SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1-CYCLOPROPYL-1,4-DIHYDRO-4-OXO-3-PYRIDINECARBOXYLIC ACID

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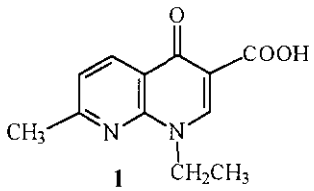
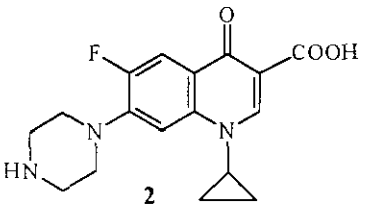
Abstract - 1-Cyclopropyl-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid was prepared in four reaction steps and tested for antibacterial activity.

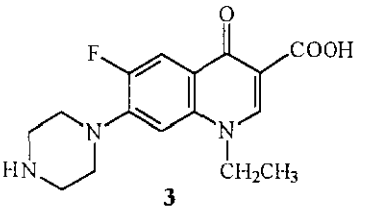
Quinolone antibiotics have become widely used therapeutics in the treatment of bacterial infectious diseases. As compared to earlier used quinolones, ciprofloxacin (**2**) has been found to be considerably more active against most gram-positive and gram-negative bacteria.[1]



1 Nalidixic acid

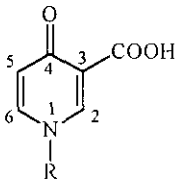


2 Ciprofloxacin



3 Norfloxacin

4



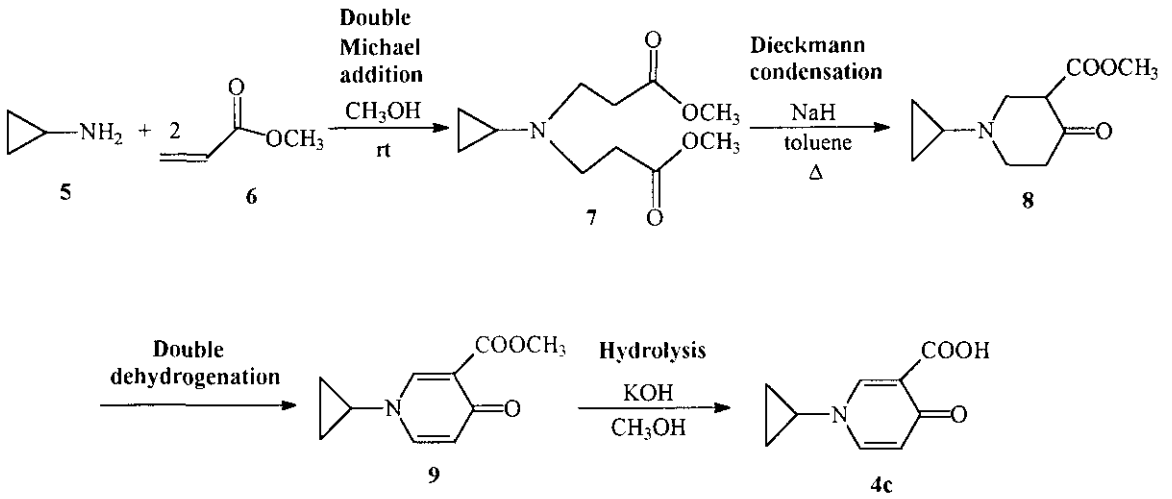
4a: R = H
4b: R = Alkyl
4c: R = Cyclopropyl

We planned to investigate the antibacterial activity of 1-cyclopropyl-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid (**4c**), which has a partial structure of **2**. Although similar compounds with other substituents R have been found to be inactive[2] we still hoped for some antibacterial activity of **4c**, due to its cyclopropyl group, which was selected because it led to improved potency of ciprofloxacin relative to norfloxacin.[3] Moreover, we planned to investigate a possible synergism of **4c** when combined with quinolones.

