Preparation and Properties *In Vitro* and *In Vivo* of Antitubercular Pyrroles

Michael J. Hearn,[a]* Michaeline F. Chen,[a] Marianne S. Terrot,[a]
Eleanor R. Webster,[a] and Michael H. Cynamon[b]

[a]Department of Chemistry, Wellesley College, Wellesley, Massachusetts 02481
[b]Veterans Affairs Medical Center, Syracuse, New York 13210
*E-mail: mhearn@wellesley.edu

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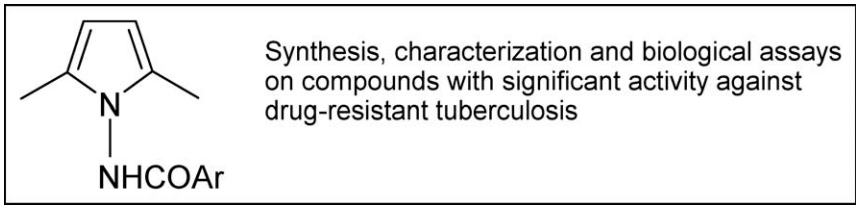

1-Acylamino-2,5-dimethylpyrroles were prepared in the exploration of heterocyclic structures useful for their antitubercular activity. The pyrroles were conveniently formed from the reaction of aromatic acid hydrazides with hexane-2,5-dione in water or ethanol, without resorting to acid catalysis. In each case, the procedure provided a single pyrrole in pure form, and the product was identified without difficulty on the basis of highly characteristic spectrometric features. Some members of this class have significant activities against drug-resistant tuberculosis *in vitro* and offer substantial protection in a rigorous mouse model of the disease.

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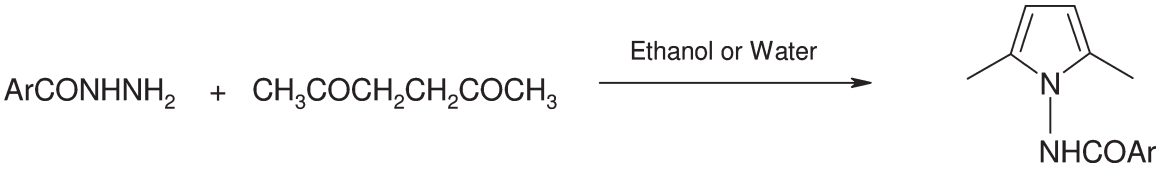
INTRODUCTION

Worldwide, tuberculosis ranks first among the causes of death from a communicable disease [1]. This killer's designation as an international public health threat by the World Health Organization more than a decade ago [2] was followed by the mobilization of considerable resources from governments, pharmaceutical companies, philanthropic foundations, and public–private partnerships [3]. Even so, advances thus far against the disease have been hard-won, and fully one-third of the earth's population remains infected with the causative bacillus, *Mycobacterium tuberculosis* [4]. In industrialized countries, the unexpected reemergence of tuberculosis has come with substantial costs to the public health infrastructure. Among the populations of developing nations, the disease has persisted, and millions of deaths result each year from such infection [5]. Although tuberculosis can strike persons of any age, the sinister natural history of the disease makes it particularly devastating to those of middle years, during the most productive period of their lives. For those who do not themselves succumb, there is social and economic hardship as individuals, families, and communities struggle to deal with the collateral burdens of widespread infection. Progress in the development of strong new treatment regimens for tuberculosis has now been made both more difficult and more necessary by the emergence of strains of *M. tuberculosis* that are drug resistant or exceptionally virulent [6–9]. In the case of the most widely prescribed tuberculosis medication, isoniazid (isonicotinic acid hydrazide, INH), resistant strains have appeared that require drug concentrations 1000 times greater than those for susceptible bacteria. This renders the drug ineffective as a therapeutic mainstay for patients infected with those strains.

Against the urgency and seeming intransigence of this situation, there is increasing evidence that heterocyclic compounds will serve as significant leads for the discovery of robust new antitubercular medications and as valuable probes for gaining a better understanding of the life cycle of the pathogen [10–13]. Much, however, remains to be learned about the scope of heterocyclic structures that will possess this valuable antimicrobial activity [14,15]. As early as 1953, it had been reported that some pyrroles had antimycobacterial activity *in vitro* [16–20], but this perceptive observation was not given concerted follow-up at the time. More recently, significant work has resumed on antitubercular drug design using pyrroles as templates for synthesis [21–23], including elegant molecular modeling studies in conjunction with laboratory experiments [24,25]. Although much ground is still to be covered, it seems clear that the chemistry of pyrroles will provide both important lead compounds in the search for new antitubercular

Scheme 1



medications and tools for probing the complex interactions among pathogen, mammalian host, and drugs in tuberculosis treatment modalities.

