Review

DOI: 10.5582/ddt.2016.01067

Nitric oxide donor hybrid compounds as promising anticancer agents

Qin-ge Ding, Jie Zang, Shuai Gao, Qianwen Gao, Wenwen Duan, Xiaoyang Li, Wenfang Xu*, Yingjie Zhang*

Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Ji'nan, Shandong, China.

Summary **Nitric oxide (NO) plays important roles in cardiovascular regulation, nerve transmission delivery and immune responses. In the last semicenturry, it has been proved that though low concentration of NO is tumor-promoting, high concentration of NO could exhibit multiple antitumor effects, which led to the research and development of kinds of NO donors and NO donor hybrid compounds as antitumor agents. Herein, the recent development of NO donor hybrid compounds is briefly reviewed.**

Keywords: NO releasing agents, antitumor, NOSs, furoxan, NO-NSAIDs

1. Introduction

Nitric oxide (NO), identified in the 1980s as a vasoactive small molecule, can regulate various pathological and physiological processes in cardiovascular, nerve transmission delivery and immune systems (*1,2*). Besides, NO also plays roles in other physiological functions such as cellular redox and anti-pathogenic responses (*3-6*).

The functions of NO are dependent on the interaction with cell factors. Two signal pathways have been discussed for mechanisms of NO. In the NO/sGC/cGMP pathway, endogenous NO synthesized by NO synthases (NOSs) activates soluble guanylate cyclase (sGC) in cells such as muscles, neurons and leukocytes. Active sGC catalyzes the synthesis of cyclic GMP (cGMP) (*7*). Then cGMP activates three effector molecules: the cGMP-dependent protein kinase G (PKG), cGMP-regulated phosphodiesterases, and cGMP-gated ion channels (*8*). These effectors lead to a cascade of effects including smooth muscle relaxation, inflammatory pain and platelet anticoagulation (*9*). In the cGMP-independent pathway, the functions of NO are based on NO-mediated protein modification, including *i*) binding to metal centers; *ii*) nitrosylation of thiol and amine groups; *iii*) nitration of tyrosine, tryptophan, amine, carboxylic acid, and phenylalanine groups; and *iv*) oxidation of thiols (both cysteine and methionine residues) and tyrosine (*10*).

2. NO-donor hybrid compounds as anti-cancer agents

Because of the promising antitumor effects of NO (*11*), numerous NO-releasing agents (also called NO-donors) have been developed as antitumor agents, including organic nitrates, synthetic peroxynitrite (*12*), 3-morpholinosydnonimine (*13*), furoxans and benzofuroxans and hydroxylamines (*14*). Increasing research showed that NO donors were effective on various malignant tumors, such as myeloma, breast cancer, ovarian cancer, prostate cancer and pancreatic cancer (*15-17*). Moreover, it is worth noting that more and more NO-donor hybrids have been designed, synthesized and evaluated as antitumor agents (Table 1), which will be discussed in the following part of this review.

2.1. *NO-nucleoside hybrids*

Novel NO-releasing 5-FU hybrid (compound 1a, Figure 1) was designed by Cai TB *et al*. with an aim to reduce the toxicity of nucleoside agents. This hybrid showed stronger cytotoxicity on tumor cells and less toxicity

Released online in J-STAGE as advance publication December 18, 2016.

**Address correspondence to:*
Dr. Yingjie Zhang and Dr. Wenfang Xu, Department of Medicinal Chemistry, School of Pharmacy, Shandong University, 44, West Culture Road, 250012, Ji'nan, Shandong, China.
E-mail: zhangyingjie@sde.edu.cn

Table 1. NO-donor hybrids dependent on different antitumor agents

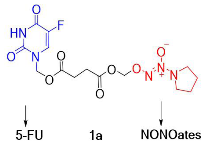
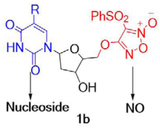
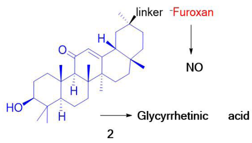
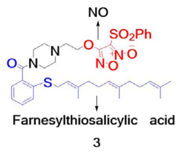
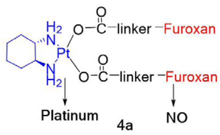
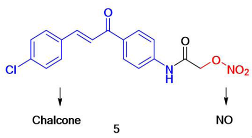
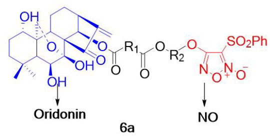
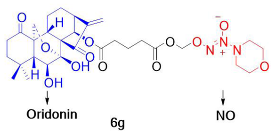
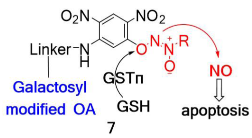
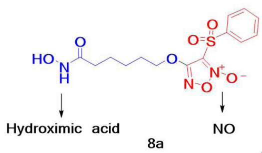
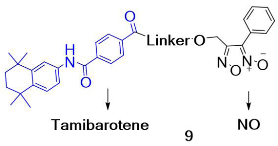
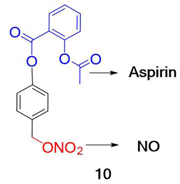
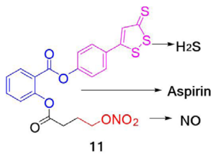
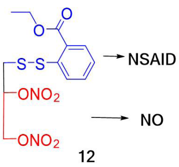
Compound NO.	NO-donor type	Ligand agents	Structure	Cell lines	*Ref.*
1a	NONOates	5-FU		DU-145, HeLa	*18*
1b	Furoxan	Nucleoside		143B, EMT-6, KBALB-STK, KBALB, 143B-LTK, Hs578Bst	*19*
2	Furoxan	Glycyrrhetinic acid		HCC cells	*20*
3	Furoxan	Farnesylthiosalicylic acid (FTS)		HCC cells	*21*
4	Furoxan	Platinum		SGC-7901,MCF-7, HepG2, HCT-116	*23,24*
5	Furoxan	Chalcone		60 human tumor cell lines: molt-4, HL-60, A549…	*25,26*
6	Furoxan	Oridonin		K562, MGC-803, Bel-7402	*28,29*
	NONOates	Oridonin		Bel-7402	*30*
7	Diazeniumdiolate	Oleanolic Acid		HepG2, HCC, H22.	*31*
8	Furoxan	HDACis		HEL	*35,36*
9	Furoxan	Tamibarotene		NB4, HL-60	*37*

