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Synthesis and biological evaluation of diversely substituted indolin-2-ones

Fadoua Bouchikhi^a, Emilie Rossignol^a, Martine Sancelme^a, Bettina Aboab^a, Fabrice Anizon^a, Dorian Fabbro^b, Michelle Prudhomme^a and Pascale Moreau^a

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Abstract

The synthesis of indolin-2-one derivatives substituted in the 3-position by an aminomethylene group bearing either an ornithine or a lysine residue is described. The inhibitory activities of these compounds toward a panel of eight kinases were examined. Furthermore, the antibacterial activities of the prepared compounds were tested against two Gram-positive bacteria *Bacillus cereus* and *Streptomyces chartreusis*, a Gram-negative bacterium *Escherichia coli* and a yeast *Candida albicans*.

Keywords: Indolin-2-ones; Kinases' inhibitors; Antimicrobial agents

1. Introduction

Increased emergence of bacterial resistance to antibiotic therapy has created an urgent need for the development of new antibacterial agents. As part of our ongoing studies concerning the preparation of potential biologically active compounds, we were interested in the synthesis of indolin-2-one derivatives [1] and [2]. Since various kinases are involved in the growth of microorganisms and because many oxindoles are kinases' inhibitors [3], [4], [5], [6], [7] and [8], these compounds could exert antibacterial activities as it was shown previously for several oxindole derivatives (Fig. 1) [9], [10] and [11].

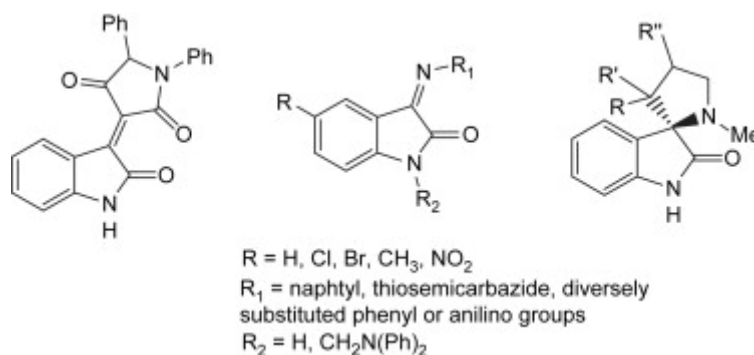


Fig. 1

We have reported, in a previous paper, the synthesis and antiproliferative activities, toward various human tumor cell lines, of indolin-2-one derivatives substituted in the 3-position by an aminomethylene group bearing different amino acid moieties (Scheme 1) [2]. The amino acid side chains were either flexible (compound **A**) or more rigid and differently oriented (compounds **B** and **C**). The results obtained in these previous structure–activity relationship studies have shown that only compound **A**, bearing a flexible side chain which allows the folding of this chain, has exhibited antiproliferative activities toward a panel of various human solid tumor cell lines [2]. Therefore, in order to evaluate the influence of the chain length on the biological activities, indolin-2-one derivatives substituted in the 3-position by an aminomethylene group bearing either an ornithine or a lysine residue were synthesized (Fig. 2). Moreover, as already shown in the literature, the substitution of the aromatic moiety by a halogen atom could affect the pharmacological profile in this series [8] and [9]. Therefore, to get an insight on the effect of bromination of the aromatic moiety, on the biological activities, compounds **6** and **8**, brominated in the 5-position were also prepared. The inhibitory potencies of the prepared compounds toward a panel of eight kinases were examined. The kinases tested were either receptor tyrosine kinase (RTKs), non-

receptor tyrosine kinases (CTKs) or serine/threonine kinases (STKs). The RTKs tested were KDR, IGF-1R, c-Met, and Ret. The CTKs tested were Src and Abl. The STK tested were PKA and CDK2. Finally, the antibacterial activities of the various compounds against two Gram-positive bacteria *Bacillus cereus* and *Streptomyces chartreusis*, a Gram-negative bacterium *Escherichia coli* and a yeast *Candida albicans* are reported.

