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Synthesis and Biological Evaluation of Novel Phenylcarbazoles as Potential Anticancer Agents

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We here report the synthesis and biological evaluation of new phenylcarbazole derivatives designed as potential anticancer agents. Indole and hydroxyindole were used to generate three scaffolds that were successively exploited to introduce various substituents on the maleimide moiety. The synthesis includes a final intramolecular key Heck-type reaction, which was carried out with a triflate derivative or with a bromophenyl derivative. Each step was optimized and the complete chemical strategy is detailed. Several compounds showed a marked cytotoxicity against CEM human leukemia cells with IC_{50} values in the 10−100 nM range. Precise structure−activity relationships were delineated. Cell cycle analysis, topoisomerase I inhibition, and interaction with DNA were evaluated, and inhibition of CDK activity was also investigated. Although binding of the drugs to DNA likely contributes to the cytotoxic action, the exact molecular targets of these molecules remain undiscovered. The efficient chemical routes reported here for the design of highly cytotoxic compounds provide novel opportunities to identify antitumor agents in the phenylcarbazole series.

Introduction

Indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole alkaloids form a class of compounds endowed with potent antitumor, antiviral, and/or antimicrobial activities.[1] This family has raised considerable attention because of the central role of these molecules in the regulation of cell cycle progression and specific enzyme inhibition.[2,3] Structure−activity relationships (SAR) in the indolocarbazole series have been extensively studied in the context of topoisomerase I inhibition and tumor cell killing. Compounds bearing a pyrroloindolocarbazole and equipped with one *N*-glycosidic bond, such as the antibiotic rebeccamycin, generally function as DNA topoisomerase I inhibitors. A few analogues, such as NB-506 and J-107088 (also known as edotecarin), have entered clinical trials for cancer treatment.[4−6] More recently, fluorinated derivatives of such molecules have been reported and their topoisomerase I-dependent anticancer activity looks promising.[7−9]

For our part, we have recently described the bioisosteric replacement of an indole moiety by a 7-azaindole unit, affording the first symmetrical and dissymmetrical 7-azaindolocarbazoles **I** and **II**.[10] In the same vein, the cytotoxic properties of the *N*-glycosylated derivatives of **I** and **II** have been reported by others.[11,12] For these different molecules, the role of topoisomerase I inhibition in the cytotoxic action seems relatively minor compared to the NB-506-type series, and evidence suggests that other signaling proteins, particularly kinases, may play a role.

To develop selective kinase inhibitors, fitting in the ATP binding site, modifications of the aromatic heterocyclic indolocarbazole moiety appeared as a valid alternative to the synthesis of glycosylated compounds. In addition, most of the aryl carbazoles designed so far for kinase inhibition are equipped with an unsubstituted maleimide, whereas in general the most cytotoxic compounds bear hydrophilic side chains. It is important to mention also that closely related (het)arylcarbazoles (type **III**) have been described as inhibitors of cyclin D1/CDK4.[13,14] This strategy was previously adopted for granulatimide and isogranulatimide (Figure 1) acting as G2 checkpoint inhibitors.[15−17] Cyclin-dependent kinases (CDKs) were also targeted with indolocarbazoles such as bis *N*-indolyl alkylated arcyriaflavins recently described as CDK1, CDK2, and CDK4 inhibitors.[19,20] In the NH maleimide series, indole versus (hetero)arylcarbazoles replacement represents also an interesting strategy.

With this in mind, we envisaged the synthesis of different naphthalenic compounds (**V**).[21] The replacement of one of the two indoles by a naphthalene ring produced a highly cytotoxic naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole derivative (**V**), suggesting that the naphthalene is effectively a suitable bioisostere for indole. However, no kinase inhibition was detected in that series. At the same time, we developed a phenylcarbazole series (**IV**) in order to broaden our SAR knowledge. Recently, this approach has been followed by a group at Eli Lilly for similar aryl or heteroaryl carbazoles. The activity of only one phenylcarbazole and the synthesis of two other analogues were reported, and most of this work consisted of the variation of one aromatic moiety.[13] This interesting data prompted us to present here our phenylcarbazole series **IV**. A new efficient synthesis of such compounds, including indolic substitution and maleimide variations, is presented together with preliminary pharmacological studies.

Chemistry

Various synthetic methods have been used to synthesize a representative panel of symmetrical or unsymmetrical indolocarbazoles.[22] Lilly's group choose to build, on one hand, the indolic glyoxalates and, on the other hand, a 2-bromophenyl acetamidate, leading to the indoylarylmaleimide.[23] Final benzannulation was performed by palladium intramolecular Heck reaction (70% yield). One disadvantage of this approach is the

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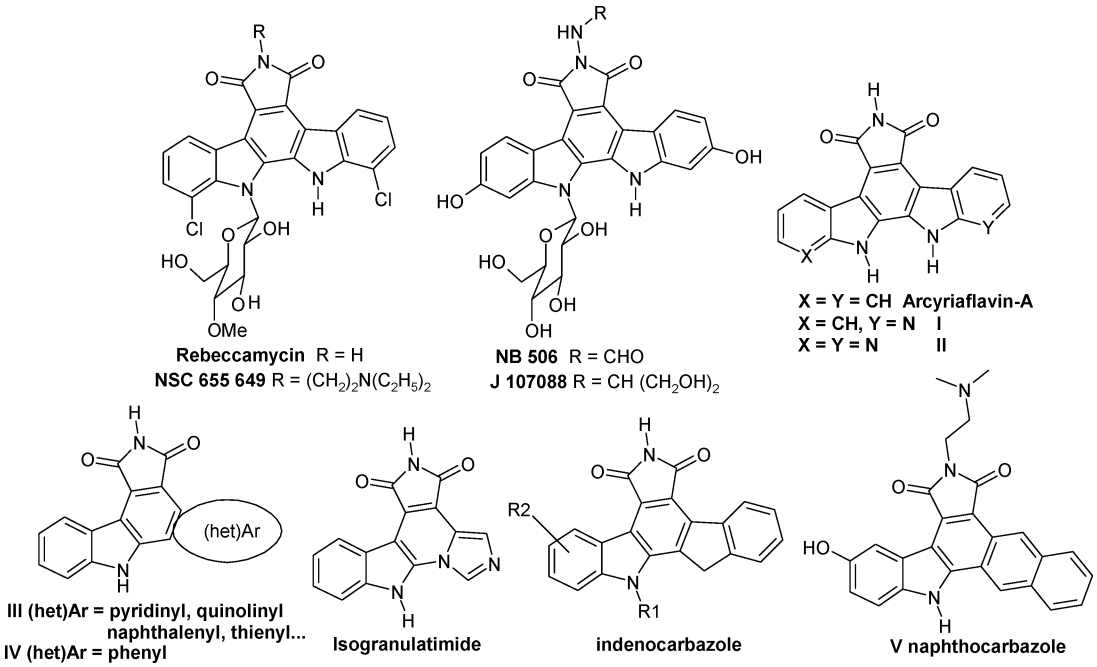
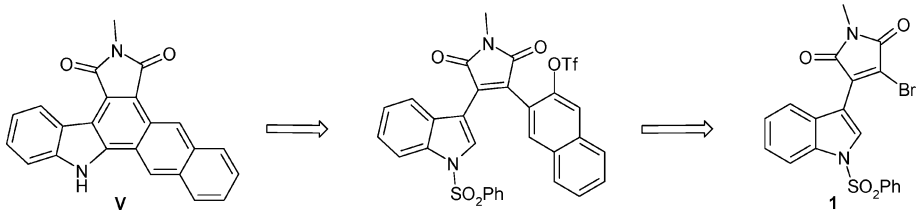


