A CONVENIENT ROUTE TO 1,4-DIHYDRO-3-CYANO-10-METHYL-PYRIDO[3,2-g]-QUINOLINE DERIVATIVES AS KEY-INTERMEDIATES FOR THE SYNTHESIS OF NOVEL MDR REVERSAL AGENTS

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ABSTRACT: The synthesis of 1,4-dihydro-3-cyano-10-methyl-pyrido[3,2-*g*]quinolin-4-one, **3**, was performed by condensation of 7-amino-8-methyl-quinoline with ethyl(ethoxymethylene)cyanoacetate, followed by the cyclization of the intermediate by intramolecular electrophilic substitution. The thiation of **3** gave the 1,4-dihydro-3-cyano-10-methyl-pyrido[3,2-*g*]quinolin-4-thione while the chlorination gave the 4-chloro-3-cyano-10-methyl-pyrido[3,2-*g*]quinoline. These derivatives could be used as suitable intermediates in the preparation of MDR reversal drugs.

INTRODUCTION

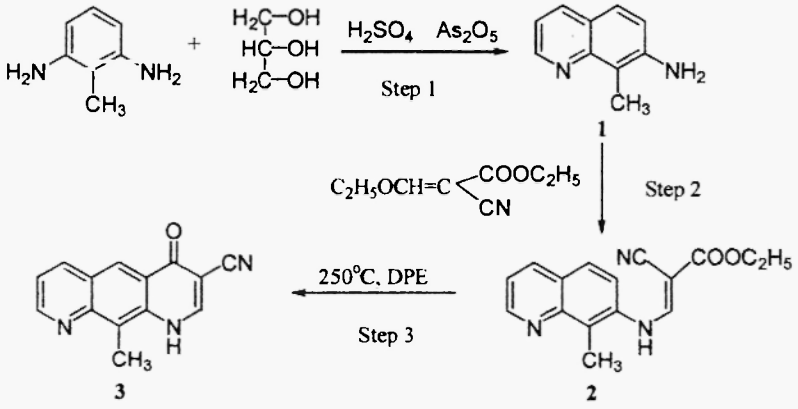
The capability of eukaryotic and prokaryotic cells to reduce their susceptibility towards structurally unrelated cytotoxic drugs is known as multidrug resistance (MDR) (1,2). This kind of resistance based on the increase of drug efflux, is responsible of several nosocomial infections which are of dramatic importance at the present moment in particular as far as Gram(-) bacteria are concerned in (3). Synthesis of symmetrically 4,6-bis-substituted amino-, oxo- and thio-pyrido[3,2-*g*]quinoline derivatives was previously reported (4,5) and their MDR reversal activity (6) was demonstrated. Hence, to synthesize unsymmetrically substituted derivatives was needed with the aim to investigate the biological role of side-chains branched on extracyclic nitrogen, oxygen or sulfur heteroatoms.

With respect to this, an easy way to prepare 3-cyano-10-methyl-pyrido[3,2-*g*]quinolin-4-one, **3**, 3-cyano-10-methyl-pyrido[3,2-*g*]quinolin-4-thione, **4**, and 4-chloro-3-cyano-10-methyl-pyrido[3,2-*g*]quinoline, **5**, as valuable intermediates is described.

RESULTS AND DISCUSSIONS

The synthesis of 3-cyano-10-methyl-pyrido[3,2-*g*]quinolin-4-one, **3**, was performed in three steps as shown in scheme 1.

In the first step, 7-amino-8-methyl-quinoline, **1**, was synthesized according to previously reported methods (7,8). Thus **1** was obtained in 47% yield and was purified by crystallization from water to a degree of purity superior to those reported.

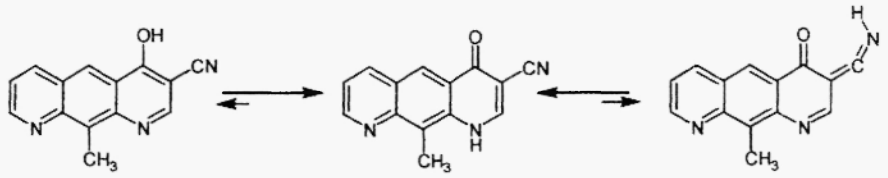


Scheme 1

In the second step, the reaction of **1** with ethyl(ethoxymethylene)cyanoacetate was performed using ethanol as solvent. The stretching vibration of the C≡N bond produces intense absorption band situated at 2211.07 cm^{-1} in the IR spectrum of **2**. The extended conjugation of the electronic system determines the unusual low value for the wavenumber characterizing the carbonyl group stretching vibration situated at 1669.61 cm^{-1}, while the stretching vibration of the homogenous double bond absorbs intensely at 1635.42 cm^{-1} due to its polarization. In the ^{1}H-NMR spectrum, the splitting due to the couplings in the –HN-CH= system is observed and for the vicinal coupling constant ^{3}J a value of 13 Hz was measured.

