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Short communication

2-Phenyl-6(7)-*R*-substituted quinoxalines *N*-oxides. Synthesis, structure elucidation and antimicrobial activity*

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Summary — A certain number of isomeric 2-phenylquinoxalines N-oxides bearing substituents at position 6/7 were prepared in order to investigate anti-microbial activity. The effect of some electron–withdrawing groups seem to increase an anti-*Trichomonas vaginalis* activity *in vitro*. Structure elucidation of 6-acetyl-2-phenylquinoxaline was achieved by an unambiguous synthesis. ^{1}H NMR spectra of N-oxides were examined in detail to establish structure of parent quinoxalines.

Résumé — N-oxydes de 2-phényl-6(7)-*R*-substituées quinoxalines. Synthèse, démonstration de la structure de quelques composés et évaluation de leur activité anti-microbienne. *Une série de N-oxydes de 2-phényl-6(7)-*R*-substituées quinoxalines a été préparée et la structure de la 2-phényl-6-acétylquinoxaline a été démontrée. L'activité anti-microbienne* in vitro *de ces composés vis-à-vis d'une variété de bactéries Gram positif, Gram négatif, de* Trichomonas vaginalis *et de* Candida parapsilosis *a été évaluée. On a trouvé que l'activité vis-à-vis de* Trichomonas vaginalis *est plus marquée pour les produits ayant un substituant électro-attracteur en position 6 de la quinoxaline. Les spectres RMN des N-oxydes de quinoxalines ont été examinés en détail pour établir la structure des quinoxalines de départ.*

quinoxaline-N-oxides / anti trichomonas vaginalis activity / NMR structure elucidation

Introduction

In a previous paper [1] some of us have reported the synthesis of new 6-trifluoromethylquinoxalines bearing substituents at the 2- and/or 3 positions and their mono N-oxides to be evaluated as antimicrobial agents. Microbiological testing has now shown that 3-phenyl-7-trifluoromethylquinoxaline N-oxide, therein described, possesses an interesting anti *Trichomonas vaginalis* and anti *Candida parapsilosis* activity *in vitro* (cf compound **5c** in table I). These results prompted us to continue an investigation on a series of similar compounds bearing different substituents at the position 6/7 of 2-phenylquinoxaline which might influence the above-mentioned activities. From a medicinal chemistry point of view it might be expected that a bioequivalence of CF_3 group with Cl, NO_2 and $COCH_3$ found in several other cases as well as the effect of electron donor substituents could be profitable in widening the range of antimicrobial activity. In this paper we wish to report the results of microbiological screening of the compounds listed in table I and the routes necessary for the structure elucidation of one of these **3g** as well as the observations on ^{1}H NMR spectra of the *N*-oxides obtained which allowed us to unambiguously define the structures of isomeric parent quinoxalines isolated from the reaction mixture formed according to scheme 1.

Chemistry

The quinoxalines necessary for our purpose were prepared using a successful procedure, previously described [1] and reproduced in scheme 1. In refluxing ethanol 1,2-diamino-4-R-benzenes **1 a–f** and phenylglyoxal **2** gave a mixture of 2 isomers whose ratio can be influenced by acid catalysis as we have recently reported [2]. In this work we described only the new quinoxalines **3g**, **4g** and **3b**, **3i** and we refer to the mentioned note for the other. Isomers **3g**, **4g** were obtained in 4:1 ratio under neutral conditions and this was not modified either under acid or basic catalysis as we have observed in other cases [2].

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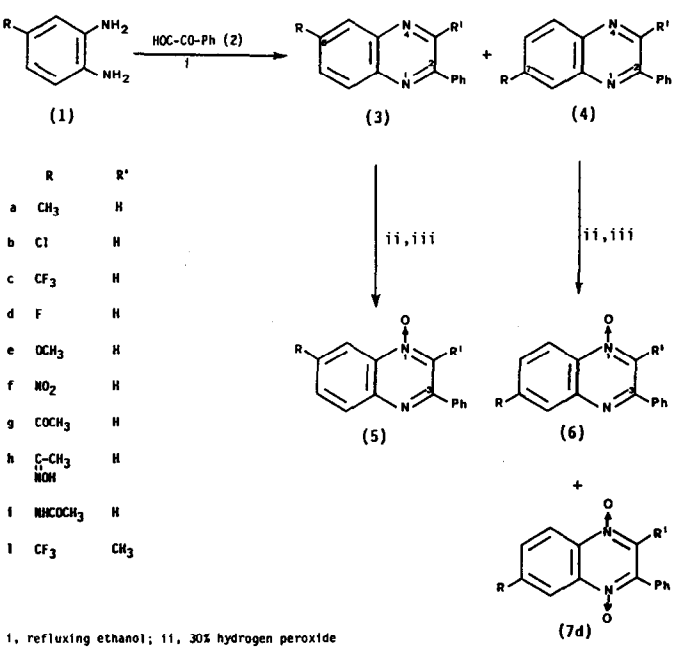
Table I. Anti-microbial activity of quinoxaline *N*-oxides **5a–6l** (Mic = μg/ml).

