Synthesis and Characterization of New 1-(4-Methylpiperazin-1-yl)-thioureas as Potential Antitubercular Agents

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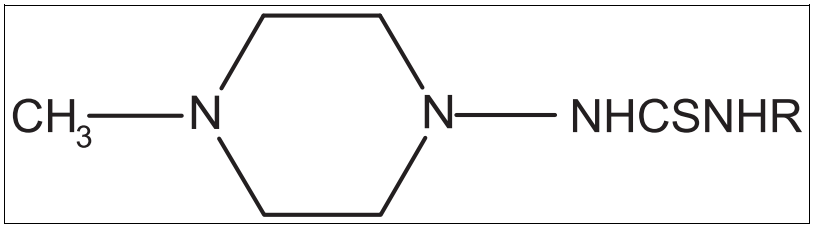

Synthesis, characterization, and preliminary biological assessments of compounds with antitubercular activity.

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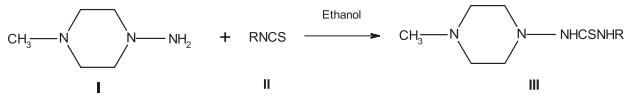
INTRODUCTION

Well into the twenty-first century, tuberculosis remains unchallenged as the primary agent of death among infectious diseases [1,2]. Despite the deployment of massive resources on the part of pharmaceutical companies, governments, public-private partnerships and the World Health Organization, the disease continues to kill one and one-half million people each year. Globally, some three billion persons are infected. Adding to the urgency of the situation has been the recent appearance of strains of the causative agent, *Mycobacterium tuberculosis* (Mtb), which are unusually virulent or highly drug resistant [3,4]. Of special concern among the latter strains are ones which thrive even in the presence of the best drugs available today. The rise of extensively drug-resistant tuberculosis may thus threaten to return therapy of the disease to the pre-antibiotic age [5]. The poor status of current progress against tuberculosis is due to a number of factors, including the small number of novel materials in the drug discovery pipeline. While it is evident that the continuing problem of worldwide tuberculosis is multifactorial in its origins, any realistic estimate of the situation must include the innate intractability of Mtb itself towards chemotherapy [6]. Among diseases caused by bacterial pathogens, tuberculosis is unique in that it requires chemotherapy of 6-20 months. This prolonged chemotherapy may be accompanied by pharmacokinetic interactions with other drugs or a number of adverse drug events [7]. The organism has proven its resilience to succeeding generations of chemists, microbiologists and physicians, underscoring the gravity of the problem. Some recent discoveries, however, are beginning to redress the balance in the fight against tuberculosis, and among these discoveries are effective new compounds bearing heterocyclic structures [8–13]. While research in this productive field holds the promise of finding new chemotherapeutics for the clinic, the study of the interactions of new heterocyclic materials with Mtb may also lead to a better understanding of the life processes of the pathogen. Novel heterocyclic compounds may thus serve as valuable biological probes in combatting tuberculosis [13–18].

As part of our ongoing investigation of the scope of antitubercular heterocyclic systems containing the thiourea moiety [19,20], we now report our results on the preparation and properties of new 1-(4-methylpiperazin-1-yl)thioureas, including preliminary findings on their activities against Mtb. In the event, the reaction of 1-amino-4-methylpiperazine (**I**) with a variety of isothiocyanates (**II**) in ethanol or toluene formed the corresponding thioureas (**III**) as stable crystalline solids in good yield and purity (Scheme 1). The reactions were exceptionally clean, requiring either no purification or, at most, washing with small portions of ether. The tractable products could be characterized in convenient ways by standard spectrometric techniques and elemental analysis. In a representative example, compound **I** was treated with 3,4-dimethoxyphenethyl isothiocyanate (**IIr**, R = 3,4-$(OCH_3)_2C_6H_3CH_2CH_2$, 1.01 equivalents) in refluxing absolute ethanol for thirty minutes. The reaction mixture was cooled slowly to room temperature, inducing the precipitation of the product, which was isolated to give **IIIr** (R = 3,4-$(OCH_3)_2C_6H_3CH_2CH_2$) as a white crystalline solid (76%) in pure form (mp 141-142°C. *Anal.* Calculated for $C_{16}H_{26}N_4O_2S$: C, 56.78; H, 7.74. Found: C, 57.17; H, 7.75. HR-MS (fast atom bombardment, MH^+) calculated for $C_{16}H_{27}N_4O_2S$: 339.1855. Found: 339.1850.). Our results on the preparation of these compounds are summarized in Table 1, with yields averaging 80%. Table 1 also includes the calculated logarithm of the partition coefficient (C log P) between n-octanol and water for each of the compounds (see Experimental for calculation method). The value of

Scheme 1



I II III

C log P is one characteristic used to evaluate the potential of a compound to behave as a drug, providing a predictive indication of a material's solubility, permeation and absorption [21]. The geometric mean of the C log P values in Table 1 is 2.04, which lies well within the C log P distribution of currently traded drugs [22].

The spectrometric features of the 1-(4-methylpiperazin-1-yl)thioureas, were highly characteristic, as shown in Table 2. Foremost among these were 1) N-H bands in accord with the thiourea moiety near 3275 and 3140 cm^{-1} in the infrared spectrum, 2) N-H peaks near δ 9.5 and 9.0 ppm in the hydrogen NMR spectrum and 3) a signal for the thiocarbonyl unit near δ 178 ppm in the carbon NMR spectrum [23]. Infrared spectroscopy was particularly helpful in making a ready bench assessment of the completion of our reactions, since the spectra of the products were so different from those of the reactants. Had they been present in the crystallized products, for example, even small residual amounts of the parent mustard oils (**II**) would have been promptly detected, due to their exceptionally intense bands in the 2200 cm^{-1} region of the infrared spectrum [24,25], but such bands were not observed after as little as 30 to 60 minutes of reaction time. Products **III** also displayed the characteristic infrared absorptions associated with the strong coupling of the C=S stretching mode with the C-N stretching mode in the ranges of 1570-1395, 1420-1260 and 1140-940 cm^{-1} [26].

Table 2

Spectrometric Features of 1-(4-Methylpiperazin-1-yl)thioureas **III**.

