5-Substituted [1]pyrindine derivatives with antiproliferative activity

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · DECEMBER 2009

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2009.11.028 · Source: PubMed

7 AUTHORS, INCLUDING:

Mary-Lorène Goddard
Université de Haute-Alsace
13 PUBLICATIONS **191** CITATIONS
SEE PROFILE

Odile Mondésert
Institute for Advanced Technology in Life S…
27 PUBLICATIONS **1,211** CITATIONS
SEE PROFILE

Emmanuelle Braud
Université René Descartes - Paris 5
19 PUBLICATIONS **196** CITATIONS
SEE PROFILE

European Journal of Medicinal Chemistry 45 (2010) 896–901

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

5-Substituted [1]pyrindine derivatives with antiproliferative activity

Stéphanie Kolb [a,b,1], Mary-Lorène Goddard [a,b,1], Ali Loukaci [a,b], Odile Mondésert [c,d], Bernard Ducommun [c,d,e], Emmanuelle Braud [a,b], Christiane Garbay [a,b,*]

[a] Université Paris Descartes, UFR Biomédicale, Laboratoire de Pharmacochimie Moléculaire et Cellulaire, 45 rue des Saints-Pères, Paris, F-75006, France
[b] INSERM U648, Paris, F-75006, France
[c] Université de Toulouse, LBCMCP; 118 route de Narbonne, F-31062 Toulouse, France
[d] CNRS, LBCMCP-UMR5088, F-31062 Toulouse, France
[e] CHU Purpan, TSA 40031, F-31059 Toulouse, France

ARTICLE INFO

Article history:
Received 2 December 2008
Received in revised form
6 November 2009
Accepted 12 November 2009
Available online 6 December 2009

Keywords:
Cancer
Cytotoxic agents
Pyrindine derivatives

ABSTRACT

We report herein the synthesis of 5-substituted [1]pyrindine derivatives and the evaluation of their antiproliferative properties on HeLa cells, a cervical carcinoma tumor cell line, and on the melanoma A2058 cell line. The most efficient compounds display cytotoxicity against tumor cells in the micromolar range but have interestingly no effect against the normal human fibroblasts CRL-2796. Generally, these pyrindines are active on both tumor cell lines. Compounds bearing large substituents with structural rigidity at position 5 such as phenyl-furyl show no inhibition of cell growth.

1. Introduction

Pyrindine systems have been reported in a variety of natural products displaying a wide range of biological activities. For example, louisianin C [1] and incarvilline [2] which possess the [2]pyrindine core are alkaloid compounds with anti-angiogenic and potent analgesic properties, respectively (Fig. 1). Molecules bearing the [1]pyrindine moiety have also been described such as streptazolin [3,4], a tricyclic compound displaying antibiotic and antifungal properties and its by-product, the 5-*O*-(β-D-xylopyranosyl)-streptazolin [5] which shows *in vitro* cytotoxic activity against several human cancer cell lines (Fig. 1).

We recently reported in silico/*in vitro* screening experiments targeting the dual-specificity phosphatases CDC25 to identify original scaffolds with CDC25 inhibitory activity [6]. These phosphatases play critical roles in cell cycle regulation by activating Cdk–cyclin complexes and are considered particularly attractive targets for the treatment of cancer [7,8]. Among the most potent compounds isolated from the screening, pyrindine **1** inhibited the phosphatase activity of the recombinant MBP-CDC25B protein with an IC_{50} value of 19.0 μM [6].

To date, a few compounds belonging to the family of [1]pyrindines of type I have been found to exhibit biological activity but only as protein kinase inhibitors (Fig. 1). Using virtual screening methods, Forino *et al.* identified compound **2** with Akt1 kinase inhibitory activity [9,10]. Subsequent evaluation of analogues of **2** allowed for the discovery of two additional inhibitors, compounds **1** and **3** with IC_{50} of 60.2 and 126 μM, respectively. Finally, a recent patent related that compound **4** could inhibit B-Raf kinase activity with an IC_{50} value superior to 20 μM [11].

Therefore, since the biological properties of these pyrindine derivatives have been poorly studied, we decided to develop a series of compounds of type I to improve the inhibitory activity toward CDC25 phosphatases (Fig. 1). Since inhibitors of CDC25 activity are intended to present cytotoxic properties, these compounds were also evaluated against two human cancer cell lines as well as a normal human cell type.

2. Chemistry

The 5-substituted [1]pyrindines **8** and **11**–**29** were synthesized in a two-step sequence starting with the preparation of the common aminopyrindinecarbonitrile scaffold **5a**,**b** obtained by reacting two equivalents of malononitrile with 2,5-hexanedione in the presence of piperidine in refluxing ethanol (Scheme 1) [12,13].

