Journal of Enzyme Inhibition and Medicinal Chemistry, October 2007; 22(5): 577–583

Synthesis and evaluation of the antimicrobial activity of novel quinazolinones

MARIA DE FATIMA PEREIRA, ROMAIN CHEVROT, ERIC ROSENFELD, VALERIE THIERY, & THIERRY BESSON

Laboratoire de Biotechnologies et Chimie Bio-organique, FRE CNRS 2766, UFR Sciences Fondamentales et Sciences pour l'Ingénieur, Bâtiment Marie Curie, Université de la Rochelle, F-17042 La Rochelle cedex 1, France

(Received 30 October 2006; accepted 10 January 2007)

Abstract
A simple and efficient microwave-assisted methodology for regioselective alkylation of exocyclic nitrogen of cyclic amidines was developed and novel N-alkylated 3,4-dihydropyrazino[2,1-b]quinazolin-6-ones were prepared. Although none of the molecules tested have any specific anti-quorum sensing (–QS) activity, our result validates the growth tests devised to control the bias of the anti-QS tests. Among the molecules studied, compound 2b exhibits interesting activity against the Gram-negative bacteria Escherichia coli and Shigella sonnei.

Keywords: *Quinazolinones, microwave-assisted chemistry, quorum sensing, anti-microbial activity*

Introduction

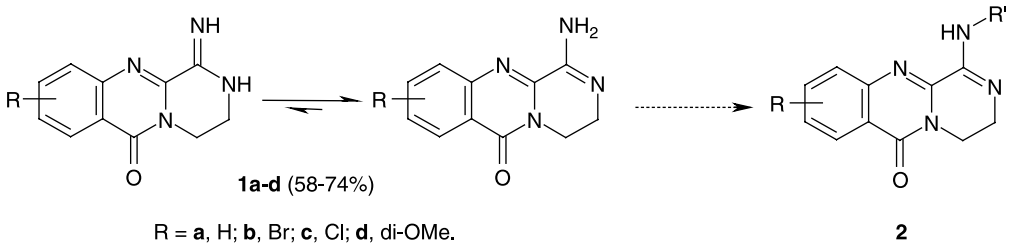
The selective pressure exerted by antibiotic treatments has made many pathogenic bacteria multi-resistant to common antibiotics. New drugs are needed to resolve this serious public health problem. Searching for new biological targets, a special attention was recently paid to the bacterial communication system named *quorum* sensing (QS). The bacteria that possess a QS system release in their environment signal molecules called autoinducers. When a threshold autoinducer concentration is reached, all the producing bacteria trigger collectively the expression of target genes responsible for the production of virulence factors essential for the pathogenicity [1–3]. Moreover, QS is dispensable for life functions, and it is well admitted that its specific inhibition does not implicate the development of bacterial resistances [4]. Molecules that inhibit QS have been called anti-pathogenic drugs since they can disturb the production of the virulence factors [5]. Recently, synthetic agents derived from algal halogenated furanones were shown to affect *Pseudomonas aeruginosa* pathogenicity in this way [6,7,8].

The occurrence of the quinazoline skeleton in various natural and synthetic products has generated interest of many groups because of their useful biological properties [9]. As a part of our ongoing research program dealing with the preparation and pharmacological evaluation of some original quinazoline derivatives [10–14], we recently described novel 3,4-dihydropyrazino[2,1-*b*]quinazolin-6-one derivatives (**1a–d**). During this preliminary work, we unambiguously established the 3D structure of compounds **1** and we confirmed the amidine isomerization in the solid state by X-ray crystallography.

After a rapid evaluation of these novel molecules on QS, it was suggested that compound **1a** might possess a moderate anti-QS activity and that modification on the benzenic moiety of the quinazolinone ring seems to have no real incidence on the expected activity. Considering this 2,3-condensed quinazolin-4-one as a possible lead compound, we focused our efforts on the synthesis of various *N*-substituted amidines (*e.g.* 2 in Scheme 1) in which the substituent will be present specifically on the exogenic nitrogen atom.

Correspondence: T. Besson, UMR CNRS 6014, Laboratoire de Chimie Pharmaceutique, UFR Médecine-Pharmacie, Université de Rouen, 22 Boulevard Gambetta, 76183 Rouen cedex 1. Tel: 33 023 5148399. Fax: 33 023 5148423. E-mail: thierry.besson@univ-rouen.fr

ISSN 1475-6366 print/ISSN 1475-6374 online © 2007 Informa UK Ltd.
DOI: 10.1080/14756360701425345



Scheme 1. Structure of quinazolinones.

