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Synthesis of new 4-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]quinoxaline and 5-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine derivatives, as potential bacterial multidrug resistance pump inhibitors*

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Abstract

The synthesis of new 4-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l** is described in five or six steps starting from various substituted nitroanilines **2a-e**. The bioisostere 5-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **1m** was also prepared. The new derivatives were evaluated as efflux pump inhibitors (EPIs) in a model targeting the NorA system of *Staphylococcus aureus*. The antibiotic susceptibility of two strains overproducing NorA, SA-1199B and SA-1, was determined alone and in combination with the neo-synthesised compounds by the agar diffusion method and MIC determination, in comparison with reserpine and omeprazole taken as reference EPIs. A preliminary structure-activity relationship study firstly allowed to clarify the influence of the substituents at positions 7 and/or 8 of the pyrrolo[1,2-*a*]quinoxaline nucleus. Methoxy substituted compounds, **1b** and **1g,** were more potent EPIs than the unsubstituted compounds (**1a** and **1f**), followed by chlorinated derivatives (**1c-d** and **1h**). Moreover, the replacement of the *N*,*N*-diethylamino group (compounds **1a-e**) by a bioisostere such as pyrrolidine (compounds **1f-h**) enhanced the EPI activity, in contrast with the replacement by a piperidine moiety (compounds **1i-k**). Finally, the pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine compound **1m** exhibited a higher EPI activity than its pyrrolo[1,2-*a*]quinoxaline analogue **1a**, opening the way to further pharmacomodulation.

Keywords: *MDR, 4-[2-(alkylamino)ethylthio]pyrrolo[1, 2-a]quinoxaline, Staphylococcus aureus, NorA efflux pump inhibitors*

Introduction

Active efflux is a mechanism of cell detoxification, reported in an increasing variety of organisms, including bacteria [1–3]. Probably all bacterial species possess an array of intrinsic efflux systems capable to export a wide range of structurally unrelated antibiotics and biocides, the so-called "Multi-Drug Resistant" (MDR) efflux pumps, resulting in a reduced intracellular accumulation and thus drug insensitivity [4]. *Staphylococcus aureus* is a major human pathogen, responsible for skin and soft tissues infections, and septicaemia. Among the MDR efflux pumps present in this species, NorA, which belongs to the Major Facilitator Superfamily (MFS) is considered both as the most efficient and as

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representative of the MDR systems in the Gram positive bacteria. In particular, NorA promotes the active efflux of fluoroquinolones, an important class of broad-spectrum antimicrobials with a potent activity [5–7]. Furthermore, this system protects the cell against chloramphenicol, a wide range of organic compounds like ethidium bromide, rhodamine, and amphipathic cations such as benzalkonium chloride and cetrimide [8,9].

Strategies to combat efflux-mediated resistance are based on the search for either new antibiotics bypassing the efflux systems or efflux pump inhibitors (EPIs). The latter option is attractive since a single EPI active against MDR pumps and used as adjunct therapy should (i) decrease intrinsic resistance, (ii) reverse acquired resistance and (iii) reduce the emergence of highly resistant mutants towards a series of existing antibiotics. It has been previously shown that drug efflux can be inhibited by the toxic protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), but also by drugs which are in clinical use for other indications such as the competitive pump blocker reserpine [10–16,20], omeprazole [10], verapamil [10,17], paroxetine [13,18] and chlorpromazine [12]. On the other hand, the screening of natural or chemical compounds has allowed to identify compounds endowed with an EPI activity. In a library of synthetic compounds, thioquinolines and quinolines derivatives have been found to behave as EPIs on several prokaryotic and eukaryotic systems [19–21].

The pyrrolo[1,2-*a*]quinoxaline nucleus, previously developed as a template for the design of new compounds active on many biological targets, is an analogue of the quinoline or quinoxaline moiety [23–25]. In this context and as part of a programme on the development of antibacterial agents, the aim of the present study was to synthesise new 4-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]quinoxaline derivatives, which are structural analogues of the thioquinolines, and to assess their *in vitro* activity as EPIs in a model targeting the NorA efflux pump of *S. aureus* (Figure 1) [19–22].

Materials and methods

Chemistry

Instrumentation. Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and reported uncorrected. Infrared (IR) spectra were determined in KBr discs on a BRUKER IFS-25 spectrometer. NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer (300 MHz). Chemical shifts refer to tetramethylsilane which was used as an internal standard. Elemental analyses were conducted by CNRS, Vernaison, France and the results were within ± 0.3% of their calculated values.

*1-(5-Phenyl-2-nitrophenyl)pyrrole (**3e**)*. A mixture of 5-chloro-2-nitroaniline **2e** (0.018 mol) and 2,5-dimethoxytetrahydrofuran (0.018 mol) in acetic acid (40 mL) was refluxed for 1 h with vigorous stirring. After cooling, the reaction mixture was poured into water (100 mL). The precipitate was filtered, washed with water and dissolved in diethyl ether (110 mL), dried over magnesium sulfate and evaporated to dryness under reduced pressure to give a crude oil which was extracted with petroleum ether to yield **3e**. Yield: 65%, orange oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 6.43 (dd, *J* 2.20 and 2.20 Hz, 2H, H-β), 6.89

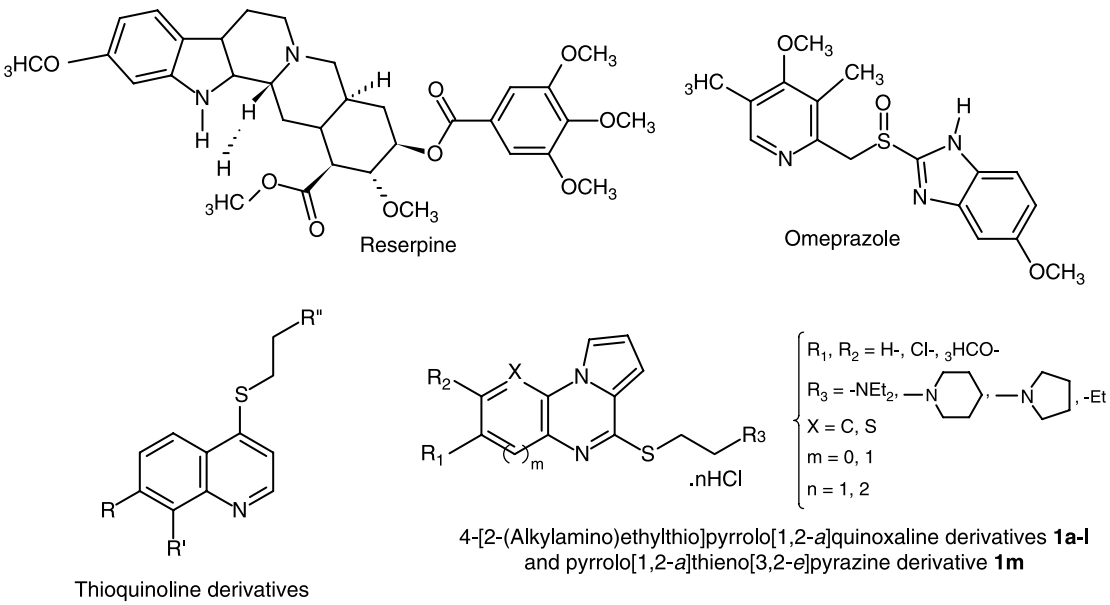


Figure 1. Structure of reserpine, omeprazole, thioquinoline derivatives and new compounds **1a–m**.

(dd, J 2.20 and 2.20 Hz, 2H, H-α), 7.51-7.55 (m, 3H, H-4, H-3' and H-5'), 7.63-7.70 (m, 4H, H-2', H-6', H-4' and H-6), 7.99 (d, J 8.10 Hz, 1H, H-3). Anal. Calcd for $C_{16}H_{12}N_2O_2$: C, 72.71; H, 4.58; N, 10.60. Found: C, 72.89; H, 4.67; N, 10.53.

