We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Therapeutic Applications and Mechanisms of YC-1: A Soluble Guanylate Cyclase Stimulator

Chieh-Hsi Wu, Chun-Hsu Pan and Ming-Jyh Sheu

Abstract

Nitric oxide (NO) is an essential endogenous vasodilator to maintain vascular homeostasis, whose effects are mainly mediated by NO-dependent soluble guanylate cyclase (sGC) which catalyzes the synthesis of cyclic guanosine monophosphate (cGMP), a critical mediator of vascular relaxation. YC-1, a novel NO-independent sGC stimulator, was first introduced as an inhibitor of platelet aggregation and thrombosis. Accumulating studies revealed that YC-1 has multiple medication potentials to use for a broad spectrum of diseases ranging from cardiovascular diseases to cancers. In contrast to NO donors, YC-1 has a more favorable safety profile and low medication tolerance. In this chapter, we introduce canonical and pathological roles of NO, review activations, and regulatory mechanisms of YC-1 on NO-independent sGC/cGMP pathway and present the potential pharmacological applications and molecular mechanisms of YC-1.

Keywords: nitric oxide, soluble guanylate cyclase, YC-1

1. Introduction

Since the discoveries of the biological effects of NO on physiological actions mediated by cGMP, delineation of the molecular mechanism of NO actions and understanding of NO activation of guanylate cyclase (GC) and the subsequent signal processes have been greatly advanced [1]. NO can function as an intracellular messenger, an autacoid, a paracrine substance, a neurotransmitter, or as a hormone that can be carried to distant sites for effects [1, 2]. It is therefore a unique simple molecule with diversified physiological functions.

2. Canonical function of NO

NO, initially known as the endothelium-derived relaxing factor (EDRF), is a gas molecule and free radical with an unpaired electron which has been shown to be involved in an ever-growing list of biological processes. NO generated in the tissue binds to major physiological target, haem moiety of GC, activating the cGMP cascade. The GC family is composed of two members including membrane-bound GC and soluble GC (sGC). Membrane-bound GC is a receptor responsive to atrial natriuretic peptide (ANP), and sGC acts as the NO sensor. NO exerts its biological

effects by activating sGC to increase the cGMP level and vascular effects known to be mediated by cGMP such as vasodilation, inhibition of platelet aggregation, and inflammatory reaction. Cyclic GMP modulates a number of signaling processes downstream of NO. The NO-cGMP cascade can be regulated by pharmacological modulation of protein kinases, phosphodiesterases (PDE), and ion channels to alter vascular tones as well as endothelial and vascular smooth muscle cell growth. Pharmacological alteration of the NO level has been a major strategy to develop therapeutic agents for cardiovascular diseases.

Deguchi and his colleagues found that GC activity in the supernatant of neuroblastoma and brain preparations were activated by L-arginine which has been identified as an endogenous activator of sGC [3]. Hibbs et al. noted the latter that the cytotoxic properties of macrophages in co-cultures with tumor cells could be enhanced with L-arginine but suppressed by N-N-methyl-arginine (LNMA), an inhibitor of nitric oxide synthase (NOS) [4]. This cytotoxicity action was accompanied by accumulation of nitrite in the conditioned medium. These important studies provide the insight to identify a pathway of L-arginine metabolism that could produce NO and nitrite.

NOS is a group of isozymes which convert L-arginine to L-hydroxyarginine and subsequently to NO and L-citrulline through cofactors including reduced nicotinamide-adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydrobiopterin (BH4) [5]. The first NOS isoform to be identified was the neuronal NOS (nNOS or named as NOS-1) [6]. This was followed shortly thereafter by inducible NOS (iNOS), also known as type II NOS (NOS-2) [7], and then by endothelial NOS (eNOS or named as NOS-3) [8]. NOS can also be inactivated by asymmetric dimethyl arginine (ADMA), an endogenous and competitive inhibitor of NOS [9–11].

3. Pathological role of NO

NO is essential in the maintenance of vascular homeostasis including smooth muscle relaxation, inhibition of platelet aggregation, attenuation of vascular smooth muscle cell (VSMC) proliferation, neurotransmission, and immune defense [12]. Therefore, the impaired NO pathway has been implicated in endothelial dysfunction and pathogenesis of a number of diseases featuring inflammatory reaction. These include arthritis, myocarditis, colitis, and nephritis. Altered NO synthesis has been noted in selected pathologic conditions such as amyotrophic lateral sclerosis, cancer, diabetes, and neurodegenerative diseases [13, 14]. In general, physiological NO actions on target tissues are brief, reversible, and dependent on the downstream cGMP-dependent signaling events. Conversely, the pathological actions noted with excessively and sustained NO production involved NO interaction with superoxide to generate peroxynitrite, a highly reactive free radical which exhibits the toxic actions of potent oxidants. Peroxynitrite, independent of the cGMP signaling events, has been implicated in oxidative injury noted in a number of disease models [15–17]. In addition to its free radical actions, peroxynitrite inactivates prostacyclin synthase to reduce prostacyclin levels, leading to vascular dysfunction [18].

ADMA, a risk factor for cardiovascular diseases, inhibits NOS to reduce biosynthesis of NO, resulting in impaired blood flow, accelerated atherogenesis, and suppressed angiogenesis [19]. ADMA is involved in the development of endothelial dysfunction. In essential hypertension patients, the L-arginine and ADMA levels are elevated and inversely related to endothelial function [20, 21]. Endothelial function depends on the integrity of eNOS and the availability and vascular signaling of

NO. In clinical settings, endothelial dysfunction is important because it may develop hypertension and atherosclerosis and therefore is a predictor in ensuing cardiovascular diseases [22]. In hyperhomocysteinemia, an increase in ADMA has been linked to impaired vascular endothelial function. Elevated homocysteine levels exert inhibitory effects on the expression or activation of dimethylarginine dimethylaminohydrolase (DDAH) [23–27]. Two isoforms of DDAH, DDAH-1 and DDAH-2, were identified in tissues expressing nNOS and eNOS, respectively [28]. Both DDAH isoforms are expressed widely in different organs, with higher content found in the liver and kidney [29]. Similarly, endothelial dysfunction has also been found in hypercholesterolemia. Several studies indicated that hypercholesterolemia may cause a decline in DDAH activity and an increase in the ADMA level [30, 31]. Böger et al. also found that exposure of cultured endothelial cells to oxidized low-density lipoprotein (oxLDL) cholesterol resulted in ADMA accumulation in the culture medium [31]. Oxidized LDL could cause endothelial dysfunction in complex mechanisms including reduction of eNOS expression [31], to trigger endothelial apoptosis [32] and to inhibit vascular endothelial growth factor (VEGF)-induced endothelial proliferation [33]. Furthermore, oxLDL impairs NO-induced stimulation of cGMP accumulation [34]. Patients with cardiac syndrome X (CSX) have higher levels of ADMA and increased mean common carotid intima-media thickness that are ascribed to ADMA effects on NO bioavailability resulting in endothelial dysfunction and subsequently impede microvascular circulation, which are the leading mechanisms in the development of CSX [10, 35, 36]. ADMA also plays important roles in endothelial dysfunction in subjects with chronic kidney failure [9, 37, 38]. ADMA is metabolized to L-citrulline *via* the action of DDAH-1, which is highly expressed in the kidney [29]. There is a strong association between impairment of renal function and elevation of ADMA content [9, 39]. Microangiopathy-related cerebral damage (MARCD) is a cerebrovascular disease caused by arteriosclerosis in deep white matter, which includes lacunar infarction and white matter hyperintensity [40]. Arteriosclerosis in deep white matter resulting from acute and chronic ischemia is probably responsible for the development of MARCD [41]. Several potential risk factors for arteriosclerosis have been evaluated in patients with MARCD [42, 43]. NO is involved not only in regulating cerebral blood flow but also in preventing arteriosclerosis by inhibiting fibrosis and proliferation of smooth muscle cells in the arterial wall [44]. In fact, NOS inhibitors and functional single-nucleotide polymorphisms in the eNOS gene have been shown to be correlated with MARCD [44, 45]. Excessive NO production could also be a problem in the progression of the disease such as glaucoma. Increased NO generated by iNOS in astrocytes and microglia in the optic nerve head of patients with glaucoma may contribute to the optic neuropathy associated with this disease. The pharmacological use of an inhibitor of iNOS, aminoguanidine, significantly prevents the loss of retinal ganglion cells [46].

