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Exogenous nitric oxide donors in the series of C-nitro compounds

V G Granik, N B Grigoriev

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Abstract. The published data on nitrogen(II) oxide donors related to C-nitro compounds are generalized and described systematically. The key classes of these compounds and the possible mechanisms of their transformations under hydrolytic conditions and in living organisms are considered. The attention is focused on C-nitro compounds as potential medicinal agents for treating a broad range of diseases (cardiovascular diseases, tuberculosis, cancer, *etc.*). The bibliography includes 106 references.

I. Introduction

In the recent decades, the biological sciences have made achievements that led to fundamental changes in our concepts of functioning of different biological systems. It was found that nitrogen(II) oxide is one of the universal and crucial regulators of cell metabolism.[1] Surprisingly, a toxic gas, moreover, the molecule that is a free radical, a short-lived and highly reactive compound, appeared to be continuously produced enzymatically in mammals and to possess a key effect on physiological and pathophysiological processes. It is of no surprise that the amount of original papers and reviews dedicated to NO is rapidly growing (see, *e.g.*,[2–13]).

Nitric oxide is involved in regulation of vascular tone, inhibits platelet aggregation and their adhesion to blood vessel walls, is a central and vegetative nervous system agent governing the functions of respiratory, gastrointestinal and urogenital systems.[1] Nitric oxide plays an important role in neurotransmission, immune system regulation and protection of the organism against bacterial infections; it takes a key part in mantaining homeostasis of a living organism (*i.e.* relative dynamic constancy of the internal environment — blood, lymph, tissue fluids) and defines the stability of the basic physiological functions of the organism — blood circulation, breath, heat exchange, metabolism, *etc.* Breath regulation, immune status, neurotransmission are also NO-dependent; in addition, NO is a cytotoxic and cytostatic agent.

Various nitric oxide donors have been found and biologically characterized to a greater or lesser extent. These include nitric acid esters (nitrates), *N*- and *C*-nitroso compounds, various mesoionic substances, NONOates, *S*-nitrosothiols, sodium nitroprusside, furoxanes, oximes, guanidines, nitrites. The so-called hybrid NO donors are also known, which comprise medicinal agents or highly biologically active compounds modified by introducing an NO-donating group. Chemical and biological data on nitric oxide donors are summarized in several reviews.[14–39] There is evidence that some C-nitro compounds also belong to NO donors, but the few publications have not yet attracted much attention, and the above-cited reviews do not even mention, as a rule, such compounds as the nitric oxide donors. Meanwhile, nitro compounds play a prominent role in organic chemistry, they are actively assayed in the search for biologically active substances. This in turn necessitates studying nitro compounds as NO donors, that is as prospective and promising subjects for biological research.

The present review is devoted to organic C-nitro compounds as the nitric oxide generators and their modification as a rational approach to the search for new potential drugs. It should be stressed once again that numerous pathologies, including cardiovascular, infectious and inflammatory diseases, thrombosis, malignant tumours, urogenital diseases, brain damage resulting from the stroke, *etc.*, can largely be

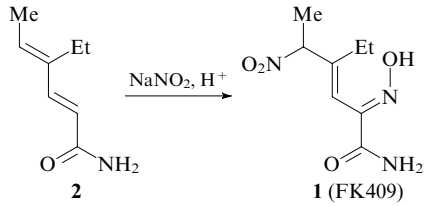
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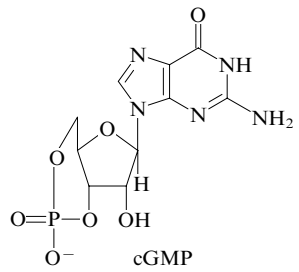
caused by shortage of nitrogen(II) oxide in the organism. It is thus natural that one of the most important trends in medicinal chemistry is the search for compounds that can generate nitric oxide in the organism, *i.e.*, xenobiotics that can undergo transformations (associated or not associated with the activity of NO-synthases[40]) that lead to the formation of nitric oxide.

II. Nitro oximes

To begin, consider a study[33] devoted to compound **1** (FK409) comprising both the *C*-nitro and the oxime group, so that a question arises which group is responsible for the NO release in the organism. Compound **1** is semisynthetic: it is obtained from precursor **2**, which has been isolated from the culture broth of the soil microorganism *Streptomyces griseosporeus*. Biological studies have shown (3*E*)-4-ethyl-2-hydroxyimino-5-nitrohex-3-enamide (**1**) to be a very efficient vasodilator with a pharmacological profile similar to that of nitroglycerine.

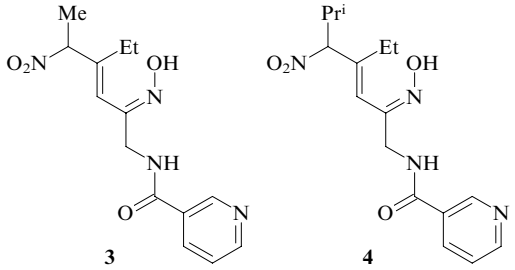


In vivo studies in dogs demonstrated that compound **1** is more efficient in curing stenocardia than nitroglycerine. Clinical trials showed that at a dose of 40 mg mend was observed in 60% – 70% of patients. Unfortunately, a distinct side effect (anemia) was manifested at this dose, so the clinical trials were stopped. However, the obtained results promoted further studies in this direction. A possibility for the improvement of the pharmacokinetic profile of compound **1** was considered so as to prolong its action and to lower the risk of side effects. Investigations into the mechanism of action of **1** showed a relationship between its pharmacological activity and the increase in the level of cyclic guanosine monophosphate (cGMP). This in turn has lead to an idea that the the biological effects mentioned are caused by release of nitric oxide upon administration of **1**.[1]



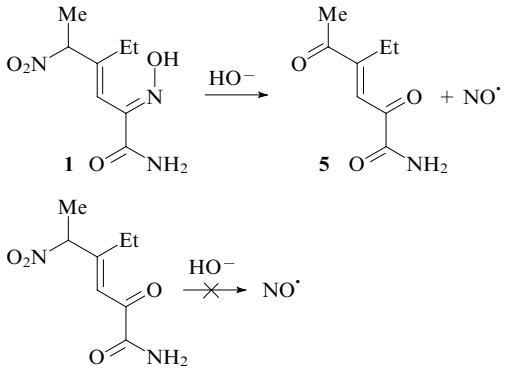
Elimination of NO upon decomposition of compound 1 in solution was proved by physicochemical methods.

The next stage was synthesis of compounds similar structurally to compound **1** that would possess analogous biological activity but devoid of the aforementioned side effect. It was suggested that this effect is caused by an accelerated release of NO. It was shown that two related nitro oximes **3** and **4** release nitric oxide 3 and 88 times more slowly, respectively, than compound **1**. The decrease in the NO release rate leads to reduced *in vivo* biological activity. The oral absorption of both compounds (**3**, **4**) occurs at the same rate as the absorption of **1**, and the oral bioavailability is comparable to that of nitro oxime **1** (this was determined by measuring the NO_x concentration in blood plasma and urine). The hypotensive action of **3** and **4** at a dose of 10 mg kg^{-1} live weight was tested *in vivo* in rats. The activities of the studied compounds turned to be as follows: **1** > **3** > **4**.

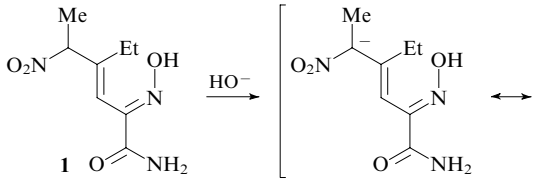


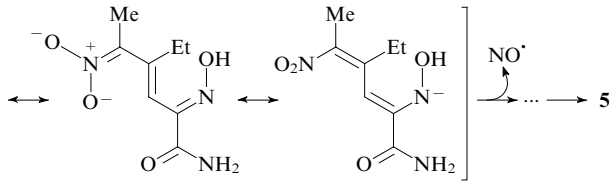
Compound **4** does not cause hypotension in the mentioned dose, and hypotension induced by compound **3** is developed with a distinct lag (3 h) compared to that of compound **1**. Thus, the chosen strategy according to which the reduction of the nitric oxide release rate suppresses the biological activity *in vitro* and prolongs the action *in vivo* appears to be efficient. A comparison of the action of compounds **1** and **3** shows that the two undesirable effects of the former — short action time and hypotension — were overcome.

Though the authors rightfully point to the need of further investigation of the mechanism of NO formation, some of their data seem to be quite interesting. Thus, it was noticed[41] that the major decomposition product of nitro oxime **1** in an alkaline phosphate buffer is diketone **5**. Removal of the oxime group from the structure of **1** leads to complete inhibition of nitric oxide formation; this can be interpreted as evidence for the oxime part of the molecule to be responsible for NO formation.



At the same time, the formation of diketone **5** devoid of both the nitro group and the oxime group upon decomposition of nitro oxime **1** requires care in answering a question of which group is the genuine nitric oxide donor. The following scheme of decomposition of compound **1** has been suggested.[33]

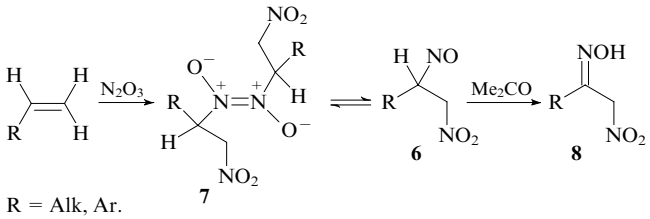




To summarize the discussion of the nitro oximes as NO donors it should be noted that biological tests of compounds **1**, **3**, **4** have shown that they do not exhibit tolerance (reduction of sensibility), which is typical in particular of *O*-nitro compounds (nitrates).

III. Nitro nitroso compounds

In this section, we discuss the nitro nitroso compounds (pseudonitrosites) **6**, which typicaly exist in the solid state as the corresponding dimers, azodioxides **7**.[42] The synthesis of these compounds was carried out as follows:



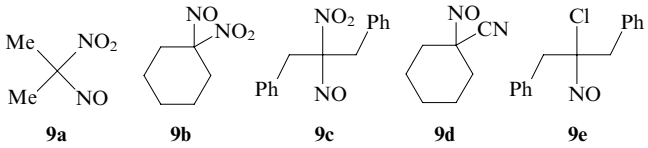
R = Alk, Ar.

A number of pseudonitrosites are known to possess antithrombotic activity, which presumably is the result of nitric oxide release. The electron-withdrawing groups, *e.g.*, the nitro group, vicinal to the nitroso group are believed to favour release of NO. It was also shown that nitro oxime **8** is formed in acetone. Biological studies of the equilibrium system **6** ⇄ **7** demonstrated that such compounds exhibited comparatively strong antiplatelet activity. Upon oral administration of these substances to rats, small antithrombotic effects were observed; the decomposition of one of these compounds was accompanied by nitric and nitrous oxide formation. However, it was pointed out that the absence of vasodilatory effects, *i.e.*, hypotension, suggests the predominance of the *in vivo* enzymatic over thermal decomposition.[43] It should be noted that NO donors generally exhibit vasodilatory effect, and their hemodynamic profile correlates with their activity as the NO donors. It was suggested [42, 43] that the results of the biological tests are due to NO formation, although no scheme was forwarded on how it is formed and what are the groups responsible for it. The decomposition of a model compound used in these experiments, pseudonitrosite **6** (R = Ph), in ethanol was shown to produce 0.18% NO and 3.8% N_2O. It should be noted that N_2O is also formed from nitroxyl (HNO), which undergoes dimerization into HON═NOH, and the latter is decomposed with the formation of H_2O and N_2O. Nitroxyl is known [1] to be an NO donor as well.

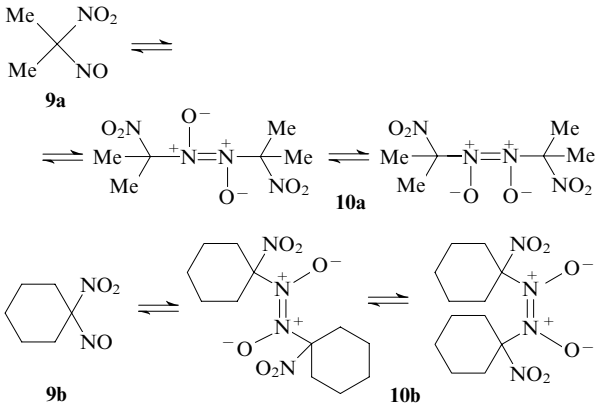
Similar results were obtained in experiments on related compounds [44, 45].