*1-(5-Phenyl-2-aminophenyl)pyrrole (**4e**)*. To a solution of 1-(5-chloro-2-nitrophenyl)pyrrole **3e** (0.012 mol) in ethanol (45 mL) was added $BiCl_3$ (0.018 mol). Sodium borohydride (0.10 mol) was added portion-wise at 0°C to the reaction mixture which was then stirred at room temperature for 2 h. The reaction mixture was then poured into an aqueous hydrochloric acid solution (1M, 45 mL) and stirred for 1 h. Ethanol was evaporated under reduced pressure. The residue was made alkaline with 30% aqueous ammonium hydroxide solution and then extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The oily residue was then extracted with petroleum ether to give **4e**. Yield: 37%, pale-orange oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 3.81 (bs, 2H, NH_2), 6.40 (dd, J 2.15 and 2.15 Hz, 2H, H-β), 6.93 (dd, J 2.15 and 2.15 Hz, 2H, H-α), 7.28-7.47 (m, 6H, H-3, H-4, H-6, H-3', H-4' and H-5'), 7.55-7.59 (m, 2H, H-2' and H-6'). Anal. Calcd for $C_{16}H_{14}N_2$: C, 82.02; H, 6.02; N, 11.96. Found: C, 81.87; H, 5.81; N, 12.13.

*8-Phenyl-5H-pyrrolo[1,2-a]quinoxalin-4-one (**5e**)*. To a solution of compound **4e** (4.5 mmol) in toluene (20 mL) was added triphosgene (1.5 mmol). The reaction mixture was refluxed for 4 h, and nitrogen was bubbled in to drive off excess of phosgene. The solution was then set aside for 30 min. The heavy crystalline precipitate was filtered off and washed with diethyl ether to give **5e**. Yield: 36%, beige crystals, mp > 260°C; IR ν_{max} (KBr)/cm^{-1} 3300 (NH), 1685 (CO). ^{1}H NMR δ (300 MHz, d_6-DMSO) 6.71 (dd, J 3.80 and 2.80 Hz, 1H, H-2), 7.06 (dd, J 3.80 and 1.40 Hz, 1H, H-3), 7.39 (t, J 7.45, 2H, H-3' and H-5'), 7.48 (d, J 8.40 Hz, 1H, H-6), 7.51 (t, J 7.45 Hz, 1H, H-4'), 7.62 (dd, J 8.40 and 1.80 Hz, 1H, H-7), 7.80 (d, J 7.45 Hz, 2H, H-2' and H-6'), 8.35 (d, J 1.80 Hz, 1H, H-9), 8.41 (dd, J 2.80 and 1.40 Hz, 1H, H-1), 11.32 (s, 1H, NH). Anal. Calcd for $C_{17}H_{12}N_2O$: C, 78.44; H, 4.65; N, 10.76. Found: C, 78.65; H, 4.78; N, 10.97.

*4-Chloro-8-phenylpyrrolo[1,2-a]quinoxaline (**6e**)*. A solution of 8-chloro-5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one **5e** (1.35 mmol) in $POCl_3$ (6 mL) was refluxed for 4 h. After removing excess of reactive under *vacuum*, the residue was carefully dissolved in water at 0°C and the resulting solution was made basic with 30% aqueous ammonium hydroxide solution. The precipitate was filtered, dried and recrystallized from ethyl acetate to give **6e**. Yield: 72%, beige crystals, mp = 128°C; ^{1}H NMR δ (300 MHz, $CDCl_3$) 6.93 (dd, J 4.00 and 2.75 Hz, 1H, H-2), 7.09 (dd, J 4.00 and 1.30 Hz, 1H, H-3), 7.45 (t, J 7.35 Hz, 1H, H-4'), 7.53 (t, J 7.35 Hz, 2H, H-3' and H-5'), 7.68 (dd, J 8.40 and 1.90 Hz, 1H, H-7), 7.70 (d, J 7.35 Hz, 2H, H-2' and H-6'), 7.96 (d, J 8.40 Hz, 1H, H-6), 8.00 (d, J 1.90 Hz, 1H, H-9), 8.04 (dd, J 2.75 and 1.30 Hz, 1H, H-1). Anal. Calcd for $C_{17}H_{11}ClN_2$: C, 73.25; H, 3.98; N, 10.05. Found: C, 73.20; H, 4.11; N, 10.25.

*Synthesis of Pyrrolo[1,2-a]quinoxaline-4-thiols (**8a-c**)*. A mixture of 1-(2-aminophenyl)pyrrole **4a-c** (25.3 mmol) in ethanol (120 mL) and CS_2 (89 mmol) and sodium hydroxide (50.6 mmol) in water (6 mL) was refluxed for 48 h. The product obtained after removal of ethanol was purified by dissolving in a hot 5% aqueous sodium hydroxide solution, filtering and acidifying the filtrate with acetic acid. Repeated washings with water, then with ethanol, and crystallization from ethanol gave pyrrolo[1,2-*a*]quinoxaline-4-thiols **8a-c**.

*Pyrrolo[1,2-a]quinoxaline-4-thiol (**8a**)*. Yield: 56%, beige crystals, mp = 274°C [26]; IR ν_{max} (KBr)/cm^{-1} 2380 (SH). ^{1}H NMR δ (300 MHz, d_6-DMSO) 6.79 (dd, J 4.00 and 2.70 Hz, 1H, H-2), 7.29 (dd, J 4.00 and 1.40 Hz, 1H, H-3), 7.34 (m, 2H, H-7 and H-8), 7.55 (dd, J 8.00 and 1.25 Hz, 1H, H-6), 8.11 (dd, J 8.00 and 1.25 Hz, 1H, H-9), 8.32 (dd, J 2.70 and 1.40 Hz, 1H, H-1), 12.90 (s, 1H, SH).

*7-Methoxypyrrolo[1,2-a]quinoxaline-4-thiol (**8b**)*. Yield: 61%, yellow crystals, mp = 245°C; IR (KBr), cm^{-1}: 2390 (SH); ^{1}H NMR δ (300 MHz, d_6-DMSO) 3.81 (s, 3H, CH_3O), 6.75 (dd, J 4.05 and 2.75 Hz, 1H, H-2), 6.98 (dd, J 9.05 and 2.75 Hz, 1H, H-8), 7.12 (d, J 2.75 Hz, 1H, H-6), 7.26 (dd, J 4.05 and 1.50 Hz, 1H, H-3), 8.08 (d, J 9.05 Hz, 1H, H-9), 8.26 (dd, J 2.75 and 1.50 Hz, 1H, H-?), 8.32 (dd, J 2.70 and 1.40 Hz, 1H, H-1), 12.80 (s, 1H, SH). Anal. Calcd for $C_{12}H_{10}N_2OS$: C, 62.59; H, 4.38; N, 12.17. Found: C, 62.72; H, 4.37; N, 12.25.

*8-Chloropyrrolo[1,2-a]quinoxaline-4-thiol (**8c**)*. Yield: 28%, beige crystals, mp = 293°C; IR (KBr), cm^{-1}: 2360 (SH); ^{1}H NMR δ (300 MHz, d_6-DMSO) 6.82 (dd, J 4.00 and 2.75 Hz, 1H, H-2), 7.32 (dd, J 4.00 and 1.45 Hz, 1H, H-3), 7.44 (dd, J 8.70 and 2.10 Hz, 1H, H-7), 7.56 (d, J 8.70 Hz, H-6), 8.36 (d, J 2.10 Hz, 1H, H-9), 8.41 (dd, J 2.75 and 1.45 Hz, 1H, H-1), 13.00 (s, 1H, SH). Anal. Calcd for $C_{11}H_7ClN_2S$: C, 56.29; H, 3.01; N, 11.94. Found: C, 56.49; H, 2.95; N, 12.04.