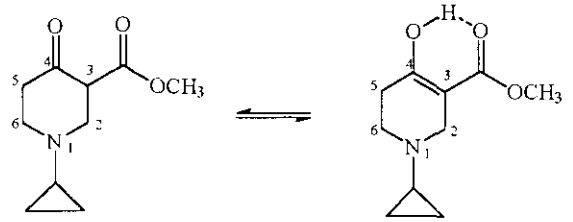
A reported general method allows the preparation of the carboxylic acids (**4b**) (and their 2-, 5- or 6-substituted derivatives)[2, 4, 5] by alkylation of the *N*-unsubstituted **4a**. However, this way of preparation was

not considered for **4c**, as in contrast to ordinary alkyl halides, cyclopropyl halides or tosylates are not susceptible to nucleophilic substitution. If they react, they only afford the corresponding allyl derivatives.[6] 2-, 5- Or 6-substituted derivatives of 1-alkyl-1,4-dihydro-4-pyridinone-3-carboxylic acids have been prepared by alternative methods.[7-10] However, for the synthesis of **4c**, either the starting material was not commercially available or its preparation would have involved too many reaction steps.

We report here a simple preparation of **4c** involving only four reaction steps according to the following Scheme:



Thus, cyclopropylamine and an excess of methyl acrylate in methanol gave, *via* a Michael addition, the symmetric diadduct (**7**) (99% yield).[11] 7 was subsequently cyclized in a Dieckmann condensation with sodium hydride in refluxing toluene to 4-piperidinone (**8**) (88% yield).[12] Compound (**8**) exists in two tautomeric forms.



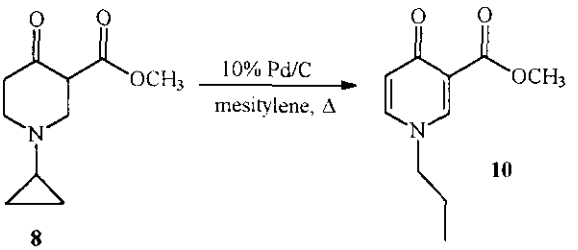
ketonic form **enolic form**

According to the ^{1}H-NMR spectrum, the enolic form is stabilized by a hydrogen bond and predominates in the equilibrium mixture (ketonic form / enolic form = *1 : 2 in $CDCl_3$*).

In a reported preparation[13] of highly substituted 4-pyridinones, ceric sulfate was successful in the dehydrogenation of suitable 4-piperidinones. However, the double dehydrogenation failed with a lower substituted piperidinone.

Therefore we investigated several other dehydrogenation reagents to perform reaction step **8** → **9**.

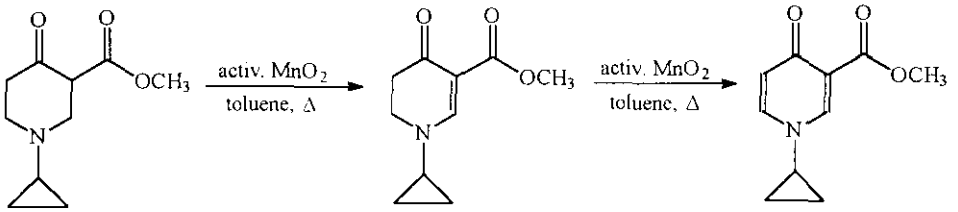
1. In a reaction using 10% Pd on carbon in refluxing mesitylene,[14] both double bonds were introduced efficiently. However, the isolated product (in 53% yield) was not the expected **9**, but the n-propyl derivative (**10**).



8 **10**

Obviously, the abstracted hydrogen was transferred by the catalyst to the cyclopropyl residue. A lower temperature (refluxing xylene) did not lead to a reaction of **8**.

2. Activated manganese dioxide[15] was successful to perform reaction **8** → **9** in low yield. By this method 4-piperidinone (**8**) was oxidized in refluxing toluene *via* the intermediate (**11**) to the desired 4-pyridinone (**9**).



8 **11** **9**

After 2 h, the manganese dioxide was removed by filtration and the intermediate product (**11**) was isolated by flash chromatography on silica gel with chloroform-methanol (25:1) as a pale yellow solid in 29% yield. Compound (**9**) was obtained as a white solid in only 5% yield. Prolonging the reaction time to 27 h did not afford a much higher yield (17%) of **9**. Therefore we searched further for a more convenient dehydrogenation method.

