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Khosrow KASHFI
NOSH-Aspirin vs Cancer

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NEW ASPIRIN FIGHTS CANCER

A potent new weapon against cancer has been developed by scientists

By Sarah Westcott

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A POTENT “super aspirin” that can cause cancer cells to self-destruct has been developed by scientists.

The hybrid version is much more powerful than the conventional painkiller but far less toxic.

Prolonged use of traditional aspirin can cause stomach ulcers and kidney failure.

But the new compound, known as NOSH, can be used in lower doses and has fewer side effects.

In tests on mice, it has been shown to shrink cancer cells by 85 per cent.

The pill is effective against 11 different forms of cancer, including colon, pancreatic, prostate, breast and leukaemia, researchers have found.
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“If what we have seen in animals can be translated to humans it could be used in conjunction with other drugs to shrink tumours before chemotherapy or surgery".

Hailing the breakthrough yesterday, Professor Khosrow Kashfi said: “If what we have seen in animals can be translated to humans it could be used in conjunction with other drugs to shrink tumours before chemotherapy or surgery.”

Professor Kashfi, of the Sophie Davis School of Biomedical Education at The City College of New York, added: “The key components of this new compound are that it is very, very potent and yet it has minimal toxicity to normal cells.”

Previous research has shown that ordinary aspirin can reduce the size of some tumours by up to half. But prolonged use of the old form of the drug can have serious side effects such as excessive bleeding.

Professor Kashfi said: “There’s a lot of data on aspirin showing that when taken on a regular basis, on average it reduces the risk of development of colon cancer by about 50 per cent compared to non-users.”

Only 24 hours after treating a culture of cancer cells, the NOSH aspirin demonstrated 100,000 times greater potency than aspirin alone.

Professor Kashfi said: “At 72 hours it is about 250,000 times more potent in an in-vitro cell culture against human colon cancer. So you need a lower amount to get the same result.”

The new drug is a hybrid of two compounds, one of which releases nitric oxide to protect the stomach lining and the other releases hydrogen sulfide to increase its cancer-fighting ability.

Lower doses would minimise or potentially eliminate its side effects.

In a second study, when mice bearing human colon cancer tumours on their flanks were given NOSH aspirin, the compound caused cancer cells to self-destruct, inhibited the proliferation of the cells and significantly reduced tumour growth without any signs of toxicity.

Writing in the journal ACS Medicinal Chemistry Letters, Professor Kashfi said any working therapy for humans was still years away, but toxicity testing and clinical trials would be the next step.

His findings will be presented at the annual meeting of the American Association for Cancer Research in Chicago next month.

Dr Kat Arney, science information manager at Cancer Research UK, said: “Scientists have been investigating the cancer-fighting properties of aspirin for many years, although prolonged use can cause side effects such as stomach bleeds.

“It will be interesting to see how this particular compound progresses, although much more research is needed to show whether it’s safe and effective for use in humans.”

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COMPOUNDS AND COMPOSITIONS FOR TREATING DYSPROLIFERATIVE DISEASES, AND METHODS OF USE THEREOF

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EC: C07C203/04 C07C205/37
IPC: A61K31/21
A61K31/24 C07C203/04

Compounds are disclosed with activity towards killing dysproliferative cells in vitro and treating cancer in vivo. Cancers such as cancer of the colon, pancreas, prostate, lung, breast, urinary bladder, skin and liver are exemplary. Compounds, pharmaceutical compositions and methods of use are described.

The invention is directed to compounds that inhibit the growth of dysproliferative cells and can be used to treat cancer. The invention is further directed to the synthesis and uses for said compounds as well as compositions comprising said compounds.

BACKGROUND OF THE INVENTION

Dysproliferative diseases including neoplasms such as cancer remain a major health problem accounting for significant morbidity and mortality in the US and nearly all of the rest of the world.

Despite substantial progress in the last two decades, there remain many cancers for which currently available methods are either partially or totally ineffective. Thus novel agents or methods are needed either to prevent the development of cancer, or, in the case where neoplasia has already developed, to render the host organism cancer-free or to reduce its neoplastic burden to a level compatible with life or at least to facilitate the use of concomitant therapies.

