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Abstract

The synthesis of new meridianin derivatives substituted at the C-5 position of the 2-aminopyrimidine ring by various aryl groups and substituted or not by a methyl group on the indole nitrogen is described. These compounds were tested for their kinase inhibitory potencies toward five kinases (CDK5/p25, CK1δ/ε, GSK-3α/β, Dyrk1A and Erk2) as well as their in vitro antiproliferative activities toward a human fibroblast primary culture and two human solid cancer cell lines (MCF-7 and PA 1).

Keywords: Meridianins; Suzuki cross-coupling; Kinase inhibitors; Dyrk1A inhibitors

1. Introduction

Meridianins are marine alkaloids isolated and characterized from the south Atlantic tunicate *Aplidium meridianum*.[1] Meridianins A–G[[1], [2] and [3]] (Fig. 1), indole derivatives substituted in the C-3 position by a 2-aminopyrimidine ring, were successfully evaluated for their ability to inhibit various protein kinases[3] and to display antitumour activity.[4] The closely related meriolins, a family of 7-azaindole synthetic meridianin analogues, which exhibit potent kinase inhibitory activities and antiproliferative properties were also described.[[5] and [6]]

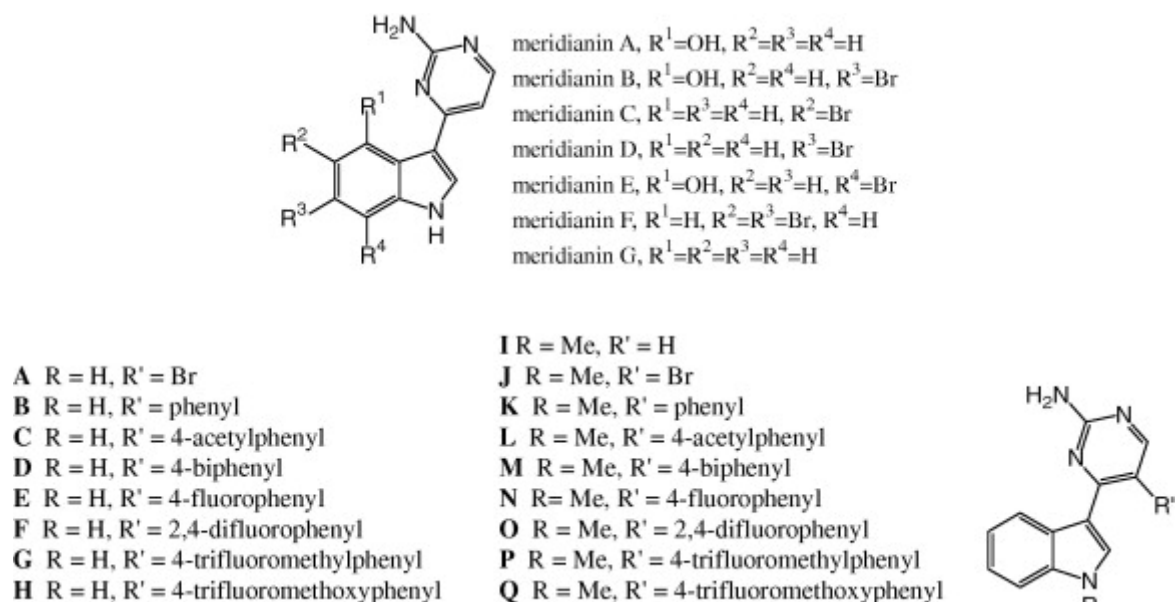

meridianin A, R¹=OH, R²=R³=R⁴=H
meridianin B, R¹=OH, R²=R⁴=H, R³=Br
meridianin C, R¹=R³=R⁴=H, R²=Br
meridianin D, R¹=R²=R⁴=H, R³=Br
meridianin E, R¹=OH, R²=R³=H, R⁴=Br
meridianin F, R¹=H, R²=R³=Br, R⁴=H
meridianin G, R¹=R²=R³=R⁴=H

A R = H, R' = Br
B R = H, R' = phenyl
C R = H, R' = 4-acetylphenyl
D R = H, R' = 4-biphenyl
E R = H, R' = 4-fluorophenyl
F R = H, R' = 2,4-difluorophenyl
G R = H, R' = 4-trifluoromethylphenyl
H R = H, R' = 4-trifluoromethoxyphenyl

I R = Me, R' = H
J R = Me, R' = Br
K R = Me, R' = phenyl
L R = Me, R' = 4-acetylphenyl
M R = Me, R' = 4-biphenyl
N R= Me, R' = 4-fluorophenyl
O R = Me, R' = 2,4-difluorophenyl
P R = Me, R' = 4-trifluoromethylphenyl
Q R = Me, R' = 4-trifluoromethoxyphenyl

Figure 1. Meridianins A–G and derivatives **A–Q** described by our group.