3. The most satisfactory results were obtained with DDQ[16] as an oxidizing agent. By using this reagent at room temperature in dioxane, either the intermediate (**11**) (65% yield) or the pyridinone (**9**) (67% yield), depending on the equiv. of DDQ used, was obtained after flash chromatography.

Finally, the carboxylic ester (**9**) was hydrolyzed in refluxing methanolic KOH to give 1-cyclopropyl-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid (**4c**) in 91% yield.

The new compound (**4c**) was investigated by the agar diffusion test. **4c** did not show antibacterial activity against *Staphylococcus aureus* DSM 1104, *Staphylococcus aureus* 25466, *Staphylococcus* 25768, *Staphylococcus* Innsbruck, *Escherichia coli* DSM 1103, *Escherichia coli* TEM 1, *Enterobacter cloacae* 30055, *Enterococcus*, *Pseudomonas aeruginosa* DSM 1117, *Pseudomonas aeruginosa* resistant. A synergistic effect was not observed when a combination of **4c** and ciprofloxacin was tested against ciprofloxacin resistant *Staphylococcus aureus*.

Due to its high stability under physiological pH-conditions,[17] a decomposition cannot be responsible for the lack of biological activity of **4c**. Thus, as reported by Georgopapadakou *et al.*,[18] an aryl substituent seems to be a prerequisite for antibacterial activity of quinolones.

EXPERIMENTAL

General: Methyl acrylate was freshly distilled prior to use. Unless otherwise stated, chemicals obtained from commercial sources were used without further purification. All moisture- and air-sensitive reactions were conducted under a nitrogen atmosphere. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker ARX-300 spectrometer working at 300 MHz (^{1}H-NMR) and 75.5 MHz (^{13}C-NMR). IR spectra were obtained on a Perkin-Elmer 1420 Ratio Recording spectrophotometer. Electron Ionization (EI) MS spectra were performed on a Finnigan MAT 90 instrument. Melting points are uncorrected.

3,3'-Cyclopropyliminodipropanoic acid methyl ester (7): The general procedure of Reitsema and Hunter[11] was followed for the preparation of 7. In a flame-dried, 100 mL round-bottomed flask, fitted with a N_2 inlet, rubber septum, and magnetic stir bar, to cyclopropylamine (**5**, 2.6 mL, 37.0 mmol) in methanol (50 mL) methyl acrylate (11.67 mL, 129.5 mmol) was added dropwise over a 10 min period at 0°C. The reaction mixture was allowed to warm up to rt and was subsequently stirred for 45 h. The methanol and excess methyl acrylate were removed in a rotary evaporator and the residue was distilled to afford 7 (8.43 g , 99%) as a colorless liquid, bp 82-83 °C (0.005 mm), IR (CH_2Cl_2): 1725 cm^{-1} vs (C=O), ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.37-0.50 (m, 4H, 2 cyclopropyl-CH_2), 1.69 -1.75 (m, 1H, cyclopropyl-CH), 2.53 (t, J = 7.3 Hz, 4H, 2 -CH_2COOMe), 2.92 (t, J = 7.3 Hz, 4H, 2 -CH_2-N), 3.67 (s, 6H, 2 -OCH_3); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 6.9 (2 cyclopropyl-$\underline{C}H_2$), 32.3 (2 -$\underline{C}H_2$-COOMe), 35.8 (cyclopropyl-$\underline{C}$H), 50.6 (2 -$\underline{C}H_2$-N), 51.5 (2 -O$\underline{C}H_3$), 173.2 (2 $\underline{C}$OOMe); MS (m/z, relative intensity) 229 (M^+, 20), 156 ([C_3H_5-N-(C_2H_4-COOMe)-CH_2]$^+$, 100); Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.63; H, 8.35; N, 6.11. Found: C, 57.55; H, 8.06; N, 6.12.

