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Review Article

Nitric Oxide: Synthesis, Pathophysiology and Application on Oncology and Cardiovascular Diseases

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ABSTRACT

Nitric oxide (NO) is a ubiquitous, water-soluble, free radical gas that plays a crucial role in various physiological and pathological processes. Over the past decades, NO has emerged as a molecule of interest in carcinogenesis and tumor growth progression. On the other hand, it also appears as a potential anti-oncogenic agent. Strategies for manipulating *in vivo* production and exogenous delivery of this molecule for therapeutic gain are being investigated. However, further validation and experimental/clinical trials are required to develop novel strategies based on NO for cancer treatment and prevention. On the other hand, NO mediates multiple physiological and pathophysiological processes in the cardiovascular system. Moreover, pharmacological compounds that release NO have been useful tools for evaluating the pivotal role of NO in cardiovascular physiology and therapeutics. This review discusses the actions of NO in cancer, and the different mechanisms by which NO acts in different cancers such as breast, cervical, gastric, colorectal, and head and neck cancers are addressed. It also offers an insight into the role of NO in the management of cardiovascular diseases.

1. Introduction

Once merely considered a toxic pollutant, as it was one of the chemicals responsible for environmental pollution in the atmosphere especially regarding the automotive traffic in cities. In recent decades, NO has attracted tremendous interest in a broad field of basic and applied research as one of the most important physiological signaling molecule in the body since its identification as an endothelium-derived relaxing factor in 1987. Also, in 1992, NO was announced as the molecule of the year. Murad, Fruchgott and Ignarro, the discoverers of NO as a signal molecule in the biological system were awarded Nobel Prize in medicine in 1998. It is a heterodiatomic, free radical that exists as a

colorless gas at r. t. [1]. Nitric oxide has a boiling point of -151.7 ˚C and a maximum solubility in water at standard temperature and pressure [2, 3]. NO can undergo multiple oxidation and reduction reactions at physiological conditions (**Figure 1.**) [1, 2]. One of the most biochemical reactions in the biological System is NO's reaction with several ROS, such as superoxide anion ($\cdot O^{2-}$). This reaction results in the formation of peroxynitrite ($\cdot ONOO^-$), a highly oxidizing and nitrating reactive nitrogen species. It is responsible for mediating protein oxidation reactions under physiological conditions. Also, it has been implicated in cellular signaling roles at low levels and is cytotoxic at elevated concentrations. In addition, NO can form S-nitrosothiols (RSNO) by adding a NO group to the thiol side chain of cysteine residues within proteins and peptides in a process termed as S-nitrosylation. [1,4].

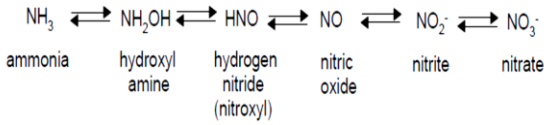


Figure 1: Redox reactions for nitrogen oxide [1]

1. 1. Biosynthesis of nitric oxide

In vivo, NO is generated during the NADPH-dependent, 5-electron oxidation of L-arginine to L-citrulline by nitric oxide synthase activity (**Figure 2.**) [5,6]. Nitric oxide synthase (NOS) has been identified as dimeric heme-containing monooxygenase in mammals. There are three main isoforms of NOS: neuronal nitric oxide synthase (nNOS or NOS1), inducible nitric oxide synthase (iNOS or NOS2), and endothelial nitric oxide synthase (eNOS or NOS3). The constitutive nNOS enzyme has been identified in multiple tissues including the lung, skeletal muscle, and genitourinary tract. Also, it is mainly expressed in the neurons of the central and peripheral nervous system [4]. It is upregulated in times of stress and after physical and/or mechanical injuries to the nervous system, resulting in a short burst of NO production. iNOS is mainly expressed in response to immune activation of many cell types, including hepatocytes, macrophages, epithelial cells, fibroblasts, endothelial cells, and neutrophils. Once induced *via* cytokine or endotoxin stimulation, iNOS produces sustained high NO levels. eNOS is a membrane-bound protein and it is commonly located in venous and arterial endothelial cells [1]. It delivers a short burst of NO production upon stimulation. It is expressed in many cell types, including neuronal cells and platelets [7].

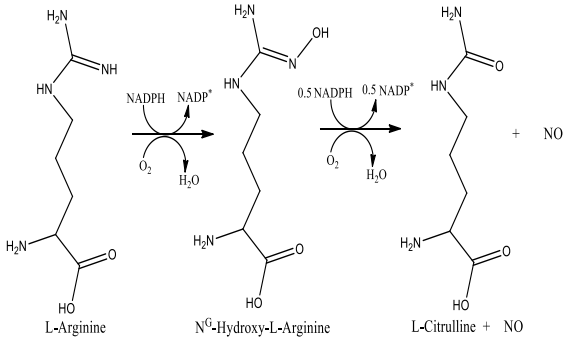


Figure 2: Biosynthesis of nitric oxide [5]

