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Synthesis and biological activity of thiobasidalin

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Abstract – Thiobasidalin **2**, the thiolactone analogue of the antibiotic basidalin **1**, is synthesized starting from easily accessible thiotetronic acid **3** via a straightforward reaction sequence employing the chemoselective lithium aluminum hydride reduction of the acid pyrazolide **31** as the final key step. Antimicrobial tests reveal that thiobasidalin **2** as well as a number of its synthetic congeners display considerable activity against both eucaryontes and procaryontes. © Elsevier, Paris

thiobasidalin / acyl pyrazolide / chemoselective reduction / antimicrobial activity

1. Introduction

In 1983, H. Iinuma et al. [1] reported on the isolation of the lactone antibiotic basidalin **1** (*figure 1*) from a culture broth of the fungus *Leucoagaricus naucina* (Fr.) Sing. The structure of **1** was elucidated by X-ray diffraction analysis [1]. Later, basidalin was also harvested from the mycelium of another mushroom [2]. The metabolite was shown to display weak antibacterial activity against *Aeromonas salmonicida* and *Vibrio anguillarum*. In addition, basidalin dose-dependently prolonged the survival time of mice that were previously inoculated with mouse leukemia L1210 cells [1].

In view of these biological effects as well as the relatively simple chemical structure of this lactone antibiotic it is surprising that there is no literature report on the synthesis of basidalin **1**, whereas its geometric isomer, (*E*)-basidalin [3] has been prepared. Basidalin, by now accessible by fermentation [4], has been converted to its dihydro and tetrahydro derivatives [1]. Nevertheless, very little is known about structure–activity relations (SAR) concerning this basic structure. Hence we decided to synthesize the thiolactone analogue of **1** which, for brevity's sake, we name thiobasidalin **2**.

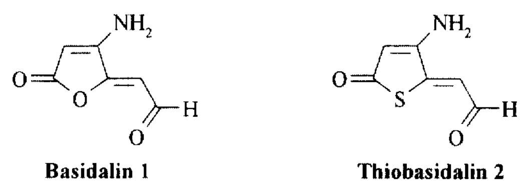

Figure 1. Structure of basidalin **1** and thiobasidalin **2**.

2. Chemistry

Based upon earlier studies with thiolactones [5] we first attempted to synthesize thiobasidalin by introduction of an appropriately functionalized side chain into the thiotetronic acid derivative **3** [6]. Hence thiolactone **3** was condensed in a *Knoevenagel* reaction with the monoprotected glyoxal **4** [7] to give the hydrazone **5** (*figure 2*). Enol **5** was converted with diazomethane into its ether **6** which underwent regioselective substitution with gaseous ammonia affording enamino ester **7** in an overall yield of 90%. In situ-prepared aluminum iodide [8] selectively brought about ester cleavage to furnish β-enamino acid **8**. Both thiolactones **7** and **8** display a characteristic splitting of the amino proton NMR signal indicating the presence of an intramolecular hydrogen bond to the neighbouring carbonyl group, which may explain why all attempts to decarboxylate **7** or **8** failed.

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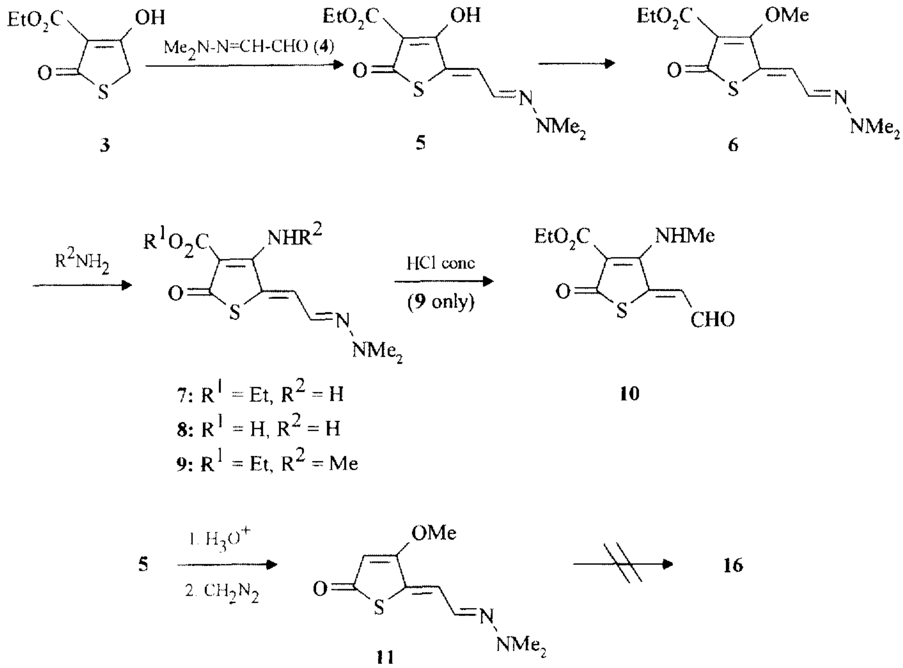

Figure 2.

Next we investigated methods to cleave the terminal dimethyl hydrazone (DMH) protective group, employing thiotetronic acid amide **9** [9] as a model compound, which in turn was easily obtained by aminolysis of enol ether **6**. Finally, exposure of **9** to concentrated hydrochloric acid gave rise to enal **10** [9] thus substantiating the DMH group as a propitious aldehyde mask.

Following this line, enol ether **11** was prepared by acidic saponification–decarboxylation of **5** and subsequent etherification. Unfortunately, here the nucleophilic displacement of the methoxy group by ammonia or primary amines failed. Therefore this route was abandoned in favor of introduction of the side chain into the fully functionalized thiolactone nucleus **15**.

The enamine **15** was prepared from thiotetronic acid **12** [6]. Since direct conversion of the hydroxyl group to the required amino function failed, enol **12** was activated for nucleophilic attack by tosylation to give the 'mixed anhydride' **13** (*figure 3*). Whereas ammonolysis of **13** led to a mixture of products, containing, among others, enol **12**, displacement with sodium azide in methanol proceeded cleanly to furnish the remarkably stable vinyl azide **14** [10]. Reduction of **14** to the desired thiotetronic acid amide **15** was accomplished by treatment with excess stannous chloride in methanol [11] in almost quantitative yield. Olefination of **15** with **4** required fairly drastic conditions, presumably due to the CH acidity weakening effect of an enaminone-like resonance stabilization. The desired hydrazone of thiobasidalin **16** could be obtained as a single stereomer albeit in moderate yield. However, in this case all efforts to obtain thiobasidalin **2** by cleavage of the hydrazone moiety, were unsuccessful.

Another derivative of thiobasidalin **2** was obtained by introduction of an unsaturated side chain via a nitrovinylation reaction [12]. Condensation of the potassium salt of thiotetronic acid amide **15** with 1-dimethylamino-2-nitroethylene [13] produced the *aci*-nitronate salt **17**. Neither exposure of **17** to strong acid (*Nef* reaction [14, 15]) nor reduction with $TiCl_3$ [16] brought about formation of the aldehyde **2**. The nitronate **17** was thereupon alkylated with methyl iodide [17] to furnish a mixture of the two isomeric nitronic acid esters **18** and **19** whose stereochemistry with respect to the C=N bond could not be clarified unambigously [18]. Upon refluxing in dioxane both nitronates cleanly underwent the expected oxido-reduction [17] to give gaseous formaldehyde and the thiobasidalin oximes as a *syn/anti*-mixture **20/21**. Attempts to liberate thiobasidalin **2**, employing a

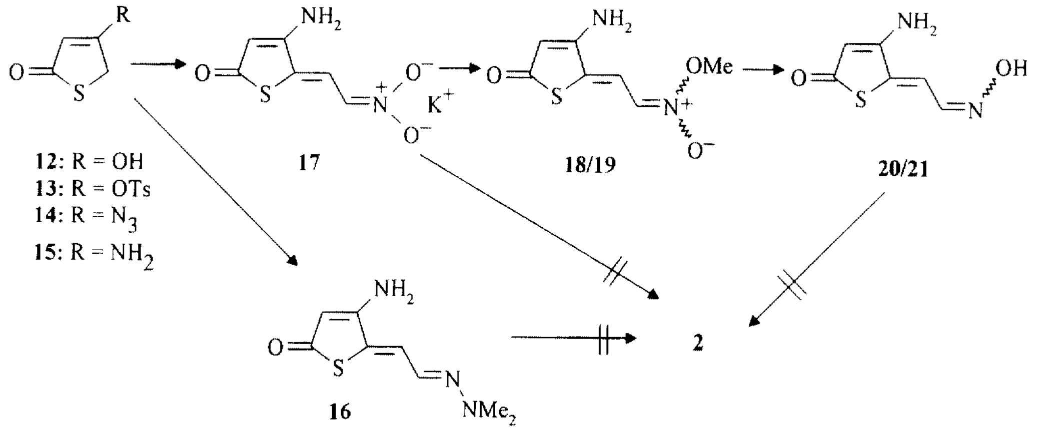

Figure 3.

series of reagents, e.g. $TiCl_3$ [19], baker's yeast [20] or formaldehyde-assisted hydrolysis [21] proceeded without success.