There has been significant progress in understanding the fundamental processes underlying the development of neoplasia. In its essence, neoplasia, including cancer, can be viewed as the inappropriate accumulation of cells, in violation of the exquisite balance between cell renewal and cell death. For neoplasia to develop, either cell renewal must be increased or cell death decreased or both. A corollary to this relationship is that an agent that affects these processes favorably for the host organism (and, consequently, unfavorably for the neoplasm), is a potential antineoplastic drug.

One approach to develop new antineoplastic agents is to synthesize novel chemical compounds and screen them for their effect on cell growth. This is achieved by determining the number of a given set of cells following their exposure to the agent under evaluation and comparing it to that of untreated control cells. For an agent to have antineoplastic properties, it must inhibit the growth of neoplastic cells compared to untreated control, so that its sustained or repeated application will progressively diminish the tumor mass, ultimately leading to the extinction of neoplasia. It is also a logical extension of these considerations that other diseases such as, for example, psoriasis in which cell kinetic abnormalities, in other words abnormalities in cell renewal or cell death, contribute to their pathogenesis, will be amenable to treatment by such agents.

It is toward the identification of novel compounds with antineoplastic properties, and the identification of unexpected antineoplastic activity in compounds otherwise known in the art, that the present application is directed....

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http://www1.ccny.cuny.edu/prospective/med/faculty/kkashfi.cfm

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NOSH-Aspirin: A Novel Nitric Oxide–Hydrogen Sulfide-Releasing Hybrid: A New Class of Anti-inflammatory Pharmaceuticals

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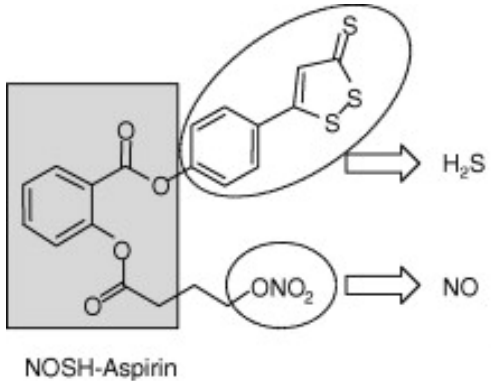
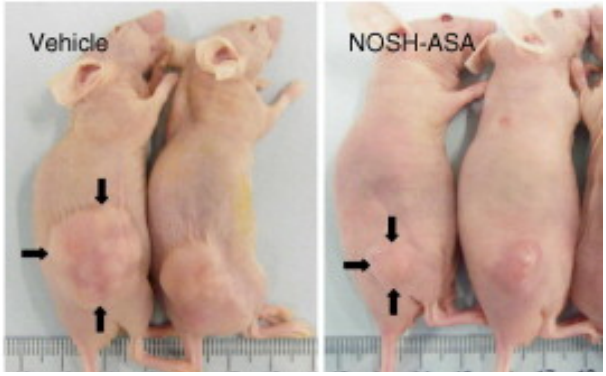
Abstract -- A series of new hybrids of aspirin (ASA), bearing both nitric oxide (NO) and hydrogen sulfide (H2S)-releasing moieties were synthesized and designated as NOSH compounds (1–4). NOSH-1 (4-(3-thioxo-3H-1,2-dithiol-5-yl) phenyl 2-((4-(nitrooxy)butanoyl)oxy) benzoate); NOSH-2 (4-(nitrooxy)butyl (2-((4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)carbonyl)phenyl)); NOSH-3 (4-carbamothioylphenyl 2-((4-(nitrooxy)butanoyl)oxy)benzoate); and NOSH-4 (4-(nitrooxy)butyl 2-(5-((R)-1,2-dithiolan-3-yl)pentanoyloxy)benzoate). The cell growth inhibitory properties of compounds 1–4 were evaluated in eleven different human cancer cell lines of six different tissue origins. These cell lines are of adenomatous (colon, pancreatic, lung, prostate), epithelial (breast), and lymphocytic (leukemia) origin. All NOSH compounds were extremely effective in inhibiting the growth of these cell lines. NOSH-1 was the most potent, with an IC50 of 48 ± 3 nM in HT-29 colon cancer cells. This is the first NSAID-based compound with such potency. This compound was also devoid of any cellular toxicity, as determined by LDH release. NOSH-1 was comparable to aspirin in its anti-inflammatory properties, using the carrageenan rat paw edema model.