1-Cyclopropyl-4-oxo-3-piperidinecarboxylic acid methyl ester (8): In a dry, three-necked 250 mL round-bottomed flask, equipped with a N_2 gas inlet adapter, reflux condenser, septum and a magnetic stir bar, 2.2 g ($\cong$ 60%, $\cong$ 1.3 g, 55 mmol) of sodium hydride in paraffin oil was washed twice with toluene (80 mL) and suspended in toluene (80 mL). To this well-stirred suspension the diester (7) (5.73 g, 25 mmol) was added dropwise at rt. The reaction was initiated with methanol (100 µL) and the mixture refluxed,

whereupon a white solid was formed. After 3 h, the starting material was consumed and at 0 °C 1N HCl (55 mL, 55 mmol) was gradually added to the reaction mixture, whereupon the white solid was dissolved. The aqueous layer was adjusted to pH 11 by addition of solid K_2CO_3 and extracted with ether (3 x 70 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Distillation of the residue afforded 4.33 g (88%) of **8** as a colorless liquid, bp 71-74 °C (0.005 mm); IR (CH_2Cl_2): 1740 cm^{-1} m (ketonic form: C=O, ester), 1712 m (ketonic form: C=O, ring), 1662 vs (enolic form: C=O), 1622 s (enolic form: C=C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.43-0.56 (m, 4H, 2 cyclopropyl-CH_2), 1.73 -1.83 (m, 1H, cyclopropyl-CH), enolic form: 2.39 (tt, ^3J = 6.0 Hz, $^5J_{homoallylic}$ = 1.7 Hz, 1.36H, 2 H-5), 2.82 (t, ^3J = 6.0 Hz, 1.36H, 2 H-6), 3.31 (t, $^5J_{homoallylic}$ = 1.7 Hz, 1.36H, 2 H-2), 3.76 (s, 2.04H, -OCH_3), 11.92 (s, 0.68 H, OH), ketonic form: 2.49-2.59 (m, 0.64H, 2 H-5), 2.87-3.06 (m, 0.64H, 2 H-6), 3.10-3.25 (m, 0.64H, 2 H-2), 3.44-3.47 (m, 0.32H, H-3), 3.74 (s, 0.96H, -OCH_3); ^{13}C-NMR (75.5 MHz, $CDCl_3$): enolic form: δ = 6.2 (2 cyclopropyl-$\underline{C}H_2$), 29.3 (C-5), 37.7 (cyclopropyl-$\underline{C}H$), 49.4/49.6 (C-2/C-6), 51.4 (OCH_3), 96.7 (C-3), 170.3/171.4 ($\underline{C}OOMe$/C-4), ketonic form: 6.8 (2 cyclopropyl-$\underline{C}H_2$), 37.0 (cyclopropyl-$\underline{C}H$), 40.8 (C-5), 52.2 (OCH_3), 53.1 (C-6), 55.5 (C-2), 56.5 (C-3), 169.3 ($\underline{C}OOMe$), 204.3 (C-4); MS (m/z, relative intensity) 197 (M^+, 38), 165 (100); Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.90; H, 7.67; N, 7.10. Found: C, 60.72; H, 7.50; N, 7.14.