Figure 1. Modifications of the indolocarbazole skeleton.

Scheme 1. Clockwise Retrosynthesis of Naphthocarbazoles



need for various acetamidate and glyoxalate skeletons not directly available and another one was the decrease of yield using substituted indoles. An alternative and more convenient synthesis consisted of the selective 3-alkylation of indole with 2,3-dibromomaleimide, using Grignard reagents to produce 3-indoylmaleimides.[24] The second (hetero)aromatic group could be introduced via palladium(0) catalyzed Stille or Suzuki reactions, whereas the final benzannulation required an intramolecular Heck reaction.

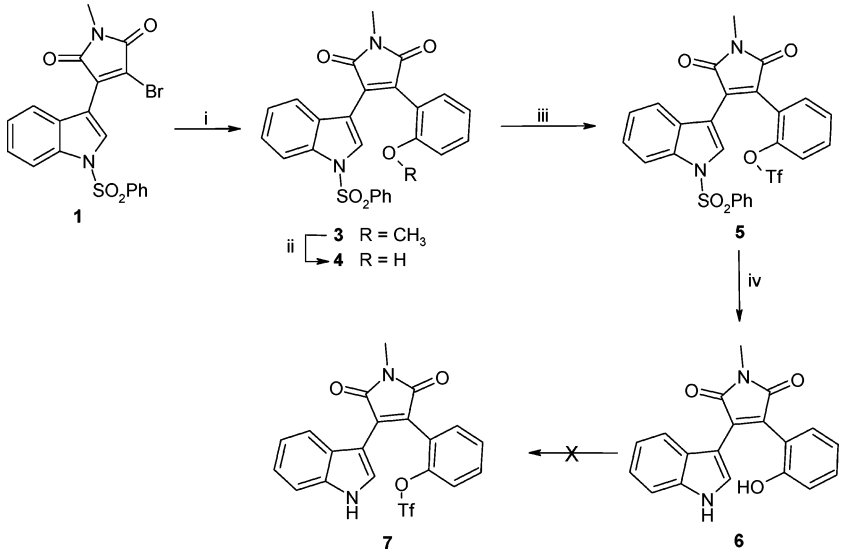
In our previous paper,[21] we described the synthesis of new naphthocarbazoles **V** (Scheme 1) in only four steps using a clockwise synthetic sequence starting from indole and involving (i) the introduction of the maleimide, (ii) a Suzuki cross-coupling reaction on the indolic bromomaleimide for the introduction of the functionalized naphthalene moiety, (iii) the activation of the naphthyl as a naphthyl triflate, and (iv) an intramolecular Heck reaction directly followed by a cleavage of the protecting group. This strategy also represents a major simplification in the synthesis of parent indolocarbazole derivatives.

Following this strategy, we began the synthesis of phenylcarbazole by treating compound **1** with 2-methoxyphenylboronic acid **2** in the presence of $Pd(OAc)_2$, dioxane, water, and K_2CO_3 as a base at 80 °C according to a Suzuki cross-coupling reaction; compound **3** was quantitatively obtained (Scheme 2). Compound **4** was obtained in a 94% yield by demethylation of **3** by using BBr_3 in dichloromethane. The hydroxy group of **4** was then converted to the triflate **5** with an 85% yield (Scheme 2).

Several attempts of cyclization of **5**, using our previously described conditions, were unproductive.[19,21] Only the starting material and detriflated compound **4** were isolated whatever the reaction time and temperature, thus indicating a complete absence of insertion of the palladium entity in the C−OTf bond. Despite a reported successful condensation of 4-indolyl triflate at the position-2 of an indole derivative in the presence of $Pd(OAc)_2$ with dppe as a ligand and an excess of triethylamine at 110 °C,[26] we were unable to apply these conditions to **5** to obtain the desired cyclized product. As such, we tried to cleave solely the benzenesulfonyl group to modify the electronic and steric behavior of the 2,3-ethylenic bond of the pyrrole moiety implicated in the formation of the six-membered ring. Compound **5** was reacted with 2.2 equiv of Bu_4NF in refluxing THF.[27] Unfortunately, the triflate group was also cleaved and the fully deprotected compound **6** was generated in a 77% yield. All our efforts to obtain the triflate derivative **7** from **6** led to a degradation of the mixture. Therefore, we decided to perform the first Suzuki reaction using 2-bromophenyl boronic acid **8** on either the unprotected *N*-indolic compound **9** or the protected compound **1** (Scheme 3). Two procedures differing by the nature of the palladium catalyst ($Pd(OAc)_2$ or $Pd(PPh_3)_4$ (Table 1, entries 1 and 2) were carried out with compound **9**. The expected derivative **12** was only obtained by using $Pd(OAc)_2$ in a moderate yield (50%). In this case, the presence of an unsubstituted indolic nitrogen atom was detrimental to the synthesis. In contrast, the benzenesulfonyl protected compound **1**, treated with **8**, afforded the desired compound **11** in an excellent yield (98%) with a trace amount (1%) of the cyclized compound **13** (entry 3), indicating the possibility of a one-pot Suzuki−Heck tandem reaction (entries 4−6).

