Synthesis and biological evaluation of new penta- and heptacyclic indolo- and quinolinocarbazole ring systems obtained *via* Pd^0 catalysed reductive *N*-heteroannulation†

Marie Laronze-Cochard,[a] Fabien Cochard,[a] Etienne Daras,[a] Amélie Lansiaux,[b] Bertrand Brassart,[c] Enguerran Vanquelef,[a,d] Elise Prost,[e,f] Jean-Marc Nuzillard,[e] Brigitte Baldeyrou,[b] Jean-François Goosens,[g] Olivier Lozach,[h] Laurent Meijer,[h] Jean-François Riou,[i] Eric Henon*[j] and Janos Sapi*[a]

Received 19th May 2010, Accepted 14th July 2010
DOI: 10.1039/c0ob00149j

A short route, involving a tetramolecular condensation reaction and a Pd/C catalyst–H_2-mediated reductive *N*-heteroannulation as the key-steps, has been found for the synthesis of some new penta- and heptacyclic indolo- (**12**), quinolino- (**13**) and indoloquinolinocarbazole (**11**) derivatives. HF-DFT (B3LYP) energy profiles and NMR calculations were carried out to help in the understanding of the experimental results. *N*-Alkylated indoloquinolinocarbazoles (**16b**, **17a**, **17b** and **18**) were prepared and screened essentially toward some cancer-(G-quadruplex, DNA, topoisomerase I) and CNS-related (kinases) targets. Biological results evidenced **13** as a potent CDK-5 and GSK-3β kinases inhibitor, while di- or triaminopropyl-substituted indoloquinolinocarbazoles **17b** or **18** targeted rather DNA-duplex or telomeric G-quadruplex structures, respectively.

Introduction

The carbazole or benzo[*b*]indole ring system is widely represented in natural or synthetic biologically active substances.[1] Many of the simple functionalised carbazole alkaloids[2] display antibacterial, antifungal, antiviral, anti-inflammatory and antitumor properties. Among such structures, some have proved to be active against metabolic[3] and neurodegenerative[4] disorders. Synthetic analogs such as 3-aryl, 3,6-diaryl, or 4-alkoxycarbazole derivatives have been developed for their antitumoral or antihypertensive activities, respectively.[5]

Heterocycle, especially aza-heterocycle-annulated natural carbazole alkaloids have been reported as potential anticancer agents interacting with various targets.[6] Ellipticine,[7] a pyrido[4,3-*b*]carbazole-type alkaloid has been identified as a strong DNA interfering substance with topoisomerase II inhibitory properties. Indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole rebeccamycin[8] was found to be a selective topoisomerase I inhibitor. Staurosporine was identified as a potent but non-selective kinase inhibitor (protein kinase C[9] (PKC), glycogen synthase kinase-3β[10] (GSK-3β)) and also implicated in cell signaling process, while its hydroxy analog UCN-01[11] or granulatimide[12] proved to be more selective cell-cycle controlling agents inhibiting cyclin dependant kinases (CDKs) and/or check-point kinase 1 (Chk1) (Fig. 1). Such polycyclic natural products have also been selected as leads for structure-based drug discovery programs (cancer, type II diabetes and CNS diseases).[13]

Thus, simple aryl-substituted pyrrolo[3,4-*c*]carbazole analogs (**I**) have been reported as inhibitors of checkpoint kinase Wee1,[14] while aryl or heteroaryl fused counterparts (**II**) were found to be potent against other kinases (CDK-1,[15] CDK-5,[15] GSK-3β,[15] Cyclin D1/CDK-4,[16] Chk-1[13c,17]). Closely related aryl-indolylmaleimides[18] (**III**) were developed as potent inhibitors of GSK-3β, an enzyme involved with CDK-5 in Alzheimer disease.[19] Isomer pyrrolo[2,3-*a*]carbazole derivatives (**IV**) have been identified as potential CDK-1[20] or Pim-kinase[21] inhibitors. Substituted indolo[3,2-*c*]quinoline-type derivatives have been reported as antineoplastic agents inducing G2/M cell cycle arrest and apoptosis. Pentacyclic indolo[3,2-*c*]quinoline derivative (**V**)[22] and

[a]*Université de Reims-Champagne-Ardenne, Institut de Chimie Moléculaire de Reims, CNRS UMR 6229, Groupe BSMA, UFR de Pharmacie, 51 rue Cognacq-Jay, F-51096 Reims, Cedex, France. E-mail: janos.sapi@univ-reims.fr; Fax: +33(0)326918029*
[b]*Laboratoire de Pharmacologie Antitumorale, INSERM U-837, Centre Oscar Lambret, Université Nord de France, Institut de Recherches sur le Cancer de Lille, IMPRT, 1 Place de Verdun, F-59045 Lille, Cedex, France*
[c]*Université de Reims-Champagne-Ardenne, CNRS UMR 6237, IFR 53 Biomolécules, UFR des Sciences Exactes et Naturelles, BP 1039, F-51687 Reims, Cedex 2, France*
[d]*Université de Picardie Jules Verne, Laboratoire des Glucides, CNRS UMR 6219, Equipe THERA, Faculté de Pharmacie, 1 rue des Louvels, F-80037 Amiens, Cedex 1, France*
[e]*Université de Reims-Champagne-Ardenne, Institut de Chimie Moléculaire de Reims, CNRS UMR 6229, Groupe IS, UFR des Sciences Exactes et Naturelles, Bat. 18., BP 1039, F-51687 Reims, Cedex 2, France*
[f]*Université Paris Descartes, Synthèse et Structure de Molécules d'Intérêt Pharmacologique, CNRS UMR 8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, F-75270 Paris, Cedex 06, France*
[g]*Université de Lille 2, EA 4034, Laboratoire de Chimie Analytique, Faculté des Sciences Pharmaceutiques et Biologiques, 3 rue du Professeur Laguesse, BP 83, F-59006 Lille, Cedex, France*
[h]*CNRS, Protein Phosphorylation & Human Disease Group, USR3151, Station Biologique, BP 74, F-29682 Roscoff, France*
[i]*Régulation et Dynamique des Génomes, INSERM U565, CNRS UMR 5153, USM 503, Muséum National d'Histoire Naturelle, 43 rue Cuvier, CP26, F-75231 Paris, Cedex 5, France*
[j]*Université de Reims-Champagne-Ardenne, Institut de Chimie Moléculaire de Reims, CNRS UMR 6229, Groupe BSMA, UFR des Sciences Exactes et Naturelles, Bat. 18., BP 1039, F-51687 Reims, Cedex 2, France. E-mail: eric.henon@univ-reims.fr; Fax: +33(0)326918497*

† Electronic supplementary information (ESI) available: Computational study of the key intermediate **8a**; Synthesis and analytical data of **6b**, **6c**, **8b**, **8c**, **17a**; Biological evaluation of **11**, **13**, **14**, **16b**, **17a–b**, **18**; References; Copies of NMR Spectra of **6b–c**, **8a–c**, **10**, **11**, **12**, **13**, **14**, **16b**, **17a–b**, **18**; Computational data of **8a**. See DOI: 10.1039/c0ob00149j

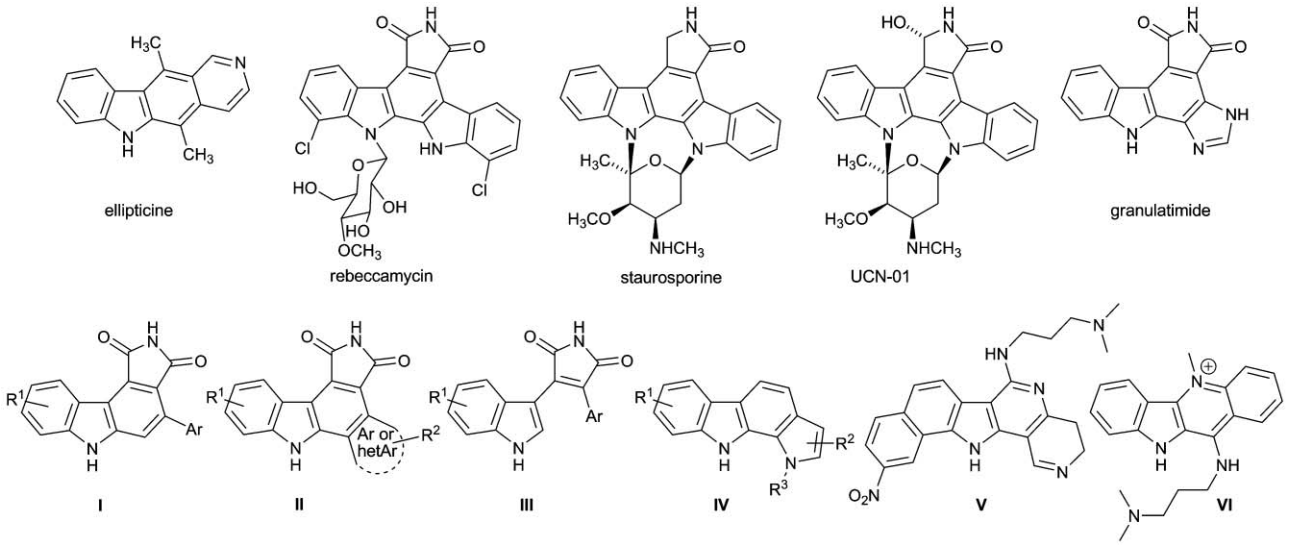


Fig. 1 Selected examples of natural and synthetic carbazoles displaying anticancer properties.

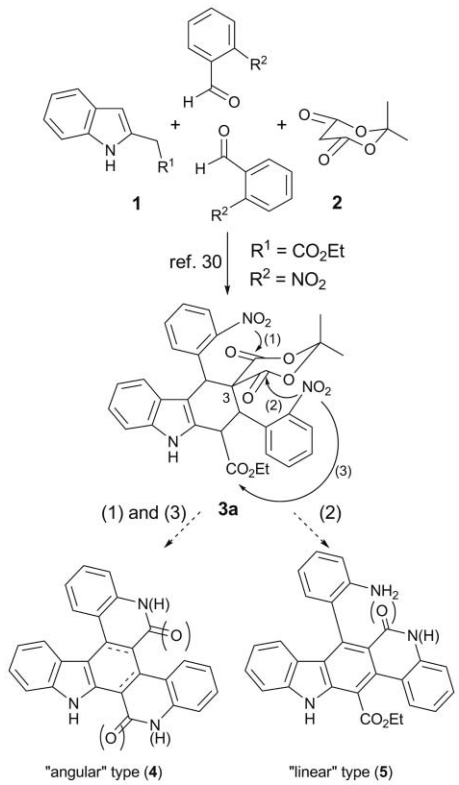
5-*N*-methylated quinoline (indolo[3,2-*b*]quinolinium) derivatives (**VI**)[23]) have displayed potent telomeric G-quadruplex binding affinities and *in vitro* telomerase inhibitory activities.

In addition to medicinal chemistry applications carbazole derivatives have emerged as promising chemically stable materials for electronic devices.[24] Substituted indolo[3,2-*b*]carbazole, poly(indolo[3,2-*b*]carbazole) or poly(2,7-carbazole) derivatives have been described as organic semiconductive materials, organic thin-film transistors or components for photovoltaic cells.[25]

The prevalence of the carbazole core in medicinal chemistry and in organic materials has triggered considerable synthetic efforts during the past years towards the preparation of this important structural unit. Apart from the well-documented[26] cyclisation of *ortho*-nitrogen-substituted diphenyl derivatives *via* nitrene, recent development of organometallic chemistry has provided new opportunities for metal-catalysed synthesis of functionalised carbazoles. Thus, carbazoles have been prepared by Pd-catalysed intramolecular arylation of *N*-(*o*-halogenophenyl)aniline derivatives obtained by cross-coupling or Buchwald–Hartwig amination method.[27] Nozaki and colleagues have developed a double *N*-arylation approach of 2,2′-dihalogeno- or 2,2′-*bis*(trifluoromethansulfonyloxy) biphenyls[28] while another strategy consists of Pd-catalysed oxidative aryl–aryl coupling of *N*-arylaniline derivatives.[29]

In this context development of simple synthetic approaches for the preparation of new heterocycle fused carbazole ring systems continues to be a challenge in organic chemistry.

