Current Status and Future Possibilities of Nitric Oxide-Donor Drugs: Focus on *S*-Nitrosothiols

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Abstract: Drugs which release nitric oxide (NO) have great therapeutic potential. The organic nitrates and sodium nitroprusside have been used in cardiovascular therapeutics for many years, but several drawbacks limit their usefulness. In this review, we consider novel and potential future developments in NO-donor drugs, and the possible clinical usefulness of such compounds.

Keywords: Nitric oxide, nitric oxide donors, *S*-nitrosothiols, therapeutics.

INTRODUCTION

There are many potential uses for nitric oxide (NO) as a therapeutic agent. It can inhibit platelet function, reduce vascular tone and act as a neurotransmitter, and is also involved in host defence [1]. NO itself can be administered directly by inhalation, and has been used in the treatment of adult respiratory distress syndrome and other cardiopulmonary conditions associated with chronic pulmonary hypertension. It is also used to treat persistent pulmonary hypertension of the newborn. In higher concentrations than those usually administered, inhalation of NO leads to methemoglobinemia, but in clinical practice it is the build-up of toxic levels of nitrite which limits its use [2].

Current interest in NO-donor drugs has focused on the design and synthesis of new drugs with improved pharmacokinetic properties. Organic nitrates, which have been used for many years to treat patients with ischemic heart disease, are believed to act by generating NO. Unfortunately, the development of tolerance to these compounds limits their use as NO donors. Sodium nitroprusside, another NO donor in use therapeutically, can lead to cyanide toxicity. There is considerable interest in the development of new compounds to act as NO donors. The NONOates are potentially useful compounds which generate NO spontaneously. Although they have been studied extensively *in vitro*, much less is known about their properties *in vivo*. The *S*-nitrosothiols (RSNOs), which occur endogenously, are degraded to release NO both enzymatically and non-enzymatically, and have also been administered to humans in small clinical trials.

In this review, we will consider the properties of NO-donor drugs in relation to their potential use as therapeutic agents. We will focus particularly on the RSNOs since, amongst the newer NO-donor drugs, these have been the most studied in terms of therapeutic effects.

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ENDOGENOUS FORMATION OF NITRIC OXIDE

Endogenous formation of NO in animals and humans is now well recognized. NO is enzymatically synthesized from L-arginine (Scheme **1**); it plays an important role as a regulator of metabolism in cells and tissues [1,3-6]. NO functions as the main cytostatic/cytotoxic effector of the cellular immune system [7-9]. NO exerts both autocrine and paracrine actions, that is to say, being synthesized in certain cells it can influence metabolic processes in these and adjacent cells. In spite of its high chemical reactivity, NO molecules can be transported to distances exceeding cell size by several-fold [10]. During this transportation free NO molecules can be bound by various endogenous scavengers, for example, by hemoglobin; hemoglobin catalyzes the oxidation of NO by molecular oxygen. Superoxide ions also oxidize NO very effectively. These factors can significantly attenuate or even abolish the paracrine effect of NO. Only the reversible incorporation of NO into compounds that can transport it from donor to target cells can prevent or diminish these processes. This is illustrated by the debate surrounding the identity of the so-called "endothelium-derived relaxing factor" (EDRF) produced by blood vessels [11]. In 1987 it was demonstrated that EDRF contains NO and it is this particular component which is responsible for the vasodilator activity of EDRF. Moreover, it was demonstrated that the efficacy of free NO added to isolated vessels in amounts equal to that found in EDRF was identical to the efficacy of EDRF. On the basis of their very similar pharmacological profiles, it was concluded that EDRF and NO are identical [12,13]. However, in other studies, the vasodilator activity of EDRF (expressed per amount of NO detected in EDRF) was found to be one order of magnitude greater than that of free NO [14-16]. This suggested that EDRF may be a nitroso-compound rather than free NO, such as *S*-nitrosocysteine (cys-NO), which protects NO against oxidation and releases it during contact with target cells. Similar conclusions were drawn by Vedernikov *et al*. [17], who compared the capacities of EDRF and NO to form dinitrosyl iron complexes (DNICs).

The suggestion that cys-NO may be the form in which NO is packaged in EDRF was due to their almost identical vasodilator efficacy [14]. Vanin proposed DNICs with thiol-containing ligands (cysteine or glutathione, GSH) as another possible candidate for EDRF [18]. It was shown that NO

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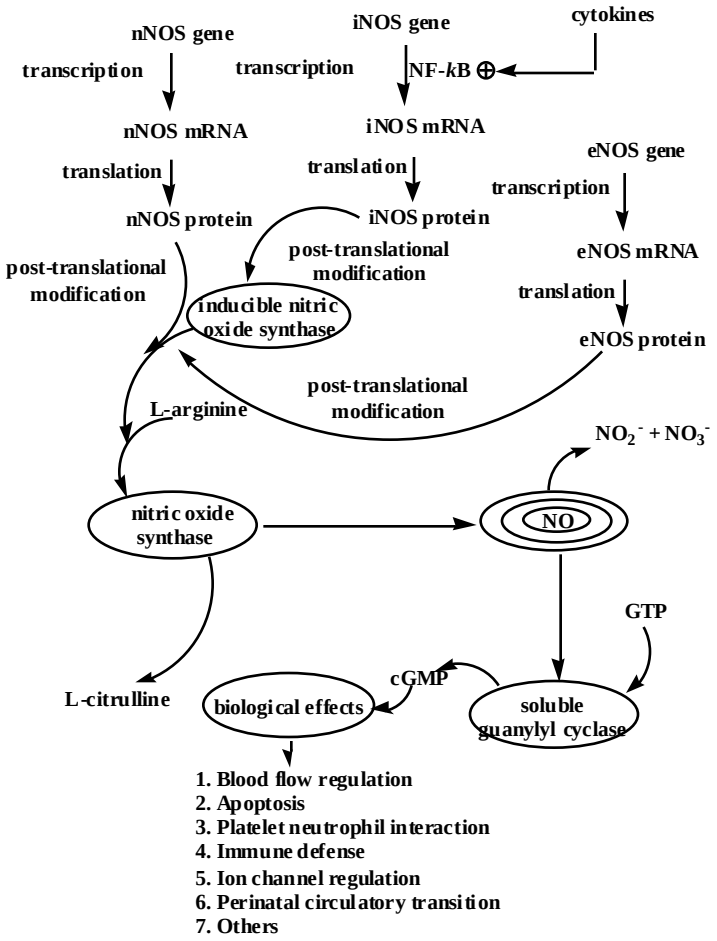


Scheme 1. Pathway for NO biosynthesis. NO synthase, of which three isoforms are known to exist, catalyzes the oxidation-reduction reaction of L- arginine, in the presence of molecular oxygen, to form the free radical NO and L-citrulline. NO has a short half-life and is oxidized to the inactive end-products nitrite and nitrate. iNOS expression is upregulated by cytokines through the transcription factor NF-κB. Transcriptional and post-transcriptional mechanisms are both important in iNOS regulation, whereas eNOS and nNOS regulation occurs through changes in $[Ca^{2+}]$ or in phosphorylation of the enzyme.

incorporation into such compounds effectively protected it against the oxidizing effect of superoxide, and this resulted in a significant potentiation of its vasodilator effect [19].

