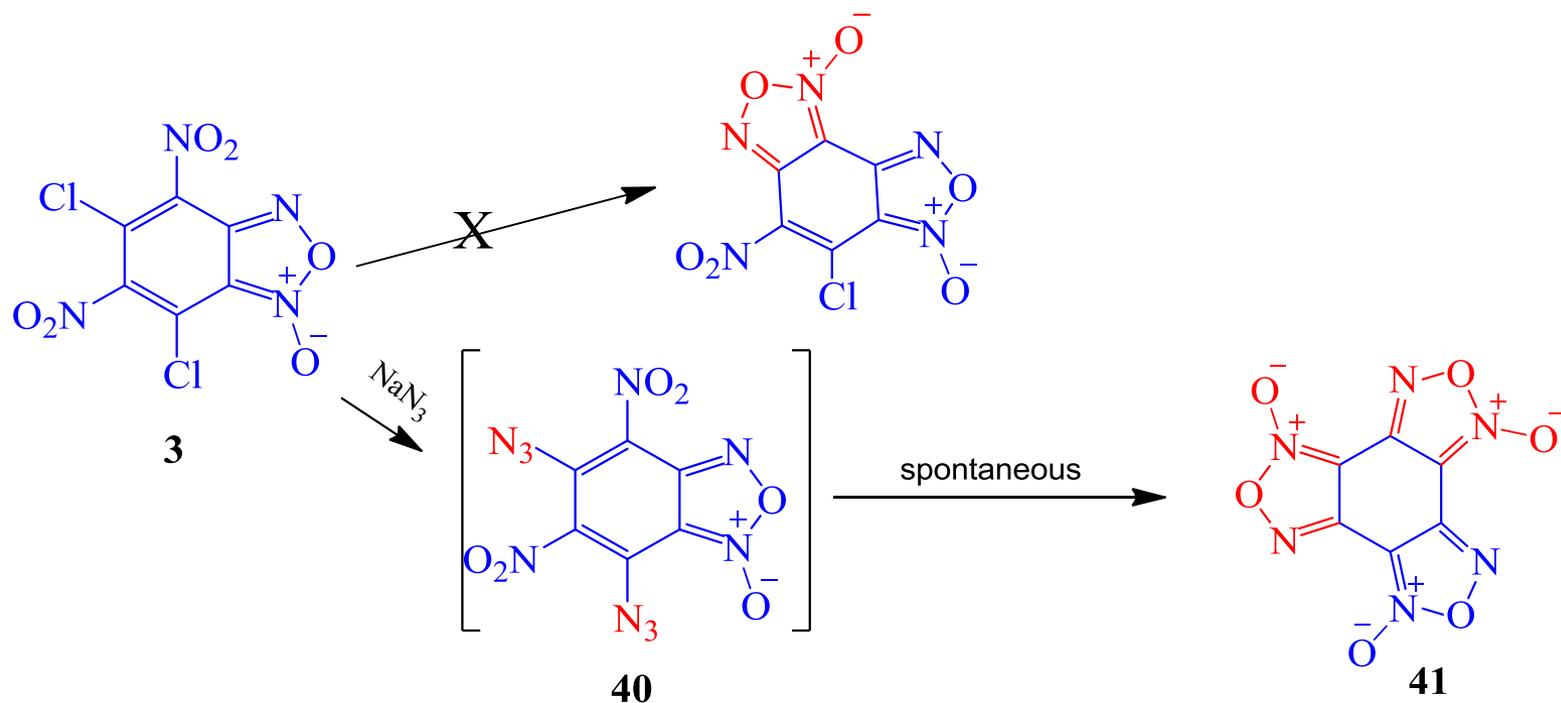


C. Jovené, M. Jacquet, E. A. Chugunova, S. V. Kharlamov, R. Goumont. Synthesis and 1-oxide/3-oxide interconversion of 4-substituted benzodifuroxans: a thorough NMR and theoretical study of the structure of 4-fluoro- and 4-chloro-benzodifuroxan // *Tetrahedron*. 2016. Vol. 72. Iss. 15. P. 2057-2063.