1. 2. Physiological and pathophysiological effects of nitric oxide

NO functions both directly and indirectly as an intercellular signaling molecule, playing a vital role in regulating numerous physiological activities, including smooth muscle relaxation, neurotransmission and the immune response. The major physiological target of NO is soluble guanylate cyclase (sGC). Soluble guanylate cyclase catalyzes the conversion of guanosine triphosphate (GTP) to the second messenger, 3`,5`-cyclic guanosine monophosphate (cGMP) resulting in the activation of cGMP target proteins. These target proteins include a variety of cGMP-dependent protein kinases, ion channels, cellular receptors, phosphatases and phospholipase C (PLC), resulting in multiple physiological activities. NOS inhibitors, including L-NAME (*N*-nitro-L-arginine methyl ester), L-NMMA (*N*-monomethyl–L–arginine) and through NO-scavenging by molecules like superoxide radical ($\cdot O^{2-}$), methylene blue and hemoglobin [8]. The vascular tone essential for the regulation of blood flow and pressure within the cardiovascular system is dependent on the generation of NO by vascular endothelial cells. The eNOS isoform can be stimulated by multiple factors, including bradykinin, histamine, serotonin and acetylcholine. Following this activation, NO

diffuses to adjacent vascular smooth muscle and other target cells where it binds and activates sGC resulting in an accumulation of the second messenger, cGMP. Among other effects, cGMP accumulation initiates Ca^{2+} uptake by the sarcoplasmic reticulum, resulting in vasorelaxation [9]. By the same mechanism, NO also plays an essential role in regulating platelet function by inhibiting platelet aggregation. Platelet NO has been implicated in the pathogenesis of several vascular disorders associated with platelet activation, such as atherosclerosis, coronary artery diseases, essential hypertension, diabetes mellitus and pre-eclampsia [10]. Moreover, CNS has a vibrant capacity to synthesize NO in response to nerve stimulation, which can diffuse over a much larger area to affect a broader range of targets [11]. In the CNS, NO has known activity in memory formation, regulating cerebral blood flow, feeding, drinking [12], learning [13], motor coordination [14], pain [15] and in the peripheral nervous system, functions in gastrointestinal and genitourinary motility/relaxation. There is evidence for NO's role in regulating renal blood flow and glomerular filtration, reduction of renovascular resistance, stimulation of renin excretion, and inhibition of tubular sodium reabsorption. It also plays a role in the pathogenesis of several renal diseases, such as diabetic nephropathy, acute and chronic renal failure [16]. In addition, there is evidence supporting NO as a critical constituent in the complex wound healing cascade as a cytostatic and cytotoxic agent. [17]. Research on eNOS knockout mice shows delayed wound healing which demonstrating that eNOS is needed for efficient neoangiogenesis during wound healing [18].

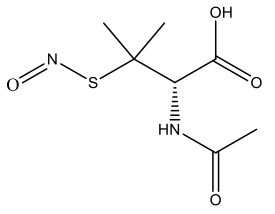
More recent research illustrates that treatment of non-healing diabetic foot wounds with NO donors results in increased NO production and decreased matrix metalloproteinase-8 (MMP-8) and 9 (MMP-9) expression that leads to reducing excessive degradation of the extracellular matrix critical for wound healing [19]. Nitric oxide can increase gastric blood flow, inhibit neutrophil adherence, and protect the gastric mucosa against damage induced by irritants [20,21]. The hypothesis that NO-releasing NSAIDs would have reduced toxicity in the gastrointestinal tract was initially tested using a new synthetic entity of Flurbiprofen and Ketoprofen in 1994 [22]. Also, NO inhalation has been shown to improve cardiopulmonary function, relaxing airway smooth muscle and thus acting as a bronchodilator in patients with pulmonary artery hypertension and acute respiratory distress [23]. In patients with hypercholesterolemia, NO donors inhibit smooth muscle cell proliferation, neutrophil/platelet aggregation, adhesion to endothelial cells and block the oxidation of low-density lipoproteins (LDL), preventing the formation of foam cells in the vascular wall [24]. Nitric oxide has a role in both acute and chronic inflammation. NOS3 is involved in the vasodilatation associated with acute inflammation. In addition, recent studies have shown that NO stimulates the synthesis of inflammatory prostaglandins by activating COX-2 [24]. Thus, inhibition of the NO pathway may benefit inflammatory diseases, including joint disorders [25]. Besides many biological activities of NO, some studies showed some side effects. The toxicity of NO may be related to its further oxidation to nitrogen dioxide (NO_2) in the presence of high oxygen concentrations. Even low concentrations of NO_2 (2 ppm) are highly toxic in animal models. Therefore, it is vital to keep NO_2 formation low during NO therapy. Also, laboratory studies have suggested potential additional toxic effects of chronic low doses of inhaled NO—the peroxynitrite formation by interaction with superoxide at low levels with cytotoxicity at elevated concentrations. The resulting chemical intermediates can stress the cell leading to irreversible damage to DNA, proteins and lipids [26]. Methemoglobinemia development is a significant complication of inhaled NO at higher concentrations. So, methemoglobin concentrations should be monitored intermittently during NO inhalation. In addition, inhaled NO can inhibit platelet function, increase bleeding time, and promote pulmonary edema formation in patients with impaired left ventricle function. So careful cardiac output and left atrial pressure monitoring is important in this situation [27].

1. 3. Nitric oxide donors

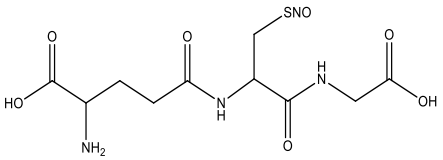
With such variable biological functions, it is no surprise that various forms of NO synthesis or activity dysfunction can result in pathological conditions. While, these conditions can result from excessive and insufficient NO concentrations, the second case is more readily corrected. Due to the gaseous nature of NO, short duration of action and the associated difficulty of handling, multiple classes of NO-donating drugs have been developed that can be tailored to a specific application for localized or systemic NO delivery to compensate for insufficient endogenous NO synthesis, such as: S-nitrosothiols, diazeniumdiolates, sydnonimine, sodium nitroprusside, organic nitrates, oximes and furoxans [28].

• S-Nitrosothiols (RSNO)

They are nitrosated derivatives of sulphydryl-containing compounds. Some of which have been identified as endogenous vasodilators. Also, RSNO does not require biotransformation to activate guanylate cyclase, suggesting that RSNO may not induce self-tolerance. One drawback to these drugs is the difficulty in predicting the *in vivo* stability of the drugs due to unpredictable decomposition in heat, light, metal ions, and thiols. Most existing RSNOs, including S-nitroso-*N*-acetyl penicillamine **I** and S-nitrosoglutathione **II** [28].