Some years ago we found that tetramolecular condensation between 2-substituted indoles **1**, Meldrum's acid **2** and two equivalents of aromatic aldehydes smoothly afforded the corresponding 1,3-diaryl substituted tetrahydrocarbazoles bearing a spiro Meldrum's acid appendage on the C-3 atom.[30] In continuation of our work aiming at DNA-interfering carbazoles[31] tetramolecular condensation product **3a** obtained with 2-(indol-2-yl)ethyl acetate and *o*-nitrobenzaldehyde seemed to be a versatile intermediate for the preparation of polycyclic aza-heterocycles (**4**, **5**) by simple functional group transformations such as intramolecular cyclisation(s)/decarboxylation/aromatisation (Scheme 1).



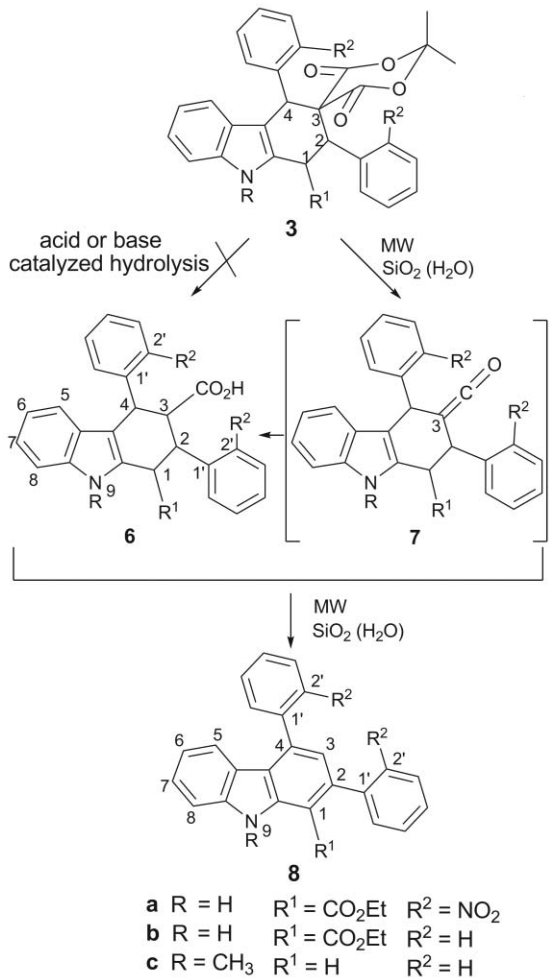
Scheme 1 Proposed synthetic pathway to "angular" (**4**) and "linear" (**5**) heterocycle annulated carbazoles.

Herein we disclose our results leading to the preparation of some new penta- and heptacyclic indolo- and quinolinocarbazoles by simple Pd–C/H_2-mediated reductive cyclisation of conformationally restricted *o*-nitrobiaryl derivatives, as the key step. A DFT computational approach was used to confirm the observed atropoisomers for the key intermediate **8a**. *N*-Alkylation reactions of indoloquinolinocarbazoles **11** and **14** and preliminary biological *in vitro* evaluation of carbazoles (**16b–18**) were carried out.

Results and discussion

(i) Synthesis of 2,4-diarylcarbazole derivatives 8a–c

Firstly, diastereomerically homogenous tetrahydrocarbazole **3a**[30] was subjected to catalytic hydrogenation (10% Pd–C, H_2, 4 bar) prospecting for an intramolecular cyclisation between the *in situ* formed aromatic amine function(s) and the Meldrum's acid core, followed by spontaneous decarboxylation.[32] Such kind of domino transformations have recently allowed the preparation of chiral pyrano-, and pyrrolidino-fused tryptamines.[33] Unfortunately, catalytic hydrogenation under varying conditions led to a very complex and inseparable mixture. After this unsuccessful reaction we envisaged the transformation of the Meldrum's acid moiety to the corresponding monoacid function (**6a**) by hydrolysis and decarboxylation (Scheme 2).[34] With such an acid in hand two different pentacyclic derivatives (**4** and **5**) could be envisaged by aryl nitro group reduction and subsequent cyclisation.



Scheme 2 Preparation of 2,4-diaryl substituted carbazoles (**8a–c**).

An "angular" type heptacyclic system **4** could be generated by a double cyclisation (paths 1 and 3), while a "linear" type pentacycle **5** may result from a monocyclisation according to path 2 (Scheme 1). To this end, Meldrum's acid containing derivative **3a** was refluxed with sodium ethylate in ethanol, in aqueous hydrochloric acid or in 30% aqueous sodium hydroxide, in vain. We have never obtained the corresponding acid **6a** probably due to the highly congested and hydrophobic environment around the C-3 carbon atom. Since thermolysis of Meldrum's acid derivatives is known to afford ketenes,[34] tetramolecular condensation product **3a** adsorbed on silicagel was heated under microwave irradiation[35] (CEM Discover® (300 W), 200 °C) aiming at the corresponding carboxylic acid **6a**, accessible *via* the postulated ketene **7a** by hydratation (Scheme 2). Surprisingly, instead of monoacid **6a** 2,4-diaryl substituted carbazole **8a** was isolated (41%) as a result of a thermally-induced decarboxylation–aromatisation process. The ^{1}H NMR spectrum of the chromatographically homogenous derivative **8a** featured great complexity. Thus, three separable peaks have been found for the indole NH proton in 50, 29 and 21% ratio at $\delta = 10.60$, 10.40 and 10.15 ppm, respectively, while only two distinct signals were present for both ester methyl and methylene protons. A careful analysis of the ^{1}H NMR spectrum emphasised the presence of three isomers. Methylene protons displayed two groups of quadruplex at $\delta = 4.76$ and 4.08 ppm with an integrated ratio of 1 to 4. Comparing the integration of NH protons to that of the deshielded quadruplex ($\delta = 4.76$ ppm) we evidenced that this latter could unambiguously be attributed to the minor isomer (a3 - 21%) while methylene protons of the two major isomers (a1 - 50%, a2 - 29%) appeared at $\delta = 4.08$ ppm. Moreover, the corresponding relative intensities have not been altered by prolonged heating (180 °C, overnight). Altogether, these data give support to the coexistence of several long lifetime isomers. A theoretical approach was proposed to analyse these observed NMR results.

Neither lower microwave performance, nor use of alumina as solid support allowed the isolation of monoacid **6a** in the nitro series. On the contrary, irradiation of tetramolecular adducts **3b**,**c** adsorbed on silicagel afforded the corresponding acids **6b**,**c** (mixture of diastereomers) in 71–86% yield depending on the microwave oven type. Higher irradiation temperature and longer reaction time permitted a direct transformation of **3b**,**c** to aromatic carbazoles **8b**,**c** in moderate yield. It is important to note that the above mentioned decarboxylation–aromatisation could be carried out without solvents and heavy metal catalysis[36] meeting the requirements of "green chemistry procedures".

(ii) Computational study[37,38,39] of the key intermediate 8a

The NMR spectra of **8a** have provided evidence for the existence of several stable isomers, even at high temperature (180 °C). Complementary to the experimental work, computations were performed to help in the understanding of these results. The details of the quantum mechanical calculations are reported in the Electronic Supplementary Information.†

Geometry variations of **8a** were considered by examining the internal rotation involving the nitrophenyl groups and the ester group. Four conformers were found (Fig. 2) with the ethyl group lying in the plane of symmetry of the carbazole tricyclic structure. Due to steric hindrance the carbazole and nitrophenyl groups are non-planar with rotation dihedral angles varying between 67 and 89°. The H···O distance between the carbazole NH group and an ester oxygen atom was found to be short (about 2 Å) in the four isomers, showing a significant interaction. The **S** and **A** notations have been reserved for the structures with the two nitro functions on the same side (*syn*) or on opposite sides (*anti*) of the carbazole structure, respectively. The * notation refers to geometries with the

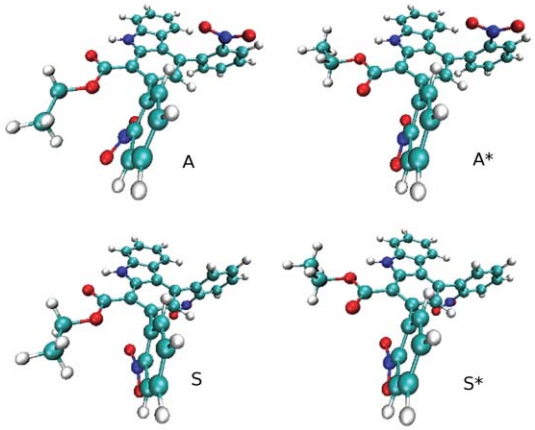


Fig. 2 Optimised **8a** structures. **A** and **S** stand for *anti* and *syn*; the * notation refers to the spatial orientation of the ester; geometries were fully optimised within the HF-DFT(B3LYP/6-31G**) toluene PCM model.

ester carbonyl group directed in the opposite direction to the NH function.

To achieve a deeper interpretation of these NMR data, chemical shifts were subsequently computed for all stable conformers relative to the absolute shielding constants of TMS (see ESI†).

The theoretical study combined with the experimental data evidenced that the relative spatial orientation of the ester function with respect to the neighbouring nitrophenyl group was responsible for the peak separation observed in the NMR spectrum of **8a**. It is interesting to note that the most stable atropoisomer (**A**) gave signals for CH_2 (or CH_3, or NH) that presumably correspond to the major conformer in the experimental ^{1}H NMR spectrum. Furthermore, the 50, 29, 21% ratio (from NMR data) fits well with the relative stability determined for **A**, **S** and **A*** (within the DFT precision). This is of special importance in view of understanding the yields observed for products **11** and **12** (see below).

(iii) Synthesis of new polycyclic indolo- and quinolinocarbazole derivatives (10, 11, 12, 13, 14, 15)

Respecting our objectives 2,4-*bis*(*o*-nitrophenyl) substituted carbazole **8a** appeared to be a proper intermediate for the preparation of both "angular" (**4**) and "linear" type (**5**) indole-heterocycles. First of all, the well-documented trivalent phosphorous-mediated (PPh_3, $(EtO)_3P$) reductive cyclisation was attempted.[40] In accordance with some reported observations,[41] in our case deoxygenation of **8a** and subsequent nitrene insertion occurred with low yields affording a multitude of side products. As an alternative to Cadogan-reaction transition metal catalysed methods using carbon monoxide as reductant have recently been developed for the synthesis of indoles[42] or carbazoles[43] from *o*-nitrostyrenes or *o*-nitrobiphenyls, respectively. However, in some cases formation of side-products or harsh reaction conditions (high temperature and high pressure of CO) have been reported, limiting the synthetic application of this method.

The above mentioned inconveniences were circumvented by Alajarín and colleagues reporting a Pd/C–hydrogen-mediated reductive cyclisation of *o*-nitrophenylaryl derivatives.[44] This simple approach retained our attention even if under such conditions a competition between simple reduction and reductive insertion should be considered.

In fact, when 2,4-*bis*(*o*-nitrophenyl) substituted carbazole **8a** dissolved in a mixture of ethanol and toluene was submitted to medium pressure hydrogenation conditions (10% Pd–C, H_2 10 bar, room temperature, 25 h) a mixture of polycyclic compounds was formed. Purification by column chromatography allowed the isolation of two new heptacyclic and two new pentacyclic derivatives (Scheme 3).

In the series of the so-called "angular" compounds (type **4**) reductive *N*-heteroannulation was accompanied by lactamisation between the amino and ester groups affording a new indolo-quinolinocarbazole **11** in 55% yield. It is interesting to note, that in the first phase of the reaction lactim ether **10** was formed, perhaps *via* an initial insertion of the nitrene moiety into the carbonyl group, and **10** progressively converted into lactam **11**. The expected indolo[3,2-*b*]carbazole derivative **12** was obtained with reproducible yield (17%) resulting from reductive insertion on the C-3 carbon atom. Less than 1% of pentacyclic quinolinocarbazole **13** was generally observed.

The structures of the new polycyclic systems were unambiguously ascertained by exhaustive NMR studies (HSQC, HMBC, COSY, ROESY) and MS analyses. Thus, "angular-type" molecules **10** and **11** were characterised by three deshielded protons H-10, H-9, H-4 (observed between $\delta = 8.80$ and 9.30 ppm in DMSO-d_6) and by a strong ROE effect between H-10 and H-9 in ROESY experiment, while "linear" molecule **12** shows only one downfielded proton (H-7: $\delta = 8.75$ ppm in DMSO-d_6).

