Focus on Recent Approaches for the Development of New NO-Donors

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Abstract: Recent research developments in the field of NO-donor compounds have concerned conjugation of NO-donor moieties with antioxidant groups, NO-donor targeting, design of NO-donor hybrid drugs and of NO-delivery systems. These new approaches are illustrated and discussed through selected examples.

Keywords: Nitric oxide, NO-donors, NO-donor antioxidants, NO-donor targeting, drug/NO-donor hybrids, NO-delivery systems.

INTRODUCTION

Nitric oxide, the nitrogen monoxide radical $NO^{\cdot}$, is a physiological messenger that is almost ubiquitous in cells and tissues [1,2]**.** It is formed from L-arginine under the action of a family of enzymes called the NO synthases (NOS). At present, three different isoforms of this family are known. Two are constitutive isoforms, e-NOS and n-NOS, and the third, i-NOS, is an inducible isoform (Fig. (**1**)).

Nitric oxide displays diverse potent physiological actions [1,2] (Fig. (**1**). As regards the cardiovascular system it helps to maintain micro- and macro-vascular homeostasis through several mechanisms, including vasodilatation, inhibition of platelet aggregation and modulation of platelet and leukocyte adhesion to the endothelial wall. In the central nervous system it plays roles in learning and memory formation. There is an evidence that it is also involved in the vision, olfaction, feeding behaviour and nociception. In the peripheral nervous system it regulates a number of gastrointestinal, respiratory and genitourinary reflexes as neurotransmitter at the endings of non-adrenergic non-cholinergic (NANC) nerves. It is produced in all these compartments in nanomolar concentrations, and triggers its effects by a common mechanism that implies activation of the soluble guanylate cyclase (sGC). Nitric oxide is also one of the final effectors in the immune system, where it is produced in micromolar concentrations, in particular by macrophages. It diffuses into the pathogen and kills it, both by producing reactive species and by inhibiting enzymes essential to the life of the pathogen.

Today there is widespread interest in a class of products collectively called NO-donors. NO-donors are substances that release NO under physiological conditions. In this connection the term NO is frequently used as a family name, embracing not only nitric oxide ($NO^{\cdot}$) but also its two redox-forms, the nitroxyl (NO^{-}) and nitrosonium (NO^{+}) ions [3]. Both these forms may play important roles in the complex signalling system related to $NO^{\cdot}$ [4]. These products offer the opportunity to bring exogenous NO to different compartments of the organism and consequently to modulate their NO levels for therapeutic gain. This paper, after a short introduction to the principal NO-donor structures, discusses some new research strategies in this field, giving selected examples.

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NO-DONORS

The most important NO-donor compounds are illustrated in Fig. (**2**).

Organic esters of nitric and nitrous acids, molsidomine (a sydnonimine) and sodium nitroprusside (SNP, an iron NO-complex) are used to treat a number of cardiovascular diseases [5]. They can be considered as accidental NO-donors since they were introduced into therapy many years before it was clear that NO is involved in their mechanism of action. The organic esters of nitric acid are the most important of these compounds. The prototype nitrate is glyceryl trinitrate (GTN). NO-release from nitrates in physiological solution is thiol dependent and the exact mechanism is not yet fully understood [6]. Both non-enzymatic and enzymatic processes may be involved in *in vivo* NO formation [7,8]. The major limit to the practical use of these products is the early development of tolerance [9].

Nitrosothiols (RSNO) [10] might be interesting alternatives to organic nitrates for the treatment of circulatory diseases, since they do not generate tolerance and display some tropism for arterials and platelets [11,12]. They release $NO^{\bullet}$ when dissolved in water solution at physiological pH, following Eq. (**1**).

$$2\ RSNO \longrightarrow RSSR + 2\ NO^{\bullet} \qquad \textbf{Eq. (1)}$$

The problem with these products is their stability, which may be influenced by a number of factors [10], including the presence of transition metals, which catalyse their decomposition [13,14]. Catalysis by Cu(I) was studied in depth, and the proposed mechanism is reported below (Eq. (**2a**)). Cu(II) also catalyses the decomposition, through its intermediate reduction to Cu(I) (Eq. (**2b**)). In this case the initial thiolate anion needed to start the reaction could derive from slow hydrolysis of the nitrosothiol.

$$Cu^{+} + RSNO \rightleftharpoons Y \longrightarrow Cu^{2+} + RS^{-} + NO \qquad \textbf{Eq. (2a)}$$

$$Cu^{2+} + RS^{-} \rightleftharpoons X \longrightarrow Cu^{+} + RS^{\bullet} \qquad \textbf{Eq. (2b)}$$

In these equations, X and Y are intermediates whose structures are not as yet fully determined.

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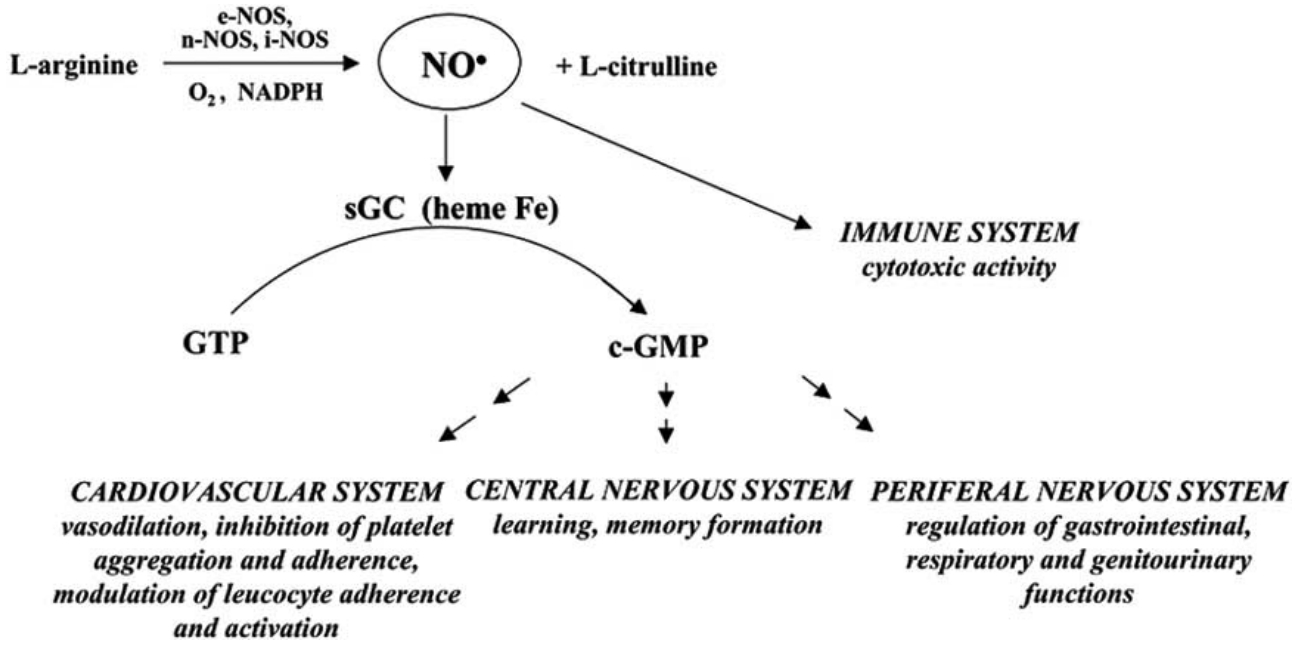


Fig. (1). Biosynthesis and biological effects of nitric oxide (NO·).

Interestingly, recent reports show that the sensitivity of nitrosothiols to copper catalysis can be modulated by appropriate structural modifications (Fig. (**3**)).

For example, product **1,** obtained by substitution of valeryl for the acetyl group present in *S*-nitroso-*N*-acetylpenicillamine (SNAP) [12], or derivative **2,** obtained by conjugation of SNAP with glucosamine tetra-acetate by an amide bond [15], are more stable than the parent compound to metal ion catalytic effects. Conjugation with amino acids (structures **3**) also increases the stability of SNAP to the catalytic effects of copper ions [16,17].

Another important class of NO-donors are the N-diazeniumdiolates (NONOates) [18], they release NO· in a quantitative manner when dissolved in physiological solution, following Eq. (**3**).

