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Synthesis, characterization and in vitro evaluation of various sulfonamide chemical delivery systems †

Marcus E. Brewster [1,2], Margaret Deyrup [2,*], Kazimierz Seyda [2,+] and Nicholas Bodor [1,2]

[1] *University of Florida, Center for Drug Design and Delivery, College of Pharmacy, Box J-497 JHMHC, Gainesville, FL 32610 (U.S.A.) and* [2] *Pharmatec, Inc., P.O. Box 730, Alachua, FL 32615 (U.S.A.)*

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Summary

Dihydropyridine ↔ pyridinium salt type chemical delivery systems were prepared for several sulfonamides found useful in the treatment of cerebral toxoplasmosis. Sulfadiazine, sulfamethoxazole, sulfamerazine and sulfamethazine were considered and both aniline (N^4) and sulfamide (N^1) derivatization were performed. The sulfamethoxazole derivative in which a reduced nicotinamide moiety was attached at the N^1-site provided a compound which rapidly oxidized in various matrices and was highly lipophilic. In addition, studies in rat brain homogenates illustrated appropriate conversion of the chemical delivery system with ultimate release of the active sulfa drug.

Introduction

A serious and often deadly opportunistic infection associated with acquired immune deficiency syndrome (AIDS) is cerebral toxoplasmosis (Luft et al., 1984; Levy et al., 1985; Navia et al., 1986; Tuazon, 1989; Rossitch et al., 1990). This malady is, in fact, the most common opportunistic infection occurring in the central nervous system (CNS) of patients stricken with AIDS and is expected to affect between 20000 and 40000 people by 1991 (Luft and Remington, 1988). The prognosis for those with cerebral toxoplasmosis is poor with more than 70% of individuals contracting the disease succumbing to the infection (Luft and Remington, 1988).

Toxoplasma gondii is an obligate intracellular parasite which is ubiquitously distributed in the world (Frenkel, 1985). This protozoan infects humans either congenitally or via a livestock vector. The latter occurs when raw or undercooked meat is consumed (McCabe and Remington, 1988). Toxoplasmosis is a common infection as indicated by seroprevalence studies which show that be-

Correspondence: M.E. Brewster, University of Florida, Center for Drug Design and Delivery, College of Pharmacy, Box J-497 JHMHC, Gainesville, FL 32610, U.S.A.

* *Present address:* Xenon Vision, Inc., Alachua, FL 32615, U.S.A.

\+ *Present address:* University of South Carolina, College of Pharmacy, Columbia, SC, U.S.A.

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tween 20 and 80% of the population expresses antitoxoplasma antibodies (Levy et al., 1985). While infection is widespread, it is usually benign as it is well controlled by T-lymphocytes and activated macrophages. In AIDS, this arm of the immune system is annihilated causing toxoplasmosis to run a more fulminate course.

In the AIDS patient, toxoplasmosis usually occurs in the brain (Navia et al., 1986). This localization may result from the immunological isolation of the CNS. In any case, *T. gondii* causes lesions in the brain the clinical manifestations of which include seizures, hemiparesis and aphasia.

Treatment of cerebral toxoplasmosis has relied on classical sulfonamides such as sulfadiazine and to a lesser extent, sulfamethoxazole, sulfamerazine and sulfamethazine in combination with pyrimethamine (Leport et al., 1988; McCabe and Oster, 1989; Sande, 1989). This regimen acts synergistically to inhibit folic acid synthesis, a biochemical process which is unique to the parasite. Typically, 4 g of sulfadiazine and 25–100 mg of pyrimethamine are administered daily to suppress infection (McCabe and Oster, 1989). While such combination therapy is initially effective in reversing clinical symptoms and shrinking cerebral lesions, it is also associated with various severe toxicities (Handler et al., 1983). These side effects, which are predominantly due to the sulfonamide component, include rashes such as Stevens-Johnson syndrome, fever, and various hematopoietic alterations such as hemolytic or aplastic anemia, agranulocytosis, thrombocytopenia and eosinophilia (Carrol et al., 1966; Benson and Harris, 1986). In addition, it appears that these toxicities occur with significantly higher frequency in the AIDS patient than in the population as a whole. It was estimated that in a recent study, more than 70% of AIDS patients who received sulfonamides for toxoplasmosis develop some intolerance to the drug (Leport et al., 1988).

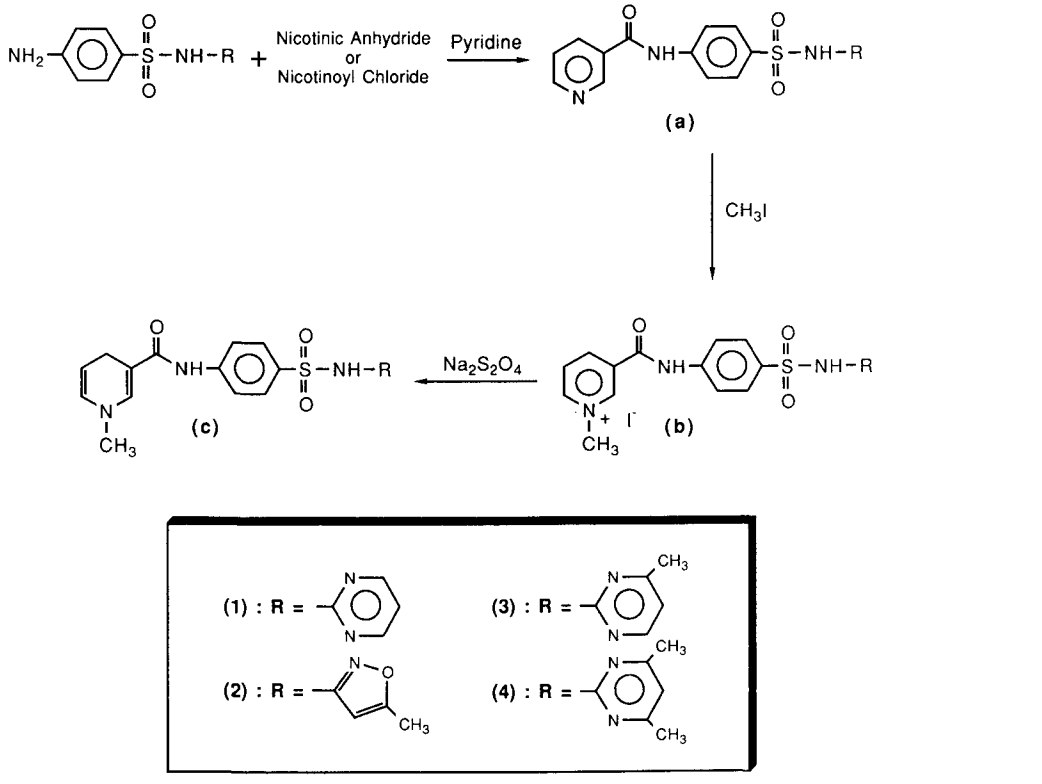
Targeting of sulfonamides to the CNS may, therefore, improve the therapeutic index of these agents. Sulfadiazine and other sulfa drugs used in the treatment of cerebral toxoplasmosis are of sufficient lipophilicity that they readily pass the blood-brain barrier (BBB). Thus, the seminal issue in developing delivery systems for these agents is selectivity. One method which may provide such specificity is the chemical delivery system (CDS) (Bodor et al., 1981; Bodor and Brewster, 1983; Bodor, 1987; Bodor and Kaminski, 1987). This approach tethers a biooxidizable redox carrier to the drug of interest. After administration, the modified drug distributes extensively in the body but exerts minimal pharmacological activity. Importantly, the molecular carrier is designed to undergo oxidative conversion to form a polar, charged salt which is readily eliminated from most tissues and the systemic circulation. In the CNS, the now polar entity is sequestered behind the lipoidal BBB with the result being that central levels of the charged drug-carrier conjugate are relatively high and sustained relative to peripheral concentrations. The 'locked' in drug precursor then degrades releasing the active principle to exert pharmacological action.

The CDS method has been extensively applied and evaluated (Bodor and Simpkins, 1983; Brewster et al., 1987; Raghavan et al., 1987). One of the most useful carrier groups which has been found are derivatives of 1,4-dihydronicotinic acids. Conjugates resulting from this carrier group are often stable to formulation and nontoxic (Brewster et al., 1988a,b).

The present communication examines the applicability of the CDS concept to sulfonamides with proven efficacy towards cerebral toxoplasmosis. This class of compounds has not been previously manipulated for the purposes of generating a redox delivery system so the requisite chemistry had to be developed. A discussion of these methods as well as the physical properties and in vitro behavior of various derivatives are presented.

