Sulphur-substituted Pyrrolo[3,4-b]quinolines: Synthesis, Chemistry and Antimicrobial Activity

Theodorus van Es[a*], Benjamin Staskun[b,c*] and Sandy van Vuuren[d]

[a]*Department of Biochemistry and Microbiology, Cook College, Rutgers The State University of New Jersey, 08903-0231, USA.*

[b]*School of Chemistry, University of the Witwatersrand, Johannesburg, South Africa.*

[c]*Visiting Associate, Department of Chemistry, Macquarie University, NSW 2109, Australia.*

[d]*Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 2193 South Africa.*

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ABSTRACT

2,3-Dihydro-2-propyl-3-propylimino-9-thioxo-pyrrolo[3,4-b]quinolin-1-one reacts with an aliphatic primary, secondary or tertiary amine to form a 1:1 substrate:amine (thiolate) complex. It also readily undergoes S-alkylation (with diazomethane or with an alkyl halide), S-acetylation (with acetic anhydride), hydrogenolysis (Raney nickel) with removal of sulphur, and acid-catalysed hydrolysis (with selective replacement of the 3-propylimino function by oxygen). Two novel di-(pyrrolo[3,4-b] quinolinyl) sulphide reaction products are described and their structures established. Treatment of these 'dimers' and related 9-alkylthio-substituted pyrroloquinolines with an aliphatic amine provides a convenient access to 'simple' or 'mixed' 2-alkyl-9-alkylamino-3-alkylimino-pyrroloquinoline derivatives. Preliminary antimicrobial (*in vitro*) tests indicate that: (a) the weak antimicrobial activity of the aforementioned 9-thioxo-pyrroloquinoline substrate against three selected Gram-positive pathogens is significantly enhanced in its amine complexes, and by the presence of a 6-fluoro atom in the quinoline moiety, and (b) that substitution of the 4-oxo-function in 4-oxo-3-quinolinecarboxylic acids by an ethylimino group leads to a marked reduction in antimicrobial properties.

KEY WORDS

2-Alkyl-3-alkylimino-9-thioxo-2,3-dihydro-pyrrolo[3,4-b]quinolin-1-ones, pyrrolo[3,4-b]quinoline (1:1) amine complexes, 6-fluoro-derivatives, antimicrobial activities, di-[(pyrrolo[3,4-b]quinolinyl)] sulphides, 9-alkylthio-substituted pyrrolo[3,4-b] quinolines.

1. Introduction

The pyrrolo[3,4-b]quinoline ring system is present in compounds associated with a wide variety of pharmacological properties as is exemplified with hypnotic[1] and cytotoxic[2] effects, and with analgesic[3] and antiviral[4] activities. It is also known[5] that nuclear-substituted 4-thioxoquinolines are significant antimicrobials. Accordingly, an interest in analogous pyrroloquinoline structures has arisen from efforts to obtain new biomedically relevant products.[4] In previous contributions[6,7] we showed that aminolysis of 3,3,9-trichloro[3,4-b] quinolinone **1a** offers a convenient and relatively simple methodology to access an assortment of hitherto undocumented 2-alkyl-3-alkylimino-9-thioxo-pyrrolo[3,4-b]quinolines **2**, and related derivatives **3**, including amine complexes of **2**, namely **4**. Compounds **2** and **4** may be viewed as unusual derivatives of 4-thioxoquinoline **5**, and in the light of the aforementioned observations the question arose as to whether certain of the title compounds might likewise possess antimicrobial activity. Here we present further developments in the chemistry and biological properties in this new area of sulphur-substituted pyrrolo[3,4-b] quinolines.

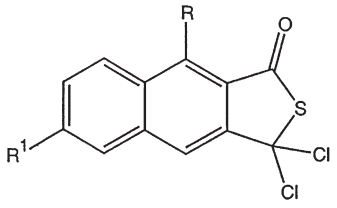
2. Results and Discussion

Utilizing a convenient general procedure[6], 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** was stirred briefly with excess of diethylamine, triethylamine and tert-butylamine, respectively, at room temperature, to yield in each case the appropriate substrate-amine (1:1) complex **4**. The latter products were relatively unstable and tended to decompose at room temperature on prolonged storage, and more rapidly on heating. In the mass spectrum the highest m/z value was that of component **2a**. Treatment with dilute acid at room temperature instantly disrupted **4** to **2a** and amine. ^{1}H NMR spectral analysis, coupled with a quantitative volumetric determination of the amount of amine present in the complex **4**, served to reveal the latters purity, and to establish a 1:1 substrate:amine composition.

In the absence of a crystal suitable for X-ray study the location and nature of the bonding between the components in complex **4** was inferred from the following observations: (a) 4-thioxo quinoline **5** and propylamine formed a comparable (1:1) complex, whereas no similar product resulted from the sulphur-free pyrroloquinolines **7** and **3a**, which was indicative of S being the focus of bonding in **4**; (b) recent X-ray crystallographic studies[6] of 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** and 2-propyl-9-thioxo-pyrroloquinoline-1,3-dione **6a** located the enolizable proton in **2a** on the 3-propylimino nitrogen atom, and on the sulphur atom in **6a**; such a proton in the course of complex **4** formation is expected to transfer to the more basic nitrogen of an amine, thereby resulting in a thiolate salt as depicted in **9**[8].

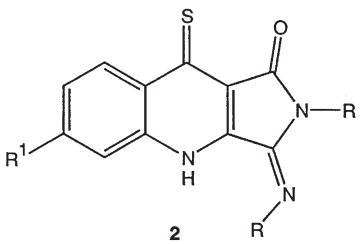
Investigative reactions to gauge the chemical reactivity and functional group transformations in 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** and related compounds showed a

* To whom correspondence should be addressed. E-mail: vanes@aesop.rutgers.edu / benmina@optusnet.com.au



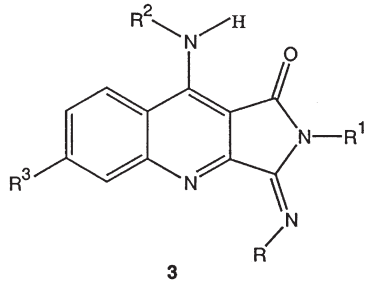
1

a R=Cl, R^1=H
b R=R^1=H
c R=Cl, R^1=F



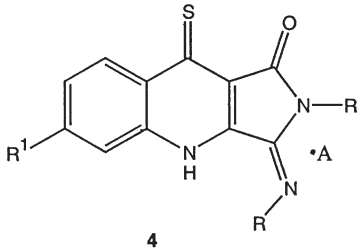
2

a R=Pr, R^1=H
b R=Et, R^1=H
c R=Pr, R^1=F



3

a R=R^1=R^2=Pr, R^3=H
b R=R^1=Pr, R^2=*iso*Pr, R^3=H
c R=R^1=Pr, R^2=Et, R^3=H
d R=R^1=R^2=Pr, R^3=F



4

a R=Pr, R^1=H; A=$PrNH_2$
b R=Pr, R^1=H; A=*iso*$PrNH_2$
c R=Pr, R^1=H; A=Et_2NH
d R=Pr, R^1=H; A=Et_3N
e R=Pr, R^1=H; A=*tert*-$BuNH_2$
f R=Pr, R^1=F; A=$PrNH_2$

