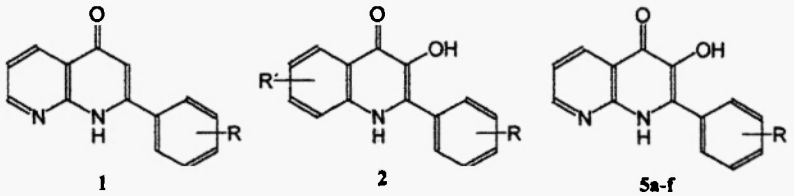
Synthesis of 3-Hydroxy-2-Phenyl-1,8-Naphthyridin-4(1*H*)-one derivatives

Simón E. López,[1*] Mónica E. Rosales,[1] José Salazar,[1] Neudo Urdaneta,[1] Rosa Ferrer,[2]
Jorge E. Angel,[2] and Jaime E. Charris[3]. *E-mail: slopez@usb.ve

[1] Departamento de Química, Universidad Simon Bolívar, Caracas 1080-A, Apartado 89000, VENEZUELA.
[2] Departamento de Quimica, Facultad Experimental de Ciencias, La Universidad del Zulia, Maracaibo, VENEZUELA.
[3] Laboratorio de Síntesis Orgánica, Facultad de Farmacia, Universidad Central de Venezuela, Caracas, VENEZUELA.

Abstract: As part of our research program directed towards the design and synthesis of novel heterocycles with potential cytotoxic activity, we synthesized some 2-phenyl-3-hydroxy-1,8-naphthyridin-4(1*H*)-one derivatives, structurally related to the antimitotic 2-phenyl-1,8-naphthyridin-4(1*H*)-ones. To the best of our knowledge, this is the first example of preparation of 3-hydroxy derivatives from the 1,8-napththyridine nucleus. A preliminary cytotoxic evaluation of two of these compounds is also included.

The 1,8-naphthyridines are an interesting class of members of the diazanaphthalene series [1,2]. Attention on these heterocycles in the last fifteen years have been focused on the preparation of biologically important 3-carboxylic acid 1,8-naphthyridin-4(1*H*)-one antibacterials related to nalidixic acid [3]. Recently a number of 2-phenyl-1,8-naphthyridin-4(1*H*)-ones **1** were prepared and tested for their cytotoxic activity towards a large number of cancer cell tumor lines [4]; these compounds were also capable of inhibit tubulin polymerization, demonstrating their potential as novel antimitotic agents [5]. A 3-hydroxy substitution on the isosteric 2-phenyl flavones and 2-phenyl 4(1*H*)-quinolones **2** has proved its value for the antimitotic activity [6]. Thus, an hydroxy group located at C-3 of 2-phenyl-1,8-naphthyridine-4(1*H*)-ones **5a-f** may lead for novel cytotoxic compounds.



1 **2** **5a-f**

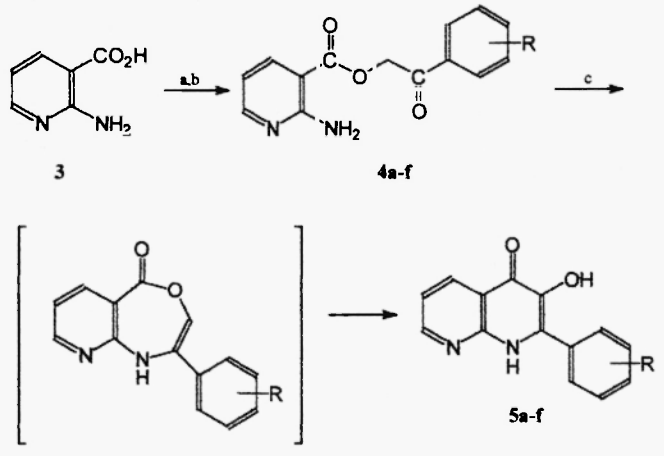
A limited number of procedures have been reported for the related and relatively unexplored 3-hydroxy-4(1*H*)-quinolones; these include a cyclization-dehydratation of an α-alcoxy-2-N-acylamino substituted acetophenone by an intramolecular anion nucleophilic attack [6] and a cyclization of phenacyl anthranylate esters in hot polyphosphoric acid to a 3*H*-benc(*e*)(1,4)oxacepin-5-one whose *in situ* rearrangment give the more stable 3-hydroxy-4(1*H*)-quinolone [7-8]. The simplicity of this last have attracted our attention due to the readily available 2-amino-nicotinic acid as a source of starting material for the required phenacyl nicotinic esters, necessary for a possible rearrangement to novel 3-hydroxy-1,8-naphthyridin-4(1*H*)-ones (Scheme 1).

