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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2633–2635

Propargylic sulfones possessing a 2-nitroimidazole function: novel hypoxic-cell radiosensitizers with intracellular non-protein thiol depletion ability

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Received 16 December 2003; revised 17 February 2004; accepted 17 February 2004

Abstract—Propargylic sulfones (**1a**–**c**) containing a 2-nitroimidazole structure were synthesized, and their non-protein thiol (NPSH) depletion abilities were investigated. Propargylic sulfones **1a**,**c** containing an electron withdrawing *p*-nitrophenyl group showed high reactivity toward capturing glutathione (GSH), a typical intracellular NPSH, in phosphate buffer. Among the three propargylic sulfones **1a**–**c**, carboxylic acid derivative **1c** showed the most potent radiosensitizing activity toward hypoxic EMT6/KU tumor cells. In view of these results and the partition coefficients between 1-octanol and water, we concluded that appropriate NPSH-depletion ability and lipophilicity are both important in achieving potent hypoxic-cell radiosensitization by propargylic sulfones possessing a 2-nitroimidazole function in biological systems.

Endogenous non-protein thiols (NPSH) play a crucial function in determining the response of biological cells to several types of radiation.[1] The reduced form of glutathione (GSH), which is a typical endogenous NPSH and exists abundantly in cells, is known to protect intracellular molecules from radiation.[2–6] Therefore, depletion of GSH in tumor cells is recognized as being effective for radiation therapy.[7–9] Several strategies have been proposed to enhance the effect of radiation therapy, including oxidation of GSH to the oxidized form (GSSG),[10] conjugation of functional compounds to GSH through a covalent bond,[11] and inhibition of intracellular GSH synthesis.[7] Previously, we reported a series of nitroazole derivatives containing an α,β-unsaturated carbonyl group in the side chains that are able to deplete GSH in tumor cells, thereby resulting in more enhanced hypoxic-cell radiosensitization in vitro relative to a well-documented nitroimidazole radiosensitizers, such as misonidazole.[12–14]

On the other hand, propargylic sulfones have been identified as prodrugs that undergo isomerization to allenic sulfones with alkylating reactivity for a variety of nucleophiles including DNA bases and thiols.[15–18] In view of such an alkylating mechanism, propargylic sulfones may be applicable to a family of NPSH-depleting agents. To expand our field of developing hypoxic-cell radiosensitizers with NPSH-depletion ability, we synthesized three propargylic sulfones (**1a**–**c**) possessing a 2-nitroimidazole structure, which has a well-identified radiosensitizing function (Fig. 1). Among the propargylic sulfones synthesized, carboxylic acid derivative **1c** exhibited efficient intracellular GSH-depletion ability and radiosensitizing activity toward hypoxic EMT6/KU cells. The propargylic sulfone **1c** had a very low lipophilicity as measured by the partition coefficient between 1-octanol and water, which should be a key property for achieving efficient radiosensitizing activity.

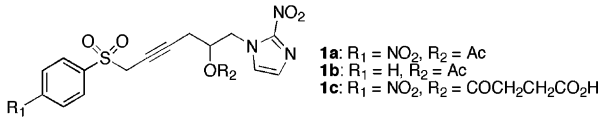

Figure 1. Structure of propargylic sulfones possessing a 2-nitroimidazole function.

Keywords: Propargylic sulfone; NPSH depletion; Radiosensitizer; Hypoxic-cell.

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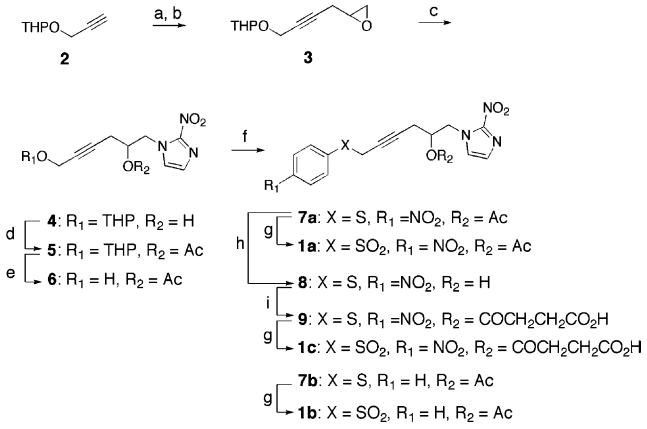
doi:10.1016/j.bmcl.2004.02.060



Scheme 1. Reagents and conditions: (a) *n*-BuLi, boron trifluoride diethyl etherate, THF, −78 °C, and then epichlorohydrin, −78 °C, 58%; (b) NaOH, H_2O–EtOH, rt, 67%; (c) 2-nitroimidazole, K_2CO_3, EtOH, 70 °C, 72%; (d) Ac_2O, DMAP, CH_2Cl_2, rt, 88%; (e) PPTS, MeOH, rt, 94%; (f) (i) MsCl, Et_3N, CH_2Cl_2, rt; (ii) 4-nitrothiophenol (for **7a**), thiophenol (for **7b**), NaH, rt, 80% (for **7a**), 26% (for **7b**); (g) mCPBA, CH_2Cl_2, 0 °C, 80% (for **1a**), 73% (for **1b**), 30% (for **1c**); (h) NaOH, CH_2Cl_2–MeOH–H_2O, rt, 88%; (i) succinic anhydride, DMAP, CH_2Cl_2, rt, 94%.