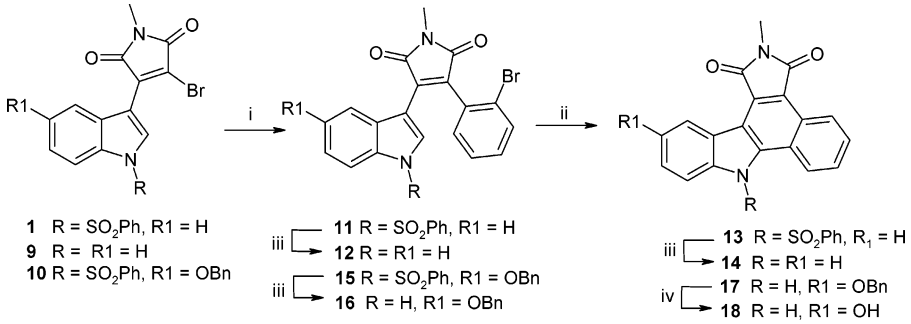
One of the differences between the Suzuki (entry 3) and the Heck-type cross-coupling reactions was the presence or the absence of an additional ligand for the catalyst, whereas both procedures involved the use of palladium acetate. So triphenyl-

Scheme 2[a]



[a] (i) 2-Methoxyphenylboronic acid **2** (1.1 equiv), dioxane, water, K_2CO_3 (2 equiv), $Pd(OAc)_2$ 10%, 80 °C, 4 h, quant.; (ii) BBr_3 (7.5 equiv), CH_2Cl_2, rt., 1 h, 94%; (iii) Tf_2O (1 equiv), NEt_3 (3 equiv), CH_2Cl_2, rt, 2 h, 85%; (iv) Bu_4NF (2 equiv), THF, reflux, 2 h, 77%.

Scheme 3[a]



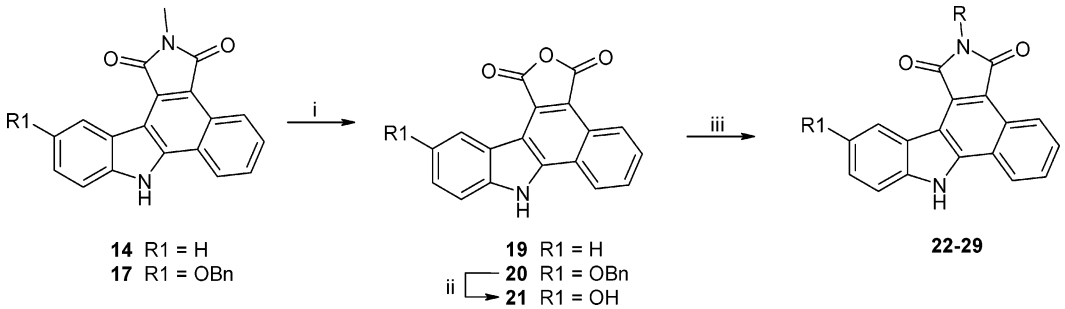
[a] (i) From **1** $Pd(OAc)_2$ (0.1 equiv), 2-BrPhB$(OH)_2$ **8** (3 equiv), K_2CO_3 (4 equiv, dioxane/water 4/1, 100 °C, 8 h, 98%; from **9**, idem, 4 h, **9** 50%; from **10**, **8** (1.5 equiv), **15** 58%, and **16** 31%; (ii) from **12**, $Pd(PPh_3)_4$ 5%, AcOK (1.1 equiv), DMA, 130 °C, 4 h, quant.; from **16**, idem, quant.; (iii) from **11**, Bu_4NF (1.2 equiv), THF, reflux, 2 h, 93%; from **13**, idem, quant.; from **15**, idem, 86%; (iv) BBr_3 (1.0 equiv), CH_2Cl_2, rt, 1 h, quant.

Table 1. Suzuki and Heck Procedures

entry	compds	catalyst (equiv)	additives (equiv)	solvent; temp, °C	time, h	product (yield %)
1	**9**	$Pd(OAc)_2$ (0.1)	K_2CO_3 (4)	dioxane/water; 100	4	**12** (50)
2	**9**	$Pd(PPh_3)_4$ (0.2)	$NaHCO_3$ satd	toluene, EtOH; reflux	12	degradation
3	**1**	$Pd(OAc)_2$ (0.1)	K_2CO_3 (4)	dioxane/water; 100	8	**11** (98), **13** (1)
4	**1**	$Pd(OAc)_2$ (0.2)	K_2CO_3 (4), PPh_3 (0.4)	dioxane/water; 100	24	**11** (51), **13** (10)
5	**1**	(i) $Pd(OAc)_2$ (0.1) (ii) $Pd(OAc)_2$ (0.1)	(i) K_2CO_3 (4), (ii) PPh_3 (0.2)	dioxane/water; 100	(i) 6 (ii) 18	**11** (70), **13** (20)
6	**1**	(i) $Pd(OAc)_2$ (0.1) (ii) $Pd(OAc)_2$ (1.0)	(i) K_2CO_3 (4) (ii) PPh_3 (2)	dioxane/water; 100	(i) 6 (ii) 12	**11** (34) **13** (20)
7	**11**	$Pd(OAc)_2$ (1.0)	PPh_3 (2)	dioxane; 90	6	**13** (83)
8	**11**	$Pd(PPh_3)_4$ (0.2)	AcOK (1.1)	DMA; 130	6	**13** (15), **12** + **11** (50)
9	**12**	$Pd(PPh_3)_4$ (0.2)	AcOK (1.1)	DMA; 130	0.5	**14** (quant.)
10	**12**	$Pd(PPh_3)_4$ (0.05)	AcOK (1.1)	DMA; 130	4	**14** (quant.)
11	**10**	$Pd(OAc)_2$ (0.1)	K_2CO_3 (4)	dioxane/water; 100	8	**15** (58), **16** (31)
12	**16**	$Pd(PPh_3)_4$ (0.05)	AcOK (1.1)	DMA; 130	4	**17** (quant.)