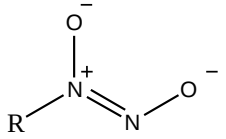
I



II

• Diazeniumdiolates (NONOates)

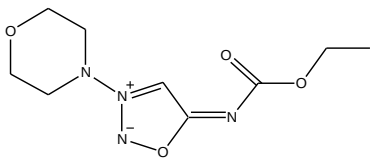
Molecules that bear the $[N(O)NO]^-$ functional group and can be chemically classified by the atom they are attached to, i. e. they can be C-, N-, O- or S-bound. They decompose spontaneously at physiological conditions to generate up to the theoretical two moles of NO per donor molecule. Distinguish themselves from other classes of NO donors by their spontaneous generation of NO in the biological system, their wide range of NO release rates, their structural diversity and thiols- or biological tissue-independent mode of bioactivation, and thus apparent lack of tolerance development which makes them attractive compounds in numerous clinical applications. NONOates are prepared by exposing a solution of the specified nucleophile to NO gas without air [28].



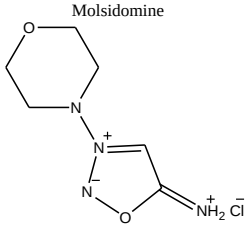
General structure of diazeniumdiolates

• Sydnonimine

Molsidomine is an example of a sydnonimine. This compound functions as a prodrug for treating coronary heart diseases that are enzymatically cleaved in the liver to produce the active drug (SIN-1, linsidomine) that further decomposes to the actual NO-releasing metabolite. Also, it provides an advantage in that even after prolonged exposure to SIN-1, no tolerance development was observed, and it displays rapid absorption following oral administration [28].



Molsidomine

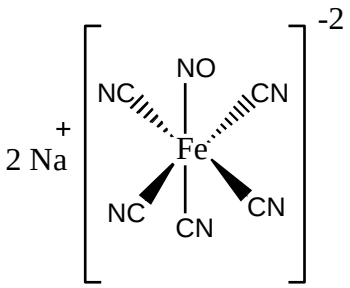


Linsidomine (SIN-1)

• Sodium Nitroprusside (SNP)

SNP is non-enzymatically metabolized in the body, producing one NO and five CN- radicals per donor molecule. From this point, NO displays its characteristic interaction with sGC to illicit its vasodilatory and other effects. Typically, SNP is employed for emergency

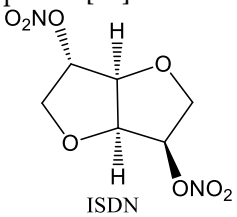
treatment of severe hypertension following the failure of different drug treatments and to induce hypotension during surgery to reduce blood loss [28].



SNP

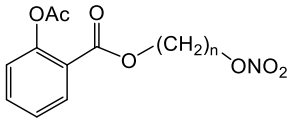
• Organic Nitrates

Organic nitrates are usually used as NO donor drugs and belong to the metabolism-dependent NO donor class. They have been used for treating angina pectoris, acute myocardial infarction and acute and chronic congestive heart failure for over a century. Vasodilating activity and preload reduction caused by these nitrates are mainly attributed to NO's release. Among them are isosorbide mononitrate (ISMN), isosorbide dinitrate (ISDN), isosorbide 5-mononitrate (5ISMN), nicorandil, and most notably, GTN [29,30]. GTN is the best-studied candidate and is mainly used in the case of acute angina pectoris [31]. NO is released from this class of NO-releasing drugs by converting nitrate into nitrite ion *via* the mercapto groups present in the plasma, which serves as a thiol source. The significant limitations of this class include the requirement of highly specific enzymatic drug metabolism, which can limit bioavailability and tolerance development [32].

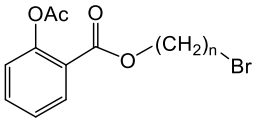


ISDN

There are several studies dealing with the preparation of organic nitrates. For example, simple nitroxyalkyl esters **III** were synthesized by **Ferris *et al.*** [33] *via* stirring acetylsalicoylbromide **IV** with the appropriate bromoalcohol in the presence of triethylamine followed by Br-displacement using $AgNO_3$ in acetonitrile.

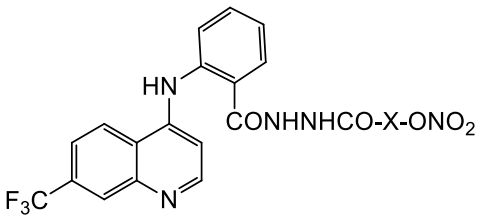


III



IV

Also, a novel 4-substituted-7-trifluor-omethylquinolinederivatives **V** having NO-releasing properties were synthesized by the reaction of 4-chloro-7-trifluoromethylquinoline with anthranilic acid followed by reaction with the reagents; thionyl chloride, hydrazine hydrate, chloroacid chlorides, and finally silver nitrate [34].



X = $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH(CH_3)-$

V

• Oximes

Oximes are compounds bearing C=NOH, like *N*-hydroxyguanidines, amidoximes, ketoximes, and hydroxamic acids. NO is released *via* a two-step bioactivation process [35]. The oxime structure is first hydrolyzed enzymatically to hydroxyimine. Then, further oxidized in the second step to the corresponding active ketone drug by microsomal cytochrome P450 (CYP) enzymes and the presence of mercapto groups present in the plasma [36]. Because the CYP-catalyzed oxidation reaction of hydroxyimine, which also leads to NO biosynthesis in biological systems, occurs *in vivo*, mainly in the liver, oximes can be used to improve site-specificity, especially liver-targeting, of a parent drug [37, 38].

The target NO-releasing oxime compounds were formed by refluxing a mixture of the appropriate ketones and hydroxylamine hydrochloride in ethanol [39].