Results and Discussion

The sulfonamides which were studied included sulfadiazine, sulfamethoxazole, sulfamerazine and sulfamethazine. In these derivatives, two potential synthetic handles for attaching the carrier systems were considered including the exocyclic N^4-aniline amino group and the N^1-sulfamido group. Simple N^4-nicotinamides were first considered (Scheme 1). These compounds would be inert antimicrobi-

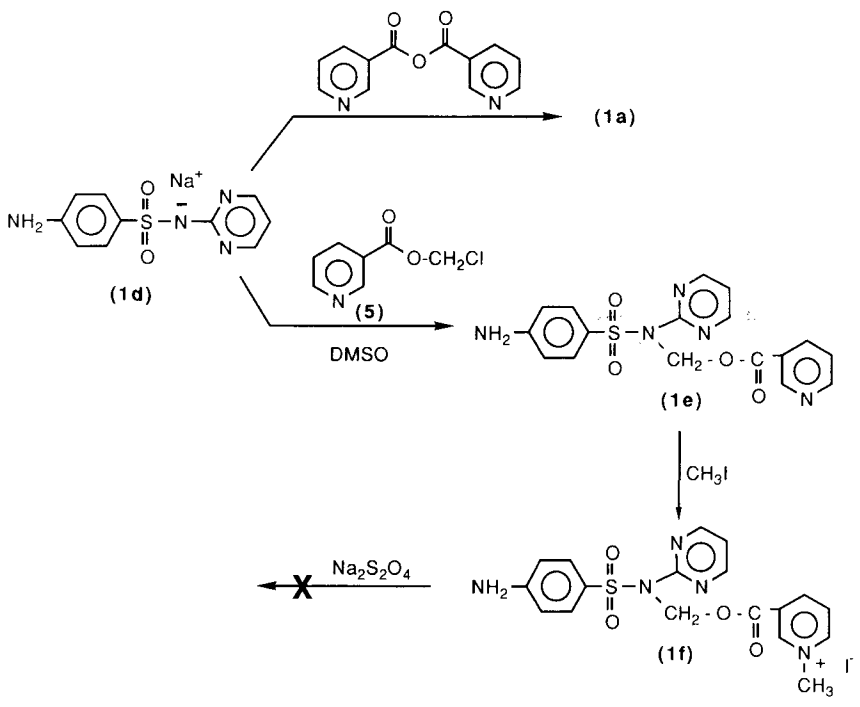


Scheme 1.

ally as structure-activity studies have clearly shown that N^4-substitution mitigates biological activity. In generating the amides, sulfadiazine (**1**), sulfamethoxazole (**2**), sulfamerazine (**3**) or sulfamethazine (**4**) were each treated with either nicotinoyl chloride or nicotinic anhydride in dry pyridine. The corresponding N^4-nicotinamides (**1a, 2a, 3a** and **4a**) were then methylated using methyl iodide to generate the N^4-(1-methylnicotinamide) iodides (**1b, 2b, 3b** and **4b**). Reaction of these salts with sodium dithionite in a basic aqueous medium gave rise to the corresponding N^4-(1-methyl-1,4-dihydronicotinamides) for sulfadiazine (**1c**), sulfamethoxazole (**2c**), sulfamerazine (**3c**) and sulfamethazine (**4c**).

Attempts were next made to derivatize the N^1-sulfamide position of sulfadiazine. Preparation of the sodium salt of sulfadiazine (**1d**) was performed using methanolic sodium hydroxide. Reaction of (**1d**) with nicotinic anhydride in pyridine, dimethylsulfoxide (DMSO), methanol or dimethylformamide (DMF) led only to isolation of the N^4-amide (**1a**). Protection of the aniline position via acetylation, phthaloylation or formation of a benzaldehyde Schiff base provided intermediates which were unreactive. While acylation was unsuccessful, alkylation of (**1d**) could be carried out using chloromethyl nicotinate (**5**) in a DMSO solvent (Scheme 2). The resulting acyloxyalkyl derivative (**1e**) was then quaternized with methyl iodide in tetrahydrofuran to give the pyridinium salt (**1f**). Use of other solvents led to product decomposition. Unfortunately, reduction of (**1f**) to the dihydronicotinate using sodium dithionite and a variety of bases and solvents failed.

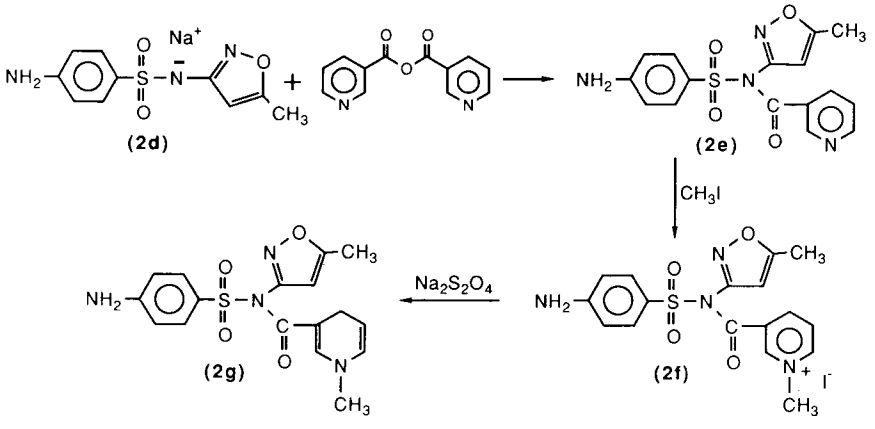
The poor reactivity of the anion of sulfadiazine may relate to its stabilization by the electron-withdrawing pyrimidine group. Use of other compounds substituted with N^1-groups which are inductively less electron-attracting may provide for somewhat more facile compound preparation. This appeared to be the case in the preparation of N^1-derivatives of sulfamethoxazole (Scheme 3). Reaction of the sodium salt of sulfamethoxazole



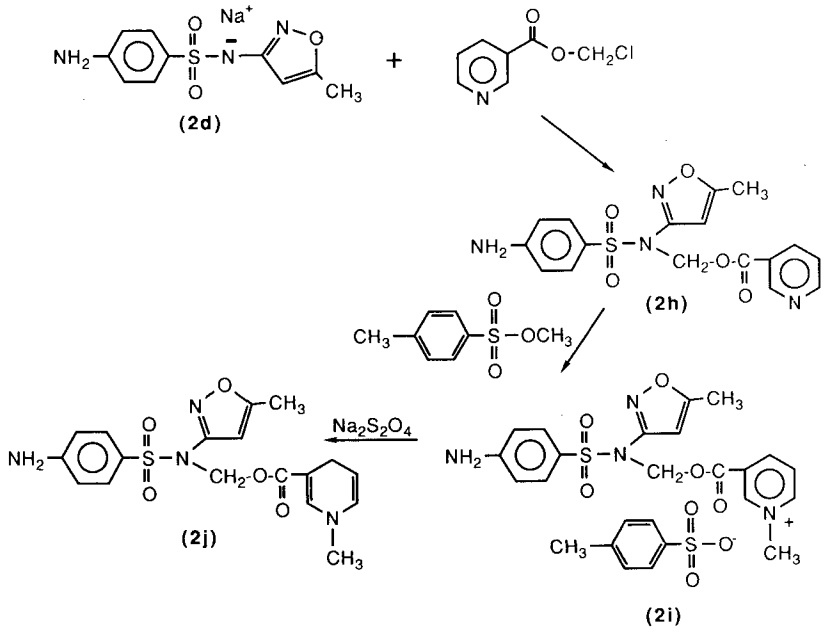
Scheme 2.

(**2d**) with nicotinic anhydride in pyridine for 3 h afforded the desired amide (**2e**) in good yield. If longer reaction times were used, N^4-N^1 equilibration occurred and mixtures of both the N^4-amide and N^1-sulfamide were obtained. Treatment of (**2e**) with methyl iodide gave the quaternary salt (**2f**) which upon sodium dithionite reduction gave the CDS (**2g**).

Finally, reaction of chloromethyl nicotinate (**5**) with (**2d**) in DMSO gave rise to the N^1-sulfamidomethylnicotinate (**2h**) in good yield (Scheme 4). While use of methyl iodide to generate the



Scheme 3.