IV. Geminally substituted nitroso compounds

Pseudonitroles **9a**–**c** and related compounds bear the cyano group (**9d**) or a chlorine atom (**9e**) instead of the nitro group in the geminal position to the nitroso group have been considered.[46]



Compounds **9a**–**e** activate soluble guanylate cyclase (sGC) in a standard RFL-6 cell culture, which contains considerable amounts of soluble guanylate cyclase, the principal receptor for NO. Compounds **9a**,**b** exist as dimers **10a**,**b** in the solid state, while solid **9c**–**e** are monomeric.



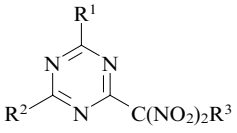
It was a critical objective of the cited work to determine whether these compounds can be a source of nitric oxide (a complete experiment was carried out with compound **9a** as the most soluble in the series). It was shown that storage of any of the studied compounds in a phosphate buffer (pH 7.4) for 1 h at 37 °C produced the nitrite anion, a conventional NO marker, detected using the Griess reaction.[47] Gas chromatography was used to detect N_2O, the dimerization and dehydratation product of nitroxyl (HNO). The increase in the cGMP basal level was established by radioimmune assay. Compounds **9** form the following series according to the amount of nitrite ion formed in 1 h: **9a** > **9c** > **9b** > **9d** > **9e**.

All the studied compounds exhibited vasodilatory effect. Of the four compounds tested *in vivo* (**9a**–**d**), pseudonitroles **9a**–**c** manifested distinct effect on the cardiovascular system, the efficacy of compounds **9a, b** being about three times that of **9c**. All three pseudonitroles exert minimal influence on the cardiac rate. On the whole, it was concluded that the hemodynamic profile of the studied substances was in accord with their NO-releasing effect.

Unfortunately, in the studies cited the issue of which structural parts (the oxime, nitroso or nitro groups) are responsible for NO release has not been considered, and the mechanism of nitric oxide elimination has not been addressed.

V. Polynitromethyl derivatives of 1,3,5-triazines

Polynitromethyl derivatives of 1,3,5-triazines **11** bearing geminal nitro groups are another class of NO-donating *C*-nitro compounds.



11a–g

Compound **11**	R^1	R^2	R^3
a	NH_2	NH_2	CH_2OH
b	NEt_2	$C(NO_2)_2CH_2OH$	CH_2OH
c	NHMe	NHMe	CH_2OH
d	OMe	NMe_2	F
e	NMe_2	NMe_2	CH_2CH_2CN
f	NHMe	NHMe	$CH_2CH_2CO_2Me$
g	NEt_2	NEt_2	CH_2OH

Nitric oxide elimination from compounds **11** under conditions of electrochemical reduction has been studied.[48] A polarographic wave of nitrous acid reduction was detected in weakly acidic non-buffered solutions, which is evidence of nitro group elimination in preceding reduction steps. The half-wave potentials can be used to evaluate the ability of a particular compound to serve as an NO donor (Table 1). Compounds **11c**,**d** bearing additional electron-withdrawing groups exhibited the highest activity.

A general scheme of nitric oxide elimination from triazine derivatives **11** was proposed:

$$RC(X)(NO_2)_2 + 2e^- + H^+ \longrightarrow RC(X){=}NO_2^- + HNO_2,$$

$$HNO_2 + e^- + H^+ \longrightarrow NO + H_2O.$$

The direction of electrochemical reduction is in many cases essentially the same as for chemical processes. In this case, the reduction with nitro group elimination was confirmed by the positive Griess reaction. Moreover, polynitro compounds **11** induce activation of soluble guanylate cyclase, which is typical of nitric oxide donors. It should be noted that *gem*-fluorodinitroethane reduction in aqueous-organic medium at a mercury electrode has been reported earlier. The two-electron nitro group abstraction occurred in the first step.[49] The nitro group abstraction in the first step was also observed in the chemical reduction of *gem*-fluorodinitroethane.[50] The most active compounds **11c**,**d** were reduced with the nitro group abstraction not only under the action of ascorbic acid, but also with potassium ferricyanide, which has been confirmed by formation of a polarographically active species, the nitroprusside anion.

Table 1. Half-wave potentials of the first reduction step of polynitromethyltriazine derivatives **11a–g**.[48]

Compound	$E_{1/2}$ /mV [a]	
	in buffer H_2O-DMF (10%) (pH 5)	in solution of $Bu^n_3NHClO_4$ in DMF (dry) (0.02 mol litre^{-1})
11a	−0.54	−1.10
11b	−0.45	−0.85
11c	+0.21	−0.60
11d	+0.03	−0.70
11e	−0.82 [b]	−1.30
11f	−0.84 [b]	−0.94
11g	−0.42	−1.04

[a] Relative to the saturated calomel electrode (s.c.e.);
[b] in these cases, 5% DMF was used.

VI. Nitrolic acids

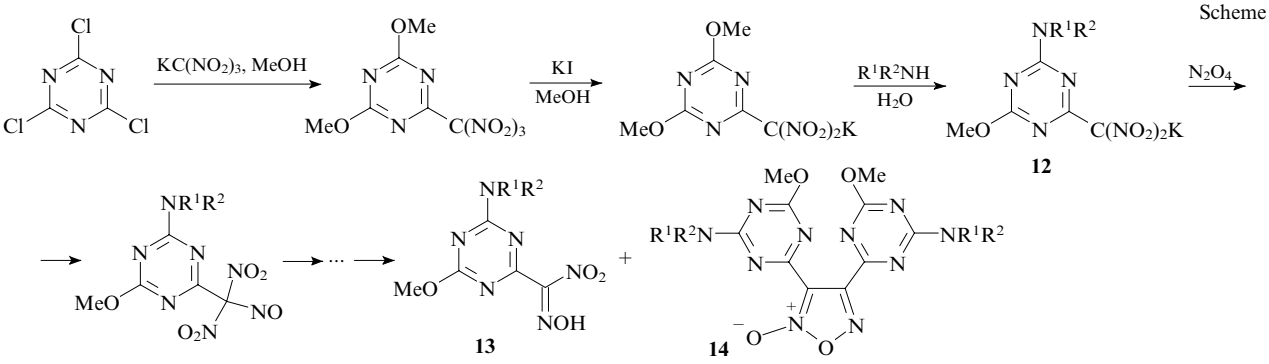
Nitrolic acids $RC(=NOH)NO_2$, with the oxime and nitro groups attached to the same carbon atom, are a promising class of new pro-drugs. Antithrombotic and hypotensive activity was reported for some nitrolic acids.[51] Imidazolenitrolic acids have recently been shown to exert ocular effect (reduction of intraocular pressure).[52, 53] Logically, the structure of nitrolic acids suggests them to be NO donors. It was shown experimentally that some nitrolic acids decompose with HNO formation even at ambient temperature.[54]

The reactions of salts **12** with nitrogen dioxide result mainly in 1,3,5-triazinenitrolic acids **13** (Scheme 1).[55] Along with nitrolic acids, furoxanes **14**, the well-known NO donors, also formed.[1]

The behaviour of nitrolic acids **13** under conditions of electroreduction at the dropping mercury electrode at various pH was studied. It was shown that the process is accelerated in the presence of oxygen. A stimulating effect of nitrolic acids **13** on the activity of human platelet soluble guanylate cyclase was demonstrated.

It was established that nitrolic acids **13** are active nitric oxide donors. Theoretically, one molecule of a nitrolic acid can generate two molecules of NO (due to the presence of $=NOH$ and NO_2 groups). However, acids **13** undergo hydrolysis with the formation of a single nitrite ion. Polarography is a useful tool for studying this process.[56, 57] It was shown that the first reduction wave of the nitro group in compounds **13** in alkaline solutions decreased until it

Scheme 1



$R^1 = R^2 = Me$; $R^1–R^2 = (CH_2)_4$, $(CH_2)_5$, $(CH_2CH_2)_2O$.

completely vanished. The process is pH-dependent, its rate increases with pH. This suggests that hydrolysis of these compounds occurs with elimination of the nitro group in the form of the nitrite anion. The formation of NO_2^- in both the phosphate buffer (pH 8) and 0.01 M NaOH was confirmed by the positive Griess reaction.[58] The electron-withdrawing nature of the 1,3,5-triazine ring determines probably the behaviour of nirolic acids **13** in media with different pH. Thus, they readily decompose in alkaline and neutral solutons and are stable only in highly acidic (1 M $HClO_4$) media. Note should be taken that alkanenitrolic acids are relatively stable also in weakly alkaline solutions. It had been shown[56] that polarographic waves of alkanenitrolic acids in acidic and alkaline solutions are related to reduction of the nitro group.

Nitro group elimination (the Griess reaction was used to detect nitrite ion formation) from compounds **13** occurs faster in the presence of oxygen than in its absence. In partially deoxygenated ethanolic solutions of nitrolic acids **13**, in the phosphate buffer (pH 8) or in 0.01 M NaOH, 10% – 15% of the nitrite anion is detected in 15 – 20 min, and 20% is detected after 24 h (assuming that 1 mole of nitrolic acid releases 1 mole of NO).

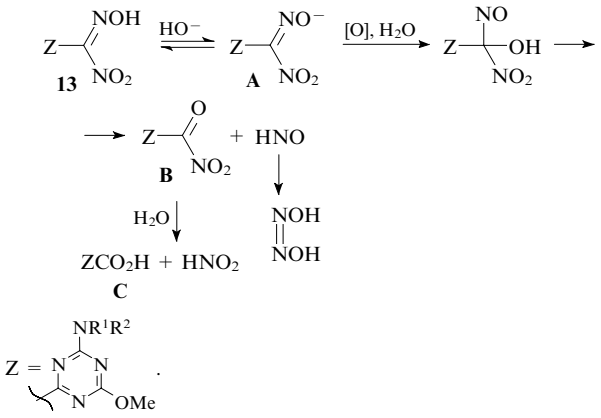
In deaerated 10% aqueous ethanol in the presence of AcOH, 2% – 6% of the nitrite ion is detected after 2.5 h in the contact with air. In 0.1 M HCl solution in 10% aqueous ethanol, no formation of nitrite is observed. But in a non-deaerated buffer at pH 10, nitrolic acids **13** release 80% – 90% of the nitrite anion in 1 h. Thus, the presence of oxygen in a basic solution is essential for nitrite ion generation from nitrolic acids. Generally, alkaline hydrolysis of nitrolic acids **13** carried out in the presence of oxygen causes efficient NO formation.

The nitrite anion is currently regarded as the 'depot' for the NO-like biological activity, thus complementing the NO-synthase pathway.[59] In the organism, the nitrite anion can be transformed into nitric oxide by different pathways, including its reaction with haemoglobin, myoglobin, xanthine oxidoreductase, ascorbate, polyphenols, protons. Reduction of nitrite to nitric oxide and NO-modified proteins that takes place during physiological and pathological hypoxia contributes to physiological hypoxia signalling, vasorelaxation, modulation of cell respiration and cell response to ischemic stress.[59]

The ability of nitrolic acids to activate soluble guanylate cyclase is seemingly caused by exogeneous NO generation (as it was shown in the studies of the nitrite anion formation).[1] A correlation is shown for the amount of liberated NO and the degree of activation of guanylate cyclase.[1] Several alternative routes of NO generation (to be correct, several NO precursors) from nitrolic acids exist.

Thus, it is known that under thermolysis or action of bases, nitrolic acids eliminate nitrous acid and transform into nitrile oxides, which further undergo dimerization into furoxanes **14** (see Scheme 1),[60,61] or hydrolysis into the corresponding carboxylic acids.[62 – 67]

On the other hand, it should be stressed again that in the studied system no nitrite anion was detected in acidic media, hence, no nitric oxide is formed. At pH $\geqslant$ 8, its formation is strongly activated, *i.e.*, the process requires preliminary transformation of the nitrolic acid into the anion. It is logical to assume that the anion **A** is then oxidized with atmospheric oxygen and after several steps forms the nitro carbonyl compound **B** and nitroxyl.



It is well-known that nitroxyl can either undergo oxidation or dimerization in the organism.[1,68] However, in a chemical experiment nitroxyl is not oxidized to NO, *i.e.*, only the nitro group transformation products were isolated. The final reaction product is triazinecarboxylic acid **C**, this has also been synthesized by an independent route.

