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Short communication

Synthesis and preliminary biological evaluation of new derivatives of the marine alkaloid leucettamine B as kinase inhibitors

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ABSTRACT

New derivatives of the marine alkaloid leucettamine B were prepared in five steps with overall yields ranging from 23 to 30%. The key step of our strategy has been the sulfur/nitrogen displacement under solvent-free microwave irradiation of (5*Z*) 5-benzo[1,3]-dioxo-5-ylmethylene-2-ethylsulfanyl-3,5-dihydroimidazol-4-one **3** with a mono-protected ethylenediamine **2**. After deprotection of the *N*-Boc group, the amino derivative of leucettamine B **5** was subjected to reductive amination in two steps with retention of configuration of the double bond, to lead to eight new analogs of leucettamine B. The effect of these compounds on CK1α/β, CDK5/p25, and GSK-3α/β were investigated.

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1. Introduction

The exploration of marine natural products, in particular those coming from sponges, continues to make promising contributions to structurally diverse metabolites that display various types of biological activity. The 2-aminoimidazolone core is present in an important number of natural products and constitutes an attractive scaffold to be used for exploiting chemical diversity and generating a broad drug-like screening library. Among these, dispacamide, extracted from Caribbean *Agelas* sponges, shows antihistaminic activity [1] (Fig. 1). Polyandrocarpamine A [2], an alkaloid from a Fijian ascidian, displays selective cytotoxicity towards the SF 268 cell line (central nervous system) with a GI_{50} value of 65 μM [3]. Leucettamine B isolated in 1993 from the sponge *Leucetta microraphis* Hæckel (*Alcarea* class) of the Argulpelu Reef in Palau [4], has been successfully synthesized by the Bergman's group [5] but no biological activity has been reported.

Due to the biological activity associated with the 2-amino imidazolone moiety, we focused our effort on the synthesis of possible bioactive molecules based on *N*-functionalized Leucettamine B derivatives bearing an arylmethyl group linked to the 2-amino function *via* a short aminoalkyl chain (Fig. 2). For this project, we envisaged to prepare an advanced scaffold on which we could bring molecular diversity by using simple reactions such as reductive amination. This intermediate **5** could be built from (5*Z*) 5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-ethylsulfanyl-3,5-dihydroimidazol-4-one **3** [6] and an appropriate symmetric alkyldiamine as precursor of the side chain appended on the 2-amino imidazolone core. In this paper, we report our initial results concerning this strategy for the synthesis of a library of new leucettamine B analogs and their biological evaluation as protein kinase inhibitors. The 518 protein kinases of the human kinome constitute a wide family of disease-relevant targets for the identification and optimization of potential therapeutic agents.

2. Chemistry

As illustrated in Scheme 1, the synthesis started with the preparation of the mono *N*-Boc protected diamine **2** from commercial ethylenediamine **1** and di-*tert*-butyldicarbonate. Complete conversion was observed after 1 h at room temperature

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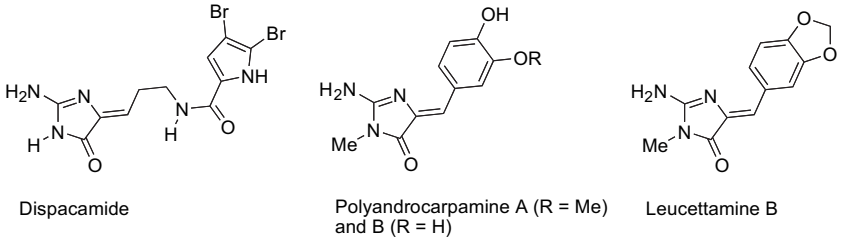

Fig. 1. Structure of alkaloids isolated from marine sponges.

in MeOH according to a modified literature method [7], to obtain the desired product **2** in 65% yield. With the mono *N*-Boc protected ethylenediamine **2** in hand, the key step of our strategy is the guanylation reaction of compound **3**, providing access to the guanidine moiety of 2-amino imidazolone core. In order to be able to carry out such sulfur/nitrogen displacement in a faster and more efficient way than the conventional thermal process [8], we examined the influence of focused microwave irradiations [9] (Synthewave® 402 reactor [10], Merck Eurolab) with different reaction times and various powers. After several experiments, we found that sulfur/nitrogen displacement under microwave irradiation (μω) gave good conversion after 2 h at 105 °C with a stoichiometry of 1/5 of **3**/**2** to produce the desired product **4** (69%).

For the cleavage of *N*-Boc group, the use of trifluoroacetic acid in dichloromethane is often efficient, but in our case, we observed the formation of some impurities. We thus preferred to use hydrochloric acid in an organic solvent [11]. An efficient deprotection of **4** was achieved using 5 equiv of acetyl chloride and 5 equiv of methanol in MeCN at room temperature [12] for 24 h. After washing with dry MeOH, the salt **5** was obtained in 98% yield and the structure was ascertained by high-resolution mass spectrometry (*m*/*z*: 288.1218 uma), proton and carbon NMR.

In order to obtain a large number of compounds suitable for the biological screening, we privileged the transformation of the salt **5** into *N*-functionalized derivatives of leucettamine B **8** by reductive amination in two steps.