Compound	Staphilococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Trichomonas vaginalis	Candida parapsilosis
(5a)	250	125	250	62.5	>500
(5b)	250	250	125	62.5	>500
(5c)	>125	>125	125	15.6	31.8
(5d)	>500	>500	>500	250	500
(5e)	>500	500	500	125	500
(5f)	125	125	250	62.5	250
(5g)	125	250	62.5	30.2	125
(5h)	>500	>500	>500	250	>500
(5i)	125	>500	>500	31.2	>500
(5L)	>125	>125	>125	>125	>125
(6a)	125	62.5	62.5	62.5	125
(6b)	125	125	125	62.5	>500
(6c)	>125	>125	>125	>125	>125
(6d)	500	500	500	500	500
(6e)	>500	500	500	500	500
(6f)	>500	500	500	250	500
(6g)	125	125	250	15.6	125
(6L)	>125	>125	>125	>125	>125



i, refluxing ethanol; ii, 30% hydrogen peroxide solution in 100% formic acid; iii, as under ii but replacing formic acid with glacial acetic acid.

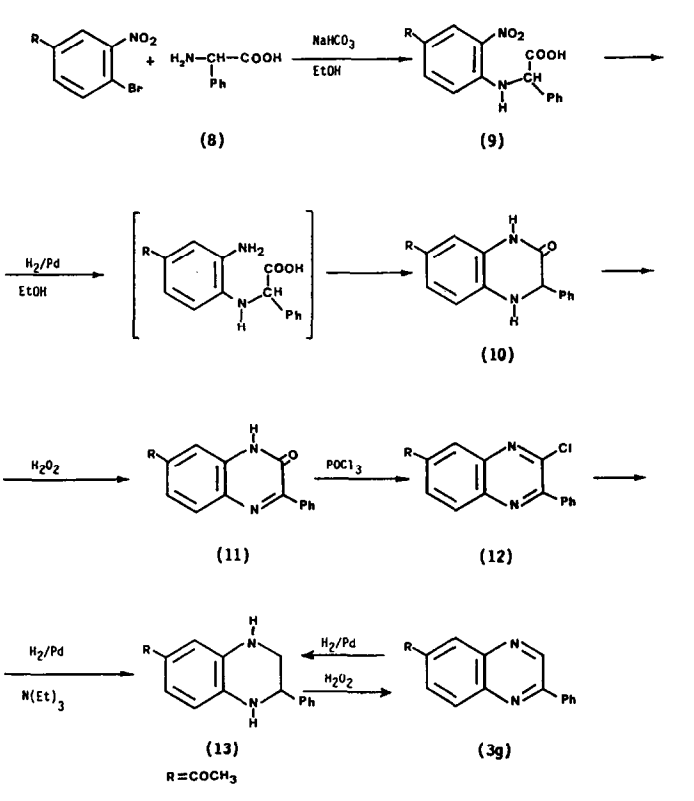
Scheme 1.

Compounds **3h**, **3i** were prepared according to scheme 3.

Spectroscopic differences for these isomers, as observed in similar cases [1], were negligible and this prompted us to envisage an unambiguous synthesis for the isomer **3g**.

From the sequence of the reactions in scheme 2 it soon appears evident that **3g** cannot be obtained in a straightforward manner from 12 because hydrogenation gave derivative **13**. This compound in turn on oxidation with H_2O_2 yielded **3g** identical to the higher melting isomer separated from the reaction mixture. This reaction is reversible and hydrogenation of **3g** afforded **13** in good yield. Oxime **3h** undergoes Beckman rearrangement to give **3i**, which was identical to a specimen derived from the hydrogenation and acetylation **3f**, thus indirectly confirming the structure assigned to this nitro compound in a previous paper [2] on the basis of the identity of its mp and ^{1}H NMR spectrum with that described in the literature [3].