4. Novel compounds for NO-independent sGC/cGMP activation

Organic NO donors such as nitrite and nitroglycerin are successful examples in clinical practice for more than a century. However, formation of harmful intermediate, peroxynitrite, and the long-term treatment with NO donors resulting in drug resistance limit the clinical applications of NO donor compounds. To overcome these obstacles, the novel agents for triggering sGC/cGMP cascade in NO-independent manner have been developed.

A series of 1-(substituted benzyl)-3-(substituted aryl)-condensed pyrazole derivatives were synthesized and identified as class novel antiplatelet agents [47, 48]. As one of the most promising analogues, 1-benzyl-3-(5'-hydroxymethyl-2'-furyl)

indazole (YC-1) was selected for further investigation. The physiological property of YC-1 in stimulation of sGC was demonstrated by Ko and colleagues [49]. Potential regulatory mechanisms of YC-1 on cardiovascular protections were summarized in **Figure 1**. Ko et al. showed that YC-1 is an antithrombotic agent. It inhibits platelet aggregation by increasing platelet cGMP levels in an NO-independent manner. YC-1 action was noted to exert its antiplatelet effect through the activation of NO-independent sGC/cGMP pathway [50]. Nearly, all the newer generations of sGC stimulator except acryl-acrylamide family have been derived based on YC-1 as the parent compound [51]. YC-1 and its successors all require the presence of a reduced haem moiety within sGC to stimulate sGC, but they also act in synergy with NO by binding NO or iron-free precursor of haem to structurally resemble the NO-haem complex and stabilize sGC in its active configuration [52–54]. Stasch et al. also reported that YC-1 and its derivate, BAY 41-2272, bind to regulatory sites (cys 238 and cyst 243 regions) in the $\alpha 1$ -subunit of sGC, resulting in conformational change and subsequent activation of recombinant sGC by NO-independent but haem-dependent mechanism [55]. Mulsch et al. also noted that the combined effect of nitrovasodilators and YC-1 in cultured VSMCs and isolated rabbit aortic rings reflected the direct synergistic action of YC-1 and NO on the sGC [56]. Wohlfart et al. reported that YC-1 can stimulate synthesis and release NO in endothelial cells independent of raising the cGMP content in a calcium-dependent manner [57]. In addition, YC-1 inhibits the cGMP-specific phosphodiesterase type 5 (PDE-5)

Figure 1. Schematic overview of regulatory mechanisms of YC-1 on cardiovascular protections. cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ECs, endothelial cells; FAK, focal adhesion kinase; GMP, guanosine monophosphate; GTP, guanosine triphosphate; HO-1, heme oxygenase-1; Hsp70, heat shock protein 70; iNOS, inducible nitric oxide synthase; MMP2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; oxLDL, oxidized low-density lipoprotein; PDE5, phosphodiesterase 5; PKG, protein kinase G; sGC, soluble guanylyl cyclase; TGF-β, transforming growth factor-beta; VASP, vasodilator-stimulated phosphoprotein; VSMC, vascular smooth muscle cells.

in platelets and in aortic extracts to raise cGMP levels and prolong its duration of action [58, 59]. The vasodilator-stimulated phosphoprotein (VASP) has been reported to be involved in cGMP- and cAMP-mediated antiplatelet actions [60]. Becker et al. noted that VASP is the target of YC-1 since VASP phosphorylation can be directly increased through stimulation of the cGMP/protein kinase G/VASP pathway [61].

5. Additional pharmacological activities of YC-1

In addition to the effects in antiplatelet aggregation and antithrombosis, YC-1 has been demonstrated to provide several beneficial effects including cardiovascular protections; antitumor, neuroprotective, and anti-inflammatory effects; as well as optical protections.

5.1 Cardiovascular protections

YC-1 inhibits VSMC proliferation, similar to specific guanylate cyclase inhibitors, suggesting that the antiproliferative effect of YC-1 is mediated by cGMP [62]. A similar conclusion has also been drawn by other investigators [63, 64]. As shown in **Figure 1**, NO-/cGMP-dependent processes have been suggested to modulate VSMC phenotype and the arterial response to endovascular injury [65, 66]. It has been reported that YC-1 can upregulate expression of iNOS and inducible heme oxygenase-1 (HO-1) at the transcriptional and translational level as well as stimulate sGC and cGMP production in the balloon-injured artery [63]. These results support the proposal that YC-1 can be developed as a potent new therapeutic agent for reducing restenosis *via* endogenous carbon monoxide (CO)- and/or NO-mediated cGMP-dependent processes. Wu et al. found that two important modulators, transforming growth factor (TGF)- β 1 and focal adhesion kinase (FAK), responsible for VSMC proliferation and migration were reduced in content in the cultured VSMC treated with YC-1. The effect of YC-1 on preventing balloon injury-induced vascular stenosis has also been demonstrated in a rat carotid angioplasty model [64]. Liu et al. also found that YC-1 can inhibit neointima formation in balloon-injured rat carotid through suppressing the expression and actions of matrix metalloproteinase (MMP)-2 and MMP-9 [67]. YC-1 can also prevent oxLDL-mediated apoptosis by inducing heat shock protein 70 (Hsp70) expression in VSMCs suggesting its cytoprotective effect in vascular diseases [68]. Similarly, Hsp70 overexpression has also been involved in protective effect of YC-1 on heat stroke [69]. In vivo evidence shows that YC-1 and zaprinast, an inhibitor of cGMP-selective PDE, inhibit injury-induced vascular remodeling through anti-mitogenic and pro-apoptotic actions in a rat carotid artery balloon injury model [70]. Moreover, YC-1 has also been found to induce cell cycle arrest of HUVEC through upregulation of p21 and p27 protein *via* inhibition of the cyclin/cyclin-dependent kinase (CDK) system. This finding suggests that YC-1-induced antiproliferation effect in HUVEC is *via* a cGMP-independent manner [71]. Besides, the prevention effects of YC-1 on the development of hypoxia-induced pulmonary arterial hypertension (PAH), right ventricular hypertrophy (RVH), and pulmonary vascular remodeling has been clearly mentioned in animal model [72].