* Corresponding author. Université Paris Descartes, UFR Biomédicale, Laboratoire de Pharmacochimie Moléculaire et Cellulaire, 45 rue des Saints-Pères, Paris, F-75006, France. Tel.: +33 142 864 080; fax: +33 142 864 082.
E-mail address: christiane.garbay@parisdescartes.fr (C. Garbay).
[1] These authors contributed equally to the work.

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.11.028

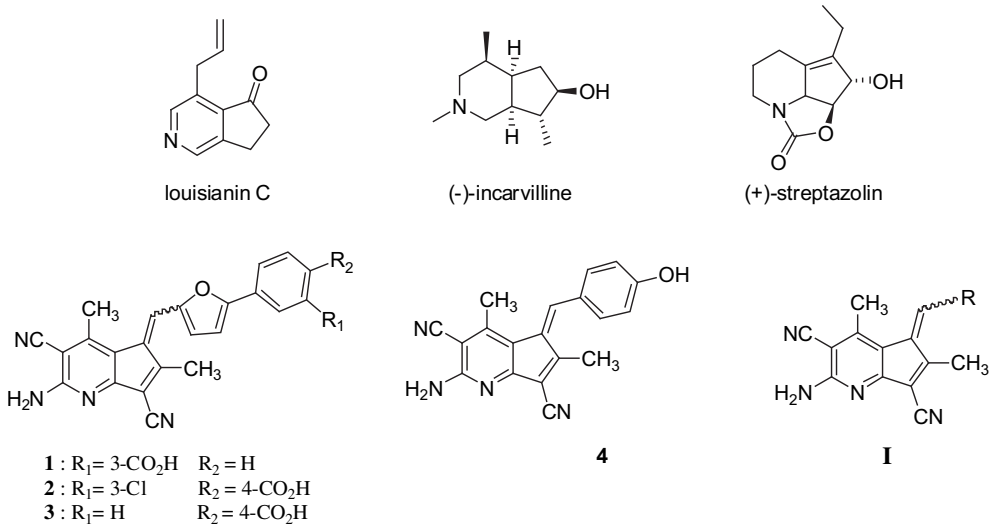


Fig. 1. Pyrindine system containing agents.

^{1}H NMR spectrum obtained in DMSO revealed that an equilibrium was observed between both tautomeric forms **5a** and **5b** in a ratio of 32/68 respectively as previously reported [12].

The final compounds were synthesized using a Knoevenagel condensation that was first described by Junek [13]. Thus, reaction of the equilibrating scaffolds **5a**, **b** with the appropriate aldehydes in the presence of piperidine in methanol afforded the final compounds **8** and **11**–**29** with yields ranging from 23 to 62% (Scheme 1).

This synthetic path led to mixtures of *Z*/*E* isomer. NMR experiments were conducted on compound **27** to fully assign each isomer its own ^{1}H NMR spectral data. Briefly, the HMBC spectrum showed three-bond correlations from C3 to NH_2 and 4-Me while complementary NOESY spectral data helped determine the correct stereochemistry by correlating the vinylic H and 4- or 6-Me (Fig. 2). *Z*/*E* ratio was subsequently determined for each compound from ^{1}H NMR spectra and Tables 1 and 2. Almost all pyrindines **I** showed comparable proportions of each isomer except compounds **8**, **14**, **21** and **26** with 70, 68, 35 and 93% of the *E* form, respectively.

3. Results and discussion

The 27 pyrindine derivatives reported in Tables 1 and 2 were first evaluated for their inhibitory activity on an enzymatic *in vitro* assay using the recombinant MBP-CDC25B fused protein [14]. Unfortunately, none of the compounds was efficient at inhibiting CDC25 phosphatase activity except compound **1** as previously mentioned [6].

Nonetheless, for a preliminary study, these molecules were assessed as mixtures of both isomers for their antiproliferative activity using a cell viability colorimetric assay against two human cancer cell lines, cervical carcinoma HeLa and melanoma A2058. Cytotoxic activity was also evaluated on the non tumor human CRL-2796 cell line. The results are reported in Tables 1 and 2. The glutathione-depleting compound menadione whose antiproliferative activity has already been demonstrated against some cancer cell lines [14] was used as a control and displayed comparable cytotoxicity against the tumor and normal cell types.

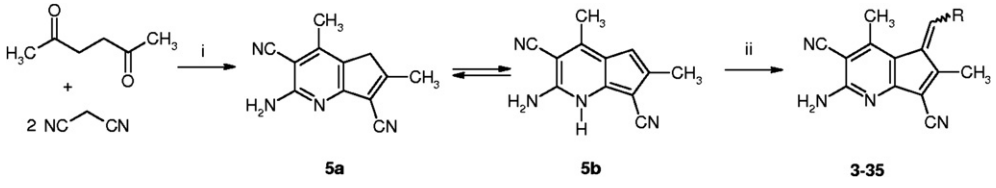
Interestingly, no pyrindine derivatives exhibited cytotoxic activity against the normal CRL-2796 cells regardless the substitution of the pyrindine system, except compound **22**.