The formation of *N*-oxides **5** and **6** was accomplished with 30% hydrogen peroxide solution in formic acid and in one case **5i** in glacial acetic acid. This type of oxidation is selective, leading to sterically less hindered N-oxides as can be deduced from ^{1}H NMR spectra which show a shielding effect of this group upon the chemical shift of C-3 proton (table II). However, in the case of the oxime **3b**, the oxidation did not produce the expected N-oxide **5h** but restored the keto group to give **5g**. Therefore **5h** was obtained on treating **5g** with hydroxylamine, as indicated in



Scheme 2.

Table II. Proton chemical shifts (J in Hz) for quinoxalines *N*-oxides **5a–l**, **6a–g** and the parent quinoxalines **3a–i** and **4a–g**. *Sharp singlet; [a]collapses into a singlet by irradiation at 7.579–7.523; [b]collapses into a singlet when irradiated at 7.425; [c]collapses into a doublet (J 2.6) when irradiated at 8.024; [d]collapses into a singlet and [e]simplifies into an apparent singlet when irradiation occurs at 7.428; [f]collapses into a singlet when irradiated at midpoint of multiplet 8.217–8.078; [g]collapses into a singlet when decoupled at these points; [h]collapses into a singlet when decoupled at 8.191–8.159 multiplet; **this resonance was erroneously reported in [2] at 8.807.