In the third reaction step, the cyclization of **2** took place by intramolecular electrophilic aromatic substitution. The reaction was performed by heating the diluted solution of **2** in diphenylether under argon atmosphere at 240°C. The 3-cyano-10-methyl-pyrido[3,2-*g*]quinolin-4-one **3** thus obtained

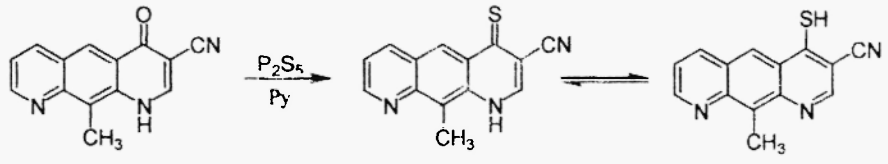
in 70% yield was structurally investigated by means of FT-IR and NMR spectroscopy because of the three possible tautomeric forms portrayed in scheme 2.



3b **3a** **3c**

Scheme 2

According to NMR spectra, there are no evidences for tautomeric forms **3b** and **3c**. In the **3a** tautomeric form, the mobility of the proton in the –NH- (vinylogue amide) group might explain the absence of a coupling constant with the neighboring proton situated at position 2 of the heterocycle, which appears as a singlet at 8.65 ppm. Structure **3a** is supported by the ^{13}C-NMR spectrum fully assigned using the 2D correlation spectra HMQC and HMBC, which contains the signal situated at 175.7 ppm due to a carbonyl group. The H-C long range heterocorrelation spectrum reveals this carbonyl group neighboring the protons situated at position 2 (8.65 ppm, singlet) and position 5 (8.71 ppm, singlet) of the heterocycle. The stretching vibration of the C≡N bond produces an absorption band situated at 2219.69 cm^{-1} in the solid state FT-IR spectrum of compound **3**. The intense absorption band situated at 1604.3 cm^{-1} was assigned to the carbonyl group stretching vibration. This extremely low value is due to the extended conjugation of the electronic π system. A broad band situated at 3000-3300 cm^{-1} suggests the presence of intermolecular hydrogen bond associations. Thiation of **3** with di-phosphorus pentasulfide in pyridine solution led to 3-cyano-10-methyl-pyrido[3,2-*g*]quinolin-4-thione, **4** (scheme 3).

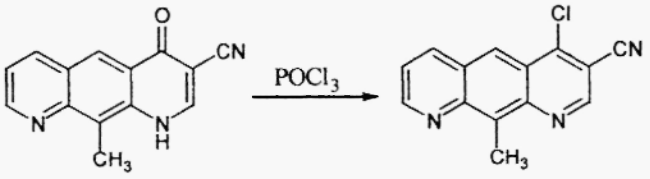


3a **4a** **4b**

Scheme 3

Tautomeric forms of **4** cannot be clearly distinguished by FT-IR spectroscopy. However the intense absorption band situated at 1351 cm^{-1} (vinylogue thioamide system) was assigned to the C=S double bond in **4a**. The low intensity absorption band situated at 3400 cm^{-1} could be due to the N-H stretching vibration while the stretching vibration of the C≡N bond determines the absorption band situated at 2226.59 cm^{-1}.

The C-S stretching vibration in **4b** which is situated in the 600-700 cm^{-1} region of the spectrum, is ambiguous owing to the fact that this region contains also skeleton vibrations of the heterocyclic system. A broad band of low intensity suggesting hydrogen bonding, is observed in the 2800-3200 cm^{-1} region. In contrast, the ^{1}H-NMR spectrum of **4** in DMSO-d_6 solution shows the presence of a mixture of tautomeric forms in 3:1 ratio with differences in the chemical shifts of similar protons of about 0.05 ppm. The thione form **4a** is supported by the 2D HMBC spectrum in which H-C long range correlation shows the presence of C=S group as a cross peak between a deshielded ^{13}C atom situated at 196 ppm in correlation with the two neighboring protons situated in position 2 (8.6 ppm, singlet) and 5 (9.1 ppm, singlet). The same spectrum enabled us to assign the singlet situated at 8.6 ppm to the proton in the neighboring position of the nitrile group (δ_{CN} = 106 ppm). Chlorination of **3** with phosphorus oxychloride led to 4-chloro-3-cyano-10-methyl-pyrido[3,2-*g*]quinoline **5** in 94% yield (scheme 4). Due to its high reactivity **5** could be used as an efficient intermediate in preparing novel derivatives capable to reverse MDR of pathogenic enterobacteriaceae strains (unpublished results).