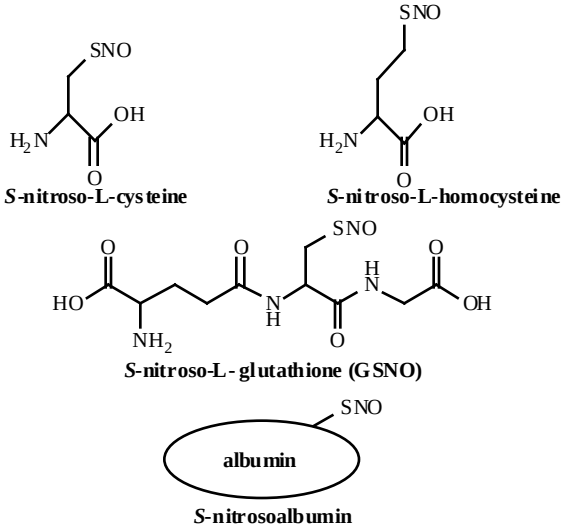
The incorporation of NO produced from L-arginine in animal cells and tissues into both RSNOs and DNICs with thiol-containing ligands has been demonstrated in numerous studies [20-28]. This, together with data on the physicochemical and functional properties of RSNOs and DNICs, suggests that these compounds can function in NO transportation and stabilization not only in blood vessels, but also in other tissues.

NITRIC OXIDE-DONOR COMPOUNDS

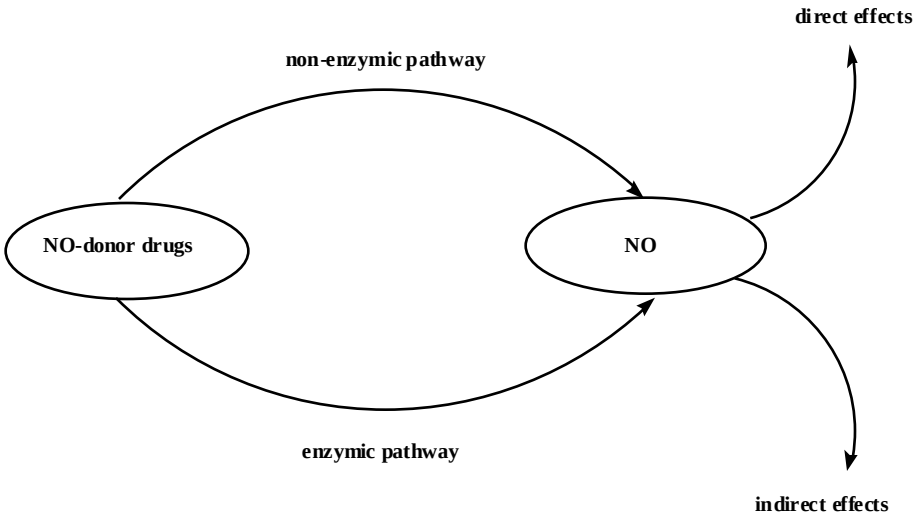
NO can be generated *in vivo* or *in vitro* from different sources, as follows.

Endogenous NO Donors

S-Nitrosothiols (Scheme **2**) are formed by *S*-nitrosation of thiols or cysteine residues of proteins and have the general formula RSNO, where R is usually a protein, peptide or amino acid. Examples of endogenous RSNOs are *S*-nitrosoalbumin (SNO-albumin), *S*-nitrosoglutathione (GSNO) and *S*-nitrosocysteine (cys-NO). Protein RSNOs tend to be more stable than RSNOs with low molecular masses [29].



Scheme 2. Endogenous NO-donor compounds. These are all examples of RSNOs.



Scheme 3. Simplified pathways for the decomposition of NO-donor drugs *in vivo* and *in vitro*.

RSNOs were first described as occurring endogenously in the early 1990s [20,21]. The precise amounts of these compounds *in vivo* remains a matter for debate, as a reliable and reproducible method for their detection has not been fully developed; nevertheless, they have been measured in the sub-micromolar range in plasma [20,30] and bronchoalveolar lavage fluid [21].

Degradation of RSNOs by heat, ultraviolet light, vitamin C and trace amounts of copper ions to produce NO and the corresponding disulphide can be enhanced by the presence of thiols, high oxygen tension and pH > 2. Enzymatic cleavage by γ-glutamyl transpeptidase [31], glutathione peroxidase [32] and xanthine oxidase [33] has also been demonstrated. Finally, *S*-nitrosothiols can also participate in transnitrosation reactions, whereby the NO^+ group is transferred to another thiol. This can then result in the release of NO if the resultant compound is more susceptible to decomposition than the parent compound [29].

