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Synthesis and Biological Evaluation of a Novel Phenyl Substituted Sydnone Series as Potential Antitumor Agents

Christopher S. Dunkley and Charles J. Thoman*

Department of Chemistry and Biochemistry, University of the Sciences in Philadelphia, 600 S. 43rd St., Philadelphia, PA 19104, USA

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Abstract—A series of compounds containing an N-(4′-substituted-3′-nitrophenyl)sydnone moiety with potential antitumor activity was prepared based on active analogues. The rationale behind the design of these compounds is presented along with the 4-step synthetic route to the derivatives in the 4′-position of the phenyl sydnone framework. Out of the six novel compounds, the 4′-fluoro derivative has an improved activity against all three cell lines as compared to the earlier leads.

Since their discovery mesoionic compounds have shown a variety of biological activities including antitumor.[1–4] It is thought that the ionic resonance structures of the heterocyclic ring promote significant interactions with biological molecules. In 1992 a series of 4′-substituted-3′-nitrophenylsydnones were synthesized and evaluated by Grynberg et al.[5] for anticancer activity and it was found that the 4′-chloro and the 4′-pyrrolidino compounds significantly enhanced the survival of Sarcoma 180 (S180), Ehrlich carcinoma (Ehrlich) and Fibrous histiocytoma (B10MCII) tumor bearing mice (Fig. 1). It was also found that the larger hetero rings; *p*-piperidino and *p*-morpholino, were less potent.

Herein we report the design, synthesis, and biological evaluation of six *para*-substituted analogues of Grynberg's molecules. These were tested for anti-carcinogenic activity against Sarcoma 180 (S180), Ehrlich carcinoma (Ehrlich) and fibrous histiocytoma (B10MCH) tumor bearing mice. Since the 4′-Cl derivative was the most active of Grynberg's compounds, the chlorine was replaced with a fluorine to ascertain whether a stronger inductive effect or the substituent's size were important in determining activity. The 4′-pyrrolidino-derivative was also active against two of the strains, leading us to synthesize the two benzo derivatives of it (the indolino and the isoindolino-). In addition, we made the open-ring analogue of the 4′-pyrrolidino moiety, the diethylamino- group. Finally, since the six-membered analogue (4′-piperidino) was inactive, we prepared the 4′-azetidino compound to see if ring size plays a role in activity.

It should be noted that no mechanism has been definitively determined for the activity of these compounds, but we may speculate about one. Fliedner[6] found that the acidity of (3-sydnonyl)acetic acid ($pK_a = 1.70$) was almost identical to that of nitroacetic acid ($pK_a = 1.68$), which means that the 3-sydnonyl moiety is as strong an electron withdrawing group as the nitro. In nucleophilic aromatic substitution, activation of a leaving group (often chloro) is promoted by nitro groups in the ortho and para positions. We propose that the facile substitution of the nucleophiles for the chloro in these studies occurs because the *o*-nitro and *p*-(3-sydnonyl) groups are acting like *o*,*p*-dinitro groups in normal nucleophilic aromatic substitutions. This may also explain the mode of activity of the active sydnone compounds against cancerous cells; nucleophilic groups on DNA or some other biological compound might be attacking the sydnone compound in the same way, becoming attached to it and somehow deactivated by it.

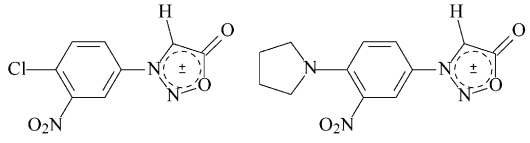

Figure 1. Structures of N-(4′-chloro and 4′-pyrrolidino-3′-nitrophenyl)sydnones.