Table 1. NO-donor hybrids dependent on different antitumor agents (continued)

Compound NO.	NO-donor type	Ligand agents	Structure	Cell lines	*Ref.*
10	Nitrate ester	Aspirin		HT 29	*40*
11	Nitrate ester	H2S- Aspirin		HT-29	*41,42*
12	Nitrate ester	NSAID		RKO, Caco-2, HT-29, RAW 264.7, SW480	*43*

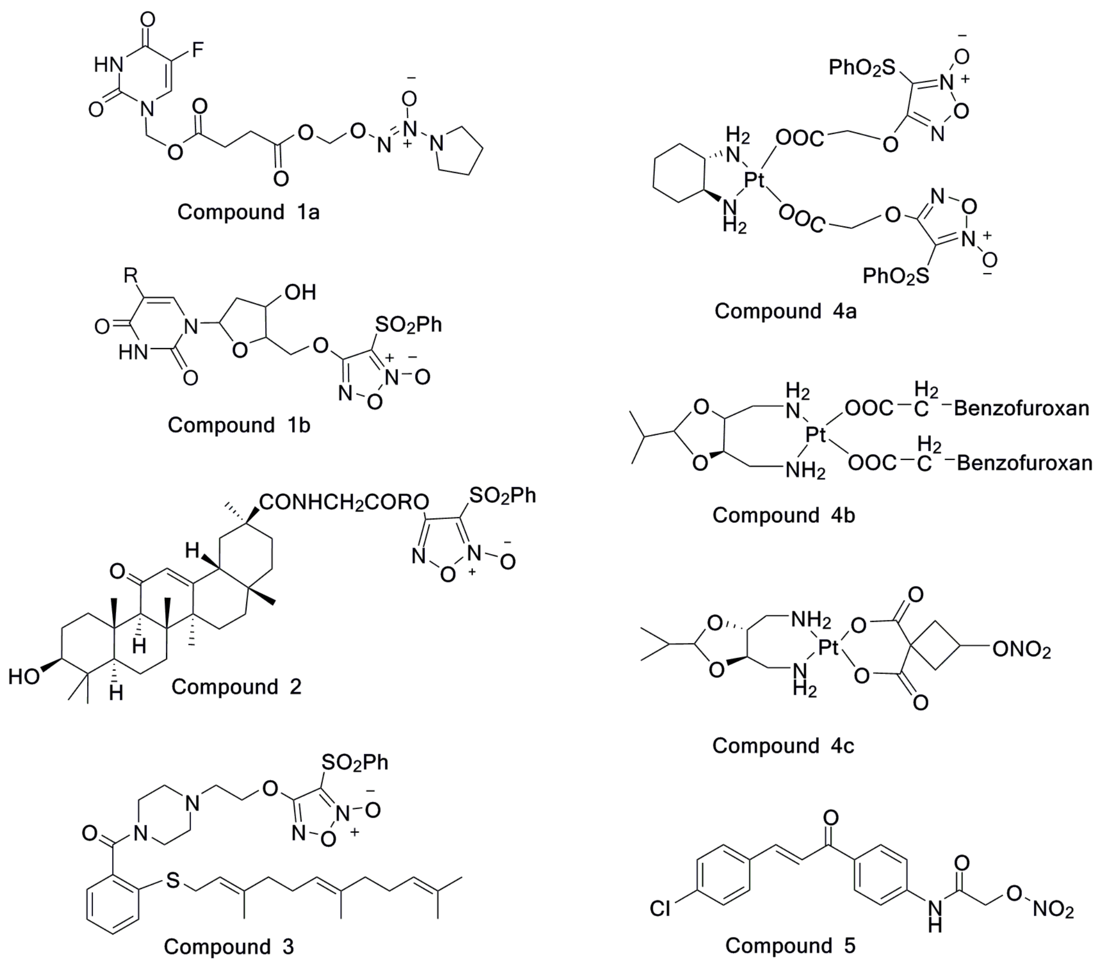


Figure 1. The structure of NO donor hybrids with chemotherapeutic drugs (compound 1-5).

on normal cells than its parent agent 5-FU in prostate and HeLa cancer cells (*18*). The latter research from Moharram S *et al*. showed that a NO-nucleoside hybrid (compound 1b, Figure 1) with the ability to release NO and nucleoside simultaneously leading to high cellular cytotoxic effects by inducing DNA alkylation (*19*).