Table 1. Endogenous and Exogenous NO-Donor Compounds

Endogenous NO-donors	Exogenous NO-donors
Endogenous S-nitrosothiols:	*Organic NO-donors:*
GSNO, CysNO, SNO-albumin, other	*S*-nitroso compounds
	O-nitrite compounds
	N-nitroso compounds
	C-nitroso compounds
	Heterocyclic NO-donor compounds
	Inorganic NO-*donors*:
	Transition metal-NO donor complexes

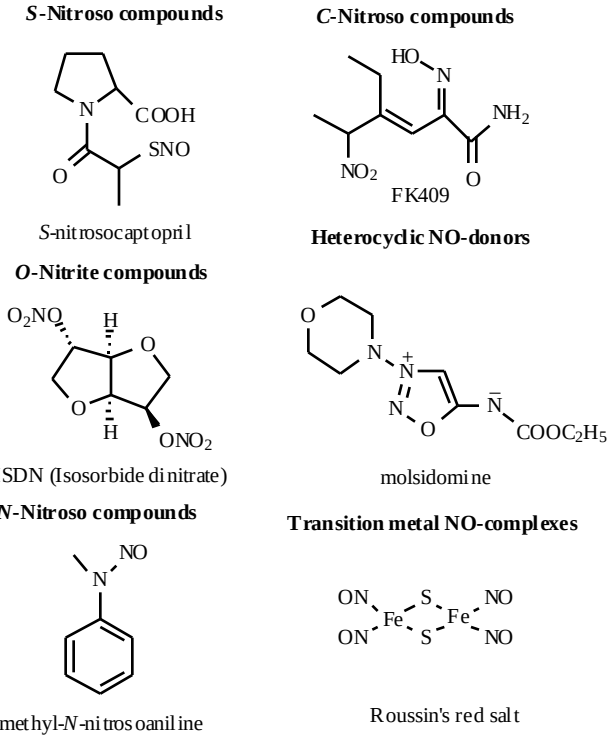
The properties of RSNOs are similar to those of NO, in that they are involved in smooth muscle cell relaxation, platelet inhibition, immunosuppression, neurotransmission and host defence. Many RSNOs have now been synthesized chemically, and at present they show the most promise as clinically useful agents, as will be discussed later.

Exogenous NO Donors

Exogenous NO donors generate NO *in vivo* and *in vitro* by two main types of pathway, enzymatic and non-enzymatic (Scheme **3**). Currently available NO-donor drugs derive from six classes of chemical compound (Table **1**).

1. S-Nitroso Compounds

Synthetic RSNOs (Scheme **4**) have great potential as therapeutic agents. They may be particularly useful alternatives to organic nitrates, as they do not lead to the development of tolerance. However, interactions with



Scheme 4. Examples of established NO-donor drugs.

copper, thiols, ascorbic acid and other reducing agents may influence their stability and ability to act as useful drugs. To date, RSNOs have only been used parenterally in both animal studies and small human clinical trials. Further study of their pharmacology is needed, and in particular the development of orally available preparations will enhance their therapeutic usefulness. Most of these compounds are unstable in their pure form, making their isolation difficult, and this has impeded the elucidation of their chemistry [34-38], although newer compounds have recently been synthesized which are more stable both in solid form and in solution [37].

2. O-Nitrite Compounds (Organic Nitrates)

Organic nitrates (Scheme **4**) have long been used as vasodilators for the treatment of angina pectoris. The release of NO from organic nitrates involves both non-enzymatic and enzymatic pathways [36]; the latter have not as yet been unambiguously identified.

3. N-Nitroso Compounds

N-Nitroso compounds (Scheme **4**) currently in existence comprise the following four types of compound.

(i) N-NO(NO) Donors

Compounds with the formula $R_2N[N(O^-)NO]$ (so-called amine NONOate) [39-41] have several interesting properties. In particular, they release NO spontaneously in a pH-dependent fashion (i.e. at neutral or acidic pH, but not alkaline pH), with no requirement for redox or light activation. Diazeniumdiolates are examples of such compounds: these are products of NO following its reaction with nucleophiles such as primary amines, secondary amines or polyamines. They were first prepared more than thirty years ago [42-46]. Several reviews on diazeniumdiolates have recently been published [47-49].

(ii) N-Hydroxy-N-Nitrosamines

Cupferron has been widely employed as a metal chelating agent and as a polymerization inhibitor [50,51]. It has also been demonstrated to generate NO upon enzymatic oxidation [52], as well as in electrochemical and chemical reactions [50,53]. Cupferron has also been found to decompose spontaneously at room temperature, releasing NO [54].

(iii) N-Nitrosoamides

These compounds are unstable and are known carcinogens. *N*-nitrosoureas constitute a large number of important antitumor drugs and have been well reviewed previously [55].

(iv) Secondary Amine-NO Complex

Nitrosamines, which are well-known carcinogens, exhibit functions very similar to those of NO such as vasodilatation and soluble guanylyl cyclase stimulation. It is believed that metabolic activation is required in order for them to exert their carcinogenic activity and to release NO [56-58]. Dephostatin is an inhibitor of protein tyrosine phosphatase [59-61], and it has been proposed that NO transfer is involved in its action [62,63].

4. C-Nitroso Compounds

C-Nitroso compounds (Scheme **4**) can undergo homolytic cleavage of the C-NO bond to generate NO upon exposure to light [64]. It is possible, therefore, that such compounds could be used in combination with laser therapy, to allow targeted delivery of NO. Whether such delivery will prove practical and possible remains to be seen.

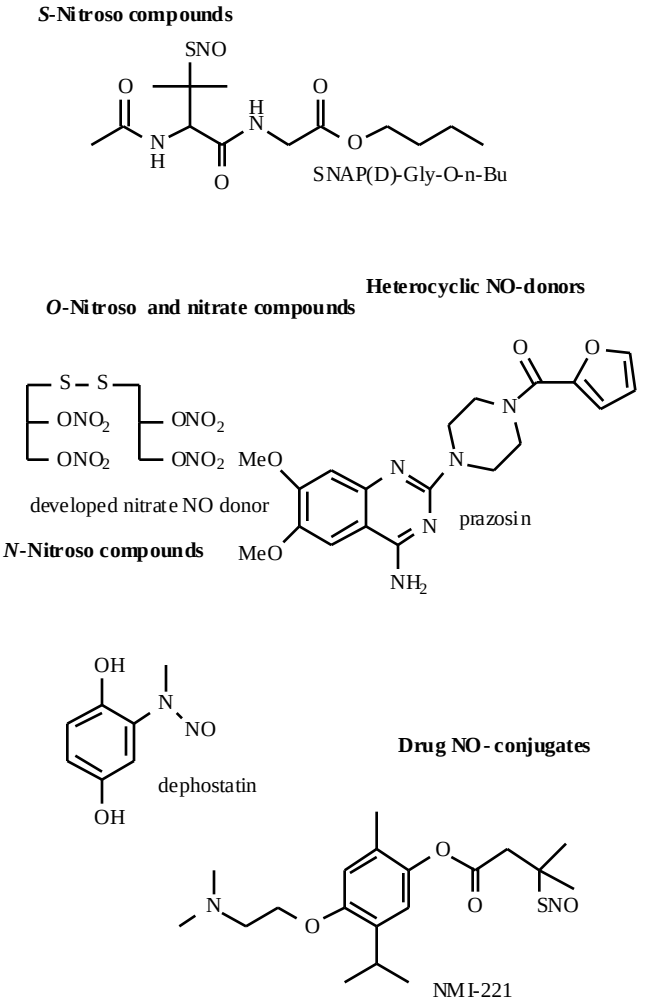
5. Heterocyclic NO Donors

Oxadiazoles (furoxans) and oxatriazoles (Scheme **4**) are two classes of important heterocyclic NO donor. Furoxans require the presence of thiols in order to release NO [65]. Lack of physiological tolerance makes this type of NO donor potentially attractive in the treatment of cardiovascular diseases.