3a **5**

Scheme 4

CONCLUSIONS

In conclusion, one must emphasize that novel pyridoquinolino substrates able to play a highly attractive part in the synthesis of new MDR reversal agents, can be easily obtained by the route proposed above. Moreover, structural investigations of compounds obtained demonstrated the strong influence of the nitrile group as substituent upon the pyrido[3,2-*g*]quinolin-4-(thi)one structure. The electron withdrawing capability of this group when situated in position 3 of the heterocyclic nucleus, appears to be competitive with that of the (thio)carbonyl bond situated in position 4 in the vinylogue electromere conjugation effect with the lone pair of unshared electrons of the nitrogen atom.

Finally the 4-chloro-derivative, **5**, is characterized by an enhanced reactivity due to the neighboring nitrile group so that this compound has to be considered as a very useful alkylating reagent for drug preparation.

EXPERIMENTAL

Melting points were measured on an Electrothermal digital apparatus and are given uncorrected. FT-IR spectra were obtained with a Bruker-Vector 22 spectrometer. NMR spectra were recorded on a 400 MHz Bruker spectrometer.

7-Amino-8-methyl-quinoline, (1).

The solution obtained from 2,6-diaminotoluene (3.66g, 30mmol), glycerol and arsenic acid freshly prepared by dissolving arsenic (V) oxide (15.4g) in water (13mL), was slowly heated at 100-110°C under vigorous stirring; then sulfuric acid (36mL) diluted with water (30mL) was added in small portions. The mixture was maintained for 4 hours under vigorous stirring at 130-140°C. After cooling at room temperature, the reaction mixture was poured into ice-water mixture (150mL) and alkalinized at pH 9-10. The precipitate thus obtained was filtered, washed with cold water and crystallized from water. Yield: 47 % ; m.p.= 128°C (ref. [5]:120 °C, ref. [6]: 125-127 °C). IR: ν_{NH2} = 3334, 3428 cm^{-1}. δ_H ($CHCl_3$-d_1): 2.59 (s, 3H, -CH_3), 4.0 (s, 2H, -NH_2), 7.0 (d, 1H, J = 8.8 Hz, H_6), 7.14 (dd, 1H, J = 4.4 Hz, J = 8.2 Hz, H_3), 7.49 (d, 1H, J = 8.8 Hz, H_5), 7.9 (dd, 1H, J = 2 Hz, J = 8.2 Hz, H_4), 8.8 (dd, 1H, J = 2 Hz, J = 4.4 Hz, H_2). δ_C: 10.25 (CH_3); 115.03 (C_8); 117.32 (C_3); 118.38 (C_6); 122.56 (C_7); 126.17 (C_5); 136.06 (C_4); 144.79 (C_{4a}); 148.26 (C_{8a}); 149.61 (C_2).

(8-Methyl-quinoline-7-aminomethylene) cyanoacetate ethyl ester (2).

Compound **1** (1.58g, 10mmol) was dissolved in absolute ethanol (10mL), ethyl (ethoxymethylene) cyanoacetate (10mmol) was added. The mixture was refluxed for 2 hours. After cooling, the white precipitate was filtered, washed with small amounts of cool ethanol and dried before crystallization from ethanol. Yield = 65% ; m.p. = 206 °C. IR: ν_{CN} = 2211.07 cm^{-1}, ν_{CO} = 1669.61 cm^{-1}, $\nu_{C=C}$ = 1635.42 cm^{-1}. δ_H ($CHCl_3$-d_1): 1.24 (t, 3H, J = 7 Hz, -CH_3), 2.84 (s, 3H, -CH_3), 4.35 (q, 2H, J = 7 Hz, -CH_2-), 7.39 (dd, 1H, J = 4 Hz, J = 8.8 Hz, H_3), 7.42 (d, 1H, J = 8.8 Hz, H_5), 7.77 (d, 1H, J = 8.8 Hz, H_6), 8.01 (d, 1H, J = 13 Hz, -CH=), 8.12 (dd, 1H, J = 1.6 Hz, J = 8.2 Hz, H_4), 8.96 (dd, 1H, J = 1.6 Hz, J = 4 Hz, H_2), 11,31 (d, 1H, J = 13 Hz, NH). δ_C: 10.92 (CH_3); 14.33 (CH_3); 61.44 (CH_2); 76,3 (CN); 115,00 (C_2); 117.81 (C_5); 120.60 (C_3); 124.43 (C_{4a}); 125.97 (C_8); 127.51 (C_6); 136.33 (C_4); 136.43 (C_7); 150.68 (C_2); 151.81 (CH); 167.74 (COO).