Scheme 4.

pyridinium salt invariably led to decomposition, surrogates such as methyl *p*-toluenesulfonate or dimethylsulfate provided the object compound (**2i**) in a highly pure form. Reduction of (**2i**) with sodium dithionite gave rise to the appropriate dihydronicotinate (**2j**).

Characterization

The CDS approach requires that the reduced transport form be sufficiently lipophilic to breech the BBB and coincidentally achieve a high and uniform tissue distribution. One of the most useful predictors for the extent of tissue distribution is lipophilicity and thus this parameter was considered (Levin, 1980). Relative lipophilicities for a series of redox derivatives as well as sulfadiazine, sulfamethoxazole and sulfamerazine were examined using an R_m method (Biagi et al., 1969). This measure uses the retention of compounds on C-18 reversed phase thin-layer chromatographic (TLC) plates in various aqueous acetone mobile phases as an indication of lipophilicity. The obtained R_m values for (**1c**), (**2c**), (**3c**), (**2g**), (**2j**), as well as (**1**), (**2**) and (**3**) are presented in Table 1. As shown, the attachment of the 1-methyl-1,4-dihydronicotinoyl group to the N^4-aniline resulted in an increase in conjugate lipophilicity compared with the unsubstituted compounds. This increase was modest and ranged from approx. 1.1 in the case of (**3c**) to 5 in the case of (**2c**). Acylation of the N^1-sulfamide group of (**2**) provided a compound (**2g**) which demonstrated 100-fold increase in lipophilicity compared to the parent sulfamethoxazole and 20-fold increase compared to the N^4-amide (**2c**). The most lipophilic derivative was the nicotinate (**2j**) which was almost 1000-times more lipoidal than sulfamethoxazole, 200-fold more

TABLE 1

Extrapolated R_m values (100% water) for a series of sulfonamide chemical delivery systems and for various sulfa drugs

Compound	R_m (100%)	r
(**1c**)	0.25	0.981
(**2c**)	0.40	0.977
(**3c**)	0.41	0.998
(**2g**)	1.74	0.990
(**2j**)	2.69	0.987
(**1**)	−0.18	0.952
(**2**)	−0.25	0.972
(**3**)	−0.37	0.999

TABLE 2

Second-order rate constants (k_0, s^{-1} M^{-1}) for ferricyanide-mediated oxidation of various sulfonamide chemical delivery systems

Compound	k_0 (s^{-1} M^{-1})	r
(1c)	15.88	0.999
(2c)	15.36	0.999
(3c)	18.72	0.999
(2g)	56.10	0.999
(2j)	0.57	0.997

TABLE 3

Apparent first-order half-lives of disappearance for various sulfonamide chemical delivery systems in isotonic pH 7.4 buffer and 20% rat brain homogenate

Compound	Buffer		Brain homogenate	
	$t_{1/2}$ (min)	r	$t_{1/2}$ (min)	r
(1c)	17.0	0.998	8.0	0.993
(2c)	20.1	0.999	7.4	0.999
(2g)	37.8	0.995	15.1	0.998

lipophilic than the corresponding N^4-amide (**2c**) and almost 10-fold more lipoidal than the N^1-sulfamide (**2g**).

Oxidative stability of the prepared compounds was investigated by examining the reactivity of the dihydropyridine derivatives to potassium ferricyanide (Powell et al., 1984; Brewster et al., 1989). As summarized in Table 2, the compounds fell into three groups based on their second order rate constants for oxidation. The most stable derivative was the nicotinoyloxymethylsulfonamide (**2j**) followed by the group of structurally-related N^4-nicotinamides (**1c, 2c** and **3c**). These latter compounds were approximately 30-fold more reactive than the nicotinate ester. The most labile derivative was (**2g**) which reacted approx. 3-times faster than the N^4-amides and 100-times more rapidly than (**2j**).

This reactivity order (**2g**) > (**1c**) ≅ (**2c**) ≅ (**3c**) > (**2j**) can be rationalized based on the degree of electron delocalization in the dihydropyridine nucleus. Esters are more electron-withdrawing than amides due primarily to competition of amide resonance which tends to localize electron density in the dihydropyridine ring. Substitution of the amide nitrogen tends to favor even more highly the imine tautomer and thus exacerbate electronic localization. This means that in the sequence ester, amide, substituted amide the degree of electron delocalization from the dihydropyridine decreases and as a result reactivity increases (Brewster et al., 1989).

In vitro evaluation

The interaction of various CDS in 20% brain homogenate and pH 7.4 buffer was next considered. The N^4-nicotinamide derivatives (**1c**) and (**2c**) degraded with similar rates in pH 7.4 buffer as shown in Table 3. The N^1-sulfamide (**2g**) disappeared with a half-life approximately twice that of the N^4-derivatives. All compounds were more labile in the biological matrix consistent with enzymatically-mediated degradation. In this medium, (**1c**) and (**2c**) were most readily oxidized while (**2g**) was more stable. Interestingly, this is the reverse rank order of reactivity which occurred in the chemical oxidant study.

Chromatographic analysis of the tissue homogenate study indicated that compounds (**1c**) and (**2c**) readily disappeared in a pseudo-first order fashion with concomitant appearance of the corresponding quaternary salts (**1b**) and (**2b**), respec-

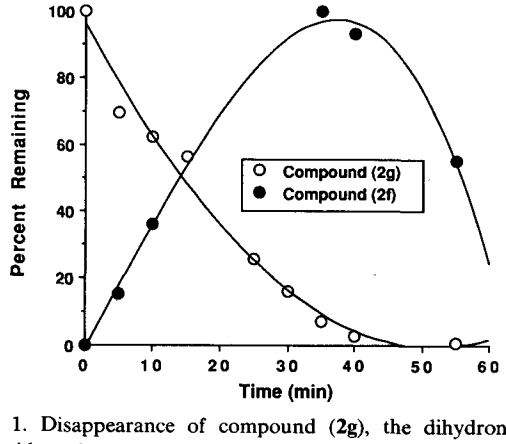


Fig. 1. Disappearance of compound (**2g**), the dihydronicotinamide and appearance of the corresponding quaternary salt (**2f**). Values are given as percent of compound remaining. In addition, the parent compound, sulfamethoxazole (**2**), appeared beginning at 35 min and was present at increasing concentrations through the end of the experiment (55 min).

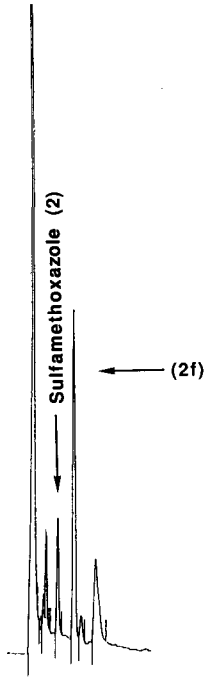


Fig. 2. Appearance of (**2f**) and (**2**) in 20% rat brain homogenate 55 min after addition of the sulfamethoxazole-CDS (**2g**).

tively. In either case, the parent compounds (**1**) or (**2**), respectively, could not be detected. Examination of (**2g**) in brain homogenate showed not only linear disappearance of (**2g**) and appearance of (**2f**) but also progressive appearance of significant concentrations of the parent drug, (**2**) (Figs 1 and 2).

The stability of the N^4-amides in biological media is consistent with various published reports which show they are very resistant to enzymatic hydrolysis. Thus, while N^4-derivatization attenuates biological activity (Roblin et al., 1940; Siuda and Cihonski, 1972), a requirement for a successful prodrug, their in vivo stability prevents rapid sulfonamide regeneration (Larsen et al., 1987). The case for N^1-substitution is different. Larsen and Bundgaard found that N^1-acyl derivatives of *N*-methyl-*p*-toluenesulfonamide underwent facile enzymatic hydrolysis generating the acid and sulfonamide readily (Larsen et al., 1988). Thus the N^1-benzoyl derivative of *N*-methyl-*p*-toluenesulfonamide was shown to hydrolyze in human plasma with a half-life of less than 2 h. This work is confirmed here in that acylation of the substituted N^1-sulfamide group resulted in the formation of a hydrolytically labile functionality. As a result, subsequent to oxidation of (**2g**) to (**2f**), hydrolysis of the charged amide occurred. This provides evidence that these CDS will act as delivery forms for the peripherally toxic sulfonamides.

In conclusion, synthesis, characterization and in vitro studies of a variety of potential CDS's for sulfa drugs were presented. The data show that the N^1-derivatized agents demonstrate the greatest potential as CDS since they are relatively lipophilic, readily convert to the quaternary depot form and that they have been shown to release the active principle in brain homogenates.

