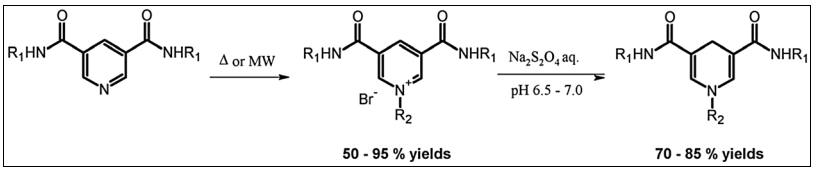
Efficient Synthesis of 3,5-Dicarbamoyl-1,4-dihydropyridines from Pyridinium Salts: Key Molecules in Understanding NAD(P)$^+$/NAD(P)H Pathways

Paolo Mellini,[a] Daniela De Vita,[a]* Barbara Di Rienzo,[a] Salvatore La Rosa,[b] Alessandro Padova,[b] Luigi Scipione,[a] Silvano Tortorella,[a] and Laura Friggeri[a]

[a]Chimica e Tecnologie del Farmaco, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
[b]Siena Biotech SpA, Strada del Petriccio e Belriguardo 35, 53100 Siena, Italy
*E-mail: daniela.devita@uniroma1.it

Received November 5, 2012
DOI 10.1002/jhet.2031
Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com).



3,5-Dicarbamoyl-1,4-dihydropyridines were prepared in high yields using a green protocol by reduction of the corresponding pyridinium salts in aqueous buffered sodium dithionite solutions. The pH value is a fundamental parameter for the reduction step and depends on the nature of substituent groups at positions 1, 3, and 5 of the pyridinium salts. These 3,5-dicarbamoyl dihydropyridines show a lower tendency towards oxidation and a higher stability than *N*-benzyl-3-carbamoyl-1,4-dihydropyridine at low pH values.

J. Heterocyclic Chem., **000**, 00 (2014).

INTRODUCTION

Nicotinamide adenine dinucleotide cofactors, including NAD$^+$, NADP$^+$, and the reduced forms (NADH and NADPH), have long been known as key molecules in energy metabolism and mitochondrial functions. Moreover, a rapidly growing body of information has suggested that NAD(P)$^+$ and NAD(P)H also play a crucial role in various biological processes such as calcium homeostasis, cellular oxidative balance, sirtuins modulation [1], gene expression, immunological functions, and aging [2]. In this context, 3-carbamoyl-1,4-dihydropyridines have always been considered affordable NAD(P)H synthetic models.

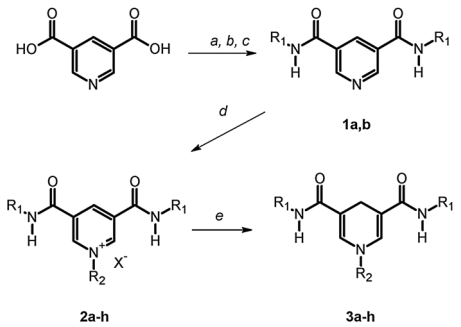
Over the past few years, the chemistry of the 1-benzyl-3-carbamoyl-1,4-dihydropyridine (**4**) and its derivatives have been investigated [3,4], but the application in organic and medicinal chemistry for these compounds is still limited because of their tendency towards the oxidation as well as the poor stability at low pH value; because of enamine reactivity, they easily add water across the 5,6-double bond [5–7]. In this scenario, the identification of new carbamoyl-1,4-dihydropyridines with a better applicability could be intriguing. Our interest in developing new NAD(P)H mimetic compounds prompted us in preparing new series of dihydropyridines (DHP) stable towards the oxidation and in acidic aqueous solutions, using efficient and friendly procedures (Scheme 1).

RESULTS AND DISCUSSION

Pyridine-3,5-dicarboxamide (**1a**) and *N*,*N*′-dimethylpyridine-3,5-dicarboxamide (**1b**) have been prepared from pyridine-3,5-dicarboxylic acid by using oxalyl chloride, instead of thionyl chloride for generating the acylchlorides [8,9] and subsequent amination by gaseous ammonia or methylamine. In our initial study, various conditions were used for the synthesis of pyridinium salts such as the classical thermal way, simply by dissolving the pyridine and the alkylating agent in dimethyl sulfoxide (**2g** and **h**) and heating the mixture for 5 h, or otherwise without solvents (**2a** and **b**) when the alkylating agent was liquid. The best reaction conditions for the synthesis of pyridinium salts were obtained by microwave-assisted procedure, whenever the thermal way did not give rise to significant yield of products (**2c–f**). It should be pointed out that by using microwave irradiation, some advantages have been demonstrated: (i) desired products were obtained in high yields (84–93%); (ii) the shorter reaction times (40 min); and (iii) decreased amount of alkylating agent. To verify the advantages of the microwave irradiation rather than conventional heating, we have carried out the microwave procedure even if the thermal heating has led to significant yields of product. Regarding **2a**, microwave-assisted condition showed a drastic reduction of both reaction time (40 min instead 8 h) and the used amount of benzyl bromide (4 eq instead of 8 eq) with a yield improvement up to 80%.