• Furoxans

Furoxans (1,2,5-oxadiazole-2-oxides) represent important heterocyclic NO-donating prodrugs [40]. Furoxans show a variety of NO-related bioactivities like cytotoxicity, mutagenicity, immunosuppression [41], central muscle relaxant properties, anticonvulsive effects, monoamine oxidase inhibition [42], direct vasodilation and blood pressure lowering activities [43]. These compounds can produce NO under the action of thiol cofactors. The nucleophilic attack by a thiolate anion at C-3 and/or C-4 of the furoxan ring is followed by ring-opening, which results in the formation of a nitrosothiol (RSNO) and subsequent release of NO after the decomposition of RSNO. Decomposition of RSNO to liberate NO can be affected thermally, photochemically or by using metals such as mercury in an aqueous system [44]. In addition, furoxans possess relatively favorable pharmacological properties since they frequently release NO slowly, resulting in a longer duration of action [94].A reported procedure by **Gasco *et al.*** was used for the synthesis of 3-phenyl-4-furoxanmethanol by treatment of cinnamyl alcohol in glacial acetic acid with sodium nitrite (**Figure 3.)** [46].

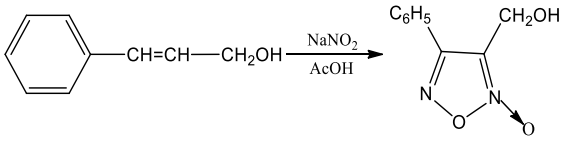


Figure 3: Synthesis of 3-phenyl-4-furoxa-nmethanol [46].

1.4. Role of nitric oxide in cancer

Cancer is a complex disease consisting of several hundred disease entities with both common and unique features. The incidence of cancer is only second to heart disease. Treatment of cancer remains one of the biggest challenges as one of the major problems in cancer treatment is the development of resistance and refractoriness to conventional therapeutics. Significant progress has been made to overcome the resistance of tumor cell therapeutics. The approaches are genetic manipulations, vaccine development and exploitation of the host immune antitumor response [47]. In most cases, the overall development of tumor cell resistance to therapeutics results from specific mechanisms to overcome cell death or apoptosis. Therefore, the possibility of interfering with the apoptotic signaling pathways may cause either direct induction of cell death and/or cell sensitization to cytotoxic stimuli [48]. Nitric oxide is a ubiquitous molecule with diverse cellular effects that depend on the source, concentration, latency, cell type and phenotype, as illustrated in **Figure 4.** [47].

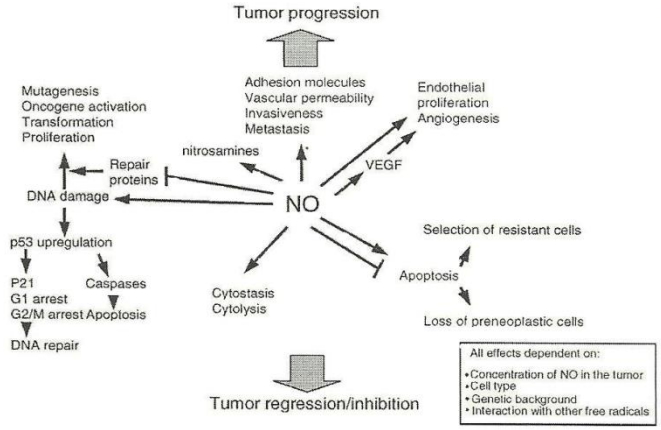


Figure 4: Dualistic effects of nitric oxide in carcinogenesis [47].

The apparent dichotomous nature of NO in tumor biology could be attributable to different levels of NO production, which is controlled, at the tissue level, by NO-synthase isoforms, where low physiological concentrations (nanomolar) of NO have been shown to increase tumorigenesis by favoring the pro-growth pathways that protect against apoptosis. Higher concentrations, as produced under pathophysiological conditions by NOS2, induce tumoricidal effects as it causes DNA damage and direct cell death [48].

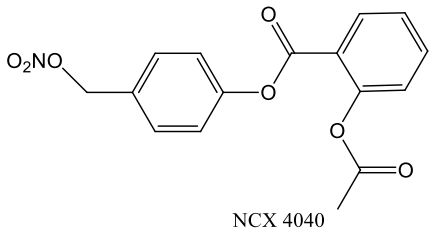
Mechanisms of NO-mediated apoptosis include inhibition of mitochondrial respiration, activation of caspase signaling, and accumulation of the tumor suppressor protein (p53). It is a critical protein involved in maintaining genomic stability and homeostasis. In response to increased regulation of p53, NOS2 activity increases in tumor cells and elevates NO concentration, thereby leading to p53-mediated growth arrest and apoptosis. Furthermore, the wild-type p53-induced transcription of NOS2, demonstrated both *in vitro* and *in vivo*, serves as a safeguard against DNA damage, thereby decreasing the likelihood of NO-induced DNA damage [49].

A study confirmed that NOS2 over-expressing murine melanoma cells displayed poor tumor growth and survival because of NO-induced apoptosis *in vitro* and consequently lost their ability for metastasis *in vivo* [50]. In addition, NOS2 knockout mice have been found to promote intestinal tumorigenesis in colon cancer mouse models [51]. New cancer therapeutics that induce NO or NO donors are the recently emerged novel agents. The application of NO donors as anticancer therapeutics is now greatly appreciated in the past as NO was primarily used to treat blood vessel-related diseases and other non-cancer-related applications. The demonstration of NO-mediated cytotoxicity directly on cancer cells and/or indirectly in the tumor microenvironment through its antiproliferative and chemo-sensitizing roles, presents new challenges for its optimal use in cancer therapy [48]. A novel NO donor may be administered orally and thus more applicable to treatment. Furthermore, for certain tumors, it is also possible to administer NO-donors intratumorally, thus reducing the systemic toxic effects that may arise from its route of administration. So, it is expected that the application of NO donors in cancer therapy will be added to the armamentarium of cancer therapeutics in the future [47].