In the present work, we describe the synthetic route to the expected tricyclic compounds (3) and we first examine their anti-QS activities by optimising the protocol described by McLean et al [15]. We used three model Gram-negative bacteria that synthesise distinct acylated homoserine lactones (HSL) as autoinducer molecules: the opportunistic pathogen *Chromobacterium violaceum* synthesises a *N*-hexanoyl-homoserine lactone (C6-HSL) [16], the plant pathogen *Agrobacterium tumefasciens* a *N*-octanoyl-homoserine lactone (C8-HSL) [17], and the opportunistic pathogen *P. aeruginosa* two different HSLs, a *N*-butyrylhomoserine lactone (C4-HSL) and a *N*-3-oxododecanoyl-homoserine lactone (C12-oxo-HSL) [18]. Using our methodology, we are able to discriminate between the specific anti-QS activities and growth inhibitions. Finally, we characterised the anti-microbial properties of one quinazolinone derivative of interest.

Materials and methods

Chemistry

Instrumentation. Commercial reagents were used as received without additional purification. Melting points were determined using a Kofler melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer Paragon 1000PC instrument. ^{1}H and ^{13}C-NMR were recorded using a JEOL NMR LA400 (400 MHz) spectrometer (Centre Commun d'Analyses, Université de la Rochelle). Chemical shifts (δ) were reported in part per million (ppm) downfield from tetramethylsilane (TMS) which was used as internal standard. Coupling constants J are given in Hz. The mass spectra (HRMS) were recorded on a Varian MAT311 spectrometer in the "Centre Régional de Mesures Physiques de l'Ouest" (CRMPO), Université de Rennes. Column chromatography experiments were performed by using Merck silica gel (70–230 mesh) at medium pressure. Light petroleum refers to the fraction boiling point 40–60°C. Other solvents were used without purification. Analytical thin layer chromatography (T.L.C.) was performed on Merck Kieselgel 60 F254 aluminium backed plates. Focused microwave irradiations were carried out with a Smith-Synthetizer™ (Personal Chemistry, AB) focused microwave reactor (300 W, 2450 MHz, monomode system). The Smith-Synthetizer™ (Personal Chemistry, AB) was a single mode cavity, producing controlled irradiation at 2450 MHz [19]. Reaction temperature and pressure were determined using the built-in, on-line IR and pressure sensors. Microwave-assisted reactions were performed in sealed Smith process vials (0.5–5 mL, total volume 10 mL) under air with magnetic stirring. The microwave output power was regulated by the software algorithm so that the selected maximum temperature was maintained for the desired reaction/irradiation time. After the irradiation period, the reaction vessel was cooled rapidly to ambient temperature by compressed air (gas-jet cooling). The minimal reaction times were determined by performing sequential series of identical reactions at constant temperature and with continuous heating, but with different irradiation times. Completion of the reaction was estimated by T.L.C. after each individual heating period.

Synthesis of 3,4-dihydropyrazino[2,1-b]quinazolin-6-one derivatives. Spectral data for compound **1a** are consistent with results published in reference 14.

Synthesis of N-substituted quinazolinone derivatives. To a stirred suspension of quinazolinone **1a** (0.2 mmol) and PTSA (0.22 mmol) in THF (3 mL) was added dropwise 0.4 mmol of alkylating agent. The mixture was irradiated for 5 min in a sealed tube. The irradiation was programmed to obtain a constant temperature (150°C). The solvent was removed under reduced pressure. Products **2a–e** were obtained after purification by column chromatography with dichloromethane/methanol (99/1) as eluent.

*1-Propylamino-3,4-dihydropyrazino[2,1-*b*]quinazolin-6-one (2a)*. This compound was prepared from 1a.Yield: 95%, white solid, mp (84°C. (Found M^+: 256.1323, $C_{14}H_{16}N_4O$ requires 256.1324); υ_{max} (KBr)/cm^{-1} 772, 1473, 1670, 2876, 2966, 3409 (NH); ^{1}H-NMR δ (400 MHz, d_6-DMSO) 0.90 (t, 3H, J 7.40 Hz, CH_3), 1.53–1.65 (m, 2H, CH_2–CH_2–CH_3), 3.18–3.24 (m, 2H, NH–CH_2–CH_2), 3.64 (t, 2H, J 6.00 Hz, CH_2), 4.00 (t, 2H, J 6.00 Hz, CH_2), 6.80 (s, 1H, NH), 7.59 (t, 1H, J 7.60 Hz, H arom.),

7.75 (d, 1H, J 7.60 Hz, H arom.), 7.86 (t, 1H, J 7.60 Hz, H arom.), 8.16 (d, 1H, J 7.60 Hz, H arom.); δ_C (100 MHz, d_6-DMSO) 11.83; 22.04; 42.55; 43.18; 121.56; 126.62; 128.16; 128.40; 135.21; 140.83; 146.29; 150.34; 160.45.