Within this context, we wished to explore, using up-to-date methods, both the preparative chemistry and the antitubercular behavior of a class of acylaminopyrroles derived from acid hydrazides and diketones. We now report on the convenient preparation and useful antimycobacterial properties of a series of these compounds (**III**). We have found that the compounds are readily formed from aromatic carboxylic acid hydrazides (**I**) and 2,5-hexanedione (**II**) in excellent purity as stable highly crystalline solids (see Scheme 1). The reactions take place to give a single product without complication, and the resulting 1-acylamino-2,5-dimethylpyrroles may be characterized in clear-cut ways by standard spectrometric techniques. In a representative example, compound **II** was treated with INH (**I**, Ar = 4-C_5H_4N, 1.00 equivalent) in refluxing water for 2 h. After cooling and standing over night, the needles that formed were filtered off to give **III** (Ar = 4-C_5H_4N) in analytically pure form, with the distinguishing spectrometric features of the acylaminopyrrole. Notably, the features included: (1) the N—H band appropriate for the amide link near 3225 wavenumbers in the infrared spectrum; (2) the amide I and amide II peaks at 1673 and 1537 wavenumbers; (3) the N—H signal at δ 11.5 ppm in the hydrogen NMR spectrum, integrating for one hydrogen, quenched by the addition of D_2O; (4) the peak at δ 5.7, a singlet integrating for two hydrogens for the pyrrole ring protons; and (5) a signal at δ 11.3 ppm in the carbon nmr spectrum for the methyl groups at the 2- and 5-positions. The overall yield of the product was 67%. Our results on the preparation of these pyrroles **III** are summarized in Table 1.

In general, the preparation of a pyrrole by the reaction of a 2,5-dione with an amine, the Paal-Knorr synthesis [26], is sensitive to both the nucleophilicity of the amine and the specific reaction conditions employed. These issues have raised considerable interest within the synthesis community [27], and some trends have become apparent. Among aliphatic amines, for example, the steric environment of the carbon bearing the nitrogen appears to be crucial, with yields falling drastically as the carbon becomes more highly substituted [28]. Among substituted anilines, low reactivity is observed for those amines in which the aromatic ring is substituted with electron-withdrawing groups; thus 2,5-dichloroaniline fails to react with hexane-2,5-dione, whereas *o*-phenylenediamine is highly nucleophilic and reacts rapidly with two equivalents of the hexane-2,5-dione to produce a 1,2-dipyrrolyl benzene [28]. With respect to reaction conditions, some researchers have pointed out the accelerating role of acid catalysis [29], while others have noted that the amount of acid used, if any, should be adequate to allow for activation of the carbonyl functions of the dione but not enough to fully protonate the amine function and thereby render it nonnucleophilic [30]. In the present work, we found that in each case the terminal nitrogen of the hydrazide moiety was sufficiently nucleophilic to permit reaction in water or ethanol, without resorting to acid catalysis. This was true even for examples in which a strongly electron-withdrawing group was present, such as **IIIf**. The ability to carry out the reaction under neutral conditions considerably simplified the work-up and isolation of the products.

Table 1

Pyrroles **III**.

Entry	Compound	Ar	% yield	mp (°C)	ν^{max} N—H (cm^{-1})	^{1}H NMR, δ	^{13}C NMR, δ
1	**IIIa**	4-C_5H_4N	67	151–153	3224	11.5	165
2	**IIIb**	C_6H_5	89	175–177	3261	11.3	166
3	**IIIc**	4-ClC_6H_4	69	185	3274	11.3	165
4	**IIId**	4-$CH_3C_6H_4$	91	187–189	3281	11.2	166
5	**IIIe**	4-BrC_6H_4	77	207–210	3246	11.3	165
6	**IIIf**	4-$NO_2C_6H_4$	75	218–221	3278	11.3	165
7	**IIIg**	2-OH-4-$NH_2C_6H_3$	30	173–174	3270	10.9	162

Table 2

In vitro activities: Minimum inhibitory concentrations in fully susceptible Mtb Erdman.