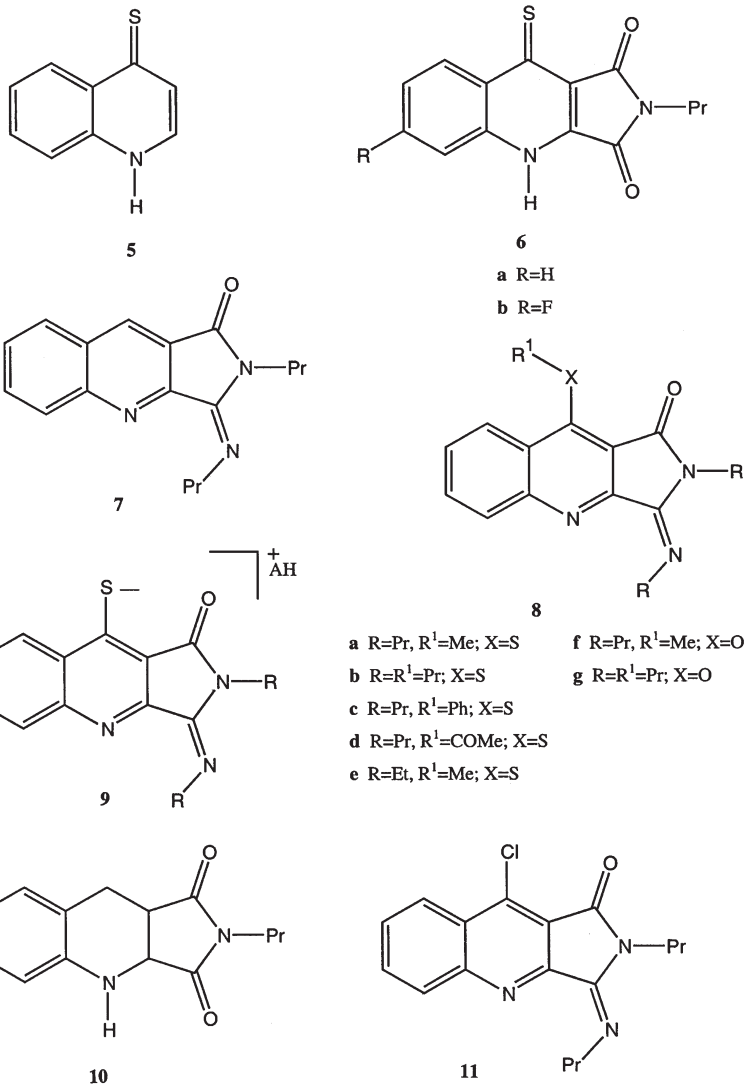
promising potential to access a diversity of pyrrolo[3,4-b] quinolines derivatives, as is illustrated with the following: Raney nickel hydrogenolysis at room temperature effected the removal of the sulphur from 2-propyl-9-thioxo-pyrroloquinoline-1,3-dione **6** to provide *tetra*hydro-pyrroloquinoline derivative **10**. Alkylation of the sulphur in substrate **2a**, and likewise in its propylamine complex **4a**, with either diazomethane or methyl iodide, gave the 9-methythio-2-propyl-3-propylimino derivative **8a** as was established by a NOE experiment. Acetic anhydride and pyridine readily converted substrate **2a** and its complex **4a** to the 9-acetylthio-2-propyl-3-propylimino derivative **8d**. Treatment of **4** with aqueous acid at room temperature instantly and quantitatively separated the amine in the complex to provide component **2**, whereas with warm (~50°C) acid the 3-alkylimino moiety in pyrroloquinolines **2**, **3**, **4**, **7** and **8** was hydrolyzed with production of the corresponding 1,3-dione derivative **12**. This synthetically useful outcome is exemplified with 9-methylthio 2-propyl-3-propylimino-pyrroloquinoline **8a** and hydrochloric acid at 50°C to give (70%) 9-methylthio-2-propyl-pyrroloquinoline-1,3-dione **12a**, The proposal[7] that complex **4a** may be formed from 9-chloro-pyrroloquinoline **11** and hydrogen sulphide in propylamine has now been verified.

Two sulphur-containing products each significantly different in structure from any of the previously obtained sulphur-substituted pyrroloquinolines, were isolated in the course of preparing 4-chloro-N-propyl-9-propylcarbamoyl-3-quinoline carboxylic acid amide **13** from trichlorothienoquinoline **1a** and propylamine[7], and were separated on a silica column. The ^{1}H NMR of one product (m.p. 251–253°C) exhibited signals (number, multiplicities and chemical shifts) requisite for two propyl groups and four aromatic protons (total 18 protons), whereas the HRMS established a molecular formula of $C_{34}H_{36}N_6O_2S$. Such data are compatible with a dimer structure **14**, The ^{1}H NMR of the other product (m.p. 230–232°C) contained signals for three disparate propyl groups and eight aromatic protons, whilst the HRMS established a molecular formula $C_{31}H_{29}N_5O_2S_2$ according with a structure assignment **15**. Assignments **14** and **15** were separately substantiated by unambiguous synthesis: thus alkylation of 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** with 9-chloro-2-propyl-3-propylimino-pyrroloquinoline **11** gave (~100%) dimer **14** free of dimer **15**, while alkylation of substrate **2a** with 9-chloro-2-propyl-3-thioxo-pyrroloquinoline **16** provided dimer **15** (~100%) also exclusively. Dimer **14** was unexpectedly produced, in addition to 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a**, on refluxing complex **4a** in toluene[9].

Dimer **14** either alone or when together with **15**, on stirring with excess propylamine at room temperature rapidly yielded a mixture of complex **4a** and 9-propylamino-pyrroloquinoline **3a**, indicative of a facile cleavage of the thioether bond in **14**/**15** (and of aminolysis of the thioxo function in **15** in an as yet undetermined sequence of events). This observation was extended to examining the aminolysis of related and representative 9-alkylthio-substituted pyrroloquinolines, *viz.* **8a**, **8b** and **8c**. The results (Table 1) confirmed a facile aminolysis with primary alkylamines and provided a convenient preparation of both

Table 1 Aminolysis of 9-alkylthio-pyrrolo[3,4-b]quinolines 8.