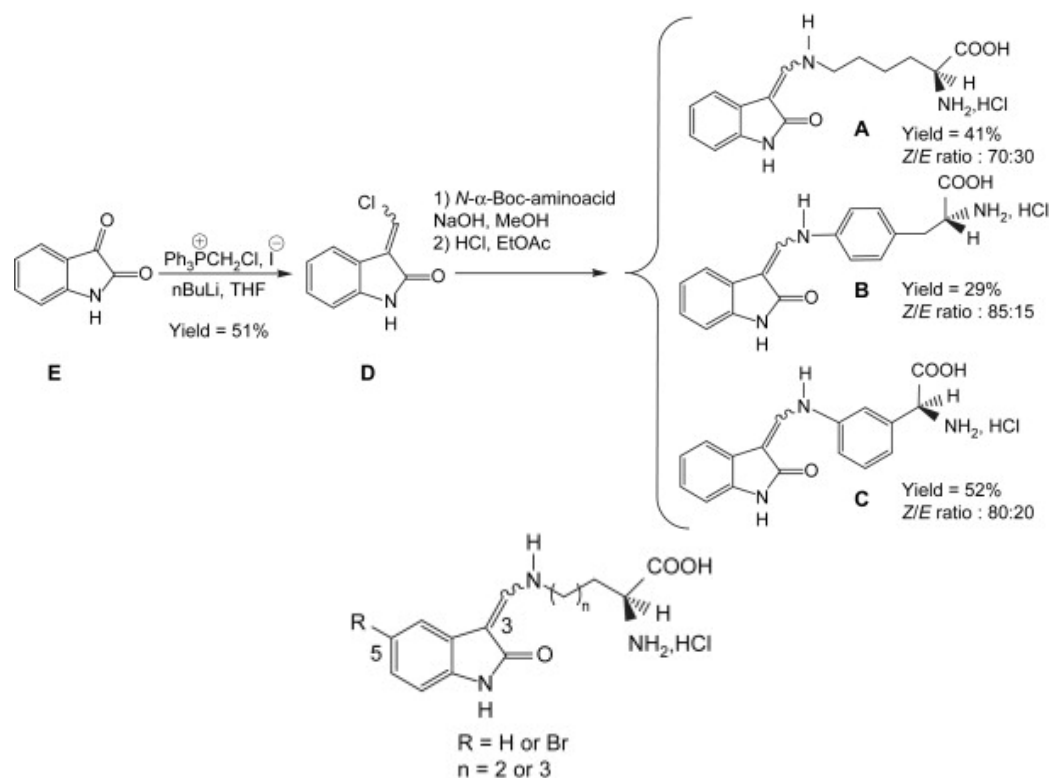
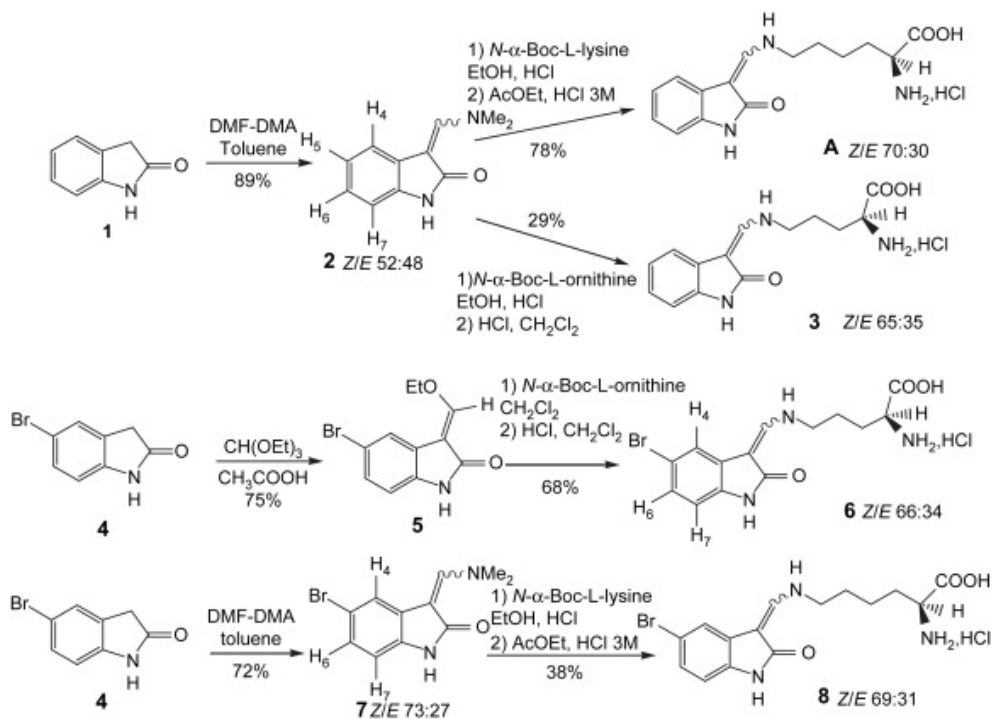


Fig. 2.

2. Chemistry

As described in our previous paper [2], the first indolin-2-one derivatives substituted in the 3-position by an aminomethylene group bearing different amino acid moieties were synthesized, in moderate yields, in three steps via the chloromethylene analogue **D**. Compound **D** was prepared in 51% yield by a Wittig reaction performed with chloromethyl triphenylphosphonium iodide in the presence of isatine **E** (Scheme 1). In order to improve the synthesis of these compounds, different approaches requiring either the corresponding 3-(*N,N*-dimethylaminomethylene)indolin-2-ones or 3-ethoxymethyleneindolin-2-ones were investigated. Compound **A** which was prepared in 21% overall yield via the chloromethylene derivative [2] was obtained in 70% overall yield from 3-(*N,N*-dimethylaminomethylene)indolin-2-one **2** (Scheme 2). Compound **2** was prepared as a mixture of *Z/E* isomers in 89% yield by treatment of indolin-2-one (**1**) with dimethylformamide-*N,N*-dimethylacetal (DMF-DMA) in toluene as described in the literature for 4-hydroxyquinolinone derivatives [12]. Compound **2** *Z/E* isomeric ratio (52:48) was determined from the ^1H NMR spectrum, in $\text{DMSO-}d_6$, on the H_6 signal (*E* isomer) and the H_4 signal (*Z* isomer). The *Z/E* assignment was made according to the NMR data described for compound **2** by Joseph-Nathan and co-workers [13]. The coupling reaction with *N*- α -Boc-L-lysine was performed in the presence of HCl in ethanol. After deprotection of the α -amino group in acidic medium, compound **A** was obtained in 78% yield as a *Z/E* isomeric mixture with the same *Z/E* isomer ratio (70:30) as the one obtained in the previously described synthetic pathway [2].