2.2. *The GA-NO-donor hybrids*

The glycyrrhetinic acid (GA) and its derivative glycyrrhizin acid both showed protective activity on hepatocytes. Dependent on the researches of Lai Y *et al*., the hybrid (compound 2) of glycyrrhetinic acid and furoxan (Figure 1) showed selective NO-releasing activity in HCC cells (*20*). Compound 2 induced selective cytotoxicity against human HCC cells with little untoward effect on normal hepatocytes due to the cytotoxicity of NO against liver tumor cells and the protective effects of GA on normal hepatocytes.

2.3. *Nitric oxide-releasing derivatives of farnesylthiosalicylic acid*

Farnesylthiosalicylic acid (FTS) was a Ras inhibitor that inhibited tumor cell proliferation. But its therapeutic efficacy was limited because of its high cytotoxicity on normal cells (*21*).

A combination of FTS and furoxan led to a kind of furoxan hybrid derivative 3 (Figure 1), which showed highly selective cytotoxicity against HCC through cooperative effects of high levels of NO and FTS but not in normal liver cells. The evaluation of phosphorylation of AKT and ERK showed that compound 3 induced stronger inhibition of Akt/ERK phosphorylation than FTS due to the production of NO (*21*).

2.4. *NO donors-Pt hybrids*

Platinum-based antitumor drugs, such as cisplatin, carboplatin and oxaliplatin have been widely used in clinical cancer therapy (*22*). The research of

Zhao J *et al*. found that hybrids of furoxan and Pt (4a, 4b, 4c, Figure 1) were more effective against gastric carcinoma cell line SGC-7901 and colonic carcinoma cell line HCT-116 *in vitro* compared with carboplatin, oxaliplatin alone or a combination of oxaliplatin and furoxan (*23*). Moreover, it was demonstrated that the hybrids had better stability and less adverse effects than Platinum agents (*24*).

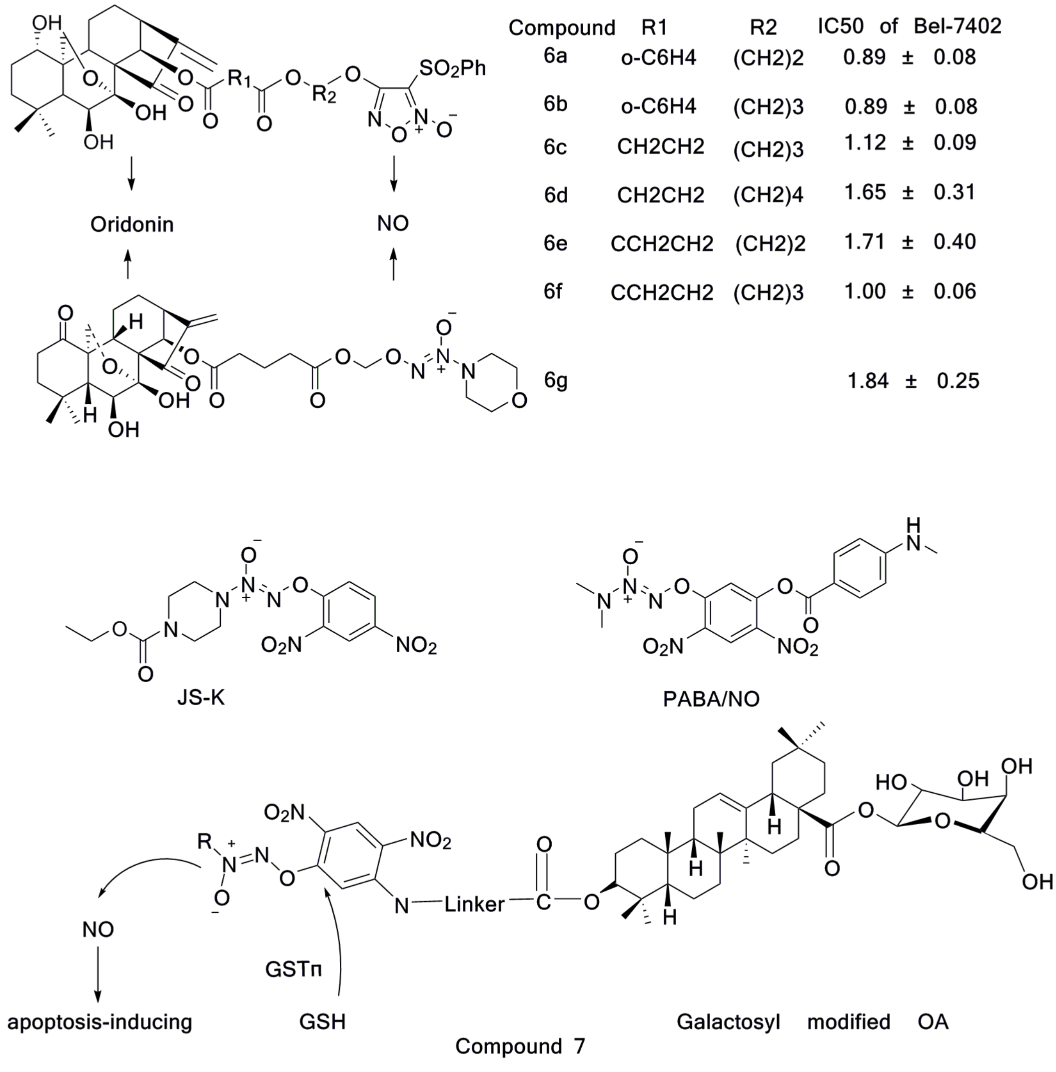


Figure 2. The structure of furoxan-oridonin (compound 6) and NO -OA hybrids (compound 7).