Materials and Methods

Chemistry

Elemental analyses of compounds synthesized were performed by Atlantic Microlabs, Atlanta, GA. Melting points were determined using a Hoover-Thomas melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL 200 or XL 300 spectrometer using either a ^1H (200 or 300 MHz, FT mode) or ^{13}C (50 or 75 MHz, FT mode) probe. Samples were dissolved in an appropriate deuterated solvent and chemical shifts reported as parts per million (δ) relative to an internal standard (tetramethylsilane). Coupling constants (J) are reported in hertz. Ultraviolet spectra (UV) were recorded on a Hewlett Packard 8451A diode array spectrophotometer. Various intermediates and most final products were purified on alumina using either an open column or radial (Chromatotron®) method. Samples were dissolved in methylene chloride and applied to the column or disc of activated, basic Brockmann Activity grade I aluminum oxide (58 Å, Aldrich Chemical Co.) which had been prepared with methylene chloride. All chemicals were of reagent grade. Sulfonamides were obtained from Sigma Chemical Co., St. Louis, MO.

*N^4-(3'-Carbonylpyridinyl)-N^1-(2''-pyrimidinyl) sulfanilamide (**1a**)*

Sulfadiazine (12.5 g) was dissolved in 60 ml of dry pyridine and 9.2 g of nicotinoyl chloride hydrochloride were added at once. The reaction mixture was stirred for 7 h at room temperature. Pyridine was then removed under vacuum and the residue was treated with 500 ml of 2% sodium bicarbonate and the mixture was stirred for 48 h. A white solid precipitated out and was filtered, washed with water (300 ml) and dried in the vacuum oven. The product was obtained as a white solid (19.5 g, 98% yield). M.p. 285–287 °C. Found C, 51.32; H, 3.98; N, 18.92; S, 8.62. For $C_{16}H_{13}N_5O_3S$ required: C, 51.46; H, 4.05; N, 18.75; S, 8.58. ^{1}H NMR (DMSO + $CDCl_3$): 10.76 (1 H, s, NH-1), 9.14 (1 H, d, $J = 2$ Hz, H-2 pyr), 8.77 (1 H, d, $J = 1$ Hz, H-6 pyr), 8.49 (2 H, d, $J = 4.8$ Hz, H-4'',6''), 8.31 (1 H, d, $J = 8$ Hz, H-4 pyr), 8.02–8.01 (4 H, m, H-2,3,5,6), 7.57–7.53 (1 H, m, H-5 pyr), 7.01 (1 H, t, $J = 5$ Hz, H-5''). ^{13}C (DMSO + $CDCl_3$): 174.49 (CO), 158.14 (C-4'',6''), 157.01 (C-2''), 152.21 (C-2 pyr), 148.76 (C-6 pyr), 142.85 (C-4), 135.54 (C-3 pyr), 134.69 (C-4 pyr) *, 130.14 (C-1) *, 128.73 (C-2,6), 123.33 (C-5 pyr), 119.48 (C-3,5), 115.60 (C-5''). [* Assignments interchangeable.]

*N^4-(N-Methyl-3'-carbonylpyridinium)-N^1-(2''-pyrimidinyl)sulfanilamide iodide (**1b**)*

Compound (**1a**) (7.8 g) was dissolved in 250 ml of dry nitromethane and an excess of methyl iodide was added. The solution was refluxed for 5 h. After cooling to the room temperature, yellow crystals formed. The product was filtered, washed with dry nitromethane (100 ml) and dried over phosphorus pentoxide. Yield was 9.5 g (87%). Found: C, 40.98; H, 3.31; I, 25.40; N, 14.13; S, 6.55. For $C_{17}H_{16}IN_5O_3S$ required: C, 41.06; H, 3.24; I, 25.52; N, 14.08; S, 6.45. ^{1}H NMR (DMSO): 11.15 (1 H, s, NH-1), 9.55 (1 H, s, H-2 pyr), 9.20 (1 H, d, $J = 6$ Hz, H-6 pyr), 9.05 (1 H, d, $J = 8$ Hz, H-4 pyr), 8.54 (2 H, d, $J = 4.5$ Hz, H-4'',6''), 8.37–8.33 (1 H, m, H-5 pyr), 8.08–7.97 (4 H, q, $J = 8$ Hz, H-2,3,5,6), 7.08 (1 H, m, H-5''), 4.48 (3 H, s, Me). ^{13}C NMR (DMSO): 160.86 (CO), 158.26 (C-4'',6''), 156.71 (C-2''), 147.48 (C-2 pyr) *, 145.74 (C-6 pyr) *, 143.42 (C-4), 141.85 (C-4 pyr), 135.48 (C-3 pyr), 133.41 (C-1), 128.83 (C-2,6), 127.24 (C-5 pyr), 119.73 (C-3,5), 115.70 (C-5''), 48.31 (N-Me).

*N^4-(N-Methyl-3'-carbonyl-1',4'-dihydropyridine)-N^1-(2''-pyrimidinyl)sulfanilamide (**1c**)*

The quaternary salt (**1b**) (6.7 g) was dissolved in a mixture of degassed and deionized water (250 ml) and methylene chloride (200 ml). A mixture of sodium dithionite (21 g) and sodium bicarbonate (14.7 g) were added and the suspension was stirred under argon for 6 h at 0 °C. The solid was then filtered, washed with methylene chloride (100 ml), subjected to chromatographic purification and dried under phosphorus pentoxide at room temperature. Yield was 3.6 g (72%). Found: C, 54.90; H, 4.65; N, 18.60; S, 8.47. For $C_{17}H_{17}N_5O_3S$ required: C, 54.98; H, 4.58; N, 18.87, S, 8.63. UV(MeOH): 276, 378 nm. ^{1}H NMR (DMSO): 9.21 (1 H, s, NH-1), 8.45 (2 H, d, $J = 5$ Hz, H-4'',6''), 7.96 (1 H, d, $J = 1.5$ Hz, NH-4), 7.88–7.77 (4 H, m, H-2,3,5,6), 7.12 (1 H, s, H-2 pyr), 6.97 (1 H, t, $J = 5$ Hz, H-5''), 5.83 (1 H, d, $J = 5$ Hz, H-6 pyr), 4.72–4.45 (1 H, m, H-5 pyr), 3.08 (2 H, s, 2H-4 pyr), 2.96 (3 H, s, N-Me). ^{13}C NMR (DMSO): 166.36 (CO), 158.06 (C-2'',6''), 157.42 (C-2''), 143.91 (C-4), 140.09 (C-2 pyr), 133.06 (C-1), 129.87 (C-6 pyr), 128.40 (C-2,6), 118.48 (C-3,5), 115.22 (C-5''), 102.54 (C-5 pyr), 99.19 (C-3 pyr), 40.19 (N-Me), 21.75 (C-4 pyr).

*N'-(3'-Carboxymethylpyridinyl)-N^1-(2''-pyrimidinyl)sulfanilamide (**1e**)*

Chloromethyl nicotinate (1.7 g, 10 mmol) was dissolved in 15 ml of dry DMSO and 2.88 g (10 mmol) of sulfadiazine potassium salt (**1d**) in 15 ml of DMSO were added to it. The reaction mixture was stirred at room temperature for 5 h. After this time, 40 ml of deionized water were added and the precipitate that formed was filtered, washed with an additional amount of water and dried in the vacuum oven. Yield of the product was 1.8 g (47%). M.p. 165–168 °C. Found: C, 52.84; H, 3.99; N, 17.89; S, 8.22. For $C_{17}H_{15}N_5O_4S$ required: C, 52.98; H, 3.92; N, 18.17; S, 8.32. ^{1}H NMR (DMSO): 8.99 (1 H, d, $J = 1.4$ Hz, H-2 pyr), 8.84 (1 H, dd, $J = 4.7$, 1.4 Hz, H-6 pyr), 8.66 (2 H, d, $J = 4.7$ Hz, H-4'',6''), 8.20 (1 H, d,

$J = 8.2$ Hz, H-4 pyr), 7.81 (2 H, d, $J = 8.8$ Hz, H-2,6), 7.61 (1 H, m, H-5 pyr), 7.21 (1 H, t, $J = 5$ Hz, H-5″), 6.61 (2 H, d, $J = 8.8$ Hz, H-3,5), 6.52 (2 H, s, CH_2), 6.25 (2 H, s, NH_2). ^{13}C NMR (DMSO): 163.9 (CO), 158.13 (C-4″,6″), 157.03 (C-2″), 153.86 (C-4), 153.73 (C-2 pyr), 149.83 (C-6 pyr), 136.76 (C-4 pyr), 131.01 (C-2,6), 125.04 (C-1), 123.88 (C-5 pyr), 123.24 (C-3 pyr), 116.99 (C-5″), 111.91 (C-3,5), 71.18 (CH_2).