Results and discussion

Starting from 2-amino-nicotinic acid **3**, its potassium salt was formed and reacted with substituted α-bromoacetophenones to obtain the corresponding phenacyl nicotinic esters **4a-f** by nucleophilic displacement. These esters were then poured on to preheat (50 °C) polyphosphoric acid and heated at 120 °C for two hours, giving the required 3-hydroxy-1,8-naphthyridin-4(1*H*)-ones **5a-f** in good yields (Table 1). Although not isolated, we supposed a 2-phenyl-1,5-dihidropyrido[2,3-e][1,4]-oxazepin-5-one as a reaction intermediate which then rearrange to the 3-hydroxy-

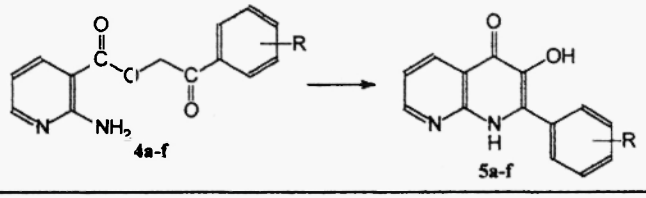
1,8-naphthyridin-4(1*H*)-one; this intermediate is quite similar to that 3*H*-benc(*e*)(1,4)oxacepin-5-one postulated for the synthesis of 3-hydroxy-quinolones under the same conditions. To our known, this is the first report of 3-hydroxy substitution on the 1,8-naphtyridine nucleus.

Scheme 1



Conditions: a. K_2CO_3/dimethylformamide, 90°C, 2h.; b. substituted 2-bromoacetophenone/ dimethylformamide, 50°C, 3h.; c. polyphosphoric acid, 120°C, 2h.

Table 1



Compd. No.	R	mp (°C)	Yield (%)
5a	H	287-289	72
5b	4-Br	>300	65
5c	3-Br	>300	70
5d	4-Cl	>300	80
5e	4-CH_3	290-291	83
5f	4-NO_2	a	a

a. Not isolated, a complex mixture of products were detected on the TLC.

Preliminary Cytotoxic Evaluation

The preliminary cytotoxic evaluation of compounds **5a** and **5e** was carried out employing the The Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay over the Vero (green monkey kidney) and HEP2 (human larynx cancer) cell lines [9,10]. Results outline in Table 2 are expressed as IC_{50} (μM) values. These results indicate that compound **5e** (IC_{50}=3 μM in HEP2 cancer cells) should be a good candidate for further evaluation in other cancer cell lines. Low solubility of compounds **5b**, **5c** and **5d** in diluting media did not permit their biological evaluation.

Table 2

	Vero	HEP2
Compound No.	**IC_{50} (μM)**	**IC_{50} (μM)**
5a	>10	>10
5e	>10	3

Experimental

Chemistry

Melting points were determined with a Fischer-Johns micro hot-stage apparatus and are uncorrected. IR spectra were recorded on a Magna Nicolet FT/IR 750 Series spectrometer as potassium bromide discs. ^{1}H NMR spectra were recorded on a JEOL Eclipse Plus (400 MHz) spectrometer in deuterated solvents; δ values in ppm relative to tetramethylsilane are given. When reported, mass spectra were recorded on a Varian Saturn 2000 Mass Selective Detector with EI(70 eV) connected to a gas chromatograph Varian Chrompack CP-38000 (GC/MS/MS). Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA , USA); the results fell in the range ± 0.4% of the required theoretical values. Silica gel plates ALUGRAM® SIL G/UV_{254} (Macherey-Nagel GmbH & Co., Germany) were used for TLC testing. Reagents were obtained from Aldrich (Milwaukee, MI, USA) or Merck (Darmstadt, Germany) and used without further purification. 1,3′-dibromoacetophenone was prepared according to the literature procedure [11]. Solvents were distilled prior to use.