NOSH-Aspirin

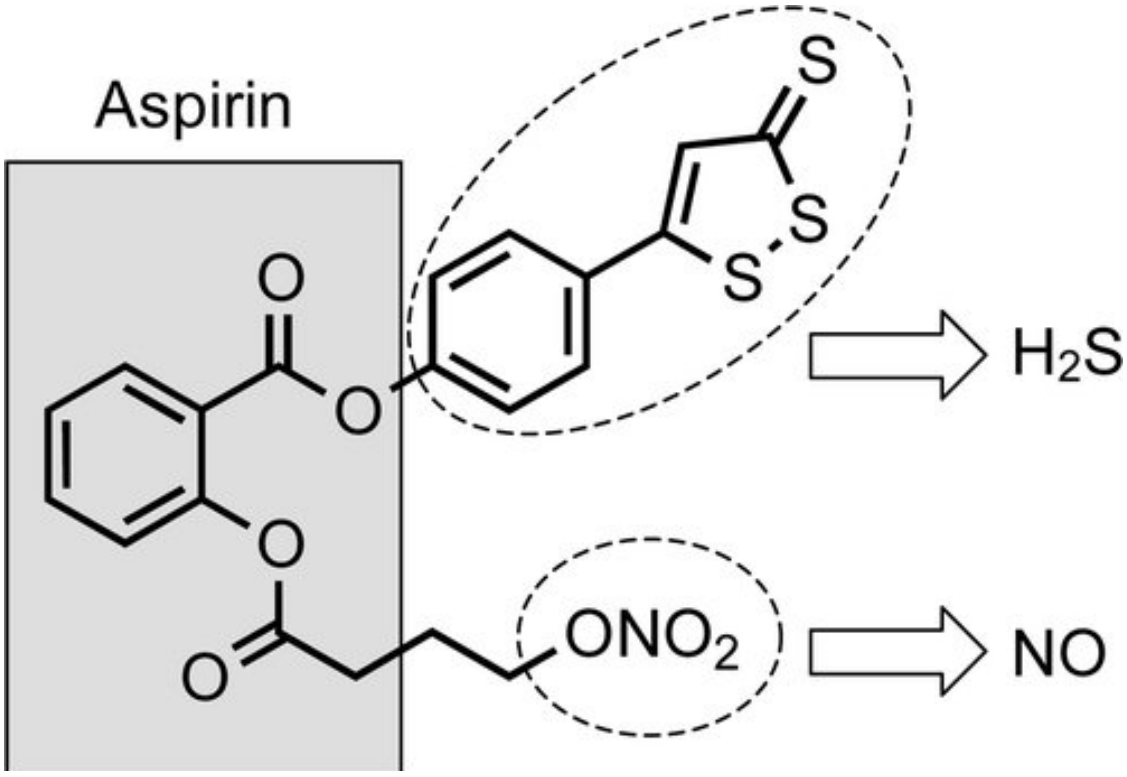


Nonsteroidal anti-inflammatory drugs (NSAIDs), in general, and aspirin, in particular, are recognized as the prototypical chemopreventive agents against many forms of cancers.(1) However, long-term use of NSAIDs may lead to serious side effects, including gastrointestinal and renal.(1) The search for “better NSAIDs” has led to the development of selective cyclooxygenase-2 inhibitors (Coxibs) and nitric oxide-releasing NSAIDs (NO-NSAIDs). Several large-scale clinical trials have shown that long-term use of coxibs is associated with an increased risk of adverse myocardial events.(2)

The development of NO-NSAIDs was based on the observation that NO has some of the same properties as prostaglandins within the gastric mucosa. Therefore, coupling an NO-releasing moiety to an NSAID might deliver NO to the site of NSAID-induced damage, thereby decreasing gastric toxicity. Animal and human studies have shown that many NO-NSAIDs are indeed safer to the GI mucosa than the parent NSAID.(3, 4)

Recently, a new class of NSAIDs possessing a hydrogen sulfide (H2S)-releasing moiety (HS-NSAIDs) have been described in the literature.(5-9) We have shown that these compounds can be useful in controlling cancer.(10-12) However, NO-NSAIDs and HS-NSAIDs have several drawbacks, limiting their development as pharmaceuticals. For example, HS-NSAIDs have relatively high IC50s for cell growth inhibition. Some NO-NSAIDs can form quinone methide intermediates, questioning the role of NO in their biological activity.(13-15) Others yet have high IC50s for cell growth inhibition.(16) Therefore, we postulated that a new hybrid that incorporated the active parts of each compound might be more potent than either one alone. Our hypothesis has proved to be correct. Here we describe the synthesis of four NOSH (nitric oxide-, hydrogen sulfide-releasing) compounds that release both H2S and NO (Figure 1). One of the compounds, NOSH-1, has IC50s for cell growth inhibition in the low nanomolar range and shows strong anti-inflammatory properties.
figure