*1-Isopropylamino-3,4-dihydropyrazino[2,1-*b*]quinazolin-6-one (2b)*. This compound was prepared from 1a. Yield: 60%, white solid, mp (91°C. (Found M^+: 256.1323, $C_{14}H_{16}N_4O$ requires 256.1324); υ_{max} (KBr)/cm^{-1} 692, 1243, 1474, 1671, 2972, 3398 (NH); ^{1}H-NMR δ (400 MHz, d_6-DMSO) 1.18 (d, 1H, 6.40 Hz, $2CH_3$), 3.64 (t, 2H, J 6.40 Hz, CH_2), 3.93–4.01 (m, 3H, J 6.40 Hz, CH_2 and NH), 6.46 (d, 1H, J 8.40 Hz, CH), 7.59 (t, 1H, J 7.60 Hz, H arom.), 7.78 (d, 1H, J 8.00 Hz, H arom.), 7.87 (t, 1H, J 8.00 Hz, H arom.), 8.16 (d, 1H, J 8.00 Hz, H arom.); δ_C (100 MHz, d_6-DMSO) 22.14; 38.38; 41.54; 43.09; 121.38; 126.28; 127.69; 127.74; 134.62; 140.50; 145.99; 148.82; 159.80.

*1-Butylamino-3,4-dihydropyrazino[2,1-*b*]quinazolin-6-one (2c)*. This compound was prepared from 1a. Yield: 81%, white solid, mp (71°C. (Found M^+: 270.1494, $C_{15}H_{18}N_4O$ requires 270.1481); υ_{max} (KBr)/cm^{-1} 774, 1330, 1682, 2860, 2925, 3421 (NH); ^{1}H-NMR δ (400 MHz, d_6-DMSO) 0.91 (t, 3H, J 7.10 Hz, CH_3), 1.25–1.40 (m, 2H, CH_2–CH_3), 1.50–1.60 (m, 2H, CH_2–CH_2–CH_2), 3.20–3.25 (m, 2H, NH–CH_2–CH_2), 3.60–3.65 (m, 2H, CH_2), 3.95–4.05 (m, 2H, CH_2), 6.75 (s, 1H, NH), 7.57 (t, 1H, J 8.00 Hz, H arom.), 7.73 (d, 1H, J 8.00 Hz, H arom.), 7.83 (t, 1H, J 8.00 Hz, H arom.), 8.11 (d, 1H, J 8.00 Hz, H arom.); δ_C (100 MHz, d_6-DMSO) 14.16; 20.24; 31.13; 38.88; 43.43; 121.76; 126.66; 128.10; 128.17; 135.03; 140.91; 146.42; 150.18; 160.23.

*1-Benzylamino-3,4-dihydropyrazino[2,1-*b*]quinazolin-6-one (2d)*. This compound was prepared from 1a. Yield: 66%, white solid, mp (110°C. (Found M^+: 304.1333, $C_{18}H_{16}N_4O$ requires 304.1324); υ_{max} (KBr)/cm^{-1} 692, 774, 1474, 1586, 1672, 2858, 2938, 3406 (NH); ^{1}H-NMR δ (400 MHz, d_6-$DMSOD_2O$) 3.65 (t, 2H, J 6.00 Hz, CH_2), 4.01 (t, 2H, J 6.00 Hz, CH_2), 4.48 (d, 2H, J 6.00 Hz, CH_2–NH), 7.18–7.13 (m, 1H, H arom.), 7.30 (t, 2H, J 7.60 Hz, H arom.), 7.36 (d, 2H, J 7.60 Hz, H arom.), 7.56–7.63 (m, 1H, H arom.), 7.76 (d, 1H, J 7.60 Hz, H arom.), 7.83–7.90 (m, 1H, H arom.), 8.17 (dd, 1H, J 8.20 Hz et J 1.20 Hz, H arom.); δ_C (100 MHz, d_6-DMSO + D_2O) 38.27; 42.97; 43.65; 121.33; 126.13; 126.38; 127.10; 127.51; 127.58; 127.99; 134.42; 139.90; 140.33; 145.91; 149.56; 159.62.