6. Transition Metal-NO Complexes

Transition metal-NO complexes (Scheme **4**) act as NO^+, NO^- or $NO\cdot$ donors. Compounds such as Roussins's salts are nitrosyl complexes of iron-sulfur clusters that can release NO [66,67]. The mechanism by which NO is released is presently unclear.

Current interest in NO-donor drugs has focused on the design and synthesis of new compounds with enhanced pharmacokinetic and NO-releasing properties *in vivo*, such as those listed in Scheme **5**. A large number of such compounds have now been synthesized, which exhibit widely different chemical properties. Their potential clinical usefulness remains to be determined.



Scheme 5. Examples of some novel NO-donor drugs.

POTENTIAL USES OF NITRIC OXIDE-DONOR DRUGS

Nitrate Tolerance

The main limitation to the use of organic nitrates is the development of tolerance, the mechanism of which is not well understood. The exception is pentaerythrityl tetranitrate which, unlike other nitrates, gives rise to little or no nitrate tolerance [68]. Many theories have been proposed for the development of tolerance to nitrates, including depletion of reduced thiol groups, increased vascular superoxide production, reflex activation of the renin–angiotensin system and increased activity of guanosine cyclic -3',5'-monophosphate (cGMP) phosphodiesterase [69]. Studies assessing the use of angiotensin converting-enzyme inhibitors, diuretics, thiol donors and antioxidants to prevent this phenomenon have produced conflicting results. At present, the only reliable way to prevent the development of nitrate tolerance is to provide a nitrate-free interval in the dosing regime [70].

RSNOs may be useful alternatives to organic nitrates as NO donors. They cause both arterial and venous dilatation, unlike nitrates which cause predominantly venous dilatation, and do not appear to induce tolerance in animals when given at therapeutic doses. Furthermore, they are effective, *in vitro*, in systems rendered tolerant to organic nitrate [71]. At present, the main limitation to their widespread use in this regard is their lack of bioavailability following oral administration, so that they can only be administered parenterally.

Platelet Inhibition

Platelets play a significant role in both the development and the clinical presentation of vascular disease. Currently available agents that reduce platelet activation and adhesion, including aspirin, clopidogrel and glycoprotein IIb/IIIa inhibitors, result in improved outcomes in patients with acute coronary syndromes; however, absolute levels of morbidity and mortality remain high. RSNOs have been shown to inhibit platelet function [72]. cGMP is involved in the anti-platelet effects of RSNOs, but, for GSNO at least, other mediators also appear to be involved [73]. Unlike organic nitrates, which can also inhibit platelet activity but only at high doses, RSNOs can achieve effective platelet inhibition in humans at doses that have little effect on vascular tone [74].

Platelet activation, as measured by levels of expression of the adhesion molecule P-selectin and of the glycoprotein IIb/IIIa receptor, is increased in acute coronary syndromes. This phenomenon is seen even in the presence of aspirin. In a small clinical trial it was found that GSNO significantly lowered levels of platelet activity; glyceryl trinitrate also achieved this effect but, because it also induced hypotension, it was less well tolerated [75].

Revascularization procedures, using balloon angioplasty and stenting or coronary artery bypass grafting, result in platelet activation. This is believed to play a role in the restenotic process observed following percutaneous intervention and in graft failure after surgery. SNO-albumin delivered locally to an area of balloon-injured rabbit femoral artery reduced platelet deposition and the subsequent development of neointimal hyperplasia [76]. Local delivery of SNO-albumin using stent-based rather than catheter-based therapy was also found to reduce platelet adhesion following deployment into pig carotid arteries [77]. In a small clinical study, intracoronary infusion of GSNO prior to percutaneous transluminal coronary angioplasty prevented the increases in platelet expression of P-selectin and glycoprotein IIb/IIIa usually seen within minutes of the procedure, without altering blood pressure [78]. Coronary artery bypass grafting is also associated with platelet activation and consumption, which can lead to post-operative thrombosis and, if consumption is excessive, bleeding [79]. GSNO decreased the uptake of platelets by both arteries and veins *in vitro* and in patients undergoing coronary bypass grafting [79], even though levels of platelet P-selectin and glycoprotein IIb/IIIa expression do not appear to be affected in this clinical setting [80].

One of the main complications from carotid endarterectomy is cerebral infarction, often caused by platelet emboli. The procedure itself results in the removal of the endothelium, leaving a potent thrombogenic surface on which platelet adhesion and aggregation can occur. Asymptomatic microemboli can be detected by Doppler ultrasonography, and their frequency correlates with the risk of early stroke. In small studies in patients undergoing carotid endarterectomy, intravenous GSNO given either peri-operatively [81] or post-operatively [82] reduced the frequency of microemboli compared with placebo. Unfortunately, neither study was powered to detect a difference in clinical outcomes.

Modulation of Apoptosis

Caspases, a family of interleukin-1β converting enzymes, have been implicated as a common effector for a variety of pro-apoptotic signals. Recently, caspase 1 and 3 have been shown to be inhibited by NO, resulting in the inhibition of TNF-α-induced apoptosis [83]. Caspases contain a critical cysteine residue in their active site [84,85], and it has been suggested that NO might inhibit apoptosis by nitrosating this residue [84,86]. Indeed, *in vitro* incubation with NO donors leads to decreased caspase-3-like enzyme activity [83,87]. Recent animal studies have indicated that *in vivo* administration of NO donors prevents apoptosis *via S*-nitrosylation of the cys163 residue of caspase-3 [88]. Whether this action will translate into useful therapeutic effects remains to be seen.