The synthetic route to propargylic sulfones possessing a nitroimidazole function is shown in Scheme 1. The propargylic sulfides **7a**,**b** were readily obtained from acetylene **2**. Oxidation of **7a**,**b** by mCPBA produced the propargylic sulfones **1a**,**b**.[19,20] Carboxylic acid derivative **1c**[21] was obtained by the coupling of succinic anhydride with alcohol **8**, which was prepared by hydrolysis of **7a** followed by oxidation.

These propargylic sulfones possessing a 2-nitroimidazole function **1a**–**c** and a control compound without a sulfonyl group **7a** were subjected to evaluation of their reactivity with a typical NPSH, namely glutathione, in phosphate buffer at pH 8.5. The second-order rate constants (k_2) for the nucleophilic addition of GSH to **1a**–**c** and **7a** are listed in Table 1.[22] As expected, propargylic sulfones **1a** and **1c** containing an electron-withdrawing *p*-nitrophenyl group reacted efficiently with GSH. In contrast, propargylic sulfone without a nitro group **1b** and control compound **7a** showed low GSH-depletion reactivity. We also investigated the reactivity of **1a**–**c** toward GSH in phosphate buffer at pH 7.5 where the effect of propargylic sulfones on intracellular GSH-depletion was evaluated. Both **1a** and **1c** showed higher GSH-depletion reactivity than **1b** at pH 7.5, while the corresponding rates were lower than those at pH 8.5. It has been demonstrated that both the electron-withdrawing sulfonyl and 2-nitrophenyl groups increase the acidity of the propargylic proton, thereby resulting in efficient conversion to the corresponding allenic isomer.[23] Thus, introduction of electron-withdrawing groups to the propargylic sulfones is indispensable for obtaining efficient GSH-depletion ability.

Table 1. Second-order rate constants (k_2) for the reaction of propargylic sulfones with GSH in phosphate buffer and 1-octanol-to-water partition coefficients ($P_{o/w}$) for propargylic sulfones

Propargylic sulfones	k_2 ($mM^{-1}\,h^{-1}$)		$P_{o/w}$
	pH 8.5	pH 7.5	
1a	17.0	3.0	15.0
1b	0.7	0.2	22.2
1c	8.6	1.1	2.2
7a	0.8	—[a]	—[a]

[a] Not determined.

Upon 60 h incubation of **1a** and GSH in phosphate buffer (pH 8.5) at 37 °C, the reaction mixture was analyzed by mass spectroscopy. The ESI/MS indicated the formation of **1a**-GSH adduct ($[M–H]^-$, 742).

We next examined the effect of propargylic sulfones **1a** and **1c** on intracellular GSH-depletion and radiosensitization of hypoxic EMT6/KU cells.[24] The cells were exposed to varying X-ray doses under hypoxic conditions and survival was determined by colony assay. The survival data were fitted to a single-hit multi-target model: $S = 1 - (1 - e^{-D/D_0})^n$, where D is the radiation dose, n is the extrapolation number, and D_0 is the mean lethal dose. Figure 2 shows the profile of the dose–survival curve for EMT6/KU cells incubated with **1a** or **1c** for 4 h followed by X-irradiation. The mean lethal dose was decreased by the treatment of hypoxic EMT6/KU cells with **1a** or **1c** from 3.6 Gy for the control to 1.6 and 1.5 Gy, respectively. A shoulder observed at lower dose as a nonlinear portion of the survival curve almost disappeared, indicating that **1a** and **1c** acted as efficient sensitizers of hypoxic cells even at a low radiation dose. The value of n for **1a** and **1c** decreased from 5.6 for the control to 3.4 and 2.6, respectively. To get molecular structural insights into intracellular GSH-depletion reactivity, we characterized radiosensitization of hypoxic cells in the presence of a reference compound **7a**. While **7a** could sensitize hypoxic cells, alteration of n value was not achieved in the dose–survival curve (data not shown). As reported previously, depletion of the intracellular NPSH level upon treatment with NPSH reactive species and irradiation leads to a decrease in the n value.[14] In view of this, it is reasonable to conclude that propargylic sulfones **1a** and **1c** can decrease the GSH level in the cell, as in phosphate buffer, thereby resulting in efficient radiosensitization. The sensitizer

