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Received November 27, 1991

The reaction of 2-chloromethyl-3-ethoxycarbonyl-4-phenylquinoline with phenylhydrazine, afforded 2,3-dihydro-9-phenyl-2-phenylamino-1*H*-pyrrolo[3,4-*b*]quinolin-1-one derivatives **2** along with a major amount of 3-ethoxycarbonyl-4-phenylquinoline-2-carboxaldehyde phenylhydrazone **4a**. The geometric isomers of phenylhydrazone **4a**, displaying solvent-dependent *E-Z* isomerism, were isolated, characterized by ¹H-nmr and mass spectra, and the *Z*-form easily cyclized to pyridazino[4,5-*b*]quinoline derivative **5a**. Analogously, compounds **2b**, **2c**, **4b**, **4c**, **5b** and **5c** were obtained. The title compounds were tested as potential ligands for central and peripheral-type of benzodiazepine receptors, and the results are reported.

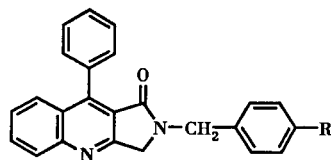
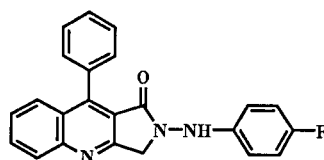
J. Heterocyclic Chem., **29**, 1111 (1992).

The central benzodiazepine receptor (CBZR) in the brain has been extensively studied since its discovery in 1977 [1], but there exists another class of pharmacologically and biochemically distinct benzodiazepine recognition site in many tissues and cell types, including kidney, heart, liver, lung [2] and even in the brain [3]. This site has been generally termed "peripheral-type" and can be differentiated by selective ligands such as Ro-5-4864, a 4'-chloro-substituted diazepam [4] and PK 11195, an isoquinoline carboxamide derivative [5], both being active in the nanomolar range to peripheral-type benzodiazepine receptor (PBZR). The common structural features, shared by these two compounds, such as the presence of a carboxamido group, a freely rotating substituted aromatic ring and an overall planar topography, were assumed to be basic requirements for the peripheral binding [6].

According to these findings, very recently, we reported [7] the synthesis and PBZR binding studies of pyrrolo[3,4-*b*]quinoline derivatives **1**. Among these, compounds **1b** and **1c** displayed good affinity for the peripheral binding site. In order to further investigate the structural prerequisites essential for peripheral binding, we wish now to

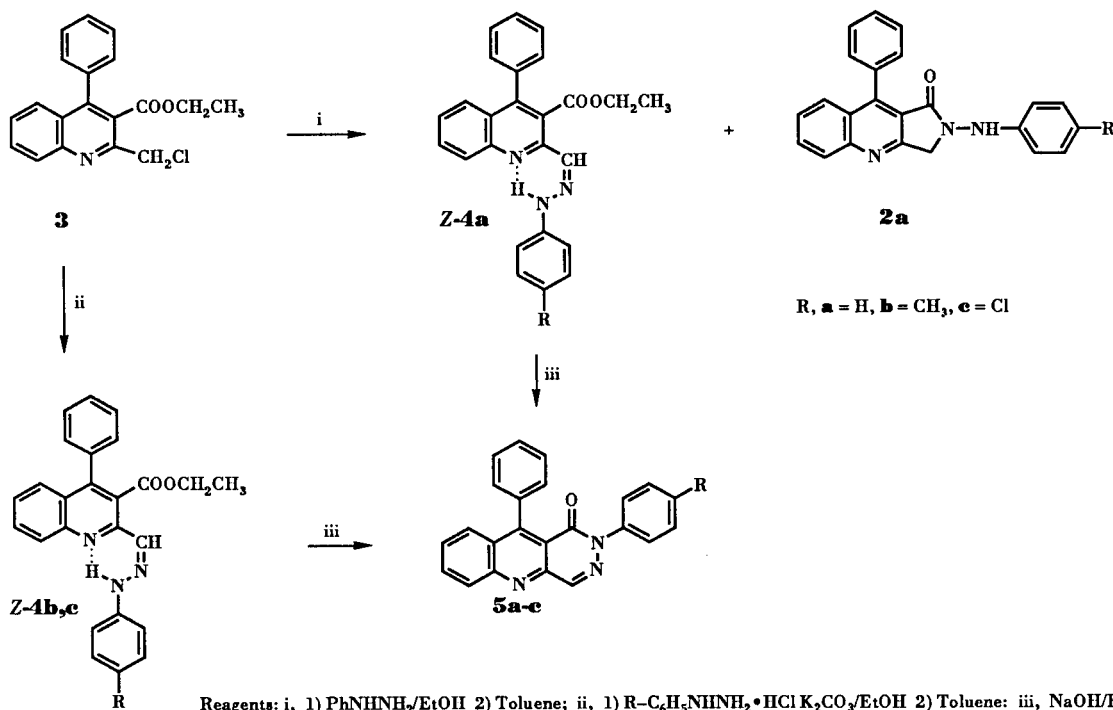
report the synthesis of compounds **2**, in which the benzylic moiety is replaced by the isosteric phenilaminic group.