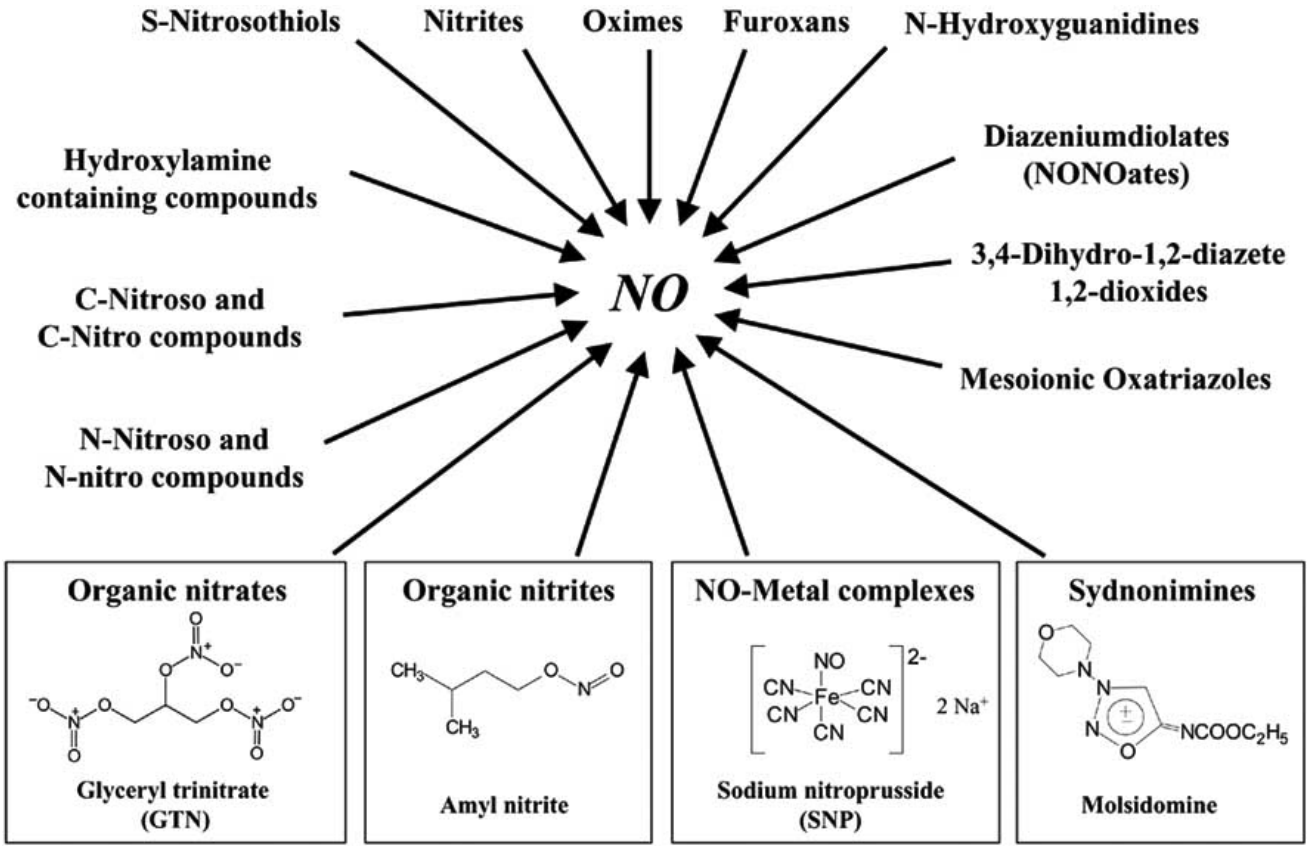
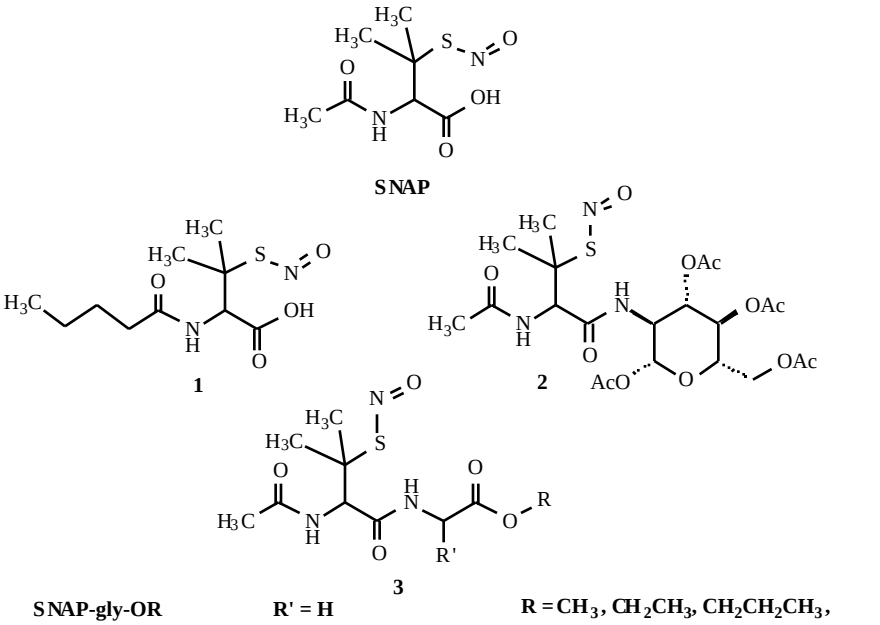


Fig. (2). The most important NO-donor compounds and some examples of NO-donor drugs (in boxes).



SNAP-gly-OR	**R' = H**	**R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, $CH(CH_3)_2$, $(CH_2)_3CH_3$, $C(CH_3)_3$**
SNAP-ala-O-Me	**R' = CH_3**	**R = CH_3**
SNAP-leu-OMe	**R' = $CH_2CH(CH_3)_2$**	**R = CH_3**
SNAP-iso-OMe	**R' = $CH(CH_3)CH_2CH_3$**	**R = CH_3**
SNAP-glu-OMe	**R' = $CH_2CH_2COOCH_3$**	**R = CH_3**
SNAP-val-OMe	**R' = $CH(CH_3)_2$**	**R = CH_3**
SNAP-phe-OMe	**R' = CH_2Ph**	**R = CH_3**
SNAP-met-OMe	**R' = $CH_2CH_2SCH_3$**	**R = CH_3**
SNAP-asp-OMe	**R' = CH_2COOCH_3**	**R = CH_3**

Fig. (3). Examples of structural modifications of SNAP.

$$R'R''NN(O^-)NO \xrightarrow{H^+} R'R''NH + 2\,NO^{\bullet} \qquad \textbf{Eq. (3)}$$

The release is spontaneous and follows first order kinetics. The half-lives of these reactions are greatly influenced by the nature of R in the starting materials. Thus these materials can be considered kinetically controlled sources of pure nitric oxide. For the moment they are not used in therapy but are important pharmacological tools and promising drugs [19].

An extensive general overview of all NO-donors was recently published by Wang et al. [20], while chemical aspects have in particular been discussed by Feelisch [21] and by Granik [22]. We refer the reader to these papers for a complete bibliography on the subject and for references to other significant recent reviews.

NEW APPROACHES IN THE FIELD OF NO-DONORS

The search for new NO-donors that produce a single redox form of NO following defined kinetics, is an important on-going research goal in this area. In parallel, some new research approaches have arisen, concerning the design of NO-donors joined to antioxidant moieties, NO-donor targeting, drug/NO-donor hybrids and NO-delivery systems.

Conjugation of NO-Donors with Antioxidant Moieties

Atherosclerosis is a disease closely correlated with endothelial dysfunction following an increase in plasma lipids, peroxidation of low-density lipoproteins (LDLs) and impaired $NO^{\cdot}$-mediated bioactions [23-25]. The endothelium produces other reactive species in addition to nitric oxide, among them superoxide anion, $O_2^{-\cdot}$ (SO). This anion is rapidly converted by the enzyme superoxide dismutase (SOD) to hydrogen peroxide H_2O_2 that via Fenton-like reactions, produces the highly reactive and toxic hydroxyl radical, $OH^{\cdot}$. SO also reacts rapidly in a diffusion-controlled reaction with $NO^{\cdot}$, giving peroxynitrite anion (^-OONO), a source of hydroxyl and nitrogen dioxide ($NO_2^{\cdot}$) radicals (Eq. (**4a)** and (**4b**))

$$O_2^{-\bullet} \xrightarrow{SOD} H_2O_2 \longrightarrow OH^{\bullet} \qquad \textbf{Eq. (4a)}$$

$$NO^{\bullet} + O_2^{-\bullet} \xrightarrow{H^+} HOONO \longrightarrow OH^{\bullet} + NO_2^{\bullet} \qquad \textbf{Eq. (4b)}$$

These reactive species, when produced in high concentrations following an increased flux of SO, when they are no longer controlled by antioxidant defence mechanisms, induce oxidative stress. This causes enhanced LDL peroxidation, which is the first step in the formation of foam cells, precursors of atherosclerotic plaques. In an atherosclerotic blood vessel, decreased production and increased destruction of nitric oxide can occur, as well as impaired target cell responsiveness. Consequently, $NO^{\cdot}$-mediated bioactions are impaired. All this creates an environment that favours the development and progression of atherosclerosis. Experimental evidence supports the hypothesis that the treatment of hypercholesterolemic animals and human subjects with appropriate amounts of

antioxidants reduce LDL oxidation and improves endothelium-dependent relaxation. In addition, responsiveness to exogenous $NO^{\cdot}$ in atherosclerotic vessels seems to be preserved. Undoubtedly, further studies will be required to define the antioxidant action mechanism [24]. A new research line is emerging in this connection, whose objective is to obtain NO-donors linked to appropriate radical scavengers. These products should ensure increased NO levels, owing to the presence in the molecule of an NO-donor moiety, and reduced levels of SO, due to the presence of the antioxidant moiety, thus avoiding the formation of peroxynitrite anion and other oxidant metabolites. The design of these products is conducted by linking the scavenger and the NO-donor substructures through chemical bonds that can be degraded under physiological conditions, but preferably the linkage should be stable to enzymatic degradation.

A typical example of this approach is the design of a series of NO-releasing morpholine derivatives [26] (structures **4a-d**, Fig. (**4**)). These products were obtained by joining substituted morpholine radical scavengers endowed with hypolipidemic activity [27] to an NO-donor nitroxy function. The resulting products were found to inhibit LDL peroxidation induced by Fe^{2+} and ascorbic acid in the rat hepatic microsomal fraction. In addition they were capable of reducing cholesterol, triglyceride and LDL plasma levels versus controls when administered i.p. to rats. The ability of $NO^{\cdot}$ to affect the production of apolipoprotein B [28], which is the principal protein component of LDL, is also probably one of the reasons for this decrease.

Nitroxides are another family of radical scavengers used in this approach (Figure (**4**)). These species are stable for long periods and prevent oxidative damage in cells and organs, as well as in the whole animal [29]. In addition, they inhibit peroxyl radical-mediated DNA scission [30] and DNA damage induced by reactive nitrogen species [31]. Nitrate of Tempol (**5a**) and of hydroxymethyl-PROXYL (**5b**) have been studied in depth [32,33]. They were found to be vasodilators not producing tolerance *in vivo*, reportedly due to their ability to trap SO. Such products could be useful in the treatment of several oxidative stress-mediated diseases.