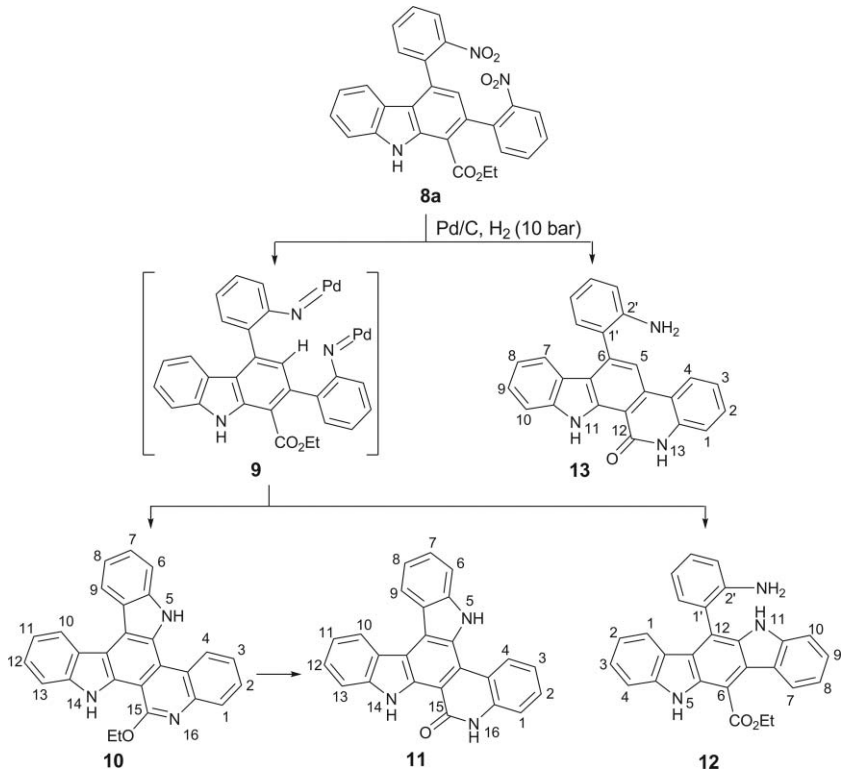
Analysis of the experimental results evidenced that in our conditions, exhaustive reduction of nitro groups was avoided and completely reduced derivative **13** was isolated in very low yield (1%). Our DFT theoretical results suggested that the ratio of **11** and **12** could be related to the different atropoisomers of the starting structure **8a**. Actually, the geometrical requirement for the nitrene insertion should be the proximity of the reacting nitrogen atom with the targeted C–H or C═O bonds. Even though the detailed mechanism leading from **8a** to **11** and **12** is unknown, the analysis of the N–C distances in **8a** shed light on the possible conformer dependence of nitrogen insertion.

As can be seen from Table 1, conformers **A** and **A*** would favour the formation of **11** rather than **12** because the reacting nitrogen atom N_a is significantly too far from the C-3 carbon atom ($\simeq 4$ Å) with respect to the ester carbon atom ($\simeq 3.6$ Å) in these species. In contrast, conformer **S** may only favour the formation of **12** bringing the nitrogen atom N_a closer to the C-3 carbon atom.

From this, **8a** would give predominantly **11** as found experimentally. These results provide additional support that several isomers of **8a** coexist in thermal equilibrium. This qualitative analysis should be of course completed by a full study of the reaction mechanism for a final clarification of these hypotheses. From a mechanistic point of view, a palladium-bound nitrene intermediate

Table 1 Selected distances (in Å) in the B3LYP/6-31G** geometry of molecule **8a**

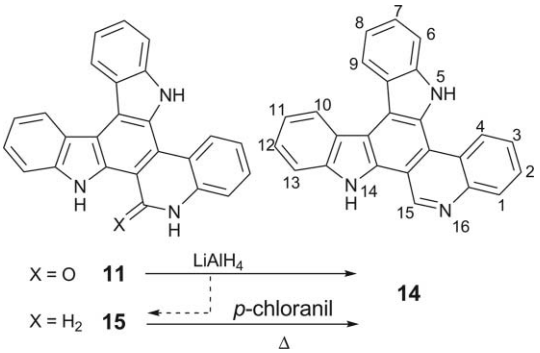
Distance	A	S	A*
$O_2N_a \cdots C{=}O_{ester}$	3.65	4.09	3.62
$O_2N_a \cdots C{-}3$	3.97	3.70	3.93
$O_2N_b \cdots C{-}3$	3.36	3.98	3.42



Scheme 3 Pd/C–H_2-mediated reductive *N*-heteroannulation toward new indoloquinolino- (**10**,**11**), indolo-(**12**), and quinolinocarbazoles (**13**).

(**9**) can be involved similar to Söderberg *et al.*'s proposition.[45] However, a competitive pathway *via* partially deoxygenated intermediates may also be considered even if their insertion into aromatic rings requires higher activation energy.[46]

Fully aromatic heptacyclic derivative **14** was obtained by the partial reduction of the lactam carbonyl group of **11** (Scheme 4). As classical methods ($POCl_3$, P_4S_{10}, Lawesson's reagent, BH_3–Me_2S) failed, we turned to $LiAlH_4$ used in large excess. Lactam **11** heated at 60 °C in DME was transformed to the corresponding heptacycle **14** (71%) sometimes isolated together with a small quantity of dihydropyridine derivative (**15**). Due to its instability this latter could only be detected by MS analysis of the crude mixture (peak (ESI) at *m*/*z* 358 $[M+H]^+$). Nevertheless, the mixture of **14** + **15** could be smoothly converted into **14** by heating in the presence of *p*-chloranil.



Scheme 4 Preparation of 5*H*,14*H*-indolo[2,3-*c*]quinolino[4,3-*a*]carbazole (**14**).

(iv) Synthesis of *N*-alkylated indolo[2,3-*c*]quinolino[4,3-*a*]carbazoles (16b) and (17a,b) and of *N*-substituted 15-oxo-5*H*,14*H*-15,16-dihydroindolo[2,3-*c*]quinolino[4,3-*a*]carbazole (18)

In order to enhance the solubility and provide a better interaction with DNA minor groove's phosphates aminoalkyl side-chains have been introduced according to the Scheme 5.

Two different side-chains were selected with tertiary amine functions attached by two or three carbons to the aromatic ring-system.

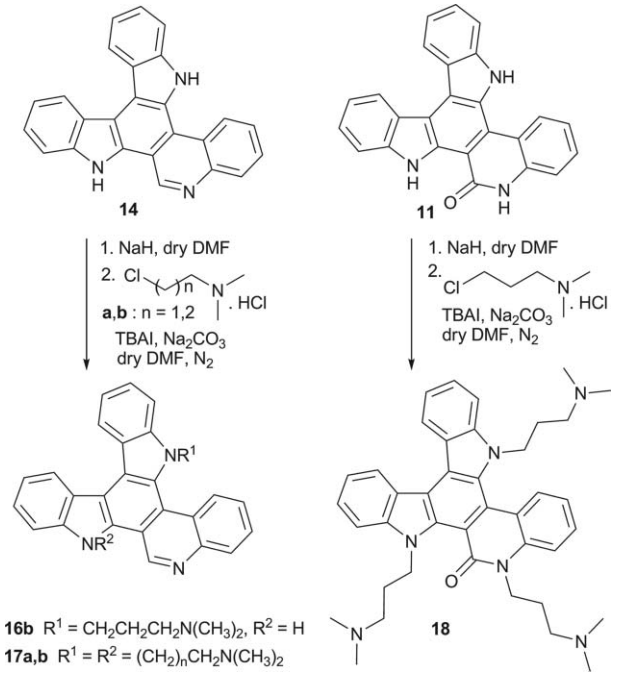
Treatment of heptacyclic derivative **14** with 6 eq. of sodium hydride and 3-chloropropyldimethylamine hydrochloride led to monoalkylated (**16b**) and dialkylated derivatives (**17b**) in 12 and 55% yield, respectively.

When **14** was reacted with sodium hydride and 2-chloroethyldimethylamine hydrochloride under the same conditions disubstituted compound **17a** was isolated in 41% yield.

In the "lactam-series" **11** was submitted to alkylation using a larger excess (9 eq.) of 3-chloropropyldimethylamine hydrochloride and sodium hydride to afford trialkylated derivative **18** (53%) and an inseparable mixture of mono- and dialkylated derivatives (approx. 17%).

(v) Biological multitarget screening of compounds (10–14 and 16b–18)

With these new polycyclic indole-heterocycles in hand we were interested in conducting a preliminary biological screening toward some CNS or cancer targets (kinases, telomeric G-quadruplex, DNA-binding, cytotoxicity tests).



Scheme 5 Synthesis of *N*-alkylated compounds **16b**, **17a**, **17b** and **18**.

Kinases inhibition. Firstly we tested the main penta- (**12**, **13**) and heptacyclic derivatives (**10**, **11**, **14**) and their four aminoalkyl-substituted counterparts (**16b**, **17a**, **17b** and **18**) as potential inhibitors of DYRK1A, cyclin-dependent kinase-5 (CDK-5)[10a,58] and glycogen synthase kinase-3β (GSK-3β)[59] enzymes, implicated in diverse CNS disorders. Among the screened products only **13** showed significant inhibitory activity for CDK-5 and GSK-3β kinases with IC_{50} = 0.93 μM and IC_{50} = 1.2 μM, respectively (see ESI†).

Telomeric G-quadruplex binding affinity measurement. The telomeric G-quadruplex binding capacity of the four aminoalkyl-substituted compounds (**16b**, **17a**, **17b** and **18**) was firstly evaluated by FRET (fluorescence resonance energy transfer) melting experiments[60] onto telomeric sequence (F21D) at a 20 μM ligand concentration. At this concentration, mono- and disubstituted compounds **16b**, **17a**, **17b** showed modest stabilisation properties toward G-quadruplex DNA (ΔT_m value <5 °C), whereas the trisubstituted one **18** exhibited a significant ΔT_m value (>10 °C) with a dose-response effect (Table 2). In the case of **18**, the presence of a third aminoalkylated branched chain might be responsible for a specific position of the molecule allowing thus more interactions with guanine tetrad and/or with potassium channel. However, even if compound **18** showed the best affinity of the series toward G-quadruplex DNA, it exhibited lower stabilisation ability than the reference tri-substituted acridine derivative **BRACO-19**[6i] (ΔT_m = 27.7 °C at 5 μM).

DNA duplex binding affinity measurements. To evaluate the extent of DNA binding, the variations of melting temperature of DNA (calf thymus DNA or CT-DNA and poly(dAdT)$_2$) were measured and depicted in Fig. 3 and Table 3. Moreover, calf thymus DNA binding affinities (K_{app}) were measured from the intrinsic fluorescence of compounds **11**, **18**, **14**, **17a** and **17b**, and DNA titration (Table 3).

Table 2 Telomeric G-quadruplex stabilisations measured by FRET

	FRET ΔT_m/°C		
No[s]	20 μM	10 μM	5 μM
16b	2.6	—	—
17a	4.7	1.7	0.7
17b	3.7	—	—
18	10.7	8.2	4.0
BRACO-19	—	—	27.7

—: Not Tested.

Table 3 Binding capacity to DNA and cytotoxicity measurements

Compounds	ΔT_m[a]	K_{app} 10^5 M^{-1} [b]	IC_{50} (HL-60)/μM[c]
11	1.7	0.93 ± 0.07	ND
18	11.1	7.51 ± 0.58	10.5 ± 1.98
14	1.8	0.74 ± 0.06	ND
17a	6.7	4.22 ± 0.62	7.3 ± 0.64
17b	15.2	7.79 ± 0.66	6.0 ± 0.21
Camptothecin			0.01 ± 0.003

[a] ΔT_m = T_m of drug–DNA complex – T_m of CT-DNA alone in °C. T_m measurements were performed in BPE buffer, in 1 cm quartz cuvettes at 260 nm with a heating rate of 1 °C min^{-1}. The T_m values were obtained from first-derivative plots (ratio 0.5). [b] Apparent affinity constant (K_{app}) calculated from intrinsic fluorescence of compounds (1 μM in BPE; λ_{exc} = 380 nm and λ_{em} = 510 nm) and CT-DNA titration n = 3. [c] drug concentration (μM) that inhibits HL-60 cell growth by 50% after 72 h of incubation ND: Not Determined.