Earlier the synthesis of the 2-substituted pyrrolo[3,4-*b*]quinoline nucleus was reported in several papers [8] but only a few described the preparation of 2-amino derivatives [9,10]. Moreover, we have reported [11] the synthesis of 2-amino-2,3-dihydro-9-phenyl-1*H*-pyrrolo[3,4-*b*]quinolin-1-one, accomplished by reaction of **3** with hydrazine hydrate. In the same manner the synthesis of compound **2** was performed, but when compound **3** was made to react with a large excess of phenylhydrazine in boiling ethanol, a yellow solid was obtained. Its characterization by ¹H-nmr revealed that it was a mixture of two products: a pyrrolo[3,4-*b*]quinoline derivative and an open intermediate in which the ester group was still present. The attempts to purify the mixture by column chromatography were unsuccessful and in order to obtain the cyclized compound **2a** as the sole product, we refluxed the yellow solid in toluene. In this way it was possible to separate the expected pyrrolo[3,4-*b*]quinoline derivative **2a** from the yellow phenylhydrazone **4a**, to which the *Z* configuration was assigned by the chemical shift (14.95 ppm) of the hy-

**1a-c****2a-c**

R, **a** = H, **b** = CH₃, **c** = Cl

Scheme 1



drazone proton. This fact could be explained taking into account the formation of putative intramolecular hydrogen bonding, between the hydrazone proton and the nitrogen atom of the quinoline nucleus.

The existence of two geometric isomers of compound **4a** was demonstrated by ^1H -nmr spectroscopic studies of the *Z* isomer **4a** in different deuterated solvents; in fact, when the spectra were observed in deuteriochloroform **Z-4a** was the sole and stable isomer, while in DMSO-d_6 the mixture of two isomers suddenly appeared, and the interconversion from **Z-4a** into *E-4a* isomer was complete in a week. Probably an equilibrium does exist between the two isomers of phenylhydrazone **4a** depending on the characteristic of the solvent. In fact, in solvent less amenable to hydrogen-bonding (e.g. toluene, chloroform), the **Z-4a**

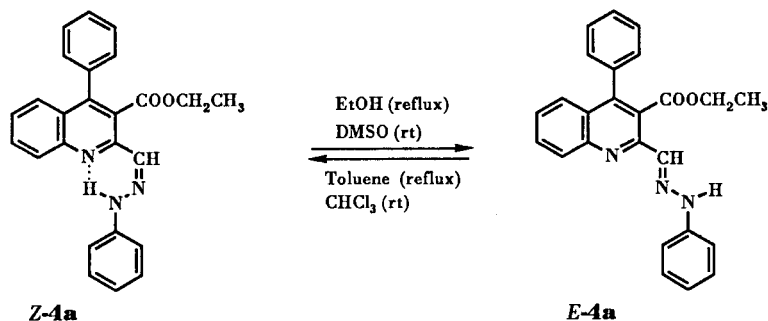
isomer with intramolecular hydrogen-bond is the preferred one, while in hydrogen-bonding solvents (e.g. ethanol, dimethyl sulfoxide) the *E-4a* isomer was favoured.

These findings were confirmed refluxing the *Z*-isomer of the phenylhydrazone **4a** in ethanol to give the *E*-isomer (Scheme 2), which was in turn boiled in toluene to afford the expected *Z*-isomer, as above described.