Since protein kinases are attractive targets for the discovery of biologically active compounds of potential therapeutic interest, our aim is to identify new ATP-competitive kinase inhibitors. Thus, we were recently interested in meridianin derivatives substituted at the C-5 position of the 2-aminopyrimidine ring. In recent papers, we described the synthesis and biological activities of meridianin G derivatives, substituted or not on the indole nitrogen with a methyl group, and bearing various aryl moieties at the C-5 position of the pyrimidine ring (compounds **A–H** and **J–Q**, Fig. 1).[[7] and [8]] It was shown that the substitution at this C-5 position and the methylation of the indole nitrogen were critical for either kinase inhibitory potencies

or in vitro antiproliferative activities. The meridianin derivatives **A–H** and **K–Q** were tested toward a panel of 9 protein kinases (KDR, IGF-1R, c-Met, RET, c-Src, c-Abl, PKA, CDK2/cyclin A and HER-1) and their in vitro antiproliferative activities were examined toward a human fibroblast primary culture and two human solid cancer cell lines (MCF-7 and PA 1). Despite weak kinase inhibitory potencies, compounds **G**, **H**, **L** and **M** exhibited high in vitro antiproliferative activities toward PA 1 cells. It was found that these compounds do not interfere with PA 1 cell-cycle and may be considered as direct cytolysis or apoptosis inducers. Considering these first biological results, it was of interest to pursue the study with new aryl substituents on the pyrimidine ring in order to identify the substitution pattern providing the highest biological potencies.

In this paper, we described the synthesis of new meridianin analogues diversely substituted at the C-5 position of the pyrimidine ring and methylated or not on the indole nitrogen. Moreover these compounds were tested for their kinase inhibitory potencies toward five kinases (CDK5/p25, CK1δ/ε, GSK3α/β, Dyrk1A and Erk2) as well as their in vitro antiproliferative activities toward a human fibroblast primary culture and two human solid cancer cell lines (MCF-7 and PA 1).

2. Chemistry

Compounds **1–9** were synthesized from brominated derivatives **A** or **J**, methylated or not on the indole nitrogen. **A** and **J** were prepared according to previously described procedure (Scheme 1).[7] Suzuki cross-coupling was performed using various commercially available arylboronic acids, tetrakis(triphenylphosphine)palladium(0) and sodium carbonate in a H_2O/EtOH/toluene medium. Compounds **1–9** were obtained in 31−60% isolated yields. Due to purification difficulties, the non-methylated analogues of compounds **7–9** could not be isolated as pure compounds.
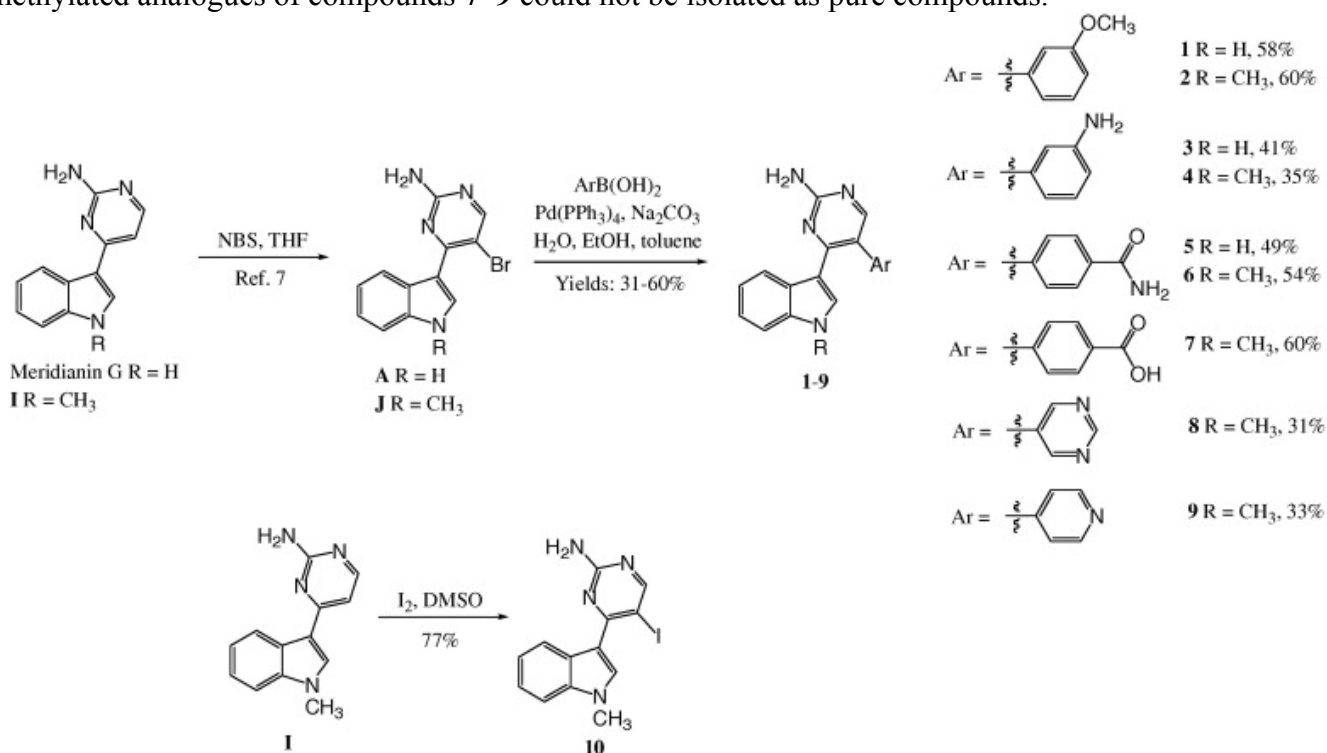
Scheme 1. Synthesis of compounds **1–10**.