Entry	Compound	MIC (μg/mL)[a]
1	**IIIa**	3.2[b]
2	**IIIb**	16
3	**IIIc**	32
4	**IIId**	>8
5	**IIIe**	32
6	**IIIf**	32
7	**IIIg**	2.6[c]

[a] INH standard control MIC 0.06 μg/mL.
[b] Geometric mean of three determinations.
[c] Geometric mean of five determinations.

With respect to biological assessment, the pyrroles displayed a range of activities against both laboratory strains and drug-resistant clinical isolates of *M. tuberculosis*. Activities *in vitro* were initially determined for the pyrroles as minimum inhibitory concentration (MIC) values against *M. tuberculosis* strain Erdman (Mtb Erdman). The MIC represents the minimum concentration of the compound necessary to inhibit growth. Mtb Erdman is a fully drug-susceptible laboratory strain, often used in primary antimycobacterial assays. The determinations were referred to the known antitubercular INH as a standard control. Individual MIC values are reported in Table 2. The geometric mean of the MICs for the pyrroles tested was 12 μg/mL. For comparison purposes, tuberculosis drugs currently used in the clinic have MIC values typically ranging from 0.015 μg/mL (rifabutin) to 50 μg/mL (pyrazinamide) [31]. Of particular interest were compounds **IIIa** and **IIIg** with the lowest MICs of 3.2 and 2.6 μg/mL, respectively. These highly effective pyrroles were selected for further evaluation in a panel of three clinical strains isolated from patients showing significant drug resistance to INH, and the results are shown in Table 3. Compound **IIIa** was slightly more effective than INH itself, and compound **IIIg** clearly maintained its strong potency in the drug-resistant bacteria, demonstrating essentially the same activity *in vitro* for both INH-susceptible and INH-resistant strains.

Table 3

In vitro activities: Minimum inhibitory concentrations in drug-resistant clinical isolates.

		Clinical isolates		
Entry	Compound	303	1889	35829
1	**IIIa**	4	4	8
	INH control	8	4	>8
2	**IIIg**	2	1	2
	INH control	64	8	>64

Table 4

In vivo activities of pyrroles.

Group	Log CFU/Lung
Untreated controls	6.53 ± 0.16[a]
INH controls	5.34 ± 0.22[a]
IIIa	5.01 ± 0.73[a]
IIIg	5.53 ± 0.16[b]

[a] Six mice/group.
[b] Four mice/group.

For testing of compounds **IIIa** and **IIIg** *in vivo*, we used a short-course therapy model in mice, which has previously been described in detail [32]. This method provides an exacting test of the activities *in vivo* of investigational drugs and has the benefit of cutting in half the time required to produce data in animal studies, compared to the more traditional murine models. Our results are given in Table 4, in which the data are presented as the logarithms of bacterial colony-forming units (log CFU) per mouse lung, with and without administration of the candidate drugs. No overt toxicity or ill effects were observed when the drugs were administered at the therapeutic doses (see Experimental). For reference purposes, highly active drugs known to be tuberculocidal, such as INH, generally display a drop of one or more log CFU *versus* untreated controls when examined in the model. On the other hand, less-active drugs that have been characterized as tuberculostatic, as opposed to tuberculocidal, such as *p*-aminosalicylic acid (PAS), typically lead to a lowering of one-half log CFU *versus* untreated controls (see Experimental). In comparing the data for **IIIa** and **IIIg** with the untreated controls, there has been a reduction of 1.00 log CFU or more in each case, suggesting that both compounds **IIIa** and **IIIg** are bactericidal in tuberculosis-infected mice. Overall, the results *in vitro* and *in vivo* warrant further development of the antitubercular properties of pyrroles derived from aromatic acid hydrazides.

In conclusion, we have found that 1-acylamino-2,5-dimethylpyrroles, of interest in the investigation of heterocyclic structures for their antitubercular activity, can be conveniently prepared from the reaction of acid hydrazides with hexane-2,5-dione in water or ethanol without an acid catalyst. In each case, the process leads to a single pyrrole in pure form, product isolation is straightforward, and the resulting compound is readily identified on the basis of highly characteristic spectrometric features. Some members of this class have useful activities against drug-resistant tuberculosis *in vitro* and offer excellent protection in an animal model of the disease.