The experience that the removal of carbonyl protective groups such as hydrazone or oxime proved impossible in our hands led to the assumption that the aldehydic function of **2** had to be introduced in the final step of the synthesis, e.g. by reduction of the corresponding unsaturated acid **29** as outlined in *figure 4*.

Alkylation of the thiotetronic acid derivative **3** with triethyloxonium tetrafluoroborate gave rise to cyclic O,S ketene acetal **22**. The high methylene activity of **22** rendered alkylidenation with ethyl glyoxalate without any catalyst feasible to furnish diester **23** in high yield and purity. Simultaneous acidic cleavage of the ketene acetal and the ester moiety followed by decarboxylation brought about the thiotetronic acid **24**. Treatment of **24** with *p*-toluenesulfonyl chloride in the presence of triethylamine cleanly gave enol tosylate **25**, which then was converted to vinyl azide **26**. Subsequent reduction of this thiotetronic acid azide with excess stannous chloride furnished enamine **27** in excellent yield and purity.

A series of reagents known to convert α,β-unsaturated esters into either aldehydes [22] or allylic alcohols [23] were tested, but none of them were successful. Upon treatment with sodium borohydride the enamine **27** rapidly underwent saturation of the double bond even in the presence of $CeCl_3$ (Luche's procedure [24]) to give the ester **28**. Equivalent amounts of lithium aluminum hydride or aluminum hydride, reported to favor 1,2-attack [26], left ester **27** unaffected.

The same resistance to reducing agents was observed with the acid **29**, which failed to react with lithium aluminum hydride [26] as well as with thexylbromoborane [27], bis(*N*-methylpiperazino)aluminum hydride [28] or aluminum hydride [26]. We thereupon decided to convert **29** to the corresponding phenylthio ester **30** employing the diphenyl disulfide/triphenylphosphine system [29]. Unfortunately, however, thiolactone **30** underwent extensive decomposition upon treatment with deactivated Raney nickel, known to desulfurize selectively thiol esters in the presence of other sulphur-containing functional groups [30]. Two alternative reagents, namely zinc borohydride and triethylsilane, also applied in transformations of thiol esters to allylic alcohols or aldehydes [31, 32], either left **30** unchanged or led to a myriad of decolourised products, respectively.

Several attempts to synthesize the more reactive acid chloride or acyl imidazolide [33] from **29** proceeded in vain, but finally we were successful in preparing the 3,5-dimethyl pyrazolide **31** [34] directly from **29** by use of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho *p*-toluenesulfonate ('Morpho CDI', [35]) as the condensing agent in good yield.

Surprisingly, amide **31** was unstable in solution, slowly giving rise to an isomer, later shown to be the (*E*)-configured pyrazolide **32**. The isomerisation did not occur in the dark nor could it be effected radically [36] or by a nucleophilic addition–elimination sequence [37]. According to energy calculations [38] **31** is thermodynamically more stable than **32**. The driving force of its isomerisation may be the formation of a seven-membered proton chelate between one of the enamine protons and the amide carbonyl group [40, 41]. The magnetic inadequacy of the NH_2 hydrogens of **32** is clearly indicated by split ^1H NMR signals. In contrast, the NH_2 signal of **31** appears unresolved. With a UV 254 nm source the photoiso-

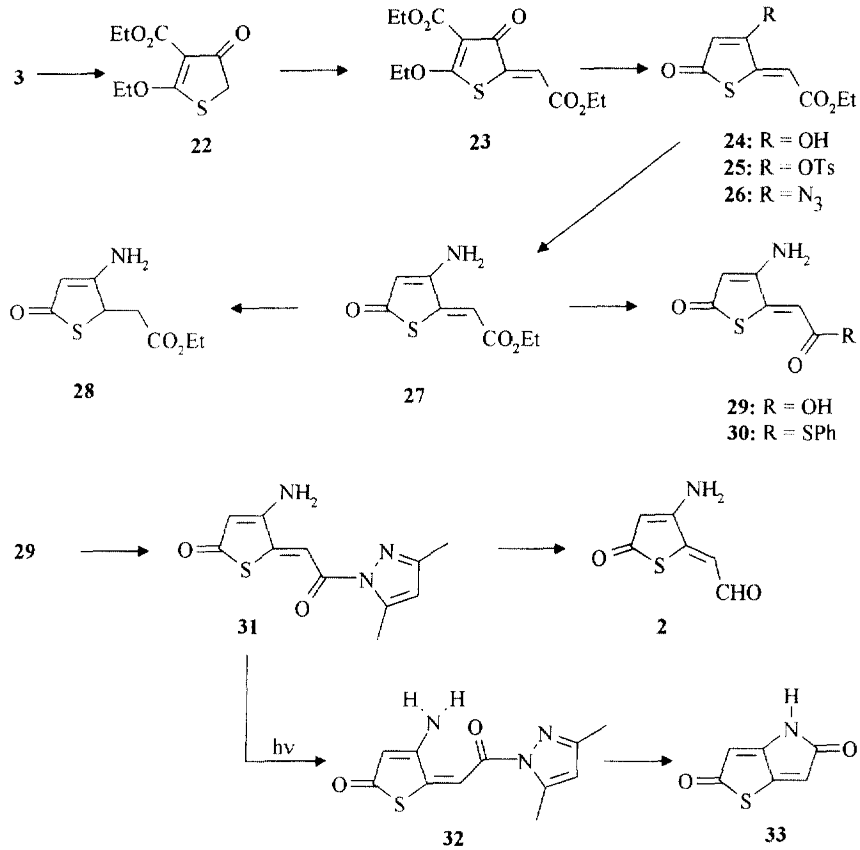

Figure 4.

merisation of both **31** or **32** solutions led to a photostationary equilibrium containing 5% of **31**.

In order to confirm the assumed configuration of **32** the compound was treated with dilute hydrochloric acid to produce the hitherto unknown thienolactam **33** [42]. Interestingly, the two vinyl proton signals in the ^{1}H NMR spectrum display a long-range coupling constant of J_5 = 1.3 Hz due to their 'W'-like fixed positioning in the now rigid hexadienedioic acid skeleton. Unfortunately, neither the pyrazolide **32** nor the lactam **33** could be reduced to give (*E*)-thiobasidalin. Even at dry ice temperature lithium aluminum hydride led to a complex mixture of products.

On the other hand, amide **31** was selectively reduced to aldehyde **2** by use of excess lithium aluminum hydride at low temperature [34]. X-ray diffraction analysis of **2** (*figure 5*) confirmed the structure as the true 1-thia heterologue of basidalin **1**.

A comparison of their crystallographic data reveals that both basidalin **1** and thiobasidalin **2** form relatively weak intermolecular hydrogen bonds by donating hydrogens (N1) to the carbonyl oxygens (O1, O2) of the neighbouring two molecules with distances of 2.074 and 2.099 Å, respectively. In addition, thiobasidalin **2** exhibits a weak intermolecular Van der Waals sulphur–sulphur interaction as indicated by the narrow

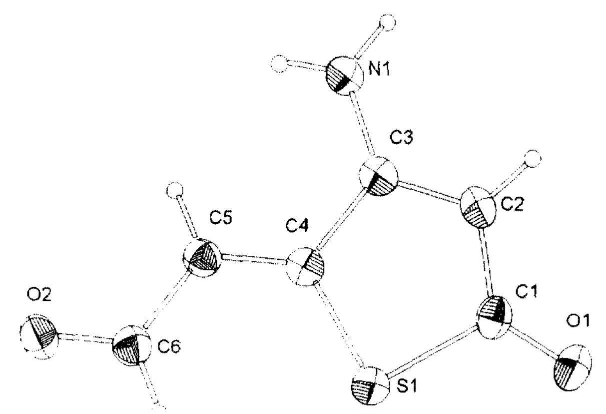

Figure 5. ORTEP-plot of thiobasidalin **2**.

