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Synthesis and in vitro anti-leukemic activity of structural analogues of JS-K, an anti-cancer lead compound

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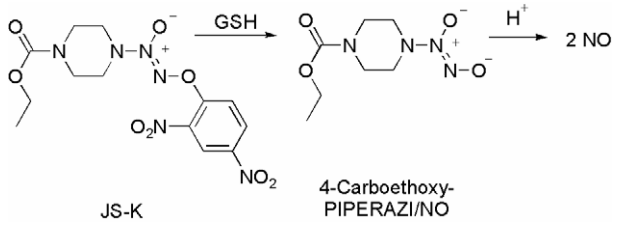
Abstract—Structural analogues of JS-K, an anti-cancer lead compound, were prepared and their in vitro anti-leukemic activity was determined. The rate of nitric oxide release from the corresponding diazeniumdiolate anions did not appear to affect the anti-leukemic activity of the prodrug forms. Two compounds with potent inhibitory activity and a potentially favorable toxicological profile were identified.

Glutathione *S*-transferase (GST), which is frequently over-expressed in cancer tissue, is a key phase II detoxification enzyme that catalyzes the conjugation of electrophilic xenobiotics with glutathione (GSH).[1] Our laboratory has developed several diazeniumdiolate-based nitric oxide (NO) prodrugs[2] that are designed as substrates for GST, notably O^2-(2,4-dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (JS-K, Scheme 1).[3]

JS-K is a potent anti-tumor agent against HL-60 human leukemia xenografts in mice, cutting their growth rate by about half and inducing significant necrosis in the tumor mass.[3a] Furthermore, this drug was found to be active against: human prostate cancer xenografts in mice;[3a] an orthotopic model of liver cancer in rats;[3d] and multiple myeloma xenografts in mice.[3e] In this study, we synthesized numerous structural analogues of JS-K and studied their in vitro anti-leukemic activity by determining the ability of these compounds to inhibit the growth of HL-60 human leukemia cells.



Scheme 1. The anti-cancer lead compound JS-K, which targets GST-overexpressing cells, and its mechanism of nitric oxide release.

It is reported that human leukemia cells are sensitive to cytotoxic effects of nitric oxide.[4] While additional pathways may be operational, nitric oxide appears to be an important component of the potent JS-K cytotoxic activity.[3] Nitric oxide release from JS-K is proposed to occur by nucleophilic aromatic displacement of the spontaneously NO-releasing diazeniumdiolate anion[2] by glutathione (Scheme 1). In the absence of strong nucleophiles such as GSH, JS-K is relatively stable in aqueous buffer; the rate constant for hydrolysis of JS-K in pH 7.4 buffer at 37 °C was reported as 1×10^{-6} s^{-1}, which translates to a half-life of over a week.[3a] In the presence of glutathione, however, JS-K nearly completely disappeared within 30 min and a second order rate constant of 1.0 M^{-1} s^{-1} was reported.[3a] The rate constant for hydrolysis of 4-carboethoxy-PIPERAZI/NO in pH 7.4 buffer

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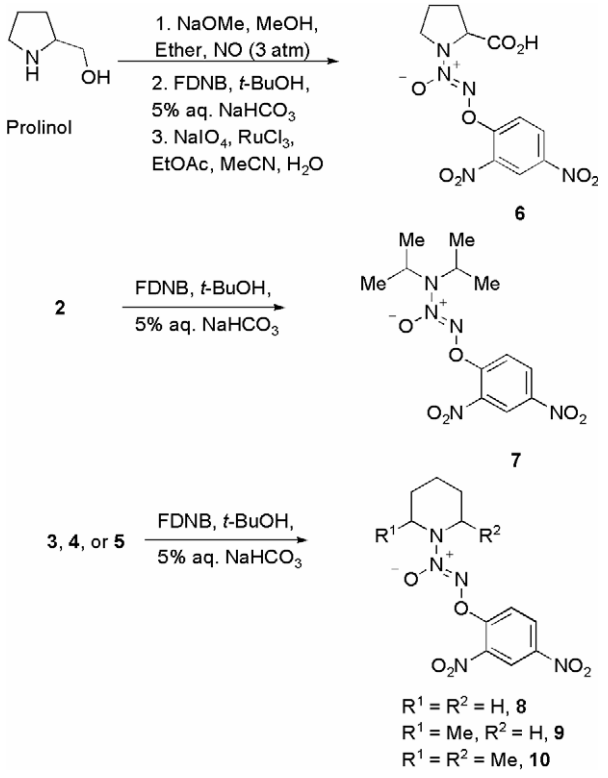
at 37 °C was reported as 1.9×10^{-3} s^{-1}, which corresponds to a half-life of 6 min (Scheme 1).[3a]