Results were similar using compounds **A** or **J** (compare **1** and **2**, **3** and **4**, **5** and **6**), and yields dropped using aryl boronic acid containing a basic nitrogen atom.

Moreover, in order to introduce various amines (aryl or alkylamines) by Buchwald type coupling, in the C-5 position of the pyrimidine ring, compound **10**, the iodo analogue of **J**, was prepared in 77% yield from methylated meridianin G (compound **I**), using iodine in DMSO.[9]

Unfortunately, we never manage to substitute this position by an amine derivative from compound **10** or its brominated analogue **J**.

In summary, the method described here allowed the introduction, in the pyrimidine C-5 position, of heteroaryl moieties or phenyl groups bearing both electron donor or electron acceptor substituents in para or meta positions.

3. Results and discussion

In vitro antiproliferative activities of compounds **1–10** were evaluated toward a human fibroblasts primary culture and two human solid cancer cell lines (MCF-7 and PA 1). The antiproliferative effect (IC_{50}) of the tested drug was assessed by the resazurin reduction test.[10] Under the conditions used, none of the newly synthesised compounds showed any significant cytotoxic activity ($IC_{50} < 50$ μM) on fibroblasts or MCF-7 cell lines. Only mild activities (IC_{50} around 30 μM) were observed on PA 1 cells with compounds **1**, **2**, **5** and **10** (Table 1). These results indicate that the substituents introduced at the C-5′ position are detrimental to the cytotoxic effect of these series on PA 1 cells.

Table 1.

Inhibitory potencies toward five protein kinases (IC_{50} in μM) and antiproliferative activity of compounds **G**, **H**, **L**, **M** and **1−10** (IC_{50} in μM)

Compounds	Kinase inhibition—IC_{50} in μM					Antiproliferative activity toward
	CDK5/p25	CK1δ/ε	GSK3α/β	Dyrk1A	Erk2	
G	11.0	8.3	1.9	2.3	0.58	0.09
H	>10	>10	3.0	2.9	3.2	0.08
L	>10	>10	1.4	5.0	>10	0.05
M	>10	>10	>10	>10	>10	0.08
1	>10	0.49	3.0	0.60	3.10	28
2	>10	2.2	3.8	3.1	>10	34
3	>10	3.3	6.0	0.64	6.9	>50
4	>10	3.1	5.0	1.9	>10	>50
5	>10	3.4	2.0	0.58	1.90	38
6	>10	4.1	0.63	2.0	7.00	>50
7	>10	>10	>10	0.9	>10	>50
8	>10	12.0	6.1	3.2	>10	>50
9	5.3	4.2	1.2	2.4	1.30	>50
10	2.6	1.1	4.2	0.4	4.0	31

The kinase inhibitory potencies of compounds **1–10** and that of previously synthesised derivatives **G**, **H**, **L** and **M** which have shown interesting antiproliferative activities toward PA1 cells were evaluated as IC_{50} values toward five protein kinases (CDK5/p25, CK1δ/ε, GSK-3α/β, Dyrk1A and Erk2).

Only two compounds were mildly active toward CDK5/p25 with IC_{50} values in the micromolar range. These derivatives are both methylated on the indole nitrogen and substituted either by a pyridyl group (compound **9**, $IC_{50} = 5.3$ μM) or by an iodine atom (compound **10**, $IC_{50} = 2.6$ μM) in the C-5 position of the aminopyrimidine moiety.