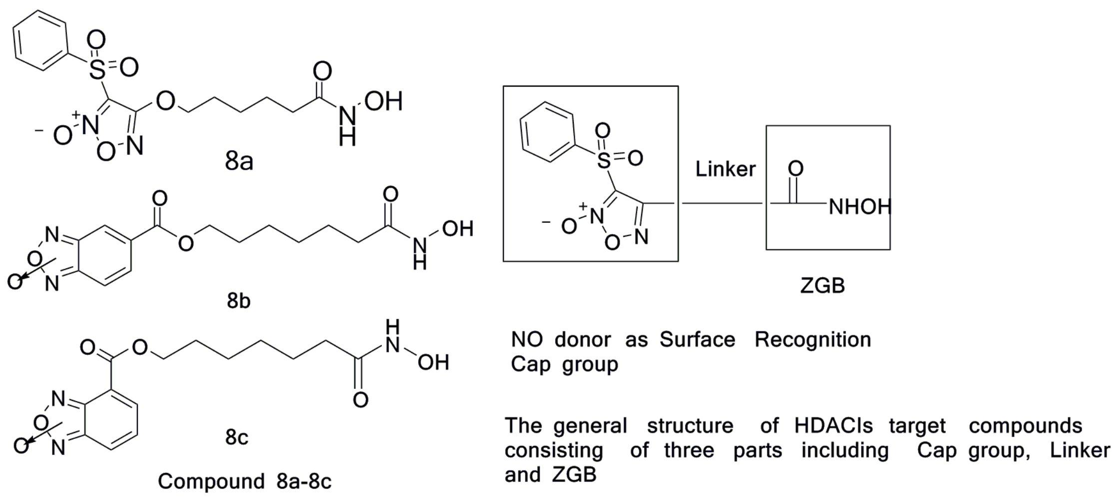


Figure 3. The structure of NO-HDACis (compound 8) and general structure of HDACis.

2.5. *NO donors-Pt hybrids*

The NO-chalcone hybrid compound 5 (Figure 1) exhibited significant activity against colorectal and melanoma cancers (*25*). The nitrate ester chimaera 5 exhibited moderate broad-spectrum antitumor activity against nine kinds of tumors, and showed high selectivity toward colon cancer, which proved that a NO donor could enhance selectivity of chalcone (*26*).

2.6. *NO-Oridonin hybrids*

Oridonin is a commercially available natural diterpenoid, and it has attracted much more attentions because of its anti-tumor activity (*27*). Li D *et al*. demonstrated that furoxan-oridonin hybrids showed improved anti-proliferative activity against several tumor cell lines due to their NO-releasing ability (*28*). The hybrids showed stronger activity when R1 was an aromatic group (6a, 6b, Figure 2) rather than an alkyl group. The anti-proliferative activity was stronger when R2 contained three carbons (*29*).

Dependent on previous studies, researchers synthesized a series of novel NO-releasing oridonin derivatives coupling diazeniumdiolates with oridonin (*30*). The hybrids inhibited tumor cell proliferation with IC_{50} ranging from 1.84 to 17.01 μM. The antitumor activity was positively correlated to the NO releasing ability of these hybrids. Stimulated by the antitumor properties of compounds 6a-6f, the hybrid 6g was a synthesized combination of oridonin and diazeniumdiolate, which exhibited stronger inhibition of Bel-7402 cells than oridonin, diazeniumdiolates and their combination after 72 h treatment (*30*).

2.7. *NO -OA hybrids*

Fu J *et al*. synthesized a series of hybrids based on oleanolic acid (OA) and O_2-(2,4-dinitrophenyl)-diazeniumdiolate (*31*). It was anticipated that those hybrids could exhibit selective cytotoxicity to tumor cells because they could only be activated to release NO in GSTπ overexpressed tumor cells. It was revealed that compound 7, which was much more stable than JS-K and PABA/NO in the absence of GSTπ (Figure 2), exhibited strong antitumor potency and low toxicity *in vivo* (*32*). Treatment with compound 7 (38.3 μM/kg) inhibited H22 tumor growth stronger than 5-FU (153.8 μM/kg).

2.8. *NO-HDACIs*

Histone deacetylases inhibitors (HDACIs) are a family of compounds that could induce cancer cell cycle arrest, differentiation, and apoptosis (*33,34*). Several HDAC inhibitors (HDACIs) have been recently approved by the FDA or CFDA for cancer therapy, including SAHA, LBH589, PXD101 and chidamide for cancer therapy.

