# The Synthesis of Aryl 4-Nitro-5-imidazolyl Sulfone Radiation Sensitizers Sterically Protected Against Glutathione Reaction



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2,6-Disubstituted aryl 1-methyl-4-nitro-5-imidazolyl sulfones **4** and aryl 1-neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl sulfones **10** have been synthesized and tested as radiation sensitizers of hypoxic carcinoma cells. These sterically crowded imidazoles show decreased displacement reactivity with glutathione at C-5, a major metabolic reaction known to deplete effective plasma drug levels in traditional aryl imidazolyl sulfone radiation sensitizers.

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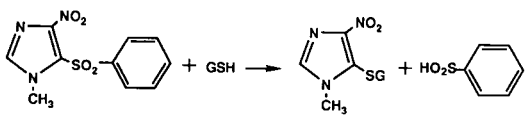
Aryl 1-methyl-4-nitro-5-imidazolyl sulfones are potent radiation sensitizers of hypoxic Chinese hamster V79 cells and of human adenocarcinoma SW1116 cells *in vitro* [1,2,3]. However, when tested *in vivo* they were inactive [4]. This lack of *in vivo* activity has been attributed to the reaction (Scheme 1) of these compounds with the thiol moiety of blood-borne glutathione before the pharmaceutical could reach the tumor and effect radiosensitization [4]. The elevated *in vitro* activity of this class of radiosensitizers is believed due in part to intra-cellular depletion of the glutathione, which acts as a natural radioprotective agent [5]. Intracellularly, this reaction is catalyzed by glutathione *S*-transferase, whereas in plasma the reaction is completely dependent upon the intrinsic nucleophilicity of glutathione at $C_5$ of the parent imidazoles. Therefore, a sterically hindered analogue which is kinetically unreactive in serum might be enzymatically reactive in the tumor cells if it were delivered intact to the malignancy. As noted earlier, this laboratory's goal is the tumor-targeted delivery of radiosensitizer drugs by the attachment to monoclonal antibodies [6]. To that end, several carboxyl-containing compounds for linking to a tumor-targeted antibody are included in this study.

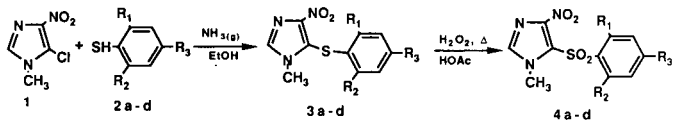
Scheme 1




G = Glutathione

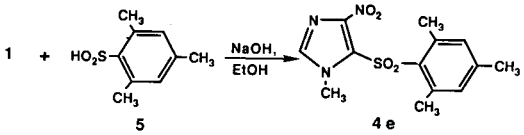
Radiosensitizers in this class were synthesized by treatment of 1-methyl-4-nitro-5-chloroimidazole (**1**) with substituted thiophenols **2** to form sulfides **3**, with subsequent oxidation of the latter to sulfones **4** (Scheme 2) [7,8,9,10]. An alternative method of preparation involved the treatment of **1** with an aryl sulfinic acid salt **5** to form the corresponding sulfone directly (Scheme 3) [7,8,10]. Since bis-*ortho* substitutions on the aryl ring might be expected to sterically decrease the glutathione reactivity of the sulfone, it was proposed that these products might survive contact with glutathione long enough to be effective radiosensitizers *in vivo*.

Scheme 2




| Thiophenol | Sulfide | Sulfone | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|---|
| 2a | 3a | 4a | $CH_3$ | $CH_3$ | $OCH_2CO_2H$ |
| 2b | 3b | 4b | Cl | $CH_3$ | H |
| 2c | 3c | 4c | Cl | Cl | H |
| 2d | 3d | 4d | Cl | $CO_2H$ | Cl |

Scheme 3




The bis-*ortho* substitution on the thiophenol did not impede the sulfide forming reaction, which was essentially complete under the conditions described here in 5 minutes. However, the oxidation of these sulfides to sulfones was slower when compared to the previously reported examples lacking *ortho* substituents [3]. Peroxide oxidation at 60° was usually effective; however, in the case of the 2,6-dichlorophenyl compound **3c** the reaction could only be driven to completion by heating to reflux.

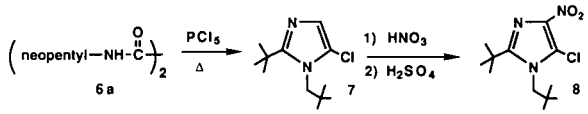
The displacement of halide from 1-methyl-4-nitro-5-chloroimidazole by an aryl sulfinate anion also took place more slowly when the nucleophile had sterically-limiting bis-*ortho* substitution. The reaction of the chloroimidazole with sodium 2,4,6-trimethylbenzene sulfinate required

14.5 hours in refluxing ethanol to achieve a comparable conversion to that obtained with a 1 hour reaction time in the analogous preparation of 4-iodophenyl 1-methyl-4-nitro-5-imidazolyl sulfone.