The synthesis of molecular hybrids is a strategy to design novel products endowed with multiple pharmacological properties to modulate more than one physiological target and/or decrease the side effects of the conventionally used drugs. Recently, several classes of hybrid compounds, combining appropriate groups with NO-releasing functions, have been obtained.

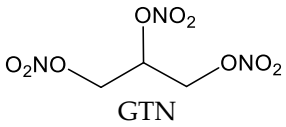
NO-Aspirin is an obvious candidate for the NO-hybrid approach. When administered orally, NCX 4040, NCX 4215, NCX 1016, and NCX 4016, these drugs not only meet or exceed the anti-inflammatory, analgesic and antithrombic activity of aspirin but also prevent and repair aspirin-induced gastrointestinal tract damage [51, 52].

NCX 4040 *in vitro* and *in vivo* studies indicate that NO-aspirin is a promising anticancer. It exerted a higher cytotoxic activity than the parental aspirin [52]. This could be attributed to the increased capacity of the spacer molecule to release the NO. In addition, it could inhibit nuclear factor (NFκB) and cyclooxygenase isoenzyme II (COX-2) in the human colon cancer cell (HT-26). Also, NO-aspirin significantly more potently inhibited the growth of Barrett's carcinoma cell line (OE-33 cells) than traditional aspirin due to activation of caspase 3 [54].



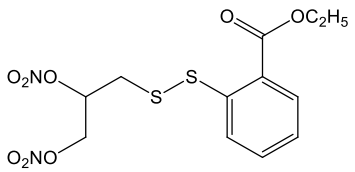
NCX 4040

Moreover, it was reported that GTN promoted beta-catenin degradation and down-regulated its transcriptional activity in colon cancer cells. In addition, NO signaling from a low-dose, sustained-release GTN patch may inhibit the hypoxia-induced progression of prostate cancer [55].



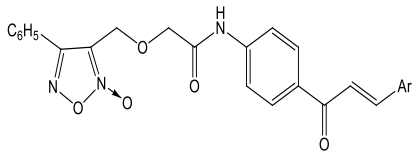
GTN

Furthermore, GT-094, a novel NO-releasing compound, showed antiproliferative activity in human colonic adenocarcinoma cells *in vitro* and reduced colon crypt proliferation and iNOS in proximal and distal colon in rat/azoxymethane model *in vivo* [56].

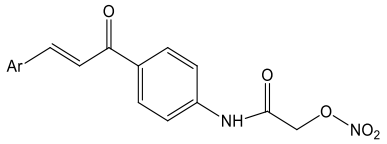


GT-094

Novel NO donating/chalcone hybrids were prepared by **Mourad *et al.*** [57], *via* binding amino chalcones with different NO-donating moieties, including; furoxans **VI**, nitrate esters **VII** and oximes **VIII**. Screening of the anticancer activity of the compounds selected by the national cancer institute (NCI) revealed that most NO-donating compounds exhibited remarkable activity against different cancer cell lines.

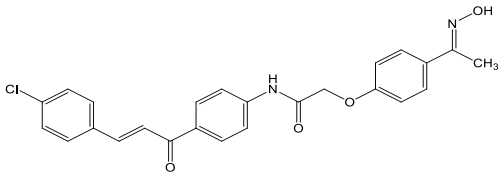


VI



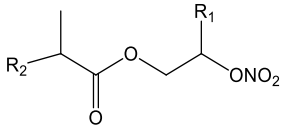
VII

Ar: 4-Cl-C_6H_4, 3`,4`-OCH_2O-C_6H_3



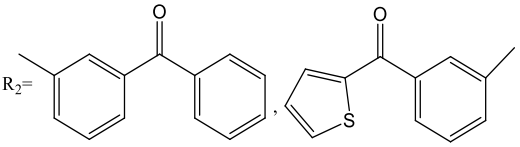
VIII

The design of profen hybrids **IX** containing a NO donor moiety connected to an aliphatic spacer led to compounds with a similar cyclooxygenase inhibition to their parent profen and with significant antiproliferative activities on prostate cancer PC3 cells [58].



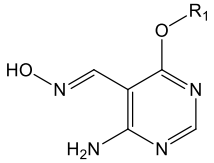
R_1= H, CH_3

R_2=



IX

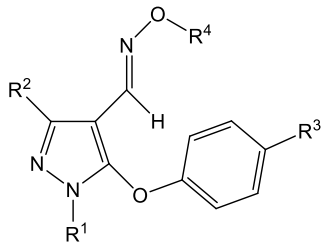
In addition, a novel 4-aminopyrimidine-5-carboxaldehyde oxime **X** scaffold with inhibitory activity against VEGFR-2 kinase has been synthesized [59]. This series scaffold had shown antiproliferative activity against several cancer cell lines and prevented cells from entering mitosis. Also, they exhibited high selectivity for vascular endothelial growth factor kinase (VEGFR-2).



R_1= $CH_3CH_2CH_2$, $CH_3CH_2CH_2CH_2$, CH_2=$CHCH_2$

X

Many multi-functionalized pyrazole oxime ethers **XI** were prepared and examined as cytotoxic agents against various human tumor cell lines. As a result, they achieved potent cytotoxicity against different human tumor cell lines such as XF 498 (CNS cancer) and HCT15 (colon cancer) [60].



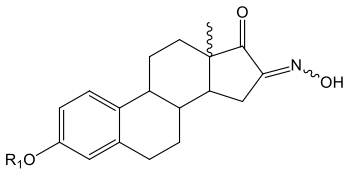
R^1, R^2 = CH_3, Ph.,.....