The classical structure of HDACIs consists of three parts: surface recognition domain, linker and zinc binding group (ZBG). Our laboratory integrated NO donors into the surface recognition domain of HDACIs to get two series of NO-donor HDACI hybrids with favorable anti-tumor activity (Figure 3) (*35,36*). The NO-donor of 8a is furoxan and the NO-donor of 8b and 8c is benzofuroxan. The most potent compound 8a had better *in vitro* and *in vivo* antitumor activity against HEL than the approved HDAC inhibitor SAHA.

2.9. *NO-Tamibarotene hybrids*

Tamibarotene (AM80), approved in Japan as a selective RARα agonist, was used for relapsed or refractory acute promyelocytic leukemia (APL). Compared with all-trans retinoic acid (ATRA), tamibarotene could cause higher differentiation and lower drug resistance in APL

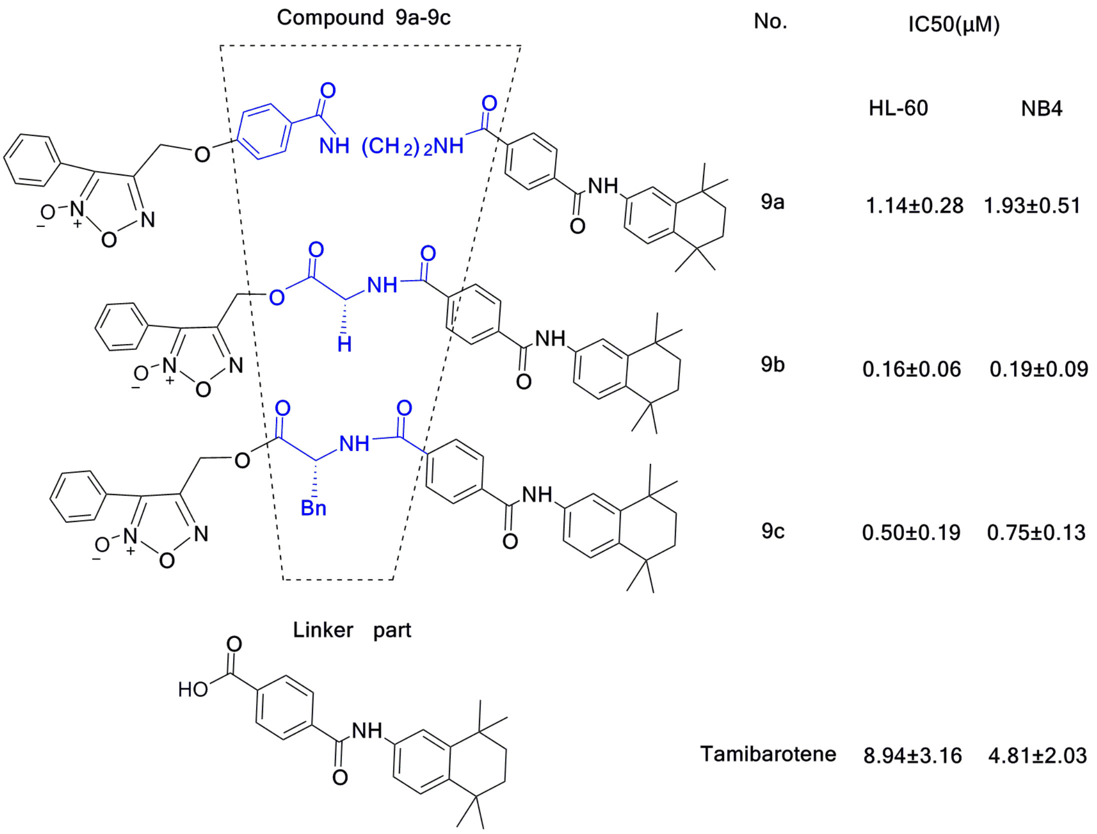


Figure 4. The structure of NO donor hybrids with tamibarotene (compound 9).

cells.

Our laboratory designed a series of novel tamibarotene derivatives by bridging tamibarotene with NO donors with various linkers (Figure 4) (*37*). The studies showed that these compounds (9a, 9b, 9c) exhibited stronger anti-leukemic activity than tamibarotene. Furthermore, the preliminary structure–activity relationships (SAR) analysis showed that biological activities could be enhanced by introduction of amino acids in the linker part of these hybrids.

2.10. *NO-aspirin*

Aspirin (ASA), a kind of non-steroidal anti-inflammatory drug (NSAIDs), showed potency in inhibiting colorectal cancer (*38*). Studies showed that the use of NO-releasing NSAIDs could reduce the gastrointestinal risk (*39*). Williams JL *et al*. synthesized a NO-NSAID hybrid (compound 10) combining aspirin and nitrate ester based NO donor (Figure 5), and compared its anti-proliferative activity in HT-29 colon tumor cells with NO-ibuprofen *in vitro* (*40*). Results showed that the IC_{50} values of compound 10 (NO-aspirin) and NO-ibuprofen were 1 μM and 42 μM, respectively, while the IC_{50} values of both aspirin and ibuprofen were over 1,000 μM. The subsequent mechanism studies showed that after 48 h treatment with 100 μM of NO-aspirin, PCNA (proliferating cell nuclear antigen) expression was reduced by 54.5% and more than 83.9% of tumor cells were blocked at G0/G1 phases. In conclusion, these data demonstrated that the NO-NSAIDs are more potent than traditional NSAIDs in anti-proliferation and apoptosis induction against colon cancer (*40*).