Considering the other kinases, the best inhibitory potencies were sub-micromolar inhibition of CK1δ/ε (compound **1**, $IC_{50} = 0.49$ μM), GSK-3α/β (compound **6**, $IC_{50} = 0.63$ μM), Dyrk1A (compounds **1**, $IC_{50} = 0.6$ μM; **3**, $IC_{50} = 0.64$ μM; **5**, $IC_{50} = 0.58$ μM; **7**, $IC_{50} = 0.9$ μM; **10**, $IC_{50} = 0.4$ μM) and Erk2 (compound **G**, $IC_{50} = 0.58$ μM). Regarding these results, this series of compounds could be particularly interesting in the development of new inhibitors of Dyrk1A kinase. Comparison of the activity of non-methylated compounds (**1**, **3**, **5**) toward Dyrk1A with that of their N–CH₃ analogues (**2**, **4**, **6**) indicate that the non-methylated analogues are more active than their methylated counterparts. Nevertheless, two methylated derivatives bearing either a *p*-carboxyphenyl moiety (**7**) or an iodine atom (**10**) at the C-5 position of the pyrimidine ring exhibited Dyrk1A sub-micromolar inhibition as well.

4. Conclusion

Encouraging preliminary biological evaluation of diversely substituted meridianins led us to synthesize new derivatives. Suzuki cross-coupling could be applied to compounds **A** or **J**, for coupling with either aromatic boronic acids bearing both electron donor and electron acceptor substituents or heteroaromatic boronic acids.

Surprisingly, the cytotoxic activity of newly synthesised derivatives **1–10** toward PA 1 cells is significantly smaller than that of the previously described compounds **G**, **H**, **L**, **M**. This could be due to a lower cellular penetration of compounds **1–10** or lower interaction, due to the modification of the aryl moiety, with the cellular target(s) involved in their antiproliferative activity.

Compounds **G**, **1**, **3**, **5–7** and **10** have shown sub-micromolar inhibition potencies toward some of the kinases tested. The interesting inhibition of Dyrk1A kinase, which is known to be involved in Alzheimer's disease[11] and Down syndrome,[12] leads us to expand our effort in the synthesis of diversely substituted meridianin derivatives as new potential inhibitors of this kinase.

5. Experimental

5.1. In vitro Kinase inhibition assays

5.1.1. Buffers

Buffer A: 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris–HCl pH 7.5, 50 µg heparin/ml.

Buffer C: 60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM phenylphosphate.

5.1.2. Kinase preparations and assays

Kinase activities were assayed in Buffer A or C, at 30 °C, at a final ATP concentration of 15 µM. Blank values were subtracted and activities expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO.

CDK5/p25 (human, recombinant) was prepared as previously described.[13] and [14] Its kinase activity was assayed in buffer C, with 1 mg histone H1 /ml, in the presence of 15 µM [γ-^{33}P] ATP (3,000 Ci/mmol; 10 mCi/ml) in a final volume of 30 µl. After 30 min incubation at 30 °C, 25 µl aliquots of supernatant were spotted onto 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper, and, 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 ml phosphoric acid/liter of water. The wet filters were counted in the presence of 1 ml ACS (Amersham) scintillation fluid.

GSK-3αβ (porcine brain, native) was assayed, as described for CDK5/p25 but in Buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine).[15] GS-1 was synthesized by Millegen (Labege, France).

CK1δ/ε (porcine brain, native) was assayed as described for CDK5/p25 but using the CK1-specific peptide substrate RRKHAAIGpSAYSITA,[16] obtained from Millegen (Labege, France).

Erk2 (rat, recombinant) was assayed as described for CDK5/p25 but using the specific substrate Ets1 (amino acids 1–138) in buffer A.

DYRK1A (rat, recombinant, expressed in *Escherichia coli* as a GST fusion protein) was purified by affinity chromatography on glutathione–agarose and assayed as described for CDK5/p25 using myelin basic protein (1 mg/ml) as a substrate.

5.2. In vitro antiproliferative assays

5.2.1. Cell cultures

Stock cell cultures were maintained as monolayers in 75 cm^2 culture flasks in Glutamax Eagle's minimum essential medium (MEM) with Earle's salts supplemented with 10% fetal calf serum, 5 ml 100 mmol/l sodium pyruvate, 5 ml of 100× non-essential amino acids and 2 mg gentamicin base. Cells were grown at 37 °C in a humidified incubator under an atmosphere containing 5% CO_2.

5.2.2. Survival assays

Cells were plated at a density of 5×10^3 cells in 150 µl culture medium in each well of 96-well microplates and were allowed to adhere for 16 h before treatment with tested drug. A stock solution 20 mmol/l of each tested drug was prepared in DMSO and kept at −20 °C until use. Then 50 µl of each tested solution were added to the cultures. A 48 h continuous drug exposure protocol was used. The antiproliferative effect of the tested drug was assessed by the resazurin reduction test.