Hence, the main route of the nitrite anion emergence is the transformation of the nitrolic acid nitro group. Although the oxime group also can serve as an NO precursor in a biological system, the results of chemical studies suggest that this pathway plays a secondary role, for the amount of NO evolved corresponds to the transformation of a single functional nitrogen-containing group.

Several pathways of NO generation are possible, each of them is presumably determined by both the decomposition conditions and the nature of the radical Z bound to the nitrolic group.

These data suggest that this class of compounds present unquestionable interest for further studies with the view of finding new efficient medicinal agents. The design and investigation of novel compounds containing a nitrolic acid fragment can result in discovering original compounds with specific biological activity.

VII. Nitric oxide formation from Bronopol

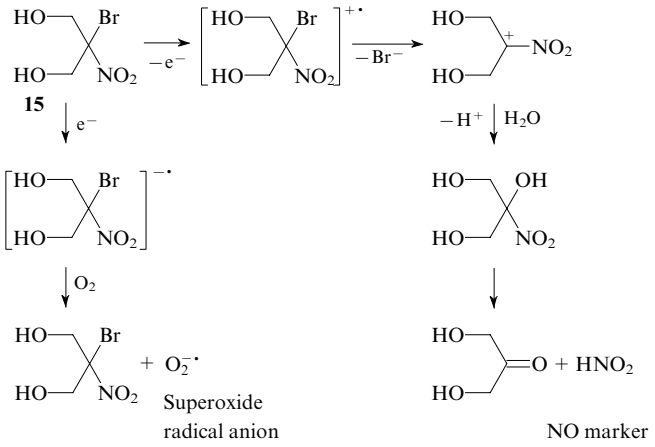
The well-known antiseptic Bronopol (2-bromo-2-nitropropane-1,3-diol, **15**) is also a *C*-nitro compound.[69]

Currently, the mechanism of biological activity of this compound is interpreted as the ability to cross-link (by virtue of the presence of the activated C – Br bond) the thiol groups of dehydrogenases to form disulfides. As a result, the cell structures of microorganisms are modified and the microbial metabolism is violated. Formaldehyde liberated due to the presence of two hydroxymethyl groups also contributes to the antimicrobial activity of Bronopol.[70]

Yet another possible mechanism of Bronopol action is associated[69] with nitric oxide release and oxidation of the Bronopol radical anion, which leads to the formation of superoxide radical anion (Scheme 2).

The subsequent processes are most probably caused by the formation of NO and peroxynitrite $ONOO^-$.

Scheme 2



Decomposition of Bronopol in the absence of oxygen and in the presence of such oxidants as hydrogen peroxide or potassium ferricyanide was carried out. NO was detected by polarography judging from the yield of the nitroprusside anion. Data for another aliphatic nitro compound, nitroethane, are given in Table 2 for comparison.

Table 2. Yields of NO (detected in the form of nitroprusside ion) in chemical transformations of Bronopol.[69]

Conditions	Yield of the nitroprusside anion (%)	
	nitroethane	Bronopol
Aqueous solution, air, 20 °C, 1 day	–	8.0
Non-deaerated buffer solution, pH 5, 80 °C, 30 min	0.6	4.0
Deaerated solution 0.01 M NaOH, 80 °C, 30 min	0.2	14.0
Non-deaerated phosphate buffer-solution, 0.04 M H_2O_2, pH 5, 80 °C, 30 min	1.0	50.0

As one can see from Table 2, Bronopol is an efficient nitric oxide donor under oxidative conditions (it is of note that nitroethane also evolves NO, though to a lesser extent). The ability of Bronopol to activate soluble guanylate cyclase, typical of NO donors, was studied (Table 3).[69]

As the concentration of Bronopol is increased, activation is changed to inhibition, possibly due to the oxidation of the thiol groups present in the heme fragments of enzymes with peroxynitrite formed or their binding due to alkylation. It should not be ruled out that it is the formation of a powerful oxidant, peroxynitrite, that leads to the above-mentioned damage of the dehydrogenase components of the bacterial cell walls, which provides for the antiseptic effect of Bronopol.

Table 3. Activation of soluble guanylate cyclase by Bronopol.[69]

Concentration of Bronopol /mol litre^{-1}	sGC specific activity [a]	sGC activation [b] (%)
0	1.39	100
10^{-6}	3.61	260
10^{-5}	6.39	460
10^{-4}	1.11	80

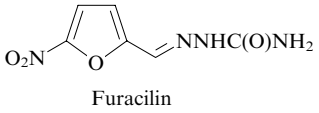
[a] sGC specific activity is measured in pmol sGMP per 1 mg of protein per 1 min; [b] 100% activation means inhibition.

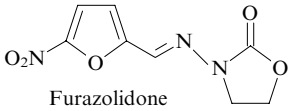
VIII. Nitro derivatives of five-membered heterocycles

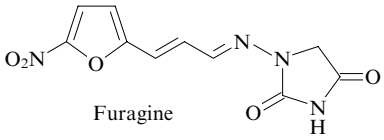
1. Compounds of the 2-nitrofuran series

Yet another group of compounds that present interest in terms of the NO-donating activity consists of the known antimicrobial chemicals of the nitrofuran series. These compounds are efficient against Gram-positive and Gram-negative bacteria. As the furan ring is known to undergo cleavage under the action of various nucleophiles, it was suggested that under reduction conditions dearomatization of nitrofuran derivatives is possible, which should, at least partly, lead to compounds able to evolve nitric oxide.[71]

The behaviour of such medicines of the 2-nitrofuran series as Furacilin (Nitrofural), Furagine, Furazolidone, were studied under reductive conditions.[71]

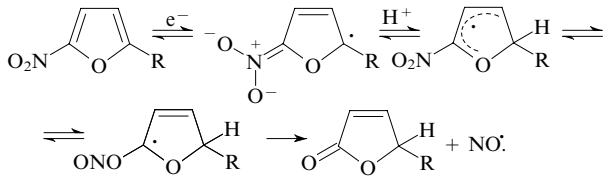

Furacilin


Furazolidone


Furagine

Heating these compounds (solutions with concentrations of 10^{-4} or 2×10^{-4} mol litre^{-1}) with potassium ferricyanide (10^{-3} mol litre^{-1}) at 60 °C and pH 5, leads, according to polarographic data, to formation of the nitroprusside anion, which shows that nitric oxide is formed. The second version of reduction of the nitrofuran derivatives made use of ascorbic acid (pH 6.5) with subsequent addition of potassium ferricyanide and also leads to the nitroprusside formation.

The radical anions that are formed upon reduction of 5-nitrofurans can undergo nitro-nitrite rearrangement.[72] A mechanism was proposed [71] for the formation of NO in the decomposition of drugs of the nitrofuran series.



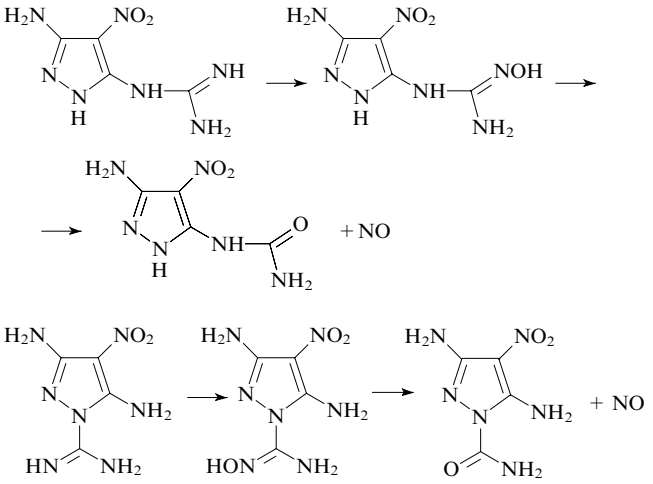
It should be noted that many of the NO effects on various systems are related to the formation of peroxynitrite ($ONOO^-$). Presumably, its antibacterial effect is connected with the inhibition of mitochondrial electron transport, which disables bacterial cell respiration. Thus, one of the reasons of the high antimicrobial activity of the discussed compounds may be their ability to release nitric oxide.

It should also be noted that the discussed antibacterials in particular, and NO donors in general, can serve as

signalling molecules in the bacterial QS system † (Ref. 73). The aforementioned compounds of the nitrofuran series, in comparison with the known nitric oxide donors (sodium nitroprusside and isosorbide 5-mononitrate), were shown to cause a considerable increase in bioluminescense of the *Escherichia coli* strains that are used to detect *N*-acylhomoserine lactones, the signalling molecules of the QS-regulatory system, in subinhibitory concentrations.[74] However, this system appeared to be non-specifically associated with the QS-system functioning.

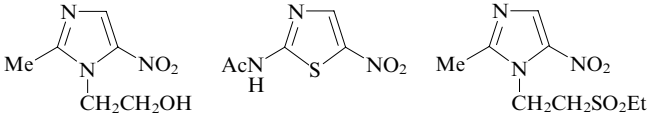
2. 2. Nitropyrazoles and nitroimidazoles with guanidine and amidine substituents

A recently published paper[75] reviews the ability of nitropyrazole derivatives with guanidine or amidine substituents to undergo transformations that lead to NO evolution. The oxidation was carried out with potassium ferricyanide in an alkaline medium, the formation of nitroprusside anion was detected by polarography. The release of nitric oxide presumably proceeds according to the general mechanism through N-hydroxylation in the first step.



3. Imidazole and thiazole derivatives: Metronidazole, Nitazole and Tinidazole

We shall further consider three well-known anti-protozoan drugs, Metronidazole, Nitazole and Tinidazole.



Metronidazole Nitazole Tinidazole

Metronidazole possesses a wide chemiotherapeutic spectrum against anaerobic protozoa and against aerobic Gram-negative and some Gram-positive bacteria. Using electrochemistry methods and EPR, Metronidazole was demonstrated to readily form a radical anion product of one-electron reduction of the nitro group, which can destruct DNA, RNA and other vital macromolecules in the cell. This radical anion is supposed to be responsible for the biological activity of the drug.[76] The mechanism of Metronidazole action, though, is not unquestionable. For instance, some researchers believe that the active species is the corresponding hydroxylamine derivative formed in the reduction.[77] It should also be noted that the nitro group of Metronidazole can be eliminated during the reduction.[76–79] Thus, the nitrite ion is formed in high yield in the electrolysis of Metronidazole,[79] and reduction in the presence of thiols and divalent iron leads to complexes that include iron ions, molecules of thio derivatives, and nitric oxide.[80]

It should be particularly noted that N-substituted 5-nitro-2-styrylimidazoles undergo decomposition in alkaline media (pH > 10) with ring opening and elimination of the nitro group in the form of the nitrite anion.[81] It was thus suggested[82] that nitric oxide release is a probable route of Metronidazole biotransformation and that this fact plays possibly an important role in the biological activity of the drug.

It was shown by polarography that alkaline hydrolysis of Metronidazole leads to the nitrite anion, precursor of nitric oxide. The rate constant for Metronidazole hydrolysis is 1.6×10^{-3} min^{-1} in 0.01 M NaOH at 80 °C. Elimination of the nitro group of Metronidazole under such conditions occurs almost quantitatively in 30 min. In biological systems (human blood), the release of NO was confirmed by the activation of soluble guanylate cyclase. The degree of activation depends, as it is typical of exogeneous NO donors, on the concentration of the compound (Table 4).

Table 4. Influence of Metronidazole on the activity of soluble guanylate cyclase.[82, 83]

Metronidazole concentration/mol litre^{-1}	sGC specific activity	sGC activation (%)
0	105 ± 12	100
10^{-7}	106 ± 5	100
10^{-6}	242 ± 18	230
10^{-5}	368 ± 24	350

The found NO-releasing property of Metronidazole prompted[82] evaluation of the effect of this chemical on such hemodynamic parameters as cardiac rate (CR) and systemic arterial blood pressure (AP) in rats and a comparison of the obtained data with the corresponding properties of such a typical NO donor as isosorbide 5-mononitrate. The action of these compounds appeared to be quite similar (Table 5).

