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Heterocyclic NO prodrugs

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Abstract

An overview of the different heterocyclic NO-releasing compounds is given. Mesoionic heterocycles like sydnone imines are one example. This class is discussed on the synthesis and the mechanism of NO formation from Molsidomine and its first metabolite SIN-1. Furthermore, 1,2,3,4 oxatriazolium olates and imidates are presented in an example of the synthesis of GEA-3175. Heterocyclic N-oxides are another group of compounds capable of NO release under certain conditions. This class is discussed in the example of furoxane carboxamides like CAS-1609, and some SAR-data show the great impact of intramolecular hydrogen bridges on their in vitro activity. Each class of compounds requires different cofactors for NO release: sydnone imines need oxidants like oxygen, furoxanes are converted to NO via reaction with thioles.

Keywords: NO-donors; Sydnone imines; Furoxanes; Molsidomine; GEA-3175; CAS-1609

1. Introduction

For a long time nitric oxide has been known as a rather toxic gas. Later it became known as one of the chemicals responsible for environmental pollution in the atmosphere, especially regarding the automotive traffic in cities. NO is also known to react with oxygen to form brown NO_x gases that are very dangerous if inhaled. Furthermore, it can combine with transition metals like iron or copper forming NO complexes, the so called prussiates.

Although sodiumnitroprusside has been used for a long time as a drug for the normalization of high blood pressure, and nitroglycerine (NTG) has been used as an antianginal drug for an even longer period of time, the physiological role of NO remained undiscovered until the mid 1980s.

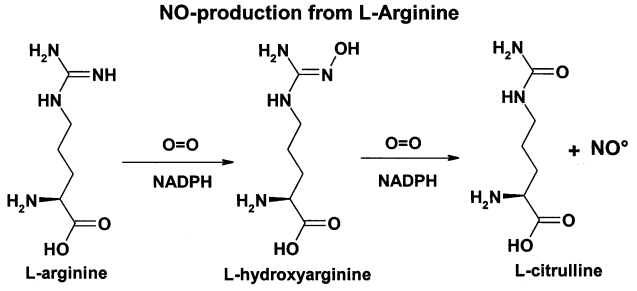
Historically, Murad et al. [1] showed in 1977 that NTG, as well as NO itself, is able to elevate cGMP via activation of the enzyme soluble guanylate cyclase (sGC).

On the other hand the sydnonimine Molsidomine (Mols), an antianginal drug, also elevated cGMP levels, as shown by Böhme et al. in 1979 [2].

In 1980 Furchgott [3] discovered an endothelial derived relaxing factor (EDRF), a relaxing substance released by the endothelium after stimulation with ACh, Bk and other agents, that in parallel to the nitrovasodilators elevates cGMP.

In 1987 Palmer et al. [4] came to the surprising conclusion that this factor is the simple molecule NO. His group and others showed in 1988 that the physiological source of NO is the amino acid L-arginine and the enzyme performing the conversion of L-arginine to NO they called NO synthase (NOS).

The chemistry of this enzyme system is rather complicated and not yet fully understood. The following scheme simplifies the process.



The overall reaction is a five electron oxidation of one of the two equivalent nitrogen atoms of the guanidino group of L-arginine. The first step, as

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demonstrated by Marletta's group [5], consists of a hydroxylation to *N*-hydroxyarginine, which is further oxidized to L-citrulline and NO, a process that requires NADPH and oxygen.

NOS exists in different isoforms and is located in many cells. In the endothelium it can be stimulated by various agonists as shown in the Furchgott experiment. It uses L-arginine as a substrate and produces L-citrulline and NO, which, because of its low molecular weight and its rather lipophilic properties penetrates cell membranes very easily. Thus, it can exert its effects on the luminal sites for example on platelets, that are deactivated, as well as in the abluminal site, where it dilates smooth muscle cells by increasing cGMP.

Nature provides NO via an oxidative process starting from the amino acid L-arginine. Interestingly, medicinal chemistry researchers have performed similar approaches in the conception and synthesis of heterocyclic compounds as NO releasers.