R^3= H, Cl

R^4= CH_2Ph-3-OMe, CH_2Ph-4-Me,.....

XI

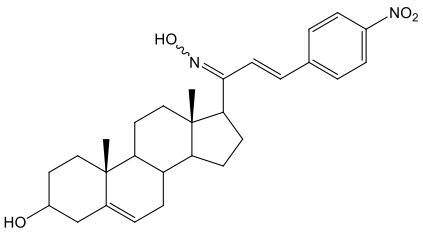
The cytotoxic effects of newly synthesized estrone-16- oxime ethers **XII** were examined by **Berényi *et al.*** on human cancer cell lines (cervix carcinoma HeLa, breast carcinoma MCF7 and skin epidermoid carcinoma A431). The results provide that the substituted estrone oximes may selectively suppress cancer cell proliferation by promoting apoptotic cell death and modulating the cell cycle progression [61].



R_1= H, Me, SO_2NH_2,.....

XII

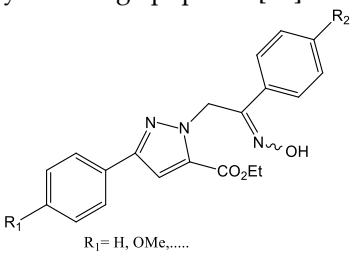
A series of novel benzylidine and benzylidine oxime derivatives of pregnenolones were synthesized and screened for anticancer activity against a panel of six human cancer cell lines. From the data, it was found that all the compounds have promising anticancer activity and compound **XIII** was the most active in this study [62].



XIII

A series of anthracenone-based oxime ethers and esters were synthesized to evaluate their antiproliferative activity. Several investigated

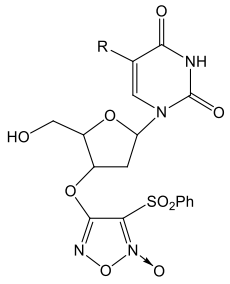
compounds displayed potent antiproliferative activity against K562 leukemia cells and proved to be potent tubulin polymerization inhibitors [63]. **Wen Zheng *et al.*** synthesized a series of novel oxime-containing pyrazole derivatives **XIV** and examined their cytotoxicity against the A549 lung cancer cell line. The evaluation of biological activity showed that these compounds could inhibit A549 lung cancer cell growth by inducing apoptosis [64].



R_1= H, OMe,.....
R_2= Cl, H,.....

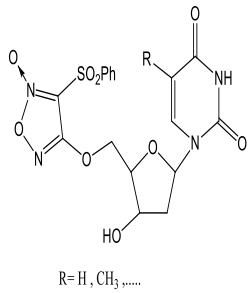
XIV

As well, a group of 3`- **XV** and 5`-O-(3-benzenesulfonylfuroxan-4-yl)-2`-deoxy uridines **XVI**were designed for the evaluation as hybrid NO donor-nucleoside anticancer agents that can simultaneously release cytotoxic NO [65].



R= H , CH_3 , ...

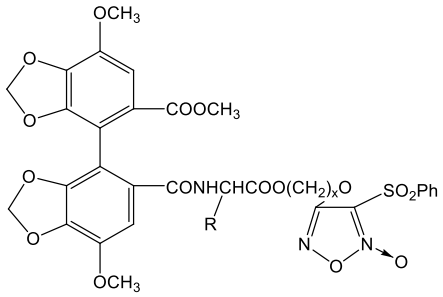
XV



R= H , CH_3 .....

XVI

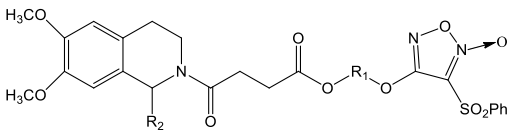
A series of novel NO-donating six alkoxyl biphenyl derivatives **XVII** were synthesized by coupling furoxan with alkoxy biphenyl skeleton using amino acids as a spacer. They were evaluated for their cytotoxicity against hepatic cell carcinoma. They showed more potent cytotoxic activities than the control 5-fluorouracil [66].



X= 2, 4 ,.....
R= H , CH_3 ,.....

XVII

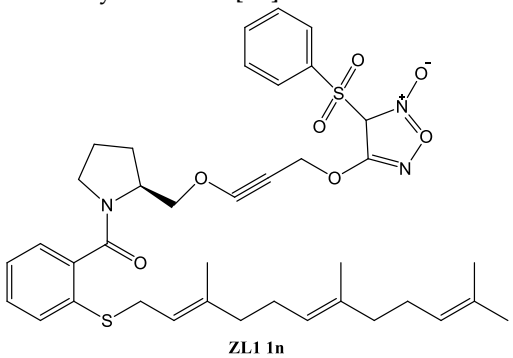
In **Zou *et al.*** study, 16 furoxan-based NO-releasing derivatives of tetrahydroisoquinoline were synthesized. Their cytotoxic activities and effects in reversing multidrug resistance have been evaluated. The results revealed that the compound **XVIII** showed higher cytotoxicity than the other synthesized compounds [67].



R_1= -CH_2C≡CCH_2, -CH_2CH_2-, ...
R_2=1-Naphthylmethyl, 3.4-dimethoxybenzyl

XVIII

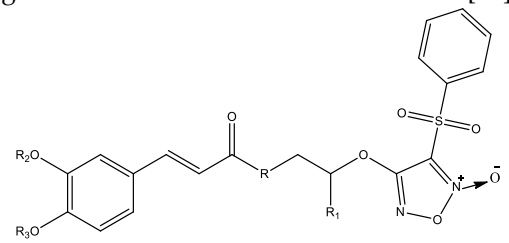
ZL11n is a novel furoxan-based NO-releasing derivative of farnesylthiosalicylic acid. This study examined the anticancer effects and the potential mechanism of action of ZL11n. It was found that ZL11n exhibited a favorable, selective cytotoxic effect in the HepG2 cell line. Furthermore, the NO yield in the ZL11n treated HepG2 cells was much higher than in the control group and the normal human liver cells. Moreover, the NO concentration was correlated to the degree of cytotoxicity observed [68].