5.2 Antitumor effects

A growing body of evidence indicates that hypoxia-inducible factor-1 (HIF-1) contributes to tumor progression and metastasis. YC-1 inhibits HIF-1-mediated

hypoxic responses [73–76]. YC-1 enhanced radiation sensitivity by inhibiting HIF-1 α expression [77]. Lau et al. also found that YC-1 suppressed both synthesis and stability of HIF-1 α , *via* regulation of murine double minute (Mdm2) protein [78]. In hypoxic gastric carcinoma cell and xenograft models, low-dose YC-1 combined with glucose and insulin can effectively inhibit anaerobic glycolysis and induce hypoxia-dependent apoptosis by suppressing HIF-1 α expression [79].

YC-1 also enhanced chemosensitivity of hepatocellular carcinoma cells to cisplatin through a Stat3-dependent manner [80]. Similarly, YC-1 also enhanced camptothecin toxicity by activating the caspase-8, the Bid pathway, and the mitochondria-mediated apoptotic pathway in ovarian carcinoma cell lines [81]. Additionally, it has also been found that YC-1 can suppress constitutive NF- κ B activation and induce apoptosis in human prostate cancer cells [82]. YC-1 inhibited VEGF- and basic fibroblast growth factor (bFGF)-mediated ERK1/ERK2 mitogen-activated protein kinase (MAPK), AKT, and protein kinase C α (PKC α) pathways in vitro and angiogenesis in in vivo models [83]. YC-1 arrested the cell cycle in G₀/G₁ in human hepatocellular carcinoma cells by upregulating p21^{CIP1/WAF1} and p27^{KIP1} expression [84]. YC-1 arrested the cell cycle at S-phase and induced apoptosis by activating checkpoint kinases in several cancer cells [85]. Similarly, YC-1 can also increase p21 protein and decrease cyclins and CDKs to induce G₀/G₁ phase arrest as well as activate caspases and disrupt the mitochondrial membrane potential to trigger mitochondria-dependent apoptosis in cisplatin-resistant human oral cancer CAR cells [86]. Additionally, apoptotic mechanism of YC-1 may also be mediated by activating JNK phosphorylation and upregulating FasL and Fas receptor clustering to activate caspase-3 and caspase-8 and then trigger mitochondria-mediated and caspase-dependent pathways in renal carcinoma cells [87]. YC-1 has been shown to downregulate several invasion-related signaling proteins, such as β -catenin, caveolin, Src, and epidermal growth factor receptor (EGFR), as well as multiple growth-related proteins, including 5'-AMP-activated protein kinase α (AMPK α), phospho-acetyl-CoA carboxylase (p-ACC), human epidermal growth factor receptor 2 (HER-2), and mammalian target of rapamycin (mTOR) in nasopharyngeal carcinoma [88]. Other anti-invasion mechanisms of YC-1 have also been identified in nasopharyngeal carcinoma (NPC) cells by reverse phase protein array [88]. Activation of beta-catenin signaling has also been evidenced to involve in inhibiting the proliferation and metastasis of hepatocellular carcinoma using combination therapy with local radiofrequency ablation and YC-1 [89]. Moreover, the previous study indicated that YC-1 has a potential effect to improve drug resistance by inhibiting multidrug-resistant protein resulting in decrease of P-glycoprotein (Pgp) efflux, whose effect is modulated by the NO-cGMP-PKG-ERK signaling pathway [90]. These observations revealed together that YC-1 exerts inhibitory effects in key signaling pathways essential for maintaining cancer or endothelial cell viability and may be developed as an antitumor agent on a broad spectrum of cancer types by facilitating apoptosis and suppressing tumor angiogenesis.

5.3 Neuroprotective and anti-inflammatory effects

The use of NO donors (e.g., NONOate) results in excessive NO production which may cause NO-induced axonal damage by inhibiting mitochondrial respiration, independent of cGMP [91]. YC-1 has been shown to protect white matter axons from NO toxicity. This axonoprotective action of YC-1 was unrelated to its activity on sGC but through a novel action on voltage-dependent Na⁺ channels in the rat isolated optic nerve [92]. Lu et al. showed YC-1 inhibition of lipopolysaccharide (LPS)-induced iNOS and cyclooxygenase-2 (COX-2) expression as well as NF- κ B activation, implying that YC-1 can be developed as an anti-inflammatory

neuroprotective agent [93]. Chien et al. reported that YC-1 promoted learning behavior in Morris water maze and avoidance tests and YC-1 pretreatment reduced scopolamine-induced learning deficit. Thereby, the NO/cGMP/PKG pathway may be involved in the learning enhancement-based experiments with intracerebro-ventricular injection of L-NAME and PKG inhibitors [94]. Similarly, YC-1 can also improve age-related learning and memory dysfunction [95]. Furthermore, YC-1 may inhibit HIF-1 α accumulation and VEGF production to protect blood-brain barrier against ischemia-/reperfusion-induced injury [96]. In addition, beneficial effect of YC-1 in ameliorating combined allergic rhinitis and asthma syndrome (CARAS) was demonstrated through reducing expressions of HIF-1 α , NF- κ B, and peroxisome proliferator-activated receptor α (PPAR α) [97].

5.4 Optical protections

Therapeutic application of YC-1 on sepsis has been mentioned. After administration with YC-1, several LPS-stimulated modulations, such as NF- κ B activation, iNOS expression, NO overproduction, and cytokine release, were markedly inhibited, thus improving survival rate of endotoxemic mice [98]. YC-1 has also been shown to inhibit HIF-1 α -induced iNOS and VEGF expressions in various tissue models. Studies showed that YC-1 inhibited optical neovascularization in the pathological stages [99, 100]. Song noted that YC-1 could prevent laser-induced choroidal neovascularization by suppressing photocoagulation-mediated HIF-1 expression [99]. The pathological retinal neovascularization could also be inhibited by YC-1 through decreasing ischemia-induced expression of HIF-1 and its downstream angiogenic mediators (e.g., VEGF) in the ischemic retina. The physiological revascularization of the retinal vascular plexuses was enhanced by YC-1 *via* inhibiting iNOS expression at mRNA and protein levels [100]. Besides, it also has been reported that YC-1 alleviated macular edema in the animal model of laser-induced experimental central retinal vein occlusion by reducing several inflammatory or angiogenesis-related factors, such as interleukin-6 (IL-6), IL-8, VEGF, and HIF-1 [101].