Dihydropyridines **3a–h** were obtained in high yields from the corresponding pyridinium salts with a green procedure, using a buffered solution under nitrogen flow, and as reducing agent sodium dithionite, which is a versatile and widely used in chemical and biochemical research. It is well known that dithionite reduction appears to be applicable to all pyridinium salts bearing electron-

Scheme 1. Reagents and conditions. *a*: CH_2Cl_2, $(COCl)_2$, reflux, 10 h; *b*: CH_3CN, $R^1NH_{2(g)}$, RT, 3–4 h; *c*: $NaHCO_{3(aq)}$ *d*: R^2X, 120°C, 8–10 h for **2a**,**b**; CH_3CN, R^2X, microwaves, 120°C, 40 min, (reactor setting: 200 psi max pressure, 300 W max power) for **2a**,**c–f** ; DMSO, R^2X 60°C, 5 h for **2g**, reflux, 5 h for **2h**; *e*: H_2O, NaH_2PO_4/Na_2HPO_4, $Na_2S_2O_4$ under N_2 flow 30 min, pH = 6.5 for **3a,c–h**, pH = 7.0 for **3b**.



1a : R^1 = H

1b : R^1 = methyl

2a : R^1 = H ; R^2 = benzyl ; X = Br

2b : R^1 = methyl ; R^2 = benzyl ; X = Br

2c : R^1 = H ; R^2 = 4-methylbenzyl ; X = Br

2d : R^1 = H ; R^2 = 4-cyanobenzyl ; X = Br

2e : R^1 = H ; R^2 = 4-trifluoromethylbenzyl ; X = Br

2f : R^1 = H ; R^2 = 4-fluorobenzyl ; X = Br

2g : R^1 = H ; R^2 = 2,6-dichlorobenzyl ; X = Br

2h : R^1 = H ; R^2 = methyl ; X = I

3a : R^1 = H ; R^2 = benzyl

3b : R^1 = methyl ; R^2 = benzyl

3c : R^1 = H ; R^2 = 4-methylbenzyl

3d : R^1 = H ; R^2 = 4-cyanobenzyl

3e : R^1 = H ; R^2 = 4-trifluoromethylbenzyl

3f : R^1 = H ; R^2 = 4-fluorobenzyl

3g : R^1 = H ; R^2 = 2,6-dichlorobenzyl

3h : R^1 = H ; R^2 = methyl

withdrawing substituents in the 3- or 3,5-positions, affording exclusively the corresponding 1,4-dihydropyridines, as final products. In the course of the reduction, formed intermediate adducts have been identified as sulfinate *S*-anions [10]. Furthermore, these adducts are stable in strong alkaline solutions, whereas at low alkaline pH values, these were protonated to sulfinate, which promptly decomposed into 1,4-dihydropyridines following SO_2 elimination [11]. In this regard, we showed that the pH value is a fundamental parameter for the reduction of different pyridinium salts. Indeed, the 1,4-dihydropyridine formation at pH = 8, which is described for 3-carbamoyl derivatives [11], did not represent the best condition in the preparation of 3,5-dicarboxamide derivatives that requires lower pH values.

This different behavior could be related to the different stability of the protonated sulfinate intermediate that depends on the nature of substituent at positions 1, 3, and 5 of the pyridine ring; electron-withdrawing groups more effectively stabilize the intermediate, which requires lower pH values to decompose: that is, pH = 7 for compound **3b** and pH = 6.5 for compounds **3a** and **c–h**.

To evaluate the different stability of 3-carboxamide and 3,5-dicarboxamide-1,4-dihydropyridines towards the oxidation, we have carried out a study of antioxidant activity of selected representative compounds (**4**, **3a** and **3h**).

The antioxidant activity was evaluated using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method according to Brand-Williams *et al*. [12] based on the ability of antioxidant compounds to react with the stable radical DPPH·. The EC_{50} was graphically extrapolated plotting the residual percentage of DPPH· concentration after 90 min of reaction versus the molar ratio [DHP]/[DPPH] (Fig. 1). The direct comparison of the obtained results shows that **3a** is not able to react with DPPH radical and **3h** instead seems having only a weak antioxidant activity (EC_{50} of 109 μM). In contrast, 1-benzyl-1,4-dihydropyridine-3-carboxamide **4** [13] has given a curve with a pronounced slope, indicating an efficacious reaction with DPPH radical with an EC_{50} of 14.4 μM. The different behavior showed by 3,5-dicarbamoyl-1,4-dihydropyridines, **3h** and particularly **3a**, indicates that these compounds possess higher stability towards oxidation with respect to 3-carbamoyl derivative **4**.

The presence of the enamine moiety in the 1,4-dihydropyridines makes them susceptible to the hydratation of 5,6-double bond because of the protonation of carbon 5 and subsequent addition of water to carbon 6; this phenomenon is strictly dependant on the H^+ concentration [14]. The electronic properties of substituents on the pyridine ring may influence the sensitivity of the 1,4-dihydropyridine in the acidic environment [7,15]. Therefore, we have decided to test the stability of 1-benzyl-1,4-dihydropyridine-3,5-dicarboxamide **3a** and 1-benzyl-1,4-dihydropyridine-3-carboxamide **4** in acidic media. The compounds **4** and **3a** were dissolved at 1.52×10^{-3} M concentration in a buffer HCl–glycine (pH = 3) and monitored with an UV/visible spectrophotometer measuring the absorbance change among 220–460 nm. For compound **4**, we have observed the immediate disappearance of the absorption maximum at 354 nm (Fig. 2, red curve) due to the 5,6-double bond hydratation.