*N^4-(N-methyl-3′-carboxymethylpyridinyl)-N^1-(2″-pyrimidinium)sulfanilamide iodide (**1f**)*

Compound (**6**) (385 mg, 1.0 mmol) was dissolved in 10 ml of dry THF and an excess of methyl iodide was added. The solution was refluxed for 4 h. After cooling, a yellow crystal was removed by filtration, washed with 10 ml more of THF and dried under vacuum giving 0.38 g (72%) of the desired product. Found: C, 40.77; H, 3.62; N, 13.22; I, 23.92; S, 6.22. For $C_{18}H_{18}N_5O_4IS$: C, 40.99; H, 3.42; N, 13.28; I, 24.09; S, 6.07. ^1H NMR (DMSO): 9.56 (1 H, s, H-2 pyr), 9.23 (1 H, d, $J = 6$ Hz, H-6 pyr), 8.95 (1 H, d, $J = 7.7$ Hz, H-4 pyr), 8.44 (2 H, d, $J = 8.8$ Hz, H-4″,6″), 8.29–8.21 (1 H, m, H-5 pyr), 7.70 (2 H, d, $J = 8.8$ Hz, H-2,6), 6.97 (1 H, m, H-5″), 6.60 (2 H, m, H-3,5), 6.51 (2 H, s, CH_2), 4.49 (3 H, s, N-Me).

*N^4-(3′-Carbonylpyridinyl)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide (**2a**)*

Exactly 5 g (20 mmol) of sulfamethoxazole were dissolved in 150 ml of dry pyridine and 3.6 g (20 mmol) of nicotinoyl chloride hydrochloride was added at once. The reaction mixture was stirred overnight at room temperature in the absence of moisture. The pyridine was removed under vacuum and the residue was stirred with 300 ml of 2% solution of sodium bicarbonate. The resulting solid was filtered, washed with water and dried in the vacuum oven over phosphorus pentoxide. Yield was 5.56 g (80%). M.p. 232°C. Found: C, 53.49; H, 3.95; N, 15.49. For $C_{16}H_{14}N_4O_4S$ required: C, 53.62; H, 3.94; N, 15.64. ^1H NMR (DMSO): 10.73 (1 H, s, NH-1), 9.16 (1 H, s, H-2 pyr), 8.75 (1 H, s, H-6 pyr), 8.30 (1 H, d, $J = 7$ Hz, H-4 pyr), 8.03–7.86 (4 H, m, H-2,3,5,6), 7.52–7.50 (1 H, m, H-5 pyr), 6.12 (1 H, s, H-4″), 2.30 (3 H, s, Me). ^{13}C NMR (DMSO): 169.57 (C-3″), 164.44 (CO), 157.66 (C-5″), 152.05 (C-2, pyr), 148.72 (C-6, pyr), 143.05 (C-4), 135.95 (C-4 pyr), 130.05 (C-1), 127.65 (C-2,6), 123.11 (C-5 pyr), 119.84 (C-3,5), 95.25 (C-4″), 12.08 (Me).

*N^4-(N-Methyl-3′-carbonylpyridinium)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide iodide (**2b**)*

Adduct (**2a**) (358 mg, 1 mmol) was dissolved in 20 ml of dry acetone and an excess of methyl iodide was added. The solution was refluxed for 6 h. The crystalline solid formed was filtered and dried in the oven. Yield was 0.3 g (60%). Found: C, 39.57; H, 3.53; N, 10.35. For $C_{17}H_{17}IN_4O_4S$ required: C, 39.39; H, 3.69; N, 10.80. ^1H NMR (DMSO): 11.06 (1 H, s, NH-1), 9.69 (1 H, s, H-2 pyr), 9.29 (1 H, s, H-6 pyr), 9.08 (1 H, d, $J = 6.5$ Hz, H-4 pyr), 8.31 (1 H, m, H-5 pyr), 6.11 (1 H, s, H-4″), 4.57 (3 H, s, N-Me), 2.30 (3 H, s, Me). ^{13}C NMR (DMSO): 169.62 (C-3″), 160.28 (CO), 157.28 (C-5″), 147.27 (C-2 pyr), 145.69 (C-6 pyr), 143.51 (C-4), 142.02 (C-4 pyr), 134.53 (C-3 pyr), 133.28 (C-1), 127.71 (C-2,6), 127.34 (C-5 pyr), 120.02 (C-3,5), 95.15 (C-4″), 48.42 (N-Me), 12.10 (Me).

*N^4-(N-Methyl-3′-carbonyl-1′,4′-dihydropyridinyl)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide (**2c**)*

1 g (10 mmol) of the quaternary salt (**2b**) was dissolved in a mixture of deionized and degassed water (50 ml) and methylene chloride (50 ml). Sodium bicarbonate (2.26 g) and sodium dithionite (3.13 g) were added and the reaction mixture was stirred under argon at 0 °C for 5 h. The solid that appeared was filtered, washed with methylene chloride (50 ml) and dried over phosphorus pentoxide. The yield was 0.5 g (67%). Found: C, 49.40; H, 4.32; N, 13.27. For $C_{17}H_{18}N_4O_4S \cdot 2H_2O$ required: C, 49.70; H, 4.42; N, 13.65. UV(MeOH): 276, 376 nm. ^1H NMR (DMSO): 9.24 (1 H, s, NH-1), 9.83–7.71 (4 H, m, H-2,3,5,6), 7.11 (1 H, s, H-2 pyr), 6.09 (1 H, s, H-4″), 5.82 (1 H, d, $J = 8$ Hz, H-6 pyr), 4.72–4.70 (1 H, m, H-5 pyr), 3.09 (2 H, 5, 2H-4 pyr), 2.36 (3 H, s, N-Me), 2.29 (3 H, s, Me). ^{13}C NMR (DMSO): 169.59 (C-3″), 166.41 (CO), 157.98 (C-5″), 144.26 (C-4), 140.17 (C-2 pyr), 132.23 (C-6 pyr), 129.78 (C-1), 127.50 (C-2,6), 118.94 (C-3,5), 102.63 (C-5

pyr), 99.15 (C-3 pyr), 95.33 (C-4″), 40.21 (N-Me), 21.74 (C-4 pyr), 12.06 (Me).

*N^1-(3′-Carbonylpyridinyl)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide (**2e**)*

The sodium salt of sulfamethoxazole (**2d**) (275 mg, 1.0 mmol) was dissolved in 10 ml of dry pyridine and 0.228 g (1 mmol) of nicotinic anhydride was added to it and the reaction mixture was stirred at room temperature for 3 h in the absence of moisture. The solvent was removed under vacuum and the residue was treated with 10 ml of water and stirred at 0–5 °C for 1 h. The solid that precipitated was filtered and dried over phosphorus pentoxide in a vacuum oven. Yield was 0.185 g (52%). M.p. 194 °C. Found: C, 53.57; H, 3.95; N, 15.57. For $C_{16}H_{14}N_4O_4S$ required: C, 53.62; H, 3.94; N, 15.63. ^{1}H NMR (DMSO): 8.66 (1 H, s, H-2 pyr), 8.63-8.61 (1 H, m, H-6 pyr), 7.87 (1 H, dd, $J = 8$ Hz, 1.5 Hz, H-4 pyr), 7.63 (2 H, m, H-3,5), 6.40 (2 H, 5, NH_2), 2.40 (3 H, s, Me). ^{13}C NMR (DMSO): 172.20 (C-3″), 166.18 (CO), 158.28 (C-5″), 154.87 (C-4), 152.46 (C-2 pyr), 148.60 (C-6 pyr), 135.82 (C-4 pyr), 131.34 (C-2,6), 129.20 (C-3 pyr), 123.37 (C-1), 120.14 (C-5 pyr), 112.36 (C-3,5), 103.29 (C-4″), 12.36 (Me).