*Corresponding author. E-mail: c.thoman@usip.edu

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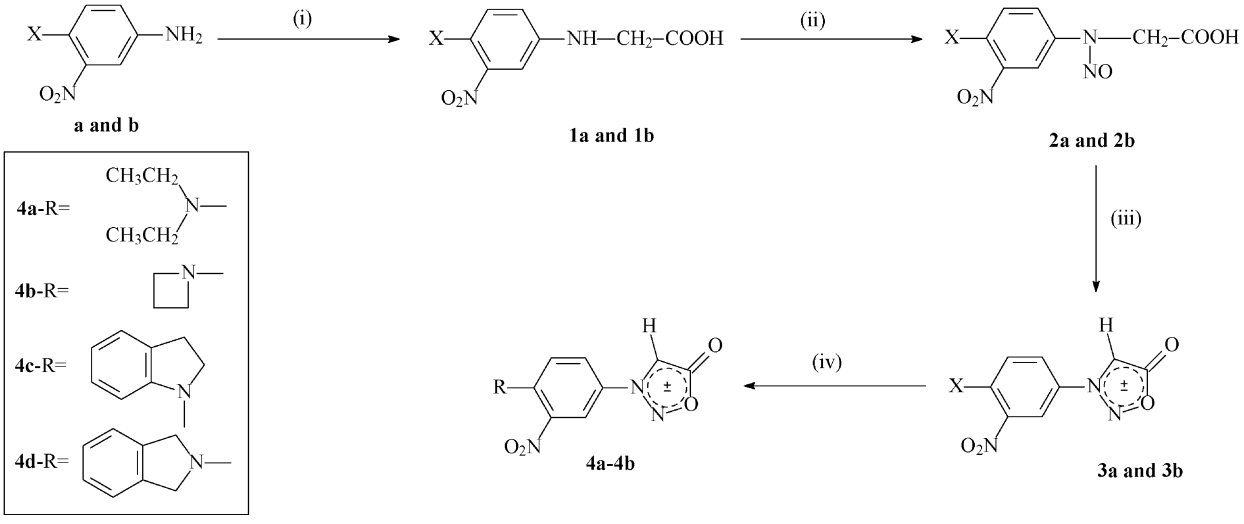
The first step, a condensation, involves neutralizing an aqueous solution of chloroacetic acid with an equimolar equivalent of NaOH and adding this solution over a period of 4 h to an aqueous solution of either 4′-chloro-3′-nitroaniline (a) or 4′-fluoro-3′-nitroaniline (b) while under refux (Scheme 1). Heating is continued for 48 h and the clear liquor is then vacuum filtered while hot, to remove any decomposition products, and refrigerated overnight. The resulting crystals are vacuum filtered and rinsed with ether to obtain compounds *N*-(4′-X-3′-nitrophenyl)glycine **1a** (X = Cl) or **1b** (X = F). A second crop of crystals is obtained by evaporating the filtrate and boiling the resulting solid in a 20% (v/v) ammonia solution which dissolves the product. Once the ammonia solution is cooled to room temperature, the starting material is removed by filtration and the filtrate is acidified with concentrated HCl and rinsed with water to afford a second crop of **1a** or **1b**.

The resulting 4-substituted-3-nitrophenylglycines **1a** and **1b** are then mechanically stirred at 0 °C while an aqueous solution of sodium nitrite is slowly added over a 30-min period. After an additional 10 min, the solid is vacuum filtered, washed with water and allowed to dry overnight to yield **2a** and **2b** quantitatively. The nitrosated compounds, **2a** and **2b**, are then treated with acetic anhydride and stirred at room temperature for 12 h to yield *N*-(4′-X-3′-nitrophenyl) sydnones **3a** (X = Cl) and **3b** (X = F). The 4′-chloro-3′-nitrophenylsydnone **3a** precipitates upon formation and is recrystallized from ethanol, but 4′-fluoro-3′-nitrophenylsydnone **3b** must be purified via column chromotography from a red paste.

Subsequently, sydnones **4a–d** are obtained by readily replacing the 4′-chloro group of **3a** with the corresponding nucleophiles.[7] This ease of replacement is due to the 1′-sydnonyl and 3′-nitro electron-withdrawing groups which drastically increase the rate of the nucleophilic aromatic substitution. Thus, the *N*-(4′-diethylamino-3′-nitrophenyl)sydnone **4a** is obtained by stirring **3a** with diethylamine neat at 40 °C for 12 h. The resulting crystals are filtered, rinsed with cold hexane and recrystallized from water.

Sydnones **4b–d** are all prepared following a slightly different protocol than that of **4a**. The three nucleophiles; azetidine hydrochloride, indoline and isoindoline, are each treated with NaH in dimethoxyethane followed by the slow addition of **3a**. Each reaction has slightly different conditions; however, the progress of each reaction is monitored using thin layer chromotography. Both the indoline and isoindoline are extracted with ethyl acetate which precipitates out the products **4c** and **4d**. These products are rinsed with cold hexanes and recrystallized from ethanol. The azetidine sydnone **4b** does not precipitate out during extraction; therefore, after concentrating the solution under reduced pressure, column chromotography is used to further purify the product.

Compounds **3a–b** and **4a–d** were prescreened at the National Cancer Institute for antitumor activity. This primary assay consists of a 3-cell line panel; MCF7 (Breast), NCI-H460 (Lung) and SF-268 (CNS). The protocol consists of inoculating and incubating each cell line on a microtiter plate. Test agents are then added at a single concentration and the culture is incubated for 48 h. End-point determinations are then made with alamar blue. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated cells. Compounds that reduce the growth of any one of the cell lines to 32% or less are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range. Although both 4′-halogen substituted sydnones passed the primary assay by inhibiting the growth of at least one cell line to less than 32%, the novel 4′-fluorosydnone derivative **3b** proved to be both more active and more versatile against each cell line (Table 1). The four nitrogen containing analogues **4a–d** proved to be neither active nor versatile against any cell line in the primary assay. In second stage testing compounds **3a** and **3b** were evaluated against a full panel of



Scheme 1. Synthetic route to sydnone derivatives **4a–d**. (i) $ClCH_2COOH$ (1 equiv), NaOH (1 equiv), reflux, 48 h; (ii) $NaNO_2$ (1.1 equiv), 0 °C, 1 h; (iii) Ac_2O neat, 40 °C, 12 h; (iv) **4a** diethylamine, 40 °C, 12 h; **4b** azetidine hydrochloride (1 equiv), NaH (1 equiv), 60 °C, 24 h; **4c** indoline (1 equiv), NaH (1 equiv), rt, 18 h; **4d** isoindoline (1 equiv), NaH (1 equiv), 50 °C, 50 min.