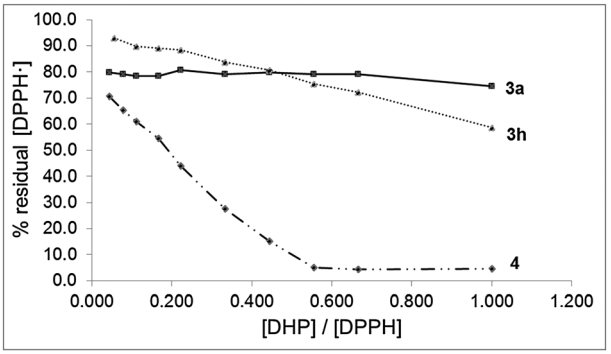


Figure 1. Residual percentages of DPPH· at the end of reaction with dihydropyridine (DHP) plotted *vs* the molar ratio [DHP]/[DPPH].

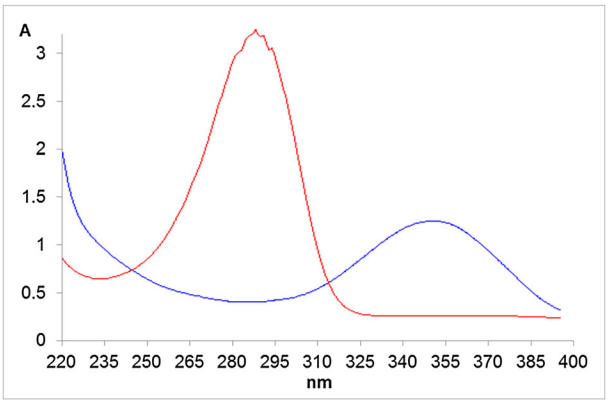


Figure 2. UV spectra of compound **4**: dissolved in methanol (blue) and immediately after dissolution in pH 3 buffer solution (red).

Differently, **3a** (Fig. 3) resulted stable and does not undergo the hydratation at the 5.6 double bond neither after 6 h at pH = 3; indeed, only little variations are evident in the absorbance values.

In conclusion, it should be noted that the presence of the second carboxamide group on C-5, because of its strong withdrawal effect, makes the 1,4-dihydropyridine system highly resistant towards the hydratation and the oxidation. A friendly and efficient microwave procedure has been developed to achieve pyridinium salts that are obtained in very low yields by thermal way; furthermore, 3,5-dicarbamoyl-1,4-dihydropyridines were obtained in high yields in aqueous buffer solution at adequate pH values. These compounds are more stable than the corresponding monocarbamoyl derivatives, thus suggesting a high versatility in applications of organic and pharmaceutical chemistry.

EXPERIMENTAL

All chemicals were of analytical grade and were purchased from Sigma-Aldrich Chemical Co. (Milan, Italy).

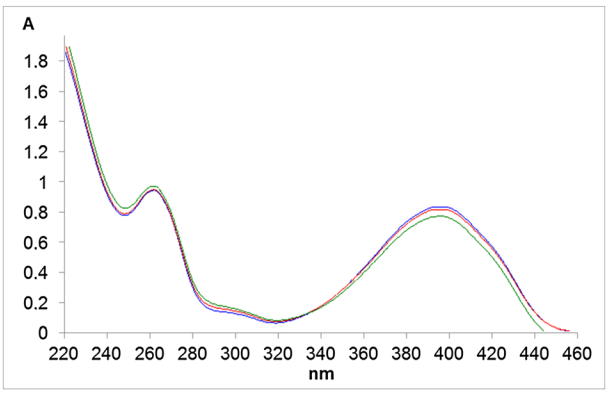


Figure 3. UV spectra of compound **3a** in pH 3 buffer solution, immediately after the dissolution (blue), after 3 h (red), and after 6 h (green).

Melting points were determined on a Tottoli apparatus and are uncorrected. IR spectra were recorded on a PerkinElmer Spectrum-One spectrophotometer equipped with a ATR detector, and band frequencies are given in wave number (cm^{-1}). ^1H and ^{13}C NMR spectra were acquired on a Bruker Avance 400 spectrometer operating at 400 and 100 MHz, respectively. Chemical shift values are reported as δ (ppm) relatively to $Si(CH_3)_4$ as internal reference; coupling constant are given in Hz. Elemental analyses were obtained by a PE 2400 (PerkinElmer) analyzer, and the analytical results were within ±0.4% of the theoretical values. UPLC-MS analyses were run using a Acquity Waters UPLC equipped with a Waters SQD (ES ionization) and Waters Acquity PDA detector, using a column BEH C18 1.7 µm, 2.1 × 50 mm. Gradients were run using 0.05% formic acid water/acetonitrile 95/5 and acetonitrile with a gradient 95/5 to 100, flow: 0.8 mL/min over 3 or 5 min, temperature: 40°C, and UV detection: 215 and 370 nm. ESI + detection in the 80–1000 *m/z* range. HPLC-MS were run using a Waters 2795 separation module equipped with a Waters Micromass ZQ (ES ionization) and Waters PDA 2996, using a X-Bridge C18 3.5 µm 2.10 × 50 mm column, gradient: 0.1% formic acid/water and 0.1% formic acid/acetonitrile with gradient 95/5 to 5/95 flow 0.8 mL/min over 10 min. Retention times (R_t) are expressed in minutes, temperature at 40°C, and UV detection at 215 and 370 nm. ESI^+ detection is in the 80–1000 *m/z* range. Microwave reactions were conducted using a CEM explorer 72 reactor.