*1-[2-(1*H*-indol-3-yl)-ethylamino]- 3,4-dihydropyrazino[2,1-*b*]quinazolin-6-one (2e)*. This compound was prepared from 1a. Yield: 58%, white solid, mp = 238°C. (Found M^+: 357.1572, $C_{21}H_{19}N_5O$ requires 357.1589); υ_{max} (KBr)/cm^{-1} 1472, 1588, 1680, 2921, 3152, 3396 (NH); ^{1}H-NMR δ (400 MHz, $CDCl_3$) 3.16 (t, 2H, J 6.80 Hz, CH_2-indol), 3.68–3.76 (m, 2H, NH–CH_2), 3.82 (t, 2H, J 6.00 Hz, CH_2), 4.15 (t, 2H, J 6.00 Hz, CH_2), 6.47 (s, 1H, NH), 7.10–7.18 (m, 2H, H arom.), 7.12 (t, 1H, J 7.80 Hz, H arom.), 7.40 (d, 1H, J 8.00 Hz, H arom.), 7.53 (t, 1H, J 7.60 Hz, H arom.), 7.63 (d, 1H, J 8.00 Hz, H arom.), 7.70 (d, 1H, J 7.60 Hz, H arom.), 7.77 (t, 1H, J 7.60 Hz, H arom.), 8.53 (s, 1H, NH), 8.30 (d, 1H, J 8.00 Hz, H arom.).

*1-Phenylamino-3,4-dihydropyrazino[2,1-*b*]quinazolin-6-one (2f)*. In a sealed vial, a stirred mixture of quinazolinone (1a) (0.053 mg, 0.25 mmol) and aniline (0.50 mL, 0.45 mmol) in NMP (2 mL) was irradiated for 15 min. The irradiation was programmed to obtain a constant temperature (220°C). The solvent was removed under reduced pressure. The product was obtained by purification by column chromatography with dichloromethane/methanol (95/5) as eluent. Yield: 62%, white solid, mp (179°C. (Found ($M-H^+$),: 289.1084, $C_{17}H_{14}N_4O$ requires 289.1089); υ_{max} (KBr)/cm^{-1} 1328, 1536, 1683, 2938, 3055, 3356 (NH); ^{1}H-NMR δ (400 MHz, $CDCl_3$) 3.96 (t, 2H, J 5.80 Hz, CH_2), 4.23 (t, 2H, J 5.80 Hz, CH_2), 7.06 (t, 1H, J 7.10 Hz, H arom.), 7.36 (t, 2H, J 7.60 Hz, H arom.), 7.55–7.63 (m, 1H, H arom.), 7.75–7.87 (m, 4H, H arom.), 8.34 (d, 1H, J 8.00 Hz, H arom.), 8.53 (s, 1H, NH); δ_C (100 MHz, $CDCl_3$) 38.22; 43.96; 119.04; 121.72; 122.69; 127.04; 127.93; 128.16; 128.95; 134.69; 139.28; 139.82; 145.88; 147.19; 160.62.

Pharmacology

Strains & growth conditions. The strains used for the anti-*quorum* sensing (–QS) tests are listed in Table I. Briefly, the biosensor strains *C. violaceum* CV017 and *P. aeruginosa lasB-gfp*(ASV) produce under QS activation a violet pigment (violacein) and a green fluorescent protein, respectively. *A. tumefaciens* NTLR4 is a mutant unable to synthesise its own autoinducer molecule (C8-HSL) but that produces the

Table I. Reporter strains used for the anti-quorum sensing (–QS) tests.

Strain	Description	Reference
Agrobacterium tumefasciens NTLR4	pTI cured, *traG*::*lacZ* fusion, Gm^r	Cha et al., 1998 [17]
Chromobacterium violaceum CV017	Smr mini-Tn*5* Hgr, violacein overproducer	Winson et al., 1994 [22]
Pseudomonas aeruginosa PA01 lasB-gfp(ASV)	*lasB::gfp(ASV)* fusion,Gm^r	Hentzer et al., 2002 [5]

β-Galactosidase enzyme when QS is activated by exogenous C8-HSL. β-Galactosidase production was revealed by the appearance of a blue pigment in the presence of the X-Gal substrate. All the strains used in this study were grown aerobically on nutrient broth. Liquid cultures (5 mL) were performed in glass tubes under continuous stirring (160 rpm). *A tumefasciens* NTLR4 and *P. aeruginosa lasB-gfp*(ASV) constructions were maintained on nutrient agar 1.2% (w/v) supplemented with 25 μg/mL and 50 μg/mL of gentamycin (Gm) respectively. *A. tumefasciens* NTLR4 and *C. violaceum* CV017 were grown at 30 °C and *P. aeruginosa lasB-gfp*(ASV) at 37°C.