Table 1. In vitro antitumor activity of sydnones **3a–b** and **4a–d** against 3-cell lines. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated cells

Compd	MCF7 (%)	NCI-H460 (%)	SF-268 (%)
3a	44	91	9
3b	0	0	0
4a	85	92	95
4b	75	95	95
4c	82	87	96
4d	75	76	95

60 tumor cell lines at a minimum of five concentrations at 10-fold dilutions and both compounds proved either inactive or caused cell death, thereby terminating further testing.

References and Notes

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7. Compounds **3b**, and **4a–b** were characterized by IR, MS, ^{1}H and ^{13}C NMR and elemental analysis. **Sydnone 3b**: mp 110–113 °C. ^{1}H NMR (acetone-d_6) δ 7.96 (dd, $J=6.3$, $J=2.7$, 1H), 8.59 (ddd, $J=3.8$, $J=2.7$, $J=9.1$, 1H), 8.80 (dd, $J=10.4$, $J=9.1$, 1H); ^{13}C NMR (acetone-d_6) δ 95.8 (s), 121.2 (d, $J=23.5$, 1C), 121.5 (d, $J=1.62$, 1C), 130.1 (d, $J=9.7$, 1C), 131.7, 138.2 (d, $J=2.10$, 1C), 157.8 (d, $J=268.6$, 1C), 169.3. calcd for $C_8H_4N_3O_4F$ (225.14): C, 42.68: H, 1.79: N, 18.67: O, 28.43: F, 8.44. Found: C, 42.74: H, 2.13: N, 17.68: F, 8.74. **Sydnone 4a**: mp 118–120 °C. ^{1}H NMR (chloroform-d_1): δ 1.23 (tr, $J=7.3$, 6H), 3.35 (q, $J=7.3$, 4H), 7.26 (d, $J=9.3$, 1H), 7.72 (dd, $J=9.2$, $J=2.7$, 1H), 8.14 (d, $J=2.7$, 1H); ^{13}C NMR (chloroform-d_1): δ 12.9, 46.6, 93.4, 120.5, 121.4, 124.0, 125.2, 139.3, 147.0, 169.1. calcd for $C_{12}H_{14}N_4O_4$ (278.27): C, 51.8: H, 5.07: N, 20.13: O, 23.00. Found: C, 1.79: H, 5.07: N, 20.14. **Sydnone 4b**: mp 139–140 °C. ^{1}H NMR (chloroform-d_1): δ 2.5 (quin, $J=1.9$, 2C), 4.18 (tr, $J=1.9$, 4H), 6.68 (s, 1H), 6.75 (d, $J=2.4$, 1H), 7.72 (dd, $J=2.4$, $J=0.7$, 1H), 8.20 (d, $J=0.7$, 1H); ^{13}C NMR (chloroform-d_1): δ 16.4, 54.5, 93.3, 116.0, 120.3, 122.7, 125.7, 134.0, 146.8, 169.1. calcd for $C_{11}H_{10}N_4O_4$ (262.22): C, 50.38: H, 3.84: N, 21.37. Found: C, 50.47: H, 3.93: N, 21.4. **Sydnone 4c**: mp 224–225 °C. ^{1}H NMR (DMSO-d_6): δ 3.18 (tr, $J=7.9$, 2H), 4.02 (tr, $J=7.9$, 2H), 6.64 (d, $J=8.0$, 1H), 6.94 (tr, $J=6.8$, 1H) 7.10 (tr, $J=6.8$, 1H), 7.31 (d, $J=8.0$, 1H), 7.88 (d, $J=9.0$, 1H), 7.89 (S, 1H), 8.16 (dd, $J=9.1$, $J=2.5$, 1H) 8.61 (d, $J=2.5$, 1H); ^{13}C NMR (DMSO-d_6): δ 28.5, 53.7, 95.1, 99.2, 120.2, 122.0, 122.9, 125.7, 126.5, 126.6, 126.7, 132.5, 138.8, 139.8, 144.9, 168.2. calcd for $C_{16}H_{12}N_4O_4$ (324.3): C, 59.26: H, 3.73: N, 17.28. Found: C, 59.04: H, 3.87: N, 17.17. **Sydnone 4d**: mp 211–212 °C. ^{1}H NMR (DMSO-d_6): δ 4.73 (s, 4H), 7.38 (m, 5H), 7.78 (s, 1H), 8.06 (dd, $J=9.2$, $J=2.4$, 1H), 8.41 (d, $J=2.4$, 1H); ^{13}C NMR (DMSO-d_6): δ 56.3, 95.2, 119.1, 120.7, 123.1, 123.3, 126.2, 128.4, 136.5, 136.6, 143.5, 169.3. calcd for $C_{16}H_{12}N_4O_4$ (324.3): C, 59.26: H, 3.73: N, 17.28. Found: C, 59.06: H, 3.84: N, 17.12.