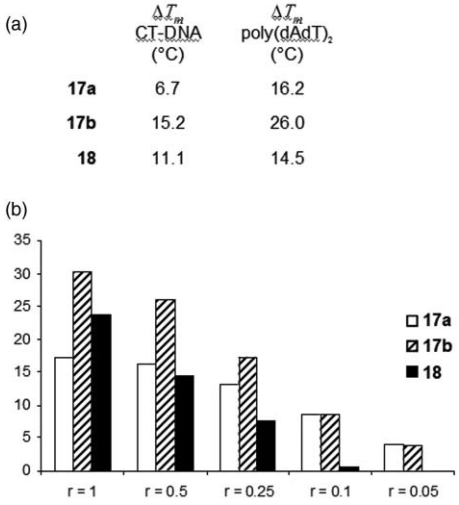


Fig. 3 Affinity measurements of di- and trisubstituted heptacyclic carbazoles (**17a**, **17b** and **18**) toward DNA.

At the *pH* of serum polycyclic derivatives without protonable functions (**10**, **11**) or with aromatic amine groups (**12**, **13**, **14**) showed no interaction with DNA. Introduction of dimethylaminoalkyl side-chain at indole or lactam nitrogen stabilizes the DNA duplex against heat denaturation. Thus, simple **11** displayed no significant effect on DNA stability, while its trisubstituted analog **18** gave an important growth of T_m values with both CT-DNA and poly(dATdT)$_2$ and a ten fold K_{app} value (Fig. 3, Table 3).

Similarly, disubstituted heptacyclic analogs (**17a**, **17b**) proved to be more efficient DNA duplex stabilizing agents. Comparison of ΔT_m data and apparent binding data of **17a** and **17b** evidenced the importance of side chain length on the DNA stabilizing capacity, with the dimethylaminopropyl moiety favored. Experiments carried out at different poly(dAdT)$_2$ ratios confirmed the affinity of **17a** and **17b** to DNA duplex.

Inhibition of the topoisomerase I activity. In parallel, we have investigated the interaction with human topoisomerase I.[61] None of these derivatives induced topoisomerase I dependent DNA cleavable complex stabilisation (see ESI†). Consequently, topoisomerase I is not a real target of these molecules even if some of them (**17a**, **17b** and **18**) showed interesting DNA binding properties.

Cytotoxicity. Cytotoxicity measurements to determine the drug concentration (in μM) required to inhibit HL-60 cell growth by 50% have also been carried out with dimethylaminoalkyl-substituted heptacyclic derivatives (**17a**, **17b** and **18**). From these results it appeared that derivatives with strong affinity to DNA showed cytotoxicity against HL-60 cell line at the micromolar range (Table 3).

Conclusions

In summary, we have found a short and efficient synthesis, based on a Pd/C–H$_2$-mediated reductive *N*-heteroannulation as the key step, for the preparation of new penta- and heptacyclic indolo-, quinolino- or indoloquinolinocarbazoles. From DFT molecular orbital computations the signal separation observed for **8a** could be attributed to the specific spatial orientation of the ethyl group relatively to the neighbouring nitrophenyl group. In addition, this study might explain the observed conformer-dependent nitrene insertion (**8a** → **11** or **12**).

Preliminary screening led to the identification of new polycyclic carbazole-type lead compounds displaying some intriguing pharmacological properties. Thus, the pentacyclic compound **13** proved to be an efficient inhibitor of CDK-5 and GSK-3β kinases implicated in Alzheimer disease.

Moreover, aminoalkyl-substituted heptacyclic carbazoles **17a**, **17b** and **18** interacted with DNA. *Tris*(aminopropyl)-substituted analog **18** also interfered with telomeric G-quadruplex structure, a promising new target in anticancer research. Pharmacomodulations of the new skeletons identified are under investigation aiming at structure–activity relationship studies and optimisation of the inhibitory potency and/or selectivity.

Experimental

Chemistry

General methods. All solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Reactions and products were routinely monitored by thin-layer chromatography (TLC) on silica gel (Kieselgel 60 F254, Merck). Column chromatography purifications were performed on CHROMAGEL® Silice 60 ACC 70–200 mm silica gel. Melting points were determined on a Reichert Thermovar hot-stage apparatus and are uncorrected. IR spectra were measured on a Perkin-Elmer Spectrum BX FTIR instrument. NMR spectra of compounds **6b–c**, **8a–c**, **10**, **12**, **13**, **14**, **16b**, **17a**,**b** and **18** were recorded on a Bruker AC 300 spectrometer. All ^{1}H NMR and ^{13}C NMR spectra are reported in δ units (ppm) using $CDCl_3$ (diluted with 10% of CD_3OD for compound **16b** (n = 2)) or DMSO-d_6 as solvent. Couplings expressed as s, br s, d, br d, ddd, t, m correspond to singlet, broad-singlet, doublet, broad-doublet, doublet of doublets of doublets, triplet and multiplet, respectively. For compounds **8b** and **8c**, the CPD experiment was used to record the ^{13}C NMR spectra; in all other cases (compounds **6b–c**, **8a**, **10**, **11**, **12**, **13**, **14**, **16b**, **17a**,**b** and **18**), the *J*-Modulated one was chosen. Partial or complete chemical shift assignment of compounds **6b–c**, **8a–c**, **10**, **12**, **13**, **14**, **16b**, **17a**,**b** and **18** was achieved using various two dimensional NMR experiments (COSY, HSQC, HMBC). For compound **11**, mono and two dimensional exhaustive NMR studies (COSY, HSQC, HMBC, ROESY) were realized using a Bruker DR X 500 spectrometer and DMSO-d_6 as solvent. Mass spectra were recorded either on an MSQ ThermoFinnigan apparatus using electronspray (ESI) in positive ion mode or on a GCT Waters apparatus using electronimpact (EI, HRMS). Microwave activated reactions were carried out in a Normalab Analis Normatron 112® oven or in a CEM Discover® (300 W) apparatus.

General procedure for the preparation of carbazoles (8a–c) by microwave irradiation

To a solution of the diastereomerically pure tetramolecular condensation product **3a**, **3b** or **3c** in CH_2Cl_2 was added silicagel. After concentration under reduced pressure the residue was heated under microwave irradiation in an open reaction vessel. *Procedure A*: Normalab Normatron 112® apparatus (1000 W); *Procedure B*: CEM Discover® apparatus. Purification of the crude solid by column chromatography on silica gel afforded the corresponding carbazoles **8a**, **8b** or **8c**.

2,4-*Bis*(2′-nitrophenyl)-1-ethoxycarbonyl-9*H*-carbazole (8a) mixture of three inseparable atropoisomers (a1/a2/a3). *Procedure B*: starting from **3a** (100 mg, 0.13 mmol) mixed with silicagel (250 mg); Irradiation: CEM Discover® (200 °C for 15 min); Purification (eluent: cyclohexane/CH_2Cl_2: 40/60→CH_2Cl_2) gave the orange foam **8a** (32 mg, 41%), as a mixture of three inseparable atropoisomers (^{1}H NMR ratio of a1/a2/a3: 50/29/21). ν_{max}(film)/cm^{-1} 3418, 1683, 1674, 1520, 1344, 1304, 1274, 1243, 1168 and 1141; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.88 (6 H, 2 × t, J^{a1} and J^{a2} 7.1, 2 × $CO_2CH_2CH_3$)^{a1,a2}, 1.65 (3 H, t, J 7.1 Hz, $CO_2CH_2CH_3$)^{a3}, 4.08 (4 H, 2 × quartet, J^{a1} and J^{a2} 7.1, 2 × $CO_2CH_2CH_3$)^{a1,a2}, 4.76 (2 H, quartet, J 7.1, $CO_2CH_2CH_3$)^{a3}, 6.78 (1 H^{a3}, d, J 8.0), 6.82–7.03 (4 H, m, 2 × H^{a2} and 2 × H^{a3}), 7.19–7.89 (26 H, m, 10 × H^{a1}, 8 × H^{a2} and 8 × H^{a3}), 8.09 (1 H^{a2}, d, J 8.0), 8.14–8.26 (2 H, m, 1 × H^{a2} and 1 × H^{a1}), 8.30 (1 H^{a3}, d, J 7.9), 8.81–8.94 (3 H, m, 1 × H^{a2} and 2 × H^{a1}), 10.15 (1 H, s, NH)^{a3}, 10.40 (1 H, s, NH)^{a2} and 10.60 (1 H, s, NH)^{a1}; δ_C(75 MHz; $CDCl_3$; Me_4Si) 13.3 (2 × $CO_2CH_2CH_3$)^{a1,a2}, 14.6 ($CO_2CH_2CH_3$)^{a3}, 60.8 ($CO_2CH_2CH_3$)^{a1}, 60.9 ($CO_2CH_2CH_3$)^{a2}, 61.4 ($CO_2CH_2CH_3$)^{a3}, 107.7, 109.6, 110.5, 110.8, 111.0^{a1}, 111.2^{a1}, 111.3, 117.5^{a1}, 118.9, 119.1, 119.2, 119.4^{a1}, 119.7^{a1}, 120.0, 120.8^{a1}, 121.0, 121.1, 121.2, 121.3^{a1}, 121.5, 121.8^{a1}, 122.4^{a1}, 122.5, 123.3^{a1}, 123.7, 124.5 (2 × CH)^{a1}, 124.6, 124.8, 125.2, 125.8^{a1}, 126.0, 126.5, 126.7, 126.8, 127.0^{a1}, 127.9, 128.8^{a1}, 129.4,

130.2, 130.8, 131.8, 131.9, 132.1[a1], 132.3, 132.5, 132.7, 132.8, 133.0[a1], 133.2[a1], 133.3, 133.7, 134.1[a1], 134.2, 134.6, 136.2[a1], 136.3, 137.4, 137.8, 138.2, 138.3, 139.5[a1], 140.2, 140.8, 141.1[a1], 141.5, 148.4 (2 × *C*–NO_2), 149.3 (2 × *C*–NO_2), 149.4 (2 × *C*–NO_2)[a1], 167.6 (2 × *C*$O_2CH_2CH_3$)[a1,a2] and 168.3 (*C*$O_2CH_2CH_3$)[a3]; *m/z* (EI) 481 (M^+ 100%); HRMS calcd. for $C_{27}H_{19}N_3O_6$ 481.1274, found 481.1248.

In some cases purification by column chromatography of the crude mixture of three atropoisomers of **8a** allowed the isolation of a small quantity of the pure atropoisomer **8a1** as a pale orange foam.

ν_{max}(film)/cm^{-1} 3426, 1680, 1522, 1349, 1307, 1277, 1246, and 1171; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.90 (3 H, t, *J* 7.1, $CO_2CH_2CH_3$), 4.11 (2 H, quartet, *J* 7.1, $CO_2CH_2CH_3$), 7.39–7.82 (10 H, m), 8.25 (1 H, dd, *J* 1.3 and 8.0), 8.88 (1 H, d, *J* 7.9), 8.91 (1 H, d, *J* 7.8) and 10.61 (1 H, s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 13.4 ($CO_2CH_2CH_3$), 60.8 ($CO_2CH_2CH_3$), 111.0, 111.2, 117.6 (4a-C), 119.4, 119.8, 121.0, 121.4 (4b-C), 121.9, 122.5, 123.3, 124.5, 124.6, 125.8, 127.0, 128.9, 132.2, 133.1, 133.2, 134.2, 136.2, 139.6, 141.1, 149.4 (2 × *C*–NO_2) and 167.6 (*C*$O_2CH_2CH_3$); *m/z* (EI) 481 (M^+ 100%); HRMS calcd. for $C_{27}H_{19}N_3O_6$ 481.1274, found 481.1267.

Preparation of polycyclic derivatives (10), (11), (12), (13) and (14)

To a solution of the three inseparable atropoisomers **8a**(a1,a2,a3) (836 mg, 1.74 mmol) in a mixture of toluene–ethanol (20 mL/50 mL) was added Pd/C 10% (124 mg). The suspension was stirred in an autoclave under hydrogen atmosphere (10 bar). After 25 h, the green suspension was filtered off on a Celite® pad and the catalyst was successively washed with toluene, CH_2Cl_2 and methanol affording a first *filtrate I*. The remaining solid was finally washed twice with distilled THF to give the second *filtrate II*. After concentration of the *filtrate I*, the dark-yellow residue (352 mg) was purified by column chromatography on silica gel to obtain successively **10** (5 mg, 0.7%), as a yellow powder (eluent: petroleum ether/CH_2Cl_2: 70/30), **12** (122 mg, 17%), as a green powder (eluent: petroleum ether/CH_2Cl_2: 50/50) and **13** (7 mg, 1%), as a pale yellow powder (eluent: CH_2Cl_2–MeOH: 99/1). Concentration of the *filtrate II in vacuo* at room temperature afforded **11** (356 mg, 55%), as a yellow powder.