A compound that seems to be particularly effective in minimising the interaction of SO with $NO^{\cdot}$ is structure **6** (Fig. (**4**)), obtained by joining a nitroxy function to a p-benzoquinone moiety [34]. The conjugation of phenol with the nitrosothiol function (**7**), also appears of interest [35]. It releases $NO^{\cdot}$ with simultaneous formation of phenolic disulphides (**8**), which are efficient anion superoxide

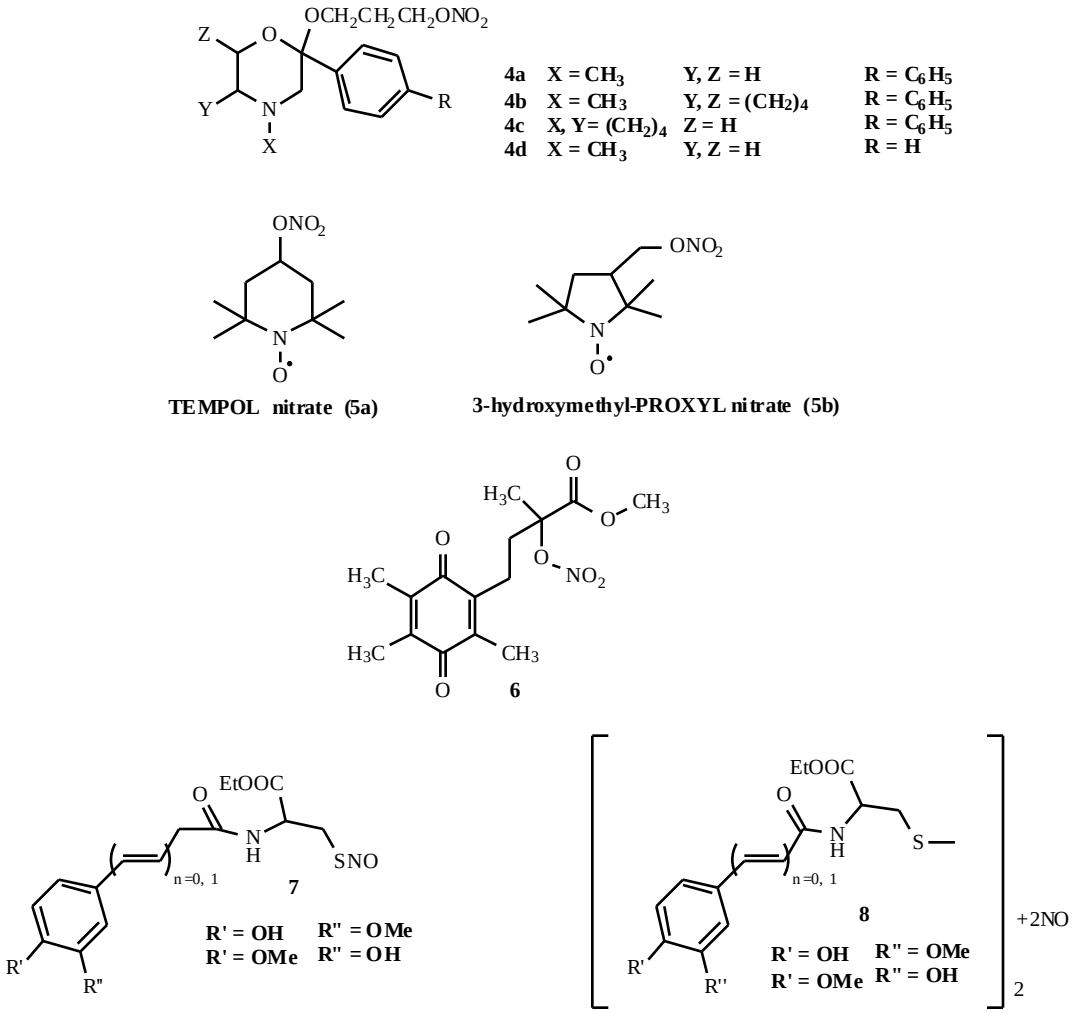


Fig. (4). NO-donors linked to radical scavengers.

scavengers. Disulphides bearing the *p*-hydroxy group appear to be more efficient $O_2^{-\bullet}$ scavengers than the *m*-isomers. The α,β-unsaturated amide link also affords more active products than the simple amide connection.

It is known that nitric oxide can produce a number of reactive species that induce oxidation and deamination of DNA [36]. Consequently, genotoxicity connected with long-term NO-donor therapy cannot be excluded [37]. The impact that the conjugation of an antioxidant moiety to an NO-donor has on the genotoxicity of the resulting product is an interesting aspect that still requires much investigation.

NO-Donor Targeting

The problem of directing drugs to their target is common to all drugs, but is paramount for NO-donors, because NO exerts a range of pharmacological actions and may also induce toxicity. NO-donor targeting has recently been addressed by a number of studies using the diazeniumdiolates. The strategy followed is based on an important finding by Keefer, who showed that diazeniumdiolates, which spontaneously release NO· as was said above, can provide stable derivatives when the O^2-terminus of the anion is linked to alkyl moieties [38]. By choosing appropriate groups that are removed by specific enzymes, it is possible to regenerate the parent unstable diazeniumdiolate, and consequently to localise NO-release to a specific compartment where the particular enzyme is abundant. For example, the vinyl pro-drug **9** (Fig. (**5**)) is selectively metabolised to the unstable parent diazenium-diolate **10** by the rat liver enzymes and consequently the bulk of NO-release is expected to be confined to this organ [39]. Since the product has been found to block tumour necrosis factor-α (TNFα) induced apoptosis and liver toxicity, it is potentially useful for the treatment of hepatic disorders such as fulminant liver failure.

Interesting stable diazeniumdiolates were obtained by adding to O^2-terminus of **10** appropriate tripeptide sequences (**11a, 11b**, Fig. (**5**)) that are specifically recognised by the prostate specific antigen (PSA) [40]. It is thought that these products will chiefly be activated to produce NO· in the prostate tissue, since PSA in blood serum is inactive owing to the presence of high concentrations of naturally inhibitory proteins.

Other interesting examples of this strategy are a number of glycosylated diazeniumdiolates (structures **12a-c**, Fig. (**5**)) [41]. These products are quite selectively accumulated in

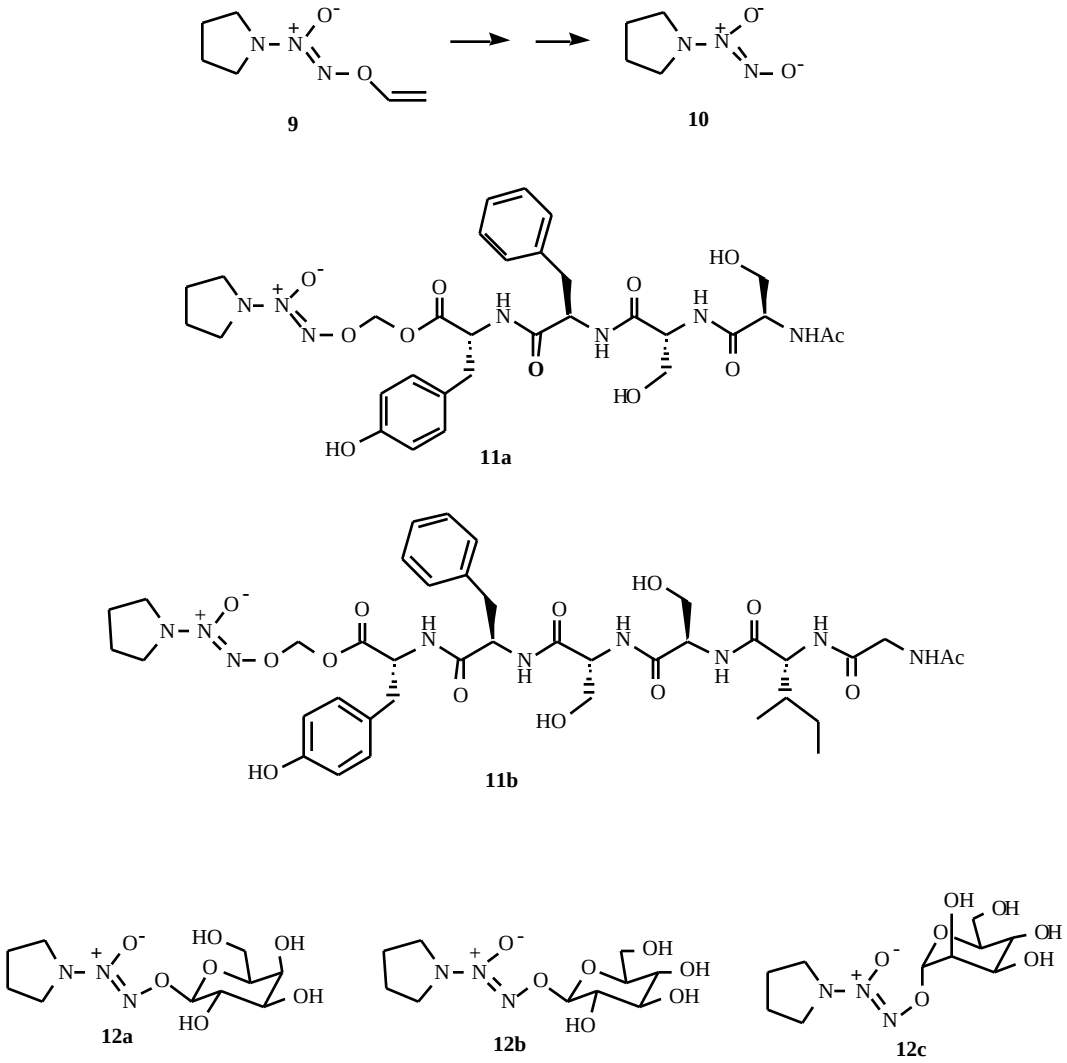


Fig. (5). Diazeniumdiolate prodrugs.