Entry	Compound	ν^{max} NH (cm^{-1})	δ ^{1}H NMR NH	δ ^{13}C NMR C=S
1	**IIIa**	3236, 3175	9.82, 9.48	180
2	**IIIb**	3268, 3105	10.00, 9.72	176
3	**IIIc**	3271, 3104	10.91, 9.58	177
4	**IIId**	3268, 3118	10.13, 9.57	179
5	**IIIe**	3264, 3113	9.54, 9.25	180
6	**IIIf**	3263, 3170	9.74, 9.43	177
7	**IIIg**	3265, 3167	9.66, 9.36	179
8	**IIIh**	3266, 3166	9.60, 9.34	177
9	**IIIi**	3284, 3123	9.19, 8.90	180
10	**IIIj**	3263, 3106	9.35, 9.05	178
11	**IIIk**	3281, 3117	9.42, 9.13	177
12	**IIIl**	3239, 3138	9.71, 9.40	177
13	**IIIm**	3308, 3138	9.50, 9.17	178
14	**IIIn**	3231, 3124	9.89, 9.70	177
15	**IIIo**	3296, 3097	9.51, 9.32	177
16	**IIIp**	3313, 3310	8.86, 8.43	180
17	**IIIq**	3327, 3119	8.77, 7.74	179
18	**IIIr**	3304, 3107	8.55, 7.46	179
19	**IIIs**	3316, 3112	9.00, 8.11	181

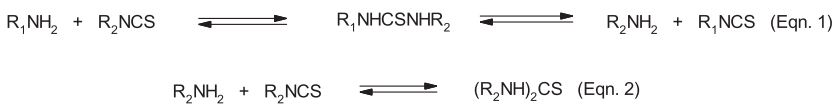
We would like to comment on the general chemical stability of the products. When heated, thioureas are sometimes observed to equilibrate with their immediate progenitors and with other amines and isothiocyanates formed from thermal breakdown (Scheme 2, Eqn. 1). This is particularly true for reactions involving weakly nucleophilic precursors (R_1NH_2), including some aromatic and heteroaromatic amines. Under such circumstances, mixtures of products may be observed, and often the reaction is driven to completion by a LeChatelier effect towards the highly crystalline

Table 1

1-(4-Methylpiperazin-1-yl)thioureas **III**.

Entry	Compound	R	% Yield	mp (°C)	C log P
1	**IIIa**	C_6H_5	58	152-153	1.99
2	**IIIb**	2-ClC_6H_4	82	188-189	2.51
3	**IIIc**	2-$NO_2C_6H_4$	89	197-198	1.95
4	**IIId**	2-CH_3O-C_6H_4	81	179-180	1.74
5	**IIIe**	3-$CH_3C_6H_4$	84	160-161	2.46
6	**IIIf**	4-$CH_3C_6H_4$	88	189-190	2.46
7	**IIIg**	4-ClC_6H_4	72	171-172	2.51
8	**IIIh**	4-IC_6H_4	88	191-192	3.25
9	**IIIi**	4-$CH_3OC_6H_4$	89	153-154	1.74
10	**IIIj**	4-$CH_3SC_6H_4$	89	184-185	2.08
11	**IIIk**	4-$(CH_3)_2NC_6H_4$	78	192-193	2.26
12	**IIIl**	4-$CH_3COC_6H_4$	65	192-193	1.30
13	**IIIm**	4-$C_6H_5OC_6H_4$	78	176-177	3.42
14	**IIIn**	2,4-$Cl_2C_6H_3$	75	194-195	3.03
15	**IIIo**	3,4,5-$(CH_3O)_3C_6H_2$	97	188-189	1.24
16	**IIIp**	$C_6H_5CH_2$	88	150-151	1.97
17	**IIIq**	$C_6H_5CH_2CH_2$	76	148-149	2.20
18	**IIIr**	3,4-$(CH_3O)_2C_6H_3CH_2CH_2$	76	141-142	1.70
19	**IIIs**	CH_2=$CHCH_2$	75	126-128	0.91

Scheme 2



and relatively insoluble symmetrical thiourea (Scheme 2, Eqn. 2) [27]. In the present reactions, however, we found that the aminoheterocycle was sufficiently nucleophilic to lead to rapid and irreversible formation of the 1-(4-methylpiperazin-1-yl)thioureas, with no tendency toward thermal fragmentations and their consequences. This observed stability appears to be typical of the thioureas derived from these strongly nucleophilic bases. Similarly, we found no evidence for any contamination of our products with the thiourethanes ($RNHCSOCH_2CH_3$) that would be formed by the side reaction of the isothiocyanates with the solvent ethanol [27]. The results of reactions run in ethanol were identical to those run in toluene (see Experimental).

In preliminary biological evaluations of the 1-(4-methylpiperazin-1-yl)thioureas, the activity of compound **IIIr** (R = 3,4-$(OCH_3)_2C_6H_3CH_2CH_2$) was representative. The substance was assayed *in vitro* against *Mycobacterium bovis BCG Tice* using the Kirby-Bauer disc diffusion method [28]. In this technique, solutions containing known weights of the compound were applied to porous disks; and the disks were laid on agar development plates having a defined cell density of the mycobacteria. The plates were incubated at 37°C for seven days and then examined for inhibition of growth using transmitted light. The antimicrobial activity of the compound was measured by the dimensions of the circular clear zone surrounding the disk in which no growth occurred, while the remainder of the plate showed a luxuriant bacterial lawn. At 200 μg, **IIIr** showed a zone of clear inhibition of diameter greater than 40 mm; at 50 μg, the diameter was greater than 30 mm; and at 20 μg, the diameter was greater than 20 mm. The size of the zone of inhibition was related to increasing amounts of the test compound and consistent with a concentration dependence of growth inhibition (see Experimental). Since a larger zone of inhibition corresponds to a more effective test compound [29–31], these results were indicative of promising activity and will warrant further investigations of the antimycobacterial character of **IIIr** and its congeners.