15-Ethoxy-5*H*,14*H*-indolo[2,3-*c*]quinolino[4,3-*a*]carbazole (10)

Yellow powder; mp 184–186 °C; [Found: C, 81.01; H, 4.68; N, 10.69. $C_{27}H_{19}N_3O$ requires C, 80.77; H, 4.77; N, 10.47%];.ν_{max} (KBr)/cm^{-1} 3463, 1604, 1591, 1458, 1419, 1392, 1326, 1309, 1230, 1216, 1141 and 1036; δ_H(300 MHz; DMSO-d_6) 1.70 (3 H, t, *J* 7.0, OCH_2CH_3), 5.07 (2 H, q, *J* 7.0, OCH_2CH_3), 7.42–7.53 (2 H, m, 8-H and 11-H), 7.56–7.67 (2 H, m, 7-H and 12-H), 7.74–7.85 (2 H, m, 2-H and 3-H), 8.03 (2 H, dl, *J* 8.5), 8.08 (1 H, d, *J* 8.1), 8.94 (1 H, d, *J* 8.1, 9-H or 10-H), 9.0 (1 H, d, *J* 8.1, 9-H or 10-H), 9.23 (1 H, d, *J* 7.6, 4-H), 11.60 (1 H, s, 5-NH) and 12.03 (1 H, s, 14-NH); δ_C(75 MHz; DMSO-d_6) 15.0 (OCH_2CH_3), 62.2 (OCH_2CH_3), 104.9, 112.7, 113.1, 115.4, 119.4, 119.8, 119.9, 121.0, 121.2, 121.5, 121.8, 122.7 (9-CH or 10-CH), 123.2 (9-CH or 10-CH), 124.9 (3-CH), 125.1, 125.6 (4-CH), 126.1, 127.7 (1-CH), 128.4 (2-CH), 129.8, 130.1, 139.4, 141.6, 143.1 (16a-C) and 158.6 (15-C). *m/z* (ESI) 402 $(M + H)^+$.

15-Oxo-5*H*,14*H*-15,16-dihydroindolo[2,3-*c*]quinolino[4,3-*a*]carbazole (11)

Yellow powder; mp >350 °C; ν_{max} (KBr)/cm^{-1} 3447, 3433, 3381, 3026, 1661, 1613, 1591 and 1320; δ_H(500 MHz; DMSO-d_6) 7.42 (1 H, t, *J* 7.5, 11-H), 7.46 (1 H, t, *J* 7.5, 8-H), 7.51 (1 H, ddd, *J* 1.9, 6.7 and 8.2, 3-H), 7.53 (1 H, t, *J* 7.5, 12-H), 7.58–7.67 (3 H, m, 1-H, 2-H and 7-H), 7.95 (1 H, d, *J* 8.1, 6-H), 8.04 (1 H, d, *J* 8.1, 13-H), 8.87 (1 H, d, *J* 8.2, 10-H), 8.96 (1 H, d, *J* 8.2, 9-H), 8.99 (1 H, d, *J* 8.2, 4-H), 11.89 (1 H, s, 5-NH), 12.09 (1 H, s, 16-NH) and 12.22 (1 H, s, 14-NH); δ_C(125 MHz; DMSO-d_6) 108.3 (14b-C), 112.8 (13-CH), 112.9 (6-CH), 115.2 (9c-C), 116.3 (1-CH), 117.5 (4a-C), 119.3 (11-CH), 119.5 (4b-C), 119.9 (8-CH), 120.7 (9d-C), 121.4 (9b-C), 121.5 (9a-C), 122.7 (3-CH), 122.7 (10-CH), 123.3 (9-CH), 125.2 (12-CH), 126.0 (4-CH), 126.4 (7-CH), 128.8 (2-CH), 129.6 (4c-C), 133.5 (14a-C), 136.5 (16a-C), 139.8 (13a-C), 141.9 (5a-C) and 162.4 (CO); *m/z* (EI) 373 (M^+ 100%); HRMS calcd. for $C_{25}H_{15}N_3O$ 373.1215, found 373.1229.

6-Ethoxycarbonyl-12-(2′-aminophenyl)-5*H*,11*H*-indolo[3,2-*b*]carbazole (12)

Green solid; mp 190–192 °C; ν_{max} (KBr)/cm^{-1} 3436, 3048, 1661, 1613, 1318, 1265, 1243, 1164 and 1146; δ_H(300 MHz; DMSO-d_6) 1.55 (3 H, t, *J* 6.7, OCH_2CH_3), 4.65 (2 H, s, NH_2), 4.75 (2 H, q, *J* 6.7, OCH_2CH_3), 6.88 (1 H, t, *J* 7.9, 5′-H), 6.92 (1 H, t, *J* 8.0, 8-H), 7.02 (d, *J* 7.9, 1 H, 3′-H), 7.10 (1 H, d, *J* 7.9, 1-H), 7.15 (1 H, t, *J* 7.9, 2-H), 7.20 (1 H, d, *J* 7.9, 6′-H), 7.35 (1 H, t, *J* 7.9, 3-H), 7.38 (1 H, t, *J* 7.9, 4′-H), 7.42 (1 H, t, *J* 8.0, 9-H), 7.60 (1 H, d, *J* 7.9, 4-H), 7.70 (1 H, d, *J* 8.0, 10-H), 8.75 (1 H, d, *J* 8.0, 7-H), 10.75 (1 H, s, 11-NH) and 11.10 (1 H, s, 5-NH); δ_C(75 MHz; DMSO-d_6) 14.7 ($CO_2CH_2CH_3$), 61.0 ($CO_2CH_2CH_3$), 104.5 (6-C), 111.4 (10-CH), 111.6 (4-CH), 115.4 (3′-CH), 117.0 (5′-CH), 117.9 (2-CH), 118.5 (8-CH), 119.7 (1′-C), 120.3 (6a-C), 120.8 (12-C), 121.4 (12a-C), 121.6 (1-CH), 121.6 (12b-C), 121.9 (6b-C), 124.9 (7-CH), 126.0 (3-CH), 126.1 (9-CH), 129.7 (4′-CH), 130.4 (6′-CH), 134.1 (11a-C), 136.2 (5a-C), 141.4 (4a-C), 142.1 (10a-C), 146.4 (2′-C) and 167.6 (*C*$O_2CH_2CH_3$); *m/z* (EI) 419 (M^+ 100%) and 373 (37); HRMS calcd. for $C_{27}H_{21}N_3O_2$ 419.1634, found: 419.1629.

6-(2′-Aminophenyl)-12-oxo-11*H*-12,13-dihydroquinolino[4,3-*a*]carbazole (13)

Pale yellow solid; mp 175–177 °C; ν_{max} (KBr)/cm^{-1} 3401, 3216, 3048, 1652, 1647, 1613, 1335 and 1318; δ_H(300 MHz; DMSO-d_6) 4.65 (2 H, s, NH_2), 6.80 (1 H, t, *J* 7.6, 5′-H), 6.92 (1 H, d, *J* 7.6, 3′-H), 7.03 (1 H, t, *J* 8.1, 8-H), 7.22 (1 H, d, *J* 7.6, 6′-H), 7.25 (1 H, d, *J* 8.1, 7-H), 7.29 (1 H, t, *J* 7.4, 3-H), 7.30 (1 H, t, *J* 7.6, 4′-H), 7.38 (1 H, t, *J* 8.1, 9-H), 7.50 (1 H, d, *J* 7.4, 1-H), 7.55 (1 H, t, *J* 7.4, 2-H), 7.90 (1 H, d, *J* 8.1, 10-H), 8.05 (1 H, s, 5-H), 8.50 (1 H, d, *J* 7.4, 4-H), 11.90 (1 H, s, 13-NH) and 12.07 (1 H, s, 11-NH); δ_H(300 MHz; DMSO-d_6) 108.9 (11b-C), 112.6 (10-CH), 114.2 (5-CH), 114.9 (3′-CH), 116.4 (1-CH), 116.5 (5′-CH), 118.5 (4a-C), 119.5 (8-CH), 120.1 (6a-C), 121.3 (7-CH), 121.4 (6b-C), 122.6 (3-CH), 124.1 (4-CH), 124.6 (1′-C), 125.7 (9-CH), 129.3 (4′-CH), 129.3 (2-CH), 129.9 (6′-CH), 133.1 (4b-C), 136.7 (13a-C), 138.7 (11a-C), 139.3 (6-C), 140.4 (10a-C), 145.8 (2′-C) and 162.1 (CO); *m/z* (EI) 375 (M^+ 100%); HRMS calcd. for $C_{25}H_{17}N_3O$ 375.1372, found: 375.1373.

5*H*,14*H*-Indolo[2,3-*c*]quinolino[4,3-*a*]carbazole (14)

To a suspension of $LiAlH_4$ (2.10 g, 55.4 mmol) in dry DME (70 mL) was added at 0 °C under nitrogen atmosphere a solution of **11** (153 mg, 0.41 mmol) in dry DME (50 mL). The orange suspension was stirred at room temperature and then heated at 60 °C (oil bath) for 11 h under nitrogen atmosphere. To the orange suspension water (17 mL) was added dropwise at 0 °C under stirring until precipitation occurred. The precipitate was washed with DME, ethyl acetate, the filtrate was dried ($MgSO_4$), filtered and concentrated *in vacuo* (180 mg). Purification by chromatography on silica gel (eluent: CH_2Cl_2–CH_3OH: 98/2) gave **14** (104 mg, 71%), as a yellow powder.

In some cases, especially in longer reactions (15–16 h at 60 °C) a mixture of **14** and its dihydro derivative (**15**) was obtained. In that case, after destruction of the excess of $LiAlH_4$ the concentrated filtrate was dissolved in a mixture of toluene (20 mL) and DME (10 mL), *p*-chloranil (376 mg, 1.53 mmol) was added to the solution and the reaction mixture was heated at 50 °C for 6 h under nitrogen atmosphere until the complete conversion of **15** into **14** (TLC monitoring). After concentration *in vacuo* the brown residue was washed with CH_2Cl_2, filtered and concentrated to give **14** (386 mg, 61%), as a yellow powder; mp >350 °C; ν_{max} (KBr)/cm^{-1} 3410, 3233, 2916, 1613, 1577, 1458, 1379, 1309 and 1243; ^{1}H NMR (300 MHz; DMSO-d_6) 7.41–7.55 (2 H, m, 8-H and 11-H), 7.62 (1 H, t, *J* 8.1, 12-H), 7.69 (1 H, t, *J* 8.1, 7-H), 7.82 (1 H, d, *J* 8.1, 13-H), 7.98 (1 H, d, *J* 8.1, 6-H), 8.03–8.18 (2 H, m, 2-H and 3-H), 8.46 (1 H, d, *J* 7.5, 1-H), 8.85 (1 H, d, *J* 8.1, 10-H), 8.92 (1 H, d, *J* 8.1, 9-H), 9.41 (1 H, d, *J* 7.5, 4-H), 10.56 (1 H, s, 15-H), 12.28 (1 H, s, 5-NH) and 13.21 (1 H, s, 14-NH); δ_C(75 MHz; DMSO-d_6) 110.4, 112.1 (13-CH), 113.3 (6-CH), 115.4 (9c-C), 118.8, 120.1 (11-CH), 120.5 (8-CH), 121.2 (9a-C), 121.4 (9d-C), 123.0 (9b-C), 123.3 (10-CH), 123.4, 123.8 (9-CH), 126.0 (2 × CH, 12-CH and 1-CH), 126.5 (4-CH), 127.8 (7-CH), 128.7 (4c-C), 129.3 (2-CH), 129.9 (3-CH), 133.0 (14a-C), 139.0 (16a-C), 139.8 (13a-C), 142.7 (5a-C) and 146.0 (15-CH); *m/z* (ESI +) 358 (M + H)$^+$; *m/z* (EI) 357 (M$^+$ 100); HRMS calcd. for $C_{25}H_{15}N_3$ 357.1266, found: 357.1259.