*Synthesis of 4-[2-(N,N-Diethylamino)ethylthio]pyrrolo [1,2-a]quinoxalines (7**a-d**), and 5-[2-(N,N-Diethylamino)ethylthio]pyrrolo[1,2-a]thieno[3,2-e] pyrazine (**10**)*. Method A: To the sodium salt of N,N-diethylaminoethylthiol prepared from the thiol (3 mmol) and sodium hydride (4.5 mmol of 60% suspension in mineral oil) in dioxane (40 mL) in 1 h was added 4-chloropyrrolo[1,2-a]quinoxaline **6a-d** or 4-chloropyrrolo[1,2-a]thieno[3,2-e]pyrazine **9** (3 mmol). The mixture was heated at 100°C for 4 h. Dioxane was evaporated under reduced pressure, and the oily residue was treaturated with water then extracted with diethyl ether. The organic layer was washed with water (100 mL), dried over sodium sulfate and evaporated to dryness. The residue was flash chromatographed on silica gel, eluting with 90% chloroform/10% methanol to give **7a-d** and **10**. Method B: To a mixture of CuI (0.12 mmol), potassium carbonate (45.3 mmol) and 4-chloropyrrolo[1,2-a]quinoxaline **6a** (2.46 mmol) under nitrogen were added 2-propanol (5 mL), ethylene glycol (4.93 mmol) and the N,N-diethylaminoethylthiol (2.46 mmol). The reaction mixture was then heated to 80°C and stirred for 24 h. After cooling, ethyl acetate (15 mL) was added. The reaction mixture was then filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with 90% chloroform / 10% methanol to afford the desired thioether **7a**. Method C: To a mixture of t-BuONa (3.69 mmol), CuI (0.25 mmol), and neocuproine (0.25 mmol) under nitrogen were added the N,N-diethylaminoethylthiol (2.71 mmol), the 4-chloropyrrolo[1,2-a]quinoxaline **6a** (2.46 mmol) and toluene (15 mL). The reaction mixture was then stirred at 110°C for 24 h. The mixture was then cooled to room temperature and filtered to remove any insoluble residues. The filtrate was concentrated in vacuo, the residue was then purified by flash chromatography on silica gel, eluting with 90% chloroform/10% methanol, to obtain the pure product **7a**. Method D: A solution of **6a** (2.46 mmol), N,N-diethylaminoethylthiol (2.46 mmol), t-BuOK (4.92 mmol), and catalytic amount of Pd $(PPh_3)_4$ (0.1 mmol) in n-butanol (25 mL) was heated at 110°C under a nitrogen stream with stirring for 5 h. The mixture was then cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure, the residue was then purified by flash chromatography on silica gel, eluting with 90% chloroform / 10% methanol, to obtain the pure product **7a**.

*4-[2-(N,N-Diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**a**)*. Yield: 42% (method A), 60% (method B), 80% (method C), 66%(method D), yellow oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 0.98 (t, *J* 7.10 Hz, 6H, $2CH_3$), 2.55 (q, *J* 7.10 Hz, 4H, $2CH_2$), 2.72 (t, *J* 7.20 Hz, 2H, CH_2), 3.37 (t, *J* 7.20 Hz, 2H, CH_2), 6.85 (m, 2H, H-2 and H-3), 7.43 (t, *J* 7.50 Hz, 1H, H-7), 7.45 (t, *J* 7.50 Hz, 1H, H-8), 7.70 (d, *J* 7.50 Hz, 1H, H-6), 8.20 (d, *J* 7.50 Hz, 1H, H-9), 8.38 (dd, *J* 2.70 and 1.45 Hz, 1H, H-1). Anal. Calcd for $C_{17}H_{21}N_3S$: C, 68.19; H, 7.07; N, 14.03. Found: C, 68.28; H, 6.95; N, 14.21.

*7-Methoxy-4-[2-(N,N-diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**b**)*. Yield: 44% (method A), orange oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.01 (t, *J* 7.15 Hz, 6H, $2CH_3$), 2.60 (q, *J* 7.15 Hz, 4H, $2CH_2$), 2.77 (t, *J* 7.40 Hz, 2H, CH_2), 3.41 (t, *J* 7.40 Hz, 2H, CH_2), 3.85 (s, 3H, CH_3O), 6.81 (dd, *J* 4.15 and 2.45 Hz, 1H, H-2), 6.83 (dd, *J* 4.15 and 1.30 Hz, 1H, H-3), 7.10 (dd, *J* 8.95 and 2.70 Hz, 1H, H-8), 7.20 (d, *J* 2.70 Hz, 1H, H-6), 8.13 (d, *J* 8.95 Hz, 1H, H-9), 8.33 (dd, *J* 2.45 and 1.30 Hz, 1H, H-1). Anal. Calcd for $C_{18}H_{23}N_3SO$: C, 65.62; H, 7.03; N, 12.76. Found: C, 65.75; H, 7.17; N, 12.79.

*8-Chloro-4-[2-(N,N-diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**c**)*. Yield: 45% (method A), yellow oil; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.00 (t, *J* 7.20 Hz, 6H, $2CH_3$), 2.54 (q, *J* 7.20 Hz, 4H, $2CH_2$), 2.73 (t, *J* 7.35 Hz, 2H, CH_2), 3.37 (t, *J* 7.35 Hz, 2H, CH_2), 6.86 (dd, *J* 3.90 and 2.85 Hz, 1H, H-2), 6.88 (dd, *J* 3.90 and 1.30 Hz, 1H, H-3), 7.43 (dd, *J* 8.65 and 2.30 Hz, 1H, H-7), 7.68 (d, *J* 8.65 Hz, 1H, H-6), 8.40 (d, *J* 2.30 Hz, 1H, H-9), 8.45 (dd, *J* 2.85 and 1.30 Hz, 1H, H-1); ^{13}C NMR δ (100 MHz, d_6-DMSO) 12.0 (CH_3), 25.7 (CH_2), 46.4 (CH_2), 51.6 (CH_2), 105.9 (C-2), 114.0 (C-3), 114.7 (C-1), 116.9 (C-9), 123.3 (C-3a), 125.5 (C-8), 127.0 (C-9a), 129.1 (C-7), 130.5 (C-6), 133.7 (C-5a), 153.9 (C-4). Anal. Calcd for $C_{17}H_{20}ClN_3S$: C, 61.15; H, 6.04; N, 12.59. Found: C, 61.02; H, 6.15; N, 12.74.

*7-Chloro-4-[2-(N,N-diethylamino)ethylthio]pyrrolo [1,2-a]quinoxaline (7**d**)*. Yield: 26% (method A), orange oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.18 (t, *J* 7.15 Hz, 6H, $2CH_3$), 2.77 (q, *J* 7.15 Hz, 4H, $2CH_2$), 2.95 (t, *J* 7.55 Hz, 2H, CH_2), 3.52 (t, *J* 7.55 Hz, 2H, CH_2), 6.81 (dd, *J* 4.00 and 2.75 Hz, 1H, H-2), 6.93 (dd, *J* 4.00 and 1.25 Hz, 1H, H-3), 7.38 (dd, *J* 8.70 and 2.35 Hz, 1H, H-8), 7.73 (d, *J* 8.70 Hz, 1H, H-9), 7.79 (d, *J* 2.35 Hz, 1H, H-6), 7.84 (dd, *J* 2.75 and 1.25 Hz, 1H, H-1). Anal. Calcd for $C_{17}H_{20}ClN_3S$: C, 61.15; H, 6.04; N, 12.59. Found: C, 61.28; H, 6.14; N, 12.49.

*8-Phenyl-4-[2-(N,N-diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**e**)*. Yield: 38% (method A), yellow oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.19 (t, *J* 7.15 Hz, 6H, $2CH_3$), 2.76 (q, *J* 7.15 Hz, 4H, $2CH_2$), 2.95 (t, *J* 7.45 Hz, 2H, CH_2), 3.55 (t, *J* 7.45 Hz, 2H, CH_2), 6.84 (dd, *J* 4.00 and 2.75 Hz, 1H, H-2), 6.94 (dd, *J* 4.00 and 1.30 Hz, 1H, H-3), 7.44 (t, *J* 7.40 Hz, 1H, H-4'), 7.52 (t, *J* 7.40 Hz, 2H, H-3' and H-5'), 7.70 (dd, *J* 8.40 and 1.90 Hz, 1H, H-7), 7.73

(d, J 7.40 Hz, 2H, H-2' and H-6'), 7.89 (d, J 8.40 Hz, 1H, H-6), 7.95 (dd, J 2.75 and 1.30 Hz, 1H, H-1), 7.99 (d, J 1.90 Hz, 1H, H-9). Anal. Calcd for $C_{23}H_{25}N_3S$: C, 73.56; H, 6.71; N, 11.19. Found: C, 73.75; H, 6.85; N, 11.36.

*5-[2-(N,N-Diethylamino)ethylthio]pyrrolo[1,2-a]thieno[3,2-e]pyrazine (**10**)*. Yield: 33% (method A), yellow oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.18 (t, $J = 7.05$, 6H, $2CH_3$), 2.75 (q, $J = 7.05$, 4H, $2CH_2$), 2.95 (t, J 7.40 Hz, 2H, CH_2), 3.51 (t, J 7.40 Hz, 2H, CH_2), 6.86 (dd, J 4.15 and 2.65 Hz, 1H, H-7), 6.92 (dd, J 4.15 and 1.30 Hz, 1H, H-6), 7.08 (d, J 5.65 Hz, 1H, H-3), 7.34 (d, J 5.65 Hz, 1H, H-2), 7.45 (dd, J 2.60 and 1.30 Hz, 1H, H-8). Anal. Calcd for $C_{15}H_{19}N_3S_2$: C, 58.98; H, 6.27; N, 13.76. Found: C, 58.85; H, 6.35; N, 13.89.

