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Preparation and properties of new antitubercular thioureas and thiosemicarbazides

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Unsymmetrical thioureas and thiosemicarbazides, valuable as lead compounds for antitubercular drug design and discovery, are prepared by the reactions of isothiocyanates with aromatic amines and acid hydrazides, respectively, in robust procedures that permit considerable structural variety. Occasional isolation of symmetrical thioureas derived wholly from the isothiocyanate components of these reactions may occur by thermal fragmentation of the initial thioureidation products.

Keywords: Thioureas; Thiosemicarbazides; Tuberculosis; Thermal fragmentation

1. Introduction

In the decade since tuberculosis was designated a global public health crisis by the World Health Organisation [1], treatment of the disease has become further complicated by the emergence of new strains of the causative organism, *Mycobacterium tuberculosis*. These new strains are either resistant to the current frontline medications or characterised by increased virulence [2–4]. Fully one-third of the human population is now infected with tuberculosis, and as many as three million deaths per year are attributed to the disease [5, 6]. The concurrent human immunodeficiency virus (HIV) pandemic is expected to continue to foster the spread of tuberculosis because the infective processes for the two conditions are synergistic [7, 8].

Faced with this crisis, researchers in drug design and discovery are attempting to find new classes of compounds that will be effective antituberculars and to re-evaluate the roles and modes of action of existing chemotherapeutic agents [9]. It is likely that sulphur-containing compounds will be significant leads for the discovery of new antitubercular medications, particularly those from the thiourea and thiosemicarbazide families (**1a** and **1b**, figure 1). Early researchers had found promising activities among these compounds [10–17], including useful results in the clinic [18, 19], but the set of compounds investigated was limited.

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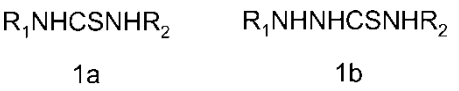


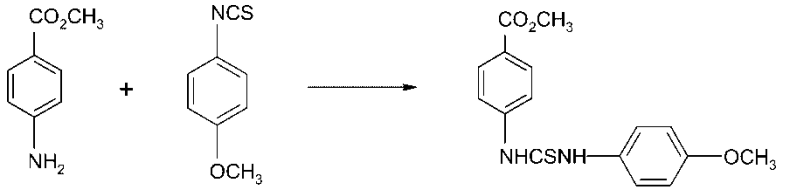
Figure 1. Unsymmetrical thiocarbonyl compounds.

The wider potential of these thiocarbonyls for treating tuberculosis was never fulfilled. Today, the urgency of the global public health impasse in treatment of the disease has kindled a renewal of interest in clarifying and amplifying the antimicrobial activities of these substances [20, 21]. In our laboratory, we have been exploring the synthesis of unsymmetrical thioureas and thiosemicarbazides that would be expected to have antimycobacterial activity [22, 23]. Our work has followed the premise that such unsymmetrical compounds may have enhanced activity compared to symmetrical counterparts [24]. We now report new work on the preparation and properties of novel thiocarbonyl compounds of interest in antitubercular drug discovery, comment on some interesting fragmentation reactions encountered in their synthesis, and provide a brief account of representative biological activity.

2. Results and discussion

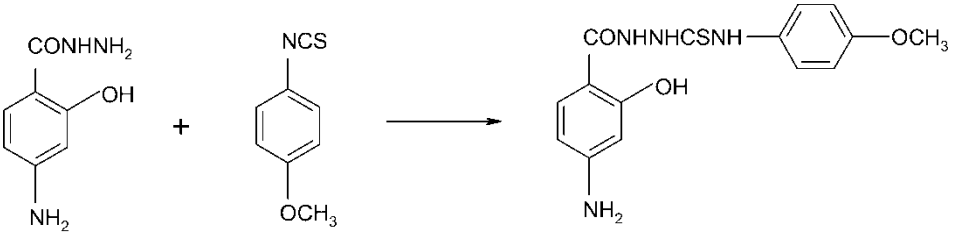
In exploring methods of preparation of the desired unsymmetrical thiocarbonyls from isothiocyanates and amino compounds, we were mindful that poor reactivity makes some amine precursors less likely to give the desired products of nucleophilic addition [25]. Generally, however, we found that the reaction of a primary aromatic amine or primary acid hydrazide with an isothiocyanate in refluxing ethanol or toluene for several hours produced the antimycobacterial thiourea or thiosemicarbazide (**1a** and **1b**, figure 1) without complication. In a typical preparative example leading to an unsymmetrical thiourea (scheme 1), methyl 4-aminobenzoate **2** reacted at reflux for four hours with 4-methoxyphenyl isothiocyanate **3** in toluene containing a few drops of triethylamine to produce compound **4** as a crystalline solid, 56%; mp 151–152 °C; ^{1}H NMR δ (ppm) 7.90–6.93 (8H, four doublets indicating two *para*-disubstituted benzenes); ^{13}C NMR δ (ppm) 179.91 (thiocarbonyl); FT-IR ν (cm^{-1}) 3215 (thiourea NH). HRMS (FAB MH$^+$): Calcd for $C_{16}H_{17}N_2O_3S$, 317.0960. Found: 317.0960. Calcd (%) for $C_{16}H_{16}N_2O_3S$: C, 60.74; H, 5.10. Found: C, 60.38; H, 4.97. In this manner, a variety of isothiocyanates led in a straightforward way to unsymmetrical thioureas **4–13** (table 1). In the same vein, primary acid hydrazides typically reacted with mustard oils to yield thiosemicarbazides **1b**. For example, 4-aminosalicylic acid hydrazide **14** (scheme 2) reacted under our conditions with one equivalent of **3** exclusively at the terminal position of the hydrazide function, giving thiosemicarbazide **15,** 90%; mp 191.5–192.5 °C; ^{1}H NMR δ (ppm) five H-bonded protons at 10.2 (brs, 1H), 9.7 (brs, 1H), 9.5 (brs, 1H), 5.8 (brs, 2H); FT-IR ν (cm^{-1}) 1247 (thiocarbonyl). Calculated (%) for $C_{15}H_{16}N_4O_3S$: C, 54.28; H, 4.85. Found: C, 53.90; H, 4.93. Our results on the preparation of the unsymmetrical thiosemicarbazides **15–23** included in this study are summarised in table 2.

Notwithstanding the reliability and robust nature of these procedures, we did occasionally isolate in fair to good yields materials that resulted from a more complex reaction. Rather than the typical nucleophilic addition, amine precursors of lower reactivity gave unexpected exchanges. For example, from the reaction of *para*-aminosalicylic acid (PAS) with phenyl isothiocyanate under conditions similar to those just described, we obtained *sym*-diphenyl thiourea (**1a**, $R_1 = R_2 = C_6H_5$, 94%). PAS is known to suffer decarboxylation at comparatively low temperatures to give *meta*-aminophenol (scheme 3) [26]. In examining the behaviour of the latter compound itself, independent treatment of pure *meta*-aminophenol with phenyl



2 **3** **4**

SCHEME 1



14 **3** **15**

SCHEME 2

isothiocyanate under our conditions once again produced *sym*-diphenyl thiourea (30%). The identity of *sym*-diphenyl thiourea as the readily isolable product of both of these reactions was confirmed by comparison with an authentic specimen prepared from aniline and phenyl isothiocyanate, according to a known method [27]. We propose (scheme 4) that the symmetrical thiourea may result from thermal fragmentation of the initial unsymmetrical thioureidation product (scheme 4, equation (1); $R_1 = 3\text{-}HOC_6H_4$, $R_2 = C_6H_4$) under our reaction conditions, followed by nucleophilic attack by the liberated amine (scheme 4, equation (2)). The resultant symmetrical thiourea is highly crystalline and relatively insoluble in the reaction medium, which may lead to its isolation through a LeChatelier effect. Although surprising that our conditions were sufficient to induce this behaviour, thermal decompositions of arylthioureas have been previously noted, albeit under more vigorous reaction conditions than ours, such as refluxing in chlorobenzene for 8–10 hours [28–30], or refluxing in carboxylic acids or anhydrides [25, 31, 32]. We further observed clear evidence for thermal fragmentation of thioureas in a gas chromatograph in tandem with a mass spectrometer. For example, when *sym*-di-4-isoamyloxyphenyl thiourea (**1a**, $R_1 = R_2 = 4\text{-}(CH_3)_2CHCH_2CH_2OC_6H_4$) was introduced

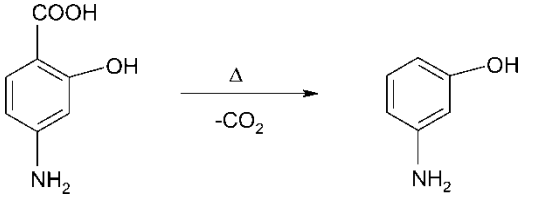
Table 1. Unsymmetrical thioureas $R_1NHCSNHR_2$.