phosphine was introduced at the beginning of the reaction in our first experiments (entry 4). Increasing the quantity of palladium acetate (0.2 equiv) and PPh_3 (0.4 equiv) slightly increased the yield of the tandem reaction (compound **13** was obtained in a 10% yield) after 24 h, but the Suzuki product **11** was obtained in only 51% yield with a complete disappearance of the starting material **1**. Therefore, we decided to introduce the second amount of palladium catalyst and triphenylphosphine after 6 h of reaction, which is the time necessary for the full Suzuki conversion of **1** into **11**. Thus, upon sequentially adding 10% catalyst and 20% of PPh_3, the reaction afforded, after 24 h, 70% of the Suzuki derivative **11** and 20% of the annulated product **13**, increasing the yields of **11** and **13**, but the Heck reaction was never completed (entry 5). Not really satisfied by this, we performed a further attempt based on our knowledge of the palladium intramolecular reaction in the naphthalene series.[21] In this case, the reaction time was reduced, in the presence of a stoichiometric amount of catalyst, and fortunately, the yields were enhanced. So adding 1 equiv of $Pd(OAc)_2$ and 2 equiv of phosphine, after 6 h of reaction, led to the same amount of cyclized compound **13** (20%) but to only 34% of compound **11** (entry 6).

Scheme 4[a]



[a] (i) KOH (5 N), EtOH, (10 equiv), reflux, then HCl, 0 °C, pH 5; from **14**, 5 h, 99%; from **17**, idem, 83%; (ii) BBr_3 (1.0 equiv), CH_2Cl_2, rt, 1 h, quant.; (iii) (a) from **19**, NH_4OH 35%, DMF, reflux, 8 h, **22** 93%; from **21**, idem, 120 °C, 24 h, **23** 96%; (b) from **19**, $NH_2(CH_2)_2N(CH_3)_2$, DMF, 120 °C, 12 h, **24** 80%; from **21**, idem, **25** 71%; (c) from **19**, $HOCH_2CH(NH_2)CH_2OH$, DMF, reflux, 12 h, **26** 75%; from **21**, idem, **27** 68%; (d) from **19**, histamine (1.5 equiv), DMF, 130 °C, 3 days, sealed tube, **28** 97%; from **21**, idem, **29** 91%.

Confronted with difficulties in enhancing the yield of the tandem cross-coupling reactions, we then decided to realize the synthesis step-by-step (entry 7). Starting from **11**, we found that a stoichiometric amount of palladium acetate led to the cyclized compound **13** in a 83% yield with a reaction time of 6 h. Several assays were also performed to optimize this step. As an example, we have changed the nature of the catalyst according to a published procedure.[23] Thus, using 0.2 equiv of $Pd(PPh_3)_4$ as a catalyst, AcOK as a base in DMA at 130 °C led to **13** in only 15% accompanied by a inseparable mixture of deprotected compound **12** and **11** (2/1, 50%, entry 8), suggesting also a thermal sensitivity of the benzenesulfonyl group in the presence of the base.

We have also cleaved the protective group of **11** by Bu_4NF in THF, which afforded **12** in a 93% yield, and applied to this compound the Heck cyclization conditions $Pd(PPh_3)_4$ (0.2 equiv), AcOK, (1.1 equiv), DMA at 130 °C. In this case, we obtained the cyclized compound **14** in a quantitative yield after a remarkably short reaction time (30 min, entry 9). Decreasing also the amount of $Pd(PPh_3)_4$ to 0.05 equiv increased the reaction time to 4 h, but the yield of **14** was not affected (entry 10). Alternatively, compound **14** was prepared from **13** using Bu_4NF in THF in only 2 h in the same yield (93%). These results showed also the difference of reactivity between deprotected and protected indoles during intermolecular Heck reaction.[28−30] Thus, compound **14** could be obtained by a Suzuki reaction on **1**, leading to **11**, followed by a deprotection reaction affording **12**, and then a quantitative Heck cyclization led to **14** in an overall yield of 91%. On the other hand, the deprotection step could be carried out after the Heck reaction, leading to **13**. By this second route the overall yield decreased slightly to 82%. Both of these methods, starting from **1**, led to the desired unprotected compound **14** in three steps.

On the basis of our previous results in the naphthalene series, we decided to introduce a hydroxyl group which is generally the most biologically effective substituent on the indolic moiety. Commercially available 5-benzyloxyindole led to compound **10** in three easy steps. The Suzuki procedure carried out with the boronic acid **8** afforded compound **15** in a 58% yield. In addition, the *N*-indolic deprotected compound **16** was isolated in a 31% yield (entry 11). This reaction was nevertheless not optimized. The synthetic sequence was directly carried on with deprotection of compound **15** into **16** in the presence of Bu_4NF in 86% yield. Using the same conditions as for **12**, the final Heck cross-coupling of **16** gave compound **17** in a quantitative yield (entry 12). Deprotection of the benzyl group was performed with BBr_3 at room temperature to afford **18** in a quantitative yield. In the 5-benzyloxyindole series, compound **17** was obtained from **9** in a 89% overall yield in three steps.

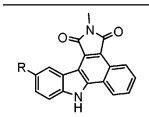
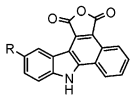
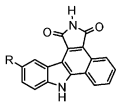
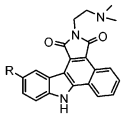
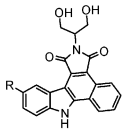
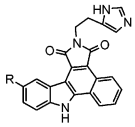
The phenylcarbazoles **14** and **17** could be easily substituted on the maleimide moiety. It should be noted that the direct displacement of the NCH_3 group by a primary amine failed. So the intermediate anhydrides **19** and **20** were prepared by treating **14** or **17** with a boiling KOH hydroalcoholic solution during a few hours and then an acidification to pH 4 (Scheme 4). These two compounds were isolated in 99 and 83% yields, respectively. Debenzylation of **20** with BBr_3 afforded the anhydride **21** in a quantitative yield. Anhydrides **19** and **21** were treated successively with ammonia or 2-dimethylaminoethylamine or 2-amino-1,3-propandiol and histamine to afford compounds **22**−**29** in moderate (51%) to excellent (97%) yields. In the case of histamine, only a slight excess of this reagent was used; 3 days of reaction in a sealed tube were necessary to complete the reaction, giving compounds **28** and **29** in excellet yields.