Earlier, we prepared a number of JS-K structural analogues and determined their in vitro anti-leukemic activity.[3d] However, a systematic study of the effect of varying the half-life of the diazeniumdiolate ion decomposition on the anti-proliferative activity of the corresponding O^2-aryl prodrug form was not conducted. Hence, our work was initiated by studying the effect of varying the rate of dissociation of the diazeniumdiolate ion to form NO on the cytotoxicity of the corresponding O^2-(2,4-dinitrophenyl) derivative, which was determined by their ability to inhibit human leukemia HL-60 cell proliferation.

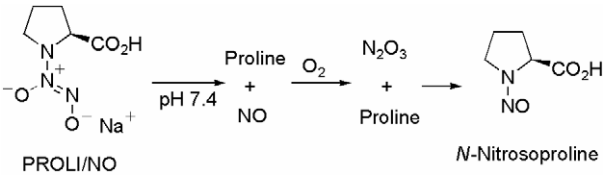
A number of diazeniumdiolate anions with a range of half-lives were identified and prepared using reported methods from the corresponding secondary amine.[5] As listed in Table 1, the reported half-life of decomposition of PROLI/NO (**1**) is 2 s while the half-lives of decomposition of **2**–**5** varied from 78 s to 50 min.[5] The O^2-(2,4-dinitrophenyl) derivatives **6**–**10** of these diazeniumdiolate anions were prepared using reported methods (Scheme 2).[5c,d]

It is noteworthy that the diazeniumdiolate ions PROLI/NO,[5a–c] **2**,[5d] and **5**[5d] are nitric oxide prodrugs with potential clinical relevance; possible byproducts of NO release from these compounds, the corresponding nitrosamines, are reported to have minimal carcinogenic activity, thus suggesting a favorable toxicological profile.[6] For example, *N*-nitrosoproline, a possible byproduct of PROLI/NO decomposition, was not found to display any carcinogenic activity in several animal models (Scheme 3).[6e–h]

Next, we prepared the O^2-aryl diazeniumdiolate **11** in three steps from *N*-(*tert*-butoxycarbonyl)piperazine using a reported method (Eq. 1).[7] This compound served as a convenient divergence point in the preparation of a variety of JS-K analogues by exposing **11** to a series of electrophiles (Scheme 4).[8]

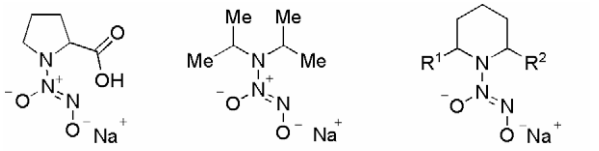


Scheme 2. Synthesis of some O^2-(2,4-dinitrophenyl) diazeniumdiolates. FDNB, 1-Fluoro-2,4-dinitrobenzene.



Scheme 3. Proposed mechanism of nitrosamine formation during diazeniumdiolate ion decomposition in aqueous buffer.

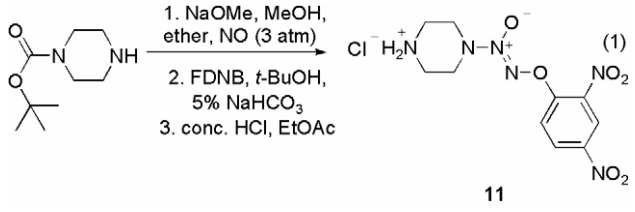
Table 1. Reported half-lives of some diazeniumdiolate ions in aqueous buffer and the corresponding O^2-arylated prodrug forms