We have first examined the reaction of salt **5** with benzaldehyde **6a** using various solvents (EtOH, MeOH, MeCN, AcOEt), at different reaction temperatures and in presence of organic or inorganic bases (Et_3N, pyridine, K_2CO_3). Among the conditions evaluated, the reaction of **5** with 2 equiv. of benzaldehyde **6a** and Et_3N (3 equiv.) in MeOH (2 ml/mmol.) at 50 °C led to the formation of aldimine **7a** in good yield after 24 h (Table 1). After removal of the solvent *in vacuo*, the pure imine **7a** was obtained after several washes with a mixture of EtOH/Et_2O (1:1). Having found suitable conditions for this aldimine synthesis, we screened a range of aromatic aldehydes **6**(**b**–**h**). As can be seen from the data presented in Table 1, the leucettamine B derivatives were easily prepared with electron-rich and electron-poor aromatic aldehydes **6** in yields ranging from 63 to 81%. Finally, the transformation of aldimines **7** into functionalized leucettamine B derivatives **8** could be readily accomplished in good yields (72–97%) using $NaBH_4$ (5 equiv.) in MeOH at 50 °C during 24 h. According to this approach, the compounds **8**(**a**–**h**) were prepared in 5 steps with overall yields ranging from 24 to 31% and the structure was confirmed by conventional techniques (^1H, ^{13}C NMR and HRMS). Extensive study of 1D and 2D NMR spectroscopy have confirmed that leucettamine derivatives **8** were obtained solely as *Z* isomer and no isomerization about the exocyclic double bound was observed during our synthesis.

3. Biology

As an initial effort to investigate their *in vitro* bioactivity, leucettamine derivatives (**5**, **7** and **8**) were tested against three protein kinases relevant to Alzheimer's disease, CK1α/β (casein kinase 1α/β), CDK5 (cyclin-dependent kinase 5)/p25 and GSK-3α/β(glycogen synthase kinase 3α/β) [13–15]. All assays were run in the presence of 15 μM ATP and appropriate protein substrates (RRKHAAIGpSAYSITA peptide for CK1α/β [13], histone H1 for CDK5/p25 [14], GS-1 (YRRAAVPPSPSLSRHSSPHQSpEDEEE) peptide for GSK-3α/β[15]). IC_{50} values were determined from dose-response curves and are provided in Table 2.

Some leucettamine analogs exhibited a moderate to potent GSK-3α/β inhibitory activity with IC_{50} ranging from 0.86 to 38 μM, and a good selectivity versus CK1α/β and CDK5/p25 (Table 2). Interestingly, replacement of an imino group present in compounds **7**(**a**–**e**) by a less rigid amino function **8**(**a**–**e**) led to a loss of activity. Substituents on phenyl group seemed to have a real influence on the activity of this family if we compare the results between the different aldimines **7**(**a**–**e**). However, the *N*-unsubstituted derivative **5** preserved a good inhibitory activity and might represent a scaffold of choice for the discovery of new selective inhibitors of GSK-3α/β. Further biological investigations are underway.

4. Conclusion

In summary, we have successfully developed the synthesis of new derivatives of the marine alkaloid leucettamine B in 5 steps from commercial ethylenediamine and with good overall yields (23–30%). One of the key steps of this approach is the sulfur/nitrogen displacement under microwave irradiation (69%), demonstrating that microwave is suitable to reduce the reaction time using solvent-free conditions. Although a limited number of different and representative substituents of the aromatic group grafted on the side chain of the 2-amino imidazolone core are presented here, it is obvious that a much larger diversity can be achieved. Biological evaluation revealed that this family of compounds could be considered as new selective inhibitory

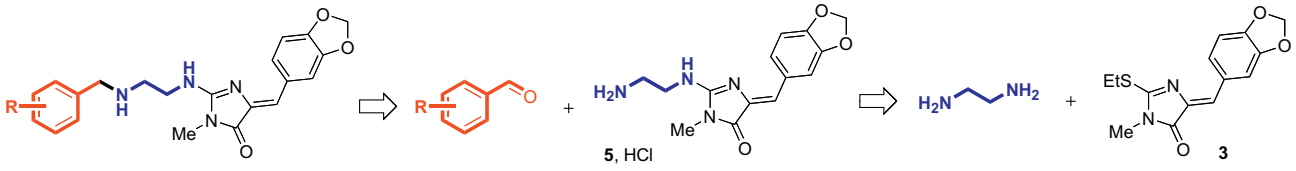

Fig. 2. Retrosynthetic strategy towards *N*-functionalized derivatives of leucettamine B.

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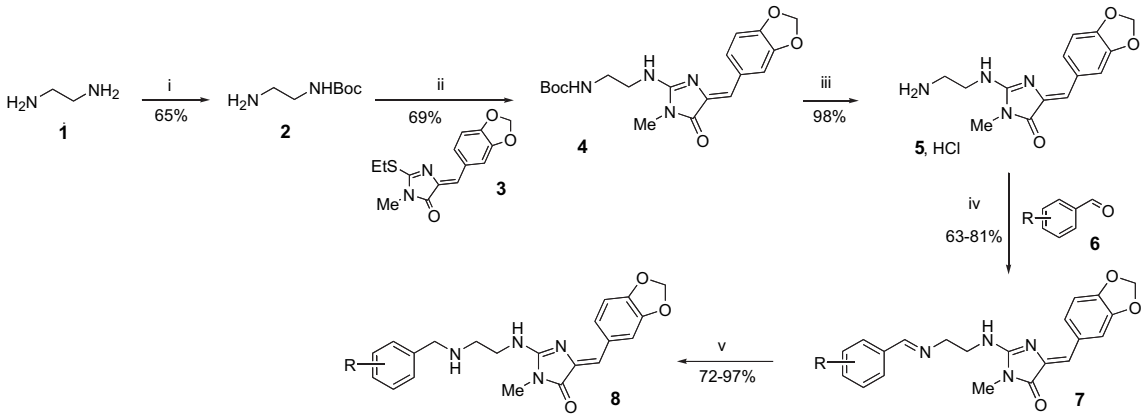