Results and Discussion

In Vitro Antiproliferative Activities. The antiproliferative activities of the compounds were tested against CEM and camptothecin-resistant CEM/C2 human leukemia cell lines. Their cytotoxicity was measured using a conventional microculture tetrazolium-based assay. IC_{50} values are collated in Table 2. The relative resistance index (i.e. the ratio $IC_{50}^{CEM/C2}/IC_{50}^{CEM}$) never exceeds 3 (versus >2000 for camptothecin).[31] Therefore, in all cases the use of the topoisomerase I-deficient CEM/C2 cell line (carrying a mutation on the *top1* gene) has only a marginal effect on the cytotoxicity of the molecules. Topoisomerase I is surely not the target of these compounds (unless they are not sensitive to this specific enzyme mutation, but this is very unlikely; see below).

The phenylcarbazole **22** was totally inactive (IC_{50} > 100 μM) in both cell lines, whereas the corresponding anhydride **19** showed a slight increase in the cytotoxic activity, ranging over 25 μM. The presence of an additional hydroxyl group on the carbazole moiety sensibly increased the cytotoxicity, and the introduction of a hydrophilic substituent induces an increase of activity by at least 2-fold (compare **22** and **19** with **23** and **21**). The introduction of various modifications on the indolic nitrogen center enhanced markedly the cytotoxic potential of the different molecules tested. Substitution on the maleimide moiety of **22** by a methyl group has no effect on its cytotoxicity, as observed with compound **14** (IC_{50} > 100 μM). In sharp contrast, the replacement the *N*-methyl group by an *N*-2-dimethylaminoethyl side chain on **24** considerably improves the efficacy of this compound to reach submicromolar activities (IC_{50} = 110 nM). However, the substitution of **22** by a ramified bis-hydroxylated side chain or by histamine was less successful. The cytotoxicities of compounds **26** and **28** were increased (IC_{50} = 3.71 and 1.58 μM, respectively) when compared to those of the unsubstituted

Table 2. In Vitro Cytotoxicity and Kinase Activity Assays IC_{50} (μM)[a]

compounds	CEM	CEMC2	RRI	CDK5	CDK1	GSK-3
 **14** R = H	> 100	> 100	ND	> 10	> 10	ND
18 R = 10-OH	1.64 ± 0.12	4.17 ± 0.095	2.5	> 10	> 10	ND
 **19** R = H	> 25	> 50	ND	> 10	> 10	ND
21 R = 10-OH	14.67 ± 1.55	32.32 ± 1.25	2.2	> 10	> 10	ND
 **22** R = H[19]	> 100	> 100	ND	8.5	80	20
23 R = 10-OH	> 25	> 25	ND	0.25	0.58	0.46
 **24** R = H	0.11 ± 0.014	0.335 ± 0.033	3	> 10	> 10	ND
25 R = 10-OH	0.0122 ± 0.0019	0.0187 ± 0.0037	1.6	> 10	> 10	ND
 **26** R = H	3.71 ± 0.66	4.95 ± 0.8	1.3	> 10	> 10	ND
27 R = 10-OH	2.84 ± 0.2	4.96 ± 0.077	1.7	> 10	> 10	ND
 **28** R = H	1.58 ± 0.07	2.92 ± 0.395	1.8	> 10	> 10	ND
29 R = 10-OH	0.715 ± 0.115	1.9 ± 0.038	2.7	> 10	> 10	ND

[a] ND = not determined; RRI = relative resistance index.

or *N*-methylated compounds **22** and **14** but did not reach the high nanomolar range observed with the *N*-2-dimethylaminoethyl compound **24**. Interestingly, the introduction of a hydroxyl group on the carbazole moiety of the substituted compounds **24** and **28** increased their cytotoxic activities 2−10-fold (IC_{50} = 12.2 and 715 nM, respectively, for compounds **25** and **29**). A remarkable level of cytotoxicity has been reached with compound **25**.

To sum up the cytotoxicity data, it appears that substitution of the maleimide moiety by a *N*-dimethylamino side chain leads to a highly cytotoxic molecule (**24**, IC_{50} = 110 nM) and this potent activity is further increased when this modification is combined with the presence of a hydroxyl group on the carbazole moiety (**25**, IC_{50} = 12.2 nM). These data confirm the idea that the presence of a basic chain on the maleimide generally improves the efficacy of the compound.[21,32] This is not entirely surprising, as the protonation of the terminal nitrogen at physiological pH must facilitate the interaction with the DNA polyanion (as well as enhancing its solubility).

Cell Cycle Effect and Mechanism of Action. The effects of the different compounds on cell cycle progression of leukemia CEM cells were investigated using propidium iodide staining and FACScan flow cytometry. No changes in the cell cycle profiles were observed after treatment of CEM cells with 1 μM of phenylcarbazole **22** or the corresponding anhydride **19**. The presence of an additional hydroxyl group on the carbazole moiety, as for compounds **23** and **21**, does not affect the accumulation of CEM cells in the different cell cycle phases (data not shown). The introduction of a methyl group or a ramified bis-hydroxylated side chain on the indolic nitrogen of **22** has also no effect on the cell cycle, as seen for compounds **14** and **26** (29% G_2/M). However, substitution on the maleimide moiety of **22** by a histamine residue induces a significant accumulation of cells in the G2/M phase of the cell cycle, reaching about 80% after treatment with 5 μM of compound **28** (Figure 2). The addition of a hydroxyl group on the carbazole moiety of those three compounds induces first an accumulation of cells in the G2/M phases, reaching 34%, 33%, and 45% for compounds **18**, **27**, and **29**, respectively, and then followed by an S phase accumulation when cells were treated with a higher concentration (5−10 μM) (Figure 2). The addition to **22** of an *N*-2-dimethylaminoethyl side chain significantly changes the profile of the CEM cell cycle when compared with the other

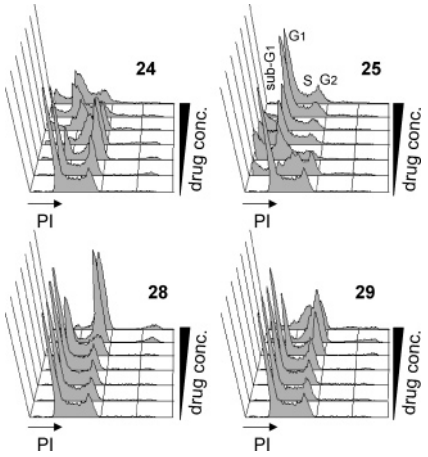


Figure 2. Cell cycle distribution in CEM cells treated for 72 h with graded concentrations (0, 0.1, 0.5, 1, 2.5, 5, and 10 μM) of compounds **24**, **25**, **28**, and **29**. Cells were analyzed with a FACScan flow cytometer. Data are the result of two independent experiments.