S–S distance of 3.883 Å. Not surprisingly, the heavier atom skeleton of thiobasidalin **2** is planar within calculational errors. The basal plane through H(N1), H'(N1) and C3 twists out from the flat five-membered ring plane by only about 1.8° compared to 6° for basidalin **1**. The enamine nitrogen N1 of **2** completely lies in the plane formed by its two bonded hydrogens and C3, whereas basidalin **1** exhibits a shallow pyramidal configuration with the nitrogen situated 0.04(1) Å above this plane. With the exception of the markedly longer S–C bonds (1.819 and 1.746 Å, respectively) in thiobasidalin the corresponding bond lengths of **1** and **2** differ only insignificantly. Both enals display an enaminone-like resonance stabilization as evidenced by the convergence of bond lengths of the incorporated β-amino acrylate system. The corresponding NMR spectra of **1** [1] and **2**, compiled in *table I*, display a pronounced diamagnetic shift of the ^{1}H and ^{13}C NMR signals of the vinylic hydrogens of basidalin **1** in comparison to its heterologue **2**.

Thiobasidalin **2** rapidly undergoes color reactions with 2,4-dinitrophenyl hydrazine and potassium permanganate as reported for **1** [1]. Reaction of thiobasidalin with hydroxylamine hydrochloride in buffered aequous ethanol cleanly produced a mixture of the aforementioned oximes **20** and **21** in a ratio of roughly 1:4 as evidenced by HPLC and NMR analysis, thus confirming their previously assigned structures. On the other hand, condensation of **2** with *N,N*-dimethyl hydrazine in the presence of acetic acid proceeded rapidly to give the hydrazone **16** as a single isomer in high yield [43].

In summary, thiobasidalin **2** is now accessible via a nine-step reaction sequence starting from in bulk-quantities available thiotetronic acid **3** in good overall yield.

Table I. NMR resonances (solvent: DMSO-d_6, values in ppm).

	Basidalin **1**[a]	Thiobasidalin **2**
^{1}H NMR	10.03 (d, J = 8 Hz)	9.86 (d, J = 4.3 Hz)
	7.68 (s, br, 2 H, NH_2)	7.87 (s, br, 2 H, NH_2)
	6.12 (d, 1 H, J = 8 Hz)	7.09 (d, J = 4.3 Hz)
	4.97 (s, 1 H)	5.29 (s, 1 H)
^{13}C NMR	189.2 (C-7)	190.7 (*C*HO)
	168.2 (C-2)	188.0 (S*C*O)
	159.5 (C-5)	165.3 (not correlated)
	158.6 (C-4)	148.8 (not correlated)
	102.8 (C-6)	117.7 (*C*HCHO)
	81.9 (C-3)	96.1 (*C*H=CNH$_2$)

[a]Atom numbering: see [1].

3. Biological assays and results

Like basidalin **1** most of the tested thiolactones exhibit antibiotic activities towards bacteria and fungi (*table II*). The highest antimicrobial effects were observed for enal **10** and nitronate **19**.

Cytotoxic activities of the thiolactones, depicted in *table II*, towards L1210 cells (mouse) were measured as described in the experimental section. Pronounced cytotoxic effects, comparable to basidalin **1** (LD_{100} = 1 μg/mL) [2], were observed only for the two nitronates **18** and **19** (LD_{100} = 1–5 μg/mL), whereas **10** caused complete lysis of cells (LD_{100}) only at concentrations of 10–20 μg/mL.

In summary, some of the thiobasidalin derivatives display high activity against both eucaryontes and procaryontes, but unfortunately with lack of selectivity.

Table II. Antimicrobial activity of thiolactones in the agar diffusion assay. Diameter of inhibition zone (in mm).

	Thiobasidalin **2**	**8**	**10**	**18**	**19**	**27**	**31**
Bacteria							
Bacillus subtilis	–	–	10[a]	8	20[a]	–	
Bacillus brevis	–	–	19[a]	–	26	–	
Micrococcus luteus	–	–	–	–	–	–	–
Enterobacter dissolvens	–	–	–	–	–	–	–
Fungi							
Mucor miehei	15[b]	–	20	15[b]	18[b]	11[b]	9[b]
Paecilomyces varioti	11[b]	–	20	11[b]	15[b]	10[b]	9[b]
Penicillium notatum	–	–	12	–	–	–	–
Nematospora coryli	16	–	22	13	15	9	10

Diameter of paper disk: 6 cm, inoculated with compounds, 50 μg/disk. –: no inhibition zone; [a]compound, 10 μg/disk; [b]inhibition zone incomplete.

4. Experimental protocols

4.1. General methods

For the plate diffusion assays [44] fungi were grown at 27 °C (*P. notatum, N. coryli*) or 37 °C in YMG-medium containing (g/L): malt extract, 10; glucose, 4; yeast extract, 4; agar, 20. Bacteria were grown in nutrient broth (Difco) containing 2% agar at 37 °C. L1210 cells (mouse lymphocytic leukemia ATCC CCL 219) were grown in F 12 medium (Gibco) containing 20% of horse serum, 20 mM HEPES-buffer, 100 µg/mL streptomycin sulfate and 65 µg/mL penicillin G. Incubation at 37 °C in a humified atmosphere containing 5% CO. Cell growth and lysis were monitored under a microscope at 24 hour intervals for three days [45]. Melting points were determined using a Gallenkamp Melting Point apparatus and are uncorrected. Flash chromatography was performed using silica gel (230–400 mesh) from Merck. ^{1}H NMR spectra were recorded at 400 MHz using Me_4Si as internal standard on a JEOL GSX 400. Mass spectra were obtained with a Hewlett Packard 5989A Mass Spectrometer employing both EI and CI mode. Infrared spectra were measured as KBr plates for solids and neat with oils using a FT-IR-Spectrometer PARAGON 1000 (Perkin-Elmer). UV analysis was performed in methanolic solutions except where otherwise noted on Uvikon 810 Anakomp 220 (Kontron) and UV/VIS Spectrometer Lambda 20 (Perkin Elmer). HPLC analysis was made employing Merck-Hitachi L-6000A/L-4000A and LiChrospher® 100 DIOL, 10 µm (Merck). Microanalyses were carried out applying an Analysator CHN-O-Rapid from Heraeus. Photochemical reactions were performed using a Pyrex mantled 125 W high-pressure mercury vapor lamp HPK from Philips employing freshly destilled solvents which were scrupulously deoxygenated by purging with dry N_2 under ultrasonification. Toluene, dichloromethane, acetonitrile and piperidine were distilled from CaH_2, methanol and ethanol from magnesium turnings under N_2. THF, diethyl ether and dioxane were distilled from sodium benzophenone ketyl under N_2 immediately prior to use. All moisture-sensitive reactions were run with flame-dried glassware.

4.2. (Z)-Ethyl[5-(2-dimethylhydrazonoethylidene)-2,5-dihydro-4-hydroxy-2-oxo]thiophene-3-carboxylate 5

A solution of thiotetronic acid **3** [6] (15.04 g, 80 mmol), glyoxal monodimethyl hydrazone **4** [7] (8.5 g, 85 mmol) and piperidine (7.23 g, 85 mmol) was refluxed in ethanol (200 mL) for 6 h. After removal of the volatiles in vacuo the resulting residue was taken up in dichloromethane (300 mL) and washed with dilute HCl. The dried (Na_2SO_4) organic extract was evaporated to dryness and the remainder recrystallized to give **5** (11.23 g, 52%). Golden platelets, m.p. 145 °C (diisopropyl ether/ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 12.57 (s, 1 H, OH), 7.46 (d, 1 H, J = 9.0 Hz), 6.72 (d, 1 H, J = 9.0 Hz), 4.41 (q, 2 H, J = 7.3 Hz), 3.20 (s, 6 H), 1.39 (t, 3 H, J = 7.3 Hz); IR ν 3300–2900 br., 2980, 2923, 1682, 1650, 1582, 1518 cm^{-1}; λ_{max} (log ε) 218 nm (4.157), 428 (4.583); Anal. Calc. for $C_{11}H_{14}N_2O_6S$ (270.31): C, 48.88; H, 5.22; N, 10.36; S, 11.86. Found: C, 48.74; H, 5.05; N, 9.99; S, 11.80. MS: 270 [M+].