Entry	Compound	R_1	R_2	% Yield	mp °C	ν^{max} N-H, cm^{-1}
1.	**4**	$4\text{-}CH_3OC_6H_4$	$4\text{-}CO_2CH_3C_6H_4$	56	151–152	3215
2.	**5**	$3\text{-}NO_2C_6H_4$	$4\text{-}CO_2CH_3C_6H_4$	57	140–143	3179
3.	**6**	$3\text{-}HO\text{-}4\text{-}CO_2PhC_6H_3$	$CH_2CH_2C_6H_5$	44	150	3256
4.	**7**	$3\text{-}HO\text{-}4\text{-}CO_2CH_3C_6H_3$	$CH_3C_6H_4$	39	156–157	3211
5.	**8**	$4\text{-}NO_2C_6H_4CO$	$3\text{-}HO\text{-}4\text{-}CO_2CH_3C_6H_3$	79	200	3284
6.	**9**	$3\text{-}ClC_6H_4CO$	$3\text{-}HO\text{-}4\text{-}CO_2CH_3C_6H_3$	81	192	3362
7.	**10**	$4\text{-}ClC_6H_4CO$	$3\text{-}HO\text{-}4\text{-}CO_2CH_3C_6H_3$	75	199–200	3284
8.	**11**	1-*cyclo*-$C_5H_{10}N$	$3\text{-}F\text{-}C_6H_4$	98	148	3274
9.	**12**	1-*cyclo*-$C_5H_{10}N$	$4\text{-}CH_3CH_2C_6H_4$	63	110	3293
10.	**13**	$4\text{-}CH_3N(CH_2)_4N(CH_2)_3$	C_6H_5	60	130–132	3145

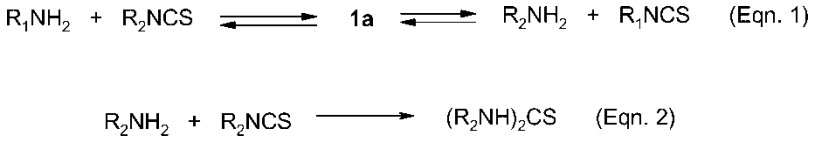
Table 2. Unsymmetrical thiosemicarbazides $R_1NHNHCSNHR_2$.

Entry	Compound	R_1	R_2	% Yield	mp °C	ν^{max} N−H, cm^{-1}
1.	**15**	4-NH_2-2-HOC_6H_3CO	4-$CH_3OC_6H_4$	90	191.5–192.5	3219
2.	**16**	4-BrC_6H_4CO	4-$CH_3OC_6H_4$	91	179–181	3224
3.	**17**	4-C_5H_4NCO	3-FC_6H_4	91	176	3263
4.	**18**	4-C_5H_4NCO	4-FC_6H_4	89	192–193	3141
5.	**19**	4-C_5H_4NCO	$CO_2CH_2CH_3$	93	202	3211
6.	**20**	4-C_5H_4NCO	4-IC_6H_4	86	176	3100
7.	**21**	4-C_5H_4NCO	2,3-$Cl_2C_6H_3$	88	200	3286
8.	**22**	4-C_5H_4NCO	3-IC_6H_4	89	194	3277
9.	**23**	2-C_4H_3SCO	$CH_2CH_2C_6H_5$	70	186	3185

into the heated injector port (250 °C) of such an instrument, two products were formed in equal amounts: isoamyloxyaniline (M^+ m/z 179, base peak m/z 109) and isoamyloxyphenyl isothiocyanate (M^+ m/z 221, base peak m/z 151).



SCHEME 3



SCHEME 4

In a related example of thiocarbonyl fragmentation, thiosemicarbazide **15**, when treated with an excess of 4-methoxyphenyl isothiocyanate (3 equivalents) under deliberately forcing conditions, gave *sym*-di-(4-methoxy)phenyl thiourea (**1a**, $R_1 = R_2 =$ 4-$CH_3OC_6H_4$, 63%, M^+ m/z 288). The identity of the latter compound was confirmed by comparison with an authentic sample prepared independently by a known route from *para*-anisidine and 4-methoxyphenyl isothiocyanate [27].

We found that strongly nucleophilic nitrogenous bases generally tended to react rapidly and irreversibly with mustard oils under mild conditions [25] to give unsymmetrical thiocarbonyls. The reaction of N-aminopiperidine with 3-fluorophenyl isothiocyanate provides such an example, giving only the stable thioureidation product **11** (98%) within several minutes of mixing the reactants. Compound **11** showed no tendency toward thermal fragmentation and exchange of the mustard oil moiety under our reaction conditions, a stability that appears to be typical of these strongly nucleophilic bases. Uncomplicated nucleophilic additions were also obtained with primary aliphatic amines, as in the formation of **13** (60%).

With regard to biological appraisal, the preliminary *in vitro* assessment of compound **6** is typical. The material was investigated in several strains of *Mycobacterium tuberculosis* and appears to have similar levels of activity in both drug-susceptible and drug-resistant organisms.

The selection criterion for the antimicrobial assay is a minimum inhibitory concentration value (MIC) of 6.25 μg/mL or less. Thus in strain $H_{37}Rv$, a fully drug-susceptible strain, the MIC was 3.13 μg/mL. In a panel of strains resistant to the specific drugs indicated, the following MIC data were obtained: rifampicin-resistant 3.13 μg/mL, thiacetazone-resistant 3.13 μg/mL, ethionamide-resistant 3.13 μg/mL, isoniazid-resistant 6.25 μg/mL, PAS-resistant 6.25 μg/mL. Thiacetazone and ethionamide are both thiocarbonyl compounds; the MIC data thus imply that compound **6** did not show extensive cross-resistance with the other thiocarbonyl drugs in this assay. Moreover, significant activity was maintained against the PAS-resistant strain, indicating that **6** may operate against the microbe by modes of action different from those of PAS. These data suggest that thiocarbonyl compounds from this cohort should be further investigated for their antitubercular properties. A full account of the biological activities of these new compounds will be the subject of a separate report in due course.

In summary, we have found that unsymmetrical thioureas and thiosemicarbazides, useful for the investigation of their antitubercular properties, may be prepared by the reactions of mustard oils with aromatic amines and acid hydrazides, respectively. In some cases, isolation of the symmetrical thiourea counterparts may occur by thermal fragmentation of the first-formed thioureidation products, a subtle process that appears to be quite sensitive to both substrate structure and reaction conditions.

3. Experimental

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, unless otherwise noted in individual procedures. Infrared (FT-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 and reagents were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin, USA) and Lancaster Synthesis Incorporated (Windham, New Hampshire, USA) and were used as received. 4-Aminosalicylic acid hydrazide was purchased from Apin Chemicals Limited Abingdon, Oxfordshire, UK, or prepared according to the method given below. 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 in dimethyl sulphoxide-d_6, recorded at 300 (^{1}H NMR) or 75 megahertz (^{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. High resolution mass spectra (HRMS, fast atom bombardment method) and low resolution mass spectra were determined at the NIH Mass Spectrometry Facility at Michigan State University, East Lansing, Michigan, USA, unless otherwise noted. *Safety Notes*: Gloves were worn during the chemical synthesis, 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 sulphur was done with due caution. No specific safety problems were encountered with the methods given below. No attempt was made to optimise yields. For biological evaluations, complete details of the methods used have been previously described [33, 34]. In brief, isonicotinic acid hydrazide (INH) was used as a positive control and was purchased from Sigma Chemical Company (St. Louis, Missouri, USA). For assessment, the test compound was dissolved in dimethyl sulphoxide and subsequently diluted in

distilled water. INH was dissolved in distilled water. Stock solutions were filter-sterilised by passage through a 0.22 μm-pore-size membrane filter and stored at −20 °C until use. *M. tuberculosis* ATCC 35801 (strain Erdman) was obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). With respect to testing against this isolate, 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, Michigan, USA) and 0.05% Tween 80, using a broth dilution method. The size of the inoculum was determined by titration and counting from triplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Maryland, USA). In some cases, results *in vitro* were also determined according to the protocols of the Tuberculosis Antimicrobial Acquisition and Co-ordinating Facility, which have been described in full [35].