Pyridine-3,5-dicarboxamide (1a). Oxalyl chloride (36.4 mmol) was dropwise added to the suspension of pyridine-3,5-dicarboxylic acid (3.59 mmol) in dichloromethane (50 mL), and the mixture was refluxed for 10 h. The obtained yellow solution was evaporated under reduced pressure to give a crude residue, which was treated with 30 mL of dry acetonitrile. Through the obtained solution, gaseous ammonia was bubbled for 3 h at room temperature. The obtained solid was filtered off, washed with acetonitrile (3 × 5 mL), water (3 × 8 mL), and treated at 70°C with aqueous saturated $NaHCO_3$ (35 mL) for 20 min. After cooling, a woolly precipitate was filtered off, washed with water (3 × 6 mL), and dried, to give pure **1a** as a brownish solid (yield 85%); mp 303–304°C (lit. [8] 303–305°C). ν_{max} (cm^{-1}) (neat): 3387, 3113, 1683, 1631, 1575. ^1H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.12 (s, 2H, H-2 and H-6), 8.62 (s, 1H, H-4), 8.24 (s br, 2H, NH_a), 7.68 (s br, 2H, NH_b). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 166.4, 151.2, 134.2, 129.9. (*Anal.* Calcd for $C_7H_7N_3O_2$: C, 50.91; H, 4.27; N, 25.44%; found C, 50.75; H, 3.98; N, 25.16%).

N,N′-dimethylpyridine-3,5-dicarboxamide (1b). Pyridine-3,5-dicarbonyl dichloride (3.59 mmol), prepared as described for **1a**, was dissolved in acetonitrile (30 mL), anhydrous methylamine was bubbled, and the solution was stirred for 4 h at room temperature.

The solvent was removed under reduced pressure, and the obtained crude residue was washed with water (4 × 10 mL) and dried. The remaining solid was treated with aqueous saturated K_2CO_3 (15 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The obtained product was purified on silica gel column chromatography (ethylacetate–methanol, 7:3 v/v) to afford pure **1b** as a yellowish solid (yield 80%); mp 215–218°C (lit. [9] 220–222°C). ν_{max} (cm^{-1}) (neat): 3285, 3075,1634; 1542, 1296. ^1H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.07 (d, 2H, H-2 and H-6, *J* = 2.2), 8.73–8.78 (m, 2H, $NHCH_3$), 8.56 (t, 1H, H-4), 2.81 (d, 6H,

NHCH$_3$). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 167.3, 151.1, 136.2, 130.4, 26,9. (*Anal*. Calcd for $C_9H_{11}N_3O_2$: C, 55.95; H, 5.74; N, 21.75%; found C, 55.75; 5.35; N, 21.48%).

1-Benzyl-3,5-dicarbamoylpyridinium bromide (2a).

Method a. a mixture of pyridine-3,5-dicarboxamide **1a** (6.05 mmol) and benzyl bromide (48.48 mmol) was refluxed and maintained under stirring for 8 h. The suspension was cooled at room temperature, and the brown solid was filtered off, washed with diethyl ether (7 × 10 mL), and dried to give pure **2a** as a light brown solid (yield 70%); mp > 300°C (lit. [9], mp > 300°C).

Method b. **2a** was also prepared by microwave-assisted synthesis using the same procedure described for **2c–f**: (yield 80%).

ν_{max} (cm^{-1}) (neat): 3358, 3158, 3056, 1674, 1598, 1443. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.71 (d, 2H, $J_{2,4} = J_{6,4} = 1.72$, H-2 and H-6), 9.40 (t, 1H, H-4), 8.63 (s br, 2H, NH_a), 8.26 (s br, 2H, NH_b), 7.63-7.44 (m, 5H, Ar), 5.97 (s, 2H, CH_2).^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 162.8, 146.6, 142.6, 134.3, 134.0, 129.9, 129.7, 129.6, 64.2 (*Anal*. Calcd for $C_{14}H_{14}BrN_3O_2$: C, 50.02; H, 4.20; Br, 23.77; N, 12.50%; found C, 50.19; H, 4.11; N, 12.32%).

1-Benzyl-3,5-bis(methylcarbamoil)pyridinium bromide (2b). **2b** was prepared using **1b** as starting reagent (2.22 mmol) following the procedure (*method a*) described for **2a**. The brown solid **2b**, crystallized by EtOH, was obtained in 50% yield; mp 258–260°C (lit. [9] 259–260°C).

ν_{max} (cm^{-1}) (neat): 3244, 3071, 2920, 1681, 1654, 1621, 1292. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.68 (s, 2H, H-2 and H-6), 9.34 (s, 1H, H-4), 9.20 (d, 2H, J = 4.64, NHCH$_3$), 7.61-7.44 (m, 5H, Ar), 5.96 (s, 2H, CH_2), 2.86 (d, 6H, NHCH$_3$).^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 162.0, 146.7, 142.4, 134.7, 134.5, 130.4, 130.1, 64.7, 27.2. (*Anal*. Calcd for $C_{16}H_{18}BrN_3O_2$: C, 52.76; H, 4.98; Br, 21.94; N, 11.54%; found: C, 52.93; H, 4.76; N, 11.35%).