Compound	MHz	Solvent	3-H*	5-H	Other resonances	Ref.
(5a)	60	$CDCl_3$	8.68	8.27	8.05÷7.15 (7H, m, Ph + 7,8-H), 2.55 (3H, s, Me)	
(3a)	"	"	9.10		8.2÷7.3 (8H, m, Ph + 5,7,8-H), 2.55 (3H, s, Me)	(3,4)
(5b)	"	"	8.71	8.47(d,2)	8.15÷7.30 (7H, m, Ph + 7,8-H)	
(3b)	"	"	9.15		8.2÷7.3 (8H, m, Ph+5,7,8-H)	
(5c)	"	"	8.80		8.3÷7.3 (8H, m Ph+5,7,8-H)	
(3c)	"	"	9.36	8.36(d,2)	8.3÷8.05 (3H, m, PhH + 5,8-H), 7.86 (1H, dd, 9 and 2, 7-H), 7.6÷7.3 (3H,m, PhH_3)	(1)
(5d)	200	"	8.848		8.243÷8.135 (2H,m,PhH_2), 8.074÷8.026 (2H,m,5,8-H)[e], 7.579÷7.523 (5H, PhH_4+7-H)	
(3d)	200	"	9.328		8.201÷8.123 (3H, m, PhH + 5,8-H), 7.752 (1H, dd, J 9 and 2, 7-H), 7.623÷7.543 (4H,m, PhH_4)	
(5e)	200	"	8.821	7.852(d,2.6)[b]	8.024(1H,d, 9.2, 8-H)[b], 8.018÷8.009 (1H,m, PhH), 7.526÷7.507(4H, m, PhH_4), 7.425 (1H, dd, 9.2 and 2.6, 7-H)[c], 3.992 (3H, s, OMe)	(2)
(3e)	200	"	9.245		8.180÷8.139 (2H, m, 5,7-H), 8.035 (1H, d, 8.81, 8-H), 7.591÷7.394 (5H, m, Ph), 3.988 (3H, s, OMe)	
(5f)	200	"	9.334	9.280(d,2.49)	8.610 (1H, dd, 9.14 and 2.49, 7-H), 8.352 (1H, d, 9.14, 8-H), 8.310÷8.261 (2H, m, PhH_2), 7.628÷7.578 (3H, m, PhH_3)	(2)
(3f)	250	"	9.501	9.034(d,2.50)	8.536 (1H,dd, 8.95 and 2.50, 7-H), 8.291 (1H, d, 8.95, 8-H), 8.291÷8.253 (1H, m, PhH), 7.646÷7.589 (4H, m, PhH_4)	
(5g)	60	"	8.72	8.95(d,1)	8.3÷7.3 (7H, m, Ph+7,8-H), 2.82 (3H,s,CH_3-CO)	
(3g)	250	"	9.419	8.690(d,1.87)	8.358 (1H, dd, 9.37 and 1.87, 7-H), 8.345÷8.229 (1H, m, PhH), 8.202 (1H,d, 9.37, 8-H), 7.643÷7.559 (4H, m, PhH_4), 2.81 (3H, s, CH_3CO)	
(5h)	60	"	9.10	8.45(d,2)	8.3÷7.8 (5H, m, PhH_2+5,7,8-H), 7.6÷7.4 (3H, m, PhH_3), 2.37 (3H, s, CH_3CO), 11.65 (1H, s, N-OH)	
(3h)	60	$CDCl_3$+DMSO-d_6 (1 : 2)	9.35		8.3÷7.8 (5H, m, PhH_2+5,7,8-H), 7.6÷7.3 (3H, m, PhH_3), 2.30 (3H, s, CH_3CO), 12.3 (1H, s, N-OH)	
(5i)	60	"(1:1)"	8.73	8.64(d,2)	8.3÷7.3 (7H, m, Ph+ 7,8-H), 2.18 (3H, s, CH_3CO), 10.4 (1H, bs, NH-CO)	
(3i)	60	"(1:1)"	9.18	8.38(d,2)	8.25÷7.3 (7H, m, Ph+7,8-H), 2.15 (3H, s, CH_3C=O), 10.3 (1H, s, NH-CO)	
			3-H	8-H		Ref.
(6a)	60	$CDCl_3$	8.61	8.32(d,9)	8.1÷7.3 (7H, PhH_5+5,7-H), 2.50 (3H, s, Me)	(3,4)
(4a)	"	"	9.10		8.3÷8.0 (8H, m, Ph+5,7,8-H), 2.55 (3H, s, Me)	(3,4)
(6b)	"	"	8.62	8.34(d,9)	8.2÷7.3 (7H, m, Ph+5,7-H)	(3,4)
(4b)	"	"	9.18		8.2÷7.85 (8H, m, Ph+5,7,8-H)	(3,4)
(6c)	"	"	8.80	8.55(d,9)	8.32 (1H,d, 2, 5-H), 8.30÷7.30 (6H, m, Ph+7-H)	(1)
(4c)	"	"	9.46		8.45÷7.30 (8H, m, Ph+5,7,8-H)	(1)
(6d)	200	"	8.797	7.771(d,9)[d]	8.615÷8.540 (1H,m,PhH), 8.078÷8.050 (2H,m, PhH+5-H)[e], 7.428 (1H,dd, J 9 and 2.36, 7-H),7.543(3H,as,PhH_3)	
(4d)	200	"	9.290		8.217÷8.078 (3H,m,PhH+5,8-H), 7.772(1H,dd,9.28 and 2.74,7-H)[f], 7.756÷7.486(4H, m,PhH_4)	
(6e)	200	"	8.707**	8.453(d,8.98)[g]	8.039 (2H, as, PhH_2), 7.524 (3H, as, PhH_3), 7.414 (1H, s, 5-H), 7.304 (1H, d,8.98, 7-H)[g]	(2)
(4e)	200	"	9.168	8.040(d,9.04)[h]	8.191÷8.159 (2H, m, 5,7-H), 7.572÷7.364 (5H, m, Ph), 3.993 (3H, s, OMe)	
(6f)	200	"	8.939	8.735(d,9.42)	9.058 (1H, d, 2.36, 5-H), 8.449 (1H, dd, 9.42 and 2.36, 7-H), 8.145÷8.096 (2H, m, PhH_2), 7.629÷7.566 (3H, m, PhH_3)	(2)
(4f)	60	"	9.36		8.94 (1H, d, 2, 5-H), 8.45 (1H, dd, 9 and 2, 7-H), 8.3÷8.0 (3H, m, PhH_2+8-H), 7.7÷7.4 (3H, m, PhH_3)	
(6g)	200	"	8.991	8.648(d,9.39)	8.745 (1H, d, 1.81, 5-H), 8.273 (1H, dd, 9.39 and 1.81, 7-H), 8.144÷8.040 (2H, m, PhH_2), 7.606÷7.573 (3H, m, PhH_3), 2.793 (3H, s, Me-CO)	
(4g)	60	$CDCl_3$+DMSO-d_6	9.33		8.65 (1H, d, 2, 5-H), 8.3÷7.9 (4H, m, PhH_2+ 7,8-H), 7.6÷7.3 (3H, m, PhH_3), 2.73 (3H, s, MeCO).	
(7d)	200	$CDCl_3$	8.500		8.783÷8.710 (1H,m,PhH_1), 8.286 (1H,dd,2.77, 7-H), 7.931÷7.882(2H,m,PhH_2),7.686÷7.547(4H,m,PhH_2+5,8-H).	

scheme 3. In one case, the formation of 1,4-dioxide was observed **7d**. The spectra of all prepared quinoxalines and their N-oxides were examined in detail (table II) at 60 or 200 MHz in order to elucidate the structures of parent quinoxalines isolated from the reaction mixture, as we have pointed out in a previous note [2]. In fact according to the observations of Bannore *et al* [4] the N-oxide group affects the C-5 and C-8 proton resonances which appear at lower field than the parent quinoxaline respectively *meta*-coupled (*J* 1–2 Hz) or as a part of an *ortho* coupled AB system (doublet, *J* 9 Hz) according to the position of R substituent in the ring.