Protection Against Ischemia/Reperfusion Injury

Reperfusion of ischemic tissue leads to local inflammation and endothelial cell dysfunction, as well as increased oxidative stress, all of which can give rise to increased tissue damage. RSNOs have been shown to improve end-organ recovery in animal models of ischemia/reperfusion injury in the heart [89,90] and liver [91]. Clinical trials are required to demonstrate their usefulness in this situation in man.

Treatment of Respiratory Diseases

Levels of RSNOs detected in bronchoalveolar lavage fluid are around 0.25μM [21]. RSNOs relax bronchial smooth muscle, inhibiting the bronchoconstrictor effects of

methacholine on human airway. As with their anti-platelet action, the bronchodilator effect of RSNOs is mediated only partially through cGMP [92].

In the airway, RSNO levels are altered in disease states. In patients with pneumonia, the mean concentration in bronchoalveolar lavage fluid has been found to be around 0.4μM, higher than that found in healthy controls [21]. In patients with asthma, NO levels in exhaled breath are increased, but airway RSNO levels are much reduced, as compared with healthy controls [93]. Since RSNOs act as bronchodilators, they may have a role to play in the treatment of asthma or chronic obstructive pulmonary disease, but as yet clinical studies are lacking.

Antimicrobial Therapy

NO and RSNOs both play an important role in host defense against infection. In a study of the effect of macrophage-derived NO on *Trypanosoma musculi*, its cytostatic action was not seen in the absence of albumin, or in the presence of antibodies to cys-NO, suggesting that the NO reacts with albumin to form SNO-albumin and that a transnitrosation reaction with cysteine occurs to form cys-NO, the active moiety [94]. RSNOs are cytotoxic to a mutant form of *Salmonella typhimurium* in which intracellular homocysteine levels are depleted; in this organism, the development of a metabolic pathway to up-regulate homocysteine production was found to confer protection against this effect [95].

Both NO and *S*-nitroso-*N*-acetylpenicillamine (SNAP) inhibit HIV-1 protease, an enzyme involved in replication of the HIV-1 virus. NO reacts with two cysteine residues in the enzyme molecule, forming cys-NO, probably *via* a transnitrosation reaction [96]. This effect of NO or SNAP on HIV-1 is cGMP-independent, and additive with that of zidovudine [97].

GSNO, SNAP and SNO-captopril inactivate a protease enzyme in the human rhinovirus, which causes the common cold; this effect again probably involves a transnitrosation reaction [98].

Thus, RSNOs may have a potential role in the treatment of conditions ranging from the common cold to AIDS. This avenue has yet to be explored in clinical studies.

Endoscopic Retrograde Cholangio-Pancreatography

RSNOs inhibit gastrointestinal smooth muscle function. Their formation in the gut is thought to occur directly as a result of nitrosation of SH groups of low-molecular-mass thiols. The topical application of *S*-nitroso-*N*-acetylcysteine to the ampulla and peri-ampullary duodenal mucosa of humans undergoing endoscopic retrograde cholangio-pancreatography caused a reduction in the frequency of sphincter contractions and in duodenal motility, without a fall in blood pressure, thus facilitating cannulation [99]. This may in the future prove a useful maneuver to inhibit motor activity in the duodenum and Sphincter of Oddi, thus facilitating ampullary cannulation.

Antitumor Therapy

The role of NO in tumor biology remains incompletely understood. NO is known to have both tumor-promoting and inhibiting effects, such that the net effect is likely to be dependent on its local concentration. Indeed, the participation of NO in the tumoricidal activity of macrophages was one the earliest roles reported for this molecule [100]. Inhibition of NO produced by macrophages lowered the antitumor response, and this seminal finding was one of the major contributions to the explosion of research focused on the function and control of NO in normal and disease states. Though NO may be an important factor in host defense against cancer, other reports suggest that NO promotes tumor growth [101,102]. It appears that the resultant action of NO depends on the stage and type of cancer, as well as on the quantity of NO produced. Thus, devising strategies to utilize NO delivery as an antitumor therapy, such as by the use of NO-donor drugs, will require further insights into factors such as timing, concentration and localization of the delivery system. It is difficult, in the current state of knowledge, to define precisely the possible role, if any, of NO-donor drugs as antitumor therapies.

Other

GSNO inhibits DNA synthesis and increases cGMP production by activated T lymphocytes [103], thus suggesting a possible role of NO donors in the prevention of T-cell-mediated inflammation. GSNO also has a cytotoxic effect on leukemia cells; irradiation of GSNO with visible light (340 or 545nm), which promotes breakdown of RSNOs to release NO, resulted in enhancement of this effect, and oxyhemoglobin, a NO scavenger, decreased it [104]. Thus, GSNO or indeed other RSNOs, alone or with adjunctive phototherapy, may have a potential future role in the treatment of leukemias [104].

THE FUTURE OF NO-DONOR DRUGS

Many different NO-donor drugs are now being developed, as has been discussed here, and many of these show promise as possible future therapeutic agents [105,106]. Their chemical properties will in large part affect their potential clinical usefulness. The ideal agents will possess the following chemical properties: (a) stability, with no release of NO during storage but the ability to release NO *in vivo*; (b) lipophilicity, in order to increase penetrance into cells; (c) high degree of purity following chemical synthesis; (d) lack of toxicity; (e) good oral bioavailability; and (f) long duration of action, to facilitate dosing. NO-donor drugs currently available in clinical practice do not, as yet, meet these goals; they also have other problems associated with them, including the development of physiological tolerance (in the case of the organic nitrates), toxicity of breakdown products (sodium nitroprusside) and lack of tissue specificity. More recently synthesized NO-donor compounds overcome many of these difficulties, as has been described in this review, but their clinical utility is as yet unestablished, partly because they have only been synthesized relatively recently, and partly because most of them are poorly absorbed from the gut, although methods for improved oral delivery of these drugs are currently being designed. A challenge for the organic chemist is to develop hybrid NO donors that can be targeted to areas of early atherosclerosis; such drugs might prove useful in the prevention of atherosclerosis progression and, ultimately, thrombosis,

myocardial infarction and stroke. Only time will tell if such strategies can be translated into reality.