2.11. *NBS-1120*

NBS-1120 (compound 11, Figure 5), standing for a series of compounds which linked NO-donor nitrate ester and H2S-aspirin in one molecule, also called NOSH-aspirins, could release gasotransmitters NO and H2S simultaneously (*41*).

The lower recurrence of colon cancer and less adverse effects of 11 than parent agent aspirin were recognized. Compound 11 showed anti-inflammatory and anti-proliferative abilities on HT-29 colon tumor cells *in vitro*. It caused G0/G1 cell cycle arrest, inhibited tumor growth and increased apoptosis at high concentrations (*42*). In animal tests, compound 11 reduced tumor volume (96% reduction) and tumor mass (97% reduction) at 50 mg/kg, whereas aspirin inhibited tumor volume (70% reduction) and tumor mass (65% reduction) at the same dosage. Less lipid peroxidation and more SOD activity were observed in animals treated by 11. These effects might decrease the side effects of compound 11 in the gastric mucosal tissue.

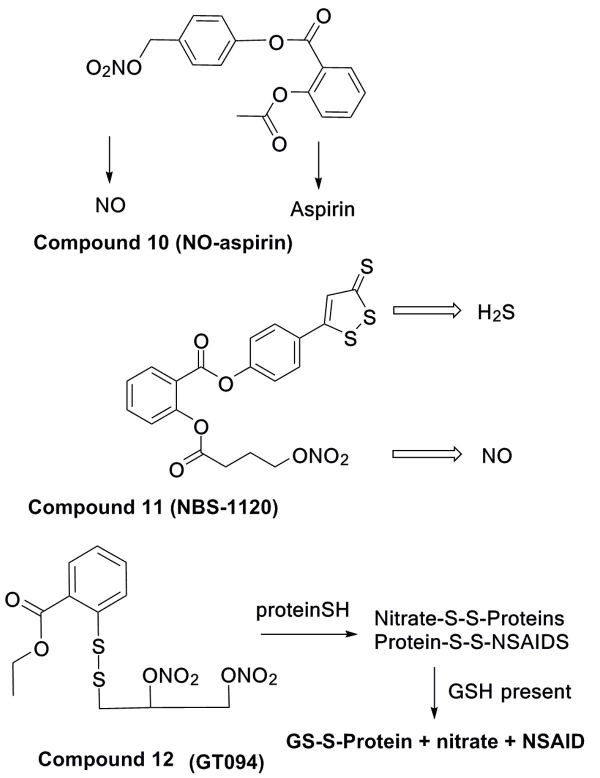


Figure 5. The structure of NO donor hybrids with NSAIDs (compound 10-12).

These results collectively showed that NOSH-aspirin derivatives were superior to aspirin in both efficacy and safety as chemotherapeutics.

2.12. *GT-094*

Compound 12 (GT-094) was a novel NO hybrid combining nitrate ester and NSAID *via* disulfide (Figure 5) (*43*). Studies showed that compound 12 inhibited tumor cell growth in both Caco-2 and HT-29 cells (*43*). Through a 28-week study, Hagos GK *et al*. found compound 12 could reduce weight and multiplicity of tumors in rats contrasted with the NO donor azoxymethane. Moreover, 12 could reduce iNOS expression more potently than azoxymethane alone.

Significant inhibition of proliferation has been shown in RKO and SW480 cancer cells after 24 h treatment with compound 12. Compound 12 down-regulated expressions of pro-survival genes such as hepatocyte growth factor receptor (c-Met), epidermal growth factor receptor (EGFR), Bcl-2, and vascular endothelial growth factor receptors (VEGFR1 and VEGFR1). It also decreased the overexpression of Sp1, Sp3 and Sp4 in colon cancer cells by down-regulating microRNA-27a (miR-27a) and up-regulating ZBTB10 (*44*).

3. Conclusion

It has been validated with increasing evidence that high concentration of NO could exhibit potent antitumor activities. Moreover, NO donors combined with other antitumor agents exhibited synergetic or additional antitumor effects, which stimulated research and development of NO donor hybrids as novel anticancer agents. Due to the multiple biological effects of NO in cardiovascular, nerve transmission and immune systems, further research should focus on characterizing the pharmacokinetics profiles and systemic toxicity of these NO donor hybrids to identify promising antitumor leads with higher potency and less side effects.

Acknowledgements

This work was supported by Major Project of Science and Technology of Shandong Province (2015ZDJS04001), Young Scholars Program of Shandong University (YSPSDU, Grant NO. 2016WLJH33).