5.5 Other activities

Wang and his colleagues evidenced that YC-1 inhibited bone resorption and induced extrinsic apoptosis of osteoclasts to reduce bone loss, which implied that YC-1 has potential application for use as an antiresorptive drug in postmenopausal osteoporosis [102]. Besides, YC-1 and its derivatives also have been mentioned to improve hepatic fibrosis, which mechanisms may be caused by inhibiting liver neutrophil infiltration as well as decreasing in TNF- α signaling and macrophage aggregation [103, 104].

6. Clinical significance of YC-1

Extensive studies have been conducted to explore possible systemic actions of YC-1 in disease models in animals to demonstrate that YC-1 has versatile physiological activities to be a potent candidate drug for a number of vascular disorders. In the cardiovascular and hematological systems, it has been reported that local extravascular administration of YC-1 could prevent neointima formation in a rat carotid artery model of balloon angioplasty [63, 64, 67]. In a study of experimental thrombosis model, YC-1 conferred beneficial effect through its anti-aggregating and pro-fibrinolytic effects [105]. BAY 63-2521 (riociguatTM), a NO-independent but heme-dependent sGC stimulators like TC-1, is currently in clinical development

for the treatment of pulmonary arterial hypertension with the only reported significant side effect to be a decrease in systemic arterial diastolic pressure [106, 107]. Similarly, intravenous administration of YC-1 has been shown to lower mean arterial blood pressure in normotensive and hypertensive rat [108]. For anticancer therapy, Lau et al. demonstrated that intraperitoneal injection of YC-1 enhances cisplatin chemosensitivity of hepatocellular carcinoma cells in nude mice xenograft tumor model, suggesting that YC-1 may be as an adjuvant agent for anticancer therapy [80]. Furthermore, oral administration of YC-1 can also decrease tumor mass in human renal cancer xenograft mice model [87]. In Morris water maze and avoidance test of mice, Chien et al. showed that YC-1 may be a good candidate for the improvement of learning and memory [94, 109]. Hwang et al. demonstrated that YC-1 can potentiate the relaxant responses of exogenous or endogenous NO through the elevation of cGMP in guinea-pig trachea [110]. The above in vivo studies all demonstrated the relevance of YC-1 in association with NO.

7. Conclusions

Accumulating evidences have shown that the administration of YC-1 may have beneficial pharmacological or physiological functions in diseased states for clinical applications. In the future, less toxic and more effective candidates would be the focus of further investigations through structural modification of YC-1 or its derivatives and better understanding of the molecular mechanisms of its actions.

Conflict of interest

The authors state that they have no conflict of interest.

Author details

Chieh-Hsi Wu^{1*}, Chun-Hsu Pan¹ and Ming-Jyh Sheu²

¹ School of Pharmacy, Taipei Medical University, Taipei, Taiwan

² School of Pharmacy, China Medical University, Taichung, Taiwan

*Address all correspondence to: chhswu@tmu.edu.tw

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Martin E, Davis K, Bian K, Lee YC, Murad F. Cellular signaling with nitric oxide and cyclic guanosine monophosphate. *Seminars in Perinatology*. 2000;**24**:2-6. DOI: 10.1016/S0146-0005(00)80045-2
- [2] Murad F. Nitric oxide signaling: Would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone? *Recent Progress in Hormone Research*. 1998;**53**:43-59. discussion 59-60
- [3] Deguchi T, Yoshioka M. L-Arginine identified as an endogenous activator for soluble guanylate cyclase from neuroblastoma cells. *Journal of Biological Chemistry*. 1982;**257**:10147-10151
- [4] Hibbs JB Jr, Taintor RR, Vavrin Z. Macrophage cytotoxicity: Role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science*. 1987;**235**:473-476. DOI: 10.1126/science.2432665
- [5] Ignarro LJ, Buga GM, Wei LH, Bauer PM, Wu G, del Soldato P. Role of the arginine-nitric oxide pathway in the regulation of vascular smooth muscle cell proliferation. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**:4202-4208. DOI: 10.1073/pnas.071054698
- [6] Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature*. 1991;**351**:714-718. DOI: 10.1038/351714a0
- [7] Nathan C, Xie QW. Nitric oxide synthases: Roles, tolls, and controls. *Cell*. 1994;**78**:915-918. DOI: 0092-8674(94)90266-6
- [8] Janssens SP, Simouchi A, Quertermous T, Bloch DB, Bloch KD. Cloning and expression of a cDNA encoding human endothelium-derived relating factor/nitric oxide synthase. *Journal of Biological Chemistry*. 1992;**267**:22694
- [9] Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet*. 1992;**339**:572-575. DOI: 0140-6736(92)90865-Z
- [10] Cooke JP. Does ADMA cause endothelial dysfunction? *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2000;**20**:2032-2037. DOI: 10.1161/01.ATV.20.9.2032
- [11] Ueda S, Yamagishi S, Matsumoto Y, Fukami K, Okuda S. Asymmetric dimethylarginine (ADMA) is a novel emerging risk factor for cardiovascular disease and the development of renal injury in chronic kidney disease. *Clinical and Experimental Nephrology*. 2007;**11**:115-121. DOI: 10.1007/s10157-007-0471-x
- [12] Murad F. Discovery of some of the biological effects of nitric oxide and its role in cell signaling. *Bioscience Reports*. 1999;**19**:133-154. DOI: 10.1023/A:1020265417394
- [13] Bian K, Murad F. Nitric oxide (NO)—Biogenesis, regulation, and relevance to human diseases. *Frontiers in Bioscience*. 2003;**8**:d264-d278. DOI: 10.2741/997
- [14] Murad F. Shattuck Lecture. Nitric oxide and cyclic GMP in cell signaling and drug development. *New England Journal of Medicine*. 2006;**355**:2003-2011. DOI: 10.1056/NEJMsa063904
- [15] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases:

The role of oxidant stress. *Circulation Research*. 2000;**87**:840-844. DOI: 10.1161/res.87.10.840

[16] Wilcox CS. Redox regulation of the afferent arteriole and tubuloglomerular feedback. *Acta Physiologica Scandinavica*. 2003;**179**:217-223. DOI: 10.1046/j.0001-6772.2003.01205.x

[17] Bian K, Doursout MF, Murad F. Vascular system: Role of nitric oxide in cardiovascular diseases. *Journal of Clinical Hypertension*. 2008;**10**:304-310. DOI: 10.1111/j.1751-7176.2008.06632.x

[18] Ullrich V, Kissner R. Redox signaling: Bioinorganic chemistry at its best. *Journal of Inorganic Biochemistry*. 2006;**100**:2079-2086. DOI: 10.1016/j.jinorgbio.2006.09.019

[19] Calver A, Collier J, Leone A, Moncada S, Vallance P. Effect of local intra-arterial asymmetric dimethylarginine (ADMA) on the forearm arteriolar bed of healthy volunteers. *Journal of Human Hypertension*. 1993;**7**:193-194