The values of the proton chemical shifts for the isomeric couples of quinoxalines **3a**, **3b**, **4a**, **4b** and their N-oxides match well with those reported by Bannore *et al* [4] while some discrepancies arose from the data for **5e** and **5f** described by Kano *et al* [3]. The spectra of these derivatives have now been recorded at 200 MHz and the assignments are unambiguous. Compound **6e** was reported to have an mp of 160–161°C, while our compound had an mp of 142–144°C and the isomer **5e** 184–186°C. It seems very likely that compound **6e** described by Japanese authors [3] dealt with a mixture of isomeric N-oxides.

In N-oxides **5d**, **6d**, C-3 protons move upfield while the *peri* protons are shifted more upfield than the parent quinoxalines, making NMR spectra more complicated by overlapping of these resonances with those of other aromatics. Decoupling experiments on 200 MHz spectra were performed and the assignments were thus unambiguously determined.

Results and Discussion

In table I arelisted the results of the *in vitro* microbiological screening of N-oxides **5a-l**, **6a-g** and **6l** against Gram positive and Gram negative bacteria as well as *Trichomonas vaginalis* and *Candida parapsilosis*.

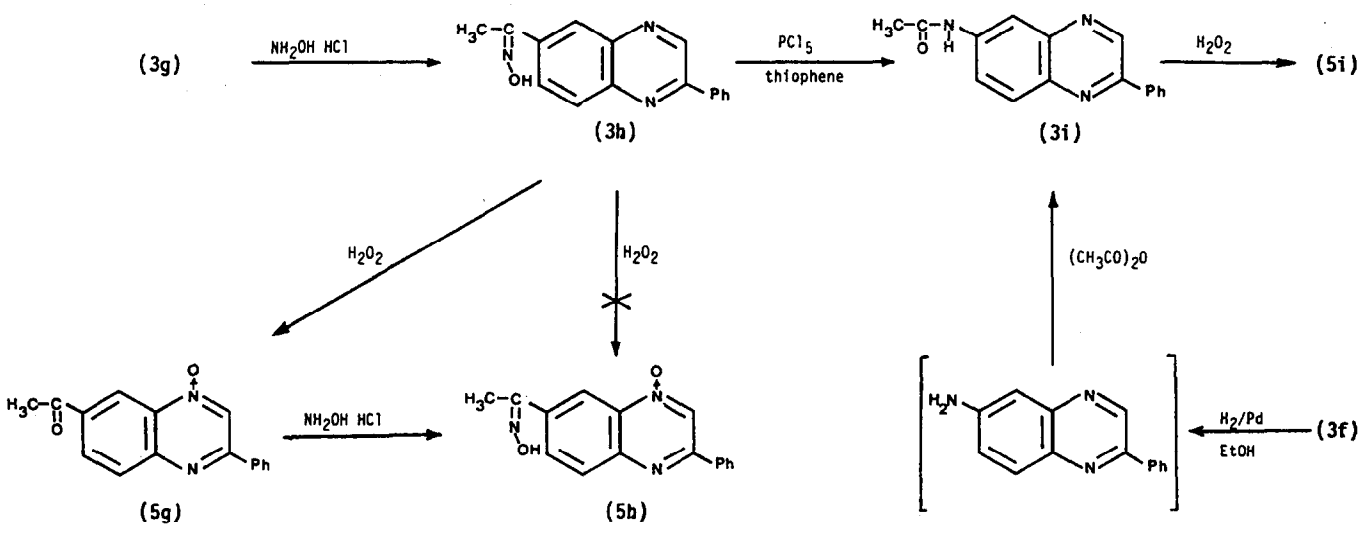
The data indicate a negligible activity against bacteria for the couple of isomeric quinoxalines N-oxides except for compound **6a** (R=CH_3,R'=H) which showed a certain equivalent MIC (62.5 μg/ml) against *E coli*, *P aeruginosa* and *T vaginalis*. The highest activity in the series was found against *Trichomonas vaginalis* for 9 compounds over 18 where MIC ranged from 15.6 μg for **5c**, **6g** to 31.2 for **5g**, **5i** and was equivalent for **5a**, **5b**, **5f**, **6a**, **6b** (62.5 μg/ml).

The structure–activity relationship indicates that electron-withdrawing groups like CF_3 and $COCH_3$ seem bioequivalent and among the couple of isomeric quinoxaline *N*-oxides isomer **5c** was much more active than **6c**, while isomer **5g** had half the activity of **6g**.