*Synthesis of 4-[2-(Pyrrolidino)ethylthio]pyrrolo[1,2-a]quinoxalines (7**f-h**), 4-[2-(Piperidino)ethylthio]pyrrolo[1,2-a]quinoxalines (7**i-k**), and 4-Butylthio]pyrrolo[1,2-a]quinoxaline (7**l**)*. To a solution of the pyrrolo[1,2-a]quinoxaline-4-thiol **8a-c** (5 mmol) in DMF (25 mL) was added sodium hydride (7.5 mmol of 60% suspension in mineral oil). The mixture was stirred at room temperature for 2 h, and then treated with 2-pyrrolidinoethyl chloride, or 2-piperidinoethyl chloride or butyl chloride (5.5 mmol) in DMF (8 mL). The temperature was raised to 90°C and the mixture kept stirred for 18 h. The solvent was then removed under reduced pressure. After treatment of the residue by an excess of HCl (2 M), the solution was filtered. The filtrate was extracted with diethyl ether and the aqueous phase made alkaline with 30% aqueous ammonium hydroxide solution, then extracted with diethyl ether (100 mL). The organic layer was washed with water (80 mL), dried over sodium sulfate and evaporated to dryness. The residue was flash chromatographed on silica gel, eluting with 95% dichloromethane / 5% methanol to give **7f-l**.

*4-[2-(Pyrrolidino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**f**)*. Yield: 80%, orange oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.87 (m, 4H, $2CH_2$ pyrrolidine), 2.73 (m, 4H, $2CH_2$ pyrrolidine), 2.92 (t, J 7.40 Hz, 2H, CH_2), 3.63 (t, J 7.40 Hz, 2H, CH_2), 6.84 (dd, J 4.00 and 2.75 Hz, 1H, H-2), 6.94 dd, J 4.00 and 1.30 Hz, 1H, H-3), 7.45 (m, 2H, H-7 and H-8), 7.82 (d, J 7.55 Hz, 1H, H-6), 7.87 (d, J 7.55 Hz, 1H, H-9), 7.89 (dd, J 2.75 and 1.30 Hz, 1H, H-1). Anal. Calcd for $C_{17}H_{19}N_3S$: C, 68.65; H, 6.44; N, 14.13. Found: C, 68.68; H, 6.61; N, 14.23.

*7-Methoxy-4-[2-(pyrrolidino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**g**)*. Yield: 57%, orange oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.92 (m, 4H, $2CH_2$ pyrrolidine), 2.79 (m, 4H, $2CH_2$ pyrrolidine), 2.99 (t, J 7.45 Hz, 2H, CH_2), 3.66 (t, J 7.45 Hz, 2H, CH_2), 3.92 (s, 3H, CH_3O), 6.77 (dd, J 4.05 and 2.70 Hz, 1H, H-2), 6.90 (dd, J 4.05 and 1.30 Hz, 1H, H-3), 7.04 (dd, J 8.95 and 2.85 Hz, 1H, H-8), 7.33 (d, J 2.85 Hz, 1H, H-6), 7.72 (d, J 8.95 Hz, 1H, H-9), 7.82 (dd, J 2.70 and 1.30 Hz, 1H, H-1). Anal. Calcd for $C_{18}H_{21}N_3SO$: C, 66.02; H, 6.46; N, 12.83. Found: C, 65.85; H, 6.56; N, 12.98.

*8-Chloro-4-[2-(pyrrolidino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**h**)*. Yield: 79%, orange crystals, mp = 37°C; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.87 (m, 4H, $2CH_2$ pyrrolidine), 2.77 (m, 4H, $2CH_2$ pyrrolidine), 2.96 (t, J 7.40 Hz, 2H, CH_2), 3.62 (t, J 7.40 Hz, 2H, CH_2), 6.82 (dd, J 4.00 and 2.80 Hz, 1H, H-2), 6.93 (dd, J 4.00 and 1.30 Hz, 1H, H-3), 7.37 (dd, J 8.65 and 2.20 Hz, 1H, H-7), 7.76 (d, J 8.65 Hz, 1H, H-6), 7.79 (d, J 2.20 Hz, 1H, H-9), 7.81 (dd, J 2.80 and 1.30 Hz, 1H, H-1). Anal. Calcd for $C_{17}H_{18}ClN_3S$: C, 61.52; H, 5.47; N, 12.66. Found: C, 61.68; H, 5.64; N, 12.77.

*4-[2-(Piperidino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**i**)*. Yield: 64%, yellow oil; ^{1}H NMR δ (300 MHz, $CDCl_3$) 1.35 (m, 2H, CH_2 piperidine), 1.48 (m, 4H, $2CH_2$ piperidine), 2.43 (m, 4H, $2CH_2$ piperidine), 2.59 (t, J 7.30 Hz, 2H, CH_2), 3.46 (t, J 7.30 Hz, 2H, CH_2), 6.86 (m, 2H, H-2 and H-3), 7.46 (m, 2H, H-7 and H-8), 7.74 (d, J 7.45 Hz, 1H, H-6), 8.18 (d, J 7.45 Hz, 1H, H-9), 8.38 (dd, J 2.70 and 1.35 Hz, 1H, H-1). Anal. Calcd for $C_{18}H_{21}N_3S$: C, 69.41; H, 6.79; N, 13.49. Found: C, 69.59; H, 6.60; N, 13.77.

*7-Methoxy-4-[2-(piperidino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**j**)*. Yield: 61%, orange oil; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.50 (m, 2H, CH_2 piperidine), 1.70 (m, 4H, $2CH_2$ piperidine), 2.60 (m, 4H, $2CH_2$ piperidine), 2.80 (t, J 7.60 Hz, 2H, CH_2), 3.60 (t, J 7.60 Hz, 2H, CH_2), 6.77 (dd, J 4.00 and 2.70 Hz, 1H, H-2), 6.90 (dd, J 4.00 and 1.30 Hz, 1H, H-3), 7.05 (dd, J 8.95 and 2.80 Hz, 1H, H-8), 7.35 (d, J 2.80 Hz, 1H, H-6), 7.72 (d, J 8.95 hz, 1H, H-9), 7.80 (dd, J 2.70 and 1.30 Hz, 1H, H-1). Anal. Calcd for $C_{19}H_{23}N_3SO$: C, 66.83; H, 6.79; N, 12.31. Found: C, 66.97; H, 6.58; N, 12.47.

*8-Chloro-4-[2-(piperidino)ethylthio]pyrrolo[1,2-a]quinoxaline (7**k**)*. Yield: 37%, orange crystals, mp = 66°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.45 (m, 2H, CH_2 piperidine), 1.65 (m, 4H, $2CH_2$ piperidine), 2.60 (m, 4H, $2CH_2$ piperidine), 2.75 (t, J 7.60 Hz, 2H, CH_2), 3.60 (t, J 7.60 Hz, 2H, CH_2), 6.80 (dd, J 3.95 and 2.80 Hz, 1H, H-2), 6.90 (dd, J 3.95 and 1.20 Hz, 1H, H-3), 7.38 (dd, J 8.65 and 2.15 Hz, 1H, H-7), 7.72 (d, J 8.65 Hz, 1H, H-6), 7.78 (m, 2H, H-1 and H-9). Anal. Calcd for $C_{18}H_{20}ClN_3S$: C, 62.50; H, 5.83; N, 12.15. Found: C, 62.74; H, 5.97; N, 12.32.

*4-(Butylthio)pyrrolo[1,2-a]quinoxaline (7**l**)*. Yield: 48%, yellow crystals, mp = 110°C; ^{1}H NMR δ

(300 MHz, d_6-DMSO) 1.00 (t, 3H, $J = 7.30$, CH_3), 1.58 (sextuplet, J 7.30 Hz, 2H, CH_2), 1.84 (qt, J 7.30 Hz, 2H, CH_2), 4.23 (t, J 7.30 Hz, 2H, CH_2), 6.96 (m, 1H, H-2), 7.25 (m, 1H, H-3), 7.46 (m, 1H, H-8), 7.53 (m, 1H, H-7), 7.86 (d, J 7.25 Hz, 1H, H-6), 8.08 (m, 1H, H-1), 9.24 (d, J 8.85 Hz, 1H, H-9). Anal. Calcd for $C_{15}H_{16}N_2S$: C, 70.27; H, 6.29; N, 10.93. Found: C, 70.36; H, 6.41; N, 11.05.