Additional retardation of glutathione reactivity might be expected if the imidazole's *N*-methyl group were replaced with a bulkier group. Neopentyl moieties, compared to methyls, are known to substantially diminish nucleophilic displacement kinetics of proximally-substituted leaving groups [11]. No simple direct alkylations of imidazoles with bulky alkyl groups have been reported, but *N,N'*-dialkyloxamides are known to yield 1,2-dialkyl-5-chloroimidazoles by the Wallach chlorocyclization reaction [12,13]. For example, *N,N'*-diethyloxamide and phosphorus pentachloride yield 1-ethyl-2-methyl-5-chloroimidazole [14]. However, there are no reports on the use of this reaction to place *tert*-butyl or neopentyl groups on the ring.

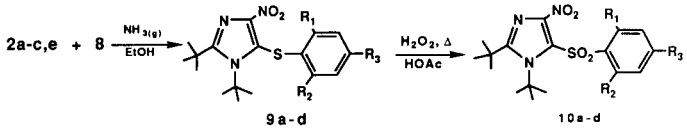
*N,N'*-Dineopentyloxamide (**6a**) prepared from diethyl oxalate and neopentylamine was cyclized to 1-neopentyl-2-*tert-butyl-5-chloroimidazole* (**7**), and nitrated to give 1-neopentyl-2-*tert*-butyl-4-nitro-5-chloroimidazole (**8**) in 50% yield (Scheme 4). This cyclization required more drastic conditions than the comparable reactions with the less sterically hindered *N,N'*-dialkyloxamides. The chloroimidazole was then employed in the syntheses of the sterically hindered radiosensitizers.

Scheme 4




The 5-chloro group was easily displaced from 1-neopentyl-2-*tert*-butyl-4-nitro-5-chloroimidazole by thiophenols and the corresponding sulfides **9** readily oxidized to the sulfones **10** (Scheme 5). As before, sulfides bearing bis-*ortho* substituents on the aryl ring required more vigorous oxidation conditions than the unhindered *para*-substituted aryl compounds.

Scheme 5




| Thiophenol | Sulfide | Sulfone | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|---|
| 2a | 9a | 10a | $CH_3$ | $CH_3$ | $OCH_2CO_2H$ |
| 2b | 9b | 10b | Cl | $CH_3$ | H |
| 2c | 9c | 10c | Cl | Cl | H |
| 2e | 9d | 10d | $CO_2Me$ | H | H |

The attempted displacement reaction of 1-neopentyl-2-*tert*-butyl-4-nitro-5-chloroimidazole with sodium aryl sulfinates as described for the 1-methyl analogues was unsuccessful with hindered as well as unhindered sulfinates bearing phenyl, 4-iodophenyl, and 2,4,6-trimethylphenyl moieties. The *N*-neopentyl group rendered the C-5 chloro group inert to this displacement reaction.

The Wallach chlorocyclization reaction was also attempted on *N-tert*-butyl-*N'*-methyloxamide (**6b**), prepared from ethyl oxalyl chloride, *tert*-butylamine, and methylamine, in order to synthesize 1-*tert*-butyl-5-chloroimidazole by treatment with phosphorus pentachloride. However, after aqueous work up only the starting oxamide was recovered.

Second-order rate constants for the reaction of the sulfones in the 1-methyl series with glutathione at physiological *p*H and temperature were determined spectrophotometrically using the method of Stratford [5]. As the compounds reacted with glutathione the absorbance at