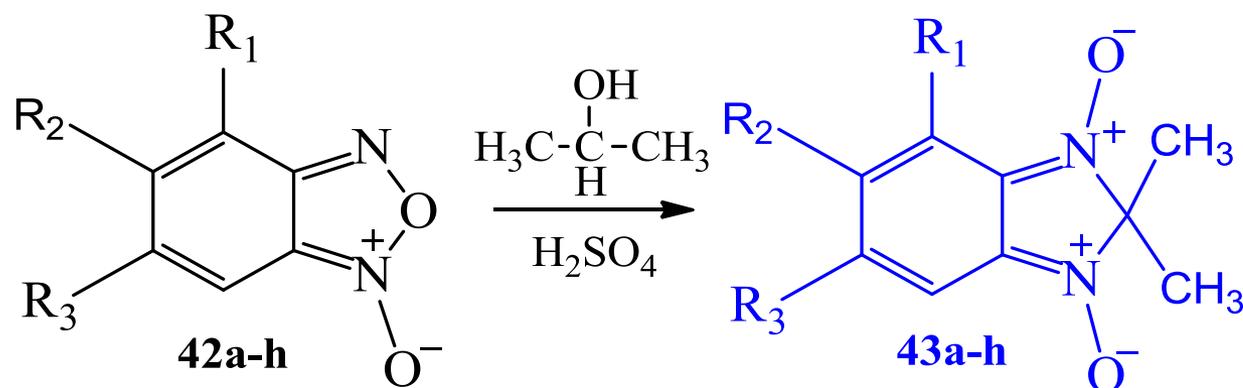
# Synthesis of benzotrifuroxan

Attempts to prepare 5-chloro-6-nitrobenzodifuroxan from the reaction of 5,7-dichloro-4,6-dinitrobenzodifuroxan with sodium azide have failed and, surprisingly, the formation of the powerful hydrogen-free explosive – benzotrifuroxan (BTF) through spontaneous cyclization at 0 °C was observed. This reaction takes place instantaneously, even at low temperature through a spontaneous cyclization.



# Synthesis of 2*H*-benzimidazole 1,3-dioxides based on the interaction between benzofuroxans and isopropyl alcohol

Benzofuroxans and their derivatives are not only established themselves as biologically active substances of different spectrum of action, but also attracted attention as a precursors for the synthesis of a number of heterocyclic compounds. Novel 2*H*-benzimidazole 1,3-dioxides (43) were obtained upon the interaction of benzofuroxans with alcohols in sulfuric acid.