Scheme 2.

To get an insight into the influence of the chain length on the biological activity, an ornithyl analogue **3** was prepared using a similar procedure as the one described above for compound **A** (Scheme 2). The required *N*- α -Boc-L-ornithine was prepared by hydrogenolysis of the commercially available *N*- α -Boc-*N*- δ -benzyloxycarbonyl-L-ornithine [14]. In this case, after the coupling reaction, the final deprotection step of the α -amino function was carried out in the presence of HCl gas generated from concentrated sulphuric acid and sodium chloride. Compound **3** was obtained as a mixture of *Z/E* isomer in a 65:35 ratio. This ratio was determined from the ^1H NMR spectrum on the signal of the vinylic protons, respectively, at 7.93 and 7.37 ppm. Unfortunately, due to purification problems the coupling/deprotection sequence was performed in only 29% yield of pure isolated **3**. Accordingly, we decided to attempt another approach for the preparation of the bromo-analogue **6**, involving the 5-bromo-3-ethoxymethyleneindolin-2-one (**5**) instead of the dimethylaminomethylene intermediate [7]. 5-Bromoindolin-2-one (**4**) was obtained by bromination of the corresponding indolin-2-one as previously described [15]. The required 5-bromo-3-ethoxymethyleneindolin-2-one (**5**) was obtained as a single isomer in 75% yield by heating the 5-bromoindolin-2-one (**4**) in the presence of triethylorthoformate in acetic acid. The *E* configuration of the exocyclic double bond was determined from the value of the long range ^1H - ^{13}C coupling constant between the vinylic proton and the ^{13}C nucleus of the carbonyl function. Indeed, the configuration of the double bond of α,β -unsaturated carbonyl compounds can be determined on the basis of this long range 3J (^1H , $^{13}\text{C}=\text{O}$) heteronuclear coupling constant. The $^3J(\text{H},\text{C})$ of ^1H and $^{13}\text{C}=\text{O}$ nuclei with a *cis* orientation with respect to the double bond are usually smaller (2–6 Hz) than those with a *trans* orientation (8–12 Hz) [16]. In our case, the $^3J(\text{H},\text{C})$ value of 4 Hz indicated a *cis* orientation of ^1H and ^{13}C nuclei corresponding to the *E* configuration of the exocyclic double bond.

Compound **6** was then obtained in 68% yield by coupling **5** with *N*- α -Boc-L-ornithine in CH_2Cl_2 followed by deprotection of the α -amino function with HCl gas as described for compound **3**. Since the signals of the vinylic protons were superimposed on other signals, compound **6** *Z/E* isomeric ratio (66:34) was determined from the ^1H NMR spectrum on the signal of H_6 protons, respectively, at 7.00 and 7.07 ppm.

Then we planned to use this method for optimizing the preparation of compound **3**. Unfortunately, when indolin-2-one (**1**) was treated with triethylorthoformate in acetic acid, 3-ethoxymethyleneindolin-2-one was obtained in a poor yield excluding further use of this synthetic intermediate.

For the preparation of compound **8**, the brominated analogue of **A**, both approaches were carried out. Unfortunately, compound **8** could not be isolated when the coupling/deprotection sequence was performed with the ethoxymethylene intermediate **5** and *N*- α -Boc-L-lysine. Therefore, compound **8** had to be prepared *via* the corresponding *N,N*-dimethylaminomethylene intermediate. So, compound **4**, by reaction with DMF-DMA in toluene gave the dimethylaminomethylene derivative **7** in 72% yield as a mixture of *Z/E* isomers in a 73:27 ratio. The *Z/E* isomeric ratio was determined from the ^1H NMR

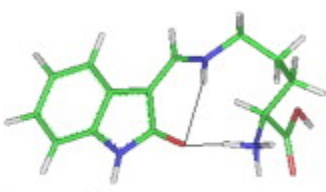
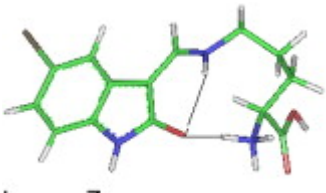
spectrum on the signal of the H₆ protons, respectively, at 6.95 and 7.05 ppm. The exocyclic double bond configuration for the two isomers of compound **7** was determined on the basis of the ¹H–¹³C coupling constant between the vinylic proton and the ¹³C nucleus of the carbonyl group as described for compound **5**. For compound **7** isomeric mixture, a coupling constant value of 9 Hz was determined for the major isomer and of 5 Hz for the minor isomer. Therefore, the major isomer had a *Z* configuration, as previously described for its analogue **2**.

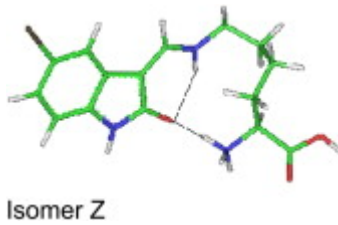
The coupling step between compound **7** and *N*- α -Boc-L-lysine was carried out with HCl in ethanol to lead, after removal of the *tert*-butoxycarbonyl protective group, to compound **8** in 38% yield. Compound **8** was obtained as a mixture of *Z/E* isomer in a 69/31 ratio. This ratio was determined from the ¹H NMR spectrum on the signal of the H₆ protons, respectively, at 6.99 and 7.06 ppm.