General Procedure for the Preparation of Substituted-benzoylmethyl-2-amino-pyridine-3-carboxylates (**4a-f**).

A mixture of 2-amino-nicotimc acid **3** (0.3 g, 2.17 mmol) and potassium carbonate (0.29 g, 2.39 mmol) in dimethylformamide (6 mL) was stirred at 90 °C for 2 hours. Once the carboxylate was formed, the mixture was allowed to cool at room temperature, and following it was added the corresponding 2-bromo substituted acetophenone (2.17 mmol) with stirring for 3 additional hours at 50 °C. The mixture was allowed to cool at room temperature and added to crushed ice-water, giving a solid which was then recrystallized and dried under vacuo.

Benzoylmethyl-2-amino-pyridine-3-carboxylate (**4a**).

0.52 g (recrystallized from ethanol-water), 94 % yield, mp: 160-162 °C; IR (KBr, cm^{-1}): ν = 3500 (NH_2), 1710 (C=O), 1656 (C=O). ^{1}H nmr (deuterio-chloroform): δ 5.54 (s, 2H, CH_2), 6.40 (bs, 2H, NH_2), 6.65 (dd, 1H, Ar-H, J= 8

Hz, 3 Hz), 7.51 (dd, 1H, Ar-H, J= 7.7, 7.7 Hz), 7.63 (m, 2H, Ar-H), 7.95 (m, 2H, Ar-H), 8.25 (dd, 1H, Ar-H, J= 2, 6.6 Hz), 8.29 (dd, 1H, Ar-H, J=2, 7.7. Hz). *Anal.* Calcd. for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.47 ; H, 4.84 ; N, 10.80.

4-Bromo-benzoylmethyl-2-amino-pyridine-3-carboxylate (**4b**).

0.33 g (recrystallized from ethanol-water), 45 % yield, mp: 179-181 °C; IR (KBr, cm^{-1}): ν = 3550 (NH_2), 1700 (C=O), 1650 (C=O). ^1H nmr (deuterio-chloroform): δ 5.49 (s, 2H, CH_2), 6.39 (bs, 2H, NH_2), 6.66 (m, 1H, Ar-H), 7.65 (d, 2H, Ar-H, J= 8 Hz), 7.82 (d, 2H, Ar-H, J= 8 Hz), 8.26 (m, 2H, Ar-H). *Anal.* Calcd. for $C_{14}H_{11}BrN_2O_3$: C, 50.17; H, 3.31; N, 8.36. Found: C, 50.35; H, 3.27 ; N, 8.23.

3-Bromo-benzoylmethyl-2-amino-pyridine-3-carboxylate (**4c**).

0.39 g (recrystallized from ethanol-water), 54 % yield, mp: 177-179 °C; IR (KBr, cm^{-1}): ν = 3450 (NH_2), 1700 (C=O), 1650 (C=O). ^1H nmr (deuterio-chloroform): δ 5.52 (s, 2H, CH_2), 6.45 (bs, 2H, NH_2), 6.67 (dd, 1H, Ar-H, J= 5.1, 7.9 Hz), 7.40 (dd, 1H, Ar-H, J= 7.9, 7.9 Hz), 7.76 (dd, 1H, Ar-H, J= 1.1, 8.0 Hz), 7.88 (d, 1H, Ar-H, J= 7.7 Hz), 8.09 (dd, 1H, Ar-H, J= 1.8, 1.8 Hz), 8.24 (dd, 1H, Ar-H, J= 1.8, 4.5 Hz), 8.29 (dd, 1H, Ar-H, J= 1.5, 7.9 Hz). *Anal.* Calcd. for $C_{14}H_{11}BrN_2O_3$: C, 50.17; H, 3.31; N, 8.36. Found: C, 50.03; H, 3.12; N, 8.47.