Depending on the amount produced by the different isoenzymes and in different cell systems, NO can exert beneficial as well as toxic effects.

At low concentrations produced by the enzyme subtypes that are located in the endothelium and in the brain, ecNOS and ncNOS, it has beneficial effects like vasodilation and antiaggregatory effects on platelets.

According to this it makes sense to administer[KS1] NO prodrugs that are able to release NO slowly compensating certain conditions where the endogenous NO production of the endothelium is insufficient, for example in diseases like coronary heart disease or platelet and endothelial dysfunction.

For other cases it can be beneficial to reduce NO production of iNOS, an isoform of NOS that is induced in inflammation and septic shock, and that after induction produces huge amounts of NO leading to dangerous conditions of hypotension in septic shock and to cell damage.

Among the oldest NO donors known there are the so-called nitrates and nitrites, alkyl esters of nitric and nitrous acid. NTG, first synthesized at the University of Turin by Sobrero in 1846 [6], has been produced and used as an explosive compound by the famous Swedish company Dynamit Nobel for more than 100 years. Its antianginal effects were discovered as early as late last century.

Later, a diminished efficacy of nitrates after a prolonged administration was observed, a phenomenon called nitrate tolerance. It was explained by the fact that nitrates need the reaction with thiole groups for NO formation, depleting or decreasing this way the thiole content of the cells and thus leading to autoinhibition of their metabolism to NO.

For the conception of a NO prodrug one has to consider several points:

1. The site of NO release: intracellular or extracellular.
2. Kinetics: short acting compounds for acute treatment, slow acting for prevention.
3. Chemical properties allowing solutions for i.v. application or sufficient half life for oral preventive treatment.
4. The redox state of the NO species released: NO^0, NO^+, NO^-: their interconversion and nitrosating potential.
5. Cofactors necessary for NO formation: thioles, oxygen, enzymes, pH.
6. Stability toward light, heat and pH.
7. The rate of NO release: slow release to avoid toxicity.

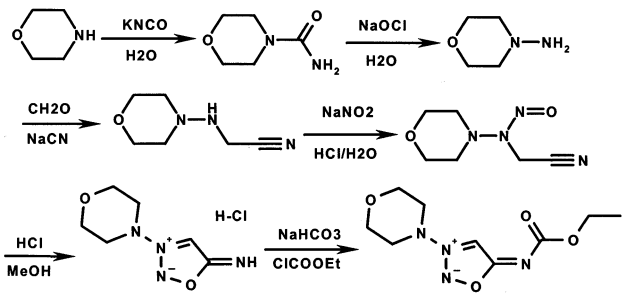
2. Mesoionic heterocycles

2.1. Sydnonimines

Mesoionic compounds called sydnones were first synthesized at the University of Sydney, Australia [7], and were named after the city of invention.

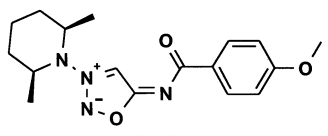
The synthesis of sydnonimines was first described by Brookes and Walker [8], the most active 3-amino sydnonimines were first synthesized by Matsuda et al. [9]. Their preparation is exemplified in the following scheme of the synthesis of Molsidomine, Mols, a derivative on the market as an antianginal drug since 1977.

Synthesis of Molsidomine

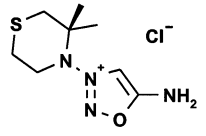


Mols was invented by researchers at the Takeda company in Japan [10] and licensed in by Cassella AG. The initial Takeda synthesis of the intermediate 1-aminomorpholine via nitrosation of morpholine to nitrosomorpholine followed by reduction to aminomorpholine, was changed by Cassella AG as indicated in the scheme to avoid the carcinogenic nitroso intermediate in the production process.

Similarly, many other sydnonimines have been prepared and tested in the Cassella laboratories. Two of them showed promising pharmacological properties.