Concerning the cytotoxic effects on cancer cell lines, results in Table 1 clearly showed that when the pyrindine core was substituted by a phenyl-furylmethylene moiety, the corresponding compounds **1**, **3**, **6**, **7**, **9** and **10** did not display any relevant antiproliferative activity on both cancer cell lines at 100 μM. No effect was either observed in case of compound **8** bearing the 5-bromofuryl moiety.

Pyridinyl derivatives **11**–**12** were found to be equally active on both cancer cell lines, **12** being slightly more potent. In contrast, 2-pyridinyl **13** displayed no cytotoxic activity. Compound **14** was the most efficient cytotoxic agent against HeLa cells with an IC_{50} value of 8.2 μM, ten-fold higher than that against A2058. No cytotoxic effect was observed for methylnaphthylderivative **15** whereas derivatives **16** and **17** displayed comparable potency and appeared more active toward A2058.

Pyrindines **18**–**21** bearing a susbtituent at position 4 of the phenyl ring displayed cytotoxicity. The 3-hydroxyl derivative **22** exhibited comparable IC_{50} values against both cancer cell lines whereas its congener **23** revealed totally inactive. Bromo derivatives **24**–**26** inhibited HeLa growth but only **25** was efficient against A2058 with an IC_{50} value of 4.2 μM. Potent cytotoxic activity was observed in the case of nitro derivatives **27** and **28** against A2058 whereas no efficiency was found on HeLa cells. Finally compound **29** revealed selective toward HeLa cells whereas **30** displayed comparable antiproliferative activities toward both cell lines.

In conclusion, [1]pyrindine derivatives substituted in the 5-position by a diversity of aromatic groups did not inhibit CDC25



Scheme 1. Reagents and conditions: (i) piperidine, abs. EtOH, reflux, 1 h (45%); (ii) RCHO, piperidine, MeOH, rt, 24 h (23–92%).

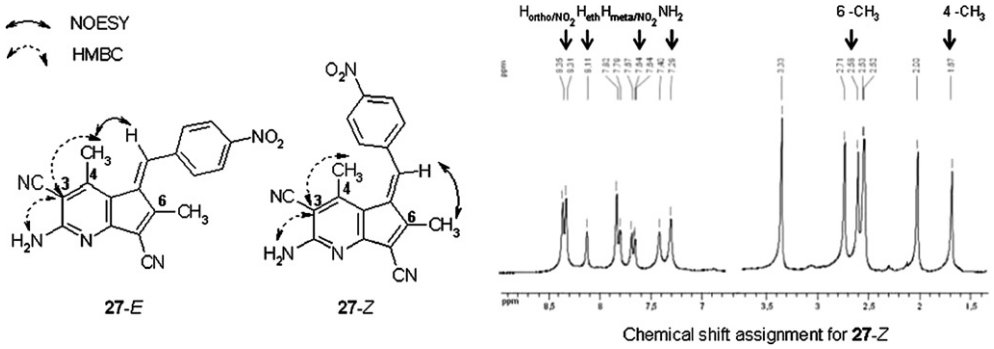


Fig. 2. Identification of *Z*- and *E*-isomers of compound **27**.

phosphatase activity. Nevertheless, they provided antiproliferative activities against HeLa and A2058 cell lines and induced no cytotoxicity toward normal CRL-2796 cells. Moreover, one can note that some of these pyrindines were more active on A2058 cells, known to be resistant to chemotherapy while others displayed cytotoxicity on HeLa cells only. Further experiments will therefore be carried out in order to identify the main biological targets of these promising candidates for the development of novel anticancer agents.

4. Materials and methods

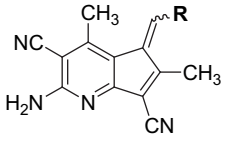
4.1. General

Kieselgel 60F_{254} plates (Merck) were used for analytical thin layer chromatography and were visualized with ultraviolet light (254 nm). All the commercial reagents and solvents were of analytical grade and purchased from Sigma and Carlo Erba – SDS, respectively. NMR spectra were performed on a Bruker WMFT−250 MHz spectrometer at the Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris Descartes, Paris, France. Chemical shifts are expressed in parts per million (ppm) with TMS used as an internal standard and coupling constants *J* are given in Hertz. Mass spectra were determined on an LCQ Advantage spectrometer (ThermoElectron, France) at the Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris Descartes, Paris, France. Compounds **1**, **3**, **6**, **7**, **9**, **10** and **30** were purchased from Akos, Germany.