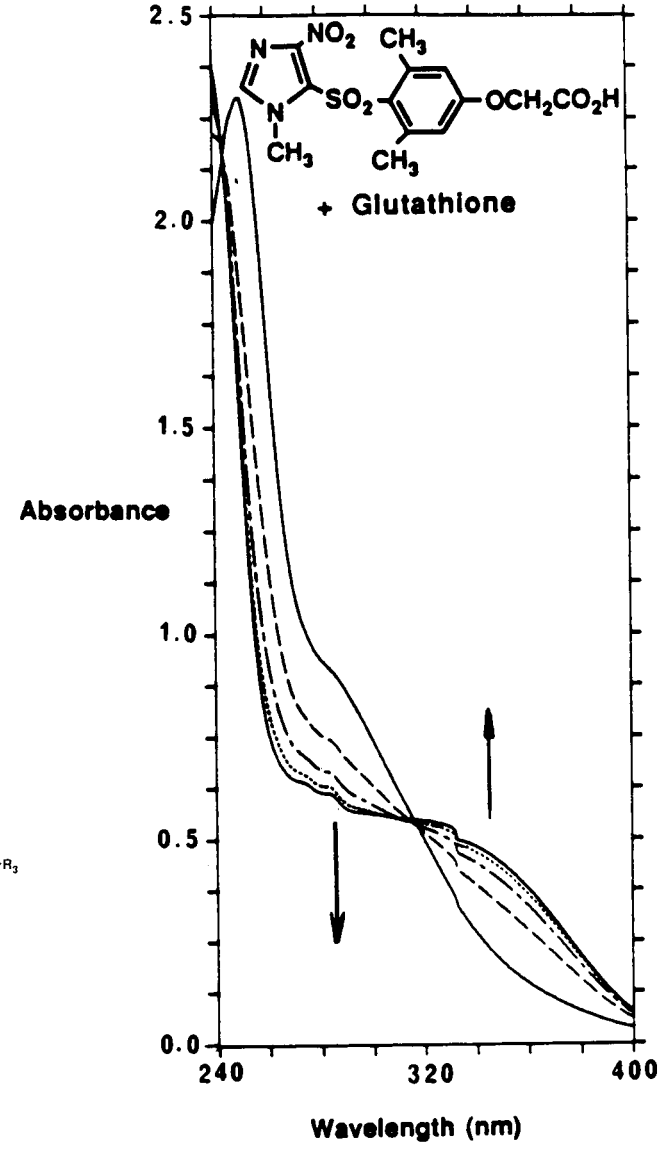



Figure 1

250-300 nm decreased and a product with absorption at 340-380 nm appeared. Stratford has shown that this spectral change is due to the formation of a common product from the second-order displacement of the 5-position substituent by glutathione. Thus, when relatively high concentrations of glutathione were used (at least 10 times the concentration of the drug), both the decrease in the peak at 250-300 nm and the increase in the peak at 340-380 nm were found to be first-order with respect to drug concentration. The isobestic nature of this reaction is shown in Figure 1. Second-order rate constants and half-lives for this reaction at a 1.0 m*M* concentration of glutathione, which is the normal physiological concentration in blood serum, are shown in Table 1.

Table 1

| Compound | $k_2$(l/mol-sec) | $t_{1/2}$(1.0 mM GSH) |
|---|---|---|
| 4a | 1.03 | 10.3 min |
| 4b | 1.63 | 7.1 min |
| 4c | 2.40 | 4.8 min |
| 4d | 0.58 | 20 min |
| 4e | 0.83 | 13 min |

In the more lipophilic *N*-neopentyl series only **10a** could be solubilized sufficiently in buffer to measure the glutathione kinetics. The steric hindrance of the neopentyl group totally inhibited the glutathione displacement, with no spectral evidence of reaction even after one week at glutathione concentrations up to 10 m*M*.

Biological testing of these compounds for radiosensitization of Chinese hamster V-79 cells is underway and will be reported in a subsequent publication. Preliminary bioevaluation shows that all of these nitroimidazoles are highly cytotoxic even in the absence of radiation. These agents had to be screened at concentrations below 0.75 m*M* in order to discriminate the radiation-induced toxicity from the inherent chemotoxicity. Those analogues which were members of the *N*-methyl series displayed radiation sensitization with sensitizer enhancement ratios [1] in the range of 1.2 to 2.7. Members of the *N*-neopentyl series displayed virtually no radiosensitizing potency but were extremely cytotoxic, even at concentrations as low as 50 $\mu M$.

## EXPERIMENTAL

Melting points were obtained on a Thomas-Hoover melting point apparatus are uncorrected. The $^1$H-nmr spectra were obtained on a JEOL FX90Q spectrometer with TMS as the internal standard. The glutathione displacement kinetic data were obtained using a Perkin Elmer Lambda 5 UV-Visible spectrophotometer equipped with a cell-temperature controller. Elemental analyses were performed by the Robertson Microanalytical Laboratory, Florham Park, N.J.

General Procedure for Preparation of Aryl 1-Methyl-4-nitro-5-imidazolyl Sulfides (**3**) from 1-Methyl-4-nitro-5-chloroimidazole (**1**) and a Thiophenol **2**.