4.3. (Z)-Ethyl[5-(2-dimethylhydrazonoethylidene)-2,5-dihydro-4-methoxy-2-oxo]thiophene-3-carboxylate 6

An ethereal solution of diazomethane was added to an ice-cooled solution of **5** (10.0 g, 37 mmol) in THF (100 mL). After N_2 evolution had ceased the mixture was concentrated to dryness in vacuo to give **6** (9.78 g, 93%). Orange needles, m.p. 70 °C (diisopropyl ether). ^{1}H NMR ($CDCl_3$) δ 7.24 (d, 1 H, J = 9.0 Hz), 6.71 (d, 1 H, J = 9.0 Hz), 4.33 (q, 2 H, J = 7.3 Hz), 4.06 (s, 3 H), 3.14 (s, 6 H), 1.34 (t, 3 H, J = 7.3 Hz); IR ν 2936, 1694, 1671, 1580, 1514 cm^{-1}; λ_{max}(log ε) 214 nm (4.050), 266 (3.836), 413 (4.559); Anal. Calc. for $C_{12}H_{16}N_2O_4S$ (284.33): C, 50.69; H, 5.67; N, 9.85; S, 11.28. Found: C, 50.80; H, 5.66; N, 9.76; S, 11.26. MS: 284 [M+].

4.4. (Z)-Ethyl[4-amino-5-(2-dimethylhydrazonoethylidene)-2,5-dihydro-2-oxo]thiophene-3-carboxylate 7

Dry gaseous ammonia was bubbled through a cooled (–10 °C) solution of **6** (14.77 g, 52 mmol) in ethyl acetate/ethanol (200 mL, 1:1) until precipitation of **7** was complete. The resulting solid was filtered off and recrystallized to give **7** (13.57 g, 97%). Orange crystals, m.p. > 200 °C dec. (ethyl acetate/ethanol). ^{1}H NMR ($CDCl_3$) δ (s, br, 1 H, NH), 8.59 (s, br, 1 H, NH), 7.63 (d, 1 H, J = 9.0 Hz), 6.70 (d, 1 H, J = 9.0 Hz), 4.17 (q, 2 H, J = 7.3 Hz), 3.11 (s, 6 H), 1.22 (t, 3 H, J = 7.3 Hz); IR ν 3445, 3284, 3028, 2987, 1701, 1634, 1605, 1587, 1526 cm^{-1}; λ_{max}(log ε) 224 nm (4.171), 243 (4.254), 379 (4.478), 402 (4.540); Anal. Calc. for $C_{11}H_{15}N_3O_3S$ (269.32): C, 49.06; H, 5.61; N, 15.60; S, 11.97. Found: C, 49.08; H, 5.46; N, 15.28; S, 11.87. MS: 269 [M+].

4.5. (Z)-[4-Amino-5-(2-dimethylhydrazonoethylidene)-2,5-dihydro-2-oxo]thiophene-3-carboxylic acid 8

To a refluxing suspension of aluminum foil (2.6 g, 96 mmol) in dry acetonitrile (150 mL) under N_2 was carefully added portionwise iodine (28.0 g, 110 mmol) and the resulting mixture was refluxed for 1 h. Enamino ester **7** was added all at once and after 15 min the heterogenous mélange was allowed to reach room temperature. The volatiles were evaporated and the remainder was taken up in ethyl acetate (200 mL), washed successively with dil HCl, sat aq H_2SO_3 solution and finally brine. After drying (Na_2SO_4) the solvent was removed in vacuo to give **8** (0.88 g, 17%). Curry-coloured powder, m.p. 198 °C (ethyl acetate/acetonitrile), brown ferric chloride test. ^{1}H NMR (D6-DMSO) δ 11.88 (s, 1 H, OH), 9.09 (s, 1 H, NH), 8.66 (s, 1 H, NH), 7.75 (d, 1 H, J = 8.5 Hz), 6.73 (d, 1 H, J = 8.5 Hz), 3.14 (s, 6 H); IR ν 3500–2500, 3364, 3285, 3139, 1686, 1642, 1597, 1574, 1528, 1494 cm^{-1}; λ_{max}(log ε) 224 nm (4.115), 244 (4.192), 380 (4.337), 410 (4.483); Anal. Calc. for $C_9H_{11}N_3O_3S$ (241.27): C, 44.80; H, 4.60; N, 17.42; S, 13.29. Found: C, 44.85; H, 4.58; N, 17.36; S, 13.59. MS: 241 [M+].

4.6. (Z)-Ethyl[5-(2-dimethylhydrazonoethylidene)-2,5-dihydro-4-methylamino-2-oxo]thiophene-3-carboxylate 9

To a cooled (–10 °C) solution of **6** (284 mg, 1 mmol) in ethyl acetate/ethanol (30 mL, 1:1) was added dropwise methylamine (8.03 M in ethanol, 0.25 mL, 2 mmol). After 1 h at room temperature the volatiles were removed in vacuo to give **9** (270 mg, 95%). Deep yellow needles, m.p. 187 °C (diisopropyl ether/ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 10.04 (s, br, 1 H, NH), 7.33 (d, 1 H, J = 8.7 Hz), 6.75 (d, 1 H, J = 8.7 Hz), 4.31 (q, 2 H, J = 7.0 Hz), 3.38 (d, 3 H, J = 5.8 Hz), 3.15 (s, 6 H), 1.36 (t, 3 H, J = 7.0 Hz); IR ν 3239, 2923, 1683, 1663, 1634, 1596 cm^{-1}; λ_{max}(log ε) 206 nm (4.178), 248 (4.026), 282 (4.058), 407 (4.102); Anal. Calc. for $C_{12}H_{17}N_3O_3S$ (283.35): C, 50.87; H, 6.05; N, 14.83; S, 11.32. Found: C, 50.83; H, 6.08; N, 14.85; S, 10.94. MS: 283 [M+].

*4.7. (Z)-[4-Ethoxycarbonyl-2,5-dihydro-3-methylamino-5-oxo-2-thienylidene]acetaldehyde **10***

A mixture of **9** (142 mg, 0.5 mmol) in chloroform (20 mL) and conc HCl (0.2 mL, 2.4 mmol) was rapidly stirred at room temperature for 1 h. Thereupon the organic layer was washed thrice with water, rapidly with 2% $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated. The remainder was recrystallized to give **10** (48 mg, 40%). Yellow needles, m.p. 122 °C (diisopropyl ether/ethanol). ^{1}H NMR ($CDCl_3$) δ 10.30 (s, br, 1 H, NH), 10.04 (d, 1 H, J = 5.1 Hz), 6.93 (d, 1 H, J = 5.1 Hz), 4.34 (q, 2 H, J = 7.2 Hz), 3.44 (d, 3 H, J = 5.5 Hz), 1.37 (t, 3 H, J = 7.2 Hz); IR ν 3119, 2985, 2824, 2737, 1675, 1603, 1568 cm^{-1}; λ_{max}(log ε) 205 nm (4.077), 290 (4.168), 331 (3.371); Anal. Calc. for $C_{10}H_{11}NO_4S$ (241.27): C, 49.78; H, 4.60; N, 5.81; S, 13.29. Found: C, 49.78; H, 4.74; N, 5.81; S, 13.29. MS: 241 [M$^+$].