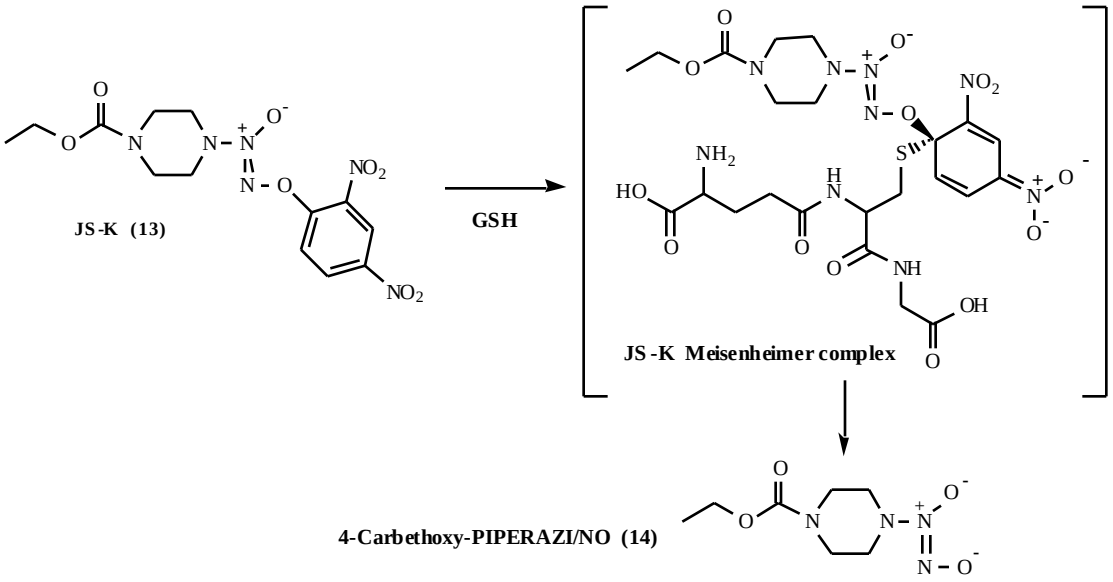


Fig. (6). Stable arylated diazeniumdiolates which release NO• under the action of glutathione (GSH) or glutathione S-transferase (GST).

solid tumor cell lines and in leukaemia cells due to an over-expression in these cells of the sugar transporter family of transmembrane proteins (GLUTs), which facilitate transport of monosacharrides in mammal cells. The products in the cells are activated to release NO· by cellular glycosidases.

Very recently, the discovery of the unusual sensitivity of acute myeloid leukaemia (AML) cells to the cytotoxic effects of NO· induced Keefer's group to screen a series of stable arylated diazeniumdiolates designed to release NO· under the action of glutathione (GSH) or glutathion *S*-transferase (GST) [42]. This is because expression of the three major GST isoforms (α, μ, π) is high in AML cases. JS-K O^2-(2,4-dinitrophenyl)-1-[(4-ethoxycarbonyl)piperazin-1-yl]diaz-en-1-ium-1,2-diolate (**13**, Fig. (**6**)) emerged as the most active antiproliferative agent against HL-60 cells implanted in mice. This material produces the instable diazeniumdiolate **14** by an interaction with GSH, through the intermediate formation of a Meisenheimer complex. This is a promising lead, and further structural modifications should be attempted to optimise solubility and pharmacokinetic profile of this product.

Series of glyco-*S*-nitrosothiols have also been designed with a view achieving specific targeting of NO to tumour cells [43,44,45]. In these products, monosaccharides such as fructose, glucose, and mannose have been linked to *S*-nitroso-*N*-acetylpenicillamine (Fig. (**7**)). Both glucose-2-SNAP (**15a**) and fructose-2-SNAP (**15b**) as well as mannose derivatives (**15d-f**) were found to display *in vitro*

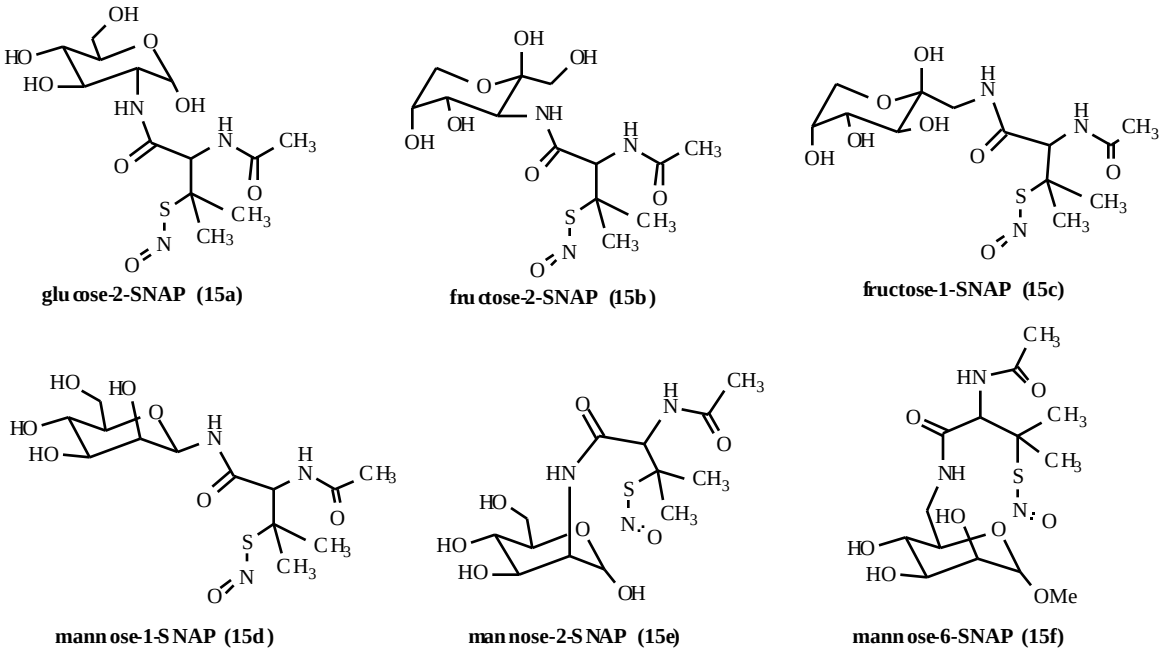


Fig. (7). Glyco-S-nitrosothiols.

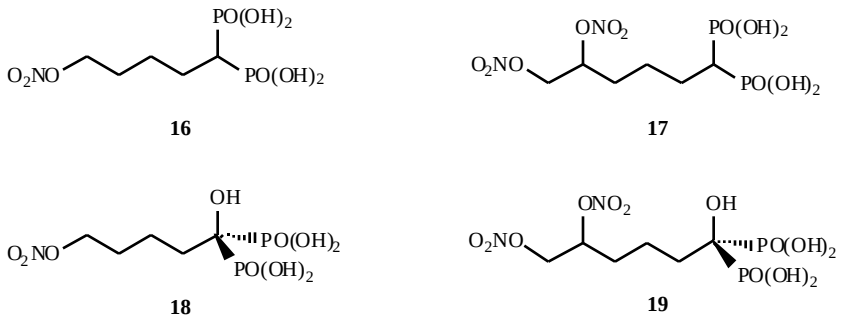


Fig. (8). NO-donor bisphosphonates.

cytotoxicity against DU-145 human prostate cancer cells more efficiently than SNAP itself [45,46]. Increased cytotoxicity against these cells was observed with fructose-1-SNAP (**15c**) [47].

Another possibility is to target NO-donors to a specific compartment by linking these drugs to vectors that exhibit high affinity for that compartment. A very recent example of this approach is the conjugation of nitrooxy functions with bisphosphonates in order to target the resulting products to the bone [Lazzarato, L.; Lolli, M.L.; Balbo, S.; Deleide, G.; Fruttero, R. Atti, Vol. 2, Poster, XXI Congresso Nazionale Della Società Chimica Italiana, Torino, 22-27 Giugno 2003; FA-CP-033]. Bisphosphonates are the organic analogues of pyrophosphate and display an exceptional affinity for bone mineral content [48]. They are commonly used to inhibit bone resorption in a variety of disorders of mineral metabolism. The resulting NO-donor products (**16-19**, Fig. (**8**)) have shown a high affinity for hydroxyapatite (HAP) *in vitro*. The ^{99m}Tc-labelled derivatives **17**, and **19** were found to accumulate preferentially in the skeleton of rats, versus the blood and muscle. They also inhibited in a dose dependent manner the osteoclast differentiation induced by receptor activator of NF-κB ligand (RANKL). Preliminary results indicate this action is NO-dependent, since it is reversed by ODQ [Günther, H. Private communication, 2003].

Conjugation of the $-ONO_2$ group through an appropriate spacer with ursodeoxycholic acid, a minor component of the total bile acid pool, generates the product **20** (Fig. (**9**)), which selectively delivers NO to the liver [49] and could be useful in the management of certain liver diseases [50].

Drug/NO-Donor Hybrids

The molecular combination of two mutually complementary biological activities into a single molecule is an old approach to design new drugs [51-54]. In the recent past it has been extensively applied in the field of NO-donors. The desired structure is obtained by joining either a known drug, or an appropriate part of it with an NO-donor moiety, by a number of means. The link between the two entities is generally stable, but in some cases may undergo chemical or enzymatic cleavage. The resulting new drug may be endowed with dual action, that of the parent drug and that related to its ability to release NO. The principal advantage of a "hybrid" drug over the simultaneous administration of the two drugs separately is expected to be that of improved pharmacokinetic behaviour [52]. The two drugs, if administered as a single hybrid, are absorbed as a single unity and, when the link is stable, they are also distributed and excreted as a single unity. Apart from some old "accidental" NO-donor hybrids, namely products designed before the involvement of NO in their action was understood, there are many recent examples of this approach.