Amine reactant	9-Alkylamino group in **8**	Product **3** (crude) yield, reaction time
n-propylamine	methyl, **8a**	**3a**, quantitative, ~30 min
n-propylamine	*n*-propyl, **8b**	**3a**, quantitative, ~15 min
*iso*propylamine	*n*-propyl, **8b**	**3b**, quantitative, ~20 h
ethylamine	*n*-propyl, **8b**	**3c**, quantitative, ~6 h
n-propylamine	phenyl, **8c**	**3a**, quantitative, ~10 min
tert-butylamine	*n*-propyl, **8b**	no reaction, 24 h
diethylamine	*n*-propyl, **8b**	no reaction, 65 h
aniline	*n*-propyl, **8b**	no reaction, 1 week



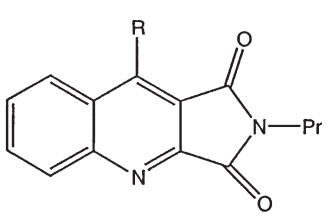
simple and mixed[7] 2-alkyl-9-alkylamino-3-alkylimino-substituted pyrroloquinolines **3**. Similar treatment of several representative non-heteroaromatic substrates, *viz.* diphenyl sulphide, methyl 2-(methylthio)benzoate, ethyl 2-(phenylthio)acetate and 1-phenyl-2-(*n*-propylthio)ethenone for ~6 h showed no detectable displacement of the –SR group. It is suggested that sulphide cleavage in the aforementioned pyrroloquinolines is effected more readily because of a more effective delocalization of charge and concomitant resonance stabilization in the proposed intermediate **A**.

Treating 9-alkylthio-pyrroloquinolines **8** with alkoxide ion provided the corresponding 9-alkoxy derivative as exemplified with the 9-methoxy- and 9-propoxy-compounds **8f** and **8g**. Compound **8f** was also obtained in high yield on refluxing 9-methylthio-pyrroloquinoline **8a** in methanol containing anhydrous sodium acetate (perhaps owing to production of methoxide ion *in situ),* and by reacting 9-chloro-pyrrolo quinoline **11** with sodium methoxide. However, no significant reaction occurred between **8a** and ammonia, and azide-, iodide-, cyanide- or *tert*-butoxide ions. The aforementioned 9-alkylthio- to 9-alkoxy- conversion could be reversed: thus treating **8f** with excess of propylthiolate ion afforded the 9-propylthio-pyrrolo quinoline **8b**. Warming 9-methoxy- and 9-propoxy-pyrrolo quinolines, *viz.* **8f** and **8g**, with aqueous acid, led to hydrolysis of both 9-alkoxy and 3-propylimino functions in each to yield pyrroloquinoline-1,3,9-trione derivative **17**.

The availability[10] of 3,3-dichloro-6-fluoro-thienopyrrolo [3,4-b]quinolin-1-one **1c** prompted an examination of (amongst others) the fate of the fluorine substituent during aminolysis with propylamine under the general reaction conditions. In the event the F remained unaffected, the products being complex **4f** (53%) (formed between equimolar amounts of 6-fluoro-2-propyl-3-propylimino-9-thioxo-pyrroloquinolinone **2c** and propylamine) and 6-fluoro-2-propylamino-3-propylimino-pyrroloquinolinone **3d** (12%). Separation of the propylamine from complex **4f** was effected with acid to furnish component **2c**, whereas with warm acid the product was the pyrroloquinolin-1,3-dione **6b** (97%).

3.1 Antimicrobial Results

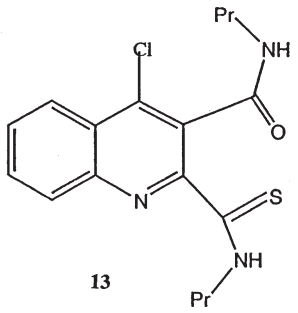
Preliminary Disc Diffusion (DD) and Minimum Inhibitory Concentration (MIC) studies of the antimicrobial efficacies of compounds **2** and **4** against three representative Gram-positive pathogens showed promise. The experimental details (*vide infra*), and the accumulated outcomes (Table 2) although limited, allowed for the following tentative inferences and conclusions: 9-thioxo-pyrroloquinolinone **2a** exhibited a weak activity against three representative Gram-positive organisms, *S. aureus, B. cereus* and *S. epidermidis* (Table 2), and did not inhibit the growth of *E. coli*; **2a** was seemingly a more selective and less effective antimicrobial than 4-thioxoquinolone **5**. It is noteworthy and significant, however, that the activity of **2a** towards



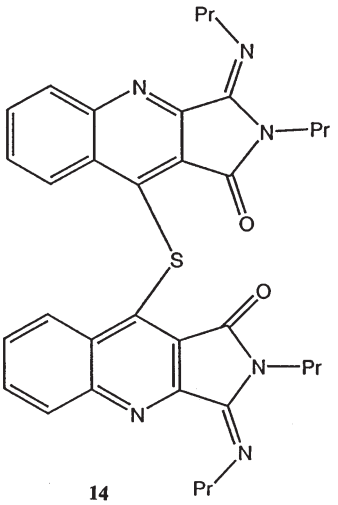
12

a R=MeS

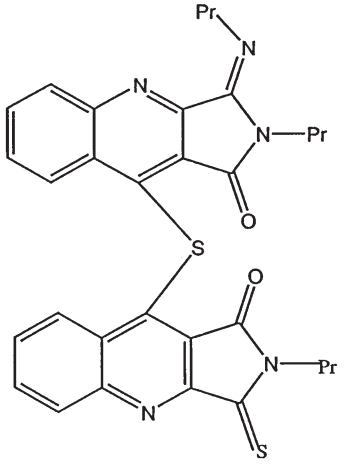
b R=PrNH



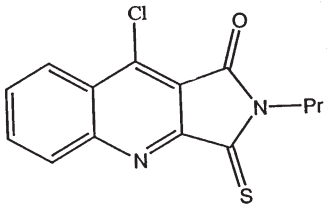
13



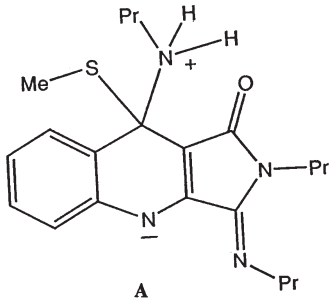
14



15



16



A

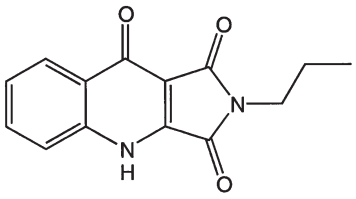
the aforementioned Gram-positive organisms was markedly enhanced (a) when complexed to an aliphatic amine (propylamine, isopropylamine, diethylamine, triethylamine and *tert*-butylamine, respectively), as in **4**, and (b) when it bore a 6-flouro-substituent, as in **2c**. Enhancement (a) is indicated from comparison of the MIC values (μg mL^{-1}) of **2a** with the corresponding ones in its complexes **4a**, **4b**, **4c**, **4d** and **4e**, while enhancement (b) is apparent from comparing the MIC values (against *S. aureus*) of **2a** (78) and its propylamine complex **4a** (19.5) with those of the corresponding 6-fluoro derivatives, *viz.* **2c** (16) and **4f** (<13), respectively[11]. The amine utilized in complex **4**, showed no measurable activity in the DD and MIC tests.

Table **2** also lists the (disc diffusion) outcomes of in *vitro testing* of two recently[12] synthesized 1-alkyl-4-alkylimino-1,4-dihydro--3-quinolinecarboxylic acids, *viz.* **18a** and **18b**, Whereas an extensive literature[13] exists devoted to the antimicrobial efficacies of the ubiquitous 4-oxo-quinolines of type **19**, there is little if any such information available regarding the related 4-imino analogues **18**. The outcomes, albeit very limited, suggest that replacing the 4-oxo function in **19** by an ethylimino group leads to a marked diminution in efficacy, at least towards the bacteria listed. Nevertheless, the 4-iminoquinolines **18** have the potential to function as prodrugs owing to their susceptibility to alkaline hydrolysis with production of the active corresponding 4-oxoquinoline **19** (anion)[12].