[20] Takiuchi S, Fujii H, Kamide K, Horio T, Nakatani S, Hiuge A, et al. Plasma asymmetric dimethylarginine and coronary and peripheral endothelial dysfunction in hypertensive patients. *American Journal of Hypertension*. 2004;**17**:802-808. DOI: 10.1016/j.amjhyper.2004.05.005

[21] Perticone F, Sciacqua A, Maio R, Perticone M, Maas R, Boger RH, et al. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. *Journal of the American College of Cardiology*. 2005;**46**:518-523. DOI: 10.1016/j.jacc.2005.04.040

[22] Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, et al. Prognostic significance of endothelial dysfunction in hypertensive

patients. *Circulation*. 2001;**104**:191-196. DOI: 10.1161/01.CIR.104.2.191

[23] Böger RH, Lentz SR, Bode-Böger SM, Knapp HR, Haynes WG. Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e) in aemia in humans. *Clinical Science*. 2001;**100**:161-167. DOI: 10.1042/cs1000161

[24] Meigs JB, Jacques PF, Selhub J, Singer DE, Nathan DM, Rifai N, et al. Fasting plasma homocysteine levels in the insulin resistance syndrome: The Framingham offspring study. *Diabetes Care*. 2001;**24**:1403-1410. DOI: 10.2337/diacare.24.8.1403

[25] Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP. Homocysteine impairs the nitric oxide synthase pathway: Role of asymmetric dimethylarginine. *Circulation*. 2001;**104**:2569-2575. DOI: 10.1161/hc4601.098514

[26] Tyagi N, Sedoris KC, Steed M, Ovechkin AV, Moshal KS, Tyagi SC. Mechanisms of homocysteine-induced oxidative stress. *American Journal of Physiology. Heart and Circulatory Physiology*. 2005;**289**:H2649-H2656. DOI: 10.1152/ajpheart.00548.2005

[27] Frey D, Braun O, Briand C, Vasak M, Grutter MG. Structure of the mammalian NOS regulator dimethylarginine dimethylaminohydrolase: A basis for the design of specific inhibitors. *Structure*. 2006;**14**:901-911. DOI: 10.1016/j.str.2006.03.006

[28] Leiper JM, Santa Maria J, Chubb A, MacAllister RJ, Charles IG, Whitley GS, et al. Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases.

Biochemical Journal. 1999;**343**
(Pt 1):209-214. DOI: 10.1042/bj3430209

[29] Nijveldt RJ, Teerlink T, van Guldener C, Prins HA, van Lambalgen AA, Stehouwer CD, et al. Handling of asymmetrical dimethylarginine and symmetrical dimethylarginine by the rat kidney under basal conditions and during endotoxaemia. *Nephrology, Dialysis, Transplantation*. 2003;**18**:2542-2550. DOI: 10.1093/ndt/gfg452

[30] Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. Novel mechanism for endothelial dysfunction: Dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation*. 1999;**99**:3092-3095. DOI: 10.1161/01.CIR.99.24.3092

[31] Böger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, et al. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: Involvement of S-adenosylmethionine-dependent methyltransferases. *Circulation Research*. 2000;**87**:99-105. DOI: 10.1161/res.87.2.99

[32] Harada-Shiba M, Kinoshita M, Kamido H, Shimokado K. Oxidized low density lipoprotein induces apoptosis in cultured human umbilical vein endothelial cells by common and unique mechanisms. *Journal of Biological Chemistry*. 1998;**273**:9681-9687. DOI: 10.1074/jbc.273.16.9681

[33] Ma FX, Zhou B, Chen Z, Ren Q, Lu SH, Sawamura T, et al. Oxidized low density lipoprotein impairs endothelial progenitor cells by regulation of endothelial nitric oxide synthase. *Journal of Lipid Research*. 2006;**47**:1227-1237. DOI: 10.1194/jlr.M500507-JLR200

[34] Chin JH, Azhar S, Hoffman BB. Inactivation of endothelial derived relaxing factor by oxidized lipoproteins.

Journal of Clinical Investigation. 1992;**89**:10-18. DOI: 10.1172/JCI115549

[35] Okay K, Cengel A, Sahinarslan A, Tavit Y, Turkoglu S, Biberoglu G, et al. Plasma asymmetric dimethylarginine and L-arginine levels in patients with cardiac syndrome X. *Coronary Artery Disease*. 2007;**18**:539-544. DOI: 10.1097/MCA.0b013e3282f08ece

[36] Sen N, Poyraz F, Tavit Y, Yazici HU, Turfan M, Hizal F, et al. Carotid intima-media thickness in patients with cardiac syndrome X and its association with high circulating levels of asymmetric dimethylarginine. *Atherosclerosis*. 2009;**204**:e82-e85. DOI: 10.1016/j.atherosclerosis.2008.09.029

[37] Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. *Lancet*. 2001;**358**:2113-2117. DOI: 10.1016/S0140-6736(01)07217-8

[38] Kielstein JT, Boger RH, Bode-Boger SM, Frolich JC, Haller H, Ritz E, et al. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. *Journal of the American Society of Nephrology*. 2002;**13**:170-176

[39] Fliser D, Kronenberg F, Kielstein JT, Morath C, Bode-Boger SM, Haller H, et al. Asymmetric dimethylarginine and progression of chronic kidney disease: The mild to moderate kidney disease study. *Journal of the American Society of Nephrology*. 2005;**16**:2456-2461. DOI: 10.1681/ASN.2005020179

[40] Schmidt R, Fazekas F, Hayn M, Schmidt H, Kapeller P, Roob G, et al. Risk factors for microangiopathy-related cerebral damage in the Austrian stroke prevention study. *Journal of the Neurological Sciences*. 1997;**152**:15-21. DOI: 10.1016/S0022-510X(97)00137-8