General procedure for the synthesis of pyridinium salts 2c–f. The appropriate 4-substituted benzyl bromide was added to a suspension of **1a** (1.21 mmol) in acetonitrile (5 mL), and the mixture was heated at 120°C, (reactor setting: 300 W max power and 200 psi max pressure) for 40 min in a microwave reactor. The resulting solution was cooled at room temperature, 15 mL diethyl ether were added, and the obtained precipitate was collected and washed with diethyl ether (5 × 4 mL) to give pure salt as a solid.

3,5-Dicarbamoyl-1-(4-methylbenzyl)pyridinium bromide (2c). **2c** was prepared using 4-methylbenzyl bromide (4.86 mmol) as alkylating agent, and it was obtained in 92% yield as a white solid. mp 265–267°C. ν_{max} (cm^{-1}) (neat) 3332, 3161, 3060, 1678, 1610.

^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.74 (s, 2H, H-2 and H-6), 9.46 (s, 1H, H-4), 8.67 (s br, 2H, NH_a), 8.25 (s br, 2H, NH_b), 7.55 (d, 2H, J = 7.8, Ar), 7.26 (d, 2H, J = 7.8, Ar), 5.96 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 162.9, 146.5, 142.5, 139.7, 134.3, 131.1, 130.2, 129.8, 64.1, 21.2. (*Anal*. Calcd for $C_{15}H_{16}BrN_3O_2$: C, 51.44; H, 4.60; Br, 22.82; N, 12.00%; found: C, 51.17; H, 4.45; N, 11.69%.

3,5-Dicarbamoyl-1-(4-cyanobenzyl)pyridinium bromide (2d). **2d** was prepared using 4-(bromomethyl)benzonitrile (4.85 mmol) as alkylating agent, and it was obtained in 84% yield as a white solid. mp 263–266°C. ν_{max} (cm^{-1}) (neat): 3369, 3203, 3144, 3055, 2239, 1682, 1614. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.85 (s, 2H, H-2 + H-6), 9.52 (s, 1H, H-4), 8.69 (s br, 2H, NH_a), 8.26 (s br, 2H, NH_b), 7.93 (d, 2H, J = 8.1 Hz, Ar), 7.85 (d, 2H, J = 8.1 Hz, Ar), 6.15 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 162.8, 147.0, 142.9, 139.1, 134.4, 133.4, 130.6, 118.8, 112.6, 63.4. (*Anal*. Calcd for $C_{15}H_{13}BrN_4O_2$: C, 49.88; H, 3.63; Br, 22.12; N, 15.51%; found: C, 49.76; H, 3.29; N, 15.32%).

3,5-Dicarbamoyl-1-[4-(trifluoromethyl)benzyl]pyridinium bromide (2e). **2e** was prepared using 4-(trifluoromethyl)benzyl bromide (4.84 mmol) as alkylating agent, and it was obtained in 90% yield a as white solid. mp 269–271°C. ν_{max} (cm^{-1}) (neat): 3299, 3137, 1694, 1671. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.88 (s, 2H, H-2 and H-6), 9.54 (s, 1H, H-4), 8.70 (s br, 2H, NH_a), 8.28 (s br, 2H, NH_b), 7.90 (d, 2H, J = 8.2, Ar), 7.81 (d, 2H, J = 8.2, Ar), 6.17 (s, 2H, CH_2).^{13}C NMR (100 MHz, $(CD_3)_2SO$): 162.8, 147.0, 142.8, 138.5, 134.4, 130.1, 130.0 (q, J_{C-F} = 31 Hz), 126.4 (q, J_{C-F} = 3 Hz), 125.7, 63.4. (*Anal*. Calcd for $C_{15}H_{13}BrF_3N_3O_2$: C, 44.57; H, 3.24; Br, 19.77; F, 14.10; N, 10.40%; found: C, 44.73; H, 2.93; N, 10.59%.

3,5-Dicarbamoyl-1-(4-fluorobenzyl)pyridinium bromide (2f). **2f** was prepared using 4-fluorobenzyl bromide (4.84 mmol) as alkylating agent, and it was obtained in 93% yield as a white solid. mp 270–271°C. ν_{max} (cm^{-1}) (neat): 3291, 3133, 3064, 1673, 1624. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.82 (s, 2H, H-2 and H-6), 9.50 (s, 1H, H-4), 8.70 (s br, 2H, NH_a), 8.28 (s br, 2H, NH_b) 7.81–7.77 (m, 2H, Ar), 7.31–7.27 (m, 2H, Ar), 6.02 (s, 2H, CH_2).

^{13}C NMR (100 MHz, $(CD_3)_2SO$): 163.1 (d, J_{C-F} = 244.4), 162.9, 146.7, 142.6, 134.3, 132.4 (d, J_{C-F} = 8), 130.3, 116.5 (d, J_{C-F} = 21.8), 63.4. (*Anal*. Calcd for $C_{14}H_{13}BrFN_3O_2$: C, 47.48; H, 3.70; Br, 22.56; F, 5.36; N, 11.86%; found: C, 47.36; H, 3.51; N, 11.61%).