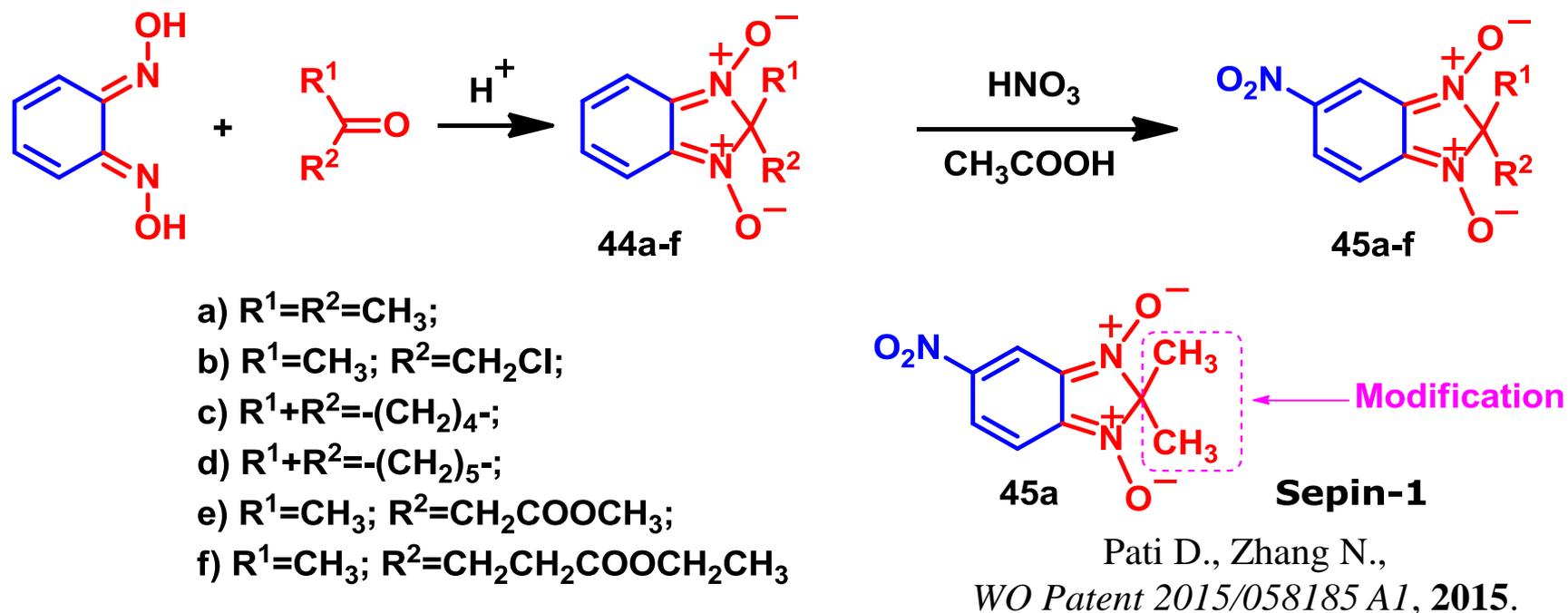


- a, R<sub>1</sub>=R<sub>3</sub>=H, R<sub>2</sub>=CH<sub>3</sub>;
- b, R<sub>1</sub>=R<sub>3</sub>=H, R<sub>2</sub>=CHNOH;
- c, R<sub>1</sub>=R<sub>3</sub>=H, R<sub>2</sub>=COH;
- d, R<sub>1</sub>=R<sub>3</sub>=H, R<sub>2</sub>=CF<sub>3</sub>;
- e, R<sub>1</sub>=R<sub>3</sub>=H, R<sub>2</sub>=COOH,
- f, R<sub>1</sub>=R<sub>3</sub>=Cl, R<sub>2</sub>=H;
- g, R<sub>1</sub>=Cl, R<sub>2</sub>=H, R<sub>3</sub>=NO<sub>2</sub>;
- h, R<sub>1</sub>=Br, R<sub>2</sub>=H, R<sub>3</sub>=NO<sub>2</sub>;



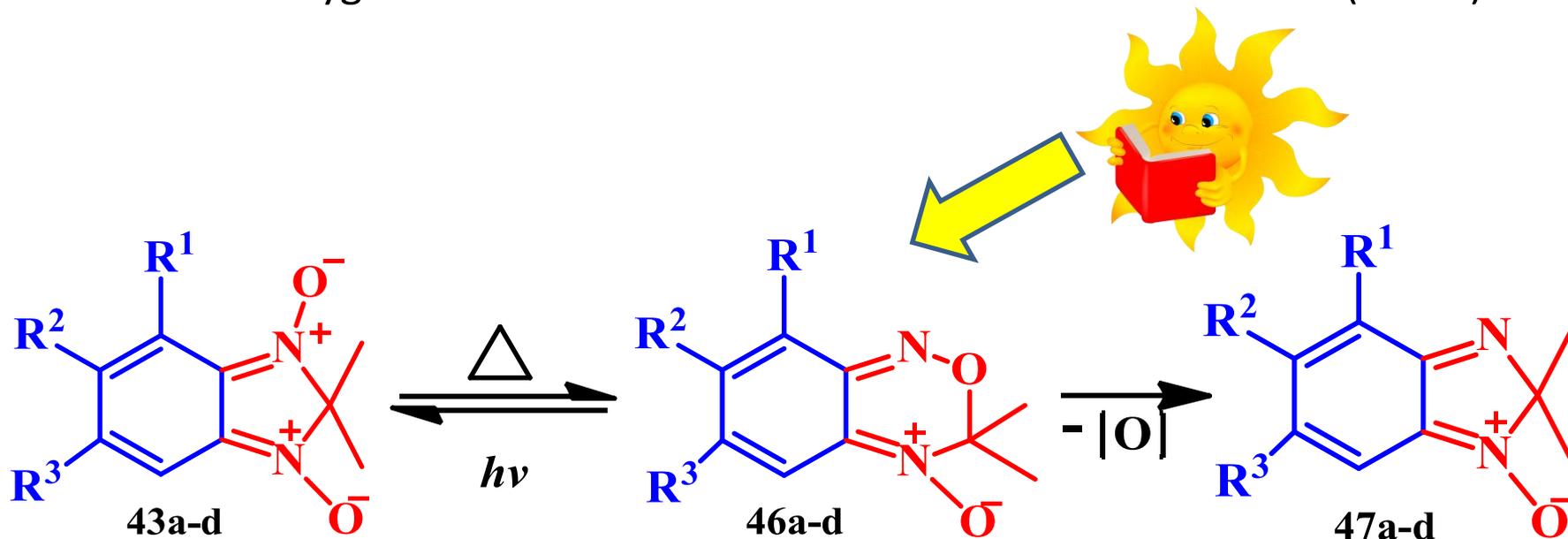
# Synthesis of 2H-benzimidazole 1,3-dioxides by reaction of *o*-benzoquinone dioximes with ketones

