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

<table>
<thead>
<tr>
<th>Compound NO.</th>
<th>NO-donor type</th>
<th>Ligand agents</th>
<th>Structure</th>
<th>Cell lines</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>NONOates</td>
<td>5-FU</td>
<td>DU-145, HeLa</td>
<td>18</td>
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<tr>
<td>1b</td>
<td>Furoxan</td>
<td>Nucleoside</td>
<td>143B, EMT-6, KBALB-STK, KBALB, 143B-LTK, Hs578Bst</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Furoxan</td>
<td>Glycyrrhetinic acid</td>
<td>HCC cells</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Furoxan</td>
<td>Farnesylthiosalicylic acid (FTS)</td>
<td>HCC cells</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Furoxan</td>
<td>Platinum</td>
<td>SGC-7901, MCF-7, HepG2, HCT-116</td>
<td>22,24</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Furoxan</td>
<td>Chalcone</td>
<td>60 human tumor cell lines: molt-4, HL-60, A549…</td>
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<td></td>
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<tr>
<td>6</td>
<td>Furoxan</td>
<td>Oridonin</td>
<td>K562, MGC-803, Bel-7402</td>
<td>28,29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NONOates</td>
<td>Oridonin</td>
<td>Bel-7402</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Diazeniumdiolate</td>
<td>Oleanolic Acid</td>
<td>HepG2, HCC, H22.</td>
<td>31</td>
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</tr>
<tr>
<td>8</td>
<td>Furoxan</td>
<td>HDACis</td>
<td>HEL</td>
<td>35,36</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Furoxan</td>
<td>Tamibarotene</td>
<td>NB4, HL-60</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>
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-ordironin (compound 6) and NO-OA hybrids (compound 7).
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...
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 IC₅₀ values of compound 10 (NO-aspirin) and NO-ibuprofen were 1 μM and 42 μM, respectively, while the IC₅₀ 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.
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), Bel-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.

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