3,5-Dicarbamoyl-1-(2,6-dichlorobenzyl)pyridinium bromide (2g). A mixture of **1a** (1.57 mmol) and dimethylsulfoxide (6.5 mL) was heated until complete dissolution, and then, 2,6-dichlorobenzyl bromide (3.17 mmol) was added. The obtained solution was heated to 60°C for 5 h, cooled at room temperature, and then water was dropwise added until complete precipitation of the salt. The precipitate was collected by filtration, washed with diethyl ether (4 × 2 mL), and dried under reduced pressure to give pure **2g** (72% yield) as light yellow solid. mp 268–269°C. ν_{max} (cm^{-1}) (neat): 3334, 3140, 3061, 1675, 1610. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.44 (s, 1H, H-4), 9.36 (s, 2H, H-2 and H-6), 8.67 (s br, 2H, NH_a), 8.28 (s br, 2H, NH_b), 7.69–7.61 (m, 3H, Ar), 6.25 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 162.7, 146.4, 142.9, 137.1, 134.1, 133.8, 130.0, 128.3, 59.9. (*Anal*. Calcd for $C_{14}H_{12}BrCl_2N_3O_2$: C, 41.51; H, 2.99; Br, 19.73; Cl, 17.50; N, 10.37%; found: C, 41.27; H, 2.88; N, 10.11%.

3,5-Dicarbamoyl-1-methylpyridinium iodide (2h). A mixture of **1a** (0.61 mmol) in dimethyl sulfoxide (3 mL) was heated until completed dissolution, and then, iodomethane was added (3.10 mmol). The obtained solution was refluxed for 5 h, cooled at room temperature, and then, water was added dropwise until complete precipitation of the salt. The precipitate was collected and washed with diethyl ether (4 × 2 mL), dried under reduced pressure, and crystallized with ethanol to give pure **2h** as a yellow solid (49% yield). mp 275–279°C. ν_{max} (cm^{-1}) (neat): 3339, 3067, 1672, 1624. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 9.50 (s, 2H, H-2 and H-6), 9.28 (s, 1H, H-4), 8.53 (s br, 2H, NH_a), 8.21 (s br, 2H, NH_b), 4.43 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 163.0, 147.6, 141.7, 133.5, 49.1.(*Anal*. Calcd for $C_8H_{10}IN_3O_2$: C, 31.29; H, 3.28; I, 41.33; N, 13.68%; found: C, 31.61; H, 3.41; N, 13.51%).

1-Benzyl-1,4-dihydropyridine-3,5-dicarboxamide (3a). A solution of sodium dithionite (1.21 mmol) in NaH_2PO_4/Na_2HPO_4 buffer (pH = 6.5, 5.6 mL) under nitrogen atmosphere was cooled in an ice bath. Then, a solution of **2a** (0.60 mmol) dissolved in 7.0 mL of NaH_2PO_4/Na_2HPO_4 (pH = 6.5) was dropwise added. The reaction was maintained under stirring for 40 min, and after this time, the obtained yellow precipitate was collected by

filtration, washed with cold water (3 × 1.5 mL), and dried under reduced pressure, to give pure **3a** (72% yield). mp 195–197°C. ν_{max} (cm^{-1}) (neat): 3350, 3175, 1687, 1644, 1557. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.40–7.29 (m, 5H, Ar), 6.98 (s, 2H, H-2 and H-6), 6.72 (s br, 4H, NH_2), 4.44 (s, 2H, CH_2), 3.07 (s, 2H, H-4 and H-4′). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 168.8, 138.5, 136.3, 129.2, 128.1, 127.8, 106.3, 56.7, 23.0. UPLC-MS (3 min run): mass (ES) *m/z*: 258 (M + 1); R_t = 0.98 min (*Anal*. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.35; H, 5.88; N, 16.33%; found: C, 65.01; H, 5.53; N, 16.15%).

1-Benzyl-*N*,*N*′-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (3b). **3b** was prepared following the same procedure described for **3a**, using 0.096 g of sodium dithionite (0.55 mmol), 4.0 mL of NaH_2PO_4/Na_2HPO_4 buffer (pH = 7.0), and 0.10 g of **2b** (0.27 mmol) dissolved in 6.0 mL of NaH_2PO_4/Na_2HPO_4 buffer (pH = 7.0). Pure **3b** was obtained as yellow powder in yield of 81%. mp 92–95°C. ν_{max} (cm^{-1}) (neat): 3306, 1686, 1539. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.39–7.26 (m, 5H, Ar), 7.19 (d, 2H, *J* = 4.4 Hz, NHCH$_3$), 6.92 (s, 2H, H-2 and H-6), 4.45 (s, 2H, CH_2), 3.09 (s, 2H, H-4 and H-4′), 2.61 (d, 6H, NHCH_3). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 167.4, 138.6, 135.5, 129.2, 128.0, 127.7, 106.2, 56.7, 26.4, 22.7. UPLC-MS (3 min run): mass (ES) *m/z*: 286 (M + 1); R_t = 1.09 min (*Anal*. Calcd for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73%; found: C, 67.11; H, 6.62; N, 14.63%).