5.2.3. Resazurin reduction test

Plates were rinsed with 200 μl PBS at 37 °C and emptied by overturning on absorbent toweling. Then 150 μl of a 25 μg/ml solution of resazurin in MEM without phenol red was added to each well. Plates were incubated for 1 h at 37 °C in a humidified atmosphere containing 5% CO_2. Fluorescence was then measured on an automated 96-well plate reader (Fluoroscan Ascent FL, Labsystem) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Under the conditions used, fluorescence was proportional to the number of living cells in the well. The IC_{50}, defined as the drug concentration required to inhibit cell proliferation by 50%, was calculated from the curve of concentration-dependent survival percentage, defined as fluorescence in experimental wells compared with fluorescence in control wells, after subtraction of the blank values.

5.3. Chemistry

IR spectra were recorded on a Perkin–Elmer Paragon 500 spectrometer $\bar{v}_{max}$ in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), chemical shifts δ are noted in ppm and the following abbreviations are used: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), doublet of doublet of doublet (ddd), doublet of triplet (dt), multiplet (m), broad signal (br s). Mass spectra (ESI+) were determined on a high resolution Waters Micro Q-Toff apparatus. Chromatographic purifications were performed on flash silica gel Geduran SI 60 (Merck) 0.040–0.063 mm column chromatography. TLC were performed on fluorescent silica gel plates (60 F254 from Merck).

5.3.1. General procedure for the preparation of compounds 1–9

A solution of Na_2CO_3 (2.5 equiv) in H_2O (2 ml/mmol) was added to a suspension of bromide **A** (288 mg, 1.0 mmol) or **J** (302 mg, 1.0 mmol), the required boronic acid (1.1 equiv), Pd(PPh₃)₄ (57.8 mg, 5 mol %) in EtOH (3 ml/mmol) and toluene (3 ml/mmol). The reaction mixture was refluxed overnight under argon and the cooled resulting mixture was evaporated onto silica gel. Purification as indicated provided the desired compounds.

5.3.2. 4-(1*H*-Indol-3-yl)-5-(3-methoxyphenyl)pyrimidin-2-amine (1)

Purification of the residue by flash chromatography (cyclohexane/EtOAc 7:3) provided **1** (185 mg, 58%) as a yellow powder.
Mp 98−100 °C; HRMS (ESI+) calcd for $C_{19}H_{17}N_4O$ (M+H)$^+$ 317.1402, found 317.1385; IR (KBr): 3650−3150, 1605 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.71 (3H, s, CH₃), 6.52−6.58 (2H, br s, NH₂), 6.69 (1H, d, J = 3.0 Hz), 6.83−6.86 (2H, m), 6.91−6.95 (1H, m), 7.06 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz; J_3 = 1.0 Hz), 7.12 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.31 (1H, t, J = 8.0 Hz), 7.34 (1H, d, J = 8.0 Hz), 8.00 (1H, s), 8.46 (1H, d, J = 8.0 Hz), 11.23−11.33 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 55.0 (OCH₃), 111.4, 112.6, 114.9, 120.0, 121.8, 121.9, 122.9, 128.9, 129.8, 157.8 (CH), 112.7, 120.9, 126.2, 135.8, 140.3, 159.5, 159.8, 162.5 (C).

5.3.3. 4-(1-Methyl-1*H*-indol-3-yl)-5-(3-methoxyphenyl)pyrimidin-2-amine (2)

Purification of the residue by flash chromatography (cyclohexane/EtOAc 7:3) provided **2** (198 mg, 60%) as a yellow powder.
Mp = 82−84 °C; HRMS (ESI+) calcd for $C_{20}H_{19}N_4O$ (M+H)$^+$ 331.1559, found 331.1546; IR (KBr): 3650−3150, 1604 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.61 (3H, s, NCH₃), 3.72 (3H, s, OCH₃), 6.56−6.59 (2H, br s, NH₂), 6.73 (1H, s), 6.83 (1H, ddd, J_1 = 7.5 Hz, J_2 = 1.5 Hz, J_3 = 1.0 Hz), 6.86 (1H, dd, J_1 = 2.5 Hz, J_2 = 1.5 Hz), 6.93 (1H, ddd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, J_3 = 1.0 Hz), 7.08 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.18 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.29 (1H, t, J = 8.0 Hz), 7.40 (1H, d, J = 8.0 Hz), 8.03 (1H, s), 8.35 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 32.8 (CH₃), 55.0 (OCH₃), 109.8, 112.7, 114.8, 120.2, 121.7, 121.9, 122.8, 129.7, 132.6, 158.1 (CH), 112.0, 120.8, 126.5, 136.4, 140.0, 159.4, 159.5, 162.4 (C).