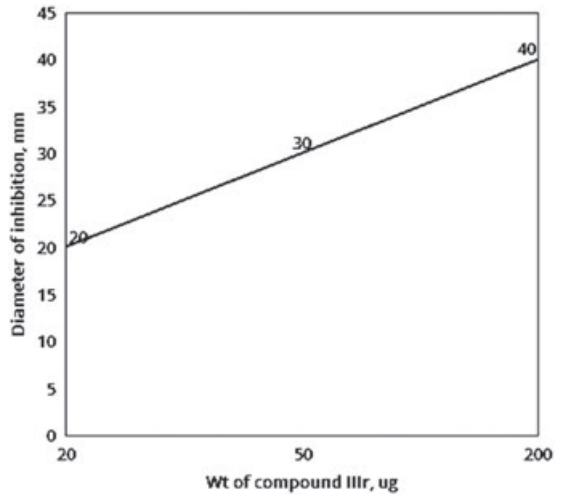
In summary, we found that 1-(4-methylpiperazin-1-yl) thioureas, useful for the study of the antitubercular activities of new heterocyclic structures, could be prepared in good yield by the reaction of 1-amino-4-methylpiperazine with isothiocyanates in ethanol or toluene. The compounds were isolated from the reaction mixture in pure form and required, at most, washing with small portions of ether to obtain the analytical samples. The stable thioureas were produced without contaminants resulting from thermal fragmentation or thiourethane formation and could be reliably identified on the basis of their salient spectrometric features. Preliminary microbiological data suggested that further examination of the antitubercular properties of these compounds will be justified.

EXPERIMENTAL

General methods. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, Tennessee, USA. Melting points (mp, °C) were taken in open capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Cambridge, MA, USA), and are corrected. Infrared (IR) spectra were recorded 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 Sigma-Aldrich Chemical Company (Milwaukee, Wisconsin, USA) and Alfa Aesar Incorporated (Windham, New Hampshire, USA) and were used as received. 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 megahertz (^{1}H NMR) or 75 megahertz (^{13}C NMR) and are reported in parts per million delta (δ) downfield from internal tetramethylsilane as reference; all aromatic coupling constants J were in the range of 7-9 Hz. 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. 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. Reactions were conveniently carried out on 10 mmole scale in a 100 mL round bottom flask fitted for reflux with a temperature-controlled heating mantle, magnetic stirrer and reflux condenser, using approximately 20 mL of absolute ethanol or toluene as solvent. Upon cooling to ambient temperature, the product formed as a crystalline solid, which was filtered off and dried. Often the product formed was analytically pure; whenever this was not the case, the analytical sample was obtained by washing the compound with small portions of ether, as specified in the individual procedures. *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. Calculated values of the logarithms of the partition coefficients (C log P values) were obtained using HyperChem Professional, Version 8.5, following geometry optimization of structures.

Biological assessments. For screening, Kirby-Bauer disk diffusion testing was used [32]. The 1-(4-methylpiperazin-1-yl)thioureas (20 mg) were dissolved in enough DMSO to prepare solutions that had a concentration of 20 mg/mL. The solutions were then applied to 6-mm filter paper disks such that the total weight of test compound per disk was 200, 50 or 20 micrograms. The disks were laid on 7H10 agar plates having a cell density of *M. bovis BCG Tice* of three MacFarland units. *M. bovis BCG Tice* was obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). The plates were incubated at 37°C for seven days and then read using transmitted light. The antimicrobial activity of the compound was measured by the dimensions of the circular clear zone surrounding the disk in which no growth occurred, while the remainder of the plate showed a luxuriant bacterial lawn. At 200 μg, **IIIr** showed a diameter of inhibition of greater than 40 mm; at 50 μg, the diameter was greater than 30 mm; and at 20 μg, the diameter was greater than 20 mm. A plot of the diameter of inhibition versus weight of **IIIr** suggests a linear concentration dependence of growth inhibition.



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In the same vein, at 200 μg, compound **IIIm** displayed a diameter of inhibition of greater than 10mm.

1-(4-Methylpiperazin-1-yl)-3-phenylthiourea (IIIa). The reaction of 1.16 g (10.1 mmol) of 1-amino-4-methyl piperazine with 1.49 g (11.0 mmol) of phenyl isothiocyanate in absolute ethanol was completed during 30 minutes of reflux. The mixture was cooled slowly to room temperature, and the ethanol was allowed to evaporate, resulting in a white solid, which was filtered off (1.45 g, 58%). Three ether washes of the white solid produced the analytical sample, mp 152-153°C, identified as the stable hydrate; IR 3506 (m), 3236 (w), 3175 (w), 2983 (w), 2948 (w), 2844 (w), 2802 (w), 1669 (w), 1601 (m), 1591 (m), 1543 (s), 1500 (s), 1465 (w), 1446 (m), 1390 (w), 1371 (m), 1328 (w), 1282 (m), 1266 (s), 1198 (m), 1159 (w), 1135 (m), 1175 (w), 1158 (w), 1089 (w), 1077 (w), 1050 (w), 1030 (w), 1005 (s), 930 (w), 899 (w), 834 (s), 761 (s), 740 (m), 693 (s), 672 (s); ^{1}H NMR (DMSO-d_6) δ 9.82 (1H, s), 9.48 (1H, s), 7.82 (2H, d), 7.54 (2H, t) 7.36 (1H, t), 3.04 (6H, m), 2.41 (5H, m); ^{13}C NMR (DMSO-d_6) δ 180.0, 141.6, 130.6, 127.3, 127.2, 56.8, 56.3, 48.1; HR-MS (fast atom bombardment, MH^+) calculated for $C_{12}H_{19}N_4S$ 251.1330, found 251.1328.

Anal. Calcd. for $C_{12}H_{18}N_4S \cdot H_2O$: C, 53.73; H, 7.45. Found: C, 53.66; H, 7.48.