Oxidation procedure 1 with Pd/C: 1,4-Dihydro-4-oxo-1-propyl-3-pyridinecarboxylic acid methyl ester (10): In a flame-dried, 25 mL round-bottomed flask, fitted with a N_2 gas inlet, reflux condenser and a magnetic stirrer, **8** (300 mg, 1.521 mmol) was dissolved in mesitylene (9 mL). To this solution 10% Pd on carbon (450 mg) was added and the mixture warmed to reflux. After 25 min, the starting material was consumed and the catalyst was filtered off and washed with methanol (3 x 9 mL). The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel with $CHCl_3$-MeOH (25:1) to afford **10** as a white solid (156 mg, 53%), mp 95-96 °C (Et_2O); IR (KBr): 1727 cm^{-1} vs (C=O, Ester), 1647 vs (C=O, Ring), 1579 vs (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.99 (t, J= 7.4 Hz, 3H, propyl-CH_3), 1.86 (tq, J_t= 7.2 Hz, J_q= 7.4 Hz, 2H, CH_3-$C\underline{H}_2$-), 3.81 (t, J = 7.2 Hz, 2H, C_2H_5-$C\underline{H}_2$ -N), 3.90 (s, 3H, -OCH_3), 6.52 (d, $J_{5/6}$ = 7.7 Hz, 1H, H-5), 7.26 (dd, $J_{6/5}$ = 7.7 Hz, $J_{6/2}$ = 2.5 Hz, 1H, H-6), 8.18 (d, $J_{2/6}$ = 2.5 Hz, 1H, H-2); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 10.7 (propyl-CH_3), 24.0 (CH_3-$\underline{C}H_2$-CH_2-N), 52.3 (OCH_3), 59.1 (CH_3-CH_2-$\underline{C}H_2$-N), 118.5 (C-3), 123.1 (C-5), 138.8 (C-6), 146.0 (C-2), 166.0 ($\underline{C}OOMe$), 175.2 (C-4); MS (m/z, relative intensity) 196 ($[M+H]^+$, 3), 195 (M^+, 22), 137 ([M - O=CH_2 (McLafferty), - C=O]$^+$,100); Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.18. Found: C, 61.47; H, 7.03; N, 7.10.

Oxidation procedure 2 with activated MnO_2: 1-Cyclopropyl-1,4-dihydro-4-oxo-3-pyridine-carboxylic acid methyl ester (9) and 1-cyclopropyl-4-oxo-1,4,5,6-tetrahydro-3-pyridinecarboxylic acid methyl ester (11); Method A: In a dry, 10 mL round-bottomed flask, fitted with a reflux condenser,

a N_2 gas inlet and a magnetic stir bar, **8** (100 mg, 0.507 mmol) was dissolved in toluene (5 mL). After adding activated MnO_2 (750 mg), the reaction mixture was warmed to reflux and stirred for 3 h. Then the resulting mixture was passed through a pad of Celite and concentrated under reduced pressure. The residue was flash chromatographed on silica gel with $CHCl_3$-MeOH (25:1) to yield **11** (29 mg, 29%) as a pale yellow solid. Further elution afforded **9** (5 mg, 5%) as a white solid.

Method B: According to the above procedure, compound **(9)** was obtained in a 17% yield after a refluxing period of 27 h.

Oxidation procedure 3 with DDQ; Method A: 1-Cyclopropyl-4-oxo-1,4,5,6-tetrahydro-3-pyridine-carboxylic acid methyl ester (11): A flame-dried, 25 mL round-bottomed flask fitted with a N_2 inlet, rubber septum, and a magnetic stirrer, was filled with a solution of **8** (100 mg, 0.507 mmol) in dioxane (4 mL). Then a solution of DDQ (126 mg, 0.555 mmol) in dioxane (3 mL) was added at rt over a period of 45 min, whereupon a pale gray to yellow solid was formed. After an additional 15 min of stirring, the solid was filtered off and washed with dioxane (2 x 5 mL). The filtrate was concentrated *in vacuo* and the residue purified by flash chromatography on basic alumina with $CHCl_3$-MeOH (75:1) to give **11** (64 mg, 65%) as a pale yellow solid, mp 71-72 °C (Et_2O); IR (CH_2Cl_2): 1720 cm^{-1} vs (C=O, ester); 1660 vs (C=O, ring); 1590 vs (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.87-0.93 (m, 4H, 2 cyclopropyl-CH_2), 2.53 (t, J = 7.7 Hz, 2H, 2 H-5), 2.92 -2.98 (m, 1H, cyclopropyl-CH), 3.65 (t, J = 7.7 Hz, 2H, 2 H-6), 3.78 (s, 3H, OCH_3), 8.25 (s, 1H, H-2); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 6.7 (2 cyclopropyl-$\underline{C}H_2$), 36.1 (C-5), 37.1 (cyclopropyl-$\underline{C}H$), 47.9 (C-6), 51.4 (OCH_3), 100.9 (C-3),159.7 (C-2), 166.0 ($\underline{C}OOMe$), 186.8 (C-4); MS (m/z, relative intensity) 196 ([M+H]$^+$,15), 195 (M$^+$, 100); Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.18. Found: C, 61.29; H, 6.83; N, 7.11.