References

1. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A. 1987; 84:9265-9269.
2. Ignarro LJ, Napoli C, Loscalzo J. Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide: An overview. Circul Res. 2002; 90:21-28.
3. Geller DA, Billiar TR. Molecular biology of nitric oxide synthases. Cancer Metastasis Rev. 1998; 17:7-23.
4. Quinn AC, Petros AJ, Vallance P. Nitric oxide: An endogenous gas. Br J Anaesth. 1995; 74:443-451.
5. Jones ML, Ganopolsky JG, Labbé A, Wahl C, Prakash S. Antimicrobial properties of nitric oxide and its application in antimicrobial formulations and medical devices. Appl Microbiol Biotechnol. 2010; 88:401-407.
6. Bogdan C. Nitric oxide and the immune response. Nat Immunol. 2001; 2:907-916.
7. Dangel O, Mergia E, Karlisch K, Groneberg D, Koesling D, Friebe A. Nitric oxide-sensitive guanylyl cyclase is the only nitric oxide receptor mediating platelet inhibition. J Thromb Haemost. 2010; 8:1343-1352.
8. Friebe A, Koesling D. Regulation of nitric oxide-sensitive guanylyl cyclase. Circul Res. 2003; 93:96-105.
9. Switzer CH, Cheng RY, Ridnour LA, Glynn SA, Ambs S, Wink DA. Ets-1 is a transcriptional mediator of oncogenic nitric oxide signaling in estrogen receptor-negative breast cancer. Breast Cancer Res. 2012; 14:1-13.
10. Gow AJ, Farkouh CR, Munson DA, Posencheg MA, Ischiropoulos H. Biological significance of nitric oxide-mediated protein modifications. Am J Physiol Lung Cell Mol Physiol. 2004; 287:262-268.
11. Mocellin S. Nitric oxide: Cancer target or anticancer agent? Curr Cancer Drug Targets. 2009; 9:214-236.
12. Robinson KM, Beckman JS. Synthesis of peroxynitrite from nitrite and hydrogen peroxide. Methods Enzymol. 2005; 396:207-214.
13. Feelisch M, Ostrowski J, Noack E. On the mechanism of NO release from sydnonimines. J Cardiovasc Pharmacol. 1989; 14 (Suppl 11):S13-S22.
14. Miranda KM, Dutton AS, Ridnour LA, Foreman CA,

Ford E, Paolocci N, Katori T, Tocchetti CG, Mancardi D, Thomas DD. Mechanism of aerobic decomposition of Angeli's salt (sodium trioxodinitrate) at physiological pH. J Am Chem Soc. 2005; 127:722-731.