4.2. 2-Amino-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**5**)

To a solution of malononitrile (6.7 mL, 106 mol) in ethanol (85 mL) cooled to 0 °C were added piperidine (12.6 mL, 127.8 mmol) and 2,5-hexanedione (5.00 mL, 42.6 mmol). The reaction mixture was heated under reflux for 1 h. The precipitate was filtered and washed with ethanol to give **5** as a brown powder (4.03 g, 45%). Compound **5a**: ^{1}H NMR (DMSO–d_6, 250 MHz) δ 2.34 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 3.63 (s, 2H, CH_2), 6.85–6.88 (m, 2H, NH_2). Compound **5b**: ^{1}H NMR (DMSO–d_6, 250 MHz) δ 2.25 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 6.03 (s, 1H, C–H), 6.85–6.88 (m, 2H, NH_2), 12.75 (s, 1H, NH).

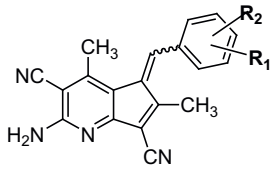
Table 1
Antiproliferative activity against tumor and normal cell lines of compounds **1**–**17**. IC_{50} values are expressed with standard error.



Entry	R	Yield (%)	*Z*/*E*	$IC_{50} \pm$ SEM (μM)		
				HeLa	A2058	CRL-2796
	Menadione	–	–	24.7 ± 2.5	13.4 ± 1.8	21.0 ± 2.0
1	2-[5-(3-carboxyphenyl)]furyl	–[a]	–[b]	n.a.	n.d.	n.d.
3	2-[5-(4-carboxyphenyl)]furyl	–[a]	–[b]	n.a.	n.d.	n.d.
6	2-[5-(2-carboxyphenyl)]furyl	–[a]	–[b]	n.a.	n.a.	n.a.
7	2-[5-(3-ethoxycarbonylphenyl)]furyl	–[a]	–[b]	n.a.	n.a.	n.a.
8	2-(5-bromo)furyl	92	30/70	n.a.	n.a.	n.a.
9	2-(5-phenyl)furyl	–[a]	–[b]	n.a.	n.a.	n.a.
10	2-[5-(3-bromophenyl)]furyl	–[a]	–[b]	n.a.	n.a.	n.a.
11	4-pyridinyl	41	40/60	24.6 ± 3.2	37.3 ± 1.2	n.a.
12	3-pyridinyl	76	40/60	13.2 ± 0.6	12.2 ± 0.4	n.a.
13	2-pyridinyl	49	40/60	n.a.	n.a.	n.a.
14	4-quinolinyl	86	32/68	8.2 ± 0.2	79.9 ± 2.1	n.a.
15	4-methylnaphthyl	68	45/55	n.a.	n.a.	n.a.
16	1,3-benzodioxol-5-yl	70	55/45	34.5 ± 3.2	10.0 ± 0.1	n.a.
17	Phenyl	70	45/55	38.9 ± 0.6	8.5 ± 0.3	n.a.

[a] Compound purchased from Akos, Germany.
[b] Quantity not sufficient to establish *Z*/*E* ratio. n.d.: not determined. n.a.: no activity at 100 μM.

Table 2
Antiproliferative activity against tumor and normal cell lines of compounds **18**–**30**. IC_{50} values are expressed with standard error.



Entry	R_1	R_2	Yield (%)	*Z*/*E*	IC_{50} ± SEM (μM)		
					HeLa	A2058	CRL-2796
18	4-Me	H	31	50/50	17.6 ± 0.3	7.9 ± 0.2	n.a.
19	4-Ph	H	82	53/47	17.0 ± 2.3	13.3 ± 0.2	n.a.
20	4-SMe	H	77	55/45	39.4 ± 1.2	34.5 ± 3.8	n.a.
21	4-$N(Me)_2$	H	87	65/35	11.3 ± 0.7	28.5 ± 1.3	n.a.
22	3-OH	H	69	44/56	25.8 ± 0.9	17.6 ± 1.1	54.7 ± 1.3
23	2-OH	H	86	52/48	n.a.	n.a.	n.a.
24	4-Br	H	44	47/53	27.1 ± 0.8	n.a.	n.a.
25	3-Br	H	23	40/60	12.5 ± 0.9	4.2 ± 0.7	n.a.
26	2-Br	H	39	7/93	36.6 ± 0.9	n.a.	n.a.
27	4-NO_2	H	66	46/54	99.0 ± 1.0	12.2 ± 0.6	n.a.
28	3-NO_2	H	67	44/56	97.2 ± 6.6	19.8 ± 1.9	n.a.
29	4-(4-Cl-Ph)O	H	68	53/47	15.2 ± 1.1	n.a.	n.a.
30	4-[(2-CN-Ph)CH_2]O	3-Br	–[a]	–[b]	15.8 ± 2.4	13.1 ± 1.0	n.a.

[a] Compound purchased from Akos, Germany.
[b] Quantity not sufficient to establish *Z*/*E* ratio. n.a.: no activity at 100 μM.