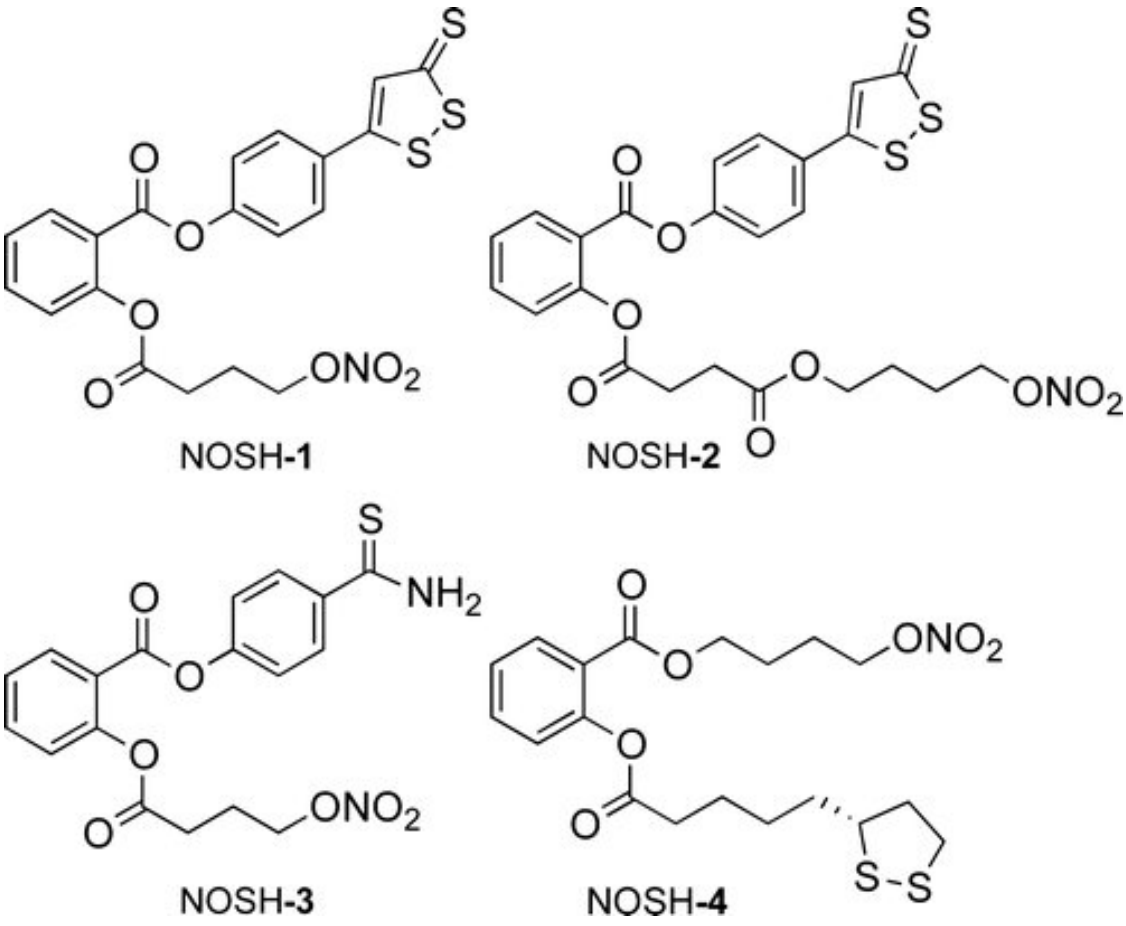
Figure 1. Chemical structures of NOSH compounds.



The NOSH compounds reported here were developed by using aspirin as a scaffold to which NO and H2S releasing moieties were coupled with one of the 1, 2 positions. We used nitrate (-ONO2) for NO release and attached it to the aspirin through an aliphatic spacer, while one of the following H2S-releasing moieties, 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione (ADT-OH), or 4-hydroxy benzothiazamide (TBZ) or lipoic acid were directly coupled to aspirin (NOSH-1–4, Figure 1). Salicylaldehyde was used as the starting material for NOSH-1–3, and aspirin was used for NOSH-4.