A solution prepared from equimolar amounts of 1-methyl-4-nitro-5-chloroimidazole **1** [17] and the appropriate thiophenol **2** was dissolved in anhydrous ethanol (25 ml per gram of **1**) and heated to 50°. The solution was stirred magnetically while a slow stream of ammonia gas was introduced, causing the formation of a dense precipitate. Heating was discontinued after 5 minutes and the ammonia stream and stirring were continued for an additional 45 minutes. The ammonia stream was removed and the solution was allowed to stand for 3 hours at ambient temperature. Water (2 ml per ml of ethanol used as solvent for the reaction) was then added to precipitate the product and dissolve the ammonium chloride by-product. The products were recrystallized from aqueous ethanol or chloroform/hexane. The yields and physical properties of the individual sulfides prepared are described below.

4-Carboxymethoxy-2,6-dimethylphenyl 1-Methyl-4-nitro-5-imidazolyl Sulfide (**3a**).

The title compound was prepared from **1** and 3,5-dimethyl-4-mercaptophenoxyacetic acid (**2a**) [18] as a yellow solid, 88%, mp 227-229°; $^1$H-nmr (DMSO-$d_6$): δ 2.24 (s, 6H, Ar-$CH_3$), 3.78 (s, 3H, N-$CH_3$), 4.69 (s, 2H, O-$CH_2$), 6.81 (s, 2H, Ar-H), 7.90 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{14}H_{15}N_3O_5S$: C, 49.84; H, 4.48; N, 12.46. Found: C, 49.87; H, 4.49; N, 12.25.

2-Chloro-6-methylphenyl 1-Methyl-4-nitro-5-imidazolyl Sulfide (**3b**).

Compound **3b** was prepared from **1** and 2-chloro-6-methylthiophenol (**2b**) as a yellow solid, 85%, mp 201-205°; $^1$H-nmr (DMSO-$d_6$): δ 2.48 (s, 3H, Ar-$CH_3$), 3.51 (s, 3H, N-$CH_3$), 7.83 (m, 3H, Ar-H), 7.99 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{11}H_{10}ClN_3O_2S$: C, 46.56; H, 3.55; N, 14.81. Found: C, 46.91; H, 3.58; N, 14.22.

2,6-Dichlorophenyl 1-Methyl-4-nitro-5-imidazolyl Sulfide (**3c**).

Compound **3c** was prepared from **1** and 2,6-dichlorobenzenethiol (**2c**) in 92% yield, mp 210-215°; $^1$H-nmr (DMSO-$d_6$): δ 3.62 (s, 3H, N-$CH_3$), 7.32-7.67 (m, 3H, Ar-H), 8.08 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{10}H_7Cl_2N_3O_2S$: C, 39.49; H, 2.32; N, 13.82. Found: C, 39.60; H, 2.13; N, 13.53.

2,4-Dichloro-6-carboxyphenyl 1-Methyl-4-nitro-5-imidazolyl Sulfide (**3d**).

Compound **3d** was prepared from **1** and 2,4-dichloro-6-carboxybenzenethiol (**2d**) [19] in 53% yield, mp 240-245°; $^1$H-nmr (DMSO-$d_6$): δ 3.51 (s, 3H, N-$CH_3$), 7.57-7.80 (dd, 2H, Ar-H), 7.96 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{11}H_7Cl_2N_3O_4S$: C, 37.94; H, 2.03; S, 12.07. Found: C, 37.85; H, 2.45; S, 11.85.

General Procedure for the Oxidation of Aryl 1-Methyl-4-nitro-5-imidazolyl Sulfides **3** to Sulfones **4**.

The sulfide **3** was dissolved in glacial acetic acid (40 ml per gram of **3**) and preheated to 60°. Aqueous 30% hydrogen peroxide (one ml per ml of acetic acid) was added dropwise and stirring was continued at 60° until the yellowish color of the solution began to lighten. The temperature was then raised stepwise to 80° and in some cases to reflux. The reaction conditions were determined by following the reaction by thin-layer chromatography on silica using ethyl acetate as a mobile phase. Once the reaction had gone to completion, water (2 ml per ml of reaction

mixture) was added causing the sulfone to precipitate as a white solid. The product was recrystallized from aqueous ethanol or chloroform/hexane. The conditions of the reaction yields for each sulfone prepared along with their physical properties are described below.

4-Carboxymethoxy-2,6-dimethylphenyl 1-Methyl-4-nitro-5-imidazolyl Sulfone (**4a**).