4.3. General procedure for the synthesis of pyrindine derivatives

To a solution of compound **5** (1 eq) and the appropriate aldehyde (2 eq) in ethanol were added two drops of piperidine. The reaction mixture was stirred at room temperature for 24 h. The precipitate was filtered and washed with ethanol.

4.4. (Z/E)-2-Amino-5-[(5-bromo-2-furyl)methylene]-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*8*)

Compound **8** was obtained from compound **5** (900 mg, 4.28 mmol) and 5-bromo-2-furaldehyde (1.5 g, 8.56 mmol) following the general procedure as a red solid (1.45 g, 92%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 2.13 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 6.93 (d, 1H, *J* = 3.7, Har), 6.97 (d, 1H, *J* = 3.7, Har), 7.10 (d, 1H, *J* = 3.7, Har), 7.14 (s, 2H, NH_2), 7.29 (s, 2H, NH_2), 7.36 (d, 1H, *J* = 3.7, Har), 7.40 (s, 1H, C–H), 7.57 (s, 1H, C–H); MS (ESI) *m*/*z* 368.0 $[M + H]^+$.

4.5. (Z/E)-2-Amino-4,6-dimethyl-5-(pyridin-4-ylmethylene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*11*)

Compound **11** was obtained from compound **5** (120 mg, 0.57 mmol) and 4-pyridinecarboxaldehyde (109 μL, 1.14 mmol) following the general procedure as a brown solid (70 mg, 41%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.64 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.28 (s, 2H, NH_2), 7.33–7.38 (m, 4H, NH_2 and Har), 7.48–7.50 (m, 2H, Har), 7.70 (s, 1H, C–H), 8.00 (s, 1H, C–H), 8.67–8.68 (m, 4H, Har); MS (ESI) *m*/*z* 297.5 $[M - H]^-$.

4.6. (Z/E)-2-Amino-4,6-dimethyl-5-(pyridin-3-ylmethylene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*12*)

Compound **12** was obtained from compound **5** (200 mg, 0.95 mmol) and 3-pyridinecarboxaldehyde (179 μL, 1.9 mmol) following the general procedure as an orange solid (216 mg, 76%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.66 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 7.24 (s, 2H, NH_2), 7.32 (s, 2H, NH_2), 7.49–7.56 (m, 2H, Har), 7.78–7.83 (m, 2H, Har and C–H), 7.93–7.96 (m, 1H, Har), 8.08 (s, 1H, C–H), 8.59–8.69 (m, 4H, Har); MS (ESI) *m*/*z* 298.3 $[M - H]^-$.

4.7. (Z/E)-2-Amino-4,6-dimethyl-5-(pyridin-2-ylmethylene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*13*)

Compound **13** was obtained from compound **5** (120 mg, 0.57 mmol) and 2–pyridinecarboxaldehyde (108 μL, 1.14 mmol) following the general procedure as an orange solid (84 mg, 49%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.63 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 7.25 (s, 2H, NH_2), 7.31 (s, 2H, NH_2), 7.417.46 (m, 2H, Har), 7.55 (d, 1H, *J* = 7.5, Har), 7.66–7.72 (m, 3H, Har and C–H), 7.90–7.99 (m, 4H, Har and C–H), 8.72 (s, 2H, Har); MS (ESI) *m*/*z* 300.3 $[M + H]^+$.

4.8. (Z/E)-2-Amino-4,6-dimethyl-5-(quinolin-4-ylmethylene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*14*)

Compound **14** was obtained from compound **5** (200 mg, 0.95 mmol) and 4-quinolinecarboxaldehyde (299 mg, 1.9 mmol) following the general procedure as a red solid (285 mg, 86%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.29 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 7.28–7.36 (m, 4H, 2 × NH_2), 7.59–7.74 (m, 4H, Har), 7.83–7.90 (m, 3H, Har and C–H), 8.04 (d, 1H, *J* = 7.9, Har), 8.12–8.15 (m, 3H, Har), 8.33 (s, 1H, C–H), 8.95–8.99 (m, 2H, Har); MS (ESI) *m*/*z* 348.7 $[M - H]^-$.

4.9. (Z/E)-2-Amino-4,6-dimethyl-5-[(4-methyl-1-naphthyl)methylene]-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*15*)

Compound **15** was obtained from compound **5** (200 mg, 0.95 mmol) and 4-methyl-1-naphthaldehyde (324 mg, 1.9 mmol) following the general procedure as a brown solid (234 mg, 68%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.37 (s, 3H, CH_3), 1.84 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 2.74 (s, 6H, 2 × CH_3), 2.81 (s, 3H, CH_3), 7.19–7.22 (m, 4H, 2 × NH_2), 7.43–7.47 (m, 3H, Har), 7.60–7.71 (m, 4H, Har), 8.04 (d, 2H, *J* = 8.5, Har), 8.13–8.19 (m, 3H, Har), 8.24 (s, 1H, C–H), 8.45 (s, 1H, C–H); MS (ESI) *m*/*z* 361.2 $[M - H]^-$.