Experimental protocols

Chemistry

Melting points were determined using Köfler apparatus and are uncorrected. UV spectra were recorded in ethanol solution in nm (log ε) with a Perkin–Elmer Lambda 5 instrument. IR spec-



Scheme 3.

tra were for nujol mulls and were measured with Perkin–Elmer 781 instrument. ^{1}H NMR spectra were recorded at 60 and 200 MHz with TMS as internal standard using R-24 A Perkin–Elmer–Hitachi and Varian XL-200 spectrometers respectively. Elemental analyses (C, H, N) were performed at Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Padua, and were within ±0.4% of the calculated values.

2-Phenylquinoxalines 3 and 4
These compounds necessary for the conversion into N-oxides of scheme 1 were obtained using the same procedure previously described by us [1, 2]. Compounds **3g**, **4g**, **3h**, **3i** are now described for the first time.

6-Acetyl-2-phenylquinoxaline 3g and 7-acetyl-2-phenylquinoxaline 4g
i) A mixture of equimolar amount (10 mmol) of **1g** and **2** was heated in ethanol under reflux for 3 h. After evaporation of the solvent *in vacuo* the residue was recristallised from aqueous ethanol to give compound **3g**, as white powder (1.6 g, 61% yield), mp 201–203°C, Anal $C_{16}H_{21}N_2O$+0.125 H_2O (C, H, N). UV: λ 208 (4.41), 249 (4.40), 267 (4.36), 344 (4.18). The ethanol mother liquors concentrated up to half volume gave a crystalline compound **4g** (0.3 g, 18% yield) mp 133–134°C, Anal $C_{16}H_{21}N_2O$+0.125 H_2O (C, H, N); UV: λ 209 (4.34), 249 (4.37), 276 (4.37), 346 (4.00).
ii) An identical run as above using alternatively one mol equivalent of 2 M HCl solution or 2 M NaOH solution did not affect the ratio of isomers formed under i).

Quinoxalines N*-oxides 5a–l and 6a–l*
General procedure. To an identical amount of quinoxaline **3** or **4** (0.5 g, 2.23-1.9 mmol) was added anhydrous formic acid (8 ml, 206 mmol) and 30% hydrogen peroxide solution (2 ml) under stirring. The mixture was warmed up to 55–60°C overnight. After cooling the resulting suspension was diluted with water and a yellow solid collected and thoroughly washed with water. On recrystallisation from aqueous ethanol pure compounds listed in table III were obtained. In the case of **6d** from the mother liquors a small portion of 1,4-dioxide **7d** was isolated.

N*(2-nitro-4-acetyl)phenylglycine 9*
3-Nitro-4-bromoacetophenone (Aldrich) (2.2 g, 9 mmol) and phenylglycine **8** (1 g, 6.6 mmol) in ethanol (45 ml) were added to a solution of sodium hydrogencarbonate (1.5 g) in water (28 ml) and the mixture was heated under reflux for 24 h. The solvent was evaporated and the resulting alkaline solution extracted with ether. The organic layer, dried over anhydrous sodium sulfate and evaporated to dryness gave unreacted 3-nitro-4-bromoacetophenone (0.3 g). The alkaline solution on careful acidification with 2 M HCl aqueous solution precipitated a solid which was filtered off and washed with water. Recrystallisation from ethanol yielded **9** (1.6 g, 77% yield) as yellow needles, mp 207–209°C. Anal $C_{16}H_{14}N_2O_5$ (C, H, N). IR (Nujol) 3360, 1735, 1645, 1560 cm^{-1}; ^{1}H NMR ($CDCl_3$+DMSO–d_6): δ. 9.30 (1H, br s, NH collapses with D_2O), 8.65 (1H, d, *J* 2, 3-H), 7.70 (1H, dd, *J* 9 and 2, 5-H), 7.45 (1H, br s, COOH, collapses with D_2O), 7.50÷7.10 (5H, m, Ph), 6.55 (1H, d, *J* 9, 6-H), 5.25 (1H, d, *J* 6, HN-C*H*-Ph, collapses into a singlet with D_2O), 2.45 (3H, s, CH_3CO).