Anti-quorum sensing and anti-bacterial tests. Anti-*quorum* sensing (−QS) tests were systematically accompanied with anti-bacterial tests in order to confirm the effect of each molecule. For both tests, sterile glass wells (4 mm × 8 mm, Polylabo®) were placed on nutrient agar plates and pre-filled with 20 μL of sterilized milliQ water. This step was required for the good diffusion of the molecules added in the wells from stock solutions prepared in methanol. 20 μL of each stock solution (20 mM) were added to the wells. For diffusion and drying, plates were stored 3 h at ambient temperature. Wells were then removed and the plates were overlaid with 5 ml of soft agar (0,6% w/v) medium seeded with different volumes of stationary phase cultures of the indicator strains.

(i) For anti-QS tests, the overlays were seeded with 5 mL of the biosensors CV017 and NTLR4, and 5 μL of a 1:1000 dilution of PA01 *lasB-gfp*(ASV). The overlay seeded with NTLR4 was supplemented with 10 μL of C8-HSL (2 g/L) and 30 μL of X-Gal (20 g/L). Detection of the green fluorescent protein was performed using the Dark Reader® from Clare Chemical. (ii) For anti-bacterial tests, the overlays were seeded with 5 μL of stationary phase cultures diluted (1:10 for CV017 and NTLR4, and 1:1000 for PA01 *lasB-gfp*) or not (other strains). Using these inoculi, growth inhibition (partial or total) could be determined easier.

For both tests, plates were incubated overnight and analysed visually for the presence or not of halos of QS inhibition and/or growth inhibition. (i) A QS inhibition halo was defined as a clear zone where the biosensor does not produce its QS-dependent pigment or fluorescence, and (ii) a growth inhibition halo was a clear zone where growth was inhibited or absent.

Each molecule was tested in duplicate on three independent cultures.