1-(4-Methylpiperazin-1-yl)-3-(2-chlorophenyl)thiourea (IIIb). 1-Amino-4-methylpiperazine (1.21 g, 10.5 mmol) reacted with 2-chlorophenyl isothiocyanate (1.76 g, 10.4 mmol) in absolute ethanol over 30 minutes. The mixture was cooled to room temperature and the resulting white solid was filtered off to give IIIb (2.42 g, 82%), identified as the stable hydrate, mp 188-189°C; IR 3268 (m), 3105 (w), 2940 (w), 2845 (w), 2795 (w), 1593 (s), 1515 (s), 1458 (m), 1443 (m), 1381 (w), 1367 (w), 1278 (m), 1264 (s), 1202 (s), 1155 (m), 1134 (m), 1082 (m), 1073 (m), 1055 (m), 1003 (s), 939 (w), 852 (w), 829 (m), 784 (w), 725 (s), 680 (w); ^{1}H NMR (DMSO-d_6) δ 10.00 (1H, s), 9.72 (1H, s), 8.40 (1H, d), 7.63 (1H, d), 7.45 (1H, t), 7.31 (1H, t), 2.96 (6H, m), 2.29 (5H, m, apparent br s); ^{13}C NMR (DMSO-d_6) δ 176.3, 134.8, 128.0, 125.9, 125.2, 125.1, 53.4, 52.5, 44.4; HR-MS (fast atom bombardment, MH^+) calculated for $C_{12}H_{18}ClN_4S$ 285.0941, found 285.0948.

Anal. Calcd. for $C_{12}H_{17}ClN_4S \cdot 0.25\ H_2O$: C, 49.82; H, 6.10. Found: C, 50.00; H, 6.02.

1-(4-Methylpiperazin-1-yl)-3-(2-nitrophenylthiourea (IIIc). The reaction of 1.18 g (10.2 mmol) of 1-amino-4-methylpiperazine with 1.80 g (10.0 mmol) of 2-nitrophenyl isothiocyanate in absolute ethanol was completed after one hour of reflux. The reaction mixture was allowed to cool to room temperature, depositing 2.17 g (89%) of yellow crystals, which were filtered off. Three sequential washes of the yellow solid with small portions of ether produced the analytical sample, mp 197-198°C; IR 3271 (m), 3104 (m), 2969 (w), 2952 (m), 2838 (w), 2801 (w), 1607 (m), 1580 (m), 1523 (w), 1504 (s), 1452 (s), 1383 (w), 1366 (m), 1337 (s), 1317 (w), 1260 (s), 1197 (s), 1159 (w), 1134 (s), 1075 (m), 1046 (m), 1004 (s), 935 (m), 926 (w), 854 (m), 832 (m), 780 (s), 741 (m), 706 (s), 670 (m); ^{1}H NMR (DMSO-d_6) δ 10.91 (1H, s), 9.58 (1H, s), 8.48 (1H, d), 7.87 (1H, d), 7.49 (1H, t), 7.09 (1H, t), 2.62 (6H, m), 2.02 (5H, m, apparent br s); ^{13}C NMR (DMSO-d_6) δ

177.3, 140.4, 134.1, 133.9, 125.9, 125.1, 124.6, 54.5, 54.2, 45.4; HR-MS (fast atom bombardment, MH^+) calculated for $C_{12}H_{18}N_5O_2S$ 296.1181, found 296.1184.

Anal. Calcd. for $C_{12}H_{17}N_5O_2S$: C, 48.80; H, 5.80. Found: C, 48.54; H, 5.66.

1-(4-Methylpiperazin-1-yl)-3-(2-methoxyphenyl)thiourea (IIId). 1-Amino-4-methylpiperazine (1.14 g, 9.90 mmol) was refluxed with 2-methoxyphenyl isothiocyanate (1.63 g, 9.87 mmol) in absolute ethanol for an hour. The mixture was cooled to room temperature, forming a solid, filtration of which gave the white crystalline product in analytically pure form (2.23 g, 81%), mp 179-180°C; IR 3268 (m), 3118 (w), 2948 (w), 2932 (w), 2838 (m), 2800 (m), 1597 (m), 1533 (s), 1489 (m), 1457 (s), 1436 (m), 1385 (w), 1372 (w), 1340 (w), 1311 (w), 1282 (m), 1268 (s), 1233 (s), 1195 (s), 1174 (w), 1156 (m), 1138 (m), 1111 (w), 1090 (m), 1078 (w), 1046 (m), 1028 (s), 1005 (s), 940 (m), 919 (m), 830 (m), 786 (m), 751 (s), 658 (w); ^{1}H NMR (DMSO-d_6) δ 10.13 (1H, s), 9.57 (1H, s), 8.82 (1H, d)), 7.23 (2H, m), 7.06 (1H, t), 4.01 (3H, s), 2.94 (6H, m), 2.24 (5H, m); ^{13}C NMR (DMSO-d_6) δ 178.5, 151.8, 130.3, 126.6, 123.3, 122.2, 113.3, 58.5, 57.1, 55.9, 47.9; HR-MS (fast atom bombardment, MH^+) calculated for $C_{13}H_{21}N_4OS$ 281.1436, found 281.1433.

Anal. Calcd. for $C_{13}H_{20}N_4OS$: C, 55.69; H, 7.19. Found: C, 55.99; H, 7.07.

1-(4-Methylpiperazin-1-yl)-3-(3-tolyl)thiourea (IIIe). The reaction of 1.20 g (10.4 mmol) of 1-amino-4-methyl piperazine with 1.26 g (8.44 mmol) of 3-tolyl isothiocyanate in absolute ethanol was completed in one hour of reflux. The mixture was cooled to room temperature and excess solvent was allowed to evaporate. The white crystalline product was filtered off and dried (1.88 g, 84%). The analytical sample was obtained by washing the product with three small portions of ether, and the compound was identified as the stable hydrate, mp 160-161°C; IR 3264 (m), 3113 (w), 2942 (m), 2831 (w), 2795 (w), 1608 (m), 1556 (m), 1524 (s), 1504 (s), 1458 (s), 1380 (m), 1369 (m), 1323 (s), 1280 (s), 1269 (s), 1216 (s), 1158 (m), 1137 (s), 1074 (m), 1046 (m), 1006 (s), 969 (w), 860 (m), 817 (m), 780 (w), 722 (s), 691 (s); ^{1}H NMR (DMSO-d_6) δ 9.54 (1H, s), 9.25 (1H, s), 7.47 (1H, d), 7.38 (1H, s), 7.20 (1H, t), 6.95 (1H, d), 2.81 (6H, m), 2.30 (5H, m), 2.19 (3H, s); ^{13}C NMR (DMSO-d_6) δ 180.0, 141.6, 140.0, 130.6, 128.0, 127.7, 124.3, 56.9, 56.5, 48.2, 23.7; HR-MS (fast atom bombardment, MH^+) calculated for $C_{13}H_{21}N_4S$ 265.1487, found 265.1483.