4.10. (Z/E)-2-Amino-5-(1,3-benzodioxol-5-ylmethylene)-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*16*)

Compound **16** was obtained from compound **5** (105 mg, 0.5 mmol) and piperonal (150 mg, 1 mmol) following the general procedure as a brown solid (120 mg, 70%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.84 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 6.13 (s, 2H, CH_2), 6.15 (s, 2H, CH_2), 6.85–7.20 (m, 10H, Har and 2 × NH_2), 7.70 (s, 1H, C–H), 8.02 (s, 1H, C–H); MS (ESI) *m*/*z* 341.4 $[M - H]^-$.

4.11. (Z/E)-2-Amino-5-benzylidene-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (*17*)

Compound **17** was obtained from compound **5** (105 mg, 0.5 mmol) and benzaldehyde (0.1 mL, 1 mmol) following the general procedure as a yellow solid (149 mg, 70%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.68 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 7.18 (s, 2H, NH_2), 7.25 (s, 2H, NH_2), 7.40–7.49 (m, 10H, Har), 7.81 (s, 1H, H, C–H), 8.14 (s, 1H, C–H); MS (ESI) *m*/*z* 297.3 $[M - H]^-$.

*4.12. (Z/E)-2-Amino-4,6-dimethyl-5-(4-methylbenzylidene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**18**)*

Compound **18** was obtained from compound **5** (200 mg, 0.95 mmol) and p-tolualdehyde following the general procedure as a brown solid (92 mg, 31%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.73 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.41 (s, 6H, 2 × CH_3), 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.15 (s, 2H, NH_2), 7.22 (s, 2H, NH_2), 7.28–7.32 (m, 5H, Har), 7.40–7.43 (m, 3H, Har), 7.76 (s, 1H, C–H), 8.10 (s, 1H, C–H); MS (ESI) m/z 313.4 $[M + H]^+$.

*4.13. (Z/E)-2-Amino-5-(biphenyl-4-ylmethylene)-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**19**)*

Compound **19** was obtained from compound **5** (200 mg, 0.95 mmol) and 4-biphenylcarboxaldehyde (347 mg, 1.9 mmol) following the general procedure as an orange solid (292 mg, 82%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.80 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 7.19 (s, 2H, NH_2), 7.27 (s, 2H, NH_2), 7.39–7.53 (m, 8H, Har), 7.62 (d, 2H, $J = 8.3$, Har), 7.77–7.86 (m, 9H, Har and C–H), 8.16 (s, 1H, C–H); MS (ESI) m/z 373.2 $[M - H]^-$.

*4.14. (Z/E)-2-Amino-4,6-dimethyl-5-[4-(methylsulfanyl)benzylidene]-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**20**)*

Compound **20** was obtained from compound **5** (200 mg, 0.95 mmol) and 4-(methylthio)benzaldehyde (253 μL, 1.90 mmol) following the general procedure as a red solid (252 mg, 77%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.80 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.51–2.56 (m, 9H, 3x CH_3), 2.71 (s, 3H, CH_3), 7.15 (s, 2H, NH_2), 7.23 (s, 2H, NH_2), 7.33–7.37 (m, 6H, Har), 7.48 (d, 2H, $J = 8.2$, Har), 7.74 (s, 1H, C–H), 8.07 (s, 1H, C–H); MS (ESI) m/z 343.7 $[M - H]^-$.

*4.15. (Z/E)-2-Amino-5-[4-(dimethylamino)benzylidene]-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**21**)*

Compound **21** was obtained from compound **5** (200 mg, 0.95 mmol) and 4-dimethylaminobenzaldehyde (284 mg, 1.9 mmol) following the general procedure as a dark red solid (282 mg, 87%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 2.09 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 3.15 (s, 12H, 4 × CH_3), 6.89–6.93 (m, 4H, Har), 7.02 (s, 2H, NH_2), 7.11 (s, 2H, NH_2), 7.41–7.45 (d, 2H, $J = 10$, Har), 7.53–7.57 (d, 2H, $J = 10$, Har), 7.80 (s, 1H, C–H), 8.16 (s, 1H, C–H); MS (ESI) m/z 340.4 $[M - H]^-$.

*4.16. (Z/E)-2-Amino-5-(3-hydroxybenzylidene)-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**22**)*

Compound **22** was obtained from compound **5** (120 mg, 0.57 mmol) and 3-hydroxybenzaldehyde (139 mg, 1.14 mmol) following the general procedure as a yellow solid (123 mg, 69%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.75 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 6.77 (s, 1H, Har), 6.84–6.91 (m, 5H, Har), 7.16 (s, 2H, NH_2), 7.24–7.33 (m, 4H, Har and NH_2), 7.72 (s, 1H, C–H), 8.06 (s, 1H, C–H), 9.67 (s, 1H, OH), 9.70 (s, 1H, OH); MS (ESI) m/z 315.4 $[M + H]^+$.