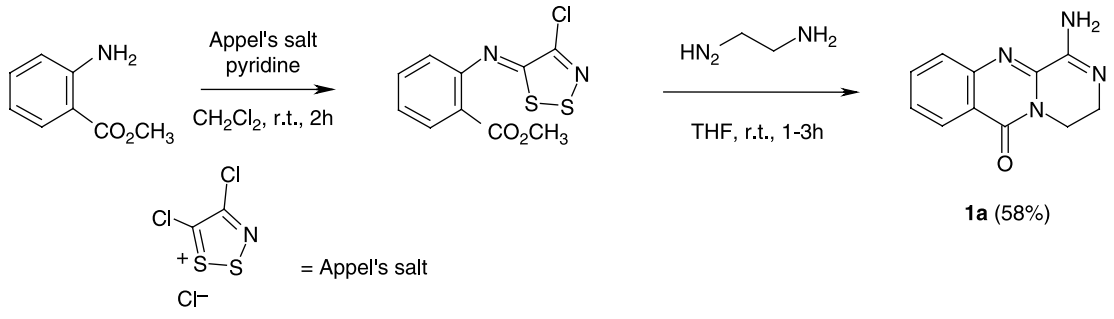
Results and discussion

Chemistry

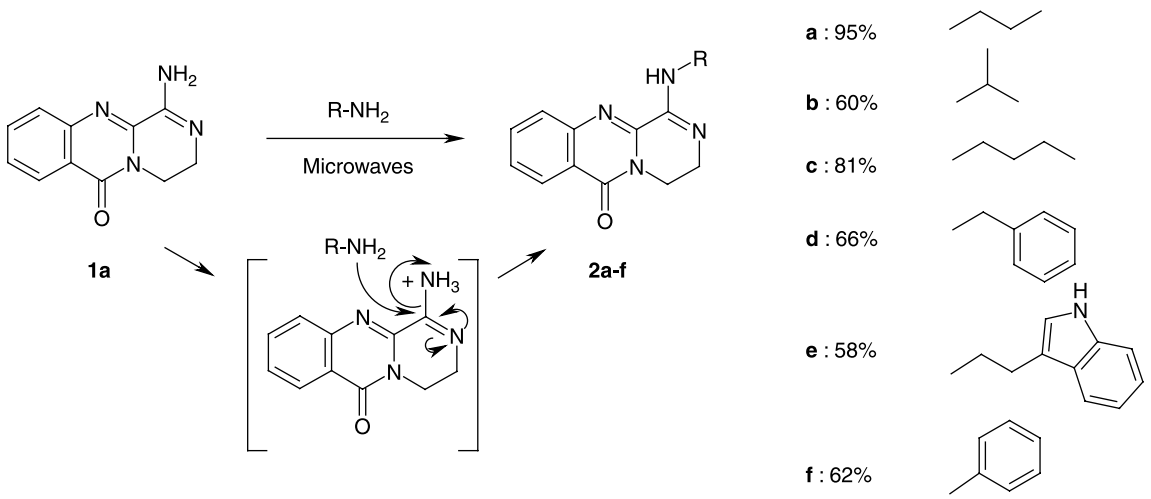
The synthesis of the 2,3-condensed (3*H*)-quinazolin-4-one precursor **1a** was performed from methyl *N*-(4-chloro-5*H*-1,2,3-dithiazol-5-ylidene)-anthranilate itself obtained by condensation of 4,5-dichloro-1,2,3-dithiazolium chloride (Appel's salt) and methyl anthranilate (Scheme 2). Stirring of a solution of the intermediate imine and ethylenediamine at room temperature in tetrahydrofurane, for 1 or 2 h, gave a 58% yield of the expected 3,4-dihydropyrazino[2,1-b]quinazolin-6-one **1a** (Scheme 2).

The second step of the synthesis is an alkylation of the heterocyclic amidine present on the studied molecule. The usual methods for alkylation of this 1,3-dinucleophile usually afforded mixtures of both the *N*-endosubstituted and the *N*-exosubstituted products. Ten years ago Bourguignon and his co-workers have developed a regioselective alkylation of the exocyclic nitrogen atom *via* N-acyl derivatives and the method was successfully applied to the synthesis of analogs of chiral amino acids [20].

The method we developed is inspired by our previous studies of the Niementowski reaction for the synthesis of novel pentacyclic quinazolin-4-ones [13,14]. It consists of a strong microwave-assisted heating (5 min) of the reactants in a sealed tube, at 220°C in the presence of 10% by weight of graphite, one of the solids most efficiently heated by microwaves. In the case studied here, the use of graphite was not suitable since the association of the liquid reagents and graphite may create hot-spots at the surface of the reactor. After exploration of various experimental conditions, we found that microwave heating of a solution of the starting compound **1a** and aliphatic



Scheme 2. Synthesis of **1a** from methyl anthranilate.



Scheme 3. Conditions: PTSA (1.1 equiv.), THF, MW, 150°C, 5 min (for **2a–e**); NMP, MW, 220°C, 15 min (for **2f**).

amines in tetrahydrofurane (THF), in the presence of 1.1 equiv. of *para*-toluenesulfonic acid (PTSA), afforded good yields of the expected *N*-alkylated 3,4-dihydropyrazino[2,1-*b*]quinazolin-6-ones (Scheme 3).

The suggested mechanism of this reaction is an addition–elimination process in which the primary amine will first attack the carbon of the amidine function, and then, eliminate the ammoniac.

Applying the conditions described above with aromatic amines (*e.g.* aniline in Scheme 3) led to different results. The synthesis of the expected products implied a more intense heating (220°C) of the starting reactants in the presence of *N*-methylpyrrolidinone (NMP) a solvent which is particularly well adapted for microwave experiments (Scheme 3).

Here we developed a simple and efficient methodology for regioselective alkylation of exocyclic nitrogen of cyclic amidines. In our case microwave heating allows very short reaction times and clean conditions for work-up.

Pharmacology

Discrimination between anti-*quorum* sensing (–QS) and anti-bacterial activities is crucial to reject false anti-QS positives. As a matter of fact, if the drug added inhibits bacterial growth under the cell density threshold, QS will not be activated even though this drug does not possess anti-QS activity. We therefore optimised the methodology described by McLean et al [15] to discriminate between anti-QS and anti-bacterial activities (see Material & Methods). We first analysed the effects of 7 quinazolinone derivatives on three model Gram-negative bacteria. We found that 5 quinazolinones inhibited the production of the QS-dependent pigment violacein in the opportunistic pathogen *C. violaceum* CV017. These apparent effects were actually due to growth inhibitions (Table II).