Nitrooxy or nitrosothiol functions have been joined to dopamine agonists, α_1–receptor ligands, non-steroidal anti-inflammatory drugs (NSAIDs), H_2-agonists, K^+-channel activators, inhibitors of the angiotensin converting enzyme (ACE), steroids, 1,4- dihydropyridine (1,4-DHP) Ca^{2+}-channel blockers and activators. These products have already been reviewed [20,55]; some of them are now under clinical investigation. The NO-NSAIDs, in which a number of NSAIDs are joined through an ester linkage to nitrooxy or nitrosothiol functions (**21-25**, Fig. (**10**)), are noteworthy from the pharmaceutical standpoint. These drugs retain the anti-inflammatory, analgesic, anti-aggregatory and anti-thrombotic properties of the parent drugs but, remarkably, they appear to have reduced gastrotoxicity, which is the major drawback of NSAIDs [56-59]. This is because nitric oxide displays gastrosparing properties [60] when produced at an appropriate rate and in appropriate amounts. This property was found to explain a number of effects on the

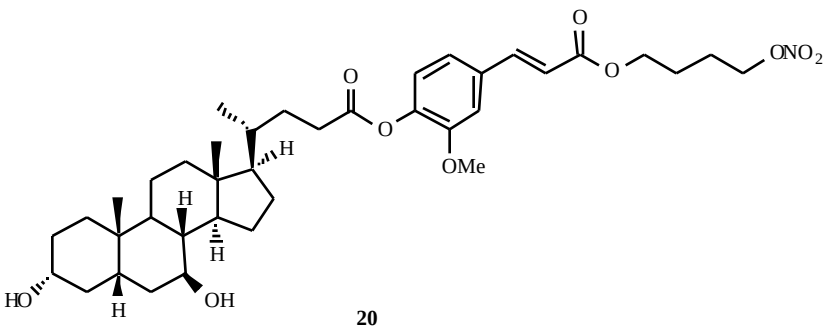
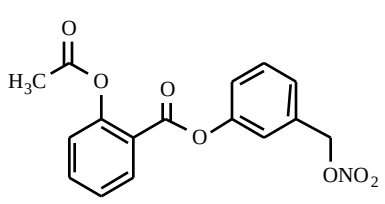
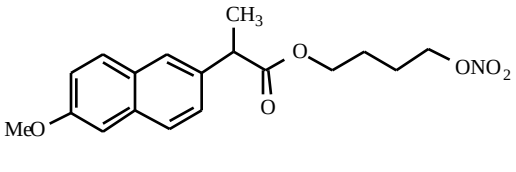


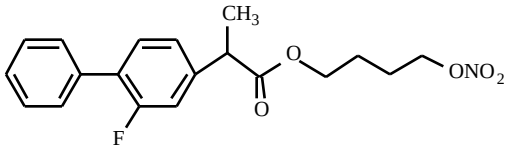
Fig. (9). NO-donor ursodeoxycholic acid.



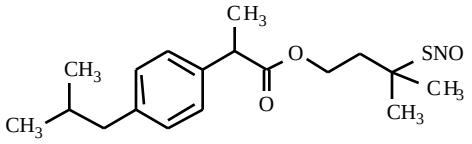
Aspirin/ONO2 (21)
NicOx*



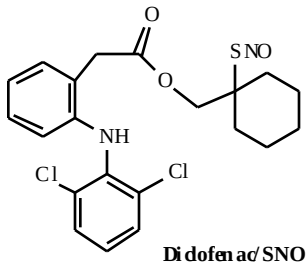
Naproxen/ONO_2 (22)
NicOx*



Flurbiprofen/ONO2 (23)
NicOx*



Ibuprofen/SNO (24)
Nitromed



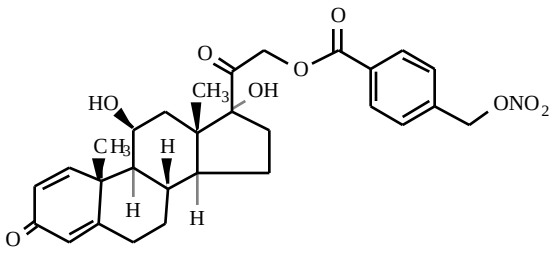
Diclofenac/SNO (25)
Nitromed

*** Under clinical developement**

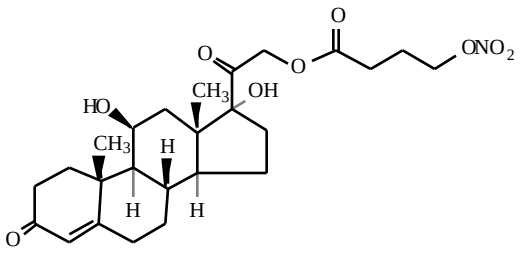
Fig. (10). Examples of NO-NSAIDs.

gastric mucosa, including the increased microcirculation and gastric release of mucous and of bicarbonate. The pharmacological behaviour of these drugs is quite complex and still requires further investigation. In particular, the role played by nitric oxide in reducing the gastrotoxicity of these drugs requires in-depth study, since another contribution to this effect could derive from the masking of the –COOH group through esterification. It is known that, in rats, the esterification of acid anti-inflammatory drugs suppresses their gastrotoxicity without adversely affecting their anti-inflammatory activity [61]. With regard to NO-aspirins, another point not yet fully clarified is whether these products are aspirin pro-drugs or behave as pro-drugs of salicylic acid. In fact it is known that esterification of the carboxylic group renders the acetyl group in aspirin extremely susceptible to enzymatic cleavage [62].

Another important class of hybrid containing nitrooxy function are NO-steroids [63]. In the main, nitrooxy derivatives of some glucocorticoids have been developed. These products display anti-inflammatory activity due to the presence of the glucocorticoid system, and additional pharmacological properties consequent on their capacity to release NO. They trigger bronchodilation and appear to be devoid of some of the typical drawbacks of glucocorticoids, such as osteoporosis, hypertension, and gastric disturbances. Promising examples of these drugs are NCX 1015 and NCX 1004 (**26** and **27,** Fig. (**11**)), obtained by esterification of the 21-hydroxy group with an appropriate acid containing the



NO-prednisolone (NCX 1015)* (26)



NO-hydrocortisone (NCX1004)* (27)

*** Under clinical developement**

Fig. (11). NO-steroids.

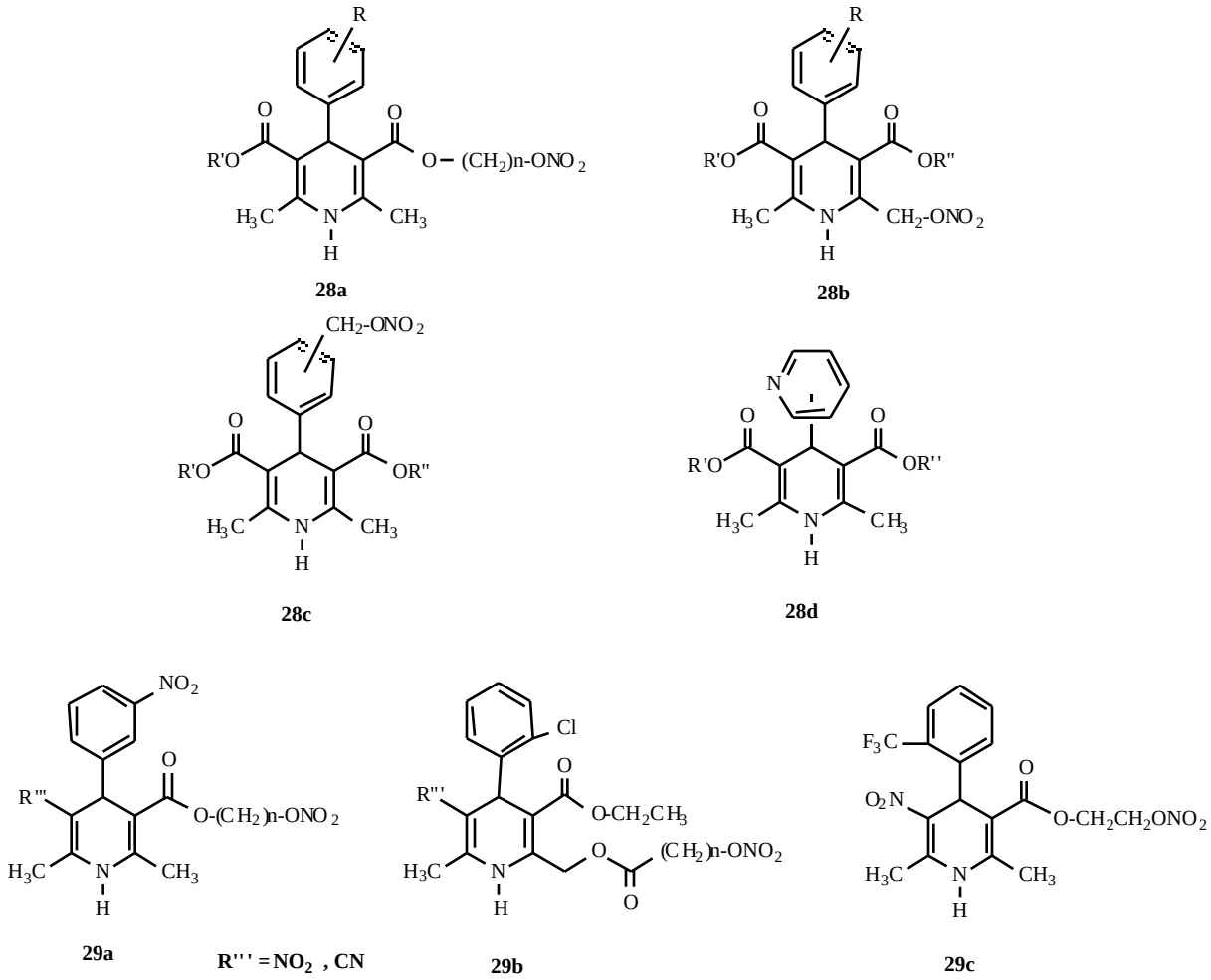


Fig. (12). NO-donor calcium blockers (**28a-d**) and activators (**29a-c**).

nitrooxy function [64,65]. The structural modulation of the lateral ester chain in NCX 1015 confers different solubility and different pharmacokinetic properties but retains the binding profile of the parent drug [66].