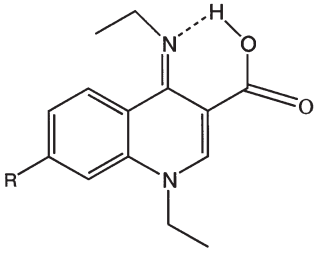
In summary, we have shown that subjecting the title compounds, exemplified by **2a**, to, amongst others, Raney nickel catalysed reduction, acid hydrolysis, and alkylation reactions, respectively, provides access, in acceptable yields, to a diversity of hitherto undocumented pyrrolo[3,4-b]quinoline derivatives suitable for further synthetic procedures and biological testing. Preliminary results indicate that complexing **2a** to an alkylamine leads to a more effective antimicrobial agent against certain Gram-positive pathogens.

4. Experimental

For general experimental details and techniques see refs 7 and 12. High-resolution mass spectra (HRMS) were recorded on a VG 70-SEQ mass spectrometer.^{1}H NMR spectra were recorded

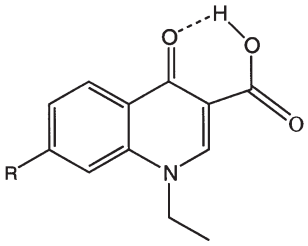


17



18

a R=Me-NC_4H_8N-
b R=Cl



19

a R=Me-NC_4H_8N-
b R=F

on a Bruker AC-200 spectrometer (200.13 MHz; in $CDCl_3$ solvent unless otherwise noted; δ (ppm) values refer to proton chemical shifts., J values are in Hz). Compounds **1a** and **1c** are described in ref. 10, **2a**, and **2b** in ref. 6, and **3c**, 4a, **8b**, **8c**, **11**, **13** and **16** in ref. 7. Yields refer to the crude product unless otherwise indicated; no serious attempts were made at optimization.

*Complex **4** formation*

The general procedure is illustrated with complex **4b**

2-Propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** (m.p. 143-144°C)[6] (113 mg, 0.36 mmol) was added to 2-aminopropane (1 mL, large mmol excess) with stirring at room temperature. After 15 min reaction the mixture was treated with water and extracted with chloroform. Evaporation of the extract under reduced pressure and temperature gave a residue of complex **4b** (117 mg, 87%). Needles (from ethyl acetate); (81 mg, melting with decomposition); δ 0.94(3H, t, J7.4), 1.06(3H, t, J7.3), 1.13(6H, d, J6.4), 1.7–1.8(4H, m), 3.3(1H, m), 3.77(2H, t, J7.2), 4.5(~2H, br peak, removed by D_2O), 4.54(~3H, t, J7.0), 7.58(1H, m, ArH), 7.73(1H, m, ArH), 8.07(1H, d, J8.2, ArH), 8.75(1H, d, J8.2, ArH). Volumetric analysis [(as with **4d** (*vide infra*)] established a 1:1 substrate:amine composition.

Table 2 Antimicrobial activity of compounds **2, 4, 5, 18** and **19** against selected pathogens.

Compound	*S. aureus*		*B. cereus*		*S. epidermidis*	
	DD[a]	MIC[b]	DD	MIC	DD	MIC
2a	8	78	8	c	c	c
2b	6	d	14	c	c	c
2c	10	16	c	c	13	16
4a	15	19.5	12	12	10	20
4b	14	20	c	c	14	39
4c	13	39	c	c	12	20
4d	13	<13[e]	c	c	12	<3[e]
4e	12	<13[e]	c	c	12	<13[e]
4f	10	<13[e]	12	31	16	156
5	14	3.8	10	3.8	30	7.6
18a	c	c	c	c	c	c
18b	c	c	c	c	c	c
19a	22	d	18	d	d	d
19b	c	d	14	d	d	d
Ciprofloxacin	22	3.1	33	3.1	d	0.39
DMSO	c	>400	c	>400	c	>400

[a] Disc Diffusion value; diameter of zone inhibition in mm including the 6 mm disc.
[b] Minimum inhibitory concentration; μg mL^{-1}.
[c] Organisms were not inhibited.
[d] Not determined, especially when antimicrobial activity is lacking in DD test.
[e] Indicative rather than specific value.

*Complex **4c***

Similar treatment of substrate **2a** (108 mg, 0.34 mmol) with diethylamine (1 mL) gave a syrup (125 mg, 0.33 mmol). This product was repeatedly triturated with cold benzene to provide a brown gum, soluble in hot hexane which analysed (^{1}H NMR) as a 1:1 substrate:amine complex; δ 0.91(3H, t, J7.4), 1.06(3H, t, J7.3), 1.27(6H, t, J7.2), 1.59–1.85(4H,m), 3.02(4H, q, J7.2), 3.73(2H, t, J7.3), 4.54(2H, t, J7.0), 5.75(2H, br peak removed by D_2O), 7.54(1H, m, ArH), 7.68(1H, m, ArH), 8.02(1H, d, J8.1, ArH), 8.8(1H, d, J8.2, ArH).

*Complex **4d***

Similar treatment of substrate **2a** (318 mg, 1.015 mmol) with triethylamine (1 mL, large mmol excess) provided complex **4d** as a brown gum (423 mg, after extended stay in a vacuum dessicator; ~100%); δ 0.93(3H, t, J7.4), 1.05(3H, t, J7.3), 1.30(9H, t, J7.3), 1.64–1.85(4H, m), 3.17(6H, q, J7.3), 3.74(2H, t, J7.2), 4.54(2H, t, J7.1), 7.5(1H, m, ArH), 7.65(1H, m, ArH), 8.0(1H, d, J8.2, ArH), 8.93(1H, d, J8.3, ArH). Product **4d** (415 mg, 1.00 mmol) was dissolved in methanol (5 mL) to which solution was added 1.00 mL of 2.188 M HCI. Sparingly soluble substrate **2a** was collected by filtration (290 mg, ~93% recovery) after which the filtrate and washing were combined and titrated (Methyl Red) with 0.1030 M NaOH which established that 1.10 mmol of triethylamine had been present, and confirmed a 1:1 complex composition.

*Complex **4e***

Similar treatment of substrate **2a** (173 mg, 0.55 mmol) and *tert*-butylamine (1 mL) gave compound **4e** (210 mg, ~100%; theory for a 1:1 complex: 214 mg). Needles (125 mg; from hexane), m.p.124–138°C (decomp.); δ 0.95 (3H,t,J7.4), 1.07(3H, t, J7.4), 1.21(9H, s), 1.65–1.85 (4H, m), 3.78(2H, t, J7.2), 4.45–4.6 (~5H, m, simplifies to 2H, t, J6.8, with D_2O), 7.55 (1H, m, ArH), 7.71(1H, m, ArH), 8.04 (1H, d, J8.3, ArH), 8.80(1H, d, J8.4).