3.1 *1-(4-Methoxy)phenyl-3-(4-methoxycarbonyl)phenyl thiourea 4*

Methyl 4-aminobenzoate (1.51 g, 10.0 mmoles) was warmed in 25 mL of toluene. To this warm rapidly stirring homogeneous mixture was added dropwise a solution of 4-methoxyphenyl isothiocyanate (1.65 g, 10.0 mmoles) dissolved in toluene (5 mL). The isothiocyanate was washed in with further toluene (5 mL). A solution of triethylamine (6 drops) in toluene (5 mL) was then added. The warm mixture was brought to reflux, and boiling was continued for 4 hours. Heating was stopped and the mixture was allowed to stand over night, depositing 0.50 g of the title compound after filtration. Toluene was distilled from the mother liquor to approximately half volume to give a second crop (0.71 g) for a combined yield of 1.21 g (56%). The first crop was washed lavishly with ether (3 × 10 mL) to obtain the analytical sample, mp 151–152 °C; ^{1}H NMR δ (ppm): 10.31–9.40 (4 broad singlets, 2H), 7.90 (m, 2H), 7.71 (m, 2H), 7.32 (m, 2H), 6.93 (m, 2H), 3.81–3.72 (2 overlapping singlets, 6H); ^{13}C NMR δ (ppm): 179.91, 166.16, 156.86, 144.24, 132.20, 130.05, 126.44, 125.15, 124.60, 114.11, 55.58, 52.26; FT-IR ν (cm^{-1}): 3215 (m), 3003 (m), 2960 (m), 2838 (m), 1717 (s), 1608 (m), 1534 (s), 1507 (s), 1465 (s), 1434 (s), 1417 (m), 1332 (s), 1276 (s), 1225 (vs), 1181 (s), 1166 (s), 1101 (s), 1031 (s), 969 (m), 925 (m), 834 (s), 820 (sh, s), 787 (m), 761 (m), 724 (s), 711 (s). HRMS (FAB MH^{+}): Calcd for $C_{16}H_{17}N_2O_3S$, 317.0960. Found: 317.0960. Calcd (%) for $C_{16}H_{16}N_2O_3S$: C, 60.74; H, 5.10. Found: C, 60.38; H, 4.97.

3.2 *1-(3-Nitro)phenyl-3-(4-methoxycarbonyl)phenyl thiourea 5*

Methyl 4-aminobenzoate (1.51 g, 10.0 mmoles) was warmed in 40 mL of toluene. To this warm rapidly stirring homogeneous mixture was added a solution of 3-nitrophenyl isothiocyanate (1.80 g, 10.0 mmoles) dissolved in toluene (13 mL) containing 5 drops of triethylamine. The mixture was brought to reflux for several minutes, then allowed to cool for 24 hours, producing pale yellow rosettes (1.27 g). Toluene was distilled from the mother liquor to approximately half volume to give a second crop of yellow rosettes (0.62 g), for a combined yield of 1.89 g (57%) of the title compound. The first crop was washed lavishly with ether (3 × 10 mL) to obtain the analytical sample, mp 140–143 °C; ^{1}H NMR δ (ppm): 10.41 (m, 2H), 8.53 (m, 1H), 7.92 (m, 4H), 7.65 (m, 3H), 3.84 (s, 3H); ^{13}C NMR δ (ppm): 180.49, 179.97, 147.90, 144.03, 141.00, 130.15, 129.96, 125.31, 122.34, 119.26, 118.21, 52.33; FT-IR ν (cm^{-1}) 3179 (m), 3088 (m), 3026 (m), 2956 (m), 1711 (s), 1591 (m), 1527 (s), 1484 (m), 1435 (s), 1414 (m), 1346 (s), 1325 (s), 1293 (s), 1255 (s), 1190 (s), 1174 (s), 1109 (s), 1015 (m), 963 (m), 921 (w), 904 (w), 879 (w), 850 (s), 827 (m), 800 (m), 769 (s), 739 (s). HRMS (FAB MH^{+}):

Calcd for $C_{15}H_{15}N_3O_4S$, 332.0705. Found: 332.0706. Calcd (%) for $C_{15}H_{14}N_3O_4S$: C, 54.37; H, 3.95. Found: C, 54.02; H, 3.94.

3.3 *1-(3-Hydroxy-4-phenoxycarbonyl)phenyl-3-(2-phenyl)ethyl thiourea 6*

Phenyl 4-aminosalicylate (2.29 g, 10.0 mmoles) was mixed with ethanol (10 mL) and brought to reflux, giving a clear water-white solution. β-Phenethyl isothiocyanate (1.63 g, 10 mmoles) was dissolved in ethanol (10 mL) and added dropwise to the ester solution at such a rate that refluxing was maintained. The mixture was refluxed for 1.5 hours and allowed to cool to room temperature, slowly depositing several crops of the title material (1.72 g, 44%). The analytical sample was obtained by washing the first crop with ether (3 × 4 mL) and drying on a porous clay plate, mp 150 °C; ^{1}H NMR δ (ppm) 10.39 (brs, 1H), 9.98 (brs, 1H), 8.19 (brs, 1H), 7.90 (d, J = 8 cps, 1H), 7.54–7.20 (m, 11H), 7.01 (d, J = 8 cps, 1H), 3.74 (*ab* m, 2H), 2.92 (t, J = 6 cps, 2H); ^{13}C NMR δ (ppm) 180.17, 167.08, 161.66, 150.47, 147.13, 139.48, 131.54, 129.93, 129.01, 128.80, 126.60, 126.46, 122.35, 112.60, 107.89, 107.20, 45.61, 34.42; FT-IR ν (cm^{-1}) 3256 (m), 3060 (m), 1670 (s), 1627 (sh, m), 1611 (m), 1533 (s), 1494 (m), 1467 (m), 1454 (m), 1402 (m), 1340 (s), 1309 (m), 1280 (s), 1254 (vs), 1185 (vs), 1153 (vs), 1096 (s), 1063 (s), 999 (m), 979 (m), 922 (m), 860 (m), 816 (m), 788 (m), 772 (s), 747 (vs), 709 (sh, s); HRMS (FAB MH$^+$): Calcd for $C_{22}H_{21}N_2O_3S$, 393.1273. Found, 393.1275. Calcd (%) for $C_{22}H_{20}N_2O_3S$: C, 67.32; H, 5.13. Found: C, 67.23; H, 5.19.

3.4 *1-(3-Hydroxy-4-methoxycarbonyl)phenyl-3-(4-methyl)phenyl thiourea 7*

To methyl 4-aminosalicylate (1.67 g, 10.0 mmoles) refluxing in a mixture of toluene (30 mL) and methanol (8 mL) was added 4-tolyl isothiocyanate (1.64 g, 11 mmoles) in toluene (5 mL). The mustard oil was rinsed in with a further 5 mL of toluene. The mixture was allowed to reflux for three hours, heating was stopped and the mixture permitted to stand over night. The solution was concentrated to half volume, producing a solid, which was hot filtered (0.308 g). Upon standing the mother liquor slowly produced several further crops of the thiourea (total yield 1.23 g, 39%). The analytical sample was prepared by washing the material of the first crop with ether (3 × 15 mL), mp 156–157 °C; ^{1}H NMR δ (ppm) 10.60 (brs, 1H), 10.00 (brs, 2H), 7.71 (d, J = 8 cps, 1H), 7.40 (brs, 1H), 7.35 (d, J = 8 cps, 2H), 7.15 (d, J = 8 cps, 2H), 7.07 (d, J = 8 cps, 1H), 3.89 (s, 3H), 2.30 (s, 3H); ^{13}C NMR δ (ppm) 179.05, 169.01, 160.74, 146.37, 136.43, 134.10, 130.24, 128.96, 123.83, 112.90, 108.44, 107.50, 52.22, 20.50; FT-IR ν (cm^{-1}) 3335 (m), 3211 (m), 1668 (m), 1623 (m), 1600 (s), 1547 (vs), 1508 (m), 1494 (m), 1438 (s), 1407 (m), 1349 (s), 1282 (m), 1251 (vs), 1196 (m), 1167 (vs), 1100 (s), 1019 (m), 994 (m), 960 (m), 866 (m), 820 (sh, m), 812 (m), 775 (vs), 726 (m), 691 (s). HRMS (FAB MH$^+$): Calcd for $C_{16}H_{17}N_2O_3S$, 317.0960. Found, 317.0958. Calcd (%) for $C_{16}H_{16}N_2O_3S$: C, 60.74; H, 5.10. Found: C, 60.69; H, 5.30.