*4.8. (Z)-[5-(2-Dimethylhydrazonoethylidene)-2,5-dihydro-4-methoxy]-2-thiophenone **11***

A solution of **5** (4.05 g, 15 mmol) and oxalic acid (0.1 g, 1.1 mmol) in dioxan/water (100 mL, 98:2) was refluxed for 75 min under N_2 and thereafter diluted with THF (50 mL). The solution was cooled in an ice-water bath and an etheral solution of diazomethane was slowly added dropwise. Upon completion of the reaction the volatiles were removed in vacuo and the resulting residue was purified by flash chromatography to give **11** (2.35 g, 74%). R_f 0.14 (hexane/diethyl ether, 1:1). Yellow needles, m.p. 124 °C (hexane/diisopropyl ether). ^{1}H NMR ($CDCl_3$) δ 7.09 (d, 1 H, J = 9.0 Hz), 6.73 (d, 1 H, J = 9.0 Hz), 5.49 (s, 1 H), 3.89 (s, 3 H), 3.10 (s, 6 H); IR ν 2889, 1661, 1591, 1565, 1522 cm^{-1}; λ_{max}(log ε) 218 nm (3.854), 264 (3.755), 289 (3.601), 378 (4.446), 396 (4.419); Anal. Calc. for $C_9H_{12}N_2O_2S$ (212.27): C, 50.92; H, 5.70; N, 13.20; S, 15.11. Found: C, 50.91; H, 5.67; N, 13.02; S, 15.43. MS: 212 [M$^+$].

*4.9. 2,5-Dihydro-4-hydroxy-2-thiophenone **12***

A solution of **3** [6] (18.8 g, 100 mmol) in nitromethane (100 mL) and H_2O (10 mL) was refluxed for 2 h and thereupon evaporated under reduced pressure to give after recrystallization from H_2O pure **12** (9.40 g, 81%). Colourless crystals, m.p. 116 °C (Lit. m.p. 115–117 °C [6]).

*4.10. 2,5-Dihydro-4-(4-toluenesulfonyloxy)-2-thiophenone **13***

p-Toluenesulfonyl chloride (20.9 g, 110 mmol) was added to a solution of **12** (11.6 g, 100 mmol) in aqueous sodium bicarbonate [$NaHCO_3$ (12.6 g, 150 mmol) in H_2O (500 mL)] and the mixture was rapidly stirred for 24 h at room temperature. The originated solid was filtered off, washed with water and recrystallized to give **13** (18.1 g, 67%). Colourless crystals, m.p. 139–140 °C (diisopropyl ether/ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 7.86 (d, 2 H, J = 8.1 Hz), 7.43 (d, 2 H, J = 8.1 Hz), 6.13 (t, 1 H, J = 1.3 Hz), 3.96 (d, 2 H, J = 1.3 Hz), 2.50 (s, 3 H); IR ν 3098, 2974, 2930, 1693, 1671, 1618, 1593, 1492; λ_{max}(log ε) 230 nm (4.315), 260 sh (3.626); Anal. Calc. for $C_{11}H_{10}O_4S_2$ (270.33): C, 48.87; H, 3.73; S, 23.72. Found: C, 49.26; H, 3.84; S, 23.26. MS: 270 [M$^+$].

*4.11. 4-Azido-2,5-dihydro-2-thiophenone **14***

Sodium azide (3.0 g, 46 mmol) was added all at once to a stirred solution of **13** (9.18 g, 34 mmol) in THF/methanol (150 mL, 1:2) at room temperature. After 2 h the mixture was diluted with water and extracted with ethyl acetate (3 x 75 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. The remainder was purified by flash chromatography to give **14** (3.60 g, 75%). Light yellow platelets, m.p. 65 °C (diethyl ether). ^{1}H NMR ($CDCl_3$) 5.97 (s, 1 H), 3.95 (s, 2 H); IR ν 3055, 2923, 2165 sh, 2102 br, 1665, 1590 cm^{-1}; λ_{max}(log ε) 211 nm (3.521), 259 (4.115); Anal. Calc. for $C_4H_3N_3OS$ (141.15): C, 34.04; H, 2.14; N, 29.77; S, 22.72. Found: C, 33.97; H, 2.21; N, 29.77; S, 22.84. MS: 141 [M$^+$].

*4.12. 4-Amino-2,5-dihydro-2-thiophenone **15***

A solution of $SnCl_2$ (7.0 g, 37 mmol) in dry MeOH (75 mL) was slowly added dropwise to an ice-cooled solution of **14** (3.67 g, 26 mmol) in MeOH (75 mL). After N_2 evolution had ceased the mixture was evaporated in vacuo and the residue taken up in ethyl acetate, washed sequentially with diluted HCl, rapidly with diluted NaOH and finally with brine. After drying (Na_2SO_4) the solvent was removed in vacuo to give **15** (2.90 g, 97%). Yellow crystals, m.p. 197–198 °C dec. (acetonitrile). ^{1}H NMR (CD_3CN) δ 5.88 (s, br, 2 H, NH_2), 5.10 (s, 1 H), 3.92 (s, 3 H); IR ν 3366, 3191, 1663, 1617, 1567 br cm^{-1}; λ_{max}(log ε) 235 nm (4.036), 283 (4.218); Anal. Calc. for C_4H_5NOS (115.16): C, 41.72; H, 4.38; N, 12.16; S, 27.85. Found: C, 41.93; H, 4.40; N, 11.94; S, 28.00. MS: 115 [M$^+$].

*4.13. (Z)-4-Amino-5-(2-dimethylhydrazonoethylidene)-2,5-dihydro-2-thiophenone **16***

A solution of **15** (0.92 g, 8 mmol), glyoxal dimethyl hydrazone **4** [7] (1.02 g, 10 mmol) and piperidine (0.86 g, 10 mmol) in ethanol (30 mL) was refluxed for 90 min. The volatiles were thereupon evaporated in vacuo and the remaining residue was washed with hot diisopropyl ether/ethyl acetate (10 mL, 1:1). Recrystallization gave pure **16** (158 mg, 10%). Fine golden needles, m.p. 258 °C dec. (ethyl acetate/ethanol). Greenish brown ferric chloride test. ^{1}H NMR (DMSO-d_6) δ 7.35 (s, 2 H, NH_2), 7.24 (d, 1 H, J = 9.0 Hz), 6.70 (d, 1 H, J = 9.0 Hz), 5.05 (s, 1 H), 3.03 (s, 6 H); IR ν 3375, 3201, 1649, 1578, 1551, 1525 cm^{-1}; λ_{max}(log ε) 246 nm (4.035), 371 (4.475), 391 (4.373); Anal. Calc. for $C_8H_{11}N_3OS$ (197.26): C, 48.71; H, 5.62; N, 21.30; S, 16.26. Found: C, 48.77; H, 5.66; N, 21.21; S, 15.62. MS: 197 [M$^+$].

*4.14. (E/Z)-2-(3-Amino-2,5-dihydro-5-oxo-2-thienylidene)ethanenitronic acid methyl ester **18** and **19***

Method A: A hot solution of 1-dimethylamino-2-nitroethylene [13] (6.5 g, 56 mmol) in dry ethanol (50 mL) was carefully added all at once to a hot solution of **15** (6.2 g, 54 mmol) in ethanolic potassium ethoxide [potassium (2.50 g, 64 mmol) in dry ethanol (100 mL)] and the mixture refluxed for 10 min. After cooling in an ice-water bath under N_2 the originated solid **17** was removed by filtration, washed with dry diethyl ether and resuspended in dry ethanol (100 mL). After addition of methyl iodide (10 mL, 160 mmol), the mixture was refluxed for 5 h, then adsorbed onto silica gel prior to flash chromatography to give in the order of elution first **18** (0.67 g, 6.2%) and then **19** (0.34 g, 3.2%).

Method B: The potassium nitronate salt **17**, prepared as described above from **15** (1.5 g, 13 mmol), 1-dimethylamino-2-nitroethylene [13] (1.74 g, 15 mmol) in ethanol (25 mL), potassium ethoxide [from 0.62 g (16 mmol) K and ethanol (30 mL)], was dissolved in water (100 mL) and, after cooling to 0 °C, acidified with diluted HCl. The resulting yellow solution was extracted with ethyl acetate (3 x 40 mL), the organic extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was taken up in methanol (50 mL), chilled in an ice-water bath and dropwise charged with an ethereal solution of diazomethane until gas evolution has ceased. The volatiles were removed under reduced pressure and the remainder was purified by flash chromatography to give a mixture of **18** (282 mg, 11%) and **19** (240 mg, 9%).