Scheme 1. Reagents and reaction conditions: (i) $(Boc)_2O$ 0.5 equiv., MeOH, 0 °C, 10 min. then 25 °C, 1 h. (ii) **2** 5 equiv., μω, 105 °C, 2 h. (iii) MeCOCl 5 equiv., MeOH 5 equiv., MeCN, 25 °C, 24 h. (iv) **6** 2 equiv., Et_3N 3 equiv., MeOH, 50 °C, 24 h. (v) $NaBH_4$ 5 equiv., MeOH, 50 °C, 24 h.

scaffold of GSK-3. This work should enable further biological evaluations, analogs synthesis through the synthesis procedure described here, and studies of the structure–activity relationship (SAR). These studies are on going in our group.

5. Experiment protocols

5.1. General

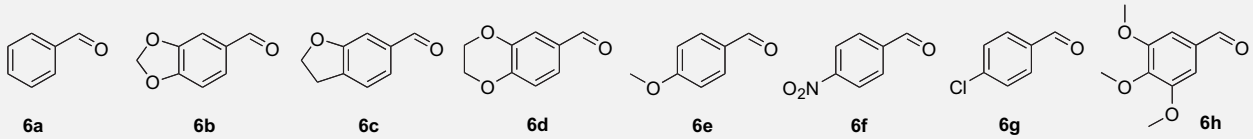
Melting points were determined on a Kofler melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was accomplished on 0.2-mm precoated plates of silica gel 60 F-254 (Merck) and visualization was made with ultraviolet light (254 and 312 nm) or with a fluorescence indicator. ^1H NMR spectra were recorded on BRUKER AC 300 P (300 MHz) and ^{13}C NMR spectra on BRUKER AC 300 P (75 MHz) spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard. Data are given in the following order: δ value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons, coupling constants *J* is given in Hertz. The mass spectra (HRMS) were taken on a VARIAN MAT 311 at an ionizing potential of 70 eV in the Centre Régional de Mesures Physiques de l'Ouest (CRMPO, Rennes). Reactions under microwave irradiations were realized in the Synthewave® 402 apparatus (Merck Eurolab, Div. Prolabo, France). The microwave instrument consists of a continuous focused microwave power output from 0 to 300 W. All experiments were performed using stirring option. The target temperature was reached with a ramp of 5 min and the chosen microwave power remained constant to keep the mixture at this temperature. The reaction temperature is monitored using calibrated infrared sensor and the reaction time include the ramp period. Acetonitrile was distilled over calcium chloride after standing overnight and stored over molecular sieves (3 Å). Solvents were evaporated with a BUCHI rotary evaporator. All reagents were purchased from Acros, Aldrich Chimie, Fluka France and used without further purification. The starting (5*Z*) 5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-ethylsulfanyl-3,5-dihydroimidazol-4-one **3** was synthesized according to our previous method [6].

5.2. Synthesis of (5Z)-5[(1,3-benzodioxol-5-yl)methylene]-3-methyl-2-[(2-tert-butylamino)ethylcarbamate]-3,5-dihydro-4H-imidazol-4-one (*4*)

A mixture of (5*Z*) 5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-ethylsulfanyl-3,5-dihydroimidazol-4-one **3** (1.3 g, 4.5 mmoles) and *N*-Boc protected ethylenediamine **2** (3.6 g, 22.5 mmoles, 5 equiv.) was placed in a cylindrical quartz reactor (Ø = 1.8 cm). The

Table 1

Yield of product (%)[a]				Reductive amination yield (%)[b]	Overall yield (%)[c]
7		**8**			
7a	71	**8a**	88	62	27
7b	70	**8b**	94	66	30
7c	72	**8c**	72	52	23
7d	67	**8d**	85	57	25
7e	77	**8e**	87	67	29
7f	81	**8f**	81	66	29
7g	71	**8g**	90	65	29
7h	63	**8h**	97	61	27



[a] Isolated yield.
[b] Yield for **5** –> **8**.
[c] Overall yield calculated from commercial ethylenediamine **1**.