5.3.4. 4-(1*H*-Indol-3-yl)-5-(3-aminophenyl)pyrimidin-2-amine (3)

Purification of the residue by flash chromatography (cyclohexane/EtOAc 4:6) provided **3** (125 mg, 41%) as a yellow powder.

Mp >250 °C, decomposition; HRMS (ESI+) calcd for $C_{18}H_{16}N_5$ (M+H)$^+$ 302.1406, found 302.1397; IR (KBr): 3550–3100, 1614 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 5.09−5.16 (2H, br s, NH$_2$), 6.42 (1H, ddd, J_1 = 7.5 Hz, J_2 = 1.5 Hz, J_3 = 1.0 Hz), 6.45−6.51 (3H, br s, 1CH+NH$_2$), 6.57 (1H, ddd, J_1 = 8.0 Hz, J_2 = 2.5 Hz, J_3 = 1.0 Hz), 6.75 (1H, d, J = 3.0 Hz), 7.05−7.16 (3H, m), 7.34 (1H, d, J = 8.0 Hz), 7.91 (1H, s), 8.62 (1H, d, J = 8.0 Hz), 11.25−11.35 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 111.3, 112.9, 114.7, 116.8, 120.0, 121.8, 123.2, 129.2, 129.3, 157.4 (CH), 112.7, 121.7, 126.4, 135.8, 139.6, 149.1, 159.8, 162.3 (C).

5.3.5. 4-(1-Methyl-1*H*-indol-3-yl)-5-(3-aminophenyl)pyrimidin-2-amine (4)

Purification of the residue by flash chromatography (cyclohexane/EtOAc 4:6) provided **4** (110 mg, 35%) as a yellow powder.

Mp = 183−185 °C; HRMS (ESI+) calcd for $C_{19}H_{18}N_5$ (M+H)$^+$ 316.1562, found 316.1548; IR (KBr): 3600–3100, 1651, 1602 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.60 (3H, s, CH$_3$), 5.08−5.16 (2H, br s, NH$_2$), 6.41 (1H, d, J = 7.5 Hz), 6.48 (1H, t, J = 1.5 Hz), 6.48−6.52 (2H, br s, NH$_2$), 6.58 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz), 6.73 (1H, s), 7.06 (1H, t, J = 7.5 Hz), 7.11 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.19 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.40 (1H, J = 8.0 Hz), 7.93 (1H, s), 8.53 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 32.9 (CH$_3$), 109.8, 112.8, 114.6, 116.8, 120.3, 121.9, 123.2, 129.3, 132.9, 157.6 (CH), 112.0, 121.7, 126.7, 136.4, 139.3, 149.0, 159.3, 162.3 (C).

5.3.6. 4-[2-Amino-4-(1*H*-indol-3-yl)pyrimidin-5-yl]benzamide (5)

Purification of the residue by flash chromatography (cyclohexane/EtOAc 4:6) provided **5** (160 mg, 49%) as a yellow powder.

Mp >250 °C; HRMS (ESI+) calcd for $C_{19}H_{16}N_5O$ (M+H)$^+$ 330.1355, found 330.1344; IR (KBr): 3600–3100, 1665, 1610 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 6.62−6.68 (2H, br s, NH$_2$), 6.67 (1H, d, J = 3.0 Hz), 7.05 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.12 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.5 Hz), 7.33−7.40 (4H, m), 7.89 (2H, d, J = 8.5 Hz), 7.98−8.02 (1H, br s, NH), 8.05 (1H, s), 8.37 (1H, d, J = 8.0 Hz), 11.26−11.32 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 111.5, 120.1, 121.9, 122.6, 127.9 (2C), 128.9, 129.2 (2C), 158.0 (CH), 112.7, 120.4, 126.1, 132.6, 135.9, 142.0, 159.9, 162.6 (C), 167.5 (C=O).

5.3.7. 4-[2-Amino-4-(1-methyl-1*H*-indol-3-yl)pyrimidin-5-yl]benzamide (6)

Purification of the residue by flash chromatography (cyclohexane/EtOAc 4:6) provided **6** (184 mg, 54%) as a yellow powder.