CAS 936



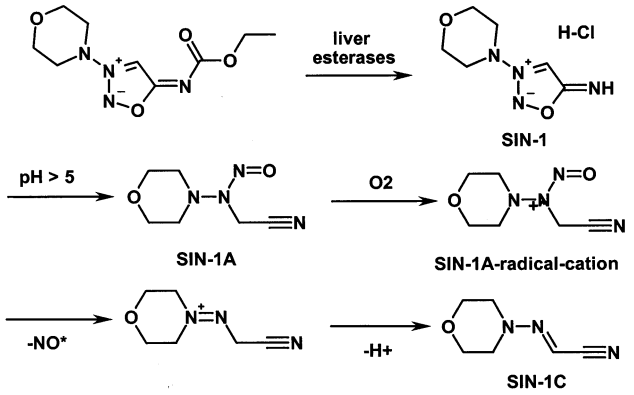
C-4144

CAS 936 [11,12] represents an acyclated sydnonimine of the Molsidomine type with a prolonged duration of action.

C-4144 [13] is a sydnonimine hydrochloride that due to its balanced properties regarding photolytic and thermal stability and kinetics showed a promising pharmacological profile.

The metabolism and decay of Mols was first investigated by researchers of the Takeda company [14]. They found out that the first activating step is an enzymatic hydrolysis by liver esterases leading to SIN-1, which at neutral pH undergoes a ring opening reaction to SIN-1A. For the formation of SIN-1C, a beta elimination of HNO was postulated. Whereas SIN-1 and SIN-1A showed similar antianginal effects as Mols, SIN-1C had no effect.

Metabolism and degradation of Molsidomine



We started our investigations on the mechanism of NO formation from Mols in the early 1980s [15] and found the following:

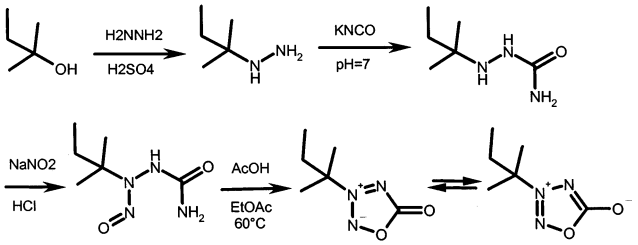
1. The decomposition of SIN-1A is a pH-independent reaction.
2. Oxygen, or an oxidative process, is involved in this step and there is a correlation of oxygen consumption and NO formation in buffered SIN-1 solutions [16].
3. NO, but not HNO, is formed from SIN-1A together with SIN-1C.

The NO formation as well as the good activity of a dimethylated derivative of SIN-1A, in which a beta elimination leading to HNO appears impossible, can be easily explained with this new mechanism.

2.2. 1,2,3,4-Oxatriazolium-5-olates

Replacement of one ring carbon atom in sydnones by nitrogen results in the so called 1,2,3,4-oxatriazolium–olates or –imidates, a further interesting mesoionic structure able to release NO and to lower blood pressure [17,18]. The synthesis of the *tert*-amyl derivative [19] is demonstrated in the following scheme and consists of a nitrosation of the semicarbazid intermediate and the subsequent cyclization under acidic conditions.

Synthesis of 1.2.3.4-Oxatriazolium-olates



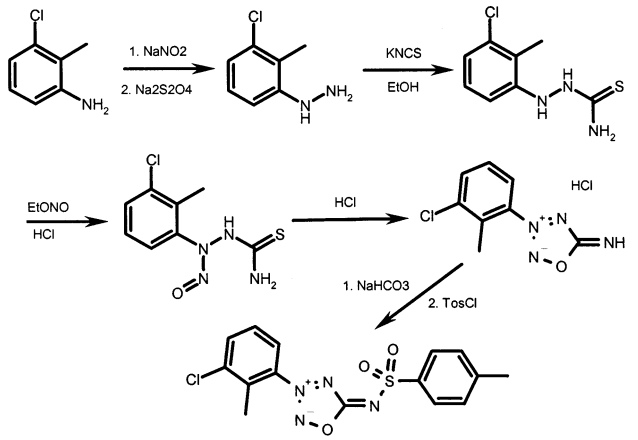
The 3-*tert*-amyl derivative was very effective in inhibiting platelet aggregation and also in preload reduction in animal models but due to mutagenic effects in in vitro models, its further development was stopped. A possible reason for that is a too fast NO release under the assay conditions.