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Table 2
Kinase inhibition[a] values (IC_{50} in μM) for compounds **5**, **7** and **8**.[b]

Compound	CK1	CDK5/p25	GSK-3α/β
8a	>100	>100	> 10
8b	>100	>100	> 10
8c	>100	>100	> 10
8d	>100	>100	> 10
8e	>100	>100	> 10
5	>100	> 100	1.4
7a	>100	>100	7.0
7b	>100	>100	0.86
7c	>100	>100	15.0
7d	>100	>100	38.0
7e	>100	>100	7.3
Leucettamine B	>100	>100	15

[a] Kinase inhibition experiments were carried out as described previously [15–16]. The final ATP-concentration in the test was 15 μM.
[b] The compounds, which do not appear in this table, have no activity towards these three kinases (>100).

reactor was then introduced into a Synthewave® 402 Prolabo microwave reactor. The stirred mixture was irradiated (after a ramp of 5 min from 20 to 105 °C) at 105 °C (power level: 30%, 90 Watt) with a reaction time of 2 h. After microwave dielectric heating, the crude reaction mixture was allowed to cool down at room temperature and Et_2O (10 ml) was added in the cylindrical quartz reactor. The resulting insoluble product **4** was filtered and dried under high vacuum (10^{-2} Torr) for 6 h (yield: 69%).

Mp = 108–110 °C. ^{1}H NMR (300 MHz, DMSO) δ: 7.92 (s, 1H, Ar), 7.62 (brs, 1H, NH), 7.42 (d, 1H, *J* = 8 Hz, Ar), 7.00 (br s, 1H, NH), 6.90 (d, 1H, *J* = 8 Hz, Ar), 6.37 (s, 1H, CH═), 6.02 (s, 2H, OCH_2O), 3.45 (t, 2H, *J* = 6 Hz, CH_2N), 3.24 (t, 2H, *J* = 6 Hz, CH_2N), 3.03 (s, 3H, NCH_3), 1.36 (s, 9H, 3 CH_3). ^{13}C NMR (75 MHz, DMSO) δ: 169.5, 158.3, 155.7, 147.2, 146.7, 138.7, 130.3, 125.2, 112.9, 109.6, 108.2, 101.0, 79.1, 41.2, 38.6, 28.2, 25.5. HRMS, *m*/*z*: 388.1743 found (calculated for $C_{19}H_{24}N_4O_5$, $M^{+\cdot}$ requires: 388.1747).

5.3. Synthesis of (5Z) 5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[(2-aminoethyl)amino]-3,5-dihydro-4H-imidazol-4-one hydrochloride (**5**)

In a 100 ml two-necked round-bottomed flask, provided with a magnetic stirrer and reflux condenser, (5*Z*)-5[(1,3-Benzodioxol-5-yl)methylene]-3-methyl-2-[(2-tert-butylamino)ethylcarbamate]-3,5-dihydro-4*H*-imidazol-4-one **4** (1 g, 2.6 mmoles) was mixed in acetonitrile pa (70 ml). To this slurry was added dropwise successively methanol (0.63 ml, 15.46 mmoles) and freshly distilled acetyl chloride (1.1 ml, 15.46 mmoles). After stirring at room temperature for 24 h and elimination of the solvent in a rotary evaporator under reduced pressure, the crude residue was submitted to washing with ethanol (10 ml). The desired product **5** was obtained as yellowish powder and was further dried under high vacuum (10^{-2} Torr) at 25 °C for 9 h (yield: 98%).

Mp = 204–206 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.47 (br s, 3H, NH_2 + NH), 7.92 (s, 1H, Ar), 7.61 (s, 1H, NH), 7.41 (d, 1H, *J* = 9 Hz, Ar), 6.98 (d, 1H, *J* = 9 Hz, Ar), 6.80 (s, 1H, CH═), 6.10 (s, 2H, OCH_2O), 3.93 (t, 2H, *J* = 5 Hz, CH_2N), 3.25 (s, 3H, NCH_3), 3.20 (t, 2H, *J* = 5 Hz, CH_2N). ^{13}C NMR (75 MHz, DMSO) δ: 164.5, 156.2, 148.5, 147.6, 126.8, 126.7, 126.5, 117.3, 109.9, 108.6, 101.6, 40.4, 37.9, 26.8. HRMS, *m*/*z*: 288.1218 found (calculated for $C_{14}H_{16}N_4O_3$, $M^{+\cdot}$ requires: 288.1222).

5.4. General procedure for synthesis of compounds **7**(**a**–**h**) from product **5** and aldehydes **6**(**a**–**h**)

In a 10 ml two-necked round-bottomed flask, provided with a magnetic stirrer and reflux condenser, (5*Z*) 5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[(2-aminoethyl)amino]-3,5-dihydro-4*H*-imidazol-4-one hydrochloride **5** (0.25 g, 0.77 mmoles) and triethylamine (0.32 ml, 2.31 mmoles) were mixed in methanol pa (5 ml). To this solution was added dropwise a solution of aldehyde (1.54 mmoles) in 1.5 ml of methanol pa over a period of 30 min. The mixture was heated at 50 °C for 24 h (monitored by TLC) then cooled down to room temperature. The solvent was evaporated *in vacuo* and 5 ml of EtOH/Et_2O (1:1) were added in the crude reaction mixture. After vigorous stirring at 25 °C for 30 min, the precipitate that has formed was filtered off and further dried in a dessicator over $CaCl_2$ under high vacuum (10^{-2} Torr) for 6 h. The expected compound **7** was further used without purification and can be stored at 4 °C under nitrogen.