3-cyano-10-methyl-pyrido[3,2-g]quinoiin-4-one (3).

Compound **2** (2.81g, 10mmol) and diphenylether (20mL) were slowly heated under argon atmosphere at 250°C. The temperature was maintained for 2 hours. After cooling the reaction mixture was poured into a large amount of petroleum ether and the precipitate thus obtained was filtered, washed with petroleum ether and methanol. Yield = 70% ; m.p = 292 °C. IR: ν_{CN} = 2219.69 cm^{-1}, ν_{CO} = 1604.3 cm^{-1}, ν_{OHasoc} = 3000-3300 cm^{-1}. δ_H (DMSO-d_6) : 2.93 (s, 1H, -CH_3), 7.57 (dd, 1H, J = 4 Hz, J = 8.4 Hz, H_7), 8.59 (dd, 1H, J = 1.6 Hz, J = 8.4 Hz, H_6), 8.65 (s, 1H, H_2), 8.71 (s, 1H, H_5), 9.06 (dd, 1H, J = 1.6 Hz, J = 4 Hz, H_8). δ_C : 11.19 (-CH_3);

91.41 (-CN); 116.91 (C_3); 121.56 (C_7); 123.94 (C_{10}); 124.52 (C_5); 124.96 (C_{5a}); 125.01 (C_{4a}); 136.26 (C_{10a}); 138.43 (C_6); 146.49 (C_{9a}); 148.51 (C_2); 153.18 (C_8); 175.77 (C_4).

3-cyano-10-methyl-pyrido[3,2-g]quinolin-4-thione (4).

Compound **3** (0.235g, 1mmol) was dissolved in anhydrous pyridine (10mL); then P_4S_{10} (0.22g, 0.5mmol) was added. The mixture was heated at 80°C for 4 hours under vigorous stirring. The reddish colored solution was poured, into water (50 mL) after cooling. The brick-red precipitate was filtered, washed with water and dried. Yield = 96% ; m.p. = 196°C (with decomposition). IR (solid): ν_{CN} = 2226.59cm^{-1}, ν_{CS} = 1351.94cm^{-1}, $\nu_{C=C}$ = 1596.99 cm^{-1}. δ_H (DMSO-d_6): 2.99 (s, 3H, -CH_3), 7.6 (dd, 1H, J = 6.4 Hz, J = 7.2 Hz, H_7), 8.60 (s, 1H, H_2), 8.64 (dd, 1H, J = 7.2 Hz, J = 1.8 Hz, H_6), 9.10 (dd, 1H, J = 6.4 Hz, J = 1.8 Hz, H_8), 9.11 (s, 1H, H_5).

4-chloro-3-cyano-10- methyl-pyrido[3,2-g]quinoline (5).

Compound **4** (0.235g, 1mmol) and phosphorus oxychloride (10mL) were gradually heated at 100°C . Temperature was maintained for 2 hours. The reaction mixture was cooled and poured cautiously into a mixture of concentrated ammonia solution (100mL) and ice (100g) under stirring. The precipitate was filtered and dried over potassium hydroxide in vacuum, at room temperature. Yield = 94% ; m.p = 264°C (with decomposition). IR (solid): ν_{CN} = 2202,95cm^{-1}. δ_H (DMSO-d_6 solution): 3.02 (s, 3H, -CH_3), 7.38 (dd, 1H, J = 8.4 Hz, J = 4 Hz, H_7), 8.34 (s, 1H, H_2), 8.44 (dd, 1H, J = 8.4 Hz, J = 1,2 Hz, H_6), 8.60 (s, 1H, H_5), 8.93 (dd, 1H, J = 4Hz, J = 1.2 Hz, H_8).

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