*Synthesis of 4-[2-(N,N-Diethylamino) ethylthio] pyrrolo[1,2-a]quinoxalines, 4-[2-(Pyrrolidino) ethylthio] pyrrolo[1,2-a]quinoxalines, 4-[2-(Piperidino)ethylthio] pyrrolo[1,2-a]quinoxalines, 4-(Butylthio)pyrrolo[1,2-a]quinoxalines and 5-[2-(N,N-Diethylamino) ethylthio] pyrrolo[1,2-a]thieno[3,2-e]pyrazine Hydro- chloride (**1a-m**)*. To solution of amines **7a-l** and **10** (20 mmol) in diethyl ether (60 mL) was bubbled with an hydrochloric acid gas flow. The precipitate was filtered, washed with diethyl ether and dried to give **1a-m** as crystals.

*4-[2-(N,N-Diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1a**)*. Yield: 69%, beige crystals, mp = 204°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.37 (t, J 7.20 Hz, 6H, $2CH_3$), 3.25 (m, 4H, $2CH_2$), 3.39 (m, 2H, CH_2), 3.71 (m, 2H, CH_2), 4.04 (bs, 1H, NH^+), 6.91 (dd, J 3.95 and 2.80 Hz, 1H, H-2), 6.94 (dd, J 3.95 and 1.25 Hz, 1H, H-3), 7.49 (t, J 7.70 Hz, 1H, H-7), 7.56 (t, J 7.70 Hz, 1H, H-8), 7.82 (d, J 7.70 Hz, 1H, H-6), 8.28 (d, J 7.70 Hz, 1H, H-9), 8.49 (dd, J 2.80 and 1.25 Hz, 1H, H-1), 10.75 (bs, 1H, NH^+). Anal. Calcd for $C_{17}H_{23}Cl_2N_3S$: C, 54.83; H, 6.22; N, 11.28. Found: C, 55.11; H, 6.55; N, 11.46.

*7-Methoxy-4-[2-(N,N-diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1b**)*. Yield: 73%, beige crystals, mp = 172°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.32 (t, J 7.20 Hz, 6H, $2CH_3$), 3.22 (m, 4H, $2CH_2$), 3.51 (m, 2H, CH_2), 3.65 (m, 2H, CH_2), 3.86 (s, 3H, CH_3O), 4.10 (bs, 1H, NH^+), 6.87 (dd, J 4.10 and 2.85 Hz, 1H, H-2), 6.91 (dd, J 4.10 and 1.10 Hz, 1H, H-3), 7.19 (dd, J 9.05 and 2.80 Hz, 1H, H-8), 7.23 (d, J 2.80 Hz, 1H, H-6), 8.23 (d, J 9.05 Hz, 1H, H-9), 8.44 (dd, J 2.85 and 1.10 Hz, 1H, H-1), 9.93 (bs, 1H, NH^+). Anal. Calcd for $C_{18}H_{25}Cl_2N_3SO$: C, 53.73; H, 6.26; N, 10.44. Found: C, 53.98; H, 6.10; N, 10.65.

*8-Chloro-4-[2-(N,N-diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1c**)*. Yield: 77%, beige crystals, mp = 206°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.32 (t, J 7.15 Hz, 6H, $2CH_3$), 3.25 (m, 4H, $2CH_2$), 3.38 (m, 2H, CH_2), 3.70 (m, 2H, CH_2), 4.06 (bs, 1H, NH^+), 6.93 (m, 1H, H-2), 6.97 (m, 1H, H-3), 7.52 (dd, J 8.65 and 2.15 Hz, 1H, H-7), 7.81 (d, J 8.65 Hz, 1H, H-6), 8.48 (d, J 2.15 Hz, 1H, H-9), 8.55 (m, 1H, H-1), 10.78 (bs, 1H, NH^+). Anal. Calcd for $C_{17}H_{22}Cl_3N_3S$: C, 50.19; H, 5.45; N, 10.33. Found: C, 50.35; H, 5.68; N, 10.52.

*7-Chloro-4-[2-(N,N-diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1d**)*. Yield: 63%, beige crystals, mp = 221°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.22 (t, J 7.25 Hz, 6H, $2CH_3$), 3.24 (m, 4H, $2CH_2$), 3.35 (m, 2H, CH_2), 3.80 (m, 2H, CH_2), 4.03 (bs, 1H, NH^+), 6.93 (m, 1H, H-2), 6.98 (m, 1H, H-3), 7.61 (dd, J 8.85 and 2.20 Hz, 1H, H-8), 7.85 (d, J 2.20 Hz, 1H, H-6), 8.33 (d, J 8.85 Hz, 1H, H-9), 8.52 (m, 1H, H-1), 10.32 (bs, 1H, NH^+). Anal. Calcd for $C_{17}H_{22}Cl_3N_3S$: C, 50.19; H, 5.45; N, 10.33. Found: C, 50.11; H, 5.61; N, 10.20.

*8-Phenyl-4-[2-(N,N-diethylamino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1e**)*. Yield: 83%, yellow crystals, mp = 61°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.35 (t, J 7.15 Hz, 6H, $2CH_3$), 3.28 (q, J 7.15 Hz, 4H, $2CH_2$), 3.42 (t, J 7.50 Hz, 2H, CH_2), 3.75 (t, J 7.50 Hz, 2H, CH_2), 4.43 (bs, 1H, NH^+), 6.93 (m, 1H, H-2), 6.97 (m, 1H, H-3), 7.43 (t, J 7.45 Hz, 1H, H-4'), 7.54 (t, J 7.45 Hz, 2H, H-3' and H-5'), 7.83 (dd, J 8.30 and 1.95 Hz, 1H, H-7), 7.90 (m, 3H, H-6, H-2' and H-6'), 8.59 (d, J 1.95 Hz, 1H, H-9), 8.72 (m, 1H, H-1), 10.79 (bs, 1H, NH^+). Anal. Calcd for $C_{23}H_{27}Cl_2N_3S$: C, 61.60; H, 6.07; N, 9.37. Found: C, 61.69; H, 5.86; N, 9.52.

*4-[2-(Pyrrolidino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1f**)*. Yield: 69%, white crystals, mp = 170°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.93 (m, 2H, CH_2 pyrrolidine), 2.01 (m, 2H, CH_2 pyrrolidine), 3.15 (m, 2H, CH_2 pyrrolidine), 3.49 (m, 2H, CH_2), 3.65 (m, 2H, CH_2 pyrrolidine), 3.72 (m, 2H, CH_2), 4.49 ((bs, 1H, NH^+), 6.92 (m, 2H, H-2 and H-3), 7.49 (t, J 7.80 Hz, 1H, H-7), 7.55 (t, J 7.80 Hz, 1H, H-8), 7.90 (d, J 7.80 Hz, 1H, H-6), 8.26 (d, J 7.80 Hz, 1H, H-9), 8.49 (dd, J 2.50 and 1.25 Hz, 1H, H-1), 11.21 (bs, 1H, NH^+). Anal. Calcd for $C_{17}H_{21}Cl_2N_3S$: C, 55.13; H, 5.71; N, 11.35. Found: C, 55.36; H, 5.98; N, 11.14.

*7-Methoxy-4-[2-(pyrrolidino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1g**)*. Yield: 82%, white crystals, mp = 168°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.93 (m, 2H, CH_2 pyrrolidine), 2.03 (m, 2H, CH_2 pyrrolidine), 3.14 (m, 2H, CH_2 pyrrolidine), 3.50 (m, 2H, CH_2), 3.69 (m, 4H, CH_2 and CH_2 pyrrolidine), 3.87 (s, 3H, CH_3O), 4.58 (bs, 1H, NH^+), 6.85 (dd, J 4.05 and 2.60 Hz, 1H, H-2), 6.89 (dd, J 4.05 and 1.30 Hz, 1H, H-3), 7.17 (dd, J 9.00 and 2.85 Hz, 1H, H-8), 7.45 (d, J 2.85 Hz, 1H, H-6), 8.20 (d, J 9.00 Hz, 1H, H-9), 8.42 (dd, J 2.60 and 1.30 Hz, 1H, H-1), 11.14 (bs, 1H, NH^+). Anal. Calcd for $C_{18}H_{23}Cl_2N_3SO$: C, 54.00; H, 5.79; N, 10.50. Found: C, 53.84; H, 5.53; N, 10.81.