EXPERIMENTAL

General methods. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN. Melting points (mp; °C) were taken in open capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Cambridge, MA) and are corrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 Fourier transform spectrometer as KBr pellets or Nujol mulls or on a Perkin-Elmer Spectrum One Fourier transform spectrophotometer fitted with a universal attenuated total reflectance sampling accessory, reported in wavenumbers (ν, cm^{-1}). Most reactants, reagents, and solvents were obtained from Aldrich Chemical Company (Milwaukee, WI) and Lancaster Synthesis (Windham, NH) and were used as received. Methyl 4-aminosalicylate was purchased from Nantong Chang Chemicals, Peking, China. Nuclear magnetic resonance (NMR) spectra were taken on a Bruker 300 Fourier transform instrument as dilute solutions in dimethyl sulfoxide-d_6 (DMSO-d_6) or chloroform-d, recorded at 300 MHz (^{1}H NMR) or 75 MHz (^{13}C NMR) and are reported in parts per million delta (δ) downfield from internal tetramethylsilane as reference, with coupling constants given in cycles per second (cps). In some proton spectra, only signals in the region 0–10 ppm are reported. Appropriate solvent blanks were recorded to account for water and DMSO. Gas chromatography (GC) and low-resolution mass spectrometry (LR-MS) analyses were recorded with an HP 5890 Series II Plus gas chromatograph, using a crosslinked 5% diphenyl- 95% dimethylsiloxane column from Hewlett Packard, and an HP 5972 electrical ionization mass detector. High-resolution mass spectroscopy (HR-MS) data were obtained using the JEOL HX-110 double focusing mass spectrometer of the Michigan State University Mass Spectrometry Facility, courtesy of Professor D. Gage and Ms. B. Chamberlin. This magnetic sector instrument is equipped with a fast atom bombardment (FAB) ionization source. It is capable of performing high-resolution (peak matching) and tandem mass spectrometry. Safety notes: gloves were worn during the chemical syntheses, and the reactions were carried out in the hood. In general, any scale up of preparations of compounds with relatively high proportions of nitrogen and oxygen was done with due caution. No specific safety problems were encountered with the methods given below. No attempt was made to optimize yields. To monitor the progress of the pyrrole-forming reactions, a thin-layer chromatography (TLC) system was devised. In general, we used plastic sheets coated with silica gel (Merck-Darmstadt), eluted with absolute ethanol, with starting materials the subjects of parallel reference runs. Details are given in individual procedures, where applicable, but typical R_f values were 0.5 for the acid hydrazide and 0.8 for the acylaminopyrroles. The dione was not observed in these runs.

Biological assessments. *M. tuberculosis* ATCC 35801 (strain Erdman) was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Drug-resistant clinical isolates of *M. tuberculosis* (isolates 303, 1889, and 35829) were kindly provided by Dr. Sheldon Morris, United States Food and Drug Administration. The mutational origins of the drug resistance of these organisms have been characterized and data are available from the authors upon request. PAS was a gift of Jacobus Pharmaceutical Company. INH was purchased from Sigma Chemical Company (St. Louis, MO). For testing, a given investigational compound was dissolved in dimethyl sulfoxide and subsequently diluted in distilled water. INH and PAS were dissolved in distilled water. Stock solutions were filter-sterilized by passage through a membrane filter 0.22-μm pore size and stored at -20°C until use. The drugs were prepared each morning, before experimentation. With respect to testing against these isolates, the MICs of all antimicrobial agents were determined in modified 7H10 broth (7H10 agar formulation with agar and malachite green omitted; pH 6.6) supplemented with 10% Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment (Difco Laboratories, Detroit, MI) and 0.05% Tween 80 [33]. The activities of the antimicrobial agents were determined by a broth dilution method [34]. The organism was grown in the modified 7H10 broth with 10% OADC enrichment and 0.05% Tween 80 on a rotary shaker at 37°C for 5 days. The culture suspension was diluted in modified 7H10 broth to yield 100 Klett units/mL (Photoelectric Colorimeter, Manostat Corporation, New York, NY), or approximately 5×10^7 CFU/mL. The size of the inoculum was determined by titration and counting from triplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, MD) supplemented with 10% OADC enrichment. The plates were incubated at 37°C in ambient air for 4 weeks before counting of the colonies. MIC values are reproducible to within 1–2 dilutions. Results *in vitro* (strain $H_{37}R_v$) were also determined according to the fully-documented protocols of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility, of the National Institutes of Health [35].