Consequently, none of the molecules tested have any specific anti-QS activity. However, this negative result validates the growth tests devised to control the bias of the anti-QS tests. None of the quinazolinones tested affect *P. aeruginosa* growth or QS and two of them had even no activity at all. Among the 5 quinazolinones that inhibit *C. violaceum* CV017 growth, the **2b** derivative was also found to inhibit *A. tumefaciens* NTLR4 growth (Table II). The anti-bacterial potential of the **2b** derivative was then analysed in more details using a panel of Gram-positive and Gram-negative bacteria. Results of Table III indicated that the **2b** molecule had no effect on the Gram-positive bacteria tested. Conversely, this quinazolinone affected the growth of the Gram-negative bacteria *Escherichia coli* and *Shigella sonnei*. This is an attractive result since *S. sonnei* causes dysenteries (called shigellosis) hard to fight owing to multi-resistance phenomena [21]. Nevertheless, additional experiments are needed to determine whether **2b** and other quinazolinone derivatives might be used to control these infections.

In conclusion, developing a simple and very efficient microwave-assisted methodology for regioselective

Table II. Effects of the quinazolinone derivatives synthesised on growth and *quorum* sensing of *C. violaceum (CV017), A. tumefaciens (NTLR4), and P. aeruginosa [PA01 lasB-gfp(ASV)]*. The symbol + indicates the presence of an inhibition halo (see Material & Methods).

	Quinazoline Derivatives						
	1a	2a	2b	2c	2d	2e	2f
CV017 growth	+	+	+	+	+	0	0
CV017 *quorum* sensing	+	+	+	+	+	0	0
NTLR4 growth	0	0	+	0	0	0	0
NTLR4 *quorum* sensing	0	0	+	0	0	0	0
PA01 growth	0	0	0	0	0	0	0
PA01 *quorum* sensing	0	0	0	0	0	0	0

Table III. Effect of the 2b derivative on growth of several Gram-positive and Gram-negative bacteria.

Strains	Origin	Inhibition
Gram-positive		
Bacillus cereus (*)	Our laboratory	0
Bacillus megatherium	CIP 6620T	0
Bacillus subtilis (*)	CIP 5262	0
Enterococcus faecalis	CIP 103214	0
Lactobacillus acidophilus (*)	Our laboratory	0
Micrococcus luteus (*)	ATCC 4698	0
Staphylococcus aureus (*)	ATCC 25923	0
Staphylococcus saprophyticus	Our laboratory	0
Gram-negative		
Citrobacter freundii	Our laboratory	0
Enterobacter cloacae	Clinical	0
Escherichia coli	Our laboratory	++
Escherichia coli K12	ATCC 23716	+
Hafnia alvei	CIP 5731T	0
Klebsiella pneumoniae subspecies. Pneumoniae	CIP 53153	0
Proteus mirabilis	Our laboratory	0
Salmonella anatum	CIP 5630	0
Salmonella enterica subspecies. Enterica serotype wien (*)	CIP 8122	0
Salmonella enteritidis	Clinical	0
Serratia marcescens	CIP 6755	0
Shigella flexeneri	CIP 5236	0
Shigella sonnei	CIP 5255	++

(*) Incubated at 30°C instead of 37°C. The symbol +(+) indicates the presence of an inhibition halo (see Material & Methods)

alkylation of exocyclic nitrogen of cyclic amidines, we prepared *N*-alkylated 3,4-dihydropyrazino[**2,1-b**]-quinazolin-6-ones among which compound **2b** exhibits interesting activity against the Gram-negative bacteria *E. coli* and *S. sonnei*. We believe that this family constitutes a scaffold from which more potent inhibitors could be designed, opening the door to various applications.

Acknowledgements

We thank the "*Comité de Charente-Maritime de la Ligue Nationale Contre le Cancer"* and the "*Conseil Général de la Charente Maritime*" for financial support ((M.-F. P. and R. C. PhD grant respectively). We are indebted to Pr Michael Givskov (Technical University of Denmark) for providing the PA01 *lasB-gfp(ASV)* strain. We are grateful to Dr Denis Faure, (Institut des Sciences du Végétal CNRS Gif sur Yvette France) for providing the *Chromobacterium* and the *Agrobacterium* strains.

References

[1] Fuqua WC, Winans SC, Greenberg EP. *Quorum*-sensing in bacteria: LuxR-LuxI family of cell-responsive transcriptional regulators. J Bacteriol 1994;176:269–275.

[2] De Kievit TR, Iglewski BH. Bacterial *quorum*-sensing in pathogenic relationship. Infect Immun 2000;68:4839–4849.

[3] Whitehead NA, Barnard AML, Slater H, Simpson NJL, Salmond GPC. *Quorum*-sensing in gram-negative bacteria. FEMS Microbiol Rev 2001;25:365–404.