*N^1-(N-Methyl-3′-carbonylpyridinium)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide iodide (**2f**)*

0.5 g (1 mmol) of the adduct (**2e**) was dissolved in dry acetone (20 ml), methyl iodide (excess) was added and the mixture was refluxed for 7 h. The solvent was then removed under vacuum and residue recrystallized from acetone/diethyl ether and dried in the vacuum oven. Yield of the product was 0.3 g (60%). Found: C, 40.52; H, 3.63; N, 11.43; S, 6.19; I, 25.33. For $C_{17}H_{17}N_4O_4SI$ required: C, 40.80; H, 3.40; N, 11.20; S, 6.40; I, 25.40. ^{1}H NMR (DMSO): 9.38 (1 H, s, H-2 pyr), 9.09 (1 H, d, $J = 6$ Hz, H-6 pyr), 8.57 (1 H, d, $J = 8$ Hz, H-4 pyr), 8.11 (1 H, m, H-5 pyr), 7.61–7.57 (2 H, m, H-2,6), 6.78 (1 H, s, H-4″), 6.71–6.68 (2 H, m, H-3,5), 4.34 (3 H, s, N-Me), 2.43 (3 H, s, Me). ^{13}C NMR (DMSO): 172.97 (C-3″), 162.39 (CO), 157.48 (C-5″), 155.25 (C-4), 148.12 (C-2 pyr), 145.44 (C-6 pyr), 142.97 (C-4 pyr), 132.69 (C-3 pyr), 131.50 (C-2,6), 127.44 (C-5 pyr), 119.05 (C-1), 112.45 (C-3,5), 103.39 (C-4″), 48.37 (N-Me), 12.62 (Me).

*N^1-(N-Methyl-3′-carbonyl-1′,4′-dihydropyridinyl)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide (**2g**)*

The quaternary salt (**2f**) (2.1 g, 4.0 mmol) was dissolved into a biphasic mixture of degassed, deionized water (100 ml) and methylene chloride (100 ml). A mixture of sodium bicarbonate (2.52 g) and sodium dithionite (6.26 g) was then added. The reaction mixture was stirred under argon for 2 h and then the organic phase was separated, dried with magnesium sulfate and solvent removal gave 0.8 g of the product (54% yield). The crude material was then chromatographed on neutral alumina and eluted with methylene chloride. Found: C, 54.50; H, 4.85; N, 14.89; S, 8.59. For $C_{17}H_{18}N_4O_4S$ required: C, 54.54; H, 4.81; N, 14.97; S, 8.54. UV (MeOH): 276, 372 nm. ^{13}C NMR ($CDCl_3$): 168.98 (C-3″), 166.17 (CO), 159.28 (C-5″), 152.35 (C-4), 141.11 (C-2 pyr), 130.84 (C-6 pyr), 129.33 (C-2,6), 123.16 (C-1), 113.37 (C-3 pyr), 103.88 (C-3,5), 98.48 (C-5 pyr), 97.13 (C-4), 40.65 (N^+-Me), 21.93 (CH_2-pyr), 12.51 (CH_3).

*N^1-(3′-Carboxymethylpyridinyl)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide (**2h**)*

Exactly 6.87 g (25 mmol) of sulfamethoxazole sodium salt (**2d**) were dissolved in 60 ml of dry DMSO and 4.7 g (25 mmol) of chloromethyl nicotinate were added and the reaction mixture was stirred overnight at room temperature. 200 ml of deionized water were then added and the suspension was stirred in ice-water mixture for 1 h. The water was decanted and the remaining sticky residue was dissolved in acetone and dried quickly with $MgSO_4$. The acetone was removed under vacuum. The residue was crystallized from 10 ml of ethyl acetate. The yellow crystal was filtered and washed with an additional portion of ethyl acetate. Yield was 4.5 g (47%). Found: C, 52.45; H, 4.16; N, 14.44; S, 8.21. For $C_{17}H_{16}N_2O_5S$ required: C, 52.57; H, 4.15; N, 14.43; S, 8.26. ^{1}H NMR (DMSO): 8.89 (1 H, d, $J = 2$ Hz, H-2 pyr), 8.84 (1 H, dd, $J = 4.8$, 1.7 Hz, H-6 pyr), 8.06–8.03 (1 H, m, H-4 pyr), 7.62 (2 H, d, $J = 8.8$ Hz, H-2,6), 7.58–7.54 (1 H, d, $J = 8$ Hz, H-5 pyr), 6.66 (2 H, d, $J = 8.8$ Hz, H-3,5), 6.56 (1 H, s,

H-4″), 6.39 (2 H, s, NH_2), 6.16 (2 H, s, CH_2), 2.40 (3 H, s, Me). ^{13}C NMR (DMSO): 171.41 (C-3″), 164.06 (CO), 159.45 (C-5″), 154.75 (C-2 pyr *), 154.29 (C-4 *), 150.40 (C-6 pyr), 137.28 (C-4 pyr), 130.34 (C-2,6), 125.30 (C-1), 124.17 (C-5 pyr), 122.14 (C-3 pyr), 113.12 (C-3,5), 97.94 (C-4″), 73.05 (CH_2), 12.52 (Me).

*N^1-(N-Methyl-3′-carboxymethylpyridinium)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide p-toluenesulfonate (**2i**)*

Adduct (**2h**) (388 mg, 1 mmol) was dissolved in acetonitrile (20 ml) and 0.2 g (1 mmol) of methyl *p*-toluenesulfonate were added. Reaction mixture was stirred at room temperature overnight. The solid was filtered, washed with 10 ml of acetonitrile and 10 ml of diethyl ether. Yield was 70%. Found: C, 50.61; H, 4.73; N, 9.35; S, 10.72. For $C_{25}H_{26}N_4O_8S_2 \cdot H_2O$ required: C, 50.49; H, 4.74; N, 9.42; S, 10.78. 1H NMR (DMSO): 9.22 (1 H, s, H-2 pyr), 9.20 (1 H, s, H-6 pyr), 8.57 (1 H, d, $J = 8$ Hz, H-4 pyr), 8.18 (1 H, t, $J = 8$ Hz, H-5 pyr), 7.55 (2 H, d, $J = 8.6$ Hz, H-2,6), 7.48 (2 H, d, $J = 8$ Hz, H-2,6 PTS [a]), 7.12 (2 H, d, $J = 8$ Hz, H-3,5 PTS), 6.60 (2 H, d, $J = 8.7$ Hz, H-3,5), 6.53 (1 H, s, H-4″), 6.16 (2 H, s, CH_2), 4.42 (3 H, s, N-Me), 2.38 (3 H, s, Me-PTS), 2.28 (3 H, s, Me). ^{13}C NMR (DMSO): 171.04 (C-3″), 160.40 (CO), 158.82 (C-5″), 154.29 (C-4), 149.21 (C-2 pyr), 146.41 (C-6 pyr), 144.23 (C-4 pyr), 137.68 (C-1 PTS), 129.88 (C-2,6), 128.28 (C-4 PTS), 128.01 (C-3,5 PTS), 127.71 (C-5 pyr), 125.35 (C-2,6 PTS), 121.31 (C-1), 112.70 (C-3,5), 97.48 (C-4″), 73.48 (CH_2), 48.34 (N-Me), 20.66 (Me-PTS), 12.07 (Me). [[a] *p*-Toluenesulfonate = PTS.]

*N^1-(N-Methyl-3′-carboxymethyl-1′,4′-dihydropyridinyl)-N^1-(5″-methyl-3″-isoxazolyl)sulfanilamide (**2j**)*

Exactly 0.51 g (1 mmol) of the quaternary salt (**2i**) was dissolved into a mixture of degassed, deionized water (100 ml) and methylene chloride (100 ml). Sodium bicarbonate (3.3 g) and sodium dithionite (7.2 g), were added and the reaction mixture stirred under argon for 4 h. The organic layer was then separated and dried with magnesium sulfate. After solvent removal, a yellow solid (0.34 g, 81%) was obtained which was purified chromatographically (alumina). UV (MeOH): 276, 364 nm. Found: C, 52.20; H, 4.90; N, 13.45. For $C_{18}H_{20}N_4O_5S \cdot 0.5H_2O$ required: C, 52.28; H, 5.12; N, 13.55. ^1H NMR (DMSO): 7.50 (2 H, d, $J = 8.8$ Hz, H-2,6), 6.60 (2 H, d, $J = 8.8$ Hz, H-3,5), 6.41 (1 H, s, H-4″), 6.30 (1 H, s, H-2 pyr), 5.84–5.81 (1 H, dd, $J = 1.5$ Hz, H-6 pyr), 5.76 (2 H, s, CH_2), 4.73–4.68 (1 H, m, H-5 pyr), 2.87 (3 H, s, N-Me), 2.82 (2 H, s, CH_2-pyr), 2.36 (3 H, s, Me). ^{13}C NMR (DMSO): 170.57 (C-3″), 165.67 (CO), 158.93 (C-5″), 153.93 (C-4), 143.11 (C-2 pyr), 129.77 (C-2,6), 129.69 (C-6 pyr), 122.06 (C-1), 112.41 (C-3,5), 103.78 (C-5 pyr), 97.32 (C-5″), 93.72 (C-3 pyr), 71.07 (CH_2), 40.15 (N-Me), 21.27 (C-4 pyr), 12.05 (Me).