15. Kiziltepe T. JS-K has potent anti-angiogenic activity *in vitro* and inhibits tumour angiogenesis in a multiple myeloma model *in vivo*. J Pharm Pharmacol. 2010; 62:145-151.
16. Sugita H, Kaneki M, Furuhashi S, Hirota M, Takamori H, Baba H. Nitric oxide inhibits the proliferation and invasion of pancreatic cancer cells through degradation of insulin receptor substrate-1 protein. Mol Cancer Res. 2010; 8:1152-1163.
17. Royle JS, Ross JA, Ansell I, Bollina P, Tulloch DN, Habib FK. Nitric oxide donating nonsteroidal anti-inflammatory drugs induce apoptosis in human prostate cancer cell systems and human prostatic stroma *via* caspase-3. J Urol. 2004; 172:338-344.
18. Cai TB, Tang X, Nagorski J, Brauschweiger PG, Wang PG. Synthesis and cytotoxicity of 5-fluorouracil/diazeniumdiolate conjugates. Bioorg Med Chem. 2003; 11:4971-4975.
19. Moharram S, Zhou A, Wiebe LI, Knaus EE. Design and synthesis of 3'- and 5'-O-(3-benzenesulfonylfuroxan-4-yl)-2'-deoxyuridines: Biological evaluation as hybrid nitric oxide donor-nucleoside anticancer agents. J Med Chem. 2004; 47:1840-1846.
20. Lai Y, Shen L, Zhang Z, Liu W, Zhang Y, Ji H, Tian J. Synthesis and biological evaluation of furoxan-based nitric oxide-releasing derivatives of glycyrrhetinic acid as anti-hepatocellular carcinoma agents. Bioorg Med Chem Lett. 2010; 20:6416-6420.
21. Ling Y, Ye X, Zhang Z, Zhang Y, Lai Y, Ji H, Peng S, Tian J. Novel Nitric Oxide-releasing derivatives of farnesylthiosalicylic acid: Synthesis and evaluation of antihepatocellular carcinoma activity. J Med Chem. 2011; 54:3251-3259.
22. Rosenberg B, Vancamp L, Krigas T. Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode. Nature. 1965; 205:968-969.
23. Zhao J, Gou S, Sun Y, Fang L, Wang Z. Antitumor platinum(II) complexes containing platinum-based moieties of present platinum drugs and furoxan groups as nitric oxide donors: Synthesis, DNA interaction, and cytotoxicity. Inorg Chem. 2012; 51:10317-10324.
24. Zhao J, Gou S, Sun Y, Yin R, Wang Z. Nitric oxide donor-based platinum complexes as potential anticancer agents. Chemistry. 2012; 18:14276-14281.
25. Abuo-Rahma Gel-D, Abdel-Aziz M, Mourad MA, Farag HH. Synthesis, anti-inflammatory activity and ulcerogenic liability of novel nitric oxide donating/chalcone hybrids. Bioorg Med Chem. 2012; 20:195-206.
26. Mourad MAE, Abdel-Aziz M, Farag HH. ChemInform abstract: Design, synthesis and anticancer activity of nitric oxide donating/chalcone hybrids. Eur J Med Chem. 2012; 43:907-913.
27. Ning K, Zhang JH, Feng Q, Sheng C, Tashiro S, Onodera S, Ikejima T. Induction of G2/M phase arrest and apoptosis by oridonin in human laryngeal carcinoma cells. J Nat Prod. 2010; 73:1058-1063.
28. Li D, Wang L, Cai H, Zhang Y, Xu J. Synthesis and biological evaluation of novel furozan-based nitric oxide-releasing derivatives of oridonin as potential anti-tumor agents. Molecules. 2012; 17:7556-7568.
29. Li DH, Wang L, Cai H, Jiang BW, Zhang YH, Sun YJ, Xu JY. Synthesis of novel furozan-based nitric oxide-releasing derivatives of 1-oxo-oridonin with anti-proliferative activity. Chin J Nat Med. 2012; 10:471-476.
30. Xu S, Wang G, Lin Y, Zhang Y, Pei L, Yao H, Hu M, Qiu Y, Huang Z, Zhang Y, Xu J. Novel anticancer oridonin derivatives possessing a diazen-1-ium-1,2-diolate nitric oxide donor moiety: Design, synthesis, biological evaluation and nitric oxide release studies. Bioorg Med Chem Lett. 2016; 26:2795-2800.
31. Fu J, Liu L, Huang Z, Lai Y, Ji H, Peng S, Tian J, Zhang Y. Hybrid molecule from O2-(2,4-dinitrophenyl) diazeniumdiolate and oleanolic acid: A glutathione S-transferase π-activated nitric oxide prodrug with selective anti-human hepatocellular carcinoma activity and improved stability. J Med Chem. 2013; 56:4641-4655.
32. Kogias E, Osterberg N, Baumer B, Psarras N, Koentges C, Papazoglou A, Saavedra JE, Keefer LK, Weyerbrock A. Growth-inhibitory and chemosensitizing effects of the glutathione-S-transferase-π-activated nitric oxide donor PABA/NO in malignant gliomas. Int J Cancer. 2012; 130:1184-1194.
33. Venugopal B, Evans TR. Developing histone deacetylase inhibitors as anti-cancer therapeutics. Curr Med Chem. 2011; 18:1658-1671.
34. Müller S, Krämer OH. Inhibitors of HDACs − effective drugs against cancer? Curr Cancer Drug Targets. 2010; 10:210-228.
35. Duan W, Li J, Inks ES, Chou CJ, Jia Y, Chu X, Li X, Xu W, Zhang Y. Design, synthesis, and antitumor evaluation of novel histone deacetylase inhibitors equipped with a phenylsulfonylfuroxan module as a nitric oxide donor. J Med Chem. 2015; 58:4325-4338.
36. Duan W, Hou J, Chu X, Li X, Jian Z, Jin L, Xu W, Zhang Y. Synthesis and biological evaluation of novel histone deacetylases inhibitors with nitric oxide releasing activity. Bioorg Med Chem. 2015; 23:4481-4488.
37. Bian H, Feng J, Li M, Xu W. Novel antileukemic agents derived from tamibarotene and nitric oxide donors. Bioorg Med Chem Lett. 2011; 21:7025-7029.
38. Martínez C, Hermosilla G, León R, Pincheira G, Cifuentes V. Aspirin inhibits colon cancer cell and tumor growth and downregulates specificity protein (Sp) transcription factors. PloS one. 2012; 7:e48208-e48208.
39. Chang SY, Howden CW. Is no NSAID a good NSAID? Approaches to NSAID-associated upper gastrointestinal disease. Curr Gastroenterol Rep. 2004; 6:447-453.
40. Williams JL, Borgo S, Hasan I, Castillo E, Traganos F, Rigas B. Nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) alter the kinetics of human colon cancer cell lines more effectively than traditional NSAIDs: Implications for colon cancer chemoprevention. Cancer Res. 2001; 61:3285-3289.
41. Chattopadhyay M, Kodela R, Olson KR, Kashfi K. NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid is a potent inhibitor of colon cancer cell growth *in vitro* and in a xenograft mouse model. Biochem Biophys Res Commun. 2012; 419:523-528.
42. Kodela R, Chattopadhyay M, Velázquez-Martínez CA, Kashfi K. NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid has enhanced chemo-preventive properties compared to aspirin, is gastrointestinal safe with all the classic therapeutic indications. Biochem Pharmacol. 2015;

98:564-572.
43. Hagos GK, Carroll RE, Kouznetsova T, Li Q, Toader V, Fernandez PA, Swanson SM, Thatcher GR. Colon cancer chemoprevention by a novel NO chimera that shows anti-inflammatory and antiproliferative activity *in vitro* and *in vivo*. Mol Cancer Ther. 2007; 6:2230-2239.
44. Pathi SS, Jutooru I, Chadalapaka G, Sreevalsan S, Anand S, Thatcher GR, Safe S, Pathi SS, Jutooru I, Chadalapaka G. GT-094, a NO-NSAID, inhibits colon cancer cell growth by activation of a reactive oxygen species-microRNA-27a: ZBTB10-specificity protein pathway. Mol Cancer Res. 2011; 9:195-202.

(Received October 10, 2016; Revised November 1, 2016; Re-revised December 4, 2016; Accepted December 4, 2016)