The title compound was prepared from **3a** by the procedure described above using the following temperatures and heating times. The solution was stirred at 60° for 3 hours, then raised to 70° for 5 hours, and subsequently to 80° for 0.5 hour. These conditions produce **4a** in 49% yield, mp 217-220°; $^1$H-nmr (DMSO-$d_6$): δ 2.41 (s, 6H, Ar-$CH_3$), 4.00 (s, 3H, N-$CH_3$), 4.78 (s, 2H, O-$CH_2$), 7.88 (s, 2H, Ar-H), 8.19 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{14}H_{15}N_3O_7S$: C, 45.52; H, 4.09; N, 11.38. Found: C, 45.25; H, 4.08; N, 11.50.

2-Chloro-6-methylphenyl 1-Methyl-4-nitro-5-imidazolyl Sulfone (**4b**).

The general method was used to prepare **4b** from compound **3b** except that the solution was stirred at 60° for 3 hours, 70° for 18 hours, and 80° for 0.5 hour. These conditions gave the title compound as a white solid in 79% yield, mp 147-150°; $^1$H-nmr (DMSO-$d_6$): δ 2.80 (s, 3H, Ar-$CH_3$), 4.06 (s, 3H, N-$CH_3$), 7.40-7.60 (m, 3H, Ar-H), 8.25 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{11}H_{10}ClN_3O_4S$: C, 41.84; H, 3.19; N, 13.31. Found: C, 41.97; H, 3.19; N, 13.07.

2,6-Dichlorophenyl 1-Methyl-4-nitro-5-imidazolyl Sulfone (**4c**).

This compound was prepared from **3c** by the general method using the following conditions. The solution was stirred at 70° for 26 hours, 80° for 18 hours, then at reflux for 0.5 hour, giving the title compound in 96% yield as a white solid, mp 173-177° (from aqueous ethanol); $^1$H-nmr (DMSO-$d_6$): δ 4.07 (s, 3H, N-$CH_3$), 7.63-7.75 (s, 3H, Ar-H), 8.20 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{10}H_7Cl_2N_3O_4S$: C, 35.73; H, 2.10; N, 12.50. Found: C, 35.96; H, 1.94; N, 12.60.

2,4-Dichloro-6-carboxyphenyl 1-Methyl-4-nitro-5-imidazolyl Sulfone (**4d**).

The method described above was used to prepare **4d** from **3d**, except that the solution was stirred at 60° for 6 hours, 70° for 2 hours, and 80° for 0.5 hour. The title compound was collected as off-white crystals, 41%, mp 138-152° dec; $^1$H-nmr (DMSO-$d_6$): δ 3.92 (s, 3H, N-$CH_3$), 7.80-8.00 (dd, 2H, Ar-H), 7.96 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{11}H_7Cl_2N_3O_6S\cdot H_2O$: C, 33.17; H, 2.83; N, 10.55. Found: C, 33.25; H, 2.65; N, 10.25.

2,4,6-Trimethylphenyl 1-Methyl-4-nitro-5-imidazolyl Sulfone (**4e**).

A solution containing **1** (0.250 g, 1.55 mmoles), 2,4,6-trimethylbenzenesulfinic acid (**5**) [20] (0.300 g, 1.63 mmoles), ethanol (1.5 ml), and 0.25 ml of 6*N* sodium hydroxide was heated to reflux, stirred magnetically for 14.5 hours, then allowed to cool to ambient temperature. Water (20 ml) was added, precipitating 0.21 g of the title compound as a white solid, 0.21 g, 44%, mp 158-160° (from ethanol); $^1$H-nmr (DMSO-$d_6$): δ 2.25 (s, 3H, *p*-$CH_3$), 2.42 (s, 6H, *o*-$CH_3$), 4.02 (s, 3H, N-$CH_3$), 7.11 (s, 2H, Ar-H), 8.20 (s, 1H, imidazole-H).

*Anal.* Calcd. for $C_{13}H_{15}N_3O_4S$: C, 50.47; H, 4.89; N, 13.58. Found: C, 50.72; H, 4.78; N, 13.28.

*N,N'*-Dineopentyloxamide (**6a**).

Diethyl oxalate (75.5 g, 0.52 mole) was dissolved in 145 ml of 95% ethanol and chilled to 2°, then neopentylamine (90.1 g, 1.03 moles) was added dropwise at 2-8° over 75 minutes to the stirred mixture. Towards the end of the addition, a copious precipitate of product caused cessation of magnetic stirring. The cold water bath was removed and the reaction allowed to stand overnight. A first crop of 98.0 g was removed by filtration, then the filtrate was refrigerated overnight, precipitating a second crop of 10.7 g, The combined crops were recrystallized from 50% aqueous ethanol to yield the title compound, 106.7 g, 91%, mp 167-170°; $^1$H-nmr (deuteriochloroform): δ 1.08 (s, 18H, $CH_3$), 3.14 (d, 4H, $CH_2$).