4-Chloro-benzoylmethyl- 2-amino-pyridine-3-carboxylate (**4d**).

0.51 g (recrystallized from ethanol-water), 81 % yield, mp: 166-168 °C; ; IR (KBr, cm^{-1}): ν = 3550 (NH_2), 1700 (C=O), 1650 (C=O). ^1H nmr (deuterio-chloroform): δ 5.52 (s, 2H, CH_2), 6.38 (bs, 2H, NH_2), 6.66 (dd, 1H, Ar-H, J= 2.6, 7.5 Hz), 7.49 (d, 2H, Ar-H, J= 8.4 Hz), 7.90 (d, 2H, Ar-H, J= 8.4 Hz), 8.26 m, 2H, Ar-H). *Anal.* Calcd. for $C_{14}H_{11}ClN_2O_3$: C, 57.84; H, 3.81; N, 9.64. Found: C, 57.62; H, 3.73 ; N, 9.76.

4-Methyl-benzoylmethyl-2-amino-pyridine-3-carboxylate (**4e**).

0.23 g (recrystallized from ethanol-water), 40 % yield, mp: 180-182 °C; IR (KBr, cm^{-1}): ν = 3350 (NH_2), 1700 (C=O), 1600 (C=O). ^1H nmr (deuterio-chloroform): δ 2.42 (s, 3H, CH_3), 5.53 (s, 2H, CH_2), 6.41 (bs, 2H, NH_2), 6.66 (dd, 1H, Ar-H, J= 4.5, 9 Hz), 7.30 (d, 2H, Ar-H, J= 8.1 Hz), 7.86 (d, 2H, Ar-H, J= 8.1 Hz), 8.27 (m, 2H, Ar-H). *Anal.* Calcd. for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.81; H, 5.15 ; N, 10.21.

4-Nitro-benzoylmethyl-2-amino-pyridine-3-carboxylate (**4f**).

0.39 g (recrystallized from ethanol-water), 60 % yield, mp: 170-172 °C; IR (KBr, cm^{-1}): ν = 3300 (NH_2), 1700 (C=O), 1630 (C=O). ^1H nmr (deuterio-chloroform): δ 5.55 (s, 2H, CH_2), 6.61 (bs, 2H, NH_2), 6.69 (dd, 1H, Ar-H, J= 5, 8 Hz), 8.13 (d, 2H, Ar-H, J= 8.4 Hz), 8.29 (d, 2H, Ar-H, J= 8.4 Hz), 8.37 (m, 2H, Ar-H). *Anal.* Calcd. for $C_{14}H_{11}N_3O_5$: C, 55.82; H, 3.68; N, 13.95. Found: C, 55.58 ; H, 3.76 ; N, 14.04.

General Procedure for the Preparation of 3-Hydroxy-2-phenyl-susbtituted-1,8-naphthyridin-4(1*H*)-ones (**5a-e**).

The corresponding ester **4a-f** (1.0 mmol) was added to preheated polyphosphoric acid (6 mL), the solution was stirred for 2 hours at 120 °C. It was allowed to cool at room temperature, distilled water was slowly added until a precipitate was formed. The solid was filtered, and washed with abundant distilled water. The product was then recrystallized and dried under vacuo for 48 h.

3-Hydroxy-2-phenyl-1,4-dihidro-1,8-naphthyridin-4-one (**5a**).