Anal. Calcd. for $C_{13}H_{20}N_4S \cdot 0.125\ H_2O$: C, 58.56; H, 7.65. Found: C, 58.59; H, 7.54.

1-(4-Methylpiperazin-1-yl)-3-(4-tolyl)thiourea (IIIf). Refluxing 1.15 g (9.98 mmol) of 1-amino-4-methylpiperazine with 1.51 g (10.10 mmol) of 4-tolyl isothiocyanate in absolute ethanol for an hour, followed by cooling and filtration gave the white crystalline product in analytically pure form (2.31 g, 88%), mp 189-190°C; IR 3263 (m), 3170 (m), 2988 (w), 2956 (w), 2887 (w), 2840 (w), 2802 (w), 1589 (m), 1537 (s), 1500 (s), 1461 (m), 1410 (w), 1383 (w), 1372 (w), 1332 (w), 1314 (w), 1301 (w), 1286 (m), 1262 (s), 1196 (s), 1145 (s), 1133 (s), 1090 (m), 1078 (m), 1052 (w), 1020 (w), 1005 (s), 942 (w), 930 (w), 843 (m), 814 (s), 797 (m), 730 (m), 699 (w); ^{1}H NMR (DMSO-d_6) δ 9.74 (1H, s), 9.43 (1H, s), 7.68 (2H, d), 7.35 (2H, d), 3.59 (6H, m), 3.04 (5H, m), 2.42 (3H, s); ^{13}C NMR (DMSO-d_6) δ 177.4, 136.4, 133.9, 128.4, 124.6, 54.1, 53.6, 45.3, 20.5; HR-MS (fast atom bombardment, MH^+) calculated for $C_{13}H_{21}N_4S$ 265.1487, found 265.1482.

Anal. Calcd. for $C_{13}H_{20}N_4S$: C, 59.06; H, 7.62. Found: C, 58.86; H, 7.99.

1-(4-Methylpiperazin-1-yl)-3-(4-cholorophenyl)thiourea (IIIg). The reaction of 1.16 g (10.1 mmol) of 1-amino-4-methylpiperazine with 1.67 g (9.84 mmol) of 4-chlorophenyl isothiocyanate in toluene was complete during one hour of reflux. The reaction mixture was cooled to room temperature, producing a beige solid, which was filtered off (2.02 g, 72%). The analytical sample was obtained by washing the beige solid with small portions of ether, mp 171-172°C; IR 3265 (m), 3167 (m), 2983 (w), 2952 (w), 2885 (w), 2838 (w), 2794 (w), 1585 (m), 1541 (s), 1498 (s), 1453 (m), 1406 (w), 1371 (w), 1331 (w), 1305 (w), 1286 (s), 1259 (s), 1200 (s), 1178 (w), 1153 (m), 1132 (m), 1089 (s), 1077 (m), 1054 (w), 1005 (s), 935 (w), 839 (s), 829 (s), 816 (m), 786 (w), 759 (m), 715 (m), 676 (w); ^{1}H NMR (DMSO-d_6) δ 9.66 (1H, s), 9.36 (1H, s), 7.65 (2H, d), 7.37 (2H, d), 3.82 (6H, m), 2.19 (5H, m); ^{13}C NMR (DMSO-d_6) δ 177.9, 138.5, 129.0, 128.3, 126.8, 54.5, 54.2, 46.0; HR-MS (fast atom bombardment, MH^+) calculated for $C_{12}H_{18}ClN_4S$ 285.0941, found 285.0950.

Anal. Calcd. for $C_{12}H_{17}ClN_4S$: C, 50.61; H, 6.02. Found: C, 50.52; H, 5.69.

1-(4-Methylpiperazin-1-yl)-3-(4-iodophenyl)thiourea (IIIh). Treatment of 1-amino-4-methylpiperazine (1.10 g, 9.55 mmol) with 1.34 g (5.13 mmol) of 4-iodophenyl isothiocyanate in refluxing toluene for one hour, followed by cooling to room temperature and the evaporation of excess toluene, led to the crystallization of the light yellow product IIIi (1.70 g, 88%). The analytical sample was obtained by washing the material with small portions of ether, mp 186-187°C; IR 3266 (m), 3166 (m), 2979 (w), 2947 (w), 2881 (w), 2834 (w), 2800 (w), 1576 (m), 1537 (s), 1496 (s), 1460 (s), 1443 (w), 1398 (m), 1368 (w), 1328 (w), 1280 (s), 1260 (s), 1199 (m), 1157 (w), 1139 (m), 1132 (m), 1090 (m), 1077 (w), 1063 (w), 1002 (s), 929 (m), 832 (s), 840 (s), 810 (s), 786 (w), 751 (s), 707 (m), 663 (w); ^{1}H NMR (DMSO-d_6) δ 9.60 (1H, s), 9.34 (1H, s), 7.63 (2H, d), 7.35 (2H, d), 2.79 (6H, m), 2.16 (5H, m); ^{13}C NMR (DMSO-d_6) δ 177.2, 138.9, 136.5, 126.7, 88.9, 54.0, 53.6, 44.4; HR-MS (fast atom

bombardment, MH^+) calculated for $C_{12}H_{18}IN_4S$ 377.0297, found 377.0283.

Anal. Calcd. for $C_{12}H_{17}IN_4S$: C, 38.31; H, 4.55. Found: C, 38.25; H, 4.49.