3.5 *1-(4-Nitro)benzoyl-3-(3-hydroxy-4-methoxycarbonyl)phenyl thiourea 8*

Methyl 4-aminosalicylate (1.67 g, 10 mmoles) was brought to reflux in toluene (20 mL). To this was added 4-nitrobenzoyl isothiocyanate (2.08 g, 10 mmoles) as a slurry in toluene (18 mL), occasioning a vigorous reaction. The mixture was refluxed for 3.5 hours, by which time it contained a voluminous solid, and filtered while still warm to the touch. The solid was transferred to a porous clay plate and allowed to dry to give the title compound (2.97 g, 79%), mp 200 °C; ^{1}H NMR δ (ppm) 10.65 (brs, 1H), 8.34 (d, 2H, J = 7 cps), 8.15 (d, 2H, J = 7 cps), 7.80 (d, 1H, J = 8 cps), 7.71 (s, 1H), 7.24 (d, 1H, J = 8cps), 3.90 (s, 3H); ^{13}C NMR

δ (ppm) 178.40, 168.50, 166.20, 160.33, 149.79, 143.97, 138.08, 130.58, 130.26, 123.35, 114.40, 110.60, 110.40, 52.40; FT-IR ν (cm^{-1}) 3284 (m), 3037 (m), 1667 (s), 1592 (s), 1552 (s), 1524 (vs), 1488 (s), 1433 (s), 1404 (m), 1326 (s), 1300 (s), 1255 (vs), 1210 (s), 1192 (s), 1169 (s), 1140 (s), 1114 (s), 1075 (s), 1009 (m), 997 (m), 960 (m), 913 (m), 865 (s), 856 (s), 837 (s), 827 (s), 802 (vs), 781 (vs). HRMS (FAB MH^+): Calcd for $C_{16}H_{14}N_3O_6S$, 376.0603. Found: 376.0598. Calcd (%) for $C_{16}H_{13}N_3O_6S$: C, 51.20; H, 3.49. Found: C, 51.19; H, 3.52.

3.6 *1-(3-Chloro)benzoyl-3-(3-hydroxy-4-methoxycarbonyl)phenyl thiourea 9*

To methyl 4-aminosalicylate (1.67 g, 10.0 mmoles) in toluene (20 mL) at the boil was added 3-chlorobenzoyl isothiocyanate (1.98 g, 10.0 mmoles) dissolved in 4 mL of toluene. The mixture was stirred vigorously and refluxed for four hours, at which time a voluminous solid was present. While still hot, the solid was filtered off and the mother liquor run into a crystallizing dish. Solid formed immediately from the mother liquor. This solid was also filtered off and combined with the first crop. The solid was dried to produce the title thiourea (2.97 g, 81%), and the analytical sample recrystallized from ethanol, mp 192 °C; ^{1}H NMR δ (ppm) 12.68 (brs, 1H), 11.80 (brs, 1H), 10.68 (s, 1H), 8.01 (brs), 7.90 (d, J = 8 cps, 1H), 7.80 (d, J = 8 cps, 1H), 7.75–7.69 (m, 2H), 7.58 (t, J = 8 cps, 1H), 7.22 (dd, J = 8 and 2, 1H), 3.90 (s, 3H); ^{13}C NMR δ (ppm) 178.47, 168.54, 166.72, 160.36, 143.92, 134.07, 133.07, 132.81, 130.53, 130.34, 128.45, 127.43, 114.40, 110.60, 110.33, 53.39; FT-IR ν (cm^{-1}) 3362 (m), 3018 (m), 1689 (m), 1673 (m), 1597 (m), 1524 (s), 1471 (m), 1433 (m), 1347 (m), 1320 (s), 1291 (s), 1257 (s), 1245 (s), 1201 (m), 1179 (s), 1161 (s), 1146 (vs), 1093 (s), 1079 (s), 996 (m), 960 (m), 919 (m), 901 (m), 864 (m), 855 (m), 810 (m), 771 (s), 726 (vs); HRMS (FAB MH^+): Calcd for $C_{16}H_{14}N_2O_4SCl$, 365.0363. Found: 365.0360. Calcd (%) for $C_{16}H_{13}N_2O_4SCl$: C, 52.68; H, 3.59. Found: C, 52.55; H, 3.58.

3.7 *1-(4-Chloro)benzoyl-3-(3-hydroxy-4-methoxycarbonyl)phenyl thiourea 10*

Methyl 4-aminosalicylate (1.67 g, 10.0 mmoles) was warmed to 80 °C in toluene in a round bottom flask fitted for reflux. A slurry of 4-chlorobenzoyl isothiocyanate (1.98 g, 10 mmoles) in toluene (3 mL) was added with stirring, occasioning an immediate reaction and formation of a solid. The mixture was brought to reflux, and from time to time additional portions of toluene were added to maintain stirring and uniform boiling (total additional volume of toluene 27 mL). After two hours, the mixture was filtered hot. The solid obtained was rinsed (3 × 3 mL) with hot toluene and set aside. As the mother liquor cooled, additional solid was formed. This was filtered off and washed well with ethanol. The two crops of solid were dried thoroughly (first crop, 1.59 g; second crop 1.15 g; combined yield 75%). The analytical sample was obtained by washing the first crop with ether (3 × 10 mL) and drying, mp 199–200 °C (uncorr); ^{1}H NMR δ (ppm) 12.80 (brs, 1H), 11.78 (brs, 1H), 10.65 (brs, 1H), 7.99 (d, J = 8 cps, 2H), 7.80 (d, J = 8 cps, 1H), 7.74 (brs, 1H), 7.61 (d, J = 8 cps, 2H), 7.22 (dd, J = 8 and 2, 1H), 3.90 (s, 3H); ^{13}C NMR δ (ppm) 178.55, 168.54, 167.09, 160.38, 143.96, 138.06, 130.84, 130.68, 130.50, 128.49, 114.40, 110.61, 110.30, 52.38; FT-IR ν (cm^{-1}) 3284 (m), 3024 (m), 1672 (m), 1592 (m), 1506 (s), 1484 (m), 1436 (s), 1399 (m), 1323 (vs), 1272 (m), 1251 (s), 1220 (m), 1188 (vs), 1145 (vs), 1113 (m), 1095 (vs), 1009 (m), 993 (m), 959 (m), 903 (m), 859 (vs), 843 (m), 828 (m), 777 (vs), 745 (vs), 720 (vs); HRMS (FAB MH^+): Calcd for $C_{16}H_{14}N_2O_4SCl$, 365.0363. Found, 365.0360. Calcd (%) for $C_{16}H_{13}N_2O_4SCl$: C, 52.68; H, 3.59. Found: C, 52.93; H, 3.63.

3.8 *1-Piperidino-3-(3-fluoro)phenyl thiourea 11*

1-Aminopiperidine (1.00 g, 10.0 mmoles) was dissolved in toluene (10 mL) and brought to reflux. To the boiling homogeneous solution was added dropwise 3-fluorophenyl isothiocyanate (1.53 g, 10.0 mmoles) at such a rate that reflux was maintained. The isothiocyanate was washed in with a small portion of toluene (5 mL), and the mixture was refluxed for 90 minutes, then allowed to stand and cool over night to produce the title compound as a crystalline solid that deposited from the mother liquor in two crops (98% over-all), mp 148 °C; ^{1}H NMR δ (ppm) 9.66 (s, 1H), 9.34 (s, 1H), 7.73 (m, 2H), 7.42–7.20 (m, 2H), 6.89 (m, 1H), 2.90 (m, 2H), 2.51 (m, 2H), 1,70 (m, 5H), 1.05 (m, 1H); ^{13}C NMR δ (ppm) 177.20, 163.41, 160.22, 141.26, 129.78, 120.11, 111.29, 55.59, 25.42, 23.11; FT-IR ν (cm^{-1}) 3274 (m), 3164 (m), 2932 (m), 2854 (m), 2822 (m), 1610 (m), 1591 (m), 1534 (s), 1492 (vs), 1444 (s), 1376 (m), 1296 (m), 1276 (s), 1212 (vs), 1165 (m), 1144 (vs), 1090 (s), 1073 (m), 1035 (s), 1004 (m), 989 (s), 910 (m), 881 (m), 865 (s), 843 (s), 780 (vs). HRMS (FAB MH^{+}): Calcd for $C_{12}H_{17}N_3SF$, 254.1127. Found, 254.1126. Calcd (%) for $C_{12}H_{16}N_3SF$: C, 56.87; H, 6.37. Found: C, 56.88; H, 6.47. In fully drug-susceptible *Mycobacterium tuberculosis* (strain Erdman), this compound assayed to have an MIC of 32 ug/mL and thus appears to be typical of this class of piperidine-substituted unsymmetrical thioureas.