4-Thioxoquinoline-propylamine complex

A mixture of 4-thioxoquinoline **5** (142 mg, 0.88 mmol) and propylamine (1 mL, large mmol excess) was stirred at room temperature for 10 min and was then evaporated at reduced

pressure and temperature to constant mass (184 mg, theory: 194 mg for a 1:1 complex). Crystallization of a small portion from ethyl acetate gave yellow crystals, which decomposed on warming or on prolonged keeping at room temperature, with liberation of propylamine (litmus paper). The freshly prepared complex (184 mg) was dissolved in water (5 mL) containing 1.00 mL of 2.162 M HCI when 4-thioxoquinoline **5** separated as a gum which solidified and was collected by filtration and identified from its IR spectrum. The combined filtrate and aqueous washings was titrated (Methyl Red) with 0.1065 M NaOH from which volumetric data, 0.88 mmol complex contained 0.83 mmol propylamine, confirming a 1:1 substrate:amine composition for the complex.

*Formation of complex **4a** from 9-chloro-3-propylimino-pyrrolo-quinoline **11** and propylamine/hydrogen sulphide*

Substrate **11** (152 mg, 0.48 mmol) was added portion-wise (over ~15 min) with stirring to an ice-cold solution (1.5 mL) of 13% (w/w) hydrogen sulphide in propylamine. Stirring was continued at room temperature overnight. Water and chloroform were added and the chloroform extract was evaporated under reduced pressure and temperature to afford complex **4a** (41 mg, ~23%) identical (IR) with authentic[2] complex **4a**.

*2-Propyl-3a,4,9,9a-tetrahydro-pyrrolo[3,4-b]quinoline-1,3-dione **10***

To 2-propyl-9-thioxo-pyrroloquinoline-1,3-dione **6a** (150 mg, 0.55 mmol) in absolute EtOH (25 mL) was added highly active Raney nickel (Across commercial product, stored under water; *ca.* 3–5 g), and the stoppered mixture was shaken at room temperature overnight. The reaction was decanted and filtered (through a thin pad of Celite) and the residual Raney nickel was washed repeatedly with hot ethanol. The combined filtrate and washings was evaporated and the residue was treated with water and chloroform. Evaporation of the chloroform extract gave S-free product **10** (96 mg, 72%) colouress crystals (from hexane), m.p. 84–85°C (Found: M^+, 244.1223. Calc. for $C_{14}H_{16}N_2O_2$: M, 244.1212) δ 0.56(3H, t, J7.4), 1.36(2H, sextet), 2.9–3.05(2H, m), 3.3–3.5(3H, m), 4.20(1H, d, J9.4), 4.46(1H, br s, removed by D_2O), 6.7–6.85(2H, m, ArH), 7.0–7.1, (2H, m, ArH).

*9-Methylthio-2-propyl-3-propylimino-2,3-dihydro-pyrrolo[3,4-b] quinolin-1-one **8a***

(a) 2-Propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** (244 mg, 0.78 mmol) was dissolved with stirring in an excess of ice-cold ethereal diazomethane (which solution contained traces of water and ethanol from its preparation). TLC (silica gel, 2% ethanol in benzene) monitoring of the reaction showed mainly one product (of high R_f) after 1 h. The mixture was allowed to evaporate in the hood and the residue of compound **8a** (220 mg, 86%) was crystallized from hexane; colourless needless, m.p. 105–106°C (Found: C, 65.80; H, 7.01; N, 12.62; S, 9.52; M^+, 327.1407 (100%). Calc. for $C_{18}H_{21}N_3OS$: C, 65.98; H, 6.46; N, 12.88; S, 9.79; M, 327.1405) δ 0.98(3H, t, J7.4), 1.07(3H, t, J7.3), 1.71–1.88(4H, m), 2.82(3H, s), 3.85(2H, t, J7.2), 4.55(2H, t, J7.0), 7.70(1H, m, ArH), 7.85(1H, m, ArH), 8.20(1H, dd, J1.0, 8.3, ArH), 8.75(1H, dd, J1.2, 8.3, ArH).

(*b*) Substrate **2a** (100 mg, 0.32 mmol) was added with stirring to ice-cold methyl iodide (4 mL, large mmol excess) followed on with anhydrous powdered potassium carbonate (~100 mg). Stirring was continued for ~5 min or until TLC showed the absence of substrate **2a**. After a further 20 min, water was added and the reaction extracted with chloroform. Evaporation of the extract gave compound **8a** (83 mg, 80%), identical (^{1}H NMR) with diazomethane product **8a**. A NOE experiment revealed, amongst others, that irradiation of the 9-methylthio singlet at δ 2.82 enhanced the signal of the 8-H aromatic proton at δ 8.75, and had no effect on that of the 5-H proton at δ 8.20. Irradiation of the latter proton confirmed its proximity[7] to the alpha-methylene protons of the 3-propylimino group, and *vice versa.*

*9-Acetylthio-2-propyl-3-propylimino-2, 3-dihydro-pyrrolo[3,4-b] quinolin-1-one **8d***

2-Propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** (63 mg, 0.20 mmol) was dissolved in acetic anhydride (3 mL) containing pyridine catalyst (2 drops) at room temperature after which was the reaction was allowed to proceed overnight. Ice-cold aqueous sodium bicarbonate was added and the mixture was stirred to hydrolyse the excess acetic anhydride. Extraction with chloroform gave crude product **8d** (53 mg, 74%). Colourless crystals (from hexane), m.p. 113–114°C (Found: C,64.10; H,5.68; N,11.68. Calc. for $C_{19}H_{21}N_3O_2S$: C,64.20; H,5.96; N,11.82) δ 0.96(3H, t, J7.4), 1.08(3H, t, J7.4) 1.7–1.9(4H, m), 2.65(3H, s), 3.83(2H, t, J7.3), 4.56(2H, t, J7.0), 7.72(1H, m, ArH), 7.85(1H, m, ArH), 8.27(1H, d, J8.7, ArH), 8.41(1H, d, J8.3, ArH.

*2-Ethyl-3-ethylimino-9-methylthio-2,3-dihydro-pyrrolo[3,4-b] quinolin-1-one **8e***

Substrate **2d** (117 mg, 0.41 mmol) with diazomethane (*vide supra*) gave title compound **8e** (112 mg, 91%). Fine needles (from hexane), m.p. 133–134°C (Found: M^+, 299.1103. Calc. for $C_{16}H_{17}N_3OS$: M, 299.1092) δ 1.30(3H, t, J7.1), 1.42(3H, t, J7.2), 2.83(3H, s), 3.94(2H, q, J7.1), 4.64(2H, q, J7.2), 7.75(1H, m, ArH), 7.85(1H, m, ArH), 8.20(1H, dd, J0.9, 8.1 ArH), 8.75(1H, dd, J0.9, 8.2, ArH).