5.4.1. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[(2-(Z)-benzylideneamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7a**)

This compound was obtained as yellow solid in 71% yield. Mp = 200–202 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.38 (s, 1H, CH═N), 8.00 (s, 1H, Ar), 7.77–7.81 (m, 3H, Ar, NH), 7.43–7.47 (m, 4H, Ar), 6.91 (d, 1H, *J* = 8 Hz, Ar), 6.40 (s, 1H, CH═), 6.03 (s, 2H, OCH_2O), 3.88 (t, 2H, *J* = 4.5 Hz, CH_2N), 3.70 (t, 2H, *J* = 4.5 Hz, CH_2N), 3.02 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO) δ: 170.0, 162.8, 158.5, 147.7, 147.2, 139.3, 136.5, 131.2, 130.9, 129.1, 128.5, 125.7, 113.5, 110.0, 108.8, 101.5, 59.6, 42.6, 26.0. HRMS, *m*/*z*: 376.1537 found (calculated for $C_{21}H_{20}N_4O_3$, $M^{+\cdot}$ requires: 376.1535).

5.4.2. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[{2-(Z)-(1,3-benzodioxol-5-yl)methyleneaminol}ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7b**)

This compound was obtained as yellow solid in 70% yield. Mp = 210–212 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.26 (s, 1H, CH═N), 8.00 (s, 1H, Ar), 7.77 (br s, 1H, NH), 7.40 (d, 1H, *J* = 8 Hz, Ar), 7.32 (s, 1H, Ar), 7.22 (d, 1H, *J* = 8 Hz, Ar), 6.97 (d, 1H, *J* = 8 Hz, Ar), 6.91 (d, 1H, *J* = 8 Hz, Ar), 6.40 (s, 1H, CH═), 6.07 (s, 2H, OCH_2O), 6.03 (s, 2H, OCH_2O), 3.83 (t, 2H, *J* = 5 Hz, CH_2N), 3.68 (t, 2H, *J* = 5 Hz, CH_2N), 3.02 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO) δ: 169.5, 161.3, 158.0, 149.4, 147.8, 147.2, 146.7, 125.2, 124.3, 112.5, 109.5, 108.2, 108.1, 106.0, 101.4, 100.1, 58.8, 42.1, 25.5. HRMS, *m*/*z*: 421.1511 found (calculated for $C_{22}H_{21}N_4O_5$, $[M+H]^+$ requires: 421.1512).

5.4.3. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[{2-(Z)-(2,3dihydrofuran-5-yl)methyleneamino}ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7c**)

This compound was obtained as yellow solid in 72% yield. Mp = 200–202 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.26 (s, 1H, CH═N), 8.01 (s, 1H, Ar), 7.77 (br s, 1H, NH), 7.66 (s, 1H, Ar), 7.47 (d, 1H, *J* = 8 Hz, Ar), 7.40 (d, 1H, *J* = 8 Hz, Ar), 6.91 (d, 1H, *J* = 8 Hz, Ar), 6.80 (d, 1H, *J* = 8 Hz, Ar), 6.40 (s, 1H, CH═), 6.03 (s, 2H, OCH_2O), 4.56 (t, 2H, *J* = 9 Hz, CH_2O), 3.82 (t, 2H, *J* = 6 Hz, CH_2N), 3.67 (t, 2H, *J* = 6 Hz, CH_2N), 3.16 (t, 2H, *J* = 9 Hz, CH_2), 3.01 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO) δ: 169.5, 162.0, 161.6, 158.0, 147.2, 146.7, 138.8, 130.3, 129.3, 129.0, 128.0, 125.3, 124.3, 112.9, 109.5, 108.7, 108.2, 101.0, 71.5, 59.0, 42.3, 28.5, 25.5. HRMS, *m*/*z*: 418.1645 found (calculated for $C_{23}H_{22}N_4O_4$, $M^{+\cdot}$ requires: 418.1641).

5.4.4. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[{2-(Z)-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methyleneamino}ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7d**)

This compound was obtained as yellow solid in 67% yield. Mp = 202–204 °C. ^{1}H NMR (300 MHz, DMSO) δ:8.23(s,1H, CH═N), 8.00 (s, 1H, Ar), 7.76 (br s, 1H, NH), 7.41 (d, 1H, *J* = 8 Hz, Ar), 7.26 (s, 1H, Ar), 7.24 (d, 1H, *J* = 8 Hz, Ar), 6.92 (d, 1H, *J* = 4.1 Hz, Ar), 6.90 (d, 1H, *J* = 4 Hz, Ar), 6.40 (s, 1H, CH═), 6.03 (s, 2H, OCH_2O), 4.26 (m, 4H, CH_2O), 3.82 (t, 2H, *J* = 6 Hz, CH_2N), 3.66 (t, 2H, *J* = 6 Hz, CH_2N), 3.02 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO) δ: 169.5, 161.3, 158.0, 147.2,

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146.7, 145.6, 143.4, 138.7, 130.3, 129.6, 125.2, 121.5, 117.1, 116.3, 112.9, 109.5, 108.3, 101.0, 64.2, 63.9, 58.9, 42.2, 25.5. HRMS, *m/z*: 435.1675 found (calculated for $C_{23}H_{23}N_4O_5$, $[M+H]^+$ requires: 435.1669).