3.9 *1-Piperidino-3-(4-ethyl)phenyl thiourea 12*

1-Aminopiperidine (1.00 g, 10.0 mmoles) was dissolved in toluene (15 mL) and brought to reflux. To the boiling homogeneous solution was added dropwise 4-ethylphenyl isothiocyanate (1.47 g, 10.0 mmoles) at such a rate that reflux was maintained. The isothiocyanate was washed in with a small portion of toluene (5 mL), and the mixture was refluxed for 90 minutes, then allowed to stand and cool over night to produce the title compound as a crystalline solid that deposited from the mother liquor in two crops (63% over-all), mp 110 °C; ^{1}H NMR δ (ppm) 9.53 (brs, 1H), 9.20 (brs, 1H), 7.50 (d, J = 8 cps, 2H), 7.18 (d, J = 8 cps, 2H), 2.92 (m, 2H), 2.56 (m from which emerges q, J = 6 cps, 4H), 1.68 (m, 5H), 1.30–1.03 (m from which emerges t, J = 6 cps, 4H); ^{13}C NMR δ (ppm) 177.51, 140.42, 135.98, 127.55, 124.88, 55.61, 28.02, 26.09, 23.15, 16.07; FT-IR ν (cm^{-1}) 3293 (m), 3175 (m), 2932 (m), 2854 (m), 2823 (m), 1533 (vs), 1514 (vs), 1487 (vs), 1464 (s), 1442 (m), 1412 (m), 1380 (m), 1322 (m), 1270 (vs), 1256 (sh, s), 1228 (m), 1205 (s), 1177 (m), 1149 (m), 1101 (m), 1087 (m), 1063 (m), 1037 (m), 1017 (m), 992 (m), 930 (m), 910 (m), 868 (m), 832 (s), 806 (s), 777 (m), 724 (s); HRMS (FAB MH^{+}): Calcd for $C_{14}H_{22}N_3S$, 264.1534. Found, 264.1534. Calcd (%) for $C_{14}H_{21}N_3S$: C, 63.84; H, 8.04. Found: C, 63.48; H, 8.24.

3.10 *1-(3-(4-Methylpiperazinyl)propyl)-3-phenyl thiourea 13*

To 1-(3-aminopropyl)-4-methylpiperazine (0.97 g, 6.35 mmoles) in 2-propanol (5 mL) within a flask fitted for reflux was added phenyl isothiocyanate (1.13 g, 8.37 mmoles, an excess), washed in with 2-propanol (20 mL). The mixture was refluxed for 2 hours. Some solvent (5.5 mL) was removed by distillation, and the solid which was produced was filtered off and dried (1.11 g, 60%). The analytical sample was obtained by washing the solid with ether (4 × 12 mL), mp 130–132 °C; ^{1}H NMR δ (ppm) 9.50 (brs, 1H), 7.75 (brs, 1H), 7.40–7.04 (m, 5H), 4.48 (brs, 2H), 2.42–2.00 (overlapping m from which emerges a sharp s at 2.10, 13H), 1.69 (quint, J = 6 cps, 2H); ^{13}C NMR δ (ppm) 180.47, 139.50, 129.06, 124.49, 123.30, 56.01, 54.92, 53.03, 46.04, 43.12, 25.84; FT-IR ν (cm^{-1}) 3145 (m), 2938 (m), 2884 (m), 2828 (m), 2801 (m), 2772 (m), 1594 (m), 1504 (vs), 1492 (vs), 1461 (s), 1443 (s), 1393 (m), 1373 (m),

1353 (m), 1303 (m), 1284 (vs), 1251 (vs), 1237 (s), 1200 (m), 1181 (vs), 1152 (vs), 1143 (vs), 1100 (s), 1066 (m), 1052 (sh, m), 1026 (m), 1014 (m), 997 (m), 938 (vs), 923 (s), 877 (m), 847 (m), 803 (m), 787 (m), 759 (s); HRMS (FAB MH^+): Calcd for $C_{15}H_{25}N_4S$, 293.1800. Found, 293.1800. Calcd (%) for $C_{15}H_{24}N_4S$: C, 61.61; H, 8.27. Found: C, 61.72; H, 8.20.

3.11 *4-Aminosalicylic acid hydrazide 14*

Methyl 4-aminosalicylate (5.00 g) was brought to reflux in 15 mL of 2-propanol, and hydrazine hydrate (64% aqueous solution, 5 mL) was added by pipette in four portions over several minutes, then washed in with 2-propanol (5 mL). After three hours of reflux, a voluminous solid was present; and 2-propanol (20 mL) was added, occasioning the precipitation of further solid. The mixture was filtered hot. The solid obtained directly from the hot filtration was recrystallized from 2-propanol (150 mL), to give the title compound (1.63 g). From the mother liquor of the hot filtration was obtained a further portion upon cooling to room temperature, also recrystallized from 2-propanol (1.28 g), for an over-all yield of 58%. This material was suitable for further reactions without modification. The following characteristics were identical to those of the authentic specimen obtained commercially: ^{1}H NMR δ (ppm) 9.58 (brs, 1H), 7.50 (d, J = 6 cps, 1H), 6.10 (d, J = 6 cps, 1H), 5.95 (s, 1H), 5.70 (brs, 2H), 4.45 (brs, 2H); ^{13}C NMR δ (ppm) 169.74, 162.47, 154.10, 128.15, 105.92, 102.16, 99.84; FT-IR ν (cm^{-1}) 3451 (m), 3357 (m), 3308 (m), 1577 (s), 1543 (s), 1493 (m), 1437 (m), 1391 (s), 1362 (s), 1330 (m), 1289 (s), 1202 (m), 1189 (m), 1149 (m), 1099 (m), 973 (m), 951 (m), 876 (m), 857 (s), 820 (s), 806 (s), 764 (m), 748 (m), 719 (m).

3.12 *1-(4-Amino-2-hydroxy)benzoyl-4-(4-methoxy)phenyl thiosemicarbazide 15 and its conversion to sym-di-(4-methoxy)phenyl thiourea*

To a well-stirred vigorously refluxing slurry of 4-aminosalicylic acid hydrazide (1.65 g, 10 mmoles) in toluene (240 mL) was added 4-methoxyphenyl isothiocyanate (1.65 g, 10 mmoles) dissolved in toluene (3 mL). The mustard oil was washed in with a further 10 mL of toluene. An abundant solid formed rapidly. Refluxing was continued for one hour. The reaction mixture was allowed to cool slowly and to stand over night. The solid was filtered and dried to give the title thiosemicarbazide (2.98 g, 90%), the purity of which was not improved by lavish washing, first with ether and then with hot 2-propanol, mp 191.5–192.5 °C; ^{1}H NMR δ (ppm) 10.2 (brs, 1H), 9.7 (brs, 1H), 9.5 (brs, 1H), 7.5 (d, J = 6 cps, 1H), 7.2 (d, J = 6 cps, 2H), 6.8 (d, J = 6 cps, 2H), 6.1 (d, J = 6 cps, 1H), 5.9 (s, 1H), 5.8 (brs, 2H), 3.7 (s, 3H); ^{13}C NMR δ (ppm) 181, 170, 163, 156, 154, 132, 129, 128, 113, 105, 102, 99, 55; FT-IR ν (cm^{-1}) 3465 (w), 3356 (m), 3302 (m), 3219 (m), 3075 (m), 1642 (m), 1629 (m), 1591 (s), 1568 (m), 1535 (s), 1510 (s), 1481 (m), 1457 (m), 1331 (m), 1247 (vs), 1203 (m), 1178 (w), 1168 (m), 1155 (m), 1118 (w), 1025 (m), 971 (w), 929 (w), 885 (w), 841 (m), 831 (m), 822 (w), 800 (w), 771 (w), 736 (w), 692 (w). Calcd (%) for $C_{15}H_{16}N_4O_3S$: C, 54.28; H, 4.85. Found: C, 53.90; H, 4.93.