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ABBREVIATIONS

Cys-NO	=	*S*-nitrosocysteine
DNIC	=	Dinitrosyl iron complex
EDRF	=	Endothelium-derived relaxing factor
cGMP	=	Guanosine cyclic -3',5'-monophosphate
GSH	=	Glutathione
GSNO	=	*S*-nitrosoglutathione
NOS	=	Nitric oxide synthase
nNOS, eNOS, and iNOS	=	Neuronal, endothelial, and inducible nitric oxide synthase
RSNO	=	*S*-nitrosothiol
SNAP	=	*S*-nitroso-*N*-acetylpenicillamine
SNO-albumin	=	*S*-nitrosoalbumin
SNO-captopril	=	*S*-nitrosocaptopril

REFERENCES

[1] Moncada, S.; Palmer, R.M.; Higgs, E.A. *Pharmacol. Rev.*, **1991**, *43*, 109.
[2] Mizutani, T.; Layon, A.J. *Chest*, **1996**, *110*, 506.
[3] Stamler, J.S.; Singel, D.J.; Loscalzo, J. *Science*, **1992**, *258*, 1898.
[4] Nathan, C. *FASEB J*., **1992**, *6*, 3051.
[5] Dawson, T.M.; Snyder, S.H. *J. Neurosci*., **1994**, *14*, 5147.
[6] Brüne, B.; Dimmeler, S.; Molinay Vedia, L.; Lapetina, E.G. *Life Sci*., **1994**, *54*, 61.
[7] Iyengar, R.; Stuehr, D.J.; Marletta, M.A. *Proc. Natl. Acad. Sci. U.S.A.*, **1987**, *84*, 6369.
[8] Hibbs, J.B. Jr.; Taintor, R.R.; Vavrin, Z.; Rachlin, E.M. *Biochem. Biophys. Res. Commun*., **1989**, *157*, 87.
[9] Clancy, R.M.; Abramson, S.B. *Proc. Soc. Exp. Biol. Med*., **1995**, *210*, 93.
[10] Lancaster, J. R. *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*, 8137.
[11] Furchgott, R.F.; Zawadzki, J.V. *Nature*, **1980**, *288*, 373.
[12] Palmer, R.M.J.; Ferrige, A.G.; Moncada, S. *Nature*, **1987**, *327*, 524.
[13] Ignarro, L.J.; Buga, G.M.; Wood, K.S.; Byrns, R.E. *Proc. Natl. Acad. Sci. U.S.A.*, **1987**, *84*, 9265.
[14] Myers, P.R.; Minor, R.L.; Guerra, R.; Bates, J.N.; Harrison, D.G. *Nature*, **1990**, *345*, 161.
[15] Myers, P.R.; Guerra, R.; Harrison, D.G. *J. Cardiovasc. Pharmacol*., **1992**, *20*, 392.
[16] Rubanyi, G. M.; Johns, A.; Wilcox, D.; Bates, J. N.; Harrison, D. G. *J. Cardiovasc. Pharmacol*., **1991**, *17* (Suppl. 3), S41.
[17] Vedernikov, Y.P.; Mordvintcev, P.I.; Malenkova, I.V.; Vanin, A. F. In *Nitric Oxide from L-Arginine: A Bioregulatory System;* Moncada, S., and Higgs, E. A., eds. Elsevier Science Publishers B. V., Amsterdam, **1990**; pp. 373-377.
[18] Vanin, A. F. *FEBS Lett.*, **1991**, *289*, 1.
[19] Vedernikov, Y.P.; Mordvintcev, P.I.; Malenkova, I.V.; Vanin, A.F. *Eur. J. Pharmacol*., **1992**, *211*, 313.
[20] Stamler, J.S.; Jaraki, O.; Osborne, J.; Simon, D.I.; Keaney, J.; Vina, J.; Singel, D.; Valery, C.R.; Loscalzo, J. *Proc. Natl. Acad. Sci. U.S.A.*, **1992**, *89*, 7674.
[21] Gaston, B.; Reilly, J.; Drazen, J.M.; Fackler, J.; Ramdev, P.; Arnelle, D., Mullins, M.E.; Sugarbaker, D.J.; Chee, C.; Singel, D.J.; Loscalzo, J.; Stamler, J.S. *Proc. Natl. Acad. Sci. U.S.A.*, **1993**, *90*, 10957.
[22] Stamler, J.S.; Osborne, J.A.; Jaraki, O.; Rabbani, L.E.; Mullins, M.; Singel, D., Loscalzo, J. *J. Clin. Invest*., **1993**, *91*, 308.
[23] Clancy, R.M.; Levartovsky, D.; Leszczynska-Piziak J.; Yegudin, J.; Abramson, S.B. *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*, 3680.
[24] Lancaster, J.R.; Hibbs, J.B. *Proc. Natl. Acad. Sci. U.S.A.*, **1990**, *87*, 1223.
[25] Drapier, J.-C.; Pellat, C.; Henry, Y. *J. Biol. Chem.*, **1991**, *266*, 10162.