*1,2-Dihydro-2-phenyl-6-acetylquinoxalin-3(4*H*)one 10*
A mixture of **9** (2.5 g, 7.95 mmol) in ethanol (100 ml) was hydrogenated at 3 atm and in the presence of 10% Pd/C (0.25 g) within 30 min. The catalyst was removed and the solvent evaporated *in vacuo* to give **10** (1.35 g, 64% yield) as yellow flakes, mp 236–238°C, from ethanol. Anal $C_{16}H_{14}N_2O_2$. IR (nujol) 3290, 1660, cm^{-1}; UV (EtOH): λ 205 (4.26), 224 (4.18), 269 (4.23), 314 (3.85), 364 (4.13); ^{1}H NMR ($CDCl_3$+DMSO–d_6): δ 10.30 (1H, br s, N*H*-CO), 7.60÷7.05 (7H, m, Ph + 5 and 7-H), 6.72 (1H, br s, N*H*), 6.65 (1H, d, *J* 9, 8-H), 5.00 (1H, br s, 2-H, sharpens with D_2O), 2.40 (3H, s, CH_3CO).

*6-Acetyl-2-phenylquinoxalin-3(4*H*)one 11*
A mixture of **10** (1 g, 3.75 mmol) in 10% sodium hydroxide solution (20 ml) and 30% hydrogen peroxide (4 ml) was heated under stirring at 100°C for 1.5 h. After cooling the alkaline solution was neutralised with aqueous HCl solution and a solid was collected, washed with water, to give **11** (0.85 g, 84%) mp 292–295°C, as pale yellow flakes, from acetic acid. Anal $C_{16}H_{12}N_2O_2$+0.25H_2O. IR (nujol): 1680, 1660 cm^{-1}; UV (EtOH): λ 203 (4.45), 227 (4.52), 316 (4.26), 378 (4.16); ^{1}H NMR (DMSO–d_6) δ 12.30 (1H, br s, N=C—OH), 8.35÷7.20 (8H, m, aromatics), 2.58 (3H, s, CH_3-CO).

Table III. Quinoxaline N-oxides. A, aqueous ethanol; B, acetone; C, ethanol. [a]lit's mp was 123–125°C but dealt with a mixture of 2 isomeric quinoxalines N-oxides. [b]Purified by column chromatography through silica gel eluting with dichloromethane.

Compound	M.P. °C	Solvent	Yield %	Formula	Elemental analysis or lit. m.p.	Ref.
(5a)	162-63	A	65	$C_{15}H_{12}N_2O$	C,H,N [a]	(3,4,5)
(5b)	190-92	A	85	-----	196-98	(3,4,5)
(5c)	186-90	A	85	-----	186-90	(1)
(5d)	176-78	B	28	$C_{14}H_9FN_2O$	C,H,N	
(5e)	184-86	C	80	$C_{15}H_{12}N_2O_2$ +0.125 H_2O	C,H,N	
(5f)	208-10	[b]	56	-----	214	(3,4)
(5g)	209-11	C	70	$C_{16}H_{12}N_2O_2$ +0.125 H_2O	C,H,N	
(5h)	271-74		78	$C_{16}H_{13}N_3O_2$ +0.125 H_2O	C,H,N	
(5i)	255-59	A	70	$C_{16}H_{13}N_3O_2$ +0.35 H_2O	C,H,N	
(5L)	160-70	A	83	-----	160-70	(1)
(6a)	121-23	A	66	-----	124-26	(3,4,5)
(6b)	162-64	A	85	-----	162-64	(3,4,5)
(6c)	158-60	A	80	-----	158-60	(1)
(6d)	121-23	B	45	$C_{14}H_9FN_2O$	C,H,N	
(6e)	142-44	C	80	$C_{15}H_{12}N_2O_2$ +0.25 H_2O	C,H,N	
(6f)	260-62	A	75	$C_{14}H_9N_3O_3$	C,H,N	
(6g)	215-17	A	56	$C_{16}H_{12}N_2O_2$ +0.25 H_2O	C,H,N	
(6L)	87-89	A	36	-----	87-89	(1)
(7d)	202-04	B	12	$C_{14}H_9N_2O_2$	C,H,N	

6-Acetyl-3-chloro-2-phenylquinoxaline ***12***
A mixture of **11** (1 g, 3.78 mmol) and a slight excess of $POCl_3$ was stirred at 90°C for 1 h. $POCl_3$ was then removed *in vacuo* and the residue was triturated with water and filtered off to give **13** (0.9 g, 84%) as pale yellow needles, mp 143–145°C from ethanol. Anal $C_{16}H_{11}ClN_2O$. IR (nujol) 1690 cm^{-1}; UV (EtOH): λ 196 (4.07), 216 (4.31), 254 (4.54), 343 (4.01); ^1H NMR ($CDCl_3$): δ 8.50+7.10 (8H, m, aromatics), 2.69 (3H, s, CH_3-CO).