Method B: 1-Cyclopropyl-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid methyl ester (9): According to the above-mentioned procedure, a solution of DDQ (2.65 g, 11.66 mmol) in dioxane (60 mL) was added to a solution of **8** (1.0 g, 5.07 mmol) in dioxane (40 mL) over a 2 h period. After an additional 3 h of stirring, the reaction mixture was worked up analogously and the crude product purified by flash chromatography on basic alumina using $CHCl_3$-MeOH (50:1). **9** (657 mg, 67%) was obtained as a white solid. Recrystallization from ethyl acetate yielded white needles, mp 127-128 °C; IR (KBr): 1728 cm^{-1} vs (C=O, Ester), 1644 vs (C=O, Ring), 1585 vs (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.03-1.17 (m, 4H, 2 cyclopropyl-CH_2), 3.44-3.52 (m, 1H, cyclopropyl-CH), 3.89 (s, 3H, -OCH_3), 6.46 (d, $J_{5/6}$ = 7.7 Hz, 1H, H-5), 7.41 (dd, $J_{6/5}$ = 7.7 Hz, $J_{6/2}$ = 2.5 Hz, 1H, H-6), 8.30 (d, $J_{2/6}$ = 2.5 Hz, 1H, H-2); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 7.2 (2 cyclopropyl-$\underline{C}H_2$), 37.6 (cyclopropyl-$\underline{C}H$), 52.2 (OCH_3), 118.4 (C-3), 122.5 (C-5), 139.9 (C-6), 146.8 (C-2), 165.6 ($\underline{C}OOMe$), 175.4 (C-4); MS (m/z, relative intensity) 194 ([M+H]$^+$, 4), 193 (M$^+$, 28), 135 ([M - O=CH_2(McLafferty), - C=O]$^+$,100); Anal. Calcd for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.14; H, 5.74; N, 7.21.

1-Cyclopropyl-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid (4c): In a dry, 10 mL round-bottomed flask, fitted with a reflux condenser, a N_2 gas inlet and a magnetic stir bar, **9** (150 mg, 0.776 mmol) was dissolved in methanol (1.5 mL). After adding KOH (90 mg, 1.61 mmol) in methanol (3.5 mL) at 0°C and stirring for 15 min at rt, the mixture was warmed to reflux for 1.5 h. Then the solvent was removed in a rotary evaporator and the residue was dissolved in water (1.5 mL). The aqueous solution was adjusted to pH 2 by addition of 1N HCl at 0 °C, whereupon **4c** precipitated in form of a white solid. It was filtered off and washed with Et_2O. The aqueous filtrate, which still contained some product, was extracted with CH_2Cl_2 (3 x 10 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford **4c** (in total: 126 mg, 91 %) as a white solid. Recrystallization from ethanol yielded white, small needles, mp 178-179 °C; IR (KBr): 1718 cm^{-1} vs (C=O, Ester), 1634 s (C=O, Ring), 1520 s (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.02-1.20 (m, 4H, 2 cyclopropyl-CH_2), 3.83-3.90 (m, 1H, cyclopropyl-CH), 6.72 (d, $J_{5/6}$ = 7.5 Hz, 1H, H-5), 8.17 (dd, $J_{6/5}$ = 7.5 Hz, $J_{6/2}$ = 2.3 Hz, 1H, H-6), 8.58 (d, $J_{2/6}$ = 2.3 Hz, 1H, H-2), 16.28 (s, 1H, COOH); ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ = 6.6 (2 cyclopropyl-$\underline{C}H_2$), 38.8 (cyclopropyl-$\underline{C}H$), 115.0 (C-3), 118.0 (C-5), 144.5 (C-6), 146.5 (C-2), 165.7 ($\underline{C}OOH$), 178.3 (C-4); MS (m/z, relative intensity) 179 (M^+, 1), 135 ([M -CO_2]$^+$, 100); Anal. Calcd for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.49; H, 5.10; N, 7.73.

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