ZL11n

Novel furoxan-based NO-releasing derivatives of hydroxycinnamic acids **XIX** were synthesized by **Lu *et al.*** Most of these

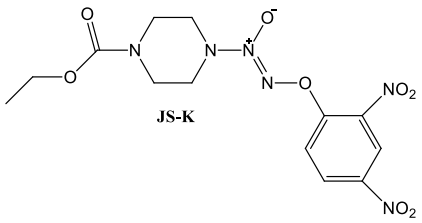
compounds displayed potent antitumor activities superior to control 5-fluorouracil in most cancer cells tested which attributed to high levels of NO released in cancer cells [69].



R= NH, $NHCH_2$, $NHCHCH_2$,.....
R_1= H, Me
R_2= Ac, H, Me
R_3= H, Ac

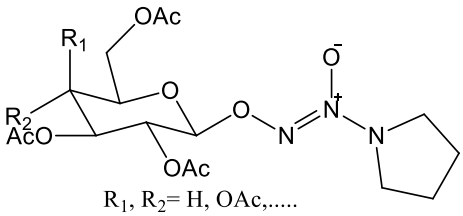
XIX

JS-K is a NO-releasing prodrug of the *O*²-arylated diazeniumdiolate family that has demonstrated pronounced cytotoxicity and antitumor properties in various cancer models both *in vitro* and *in vivo* [70].



JS-K

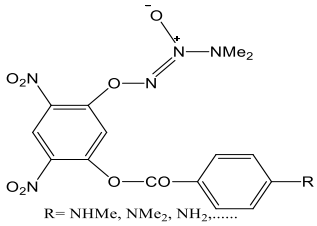
Synthetic procedures have been developed to link the nitric oxide-releasing diazeniumdiolate functional groups to a carbohydrate unit. These glycosylated diazeniumdiolates **XX** could readily release nitric oxide upon activation by glycosidases. A preliminary antitumor screen assay demonstrated that this class of compounds exhibited broad-spectrum cytotoxicity against cancer lines [71].



R_1, R_2= H, OAc,.....

XX

Moreover, several structural analogs of *O*²-(2,4-dinitro-5-(4-(*N*-methylamino)benzoyloxy)phenyl)1-(*N*,*N*-dimethylamino)diazen-1-ium-1,2-diolate **XXI**, an anticancer lead compound that is designed to release NO upon activation by glutathione, were prepared. The ability of these structural analogs to inhibit human leukemia cell proliferation was determined and it was found that compounds are releasing elevated amounts of NO displayed superior cytotoxic effects [72].



R= NHMe, NMe_2, NH_2,.....

XXI

1.5. Role of nitric oxide in the cardiovascular system

Since the discovery of NO as the endothelium-derived relaxation factor, new roles for NO in cardiovascular physiology continue to emerge as it plays many vital roles in the cardiovascular system [73]. NO is known to govern cardiac morphometric and functional properties through activation of sGC, leading to increased levels of cyclic cGMP and subsequently activation of protein kinase, phosphodiesterases, and ion channels which finally leads to vascular relaxation [74] (**Figure 5.**).

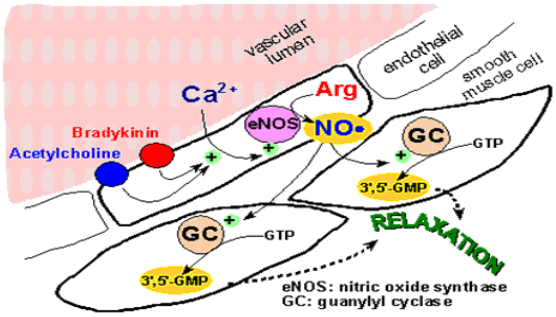
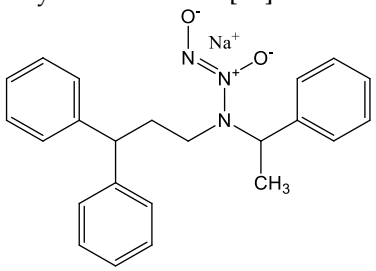


Figure 5: Endothelium-dependent vascular relaxation [74].

In addition, NO serves many critical biological functions in cardiovascular physiology. NO maintains vascular integrity by inhibiting platelet aggregation, leukocyte endothelium adhesion and vascular smooth muscle proliferation [75]. Also, NO is produced in cardiac smooth muscle, which regulates cardiac contractility [76]. Moreover, the therapeutic use of nitric oxide (NO) donors to bypass PKI resistance in cancer has never been tested in clinic yet but several arguments suggest that the combination of PKIs and NO donors may exert a potential

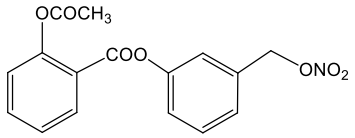
anticancer effect [77]. Diminished NO bioactivity may cause constriction of coronary arteries during exercise or mental stress and contribute to the provocation of myocardial ischemia in patients with coronary artery disease [78]. Additionally, diminished NO bioactivity may facilitate vascular inflammation that could lead to oxidation of lipoproteins and foam cell formation, the precursor of atherosclerotic plaque [79]. Numerous therapies have been investigated to assess the possibility of reversing endothelial dysfunction by enhancing NO is released from the endothelium, either through stimulation of NO synthesis or protection of NO from oxidative inactivation and conversion to toxic molecules such as peroxynitrite [80]. Accordingly, causal relationships between improved endothelial function and reduction in myocardial ischemia and acute coronary events have been investigated [81].