*4.17. (Z/E)-2-Amino-5-(2-hydroxybenzylidene)-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**23**)*

Compound **23** was obtained from compound **5** (120 mg, 0.57 mmol) and 2-hydroxybenzaldehyde (122 μL, 1.14 mmol) following the general procedure as an orange solid (154 mg, 86%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.77 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 6.87–7.04 (m, 6H, Har), 7.13–7.18 (m, 3H, Har and NH_2), 7.28–7.34 (m, 3H, Har and NH_2), 7.69 (s, 1H, C–H), 7.99 (s, 1H, C–H), 10.10 (s, 1H, OH), 10.27 (s, 1H, OH); MS (ESI) m/z 315.4 $[M + H]^+$.

*4.18. (Z/E)-2-Amino-5-(4-bromobenzylidene)-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**24**)*

Compound **24** was obtained from compound **5** (120 mg, 0.57 mmol) and 4-bromobenzaldehyde (211 mg, 1.14 mmol) following the general procedure as a brown solid (93 mg, 44%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.73 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.21 (s, 2H, NH_2), 7.29 (s, 2H, NH_2), 7.35 (d, 2H, $J = 8.1$, Har), 7.47 (d, 2H, $J = 8.1$, Har), 7.67–7.72 (m, 5H, Har and C–H), 8.04 (s, 1H, C–H); MS (ESI) m/z 378.1 $[M + H]^+$.

*4.19. (Z/E)-2-Amino-5-(3-bromobenzylidene)-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**25**)*

Compound **25** was obtained from compound **5** (120 mg, 0.57 mmol) and 3-bromobenzaldehyde (133 μL, 1.14 mmol) following the general procedure as a yellow solid (48 mg, 23%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.70 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.22 (s, 2H, NH_2), 7.32–7.50 (m, 6H, NH_2 and Har), 7.64–7.67 (m, 3H, Har), 7.75–7.76 (s, 2H, Har and C–H), 8.06 (m, 1H, C–H); MS (ESI) m/z 378.1 $[M + H]^+$.

*4.20. (E)-2-Amino-5-(2-bromobenzylidene)-4,6-dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**26**)*

Compound **26** was obtained from compound **5** (120 mg, 0.57 mmol) and 2-bromobenzaldehyde (133 μL, 1.14 mmol) following the general procedure as a yellow solid (85 mg, 39%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.94 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 7.27 (s, 2H, NH_2), 7.42–7.53 (m, 4H, Har), 7.79–7.82 (m, 1H, Har), 7.89 (s, 1H, C–H); MS (ESI) m/z 378.1 $[M + H]^+$.

*4.21. (Z/E)-2-Amino-4,6-dimethyl-5-(4-nitrobenzylidene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**27**)*

Compound **27** was obtained from compound **5** (200 mg, 0.95 mmol) and 4-nitrobenzaldehyde (287 mg, 1.9 mmol) following the general procedure as a red solid (217 mg, 66%). (E)-2-Amino-4,6-dimethyl-5-(4-nitrobenzylidene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**27**-E). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 2.00 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 7.40 (s, 2H, NH_2), 7.79–7.82 (m, 3H, Har and C–H), 8.33 (d, 2H, $J = 10$, Har). (Z)-2-Amino-4,6-dimethyl-5-(4-nitrobenzylidene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**27**-Z). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.67 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 7.29 (s, 2H, NH_2), 7.66 (d, 2H, $J = 10$, Har), 8.11 (s, 1H, C–H), 8.33 (d, 2H, $J = 10$, Har); MS (ESI) m/z 342.1 $[M - H]^-$.

*4.22. (Z/E)-2-Amino-4,6-dimethyl-5-(3-nitrobenzylidene)-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**28**)*

Compound **28** was obtained from compound **5** (200 mg, 0.95 mmol) and 3-nitrobenzaldehyde (287 mg, 1.9 mmol) following the general procedure as a red solid (218 mg, 67%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.61 (s, 3H, CH_3), 1.93 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 7.19 (s, 2H, NH_2), 7.30 (s, 2H, NH_2), 7.66–7.73 (m, 3H, Har), 7.78 (s, 1H, C–H), 7.89 (d, 1H, $J = 7.5$, Har), 8.07 (s, 1H, C–H), 8.18–8.25 (m, 3H, Har), 8.31 (s, 1H, Har); MS (ESI) m/z 342.0 $[M - H]^-$.