18: Orange crystals, m.p. 173 °C dec. (acetonitrile). R_f 0.28 (chloroform/ethyl acetate, 2:1). ^1H NMR (CD_3CN) δ 7.07 (d, 1 H, J = 10.3 Hz), 6.93 (d, 1 H, J = 10.3 Hz), 5.92 (s, br, 2 H, NH_2), 5.30 (s, 1 H), 3.81 (s, 3 H); IR ν 3418, 3346, 3227, 1635, 1578, 1541 cm^{-1}; λ_{max}(log ε) 253 nm (3.905), 355 (4.473); Anal. Calc. for $C_7H_8N_2O_3S$ (200.22): C, 41.99; H, 4.03; N, 13.99; S, 16.02. Found: C, 42.02; H, 4.07; N, 13.47; S, 15.33. MS: 200 [M^+].

19: Orange crystals, m.p. > 140 °C dec. (diisopropyl ether/ethyl acetate). R_f 0.19 (chloroform/ethyl acetate, 2:1). ^1H NMR (CD_3CN) δ 7.30 (d, 1 H, J = 10.3 Hz), 6.64 (d, 1 H, J = 10.3 Hz), 6.00 (s, br, 2 H, NH_2), 5.30 (s, 1 H), 3.85 (s, 3 H); IR ν 3411, 3342, 3223, 1627, 1574 sh, 1548 cm^{-1}; λ_{max}(log ε) 253 nm (3.983), 353 (4.436); Anal. Calc. for $C_7H_8N_2O_3S$ (200.22): C, 41.99; H, 4.03; N, 13.99; S, 16.02. Found: C, 42.53; H, 4.00; N, 13.15; S, 15.22 [46]. MS: 200 [M^+].

4.15. *(E/Z)-2-(3-Amino-2,5-dihydro-5-oxo-2-thienylidene)-acetaldoxime* ***20***

A solution of **19** (0.6 g, 3 mmol) in dry dioxane (20 mL) was refluxed under N_2 for 5 h, thereupon evaporated and flash chromatographed to give **20** (270 mg, 53%). Yellow crystals, m.p. > 170 °C dec. (diisopropyl ether/ethyl acetate). R_f 0.17 (dichloromethane/ethyl acetate, 3:2). ^1H NMR (DMSO-d_6) δ 11.99 (s, 1 H, OH), 7.77 (d, 1 H, J = 9.5 Hz), 7.63 (s, br, 2 H, NH_2), 7.22 (d, 1 H, J = 9.5 Hz), 5.16 (s, 1 H); IR ν 3411, 3352, 3241, 1660, 1621, 1557 cm^{-1}; λ_{max}(log ε) 233 nm (3.963), 316 (4.407); Anal. Calc. for $C_6H_6N_2O_2S$ (170.19): C, 42.34; H, 3.55; N, 16.46; S, 18.84. Found: C, 42.44; H, 3.66; N, 15.78; S, 17.99. MS: 170 [M^+].

4.16. *(E/Z)-2-(3-Amino-2,5-dihydro-5-oxo-2-thienylidene)-acetaldoxime* ***21***

This disproportionation reaction was performed in an analogous manner to **20** from **18** (0.8 g, 4 mmol) in dioxane (40 mL) to give aldoxime **21** (388 mg, 57%). Yellow crystals, m.p. > 170 °C dec. (ethyl acetate/acetonitrile). R_f 0.27 (dichloromethane/ethyl acetate, 3:2). ^1H NMR (DMSO-d_6) δ 12.02 (s, 1 H, OH), 7.73 (s, br, 2 H, NH_2), 7.57 (d, 1 H, J = 9.4 Hz), 7.22 (d, 1 H, J = 9.4 Hz), 5.19 (s, 1 H); IR ν 3415, 3344, 3237, 3052, 1668 sh, 1618, 1599, 1552 cm^{-1}; λ_{max}(log ε) 234 nm (4.008), 316 (4.394); Anal. Calc. for $C_6H_6N_2O_2S$ (170.19): C, 42.34; H, 3.55; N, 16.46; S, 18.84. Found: C, 42.60; H, 3.65; N, 16.06; S, 17.96. MS: 170 [M^+].

4.17. *Ethyl(2-ethoxy-4,5-dihydro-4-oxo)thiophene-3-carboxylate* ***22***

A solution of **3** (94.0 g, 0.5 mol) and triethyloxonium tetrafluoroborate (190 g, 1 mol) in dry CH_2Cl_2 (700 mL) was left to stand at room temperature for 50 h, thereupon carefully washed with sat aqueous $NaHCO_3$ solution (*Caution*: CO_2↑), dried (Na_2SO_4) and evaporated in vacuo. Trituration of the resulting residue with diisopropyl ether/ethyl acetate gave rise to **22** (32.4 g, 30%). Cream-coloured crystals, m.p. 70 °C (diisopropyl ether/ethyl acetate). ^1H NMR ($CDCl_3$) δ 4.49 (q, 2 H, J = 7.3 Hz), 4.25 (q, 2 H, J = 7.3 Hz), 3.73 (s, 2 H), 1.54 (t, 3 H, J = 7.3 Hz), 1.29 (t, 3 H, J = 7.3 Hz); IR ν 2985, 2939, 2895, 1724, 1659, 1526 cm^{-1}; λ_{max}(log ε) 213 nm (4.058), 294 (4.086); Anal. Calc. for $C_9H_{12}O_4S$ (216.26): C, 49.99; H, 5.59; S, 14.83. Found: C, 49.88; H, 5.48; S, 14.59. MS: 216 [M^+].

4.18. *(Z)-Ethyl(5-ethoxy-4-ethoxycarbonylmethylene-2,3-dihydro-3-oxo-2-thienylidene)acetate* ***23***

A solution of **22** (38.5 g, 178 mmol) and ethyl glyoxalate (40 g, 50% in toluene, 196 mmol) in toluene (500 mL) was refluxed for 1 h using a Dean–Stark apparatus. Thereupon the mixture was evaporated under reduced pressure to give **23** (46.5 g, 87%). Yellow needles, m.p. 143 °C (diisopropyl ether/ethyl acetate). ^1H NMR ($CDCl_3$) δ 7.02 (s, 1 H), 4.60 (q, 2 H, J = 7.3 Hz), 4.31 (q, 2 H, J = 7.3 Hz), 4.27 (q, 2 H, J = 7.3 Hz), 1.59 (t, 3 H, J = 7.3 Hz), 1.33 (t, 3 H, J = 7.3 Hz), 1.31 (t, 3 H, J = 7.3 Hz); IR ν 3062, 2985, 2939, 1715, 1698, 1617, 1523 cm^{-1}; λ_{max}(log ε) 209 nm (4.220), 261 (4.285), 270 (4.279), 374 (3.534); Anal. Calc. for $C_{13}H_{16}O_6S$ (300.33): C, 51.99; H, 5.37; S, 10.68. Found: C, 51.87; H, 5.33; S, 10.55. MS: 300 [M^+].

4.19. *(Z)-Ethyl(2,5-dihydro-3-hydroxy-5-oxo-2-thienylidene)-acetate* ***24***

A solution of **23** (2.7 g, 9 mmol) and conc HCl (1.0 mL, 12 mmol) in acetic acid (25 mL) was refluxed for 75 min, thereupon evaporated under reduced pressure, redissolved in toluene (50 mL) and the solvent removed once again in vacuo to give **24** (1.57 g, 87%). Slightly orange crystals, m.p. 150 °C dec. (hexane/diisopropyl ether), reddish brown ferric chloride test. ^1H NMR ($CDCl_3$) δ 10.0–9.7 (s, br, 1 H, OH), 6.38 (s, 1 H), 5.50 (s, 1 H, exchangeable with D_2O), 4.05 (q, 2 H, J = 7.3 Hz), 1.13 (t, 3 H, J = 7.3 Hz); IR ν 3270 br, 3094, 2996, 1704, 1615, 1587 cm^{-1}; λ_{max}(log ε) 293 br nm (4.338); Anal. Calc. for $C_8H_8O_4S$ (200.21): C, 47.99; H, 4.03; S, 16.02. Found: C, 47.94; H, 4.17; S, 15.40. MS: 200 [M^+].