Modifying fendiline, a coronary vasodilator, with NO generates a new lipophilic member of the diazeniumdiolate family, fendiline diazeniumdiolate (FDL-NONOates), which has increased potential as a pharmaceutical NO donor and multiple applications in the coronary health market [82].



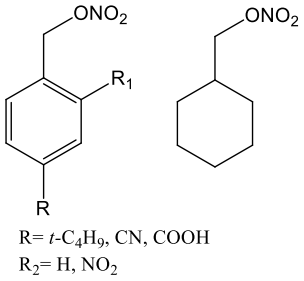
Fendiline diazeniumdiolate

In addition, NO-asprins are of particular interest concerning the prevention of severe cardiovascular disease. They show promising effects in various cardiovascular disorders, especially NCX 4016, which inhibit the proliferation of vascular smooth muscle through a mechanism involving NO release. Thus, prevent atherosclerosis, the most common cause of acute myocardial infarction [83]. Also, NCX 4016 does not cause leukocyte adherence to the vascular endothelium and can suppress the adherence induced by pro-inflammatory mediators [84].



NCX 4016

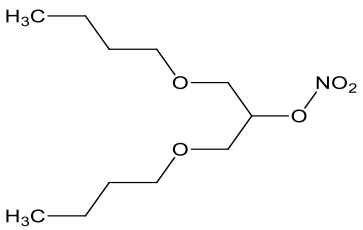
A series of substituted cyclohexyl methyl nitrate have been synthesized by **Weßler *et al.*** [85]. Vasodilating activities were measured and GTN was used as a reference. The results revealed that in intact coronary arteries, all benzylnitrates **XXII** vasodilating activities are lower compared with GTN but higher with cyclohexyl methyl nitrate (**XXIII**).



R= *t*-C_4H_9, CN, COOH
R_2= H, NO_2

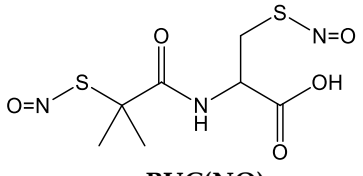
XXII **XXIII**

Moreover, **Franca-Silva *et al.*** suggested that the nitrate ester derivative NDBP is a new NO generator as it induced vasorelaxation in superior rat mesenteric artery through NO generation and activation of the sCG/cGMP/PKG pathway [86].



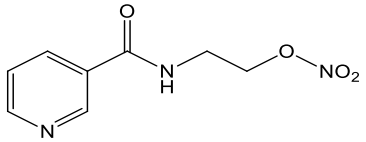
NDBP

A new RSNO, [*S*,*S*'-dinitrosobucillamine ($BUC(NO)_2$] had been synthesized by **Dahboul *et al.*** . The results revealed that this compound can release a large amount of NO into the aorta and subsequently induces vasorelaxation at lower concentrations than other previously reported RSNO [87].



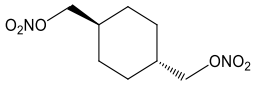
$BUC(NO)_2$

The cardiovascular effects of developed nicotinamide *N*-(2-hydroxyethyl)nicotinamide nitrate (SG-75) were examined and the results indicated that SG-75 has desirable characteristics as an antianginal agent [88].



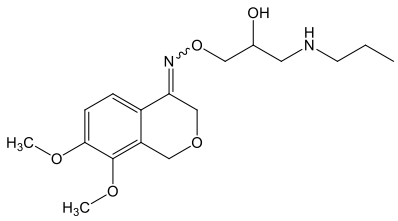
SG-75

Furthermore, **Van Woerkens *et al.*** synthesized five novel nitrate-ester derivatives. They found that CEDO 8956 [1,4-(*trans*)-di(hydroxymethyl)cyclohexane dinitrate] was the most potent vasodilator of the novel compounds with exhibiting a cardiovascular profile similar to that of GTN [89].



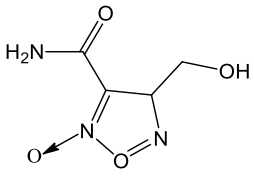
CEDO 8956

In a search for new cardiovascular drug candidates, a series of novel oxime ethers derived from a natural isochroman-4-one were synthesized. The results suggested that compound **XXIV** was the most promising derivative, which exhibited β1-adrenoceptor blocking activity as superior to propranolol [90].



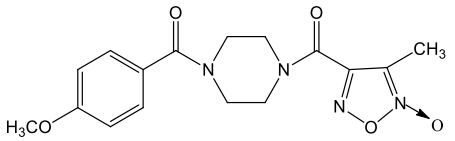
XXIV

Bohn *et al.* study; examine the cardiovascular effects of CAS 1609 (4-hydroxymethyl-furoxan-3-carboxamide) *in vitro* and *in vivo* in various animal models. The results indicated that the NO-releasing furoxan derivative (CAS 1609) is a potent, long-lasting, orally active vasodilator agent devoid of tolerance development [91].



CAS 1609

Moreover, a series of hybrid molecules incorporating the furoxan and nicorandil moieties were designed as potential NO donors with cardiovascular and cerebrovascular activities. The results suggest that furoxan-nicorandil derivative **XXV** is a valuable lead in the design of NO-donor compounds for hypertension [92].



XXV

Conclusion and future perspectives: NO plays a key role in tumor biology and therapy, and there are several approaches that have been utilized to induce anti-tumor activities, or improve the efficacy of chemotherapy and radiotherapy, from NO releasing compounds. So far at least 16 families of NO precursor and NO donor functional groups have been developed. However, only organic nitrates and SNP have clinical applications, predominantly for cardiovascular disease. Hence the development of stable and tuned NO donor compounds is a priority for drug discovery programs. However, more in-depth research is needed to reveal NO signaling mechanisms, and clinical trials are also warranted to verify NO-based prevention and treatment of tumors.

Conflicts of Interest: The authors declare no conflict of interest.

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