General procedure for the preparation of *N*-substituted indolo[2,3-*c*]quinolino[4,3-*a*]carbazoles (16b) and (17a,b) and of *N*-substituted 15-oxo-15,16-dihydroindolo[2,3-*c*]quinolino[4,3-*a*]carbazole (18)

Sodium hydride 60% was carefully introduced at −50 °C under nitrogen atmosphere to a stirred solution of **11** or **14** in dry DMF. The suspension of the halide (series **a** (*n* = 1) or series **b** (*n* = 2)), Na_2CO_3 (powder) and tetrabutylammonium iodide (TBAI) in dry DMF was dropwise added to the latter under nitrogen atmosphere. The mixture was heated, diluted with ethyl acetate and extracted by a saturated aqueous potassium carbonate solution. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Purification of the crude mixture by column chromatography on silica gel afforded the corresponding *N*-substituted carbazoles.

Preparation of carbazoles 16b and 17b

According to General Procedure: Starting from **14** (55 mg, 0.15 mmol) reacted with NaH 60% (37 mg, 0.92 mmol), 3-chloropropyldimethylamine hydrochloride (series **b**) (146 mg, 0.92 mmol), Na_2CO_3 (340 mg, 2.46 mmol), TBAI (6 mg, 0.016 mmol) in DMF (12 mL); Heating: 85 °C for 13 h; Extraction: sat. aq. K_2CO_3 (10 mL) and ethyl acetate (10 mL × 3); Purification (eluent: CH_2Cl_2–CH_3OH: 98/2→ CH_2Cl_2–CH_3OH: 60/40) gave the orange foam **16b** (8 mg, 12%) and **17b** (45 mg, 55%) as an amorphous orange solid.

5-[3′-(*N*,*N*-Dimethylamino)propyl]-14*H*-indolo[2,3-*c*]quinolino[4,3-*a*]carbazole (16b)

Orange foam; ν_{max}(film)/cm^{-1} 2919, 2844, 1595, 1581, 1455, 1378, 1361 and 1328; δ_H(300 MHz; $CDCl_3$; CD_3OD; Me_4Si) 2.18–2.32 (2 H, m, 2′-CH_2), 2.32 (6 H, s, N(Me)$_2$), 2.53 (2 H, t, *J* 7.4, 3′-CH_2), 4.72 (2 H, t, *J* 7.4, 1′-CH_2), 7.43 (2 H, t, *J* 7.7, 11-H and 8-H), 7.59 (2 H, t, *J* 8.3, 12-H and 7-H), 7.69 (1 H, d, *J* 8.3, 6-H), 7.73–7.88 (3 H, m, 13-H, 2-H and 3-H), 8.21 (1 H, d, *J* 8.7, 1-H), 8.82 (1 H, d, *J* 8.3, 10-H), 8.86 (1 H, d, *J* 8.3, 9-H), 9.00 (1 H, d, *J* 7.7, 4-H) and 9.83 (1 H, s, 15-H); δ_C(75 MHz; $CDCl_3$; CD_3OD; Me_4Si) 26.5 (2′-CH_2), 44.6 (N(Me)$_2$), 44.9 (1′-CH_2), 55.9 (3′-CH_2), 109.5 (6-CH), 111.9 (13-CH), 112.8 (4b-C or 14b-C), 117.3 (9c-C), 119.0 (4b-C or 14b-C), 119.6 (8-CH), 119.8 (11-CH), 120.3 (9b-C), 122.0 (9a-C), 122.5 (9d-C), 122.9 (10-CH), 123.5 (9-CH), 123.6 (4a-C), 124.6 (4-CH), 125.3 (12-CH), 126.1 (7-CH), 127.3 (3-CH), 127.9 (2-CH), 128.9 (1-CH), 130.2 (14a-C), 131.3 (4c-C), 141.3 (5a-C or 13a-C), 141.4 (5a-C or 13a-C), 143.0 (16a-C) and 146.8 (15-CH); *m/z* (EI) 443 (M$^+$ + 1, 4%), 442 (M$^+$, 13%), 368 (31), 129 (100); HRMS calcd. for $C_{30}H_{26}N_4$ 442.2157, found 442.2147.

5-[3′-(*N*,*N*-Dimethylamino)propyl]-14-[3″-(*N*,*N*-dimethylamino)-propyl]-indolo[2,3-*c*]quinolino[4,3-*a*]carbazole (17b)

Amorphous orange solid; ν_{max} (KBr)/cm^{-1} 3047, 2929, 2754, 1598, 1579, 1456, 1446, 1373 and 1321; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.92–1.10 (2 H, m, 2′-CH_2), 1.41 (2 H, t, *J* 7.0, 3′-CH_2), 1.68 (6 H, s, N(Me)$_2$), 2.31 (6 H, s, N(Me)$_2$), 2.32–2.40 (2 H, m, 2″-CH_2), 2.52 (2 H, t, *J* 6.9, 3″-CH_2), 4.68 (2 H, t, *J* 7.0, 1′-CH_2), 4.99 (2 H, t, *J* 6.9, 1″-CH_2), 7.46–7.56 (2 H, m, 11-H and 3-H), 7.57–7.72 (3 H, m, 12-H, 7-H and 8-H), 7.73–7.75 (1 H, m, 2-H), 7.75–7.84 (2 H, m, 13-H and 1-H), 8.26 (1 H, dd, *J* 1.1 and 8.0, 6-H), 8.80 (1 H, dd, *J* 1.1 and 8.1, 4-H), 8.97 (1 H, d, *J* 8.0, 10-H), 9.04 (1 H, d, *J* 8.0, 9-H) and 10.20 (1 H, s, 15-H); δ_C(75 MHz; $CDCl_3$; Me_4Si) 25.0 (2′-CH_2), 27.7 (2″-CH_2), 44.7 (N(Me)$_2$), 45.0 (1″-CH_2), 45.5 (N(Me)$_2$), 46.7 (1′-CH_2), 55.9 (3′-CH_2), 56.5 (3″-CH_2), 109.7 (13-CH), 112.9 (1-CH), 113.3 (4b-C), 116.8 (9c-C), 119.6 (11-CH), 120.6 (3-CH), 122.0 (9d-C), 122.8 (9a-C), 123.1 (10-CH), 123.4 (9b-C), 123.8 (14b-C), 124.1 (9-CH), 125.3 (12-CH), 125.4 (4a-C), 125.8 (8-CH), 126.2 (7-CH), 127.4 (4-CH), 127.9 (2-CH), 128.3 (6-CH), 132.3 (14a-C), 132.8 (4c-C), 141.6 (13a-C), 143.5 (16a-C), 145.8 (5a-C) and 147.5 (15-CH); *m/z* (ESI+) 528 (M + 1)$^+$; *m/z* (EI) 528 (M$^+$ + 1, 26%), 527 (M$^+$ 100%) 469 (67), 455 (41); HRMS calcd. for $C_{35}H_{37}N_5$ 527.3049, found 527.3042.

Preparation of *N*-substituted 15-oxo-15,16-dihydroindolo[2,3-*c*]quinolino[4,3-*a*]carbazole (18)

According to General Procedure: Starting from **11** (78 mg, 0.21 mmol) reacted with NaH 60% (76 mg, 1.89 mmol),

3-chloropropyldimethylamine hydrochloride (series **b**) (199 mg, 1.26 mmol), Na_2CO_3 (356 mg, 3.36 mmol), TBAI (8 mg, 0.021 mmol) in DMF (12 mL); Heating: 85 °C for 20 h; Extraction: sat. aq. K_2CO_3 (10 mL) and ethyl acetate (10 mL × 3); Purification (eluent: CH_2Cl_2–CH_3OH: 95/5→ CH_3OH/NH_4OH: 90/10) gave the inseparable mixture of mono- and dialkylated compounds (11 mg; approx. 17%) as an amorphous orange solid, and **18** (70 mg, 53%) as an amorphous orange solid.

5,14,16-*Tris*[3-(*N*,*N*-dimethylamino)propyl]-15-oxo-15,16-dihydroindolo[2,3-*c*]quinolino[4,3-*a*]carbazole (18)

Amorphous orange solid; ν_{max} (KBr)/cm^{-1} 2943, 2706, 1635, 1598, 1458, 1370 and 1115; δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.15–1.29 (2 H, m, 2‴-CH_2), 1.44–1.58 (2 H, m, 3‴-CH_2), 1.72 (6 H, s, N(Me)$_2$), 1.73–1.83 (2 H, m, 2′-CH_2 or 2″-CH_2), 2.09–2.19 (2 H, m, 2′-CH_2 or 2″-CH_2), 2.31 (6 H, s, N(Me)$_2$), 2.33 (6 H, s, N(Me)$_2$), 2.50 (2 H, t, *J* 7.3, 3′-CH_2 or 3″-CH_2), 2.53–2.67 (2 H, m, 3′-CH_2 or 3″-CH_2), 3.48 (2 H, t, *J* 6.4, 1′-CH_2 or 1″-CH_2), 4.51 (2 H, t, *J* 7.5, 1′-CH_2 or 1″-CH_2), 4.58–4.75 (2 H, m, 1‴-CH_2), 7.30 (1 H, t, *J* 7.9, 3-H), 7.41–7.54 (3 H, m, 2-H, 8-H and 11-H), 7.57–7.68 (3 H, m, 1-H, 7-H, 12-H), 7.73 (2 H, d, *J* 8.2, 6-H and 13-H), 8.27 (1 H, dd, *J* 1.1 and 7.9, 4-H), 8.94 (1 H, d, *J* 8.0, 9-H or 10-H), 9.02 (1 H, d, *J* 8.0, 9-H or 10-H); δ_C(125 MHz; $CDCl_3$; Me_4Si) 24.8 (2‴-CH_2), 25.9 (2′-CH_2 or 2″-CH_2), 27.3 (2′-CH_2 or 2″-CH_2), 41.3 (1′-CH_2 or 1″-CH_2), 44.9 (N(Me)$_2$), 45.0 (N(Me)$_2$), 45.4 (1‴-CH_2), 45.5 (N(Me)$_2$), 55.9 (3‴-CH_2), 56.4 (3′-CH_2 or 3″-CH_2), 57.0 (3′-CH_2 or 3″-CH_2), 68.7 (1′-CH_2 or 1″-CH_2), 110.6, 111.1 (6-CH or 13-CH), 112.4 (6-CH or 13-CH), 114.3 (1-CH), 119.4, 119.5 (8-CH or 11-CH), 119.9 (4a-C), 120.3 (8-CH or 11-CH), 121.7 (3-CH), 123.0 (9-CH or 10-CH), 124.3 (9-CH or 10-CH), 124.9 (9a-C or 9d-C), 125.5 (7-CH or 12-CH), 126.5 (7-CH or 12-CH), 128.2 (2-CH), 128.3 (4-CH), 132.4, 135.5 (16a-C), 136.5, 143.4 (5a-C or 13a-C), 145.7 (5a-C or 13a-C) and 160.6 (CO); *m*/*z* (EI) 629 (M^+ + 1, 13%), 628 (M^+, 51%), 543 (100), 472 (39), 458 (67), 357 (42); HRMS calcd. for $C_{40}H_{48}N_6O$ 628.3890, found 628.3906.

Computational experiments

Calculations were performed using the GAUSSIAN 03 packages.[47] The structures were fully optimised at the HF-DFT (B3LYP)[48–51] level of theory using the analytical gradients and the 6-31G** basis set. The properties of similar cyclic structures have suitably been obtained using this hybrid DFT functional.[52] Stationary points were obtained using both the gas-phase model and the Polarized Continuum Model (PCM).[53–55] Chemical shifts were predicted at the B3LYP/6-311++G(2d,p)//B3LYP/6-31G** level using the GIAO method[56,57] For details, see Electronic Supplementary Information.†

Biological evaluation

In vitro kinase inhibition assays (CDK-5/p25,[10a,58] GSK-3αβ[59] and DYRK-1A), FRET melting experiments,[60] DNA binding measurements and topoisomerase I inhibition experiments[61] were carried out according to standardized protocols (see Electronic Supplementary Information†).