The compound thus obtained (0.88 g, 0.27 mmoles) was refluxed approximately 20 hours with 4-methoxyphenyl isothiocyanate (1.33 g, 0.81 mmoles, 3 equivalents) in 15 mL of pyridine. After cooling to room temperature, the addition of cold water to bring the total volume to 55 mL produced a solid, which was filtered off. The solid was allowed to dry and then washed several times with small portions of ether to produce the title thiourea (1.18 g, 92%, M^+ m/z 288). Recrystallization from ethanol gave material identical (FT-IR, ^{1}H NMR, ^{13}C NMR) with the authentic specimen produced as follows. *para*-Anisidine (1.23 g, 10 mmoles) was weighed into a 250 mL round bottom flask and brought to reflux with absolute ethanol

(25 mL). With rapid stirring 4-methoxyphenyl isothiocyanate (1.81 g, 11 mmoles) was added dropwise over several minutes, then washed in with ethanol (10 mL). A voluminous solid began to form in less than 5 minutes. After 30 minutes of reflux, the solid within the flask was filtered off, while the mixture was still hot. The solid thus obtained was washed with alcohol and allowed to dry on the filter over night to give *sym*-di-(4-methoxy)phenyl thiourea (2.16 g, 75%), mp 188 °C, lit [36] mp 185–186 °C; ^{1}H NMR δ (ppm) 9.45 (s, 2H), 7.34 (d, J = 6 cps, 4H), 6.89 (d, J = 6 cps, 4H), 3.72 (s, 6H); ^{13}C NMR δ (ppm) 180.12, 156.46, 132.18, 126.09, 113.58, 55.17; FT-IR ν (cm^{-1}) 3216 (m), 3006 (m), 2960 (m), 2840 (m), 1609 (m), 1533 (s), 1502 (s), 1466 (s), 1454 (s), 1441 (s), 1417 (m), 1334 (s), 1282 (s), 1232 (s), 1181 (s), 1161 (s), 1099 (s), 1029 (s), 949 (m), 924 (m), 835 (s), 818 (m), 786 (m), 757 (m), 723 (s).

3.13 *1-(4-Bromobenzoyl-4-(4-methoxyphenyl) thiosemicarbazide 16*

To a warm well-stirred homogeneous solution of 4-bromobenzoic acid hydrazide (2.15 g, 10.0 mmoles) in toluene (65 mL) was added in several portions 4-methoxyphenyl isothiocyanate (1.65 g, 10.0 mmoles), and the mustard oil was rinsed in with further toluene (3 mL). Some white solid began to form. The mixture was brought to reflux, and refluxing was continued for 35 minutes. The mixture was allowed to cool to room temperature, and the very fine white solid filtered off to give the thiosemicarbazide (3.46 g, 91%). The compound was washed with ether (3 × 10 mL) and dried to give the analytical sample, mp 179–181 °C; ^{1}H NMR δ (ppm) 10.59 (brs, 1H), 9.69 (brs, 1H), 9.60 (brs, 1H), 7.89 (d, J = 8 cps, 2H), 7.71 (d, J = 8 cps, 2H), 7.20 (d, J = 8 cps, 2H), 6.88 (d, J = 8 cps, 2H), 3.76 (s, 3H); ^{13}C NMR δ (ppm) 181.61, 165.52, 157.11, 132.34, 132.08, 131.60, 130.35, 127.88, 125.96, 113.55, 55.53; FT-IR ν (cm^{-1}) 3289 (m), 3224 (m), 3088 (m), 3004 (m), 2930 (m), 2834 (m), 1641 (m), 1597 (m), 1588 (m), 1567 (m), 1547 (s), 1507 (s), 1489 (sh, m), 1449 (vs), 1421 (sh, vs), 1363 (s), 1310 (s), 1295 (vs), 1259 (m), 1233 (vs), 1177 (vs), 1168 (sh, vs), 1107 (s), 1069 (s), 1034 (s), 1006 (vs), 953 (m), 934 (m), 911 (s), 840 (s), 825 (vs), 782 (vs), 734 (vs). HRMS (FAB MH$^+$): Calcd for $C_{15}H_{15}N_3O_2SBr$, 380.0068. Found, 380.0069. Calcd (%) for $C_{15}H_{14}N_3O_2SBr$: C, 47.38; H, 3.71. Found: C, 47.37; H, 3.78.

3.14 *sym-Di-4-isoamyloxyphenyl thiourea and its thermal fragmentation*

Because this thiourea, also known as isoxyl or thiocarlide, is of considerable interest to the tuberculosis research community as both a medication and reference standard, we are providing its preparation from 4-acetamidophenol in detail, the last published reports having been made some time ago without spectrometric characterisation. Intermediate compounds were sufficiently clean to permit carrying through to the thiourea without purification. *Isoamylation*. 4-Acetamidophenol (7.25 g, 48.0 mmoles) was dissolved in absolute ethanol (32 mL) in a 100 mL round bottom flask fitted for reflux. At room temperature, the addition of potassium hydroxide (2.69 g, 48.0 mmoles) gave rise to a deep purple inhomogeneous mixture. When brought to reflux, the mixture clarified but remained deep purple in colour. Isoamyl iodide (10.0 g, 50.5 mmoles) in ethanol (10 mL) was added in several portions at such a rate that reflux was maintained. The solution was refluxed for 12 hours, concentrated to half volume, water (15 mL) added and the resulting solid filtered and dried to give 6.71 g (63%) of 4-isoamyloxyacetanilide, ^{1}H NMR δ (ppm) 9.79 (brs, 1H), 7.46 (d, J = 9 cps, 2H), 6.86 (d, J = 9 cps, 2H), 3.93 (t, J = 6 cps, 2H), 2.01 (s, 3H), 1.76 (nonet, J = 6 cps, 1H), 1.58 (q, J = 6 cps, 2H), 0.92 (d, J = 6 cps, 2H); ^{13}C-NMR δ (ppm) 168.06, 154.76, 132.73, 120.83, 115.29, 66.23, 37.83, 24.89, 24.11, 22.75; FT-IR ν (cm^{-1}) 3277 (s), 2956 (m), 2929 (m), 2870 (m), 1657 (sh, s), 1645 (vs), 1611 (m), 1556 (s), 1506 (vs), 1475 (s), 1451 (sh, m), 1410 (m), 1389 (m),

1372 (m), 1302 (m), 1266 (s), 1239 (vs), 1170 (s), 1144 (m), 1114 (m), 1060 (m), 1041 (m), 1020 (m), 982 (s), 911 (m), 874 (m). *Hydrolysis*. The 4-isoamyloxyacetanilide (6.71 g obtained above) was refluxed for 2.5 hours with a solution of concentrated sulfuric acid (3.3 mL) in water (24.3 mL). The mixture was cooled to room temperature and made alkaline with sodium hydroxide, then extracted with ether (3 × 30 mL). The ether extract was dried over anhydrous magnesium sulphate, filtered, evaporated and dried to give crude 4-isoamyloxyaniline (4.48 g, 82%). The entire portion of the crude aniline was not further worked up but was used directly to form the thiourea. *Thioureidation*. The aniline was taken up in ethanol (15 mL). Potassium ethoxy xanthate (0.22 g) was added, followed by carbon disulphide (1.8 mL). *Caution: Carbon disulphide is toxic and must be used in the hood. Eye protection and impermeable gloves are required*. The reaction mixture was stirred for 6 hours, cooled to induce crystallization and facilitate work-up, the product filtered, washed with ethanol and recrystallized from ethanol to give *sym*-di-4-isoamyloxyphenyl thiourea (isoxyl, thiocarlide, 2.73 g, 55%), mp 141.5 °C, lit [37] mp 143–145 °C, lit [10] mp 148–149 °C (mp dependent on heating rate); ^{1}H NMR δ (ppm) 9.42 (brs, 1H), 7.28 (d, J = 8 cps, 2H), 6.88 (d, J = 8 cps, 2H), 3.96 (t, J = 6 cps, 2H), 1.78 (nonet, J = 6 cps, 1H), 1.59 (q, J = 6 cps, 2H), 0.92 (d, J = 6 cps, 6H);); FT-IR ν (cm^{-1}) 3270 (m), 3060 (m), 29.54 (m), 2929 (m), 2867 (m), 1614 (m), 1595 (m), 1542 (s), 1507 (vs), 1464 (m), 1425 (m), 1414 (sh, m), 1385 (m), 1341 (s), 1298 (sh, m), 1234 (vs), 1168 (s), 1113 (m), 1060 (s), 1030 (m), 1008 (m), 983 (s), 935 (m), 920 (m), 873 (m), 825 (vs), 804 (m), 720 (s). Thermal fragmentation. A dilute solution of the thiourea in methylene chloride was injected into a Hewlett Packard 5890 Series II Plus gas chromatograph/mass spectrometer. The chromatograph contained a 30.00 m × 0.25 mm glass capillary column. The injector port was maintained at 250 °C, and the column was temperature programmed to rise from 125 °C to 325 °C at a rate of 30 °C per minute. The retention times for the 4-isoamyloxyaniline (47 area %, M^+ m/z 179, base peak m/z 109) and the 4-isoamyloxylphenyl isothiocyanate (53 area %, M^+ m/z 221, base peak m/z 151) were 4.9 and 6.1 minutes, respectively. No other products were observed.