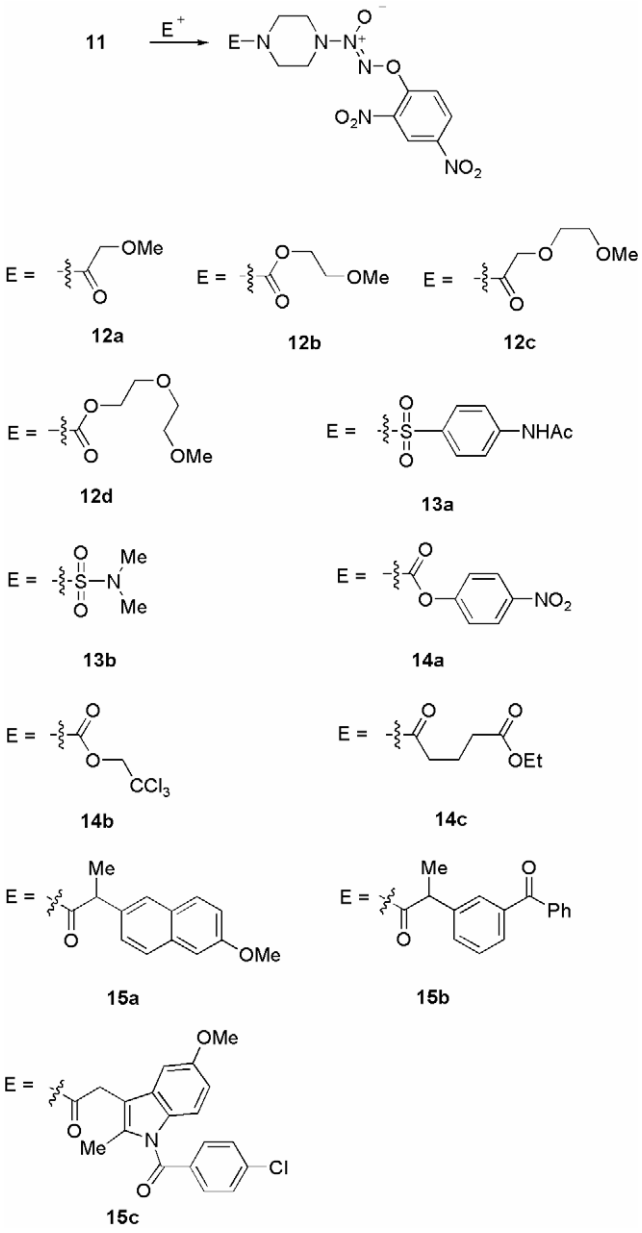


NO donor	Half-life[a]	O^2-(2,4-Dinitrophenyl) derivative
4-Carboethoxy-PIPERAZI/NO	6.0 min	JS-K
PROLI/NO, **1**	2 s	**6**
2	4.3 min	**7**
3	78 s	**8**
4	8.3 min	**9**
5	50 min	**10**

[a] In pH 7.4 buffer at 37 °C.

The methyl ethers **12a**–**12d** were prepared by treating **11** with the corresponding acyl chloride. The carbamate portion of JS-K was replaced by a sulfamate functionality in compounds **13a** and **13b**. Analogues **14a**–**14c**, where the ester group of the carbamate of JS-K was altered, were prepared in a similar fashion by reaction of the corresponding acyl chloride with **11**.





Scheme 4. Synthesis of some structural analogues of JS-K from **11**.

Diazeniumdiolate-based nitric oxide donors conjugated with non-steroidal anti-inflammatory drugs (NSAIDs) are assuming importance as multi-faceted therapeutic agents.[9] Thus, we prepared several JS-K-type NSAID-conjugates **15a**–**15c** using the precursor **11**.