1-(4-Methylpiperazin-1-yl)-3-(4-methoxyphenyl)thiourea (IIIi). The reaction of 1.29 g (11.2 mmol) of 1-amino-4-methylpiperazine with 1.69 g (10.2 mmol) of 4-methoxyphenyl isothiocyanate in absolute ethanol was carried out for 30 minutes, followed by cooling to room temperature, evaporation of excess ethanol and filtration of the resulting analytically pure beige solid (2.54 g, 89%), mp 153-154°C; IR 3284 (w), 3244 (w), 3123 (w), 2945 (w), 2833 (w), 2794 (w), 1512 (s), 1457 (m), 1440 (m), 1412 (w), 1382 (w), 1297 (w), 1263 (m), 1240 (s), 1196 (s), 1178 (w), 1157 (m), 1136 (m), 1086 (m), 1047 (w), 1028 (m), 1004 (s), 934 (w), 821 (s), 796 (w), 782 (w), 750 (m); ^{1}H NMR (DMSO-d_6) δ 9.19 (1H, s), 8.90 (1H, s), 7.15 (2H, d), 6.63 (2H, d), 3.49 (3H, s), 2.56 (6H, m), 1.93 (5H, m); ^{13}C NMR (DMSO-d_6) δ 179.69, 158.3, 133.7, 128.5, 114.9, 57.0, 55.9, 55.5, 47.3; HR-MS (fast atom bombardment, MH^+) calculated for $C_{13}H_{21}N_4OS$ 281.1436, found 281.1441.

Anal. Calcd. for $C_{13}H_{20}N_4OS$: C, 55.69; H, 7.19. Found: C, 55.57; H, 7.05.

1-(4-Methylpiperazin-1-yl)-3-(4-methylthiophenyl)thiourea (IIIj). Refluxing 1-amino-4-methylpiperazine (1.14 g, 9.90 mmol) with 1.18 g (9.98 mmol) of 4-methylthiophenyl isothiocyanate in absolute ethanol for one hour, followed by cooling to room temperature, deposited a white crystalline solid, which was filtered off and dried (2.62 g, 89%). Washing the white solid with small portions of ether produced the analytical sample, mp 184-185°C; IR 3263 (s), 3106 (w), 2944 (m), 2911 (w), 2831 (m), 2797 (w), 1503 (s), 1459 (m), 1436 (m), 1381 (w), 1302 (w), 1279 (s), 1206 (s), 1160 (m), 1139 (s), 1090 (m), 1077 (m), 1047 (w), 1006 (s), 973 (w), 931 (w), 919 (w), 838 (m), 817 (s), 783 (m), 761 (s), 724 (m), 714 (m), 671 (m); ^{1}H NMR (DMSO-d_6) δ 9.35 (1H, s), 9.05 (1H, s), 7.33 (2H, d), 7.02 (2H, d), 2.61 (6H, m), 2.26 (3H, s), 1.98 (5H, m); ^{13}C NMR (DMSO-d_6) δ 177.8, 136.6, 134.0, 126.2, 125.7, 54.5, 54.1, 45.8, 15.6; HR-MS (fast atom bombardment, MH^+) calculated for $C_{13}H_{21}N_4S_2$ 297.1208, found 297.1201.

Anal. Calcd. for $C_{13}H_{20}N_4S_2$: C, 52.67; H, 6.80. Found: C, 52.84; H, 6.56.

1-(4-Methylpiperazin-1-yl)-3-(4-dimethylaminophenyl)thiourea (IIIk). The reaction of 1.22 g (10.6 mmol) of 1-amino-4-4methylpiperazine with 1.55 g (8.70 mmol) of 4-dimethylaimnophenyl isothiocyanate in absolute ethanol at reflux was ended after one hour. Cooling the reaction mixture to room temperature led to the formation of a white crystalline solid, which was filtered off and dried (1.98 g, 798%). Washing the solid with small portions of ether gave the analytical sample, mp 192-193°C; IR 3281 (m), 3117 (m), 2944 (m), 2794 (m), 1616 (m), 1528 (s), 1505 (s), 1459 (s), 1447 (s), 1359 (s), 1269 (s), 1208 (s), 1182 (m), 1158 (m), 1138 (s), 1076 (m), 1048 (w), 1008 (s), 953 (w), 836 (m), 808 (s), 784 (m), 769 (m), 731 (m), 690 (w); ^{1}H NMR (DMSO-d_6) δ 9.42 (1H, s), 9.13 (1H, s), 7.38 (2H, d), 6.77 (2H, d), 2.98 (12H, m), 2.28 (5H, m); ^{13}C NMR (DMSO-d_6) δ 176.8, 147.2, 127.3, 125.3, 110.8, 53.2, 52.8, 44.5, 39.5; HR-MS (fast atom bombardment, MH^+) calculated for $C_{14}H_{24}N_5S$ 203.0933, found 203.0927.

Anal. Calcd. for $C_{14}H_{23}N_5S$: C, 57.30; H, 7.90. Found: C, 56.90; H, 7.71.

1-(4-Methylpiperazin-1-yl)-3-(4-acetylphenyl)thiourea (IIIl). Refluxing 1-amino-4-methylpiperazine (1.17 g, 10.2 mmol) with 4-acetylphenyl isothiocyanate (1.80 g, 10.2 mmol) in absolute ethanol for an hour, followed by cooling to room temperature, produced a white crystalline solid, which was filtered off and dried (1.92 g, 65%), in analytically pure form, mp 192-193°C; IR 3239 (m), 3138 (m), 2980 (w), 2943 (m), 2840 (m), 2790 (m), 1674 (s), 1601 (s), 1543 (s), 1514 (m), 1489 (s), 1456 (m), 1420 (w), 1358 (m), 1282 (w), 1257 (s), 1214 (s), 1179 (m), 1154 (s), 1133 (m), 1086 (w), 1076 (w), 1052 (w), 1005 (s), 957 (m), 928 (w), 857 (m), 849 (w), 831 (m), 788 (w), 769 (w), 745 (m), 687 (m); ^{1}H NMR (DMSO-d_6) δ 9.71 (1H, s), 9.40 (1H, s), 7.79 (4H, m apparent br s), 2.71 (6H, m), 2.43 (3H, s), 2.07 (5H, m apparent br s); ^{13}C NMR (DMSO-d_6) δ 196.8, 176.9, 143.4, 132.5, 128.3, 122.9, 54.0, 53.7, 45.4, 26.5; HR-MS (fast atom bombardment, MH^+) calculated for $C_{14}H_{21}N_4OS$ 293.1436, found 293.1439.

Anal. Calcd. for $C_{14}H_{20}N_4OS$: C, 57.51; H, 6.89. Found: C, 57.36; H, 6.79.