Acknowledgements

Authors thank Pr. J.-Y. Laronze (Université de Reims) for helpful discussions. Financial support of CNRS, *Région Champagne-Ardenne*, *Fond Européen pour le Développement Régional* (*FEDER*) and University of Reims is gratefully acknowledged. F.C. thanks the French Ministry of Education and Research for a fellowship. The C.R.I.H.A.N computing centre and the computational centre of the Université de Reims Champagne-Ardenne (ROMEO) are acknowledged for the CPU time donated. Spectroscopic measurements by C. Petermann (NMR), D. Patigny (MS) and P. Sigaut (MS) are gratefully acknowledged. J.-F.R. and B.B. are supported by the *Ligue Nationale contre le Cancer*, *équipe labellisée 2010*.

Notes and references

1 For some recent reviews, see: (*a*) G. H. Kirsch, *Curr. Org. Chem.*, 2001, **5**, 507–518; (*b*) H.-J. Knölker and K. R. Reddy, *Chem. Rev.*, 2002, **102**, 4303–4427; (*c*) H.-J. Knölker, *Top. Curr. Chem.*, 2005, **244**, 115–148; (*d*) C. Sanchez, C. Méndez and J. A. Salas, *Nat. Prod. Rep.*, 2006, **23**, 1007–1045; (*e*) T. A. Choi, R. Czerwonka, R. Forke, A. Jäger, J. Knöll, M. P. Krahl, T. Krause, K. R. Reddy, S. G. Franzblau and H.-J. Knölker, *Med. Chem. Res.*, 2008, **17**, 374–385; (*f*) T. Janosik, N. Wahlström and J. Bergman, *Tetrahedron*, 2008, **64**, 9159–9180.

2 (*a*) H.-J. Knölker and K. R. Reddy, in *The Alkaloids*, ed. G. A. Cordell, Academic Press, Amsterdam, 2008, vol. 65, pp. 1-430; (*b*) H.-J. Knölker, *Chem. Lett.*, 2009, **38**, 8–13.

3 F. Lehmann, S. Haile, E. Axen, C. Medina, J. Uppenberg, S. Svensson, T. Lundbäck, L. Rondahl and T. Barf, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 4445–4448.

4 K. R. Brunden, J. Q. Trojanowski and V. M.-Y. Lee, *Nat. Rev. Drug Discovery*, 2009, **8**, 783–793.

5 (*a*) E. A. Dubois, J. C. van den Bos, T. Doornbos, P. A. P. M. van Doremalen, G. A. Somsen, J. A. J. M. Vekemans, A. G. M. Janssen, H. D. Batink, G. J. Boer, M. Pfaffendorf, E. A. van Royen and P. A. van Zwieten, *J. Med. Chem.*, 1996, **39**, 3256–3262; (*b*) N. Dias, U. Jacquemard, B. Baldeyrou, C. Tardy, A. Lansiaux, P. Colson, F. Tanious, W. D. Wilson, S. Routier, J.-Y. Mérour and C. Bailly, *Biochemistry*, 2004, **43**, 15169–15178; (*c*) L. Hu, Z.-R. Li, Y. Li, J. Qu, Y.-H. Ling, J.-D. Jiang and D. W. Boykin, *J. Med. Chem.*, 2006, **49**, 6273–6282.

6 For some recent review references on carbazoles as antitumor agents, see: (*a*) M. Prudhomme, *Curr. Med. Chem.: Anti-Cancer Agents*, 2004, **4**, 509–521; (*b*) C. Asche and M. Demeunynck, *Curr. Med. Chem.–Anticancer Agents*, 2007, **7**, 247–267. For some recent review references on topoisomerase inhibitors, see: (*c*) M. Prudhomme, *Curr. Med. Chem.*, 2000, **7**, 1189–1212; (*d*) Y. Pommier, *Chem. Rev.*, 2009, **109**, 2894–2902. For some recent review references on kinase inhibitors, see: (*e*) L. Meijer, *Bull. Cancer*, 2006, **93**, 41–53; (*f*) S. Lapenna and A. Giordano, *Nat. Rev. Drug Discovery*, 2009, **8**, 547–566. For some recent references on telomerase inhibitors by G-quadruplex stabilization, see: (*g*) J. Cuesta, M. A. Read and S. Neidle, *Mini-Rev. Med. Chem.*, 2003, **3**, 11–21; (*h*) D. Monchaud and M.-P. Teulade-Fichou, *Org. Biomol. Chem.*, 2008, **6**, 627–636; (*i*) A. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, J.-F. Riou and J.-L. Mergny, *Biochimie*, 2008, **90**, 131–155; (*j*) L. Ginnari-Satriani, V. Casagrande, A. Bianco, G. Ortaggi and M. Franceschin, *Org. Biomol. Chem.*, 2009, **7**, 2513–2516; (*k*) M. Franceschin, *Eur. J. Org. Chem.*, 2009, 2225–2238.

7 G. W. Gribble, in *The Alkaloids*, ed. A. Brossi, Academic Press, London, 1990, vol. 39, pp 239-352.

8 (*a*) J. A. Bush, B. H. Long, J. J. Catino, W. T. Bradner and K. Tomita, *J. Antibiot.*, 1987, **40**, 668–678; (*b*) C. Bailly, in *Small Molecule DNA and RNA Binders*, ed. M. Demeunynck, C. Bailly and W. D. Wilson, Wiley-VCH Verlag GmbH & Co. KGaA, 2003, part 2: vol. 2, chap. 20, pp. 538-575.

9 S. Omura, Y. Iwai, A. Hirano, A. Nakagawa, J. Awaya, H. Tsuchiya, Y. Takahashi and R. Masuma, *J. Antibiot.*, 1977, **30**, 275–282.

10 (*a*) S. Leclerc, M. Garnier, R. Hoessel, D. Marko, J. A. Bibb, G. L. Snyder, P. Greengard, J. Biernat, Y.-Z. Wu, E.-M. Mandelkow, G. Eisenbrand and L. Meijer, *J. Biol. Chem.*, 2000, **276**, 251–260; (*b*) J. A.