4.20. *(Z)-Ethyl[2,5-dihydro-3-(4-toluenesulfonyloxy)-5-oxo-2-thienylidene]acetate* ***25***

Triethylamine (0.91 g, 9 mmol) was added dropwise to an ice-cooled solution of **24** (1.6 g, 8 mmol) and *p*-toluenesulfonyl chloride (1.6 g, 8.4 mmol) in dry dichloromethane (50 mL) and the resulting suspension was stirred at 0 °C for 90 min. The mixture was then washed sequentially with water and brine, dried (Na_2SO_4) and evaporated in vacuo. Purification of the obtained residue by flash chromatography gave **25** (1.73 g, 61%). Deep orange crystals, m.p. 102–103 °C (diisopropyl ether), R_f 0.50 (hexane/ethyl acetate, 3:1). ^1H NMR ($CDCl_3$) δ 7.86 (d, 2 H, J = 8.1 Hz), 7.41 (d, 2 H, J = 8.1 Hz), 6.41 (s, 1 H), 6.38 (s, 1 H), 4.24 (q, 2 H, J = 7.3 Hz), 2.48 (s, 3 H), 1.30 (t, 3 H, J = 7.3 Hz); IR ν 3095, 2998, 1686, 1592 cm^{-1}; λ_{max}(log ε) 228 nm (4.240), 284 (4.198), 332 (3.688); Anal. Calc. for $C_{15}H_{14}O_6S_2$ (354.40): C, 50.84; H, 3.98; S, 18.10. Found: C, 50.81; H, 4.02; S, 18.63. MS: 354 [M^+].

4.21. *(Z)-Ethyl(3-azido-2,5-dihydro-5-oxo-2-thienylidene)-acetate* ***26***

Sodium azide (1.65 g, 25 mmol) was added all at once to an ice-cooled suspension of **25** (7.08 g, 20 mmol) in dry methanol (100 mL) and the resulting mixture was stirred at 0 °C for 1 h. After dilution with water (500 mL) the solution was extracted with ethyl acetate (3 x 75 mL), the organic layer washed with brine, dried (Na_2SO_4) and evaporated in vacuo. Purification of the resulting residue by flash chromatography gave azide **26** (3.51 g, 78%). Yellow crystals, m.p. 72 °C (diisopropyl ether),

R_f 0.35 (hexane/ethyl acetate, 5:1). ^{1}H NMR ($CDCl_3$) δ 6.52 (s, 1 H), 6.26 (s, 1 H), 4.28 (q, 2 H, J = 7.3 Hz), 1.33 (t, 3 H, J = 7.3 Hz); IR ν 3063, 2986, 2141, 1700, 1680, 1612, 1572, 1474 cm^{-1}; λ_{max}(log ε) 225 nm (4.100), 302 (4.294), 352 (3.565); Anal. Calc. for $C_8H_7O_3S$ (225.23): C, 42.66; H, 3.13; N, 18.66; S, 14.24. Found: C, 43.04; H, 3.22; N, 18.13; S, 14.19. MS: 225 [M+].

*4.22. (Z)-Ethyl(3-amino-2,5-dihydro-5-oxo-2-thienylidene)-acetate **27***

This reduction protocol was analogous to that described for **15** employing a solution of $SnCl_2$ (5.7 g, 30 mmol) in anhydrous methanol (75 mL) and another solution of **26** (4.5 g, 20 mmol) in methanol (75 mL). Removal of solvents gave essentially pure **27** (3.78 g, 95%). Yellow crystals, m.p. 178–179 °C (diisopropyl ether/ethyl acetate). ^{1}H NMR (CD_3CN) δ 6.47 (s, 1 H), 6.07 (s, br, 2 H, NH_2), 5.39 (s, 1 H), 4.23 (q, 2 H, J = 7.3 Hz), 1.28 (t, 3 H, J = 7.3 Hz); IR ν 3431, 3343, 3221, 3061, 2984, 1707, 1610, 1562, 1537 cm^{-1}; λ_{max}(log ε) 224 nm (4.223), 301 (4.345), 304 (4.366), 401 (3.214); Anal. Calc. for $C_8H_9NO_3S$ (199.23): C, 48.23; H, 4.55; N, 7.03; S, 16.10. Found: C, 48.19; H, 4.52; N, 6.83; S, 16.72. MS: 199 [M+].

*4.23. Ethyl(3-amino-2,5-dihydro-5-oxo-2-thienyl)acetate **28***

To an ice-cooled suspension of **27** (0.2 g, 1 mmol) and Cu_2Cl_2 (0.3 g, 3 mmol) in dry MeOH (50 mL) was portionwise added $NaBH_4$ (0.38 g, 10 mmol). After stirring for 15 min at this temp the mixture was acidified with dil H_2SO_4 and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated in vacuo to give **28** (186 mg, 92%). Colourless crystals, m.p. 98 °C (diisopropyl ether). ^{1}H NMR (CD_3CN) δ 5.92 (s, br, 2 H, NH_2), 5.12 (s, 1 H), 4.50 (dd, 1 H, J_1 = 4.3 Hz; J_2 = 9.8 Hz), 4.17 (q, 2 H, J = 7.3 Hz), 3.18 (dd, 1 H, J_1 = 4.3 Hz; J_2 = 17.1 Hz), 2.70 (dd, 1 H, J_1 = 9.8 Hz; J_2 = 17.1 Hz), 1.25 (t, 3 H, J = 7.3 Hz); IR ν 3430, 3339, 3199, 2983, 2925, 1717, 1657 sh, 1618, 1558 cm^{-1}; λ_{max}(log ε) 236 nm (4.037), 286 (4.183); Anal. Calc. for $C_8H_{11}NO_3S$ (201.24): C, 47.75; H, 5.51; N, 6.96; S, 15.93. Found: C, 47.66; H, 5.18; N, 6.76; S, 16.05. MS: 201 [M+].

*4.24. (Z)-(3-Amino-2,5-dihydro-5-oxo-2-thienylidene)acetic acid **29***

A solution of K_2CO_3 (9.0 g, 65 mmol) in water (150 mL) was added to an ice-cooled solution of **27** (1.99 g, 10 mmol) in methanol (50 mL). After 3 h the mixture was acidified by addition of diluted H_2SO_4 and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo to give essentially pure **29** (1.68 g, 98%). Deep yellow crystals, m.p. 228 °C dec. (acetonitrile). ^{1}H NMR (DMSO-d_6) δ 7.70 (s, br, 2 H, NH_2), 6.79 (s, 1 H), 5.24 (s, 1 H); IR ν 3500–2500 br, 3479, 3386, 3349, 3247, 3058, 2926, 2596, 1701, 1626, 1547 cm^{-1}; λ_{max}(log ε) 224 nm (3.558), 302 (3.760), 397 (2.595); Anal. Calc. for $C_6H_5NO_3S$ (171.18): C, 42.10; H, 2.94; N, 8.18; S, 18.73. Found: C, 42.17; H, 3.07; N, 8.44; S, 18.52. MS: 171 [M+].

*4.25. (Z)-S-Phenyl-(3-amino-2,5-dihydro-5-oxo-2-thienylidene)-thioacetate **30***

A solution of **29** (1.71 g, 10 mmol), diphenyl disulphide (3.27 g, 15 mmol) and triphenyl phosphane (3.93 g, 15 mmol) in dry acetonitrile (75 mL) was refluxed under N_2 for 45 min. The volatiles were removed in vacuo and the residue purified by flash chromatography to give **30** (1.08 g, 41%). Yellow crystals, m.p. 221 °C dec. (diisopropyl ether/ethyl acetate), R_f 0.40 (diethyl ether). ^{1}H NMR (CD_3CN) δ 7.49 (m, 5 H), 6.93 (s, 1 H), 6.10 (s, br, 2 H, NH_2), 5.45 (s, 1 H); IR ν 3424, 3340, 3236, 1664, 1605, 1553, 1528 cm^{-1}; λ_{max}(log ε) 236 nm (3.520), 334 (3.721); Anal. Calc. for $C_{12}H_9NO_2S$ (263.34): C, 54.73; H, 3.44; N, 5.32; S, 24.35. Found: C, 54.59; H, 3.26; N, 5.09; S, 24.35. MS: 263 [M+].