*Anal.* Calcd. for $C_{12}H_{24}N_2O_2$: C, 63.12; H, 10.59; N, 12.27. Found: C, 63.11; H, 10.87; N, 12.27.

*N-tert*-Butyl-*N'*-methyloxamide (**6b**).

Ethyl oxalyl chloride (12.22 g, 89.5 mmoles) was dissolved in 100 ml of dry benzene and chilled to 0°. *tert*-Butylamine (13.1 g, 179 mmoles) was then added dropwise over 30 minutes and stirred for an additional 30 minutes at that temperature. The *tert*-butylamine hydrochloride by-product was removed by filtration and the filtrate warmed to 25°. Methylamine (2.77 g, 503 mmoles) as a 40% aqueous solution was added dropwise to the filtrate over 10 minutes with stirring, which was continued for 0.5 hour after addition was complete. Solid impurities were removed by filtration, then the benzene was evaporated *in vacuo*. The product was washed with 50 ml of 5% sodium bicarbonate, then collected by vacuum filtration to yield the title compound, 6.95 g, 49%, mp 119-121°; $^1$H-nmr (DMSO-$d_6$): δ 1.35 (s, 9H, C($CH_3$)$_3$), 2.68 (d, J = 8 Hz, 3H, N-$CH_3$), 3.38 (br s, 1H, NH), 4.20 (q, 1H, NH).

*Anal.* Calcd. for $C_7H_{14}N_2O_2$: C, 53.14; H, 8.92; N, 17.71. Found: C, 53.18; H, 9.01; N, 17.84.

1-Neopentyl-2-*tert*-butyl-4-nitro-5-chloroimidazole (**8**).

A dry mixture of **6a** (100 g, 0.438 mole) and phosphorus pentachloride (300 g, 1.44 moles) was heated on a steam bath for 11.5 hours, followed by heating to reflux (110°) on a heating mantle for 2 hours. The mixture was cooled, then vacuum distilled to remove the by-product phosphorus oxychloride. The residue was chilled in an ice bath and 125 ml of water was slowly added. The *p*H was adjusted to 10 with 50% aqueous sodium hydroxide, the inorganic salts were removed by filtration, and the filtrate was extracted with chloroform (4 x 250 ml). The chloroform was removed by evaporation *in vacuo* in yield 50 g of a viscous dark liquid which was vacuum distilled to yield to yield 1-neopentyl-2-*tert*-butyl-5-chloroimidazole (**7**), 36 g, 36%, bp 85-90°, 0.5 mm Hg; $^1$H-nmr (DMSO-$d_6$): δ 1.00 (s, 9H, neopentyl-$CH_3$), 1.39 (s, 9H, *tert*-butyl-$CH_3$), 4.00 (s, 2H, $CH_2$), 6.89 (s, 1H, imidazole-4-H).

A mixture of concentrated nitric acid (15 ml, density 1.51) and water (10 ml) was added dropwise to a flask containing **7** (15.0 g, 65.5 mmoles). The mixture was heated to 70° and stirred for 0.5 hour, then the water and excess acid was removed by evaporation *in vacuo* to yield the nitrate salt as a yellowish-green oil. Concentrated sulfuric acid (70 ml) was added dropwise to the salt over 0.5 hour while stirring. After addition was complete the mixture was heated with a steam bath for 2 hours. The mixture was then chilled and poured int 500 ml of ice water precipitating a brown solid which was recrystallized from chloroform/hexane to give the title product as off-white crystals, 11.25 g, 63%, mp 70-72°; $^1$H-nmr (DMSO-$d_6$): δ 1.02 (s, 9H, neopentyl-$CH_3$), 1.43 (s,

9H, *tert*-butyl-$CH_3$), 4.19 (s, 2H, $CH_2$).

*Anal.* Calcd. for $C_{12}H_{20}ClN_3O_2$: C, 52.64; H, 7.36; N, 15.35. Found: C, 52.66; H, 7.30; N, 15.33.

General Method for the Preparation of Aryl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfides **9**.

A solution prepared from **8** and the appropriate thiophenol **2** dissolved in anhydrous ethanol (30 ml per gram of **8**) was heated to 50° and stirred while ammonia gas was introduced, causing the formation of a dense precipitate. After 10 minutes the heat was removed. The ammonia stream and stirring was continued for an additional 50 minutes, after which the ammonia stream was ceased and the solution stirred at ambient temperature for 2 hours. The reaction mixture was then diluted with water (2 ml per ml of ethanol solvent) and chilled to precipitate a gummy solid. The crude products were recrystallized from chloroform/-hexane to yield the sulfides. The yields and physical constants of the compounds prepared by this method are reported below.