Future, we developed new method of synthesis of 2H-benzimidazole 1,3-dioxides with help of the interaction of *o*-benzoquinone dioxime with ketones. Further nitration of obtained compounds makes it possible to obtain a wide range of Sepin-1 analogues, patented in the literature as a separate inhibitor, with various substituents at position 2.



# Photochromism of 2*H*-benzimidazole 1,3-dioxides

The interesting property of these systems is that they can be involved into thermal reactions. Heating of 2*H*-benzimidazole 1,3-dioxides (43a-d) results in the formation of 3*H*-[2,1,4]benzoxadiazine 4-oxides (46a-d), which are unstable and easily transformed into initial 2*H*-benzimidazole 1,3-dioxides on exposure to sunlight. More prolonged heating of 3*H*-[2,1,4]benzoxadiazine 4-oxides (46a-d) causes sequential elimination of the *N*-oxide oxygen atom to form 2*H*-benzimidazole mono *N*-oxides (47a-d)

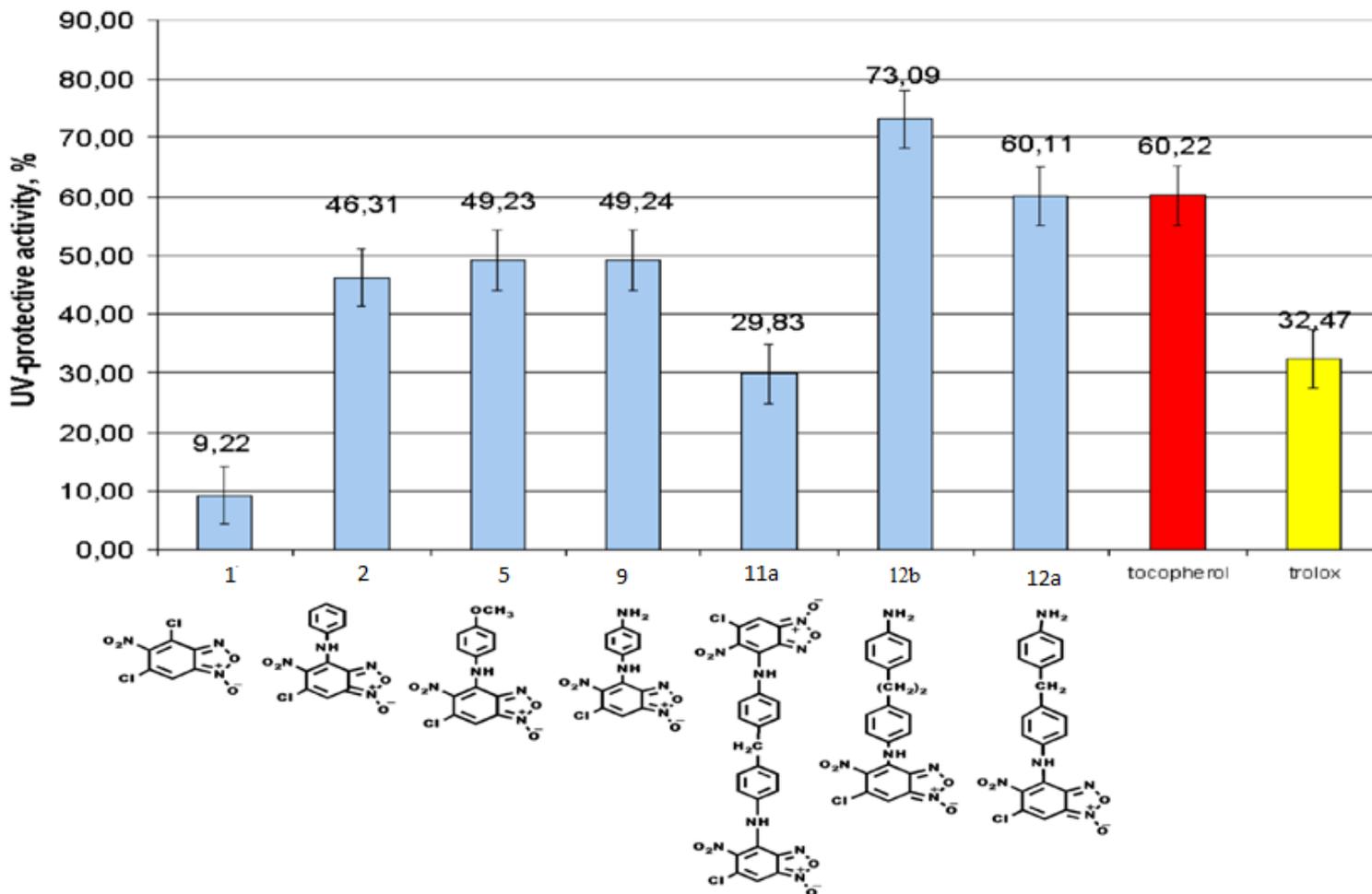


# UV-activity of benzofuroxans

Ability to suppress and prevent genotoxic effects of UV-radiation in the wavelength range between 300–400 nm was shown for compound on the base of benzofuroxans and diamines. These compounds are able to protect bacterial cells from destructive effects of the UV-radiation. Comparing the results obtained for various benzofuroxans to those