1-(4-Methylbenzyl)-1,4-dihydropyridine-3,5-dicarboxamide (3c). **3c** was prepared following the same procedure described for **3a**, using 0.15 g of sodium dithionite (0.86 mmol) and 0.15 g of **2c** (0.43 mmol). Pure **3c** was obtained as a yellow powder in 77% yield. mp 167–170°C. ν_{max} (cm^{-1}) (neat): 3343, 3179, 1688, 1640. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.19 (s, 4H, Ar), 6.96 (s, 2H, H-2 and H-6), 6.71 (s br, 4H, NH_2), 4.39 (s, 2H, CH_2), 3.07 (s, 2H, H-4 and H-4′), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 168.8, 137.3, 136.2, 135.4, 129.7, 127.8, 106.2, 56.5, 23.0, 21.1. UPLC-MS (5 min run): mass (ES) *m/z*: 272 (M + 1); R_t = 1.65 min (*Anal*. Calcd for $C_{15}H_{17}N_3O_2$: C, 66.40; H, 6.32; N, 15.49%; found: C, 66.13; H, 6.12; N, 15.15%).

1-(4-Cyanobenzyl)-1,4-dihydropyridine-3,5-dicarboxamide (3d). **3d** was prepared following the same procedure described for **3a**, using 0.14 g of sodium dithionite (0.80 mmol) and 0.15 g of **2d** (0.41 mmol). Pure **3d** was obtained as a yellow powder in 80% yield. mp 110–112°C. ν_{max} (cm^{-1}) (neat): 3351, 3205, 2231, 1686, 1644. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.87 (d, 2H, *J* = 8.31, Ar), 7.51 (d, 2H, *J* = 8.31, Ar), 6.98 (s, 2H, H-2 and H-6), 6.74 (s br, 4H, NH_2), 4.57 (s, 2H, CH_2), 3.08 (s, 2H, H-4 and H-4′). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 168.7, 144.4, 136.1, 133.1, 129.0, 128.6, 110.8, 106.8, 56.1, 22.9. UPLC-MS (5 min run): mass (ES) *m/z*: 283 (M + 1); R_t = 1.42 min (*Anal*. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85%; found: C, 63.96; H, 4.83; N, 20.13%).

1-[4-(Trifluoromethyl)benzyl]-1,4-dihydropyridine-3,5-dicarboxamide (3e). **3e** was prepared following the same procedure described for **3a**, using 0.13 g of sodium dithionite (0.74 mmol) and 0.15 g of **2e** (0.37 mmol). Pure **3e** was obtained as a yellow powder in 84% yield. mp 147–150°C. ν_{max} (cm^{-1}) (neat): 3365, 3192, 1687, 1620. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.77 (d, 2H, *J* = 8.08, Ar), 7.52 (d, 2H, *J* = 8.08, Ar), 7.00 (s, 2H, H-2 and H-6), 6.77 (s br, 4H, NH_2), 4.58 (s, 2H, CH_2), 3.09 (s, 2H, H-4 and H-4′). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 168.7, 143.4, 136.1, 128.5, 126.1, 124.5 (q, J_{C-F} = 270 Hz), 106.7, 56.1, 23.0. HPLC-MS (10 min run): mass (ES) *m/z*: 326 (M + 1); R_t = 4.28 min (*Anal*. Calcd for $C_{15}H_{14}F_3N_3O_2$: C, 55.39; H, 4.34; F, 17.52; N, 12.92%; found: C, 55.21; H, 4.08; N, 12.77%).

1-(4-Fluorobenzyl)-1,4-dihydropyridine-3,5-dicarboxamide (3f). **3f** was prepared following the same procedure described for **3a**, using 0.15 g of sodium dithionite (0.86 mmol) and 0.15 g of **2f** (0.42 mmol). Pure **3f** was obtained as a yellow powder in 74% yield. mp 180–183°C. ν_{max} (cm^{-1}) (neat): 3342, 3172, 1687, 1644. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.36 (d, 2H, Ar), 7.22 (d, 2H, Ar), 6.98 (s, 2H, H-2 and H-6), 6.73 (s br, 4H, NH_2), 4.44 (s, 2H, CH_2), 3.07 (s, 2H, H-4 and H-4′). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 168.8, 168.6 (d, J_{C-F} = 258.0 HZ), 136.1, 134.7, 129.9 (d, J_{C-F} = 8.8 Hz), 116.0 (d, J_{C-F} = 21.0 Hz), 106.5, 55.9, 13.0. UPLC-MS (5 min run): mass (ES) m/z: 276 (M + 1); R_t = 1.56 min (*Anal*. Calcd for $C_{14}H_{14}FN_3O_2$: C, 61.08; H, 5.13; F, 6.90; N, 15.26%; found: C, 60.72; H, 4.94; N, 15.02).

1-(2,6-Dichlorobenzyl)-1,4-dihydropyridine-3,5-dicarboxamide (3g). **3g** was prepared following the same procedure described for **3a**, using 0.17 g of sodium dithionite (0.98 mmol) and 0.20 g of **2g** (0.49 mmol). Pure **3g** was obtained as a yellow powder in 80% yield. mp 201–204°C. ν_{max} (cm^{-1}) (neat): 3343, 3185, 1688, 1644, 1561. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.57–7.49 (m, 3H, Ar), 6.90 (s, 2H, H-2 and H-6), 6.73 (s br, 4H, NH_2), 4.68 (s, 2H, CH_2), 3.06 (s, 2H, H-4 and H-4′). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 168.7, 137.1, 136.2, 135.7, 131.7, 129.6, 106.3, 51.9, 23.1. UPLC-MS (3 min run): mass (ES) *m/z*: 326 (M + 1); R_t = 0.74 min (*Anal*. Calcd for $C_{14}H_{13}Cl_2N_3O_2$: C, 51.55; H, 4.02; Cl, 21.74; N, 12.88%; found: C, 51.34; H, 3.71; N, 12.61%).