[26] Stadler, J.; Bergonia, H.A.; DiSilvio, M.; Sweetland, M.A.; Billiar, T.R.; Simmons, R.L.; Lancaster, J.R. *Arch. Biochem. Biophys*., **1993**, *302*, 4.
[27] Mülsch, A.; Mordvintcev, P.I.; Vanin, A.F.; Busse, R. *Biochem. Biophys. Res. Commun*., **1993**, *196*, 1303.
[28] Geng, Y.-L.; Petersson, A.-S.; Wennmalm, A.; Hannson, G. *Exp. Cell Res*., **1994**, *214*, 418.
[29] Williams, D. L. H. *Acc. Chem. Res*., **1999**, *32*, 869.
[30] Marley, R.; Feelisch, M.; Holt, S.; Moore, K. A. *Free Radical Res*., **1999**, *32*, 1.
[31] Hogg, N.; Singh, R.J.; Koronev, E.; Joseph, J.; Kalyanaraman, B. *Biochem. J*., **1997**, *323*, 477.
[32] Freedman, J.E.; Frei, B.; Welch, G.N.; Loscalzo, J. *J. Clin. Invest*., **1995**, *96*, 394.
[33] Trujillo, M.; Alvarez, M.N.; Peluffo, G.; Freedman, B. *J. Biol. Chem*., **1998**, *273*, 7828.
[34] Nguyen, T.; Brunson, D.; Crespi, C.L.; Penman, B.W.; Wishnok, J.S.; Tannenbaum, S.R. *Proc. Natl. Acad. Sci. U.S.A.*, **1992**, *89*, 3030.
[35] Butler, A.R.; Al-Sa'doni, H.H.; Megson, I.L.; Flitney, F.W. *Nitric Oxide Biol. Chem.*, **1998**, *2*, 193.
[36] Al-Sa'doni, H.H.; Ferro, A. *Clin. Sci.*, **2000**, *98*, 507.
[37] Al-Sa'doni, H.H.; Khan, I.Y.; Poston, L.; Fisher, I.; Ferro, A. *Nitric Oxide: Biol. Chem.*, **2000**, *4*, 550.
[38] 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**, *4*, 481.
[39] Maragos, C.M.; Morley, D.; Wink, D.A.; Dunams, T. M.; Saavedra, J. E.; Hoffman, A.; Bov, A. A.; Isaac, L.; Hrabie, J. A.; Keefer, L.K. *J. Med. Chem*., **1991**, *34*, 3242.
[40] Maragos, C.M.; Wang, J.M.; Hrabie, J.A.; Oppenheim, J.J.; Keefer, L.K. *Cancer Res*., **1993**, *53*, 564.
[41] Hrabie, J.A.; Klose, J.R.; Wink, D.A.; Keefer, L.K. *J. Org. Chem.*, **1993**, *58*, 1472.
[42] Drago, R.S.; Paulik, F.E. *J. Am. Chem. Soc.*, **1960**, *82*, 96.
[43] Drago, R.S.; Karstetter, B.R. *J. Am. Chem. Soc.*, **1961**, *83*, 1819.
[44] Drago, R.S.; Ragsdale, R.0.; Eyman, D.P.B.R. *J. Am. Chem. Soc.*, **1961**, *83*, 4337.
[45] Longchi, R.; Ragasdale, R.0.; Drago, R.S. *Inorg. Chem.*, **1962**, *1*, 768.
[46] Ragasdale, R.0.; Karstetter, B.R.; Drago, R.S. *Inorg. Chem.*, **1965**, *4*, 420.
[47] Keefer, L.K.; Christodoulou, D.; Dunams, T.M.; Hrabie, J.A.; Maragos, C.M.; Saavedra, J.E.: Wink, D.A. In *Chemistry of "NONOates", Unusual N-Nitroso Compounds Formed by Reacting Nitric Oxide with Nucleophiles, Nitrosamines and Related N-Nitroso Compounds:* Chemistry and Biochemistry; Loeppky, R. N. and Michedja, C. J. Eds.; ACS Symp. Series No. 553, *Am. Chem. Soc.*, Washington, D.C., **1994**; pp 136-146.
[48] Hanson, S.R.; Hutseli, T.C.; Keefer, L.R.; Mooradian, D.L.; Smith, D. J. In *Nitric Oxide Donors, A Continuing Opportunity in Drug Design, Nitric Oxide, Biochemistry, Molecular Biology, and Therapeutic Implications*; Ignarro, L.; Murad, F. Eds.; Academic Press, San Diego; **1995**; pp 383-399.
[49] Keefer, L.K. *Chemtech.*, **1998**, *28*, 30.
[50] Lawless, J.G.; Hawley, M.D. *Anal. Chem.*, **1968**, *40* (6), 948.
[51] Cupferron, Wako Pure Chemical. Industries, Ltd. **1995**.
[52] Alston, T.A.; Porter, D.J.T.; Bright, H.J. *J. Biol. Chem.*, **1985**, *260* (7), 4069.