*N^4-(3′-Carbonylpyridinyl)-N^1-(6″-methyl-2″-pyrimidinyl)sulfanilamide (**3a**)*

Exactly 2.86 g (10 mmol) of sulfamerazine sodium salt was dissolved in 50 ml of dry pyridine and 1.78 g (10 mmol) of nicotinoyl chloride hydrochloride was added. The reaction mixture was stirred overnight and then solvent was removed under vacuum, and the residue treated with 20 ml of 2% sodium bicarbonate solution. A white solid was removed by filtration, washed with water (25 ml) and dried under vacuum over phosphorus pentoxide. Yield of the product was 3.38 g (92%). M.p. 253–256 °C. Found: C, 51.68; H, 4.42; N, 17.76. For $C_{17}H_{15}N_5O_3S \cdot 1.5H_2O$ required: C, 51.50; H, 4.58; N, 17.66. ^1H NMR (DMSO): 10.99 (1 H, s, NH-1), 9.22 (1 H, s, H-2 pyr), 8.82 (1 H, d, $J = 4$ Hz, H-6 pyr), 8.42–8.36 (2 H, m, H-4 pyr, H-4″), 8.08 (4 H, m, H-2,3,5,6), 7.63–7.59 (1 H, m, H-5 pyr), 6.92 (1 H, d, $J = 5$ Hz, H-5″), 2.36 (3 H, s, Me). ^{13}C NMR (DMSO): 168.34 (C-2″), 164.71 (CO), 157.55 (C-4″), 152.43 (C-2 pyr), 148.95 (C-6 pyr), 142.78 (C-4), 135.79 (C-3 pyr), 135.24 (C-4 pyr), 130.21 (C-1), 128.99 (C-2,6), 123.57 (C-5 pyr), 119.68 (C-3,5), 114.75 (C-5″), 23.34 (Me).

*N^4-(N-Methyl-3′-carbonylpyridinium)-N^1-(6″-methyl-2″-pyrimidinyl)sulfanilamide iodide (**3b**)*

Compound (**3a**) (1.84 g, 5 mmol) was dissolved in 200 ml of dry acetone and an excess of methyl iodide was added. The solution was refluxed for 7 h. After cooling to room temperature, a yellow crystal appeared and it was filtered, washed with

dry acetone, and dried over phosphorus pentoxide. Yield 2.08 g (82%). Found: C, 40.69; H, 3.74; N, 13.04; I, 23.73, S, 6.16. For $C_{18}H_{18}IN_5O_3S \cdot H_2O$ required: C, 40.84; H, 3.88; N, 13.23; I, 23.97; S, 6.06. ^{1}H NMR (DMSO): 11.08 (1 H, s, NH-1), 9.60 (1 H, s, H-2 pyr), 9.24 (1 H, d, $J = 6$ Hz, H-6 pyr), 9.06 (1 H, d, $J = 8$ Hz, H-4 pyr), 8.36–8.30 (2 H, m, H-5 pyr and H-4″), 8.07–7.96 (4 H, q, $J = 8.6$ Hz, H-2,3,5,6), 6.88 (1 H, d, $J = 5$ Hz, H-5″), 4.52 (3 H, s, N-Me), 2.34 (3 H, s, Me). ^{13}C NMR (DMSO): 168.06 (C-2″), 160.62 (CO), 157.31 (C-4″), 156.45 (C-6″), 147.51 (C-2 pyr), 145.84 (C-6 pyr), 143.49 (C-4 pyr), 141.67 (C-4), 135.74 (C-3 pyr), 133.51 (C-1), 129.02 (C-2,6), 127.40 (C-5 pyr), 119.54 (C-3,5), 114.69 (C-5″), 48.42 (N-Me), 23.28 (Me).

*N^4-(N-Methyl-3′-carbonyl-1′,4′-dihydropyridinyl)-N^1-(6″-methyl-2″-pyrimidinyl)sulfanilamide (**3c**)*

The quaternary salt (**3b**) (1.53 g, 3 mmol) was dissolved into a mixture of deionized, degassed water (150 ml) and methylene chloride (100 ml). Sodium bicarbonate (2.01 g) and sodium dithionite (4.16 g) were added and the reaction mixture was stirred in the argon atmosphere for 7 h. The solid which was insoluble in methylene chloride or water was removed by filtration, washed with 2×50 ml portions of CH_2Cl_2 and dried over phosphorus pentoxide. Yield was 0.66 g (58%). Found: C, 56.00; H, 5.16; N, 18.20; S, 8.51. For $C_{18}H_{19}N_5O_3S$ required: C, 56.10; H, 4.94; N, 18.18; S, 8.31. UV (MeOH): 274, 376 nm. ^{1}H NMR (DMSO): 9.27 (1 H, s, NH-1), 8.31 (1 H, m, H-4″), 7.92–7.81 (4 H, m, H-2,3,5,6), 7.15 (1 H, s, H-2 pyr), 6.87 (1 H, d, H-5), 5.86 (H, d, H-6 pyr), 5.76 (H, s, NH), 4.73–4.70 (1 H, 5 pyr), 3.09 (2 H, H-4 pyr), 2.35 (3 H, s, N-CH_3), 2.31 (3 H, s, CH_3). ^{13}C NMR (DMSO): 168.12 (C-2″), 166.45 (C = 0), 157.55 (C-4″), 156.95 (C-6″), 143.97 (C-4), 140.29 (C-2 pyr), 133.18 (C-1), 130.07 (C-6 pyr), 128.76 (C-2,6), 118.52 (C-3,5), 114.68 (C-5″), 102.58 (C-5 pyr), 99.15 (C-3 pyr), 40.22 (N-Me), 23.32 (CH_3), 21.86 (C-4 pyr).

*N^4-(3′-Carbonylpyridinyl)-N^1-[2″-(4″,6″-dimethyl)pyrimidinyl]sulfanilamide (**4a**)*

Sulfamethazine (5.6 g, 20 mmol) was dissolved in 50 ml of dry pyridine and nicotinoyl chloride hydrochloride (3.55 g, 20 mmol) was added at once. The reaction mixture was stirred at room temperature for 4 h. Solvent was removed under vacuum and the residue was treated with 200 ml of 2% solution of $NaHCO_3$. Solid that formed was filtered off, washed with water, and dried over phosphorus pentoxide. Yield 7.56 g (98%). M.p. 255–258°C. Found: C, 56.44; H, 4.41; N, 18.18. For $C_{18}H_{16}N_5O_3S$ required: C, 56.60; H, 4.21; N, 18.28. ^{1}H NMR (DMSO): 10.83 (1 H, s, NH-1), 9.19 (1 H, s, H-2 pyr), 8.82 (1 H, d, $J = 4$ Hz, H-6 pyr), 8.35 (1 H, d, $J = 7.8$ Hz, H-4 pyr), 8.12–8.02 (4 H, m, H-2,3,5,6), 7.63–7.61 (1 H, m, H-5 pyr), 6.75 (1 H, s, H-5″), 2.29 (6 H, s, 2 Me). ^{13}C NMR (DMSO): 167.40 (C-2″), 164.59 (CO), 156.27 (C-4″,6″), 152.39 (C-2 pyr), 148.84 (C-6 pyr), 142.59 (C-4), 135.66 (C-4 pyr), 135.66 (C-3 pyr), 130.24 (C-1), 129.27 (C-2,6), 123.54 (C-5 pyr), 119.35 (C-3,5), 113.53 (C-5″), 22.90 (2 Me).