To shorten the side chain length, we also tried the reaction between *N*- α -Boc-2,4-diaminobutyric acid and 3-(*N,N*-dimethylaminomethylene)indolin-2-one (**2**). Nevertheless, despite several trials, we never obtained the attempted product.

The *Z/E* isomeric ratios for compounds **3**, **6** and **8** were also evaluated by SAM1 [17] quantum semi-empirical calculations, using Ampac8.0 program [18], on both isomers' global minima geometries which were found previously by conformational analysis using Monte-Carlo Multiple Method [19] with MM3 force field [20] of Macromodel 7.0 program [21]. The comparison of the *E_a* activation energy (energy difference between the reactants and the transition state) of *Z* and *E* isomers for compounds **3**, **6** and **8** showed that the *Z* configuration should be the major one (Table 1). As it was previously described for compound **A**, the results showed that, for these three compounds, the *Z* isomer was stabilized by an intramolecular hydrogen bond between the NH hydrogen and the oxygen of the oxindole carbonyl group and that the folding of the flexible side chain was stabilized by an additional hydrogen bond between the α -amino function and the oxygen atom of the oxindole moiety (Table 1).

Table 1.
Computational results of *Z/E* isomers for compounds **3**, **6** and **8**

	3Z	3E	
<i>E_a</i> activation energy (kcal mol ⁻¹)	26.0	34.0	 Isomer Z
<i>E_a</i> (<i>E</i>) – <i>E_a</i> (<i>Z</i>) (kcal mol ⁻¹)	8.0		
Theoretical ratio <i>Z/E</i>	99.9	0.1	
Experimental ratio <i>Z/E</i>	65	35	
	6Z	6E	
<i>E_a</i> activation energy (kcal mol ⁻¹)	30.0	38.0	 Isomer Z
<i>E_a</i> (<i>E</i>) – <i>E_a</i> (<i>Z</i>) (kcal mol ⁻¹)	8.0		
Theoretical ratio <i>Z/E</i>	99.9	0.1	
Experimental ratio <i>Z/E</i>	66	34	
	8Z	8E	

	3Z	3E	
E_a activation energy (kcal mol ⁻¹)	23.0	26.0	 Isomer Z
$E_a(E) - E_a(Z)$ (kcal mol ⁻¹)	3.0		
Theoretical ratio Z/E	99.0	1.0	
Experimental ratio Z/E	69	31	

Intramolecular hydrogen bonds are represented by grey lines.

3. Results and discussion

3.1. Kinase inhibition

The in vitro inhibitory potencies of compounds **A**, **3**, **6** and **8** were tested toward a panel of eight kinases. KDR, IGF-1R, c-Met, Ret, Src, Abl, and PKA were tested using the same method as the one described by Traxler et al. with AEE788 as control [22] whereas CDK2/Cyclin A assay was performed as previously described [23].

Compounds **A**, **3** and **8** did not inhibit the kinases tested ($IC_{50} > 10 \mu M$) whereas compound **6** was active toward CDK2 with an IC_{50} value of $6.6 \mu M$. Compound **6** was inactive toward the other kinases tested ($IC_{50} > 10 \mu M$).

3.2. Antimicrobial properties

The antimicrobial activities of compounds **A**, **3**, **6** and **8** were tested against two Gram-positive bacteria (*B. cereus* and *S. chartreusis*), a Gram-negative bacterium (*E. coli*) and a yeast (*C. albicans*) (Table 2). Ampicillin and Amphotericin B were used as a positive control for the bacterial strains and the yeast, respectively. The most efficient was compound **3** bearing an ornithyl side chain. This compound inhibited clearly the growth of the Gram-positive bacteria tested, particularly that of *S. chartreusis*. Moreover compound **3** inhibited weakly the growth of *E. coli* and *C. albicans*. Compound **6**, the brominated analogue of **3**, was only active on the Gram-positive bacteria tested. Compounds **A** and **8**, substituted in the 3-position by a lysine residue, had similar inhibition profiles: they both inhibited the growth of all the microorganisms tested except *E. coli*. Therefore, these preliminary results indicate that the presence of a side chain containing three methylene groups seems to be favourable to the antimicrobial activities of these compounds.

Table 2.

Antimicrobial activities of compounds **A**, **3**, **6**, **8** (300 μg per disk) against two Gram-positive bacteria (*B. cereus* and *S. chartreusis*), a Gram-negative bacterium (*E. coli*) and a yeast (*C. albicans*)

Cpds	<i>B. cereus</i>	<i>S. chartreusis</i>	<i>E. coli</i>	<i>C. albicans</i>
	ATCC 14579	NRRL 11407	ATCC 11303	IP 444
Controls	Amp. +++++	Amp. >++++ (25)	Amp. >++++ (31)	Amph. B. +++++
A	±	±	–	±
3	+	+++	±	±
6	+	++	–	–
8	±	+	–	±

The size of zones of growth inhibition was 13–16 mm (++++), 10–12 mm (+++), 8–9 (++) , 7–8 mm (+), 6–7 mm (\pm); (-): inactive. For the positive controls: Amp. (ampicillin), Amphotericin B (Amph. B.); the size of zones of growth inhibition (mm) are indicated in brackets.