0.17 g (recrystallized from dimethylformamide), 72 % yield, mp: 287-289 °C; IR (KBr, cm^{-1}): ν = 3300 (OH), 3150 (NH), 1650 (C=O). ^{1}H nmr (DMSO-*d6*): δ 7.37 (dd, 1H, H-6, J=4.4, J=8.0 Hz), 7.49-7.56 (m, 3H, H-ar), 7.80-7.82 (m, 2H, H-ar), 8.56 (dd, 1H, H-5, J=1.8, J=8.0 Hz), 8.75 (dd, 1H, H-7, J= 1.8, J=4.4 Hz). MS: m/z 238 [M^+,2], 239 [M^++1, 40]. *Anal.* Calcd. for $C_{14}H_{10}N_2O_2$: C, 70.59; H, 4.23; N, 11.76. Found: C, 70.33; H, 4.15; N, 11.57.

3-Hydroxy-2-(4-bromophenyl)-1,4-dihidro-1,8-naphthyridin-4-one (**5b**).

0.21 g (recrystallized from dimethylformamide), 65 % yield, mp: >300 °C; ν = 3300 (OH), 3150 (NH), 1650 (C=O). ^{1}H nmr (DMSO-*d6*): δ 7.36 (dd, 1H, H-6, J=4.4, J=8.0 Hz), 7.72 (d, 2H, H-ar, J=8.7 Hz), 7.77 (d, 2H, H-ar, J= 8.7 Hz), 8.55 (dd, 1H, H-5, J=1 8, 8.0 Hz), 8.74 (dd, 1H, H-7, J=1.8, 4.4 Hz). *Anal.* Calcd. for $C_{14}H_9BrN_2O_2$: C, 53.02; H, 2.86; N, 8.83. Found: C, 52.84; H, 2.94; N, 8.71.

3-Hydroxy-2-(3-bromophenyl)-1,4-dihidro-1,8-naphthyridin-4-one (**5c**).

0.22 g (recrystallized from dimethylformamide), 70 % yield, mp: >300 °C; ν = 3300 (OH), 3200 (NH), 1655 (C=O). ^{1}H nmr (DMSO-*d6*): δ 7.36 (dd, 1H, H-6, J=4.4, J=8.0 Hz), 7.49 (t, 1H, 5′-H, J= 7.7 Hz), 7.69 (d, 1H, 6′-H, J=7.7 Hz), 7.78 (d,1H, 4′-H, J=7.7 Hz), 7.96 (s, 1H, 2′-H), 8.55 (dd, 1H, H-5, J=1.5, 8.0 Hz), 8.76 (dd, 1H, H-7, J=1.5, J=4.4 Hz), 12.24 (bs, 1H, NH). *Anal.* Calcd. for $C_{14}H_9BrN_2O_2$: C, 53.02; H, 2.86; N, 8.83. Found: C, 53.25; H, 2.73; N, 8.75.

3-Hydroxy-2-(4-chlorophenyl)-1,4-dihidro-1,8-naphthyridin-4-one (**5d**).

0.22 g (recrystallized from dimethylformamide), 80 % yield, mp: >300 °C; ν = 3310 (OH), 3150 (NH), 1650 (C=O). ^{1}H nmr (DMSO-*d6*): δ 7.34 (dd, 1H, H-6, J=4.4, 8.0 Hz), 7.57 (d, 2H, 2′-H, 6′-H, J=8.4 Hz), 7.86 (d, 2H, 3′-H, 5′-H, J=8.4 Hz), 8.55 (dd, 1H, H-6, J=1.5, 8.0 Hz), 8.73 (dd, 1H, H-7, J= 1.5, 4.4 Hz). *Anal.* Calcd. for $C_{14}H_9ClN_2O_2$: C, 61.67; H, 3.33; N, 10.27. Found: C, 61.48; H, 3.16; N, 10.38.

3-Hydroxy-2-(4-methylphenyl)-1,4-dihidro-1,8-naphthyridin-4-one (**5e**)

0.21 g (recrystallized from dimethylformamide), 83 % yield, mp: 290-291 °C; ν = 3380 (NH), 3200 (NH), 1630 (C=O). ^{1}H nmr (DMSO-*d6*): δ 2.40 (s, 3H, CH_3), 7.32-7.37 (m, 3H, H-ar), 7.73 (d, 2H, H-ar, J=8.0 Hz), 8.55 (dd, 1H, H-6, J=1.8, 8.0 Hz), 8.74 (dd, 1H, H-7, J= 4.4, 1.8 Hz), 11.91 (bs, 1H, NH). MS: m/z 252 [M^+,1], 239 [M^++1, 3]. *Anal.* Calcd. for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.61; H, 4.63; N, 10.59.