*8-Chloro-4-[2-(pyrrolidino)ethylthio]pyrrolo[1,2-a] quinoxaline hydrochloride (**1h**)*. Yield: 65%, orange

crystals, mp = 199°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.89 (m, 2H, CH_2 pyrrolidine), 2.04 (m, 2H, CH_2 pyrrolidine), 3.15 (m, 2H, CH_2 pyrrolidine), 3.53 (m, 2H, CH_2), 3.64 (m, 2H, CH_2 pyrrolidine), 3.71 (m, 2H, CH_2), 4.60 (bs, 1H, NH^+), 6.93 (dd, *J* 3.90 and 2.85 Hz, 1H, H-2), 6.98 (dd, *J* 3.90 and 1.15 Hz, 1H, H-3), 7.53 (dd, *J* 8.55 and 2.05 Hz, 1H, H-7), 7.90 (d, *J* 8.55 Hz, 1H, H-6), 8.49 (d, *J* 2.05 Hz, 1H, H-9), 8.55 (dd, *J* 2.85 and 1.15 Hz, 1H, H-1), 10.69 (bs, 1H, NH^+). Anal. Calcd for $C_{17}H_{20}Cl_3N_3S$: C, 50.44; H, 4.98; N, 10.38. Found: C, 50.72; H, 5.15; N, 10.59.

*4-[2-(Piperidino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1i**)*. Yield: 85%, white crystals, mp = 204°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.43 (m, 1H, CH_2 piperidine), 1.74 (m, 5H, CH_2 piperidine), 3.01 (m, 2H, CH_2), 3.39 (m, 2H, CH_2 piperidine), 3.58 (m, 2H, CH_2 piperidine), 3.75 (m, 2H, CH_2), 3.90 (bs, 1H, NH^+), 6.91 (dd, *J* 4.00 and 2.70 Hz, 1H, H-2), 6.94 (dd, *J* 4.00 and 1.30 Hz, 1H, H-3), 7.49 (t, *J* 8.00 Hz, 1H, H-7), 7.56 (t, *J* 8.00 Hz, 1H, H-8), 7.89 (d, *J* 8.00 Hz, 1H, H-6), 8.27 (d, *J* 8.00 Hz, 1H, H-9), 8.49 (dd, *J* 2.70 and 1.30 Hz, 1H, H-1), 10.56 (bs, 1H, NH^+). Anal. Calcd for $C_{18}H_{23}Cl_2N_3S$: C, 56.25; H, 6.03; N, 10.93. Found: C, 56.39; H, 5.84; N, 11.17.

*7-Methoxy-4-[2-(piperidino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1j**)*. Yield: 71%, beige crystals, mp = 214°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.42 (m, 1H, CH_2 piperidine), 1.71 (m, 3H, CH_2 piperidine), 1.84 (m, 2H, CH_2 piperidine), 3.05 (m, 2H, CH_2 piperidine), 3.34 (m, 2H, CH_2), 3.51 (m, 2H, CH_2 piperidine), 3.70 (m, 2H, CH_2), 3.88 (s, 3H, CH_3O), 3.95 (bs, 1H, NH^+), 6.87 (dd, *J* 3.95 and 2.65 Hz, 1H, H-2), 6.91 (dd, *J* 3.95 and 1.25 Hz, 1H, H-3), 7.19 (dd, *J* 8.95 and 2.75 Hz, 1H, H-8), 7.35 (d, *J* 2.75 Hz, 1H, H-6), 8.22 (d, *J* 8.95 Hz, 1H, H-9), 8.42 (dd, *J* 2.65 and 1.20 Hz, 1H, H-1), 9.60 (bs, 1H, NH^+). Anal. Calcd for $C_{19}H_{25}Cl_2N_3SO$: C, 55.07; H, 6.08; N, 10.14. Found: C, 54.85; H, 6.25; N, 10.28.

*8-Chloro-4-[2-(piperidino)ethylthio]pyrrolo[1,2-a]quinoxaline hydrochloride (**1k**)*. Yield: 72%, beige crystals, mp = 229°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.45 (m, 1H, CH_2 piperidine), 1.78 (m, 5H, CH_2 piperidine), 3.00 (m, 2H, CH_2 piperidine), 3.24-3.39 (m, 4H, CH_2 piperidine and CH_2), 3.70 (t, *J* 7.60 Hz, 2H, CH_2), 3.78 (bs, 1H, NH^+), 6.94 (dd, *J* 3.75 and 2.85 Hz, 1H, H-2), 6.98 (dd, *J* 3.75 and 1.20 Hz, 1H, H-3), 7.53 (dd, *J* 8.95 and 2.00 Hz, 1H, H-7), 7.87 (d, *J* 8.95 Hz, 1H, H-6), 8.49 (d, *J* 2.00 Hz, 1H, H-9), 8.55 (dd, *J* 2.85 and 1.20 Hz, 1H, H-1), 9.83 (bs, 1H, NH^+). Anal. Calcd for $C_{18}H_{22}Cl_3N_3S$: C, 51.62; H, 5.29; N, 10.03. Found: C, 51.50; H, 5.36; N, 9.85.

*4-(Butylthio)pyrrolo[1,2-a]quinoxaline hydrochloride (**1l**)*. Yield: 73%, pale-yellow crystals, mp = 156°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 0.94 (t, 3H, *J* 7.35 Hz, CH_3), 1.47 (sextuplet, *J* 7.35 Hz, 2H, CH_2), 1.72 (qt, *J* 7.35 Hz, 2H, CH_2), 3.38 (t, *J* 7.35 Hz, 2H, CH_2), 4.23 (bs, 1H, NH^+), 6.89 (m, 2H, H-2 and H-3), 7.46 (t, *J* 7.90 Hz, 1H, H-8), 7.52 (t, *J* 7.90 Hz, 1H, H-7), 7.78 (d, *J* 7.90 Hz, 1H, H-6), 8.25 (d, *J* 7.90 Hz, 1H, H-9), 8.44 (dd, *J* 2.65 and 1.40 Hz, 1H, H-1). Anal. Calcd for $C_{15}H_{17}ClN_2S$: C, 61.52; H, 5.85; N, 9.57. Found: C, 61.68; H, 6.02; N, 9.53.

*5-[2-(N,N-Diethylamino)ethylthio]pyrrolo[1,2-a]thieno[3,2-e]pyrazine hydrochloride (**1m**)*. Yield: 68%, pale-yellow crystals, mp = 220°C; ^{1}H NMR δ (300 MHz, d_6-DMSO) 1.28 (t, *J* 6.40 Hz, 6H, $2CH_3$), 3.23 (m, 4H, $2CH_2$), 3.32 (m, 2H, CH_2), 3.61 (t, *J* 6.85 Hz, 2H, CH_2), 3.82 (bs, 1H, NH^+), 6.96 (m, 2H, H-6 and H-7), 7.39 (d, *J* 5.65 Hz, 1H, H-3), 7.53 (d, *J* 5.65 Hz, 1H, H-?), 8.02 (m, 1H, H-8), 9.90 (bs, 1H, NH^+). Anal. Calcd for $C_{15}H_{21}Cl_2N_3S_2$: C, 47.61; H, 5.59; N, 11.10. Found: C, 47.74; H, 5.78; N, 11.26.

Pharmacology

Bacterial strains and media. The strains of *S. aureus* used in this study included SA 1199 (a fluoroquinolone susceptible clinical isolate) and SA 1199B (its overproducing NorA mutant), generously provided as gifts from G.W. Kaatz (University of Michigan, USA) [6,14,15] and *S. aureus* ATCC 25923, a reference strain and its overproducing NorA mutant SA-1, selected in our laboratory [27]. These strains were routinely cultured on Mueller Hinton agar (MH) (BIO-RAD, Marnes-la-Coquette, France) and/or on broth adjusted to contain 20 μg/ml of Ca^{2+} and 10 μg/ml of Mg^{2+} (AES, Bruz, France), at 37°C. They were stored in 30% glycerol Brain Heart broth at −80°C.

Antibiotic and chemicals. Norfloxacin, reserpine, and omeprazole were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Solutions of reserpine, omeprazole, and the candidate inhibitors **1a-m** were extemporaneously prepared in 100% dimethylsulfoxide (DMSO), but the highest final concentration of DMSO present in all assays (4%, v/v) caused no inhibition of bacterial growth (data not shown).

Antibiotic susceptibility test. The antibiotic susceptibility of the strains was determined by the disk diffusion method in MH medium alone or supplemented with the EPIs at 1/4 MIC [28]. The two hydrophilic fluoroquinolones, norfloxacin (5 μg) and ciprofloxacin (5 μg), which are the main substrates

of NorA efflux pump were tested. After overnight incubation at 37°C, the inhibition zone diameters were measured. All tests were done at least in triplicate and the mean values were retained. Then, results were expressed as the percentages of NorA efflux pump inhibition, calculated as follows: [(diameter for the resistant strain in presence of EPI) minus (diameter for the resistant strain without EPI)] divided by [(diameter for the susceptible strain without EPI) minus (diameter for the resistant strain without EPI)] and multiplied by 100.