3.15 *1-(Isonicotinoyl)-4-(3-fluoro)phenyl thiosemicarbazide 17*

Isonicotinic acid hydrazide (0.82 g, 6.0 mmoles) was weighed into a 100 mL round bottom flask fitted for reflux and efficient stirring, mixed with absolute ethanol (20 mL) and brought to the boil, producing a clear colourless solution. To the solution at reflux was added dropwise 3-fluorophenyl isothiocyanate (0.92 g, 6.0 mmoles) dissolved in ethanol (5 mL), washed in with a further portion of ethanol (3 mL). The homogeneous reaction mixture was refluxed for 70 minutes, half of the solvent distilled off and the mixture cooled, depositing in two crops the title compound as a crystalline solid (1.59 g, 91%), mp 176 °C; ^{1}H NMR δ (ppm) 10.96 (brs, 1H), 9.99 (brs, 2H), 8.80 (d, J = 8 cps, 2H), 7.88 (d, J = 8 cps, 2H), 7.50–7.23 (m, 3H), 6.96 (m, 1H); ^{13}C NMR δ (ppm) 181.74, 165.39, 163.94, 160.75, 151.11, 141.81, 140.35, 130.43, 122.54, 113.32; FT-IR ν (cm^{-1}) 3263 (m), 3101 (m), 2940 (m), 1672 (m), 1599 (m), 1521 (s), 1495 (vs), 1448 (m), 1435 (s), 1405 (m), 1351 (m), 1309 (m), 1264 (vs), 1232 (vs), 1214 (vs), 1166 (m), 1137 (vs), 1060 (m), 1002 (m), 983 (m), 965 (m), 913 (m), 845 (s), 772 (m), 750 (s), 739 (vs), 721 (s); HRMS (FAB MH$^+$): Calcd for $C_{13}H_{12}N_4OSF$, 291.0716. Found, 291.0716. Calcd (%) for $C_{13}H_{11}N_4OSF$: C, 53.78; H, 3.81. Found: C, 53.62; H, 3.87.

3.16 *1-(Isonicotinoyl)-4-(4-fluoro)phenyl thiosemicarbazide 18*

Isonicotinic acid hydrazide (0.82 g, 6.0 mmoles) was weighed into a 100 mL round bottom flask fitted for reflux and efficient stirring, mixed with absolute ethanol (20 mL) and brought

to a boil, producing a clear colourless solution. To the solution at reflux was added dropwise 4-fluorophenyl isothiocyanate (0.92 g, 6.0 mmoles) dissolved in ethanol (5 mL), washed in with a further portion of ethanol (3 mL). Within several minutes, the reaction mixture became yellow and began to deposit a solid. The reaction mixture was refluxed for 65 minutes and the mixture cooled, depositing in two crops the title compound as a crystalline solid (1.55 g, 89%), mp 192–193 °C; ^{1}H NMR δ (ppm) 10.88 (brs, 1H), 9.89 (brs, 2H), 8.74 (d, J = 8 cps, 2H), 7.89 (d, J = 8 cps, 2H), 7.40 (m, 2H), 7.20 (m, 2H); ^{13}C NMR δ (ppm) 181.08, 164.84, 150.53, 139.87, 135.76, 128.49, 122.03, 115.25; FT-IR ν (cm^{-1}) 3141 (m), 1672 (m), 1505 (vs), 1411 (m), 1364 (m), 1311 (m), 1254 (m), 1217 (vs), 1149 (s), 1064 (m), 1001 (m), 829 (vs), 817 (vs), 735 (s), 688 (vs); HRMS (FAB MH^{+}): Calcd for $C_{13}H_{12}N_4OSF$, 291.0716. Found, 291.0720. Calcd (%) for $C_{13}H_{11}N_4OSF$: C, 53.78; H, 3.81. Found: C, 53.64; H, 3.89.

3.17 *1-Isonicotinoyl-4-ethoxycarbonyl thiosemicarbazide 19*

Isonicotinic acid hydrazide (1.37 g, 10.0 mmoles) was weighed into a flask fitted for reflux and efficient stirring, toluene (125 mL) added, and the mixture brought to a boil. The dropwise addition of ethoxycarbonyl isothiocyanate (1.31 g, 10.0 mmoles) was followed by refluxing for two hours, during which time a solid precipitated. The solid was filtered off and dried to give the thiosemicarbazide (2.49 g, 93%), mp 202 °C; ^{1}H NMR δ (ppm) 8.78 (d, J = 8 cps, 2H), 7.80 (d, J = 8 cps, 2H), 4.23 (q, J = 6 cps, 2H), 1.28 (t, J = 6 cps, 3H); ^{13}C NMR δ (ppm) 181.16, 163.66, 153.39, 150.71, 139.70, 121.81, 62.57, 14.47; FT-IR ν (cm^{-1}) 3211 (m), 2972 (m), 2719 (m), 1716 (s), 1653 (m), 1563 (m), 1492 (sh, s), 1478 (s), 1408 (m), 1362 (m), 1279 (m), 1223 (vs), 1189 (sh, vs), 1114 (m), 1041 (vs), 1002 (s), 971 (m), 920 (m), 894 (m), 881 (m), 844 (s), 813 (m), 765 (vs), 739 (vs), 678 (s). HRMS (FAB MH^{+}): Calcd for $C_{10}H_{13}N_4O_3S$, 269.0708. Found, 269.0707. Calcd (%) for $C_{10}H_{12}N_4O_3S$: C, 44.77; H, 4.51. Found: C, 44.96; H, 4.61.

3.18 *1-Isonicotinoyl-4-(4-iodo)phenyl thiosemicarbazide 20*

Isonicotinic acid hydrazide (1.04 g, 7.70 mmoles) was weighed into a 100 mL round bottom flask fitted for reflux and efficient stirring. Absolute ethanol (20 mL) was added and the mixture brought to the boil. 4-Iodophenyl isothiocyanate (2.00 g, 7.70 mmoles) was dissolved in ethanol (10 mL) on the steam bath, and the warm solution added in several portions to the refluxing hydrazide. A crystalline solid began to form immediately. The mixture was refluxed for 30 minutes, allowed to cool; and the product **19** was obtained in two crops (2.64 g, 86%), mp 176 °C; ^{1}H NMR δ (ppm) 10.90 (brs, 1H), 9.89 (two brs overlapping, 2H), 8.80 (d, J = 8 cps, 2H), 7.90 (d, J = 8 cps, 2H), 7.73 (d, J = 9f cps, 2H), 7.30 (m, 2H); ^{13}C NMR δ (ppm) 181.14, 164.79, 150.58, 139.81, 139.36, 137.10, 128.50, 122.01; FT-IR ν (cm^{-1}) 3100 (m), 2944 (m), 1668 (m), 1585 (m), 1512 (s), 1482 (vs), 1396 (m), 1372 (m), 1299 (m), 1252 (vs), 1212 (s), 1144 (s), 1062 (m), 1001 (m), 940 (m), 898 (m), 840 (m), 820 (s), 749 (vs), 707 (sh, m), 688 (s). HRMS (FAB MH^{+}): Calcd for $C_{13}H_{12}N_4OSI$, 398.9777. Found, 398.9778. Calcd (%) for $C_{13}H_{11}N_4OSI$: C, 39.21; H, 2.78. Found: C, 39.11; H, 2.77.