4-Carboxymethoxy-2,6-dimethylphenyl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfide (**9a**).

Compound **9a** was prepared from **8** and **2a** in 82% yield, mp 194-196°; $^1$H-nmr (DMSO-$d_6$): δ 1.00 (s, 9H, neopentyl-$CH_3$), 1.41 (s, 9H, *tert*-butyl-$CH_3$), 2.10 (s, 6H, Ar-$CH_3$), 4.37 (s, 2H, N-$CH_2$), 4.62 (s, 2H, O-$CH_2$), 6.70 (s, 2H, Ar-H).

*Anal.* Calcd. for $C_{22}H_{31}N_3O_5S$: C, 58.77; H, 6.95; N, 9.35. Found: C, 58.65; H, 6.70; N, 9.14.

2-Chloro-6-methylphenyl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfide (**9b**).

Compound **9b** was prepared from **8** and 2-chloro-6-methylthiophenol (**2b**) as a light yellow solid, 38%, mp 137-138° (from aqueous ethanol); $^1$H-nmr (DMSO-$d_6$): δ 1.10 (s, 9H, neopentyl-$CH_3$), 1.42 (s, 9H, *tert*-butyl-$CH_3$), 2.49 (s, 3H, Ar-$CH_3$), 4.39 (s, 2H, $CH_2$), 7.28 (s, 3H, Ar-H).

*Anal.* Calcd. for $C_{19}H_{26}ClN_3O_2S$: C, 57.63; H, 6.62; N, 10.61. Found: C, 57.32; H, 6.27; N, 10.71.

2,6-Dichlorophenyl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfide (**9c**).

Compound **9c** was prepared in 96% yield from **8** and 2,6-dichlorothiophenol (**2c**) as a yellow solid, mp 148-149°; $^1$H-nmr (DMSO-$d_6$): δ 1.01 (s, 9H, neopentyl-$CH_3$), 1.42 (s, 9H, *tert*-butyl-$CH_3$), 4.39 (s, 2H, $CH_2$), 7.26-7.62 (m, 3H, Ar-H).

*Anal.* Calcd. for $C_{18}H_{23}Cl_2N_3O_2S$: C, 51.92; H, 5.57; N, 10.09. Found: C, 51.88; H, 5.56; N, 10.02.

1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl 2-Carbomethoxyphenyl Sulfide (**9d**).

Compound **9d** was prepared from **8** and 2-carbomethoxythiophenol (**2e**) [17] as a yellow solid, 93%, mp 117-118°; $^1$H-nmr (DMSO-$d_6$): δ 1.00 (s, 9H, neopentyl-$CH_3$), 1.50 (s, 9H, *tert*-butyl-$CH_3$), 3.92 (s, 3H, O-$CH_3$), 4.17 (s, 2H, $CH_2$), 6.39-8.05 (m, 4H, Ar-H).

*Anal.* Calcd. for $C_{20}H_{27}N_3O_4S$: C, 59.23; H, 6.71; N, 10.36. Found: C, 59.10; H, 6.48; N, 10.22.

General Procedure for the Oxidation of Aryl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfides **9** to Sulfones **10**.

The sulfide **9** was dissolved in glacial acetic acid (40 ml per gram of **9**) and preheated to 60°. Aqueous 30% hydrogen peroxide (one ml per ml of acetic acid) was added dropwise and stirring was continued at 60° until the yellowish color of the solution began to lighten. The temperauture was then raised stepwise to 80° and in some cases to reflux. Once the reaction had gone to completion, water (2 ml per ml of reaction mixture) was added causing the sulfone to precipitate as a white solid. The product was collected by filtration, then the filtrate was extracted with chloroform from which a second crop of product was precipitated by addition of hexane. The product was then recrystallized from chloroform/hexane. The yields for the sulfones and their physical properties are described below.

4-Carboxymethoxy-2,6-dimethylphenyl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfone (**10a**).

The title compound was prepared from **9a** using the method described above and the following conditions. The reaction mixture was stirred for 0.5 hour at 60° followed by 4 hours at 70° and 1 hour at 80°. After work up and recrystallization, **10a** was collected in 34% yield, mp 68-68.5°; $^1$H-nmr (DMSO-$d_6$): δ 1.05 (s, 9H, neopentyl-$CH_3$), 1.48 (s, 9H, *tert*-butyl-$CH_3$), 2.25 (s, 6H, Ar-$CH_3$), 4.26-5.00 (dd, 2H, N-$CH_2$), 5.78 (s, 2H, O-$CH_2$), 6.0 (s, 2H, Ar-H).