*9-Methylthio-2-propyl-2,3-dihydro-pyrrolo[3,4-b]quinoline-1,3-dione **12a***

Acid hydrolysis (methanol + aqueous 2M HCl)[7] at 50°C, of 9-methylthio-2-propyl-pyrroloquinolin-1-one **8a** (140 mg, 0.43 mmol) for 4 h gave compound **12a** (85 mg, 70%). Crystals (from methanol), m.p. 113–114°C (Found: C, 63.13; H, 5.03; N, 9.59; S, 10.38%; M^+, 286.0782. Calc. for $C_{15}H_{14}N_2O_2S$: C, 62.89; H, 4.93; N, 9.82; S, 11.19%; M, 286.0776) δ 1.0(3H, t, J7.4), 1.8(2H, m), 2.88(3H, s), 3.81(2H, t, J7.3), 7.8 (1H, m, ArH), 7.95 1H, m, ArH), 8.37(1H, d, J8.2, ArH), 8.73(1H, d, J8.4, ArH). Compound **12a** was also obtained (178 mg, 94%) from 2-propyl-9-thioxo-pyrroloquinoline-1,3-dione **6a** (180 mg, 0.66 mmol) and methyl iodide/potassium carbonate as described for **8a**.

*2-Propyl-9-propylamino-2,3-dihydro-pyrrolo[3,4-b]quinoline-1,3-dione **12b***

Acid hydrolysis of 2-propyl-9-propylamino-3-propylimino-pyrroloquinoline **3a** (145 mg, 0.43 mmol) as for **12a** gave product **12b** (117 mg, 92%). Crystals (from ethyl acetate-hexane), m.p. 118–120°C; δ 0.97(3H, t, J7.4), 1.13(3H, t, J7.4), 1.6–1.9(4H, m), 3.69(2H, t, J7.3), 3.93(2H, q, simplified to t, J7.0 by D_2O), 7.3(1H, br peak, overlapping $CDCl_3$ signal, simplified to the latter by D_2O), 7.55(1H, m, ArH), 7.75(1H, m, ArH), 8.23(1H, m, ArH).

*Formation of dimers **14** and **15***

Complex **4a** (150 mg, 0.40 mmol) and 4-chloro-2-propyl-carbamoyl-3-quinolinecarboxylic acid amide **13** (150 mg, 0.43 mmol) were dissolved in ethyl acetate (5 mL) with warming, after which the solution was allowed to remain at room temperature for 5 days. The solid which had separated was collected by filtration (256 mg) and crystallized from ethyl acetate to obtain (185 mg) a mixture (m.p. 242–245°C) of dimers **14** and **15**, as evidenced from ^{1}H NMR ($CDCl_3$) (*vide infra*), and HPLC (*iso*propyl alcohol-hexane, 10:490, 254 nm) analysis. TLC [(acetone-benzene, 1:7)] showed the two components with dimer **14** of slightly higher Rf value. Separation of the two

dimers was achieved on a silica gel column using acetone (~3%) in chloroform as eluent.

*Formation of dimer **14***

Complex **4a** (398 mg, 1.07 mmol) in toluene (15 mL) was heated to reflux temperature for 30 min in a stream of nitrogen. The vapours were passed into a solution of 2.277 M hydrochloric acid. Back-titration with standard sodium hydroxide established 0.96 mmol of propylamine (also identified as N-benzoyl-propylamine). Evaporation of the reaction gave a mixture (336 mg) of 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** and dimer **14**, The mixture was separated by treatment with aqueous ammonia and chloroform; evaporation of the chloroform extract gave dimer **14** (122 mg, 0.21 mmol) identified from (^{1}H NMR spectrum and HPLC) comparison with authentic product (*vide infra*). The ammoniacal solution was acidified and extracted with chloroform to give 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** (206 mg, 61%), identified from its ^{1}H NMR[7] spectrum and m.p. By contrast, refluxing **2a** in toluene solution for up to 5 h led to no production of dimer **14**.

*Synthesis of dimer **14***

A solution of 2-propyl-3-propylimino-9-thioxo-pyrroloquinoline **2a** (115 mg, 0.37 mmol) and 9-chloro-2-propyl-3-propylimino-pyrroloquinoline **11** (115 mg, 0.36 mmol) in dry dioxan (10 mL) containing triethylamine (0.5 mL) was stirred at room temperature for 2 days (an arbitrary time period). Evaporation gave a residue to which was added chloroform and water. Evaporation of the chloroform extract afforded dimer **14** (238 mg, ~100%), needles (from ethyl acetate), (m.p. 251–253°C), and free of contaminant dimer **15**, as evidenced from TLC (silica gel, acetone-benzene, 1:7), ^{1}H NMR, and HPLC (*iso*propyl alcohol-hexane, 10:490; 254 nm) examination. (Found: M^+, 592.2621. Calc. for $C_{34}H_{36}N_6O_2S$: M, 592.2621) δ 0.68(6H, t, J7.4), 1.05(6H, t, J7.4), 1.50(4H, m), 1.80(4H, m), 3.58 (4H, t, J7.0), 4.54(4H, t, 7.0), 7.63(2H, m, ArH), 7.85(2H, m, ArH), 8.26(2H, dd, 0.8, 8.4, ArH), 8.46(2H, dd, 0.9, 8.5, ArH). Mixture m.p. with dimer **15** (*vide infra*), 239–242°C.

*Synthesis of dimer **15***

Similar alkylation of 2-propyl-3-propylimino-9-thioxo-pyrrolo quinoline **2a** (94 mg, 0.30 mmol) with 9-chloro-2-propyl-3-thioxo-pyrroloquinoline **16** (87 mg, 0.30 mmol) and triethylamine in dry dioxan (10 mL) gave dimer **15** (177 mg, ~100%); crystals (from ethyl acetate), m.p. 230–232°C, free of dimer **14**, as evidenced from TLC and HPLC (*vide supra*). (Found: M^+, 567.1780. Calc. for $C_{31}H_{29}N_5O_2S_2$: M, 567.1763) δ 0.65–0.75(6H, m), 1.06(3H, t, J7.0), 1.5–1.65(4H, m), 1.75–1.85(2H, m), 3.61(1H, t, J7.2), 3.88(2H, t, J7.0), 4.53(2H, t, J6.0), 7.63–7.75(2H, m, ArH), 7.8–7.95(2H, m, ArH), 8.2–8.6(4H, m, ArH).

*Aminolysis of dimer(s) **14** + **15** with propylamine*

A mixture of approx. equimolar amounts of **14** + **15** (*vide supra)* (274 mg,~0.47 mmol) and propylamine (5 mL) was stirred at room temperature for 1 h or when TLC (acetone-benzene, 1:7) revealed the absence of dimer substrate. Excess amine was evaporated under reduced pressure and temperature and the residue was treated with water and chloroform. Evaporation of the chloroform extract gave (271 mg) of complex **4a** admixed with 9-propylamino-pyrroloquinoline **3a**. The components were separated using warm ethyl acetate to afford sparingly soluble complex **4a** (153 mg, 0.41 mmol), while evaporation of the filtrate gave 2-propyl-9-propylamino-3-propylimino-pyrroloquinoline **3a** (112 mg, 0.33 mmol), as was verified from the appropriate ^{1}H NMR spectra.