Table 5. Influence of Metronidazole and isosorbide 5-mononitrate on cardiac rate in rats.[82, 83]

Studied compounds	Dose [a]	AP/mm Hg	CR/bpm
Control (0.9% NaCl solution)	0	142 ± 5.0	322 ± 9.0
Metronidazole	0.001	131 ± 5.0	300 ± 9.0
	0.01	120 ± 4.0	282 ± 10.0
	0.1	108 ± 2.0	280 ± 6.0
Isosorbide 5-mononitrate	0.001	137 ± 4.0	320 ± 9.0
	0.01	125 ± 6.0	280 ± 10.0
	0.1	97 ± 2.0	278 ± 9.0

[a] In miligrams per 1 kg of animal live weight intravenously.

† QS-system (from Quorum Sensing), a special type of gene expression regulation dependent on the density of bacterial population; it is based on the action of low-molecular-weight signalling molecules of various nature.

However, the nitro-nitrite rearrangement, which occurs in the case of nitrofuran derivatives, cannot be ruled out in this case as well. As for the possibility of NO evolution upon reduction, additional data are required to formulate the mechanism.

Analogous results were obtained in the studies of Nitazole: it also releases NO under the discussed conditions and activates soluble guanylate cyclase.[83]

Tinidazole is a close analogue of Metronidazole as regards its structure and chemotherapeutic spectrum; the mechanisms of their action are presumably similar, so both of them possess NO-donating properties.[84]

Hydrolytic transformations of Tinidazole were studied using direct current polarography, the elimination of the nitro group was detected using the Griess reacion.

The possible mechanisms of molecular action on these drugs (RNO_2), based on literature data, are presented in Scheme 3. It was believed that *N*-nitrosamines and *N*-hydroxylamines inhibit the bacterial respiratory enzymes. However, we consider that it is the last reaction in Scheme 3 that fits the best with the experimental data.

Scheme 3

$$RNO_2 \xrightarrow{\text{reduction}} RNHNO + RNHOH,$$

$$RNO_2 \xrightarrow{e^-} RNO_2^{-\bullet} \dashrightarrow O_2^{-\bullet} \xrightarrow{RNO_2^{-\bullet},\ RNO_2} H_2O_2 + HO^{\bullet} + NO_2^-,$$

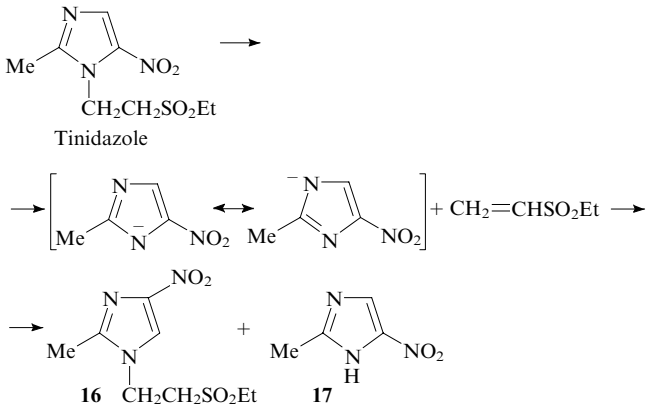
$$RNO_2 \xrightarrow{\text{reduction}} NO_2^- + N^{\bullet}O \xrightarrow{O_2^{-\bullet}} ONOO^- \xrightarrow{H^+} ONOOH.$$

Nitroimidazoles are known to readily form radical anions, the one-electron reduction products, which, as is currently believed, are responsible for the mechanism of their action.[85]

Unlike Metronidazole, Tinidazole liberates no more than 8% nitrite under the same conditions. This fact seemingly contradicts the data obtained in the studies of stability of these drugs. Thus, it was shown[86] that the hydrolysis rate constant of Metronidazole at pH 11 and 80 °C is 150 times smaller than that of Tinidazole. Nevertheless, polarography for alkaline solutions of Metronidazole and Tinidazole after heating at 80 °C for 30 min confirms the decomposition of both of them. In the case of Metronidazole, no electrochemically active compounds are formed, and in the case of Tinidazole a wave is observed with limiting current slightly higher than that of the limiting current of the wave of the initial solution. The half-wave potential has a significant negative shift, which implies that a new compound is formed, which seemingly also bears a nitro group.

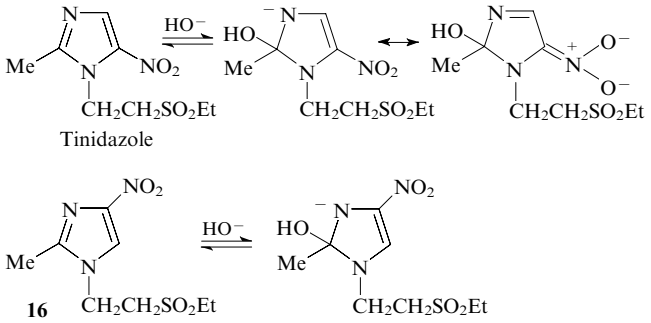
A comparison of polarograms recorded for fresh acidic solutions (immediately after dissolution of the substances) and those recorded following 2-h incubation in the basic medium and subsequent acidification shows that Metronidazole is in fact more stable in basic solutions than Tinidazole and does not undergo irreversible transformations at moderate temperatures. In the case of Metronidazole, a new compound is not detected in the solution, unlike in the case of Tinidazole.

These differences can be explained on the basis of the chemical peculiarities of imidazole derivatives that bear the 1-[2-(alkylsulfonyl)ethyl] group. It was shown that such compounds are easily transformed into two products, *viz*., isomeric 4-nitroimidazole **16** and 2-methyl-5-nitroimidazole (**17**) in alkaline solutions. The latter was the major product at high concentration of alkali, whereas at low alkalinity (for example, at the concentration of sodium hydrogencarbonate of 4 g litre^{-1}), the former product was obtained in virtually quantitative yield.



Thus, 5-nitroimidazoles (Metronidazole and Tinidazole) can apparently decompose in highly basic media with nitric oxide formation; this transformation is not typical for their 4-nitro isomers.

This can be explained, first, by the presence of two adjacent bulky groups (the nitro group and the alkylsulfonyl group) in Tinidazole, in contrast to 2-methyl-4-nitroimidazole **16**. This steric effect destabilizes the molecule and facilitates its decomposition. Second, if we assume that the attack of the hydroxyl anion, which preceeds opening of the imidazole ring of tinidazole with release of NO,[82] occurs at the 2 position, and this step is rate-limiting, than clear distinctions can be seen for Tinidazole and 2-methyl-4-nitroimidazole **16**.



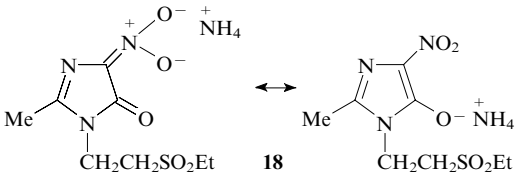
In the case of 5-nitro isomers, the anion formed is efficiently stabilized by conjugation, which cannot be realized in the case of 4-nitro derivatives. Thus, the rate of hydroxyl addition to 5-nitro derivatives is sufficiently higher.

Thus, in the case of Tinidazole, the processes that result in the nitro group elimination in basic media (similar to those occurring in solutions of Metronidazole) compete with the processes that result in 4-nitroimidazole derivatives, and the latter do not eliminated the nitro group under the described conditions. This suggestion is confirmed by the polarographic data, together with the detection of the nitrite anion in the hydrolysis products. In the case of Tinidazole, contrary to Metronidazole, more drastic conditions do not result in higher yields of the nitrite anion. This fact can be properly explained in the framework of the above-described scheme.

The elimination of the nitro group from the imidazole ring in alkaline solutions can occur under nucleophilic attack.[86] Though the increased alkalinity does increase the nucleophile (HO^-) concentration, it also accelerates the formation of 4-nitro isomer of Tinidazole, which is more thermodynamically stable than the initial substance. The elimination of the nitro group is thus favoured by relatively mild conditions, when the rate of processes that lead to the rearrangement is slow. Under mild conditions, Tinidazole eliminates nitrite much more smoothly than Metronidazole. The latter does not undergo this reaction at pH 10; in a buffer solution with pH 10 at 80 °C, Metronidazole forms only 2% – 3% of the nitrite anion in 1.5 h. At the same time, Tinidazole gives comparable amounts of nitrite in buffers with lower pH (8 – 9) even at 37 °C. The schemes of alkaline hydrolysis involving nucleophilic attack at positions 4 or 2 of the imidazole ring of Metronidazole and eliminaion of the nitro group after ring opening were suggested.[82] If the addition of the hydroxide anion takes place at position 2 (which conforms well with experimental data on the difference in Metronidazole and Tinidazole decomposition in alkaline media), the whole process can follow Scheme 4.

For Metronidazole (R = CH_2CH_2OH), acetamide, ethanolamine and glycolic acid are easily predictable hydrolysis products in addition to nitrite. Notably, these products were detected in the mixtures formed after contact of Metronidazole with biological systems of various complexity: after anaerobic incubation with xanthine oxidase, with the content of rat blind gut, with *Clostridium perfringens*, and in urine of non-sterile rats following administration of Metronidazole. The most complete results were obtained in the experiment with xanthine oxidase.[82]

The competition of chemical and metabolic processes can be demonstrated by the fact that a derivative of 4-nitroimidazole, *viz*., ammonium salt of 1-[2-(ethylsulfonyl)ethyl]-2-methyl-4-nitro-4,5-dihydroimidazol-5(1*H*)-one (**18**) was found among its metabolites.[87]



Thus, adhering to the concept of the possibility of modelling of drug biotransformations using chemical reactions and reagents, allows one to conclude that fragmentation of Tinidazole in the organism can occur with elimination of NO, and the action of this drug on protozoa and bacteria is presumably at least partially linked to its NO-donating properties.

Analogous data are available for Nitazole: it also releases NO under conditions similar to those for Metronidazole and activates soluble guanylate cyclase (Table 6).

Table 6. Influence of Nitazole on the activity of soluble guanylate cyclase.[82, 83]

Concentration of Nitazole /mol litre^{-1}	sGC specific activity	sGC activation (%)
0	105 ± 12	100
10^{-7}	179 ± 14	170
10^{-6}	147 ± 10	140
10^{-5}	148 ± 11	140

4. Miscellaneous five-membered nitroheterocycles and dinitroheterocycles

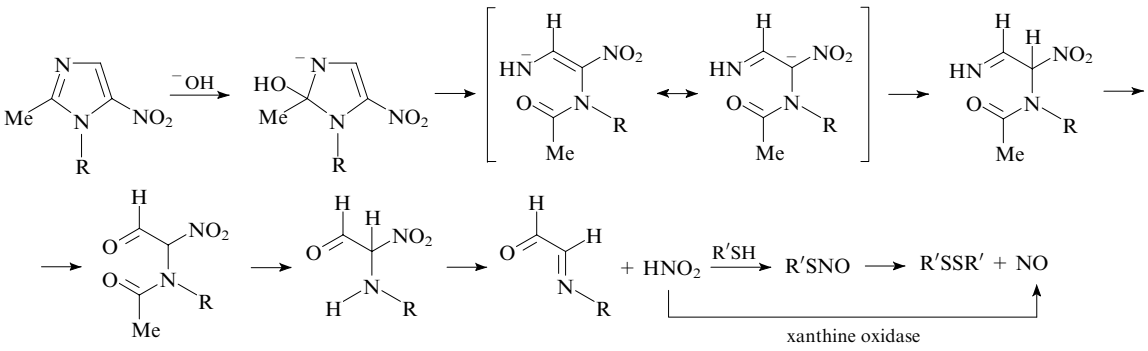
Nitroheterocycles can release NO by another pathway, namely, by ring opening at a position different from the one the nitro group is attached to, dearomatization and subsequent elimination of NO from the non-heterocyclic precursor.

Compounds the derivatives of which are discussed in the present review, are arranged in a series according to the decrease in their aromaticity:[88] benzene > 1,2,4-triazole > pyrazole > imidazole > isoxazole.

It was shown[89] that the release of the nitrite anion upon heating of mononitro derivatives in 0.1 M NaOH containing 10% ethanol at 70 °C for 3 h little depends on the nature of the heterocycle: for nitropyrazole and nitroimidazole derivatives it amounted 2%, and 3% for nitrotriazole. As was expected, exposure of an isoxazole derivative under such conditions led to a considerable decomposition of the initial molecule and the amount of NO_2^- formed reached 26%.