*5.4.5. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(Z)-(4-methoxybenzylideneamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7e**)*

This compound was obtained as yellow solid in 77% yield. Mp = 194–196 °C. ^{1}H NMR (300 MHz, DMSO) δ:8.30(s, 1H, CH=N), 8.00 (s, 1H, Ar), 7.78 (br s, 1H, NH), 7.71 (d, 2H, J = 9 Hz, Ar), 7.41 (d, 1H, J = 8.5 Hz, Ar), 7.00 (d, 2H, J = 9 Hz, Ar), 6.92 (d, 1H, J = 8.5 Hz, Ar), 6.38 (s, 1H, CH=), 6.03 (s, 2H, OCH_2O), 3.84 (t, 2H, J = 7 Hz, CH_2N), 3.80 (s, 3H, OCH_3), 3.67 (t, 2H, J = 7 Hz, CH_2N), 3.02 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO) δ: 169.5, 161.5, 161.2, 158.0, 147.2, 146.7, 138.8, 130.3, 129.6, 128.8, 125.2, 113.9, 112.9, 109.5, 108.3, 101.0, 59.0, 55.2, 42.2, 25.5. HRMS, *m/z*: 406.1667 found (calculated for $C_{22}H_{22}N_4O_4$, $M^{+\cdot}$ requires: 406.1641).

*5.4.6. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(Z)-(4-nitrobenzylideneamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7f**)*

This compound was obtained as red solid in 81% yield. Mp = 206–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ:8.46(s, 1H, CH=N), 8.26 (d, 2H, J = 8.5 Hz, Ar), 8.00 (s, 1H, Ar), 7.90 (d, 2H, J = 8.5 Hz, Ar), 7.33 (d, 1H, J = 8 Hz, Ar), 6.82 (d, 1H, J = 8 Hz, Ar), 6.68 (s, 1H, CH=), 6.00 (s, 1H, OCH_2O), 5.18 (br s, 1H, NH), 3.98 (m, 4H, CH_2N), 3.13 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.1, 160.8, 157.0, 149.2, 147.7, 141.0, 137.8, 130.1, 128.9, 128.8, 126.3, 123.9, 117.5, 110.3, 108.4, 101.1, 60.0, 42.5, 25.1. HRMS, *m/z*: 421.1351 found (calculated for $C_{21}H_{19}N_5O_5$, $M^{+\cdot}$ requires: 421.1386).

*5.4.7. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(Z)-(4-chlorobenzylideneamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7g**)*

This compound was obtained as yellow solid in 71% yield. Mp = 192–194 °C. ^{1}H NMR (300 MHz, DMSO) δ:8.40(s, 1H, CH=N), 8.00 (s, 1H, Ar), 7.78 (m, 3H, NH, Ar), 7.50 (d, 2H, J = 8 Hz, ArH), 7.40 (d, 1H, J = 8 Hz, Ar), 6.91 (d; 1H, J = 8 Hz, Ar), 6.40 (s, 1H, CH=), 6.03 (s, 2H, OCH_2O), 3.87 (t, 2H, J = 6 Hz, CH_2N), 3.70 (t, 2H, J = 6 Hz, CH_2N), 3.02 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO) δ: 170.0, 161.6, 158.7, 147.7, 147.2, 139.2, 135.7, 135.3, 130.8, 130.1, 129.2, 125.7, 113.5, 110.0, 108.8, 101.5, 59.6, 42.5, 26.0. HRMS, *m/z*: 410.1183 found (calculated for $C_{21}H_{19}N_4O_3Cl$, $M^{+\cdot}$ requires: 410.1146).

*5.4.8. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(Z)-(3,4,5-trimethoxybenzylideneamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**7h**)*

This compound was obtained as yellow solid in 63% yield. Mp = 198–200 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.28 (s, 1H, CH=N), 8.00 (s, 1H, Ar), 7.80 (br s, 1H, NH), 7.41 (d, 1H, J = 8 Hz, Ar), 7.08 (s, 2H, Ar), 6.91 (d, 1H, J = 8 Hz, Ar), 6.40 (s, 1H, CH=), 6.02 (s, 2H, OCH_2O), 3.87 (t, 2H, J = 6 Hz, CH_2N), 3.80 (s, 6H, OCH_3), 3.70 (m, 5H, CH_2N, OCH_3), 3.02 (s, 3H, NCH_3). ^{13}C NMR (75 MHz, DMSO) δ: 170.0, 162.3, 158.5, 153.5, 147.7, 147.2, 140.0, 139.3, 132.0, 130.8, 125.7, 113.4, 110.0, 108.8, 105.6, 101.5, 60.5, 59.5, 56.3, 42.5, 26.0. HRMS, *m/z*: 466.1823 found (calculated for $C_{24}H_{26}N_4O_6$, $M^{+\cdot}$ requires: 466.1852).

*5.5. General procedure for preparation of compounds **8**(**a**–**h**) by reduction of **7**(**a**–**h**)*

In a 10 ml two-necked round-bottomed flask, provided with a magnetic stirrer and reflux condenser, compound **7** (0.5 mmoles) was dissolved in methanol pa (3 ml) under vigorous stirring. To this solution was added dropwise a solution of sodium borohydride $NaBH_4$ (0.1 g, 2.5 mmoles) in 2 ml of methanol pa over a period of 30 min. The homogeneous solution was stirred at 50 °C for 24 h (monitored by TLC). After elimination of solvent in a rotary evaporator under reduced pressure, deionized water (4 ml) was added in one portion to the crude residue. The mixture was transferred to a separating funnel and was extracted with dichloromethane (3 × 5 ml). The combined organic phases were dried over magnesium sulphate $MgSO_4$, filtered and the solvent was eliminated *in vacuo*. The desired product **8** was obtained as yellowish powder and was submitted to purification by recrystallization with EtOH. Pure compound **8** was dried under high vacuum (10^{-2} Torr) for 6 h at room temperature.