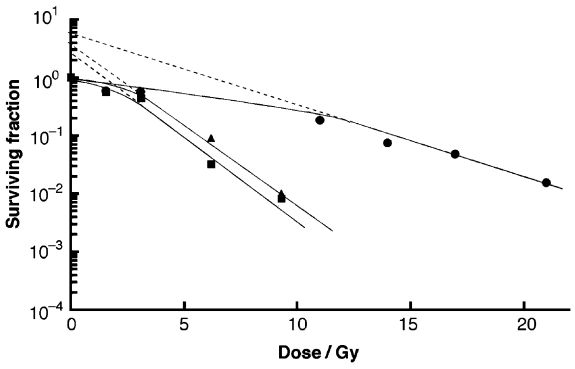


Figure 2. Survival of EMT6/KU cells upon X-irradiation after 4 h incubation with (■) 0.5 mM of **1c**, (▲) 0.5 mM of **1a**, and (●) no propargylic sulfones under hypoxic conditions (95% N_2+5% CO_2).

enhancement ratios in vitro ($SER_{in\ vitro}$) at 0.5 mM for **1a** and **1c** were 2.4 and 2.6, respectively (see Fig. 2).

It is also seen from Figure 2 that carboxylic acid derivative **1c** showed higher hypoxic-cell radiosensitizing activity than acetate **1a**, although the GSH-depletion reactivity of **1c** as measured by the second-order rate constant k_2 for nucleophilic addition (Table 1) was about half that of **1a**. By reference to the partition coefficient ($P_{o/w}$) between 1-octanol and water listed in Table 1, **1a** is of much higher lipophilicity ($P_{o/w} = 15.0$) than carboxylic acid derivative **1c** ($P_{o/w} = 2.2$). It seems that **1c** may readily permeate the cell membrane because of its appropriately low lipophilicity, while highly lipophilic **1a** is subject to trapping by the cell membrane. Thus, appropriate lipophilicity and GSH-depletion reactivity are important factors in the molecular design of a family of propargylic sulfones containing a 2-nitroimidazole function for hypoxic-cell radiosensitizers with cellular NPSH-depletion ability.

In summary, we synthesized propargylic sulfones containing a 2-nitroimidazole function to investigate their GSH-depletion reactivity and hypoxic-cell radiosensitizing activity. These compounds were confirmed to reduce the intracellular NPSH level and exhibited a very high value of $SER_{in\ vitro}$, thus, they are promising candidates as novel radiosensitizers for the treatment of hypoxic tumor cells.

Acknowledgements

We are grateful to Dr. Wei-Min Dai (Department of Chemistry, The Hong Kong University of Science and Technology) for his valuable discussions.