The process of the nitrite anion release on heating in alkaline solutions was studied under conditions that are far from physiological. The problem of hydrolytic ring opening is discussed in a review[2] and note is taken that this processes can proceed either non-enzymatically or with enzymatic catalysis. Hydrolysis of heterocycles in the organism is still not fully studied, but, *e.g*., data for thiazolidine show that its ring opening is catalyzed by 5-oxo-L-prolinase. Hydrolytic transformation of the nitro derivatives studied is a model process performed under non-physiological conditions, which, however, does not exclude the possibility of these compounds to serve as the NO sources in the organ-

Scheme 4



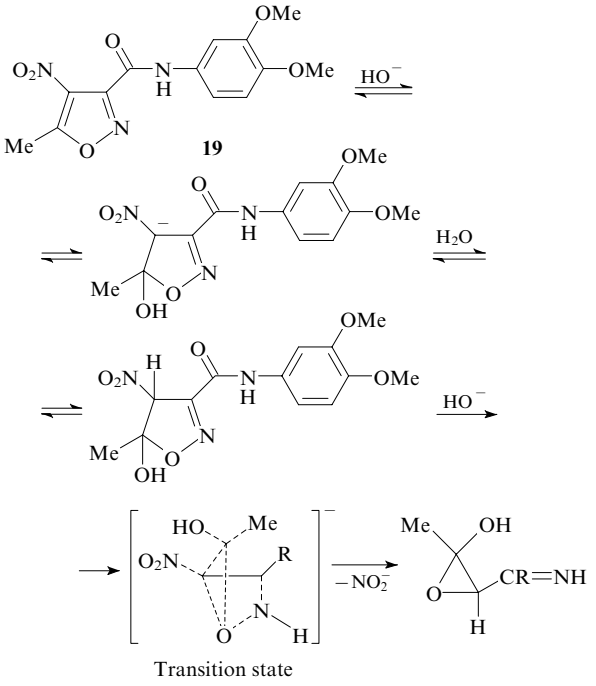
R = CH_2CH_2OH or $CH_2CH_2SO_2Et$; R′ is on endogenous thiol.

ism, too, since the reaction rates and product yields increase manifold for enzymatic reactions.

The very fact of detection of considerable amounts of the nitrite anion upon decomposition of five-membered heterocycles allows one to infer reliably that such compounds can be nitric oxide donors in a living organism. This is also indirectly proved by the data on activation of soluble guanylate cyclase by these substances.

The kinetics of NO release in the course of hydrolysis of heterocyclic *C*-nitro compounds was studied,[89] and on the basis of rate constants (k_{aver}) for these reactions measured at different temperatures, thermodynamic parameters were calculated, which were considered when suggesting the reaction mechanisms.

Thus, the following scheme was proposed for the degradation of nitroisoxazole derivative **19** in 0.1 M NaOH solution containing 10% ethanol.

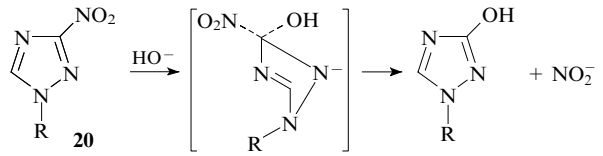


R = C(O)NHC$_6$H$_3$(OMe)$_2$-3,4.

It should be noted that regardless of the relatively high degree of decomposition of compound **19** with elimination of the nitrite anion (~16% NO_2^- is formed upon heating of the solution at 70 °C for 3 h), other routes of decomposition of this isoxazole derivative are realized. Nevertheless, a kinetic study of this hydrolytic process was carried out,[89] which allowed one to obtain the corresponding thermodynamic parameters (Table 7).

On the basis of the obtained results, the following conclusions was inferred:[86] the low enthalpy of activation is probably caused by the low aromaticity of the isoxazole ring and low enegry required to break the cyclic N—O bond. The most spectacular is the high negative entropy of activation, which points to a highly ordered transition state corresponding to elimination of the nitrite ion from the molecule. According to the above depicted supposed transition state, such a structure is really highly ordered and its formation corresponds with the thermodynamic calculations.

The high negative entropy of activation for 1,2,4-triazole derivative **20** also says for a highly ordered transition state, which can be depicted as follows:



R = $CH_2C(O)NHC_6H_4Et$-4.

Clearly, the intensity of reactions with nucleophiles should increase in the cases where a heterocycle bears several electron-withdrawing groups. Logically, the probability of nirite ion elimination under nucleophilic attack should be higher for dinitro derivatives than for mononitro compounds. Thus, for pyrazole, triazole and imidazole mononitro derivatives, the degree of elimination of nitrite reaches generally 1%–7%, for dinitropyrazoles it is 7%–28%, and for dinitroimidazoles it is even 100%–130% (the value more than 100% gives evidence that in the studied conditions the first nitro group is completely cleaved before the reaction of the second group begins).

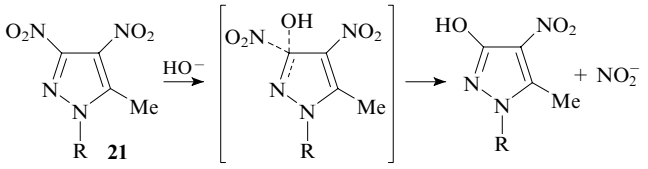
Kinetic studies of a variety of 3,4-dinitropyrazole derivatives at different temperatures also enabled the evaluation of thermodynamic parameters of the processes. The data on alkaline degradation of 5-methyl-3,4-dinitropyrazole **21** are presented in Table 7.

The obtained data on dinitropyrazole **21** (and related compounds) are interpreted as follows: the relatively high enthalpy of activation implies that cleavage of a particular bond in the transition state was almost completed, or, at least, reached a rather high degree. And the relatively low entropy of activation can be explained from the same structural considerations — the bond is almost cleaved and the transition state is close to the final structure, *i.e.*, similar in its steric parameters to the initial state.

Table 7. Rate constants [a] and thermodynamic activation parameters for the decomposition reactions of some five-membered heterocycles.[89]

Compound	k_{aver} /s^{-1}			$\Delta G^{\neq}$ /kcal mol^{-1}	$\Delta H^{\neq}$ /kcal mol^{-1}	$\Delta S^{\neq}$ /cal mol^{-1} K^{-1}
	T_1	T_2	T_3			
19	$3.1\times10^{-5}\pm4.6\times10^{-6}$	$9.3\times10^{-5}\pm1.1\times10^{-6}$	$3.9\times10^{-4}\pm6.2\times10^{-5}$	24.4 ± 2.4	11.1 ± 1.7	-41.8 ± 1.2
21	$6.0\times10^{-5}\pm7.0\times10^{-6}$	$6.4\times10^{-4}\pm7.0\times10^{-5}$	$1.2\times10^{-3}\pm1.0\times10^{-4}$	24.1 ± 2.8	26.8 ± 2.7	-8.6 ± 0.3
22	$2.3\times10^{-3}\pm3.1\times10^{-4}$	$5.0\times10^{-3}\pm5.0\times10^{-4}$	$1.22\times10^{-2}\pm9.0\times10^{-4}$	13.2 ± 1.17	12.1 ± 1.2	-30.2 ± 1.5

[a] T_1, T_2 and T_3 are 35, 40 and 50 °C, respectively, for compounds **21** and **22**, 25, 40 and 70 °C, respectively, for compound **19**.

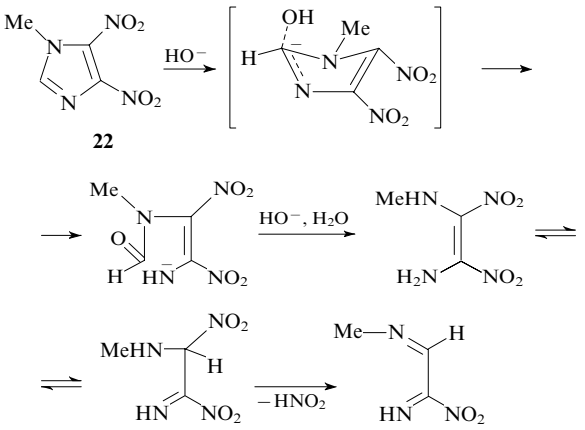


R = $CH_2C(O)NHC_6H_4OMe$-4.

Thus, the entropy of the transition state is close to that of the initial state, and $\Delta S^{\neq}$ does not differ much from zero. Another compound studied,[86] 1-methyl-4,5-dinitroimidazole (**22**), differed dramatically in behaviour from pyrazole derivative **21**, which cannot be explained by different substituents in position 1. First, imidazole **22** rapidly (approximately in 12 min) and quantitatively eliminates the nitrite anion. Second, the process is not finished at this stage, it proceeds as a slower and non-quantitative transformation of the intermediate with elimination of the second nitro group.

Seemingly, the process mechanism is completely different from that for the pyrazole derivative. In the case of imidazole, the enthalpy of activation is much lower, and the entropy is negative and has much higher magnitude. Thus, the situation reminds that of the mononitroisoxazole **19** (it should be once again noted that the imidazole ring is significantly less aromatic than the triazole and pyrazole rings).

These results could be rationalized assuming that the first step of the process is the imidazole ring opening under the attack by the hydroxide anion,[89] and that this step is the rate-determining for the whole process of nitrite elimination.

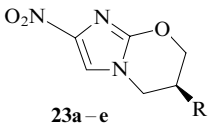


It should be noted that the rate constants for hydrolysis of the studied compounds were determined using two independent methods, spectrophotometry and polarography. In the first case, the Griess reaction was employed to evaluate the accumulation of the final product, the nitrite anion. In the second case, the analytical method allowed one to monitor the amount of the initial substance judging by lowering of the limiting current of the nitro group reduction. A good correlation of the results obtained by different methods says for reliability of the suggested hydrolysis mechanism.

Certainly, the above reaction mechanisms are to a significant extent speculative, although they are confirmed by experimental data. The main result of the study [86] is that the *C*-nitro compounds assayed (and especially dinitro compounds) definitely release nitric oxide and are of considerable interest for their biological activity examinations.

5. Bicyclic nitroimidazoles

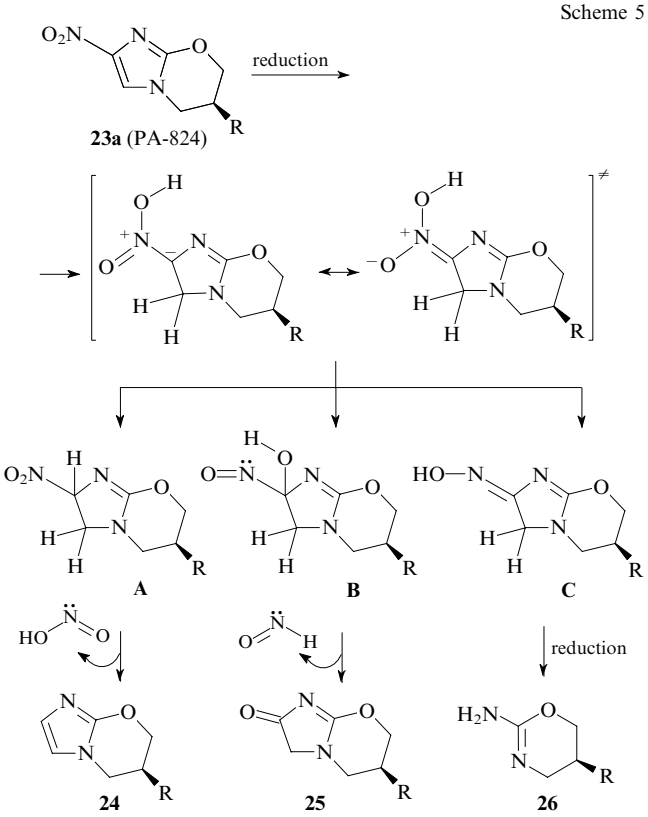
A series of bicyclic nitroimidazoles **23** were studied;[90] some of them are potential pro-drugs against tuberculosis (pro-drugs require intracellular activation to perform their functions).