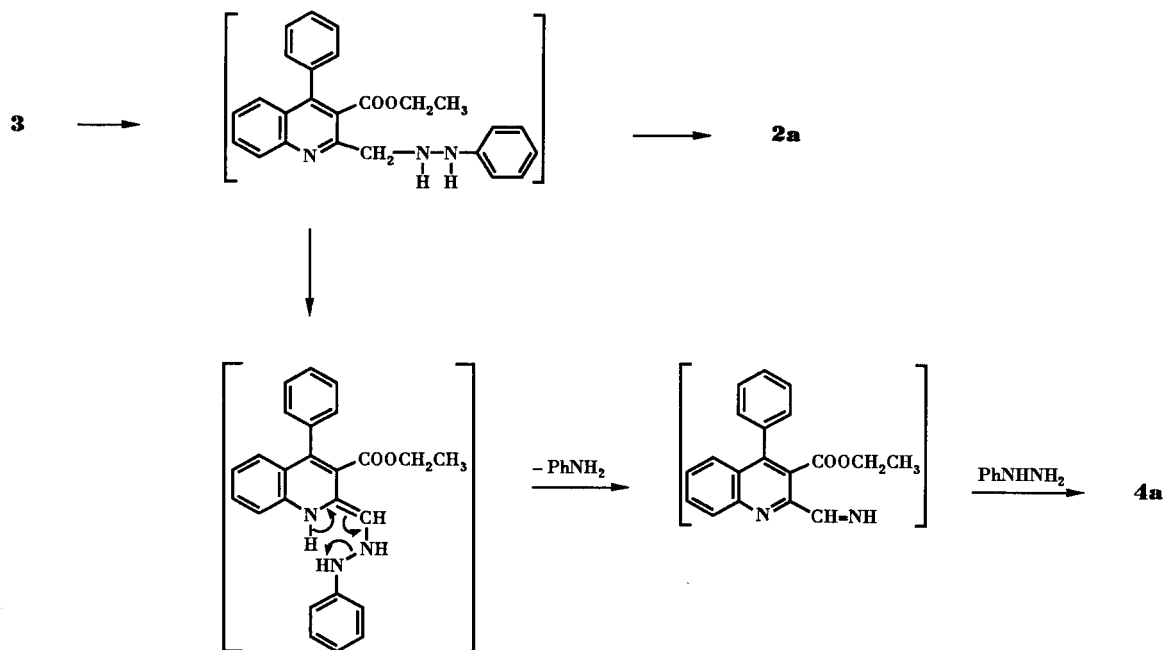
The comparison of the ^1H -nmr spectra of the *E*-isomer of the phenylhydrazone **4a** and pure pyrrolo[3,4-*b*]quinoline derivative **2a** with that of the yellow solid demonstrated that this one was a mixture formed by a large part of compound *E-4a* along with a minor amount of compound **2a**.

When compound **3** was allowed to react with phenylhydrazine the nucleophilic substitution could be followed by

Scheme 2



Scheme 3



cyclization which furnished the pyrrolo[3,4-*b*]quinoline derivative **2a**. Competitively, an oxidation process analogous to the formation of oxazones and described for similar compounds [12] could also occur. The latter process led to the hydrazone **4a** as reported in the Scheme 3. By using the substituted phenylhydrazine hydrochloride in large excess, only the oxidation process occurred and the arylhydrazones **4b,c** were the sole recovered products. Compounds **4a-c** were in turn cyclized to pyridazino[3,4-*b*]quinoline derivatives **5a-c** in quantitative yield, running the reaction in refluxing ethanol and in presence of catalytic amount of 2.5 *N* sodium hydroxide.

Furthermore, starting from compound **3**, it was possible to obtain the pyrrolo[3,4-*b*]quinoline derivatives **2b,c**, although in no satisfactory yield, using the proper phenylhydrazine hydrochlorides in slight excess and in presence of potassium carbonate.

In vitro binding studies were performed, according to well established protocols [13,14,15], to test the ability of the title compounds to displace ^3H -Ro 151788 and ^3H -PK 11195 from CBZR and PBZR, respectively [16]. While all the synthesized compounds were devoid of any biological activity for CBZR, derivatives **2b** and **2c** displayed micromolar affinity for PBZR (Table 1), comparable to that which we previously reported [7] for the above cited compounds **1**. In conclusion, the replacement of the benzyl moiety with the phenylamine group in compounds **2**, seems not to significantly alter the binding for the peripheral-type of benzodiazepine receptor, confirming the selectivity of the pyrrolo[3,4-*b*]quinolin-1-one derivatives for this recognition site.

Table 1

Results of Binding on Benzodiazepine "Peripheral" Receptor

Compound	IC ₅₀ (nM)
2a	N.A. [a]
2b	728.56
2c	801.48
4a	4432.81
4b	5154.92
4c	N.A.
5a	N.A.
5b	N.A.
5c	N.A.
PK 11195	2.94

[a] N.A., no inhibition till 10^{-5} M.