The mechanism of decomposition and NO formation possibly consists of a hydrolytic (or enzymatic) ring opening reaction leading to an unstable carbamate that eliminates carbon dioxide spontaneously forming a nitroso hydrazine, that in the presence of oxygen and especially if traces of transition metals like ferric ions are present, releases NO via an oxydative process.

2.3. 1,2,3,4-Oxatriazolium–imidates

The synthesis of 1,2,3,4-oxatriazolium–imidates is illustrated with the following synthetic process, published by researchers of the GEA company in Copenhagen, Denmark [20].

Synthesis of GEA 3175



The semicarbazide intermediate is synthesized from the corresponding hydrazine and first nitrosation with ethyl nitrite under acidic conditions affords a nitroso compound, that on acidification with HCl, cyclizes with elimination of H_2S. In the presence of bases the

oxatriazolium–imidate reacts with tosyl chloride to the N-tosylated product GEA 3175.

Little is known about the mechanism of NO release from this compound, but in analogy to sydnonimines, a similar reaction sequence can be postulated for this compound.

3. Heterocyclic N-oxides

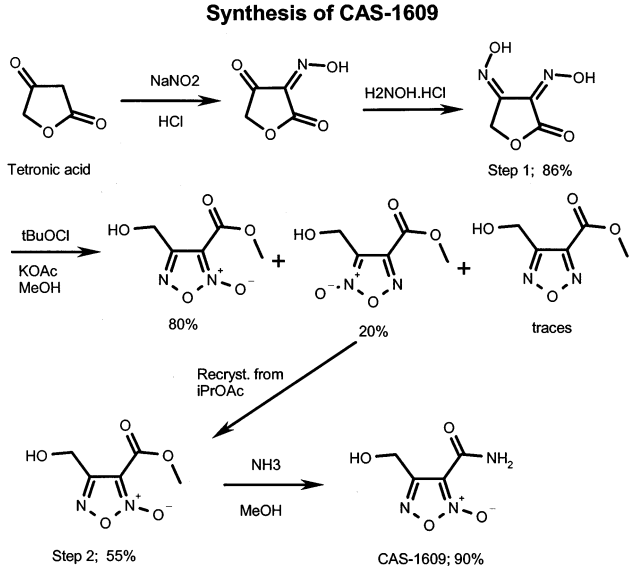
3.1. Furoxanes

Heterocyclic N-oxides like furoxanes, 1.2.3-triazol-2-oxides, pyrazol–oxides and azetin-di-N-oxides represent another class of NO donors.

1,2,5-Oxadiazole-2-oxides, the so-called furoxanes, have been extensively investigated regarding their chemistry, their stability and their activity in Professor Gasco's group at the University of Turin, Italy [21–23], and most of our knowledge in this interesting class of heterocyclic compounds has been gained in this research group [24–26]. The most frequently used synthetic pathways for their preparation are the following reactions:

1. Oxidation of [KS2][KS3]dioximes with reagents like sodium hypochlorite or $K_3Fe(CN)_6$ [27].
2. Thermolysis of *o*-nitro-azides.
3. Dimerization of nitrile-N-oxides.
4. Oxidation of *o*-amino-nitro-derivatives.
5. Reaction of alkenes with N_2O_3.

An example of the first method is the synthesis of one of our most promising furoxane derivative CAS 1609 [28].



Starting from tetronic acid the dioximino derivative can be prepared in a one pot reaction with excellent yield. The oxidation is best performed with *t*-butyl hypochloride, both isomeric furoxanes and traces of the corresponding furazan are formed. From this mixture the desired 4-hydroxymethyl ester derivative can be isolated by a simple recrystallization from isopropyl acetate. Methanolic ammonia converts it to the amide CAS 1609 in 90% yield.