1-Methyl-1,4-dihydropyridine-3,5-dicarboxamide (3h). **3h** was prepared following the same procedure described for **3a**, using 0.25 g of sodium dithionite (1.44 mmol) and 0.20 g of **2h** (0.65 mmol). Pure **3h** was obtained as a yellow powder in 71% yield. mp 220–223°C. ν_{max} (cm^{-1}) (neat): 3415, 3163, 1704, 1651, 1558. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 6.83 (s, 2H, H-2 and H-6), 6.69 (s br, 4H, NH_2), 3.03 (s, 2H, H-4 and H-4′), 2.99 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 169.1, 137.0, 105.6, 40.8, 22.7.UPLC-MS (3 min run): mass (ES) *m/z*: 182 (M + 1); R_t = 0.19 min (solvent front) (*Anal*. Calcd for for $C_8H_{11}N_3O_2$: C, 53.03; H, 6.12; N, 23.19%; found: C, 52.77; H, 5.99; N, 23.05%).

Evaluation of antioxidant activity. The antioxidant activity of DHP was spectrophotometrically evaluated against stable radical DPPH [12] (Sigma-Aldrich, Milano, Italia), measuring the absorbance decrease at 515 nm by mean of UV/visible spectrophotometer PerkinElmer (mod.Lambda 40). Methanol was used as solvent. Standard polystyrene optical cuvette (3 mL volume) were used, repeating each measure at least in triplicate. Data were processed by mean of Microsoft Excel 2010 software. A calibration curve with DPPH solutions in the concentration range 20–180 μM was obtained (r^2 = 0.996). The decrease of the absorbance of 60 μM DPPH solutions containing 30 μM 1-benzyl-3-carbamoyl-1,4-dihydropyridine (**4**) was measured in a time drive mode for 2 h; the final plateau absorbance values were reached after 60 min. The absorbance of 60 μM DPPH solution was measured, and thereafter, 30 μL of appropriate stock DHP solution were added to obtain the desired final concentration in cuvette. Each solution was stored at 25°C in a dark box for 90 min, and then, the absorbance was measured. The initial and final DPPH concentration

for each cuvette were calculated using the DPPH calibration curve equation. For each DHP, the percentages of residual DPPH remaining after 90 min were plotted versus the molar ratio [DHP]/[DPPH]. The molar ratio able to decrease by 50% the initial DPPH concentration was calculated from these plots, and subsequently, the EC_{50} values were obtained (DHP concentration needed to halve initial DPPH radical concentration). The absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily.

Acknowledgments. This study was financially supported by Sapienza University of Rome and Siena Biotech S.p.A. The authors thank MSc Letizia Fontana for her valuable contribution.

REFERENCES AND NOTES

[1] Mai, A.; Valente, S.; Meade, S.; Carafa, V.; Tardugno, M.; Nebbioso, A.; Galmozzi, A.; Mitro, N.; De Fabiani, E.; Altucci, L.; Kazantsev, A. J Med Chem 2009, 52, 5496.

[2] Xia, W.; Wang, Z.; Wang, Q.; Han, J.; Zhao, C.; Hong, Y.; Zeng, L.; Tang, L.; Ying, W. Curr Pharm Des 2009, 15, 129.

[3] Eisner, U.; Kuthan, J. Chem Rev 1972, 72, 1.

[4] Stout, D. M.; Meyers, A. I. Chem Rev 1982, 82, 223.

[5] Anderson, A. G., Jr; Berkelhammer, G. J Am Chem Soc 1958, 80, 992.

[6] Choi, K. S.; Alivisitos, S. G. Biochemistry 1968, 7, 190.

[7] Pop, E.; Brewster, M. E.; Huang, M.; Bodor, N. J Mol Struc - Theochem 1995, 337, 49.

[8] Davis, M. C.; Irvin, D. J. Synth Commun 2008, 38, 1159.

[9] Carelli, V.; Liberatore, F.; Tortorella, S.; Di Rienzo, B.; Scipione, L. J Chem Soc Perkin Trans 2002, 1, 542.

[10] Carelli, V.; Liberatore, F.; Scipione, L.; Musio, R.; Sciacovelli, O. Tetrahedron Lett 2000, 41, 1235.

[11] Carelli, V.; Liberatore, F.; Scipione, L.; Di Rienzo, B.; Tortorella, S. Tetrahedron 2005, 61, 10331.

[12] Brand-Williams, W.; Cuvelier, M. E.; Berset, C. LWT-Food Sci Technol 1995, 28, 25.

[13] Haynes, R. K.; Chan, W.; Wong, H.; Li, K.; Wu, W.; Fan, K.; Sung, H. H. Y.; Williams, I. D.; Prosperi, D.; Melato, S.; Coghi, P.; Monti, D. Chem Med Chem 2010, 5, 1282.

[14] Norris, D. J.; Stewart, R. Can J Chem 1977, 55, 1687.

[15] Hentall, P. L.; Flowers, N.; Bugg, T. D. H. Chem Commun 2001, 20, 2098.