Mp >250 °C; HRMS (ESI+) calcd for $C_{20}H_{18}N_5O$ (M+H)$^+$ 344.1511, found 344.1494; IR (KBr): 3600–3100, 1681, 1594 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.62 (3H, s, CH$_3$), 6.62−6.70 (2H, br s, NH$_2$), 6.80 (1H, s), 7.06 (1H, t, J = 7.5 Hz), 7.18 (1H, t, J = 7.5 Hz), 7.36 (2H, d, J = 8.0 Hz), 7.35−7.41 (1H, br s, NH), 7.41 (1H, d, J = 8.0 Hz), 7.87 (2H, d, J = 8.0 Hz), 7.97−8.02 (1H, br s, NH), 8.07 (1H, s), 8.20 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 32.8 (CH$_3$), 109.9, 120.2, 121.9, 122.5, 127.9 (2C), 129.0 (2C), 132.6, 158.4 (CH), 112.0, 120.3, 126.3, 132.5, 136.5, 141.7, 159.4, 162.6 (C), 167.5 (C=O).

5.3.8. 4-[2-Amino-4-(1-methyl-1*H*-indol-3-yl)pyrimidin-5-yl]benzoic acid (7)

Purification of the residue by flash chromatography (EtOAc/MeOH 9:1) provided **7** (207 mg, 60%) as a yellow powder.

Mp >250 °C; HRMS (ESI+) calcd for $C_{20}H_{17}N_4O_2$ (M+H)$^+$ 345.1352, found 345.1342; IR (KBr): 3250−2800, 1608 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.63 (3H, s, CH$_3$), 6.66−6.72 (2H, br s, NH$_2$), 6.83 (1H, s), 7.05 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.17 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.38−7.42 (3H, m), 7.91 (2H, d, J = 8.5 Hz), 8.08 (1H, s), 8.16 (1H, d, J = 8.0 Hz), 12.73−13.09 (1H, br s, COOH); ^{13}C NMR (100 MHz, DMSO-d_6): 32.8 (CH$_3$), 109.9, 120.2, 121.9, 122.4, 129.3 (2C), 129.7 (2C), 132.7, 158.4 (CH), 111.9, 120.1, 126.3, 129.1, 136.5, 143.3, 159.4, 162.6 (C), 167.2 (C=O).

5.3.9. 4-(1-Methyl-1*H*-indol-3-yl)-5,5′-bipyrimidin-2-amine (8)

Purification of the residue by flash chromatography (cyclohexane/EtOAc 2:8) provided **8** (95 mg, 31%) as a yellow powder.

Mp >250 °C; HRMS (ESI+) calcd for $C_{17}H_{15}N_6$ (M+H)$^+$ 303.1358, found 303.1344; IR (KBr): 3400−3100, 1638 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.69 (3H, s, CH$_3$), 6.80−6.87 (2H, br s, NH$_2$), 7.04 (1H, s), 7.05 (1H, t, J = 7.5 Hz), 7.18 (1H, t, J = 7.5 Hz), 7.43 (1H, d, J = 8.0 Hz), 7.99 (1H, d, J = 8.0 Hz), 8.21 (1H, s,), 8.70 (2H, s), 9.09 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6): 32.8 (CH$_3$), 110.1, 120.3, 122.0, 122.1, 132.7, 156.5 (3C), 159.1 (CH), 111.7, 114.2, 125.9, 132.5, 136.6, 160.1, 163.2 (C).

5.3.10. 4-(1-Methyl-1*H*-indol-3-yl)-5-(pyridin-4-yl)pyrimidin-2-amine (9)
Purification of the residue by flash chromatography (cyclohexane/EtOAc 4:6) provided **9** (98 mg, 33%) as a yellow powder.
Mp >250 °C; HRMS (ESI+) calcd for $C_{18}H_{16}N_5$ (M+H)$^+$ 302.1406, found 302.1393; IR (KBr): 3350−3100, 1649 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.67 (3H, s, CH$_3$), 6.76−6.83 (2H, br s, NH$_2$), 7.01 (1H, s), 7.04 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.18 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.30 (2H, m), 7.42 (1H, d, J = 8.0 Hz), 8.02 (1H, d, J = 8.0 Hz), 8.14 (1H, s), 8.49 (2H, d, J = 5.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 32.8 (CH$_3$), 110.1, 120.3, 122.0, 122.1, 124.0 (2C), 132.6, 149.8 (2C), 158.6 (CH), 111.8, 118.5, 126.1, 136.6, 146.5, 159.5, 162.9 (C).