MIC determinations. The minimum inhibitory concentrations (MICs) of norfloxacin, EPIs and their combinations were determined by an agar dilution method in MH medium [28]. Serial two-fold dilutions of the stock solutions were prepared extemporaneously and incorporated to agar plates. The bacterial inocula were obtained by suspending fresh cultures in peptone water, to an optical density equivalent to a 0.5 Mac Farland ($\approx 1.5 \times 10^8$ CFU/mL). Then, these suspensions were applied to the surface of the drug-containing media by means of a Steers replicator, yielding a final concentration of approximately 10^4 colony forming units (CFU) per spot. After incubation for 24 h in air at 37°C, the MIC was defined as the lowest concentration that inhibited any visible growth. All tests were done at least in triplicate and the mode values were retained.

Results and discussion

Chemistry

The synthesis of the new alkylthiopyrrolo[1,2-*a*]quinoxalines **1a-l** has been achieved starting from various substituted 2-nitroanilines **2a-e** in five or six steps (Scheme 1). Not commercially available 5-phenyl-2-nitroaniline **2e** was prepared *via* the Suzuki-Miyaura reaction by coupling 5-chloro-2-nitroaniline **2c** with phenylboronic acid using $Pd(OAc)_2$ as catalyst in the presence of the 2-(biphenyl)-di-*tert*-butylphosphine ligand [29]. The Clauson-Kaas reaction [30] of anilines **2a-e** with 2,5-dimethoxytetrahydrofuran (DMTHF) in acetic acid gave the pyrrolic derivatives **3a-e**, which were reduced using a $BiCl_3$-$NaBH_4$ treatment to provide the attempted 1-(2-aminophenyl)pyrroles **4a-e** [31,32]. The cyclization was then possible between the NH_2 and the C-α of the pyrrole ring by reacting compounds **4a-e** with triphosgene in toluene to give the lactams **5a-e**, which were subsequently chlorodehydroxylated with phosphorous oxychloride to obtain the chloroquinoxalines **6a-e**. Finally a nucleophilic substitution of the chlorine atom in compounds **6a-e** with an appropriate dialkylaminoethylthiol in the presence of NaH as a base furnished the pyrrolo[1,2-*a*]quinoxalines **7a-e**.

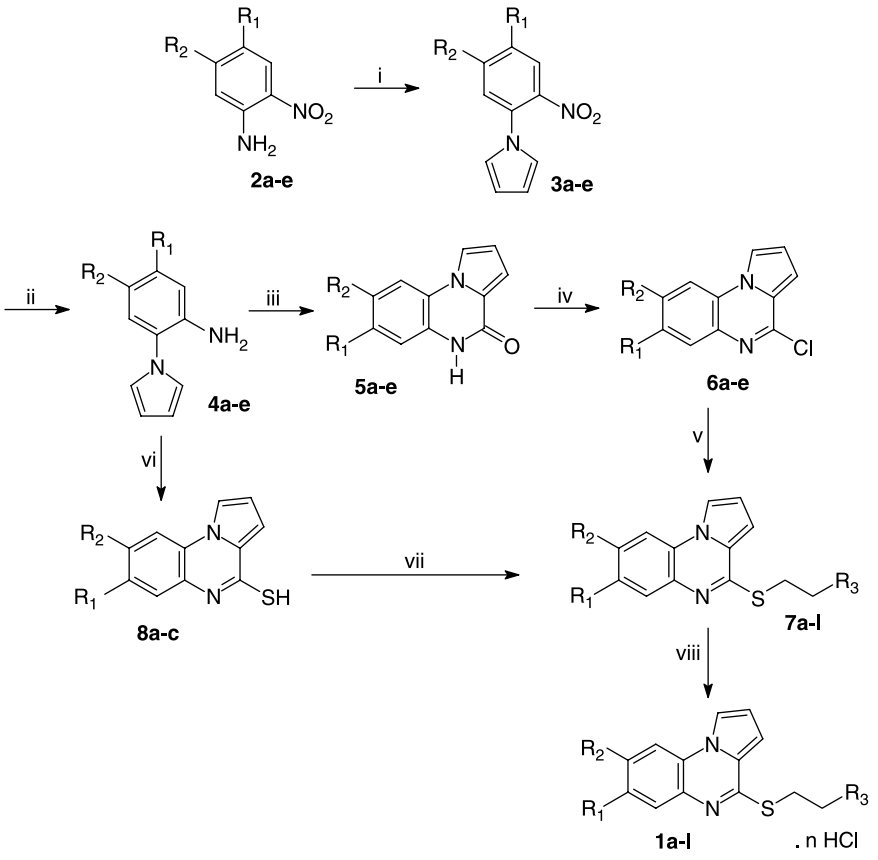
Due to the moderate yields (26–45%) obtained by this first method in the preparation of **7a-e** (method A), we decided to investigate metal-catalyzed nucleophilic substitutions of 4-chloropyrrolo[1,2-*a*]quinoxaline **6a** by the *N,N*-diethylaminoethylthiol (Table I). First, a copper-catalyzed carbon-sulfur bond formation reaction was developed (method B). Thus, optimised reaction conditions using 5 mol % CuI, K_2CO_3 (2 eq.), and ethylene glycol (2 eq.) in reagent-grade 2-propanol at 80°C under nitrogen led to **7a** (yield: 60%) [33,34]. Then, a third new method (method C) used a mild palladium-free synthetic protocol for the cross-coupling reaction of the 4-chloropyrrolo[1,2-*a*]quinoxaline **6a** with *N, N*-diethylaminoethylthiol in presence of 10 mol % CuI, 10 mol % neocuproine, and *t*-BuONa as the base, in toluene at 110°C [33–35]. Using this protocol, the arylsulfide **7a** was synthesised (yield: 80%). The reaction of **6a** with the thiolate anion, prepared by the action of *t*-BuOK on the *N,N*-diethylaminoethylthiol, in the presence of a catalytic amount of tetrakis(-triphenylphosphine)palladium in *n*-butanol (method D) was also found to be useful in the preparation of the sulfide **7a** (66%) [36,37].

A second investigated route to **7f-l** first involved the treatment of aminophenylpyrroles **4a-c** with CS_2 in alcoholic alkali to afford the expected pyrrolo[1,2-*a*]quinoxaline-4-thiols **8a-c** [26]. It was then followed by the reaction of **8a-c** with aminoalkyl halides to give the *S*-alkylated products **7f-l**. All alkylthiopyrrolo[1,2-*a*]quinoxalines **7a-l** were converted into their hydrochlorides **1a-l** by treatment with hydrochloric acid gas in diethyl ether.

A similar nucleophilic substitution with the *N,N*-diethylaminoethylthiol and 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **9** [38] furnished the pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **10**, converted in its hydrochloride **1m** (Scheme 2).

Pharmacology

In this study, a series of twelve new pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l** and one pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **1m** were synthesised from various commercially nitroanilines in order to obtain new compounds endowed with a higher bacterial EPI activity. These target derivatives were evaluated in a model targeting a typical MFS transporter of *S. aureus*, involved in the fluoroquinolone resistance of this bacterial species [5,14,17,39]. This model used the *S. aureus* strains SA 1199B and SA-1, which overexpress the NorA efflux pump. In preliminary experiments, the MIC of the reference and putative EPIs were determined. Reserpine and omeprazole were found to lack any intrinsic antibacterial activity (MIC $>$ 512 μg/mL), but the derivatives **1a-m** exhibited various MICs, ranging between 16 μg/mL (compound **1e**) and $>$512 μg/mL (compounds **1l-m**)

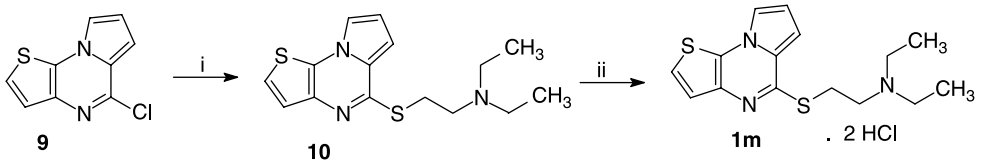


	a	b	c	d	e	f	g	h	i	j	k	l
R_1–	H–	CH_3O–	H–	Cl–	H–	H–	CH_3O–	H–	H–	CH_3O–	H–	H–
R_2–	H–	H–	Cl–	H–	C_6H_5-	H–	H–	Cl–	H–	H–	Cl–	H–
R_3–	$-NEt_2$	$-NEt_2$	$-NEt_2$	$-NEt_2$	$-NEt_2$	–N	–N	–N	–N	–N	–N	Et–
n	2	2	2	2	2	2	2	2	2	2	2	1