R = $OCH_2C_6H_4OCF_3$-4 (**a**), $NH(CH_2)_3C_6H_4OCF_3$-4 (**b**), 4-$NHCH_2C_6H_4OCH_2C_6H_4OCF_3$-4 (**c**), $NH(CH_2)_4C_6H_4OCF_3$-4 (**d**), $NHCH_2C_6H_4Bu^n$-4 (**e**), *etc.*

Compound **23a** (PA-824) is currently under clinical trials. It was established that deazaflavin-dependent nitroreductase (Ddn) transforms PA-824 into three primary metabolites, the major of which is the corresponding denitroimidazole **24** (Scheme 5). Studies on dinitroimidazoles **23** showed that the amount of metabolite **24** formed correlates well with anaerobic killing of *Mycobacterium tuberculosis*. The formation of the metabolites is accompanied by generation of reactive nitrogen species including nitric oxide. The latter is the most important effector of the anaerobic activity of these nitroimidazoles. Moreover, acceptors of NO protect the bacteria from the lethal effect of pro-drugs. Thus, compounds **23** may act as intracellular NO donors and enhance the immune system response.

Scheme 5



R = $OCH_2C_6H_4OCF_3$-4.

Drug-resistant tuberculosis is currently a severe threat to the health and lives of many people. Some hope in this area is inspired by bicyclic imidazoles PA-824 (**23a**) and OPC-67683 (an analogue of PA-824 with the imidazole ring fused with five-membered rather than six-membered heterocycle). They are currently under clinical trials. The mentioned compounds are active not only against replicating bacteria, but also against bacteria that do not replicate under hypoxic conditions. Such bacteria are extremely hard to defeat and it is these that are responsible for the long (6–8 months) treatment cycle required to avoid recurrence. Moreover, it is thought that non-replicating bacteria are responsible for latent tuberculosis, which is typical of one third of the Earth's population.

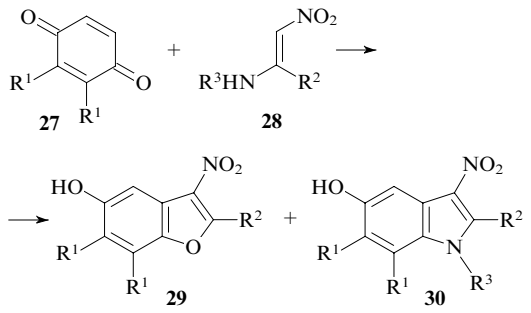
The action mechanism of pro-drugs PA-824 and OPC-67683 is complicated. Their application, presumably, inhibits its synthesis of mycolic acids, the main components of *M. tuberculosis* cell walls. However, such an effect is unlikely to be the major one, *i.e.*, be responsible for killing of non-replicating bacterial cells, as the bacteria do not produce mycolic acids in considerable amounts under anaerobic conditions. Coenzyme F and dependent on it glucose-6-phosphate dehydrogenase (Fgd1) are important for the activation of bicyclic nitroimidazoles, they reduce the bacterial resistance against their action. Chemical structure of the reduction products of compound **23a** was studied. The metabolites of enzymatic (Ddn) reduction of PA-824 are compounds **24**, **25**, **26**; they are formed *via* the intermediates **A**, **B**, **C**, respectively (see Scheme 5).

Herefore, reactive nitrogen species are the dominant factor of anaerobic activity of compound **23a**, but aerobic conditions provide for other effects.

The metabolism of compound **23a** leads to reduction of the nitro group with the formation of amino compound **26**, oxo derivative **25** (nitroxyl elimination occurs) and the main process is the elimination of the nitro group with the formation of denitro compound **24** and nitrite, the known marker of nitrogen(II) oxide. This is in good agreement with the above described effect of NO acceptors that protect bacteria from killing.

6. 3-Nitro derivatives of benzofuran and indole as potential anticancer agents

Studies of the Nenitzescu reaction (condensation of quinones with enamines) leading to 5-hydroxyindoles as the major products showed that nitro enamines can also serve as substrates of this reaction.[91] Thus benzoquinones **27** react with substituted β-nitro enamines **28** with the formation of nitrobenzofurans **29** and nitroindoles **30**, many of which are NO donors. It is of note that in this case 3-nitrofurans are obtained, which have not been tested so far as the NO donors, in contrast to 2-nitrofurans.



The NO-donating activity of 3-nitrobenzofuran and 3-nitroindole derivatives was proved judging from the possibility of nitro group elimination under conditions of alkaline hydrolysis and detection (using polarography and spectrophotometry) of the nitrite anion, which is the marker of nitric oxide.[1,69] It was established that their alkaline hydrolysis proceeded under much milder conditions than hydrolysis of the known drugs, 2-nitrofuran derivatives. It should be noted that these compounds activate soluble guanylate cyclase by the NO-dependent mechanism, which confirms the conclusion that they belong to exogenous NO donors.

Considering the cytotoxic and cytostatic properties of NO against tumor cells, it should be expected that in the nitrobenzofuran series compounds can be found with anticancer activity, which may be regarded as promising antitumour agents.[1]

Despite huge amount of studies devoted to nitric oxide, its role in the tumour biology is still contradictory. Nitric oxide participates in all stages of neoplasm development: initiation, promotion, progress, invasion and metastasis.[92–94] The enhanced production of NO and its transformation products in the living organism, *e.g.*, *S*-nitrosothiols or non-haem iron dinitrosyl complexes with low-molecular-weight thiol ligands, results in damage of the DNA structure, which can be inherited as mutations and cause cell transformations and tumour polymorphism. Other NO targets in the cell are enzymes that repair the DNA and regulate metabolism. In cancer patients, the levels of NO as the product of inducible NO-synthase (iNOS) is manifold increased in response to the action of formed cytokines — interleukin-1 and interleukin-2, interferon-γ or its combinations with the TNF-α, TNF-β, EGF and other factors. Angiogenic and metastatic effects of NO were proved by experimental and clinical data.[95] Clinical trials show a positive correlation between the tumour stage and iNOS concentration in the cells of mouth, larynx, lungs, reproductive organs and central nervous system cancer.[93,96]

At the same time, nitric oxide is an integral part of the immune system defence against cancer, with the participation of immunocompetent cells. Monocytes, macrophages and neutrophils produce nitric oxide to exhibit cytotoxic and cytostatic effects on the tumour. Thus tumour growth is accompanied by a decrease in NO production by the immune cells.[97] The high reaction rate of NO with free radicals allows it to be considered as an *in vivo* scavenger of free radicals. The diverse character of the effect of NO on tumor growth is explained by the complex action of this radical, which depends on the concentration, exposure time, heterogeneity of reactions with key reagents (oxygen and its reactive species, carbon dioxide, *etc.*) and targets (metals, thiol-containing amino acids and proteins, *etc.*), on the cancer cell genotype, *etc.* Short-term flow of NO produced by the constitutive NO-synthases are thought to directly react with biological targets, causing the formation of metal nitrosyl complexes, stimulation of cyclooxygenase, activation of soluble guanylate cyclase, whereas higher NO concentrations produced by inducible NO-synthases for prolonged periods result in nitrogen reactive species and prevailing of other reaction types, such as nitrosation, oxidation, nitration and hydroxylation.[93]

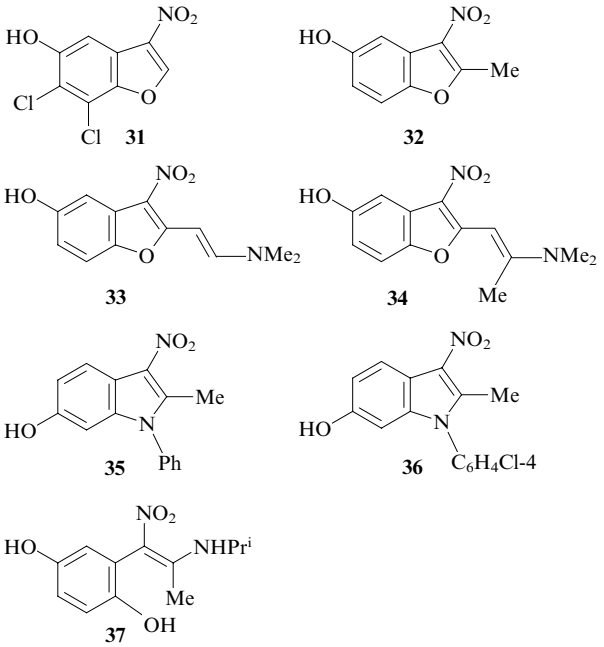
It was demonstarted that endogenous NO (as well as NO produced by exogenous donors) self-inhibits the synthesis of NO by binding the haem-containing group of NO-synthases

with nitric oxide and by suppression of NOS gene expression through NFκB transcription factor deactivation, and other mechanisms.[1] The number of publications showing that excess nitric oxide production leads to tumour cell destruction through apoptosis, is currently growing. Moreover, restoration of the suppressed expression of iNOS during cancer progress is viewed as a preventive measure against metastasis.[98, 99]

It was discovered that NO donors are able to enhance the efficiency of a variety of clinically important methods of cancer treatment.[100] Studies in mice with Ehrlich's adenocarcinoma showed that hypodermic injection of sodium nitroprusside (the NO donor, 80 μg kg^{-1}) caused reliable tumour growth slowdown (by volume and/or mass), while higher dose (4 mg kg^{-1}) potentiated its growth (28% – 124%).[101] The five-membered heterocycles (nitro derivatives of furan, imidazole, thiazole, *etc.*) are potential sources of exogenous nitric oxide. The mechanism of chemotherapeutic activity is linked with their metabolism features, which results in elimination of the nitro group in the form of the nitrite anion with subsequent transformaton into nitric oxide, a potent cytotoxic agent.

Taking into account the above data, it seemed reasonable to study the antitumour activity of 3-nitrobenzofuran (**31** – **34**) [102, 103] and 3-nitroindole derivatives (**35**, **36**) [104] synthesized by us, as well as the derivative of nitrovinylhydroquinone **37** [105] obtained from 3-nitrobenzofuran. Data on biological activity of the tested compounds were limited, and data on anti-tumour effect were absent in the literature.

The study was conducted in mice with transplanted Ehrlich's adenocarcinoma. The tested compounds are poorly water-soluble and therefore were used as aqueous suspensions. The inhibitory effect was evaluated by inhibition of the tumour growth (by mass and volume) compared with the control group. The toxicity was evaluated by early death of mice in the experimental group, and by the statuses of their organs (spleen, liver, *etc.*).



The results of the study showed that of the seven tested compounds three (nitrobenzofuran derivatives **31**, **32** and **34**) reliably inhibited tumour growth, while the other four (**33**, **35** – **37**) did not affect the neoplasm. The maximal ihnibition was recorded in a week after the start of administration of the test substances. It should be stressed that the doses administred did not cause death of mice and that the studied substances revealed low toxicities. The obtained results form the basis for further extended studies, with a wider dose spectrum, various schemes of administration and using other tumour models with the aim of revealing the maximal inhibitory activity of nitrobenzofuran derivatives against tumours.

In addition, it should be noted that a study of antiviral activity of a number of 5-hydroxy-3-nitrobenzofuran derivatives was carried out. Compounds **33** and **34** inhibited the reproduction of Venezuelan horse encephalomyelitis (VHE) in the culture of chicken embryo fibroblasts (CEF). Compound **34** showed higher activity inhibiting the reproduction of VHE in 5 μg ml^{-1} concentration. Of all compounds studied, only benzofuran **34** inhibited the reproduction of herpes virus in the CEF medium. The influence of compound **34** on the reproduction of the influenza virus FPV was insignificant.[103, 105]

IX. Novel nitric oxide donors among six-membered heterocycles

Data of Section VIII demonstrate that many five-membered N-, O- and S-heterocycles can serve as exogenous nitric oxide donors. So far, there were few or no such data on six-membered heterocycles. Transformations of 5-nitropyrimidine derivatives as potential NO donors in mammals were reviewed.[58]

Of indubitable interest are chemical and biological investigations into nitropyrimidine derivatives that bear an *ortho*-dithiocarbamoyl substituent. It should be noted that in the series of dialkyldithiocarbamates a large group of biologically active compounds was discovered. These substances exhibit fungicidal, insecticidal, herbicidal, anti-cholesteric, hypotensive, antimicrobial, anthelmintic effects, but the mechanisms of their action have been studied poorly so far. This makes topical the search for biologically active compounds in the series of nitrohetaryl derivatives of dithiocarbamates.