5.3.11. 5-Iodo-4-(1-Methyl-1*H*-indol-3-yl)pyrimidin-2-amine (10)
Iodine (1.13 g, 4.46 mmol) was added to the solution of compound **I** (1.0 g, 4.46 mmol) in DMSO (50 ml). The mixture was stirred overnight at 25 °C. The reaction was quenched with a saturated aqueous solution of sodium thiosulfate and was extracted with EtOAc. The organic phase was washed with a brine solution and then dried over MgSO$_4$, filtrated and evaporated onto silica gel. Purification by flash chromatography (cyclohexane/ethyl acetate 1:9) provided **10** (1.20 g, 3.43 mmol, 77%) as a yellow powder.
Mp = 185−188 °C; HRMS (ESI+) calcd for $C_{13}H_{12}IN_4$ (M+H)$^+$ 351.0107, found 351.0091; IR (KBr): 3650−3200, 1633 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.89 (3H, s, CH$_3$), 6.67−6.77 (2H, br s, NH$_2$), 7.16 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.25 (1H, ddd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, J_3 = 1.0 Hz), 7.51 (1H, dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 8.32 (1H, ddd, J_1 = 8.0 Hz, J_2 = 1.0 Hz, J_3 = 0.5 Hz), 8.43 (1H, s), 8.49 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6): 33.0 (CH$_3$), 110.1, 120.5, 122.1, 122.7, 133.2, 165.8 (CH), 74.7, 112.8, 126.6, 136.5, 161.9, 162.1 (C).

References and notes
1 L.H. Franco, E. Bal de Kier Joffé, L. Puricelli, M. Tatian, A.M. Seldes and J.A. Palermo, *J. Nat. Prod.* **61** (1998), p. 1130.
2 L.H. Franco and J.A. Palermo, *Chem. Pharm. Bull.* **51** (2003), p. 975.
3 M. Gompel, M. Leost, E. Bal de Kier Joffé, L. Puricelli, L.H. Franco, J. Palermo and L. Meijer, *Bioorg. Med. Chem. Lett.* **14** (2004), p. 1703. 4 M.A.A. Radwan and M. El-Sherbiny, *Bioorg. Med. Chem.* **14** (2007), p. 1206.
5 K. Bettayeb, O.M. Tirado, S. Marionneau-Lambot, Y. Ferandin, O. Lozach, J.C. Morris, S. Mateo-Lozano, P. Drueckes, C. Schächtele, M.H.G. Kubbutat, F. Liger, B. Marquet, B. Joseph, A. Echalier, J.A. Endicott, V. Notario and L. Meijer, *Cancer Res.* **67** (2007), p. 8325.
6 A. Echalier, K. Bettayeb, Y. Ferandin, O. Lozach, M. Clément, A. Valette, F. Liger, B. Marquet, J.C. Morris, J.A. Endicott, B. Joseph and L. Meijer, *J. Med. Chem.* **51** (2008), p. 737.
7 E. Rossignol, A. Youssef, P. Moreau, M. Prudhomme and F. Anizon, *Tetrahedron* **63** (2007), p. 10169.
8 E. Rossignol, E. Debiton, D. Fabbro, P. Moreau, M. Prudhomme and F. Anizon, *Anti-Cancer Drugs* **19** (2008), p. 789.
9 M.V. Jovanovic, *Heterocycles* **22** (1984), p. 1195.
10 J. O'Brien, I. Wilson, T. Orton and F. Pognan, *Eur. J. Biochem.* **267** (2000), p. 5421.
11 R. Kimura, K. Kamino, M. Yamamoto, A. Nuripa, T. Kida, H. Kazui, R. Hashimoto, T. Tanaka, T. Kudo, H. Yamagata, Y. Tabara, T. Miki, H. Akatsu, K. Kosaka, E. Funakoshi, K. Nishitomi, G. Sakaguchi, A. Kato, H. Hattori, T. Uema and M. Takeda, *Hum. Mol. Genet.* **16** (2007), p. 15.
12 I. Branchi, Z. Bichler, L. Minghetti, J.M. Delabar, F. Malchiodi-Albedi, M.C. Gonzalez, Z. Chettouh, A. Nicolini, C. Chabert, D.J. Smith, E.M. Rubin, D. Migliore-Samour and E. Alleva, *J. Neuropathol. Exp. Neurol.* **63** (2004), p. 429.
13 S. Leclerc, M. Garnier, R. Hoessel, D. Marko, J.A. Bibb, G.L. Snyder, P. Greengard, J. Biernat, E.-M. Mandelkow, G. Eisenbrand and L. Meijer, *J. Biol. Chem.* **276** (2001), p. 251.

14 S. Bach, M. Knockaert, O. Lozach, J. Reinhardt, B. Baratte, S. Schmitt, S.P. Coburn, L. Tang, T. Jiang, D.C. Liang, H. Galons, J.F. Dierick, F. Totzke, C. Schächtele, A.S. Lerman, A. Carnero, Y. Wan, N. Gray and L. Meijer, *J. Biol. Chem.* **280** (2005), p. 31208.

15 A. Primot, B. Baratte, M. Gompel, A. Borgne, S. Liabeuf, J.L. Romette, F. Costantini and L. Meijer, *Protein Exp. Purif.* **20** (2000), p. 394.

16 J. Reinhardt, Y. Ferandin and L. Meijer, *Protein Exp. Purif.* **54** (2007), p. 101.