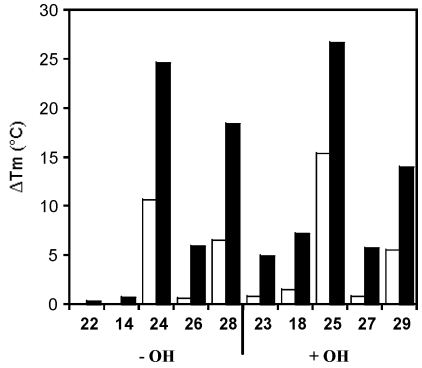


Figure 3. Variation of melting temperatures ($\Delta T_m = T_m^{DNA/molecule\ complex} - T_m^{DNA}$) for the different derivatives bound to calf thymus DNA (open bars) or poly(dAT)$_2$ (plain bars) at a drug/DNA ratio of 0.5. T_m measurements were performed in pH 7.1 BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA) at 260 nm with a heating rate of 1 °C/min.

molecules studied. Treatment with 1 μM **24** provokes an accumulation of cells in the S phase (26%) and the appearance of a small population of apoptotic sub-G1 cells (5%). The introduction of a hydroxyl group to this molecule triggers a similar but more potent effect, as 15% of the cells are apoptotic after treatment with 0.5 μM **25**. This data set might be correlated with the gain of cytotoxic activity measured when the hydroxyl group was added (IC_{50} = 110 and 12.2 nM for **24** and **25**, respectively).

To understand how those molecules induce their cellular effects, we have investigated their effect on purified human topoisomerase I. None of these compounds were able to induce topoisomerase I-dependent DNA strand breaks, as seen with the reference drug camptothecin (data not shown). The molecular assay confirms the cell data to conclude that topoisomerase I is not the target of these molecules, despite the capacity of some compounds to bind to DNA. Topoisomerase II inhibition was also investigated but no effect was detected (data not shown).

DNA binding was studied by melting temperature experiments using both calf thymus DNA (CT-DNA) and the polymer poly(dAT)$_2$ (Figure 3). In our experimental conditions, CT-DNA and poly(dAT)$_2$, respectively, melt at 66 and 43 °C. The melting temperatures (T_m) remained unchanged when nucleic acids were incubated with the phenylcarbazole **22** or its corresponding anhydride **19**.

The introduction of various chemical modifications at the indolic nitrogen center stabilizes the duplex DNA against heat denaturation. Whereas the *N*-methyl derivative **14** shows no significant effect on DNA stability, the replacement of the Me group by a longer side chain, such as a *N*-dimethylaminoethyl (**24**), a ramified bis-hydroxylated (**26**), or a histamine (**28**) group, leads to a significant increase of the T_m values with both CT-DNA and poly(dAT)$_2$. These compounds do exhibit significant interaction with nucleic acids. In all cases, the addition of a hydroxyl group on the carbazole chromophore promotes DNA binding (Figure 3). This enhanced affinity for DNA correlates with the gain of cytotoxicity and the drug capacity to block CEM cells in G2/M and S phases of the cell cycle. Therefore, it is tempting to conclude that DNA interaction plays a role in the cytotoxic action of these compounds, but this is certainly not sufficient to explain the remarkable antiproliferative activity of compounds such as **24** and **25**.

A DNase I footprinting approach was also performed to evaluate the sequence selectivity of the different compounds. Two DNA restriction fragments of 117 and 265 base pairs (bp)

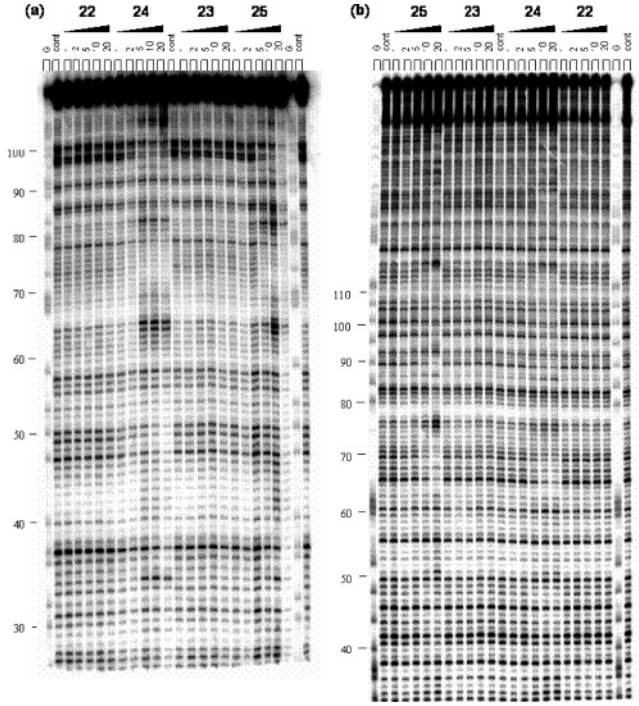


Figure 4. DNase I footprinting for compounds **22**−**25** on the 3′-end labeled (a) 117-bp and (b) 265-bp *Eco*R I−*Pvu* II DNA restriction fragments from the pBS plasmid. The products of nuclease digestion were resolved on 8% polyacrylamide gels containing 7 M urea. Control tracks (cont) contain no ligand. The concentrations (μM) of the tested products are shown at the top of the appropriate gels. Tracks labeled "G" represent dimethyl sulfate-piperidine markers specific for guanines. Numbers on the left side of the gels refer to the standard numbering scheme for the nucleotide sequence of the DNA fragment.

were subjected to DNase I digestion in the presence of increasing drug concentrations. In most cases, the molecules do not affect the DNase I cleavage pattern and show no sequence preference. However, clear modulation of the enzyme cutting was observed with **24** and **25**, both possessing the dimethylaminoethyl side chain. The footprinting gels in Figure 4 show the concentration dependence for the modulation of DNase I cleavage by these two molecules and the densitometric analysis of the gels attests to the sequence preference (Figure 5).