Salicylaldehyde (5) coupled with 4-bromobutyric acid (6) in the presence of DCC/DMAP was used to yield compound 7. The bromo moiety in compound 7 was then substituted with nitrate using AgNO3 to give compound 8. Then the aldehyde group of compound 8 was oxidized to its corresponding carboxylic group in the presence of KMnO4 to yield compound 9.(17) This was then used as the precursor for preparation of NOSH-1 and -3 using either 5-(4-hydroxyphenyl)-3H-1, 2-dithiole-3-thione (ADT-OH, 10) or 4-hydroxythiobenzamide (TBZ, 11), respectively (Scheme 1).

Scheme 1. Synthesis of NOSH-1 and NOSH-3a

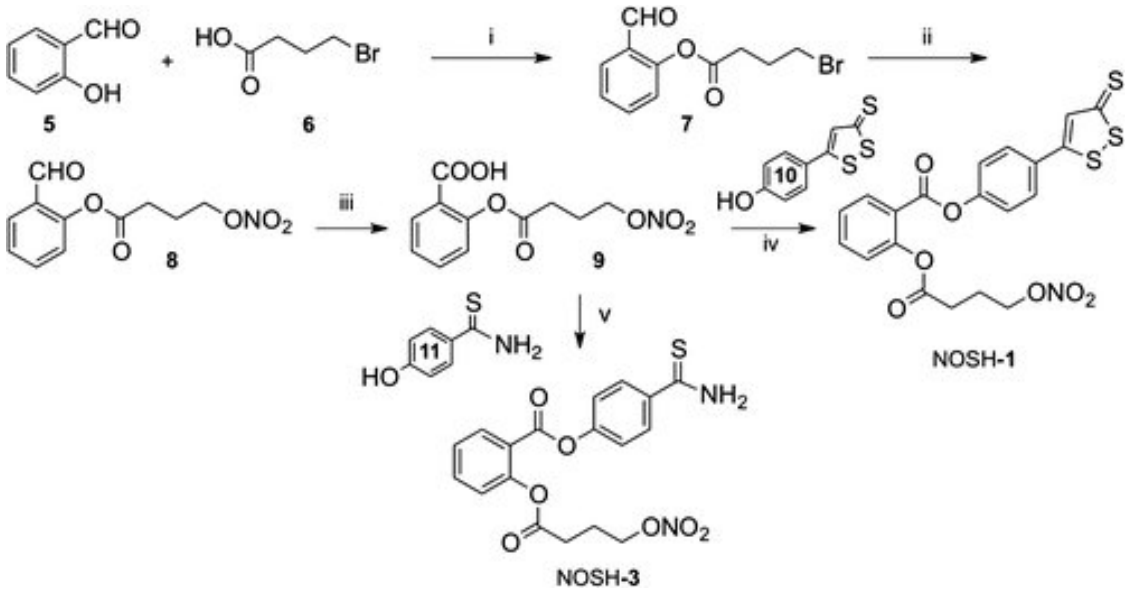


aConditions: (i) DCC/DMAP, DCM, rt, 6 h, (ii) AgNO3, CH3CN, rt, 12 h, (iii) KMNO4, acetone, 0 °C to rt, 3 h, (iv) ADT-OH (10), DCC/DMAP, DCM, rt, 6 h, (v) TBZ (11), DCC/DMAP, DCM, rt, 6 h.

For preparation of compound NOSH-2, salicyladehyde (5), succinic anhydride (12), and a catalytic amount of DMAP in methylene chloride were treated for 24 h, at room temperature, to prepare the succinic acid linked intermediate. To this intermediate in situ were added

hydroxybutyl nitrate (13) and DCC to afford compound 14. This was further oxidized by KMnO4 to its corresponding aromatic carboxylic acid (15), which was coupled to ADT-OH (10) in the presence of DCC/DMAP in methylene chloride to give NOSH-2 (Scheme 2).