[4] Rasmussen TB, Givskov M. *Quorum*-sensing inhibitors as anti-pathogenic drugs. Internat J Med Microbiol 2006;296: 149–161.

[5] Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, Manefield M, Costerton JW, Molin S, Eberl L, Steinberg P, Kjelleberg S, Høiby N, Givskov M. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J 2003;22:3803–3815.

[6] Rasmussen TB, Bjarnsholt T, Skindersoe ME, Hentzer M, Kristoffersen P, Köte M, Nielsen J, Eberl L, Givskov M. Screening for *quorum*-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. J Bacteriol 2005;187: 1799–1814.

[7] Wu H, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, Høiby N. Synthetic furanones inhibit *quorum*-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. J Antimicrob Chemother 2004;53: 1054–1061.

[8] Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, Rice SA, Eberl L, Molin S, Høiby N, Kjelleberg S, Givskov M. Inhibition of *quorum*-sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiol 2002;148:87–102.

[9] For a recent review on quinazolinones and quinazolines see. Connolly DJ, Cusack D, O'Sullivan TP, Guiry PJ. Tetrahedron 2005;61:10153–10202.

[10] Frère S, Thiéry V, Bailly C, Besson T. Novel 6-substituted benzothiazol-2-yl indolo[1,2-c]quinazolines and benzimidazo[1,2-c]quinazolines. Tetrahedron 2003;59:773–779.

[11] Alexandre FR, Berecibar A, Wrigglesworth R, Besson T. Novel series of 8H-quinazolino[4,3-b]quinazolin-8-ones via two niementowski condensations. Tetrahedron 2003;59: 1413–1419.

[12] Testart A, Thiéry V, Picot L, Lozach O, Blairvacq M, Meijer L, Murillo L, Piot JM, Besson T. Synthesis and evaluation of the antiproliferative activity of novel thiazoloquinazolinone kinases inhibitors. J Enz Inhib Med Chem 2005;20:557–568.

[13] Testart A, Logé C, Léger B, Robert JM, Lozach O, Blairvacq M, Meijer L, Thiéry V, Besson T. Thiazolo[5,4-f]quinazolin-9-ones, inhibitors of glycogen synthase kinase-3. Bioorg Med Chem Lett 2006;16:3419–3423.

[14] Pereira MF, Picot L, Guillon J, Léger JM, Jarry C, Thiéry V, Besson T. Efficient synthesis of novel pentacyclic 6,7-dihydro-5a,7a,13,14-tetraaza-pentaphene-5,8-diones. Tetrahedron Lett 2005;46:3445–3447.

[15] McLean RJC, Pierson LS, Fuqua C. A simple screening protocol for the identification of *quorum*-sensing signal antagonists. J Microbiol Met 2004;58:351–360.

[16] McLean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, Daykin M, Lamb JH, Swift S, Bycroft BW, Stewart GSAB, Williams P. *Quorum*-sensing in *chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiol 1997;143:3703–3711.

[17] Cha C, Gao P, Chen YC, Shaw PD, Farrand SK. Production of acyl-homoserine lactone *quorum*-sensing signals by gram-negative plant-associated bacteria. MPMI 1998;11:1119–1129.

[18] Venturi V. Regulation of *quorum*-sensing in *Pseudomonas*. FEMS Microbiol Rev 2006;30:274–291.

[19] Detailed descriptions of this microwave reactor with integrated robotics was recently published Detailed descriptions of this microwave reactor with integrated robotics was recently published: Schanche JS. Microwave synthesis solutions from personal Chemistry. Mol Diversity 2003;7:293–300.

[20] Bourguignon JJ, Kerbal A, Abarghaz M. Regioselective alkylation of the exocyclic nitrogen of heterocyclic amidines via the Mitsunobu reaction. Tetrahedron Lett 1995;36:6463–6466.

[21] Sivapalasingam S, Nelson JM, Joyce K, Hoekstra M, Angulo FJ, Mintz ED. High prevalence of antimicrobial resistance among *shigella* isolates in the united states tested by the national antimicrobial resistance monitoring system from 1999 to 2002. Antimicrob Agents Chemother 2006;50:49–54.

[22] Winson MK, Bainton NJ, Chhabra SR, Bycroft BW, Salmond GPC, Williams P, Stewart GSAB. Control of N-acyl-l-homoserine lactone-regulated expression of multiple phenotypes in *Chromobacterium violaceum*, Abstracts of the 94th Annual Meeting of the American Society for Microbiology, Washington, D.C: American Society for Microbiology. 1994;Abstr. H-71; p. 212.