References and notes

1. Liphard, M.; Bothe, E.; Schulte-frohlinde, D. *Int. J. Radiat Biol.* **1990**, *58*, 603.
2. Alper, T. *Cellular Radiobiology*; Cambridge University: Cambridge, 1979.
3. Astor, M. B.; Anderson, V.; Mester, A. *Pharmacol. Ther.* **1998**, *39*, 115.
4. Held, K. D. *Pharmacol. Ther.* **1988**, *39*, 123.
5. Howard-Glanders, P. *Nature* **1960**, *186*, 485.
6. Chapman, J. D.; Greenstock, C. L.; Reuvers, A. P.; Dugle, D. L. *Radiat. Res.* **1975**, *64*, 365.
7. Biaglow, J. E.; Varnes, M. E.; Clark, E. P.; Epp, E. R. *Radiat. Res.* **1983**, *95*, 473.
8. Bump, E. A.; Yu, N. Y.; Brown, J. M. *Science* **1982**, *217*, 544.
9. Bump, E. A.; Yu, N. Y.; Brown, J. M. *Int. J. Oncol. Biol. Phys.* **1982**, *84*, 39.
10. Harris, J. W.; Power, J. A. *Radiat. Res.* **1973**, *56*, 97.
11. Han, A.; Sinclair, W. K.; Kimler, B. F. *Radiat. Res.* **1976**, *65*, 337.
12. Nishimoto, S.; Zhou, L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 439.
13. Zhou, L.; Nishimoto, S. *Int. J. Radiat Biol.* **1995**, *67*, 335.
14. Zhou, L.; Ino, A.; Dai, W.-M.; Nishimoto, S. *Bioorg. Med. Chem.* **1999**, *7*, 2591.
15. Nicolaou, K. C.; Skokotas, G.; Maligres, P.; Zuccarello, G.; Schweiger, E. J.; Toshima, K.; Wendeborn, S. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1272.
16. Haruna, K.; Tanabe, K.; Ishii, A.; Dai, W.-M.; Hatta, H.; Nishimoto, S. *Bioorg. Med. Chem.* **2003**, *11*, 5311.
17. Dai, W.-M.; Lai, K. W.; Wu, A.; Hamaguchi, W.; Lee, Y. H. M.; Zhou, L.; Ishii, A.; Nishimoto, S. *J. Med. Chem.* **2002**, *45*, 758.
18. Dai, W.-M.; Li, Q.; Fong, K. C.; Chow, C. W.; Zhou, L.; Hamaguchi, W.; Nishimoto, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2789.
19. Compound **1a**: Mp 148–150 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (d, 2H, $J = 9.0$ Hz), 8.17 (d, 2H, $J = 9.0$ Hz), 7.13 (s, 1H), 7.13 (s, 1H), 5.22–5.19 (1H), 4.88 (dd, 1H, $J = 14.8$, 3.0 Hz), 4.49 (dd, 1H, $J = 14.8$, 7.5 Hz), 4.02 (s, 2H), 2.63–2.45 (2H), 1.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 151.2, 143.3, 130.1, 128.4, 126.6, 124.5, 82.8, 76.6, 70.7, 69.2, 51.1, 48.7, 22.0, 20.6, 14.2; FABMS (NBA) m/z 437 [(M+H)$^+$]; HRMS calcd for $C_{17}H_{17}O_8N_4S$ [(M+H)$^+$] 437.0767, found 437.0773.
20. Compound **1b**: ^{1}H NMR (DMSO-d_6 400 MHz) δ 7.93 (d, 2H, $J = 7.2$ Hz), 7.75 (t, 1H, $J = 7.2$ Hz), 7.65 (t, 2H, $J = 7.2$ Hz), 7.49 (s, 1H), 7.15 (s, 1H), 5.19 (m, 1H), 4.67 (dd, 1H, $J = 14.0$, 3.2 Hz), 4.49 (s, 2H), 4.37 (dd, 1H, $J = 14.0$, 8.8 Hz), 2.66–2.55 (2H), 1.86 (s, 3H); ^{13}C NMR (DMSO-d_6 100 MHz) δ 169.1, 144.6, 138.0, 134.1, 129.1, 128.2, 128.1, 127.7, 82.0, 71.5, 68.9, 51.1, 47.3, 21.4, 20.3; FABMS (NBA) m/z 392 [(M+H)$^+$]; HRMS calcd for $C_{17}H_{18}O_6N_3S$ [(M+H)$^+$] 392.0916, found 392.0923.
21. Compound **1c**: Mp 64–66 °C. ^{1}H NMR (DMSO-d_6 300 MHz) δ 8.45 (d, 2H, $J = 9.0$ Hz), 8.20 (d, 2H, $J = 9.0$ Hz), 7.51 (s, 1H), 7.11 (s, 1H), 5.17 (m, 1H), 4.54 (dd, 1H, $J = 12.8$, 3.0 Hz), 4.37 (dd, 1H, $J = 12.8$, 7.5 Hz), 3.42 (s, 2H), 2.61 (m, 6H), 2.40–2.31 (4H); ^{13}C NMR (DMSO-d_6 75 MHz) δ 173.0, 171.2, 150.7, 143.2, 130.0, 128.3, 127.8, 124.4, 82.5, 71.0, 69.1, 56.0, 51.0, 47.1, 28.4, 21.4, 18.5; FABMS (NBA) m/z 495 [(M+H)$^+$]; HRMS calcd for $C_{19}H_{19}O_{10}N_4S$ [(M+H)$^+$] 495.0822, found 495.0802.
22. The reactivity of GSH toward propargylic sulfones were measured by the Ellman's method. See: Tietze, F. *Anal. Biochem.* **1969**, *27*, 502. To an aqueous solution of 10 mM GSH and 20 mM aqueous EDTA·2Na was added a solution of propargylic sulfones in 1:9 v/v mixture of acetonitrile and phosphate buffer. The resulting mixture was incubated at 37 °C for the given periods of reaction. Aliquot (0.1 mL) was withdrawn from the mixture and was poured immediately into phosphate buffer (0.9 mL) containing 0.2 mM of the Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid), DTNB). The concentration of unreacted GSH was determined by UV absorption spectroscopy at the maximum absorption wavelength at 412 nm.
23. Dai, W.-M.; Fong, K. C.; Danjo, H.; Nishimoto, S.; Solow, M.; Mak, W. L.; Yeung, M. L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1093.
24. EMT6/KU cells were grown as monolayer in Eagle's minimum essential medium (MEM) supplemented with 12.5% fetal bovine serum (FBS) and L-glutamine. MEM was adjusted to pH 7.5 by 10% aqueous $NaHCO_3$ solution. All propargylic sulfones for evaluation were dissolved in FBS free MEM containing small amount of DMSO. Cells were seeded into glass cultural dishes, 24 h prior to experiment, to establish an exponential growth condition.