Conjugation of the nitrooxy function with 1,4-DHP system is also worthy of comment. Both NO-donor calcium blockers (**28a-d**, Fig. (**12**)) [55,67,68] and NO-donor calcium activators (**29a-c**, Fig. (**12**)) [69,70] have been obtained. The former could be interesting anti-atherosclerosis drugs also in view of their additional antioxidant properties due to the presence of 1,4-dihydropyridine nucleus [71,72]. The latter could be an innovative approach to new inotropic agents devoid of vasoconstrictor properties.

Interesting hybrid products have been produced by linking the diazeniumdiolate group to drugs and biomolecules through a piperazine ring. These structures are methylated on the O^2-terminus of the diazeniumdiolate moiety to obtain stable products. Enzymatic removal of the CH_3 group should produce unstable NO-releasing diazeniumdiolate, and consequently these products could exert some degree of tissue tropism [73]. Representative examples include diazeniumdiolate conjugated to ibuprofen

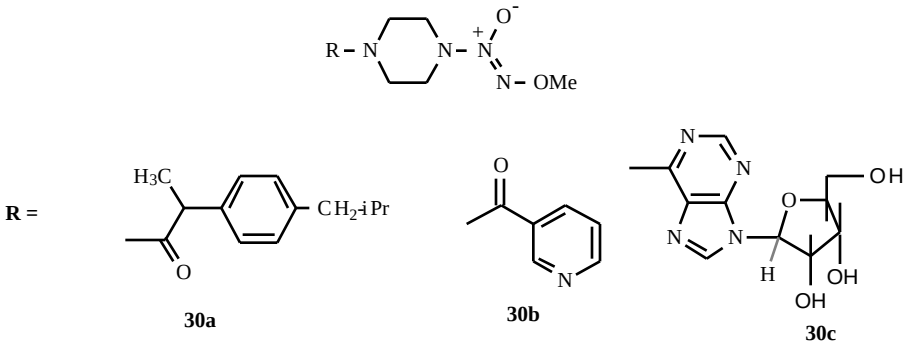
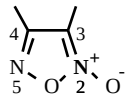


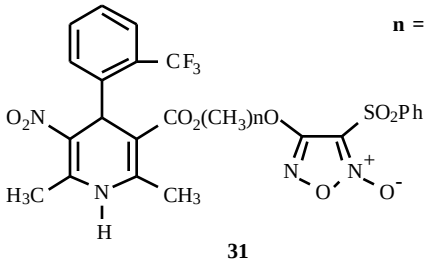
Fig. (13). Diazeniumdiolates conjugated to ibuprofen (**30a**), vitamin B_3 (**30b**) and purine riboside (**30c**).

(**30a**), to vitamin B_3 (**30b**), and to purine riboside (**30c**) (Fig. (**13**)).

The NO-donor furoxan system has also been used in the design of hybrid drugs. Furoxan is 1,2,5-oxadiazole 2-oxide (**I**, Fig. (**14**)) and is an old system whose chemistry is complex [74,75]. It has recently been found that furoxan derivatives can activate sGC and release NO in physiological solution in the presence of thiols [76,77]. The NO-release mechanism is complex and could imply the formation of more than one redox form of NO [21]. Enzymatic bioactivation may also be involved in cells and tissues [78]. Many hybrids containing NO-donor furoxans have been synthesised and studied for their dual activity. Examples of drugs hybridised with furoxan moieties include α_1-

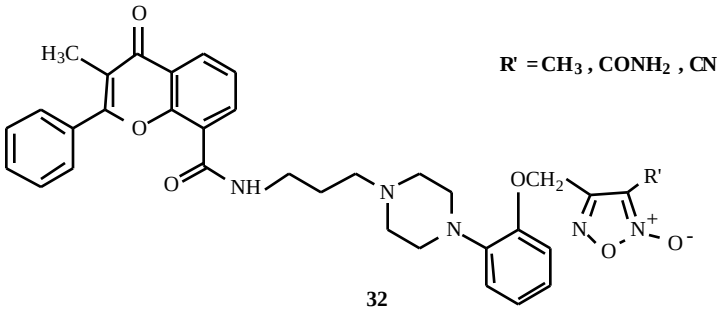


I



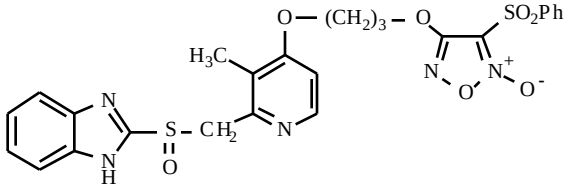
n = 2, 3, 4

31

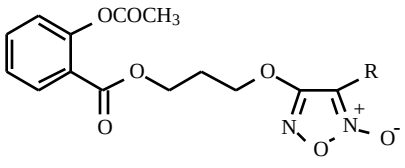


R' = CH_3 , $CONH_2$, CN

32

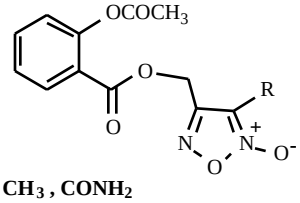


33



R = Ph SO_2, Ph

34a



R = CH_3 , $CONH_2$

34b

Fig. (14). Examples of hybrid molecules containing NO-donor furoxans.

antagonists, β_1-antagonists, K^+-channel activators, Ca^{2+}-channel blockers, NSAIDs, H_3- and H_2-antagonists. All these products have already been reviewed [20,79]. More recently, as illustrated in Fig. (**14**), NO-donor furoxans were linked to 4-phenyl-1,4-dihydropyridine calcium channel activators (**31**) [80], to REC 15/2739, an uroselective α_1-antagonist (**32**) [81], to rabeprazole a potent inhibitor of H^+/K^+ ATP-ase enzyme (**33**) [82], and to aspirin (**34a,b**) [83]. Interestingly, NO-donor furoxan aspirins displayed gastrosparing and anti-inflammatory properties close to those of their furazan analogues, which are unable to release NO. Therefore, the possible involvement of nitric oxide in the gastrosparing action of these products does not appear to be certain. For all these products, no production of aspirin was detected during the study of their metabolism in human serum. Some NO-donor furoxan aspirins are also endowed with potent anti-aggregatory activities [83].

In designing an NO-donor hybrid, one aspect to which proper attention is not always paid is the "balance" of the resulting product [52]. In a well-designed hybrid containing two pharmacophoric groups, the pharmacological properties of one do not prevail over those of the other. The furoxan system appears to be quite a flexible tool to achieve this goal. It is sufficient to change the nature of the substituent at the ring in order to modulate the NO-dependent pharmacological properties. For example, linking the methylfuroxan substructure to the Ca^{2+}-blocker 4-phenyl-1,4-dihydropyridine pharmacophore gives a product with prevalent Ca^{2+}-blocker properties; linking it with the 3-cyanofuroxan system produces a product that behaves as a vasodilator, prevalently due to its NO releasing capability. Finally, linking it with the 3-carbamoylfuroxan substructure produces a product with vasodilatating activity, due to its ability both to block Ca^{2+}-channels and to release NO [84].

Both recent and older reports have claimed that some furoxan derivatives have antitumuoral, antimicrobial, or antihelmintic activities [79,74,75]. Since it is well known that nitric oxide triggers important cytoxic effects in the human immune system and, since a number of NO-donors display antiviral [85], antimicrobial [86], and antitumoral effects [46], these properties may, at least in part, be dependent on the potential NO-mediated activity of these products. However, no clear evidence of this has yet been provided. A series of hybrid furoxan derivatives linked to metronidaziole was synthesised (**35a-c**, Fig. (**15**)). The whole series displayed potent anti–*Helicobacter pylori* activity. The potential contribution to this activity of the redox properties and/or of NO-release associated to furoxan system did not emerge clearly. In fact, the furazan (1,2,5-oxadiazole) analogues, which have different redox behaviour and do not release NO, behaved similarly [87].

The fact that the metronidazole ester of γ-nitrooxybutyrric acid has an enhanced capability *in vitro* to kill *Entamoeba histolytica* versus metronidazole has been claimed to involve NO release by the product into the parasite cell [88]. Indeed an increased level of DAF fluoresceine compared with the control was detected when *Entamoeba histolytica trophozoites* were treated with NCX 972 (**35a**, Fig. (**15**)) in the presence of DAF-2-DA (4,5-diaminofluorescein diacetate), an indicator of nitric oxide. However additional evidence is needed to confirm this, since other molecular properties of the product could justify its improved antiparasite activity.

NO-Delivery Systems

Some NO-donors have been incorporated or covalently attached, directly or through an appropriate spacer, to a number of synthetic and natural polymers. The resulting products can be obtained in different pharmaceutical forms such as films, microspheres, gels, powders, and resins, all of which release NO only to the tissue with which they come into contact. These systems can be used where local sustained release of NO is required.

The $N(O)NO^-$ group appears to be quite flexible for this purpose. Two different structural types of derivatives have been considered: diazeniumdiolates incorporated in many polymers, including poly(ethyleneglycol), and in the biodegradable poly(caprolactone), or diazeniumdiolates covalently attached to polysaccharides, to methacrylic polymers and to poly(ethylenimine) [89,90,91]. This latter product proved to trigger potent antiplatelet activity in baboons and inhibited the *in vitro* proliferation of rat aorta smooth muscle cells [91]. Similarly the stable O^2-terminus methyloxymethyl derivative of piperazine diazeniumdiolate (MOM-PIPERAZI/NO) was covalently attached directly to heparin and poly(vinylchloride) (PVC) or through an appropriate spacer to albumin [73,92]. When in contact with water the methoxymethyl group slowly hydrolyses giving the unstable parent diazeniumdiolate. The synthesis of diazeniumdiolates covalently linked to a siloxane polymer using sol-gel chemistry produced materials that actively reduce bacterial adhesion [93].