6-Acetyl-2-phenyl-1,2,3,4-tetrahydroquinoxaline ***13***
A mixture of **12** (0.4 g, 1.4 mmol), triethylamine (1.43 g, 1.4 mmol), 10% palladised charcoal (80 mg) in ethanol (60 ml) was hydrogenated at 20°C and 3 atm within 2 h. The catalyst was removed by filtration and the solvent evaporated *in vacuo* to afford a yellow residue of **13** (0.3 g, 85%), as yellow needles, mp 166–168°C from ethanol. Anal $C_{16}H_{16}N_2O$. IR (nujol): 3370, 3340, 1645 cm^{-1}; UV (EtOH): λ 205 (4.26), 244 (4.18), 269 (4.23), 315 (3.83), 364 (4.13); ^1H NMR ($CDCl_3$): δ 7.20+7.10 (8H, 2 app. s, arom), 6.40 (1H, d, *J* 9, 8-H), 4.60+4.10 (2H, m, NH+C*H*-Ph), 3.50+3.10 (3H, m, CH_2 + NH), 2.40 (3H, s, CH_3-CO).

6-Acetyl-2-phenylquinoxaline ***3g***
To **13** (0.15 g, 0.6 mmol) in methanol (5 ml) at room temperature was added a 30% hydrogen peroxide solution (0.7 ml) and concd hydrochloric acid (4 drops). The mixture was stirred for 40 min the diluted with water and made neutral with 2 M sodium hydroxide solution. The precipitate collected, washed with water and dried, gave **3g** (0.1 g, 69% yield) identical with an authentic specimen coming from the separation of the isomeric mixture of **3g**, **4g**. The hydrogenation of **3g** in an identical run as under **13** gave this compound in 80% yield whose mp, IR, UV, NMR were coincident.

Methyl-(2-phenylquinoxalin-6-yl)ketoxime ***3b***
A solution of hydroxylamine hydrochloride (1.68 g, 24 mmol) in 10% aqueous sodium hydroxide solution was added to a suspension of **3g** (1 g, 4 mmol) in ethanol (100 ml). The mixture was heated under reflux for 2 h, then allowed to cool. A solid filtered off and washed with ethanol yielded compound **3h** (0.8 g, 75%) mp 263–265°C, from acetic acid. Anal $C_{16}H_{13}N_3O$ + 0.5 H_2O. UV (EtOH): λ 216 (4.05), 271 (4.37), 354 (3.94).

6-Acetylamino-2-phenylquinoxaline ***3i***
i) From compound **3f**: — a mixture of **3f** (0.8 g, 3.18 mmol) and 10% palladised charcoal (80 mg) in ethanol (200 ml) was hydrogenated at 20°C and 3 atm within 30 min. After filtration of the catalyst and evaporation of the solvent a brown glassy solid was obtained. Trituration with acetic anhydride (2 ml) gave **3i** (0.6 g, 71%), mp 236–240°C, as brown crystals from ethanol. Anal $C_{16}H_{13}N_3O$ + 0.25 H_2O. IR (nujol): 1700 cm^{-1}; UV (EtOH): λ 214 (4.17), 272 (4.55), 332 (3.70), 370 (4.12).

ii) From **3h**: — To a suspension of **3h** (0.4 g, 1.52 mmol) in dry tiophene (4 ml) was added within 10 min PCl_5 (0.46 g, 2.2 mmol) and the mixture was stirred at room temperature for 2.5 h. The water (4 ml) was added and the stirring was continued for 3 h. Further dilution with water followed by filtration yielded **3i** (0.3 g, 75%), mp 235–240°C identical with a specimen obtained as above.

Microbiological methods

Test organisms and culture media
Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Candida parapsilosis* (hospital isolated) were cultivated in trypticase soy broth (TSB-Oxoid), while *Trichomonas vaginalis* (hospital isolated) was grown in Diamond's medium [6].

Antimicrobial assay
The antimicrobial activities of the compounds listed in table I were determined by a microdilution broth method [7] and the results are described in table III.

Each compound was dissolved in dimethylsulfoxide (DMSO), methanol, and further diluted in TSB. Inocula of bacteria and *Candida parapsilosis* were prepared from overnight growth cultures diluted such that the final inoculum size was of *ca* 10^6 cells/ml, while *Trichomonas vaginalis* inoculum was a desired volume of 48 h culture. Plastic trays inoculated adding 50 µl of diluted antimicrobial agent were incubated at 37°C and examined after 24 and 48 h for the presence of growth. The minimal inhibitory concentration (MIC) was defined as the lowest amount of compound preventing growth or definite turbidity. The inhibitory effect of the DMSO–methanol–TSB solution towards the test organisms was determined and was observed that it had no antimicrobial activity. As quality control the test organisms were assayed for susceptibility to nalidixic acid.

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