*5.5.1. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(benzylamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8a**)*

This compound was obtained as yellow solid in 88% yield. Mp = 162–164 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97 (s, 1H, Ar), 7.31 (m, 6H, Ar), 6.81 (d, 1H, J = 8 Hz, Ar), 6.65 (s, 1H, CH=), 5.98 (s, 2H, OCH_2O), 5.56 (br s, 2H, NH), 3.84 (s, 2H, CH_2N), 3.65 (t, 2H, J = 5 Hz, CH_2N), 3.12 (s, 3H, NCH_3), 2.87 (t, 2H, J = 5 Hz, CH_2N). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.2, 157.2, 147.7, 147.5, 139.9, 138.1, 130.2, 128.6, 128.1, 127.3, 126.0, 116.6, 110.2, 108.3, 101.1, 53.3, 47.4, 41.0, 25.1. HRMS, *m/z*: 378.1700 found (calculated for $C_{21}H_{22}N_4O_3$, $M^{+\cdot}$ requires: 378.1692).

*5.5.2. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-{(1,3-benzodioxol-5-yl)methylamino}ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8b**)*

This compound was obtained as yellow solid in 94% yield. Mp = 218–220 °C. ^{1}H NMR (300 MHz, DMSO) δ: 7.97 (s, 1H, Ar), 7.40 (s, 1H, Ar), 6.90 (d, 2H, J = 8 Hz, Ar), 6.76 (d, 2H, J = 8 Hz, Ar), 6.40 (s, 1H, CH=), 6.01 (s, 2H, OCH_2O), 5.93 (s, 2H, OCH_2O), 5.57 (br s, 2H, NH), 3.65 (s, 2H, CH_2N), 3.51 (t, 2H, J = 5 Hz, CH_2N), 3.03 (s, 3H, NCH_3), 2.75 (t, 2H, J = 5 Hz, CH_2N). ^{13}C NMR (75 MHz, DMSO) δ: 159.6, 158.1, 147.2, 147.1, 146.6, 145.7, 138.8, 134.9, 130.4, 125.1, 120.8, 112.6, 109.5, 108.2, 112.6, 109.5, 108.3, 108.2, 107.7, 101.0, 100.6, 52.2, 47.3, 41.2, 25.4. HRMS, *m/z*: 423.1662 found (calculated for $C_{22}H_{23}N_4O_5$, $[M+H]^+$ requires: 423.1669).

*5.5.3. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-{(2,3-dihydrobezofuran-5-yl)methylamino}ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8c**)*

This compound was obtained as yellow solid in 72% yield. Mp = 188–190 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97 (s, 1H, Ar), 7.33 (d, 1H, J = 8 Hz, Ar), 7.12 (s, 1H, Ar), 7.03 (d, 1H, J = 8 Hz, Ar), 6.80 (d, 1H, J = 8 Hz, Ar), 6.73 (d, 1H, J = 8 Hz, Ar), 6.63 (s, 1H, CH=), 5.96 (s, 2H, OCH_2O), 5.47 (br s, 2H, NH), 4.55 (t, 2H, J = 9 Hz, CH_2O), 3.73 (s, 2H, CH_2N), 3.62 (t, 2H, J = 4.5 Hz, CH_2N), 3.17 (t, 2H, J = 9 Hz, CH_2), 3.10 (s, 3H, NCH_3), 2.93 (t, 2H, J = 4.5 Hz, CH_2N). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.2, 159.4, 157.2, 147.7, 147.5, 138.1, 132.0, 130.2, 128.0, 127.3, 126.0, 124.9, 116.8, 110.3, 109.1, 108.3, 101.1, 71.3, 53.0, 47.2, 40.9, 29.7, 25.1. HRMS, *m/z*: 420.1825 found (calculated for $C_{23}H_{24}N_4O_4$, $M^{+\cdot}$ requires: 420.1798).

*5.5.4. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-{(1,4-benzodioxin-6-yl)methylamino}ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8d**)*

This compound was obtained as yellow solid in 85% yield. Mp = 186–188 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.00 (s, 1H, Ar), 7.34 (d, 1H, J = 8 Hz, Ar), 6.80 (m, 4H, Ar), 6.64 (s, 1H, CH=), 5.97 (s, 2H, OCH_2O), 5.50 (br s, 2H, NH), 4.24 (m, 4H, CH_2O), 3.71 (s, 2H, CH_2N), 3.60 (t, 2H, J = 5 Hz, CH_2N), 3.11 (s, 3H, NCH_3), 2.92 (t, 2H, J = 5 Hz, CH_2N). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.3, 157.2, 147.7, 147.5, 143.5, 142.7, 138.2, 133.4, 130.3, 126.0, 121.0, 117.2, 116.8, 110.3, 108.3, 101.1, 64.4, 64.3, 52.7, 47.1, 40.9, 25.1. HRMS, *m/z*: 436.1790 found (calculated for $C_{23}H_{24}N_4O_5$, $M^{+\cdot}$ requires: 436.1747).