*4.26. (Z)-(3-Amino-2,5-dihydro-5-oxo-2-thienylidene)acetic acid 3,5-dimethyl pyrazolide **31***

A mixture of **29** (3.42 g, 20 mmol), 3,5-dimethyl pyrazole (2.02 g, 21 mmol), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho *p*-toluenesulfonate ('Morpho CDI', 8.88 g, 21 mmol) and 4-dimethylamino pyridine (0.12 g, 1 mmol) in dry acetonitrile (200 mL) was stirred under N_2 for 16 h at room temperature, thereupon adsorbed onto silica gel and purified by flash chromatography to give **31** (3.69 g, 74%). Yellow crystals, m.p. 225 °C dec. (ethyl acetate), R_f 0.19 (chloroform/ethyl acetate, 2:1). ^{1}H NMR (CD_3CN) δ 7.90 (s, 1 H), 6.20 (s, br, 2 H, NH_2), 6.14 (s, 1 H), 5.45 (s, 1 H), 2.56 (s, 3 H), 2.26 (s, 3 H); IR ν 3406, 3340, 3234, 3067, 2927, 2850, 1684, 1608, 1561 cm^{-1}; λ_{max}(log ε) (MeCN): 208 nm (4.214), 324 (4.564), 334 sh (4.511); λ_{max}(log ε) (MeOH): 214 nm (4.248), 239 (4.174), 322 (4.421), 335 (4.428); Anal. Calc. for $C_{11}H_{11}N_3O_2S$ (249.29): C, 53.00; H, 4.45; N, 16.88; S, 12.86. Found: C, 52.97; H, 4.57; N, 16.81; S, 12.86. MS: 249 [M+].

*4.27. (E)-(3-Amino-2,5-dihydro-5-oxo-2-thienylidene)acetic acid 3,5-dimethyl pyrazolide **32***

A solution of **31** (1.0 g, 4 mmol) in dry acetonitrile (100 mL), scrupulously degassed under sonification by purging with dry N_2, was irradiated under N_2 for 3 h using a pyrex-mantled UV lamp. The mixture was shown to consist of both **31** and **32** in a ratio of 5:95 as evidenced by HPLC and NMR analysis. Thereupon the solvent was removed in vacuo and the resulting residue purified by flash chromatography to give **32** (865 mg, 87%) besides unchanged starting material **31** (46 mg, 5%). Orange crystals, subl. 200 °C/0.02 Torr, m.p. 152 °C (diisopropyl ether/ethyl acetate), R_f 0.25 (hexane/ethyl acetate, 3:1). ^{1}H NMR (CD_3CN) δ 7.86 (s, 1 H), 6.16 (s, 1 H), 5.78 (s, 1 H, NH, Integration < 1 H), 5.68 (1 H, NH, Integration < 1 H), 5.42 (s, 1 H), 2.55 (s, 3 H), 2.23 (s, 3 H); IR ν 3319, 3160, 1688, 1661, 1624, 1546; λ_{max}(log ε) (MeCN): 217 nm (4.140), 252 (4.175), 332 (4.562); Anal. Calc. for $C_{11}H_{11}N_3O_2S$ (249.29): C, 53.00; H, 4.45; N, 16.88; S, 12.86. Found: C, 53.31; H, 4.51; N, 16.51; S, 12.78. MS: 249 [M+].

*4.28. Thieno[3.2-b]pyrrole-2,5(4H)-dione **33***

Diluted HCl (2 N, 2.0 mL, 4 mmol) was added to a solution of **32** (250 mg, 1 mmol) in 1,2-dimethoxyethane (20 mL) and the mixture refluxed for 2 h. After cooling to room temperature the solution was diluted with water (30 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated in vacuo to give a residue which was purified by flash chromatography to furnish **33** (49 mg, 32%, unoptimized yield). Yellow crystals, m.p. 191 °C dec. (hexane/diisopropyl ether), R_f 0.26 (hexane/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$) δ 7.58 (s, br, 1 H, NH), 6.09 (d, 1 H, J = 1.3 Hz), 5.63 (d, 1 H, J = 1.3 Hz); IR ν 3185 br, 3077, 1712, 1639, 1563 cm^{-1}; λ_{max}(log ε) 207 nm (4.226), 292 (4.680), 384 (2.896); Anal. Calc. for $C_6H_3NO_2S$ (153.16): C, 47.05; H, 1.97; N, 9.15; S, 20.94. Found: C, 47.03; H, 2.10; N, 9.04; S, 21.10. MS: 153 [M+].

4.29. (Z)-(3-Amino-2,5-dihydro-5-oxo-2-thienylidene)acetaldehyde [Thiobasidalin, 2]

Lithium aluminum hydride (16.0 mL, 1 M in THF, 16 mmol) was slowly added dropwise under N_2 to a solution of **31** (1.0 g, 4 mmol) in dry THF (75 mL) previously cooled to –78 °C. After 7 h at this temp the mixture was poured into an ice-cooled solution of dil H_2SO_4 (*Caution*: $H_2\uparrow$) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated in vacuo leaving a residue which was purified by flash chromatography to give **2** (330 mg, 53%). Green shimmering crystals (ethyl acetate), m.p. > 158 °C dec., R_f 0.29 (dichloromethane/acetone, 7:1). ^1H NMR (CD_3CN) δ 9.88 (d, 1 H, J = 4.3 Hz), 6.70 (d, 1 H, J = 4.3 Hz), 6.11 (s, br, 2 H, NH_2), 5.41 (s, 1 H); ^1H NMR (D6-DMSO) δ 9.86 (d, 1 H, J = 4.3 Hz), 7.87 (s, br, 2 H, NH_2), 7.09 (d, 1 H, J = 4.3 Hz), 5.29 (s, 1 H); CH-COSY (D6-DMSO) δ 190.7 (*C*HO), 188.0, 165.3, 148.8, 117.7 (*C*H=CHO), 96.1 (*C*H=CNH$_2$); IR ν 3375, 3181, 2851, 1682 sh, 1634, 1568 sh, 1545 cm^{-1}; λ_{max}(log ε) 216 nm (4.098), 283 (4.177), 320 (4.081); Anal. Calc. for $C_6H_5NO_2S$ (155.18): C, 46.44; H, 3.25; N, 9.03; S, 20.66. Found: C, 46.48; H, 3.49; N, 9.00; S, 20.70. MS: 155 [M$^+$].

4.30. X-ray diffraction analysis

A suitable crystal (size 0.03 x 0.40 x 0.53 mm) of thiobasidalin **2** was obtained by slow evaporation of an ethyl acetate solution: $C_6H_5NO_2S$, M = 155.17, orthorhombic, Space group $Pna2_1$, a = 8.206(2), b = 14.142(2), c = 5.6572(6) Å, V = 656.5(2) Å^3, Z = 4, D = 1.570 Mg m^{-3}, l (Mo-Kα) = 0.71069 Å, F(000) = 320, μ = 0.420 mm^{-1}. Cell parameters were obtained by least squares refinement of 25 reflections in the range of 10 < Θ < 13. The data collection was performed at room temperature using graphite monochromized Mo-Kα radiation on a Nonius CAD4 diffractometer; ω-scan, scan width [1.21 + 0.95 tan Θ]° and a maximum measuring time of 45 s. Three standard reflections were measured every two hours and showed an intensity decay of 2.3%. The corrections for Lp, linear decay and absorption (T_{min} = 0.8428, T_{max} = 0.9946) were applied on a total of 1017 reflections, 909 unique and 823 with $I > 2\sigma I$. All non-hydrogen atoms were refined anisotropically. The hydrogens were positioned geometrically with U_i = 1.2 x U_{eq} of the adjacent non-hydrogen atom and included in the final least squares refinement. The final $R1$ was 0.0320 (w$R2$ = 0.0758) for 823 reflections and 91 variables and 1 restraint and $R1$ = 0.0372 (w$R2$ = 0.0798) for all 909 data. The structure was solved using SHELXS-86 [47] and refined by SHELXL-93 [48] against F^2. Weights: SHELXL-93. The absolute structure parameter refined to a value of 0.03(12) indicating that the absolute structure was established. The final residual electron density was 0.182 and –0.155 e Å^{-3}. The drawing was made using XPMA, ZORTEP [49]. The calculations were performed on a Pentium-PC.

Complete details of the structure investigation are available on request from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, England on quoting the names of the authors and the journal citation.

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