obtained for the natural antioxidant alpha - tocopherol (vitamin E) and for the synthetic antioxidant trolox, which are references in this domain, we have shown that some benzofuroxans quantitatively exhibit a similar protective effect, and that compounds prepared from the reaction between 4,6-dichloro-5-nitrobenzofuroxan and ethylenedianiline possess potent protective potential



# The maximum protective effect of benzofuroxans and antioxidants on E.coli MG1655 (pKatG-lux) in the wavelength range between 300-400 nanometers



## Biological activity of benzofuroxans

The *in vitro* antibacterial and antifungal activity of the benzofuroxans was investigated against several pathogenic representative Gram-negative and Gram-positive bacteria moulds and yeast. Benzofuroxans exhibit good bacteriostatic and fungistatic activity. The screening data show that benzofuroxans exhibit activity towards gram-positive bacteria especially *Staphylococcus aureus* and fungi especially *Candida albicans* with the MICs values comparable with reference drugs.

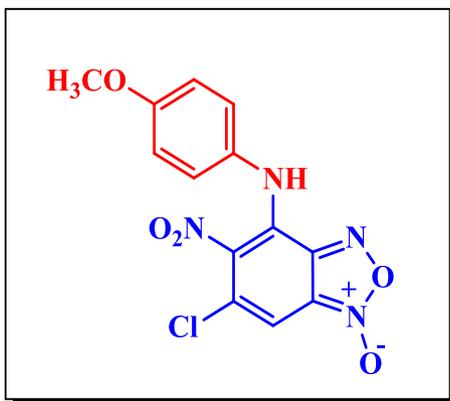
Safety of the benzofuroxans derivatives was exclusively studied for the modeled object – various bacterial biosensors. It was found that benzofuroxan derivatives do not belong to the class of the substances damaging DNA and proteins.