The differential cleavage plots (Figure 5) for the 117- and 265-bp fragments in the presence of **22**−**25** show marked differences between the molecules. The sequences protected from the enzyme cleavage (i.e. the presumptive drug binding sites) by **24** and **25** contain mixed AT and CG bp but are essentially GC-rich, such as 5′-CGCC and 5′-CGTTGT in the 117-bp fragment and the sequence 5′-CTCACT in the 265-bp fragment. Strongly enhanced DNase I-cleaved regions were also characterized in the vicinity of the protected sites (such as around sites 45 and 65 on the 117-mer). These sites reflect structural perturbations of the DNA at sequences flanking the binding sites, and this is generally a signature of intercalative drug binding.

We next tested the compounds for potential inhibition of cyclin-dependent kinases (CDK1 and CDK5) and glycogen synthase kinase-3 (GSK-3). Only compounds **22** and **23** showed a significant inhibitory activity. None showed a selectivity for CDKs or GSK-3. Despite these kinase inhibitory activities (particularly compound **23**), the compounds were inactive in the cell proliferation assay. The most active compounds on proliferation (**24** and **25**) were inactive on the kinases tested. It is thus unlikely that the antiproliferative effects of the synthesized compounds can be accounted for by a direct effect on

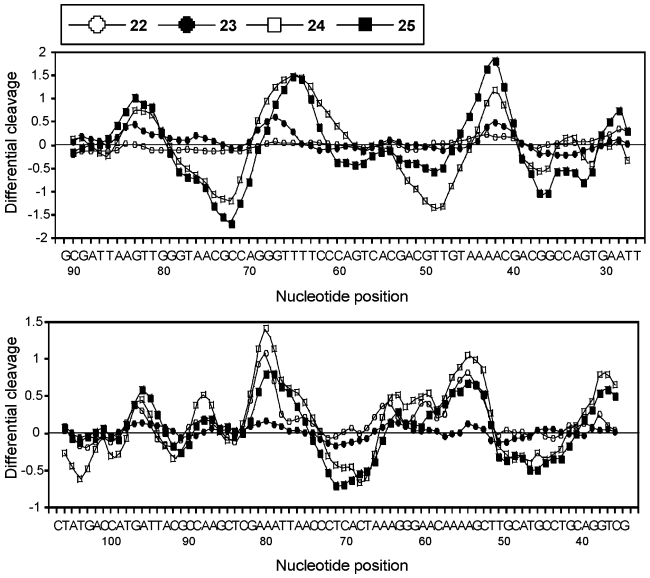


Figure 5. Differential cleavage plots indicating the susceptibility of (a) 117-bp and (b) 265-bp fragments to DNase I cutting in the presence of indicated compounds (10 μM each). Negative values correspond to a ligand-protected site and positive values represent enhanced cleavage. Vertical scales are in units of $\ln(f_a) - \ln(f_c)$, where f_a is the fractional cleavage at any bond in the presence of the drug and f_c is the fractional cleavage of the same bond in the control, given closely similar extents of overall digestion. Only the region of the restriction fragment analyzed by densitometry is shown.

CDKs or GSK-3. However, the reason for the lack of cellular effects of compounds **22** and **23** remains to be identified. These compounds constitute interesting scaffolds from which more potent and more selective inhibitors could be designed. A structural analogy can be found between known highly cytotoxic antitumor agents and the most potent compounds reported here, such as **24**, which bears a dimethylaminoethyl side chain appended to a flat hydroxylated chromophore as for the triazoloacridine drug C-1305.[33] This is also the case for compound **27**, which is structurally comparable to the indolocarbazole derivative J-107088 (edotecarin), mentioned in the Introduction.[34] In conclusion, we have devised a novel efficient synthetic strategy to prepare a family of phenylcarbazole derivatives and established structure−activity relationships. The synthesis of phenylcarbazoles was realized in only four very efficient steps starting from indoles. The strategy involving palladium-catalyzed step represents a general method that could be easily scale-up and applied to other (hetero)aromatic systems. Some of the molecules, in particular those containing an *N*-dimethylaminoethyl side chain on the maleimide nitrogen, show potent cytotoxic properties, and interaction with DNA (but not topoisomerase I) may contribute to this antiproliferative activity. DNA binding, presumably by intercalation at defined AT/GC-containing sites, likely accounts for their propensity to block CEM cell cycle progression in the G_2/M or S phases. The phenylcarbazole series certainly warrants further investigations, and the chemical routes reported here offer interesting perspectives to introduce a variety of substituents.

Experimental Section

Chemistry. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 250 instrument using $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm (δ scale) and all *J* values are in hertz. The following abbreviations are used: singlet (s), doublet (d), doubled doublet (dd), triplet (t), multiplet (m), quaternary carbon (Cq). Melting points are uncorrected. IR absorptions were recorded on a Perkin-Elmer PARAGON 1000 PC, and values are reported in cm^{-1}. MS spectra (ion spray) were performed on a Perkin-Elmer Sciex PI 300 or on Avatar 320 using an ATR (Ge) technique. Monitoring of the reactions was performed using silica gel TLC plates (silica Merck 60 F_{254}). Spots were visualized by UV light at 254 and 356 nm. Flash chromatography columns were performed using silica gel 60 (0.063−0.200 mm, Merck).

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-methoxyphenyl)-1-methylpyrrole-2,5-dione (3). A solution containing 2-methoxyphenylboronic acid **2** (150 mg, 0.99 mmol), compound **1** (400 mg, 0.9 mmol), and K_2CO_3 (224 mg, 1.8 mmol) in a mixture of dioxane (9 mL) and water (1.7 mL) was degassed by argon bubbling for 20 min. $Pd(OAc)_2$ (20 mg, 0.9 mmol) was added in one portion and the reaction mixture immersed into a preheated oil bath at 80 °C for 4 h. After cooling, water (50 mL) and EtOAc (50 mL) were added, and the solution was filtered. The precipitate was washed with EtOAc (2 × 25 mL), and the aqueous layers were separated. The organic layer was evaporated under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/EtOAc 8/2) to afford compound **3** as a yellow solid (423 mg, quant.). Mp: 142 °C. ^{1}H NMR (DMSO, 250 MHz): δ 2.66 (s, 3H, NCH_3), 3.05 (s, 3H, OCH_3), 6.50 (d, 1H, J = 7.8 Hz), 6.78 (d, 1H, J = 8.8 Hz), 6.89 (t, 1H, J = 8.8 Hz), 7.03 (t, 1H, J = 7.3 Hz), 7.24 (t, 1H, J = 7.8 Hz), 7. 37 (m, 2H), 7.62 (m, 2H), 7.70 (d, 1H, J = 7.1 Hz), 7.96 (d, 1H, J = 8.3 Hz), 8.05 (m, 3H). MS (IS): 473 $(M + 1)^+$. Anal. ($C_{26}H_{20}N_2O_5S$): C, H, N.