In brief, for the short-course therapy studies *in vivo*, 4-week-old female C_{57}BL/6 mice (Charles River, Wilmington, MA) were infected intranasally with approximately one million viable *M. tuberculosis* organisms. As a negative control, groups of infected but untreated mice were sacrificed at the initiation of therapy. The known antitubercular drug INH was used as a positive control. Treatment began 1 day post-infection and was administered for 2 days. The agents were introduced by gavage: the INH control and a given pyrrole were dosed daily at 25 and 100 mg/kg of body weight, respectively. No overt toxicity or ill effects were noted at these doses. Mice were sacrificed by carbon dioxide inhalation 3 days post-infection. Their right lungs were removed aseptically and were ground in a tissue homogenizer (IdeaWorks! Laboratory Devices, Syracuse, NY). The number of viable organisms was determined by titration on 7H10 agar plates. The plates were incubated at 37°C in ambient air for 4 weeks before counting of the bacterial colonies. Data are presented as the logarithms of colony-forming units. The complete procedure for the short-course therapy model has been reported [32] in detail. As standards of reference, such tuberculocidal drugs as INH generally display a drop of one or more log CFU versus untreated controls in the model. By way of contrast, PAS is tuberculostatic, as distinct from tuberculocidal, typically leading to a falling-off of one-half log CFU versus untreated controls. A typical data set for PAS is as follows: dose 500 mg PAS/kg/day, six mice per group, untreated controls 5.78 ± 0.38 log CFU, PAS 5.32 ± 0.22 log CFU, positive control (INH) 4.44 ± 0.62 log CFU. The use of experimental animals complied with institutional policies and federal guidelines.

1-(4-Pyridoyl)amino-2,5-dimethylpyrrole (IIIa). Isonicotinic acid hydrazide (0.82 g, 6 mmol) was dissolved in deionized distilled water (25 mL). 2,5-Hexanedione (0.68 g, 6 mmol) was added and the mixture was heated at a gentle boil with reflux for 2 h. The mixture was then allowed to cool

overnight, after which time crystals of **IIIa** formed in solution and were collected by vacuum filtration. The mother liquor was allowed to evaporate on a watch glass and the resulting crystals were collected separately. The total yield was 67%, mp 151–153°C; IR 3224, 3041, 2923, 1673, 1595, 1537, 1490, 1410, 1322, 1289; ^{1}H NMR (DMSO-d_6) δ 11.5 (s, 1 H, disappeared after D_2O shake), 8.8 (d, J = 6 cps, 2 H), 7.9 (d, J = 6 cps, 2 H), 5.7 (s, 2 H), 2.0 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 165, 151, 139, 127, 122, 104, 11.

Anal. Calcd. for $C_{12}H_{13}N_3O$: C, 66.96; H, 6.09. Found: C, 66.84; H, 6.18.

1-Benzoylamino-2,5-dimethylpyrrole (IIIb). Benzoic acid hydrazide (0.82 g, 6 mmol) was heated to boiling in 35 mL deionized, distilled water with reflux. 2,5-Hexanedione (0.82 g, 7.2 mmol) was dissolved in 10 mL deionized, distilled water and added drop wise to the reflux flask without ever halting boiling. Reflux continued for 23 h. After cooling, crystals of **IIIb** appeared in solution and were collected by vacuum filtration (89%). A second crop of crystals was harvested by evaporation of the mother liquor (combined yield 97%). After recrystallization in a mixed system of ethanol/water, crystals were clean, very fine, and just slightly off-white in color, mp 175–177°C; IR 3261, 3061, 2919, 1684, 1602, 1580, 1536, 1488, 1440, 1282; ^{1}H NMR (DMSO-d_6) δ 11.3 (s, 1 H, quenched by D_2O), 8.0 (d, second peak split, J = 6, 2 cps, 2 H), 7.7 (tt, J = 6, 2 cps, 1 H), 7.5 (t, J = 6 cps, 2 H), 5.7 (s, 2 H), 2.1 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 166, 133, 132, 129, 128, 127, 103, 11; major fragments in LR-MS *m/z* 105, 94; HR-MS (fast atom bombardment, MH^+) calculated for $C_{13}H_{15}N_2O$ 215.1184, found 215.1174.

Anal. Calcd. for $C_{13}H_{14}N_2O$: C, 72.87; H, 6.59. Found: C, 72.69; H, 6.61.