- [41] Englund E. Neuropathology of white matter lesions in vascular cognitive impairment. *Cerebrovascular Diseases*. 2002;**13**(Suppl 2):11-15. DOI: 10.1159/000049144
- [42] Shibata H, Nabika T, Moriyama H, Masuda J, Kobayashi S. Correlation of NO metabolites and 8-iso-prostaglandin F₂ α with periventricular hyperintensity severity. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2004;**24**:1659-1663. DOI: 10.1161/01.ATV.0000137415.67349.3c
- [43] Wright CB, Paik MC, Brown TR, Stabler SP, Allen RH, Sacco RL, et al. Total homocysteine is associated with white matter hyperintensity volume: The Northern Manhattan Study. *Stroke*. 2005;**36**:1207-1211. DOI: 10.1161/01.STR.0000165923.02318.22
- [44] Hassan A, Gormley K, O'Sullivan M, Knight J, Sham P, Vallance P, et al. Endothelial nitric oxide gene haplotypes and risk of cerebral small-vessel disease. *Stroke*. 2004;**35**:654-659. DOI: 10.1161/01.STR.0000117238.75736.53
- [45] Nasreen S, Nabika T, Shibata H, Moriyama H, Yamashita K, Masuda J, et al. T-786C polymorphism in endothelial NO synthase gene affects cerebral circulation in smokers: Possible gene-environmental interaction. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2002;**22**:605-610. DOI: 10.1161/01.ATV.20.9.2032
- [46] Neufeld AH. Pharmacologic neuroprotection with an inhibitor of nitric oxide synthase for the treatment of glaucoma. *Brain Research Bulletin*. 2004;**62**:455-459. DOI: 10.1016/j.brainresbull.2003.07.005
- [47] Kuo SC, Lee FY, Teng CM. 1-(Substituted benzyl)-3-(substituted aryl)-condensed pyrazole derivatives and processes of making the same. US Patent 5574168
- [48] Lee FY, Lien JC, Huang LJ, Huang TM, Tsai SC, Teng CM, et al. Synthesis of 1-benzyl-3-(5'-hydroxymethyl-2'-furyl)indazole analogues as novel antiplatelet agents. *Journal of Medicinal Chemistry*. 2001;**44**:3746-3749. DOI: 10.1021/jm010001h
- [49] Ko FN, Wu CC, Kuo SC, Lee FY, Teng CM. YC-1, a novel activator of platelet guanylate cyclase. *Blood*. 1994;**84**:4226-4233
- [50] Wu CC, Ko FN, Kuo SC, Lee FY, Teng CM. YC-1 inhibited human platelet aggregation through NO-independent activation of soluble guanylate cyclase. *British Journal of Pharmacology*. 1995;**116**:1973-1978. DOI: 10.1111/j.1476-5381.1995.tb16400.x
- [51] Stasch JP, Hobbs AJ. NO-independent, haem-dependent soluble guanylate cyclase stimulators. *Handbook of Experimental Pharmacology*. 2009:277-308. DOI: 10.1007/978-3-540-68964-5_13
- [52] Friebe A, Schultz G, Koesling D. Sensitizing soluble guanylyl cyclase to become a highly CO-sensitive enzyme. *EMBO Journal*. 1996;**15**:6863-6868
- [53] Martin E, Lee YC, Murad F. YC-1 activation of human soluble guanylyl cyclase has both heme-dependent and heme-independent components. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**:12938-12942. DOI: 10.1073/pnas.231486198
- [54] Schmidt K, Schrammel A, Koesling D, Mayer B. Molecular mechanisms involved in the synergistic activation of soluble guanylyl cyclase by YC-1 and nitric oxide in endothelial cells. *Molecular Pharmacology*. 2001;**59**:220-224. DOI: 10.1124/mol.59.2.220
- [55] Stasch JP, Becker EM, Alonso-Alija C, Apeler H, Dembowski K, Feurer A, et al.

NO-independent regulatory site on soluble guanylate cyclase. *Nature*. 2001;**410**:212-215. DOI: 10.1038/35065611

[56] Mulsch A, Bauersachs J, Schafer A, Stasch JP, Kast R, Busse R. Effect of YC-1, an NO-independent, superoxide-sensitive stimulator of soluble guanylyl cyclase, on smooth muscle responsiveness to nitrovasodilators. *British Journal of Pharmacology*. 1997;**120**:681-689. DOI: 10.1038/sj.bjp.0700982

[57] Wohlfart P, Malinski T, Ruetten H, Schindler U, Linz W, Schoenafinger K, et al. Release of nitric oxide from endothelial cells stimulated by YC-1, an activator of soluble guanylyl cyclase. *British Journal of Pharmacology*. 1999;**128**:1316-1322. DOI: 10.1038/sj.bjp.0702921

[58] Friebe A, Mullershausen F, Smolenski A, Walter U, Schultz G, Koesling D. YC-1 potentiates nitric oxide- and carbon monoxide-induced cyclic GMP effects in human platelets. *Molecular Pharmacology*. 1998;**54**:962-967. DOI: 10.1124/mol.54.6.962

[59] Galle J, Zabel U, Hubner U, Hatzelmann A, Wagner B, Wanner C, et al. Effects of the soluble guanylyl cyclase activator, YC-1, on vascular tone, cyclic GMP levels and phosphodiesterase activity. *British Journal of Pharmacology*. 1999;**127**:195-203. DOI: 10.1038/sj.bjp.0702495

[60] Aszódi A, Pfeifer A, Ahmad M, Glauner M, Zhou XH, Ny L, et al. The vasodilator-stimulated phosphoprotein (VASP) is involved in cGMP- and cAMP-mediated inhibition of agonist-induced platelet aggregation, but is dispensable for smooth muscle function. *EMBO Journal*. 1999;**18**:37-48. DOI: 10.1093/emboj/18.1.37

[61] Becker EM, Schmidt P, Schramm M, Schroder H, Walter U, Hoenicka M, et al. The vasodilator-stimulated

phosphoprotein (VASP): Target of YC-1 and nitric oxide effects in human and rat platelets. *Journal of Cardiovascular Pharmacology*. 2000;**35**:390-397. DOI: 10.1097/00005344-200003000-00007

[62] Yu SM, Cheng ZJ, Guh JH, Lee FY, Kuo SC. Mechanism of anti-proliferation caused by YC-1, an indazole derivative, in cultured rat A10 vascular smooth-muscle cells. *Biochemical Journal*. 1995;**306** (Pt 3):787-792. DOI: 10.1042/bj3060787

[63] Tulis DA, Durante W, Peyton KJ, Chapman GB, Evans AJ, Schafer AI. YC-1, a benzyl indazole derivative, stimulates vascular cGMP and inhibits neointima formation. *Biochemical and Biophysical Research Communications*. 2000;**279**:646-652. DOI: 10.1006/bbrc.2000.3942

[64] Wu CH, Chang WC, Chang GY, Kuo SC, Teng CM. The inhibitory mechanism of YC-1, a benzyl indazole, on smooth muscle cell proliferation: An in vitro and in vivo study. *Journal of Pharmacological Sciences*. 2004;**94**:252-260. DOI: 10.1254/jphs.94.252

[65] Lincoln TM, Dey NB, Boerth NJ, Cornwell TL, Soff GA. Nitric oxide-cyclic GMP pathway regulates vascular smooth muscle cell phenotypic modulation: Implications in vascular diseases. *Acta Physiologica Scandinavica*. 1998;**164**:507-515. DOI: 10.1111/j.1365-201X.1998.tb10700.x

[66] Kaul S, Cercek B, Rengstrom J, Xu XP, Molloy MD, Dimayuga P, et al. Polymeric-based perivascular delivery of a nitric oxide donor inhibits intimal thickening after balloon denudation arterial injury: Role of nuclear factor-kappaB. *Journal of the American College of Cardiology*. 2000;**35**:493-501. DOI: 10.1016/S0735-1097(99)00543-4

[67] Liu YN, Pan SL, Peng CY, Guh JH, Huang DM, Chang YL, et al. YC-1

[3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole] inhibits neointima formation in balloon-injured rat carotid through suppression of expressions and activities of matrix metalloproteinases 2 and 9. *Journal of Pharmacology and Experimental Therapeutics*. 2006;**316**:35-41. DOI: 10.1124/jpet.105.090563