*Anal.* Calcd. for $C_{22}H_{31}N_3O_7S$: C, 54.87; H, 6.49; N, 8.73. Found: C, 54.78; H, 6.22; N, 9.00.

2-Chloro-6-methylphenyl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfone (**10b**).

Compound **10b** was prepared as a white solid from **9b** by the procedure described above using the following heating temperatures and times. The reaction mixture was stirred at 60° for 1.5 hours, 4.75 hours at 70°, then 1 hour at 80° and 1 hour at reflux. The reaction yielded the title compound in 70% yield, mp 181-184°; $^1$H-nmr (DMSO-$d_6$): δ 1.01 (s, 9H, neopentyl-$CH_3$), 1.49 (s, 9H, *tert*-butyl-$CH_3$), 2.79 (s, 3H, Ar-$CH_3$), 4.30-4.98 (dd, 2H, $CH_2$), 7.45 (m, 3H, Ar-H).

*Anal.* Calcd. for $C_{19}H_{26}ClN_3O_4S$: C,. 53.32; H, 6.12; N, 9.82. Found: C, 53.08; H, 5.96; N, 9.60.

2,6-Dichlorophenyl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfone (**10c**).

The conditions under which compound **10c** was prepared from **9c** were heating at 60° for 1 hour, 5 hours at 70°, then 5 hours at reflux. The title compound was isolated as a white solid, mp 175-177°; $^1$H-nmr (DMSO-$d_6$): δ 1.02 (s, 9H, neopentyl-$CH_3$), 1.49 (s, 9H, *tert*-butyl-$CH_3$), δ 4.31-4.94 (dd, 2H, $CH_2$), 7.62 (s, 3H, Ar-H).

*Anal.* Calcd. for $C_{18}H_{23}Cl_2N_3O_4S$: C, 48.22; H, 5.17; N, 9.37. Found: C, 47.93; H, 5.02; N, 9.15.

2-Carbomethoxyphenyl 1-Neopentyl-2-*tert*-butyl-4-nitro-5-imidazolyl Sulfone (**10d**).

Compound **10d** was prepared from **9d** by the general method described above. The conditions used were heating at 60° for 1.25 hours, 70° for 1.5 hours, then 80° for 1.25 hours. The title compound was collected as a yellow solid in 83% yield, mp 131-133°; $^1$H-nmr (DMSO-$d_6$): δ 1.02 (s, 9H, neopentyl-$CH_3$), 1.51 (s, 9H, *tert*-butyl-$CH_3$), 3.78 (s, 3H, O-$CH_3$), 4.27-4.83 (dd, 2H, $CH_2$), 7.77-8.32 (m, 4H, Ar-H).

*Anal.* Calcd. for $C_{20}H_{27}N_3O_6S$: C, 54.90; H, 6.22; N, 9.60. Found: C, 54.66; H, 6.48; N, 9.36.

Determination of Glutathione Displacement Rates.

The rate of displacement of the sulfonyl group from the aryl

1-methyl-4-nitro-5-imidazoly sulfones by glutathione was measured at least three times for each compound to insure the reproducibility of the data. A 900-μl portion of the sulfone dissolved in *p*H 7.4 phosphate buffer [22] at a concentration of 50 μ*M* was placed in a 1.0 ml 10 mm pathlength quartz uv cuvette and was allowed to equilibrate to 37.0° in the temperature controlled cell holder of the spectrophotometer before the experiment was started. The uv spectrum was recorded from 200 to 400 nm, and the spectrometer was set to record the absorbance at 295 nm and 340 nm at time intervals of 15 seconds. A 100-μl aliquot of 10.0 m*M* glutathione in *p*H 7.4 buffer was added to the cell and data collection was started. It was continued until the change in absorbance remained constant to 0.001 absorbance units for at least 5 minutes, implying that the reaction had gone to completion and all the sulfone had been consumed. Since the starting concentrations in the reaction mixture were approximately 0.045 μ*M* in sulfone and 1.00 m*M* in glutathione, the glutathione was present in greater than 20-fold excess and pseudo first-order kinetics should hold, even at the end of the reaction. A pseudo first-order rate constant was calculated from the data and converted into the actual second-order rate constant by dividing by the glutathione concentration. The rate was calculated from the data at both wavelengths to insure that the spectra changes were occurring isobestically. To insure that the second-order rate constant was not dependent upon the glutathione concentration, the experiment was also run at a 2.00 m*M* or 4.00 m*M* final glutathione concentration.

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