1-(4-Chlorobenzoyl)amino-2,5-dimethylpyrrole (IIIc). 4-Chlorobenzoic hydrazide (1.02 g, 6 mmol) was dissolved in absolute ethanol (100 mL) and heated to a gentle boil with reflux. 2,5-Hexanedione (0.82 g, 7.2 mmol) was dissolved in absolute ethanol (10 mL) and added dropwise to the hydrazide solution. Reflux was continued for 50 h, during which time no solid precipitated from solution. TLC was used to monitor reaction progress and showed gradual progression from 4-chlorobenzoic hydrazide (R_f 0.5) to **IIIc** (R_f 0.8). The mixture was cooled to room temperature and kept over night. Excess ethanol was boiled off and cold water added, causing the precipitation of white solid **IIIc**. After vacuum filtration and drying, this solid dissolved easily and cleanly in DMSO, yield 69%, consistently isolated as the hemihydrate and analyzed as such, mp 185°C with decomposition; IR 3449, 3274, 2998, 2961, 1656, 1623, 1594, 1521, 1483, 1448, 1299, 1272; ^{1}H NMR (DMSO-d_6) δ 11.3 (s, 1 H, quenched with D_2O), 8.0 (dd, J = 6, 2 cps, 2 H), 7.6 (dd, J = 6, 2 cps, 2 H), 5.7 (s, 2 H), 2.0 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 165, 137, 131, 130, 129, 128, 127, 104, 11; major fragments in LR-MS *m/z* 139, 94; HR-MS (fast atom bombardment MH^+) calculated for $C_{12}H_{14}N_2OCl$: 249.0795, found 249.0801.

Anal. Calcd. for $C_{12}H_{13}N_2OCl$ x $0.5H_2O$: C, 60.58; H, 5.48. Found: C, 60.29; H, 5.19.

1-(4-Toluoyl)amino-2,5-dimethylpyrrole (IIId). 4-Toluic acid hydrazide (0.90 g, 6 mmol) was reacted with 2,5-hexanedione (0.82 g, 7.2 mmol), in a manner similar to that for **IIIc**. Reflux continued for 70 h, during which TLC was used to track reaction progress and no solid appeared in solution. After 70 h, TLC (silica gel, absolute ethanol) showed no remaining traces of the 4-toluic hydrazide. The reaction mixture was allowed to cool and placed on ice. To the reaction mixture on ice was added 50 mL of cold water, provoking the gradual precipitation of **IIId**. An additional 100 mL of cold water was necessary to keep the solution thin enough to pour into the filter. Vacuum filtration of the precipitate yielded tiny, fluffy, off-white crystals, 1.25 g (91%); mp 187–189°C; IR 3281, 2980, 2935, 1665, 1610, 1531, 1491, 1324, 1301, 1276; ^{1}H NMR (DMSO-d_6) δ 11.2 (s, 1 H, quenched with D_2O), 7.9 (d, J = 6 cps, 2 H), 7.4 (d, J = 6 cps, 2 H), 5.7 (s, 2 H), 2.4 (s, 3 H), 2.0 (s, 6 H); ^{13}C NMR (DMSO- d_6) δ 166, 143, 130, 129, 128, 127, 103, 22, 11; major fragments in LR-MS *m/z* 119, 94; HR-MS (fast atom bombardment MH^+) calculated for $C_{14}H_{17}N_2O$ 229.1341, found 229.1337.

Anal. Calcd for $C_{14}H_{16}N_2O$: C, 73.65; H, 7.06. Found: C, 73.76; H, 7.13.