1-(4-Methylpiperazin-1-yl)-3-(4-phenoxyphenyl)thiourea (IIIm). Treatment of 1-amino-4-methylpiperazine (1.16 g, 10.1 mmol) with 2.28 g (10.0 mmol) of 4-phenoxyphenyl isothiocyanate in refluxing absolute ethanol for an hour, followed by cooling to room temperature and evaporation of excess ethanol, led to the formation of 2.67 g (78%) of white crystalline solid, which was washed with small portions of ether to obtain the analytical sample,

mp 189-190°C; IR 3308 (m), 3138 (m), 2945 (w), 2842 (w), 2796 (m), 2769 (w), 1588 (m), 1529 (s), 1508 (s), 1484 (s), 1416 (w), 1387 (w), 1375 (w), 1286 (m), 1225 (s), 1195 (s), 1164 (m), 1154 (m), 1134 (w), 1085 (m), 1006 (s), 934 (w), 914 (w), 866 (m), 844 (m), 800 (m), 765 (m), 738 (m), 694 (s); ^{1}H NMR (DMSO-d_6) δ 9.50 (1H, s), 9.17 (1H, s), 7.49 (2H, d), 7.32 (2H, t), 7.05 (2H, t), 6.91 (3H, t), 2.74 (6H, m, apparent br s), 2.10 (5H, m); ^{13}C NMR (DMSO-d_6) δ 178.4, 157.9, 154.1, 135.5, 130.9, 127.4, 124.1, 119.2, 119.0, 54.9, 54.5, 46.3; HR-MS (fast atom bombardment, MH^+) calculated for $C_{18}H_{23}N_4OS$ 343.1593, found 343.1578.

Anal. Calcd. for $C_{18}H_{22}N_4OS$: C, 63.13; H, 6.48. Found: C, 63.10; H, 6.58.

1-(4-Methylpiperazin-1-yl)-3-(2,4-dichlorophenyl)thiourea (IIIn). The reaction of 1.19 g (10.3 mmol) of 1-amino-4-

methylpiperazine with 2.15 g (10.5 mmol) of 2,4-dichlorophenyl isothiocyanate in absolute ethanol was complete within 30 minutes. The mixture was cooled to room temperature, occasioning the precipitation of analytically pure white crystalline product, which was filtered off and dried (2.49 g, 75%), mp 194-195°C; IR 3231 (w), 3124 (w), 2949 (w), 2841 (w), 2797 (m), 1589 (w), 1572 (s), 1530 (s), 1511 (s), 1461 (m), 1446 (m), 1385 (m), 1372 (m), 1319 (m), 1270 (s), 1196 (s), 1156 (s), 1137 (s), 1097 (m), 1077 (m), 1051 (m), 1005 (s), 938 (w), 860 (s), 837 (s), 822 (s), 805 (s), 781 (w), 691 (m), 661 (s); ^{1}H NMR (DMSO-d_6) δ 9.89 (1H, s), 9.70 (1H, s), 8.25 (1H, d), 7.71 (1H, s), 7.46 (1H, d), 2.88 (6H, m), 2.22 (5H, m); ^{13}C NMR (DMSO-d_6) δ 177.4, 135.2, 129.4, 128.4, 127.6, 127.0, 54.3, 53.5, 45.4; HR-MS (fast atom bombardment, MH^+) calculated for $C_{12}H_{17}Cl_2N_4S$ 319.0551, found 319.0561.

Anal. Calcd. for $C_{12}H_{16}Cl_2N_4S$: C, 45.15; H, 5.05. Found: C, 45.01; H, 4.92.

1-(4-Methylpiperazin-1-yl)-3-(3,4,5-trimethoxyphenyl)thiourea (IIIo). When 1-amino-4-methylpiperazine (0.65 g, 5.64 mmol) was allowed to react over one hour with 3,4,5-trimethoxyphenyl isothiocyanate (1.44 g, 6.39 mmol) in refluxing absolute ethanol, then cooled to ambient temperature, a light grey-brown crystalline solid was formed, which was filtered off and dried (1.86 g, 97%). Washing with small portions of ether gave the analytical sample, mp 188-189°C; IR 3296 (m), 3097 (w), 2937 (w), 2842 (w), 1597 (s), 1504 (s), 1451 (s), 1419 (s), 1384 (w), 1368 (w), 1345 (m), 1298 (w), 1281 (w), 1270 (w), 1228 (s), 1192 (m), 1157 (w), 1120 (s), 1082 (m), 1045 (w), 998 (s), 932 (w), 859 (w), 843 (m), 812 (m), 779 (m), 746 (w), 714 (m), 658 (m); ^{1}H NMR (DMSO-d_6) δ 9.51 (1H, s), 9.32 (1H, s), 7.09 (2H, s), 3.81 (6H, s), 3.70 (3H, s), 2.87 (6H, m), 2.25 (5H, m); ^{13}C NMR (DMSO-d_6) δ 177.0, 152.0, 134.8, 134.3, 102.2, 60.0, 55.8, 54.0, 53.7, 45.4; HR-MS (fast atom bombardment, MH^+) calculated for $C_{15}H_{25}N_4O_3S$ 341.1647, found 341.1639.

Anal. Calcd. for $C_{15}H_{24}N_4O_3S$: C, 52.92; H, 7.11. Found: C, 52.80; H, 6.84.

1-(4-Methylpiperazin-1-yl)-3-benzylthiourea (IIIp). 1-Amino-4-methylpiperazine (1.16 g, 10.1 mmol) and benzyl isothiocyanate (1.50 g, 10.1 mmol) were weighed into a 100 mL round bottom flask fitted for stirring and reflux and brought to the boil in absolute ethanol (*ca*. 20 mL). After an hour at reflux, the solution was cooled to room temperature and the excess ethanol evaporated to give a pale yellow solid, which was filtered off and dried (2.35 g, 88%). The analytical sample was prepared by washing the yellow solid with small portions of ether, mp 150-151°C; IR 3313 (m), 3310 (m), 2944 (m), 2836 (w), 2795 (w), 1521 (s), 1494 (m), 1452 (m), 1383 (w), 1359 (w), 1279 (s), 1268 (s), 1219 (m), 1192 (m), 1158 (m), 1138 (s), 1095 (w), 1073 (m), 1047 (w), 1029 (w), 1006 (s), 967 (s), 932 (w), 846 (m), 793 (m), 731 (s), 693 (s), 662 (s); ^{1}H NMR (DMSO-d_6) δ 8.86 (1H, s), 8.43 (1H, t), 7.22 (5H, m), 4.67 (2H, m), 2.66 (6H, m), 2.09 (5H, m); ^{13}C NMR (DMSO-d_6) δ 179.8, 140.3, 128.6, 127.4, 127.0, 54.7, 54.2, 46.5, 45.9; HR-MS (fast atom bombardment, MH^+) calculated for $C_{13}H_{21}N_4S$ 265.1487, found 265.1482.