In conclusion, indolin-2-one derivatives substituted in the 3-position by an aminomethylene group bearing either an ornithine or a lysine residue were synthesized using different synthetic approaches requiring either the corresponding 3-(*N,N*-dimethylaminomethylene)indolin-2-one or 3-ethoxymethyleneindolin-2-one intermediates. The methods described here are compatible with the presence of a bromine atom in the 5-position. Furthermore, these synthetic approaches are more efficient than the one previously described via the chloromethylene intermediate.

The biological results have shown that compounds **3** and **6** were the most promising, indicating that the presence of an ornithine residue is favourable for the biological activities in this series. The preparation of various analogues of compounds **3** and **6**, diversely substituted on the aromatic ring, is currently under investigation, especially to improve their mild inhibitory potencies, compared to that of known CDK2 inhibitors (e.g.: IC₅₀ roscovitine CDK2/cyclinA = 0.7 μ M).

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Perkin–Elmer Paragon 500 spectrometer (ν in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 (¹H: 400 MHz, ¹³C: 100 MHz) or AVANCE 500 (¹H: 500 MHz, ¹³C: 125 MHz), chemical shifts δ in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), quadruplet (q), doubled doublet (dd), sextuplet (sext), multiplet (m), broad signal (br s). When necessary to identify all carbon atoms, complementary NMR experiments (HSQC, HMBC) were performed on a Bruker AVANCE 500. Mass spectra (ES) were determined on a high resolution Waters Micro Q-toff apparatus. Chromatographic purifications were performed by flash silica gel Geduran SI 60 (Merck) 0.040–0.063 mm column chromatography. For purity tests, TLC were performed on fluorescent silica gel plates (60 F254 from Merck).

4.1.1. (*Z/E*)-(2*S*)-2-amino-6-([(2-oxo-indolin-3-ylidene)methyl]amino)hexanoic acid, hydrochloride (**A**)

To a suspension of (*Z/E*)-3-(*N,N*-dimethylaminomethylene)indolin-2-one (**2**) (100 mg, 0.531 mmol) in ethanol (1.8 mL) were added 37% hydrochloric acid (44 μ L, 0.531 mmol) and *N*-(α)-Boc-l-lysine (138 mg, 0.560 mmol). The mixture was refluxed for 36 h. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate then ethyl acetate/methanol 98:2 to 7:3) to give the coupling product (167 mg) as a grey solid. Ethyl acetate (2 mL) was added to the residue (31.6 mg) and 3 M aqueous hydrochloric acid (1.26 mL) was added. The mixture was stirred at 50 °C for 4 h and then, after evaporation, the residue was precipitated in a minimum of diethylether. Elimination of the liquid phase led to compound **A** (25.6 mg, 0.0785 mmol, 78% yield for the two steps) as a grey solid.

4.1.2. (*Z/E*)-(2*S*)-2-amino-5-([(2-oxo-indolin-3-ylidene)methyl]amino)pentanoic acid, hydrochloride (**3**)

To a suspension of (*Z/E*)-3-(*N,N*-dimethylaminomethylene)indolin-2-one (**2**) (156 mg, 0.83 mmol) in ethanol (3 mL) were added 37% hydrochloric acid (69 μ L, 0.83 mmol) and *N*-(α)-Boc-l-ornithine (231 mg, 0.995 mmol). The mixture was stirred at 50 °C for 48 h. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate then ethyl acetate/methanol 98:2 to 8:2). Dichloromethane (3 mL) was added to the residue and the mixture was stirred at room temperature for 1.5 h while bubbling HCl through the mixture. Solvent was evaporated in an argon flux and the residue was precipitated in a minimum of diethylether. Elimination of the liquid phase led to compound **3** (74.1 mg, 0.238 mmol, 29% yield for the two steps) as a grey solid. HRMS (ES⁻) calcd for C₁₄H₁₆N₃O₃ (M – H)⁻ 274.1192, found 274.1192. IR (KBr): 3420, 1740, 1665, 1620. NMR of the major *Z* isomer: ¹H NMR (400 MHz, DMSO-*d*₆): 1.59–1.92 (m, 4H, CH₂), 3.31–3.42 (m, 2H, CH₂), 3.83–3.91 (m, 1H, C α H), 6.73–6.89 (m, 3H), 7.27 (d, 1H, *J* = 7.0 Hz), 7.93 (d, 1H, *J* = 13.0 Hz, CH_{vinyl}), 8.35–8.69 (br s, 3H, NH₃Cl), 8.70–8.79 (m, 1H, NH), 10.20 (s, 1H, NH_{indole}). The signal of the carboxylic acid proton is not

visible on the spectrum. ^{13}C NMR (100 MHz, DMSO- d_6): 26.4, 27.0, 47.5 (CH_2), 51.6 (C_αH), 108.6, 115.0, 119.7, 122.0, 147.8 (CH), 94.5, 125.3, 135.6 (C), 169.5, 170.8 ($\text{C}=\text{O}$).