[68] Liu YN, Pan SL, Peng CY, Huang DY, Guh JH, Kuo SC, et al. YC-1 induces heat shock protein 70 expression and prevents oxidized LDL-mediated apoptosis in vascular smooth muscle cells. *Shock*. 2008;**30**:274-279. DOI: 10.1097/shk.0b013e318162c63a

[69] Lam KK, Cheng PY, Lee YM, Liu YP, Ding C, Liu WH, et al. The role of heat shock protein 70 in the protective effect of YC-1 on heat stroke rats. *European Journal of Pharmacology*. 2013;**699**: 67-73. DOI: 10.1016/j.ejphar.2012.11.044

[70] Keswani AN, Peyton KJ, Durante W, Schafer AI, Tulis DA. The cyclic GMP modulators YC-1 and zaprinast reduce vessel remodeling through antiproliferative and proapoptotic effects. *Journal of Cardiovascular Pharmacology and Therapeutics*. 2009;**14**:116-124. DOI: 10.1177/1074248409333266

[71] Hsu HK, Juan SH, Ho PY, Liang YC, Lin CH, Teng CM, et al. YC-1 inhibits proliferation of human vascular endothelial cells through a cyclic GMP-independent pathway. *Biochemical Pharmacology*. 2003;**66**:263-271. DOI: 10.1016/S0006-2952(03)00244-2

[72] Huh JW, Kim SY, Lee JH, Lee YS. YC-1 attenuates hypoxia-induced pulmonary arterial hypertension in mice. *Pulmonary Pharmacology & Therapeutics*. 2011;**24**:638-646. DOI: 10.1016/j.pupt.2011.09.003

[73] Chun YS, Yeo EJ, Choi E, Teng CM, Bae JM, Kim MS, et al. Inhibitory effect of YC-1 on the hypoxic induction

of erythropoietin and vascular endothelial growth factor in Hep3B cells. *Biochemical Pharmacology*. 2001;**61**:947-954. DOI: 10.1016/S0006-2952(01)00564-0

[74] Shin DH, Kim JH, Jung YJ, Kim KE, Jeong JM, Chun YS, et al. Preclinical evaluation of YC-1, a HIF inhibitor, for the prevention of tumor spreading. *Cancer Letters*. 2007;**255**:107-116. DOI: 10.1016/j.canlet.2007.03.026

[75] Sun HL, Liu YN, Huang YT, Pan SL, Huang DY, Guh JH, et al. YC-1 inhibits HIF-1 expression in prostate cancer cells: Contribution of Akt/NF-kappaB signaling to HIF-1alpha accumulation during hypoxia. *Oncogene*. 2007;**26**:3941-3951. DOI: 10.1038/sj.onc.1210169

[76] Zhao Q, Du J, Gu H, Teng X, Zhang Q, Qin H, et al. Effects of YC-1 on hypoxia-inducible factor 1-driven transcription activity, cell proliferative vitality, and apoptosis in hypoxic human pancreatic cancer cells. *Pancreas*. 2007;**34**:242-247. DOI: 10.1097/01.mpa.0000250135.95144.b6

[77] Moon SY, Chang HW, Roh JL, Kim GC, Choi SH, Lee SW, et al. Using YC-1 to overcome the radioresistance of hypoxic cancer cells. *Oral Oncology*. 2009;**45**:915-919. DOI: 10.1016/j.oraloncology.2009.04.005

[78] Lau CK, Yang ZF, Lam CT, Tam KH, Poon RT, Fan ST. Suppression of hypoxia inducible factor-1alpha (HIF-1alpha) by YC-1 is dependent on murine double minute 2 (Mdm2). *Biochemical and Biophysical Research Communications*. 2006;**348**:1443-1448. DOI: 10.1016/j.bbrc.2006.08.015

[79] Wakiyama K, Kitajima Y, Tanaka T, Kaneki M, Yanagihara K, Aishima S, et al. Low-dose YC-1 combined with glucose and insulin selectively induces apoptosis in hypoxic gastric carcinoma cells by inhibiting anaerobic glycolysis.

Scientific Reports. 2017;7:12653. DOI: 10.1038/s41598-017-12929-9

[80] Lau CK, Yang ZF, Lam SP, Lam CT, Ngai P, Tam KH, et al. Inhibition of Stat3 activity by YC-1 enhances chemo-sensitivity in hepatocellular carcinoma. *Cancer Biology & Therapy*. 2007;6:1900-1907. DOI: 10.4161/cbt.6.12.4970

[81] Lee SJ, Kim YJ, Lee CS, Bae J. Combined application of camptothecin and the guanylate cyclase activator YC-1: Impact on cell death and apoptosis-related proteins in ovarian carcinoma cell lines. *Chemico-Biological Interactions*. 2009;181:185-192. DOI: 10.1016/j.cbi.2009.05.013

[82] Huang YT, Pan SL, Guh JH, Chang YL, Lee FY, Kuo SC, et al. YC-1 suppresses constitutive nuclear factor-kappaB activation and induces apoptosis in human prostate cancer cells. *Molecular Cancer Therapeutics*. 2005;4:1628-1635. DOI: 10.1158/1535-7163.MCT-05-0090

[83] Pan SL, Guh JH, Peng CY, Wang SW, Chang YL, Cheng FC, et al. YC-1 [3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole] inhibits endothelial cell functions induced by angiogenic factors in vitro and angiogenesis in vivo models. *Journal of Pharmacology and Experimental Therapeutics*. 2005;314:35-42. DOI: 10.1124/jpet.105.085126

[84] Wang SW, Pan SL, Guh JH, Chen HL, Huang DM, Chang YL, et al. YC-1 [3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole] exhibits a novel antiproliferative effect and arrests the cell cycle in G0-G1 in human hepatocellular carcinoma cells. *Journal of Pharmacology and Experimental Therapeutics*. 2005;312:917-925. DOI: 10.1124/jpet.104.077230

[85] Yeo EJ, Ryu JH, Chun YS, Cho YS, Jang IJ, Cho H, et al. YC-1 induces S cell cycle arrest and apoptosis by activating

checkpoint kinases. *Cancer Research*. 2006;66:6345-6352. DOI: 10.1158/0008-5472.CAN-05-4460

[86] Lee MR, Lin C, Lu CC, Kuo SC, Tsao JW, Juan YN, et al. YC-1 induces G0/G1 phase arrest and mitochondria-dependent apoptosis in cisplatin-resistant human oral cancer CAR cells. *Biomedicine (Taipei)*. 2017;7:12. DOI: 10.1051/bmdcn/2017070205

[87] Wu SY, Pan SL, Chen TH, Liao CH, Huang DY, Guh JH, et al. YC-1 induces apoptosis of human renal carcinoma A498 cells in vitro and in vivo through activation of the JNK pathway. *British Journal of Pharmacology*. 2008;155:505-513. DOI: 10.1038/bjp.2008.292