Thermal treatment of such compounds leads to processes involving removal of the nitro group, and the adjacent dithiocarbamoyl fragment strongly favours such processes.[58]

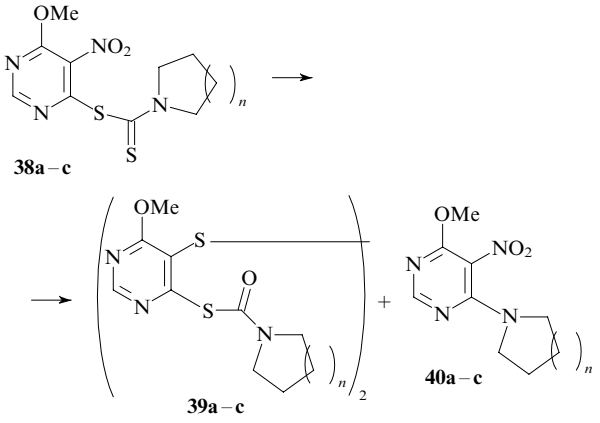
Denitration of nitropyrimidyl dithiocarbamates under hydrolytic conditions was studied.[58] Some relationships that determine the ability of the discussed compounds to serve as NO donors were found. Upon heating in a buffer with pH close to the physiological value (phosphate buffer, pH 6.86), most of the studied compounds underwent degradation with the formation of the nitrite anion.

The amount of released NO_2^- depended on the nature of the substituent in position 6 of the pyrimidine ring. The highest NO_2^- level was observed for compounds with PhO or MeO in position 6, replacement them by stronger electron donors, amino groups, greatly decreased its release.

It was shown that cleavage of 4-dithiocarbamoyl-5-nitropyrimidine proceeds by two main routes: with the formation of disulfides and 4-dialkylamino-5-nitropyrimidines.[58] Clearly, only in the former case the elimination of the nitro group and formation of nitric oxide takes place.

Thus, the appearance of disulfides should accompany release of NO in an organism.

By example of compounds **38a–c**, which have different ring size in the dithiocarbamoyl fragment, the reaction mixtures were studied using ^{1}H NMR spectroscopy. It appeared that the larger the ring the higher the proportion of disulfides in the mixture of **39** and **40**.



38a–c

39a–c **40a–c**

n = 1 (**a**), 2 (**b**), 3 (**c**).

Compound **38**	Ratio **39** : **40**
a	3 : 2
b	9 : 1
c	major product : traces

The insight into this process revealed some regularities deserving special interpretation.

1. The amount of liberated NO increased reliably on going from compound **38a** to **38b** and then to **38c**.

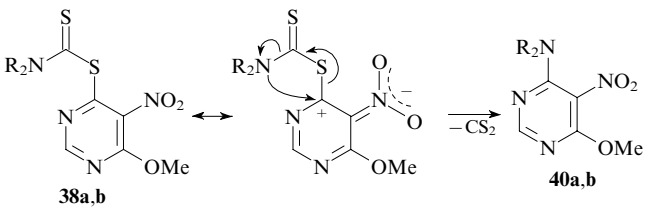
2. In the course of degradation of analogous compounds having a methyl group instead of hydrogen in position 2, the release of NO strongly dropped.

3. The release of the nitrite anion during hydrolysis took place only under aerobic conditions. In an argon atmosphere, the yield of nitrite did not exceed the determination error.

A general scheme of the processes was proposed based on the above facts (Scheme 6).

Generally, the formation of nitrites **41** can (presumably, following oxidation) result in NO^+ elimination, which in turn can bring about nitrosation of the thiolate fragment with the formation of S-nitrosothiols, the known NO donors.

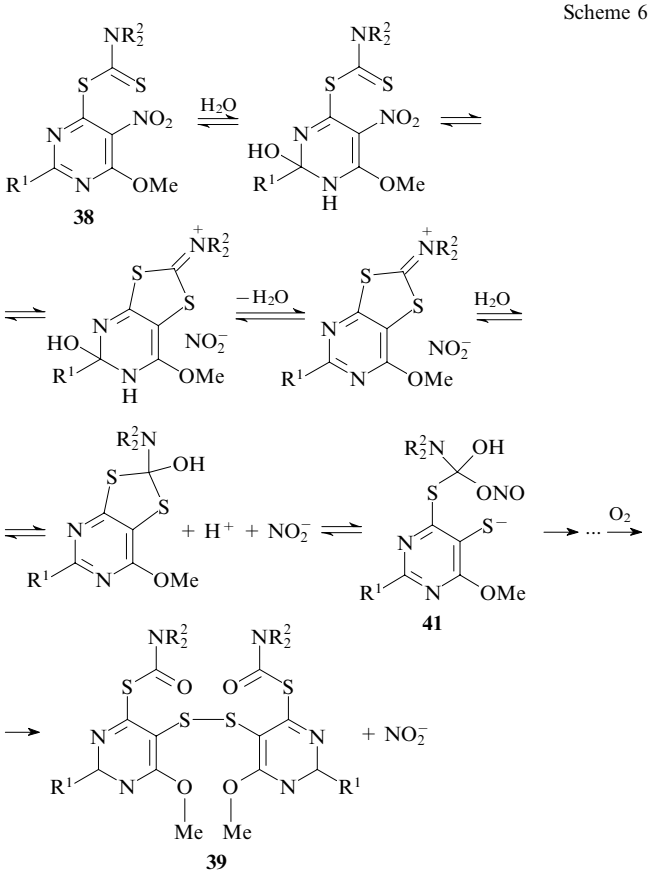
The mentioned processes are reversible, and finally the system stabilizes through irreversible oxidation with the formation of a disulfide, which requires the presence of oxygen in the solution.



38a,**b** **40a**,**b**

NR_2 = $N(CH_2)_n$ (n = 4, 5).

Scheme 6



38 **41** **39**

R^1 = H, Me; NR_2^2 = $N(CH_2)_n$ (n = 4–6).

The second process, the formation of 4-aminopyrimidine derivatives **40**, proceeds with evolution of carbon disulfide. The latter was detected in the reaction mixture by gas-liquid chromatography. This reaction mechanism involves the nucleophilic attack by the lone electron pair of the dithiocarbamate fragment on C-4 of the pyrimidine ring.

In accordance with the proposed mechanisms, it was possible to explain other peculiarities of the process. Thus the largest steric crowding of the hexahydroazepine derivative **38c** leads to its slight destabilization, and this dithiolone is oxidized into disulfide more easily than compounds **38a**,**b**.

A sharp decrease in the amount of released nitrite ion where a substituent is present in position 2 of the pyrimidine ring is probably due to that NO_2^- release occurs only after rearomatization of the pyrimidine ring, which occurs upon addition of the hydroxide at position 2. This addition is hindered where an alkyl (in particular, methyl) substituent is present in position 2.

Thus, it was demonstrated that nitro derivatives of pyrimidyl dithiocarbamates release the nitrite anion upon hydrolysis, which marks these compound as potential NO generators in the living organism.

X. Conclusion

The material discussed in this review vividly demonstrates the important role of nitrogen(II) oxide donors, in particular C-nitro compounds, as both drugs in use and potential medicinal agents against a wide spectrum of diseases. At the

same time, the role of NO in the organisms of mammals, including human, is extremely complex, various and quite underexplored. Moreover, the effect of NO on the organism can be contradictory, and is critically dependent on its concentration. Thus recent data show that nitric oxide, superoxide radical anion and a product of their reaction, peroxynitrite, can, when 'overproduced' in the organism, mediate inflammation, damage of enzymes and nucleic acids. These effects may provoke cancer.

It is thought that the free radical theory of cancerogenesis can unite different suggested mechanisms based on the fact that it is free radicals, including superoxide, hydroxyl, nitric oxide and peroxynitrite, that are responsible for damage of enzymes, membranes, DNA and RNA. Nitric oxide is a relatively stable free radical, which easily diffuses into cells and cell membranes where it reacts with such targets as haem, thiol groups and amino groups. Reactions with enzymes can inhibit cell proliferation and biosynthesis of nucleic acids and bring about changes in enzymatic activity, cytotoxicity and apoptosis. However, nitric oxide produced by macrophages is involved in destruction of intramolecular pathogenes and tumour cells. It rapidly reacts with the superoxide anion with the formation of a long-lived cytotoxic oxidant, peroxynitrite, which initiates lipid peroxidation and reacts with the thiol groups in cell membranes and with DNA components.[106]

The interest in C-nitro compounds as nitric oxide donors is caused by the various biological activity of such compounds. The structural diversity of C-nitro compounds allows varying their NO-donating properties in order to achieve one or another goal. The number of studied compounds is constantly growing, with novel potential NO donors emerging before researchers. Thus derivatives of six-membered heterocycles have recently become subjects of investigations, whereas it was only five-membered rings that have formerly been studied.

There is no doubt that new C-nitro compounds will be suggested as exogenous NO donors; the increase in the amount of potential drugs as donors of nitric oxide will proceed in parallel with the increase in our knowledge about the functions of NO and the mechanisms of its action.

References

1. V G Granik, N B Grigoriev *Oksid Azota* (Nitric Oxide) (Moscow: Vuzovskaya Kniga, 2004)
2. S Moncada, R M Palmer, E A Higgs *Pharmacol. Rev.* **43** 109 (1991)
3. M A Marletta *J. Med. Chem.* **37** 1899 (1994)
4. O W Griffith, D J Stuehr *Annu. Rev. Physiol.* **57** 707 (1995)
5. V G Granik, S Yu Ryabova, N B Grigoriev *Usp. Khim.* **66** 792 (1997) [*Russ. Chem. Rev.* **66** 717 (1997)]
6. A F Vanin *Biokhimiya* **63** 924 (1998) [a]
7. I S Severina *Biokhimiya* **63** 939 (1998) [a]
8. E B Men'shikova, N K Zenkov, V P Reutov *Biokhimiya* **65** 485 (2000) [a]
9. *Nitric Oxide. Basic Research and Clinical Application (NATO Sci. Ser.)* (Eds R J Gryglewsky, P Minuz) (Amsterdam, Berlin, Oxford, Tokyo, Washington, DC: IOS Press, 2001)
10. D Voet, J G Voet *Biochemistry* (2nd Ed.) (New York, Chichester, Bricbane, Toronto, Singapore: Wiley, 1995) p. 1290
11. F Murad *Neurotransmissions* **10** (2) 1 (1994)
12. H Al-Sa'doni, A Ferro *Clin. Sci.* **98** 507 (2000)
13. M Feelish *J. Cardiovasc. Pharmacol.* **17** (Suppl. 3) 25 (1991)
14. I V Serkov, V V Bezuglov *Usp. Khim.* **78** 442 (2009) [*Russ. Chem. Rev.* **78** 407 (2009)]
15. M O Lozins'kii, A M Borisevich, V M Britsun *Ukr. Khim. Zh.* **75** 3 (2009) [b]
16. M Kirsch, H de Groot *J. Pineal Res.* **46** 121 (2009)
17. S Huerta, S Chilka, B Bonavida *Int. J. Oncol.* **33** 909 (2008)
18. L Fang, J Lehmann *Expert Opin. Therap. Pat.* **18** 1111 (2008)
19. A Gasco, D Boschi, K Chegaev, C Cena, A Di Stilo, R Fruttero, L Lazzarato, B Rolando, P Tosco *Pure Appl. Chem.* **80** 1693 (2008)
20. J A Coulter, H O McCarthy, J Xiang, W Roedl, E Wagner, T Robson, D G Hirst *Nitric Oxide* **19** 192 (2008)
21. B Bonavida, S Baritaki, S Huerta-Yepez, M I Vega, D Chatterjee, K Yeung *Nitric Oxide* **19** 152 (2008)
22. S Fiorucci, L Santucci, E Distrutti *Digest. Liver Disease* **39** 1043 (2007)
23. B E Laursen, E Stankevicius, H Pilegaard, M Mulvany, U Simonsen *Cardiovasc. Drug Rev.* **24** 247 (2006)
24. E A Romero-Sandoval, M M Curros-Criado, G Gaitan, C Molina, J F Herrero *CNS Drug Rev.* **13** 279 (2007)
25. H Katsumi, M Nishikawa, M Hashida *Cardiovasc. Hematol. Agents Med. Chem.* **5** 204 (2007)
26. M R Miller, I L Megson *Br. J. Pharmacol.* **151** 305 (2007)
27. D McGrowder, D Ragoobirsingh, P Brown *Int. J. Pharmacol.* **2** 366 (2006)
28. P F Mannaioni, V Fabbroni, R Mastroianni, E Masini *Anti-Inflam. Anti-Allergy Agents Med. Chem.* **5** 335(2006)
29. M N Muscara, J L Wallace *Cardiovasc. Hematol. Agents Med. Chem.* **4** 155 (2006)
30. C Cena, M Bertinaria, D Boschi, M Giorgis, A Gasco *ARKIVOC* (vii) 301 (2006)
31. M Villarroya, M G Lopez, R de Pascual, A G Garcia *Cardiovasc. Drug Rev.* **23** 149 (2005)
32. D M Gooden, H Chakrapani, E J Toone *Curr. Top. Med. Chem.* **5** 687 (2005)
33. K Yoshida, Y Kita *Curr. Top. Med. Chem.* **5** 675 (2005)
34. L K Keefer *Curr. Top. Med. Chem.* **5** 625 (2005)
35. R Scatena, P Bottoni, G E Martorana, B Giardina *Expert Opin. Invest. Drugs* **14** 835 (2005)
36. S A Doggrell *Expert Opin. Invest. Drugs* **14** 823 (2005)
37. H Al-Sa'doni, A Ferro *Mini-Rev. Med. Chem.* **5** 247 (2005)
38. A Gasco, R Fruttero, B Rolando *Mini-Rev. Med. Chem.* **5** 217 (2005)
39. J Liu, M P Waalkes *Toxicology* **208** 289 (2005)
40. A R Seminara, J S Krumenasker, F Murad, in *Nitric Oxide. Basic Research and Clinical Applications (NATO Sci. Ser.)* (Eds R J Gryglewsky, P Minuz) (Amsterdam, Berlin, Oxford, Tokyo, Washington, DC: IOS Press, 2001) p. 5
41. J L Decout, B Roy, M Fontecave, J C Miller, P H Williams, D Louaux *Bioorg. Med. Chem. Lett.* **5** 973 (1995)
42. K Rehse, M Herpel *Arch. Pharm.* **331** 111 (1998)
43. K Rehse, M Herpel *Arch. Pharm.* **331** 79 (1998)
44. K Rehse, M Herpel *Arch. Pharm.* **331**104 (1998)
45. K Rehse, S Bade, A Harsdorf, B Clement *Arch. Pharm.* **330** 392 (1997)
46. A Di Stilo, C Medana, B Ferrarotti, A L Gasco, D Ghigo, A Bosia, P A Martorana, A Gasco *Pharmacol. Res.* **41** 469 (2000)
47. C Medana, A Di Stilo, S Visentin, R Fruterro, A Gasco, D Ghigo, A Bosia *Pharm. Res.* **16** 956 (1999)
48. A A Gidaspov, V V Bakharev, E V Kachanovskaya, Yu N Bulychev, V I Levina, O V Azizov, A P Arzamastsev, N B Grigoriev, V G Granik *Khim.-Farm. Zh.* (9) 12 (2003) [c]
49. V A Petrosyan, S G Mairanovskii, L V Okhlobystina, V I Slovenskii, A A Fainzil'berg *Izv. Akad. Nauk SSSR, Ser. Khim.* 1690 (1971) [d]
50. V I Slovetskii, M Sh L'vova, A A Fainzil'berg, G I Chaeva *Izv. Akad. Nauk SSSR, Ser. Khim.* 2690 (1970) [d]
51. K Rehse, M Herpel, D Piechocki *Arch. Pharm.* **329** 83 (1996)