EXPERIMENTAL

Melting points were determined in open capillaries on a Büchi apparatus and are uncorrected. Microanalyses were carried out on a Perkin-Elmer 240C Elemental Analyzer. Anhydrous sodium sulphate was used as the drying agent. Merck silica gel 60 (70-230 mesh) was used for column chromatography. The ir spectra were recorded in Nujol mulls on a Perkin-Elmer 398 spectrometer. The ^1H -nmr spectra were recorded on a Varian XL 200 spectrometer in the solvents indicated. Chemical shifts are given in ppm from TMS as the internal standard, and coupling constants (*J*) in Hz. Mass spectra (EI, 70 eV) were recorded on a VG 70-250S spectrometer. The ir, nmr and elemental analyses were performed in house. Mass spectra were performed by Centro di Analisi e Determinazioni Strutturali-Università di Siena.

Procedure for the Condensation of 3-Carboethoxy-2-chloromethyl-4-phenylquinoline (**3**) with Phenylhydrazines.

Method A.

A mixture of compound **3** (1.0 g, 3.07 mmoles) and phenylhydrazine (20.3 mmoles) in ethanol (20 ml) was heated to reflux for 60 minutes and after being cooled in a freezer overnight, a yellow solid was obtained. The solid was thoroughly washed with water, dried and added to boiling toluene (20 ml). The solution was refluxed for 4 hours and the solvent removed under reduced pressure to afford a residue which was chromatographed eluting first with light petroleum ether/chloroform (50/50 v/v) to give pure **4a** (0.6 g, yield 49%) and then with chloroform/ethyl acetate (70/30 v/v) to yield **2a** (0.23 g). Further crystallization of **2a** from ethyl acetate/chloroform gave the pure product (0.15 g, yield 14%).

3-Carboethoxy-4-phenylquinoline-2-carboxaldehyde Phenylhydrazone (**Z-4a**).

An analytical sample crystallized from benzene/cyclohexane and melted at 166-167°; ir: 1720 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 0.98 (t, 3H, CH₃), 4.09 (q, 2H, CH₂), 6.96-7.10 (m, 1H, arom), 7.31-7.57 (m, 12H, 11H arom and 1H, CH=N), 7.75-7.82 (m, 1H, arom), 8.11 (d, 1H, J = 8.3, H₆), 14.96 (s, 1H, NH); ms: m/z 395 (100, M⁺).

Anal. Calcd. for C₂₅H₂₁N₃O₂: C, 75.93; H, 5.35; N, 10.62. Found: C, 76.13; H, 5.44; N, 10.58.

2,3-Dihydro-9-phenyl-2-phenylamino-1*H*-pyrrolo[3,4-*b*]quinolin-1-one (**2a**).

Recrystallization from ethyl acetate/chloroform gave an analytical sample melting at 225° dec; ir: 3260 cm⁻¹ (NH), 1700 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 4.90 (s, 2H, CH₂), 6.44 (s, 1H, NH), 6.78-6.83 (m, 2H, arom), 6.90-6.98 (m, 1H, arom), 7.19-7.23 (m, 2H, arom), 7.43-7.61 (m, 6H, arom), 7.86-7.92 (m, 2H, arom), 8.22 (d, 1H, J = 8.8, H₅); ¹H-nmr (DMSO-*d*₆): 4.79 (s, 2H, CH₂), 6.69-6.77 (m, 3H, arom), 7.10-7.18 (m, 2H, arom), 7.41-7.72 (m, 7H, arom), 7.88-7.95 (m, 1H, arom), 8.17 (d, 1H, J = 8.7, H₅), 8.31 (s, 1H, NH); ms: m/z 351 (100, M⁺).

Anal. Calcd. for C₂₃H₁₇N₃O: C, 78.61; H, 4.88; N, 11.96. Found: C, 78.63; H, 4.89; N, 11.90.

Method B.

A mixture of compound **3** (1.0 g, 3.07 mmoles) and the proper phenylhydrazine hydrochloride (12.6 mmoles) in ethanol (20 ml) in the presence of potassium carbonate (0.87 g, 6.3 mmoles) was refluxed for 4 hours. After removal of the solvent under reduced pressure, the reaction product was dissolved in chloroform and the organic layer washed with water to neutrality and worked up as usual affording a gummy residue which on treatment with ethanol provided a yellow solid. The solid was then filtered, dried and dissolved in boiling toluene. After being refluxed for 4 hours the solution was evaporated *in vacuo* and the residue was purified by column chromatography using light petroleum ether/chloroform (50/50 v/v) to give compound **Z-4b** or **Z-4c**, respectively.