Scheme 1. Synthesis of compounds **1a-l**. Reagents: (i) DMTHF, CH_3COOH, Δ; (ii) $BiCl_3$ / $NaBH_4$, EtOH; (iii) $CO(OCCl_3)_2$, toluene, Δ; (iv) $POCl_3$, Δ; (v) *Method A:* NaH, HS-CH_2-CH_2-R_3, dioxane; *Method B:* K_2CO_3, HS-CH_2-CH_2-R_3, CuI, $HOCH_2CH_2OH$, 2-propanol; *Method C:* *t*-BuONa, HS-CH_2-CH_2-R_3, CuI, neocuproine, toluene; *Method D:* *t*-BuOK, HS-CH_2-CH_2-R_3, $Pd(PPh_3)_4$, *n*-BuOH (vi) CS_2, NaOH, EtOH; (vii) NaH, Cl-CH_2-CH_2-R_3, DMF; (viii) HCl, Et_2O.

Table I. Optimized reaction conditions for coupling of **6a** and *N,N*-diethylaminoethylthiol.

Method	Base	Catalyst	Ligand	Reaction conditions	% Yield of **7a**
A	NaH	–	–	dioxane, 100°C, 4 h	42
B	K_2CO_3	CuI	$HOCH_2CH_2OH$	2-propanol, 80°C, 24 h	60
C	*t*-BuONa	CuI	Neocuproine	toluene, reflux, 24 h	80
D	*t*-BuOK	$Pd(PPh_3)_4$	–	*n*-BuOH, reflux, 5 h	66



Scheme 2. Synthesis of compound **1m**. Reagents: (i) *Method A:* NaH, HS-CH_2-CH_2-$N(C_2H_5)_2$, dioxane; (ii) HCl, Et_2O.

(data not shown). All tested EPIs were subsequently used at concentrations devoid of bacterial toxicity (25% of their MIC) in order to observe the most important EPI effect. Then, EPI activity of all newly synthesised compounds was evaluated by the high throughput disk diffusion method and by MIC determination. All results were in agreement with the literature [5,10,15,39] and are reported in Table II.

The antibiotic resistance and inhibition profiles of SA 1199B and SA-1 reflected the overexpression of NorA efflux pump, which preferentially exports the hydrophilic fluoroquinolones [14,39]. Biological results showed that SA 1199B exhibited a high-level fluoroquinolone resistance that was not completely reversed by the EPIs, as previously described [10]. In fact, SA 1199B, commonly used to evaluate NorA expression and inhibition, is a double target (A116E in GrlA)/efflux mutant [15] and a lesser affinity of the drug for its modified target probably leads to a diminished entry by passive diffusion into the bacterial cell, that cannot be amended by EPIs. In contrast, the fluoroquinolone susceptibility of the single NorA mutant, SA-1, was totally restored by reserpine and omeprazole [27] (Table II). Thus, SA-1 is probably a better test strain for investigating NorA efflux pump inhibition. In a first step, the agar diffusion method showed that the percentage of NorA inhibition by reserpine, omeprazole and the newly synthesised compounds reached 29–100% for norfloxacin and 25–100% for ciprofloxacin, in SA-1. Two new derivatives, **1 g** and **1m,** appeared to be more active than the reference EPIs. In a second step, MIC determination of norfloxacin confirmed these results. Indeed, the addition of **1 g** and **1m** resulted in a similar or higher decrease of the norfloxacin MIC (32-fold for SA-1; 16-fold for SA-1199B) than the combination with omeprazole and reserpine (32-fold for SA-1; 8-fold for SA-1199B).

Consequently, all of these preliminary biological results allowed clarification of the influence of the substituents at positions 7 and/or 8 of the pyrrolo[1,2-*a*]quinoxaline nucleus and of the 4-alkylaminoethylthio chain on the EPI activity. Indeed, with regard to the unsubstituted 4-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]quinoxalines **1a**, **1f** and **1i**, their 7-methoxy analogues **1b**, **1 g** and **1j** were more active, whereas the chlorosubstituted compounds **1c-d**, **1 h** and **1k**, and the 8-phenyl substituted compound **1e** provided the lowest EPI effects. Moreover, the EPI activity was significantly affected by replacement of the *N*,*N*-diethylamino group by bioisostere groups, such as pyrrolidine or piperidine in the di(alkylamino)ethylthio chain. Indeed, the substitution of the diethylamino group by its isomer with restricted conformation such as pyrrolidine moiety enhanced the EPI activity, i.e. compound **1a** *versus* **1f**, and **1b** *versus* **1 g**. Replacement by a piperidine ring leading to a greater flexibility in the saturated heterocycle (compounds **1i-k**) decreased the EPI efficiency. However, the introduction of a *n*-butylthio function at position 4 of the pyrrolo[1,2-*a*]quinoxaline moiety in compound **1 l** was not found beneficial,

Table II. EPI activities of reserpine, omeprazole, 4-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l** and pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **1m** on the *S. aureus* strains, SA 1199B and SA-1.

		SA 1199B			SA-1		
		% inhibition[b]		MIC(μg/mL)[c]	% inhibition[b]		MIC(μg/mL)[c]
EPI	Concentration[a] (μg/mL)	Nor[d]	Cip[d]	Nor[d]	Nor[d]	Cip[d]	Nor[d]
–	–	–	–	64	–	–	16
Reserpine	128	33	69	8	100	100	2
Omeprazole	128	38	69	8	100	100	2
1a	64	28	62	16	79	83	4
1b	64	33	62	8	86	100	2
1c	16	0	15	64	21	17	16
1d	32	28	62	16	71	83	2
1e	4	28	62	16	71	75	2
1f	64	43	77	8	86	92	1
1 g	16	52	85	4	100	100	1
1 h	16	14	38	16	57	67	4
1i	32	14	46	16	50	58	4
1j	16	0	23	32	43	50	8
1k	16	0	31	32	43	50	8
1 l	128	0	23	64	29	25	16
1m	64	48	85	4	100	100	1

[a]In all experiments, the EPIs were used at $0.25 \times MIC_{(EPI)}$.
[b]% inhibition were calculated with the following equation: $\% = [Ø_R(AB + EPI) - Ø_R(AB)] / [Ø_S(AB) - Ø_R(AB)] \times 100$ Ø = diameter of the inhibition zone, R = Resistant strain, S = fluoroquinolone susceptible strain, AB = antibiotic, +EPI = in presence of EPI [40].
[c]MIC is the minimum inhibitory concentration of norfloxacin in absence or in presence of EPI.
[d]Nor: norfloxacin, Cip: ciprofloxacin.

suggesting the importance of a potential protonable nitrogen atom in the alkyl chain. Replacement of the pyrroloquinoxaline moiety by a pyrrolothienopyrazine moiety (compound **1m**), bioisostere of the pyrroloquinoxaline nucleus, showed the most efficient EPI activity, suggesting that an electron-rich (or electronegative) atom, such as a sulfur atom, potentialized this activity. Finally, four pyrroloquinoxaline derivatives **1b**, **1f-g** and **1m** were found more efficient than reserpine, taken as the reference EPI.

Conclusion

In the present paper, we have described the synthesis and the efflux pump inhibition activity of twelve new 4-[2-(alkylamino)ethylthio]pyrrolo[1,2-*a*]quinoxalines **1a-l** and one pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine compound **1m**. Through this study, it was observed that few pyrroloquinoxaline derivatives **1** showed a higher EPI activity than reserpine or omeprazole. Substitution of the pyrroloquinoxaline nucleus by a methoxy group increased the EPI activity, whereas introduction of a chlorine atom decreased it. The best results were observed with a pyrrolidine moiety fixed to the 4-substituted thioethyl chain. Based on these preliminary structure-activity results, it could be possible to further identify new pyrrolo[1,2-*a*]quinoxaline or pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine derivatives developed as EPIs on NorA efflux pump. Combinations of all pyrrolo[1,2-*a*]quinoxaline SAR studies might allowed to identify pharmacophore moiety and potential inhibition mechanism in order to obtain therapeutically useful derivatives.

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