52. L Oresmaa, H Kotikoski, M Haukka, J Salminen, O Oksala, E Pohjala, E Moilanen, H Vapaatalo, P Vainiotalo, P Aulaskari *J. Med. Chem.* **48** 4231 (2005)
53. L Oresmaa, H Kotikoski, M Haukka, O Oksala, E Pohjala, H Vapaatalo, P Vainiotalo, P Aulaskari *Bioorg. Med. Chem. Lett.* **16** 2144 (2006)
54. A R Butler, R Nicholson *Life, Death and Nitric Oxide* (Cambridge: Royal Society of Chemistry, 2003)
55. V V Bakharev, A A Gidaspov, E V Peresedova, V G Granik, N B Grigoriev, V I Levina, I S Severina, A Yu Shchegolev, D E Dmitriev, A B Sheremetev *Izv. Akad. Nauk, Ser. Khim.* 1900 (2009) [d]
56. V V Paramonov, V A Petrosyan, V I Slovetskii *Izv. Akad. Nauk SSSR, Ser. Khim.* 786 (1978) [d]
57. Ya P Stradyn' *Polyarografiya Organicheskikh Nitrosoedinenii* (Polarography of Organic Nitro Compounds) (Riga: Izd. AN Latv. SSR, 1961)
58. O B Ryabova, E Yu Khmel'nitskaya, V A Makarov, L M Alekseeva, N B Grigoriev, V G Granik *Izv. Akad. Nauk, Ser. Khim.* 2777 (2005) [d]
59. J O Lundberg, E Weitzberg, M T Gladwin *Nat. Rev. Drug Discov.* **7** 156 (2008)
60. L I Khmel'nitskii, S S Novikov, T I Godovikova *Khimiya Furoksanov. Stroenie i Sintez* (The Chemistry of Furoxans. Structure and Synthesis) (Moscow: Nauka, 1996)
61. A B Sheremetev, N N Makhova, W Friedrichsen *Adv. Heterocycl. Chem.* **78** 65 (2001)
62. S V Vasil'ev, O T Burbelev *Zh. Org. Khim.* **2** 1965 (1966) [e]
63. O A Rakitin, V A Ogurtsov, E A Khaibullina, T I Godovikova, L I Khmel'nitskii *Khim. Geterotsikl. Soedin.* 1283 (1993) [f]
64. T P Pillai, J H Boyer *Org. Prep. Proced. Int.* **14** 365 (1982)
65. Z S Smolyan, I Ya Lubyanitskii, K N Korotaevskii, V K Fukin, G N Matveeva, N K Petrova, E P Zil'berman *Zh. Org. Khim.* **12** 2099 (1976) [e]
66. K N Korotaevskii, E P Zil'berman, N K Petrova, I Ya Lubyanitskii, V K Fukin, G N Matveeva *Zh. Org. Khim.* **13** 1814 (1977) [e]
67. K N Korotaevskii, E P Zil'berman, I Ya Lubyanitskii *Zh. Fiz. Khim.* **51** 2958 (1977) [g]
68. *Nitric Oxide and the Cardiovascular System* (Totowa, NJ: Humana Press, 2000)
69. V I Levina, O V Azizov, N V Pyatakova, O G Busygina, I S Severina, A P Arzamastsev, N B Grigoriev, V G Granik *Vopr. Biol. Med. Farm. Khim.* (2) 54 (2001)
70. G Ya Legin *Khim.-Farm. Zh.* (4) 54 (1996) [c]
71. N B Grigoriev, G V Chechekin, A P Arzamaztsev, V I Levina, V G Granik *Khim. Geterotsikl. Soedin.* 902 (1999) [f]
72. P Cogolli, L Testaferri, M Tiecco, M Tingoli *J. Chem. Soc., Chem. Commun.* 800 (1979)
73. I A Khmel', A Z Metlitskaya *Mol. Biol.* **40** 195 (2006) [h]
74. Yu V Zaiseva, V G Granik, A S Belik, O A Koksharova, I A Khmel *Mol. Gen. Microbiol. Virol.* **25** 71 (2010)
75. A Makarov, A V Danilov, V I Levina, N B Grigoriev, M Shmidke, A Shtel'tsner, V G Granik *Vopr. Biol. Med. Farm. Khim.* (6) 45 (2002)
76. N F Russo, M Tomasz, M Muller, R Lipman *Mol. Pharmacol.* **13** 872 (1977)
77. P Kubes, J L Wallace *Mediat. Inflamm.* **4** 397 (1995)
78. R J Knox, R C Knight, D I Edwards *Biochem. Pharmacol.* **32** 2149 (1983)
79. J-J Jeong, D E Berg *Antimicrob. Agents Chemother.* **44** 3127 (2000)
80. R P Mason, P D Josephy *J. Inorg. Biochem.* **24** 161 (1985)
81. A F Cockerill, R C Harden, D N B Mallen *J. Chem. Soc., Perkin Trans. 2* 1428 (1972)
82. N B Grigoriev, V I Levina, O V Azizov, N V Pyatakova, A P Arzamastsev, I S Severina, V A Parshin, V G Granik *Vopr. Biol. Med. Farm. Khim.* (4) 10 (2002)
83. V I Levina, O V Azizov, N V Pyatakova, A P Arzamastsev, I S Severina, N B Grigoriev, V G Granik *Vopr. Biol. Med. Farm. Khim.* (4) 6 (2002)
84. M D Mashkovskii *Lekarstvennye Sredstva* (Medicinal Compounds) (Khar'kov: Torgsin, 1997) Vols 1, 2
85. C Viode, N Bettache, N Cenas *Biochem. Pharm.* **57** 549 (1999)
86. J P Salo, H Salomies *J. Pharm. Biomed. Anal.* **14** 1267 (1996)
87. L F Chasseaud, K Henrick, R W Matthews, P W Scott, S G Wood *J. Chem. Soc., Chem. Commun.* 491 (1984)
88. A F Pozharskii *Teoreticheskie Osnovy Khimii Geterotsiklov* (Theoretical Foundations of the Chemistry of Heterocycles) (Moscow: Khimiya, 1985)
89. L A Trukhacheva, N B Grigoriev, A P Arzamastsev, I L Dalinger, I A Vatsadze, G P Popova, S A Shevelev, V G Granik *Izv. Akad. Nauk, Ser. Khim.* 2719 (2005) [d]
90. A R Chopra, J-F Louet, P Saha, J An, F De Mayo, J Xu, B York, S Karpen, M Finegold, D Moore, L Chan, C B Newgard, B W O'Malley *Science* **322** 1392 (2008)
91. V G Granik *Organicheskaya Khimiya* (Organic Chemistry) (Moscow: Vuzovskaya Kniga, 2004)
92. J R Lancaster, X Keping *Cancer Res.* **66** 6459 (2006)
93. P Laudansky, T Anchim, S Wolczynski *Ginekol. Pol.* **72** 251 (2001)
94. S Tamir, S R Tanenbaum *Biochim. Biophys. Acta* **1288** F31 (1996)
95. L L Thomsen, D W Miles *Cancer Metast. Rev.* **17** 107 (1998)
96. C V Rao *Mutat. Res.* **555** 107 (2004)
97. N K Zenkov, E B Men'shikova, V P Reutov *Vestn. Ros. Akad. Med. Nauk* (4) 30 (2004)
98. N Ouyang, J L illiams, G J Tsioulias, J J Gao, M J Iatropoulos, L Kopelovich, K Kashfi, B Rigas *Cancer Res.* **66** 4503 (2006)
99. K Xie, S Huang *Free Radical. Biol. Med.* **34** 969 (2003)
100. D A Wink, J A Cook, D Christodoulou, M C Krishna, R Pacelli, S Kim, W De Graff, J Gamson, Y Vodovotz, A Russo, J B Mitcell *Nitric Oxide* **1** 88 (1997)
101. V P Deryagina, N I Ryzhova *Vestn. Ros. Onkol. Nauch. Tsent. im. N N Blokhina Ros. Akad. Med. Nauk* **18** 32 (2007)
102. V M Lyubchanskaya, V G Granik *Khim. Geterotsikl. Soedin.* 597 (1990) [f]
103. V M Lyubchanskaya, G A Bogdanova, I S Nikolaeva, M G Il'ina, A N Fomina, V G Granik *Khim.-Farm. Zh.* (3) 34 (1990) [c]
104. V M Lyubchanskaya, L M Alekseeva, V G Granik *Khim. Geterotsikl. Soedin.* 40 (1992) [f]
105. V M Lyubchanskaya, L M Alekseeva, I S Nikolaeva, M G Il'ina, A N Fomina, V G Granik *Khim.-Farm. Zh.* (4) 35 (1991) [c]
106. www medportal ru/mednovosti/news/2007/05/28/ breast-cancer

[a] — *Biochemistry (Moscow) (Engl. Transl.)*
[b] — *Ukr. Chem. J. (Engl. Transl.)*
[c] — *Pharm. Chem. J. (Engl. Transl.)*
[d] — *Russ. Chem. Bull., Int. Ed. (Engl. Transl.)*
[e] — *Russ. J. Org. Chem. (Engl. Transl.)*
[f] — *Chem. Heterocycl. Compd. (Engl. Transl.)*
[g] — *Russ. J. Phys. Chem., Ser. A (Engl. Transl.)*
[h] — *Mol. Biol. (Engl. Transl.)*