3-Carboethoxy-4-phenylquinoline-2-carboxaldehyde *p*-Tolylhydrazone (**Z-4b**).

This compound was obtained in 64% yield. Recrystallization from benzene/cyclohexane gave an analytical sample melting at

186-187°; ir: 1720 cm⁻¹; ¹H-nmr (deuteriochloroform): 0.98 (t, 3H, CH₃ ester), 2.33 (s, 3H, CH₃Ph), 4.09 (q, 2H, CH₂ ester), 7.14-7.59 (m, 12H, 11H arom and 1H, CH=N), 7.73-7.81 (m, 1H, arom), 8.09 (d, 1H, J = 8.1, H₆), 14.92 (s, 1H, NH); ms: m/z 409 (100, M⁺).

Anal. Calcd. for C₂₆H₂₃N₃O₂: C, 76.26; H, 5.66; N, 10.26. Found: C, 76.19; H, 5.77; N, 10.26.

3-Carboethoxy-4-phenylquinoline-2-carboxaldehyde *p*-Chlorophenylhydrazone (**Z-4c**).

This compound was obtained in 61% yield. An analytical sample crystallized from benzene/cyclohexane melted at 209-210°; ir: 1723 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 0.97 (t, 3H, CH₃), 4.09 (q, 2H, CH₂), 7.23-7.62 (m, 12H, 11H arom and 1H, CH=N), 7.75-7.83 (m, 1H, arom), 8.09 (d, 1H, J = 7.9, H₆), 14.97 (s, 1H, NH); ms: m/z 429 (100, M⁺).

Anal. Calcd. for C₂₅H₂₀ClN₃O₂: C, 69.85; H, 4.69; N, 9.77. Found: C, 70.10; H, 4.76; N, 9.75.

Method C.

A mixture of compound **4a** or **4b** or **4c** (1 mmole) in ethanol (20 ml), to which four drops of 2.5 *N* sodium hydroxide was added, was kept at reflux for 1 hour. The solid which precipitated on cooling was filtered, washed with water and dried to give the corresponding compound **5a** or **5b** or **5c** as a pure product.

2,10-Diphenyl-2*H*-pyridazino[4,5-*b*]quinolin-1-one (**5a**).

This compound was obtained in 84% yield. Recrystallization from benzene/ethyl acetate afforded an analytical sample melting at 216-217°; ir: 1674 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 7.25-7.65 (m, 12H, arom), 7.89-7.97 (m, 1H, arom), 8.31 (d, 1H, J = 8.6, H₆), 8.65 (s, 1H, H₄); ms: m/z 349 (100, M⁺).

Anal. Calcd. for C₂₃H₁₅N₃O: C, 79.07; H, 4.33; N, 12.03. Found: C, 79.40; H, 4.48; N, 12.11.

10-Phenyl-2-(*p*-tolyl)-2*H*-pyridazino[4,5-*b*]quinolin-1-one (**5b**).

This compound was obtained in 88% yield. An analytical sample recrystallized from benzene/ethyl acetate melted at 251-252°; ir: 1680 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 2.34 (s, 3H, CH₃), 7.18-7.61 (m, 11H, arom), 7.88-7.96 (m, 1H, arom), 8.30 (d, 1H, J = 8.5, H₆), 8.63 (s, 1H, H₄); ms: m/z 363 (100, M⁺).

Anal. Calcd. for C₂₄H₁₇N₃O: C, 79.32; H, 4.72; N, 11.56. Found: C, 79.27; H, 4.86; N, 11.31.

2-(*p*-Chlorophenyl)-10-phenyl-2*H*-pyridazino[4,5-*b*]quinolin-1-one (**5c**).

This compound was obtained in 87% yield. An analytical sample recrystallized from benzene/ethyl acetate melted at 232-233°; ir: 1680 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 7.24-7.61 (m, 11H, arom), 7.89-7.98 (m, 1H, arom), 8.30 (d, 1H, J = 8.7, H₆), 8.64 (s, 1H, H₄); ms: m/z 383 (100, M⁺).