1-(4-Bromobenzoyl)amino-2,5-dimethylpyrrole (IIIe). 4-Bromobenzoic hydrazide (1.29 g, 6 mmol) was reacted with 2,5-hexanedione (0.82 g, 7.2 mmol) in the manner described for the synthesis of **IIIc**. After 1 h of reflux, a fine suspended solid clouded the mixture, which the addition of 100 mL ethanol did not clear. The solid was not filterable from the reaction mixture. TLC analysis (silica gel, absolute ethanol) revealed a mixture of three species: 4-bromobenzoic hydrazide (R_f 0.5), **IIIe** (R_f 0.8), and a third compound (R_f 0.7) postulated to be a reactive intermediate, as suggested in the work of Amarnath et al. [36] for a different family of pyrroles. After 27 h of reflux, the solid had disappeared, and TLC monitoring began to show a preponderance of **IIIe**, with small amounts of the other two compounds still present. Reflux was stopped after 47 h, no solid having reappeared in the intervening day. Crystals were precipitated from solution by the addition of cold water to the reaction mixture on ice, with a total yield of 1.34 g (77%); mp 207–210°C; IR 3246, 1660, 1588, 1534, 1521, 1465, 1377, 1322, 1300, 1268; ^{1}H NMR (DMSO-d_6) δ 11.3 (s, 1 H, quenched with D_2O), 7.9 (dd, J = 6, 2 cps, 2 H), 7.8 (dd, J = 6, 2 cps, 2 H), 5.7 (s, 2 H), 2.0 (s, 6 H); ^{13}C NMR (DMSO-d_6) 165, 132, 131, 130, 127, 126, 104, 11; major fragment in LR-MS *m/z* 94; HR-MS (fast atom bombardment MH^+) calculated for $C_{13}H_{14}N_2OBr$ 293.0290, found 293.0281.

Anal. Calculated for $C_{13}H_{13}N_2OBr$: C, 53.26; H, 4.47. Found: C, 53.36; H, 4.57.

1-(4-Nitrobenzoyl)amino-2,5-dimethylpyrrole (IIIf). 4-Nitrobenzoic hydrazide (1.09 g, 6 mmol) was reacted with 2,5-hexanedione (0.82 g, 7.2 mmol) following the procedure for the synthesis of **(IIIc)**. Solid clouded the reflux solution after 1 h; addition of 100 mL ethanol did not improve homogeneity. After 66 h, cloudiness persisted but TLC analysis revealed the material present to be entirely **IIIf**, with no traces of starting material or an intermediate. Reflux was stopped, and the solution was filtered while hot. After cooling, cold water was used to provoke the precipitation of **IIIf** from the mother liquor, with a yield of 1.16 g (75%); mp 218–221°C (recrystallization from ethanol and water); IR 3278, 1674, 1604, 1523, 1468, 1343, 1270; ^{1}H NMR (DMSO-d_6) δ 11.3 (s, 1 H, quenched with D_2O), 8.4 (dd, J = 6, 2 cps, 2 H), 8.2 (dd, J = 6, 2 cps, 2 H), 5.7 (s, 2 H), 2.1 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 165, 150, 138, 129, 127, 124, 104, 11; major fragment in LR-MS m/z 94; HR-MS (fast atom bombardment MH^+) calculated for $C_{13}H_{14}N_3O_3$ 258.0879, found 258.0876.

Anal. Calculated for $C_{13}H_{13}N_3O_3$: C, 60.23; H, 5.05. Found: C, 60.30; H, 5.17.

1-(4-Aminosalicyloyl)amino-2,5-dimethylpyrrole (IIIg). 4-Aminosalicylic acid hydrazide (ASAH) was obtained (58%) in pure form from the hydrazinolysis of methyl 4-aminosalicylate by a method that we have previously described in detail [37]. ASAH thus obtained (1.67 g, 10.0 mmoles) was weighed into a 100 mL round-bottom flask fitted for reflux with a temperature-controlled heating mantle, reflux condenser, and magnetic stirrer. Deionized distilled water (25 mL) was added, and the mixture was brought to the boil. Just below the boiling point, the mixture was heterogeneous, but a clear solution was obtained at the boiling point. 2,5-Hexanedione (1.14 g, 10.0 mmoles) was added and the mixture was refluxed for 2 h. Heating was stopped, and stirring was continued for an hour as the mixture slowly cooled. The resulting white solid was filtered off to provide the title compound **IIIg** (0.722 g, 30%), mp 173–174°C; IR 3371, 3270, 1612, 1494, 1385, 1334, 1312, 1270, 1200, 1161, 1012, 967, 833, 786, 757, 692; ^{1}H NMR (DMSO-d_6) δ 12.1 (s, 1H), 10.9 (s, 1H), 7.6 (d, $J = 7$ cps, 1H), 6.2 (d, $J = 7$ cps, 1H), 6.1–6.0 (overlapping s, 3H), 5.7 (s, 2H), 2.0 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 169, 162, 155, 129, 127, 106, 103, 101, 99, 11; HR-MS (fast atom bombardment MH^+) calculated for $C_{13}H_{16}N_3O_2$ 246.1243, found 246.1244.

Anal. Calcd. for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16. Found: C, 63.48; H, 6.28.

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