4.1.3. (*E*)-5-bromo-3-ethoxymethyleneindolin-2-one (**5**)

A suspension of 5-bromooxindole (**4**) (1.05 g, 4.95 mmol) in acetic acid (20 mL) was warmed until complete dissolution and triethylorthoformate (8.5 mL, 51.1 mmol) was added at this temperature. The reaction mixture was stirred at reflux for 5 h. The solvent was removed under reduced pressure and the residue was co-evaporated twice with toluene. The residue was dissolved in warm toluene (20 mL), cyclohexane was added (400 mL) and the solution was left to precipitate for 12 h. The precipitate was collected by filtration to give **5** (997 mg, 3.72 mmol, 75% yield) as a yellow powder. Mp 180–182 °C. IR (KBr): 3440, 1700, 1680, 1650, 1625. HRMS (ES+) calcd for $\text{C}_{11}\text{H}_{11}^{79}\text{BrNO}_2$ ($\text{M} + \text{H}$)⁺ 267.9973, found 267.9967. ^1H NMR (400 MHz, DMSO- d_6): 1.37 (3H, t, $J = 7.0$ Hz), 4.40 (2H, q, $J = 7.0$ Hz), 6.77 (1H, d, $J = 8.0$ Hz), 7.27 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz), 7.52 (1H, d, $J = 2.0$ Hz), 7.74 (1H, s), 10.36 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO- d_6): 15.4 (CH_3), 72.1 (CH_2), 110.8, 124.2, 129.1, 158.3 (CH), 106.2, 112.5, 138.4 (C), 168.9 ($\text{C}=\text{O}$).

4.1.4. (*Z/E*)-(2*S*)-2-amino-5-([(5-bromo-2-oxo-indolin-3-ylidene)methyl]amino)pentanoic acid, hydrochloride (**6**)

To a suspension of (*E*)-5-bromo-3-ethoxymethyleneindolin-2-one (**5**) (500 mg, 1.86 mmol) in dichloromethane (10 mL) was added *N*-(α)-Boc-l-ornithine (269 mg, 1.16 mmol). The mixture was stirred at reflux for one night. The solvent was evaporated and a mixture CH_2Cl_2 /cyclohexane 1:1 was added to the residue before precipitation with a few drops of diethylether. The precipitate was filtered off to give the coupling product (415 mg) as a yellow-orange solid. Dichloromethane (8 mL) was added to the residue (227 mg) and the mixture was stirred at room temperature for 1.5 h while bubbling HCl through the mixture. Solvent was evaporated in an argon flux and the residue was precipitated in a minimum of diethylether. Elimination of the liquid phase led to compound **6** (168 mg, 0.43 mmol, 68% yield for the two steps) as a yellow-orange solid. HRMS (ES⁻) calcd for $\text{C}_{14}\text{H}_{15}^{79}\text{BrN}_3\text{O}_3$ ($\text{M} - \text{H}$)⁻ 352.0297, found 352.0307. IR (KBr): 3000–3600, 1737, 1654. NMR of the major *Z* isomer: ^1H NMR (400 MHz, DMSO- d_6): 1.59–1.91 (m, 4H, CH_2), 3.33–3.44 (m, 2H, CH_2), 3.89–3.98 (m, 1H, C_αH), 6.73 (d, 1H, $J = 8.0$ Hz), 7.00 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz), 7.48 (d, 1H, $J = 1.5$ Hz), 8.06 (d, 1H, $J = 13.5$ Hz, CH_{vinyl}), 8.38–8.53 (br s, 3H, NH_3Cl), 8.82–8.92 (m, 1H, NH), 10.32 (s, 1H, $\text{NH}_{\text{indole}}$). The signal of the carboxylic acid proton is not visible on the spectrum. ^{13}C NMR (100 MHz, DMSO- d_6): 26.2, 26.9, 47.7 (CH_2), 51.5 (CH), 110.3, 117.5, 124.0, 149.1 (CH), 93.7, 112.0, 127.9, 134.5 (C), 169.2, 170.9 ($\text{C}=\text{O}$).

4.1.5. (*Z/E*)-5-bromo-3-(*N,N*-dimethylaminomethylene)indolin-2-one (**7**)

DMF–DMA (2.66 mL, 20.0 mmol) was added to a solution of 5-bromooxindole (**4**) (2.12 g, 10.0 mmol) in toluene (40 mL). The mixture was stirred for 48 h, the precipitate was collected by filtration and washed with dichloromethane to give **7** (1.91 g, 7.2 mmol, 72% yield) as a beige solid. HRMS (ES+) calcd for $\text{C}_{11}\text{H}_{12}^{79}\text{BrN}_2\text{O}$ ($\text{M} + \text{H}$)⁺ 267.0133, found 267.0132. IR (KBr): 3445, 1675, 1615, 1590. NMR of the major *Z* isomer: ^1H NMR (400 MHz, DMSO- d_6): 3.23–3.31 (br s, 3H), 3.61–3.69 (br s, 3H), 6.62 (d, 1H, $J = 8.0$ Hz), 6.95 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz), 7.48 (d, 1H, $J = 1.5$ Hz), 7.67 (s, 1H), 10.07 (s, 1H). ^{13}C NMR (100 MHz, DMSO- d_6): 42.3, 46.5 (CH_3), 109.3, 116.8, 123.5, 149.0 (CH), 92.1, 111.7, 131.4, 134.5 (C), 165.6 ($\text{C}=\text{O}$).