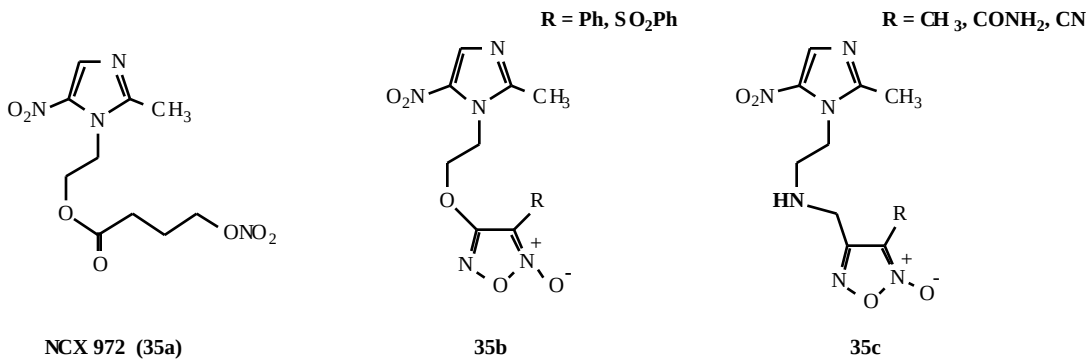
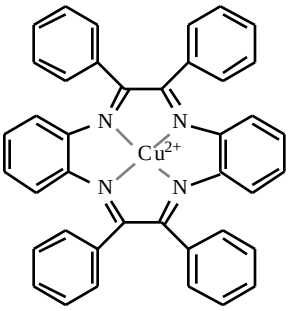


Fig. (15). NO-donor metronidazoles.

In general the most severe limit of all NO-releasing polymers is the small size of the reservoir of NO-adducts that can be loaded into the polymeric matrices. This prevents their use as long-term biomedical implants. Recent findings that the decomposition of nitrosothiols to NO· can also occur via a heterogeneous catalytic reaction at the surface of polymer films containing lipophilic Cu(II) complexes, such as the Cu(II)-dibenzo[e,k]-2,3,8,9-tetraphenyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene (Cu(II)-DTTCT) (structure **36**, Fig. (**16**)), open new perspectives in this field [94]. It is thought that when these polymers come into contact with flowing blood, they will catalyse the decomposition of the endogenous nitrosothiols present in the bloodstream, and consequently they should provide a continuous supply of NO· at appropriate physiological levels.



36

Fig. (16). Cu(II)-DTTCT complex.

Polyethylene glycol hydrogels containing covalently linked S-nitrosocysteine and NO complexes either with L-lysine or with diethylenetriamine were found to reduce platelet adhesion and smooth muscle cells proliferation [95]. These preparations were claimed to prevent restenosis and thrombosis. Finally, the S-nitrosylation of bovine serum albumin (BSA) produces poly-S-nitrosated BSA, which was found to display vasodilatory and antiplatelet activities *in vivo* and to act as antiplatelet coating on artificial surfaces [96,97,98]. A cream produced from the combination of potassium nitrite and ascorbic or salicylic acid has also been proposed for topical nitric oxide delivery in treating cutaneous leishmaniasis [99].

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REFERENCES

[1] Kerwin, J.F. Jr.; Heller, M. *Med. Res. Rev.*, **1994**, *14*, 23.
[2] Kerwin, J.F. Jr.; Lancaster, J.R. Jr.; Feldman, P.L. *J. Med. Chem.*, **1995**, *38*, 4343.
[3] Lipton, S.A. ; Stamler, J.S.; Singel, D.J. *Nature*, **1994**, *28*, 367.
[4] Stamler, J.S.; Singel, D.J.; Loscalzo, J. *Science*, **1992**, *258*, 1898.
[5] Horowitz, J.D. In *Nitric Oxide and the Cardiovascular System;* Loscalzo, J.; Vita J.A.; Eds.; Humana Press: Totowa, New Jersey, **2000**; pp. 383-409.
[6] Tatcher, G.R.; Weldon, H. *Chem. Soc. Rev.*, **1998**, *27*, 331.
[7] Schröder, H. *Adv. Drug. Res.*, **1996**, *28*, 253.
[8] Chen, Z.; Zhang, J.; Stamler, J.S. *Proc. Natl. Acad. Sci. U. S. A.*, **2002**, *99*, 8306.
[9] Abrams, J.; Elkayam, U.; Thadani, U.; Fung, H.-L. *Am. J. Cardiol.*,**1998**, *81 (1A)* 3A-14A.
[10] Wang, K.; Zhang, W.; Xian, M.; Hou, Y.-C.; Chen, X.-C.; Cheng, J.-P.; Wang, P.G. *Curr. Med. Chem.*, **2000**, *7*, 821.
[11] Richardson, G.; Benjamin, N. *Clin. Sci.*, **2002**, *102*, 99.
[12] Megson, I.L.; Morton, S.; Greig, I.R.; Mazzei, F.A.; Field, R.A.; Butler, A.R.; Caron, G.; Gasco, A.; Fruttero, R.; Webb, D.J. *Br. J. Pharmacol.*, **1999**, *126*, 639.
[13] Askew, S.C.; Barnett, D.J.; McAninly, J.; Williams, D.L.H. *J. Chem. Soc. Perkin Trans. 2*, **1995**, 741.
[14] Dicks, A.P.; Swift, H.R.; Williams, D.L.H.; Butler, A.R.; Al-Sa'doni, H.H.; Cox, B.G. *J. Chem. Soc. Perkin Trans. 2*, **1996**, 481.
[15] Megson, I.L.; Greig, I.R.; Gray, G.A.; Webb, D.J.; Butler, A.R. *Br. J. Pharmacol.*, **1997**, *122*, 1617.
[16] Butler, A.R.; Al-Sa'doni, H.H.; Megson, I.L.; Flitney, F.W. *Nitric Oxide*, **1998**, *2*, 193.
[17] Al-Sa'doni, H.H.; Khan, I.Y.; Poston, L.; Fisher, I.; Ferro, A. *Nitric Oxide*, **2000**, *4*, 550.
[18] Hrabie, J.A.; Keefer, L.K. *Chem. Rev.*, **2002**, *102*, 1135.
[19] Keefer, L.K. *CHEMTECH.*, **1998**, *28*, 30.
[20] Wang, P.G.; Xian, M.; Tang, X.; Wu, X.; Wen, Z.; Cai, T.; Janczuk. A.J. *Chem. Rev.*, **2002**, *102*, 1091.
[21] Feelisch, M.; Stamler, J.S. In *Methods in Nitric Oxide Research;* Feelisch, M. and Stamler, J.S. Eds.; John Wiley & Sons: New York, **1996**; pp. 71-115.
[22] (a) Granik, V.G.; Ryabova, S.Y.; Grigoriev, N.B. *Russ. Chem. Rev.*, **1997**, *66*, 717; (b) Granik, V.G.; Grigoriev, N.B. *Russ. Chem. Bull. Int. Ed.*, **2002**, *51*, 1375.
[23] Berliner, J.A.; Navab, M.; Fogelman, A.M.; Frank, J.S.; Demer, L.L.; Edwards, P.A.; Watson, A.D.; Lusis, A.J. *Circulation*, **1995,** *91*, 2488.
[24] Keaney, J.F. Jr.; Vita, J.A. *Prog. Cardiovasc. Dis.*, **1995**, *38*, 129.
[25] Rekka, E.A.; Chrysselis, M.C. *Mini Rev. Med. Chem.*, **2002**, *2*, 585.
[26] Chrysselis, M.C.; Rekka, E.A.; Siskou, I.C.; Kourounakis, P.N. *J. Med. Chem.*, **2002**, *45*, 5406.
[27] Chryssselis, M.C.; Rekka, E.A.; Kourounakis, P.N. *J. Med. Chem.*, **2000**, *43*, 609.
[28] Kurowska, E.M.; Carroll, K.K. *Biochim. Biophys. Acta*, **1998**, *1392*, 41.
[29] Samuni, A.M.; Barenholz, Y. *Free Radical Biol. Med.*, **2003**, *34*, 177.
[30] Offer, T.; Samuni, A. *Free Radical Biol. Med.*, **2002**, *32*, 872.
[31] Fedeli, D.; Damiani, E.; Greci, L.; Littaru, G.P.; Falcioni, G. *Mutat. Res-Gen.Tox. En.*, **2003**, *535* (2), 117.
[32] Änggård, E.E.; Haj-Yehia, A.I. U.S. Patent 6,455,542. B1, **2002**.
[33] Haj- Yehia, A.; Nassar, T.; Lotan, T.; Münzel, Benet, L.; Änggård; E.E. *Drug. Develop. Res.*, **2000**, *50*, 528.
[34] Zhang, Z.; Naughton, D.P.; Sumi, Y.; Imaizumi, A. U.S. Patent 6,346,634, **2002**.
[35] Petit, C.; Bernardes-Genisson, V.; Hoffmann, P.; Souchard, J.P.; Labidalle, S. *Braz. J. Med. Biol. Res.*, **1999**, *32*, 1407.
[36] Kelm, M. *Biochim. Biophys. Acta*, **1999**, 1411, 273.
[37] Andreassi, M.G.; Picano, E.; DelRy, S.; Botto, N.; Colombo, M.G.; Giannessi, D.; Lubrano, V.; Vassalle, C.; Bigini, A. *Mutagenesis*, **2001**, *16*, 517.
[38] Saavedra, J.E.; Dunams, T.M.; Filippen-Anderson, J.L.; Kefeer; L.K. *J. Org. Chem.*, **1992**, *58*, 1472.
[39] Saavedra, J.E.; Billiar, T.R.; Williams, D.L.; Kim, Y.-M.; Watkins, S.C.; Keefer, L.K. *J. Med. Chem.*, **1997**, *40*, 1947.
[40] Tang, X.; Xian, M.; Trikha, M.; Honn, K.V.; Wang, P.G. *Tetrahedron Lett.*, **2001**,*42*, 2625.
[41] Wu, X.; Tang, X.; Xian, M.; Wang; P.G. *Tetrahedron Lett*., **2001**, *42*, 3779.
[42] Shami, P.J.; Saavedra, J.E.; Wang, L.Y.; Bonifant, C.L.; Diwan, B.A.; Singh, S.V.; Gu, Y.; Fox, S.D.; Buzard, G.S.; Citro, M.L.; Waterhouse, D.J.; Davies, K.M.; Ji, X.; Keefer, L.K. *Mol. Cancer, Ther.*, **2003**, *2*, 409.
[43] Ramirez, J.; Yu, L.; Li, J.; Braunschweiger, P.G.; Wang, P.G. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2575.
[44] Hou, Y.C.; Wang, J.-Q.; Ramirez, J.; Wang. P.G.; *Method. Enzymol.*, **1999**, *301*, 242.
[45] Wu, X; Tang, X.; Xian, M.; Brauschweiger, P.G.; Wang; P.G. *Bioorg. Med. Chem.*, **2002**, *10*, 2303.