Biological

Compounds **5a** and **5e** were evaluated using the Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay uses the novel tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and the electron coupling reagent, phenazine methosulfate (PMS). MTS is chemically reduced by cells into formazan, which is soluble in tissue culture medium [9,10]. The measurement of the absorbance of the formazan were carried out using 96 well microplates at 492nm. The assay measures dehydrogenase enzyme activity found in metabolically active cells. Since the production of formazan is proportional to the number of living cells, the intensity of the produced color is a good indication of the viability of the cells. MTS solutions were prepared according to the manufacturer's instructions [9].

Cells ($3x10^3$ /well) were suspended in MEM (100 µL), complemented with 10% fetal serum and incubated for 48 h. at 37°C under a CO_2 (5%) atmosphere. After 2 days, zero time was taken by colorimetry adding MTS (20 µL/well), then a variety of concentrations of tested drugs were added and complemented with MEM (up to 100 µL). Drug solutions were prepared in DMSO, and the final solvent concentration was <2% DMSO (v/v), a concentration without effect on cell replication . Treated cells were incubated further for 72 additional hours measuring then final time.

Acknowledgments

This work was supported in part from Grant No. S1-99000020 of Consejo Nacional de Investigaciones Científicas y Tecnologicas (CONICIT, Venezuela). The authors thank Laboratorio de RMN from Departamento de Química-Universidad Simón Bolívar (BID-CONICIT, Project QF13) for the spectral facilities. We also thanks Professor Diana Ajami and Lic. Edgar A. Valverde (Dep. Biología Celular, USB) for the preliminary biological testing.

References

[1] J.A. Turner, *J. Org. Chem.*, **55**, 4744 (1990).

[2] For a review see: [a] W.W. Paudler, R.M., Sheeta, *Adv. Heterocycl. Chem.*, **33**, 147 (1983).

[b] M. Wosniak, H.C. van der Plas, *Heterocycles*, **61**, 318 (1982).

[3] [a] T. Miyamoto, H. Ewaga, K. Shibamori, J. Matsumoto, *J. Heterocycl. Chem.*, **24**, 1333 (1987). [b] V.D. Parikh, A.H. Fray, E.F. Kleinman, *J. Heterocycl. Chem.*, **25**, 1567 (1988).

[4] K.Chen, S.C. Kuo, M.C. Hsih, A. Mauger, C.M. Lin, E. Hamel, K.H. Lee. *J. Med. Chem.*, **40**, 2266 (1996).

[5] K.Chen, S.C. Kuo, M.C. Hsih, A. Mauger, C.M. Lin, E. Hamel, K.H. Lee. *J. Med. Chem.*, **40**, 3049 (1997).

[6] Z. Sui, V.N. Nguyen, J. Altom, J. Fernandez, J.J. Hilliard, J.I. Bernstein, J.F. Barret, K.A. Obemeng. *Eur. J. Med. Chem.*, **34**, 381 (1999).

[7] P. Hradil, J. Jirman, *Collect. Czech. Chem. Commun.*, **50**, 1357 (1995).

[8] P. Hradil, J. Hlavac, K. Lerm, *J. Heterocycl. Chem.*, **36**, 141 (1999).

[9] Cell Titer 96® AQueous Non-Radioactive Cell Proliferation Assay Technical Bulletin #TB169, Promega Corporation.

[10] G. Malich, B.Markovic, C. Winder, *Toxicol.* **124**, 179 (1997).

[11] M.C. Rebstock, E.L. Pfeiffer, *J. Am. Chem. Soc.*, **74**, 3207 (1952).

Received on March 21, 2003