Scheme 2. Synthesis of NOSH-2a



aConditions: (ia) succinic anhydride (12), DMAP, DCM, rt, 12 h, (ib) 4-hydroxybutyl nitrate (13), DCC, rt, 6 h, (ii) KMnO4, acetone, 0 °C to rt, 3 h, (iii) ADT-OH (10), DCC/DMAP, DCM, rt, 6 h.

NOSH-4 was synthesized by using lipoic acid as H2S-releasing donor. We used aspirin as the starting material and coupled it with compound 13 in the presence of DCC/DMAP to give 16. (18) This then underwent deacetylation by K2CO3 in THF/MeOH (1:1) to produce compound 17.(19) This was then coupled with (R)-lipoic acid (18) in the presence of DCC/DMAP to produce NOSH-4 (Scheme 3).

Scheme 3. Synthesis of NOSH-4a

aConditions: (i) 4-hydroxybutyl nitrate (13), DCC/DMAP, DCM, rt, 6 h, (ii) K2CO3, THF/MeOH (1:1), 15 min, rt, (iii) (R)-lipoic acid (18), DCC/DMAP, DCM, rt, 6 h.

We investigated the effects of NOSH-1–4 and ASA on the growth properties of eleven different cancer cell lines of six different histological subtypes. The cell lines were that of colon (HT-29, COX-1 and COX-2 positive; HCT 15, COX null; and SW480, COX-1 positive, low levels of endogenous COX-2), breast (MCF7, [ER(+)]; MDA MB-231 and SKBR3, [ER(-)]), T-cell leukemia (Jurkat), pancreas (BxPC3, both COX-1 and COX-2 positive; MIAPaCa-2, COX-null), prostate (LNCaP), and lung (A549). All four NOSH compounds were extremely effective in inhibiting the growth of these cell lines (Table 1). NOSH-1 was very potent, and its IC50 for cell growth inhibition ranged from 48 to 280 nM. The corresponding IC50 values for NOSH-2, -3, and -4 were 70–120, 4300–7500, and 240–800 nM, respectively. The growth inhibition by NOSH-1–4 versus traditional ASA was very high in the cell lines studied. In a fold comparison study of the IC50 values (ASA/NOSH-1–4), NOSH-1 was at least 100,000-fold more potent than ASA in HT-29 colon cancer cells. The increases in potency for NOSH-2, -3, and -4 in the same cell line were >60,000-fold, >600-fold, and >16,000-fold, respectively. In general, NOSH-1 was the most potent in all cell

lines. Cyclooxygenase (COX) represents the best-known mechanistic target of NSAIDs. An interesting aspect of growth inhibition also emerges with respect to COX expression in the cell lines examined. NOSH-1–4 showed similar effects on two colon cancer cell lines, HT-29 (expresses COX-1 and COX-2) and HCT 15 (no COX expression),(20) and on two pancreatic cancer cell lines, BxPC-3 (expresses COXs) and MIA PaCa-2 (no COX expression),(21) suggesting a COX-independent effect.

Table 1. IC50 nM for Cell Growth Inhibition at 24 ha

	colon	breast	pancreas	lung	prostate	leukemia					
NOSH	HT-29	HCT15	SW480	MDA MB231	SKBR3	MCF7	MIA PaCa2	BxPC3	A540	LNCAP	Jurkat
1	48 ± 3	50 ± 5	60 ± 4	100 ± 11	75 ± 5	280 ± 16	47 ± 5	57 ± 4	50 ± 7	88 ± 8	100 ± 8
2	80 ± 5	90 ± 6	97 ± 7	85 ± 8	88 ± 7	70 ± 5	102 ± 18	100 ± 9	120 ± 14	100 ± 12	90 ± 5
3	7500 ± 355	5900 ± 305	5300 ± 240	6000 ± 220	6500 ± 268	5700 ± 323	4800 ± 322	5500 ± 390	6500 ± 224	4300 ± 212	7000 ± 321
4	300 ± 35	520 ± 21	600 ± 25	800 ± 22	550 ± 28	280 ± 15	800 ± 39	700 ± 32	300 ± 12	500 ± 18	240 ± 11
ASA	>5,000,000?nM?at?24 h?in?all?cell?lines										
a											

Colon, breast, pancreas, lung, prostate, and leukemia cancer cell lines were treated with various concentrations of NOSH-1, NOSH-2, NOSH-3, NOSH-4, and aspirin (ASA). Cell viability was determined at 24 h, from which IC50 values were calculated. Results are mean ± SEM of at least four different experiments performed in triplicate. $P < 0.001$ for all NOSH compounds compared to ASA in all cell lines.