Furoxanes are thermally very stable compounds and they are also stable against acids and electrophiles The stability toward bases and nucleophiles is less pronounced. The question arises: how do they decompose to NO? The most probable mechanism of NO release is via nucleophilic attack of thiolates [29–32].

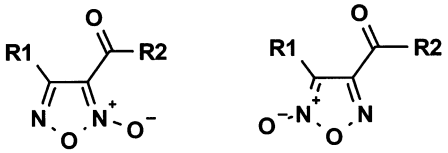
The influence of the orientation and the electronic and steric properties of the substituents of furoxane carboxamides on the activity in vitro is shown in Table 1 [33].

Compared with other NO donors furoxanes can exhibit a very desirable pharmacological profile: slow onset and long duration of action with no development of nitrate tolerance.

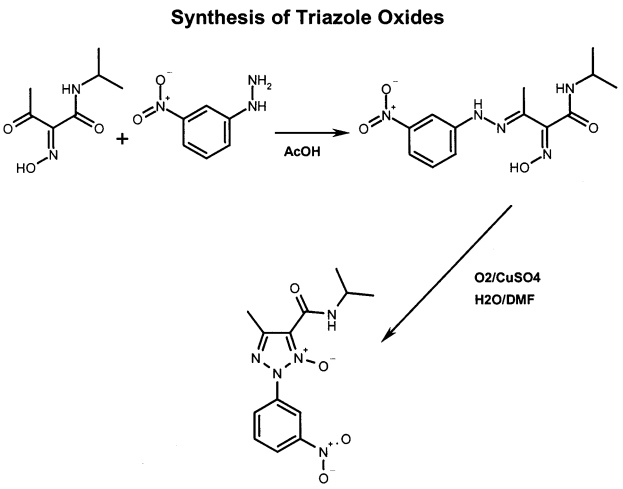
3.2. 1,2,3-Triazole-2-oxides

1.2.3-Triazole-2-oxides represent a heterocyclic class of compounds that very much resembles the furoxanes: the ring oxygen has been replaced by nitrogen. The synthesis of these heterocycles can be achieved starting from oximino acetoacetic acid amides by condensation with hydrazines and oxidation of the resulting hydrazone with cupric sulfate. The preparation of the 2-(3-nitrophenyl)-derivative is shown in the following scheme.

Table 1
Furoxanes: isolated guinea pig pulmonary artery



R1	R2	IC-50 (μM)	IC-50 (μM)
$-CH_3$	$-NH_2$	2.9	>100
$-CH_2CH_2CH_3$	$-NHCH_2CH_2N^iPr_2$	9.5	32.0
$-CH_2-OH$	$-NH_2$	12.0	>100
3-Pyridyl	$-NHCH_3$	0.7	4.0
4-NO_2–Ph	$-NHCH_2CH_2N^iPr_2$	0.04	–
$-CONH^iPr$	$-NH^iPr$	0.07	0.07
$-CONEt_2$	$-Net_2$	>100	>100

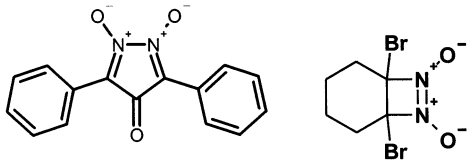


Introduction of an electron withdrawing substituent in position 2 makes the compound even more furoxane-like, but the oxidation step did not work in our hands when tosyl or acyl hydrazines were used instead of phenyl hydrazines. 2-Nitrophenyl-4-methyl-1-oxo-1,2,3-triazole-5-isopropyl amide showed weak activity on isolated guinea pig pulmonary arteries.

The similarity of this compound with the furoxan ring system suggests a similar mechanism of NO formation.

3.3. Further heterocyclic N-oxides

Pyrazol-1.2-dioxides [34] and azetidine-di-N-oxides [35] have been reported to show platelet antiaggregatory properties via release of NO.



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