4.1.6. (*Z/E*)-(2*S*)-2-amino-6-([(5-bromo-2-oxo-indolin-3-ylidene)methyl]amino)hexanoic acid, hydrochloride (**8**)

To a mixture of (*Z/E*)-5-bromo-3-(*N,N*-dimethylaminomethylene)indolin-2-one (**7**) (57 mg, 0.21 mmol) in ethanol (2 mL) were added 37% hydrochloric acid (18 μL , 0.21 mmol) and *N*-(α)-Boc-l-lysine (64 mg, 0.26 mmol). The mixture was refluxed for 48 h. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate/methanol 9:1). The residue was dissolved in ethyl acetate, precipitated with cyclohexane, filtered and washed with dichloromethane. Diethylether (3 mL) was added to the residue and the mixture was stirred at room temperature for 1.5 h while bubbling HCl through the mixture. The precipitate was collected by filtration under argon, washed with dichloromethane to give **8** (33 mg, 0.082 mmol, 38% yield) as a black solid. HRMS (ES+) calcd for $\text{C}_{15}\text{H}_{19}^{79}\text{BrN}_3\text{O}_3$ ($\text{M} + \text{H}$)⁺

368.0610, found 368.0607. IR (KBr): 3410, 1735, 1665, 1615. NMR spectra of the major *Z* isomer: ¹H NMR (400 MHz, DMSO-*d*₆): 1.30–1.52 (m, 2H, CH₂), 1.53–1.67 (m, 2H, CH₂), 1.77–1.89 (m, 2H, CH₂), 3.30–3.50 (m, 2H, CH₂), 3.84–3.93 (m, 1H, C_αH), 6.72 (d, 1H, *J* = 8.0 Hz), 6.99 (d, 1H, *J* = 7.5 Hz), 7.48 (s, 1H), 8.04 (d, 1H, *J* = 13.5 Hz), 8.19–8.50 (br s, 3H, NH₃Cl), 8.82–8.93 (m, 1H), 10.24–10.34 (br s, 1H, NH). The signal of the carboxylic acid proton is not visible on the spectrum. ¹³C NMR (125 MHz, DMSO-*d*₆): 21.2, 29.4, 29.9, 47.9 (CH₂), 51.7 (C_αH), 110.1, 117.3, 123.7, 149.0 (CH), 93.4, 111.8, 127.8, 134.3 (C), 169.1, 170.8 (C=O).

4.2. Kinases' inhibition

The in vitro kinase assays were performed in 96-well plates (30 μl) at ambient temperature for 15–45 min using the recombinant glutathione *S*-transferase-fused kinase domains (4–100 ng, depending on specific activity) prepared previously [24] and [25]. [³³P]ATP was used as phosphate donor and polyGluTyr-(4:1) peptide as acceptor. With the exception of protein kinase A for which the heptapeptide Leu-Arg-Arg-Ala-Ser-Leu-Gly (known as Kemptide Bachem; Bubendorf, Switzerland) was used as peptide substrate. Assays were optimized for each kinase using the following ATP concentrations: 1.0 μM (c-Met and RET), 5.0 μM (c-Abl), 8.0 μM (KDR, IGF-1R) and 20.0 mM (c-Src and PKA). The reaction was terminated by the addition of 20 μl 125 mM EDTA. Thirty μl (c-Abl, c-Src, IGF-1R, RET) or 40 μl (all other kinases) of the reaction mixture was transferred onto Immobilon-polyvinylidene difluoride membrane (Millipore, Bedford, MA), pre-soaked with 0.5% H₃PO₄ and mounted on a vacuum manifold. Vacuum was then applied and each well rinsed with 200 μl 0.5% H₃PO₄. Membranes were removed and washed four times with 1.0% H₃PO₄ and once with ethanol. Dried membranes were counted after mounting in a Packard TopCount 96-well frame and with the addition of 10 μl/well of Microscint.

For RET kinase assay, either glutathione *S*-transferase-wild-type RET (15 ng) or glutathione *S*-transferase-RET-Men2B protein (15 ng) were used.

The CDK2/Cyclin A assay was performed as previously described [23].

4.3. Antibioassay tests

Four strains were tested, two Gram-positive bacteria (*B. cereus* ATCC 14579, *S. chartreusis* NRRL 11407), a Gram-negative bacterium (*E. coli* ATCC 11303) and a yeast (*C. albicans* 444 from the Pasteur Institute, Paris). The antimicrobial activity was determined by the conventional paper disk (Durieux No. 268; 6 mm in diameter) diffusion method using the following nutrient media: Mueller–Hinton (Difco) for *B. cereus* and *E. coli*, Sabouraud agar (Difco) for *C. albicans* and Emerson agar (0.4% beef extract, 0.4% peptone, 1% dextrose, 0.25% NaCl, 2% agar, pH 7.0) for the *Streptomyces* strains. Paper disks impregnated with solutions in DMSO (300 μg of drug per disk) were placed on Petri dishes. Growth inhibition was examined after 24 h incubation at 27 °C.