4.23. (Z/E)-2-Amino-5-[4-(4-chlorophenoxy)benzylidene]-4,6–dimethyl-5H-cyclopenta[b]pyridine-3,7-dicarbonitrile (**29**)

Compound **29** was obtained from compound **5** (200 mg, 0.95 mmol) and 4-(4-chlorophenoxy)benzaldehyde (443 mg, 1.9 mmol) following the general procedure as a red solid (274 mg, 68%). ^{1}H NMR (DMSO–d_6, 250 MHz) δ 1.82 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 7.09–7.23 (m, 12H, Har and $2 \times NH_2$), 7.44–7.58 (m, 8H, Har), 7.76 (s, 1H, C–H), 8.10 (s, 1H, C–H); MS (ESI) m/z 423.9 $[M - H]^-$.

4.24. Cell culture and chemicals

Human cancer cell line HeLa was obtained from Aptanomics (Lyon, France). A2058 cells and CRL-2796 cells were obtained from ATCC (Rockville, MD, USA). Cells were cultured at 37 °C in Dulbecco's minimum essential medium complemented with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin in a humidified atmosphere of 5% CO_2. All compounds were solubilized in dimethyl sulfoxide so that the DMSO final concentration was <1%. The tetrazolium salt WST-1 was purchased from Roche Diagnostics (Mannheim, Germany).

4.25. Cytotoxic assay

The inhibition of cell proliferation was determined using a colorimetric assay based on the cleavage of the WST-1 tetrazolium salt by mitochondrial dehydrogenases in viable cells, leading to formazan formation. At day 0, HeLa cells, A2058 cells and CRL-2796 cells were plated in 96-well culture plates with 95 μL of medium/well at 3000, 4000 and 5000 cells/well respectively. At day 1, cells were treated with 5 μL of increasing concentrations of drug. At day 6 (HeLa), 5 (A2058) or 4 (CRL-2796), after addition of 10 μL of WST-1 per well, cells were incubated 2 h at 37 °C in a humidified atmosphere of 5% CO_2. Absorbance was measured at 430 nm with a Bio-Rad microplate reader. The results are expressed as the mean of three independent experiments with three determinations per tested concentration and per experiment. For each compound, the IC_{50} value was determined from a sigmoidal dose-response using GraphPad Prism (GraphPad Software, San Diego, CA).

Acknowledgments

We are grateful to Dr Wang-Qing Liu for mass spectra recording. This research is supported by La Ligue Nationale contre le Cancer: Equipe Labellisée 2006 (U648) and Equipe Labellisée 2008 (UMR 5088).

References

[1] T. Sunazuka, T. Zhi-Ming, Y. Harigaya, S. Takamatsu, M. Hayashi, K. Komiyama, S. Omura, J. Antibiot. 50 (1997) 274–275.
[2] Y.M. Chi, W.M. Yan, D.C. Chen, H. Noguchi, Y. Iitaka, U. Sankawa, Phytochemistry 31 (1992) 2930–2932.
[3] H. Drautz, H. Zähner, E. Kupfer, W. Keller-Schierlein, Helv. Chim. Acta 64 (1981) 1752–1765.
[4] S. Grabley, P. Hammann, R. Thiericke, J. Wink, S. Philipps, A. Zeek, J. Antibiot. 46 (1993) 343–345.
[5] C. Puder, S. Loya, A. Hizi, A. Zeeck, J. Nat. Prod. 64 (2001) 42–45.
[6] M. Montes, E. Braud, M.A. Miteva, M.L. Goddard, O. Mondesert, S. Kolb, M.P. Brun, B. Ducommun, C. Garbay, B.O. Villoutreix, J. Chem, Inf. Model. 48 (2008) 157–165.
[7] K. Kristjandottir, J. Rudolph, Chem. Biol. 11 (2004) 1043–1051.
[8] R. Boutros, V. Lobjois, B. Ducommun, Nat. Rev. Cancer 7 (2007) 495–507.
[9] M. Forino, D. Jung, J.B. Easton, P.J. Houghton, M. Pellecchia, J. Med. Chem. 48 (2005) 2278–2281.
[10] M. Pellecchia, M. Forino, D. Jung, PCT Int. Appl. WO2006094230; 2006.
[11] T.C. Gahman, H. Lang, R. Davis, S.A. Scranton, PCT Int. Appl. WO2006124874; 2006.
[12] K. Hartke, R. Matusch, Chem. Ber 105 (1972) 2584–2593.
[13] H. Junek, R.J. Schaur, Monatsh. Chem. 99 (1968) 89–95.
[14] M.C. Brezak, M. Quaranta, O. Mondésert, M.O. Galcera, O. Lavergne, F. Alby, M. Cazales, V. Baldin, C. Thurieau, J. Harnett, C. Lanco, P.G. Kasprzyk, G.P. Prevost, B. Ducommun, Cancer Res. 64 (2004) 3320–3325.