This high degree of potency raised the question as to how toxic this compound was to the cells. To assess this, we used lactate dehydrogenase (LDH) release as a measure of cellular toxicity. Cells were treated with several concentrations of NOSH-1 for 2–24 h and compared to untreated controls. Although the cytotoxicity caused by NOSH-1 was both dose- and time-dependent, this was minimal (Figure 2). At 4-times its IC50, LDH release was less than 10% at 24 h. LDH release for shorter durations of treatment (2 h, 4 h, 6 h, and 8 h) ranged between 0.5 and 4% at its IC50 and between 1 and 5% at 4-times its IC50. This demonstrates a remarkable degree of safety for a compound that is so potent.

Figure 2. Toxicity profile of NOSH-1 as measured by LDH release in HT-29 colon cancer cells.

The most common use for NSAIDs (including aspirin) is the treatment of inflammatory conditions. Therefore, we wanted to compare the COX-dependent anti-inflammatory activity of ASA to that of NOSH-1. This was done by using the rat paw edema model, as described in the Supporting Information. After inducing inflammation in rat's paw with carrageenan, animals receiving vehicle showed a fast time-dependent increase in paw volume (?V = 1.1 mL) after 2–3 h, which decreased gradually every hour thereafter until the end of the experiment (6 h) (Figure 3A). In contrast, animals receiving ASA showed a weak inflammatory response (?V = 0.4 mL) at 1 h, decreasing to about ?V = 0.35 mL over the next 2 h and then decreasing to about ?V = 0.35 mL after 6 h. The anti-inflammatory effect registered in animals treated with NOSH-1 was dose-dependent. Rats treated with low dose NOSH-1 (0.21 mmol/kg) showed a change in paw volume ?V = 0.5 mL after 1 h which

increased to ?V = 0.6 mL by 3 h and then came down to about ?V = 0.4 mL over the next 3 h. Rats treated with high dose NOSH-1 (0.52 mmol/kg), a dose which was slightly less than that of ASA (0.56 mmol/kg), showed a plateaued change in paw volume of ?V = 0.45 mL after 1–2 h, which then deceased steadily over the next 4 h to ?V = 0.35 mL, a change that was comparable to that of ASA (Figure 3A).

Figure 3. Anti-inflammatory properties of NOSH-1.

Rat paw edema was induced by carrageenan injection. (A) ASA and NOSH-1 caused a significant reduction in paw volume at all time points. Results are mean ± SEM of four rats in each group; *P < 0.05 versus vehicle treated rats at all time points. (B) ASA and NOSH-1 caused a significant reduction in PGE2 levels in the paw exudate. Results are mean ± SEM for four rats in each group; *P < 0.01versus vehicle. (C) NOSH-1 inhibits induction of COX-1 and COX-2 by carrageenan. Results show one animal is the control, four are in carrageenan injected, and two are in NOSH-1 treated at two different doses.

Prostaglandins (PGE2) are the main product of cyclooxygenase-mediated arachidonic acid metabolism.(1) Comparison of PGE2 content of paw exudates from control, ASA-treated, and NOSH-1-treated animals showed a clear and significant COX inhibition by aspirin and NOSH-1. Figure 3B shows that aspirin (0.21 mmol/kg) caused a considerable decrease in PGE2 levels (12 ± 3 pg/mg protein) compared with the control group (82 ± 2 pg/mg). Treatment with NOSH-1 reduced PGE2 levels to 42 ± 3 and 21 ± 4 pg/mg at 0.21 and 0.52 mmmol/kg, respectively. We further evaluated the effect of NOSH-1 on COX expression in paw exudates. Figure 3C shows that COX-1 was constitutively expressed in the controls; this was induced by carrageenan and inhibited to the same extent by NOSH-1 regardless of the dose. On the other hand, COX-2, which produces inflammatory PGE2, was barely detectable in the controls, was significantly induced by carrageenan, and was dose-dependently inhibited by NOSH-1.