*Aminolysis of 9-alkythio-substituted pyrrolo[3,4-b]quinoline **8***

General procedure: a mixture of the appropriate substrate **8** (generally 0.5 mmol) and amine (3 mL, large mmol excess) was stirred at room temperature with TLC and/or HPLC *(iso*propyl alcohol-hexane, 3:497) monitoring to estimate the end of reaction (*cf.* Table 1). The product was isolated by evaporation of excess amine at reduced temperature (to avoid/minimize possible[7] amine-imine exchange), and was identified by comparison (IR, mixture m.p) with authentic[7,10] product **3**. The respective liberated mercaptan could be detected from its odour but was not isolated. Table 1 lists the experimental conditions and outcomes. New *2-Propyl-9-isopropylamino-3-propylimino-2,3-dihydro-pyrrolo[3,4-b]quinolin-1-one 3b* was obtained from substrate **8a** and 2-aminopropane (Table 1). Crystals (from aqueous ethanol), m.p. 77–78°C (Found: C,70.83; H,7.60; N,16.29. Calc. for $C_{20}H_{26}N_4O$: C,70.97; H,7.74; N,16.56) δ 0.96(3H, t, J7.4), 1.05(3H, t, J7.4), 1.43(6H, d, J6.3), 1.6–1.85(4H, m), 3.73(2H, t, J7.2), 4.4–4.6(3H, m), 7.45(1H, m, ArH), 7.6–7.7(2H, m, simplifies to 1H, m, with D_2O), 8.05(1H, d, J8.3, ArH), 8.18(1H, d, J8.6).

*9-Methoxy-2-propyl-3-propylimino-2,3-dihydro-pyrrolo(3,4-b) quinolin-1-one **8f***

a) To a solution of sodium (40 mg, 1.7 mmol) in methanol (2 mL) was added 9-methylthio-pyrroloquinoline **8a** (165 mg, 0.50 mmol). The reaction was complete in ~1 h (TLC monitoring). Water was added and the mixture extracted with chloroform. Evaporation of the extract gave product **8f** (155 mg, 0.50 mmol), crystals (from aqueous ethanol), m.p. 64°C (Found: C, 69.55; H, 6.59; N, 13.47. Calc. for $C_{18}H_{21}N_3O_2$: C,69.43; H,6.80; N,13.50).

b) 9-Methylthio-compound **8a** (600 mg, 1.83 mmol) was refluxed overnight in methanol (20 mL) containing anhydrous sodium acetate (2.0 g). Isolation as in a) gave **8f** (590 mg, ~100%), m.p. 63.5-64°C (from aqueous ethanol). In the absence of sodium acetate no **8f** was formed.

c) 9-Chloro-compound **11** (25 mg, 0.071 mmol) was added at room temperature to a solution of sodium (30 mg, ~1.3 mmol) in methanol (3 mL). After 1 h at room temperature the reaction mixture was treated as in a) to give **8f**, m.p. 63.5–64°C, identical (IR, mixture m.p., with the products in a) and b).

*9-Methoxy-pyrroloquinoline **8f** transformations*

a) *Aminolysis.* Treatment of **8f** (102 mg, 0.33 mmol) with propylamine (2 mL, large mmol excess) as for substrates **8** (*vide supra*) for 12 h gave 9-propylamino-pyrroloquinoline **3a** (118 mg, 0.35 mmol), m.p. 71°C (from ethanol), identical (mixture m.p. and IR) with the literature[7] compound.

b) *Thiolysis.* Substrate **8f** (10 mg, 0.03 mmol) was added to a solution of sodium (20 mg, 0.9 mmol) in methanol (2 mL) containing 1-propanethiol (95 mg, 1.2 mmol). TLC monitoring showed reaction was complete within 20 h at room temperature, and yielded 9-propylthio-pyrroloquinoline **8b**, identical with authentic[7] compound.

c) *Hydrolysis.* Compound **8g** (70.0 mg, mmol) in methanol (1 mL) and 2M HCl (1 mL) was warmed at ~50°C for 3 h, followed by cooling, dilution with water and filtration to give *2-propyl-2,3-dihydro-pyrrolo[3,4-b]quinoline-1,3,9-trione* **17** (49.0 mg,~93%), m.p. >250°C (Found: C,65.37; H,4.54; N,10.78. Calc. for $C_{14}H_{12}N_2O_3$: C,65.62; H,4.72; N, 10.93). Similar hydrolysis of substrate **8f** afforded the identical product.

*2-Propyl-3-propylimino-9-propoxy-2,3-dihydro-pyrrolo[3,4-b] quinolin-1-one **8g***

To a solution of sodium propoxide [prepared from sodium (40 mg, 1.7 mmol) and 1-propanol (1 mL)] was added

9-methylthio-pyrroloquinoline **8a** (165 mg, 0.51 mmol). After ~1 h at room temperature? TLC showed only product **8g**. Extraction (water and chloroform) and evaporation of the extract gave (194 mg, ~100%) 9-propoxy derivative **8g**; crystals (from aqueous ethanol), m.p. 57°C (Found: C,70.81; H,7.07; N,12.45. Calc. for $C_{20}H_{25}N_3O_2$: C,70.77; H,7.42; N, 12.38) δ 0.97 (3H, t, J7.4), 1.07–1.14 (6H, m), 1.70–1.97(6H, m),3.80 (2H, t, J7.2), 4.53 (2H, t, J7.0), 4.94 (2H, t, J6.4), 7.59–7.62 (1H, m), 7.77 (1H, m), 8.10(1H, d, J8.4), 8.40(1H, d, J8.4).

*6-Fluoro-2-propyl-3-propylimino-9-thioxo-2,3-dihydro-pyrrolo[3,4-b] quinolin-1-one-propylamine complex **4f***

6-Fluoro-3,3,9-trichlorothieno[3,4-b]quinolin-1-one[10] **1c** (1.3g, 4.03 mmol) was added in small portions (over a period of 10–15 min) with stirring to ice-cold propylamine (10 mL; large mmol excess) after which the reaction was continued and worked up in the standard manner[6] to obtain product **4f** (0.82 g, 53%). Crystals (from ethyl acetate), m.p. 120–145°C (decomp.) δ 0.88–0.94 (6H, m), 1.03 (3H, t, J7.3), 1.58–1.82 (6H, m), 2.88(2H, t, J7.5), 3.71(2H, t, J7.3), 4.48(2H, t, J7.0), 4.9(~3H, br peak), 7.23(1H, m, ArH), [7.28 ($CDC1_3$)], 7.56(1H, dd, J2.6, 10.0, ArH), 8.96(1H, m, ArH). Volumetric analysis (as for complex **4d**) established an equimolar (**2c**: amine) composition. From the combined filtrate and washings was isolated 6-fluoro-2-propyl-9-propylamino-3-propylimino-2,3-dihydro-pyrrolo[3,4-b]quinolin-1-one **3d**. Crystals (from ethanol), m.p. 76–78 C (175 mg, 12%); δ 0.95(3H, t, J7.3), 1.04(3H, t, J7.3), 1.12(3H, t, J7.3), 1.7–1.9(6H, m), 3.72(2H, t, J6.8), 3.80(2H, q, simplifies to t, J6.8, with D_2O), 4.47(2H, t, J6.9), 7.20(1H, m, ArH), 7.66(1H, d, J10.1, ArH) 7.90(1H, br peak, removed by D_2O, NH), 8.29(1H, m, ArH).