[46] Hou, Y.C.; Wang, J.; Andreana, P.R.; Cantauria, G.; Tarasia, S.; Sharp, L.; Braunschweiger, P.G.; Wang; P.G. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 2255.
[47] Hou, Y.; Wu, X.; Xie, W.; Braunschweiger, P.G.; Wang, P.G. *Tetrahedron Lett.*, **2001**, *42*, 825.
[48] Fleisch, H. *Bisphosphonates in Bone Disease*, The Parthenon Publishing Group: New York, London, **1997**.
[49] Fiorucci, S.; Mencarelli, A.; Palazzetti, B.; Del Soldato, P.; Morelli, A.; Ignarro, L.J. *Proc. Natl. Acad. Sci. U. S. A.*, **2001**, *98*, 2652.
[50] Fiorucci, S.; Antonelli, E.; Morelli, O.; Mencarelli, A.; Casini, A.; Mello, T.; Palazzotti, B.; Tallet, D.; Del Soldato, P.; Morelli, A. *Proc. Natl. Acad. Sci. U. S. A.*, **2001**, *98*, 8897.
[51] Royer, R. In Actualités de Chimie. Thérapeutiques 4éme série, société de chimie thérapeutique (sct), **1976**, 37.
[52] Nicolaus, B.J.R. In *Decision Making in Drug Research*; F. Gross, Ed.; Raven Press: New York, **1983**; pp.173-186.
[53] Baldwin, J.J. In *Drug Discovery Development;* M. Williams, J.B. Malick, Eds.; Humana Press: Clifton, New Jersey, **1987**; pp. 33-71.
[54] Christiaans, J.A.M.; Timmerman, H. *Eur. J. Pharm. Sci.*, **1996**, *4*, 1.
[55] Gasco, A.; Fruttero, R.; Sorba, G. *Farmaco*, **1996**, *51*, 617.
[56] Wallace, J.L.; Del Soldato, P.; Cirino, G.; Muscarà, M.N. *Drugs*, **1999**, *2*, 321.
[57] Bandarage, U.K.; Janero, D.R. *Mini Rev. Med. Chem.* **2001**, *1*, 57.
[58] Keeble, J.E.; Moore, P.K. *Br. J. Pharmacol.*, **2002**, *137*, 295.
[59] Napoli, C.; Ignarro, L.J. *Annu.Rev. Pharmacol. Toxicol.*, **2003**, *43*, 97.
[60] Wallace, J.; Granger, D.N. *FASEB J.*, **1996**, *10*, 731.
[61] Whitehouse, M.W.; Rainsford, K.D.J. *Pharm. Pharmacol*., **1980**, *32*, 795.
[62] Nielsen, N.M.; Bundgaard, H. *J. Med. Chem.* **1989**, *32*, 727.
[63] Burgaud, J.L.; Riffaud, J.P.; Del Soldato, P. *Curr. Pharm. Design*, **2002**, *8*, 201.
[64] Tallet, D.; Del Soldato, P.; Oudart, N.; Burgaud, J.L. *Biochem. Biophys. Res. Commun.*, **2002**, *290* (1), 125.
[65] Turesin, F.; Del Soldato, P.; Wallace, J.L. *Br. J. Pharmacol.*, **2003**, *139*, 966.
[66] Baraldi, P.G.; Romagnoli, R.; Nuñez, M.; Perretti, M.; Paul-Clark, M.J.; Ferrario, M.; Govoni, M.; Benedini, F.; Ongini, E. *J. Med. Chem*., **2004**, *47*, 711.
[67] Lehmann, J.; Kahlich, R.; Meyer zum Gottesberge, C.; Fricke, U. *Arch. Pharm. Pharm. Med. Chem. (Weinheim, Ger.)*, **1997**, *330*, 247.
[68] Nguyen, J.-T., McEwen, C.-A.; Knaus, E. *Drug Dev. Res.*, **2000**, *51*, 233.
[69] Wessler, C.; Diewald, D.; Lehmann, J. *Eur. J. Pharm. Sci.*, **1998**, *6* (suppl. 1), S37.
[70] Shan, R.; Howlett, S.E.; Knaus, E.E. *J. Med. Chem.*, **2002**, *45*, 955.
[71] Rojstaczer, N.; Triggle, D.J. *Biochem. Pharmacol.*, **1996**, *51*, 141.
[72] Sobal, G.; Menzel, E.J.; Sinzinger, H. *Biochem. Pharmacol.*, **2001**, *61*, 373.
[73] Saavedra, J.E.; Booth, M.N.; Harabie, J.A.; Davies, K.M.; Keefer, L.K. *J. Org. Chem.*, **1999**, *64*, 5124.
[74] Gasco, A.; Boulton, A.J. *Adv, Heterocycl. Chem.*, **1981**, *29*, 251.
[75] Khmel'nitskii, L.I.; Novikov, S.S.; Godovikova, T.I. *Chemistry of Furoxans: Structure and synthesis.* Khmel'nitskii, L.I.; Novikov, S.S.; Godovikova, T.I. *Chemistry of Furoxans: Reaction and use.* 2nd ed; M. Nauka, **1996** (in Russian).
[76] Ghigo, R.; Heller, P.; Calvino, R.; Alessio, R.; Fruttero, R.; Gasco, A.; Bosia, A.; Pescarmona, G.P. *Biochem. Pharmacol.*, **1992**, *43*, 1281.
[77] Feelisch, M.; Shönafinger, K.; Noack, E. *Biochem. Pharmacol.*, **1992**, *44*, 1149.
[78] Hecker, M.; Vorhoff, W.; Bara, A.T.; Mordvintcev, P.I.; Busse, R. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **1995**, *351*, 426.
[79] Cerecetto, H.; Porcal, W. *Mini Rev. Med. Chem.* in press.
[80] Vo, D.; Nguyen, J.-T.; Mc.Ewen, C.-A.; Shan, R.; Knaus, E.E. *Drug Dev. Res.*, **2002**, *56*, 1.
[81] Boschi, D.; Tron, G.C.; Di Stilo, A.; Fruttero, R.; Gasco, A.; Poggesi, E.; Motta, G.; Leonardi, A. *J. Med. Chem.*, **2003**, *46*, 3762.
[82] Sorba, G.; Galli, U.; Cena, C.; Fruttero, R.; Gasco, A.; Morini, G.; Adami, M.; Coruzzi, G.; Brenciaglia, M.I.; Dubini, F. *ChemBioChem.*, **2003**, *4*, 899.
[83] Cena, C.; Lolli, M.L.; Lazzarato, L.; Guaita, E.; Morini, G.; Coruzzi, G.; McElroy, S.P.; Megson, I.L.; Fruttero, R.; Gasco, A. *J. Med. Chem.*, **2003**, *46*, 747.
[84] Di Stilo, A.; Visentin, S.; Cena, C.; Gasco, A.M.; Ermondi, G; Gasco, A. *J. Med. Chem.*, **1998**, *41*, 5393.
[85] Mannick, J.B. *Res. Immunol.*, **1995**, *146*, 693.
[86] Marcinkiewicz, J. *Immunopharmacology*, **1997**, *37*, 35.
[87] Bertinaria, M.; Galli, U.; Sorba, G.; Fruttero, R.; Gasco, A.; Brenciaglia, M.I.; Scaltrito, M.M.; Dubini, F. *Drug. Dev. Res.*, **2003**, *60*, 225.
[88] Sannella, A.; Gradoni, L.; Persichini, T.; Ongini, E.; Venturini, G.; Colasanti, M. *Antimicrob. Agents Chemother*., **2003**, *47*, 2303.
[89] Smith, D.J.; Chakravarthy, D.; Keefer, L.K.; U.S. Patent 5,691,423, **1997**.
[90] Parzuchowski, P.G.; Frost, M.C.; Meyerhoff, M. *J. Am. Chem. Soc.*, **2002**, *124*, 12181.
[91] Smith, D.J.; Chakravarthy, D.; Pulfer, S.; Simmons, M.L.; Hrabie, J.A.; Citro, M.L. *J. Med. Chem.*, **1996**, *39*, 1148.
[92] Saavedra, J.; Keefer, L. *Chem. Brit.*, **2000**, *36*, 30.
[93] Nablo, B.J.; Chen, T.-Y.; Schoenfisch, M.H. *J. Am. Chem. Soc.*, **2001**, *123*, 9712.
[94] Oh, B.K.; Meyerhoff, M.E. *J. Am. Chem. Soc.*, **2003**, *125*, 9552.
[95] Bohl, K.S.; West, J.L. *Biomaterials*, **2000**, *21*, 2273.
[96] Stamler, J.S.; Simon, D.I.; Osborne, J.A.; Mullins, M.E.; Jaraki, O.; Singel, D.J.; Loscalzo, J. *Proc. Natl. Acad. Sci. U. S. A.*, **1992**, *89*, 444.
[97] Keaney, J.F.; Simon, D.I.; Stamler, J.S.; Jaraki, O.; Scharfstein, J.; Vita, J.A.; Loscalzo, J. *J. Clin. Invest.*, **1993**, *91*, 1582.
[98] Maalej, N.; Albrecht, R.; Loscalzo, J.; Folts, J.D. *J. Am. Coll. Cardiol.*, **1999**, *33*, 1408.
[99] Davidson, R.N.; Yardley, V.; Croft, S.L.; Konecny, P.; Benjamin, N. *Trans R. Soc. Trop. Med. Hyg.*, **2000**, *94*, 319.