The in vitro anti-leukemic activity of these compounds was studied using the HL-60 human leukemia cell line by a reported procedure.[3a] The IC_{50} of JS-K was reported as 0.2–0.5 μM (Table 2, entry 1).[3a]

Although the aqueous solubility of **6** was much higher than those of the other JS-K analogues prepared in this study, its cytotoxicity was diminished (Table 2, entry 2). The other prodrugs **7**–**10** were tested in this assay and the half-life of the diazeniumdiolate anion in buffer did not appear to affect the cytotoxicity. The

Table 2. In vitro anti-leukemic activity of JS-K and its structural analogues

Entry	Compound	IC_{50} (μM)
1	JS-K	0.2–0.5
2	**6**	>50
3	**7**	3.2
4	**8**	1.7
5	**9**	2.5
6	**10**	2.0
7	**11**	5.0
8	**12a**	2.3
9	**12b**	7.2
10	**12c**	4.5
11	**12d**	8.8
12	**13a**	6.6
13	**13b**	6.8
14	**14a**	7.4
15	**14b**	8.3
16	**14c**	6.0
17	**15a**	9.8
18	**15b**	7.4
19	**15c**	9.1

derivative **7** and the piperidine analogues **8**–**10** displayed potent but comparable activity, suggesting that the differences in the rate of NO release from the corresponding diazeniumdiolate ions **2**–**5** did not affect potency of the diazeniumdiolate prodrug (Table 2, entries 3–6).

In an earlier study, it was reported that diazeniumdiolate anions with extended durations of nitric oxide release were more effective at inhibiting the growth of this human leukemia cell line than their counterparts with lesser durations of NO release.[4] In this study, three compounds, DETA/NO, with a half-life of NO release of 20 h, PAPA/NO, which has a half-life of 30 min, and MAHMA/NO, whose half-life is 2 min, were tested for their anti-proliferative activity using the HL-60 human leukemia cell line.[4] Among these compounds, it was reported that the best inhibitor of cell proliferation was DETA/NO (ID_{50} = 50 μM), followed by PAPA/NO (ID_{50} = 100 μM), and MAHMA/NO, which was found to be inactive. In contrast, our study indicates that there were no observable effects of altering the rate of NO release on the cytotoxicity of these prodrugs, suggesting a role for nitric oxide-independent cytotoxic pathways.[3] Furthermore, it is worth noting that, by releasing NO spontaneously, DETA/NO, PAPA/NO, and MAHMA/NO act on leukemia cells by a bystander effect while the compounds described in this study get activated to release NO intracellularly. Thus, the rate of NO release intracellularly may not be as important for cytotoxicity as when it is released extracellularly.

Earlier, we reported that **2** and **5** decomposed in aqueous buffer to release nearly quantitative amounts of NO, suggesting that nitrosamine formation is a minor process.[5d] Due to their potent cytotoxicity and the potentially favorable toxicological profile of their corresponding parent diazeniumdiolate anions, **7** and **10** are

noteworthy; further in vitro and in vivo testing of their anti-cancer activity will be carried out in due course.

The ether derivatives **12a**–**12d** were all found to display lower potency than JS-K but comparable to that of **11**; the highest cytotoxicity was shown by **12a**, whose IC_{50} was determined as 2.3 μM (Table 2, entries 8–11). Changing the carbamate functionality to a sulfamate group (**13a** and **13b**) or varying the ester portion of the carbamate group (**14a**–**14c**) resulted in diminished anti-proliferative activity (Table 2, entries 12–16). The IC_{50} values of all NSAID derivatives were closer to 10 μM (Table 2, entries 17–19). These results are consistent with those of our earlier study, wherein we observed that minor structural modifications led to substantial decrease in potency.[3d] For example, when the ethyl carbamate of JS-K was changed to a methyl carbamate, the potency of the resulting compound diminished from IC_{50} 0.2–0.5 to 3.5 μM.[3d]

While the molecular mechanism of the potent anti-leukemic activity of JS-K remains to be fully determined, this study further reinforces the unique features of this compound that result in a diminution of potency with structural perturbation or conjugation of other potential pharmacophores, leading us to speculate that more than one cytotoxic pathway may be involved; the nature of such alternate pathways is currently being explored in our laboratories.

Acknowledgments

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Supplementary data

Analytical data for all new compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.044.

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8. General procedure. A solution of **11** in DCM was reacted with a solution of one equivalent of the electrophile and two equivalents of triethylamine. The reaction mixture was diluted with DCM, washed with dil HCl and aq sodium bicarbonate. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure to form a solid. This was then triturated with ether, filtered, and dried under vacuum.
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