[88] Hong B, Lui VW, Hui EP, Lu Y, Leung HS, Wong EY, et al. Reverse phase protein array identifies novel anti-invasion mechanisms of YC-1. *Biochemical Pharmacology*. 2010;79:842-852. DOI: 10.1016/j.bcp.2009.10.021

[89] Zhao W, Li X, Li Z. Combination therapy with local radiofrequency ablation and YC-1 inhibits the proliferation and metastasis of hepatocellular carcinoma through activating beta-catenin signaling. *Die Pharmazie*. 2016;71:524-529. DOI: 10.1691/ph.2016.6602

[90] Hung CC, Liou HH. YC-1, a novel potential anticancer agent, inhibit multidrug-resistant protein via cGMP-dependent pathway. *Investigational New Drugs*. 2011;29:1337-1346. DOI: 10.1007/s10637-010-9496-1

[91] Garthwaite G, Goodwin DA, Batchelor AM, Leeming K, Garthwaite J. Nitric oxide toxicity in CNS white matter: An in vitro study using rat optic nerve. *Neuroscience*. 2002;109:145-155. DOI: 10.1016/S0306-4522(01)00447-X

[92] Garthwaite G, Goodwin DA, Neale S, Riddall D, Garthwaite J. Soluble guanylyl

cyclase activator YC-1 protects white matter axons from nitric oxide toxicity and metabolic stress, probably through Na(+) channel inhibition. *Molecular Pharmacology*. 2002;**61**:97-104. DOI: 10.1124/mol.61.1.97

[93] Lu DY, Tang CH, Liou HC, Teng CM, Jeng KC, Kuo SC, et al. YC-1 attenuates LPS-induced proinflammatory responses and activation of nuclear factor-kappaB in microglia. *British Journal of Pharmacology*. 2007;**151**:396-405. DOI: 10.1038/sj.bjp.0707187

[94] Chien WL, Liang KC, Teng CM, Kuo SC, Lee FY, Fu WM. Enhancement of learning behaviour by a potent nitric oxide-guanylate cyclase activator YC-1. *European Journal of Neuroscience*. 2005;**21**:1679-1688. DOI: 10.1111/j.1460-9568.2005.03993.x

[95] Komsuoglu Celikyurt I, Utkan T, Ozer C, Gacar N, Aricioglu F. Effects of YC-1 on learning and memory functions of aged rats. *Medical Science Monitor Basic Research*. 2014;**20**:130-137. DOI: 10.12659/MSMBR.891064

[96] Yeh WL, Lu DY, Lin CJ, Liou HC, Fu WM. Inhibition of hypoxia-induced increase of blood-brain barrier permeability by YC-1 through the antagonism of HIF-1alpha accumulation and VEGF expression. *Molecular Pharmacology*. 2007;**72**:440-449. DOI: 10.1124/mol.107.036418

[97] Wang X, Liu C, Wu L, Zhu S. Potent ameliorating effect of hypoxia-inducible factor 1alpha (HIF-1alpha) antagonist YC-1 on combined allergic rhinitis and asthma syndrome (CARAS) in rats. *European Journal of Pharmacology*. 2016;**788**:343-350. DOI: 10.1016/j.ejphar.2016.07.040

[98] Pan SL, Guh JH, Peng CY, Chang YL, Cheng FC, Chang JH, et al. A potential role of YC-1 on the inhibition of cytokine release

in peripheral blood mononuclear leukocytes and endotoxemic mouse models. *Thrombosis and Haemostasis*. 2005;**93**:940-948. DOI: 10.1267/THRO05050940

[99] Song SJ, Chung H, Yu HG. Inhibitory effect of YC-1, 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole, on experimental choroidal neovascularization in rat. *Ophthalmic Research*. 2008;**40**:35-40. DOI: 10.1159/000111157

[100] Deniro M, Al-Halafi A, Al-Mohanna FH, Alsmadi O, Al-Mohanna FA. Pleiotropic effects of YC-1 selectively inhibits pathological retinal neovascularization and promotes physiological revascularization in a mouse model of oxygen-induced retinopathy. *Molecular Pharmacology*. 2009;**77**:348-367. DOI: 10.1124/mol.109.061366

[101] Yan Z, An J, Shang Q, Zhou N, Ma J. YC-1 inhibits VEGF and inflammatory mediators expression on experimental central retinal vein occlusion in rhesus monkey. *Current Eye Research*. 2018;**43**:526-533. DOI: 10.1080/02713683.2018.1426102

[102] Wang JW, Yeh CB, Chou SJ, Lu KC, Chu TH, Chen WY, et al. YC-1 alleviates bone loss in ovariectomized rats by inhibiting bone resorption and inducing extrinsic apoptosis in osteoclasts. *Journal of Bone and Mineral Metabolism*. 2018;**36**:508-518. DOI: 10.1007/s00774-017-0866-z

[103] Lee TY, Leu YL, Wen CK. Modulation of HIF-1alpha and STAT3 signaling contributes to anti-angiogenic effect of YC-1 in mice with liver fibrosis. *Oncotarget*. 2017;**8**:86206-86216. DOI: 10.18632/oncotarget.21039

[104] Xiao J, Jin C, Liu Z, Guo S, Zhang X, Zhou X, et al. The design, synthesis, and biological evaluation of novel

YC-1 derivatives as potent anti-hepatic fibrosis agents. *Organic & Biomolecular Chemistry*. 2015;**13**:7257-7264. DOI: 10.1039/c5ob00710k

[105] Teng CM, Wu CC, Ko FN, Lee FY, Kuo SC. YC-1, a nitric oxide-independent activator of soluble guanylate cyclase, inhibits platelet-rich thrombosis in mice. *European Journal of Pharmacology*. 1997;**320**:161-166. DOI: 10.1016/S0014-2999(96)00911-9

[106] Belik J. Riociguat, an oral soluble guanylate cyclase stimulator for the treatment of pulmonary hypertension. *Current Opinion in Investigational Drugs*. 2009;**10**:971-979

[107] Grimminger F, Weimann G, Frey R, Voswinckel R, Thamm M, Bolkow D, et al. First acute haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension. *European Respiratory Journal*. 2009;**33**:785-792. DOI: 10.1183/09031936.00039808

[108] Rothermund L, Friebe A, Paul M, Koesling D, Kreutz R. Acute blood pressure effects of YC-1-induced activation of soluble guanylyl cyclase in normotensive and hypertensive rats. *British Journal of Pharmacology*. 2000;**130**:205-208. DOI: 10.1038/sj.bjp.0703320

[109] Chien WL, Liang KC, Fu WM. Enhancement of active shuttle avoidance response by the NO-cGMP-PKG activator YC-1. *European Journal of Pharmacology*. 2008;**590**:233-240. DOI: 10.1016/j.ejphar.2008.06.040

[110] Hwang TL, Wu CC, Teng CM. YC-1 potentiates nitric oxide-induced relaxation in guinea-pig trachea. *British Journal of Pharmacology*. 1999;**128**: 577-584. DOI: 10.1038/sj.bjp.0702830