*6-Fluoro-2-propyl-3-propylimino-9-thioxo-2,3-dihydro-pyrrolo[3,4-b] quinolin-1-one **2c***

Complex **4f** (168 mg, 0.43 mmol) was added with stirring to glacial acetic acid (1 mL) at room temperature. After 10 min reaction water was added dropwise till precipitation of product **2c** was complete. This was collected by filtration, washed with water and dried to yield 135 mg (95%) compound **2c**. Crystals (from ethyl acetate), m.p. 119–122°C (Found: C,61.51; H,5.37; N,12.55. Calc. for $C_{17}H_{18}FN_3OS$: C,61.61; H,5.47; N,12.68) δ ($CDC1_3$ + DMSO-d_6) 0.96(3H, t, J7.4), 1.05(3H, t, J7.4), 1.68(2H, m), 1.85(2H, m), 3.80(2H, t, J7.3), 4.47(2H, t, J7.1), 7.30(1H, m, ArH), 7.47(1H, m, ArH), [8.12 ($CDC1_3$)], 8.94(1H, m, ArH).

*6-Fluoro-2-propyl-9-thioxo-2,3-dihydro-pyrrolo[3,4-b]quinoline-1,3-dione **6b***

To a solution of complex **4f** (194 mg, 0.50 mmol) in methanol (5 mL) was added 2M HCl (2 mL). The reaction was kept at 50°C for 12 h and diluted with water. Product **6b** was collected by filtration, washed with water and dried (140 mg; 97%). Crystals (from methanol), m.p. 210–212°C (Found: C,57.66; H,3.73, N,9.51. Calc. for $C_{14}H_{11}FN_2O_2S$: C 57.92; H,3.82; N,9.65) δ ($CDC1_3$ + DMSO-d_6) 0.96(3H, t, J7.4), 1.7(2H, m), 3.63(2H, t, J7.3), 7.26(1H, m, ArH), [7.85($CDC1_3$)], 7.62(1H, m, ArH), 8.83(1H, m, ArH).

Antimicrobial Activity

Broad-spectrum antimicrobial screening was performed on the compounds shown in Table 2 using a disc diffusion (DD) method. From the initial screening a number of these were identified as having some antimicrobial activity. This activity was then assessed more quantitatively using a minimum inhibitory concentration (MIC) procedure.

Disc diffusion studies. Antimicrobial DD assays were performed on four reference bacterial strains: *Escherichia coli* (ATCC 11775), *Staphylococcus aureus* (ATCC 25923), *Stapthylococcus epidermidis* (ATCC 2223) and *Bacillus cereus* (ATCC 11778). Base layers of Tryptone Soya (Oxoid) agar were prepared for bacterial cultures. Spore suspensions yielding an inoculum size of approximately 1×10^6 CFU mL^{-1} were thoroughly mixed into the overlaying agar surface. With aseptic manipulation,. 6 mm discs were saturated with a solution containing the accurately weighed (8–12 mg) compound dissolved in dimethyl sulphoxide (Merck) at a starting concentration of 50 mg mL^{-1} and placed onto the set agar. Ciprofloxacin discs (1 μg, Oxoid) were used for positive bacterial controls. All plates were incubated at 37°C for 24 h. Tests were done in duplicate, and the measured inhibition zones assigned an arbitrary rating: 7–8 mm = weakly active; 10–12 mm = moderately active; 14–16 mm = good activity.

Microplate bioassay. Minimum inhibitory concentrations (MIC) were determined on most of the compounds which showed a positive antimicrobial activity (DD), using the INT microplate method as described by Eloff[14]. The respective dimethyl sulphoxide solutions, at starting concentrations of 1.25 mg mL^{-1}, were transferred into the first well of a microtitre plate. Serial dilutions were performed so that the extract concentrations of 0.625, 0.313, 0.156, 0.078, 0.039, 0.020 and 0.013 mg mL^{-1} were obtained. A fixed bacterial culture yielding an inoculum size of 1×10^6 CFU mL^{-1} was added to all wells and incubated at 37°C for 24 h. An amount of 40 μL of a 0.2 mg mL^{-1} *p*-iodonitrotetrazolium violet (INT) solution was transferred to all inoculated wells and examined to determine a colour change in relation to concentration of microbial growth after 6 h. MIC determinations were run on propylamine, triethylamine and *tert*-butylamine, respectively, utilizing a MIC microtitre plate covered with a plastic seal to avoid or at least minimize loss of amine by evaporation. These amines showed no measurable activities in the DD and MIC tests.

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References

1 J-B. Fourtillan, M. Fourtillan, J-C. Jacquesy, O. Karam, F. Zunio and M-P. Jouannetaud, French Patent (2000). *Chem. Abstr.,* **133**, 321875z.

2 H. Wang and A. Ganeson, *Tetrahedron Lett.,* 1998, **39**, 3097.

3 T. Tanaka, K. Mashimo, M. Miyazaki and M. Sato, Jap. Patent (1972). *Chem. Abstracts,* **78**, 4227.

4 J.M.D. Fortunak, A.R. Mastrocola, M. Mellinger, N.J. Sisti, J.L. Wood and Z-P. Zhuang, *Tetrahedron Lett.,* 1996, **37**, 5679. J.S.Yadav, S. Chandrasekhar, *Tetrahedron,* 1999, **55**, 5449.

5 M. Konishi, N. Fukuda, Y. Oku, H. Yamazaki, K. Imaizumi and H. Kobayashi. Jap. Patent (1996). *Chem. Abstr.*, 1996, **125**, 114509c. See also: M-G. Kayirere, A. Mahamoud, J. Chevalier, J-C. Soyfer, A. Cremieux and J. Barbe, *Eur. J. Med. Chem.*, 1998, **33**, 55.

6 T. van Es, B. Staskun and M.A.Fernandes, *S. Afr J. Chem.,* 2003, **56**, 30.

7 T. van Es and B. Staskun, *S. Afr. J. Chem.*, 2003, **56**, 40.

8 A similar (thiolate) salt is formed when 4-thioxoquinoline is treated with (liquid) ammonia. C.A. Kraus and G.F. White, *J. Amer. Chem. Soc.,* 1923, **45**, 768.

9 M.A. Francisco and A. Kurs, *J. Org. Chem.*, 1988, **53**, 596.

10 D.C. Levendis, J. Moffit, B. Staskun and T. van Es, *J. Chem. Res* (S)., 1999, 604.

11 Attention is drawn to the possibility of obtaining a reduced/inconsistent reading of antimicrobial activity when testing a particularly thermally-sensitive complex **4** in which a significant degree of dissociation (to component **2** and amine) might occur on warming in DMSO.

12 T. van Es and B. Staskun, *S. Afr. J. Chem.*, 2002, **55**, 13.

13 R. Albrecht, *Prog. Drug Res.*, 1977, **21**, 9.

14 J.N. Eloff, *Planta Med.*, 1988, 711.