Bertrand, S. Thieffine, A. Vulpetti, C. Cristiani, B. Valsasina, S. Knapp, H. M. Kalisz and M. Flocco, *J. Mol. Biol.*, 2003, **333**, 393–407.
11 (*a*) S. Akinaga, K. Sugiyama and T. Akiyama, *Anticancer Drug Design*, 2000, **15**, 43–52; (*b*) A. Tenzer and M. Pruschy, *Curr. Med. Chem.: Anti-Cancer Agents*, 2003, **3**, 35–46.
12 (*a*) R. G. S. Berlinck, R. Britton, E. Piers, L. Lim, M. Roberge, R. Moreira da Rocha and R. J. Andersen, *J. Org. Chem.*, 1998, **63**, 9850–9856; (*b*) S. Deslandes, S. Chassaing and E. Delfourne, *Mar. Drugs*, 2009, **7**, 754–786.
13 For some recent references, see: (*a*) G. Zhang, J. Shen, H. Cheng, L. Zhu, L. Fang, S. Luo, M. T. Muller, G. Eui Lee, L. Wei, Y. Du, D. Sun and P. G. Wang, *J. Med. Chem.*, 2005, **48**, 2600–2611; (*b*) S. Messaoudi, F. Anizon, P. Peixoto, M.-H. David-Cordonnier, R. M. Golsteyn, S. Leonce, B. Pfeiffer and M. Prudhomme, *Bioorg. Med. Chem.*, 2006, **14**, 7551–7562; (*c*) E. Conchon, F. Anizon, B. Aboab and M. Prudhomme, *J. Med. Chem.*, 2007, **50**, 4669–4680; (*d*) S.-M. Yang, R. Malaviya, L. J. Wilson, R. Argentieri, X. Chen, C. Yang, B. Wang, D. Cavender and W. V. Murray, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 326–331; (*e*) F. Animati, M. Berettoni, M. Bigioni, M. Binaschi, P. Felicetti, L. Gontrani, O. Incani, A. Madami, E. Monteagudo, L. Olivieri, S. Resta, C. Rossi and A. Cipollone, *ChemMedChem*, 2008, **3**, 266–279; (*f*) H. Henon, E. Conchon, B. Hugon, S. Messaoudi, R. Golsteyn and M. Prudhomme, *Anticancer Agents in Med. Chem.*, 2008, **8**, 577–597; (*g*) R. Tripathy, T. S. Angeles, S. X. Yang and J. P. Mallamo, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3551–3555; (*h*) S. Delarue-Cochin and I. McCort-Tranchepain, *Org. Biomol. Chem.*, 2009, **7**, 706–716; (*i*) I. Hers, J. M. Tavaré and R. M. Denton, *FEBS Lett.*, 1999, **460**, 433–436; (*j*) I. Dorronsoro, A. Castro and A. Martinez, *Expert Opin. Ther. Pat.*, 2002, **12**, 1527–1536; (*k*) H.-C. Zhang, K. B. White, H. Ye, D. F. McComsey, C. K. Derian, M. F. Addo, P. Andrade-Gordon, A. J. Eckardt, B. R. Conway, L. Westover, J. Z. Xu, R. Look, K. T. Demarest, S. Emanuel and B. E. Maryanoff, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3049–3053; (*l*) H.-C. Zhang, L. V. R. Boñaga, H. Ye, C. K. Derian, B. P. Damiano and B. E. Maryanoff, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2863–2868; (*m*) A. S. Wagman, K. W. Johnson and D. E. Bussiere, *Curr. Pharm. Des.*, 2004, **10**, 1105–1137; (*n*) A. Martinez and D. I. Perez, *J. Alzh. Dis.*, 2008, **15**, 181–191.
14 B. D. Palmer, A. M. Thomson, R. J. Booth, E. M. Dobrusin, A. J. Kraker, H. H. Lee, E. A. Lunney, L. H. Mitchell, D. F. Ortwine, J. B. Smaill, L. M. Swan and W. A. Denny, *J. Med. Chem.*, 2006, **49**, 4896–4911.
15 S. Routier, J.-Y. Mérour, N. Dias, A. Lansiaux, C. Bailly, O. Lozach and L. Meijer, *J. Med. Chem.*, 2006, **49**, 789–799.
16 (*a*) G. Zhu, S. Conner, X. Zhou, C. Shih, H. B. Brooks, E. Considine, J. A. Dempsey, C. Ogg, B. Patel, R. M. Schultz, C. D. Spencer, B. Teicher and S. A. Watkins, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1231–1235; (*b*) T. A. Engler, K. Furness, S. Malhotra, C. Sanchez-Martinez, C. Shih, W. Xie, G. Zhu, X. Zhou, S. Conner, M. M. Faul, K. A. Sullivan, S. P. Kolis, H. B. Brooks, B. Patel, R. M. Schultz, T. B. DeHahn, K. Kirmani, C. D. Spencer, S. A. E. L. Considine, J. A. Dempsey, C. A. Ogg, N. B. Stamm, B. D; Anderson, R. M. Campbell, V. Vasudevan and M. L. Lytle, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2261–2267.
17 H. Henon, F. Anizon, R. M. Goldsteyn, S. Léonce, R. Hofmann, B. Pfeiffer and M. Prudhomme, *Bioorg. Med. Chem.*, 2006, **14**, 3825–3824.
18 (*a*) M. P. Coghlan, A. A. Culbert, D. A. E. Cross, S. L. Corcoran, J. W. Yates, N. J. Pearce, O. L. Rausch, G. J. Murphy, P. S. Carter, L. Roxbee Cox, D. Mills, M. J. Brown, D. Haigh, R. W. Ward, D. G. Smith, K. J. Murray, A. D. Reith and J. C. Holder, *Chem. Biol.*, 2000, **7**, 793–803; (*b*) D. A. E. Cross, A. A. Culbert, K. A. Chalmers, L. Facci, S. D. Skapper and A. D. Reith, *J. Neurochem.*, 2001, **77**, 94–102; (*c*) M. Alonso and A. Martinez, *Curr. Med. Chem.*, 2004, **11**, 755–763; (*d*) T. A. Engler, J. R. Henry, S. Malhotra, B. Cunningham, K. Furness, J. Brozinick, T. P. Burkholder, M. P. Clay, J. Clayton, C. Diefenbacher, E. Hawkins, P. W. Iversen, Y. Li, T. D. Lindstrom, A. L. Marquart, J. McLean, D. Mendel, E. Misener, D. Briere, J. C. O'Tool, W. J. Porter, S. Queener, J. K. Reel, R. A. Owens, R. A. Brier, T. E. Eessalu, J. R. Wagner, R. M. Campbell and R. Vaughn, *J. Med. Chem.*, 2004, **47**, 3934–3937; (*e*) T. A. Engler, S. Malhotra, T. P. Burkholder, J. R. Henry, D. Mendel, W. J. Porter, K. Furness, C. Diefenbacher, A. L. Marquart, J. K. Reel, Y. Li, J. Clayton, B. Cunningham, J. McLean, J. C. O'Tool, J. Brozinick, E. Hawkins, E. Misener, D. Briere, R. A. Brier, J. R. Wagner, R. M. Campbell, B. D. Anderson, R. Vaughn, D. B. Benett, T. I. Meier and J. A. Cook, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 899–903; (*f*) A. Martinez, *Med. Res. Rev.*, 2008, **28**, 773–796; (*g*) Q. Ye, G. Xu, D. Lv, Z. Cheng, J. Li and Y. Hu, *Bioorg. Med. Chem.*, 2009, **17**, 4302–4312.
19 (*a*) P. Cohen and M. Goedert, *Nat. Rev. Drug Discovery*, 2004, **3**, 479–487; (*b*) O. Engmann and K. P. Giese, *Frontiers Mol. Neurosci.*, 2009, **2**, 1–5; (*c*) L. K. Chico, L. J. Van Eldik and D. M. Watterson, *Nat. Rev. Drug Discovery*, 2009, **8**, 892–909.
20 M. A. Fousteris, A. Papakyriakou, A. Koutsourea, M. Manioudaki, E. Lampropoulou, E. Papadimitriou, G. A. Spyroulias and S. S. Nikolaropoulos, *J. Med. Chem.*, 2008, **51**, 1048–1052.
21 R. Akué-Gédu, E. Rossignol, S. Azzaro, S. Knapp, P. Filippakopoulos, A. N. Bullock, J. Bain, P. Cohen, M. Prudhomme, F. Anizon and P. Moreau, *J. Med. Chem.*, 2009, **52**, 6369–6381.
22 P. Alberti, P. Schmitt, C.-H. Nguyen, C. Rivalle, M. Hoarau, D. S. Grierson and J.-L. Mergny, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1071–1074.
23 Y.-J. Lu, T.-M. Ou, J.-H. Tan, J.-Q. Hou, W.-Y. Shao, D. Peng, N. Sun, X.-D. Wang, W.-B. Wu, X.-Z. Bu, Z.-S. Huang, D.-L. Ma, K.-Y. Wong and L.-Q. Gu, *J. Med. Chem.*, 2008, **51**, 6381–6392.
24 (*a*) S. Grigalevicius, *Synth. Met.*, 2006, **156**, 1–12; (*b*) M. Belletête, N. Blouin, P.-L. T. Boudreault, M. Leclerc and G. J. Durocher, *J. Phys. Chem. A*, 2006, **110**, 13696–13704; (*c*) P.-L. T. Boudreault, S. Wakim, N. Blouin, M. Simard, C. Tessier, Y. Tao and M. Leclerc, *J. Am. Chem. Soc.*, 2007, **129**, 9125–9136; (*d*) R. M. Adhikari, R. Mondal, B. K. Shah and D. C. Neckers, *J. Org. Chem.*, 2007, **72**, 4727–4732.
25 (*a*) Y. Li, Y. Wu and B. S. Ong, *Macromolecules*, 2006, **39**, 6521–6527; (*b*) K. Kawaguchi, K. Nakano and K. Nozaki, *J. Org. Chem.*, 2007, **72**, 5119–5128; (*c*) X. Meng, W. Zhu and H. Tian, *Opt. Sci. Eng.*, 2008, **133**, 129–172; (*d*) P.-L. T. Boudreault, N. Blouin and M. Leclerc, *Adv. Polymer Sci.*, 2008, **212**, 99–124; (*e*) N. Blouin and M. Leclerc, *Acc. Chem. Res.*, 2008, **41**, 1110–1119.
26 (*a*) B. C. G. Söderberg, *Curr. Org. Chem.*, 2000, **4**, 727–764; (*b*) A. W. Freeman, M. Urvoy and M. E. Criswell, *J. Org. Chem.*, 2005, **70**, 5014–5019.
27 For some recent references, see: (*a*) Z. Liu and R. Larock, *Org. Lett.*, 2004, **6**, 3739–3741; (*b*) W. C. P. Tsang, N. Zheng and S. Buchwald, *J. Am. Chem. Soc.*, 2005, **127**, 14560–14561; (*c*) L.-C. Campeau, M. Parisisen, A. Jean and K. Fagnou, *J. Am. Chem. Soc.*, 2006, **128**, 581–590; (*d*) R. B. Bedford and M. Betham, *J. Org. Chem.*, 2006, **71**, 9403–9410; (*e*) B.-J. Li, S.-L. Tian, Z. Fang and Z.-J. Shi, *Angew. Chem., Int. Ed.*, 2008, **47**, 1115–1118; (*f*) W. C. P. Tsang, R. H. Munday, G. Brasche, N. Zheng and S. Buchwald, *J. Org. Chem.*, 2008, **73**, 7603–7610; (*g*) J. A. Jordan-Hore, C. C. C. Johansson, M. Gulias, E. M. Beck and M. J. Gaunt, *J. Am. Chem. Soc.*, 2008, **130**, 16184–16186.
28 For some recent references, see: (*a*) K. Nozaki, K. Takahashi, K. Nakano, T. Hiyama, H.-Z. Tang, M. Michiya Fujiki, S. Yamaguchi and K. Tamao, *Angew. Chem., Int. Ed.*, 2003, **42**, 2051–2053; (*b*) A. Kuwahara, K. Nakano and K. Nozaki, *J. Org. Chem.*, 2005, **70**, 413–419; (*c*) A. Kuwahara, K. Nakano and K. Nozaki, *Org. Lett.*, 2008, **10**, 1199–1202.
29 (*a*) T. Watanabe, S. Ueda, S. Inuki, S. Oishi, N. Fujii and H. Ohno, *Chem. Commun.*, 2007, 4516–4518; (*b*) B. Liégault, D. Lee, M. P. Huestis, D. R. Stuart and K. Fagnou, *J. Org. Chem.*, 2008, **73**, 5022–5028.
30 F. Cochard, M. Laronze, P. Sigaut, J. Sapi and J.-Y. Laronze, *Tetrahedron Lett.*, 2004, **45**, 1703–1707.
31 M. Laronze, M. Boisbrun, S. Léonce, B. Pfeiffer, P. Renard, O. Lozach, L. Meijer, A. Lansiaux, C. Bailly, J. Sapi and J.-Y. Laronze, *Bioorg. Med. Chem.*, 2005, **13**, 2263–2283.
32 M. Boisbrun, A. Kovács-Kulyassa, L. Jeannin, J. Sapi, L. Toupet and J.-Y. Laronze, *Tetrahedron Lett.*, 2000, **41**, 9771–9775.
33 E. Dardennes, A. Kovács-Kulyassa, M. Boisbrun, C. Petermann, J.-Y. Laronze and J. Sapi, *Tetrahedron: Asymmetry*, 2005, **16**, 1329–1339.
34 For leading references on Meldrum's acid chemistry, see: (*a*) B.-J. Chen, *Heterocycles*, 1991, **32**, 529–597; (*b*) A. E.-A. M. Gaber and H. McNab, *Synthesis*, 2001, 2059–2074; (*c*) A. S. Ivanov, *Chem. Soc. Rev.*, 2008, **37**, 789–811.
35 A. Loupy, in *Microwaves in Organic Synthesis*, Wiley-VCH, Weinheim, 2002.
36 Decarboxylation of aromatic acids is most often carried out by using copper salts and heteroaromatic (quinoline, pyridine) promoters. For a recent paper, see: A. S. Lisitsyn, *Appl. Catal., A*, 2007, **332**, 166–170, and references therein.
37 R. Andreani, C. Bombelli, S. Borocci, J. Lah, G. Mancini, P. Mencarelli, G. Vesnaverc and C. Villanie, *Tetrahedron: Asymmetry*, 2004, **15**, 987–994.
38 J. K. M. Sanders and B. K. Hunter, in *Modern NMR Spectroscopy A Guide for Chemists*, Oxford University Press, 1987.

39 H. Eyring, *J. Chem. Phys.*, 1935, **3**, 107.
40 (*a*) J. I. G. Cadogan, M. Cameron-Wood, R. K. Mackie and R. J. G. Searle, *J. Chem. Soc.*, 1965, 4831–4837; (*b*) J. I. G. Cadogan, *Synthesis*, 1969, 11–17; (*c*) J. I. G. Cadogan, in *Organophosphorous Reagents in Organic Synthesis*, ed: J. I. G. Cadogan, Academic, London, 1979, pp. 269-294.
41 (*a*) R. J. J. Sundberg, *J. Org. Chem.*, 1965, **30**, 3604–3610; (*b*) I. Puskas and E. K. Fields, *J. Org. Chem.*, 1968, **33**, 4237–4242.
42 (*a*) M. Akazome, T. Kondo and Y. Watanabe, *J. Org. Chem.*, 1994, **59**, 3375–3380; (*b*) S. Tollari, S. Cenini, A. Rossi and G. Palmisano, *J. Mol. Catal. A: Chem.*, 1998, **135**, 241–248; (*c*) B. C. G. Söderberg and J. A. Shriver, *J. Org. Chem.*, 1997, **62**, 5838–5845; (*d*) R. W. Clawson, Jr. and B. C. G. Söderberg, *Tetrahedron Lett.*, 2007, **48**, 6019–6021.
43 (*a*) M. Pizzotti, S. Cenini, S. Quici and S. Tollari, *J. Chem. Soc., Perkin Trans. 2*, 1994, 913–917; (*b*) J. H. Smitrovich and I. W. Davies, *Org. Lett.*, 2004, **6**, 533–535; (*c*) T. L. Scott, X. Yu, P. Gorugantula, G. Carreero-Martinez and B. C. G. Söderberg, *Tetrahedron*, 2006, **62**, 10835–10842.
44 M. Alajarín, A. Vidal, M.-M. Ortín and F. Tovar, *Synthesis*, 2002, 2393–2398.
45 R. W. Clawson, Jr., R. D. Deavers, III., N. G. Akhmedov and B. C. G. Söderberg, *Tetrahedron*, 2006, **62**, 10829–10834.
46 I. W. Davies, V. A. Guner and K. N. Houk, *Org. Lett.*, 2004, **6**, 743–746.
47 *Gaussian 03, Revision C.02*, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.
48 C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B: Condens. Matter*, 1988, **37**, 785–789.
49 B. Miehlich, A. Savin, H. Stoll and H. Preuss, *Chem. Phys. Lett.*, 1989, **157**, 200–206.
50 S. H. Vosko, L. Wilk and M. Nusair, *Can. J. Phys.*, 1980, **58**, 1200–1211.
51 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652.
52 F. Leroux, *ChemBioChem*, 2004, **5**, 644–649.
53 S. Miertus, E. Scrocco and J. Tomasi, *Chem. Phys.*, 1981, **55**, 117–129.
54 M. Cossi, G. Scalmani, N. Rega and V. J. Barone, *Chem. Phys.*, 2002, **117**, 43–54.
55 R. Cammi, B. Mennucci and J. Tomasi, *J. Phys. Chem. A*, 2000, **104**, 5631–5637.
56 R. Ditchfield, *Mol. Phys.*, 1974, **27**, 789–807.
57 K. Wolinski, J. F. Hilton and P. Pulay, *J. Am. Chem. Soc.*, 1990, **112**, 8251–8260.
58 S. Bach, M. Knockaert, O. Lozach, J. Reinhardt, B. Baratte, S. Schmidt, S. P. Coburn, L. Tang, T. Jiang, D. C. Liang, H. Galons, J. F. Dierick, F. Totzke, C. Schächtele, A. S. Lerman, A. Carnero, Y. Wan, N. Gray and L. Meijer, *J. Biol. Chem.*, 2005, **280**, 31208–31219.
59 A. Primot, B. Baratte, M. Gompel, A. Borgne, S. Liabeuf, J. L. Romette, F. Constantini and L. Meijer, *Protein Expression Purif.*, 2000, **20**, 394–404.
60 B. Brassart, D. Gomez, A. De Cian, R. Paterski, A. Montagnac, K. H. Qui, N. Temime-Smaali, C. Trentesaux, J.-L. Mergny, F. Guéritte and J.-F. Riou, *Mol. Pharmacol.*, 2007, **72**, 631–640.
61 C. Bailly, *Methods Enzymol.*, 2001, **340**, 610–623.