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References

- [1] S. Messaoudi, M. Sancelme, V. Polard-Housset, B. Aboab, P. Moreau and M. Prudhomme, *Eur. J. Med. Chem.* **39** (2004), pp. 453–458.
- [2] M. Sassatelli, E. Debiton, B. Aboab, M. Prudhomme and P. Moreau, *Eur. J. Med. Chem.* **41** (2006), pp. 709–716.
- [3] A.D. Laird and J.M. Cherrington, *Expert Opin. Investig. Drugs* **12** (2003), pp. 51–64.
- [4] L. Sun, C. Liang, S. Shirazian, Y. Zhou, T. Miller, J. Cui, J.Y. Fukuda, J.-Y. Chu, A. Nematalla, X. Wang, H. Chen, A. Sistla, T.C. Luu, F. Tang, J. Wei and C. Tang, *J. Med. Chem.* **46** (2003), pp. 1116–1119.
- [5] A. Andreani, A. Cavalli, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, M. Recanatini, M. Garnier and L. Meijer, *Anticancer Drug Design* **15** (2000), pp. 447–452.
- [6] D.J. Moshinsky, C.R. Bellamacina, D.C. Boisvert, P. Huang, T. Hui, J. Jancarik, S.-H. Kim and A.G. Rice, *Biochem. Biophys. Res. Commun.* **310** (2003), pp. 1026–1031.
- [7] H. Neal Bramson, J. Corona, S.T. Davis, S.H. Dickerson, M. Edelstein, S.V. Frye, R.T. Gampe Jr., P.A. Harris, A. Hassell, W.D. Holmes, R.N. Hunter, K.E. Lackey, B. Lovejoy, M.J. Luzzio, V. Montana, W.J. Rocque, D. Rusnak, L. Shewchuk, J.M. Veal, D.H. Walker and L.F. Kuyper, *J. Med. Chem.* **44** (2001), pp. 4339–4358.
- [8] A.H. Abadi, S.M. Abou-Seri, D.E. Abdel-Rahman, C. Klein, O. Lozach and L. Meijer, *Eur. J. Med. Chem.* **41** (2006), pp. 296–305.
- [9] S.K. Sridhar, M. Saravanan and A. Ramesh, *Eur. J. Med. Chem.* **36** (2001), pp. 615–625.

- [10] M.N.S. Saudi, M.M.A. El Smary and G. El Sawaf, *Pharmazie* **57** (2002), pp. 519–522.
- [11] A. Amal Raj, R. Raghunathan, M.R. Sridevikumari and N. Raman, *Bioorg. Med. Chem.* **11** (2003), pp. 407–419.
- [12] M. Abass and B.B. Mostafa, *Bioorg. Med. Chem.* **13** (2005), pp. 6133–6144.
- [13] M.S. Morales-Rios, M. Garcia-Velgara, H. Cervantes-Cuevas, C. Alvarez-Cisneros and P. Joseph-Nathan, *Magn. Reson. Chem.* **38** (2000), pp. 172–176.
- [14] P.A. Wender, T.C. Jessop, K. Pattabiraman, E.T. Pelkey and C.L. VanDeusen, *Org. Lett.* **3** (2001), pp. 3229–3232.
- [15] F. Bouchikhi, F. Anizon and P. Moreau, *Eur. J. Med. Chem.* (2007)
- [16] B. Stanovnik and J. Svete, *Chem. Rev.* **104** (2004), pp. 2433–2480.
- [17] M.J.S. Dewar, C. Jie and J. Yu, *Tetrahedron* **49** (1993), pp. 5003–5038.
- [18] Ampac8.0, © 2000 Semichem, 7128 Summit, Shawnee, KS 66216, USA.
- [19] G. Chang, C.G. Wayne and W.C. Still, *J. Am. Chem. Soc.* **111** (1989), pp. 4379–4386.
- [20] N.L. Allinger, Y.H. Yuh and J.H. Lii, *J. Am. Chem. Soc.* **111** (1989), pp. 8551–8566.
- [21] Macromodel 7.0, Shroedinger Inc, 1500 SW First Ave. Suite 1180, Portland, OR 97201, USA.
- [22] P. Traxler, P.R. Allegrini, R. Brandt, J. Brueggen, R. Cozens, D. Fabbro, K. Grosios, H.A. Lane, P. McSheehy, J. Mestan, T. Meyer, C. Tang, M. Wartmann, J. Wood and G. Caravatti, *Cancer Res.* **64** (2004), pp. 4931–4941.
- [23] R. Soni, H. Fretz, L. Muller, J. Schoepfer and B. Chaudhuri, *Biochem. Biophys. Res. Commun.* **272** (2000), pp. 794–800.
- [24] J.M. Wood, G. Bold, E. Buchdunger, R. Cozens, S. Ferrari, J. Frei, F. Hofmann, J. Mestan, H. Mett, T. O'Reilly, E. Persohn, J. Rösel, C. Schnell, D. Stover, A. Theuer, H. Towbin, F. Wenger, K. Woods-Cook, A. Menrad, G. Siemeister, M. Schirmer, K.-H. Thierauch, M.R. Schneider, J. Drevs, G. Martiny-Baron, F. Totzke and D. Marmé, *Cancer Res.* **60** (2000), pp. 2178–2189.
- [25] G. Bold, K.-H. Altmann, J. Frei, M. Lang, P.W. Manley, P. Traxler, B. Wietfeld, J. Brügggen, E. Buchdunger, R. Cozens, S. Ferrari, P. Furet, F. Hofmann, G. Martiny-Baron, J. Mestan, J. Rösel, M. Sills, D. Stover, F. Acemoglu, E. Boss, R. Emmenegger, L. Lässer, E. Masso, R. Roth, C. Schlachter, W. Vetterli, D. Wyss and J.M. Wood, *J. Med. Chem.* **43** (2000), pp. 2310–2323
- J. Med. Chem.* **43** (2000), p. 3200.