Anal. Calcd. for C₂₃H₁₄ClN₃O: C, 71.97; H, 3.68; N, 10.95. Found: C, 72.05; H, 3.69; N, 11.03.

Method D.

A mixture of compound **3** (1.0 g, 3.07 mmoles) in ethanol (20 ml), was allowed to react with a suitable phenylhydrazine hydrochloride (3.07 mmoles) in presence of an excess of potassium carbonate (2.0 g). After the mixture has been refluxed for 4 hours, an additional amount of the same phenylhydrazine hydrochloride (1.5 mmoles) was added and heating prolonged for 4 hours. The solvent was removed under reduced pressure and the residue dis-

solved in chloroform. The organic layer was washed with water, dried and concentrated *in vacuo* to give a gummy product which was purified by chromatography, using chloroform as eluent to afford **2b** or **2c**, respectively.

2,3-Dihydro-9-phenyl-2-(*p*-tolylamino)-1*H*-pyrrolo[3,4-*b*]quinolin-1-one (2b**).**

This compound was obtained in 21% yield. Recrystallization from ethyl acetate/chloroform gave an analytical sample melting at 229° dec; ir: 3260 cm⁻¹ (NH), 1690 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 2.24 (s, 3H, CH₃), 4.87 (s, 2H, CH₂), 6.40 (s, 1H, NH), 6.70-6.74 (m, 2H, arom), 7.00-7.04 (m, 2H, arom), 7.41-7.60 (m, 6H, arom), 7.81-7.90 (m, 2H, arom), 8.20 (d, 1H, J = 8.4, H_s); ms: m/z 365 (62, M⁺).

Anal. Calcd. for C₂₄H₁₉N₃O: C, 78.88; H, 5.24; N, 11.50. Found: C, 78.55; H, 5.25; N, 11.35.

2-(*p*-Chlorophenylamino)-2,3-dihydro-9-phenyl-1*H*-pyrrolo[3,4-*b*]quinolin-1-one (2c**).**

This compound was obtained in 13% yield. Recrystallization from ethyl acetate/chloroform afforded an analytical sample melting at 222°; ir: 3270 cm⁻¹ (NH), 1698 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 4.84 (s, 2H, CH₂), 6.48 (s, 1H, NH), 6.69-6.73 (m, 2H, arom), 7.14-7.18 (m, 2H, arom), 7.38-7.60 (m, 6H, arom), 7.82-7.90 (m, 2H, arom), 8.20 (d, 1H, J = 8.7, H_s); ms: m/z 385 (100, M⁺).

Anal. Calcd. for C₂₃H₁₆ClN₃O: C, 71.60; H, 4.18; N, 10.89. Found: C, 71.45; H, 4.22; N, 10.68.

Transformation of the *Z*-Isomer **4a into the Corresponding *E*-Isomer.**

A mixture of compound **Z-4a** (0.395 g, 1 mmole) in ethanol (20 ml) was heated to reflux for 5 hours and the solvent removed under reduced pressure. The residue was purified by column chromatography, using chloroform as the eluent, to give the pure *E*-isomer **4a** (0.316 g, yield 80%).

3-Carboxy-4-phenylquinoline-2-carboxaldehyde Phenylhydrazine (Z-4a**).**

Further chromatography, eluting with chloroform, afforded an analytical sample melting at 162-163°; ir: 3252 cm⁻¹ (NH), 1686 cm⁻¹ (C=O); ¹H-nmr (DMSO-d₆): 0.81 (t, 3H, CH₃), 3.97 (q, 2H, CH₂), 6.81-6.99 (m, 1H, arom), 7.03-7.19 (m, 2H, arom), 7.23-7.27 (m, 2H, arom), 7.32-7.57 (m, 7H, arom), 7.79-7.82 (m, 1H, arom), 8.04-8.08 (d, 2H, H_s and CH=N), 10.98 (s, 1H, NH); ms: m/z 395 (100, M⁺).

Anal. Calcd. for C₂₅H₂₁N₃O₂: C, 75.93; H, 5.35; N, 10.62. Found: C, 75.67; H, 5.45; N, 10.42.

Acknowledgement.

This work was supported by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica and the Consiglio Nazionale delle Ricerche. The authors wish to thank Professor F. Ponticelli for recording the mass spectra and helpful discussion. Dr. T. Mennini (Istituto Ricerche Farmacologiche "M. Negri", Milano) is also acknowledged for her collaboration.

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- * To whom correspondence and inquiries should be addressed.
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