3.19 *1-Isonicotinoyl-4-(2,3-dichloro)phenyl thiosemicarbazide 21*

Isonicotinic acid hydrazide (1.37 g, 10.0 mmoles) was weighed into a 100 mL round bottom flask equipped for reflux and good stirring. Ethanol (15 mL) was added, the mixture brought to the boil and 2,3-dichlorophenyl isothiocyanate (2.04 g, 10.0 mmoles) added dropwise as the neat liquid, then washed in with ethanol (20 mL). The reaction mixture was refluxed for

90 minutes, allowed to cool and the thiosemicarbazide **21** obtained in two crops from the mother liquor (3.01 g, 88%), mp 200 °C; ^{1}H NMR δ (ppm) 10.95 (brs, 1H), 10.05 (brs, 1H), 9.87 (brs, 1H), 8.80 (d, J = 8 cps, 2H), 7.86 (br d, 2H), 7.59 (d, J = 9 cps, 1H), 7.36 (m, 3H); ^{13}C NMR δ (ppm) 182.23, 164.94, 150.53, 139.83, 139.20, 131.99, 130.91, 130, 37, 129.03, 127.98, 122.11; FT-IR ν (cm^{-1}) 3286 (m), 3119 (m), 2947 (m), 1677 (vs), 1602 (m), 1500 (s), 1476 (vs), 1454 (s), 1421 (m), 1405 (m), 1370 (m), 1306 (m), 1241 (vs), 1190 (m), 1146 (s), 1106 (m), 1059 (m), 1002 (m), 970 (m), 908 (m), 850 (m), 801 (m), 781 (m), 754 (s), 738 (s), 700 (vs). HRMS (FAB MH$^+$): Calcd for $C_{13}H_{11}N_4OSCl_2$, 341.0031. Found, 341.0001. Calcd (%) for $C_{13}H_{10}N_4OSCl_2$: C, 45.75; H, 2.95. Found: C, 45.44; H, 2.94.

3.20 *1-Isonicotinoyl-4-(3-iodo)phenyl thiosemicarbazide 22*

Isonicotinic acid hydrazide (1.05 g, 7.70 mmoles) and isopropyl alcohol (15 mL) were brought to reflux with efficient stirring in a 100 mL round bottom flask. To this was added in several portions 3-iodophenyl isothiocyanate (2.00 g, 7.70 mmoles) dissolved in hot isopropyl alcohol (3 mL). The mixture was refluxed for 15 minutes, cooled, the solid filtered, washed with ether (15 mL) and allowed to dry on a clay plate to give thiosemicarbazide **21** (2.72 g, 89%), mp 194 °C; ^{1}H NMR δ (ppm) 10.88 (brs, 1H), 9.94 (brs, 1H), 9.84 (brs, 1H), 8.80 (d, J = 8 cps, 2H), 7.88 (m from which emerges br d, 3H), 7.54 (m, 2H), 7.40 (m, 1H); FT-IR ν (cm^{-1}) 3277 (m), 3130 (m), 2943 (m), 1676 (s), 1580 (m), 1514 (s), 1466 (vs), 1420 (s), 1406 (sh, s), 1370 (s), 1307 (m), 1266 (s), 1247 (vs), 1139 (s), 1062 (m), 995 (m), 906 (m), 847 (m), 765 (m), 751 (vs). HRMS (FAB MH$^+$): Calcd for $C_{13}H_{12}N_4OSI$, 398.9777; Found, 398.9775. Calcd (%) for $C_{13}H_{11}N_4OSI$: C, 39.21; H, 2.78. Found: C, 39.17; H, 2.964.

3.21 *1-(2-Thiophenoyl)-4-(2-phenethyl) thiosemicarbazide 23*

With efficient stirring, 2-thiophenecarboxylic hydrazide (1.42 g, 10 mmoles) was brought to reflux in toluene (45 mL), giving a homogeneous mixture, to which was added dropwise 2-phenethyl isothiocyanate (1.63 g, 10 mmoles). The mustard oil was washed in with a small portion of toluene (5 mL). Within several minutes of mixing, the mixture became milky in appearance and began to deposit a white solid. After 35 minutes of refluxing, a voluminous white solid was present. The solid was filtered off and dried to yield the title compound (2.73 g, 70%), mp 186 °C; ^{1}H NMR δ (ppm) 10.39 (brs, 1H), 9.39 (brs, 1H), 8.23 (brs, 1H), 7.89 (m, 2H), 7.32–7.12 (m, 6H), 3.68 (m, 2H), 2.88 (t, J = 6 cps, 2H); ^{13}C NMR δ (ppm) 182.03, 161.36, 139.64, 137.77, 132.07, 129.77, 128.95, 128.73, 128.36, 126.44, 45.68, 35.22; FT-IR ν (cm^{-1}) 3185 (m), 2966 (m), 1652 (m), 1527 (vs), 1493 (vs), 1453 (m), 1414 (m), 1350 (m), 1256 (vs), 1233 (sh, vs), 1147 (s), 1116 (sh, m), 1079 (m), 1063 (m), 1032 (m), 924 (m), 868 (m), 848 (s), 746 (m), 711 (vs). HRMS (FAB MH$^+$): Calcd for $C_{14}H_{16}N_3OS_2$, 306.0735. Found, 306.0735. Calcd (%) for $C_{14}H_{15}N_3OS_2$: C, 55.05; H, 4.95. Found: C, 54.77; H, 4.86.

3.22 *Reaction of 4-aminosalicylic acid with phenyl isothiocyanate: Isolation of sym-diphenyl thiourea*

4-Aminosalicylic acid (1.53 g, 10 mmoles) was weighed into a 100 mL round bottom flask fitted for reflux, mixed with absolute ethanol (35 mL) and brought to the boil. Phenyl isothiocyanate (1.35 g, 10 mmoles) was added with a dropper pipette over 5 minutes and washed in with ethanol (5 mL). The mixture was allowed to reflux for 3 hours, to stand at room temperature over night, and then the resulting solid was filtered off to give 1.07 g (94%) of *sym*-diphenylthiourea, rather than its unsymmetrical counterpart. The compound

was recrystallized from ethanol and found to be identical (FT-IR, ^{1}H NMR) to an authentic sample prepared from aniline and phenyl isothiocyanate. Thus aniline (0.52 g, 5.6 mmoles) was treated with neat phenyl isothiocyanate (0.52 g, 3.6 mmoles) in a large test tube. A voluminous solid was rapidly produced in an exothermic reaction. The solid was recrystallized in the test tube from ethanol to produce *sym*-diphenylthiourea (0.66 g, 75%), mp 152 °C, lit [27] mp 150–152 °C; ^{1}H NMR δ (ppm) 9.81 (s, 2H), 7.51 (m, 2H), 7.32 (m, 2H), 7.11 (m, 1H); ^{13}C NMR δ (ppm) 179.57, 139.42, 128.42, 124.40, 123.62; FT-IR ν (cm^{-1}): 3203 (s), 3034 (m), 1598 (w), 1527 (s), 1492 (m), 1450 (s), 1343 (s), 1313 (s), 1288 (s), 1242 (s), 1070 (m), 1022 (m), 1003 (m), 933 (m), 817 (w), 765 (s), 756 (s).

3.23 *Reaction of meta-aminophenol with phenyl isothiocyanate: Isolation of sym-diphenyl thiourea*

meta-Aminophenol (1.09 g, 10 mmoles) was reacted with phenyl isothiocyanate (1.35 g, 10 mmoles) under the same conditions as noted above for 4-aminosalicylic acid, leading to the isolation of *sym*-diphenylthiourea (0.34 g, 30%), identical (mp, FT-IR, ^{1}H NMR, ^{13}C NMR) to the authentic sample prepared as described above, rather than its unsymmetrical counterpart. Since the formation of *sym*-di-(3-hydroxy)phenyl thiourea from *meta*-aminophenol and phenyl isothiocyanate for short reaction times at room temperature is a well-documented process [27], we conclude that the nature of the product formed in these reactions may be acutely sensitive to reaction conditions.

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