[53] Balaban, A.T.; Negoita, N.; Pascaru, I. *Rev. Roum. Chim.*, **1971**, *16*, 721.
[54] Garfield, R.E.; Balaban, A.T.; Seitz, W.A.; Klein, D.J.; Lesko, M. PCT WO 96/36326.
[55] Gnewuch, C.T.; Sosnovsky, G. *Chem. Rev.*, **1997**, *97*, 829.
[56] Lijinsky, W. Ed.; *Chemistry and Biology of N-nitroso Compounds.* Cambridge University Press: Cambridge, **1992**; p 9.
[57] Lippton, H.L.; Gruetter, C.A.; Ignarro, L.J; Meyer, R. L.; Kadowitz, P. J. *Can. J. Physiol. Pharmacol.*, **1982**, *60*, 68.
[58] DeRubertis, F.R.; Graven, P.A. *Scienc*e, **1976**, *193*, 897.
[59] Imoto, M.; Kakeya, H.; Sawa, T.; Hayashi, Y.; Hamada, M.; Takeuchi, T.; Umezawa, K. *J. Antibiotics*, **1993**, *46*, 1342.
[60] Kakeya, H.; Imoto, M.; Takahashi, Y.; Naganawa, H.; Takeuchi, T.; Umezawa, K. *J. Antibiotics*, **1993**, *46*, 1716.
[61] Yu, L.; McGill, A.; Ramirez, J.; Wang, P. G.; Zhang, Z.-Y. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 1003.
[62] *Guo, Z.;* McGill, A.; Yu, L.; Li, J.; Ramirez, J.; Wang, P.G. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 573.
[63] Kubrina, L.N.; Yakubovich, L.M.; Vanin, A.F. *Izv. Akad. Nauk. USSR. Ser. Biol.*, **1988**, 844.
[64] Fontecave, M.; Pierre, J.L. *Bull. Soc. Chim. Fr*., **1994**, *131*, 620.
[65] Feelisch, M.; SchGnafinger, K.; Noack, E. *Biochem. Pharmacol.*, **1992**,*44*, 1149.
[66] Kowaluk, E.A.; Fung, H.-L.J. *Pharmacol. Exp. Ther*., **1990**, *255*, 1256.
[67] Kowaluk, E. A.; Fung, H.-L. *Eur. J. Pharmacol.*, **1990**, *176*, 91.
[68] Fink, B.; Bassenge, E.J. *Cardioavasc. Pharmacol*., **1997**, 30, 832.
[69] Glasser, S. P. *Lancet*, **1999**, *353*, 1545.
[70] Thadani, U. *Cardiovasc. Drugs. Ther*., **1997**, *10*, 735.
[71] Miller, M.R.; Roseberry, M.J.; Mazzei, F.A.; Butler, A.R.; Webb, D.J.; Megson, I.L. *Eur. J. Pharmacol.*, **2000**, *408*, 335.
[72] Radomski, M.W.; Rees, D.D.; Dutra, A.; Moncada, S. *Br. J. Pharmacol.*, **1992**, *107*, 745.
[73] Gordge, M.P.; Hothersall, J.S.; Noronha-Dutra, A.A. *Br. J. Pharmacol.*, **1998**, *124*, 141.
[74] de Belder, A.J.; MacAllister, R.; Radomski, M.W.; Moncada, S.; Vallance, P.J.T. *Res.*, **1994**, *28*, 691.
[75] Langford, E.J.; Wainwright, R.J.; Martin, J.F. *Arterioscler. Thromb. Vasc. Biol.*, **1996**, *16*, 51.
[76] Marks, D.S.; Vita, J.A.; Folts, J.D.; Keaney, Jr, J.F.; Welch, G.N.; Loscalzo, J. *J. Clin. Invest.*, **1995**, *96*, 2630.
[77] Maalej, N.; Albrecht, R.; Loscalzo, J.; Folts, J.D. *J. Am. Coll. Cardiol.*, **1999**, *33*, 1408.
[78] Langford, E.J.; Brown, A.S.; Wainwright, R.J.; de Belder, A.J.; Thomas, M.R.; Smith, R.E.; Radomski, M.W.; Martin, J.F.; Moncada, S. *Lancet*, **1994**, *344*, 1458.
[79] Salas, E.; Langford, E.J.; Marrinan, M.T.; Martin, J.F.; Moncada, S.; de Belder, A.J. *Heart*, **1998**, *80*, 146.
[80] Langford, E.J.; Parfitt, A.; de Belder, A.J.; Marrinan, M.T.; Martin, J.F. *Thromb. Haemostasis*, **1997**, *78*, 1516.
[81] Molloy, J.; Martin, J.F.; Baskerville, P.A.; Fraser, S.C.A.; Markus, H.S. *Circulation*, **1998**, *98*, 1372.
[82] Kaposzta, Z.; Baskerville, P.A.; Madge, D.; Fraser, S.; Martin, J.F.; Markus, H.S. *Circulation*, **2001**, *103*, 2371.
[83] Dimmler, S.; Haendeler, J.; Nehls, M.; Zeiher, A.M. *J. Exp. Med*., **1997**, *185*, 601.
[84] Haendeler, J.; Weiland, U.; Zeiher, A. M.; Dimmeler, S. *Nitric Oxide: Biol. Chem.*, **1998**, *1*, 282.
[85] Tenneti, L.; D'Emillia, D.M.; Lipton, S.A. *Neurosic. Lett.*, **1997**, *236*, 139.
[86] Kim, Y. M.; de Vera, M. E.; Watkins, S. C.; Billiar, T. R. *J. Biol. Chem.*, **1997**, *272*, 1402.
[87] Li, J.; Billiar, T.R.; Talanian, R.V.; Kim, Y.M. *Biochem. Biophys. Res. Commun.*, **1997**, *240*, 419.
[88] Rossig, L.; Fichtlscherer, B.; Breitschopf. K.; Haendeler, J.; Zeiher, A.M.; Muisch, A.; Dimmeler, S. *J. Biol. Chem*., **1999**, *274*, 6823.
[89] Delyani, J.A.; Nossuli, T.O.; Scalia, R., Thomas; G., Garvey, D.S.; Lefer, A.M. *J. Pharmacol. Exp. Ther*., **1996**, *279*, 1174.
[90] Konorev, E.A.; Joseph, J.; Tarpey, M.M.; Kalyanaraman, B. *Br. J. Pharmacol.*, **1996**, *119*, 511.
[91] Ikebe, N.; Akaike, T.; Miyamoto, Y.; Hayashida, K.; Yoshitake, J.; Ogawa, M.; Maeda, H. *J. Pharmacol. Exp. Ther*., **2000**, *295*, 904.
[92] Gaston, B.; Drazen, J.M.; Jansen, A.; Sugarbaker, D.A.; Loscalzo, J.; Richards, W.; Stamler, J.S. *J. Pharmacol. Exp. Ther*., **1994**, *268*, 978.
[93] Gaston, B.; Sears, S.; Woods, J.; Hunt, J.; Ponaman, M.; McMahon, T.; Stamler, J.S. *Lancet*, **1998**, *351*, 1317.
[94] Mnaimneh, S.; Geffard, M.; Veyret, B.; Vincendeau, P. *J. Immunol*., **1997**, *158*, 308.
[95] De Groote, M.; Testerman, T.; Xu, Y.; Stauffer, G.; Fang, F. *Science*, **1996**, *272*, 414.
[96] Sehajpal, P.K.; Basu, A.; Ogiste, J.S.; Lander, H.M. *Biochemistry*, **1999**, *38*, 13407.
[97] Mannick, J.B.; Stamler, J.S.; Teng, E.; Simpson, N.; Lawrence, J.; Jordan, J.; Finberg, R.W. *J. Acquired Immune Defic. Syndr.*, **1999**, *22*, 1.
[98] Xian, M.; Wang, Q.M.; Chen, X.; Wang, K.; Wang, P.G. *Bioorg. Med. Chem. Lett*., **2000**, *10*, 2097.
[99] Slivka, A.; Chuttani, R.; Carr-Locke, D.L.; Kobzik, L.; Bredt, D.S.; Loscalzo, J.; Stamler, J.S. *J. Clin. Invest.*, **1994**, *94*, 1792.
[100] Hibbs, J.B.; Vavrin, Z., Taintor, R.R. *J. Immunol.*, **1987**, *138*, 550.
[101] Jenkins, D.C.; Charles, I.G.; Thomsen, L.L.; Moss, D.W.; Holmes, L.S.; Baylis, S.A.; Rhodes, P.; Westmore, K.; Emson, P.C.; Moncada, S. *Proc. Natl. Acad. Sci. U.S.A.*, **1995**, *92*, 4392.
[102] Thomsen, L.L.; Miles, D.W.; Happerfield, L.; Bobrow, L.G.; Knowles, R.G.; Moncada, S. *Br. J. Cancer*, **1995**, *72*, 41.
[103] Merryman, P.F.; Clancy, R.M.; He, X.Y.; Abramson, S.B. *Arthritis Rheum.*, **1993**, *36*, 1414.
[104] Sexton, D.J.; Muruganandam, A.; McKenney, D.J.; Mutus, B. *Photochem. Photobiol*., **1994**, *59*, 463.
[105] Megson, I. L; Webb, D. J. *Expert Opin. Invest. Drugs*, **2002**, *11*, 587.
[106] Burgaud, J. L.; Riffaud, J. P.; Del Soldato, P. *Curr. Pharm. Des.*, **2002**, *8*, 201.