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*5.5.5. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(4-methoxybenzylamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8e**)*

This compound was obtained as yellow solid in 87% yield. Mp = 184–186 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.00 (s, 1H, Ar), 7.35 (d, 1H, J = 8 Hz, Ar), 7.24 (d, 2H, J = 8 Hz, Ar), 6.88 (d, 2H, J = 8 Hz, Ar), 6.82 (d, 1H, J = 8 Hz, Ar), 6.66 (s, 1H, CH═), 6.00 (s, 2H, OCH_2O), 5.47 (br s, 2H, NH), 3.81 (s, 3H, OCH_3), 3.78 (s, 2H, CH_2N), 3.63 (t, 2H, J = 5.5 Hz, CH_2N), 3.12 (s, 3H, NCH_3), 2.96 (t, 2H, J = 5.5 Hz, CH_2N). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.3, 158.8, 157.2, 147.7, 147.5, 138.2, 132.0, 130.2, 129.3, 126.0, 116.8, 113.9, 110.3, 108.3, 101.1, 55.3, 52.7, 47.2, 40.9, 25.1. HRMS, m/z: 408.1762 found (calculated for $C_{22}H_{24}N_4O_4$, $M^{+\cdot}$ requires: 408.1798).

*5.5.6. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(4-nitrobenzylamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8f**)*

This compound was obtained as yellow solid in 81% yield. Mp = 150–152 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.14 (d, 2H, J = 8.6 Hz, Ar), 7.96 (s, 1H, Ar), 7.62 (d, 2H, J = 8.6 Hz, Ar), 7.37 (d, 1H, J = 8 Hz, Ar), 6.90 (d, 1H, J = 8 Hz, Ar), 6.35 (s, 1H, CH═), 6.03 (s, 2H, OCH_2O), 5.50 (br s, 2H, NH), 3.90 (s, 2H, CH_2N), 3.53 (t, 2H, J = 5.5 Hz, CH_2N), 3.04 (s, 3H, NCH_3), 2.80 (t, 2H, J = 5.5 Hz, CH_2N). ^{13}C NMR (75 MHz, DMSO) δ: 169.5, 158.1, 149.5, 147.1, 146.6, 146.2, 138.8, 130.4, 128.7, 125.1, 123.1, 112.6, 109.4, 108.2, 101.0, 51.7, 47.5, 41.1, 25.5. HRMS, m/z: 245.0802 found (calculated for $C_{12}H_{11}N_3O_3$, $[M–C_9H_{10}N_2O_2]^+$ requires: 245.0800).

*5.5.7. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(4-chlorobenzylamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8g**)*

This compound was obtained as yellow solid in 90% yield. Mp = 188–190 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.00 (s, 1H, Ar), 7.60 (br s, 2H, NH), 7.40 (d, 1H, J = 7.5 Hz, Ar), 7.34 (m, 4H, Ar), 6.90 (d, 1H, J = 7.5 Hz, Ar), 6.37 (s, 1H, CH═), 6.03 (s, 2H, OCH_2O), 3.74 (s, 2H, CH_2N), 3.52 (t, 2H, J = 6 Hz, CH_2N), 3.04 (s, 3H, NCH_3), 2.77 (t, 2H, J = 6 Hz, CH_2N). ^{13}C NMR (75 MHz, DMSO) δ: 170.0, 158.6, 147.7, 147.1, 140.5, 139.3, 131.4, 130.9, 130.1, 128.4, 125.6, 113.1, 110.0, 108.7, 101.5, 52.2, 48.0, 41.6, 26.0. HRMS, m/z: 412.1302 found (calculated for $C_{21}H_{21}N_4O_3Cl$, $M^{+\cdot}$ requires: 412.1302).

*5.5.8. (5Z)-5-[(1,3-benzodioxo-5-yl)methylene]-3-methyl-2-[2-(3,4,5-trimethoxybenzylamino)ethylamino]-3,5-dihydro-4H-imidazol-4-one (**8h**)*

This compound was obtained as yellow solid in 97% yield. Mp = 184–186 °C. ^{1}H NMR (300 MHz, DMSO) δ: 8.03 (s, 1H, Ar), 7.67 (br s, 2H, NH), 7.44 (d, 1H, J = 8 Hz, Ar), 6.93 (d, 1H, J = 8 Hz, Ar), 6.67 (s, 2H, Ar), 6.40 (s, 1H, CH═), 6.05 (s, 2H, OCH_2O), 3.74 (m, 8H, CH_2N, OCH_3), 3.64 (s, 3H, OCH_3), 3.60 (t, 2H, J = 6 Hz, CH_2N), 3.10 (s, 3H, NCH_3), 2.83 (t, 2H, J = 6 Hz, CH_2N). ^{13}C NMR (75 MHz, DMSO) δ: 169.6, 158.2, 152.6, 147.2, 146.6, 138.9, 136.7, 135.9, 130.4, 125.1, 112.5, 109.4, 108.2, 104.8, 101.9, 59.8, 55.6, 52.6, 47.6, 41.0, 25.4. HRMS, m/z: 468.2018 found (calculated for $C_{24}H_{28}N_4O_6$, $M^{+\cdot}$ requires: 468.2019).

5.6. Biology

Kinase activities were assayed according to the methodology [16] developed by the Cell Cycle Group of the Station Biologique, CNRS, Roscoff, France.

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