We also determined the inhibitory effect of ASA and NOSH-1 on proinflammatory cytokine tumor necrosis factor-a (TNF-a) in plasma obtained from control and NOSH-1-treated animals. Administration of ASA (0.56 mmol/kg) increased the TNF-a concentration by about 20-fold (10 ± 1 control and 200 ± 10 pg/mL ASA); however, this rise was considerably lower in the NOSH-1 (55 ± 2 pg/mL at 0.21 mmol/kg and 40 ± 3 pg/mL at 0.52 mmol/kg) treated animals (Figure 4).

Figure 4. Effect of ASA and NOSH-1 on plasma TNF-a.
ASA caused a significant rise in plasma TNF-a; however, this rise was significantly less in the NOSH-1 treated rats. Results are mean ± SEM for four rats in each group; *P < 0.01 vs vehicle, †P < 0.01 vs ASA.

The NOSH compounds were designed to release both NO and H2S. In order to show that indeed this was the case in vivo, blood was collected from vehicle-, ASA-, and NOSH-1-treated animals at the end of the carrageenan-induced edema studies. Figure 5 shows that indeed both NO and H2S were dose-dependently significantly higher in NOSH-1-treated animals.

Figure 5. NO and H2S levels in vivo after NOSH-1 administration.
The plasma concentration of NOx and H2S was quantified as detailed in the Supporting Information. Results are mean ± SEM of four rats in each group. *P < 0.001 versus vehicle

and ASA-treated animals.

In the present study, we described the synthesis of four compounds designed to release both NO and H2S. These NOSH compounds used aspirin as a scaffold and were shown to inhibit the growth of several cancer cell lines arising from a variety of tissue types such as colon, breast, pancreas, lung, prostrate, and T cell leukemia. The compounds described here are the first to show IC50 values for cell growth inhibition that are in the nanomolar range and yet are devoid of any cellular toxicity. These NOSH compounds were more potent than ASA, with enhanced potency ranging from at least 650 to greater than 100,000-fold. Of the four NOSH compounds evaluated here, NOSH-1 was consistently the most potent in all cell lines tested, and in some cases this enhancement was in excess of 150-fold over the others. Our data indicate that the effect of these NOSH compounds may be tissue-type independent since the NOSH-1–4 were effective against adenomatous, epithelial, and lymphocytic cancer cell lines. Here we studied eleven cell lines originating from six different tissues; therefore, it may be envisaged that our findings are part of a generalized effect, especially since all cell types responded, although in a differential manner. NOSH-1 also showed strong anti-inflammatory properties that were comparable to that of ASA, as demonstrated by measuring the in vivo carrageenan-induced rat paw edema, and direct measurement of cyclooxygenase-dependent production of PGE2.

We are currently studying the molecular targets of these interesting compounds with respect to cell growth inhibition and are evaluating them in various animal models of cancer. Some on the non-Cox targets being investigated include NF-?B, reactive oxygen species, the intrinsic apoptosis pathway, and Wnt signaling.

Supporting Information

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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

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are prototypical anti-cancer agents. However, their long-term use is associated with adverse gastrointestinal effects. Recognition that endogenous gaseous mediators, nitric oxide (NO) and hydrogen sulfide (H2S) can increase mucosal defense mechanisms has led to the development of NO- and H2S-releasing NSAIDs with increased safety profiles. Here we report on a new hybrid, NOSH–aspirin, which is an NO- and H2S-releasing agent. NOSH–aspirin inhibited HT-29 colon cancer growth with IC50s of 45.5 ± 2.5, 19.7 ± 3.3, and 7.7 ± 2.2 nM at 24, 48, and 72 h, respectively. This is the first NSAID based agent with such high degree of potency. NOSH–aspirin inhibited cell proliferation, induced apoptosis, and caused G0/G1 cell cycle block. Reconstitution and structure–activity studies representing a fairly close approximation to the intact molecule showed that NOSH–aspirin was 9000-fold more potent than the sum of its parts towards growth inhibition. NOSH–aspirin inhibited ovine COX-1 more than ovine COX-2. NOSH–ASA treatment of mice bearing a human colon cancer xenograft caused a reduction in volume of 85%. Taken together, these results demonstrate that NOSH–aspirin has strong anti-cancer potential and merits further evaluation.

Highlights

NOSH–aspirin is the first dual acting NO and H2S releasing hybrid. ? Its IC50 for cell growth inhibition is in the low nano-molar range. ? Structure–activity studies show that the sum of the parts does not equal the whole. ? NOSH–aspirin reduced tumor growth by 85% in mice bearing a colon cancer xenograft.