Anal. Calcd. for $C_{13}H_{20}N_4S$: C, 59.06; H, 7.62. Found: C, 59.36; H, 7.63.

1-(4-Methylpiperazin-1-yl)-3-phenethylthiourea (IIIq). When 1-amino-4-methylpiperazine (1.15 g, 9.98 mmol) was treated with β-phenethyl isothiocyanate (1.68 g, 10.3 mmol) for one hour in refluxing absolute ethanol, followed by cooling to room temperature, a white crystalline solid was formed, which was filtered off and dried (2.10 g, 76%), in analytically pure form, mp 148-149°C; IR 3327 (m), 3119 (m), 2943 (m), 2848 (m), 2934 (m), 2805 (w), 1604 (w), 1524 (s), 1486 (m), 1455 (m), 1447 (m), 1418 (w), 1379 (w), 1363 (w), 1330 (w), 1281 (m), 1228 (m), 1176 (m), 1156 (w), 1119 (w), 1137 (m), 1086 (w), 1076 (w), 1050 (w), 947 (m), 925 (w), 889 (w), 831 (m), 786 (m), 767 (s), 759 (s), 702 (s); ^{1}H NMR (DMSO-d_6) δ 8.77 (1H, s), 7.74 (1H, t), 7.27 (2H, m), 7.16 (3H, m), 3.63 (2H, m), 2.77 (2H, m), 2.58 (6H, m, apparent br s), 2.11 (3H, s), 1.98 (2H, m); ^{13}C NMR (DMSO-d_6) δ 178.9, 139.6, 129.0, 128.6, 128.4, 55.5, 53.8, 45.7, 44.4, 35.1; HR-MS (fast atom bombardment, MH^+) calculated for $C_{14}H_{23}N_4S$ 279.1643, found 279.1636.

Anal. Calcd. for $C_{14}H_{22}N_4S$: C, 60.40; H, 7.96. Found: C, 60.17; H, 8.09.

1-(4-Methylpiperazin-1-yl)-3-(3,4-dimethoxyphenethyl)thiourea (IIIr). The reaction of 1.15 g (9.98 mmol) of 1-amino-4-methylpiperazine with 2.25 g (10.1 mmol) of 3,4-dimethoxyphenethylisothiocyanate in absolute ethanol was completed during 30 minutes of reflux. Cooling the mixture slowly to ambient temperature, produced white crystals, which were filtered off and dried (2.57 g, 76%) in analytically pure form; mp 141-142°C; IR 3304 (m), 3107 (w), 2937 (m), 2840 (m), 2794 (w), 1589 (w), 1514 (s), 1465 (m), 1443 (m), 1419 (w), 1385 (w), 1344 (w), 1292 (w), 1260 (s), 1227 (s), 1210 (m), 1193 (w), 1145 (s), 1095 (w), 1074 (w), 1034 (w), 1027 (s), 1003 (s), 924 (w), 897 (w), 856 (m), 827 (s), 786 (m), 759 (m); ^{1}H NMR (DMSO-d_6) δ 8.55 (1H, s), 7.46 (1H, m), 6.60 (2H, m), 6.47 (1H, m), 3.49 (8H, m), 2.53 (2H, m), 2.35 (6H, m), 1.89-1.75 (5H, m); ^{13}C NMR (DMSO-d_6) δ 179.2, 149.3, 147.8, 132.3, 121.1, 113.0, 112.4, 56.1, 55.9, 54.8, 54.1, 46.0, 44.8, 34.9; HR-MS (fast atom bombardment, MH^+) calculated for $C_{16}H_{27}N_4O_2S$ 339.1855, found 339.1850.

Anal. Calcd. for $C_{16}H_{26}N_4O_2S$: C, 56.78; H, 7.74. Found: C, 57.17; H, 7.75.

1-(4-Methylpiperazin-1-yl)-3-allylthiourea (IIIs). Treatment of 1-amino-4-methylpiperazine (1.19 g, 10.3 mmol) with allyl isothiocyanate (0.99 g, 9.98 mmol) in refluxing absolute ethanol for 30 minutes was followed by cooling

slowly to room temperature. The excess ethanol was evaporated, producing a tan solid, which was isolated and dried (1.61 g, 75%). Washing with small portions of ether gave the analytical sample, mp 126-128°C; IR 3316 (m), 3112 (w), 3074 (w), 2982 (w), 2943 (m), 2891 (w), 2834 (m), 2799 (w), 2056 (w), 1644 (m), 1517 (s), 1459 (m), 1439 (w), 1407 (w), 1382 (w), 1360 (w), 1280 (s), 1267 (s), 1216 (s), 1159 (w), 1136 (s), 1095 (w), 1083 (w), 1075 (m), 1047 (w), 1032 (w), 1006 (s), 965 (s), 930 (w), 912 (m), 857 (s), 840 (m), 788 (m), 659 (s); ^{1}H NMR (DMSO-d_6) δ 9.00 (1H, s), 8.11 (1H, m), 5.99 (1H, m), 5.21 (2H, m), 4.25 (2H, m), 3.55 (4H, m), 2.84 (7H, m); ^{13}C NMR (DMSO-d_6) δ 181.2, 137.8, 117.2, 56.5, 56.0, 47.8, 47.4; HR-MS (fast atom bombardment, MH^+) calculated for $C_9H_{19}N_4S$ 215.1330, found 215.1335.

Anal. Calcd. for $C_9H_{18}N_4S$: C, 50.43; H, 8.46. Found: C, 50.43; H, 8.46.

Repetition of this preparation using toluene instead of ethanol as the solvent gave a similar yield (78%), with spectrometric and analytical characteristics identical to those noted above.

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