The toxicity of the more effective benzofuroxan derivatives has been determined in mice. According to levels of acute toxicity for mammals, the benzofuroxan derivatives can be considered as moderately toxic compounds.



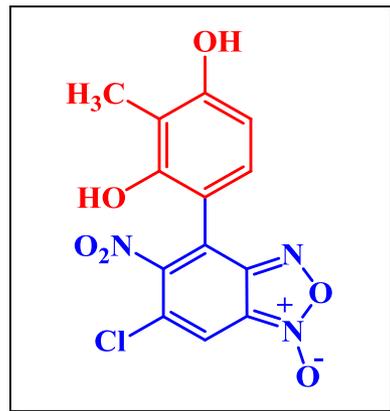
# Biological activity of benzofuroxans

## Antifungal activity

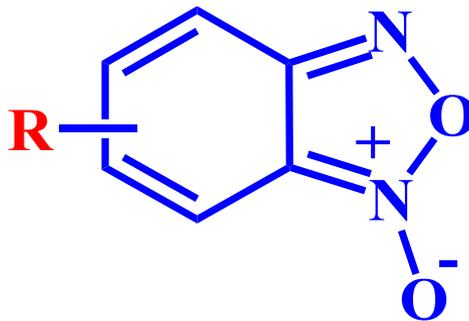


MIC = 1.9  $\mu\text{g/ml}$ , LD<sub>50</sub> = 250 mg/kg

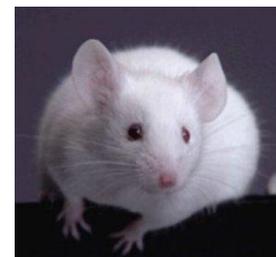
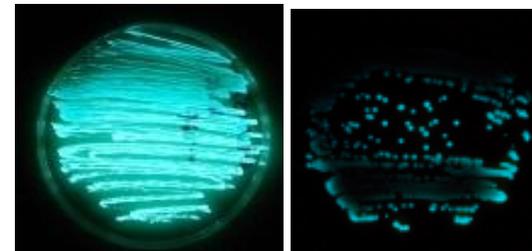
## Antibacterial activity



MIC = 0.19  $\mu\text{g/ml}$



## Absence of toxicity and genotoxicity



- Patent Ru No. 2428419 C2, publication date 10/09/2011. "New benzofuroxans having fungicidal and acaricidal activity" / AR Burirov, E.M Kasymova, E.A. Chugunova, LM Yusupova, V.V. Zobov
- The Study of the Biological Activity of Amino-Substituted Benzofuroxans E.A. Chugunova, A.D. Voloshina, R.E. Mukhamatdinova, I.V. Serkov, A.N. Proshin, E.M. Gibadullina, A.R. Burirov, N.V. Kulik, V.V. Zobov, D.B. Krivolapov, A.B. Dobrynin, R. Goumont // Letters in Drug Design & Discovery, 2014, V. 11, I 4, P. 502-512.



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## Conclusions

- Substances, possessing antibacterial and antifungal activity, were prepared on the basis of the interaction of (di)chloro(di)nitrobenzofuroxans with different aliphatic, aromatic amines, amino acids, aminoalcohol nitrates, sulfanilamides, polyene antibiotics and other nucleophiles.
- Novel 2*H*-benzimidazole 1,3-dioxides were prepared on the base of benzofuroxans upon the interaction with alcohols in sulfuric acid.
- It was shown, that under heating 2*H*-benzimidazole 1,3-dioxides are rearranged to 3*H*-[2,1,4]benzoxadiazine 4-oxide which under irradiation converted back to 2*H*-benzimidazole 1,3-dioxides. More prolonged heating causes sequential elimination oxygen atom from oxadiazine cycle to form 2*H*-benzimidazole mono N-oxide.
- A new method for the preparation of 2*H*-benzimidazole 1,3-dioxides by reaction of *o*-benzoquinonedioxime with ketones was developed. Further nitration of compounds yielded a wide range of Sepin-1 analogues (separase inhibitor) with various substituents in the 2-position.



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