*N^4-(N-Methyl-3′-carbonylpyridinyl)-N^1-[2″-(4″,6″-dimethyl)pyrimidinyl]sulfanilamide iodide (**4b**)*

2 g (5.2 mmol) of adduct (**4a**) were dissolved in acetone (30 ml) and an excess of methyl iodide was added. The solution was refluxed for 7 h. After cooling yellow crystals were filtered, washed with additional amount of acetone and dried in the vacuum oven. Yield was 2.4 g (88%). Found: C, 41.85; H, 3.89; N, 12.72; I, 22.95. For $C_{19}H_{20}IN_5O_3S \times H_2O$ required: C, 42.00; H, 4.08; N, 12.89; I, 23.35. ^{1}H NMR (DMSO): 11.12 (1 H, s, NH-1), 9.60 (1 H, s, H-2 pyr), 9.25 (1 H, d, $J = 6$ Hz, H-6 pyr), 9.08 (1 H, d, $J = 8$ Hz, H-4 pyr), 8.39 (1 H, dd, $J = 8$, 6 Hz, H-5 pyr), 8.10 (2 H, d, $J = 8.8$ Hz, H-2,6), 8.05 (2 H, d, $J = 8.8$ Hz, H-3,5), 6.77 (1 H, s, H-5″), 4.53 (3 H, s, N-Me), 2.28 (6 H, s, 2 Me). ^{13}C NMR (DMSO): 167.39 (C-2″), 160.88 (CO), 156.04 (C-4″,6″), 147.38 (C-2 pyr), 145.80 (C-6 pyr), 143.61 (C-4), 142.58 (C-4 pyr), 136.18 (C-3 pyr), 133.47 (C-1), 129.33 (C-2,6), 127.44 (C-5 pyr), 119.55 (C-3,5), 113.34 (C-5″), 48.56 (N-Me), 22.86 (2 Me).

*N^4-(N-Methyl-3′-carbonyl-1′,4′-dihydropyridinyl)-N^1-[2″-(4″,6″-dimethyl)pyrimidinyl]sulfanilamide (**4c**)*

2 g (3.8 mmol) of quaternary salt (**4b**) were dissolved in a mixture of degassed, deionized water (75 ml) and methylene chloride (50 ml). Sodium

bicarbonate (2.56 g) and sodium dithionite (5.3 g) were added and the reaction mixture was stirred under stream of argon for 3 h. The organic phase was then separated, dried with $MgSO_4$ and after solvent removal, a yellow solid was obtained (1.2 g, 80%). The solid was chromatographed on neutral alumina. Found: C, 54.60; H, 5.57; N, 16.83; S, 7.66. For $C_{19}H_{21}N_5O_3S \cdot H_2O$ required: C, 54.68; H, 5.52; N, 16.78; S, 7.67. UV (MeOH): 272.5, 376 nm. ^{1}H NMR (DMSO): 9.25 (1 H, s, NH-1), 7.88–7.78 (4 H, m, H-2,3,5,6), 7.14 (1 H, s, H-2 pyr), 6.67 (1 H, s, H-5″), 5.84–5.75 (1 H, m, H-6 pyr), 4.69 (1 H, m, H-5 pyr), 3.08 (2 H, s, 2H-4 pyr), 2.94 (3 H, s, N-Me), 2.22 (6 H, s, 2 Me). ^{13}C NMR (DMSO): 167.22 (C-2″), 166.44 (CO), 157.03 (C-4″,6″), 143.66 (C-4), 140.22 (C-2 pyr), 133.77 (C-1), 130.09 (C-6 pyr), 128.96 (C-2,6), 118.31 (C-5″), 102.55 (C-2 pyr), 99.19 (C-3 pyr), 40.20 (N-Me), 22.99 (2 Me), 21.87 (C-4 pyr).

Characterization

R_m determinations These were obtained using Baker®, Si-C18F 19C, 20 × 20 cm glass TLC plates coated with octadecylsilane reversed phase bonded to silica gel. The layer thickness was 200 μm, the particle size was approx. 20 μm and the silica was indicated with a 254 nm fluorescent dye. Compounds were dissolved in methylene chloride to generate a 5 μg/μl solution of which 1 μl was applied to the TLC plate, 2 cm above the bottom. A mobile phase consisting of various concentrations of aqueous acetone was allowed to elute 14 cm above the origin. The developed plates were then dried and the compound detected by ultraviolet illumination. The R_m values were calculated from R_f values by means of the equation:

$$R_m = \log(1/R_f - 1).$$

The extrapolated (100% aqueous) R_m was then obtained by a plot of R_m values vs the aqueous component of the mobile phase.

Ferricyanide-mediated oxidations These were performed using a published procedure. Briefly, the rate of decrease of the band III absorbance (~ 360 nm) was determined in buffered 20% aqueous acetonitrile [0.1 mM $K_4Fe(CN)_6$, 60 mM KCl and 1.0 mM K_2CO_3] containing various concentrations of $K_3Fe(CN)_6$ (1–50 mM). The sulfonamide CDS in acetonitrile were added to the ferricyanide solutions using a Hamilton syringe. The solutions were maintained at 37°C in a thermostated cell holder and contained in an anaerobic screw-top cuvette (Spectrocell, Inc.) fitted with a teflon septum. For a given ferricyanide concentration, the pseudo-first-order rate constant was determined and then these values plotted as a function of molar ferricyanide ion concentration. The slope of the resulting relationship gave rise to the second-order rate constant in s^{-1} M^{-1}. In all kinetic studies, solutions were prepared with water that had been boiled for 1 h and cooled with a stream of helium passing through it.

In vitro evaluation

Analytical methodologies for examining various sulfonamide CDSs and their degradation products utilized HPLC. In all determinations, a Spectra Physics SP 8800 pump, SP 8490 variable-wavelength detector, SP 4270 integrator and SP 8780 refrigerated auto sampler were used. A Spherisorb ODS-2, 5 μm (250 mm × 4.6 mm i.d.) (Alltech, Inc.) analytical column was used and separations were performed at ambient temperature. For quantitation of (**2g**) a mobile phase of aqueous acetonitrile (50:50) containing 0.01 M triethylamine flowing at a rate of 1 ml/min was used. The compound eluted at 5.4 min and was detected at 372 nm. For this assay, the limit of detection was approx. 4 ng. Detection of (**2f**) and (**2**) required a mobile phase of acetonitrile:0.05 M acetate buffer (pH 6) 45:55 containing 1×10^{-5} M tetrabutylammonium perchlorate. These compounds were detected at 290 nm and had retention times and limits of detection of 6.94 min, 3 ng for (**2f**) and 5.28 min, 2.5 ng for (**2**).

For (**2c**), a Spherisorb C8, 5 μm (250 mm × 4.6 mm i.d.) column was used. The mobile phase consisted of acetonitrile:0.05 M acetate buffer (pH 3.6) (50:50) with a flow rate of 1 ml/min. The compound was detected at 276 nm, the method had a limit of detection of 1 ng and the compound eluted at 7.0 min. In this system, sulfamethoxazole itself had a retention time of 5.0 min. In order to elute the quaternary metabolite of (**2c**), i.e., (**2b**), a Spherisorb ODS-2, 5 μm (250 mm × 4.6 mm i.d.) column was used. The mobile phase was a mixture

of acetonitrile and 0.05 M acetate buffer (pH 3.6) (75:55) and contained 2.5×10^{-5} M tetrabutylammonium perchlorate. The flow rate was 1 ml/min, the compound was detected at 276 nm and had a retention time of 5.4 min. Compound (**2**) eluted at 5.3 min in this system. Analysis for the sulfadiazine CDS (**1c**) also utilized a Spherisorb ODS-2 column but required a mobile phase containing acetonitrile : 0.05 M acetate buffer (pH 3.6) 30:70 and 1×10^{-5} M tetrabutylammonium perchlorate. The compound was detected at 14.4 min and its lower limit of detection was 5 ng. The pyridinium salt (**1b**) eluted at 10.12 min while sulfadiazine had a retention time of 5.03 min. The limits of detection for (**1b**) and (**1**) in this system were 34 and 1.0 ng, respectively.

Matrix degradation studies used isotonic phosphate buffer at pH 7.5 and 20% brain homogenate. In preparing the biological system, freshly obtained rat brains were homogenized with phosphate-buffered saline to give a 20% w/v matrix. The homogenate was then centrifuged. Buffer or supernatant were then equilibrated at 37°C and then spiked with a DMSO solution of the appropriate CDS. At various times post-addition, 300 μl of matrix was removed, mixed with 450 μl of cold acetonitrile and centrifuged for 3 min at $10\,000 \times g$ (Beckman Microfuge 12). The supernatant was then analyzed by HPLC. Disappearance of the sulfonamide CDS derivatives was plotted semilogarithmically as a function of time to generate the first-order rate constants.

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