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-hydroxyphenyl)-1-methylpyrrole-2,5-dione (4). To a solution containing compound **3** (500 mg, 1.06 mmol) in distillated CH_2Cl_2 (8 mL) at 0 °C was slowly added a solution of BBr_3 (1M in CH_2Cl_2, 8.0 mL, 8.0 mmol). After 1 h at room temperature, the reaction mixture was poured into ice (20 g). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic layers were evaporated under reduced pressure. Flash chromatography (petroleum ether/EtOAc 3/7) afforded compound **4** as an orange solid (455 mg, 94%). Mp: 172−174 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.05 (s, 3H), 6.76 (m, 2H), 6.97 (t, 1H, J = 7.0 Hz), 6.89 (m, 3H), 7.25 (t, 1H, J = 7.3 Hz), 7.61 (m, 2H), 7.70 (d, 1H, J = 7.3 Hz), 7.89 (m, 3H), 8.70 (s, 1H), 9.61 (s, 1H, exchangeable D_2O). MS (IS): 459 $(M + 1)^+$. Anal. ($C_{25}H_{18}N_2O_5S$): C, H, N.

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-trifluoromethylsulfonatephenyl)-1-methylpyrrole-2,5-dione (5). To a solution of compound **4** (800 mg, 1.75 mmol) and NEt_3 (730 μL, 5.26 mmol) in CH_2Cl_2 (36 mL) cooled at 0 °C was added dropwise a solution of triflic anhydride (980 μL, 1.75 mmol) in CH_2Cl_2 (5 mL). After 2 h, water (20 mL) was added, and the aqueous layers were extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were evaporated under reduced pressure, and the crude residue was purified by flash chromatography (petroleum ether/EtOAc 7/3 NEt_3 1%) to afford compound **5** as a yellow solid (875 mg, 85%). Mp: 94 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.12 (s, 3H), 6.48 (d, 1H, J = 7.8 Hz), 6.93 (t, 1H, J = 7.8 Hz), 7.27 (t, 1H, J = 7.8 Hz), 7.40 (d, 1H, J = 7.8 Hz), 7.59−7.78 (m, 6H), 7.96 (m, 3H), 8.15 (s, 1H). MS (IS): 591 $(M + 1)^+$. Anal. ($C_{26}H_{17}F_3N_2O_7S_2$): C, H, N.

3-(2-Hydroxyphenyl)-4-(1*H*-indol-3-yl)-1-methylpyrrole-2,5-dione (6). A solution of compound **5** (150 mg, 0.255 mmol) and Bu_4NF (1M in THF, 510 μL, 0.510 mmol) in dry THF (15 mL) under argon was refluxed for 2 h. After cooling, water (15 mL) was added and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were evaporated under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/EtOAc 7/3) to afford compound **6** as an orange solid (70 mg, 77%). Mp: 108 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.02 (s, 3H), 6.54 (d, 1H, J = 7.9 Hz), 6.65 (t, 1H, J = 7.9 Hz), 6.81 (m, 2H), 7.02 (t, 1H, J = 7.8 Hz), 7.13 (d, 1H, J = 6.7 Hz), 7.22 (t, 1H, J = 7.5 Hz), 7.36 (d, 1H, J = 8.1 Hz), 7.97 (d, 1H, J = 3.3 Hz), 9.41 (s, 1H, exchangeable D_2O), 11.80 (s, 1H, exchangeable D_2O). MS (IS): 319 $(M + 1)^+$. Anal. ($C_{19}H_{14}N_2O_3$): C, H, N.

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-bromophenyl)-1-methylpyrrole-2,5-dione (11). A solution containing 2-bromophenylboronic acid **8** (712 mg, 3.26 mmol), compound **1** (410 mg, 0.92 mmol), and K_2CO_3 (509 mg, 3.68 mmol) in a mixture of dioxane (5 mL) and water (1.2 mL) was degassed by argon bubbling for 20 min. $Pd(OAc)_2$ (26 mg, 0.09 mmol) was then added in one portion and the reaction mixture poured into a preheated oil bath at 100 °C for 9 h. After cooling, water (50 mL) and EtOAc (50 mL) were added, and the solution was filtered. The precipitate was washed with EtOAc (2 × 25 mL), and the aqueous layers were separated. The organic layer was evaporated under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/EtOAc 9/1) to afford compound **11** as a yellow solid (469 mg, 98%). Mp: 160 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.08 (s, 3H), 6.77 (d, 1H, J = 8.1 Hz), 7.00 (t, 1H, J = 8.1 Hz), 7.33 (t, 1H, J = 8.1 Hz), 7.44 (m, 3H), 7.63 (m, 3H), 7.73 (t, 1H, J = 7.3 Hz), 7.92 (m, 3H), 8.08 (s, 1H). MS (IS): 521−523 (M + 1)$^+$. Anal. ($C_{25}H_{17}BrN_2O_4S$): C, H, N.

3-(2-Bromophenyl)-4-(1*H*-indol-3-yl)-1-methylpyrrole-2,5-dione (12). To a solution of compound **11** (1.100 g, 2.11 mmol) in THF (20 mL) was added at room temperature a solution of Bu_4NF (1 M in THF, 2.53 mL, 2.53 mmol). After 2 h at reflux, the mixture was cooled, water (20 mL) was added, and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were evaporated under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/EtOAc 7/3) to afford compound **12** as a orange solid (747 mg, 93%). Mp: 196−198 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.11 (s, 3H), 6.42 (d, 1H, J = 8.1 Hz), 6.73 (t, 1H, J = 8.1 Hz), 7.13 (t, 1H, J = 7.6 Hz), 7.41 (m, 4H), 7.81 (dd, 1H, J = 1.6 Hz, J = 3.1 Hz), 8.13 (d, 1H, J = 1.6 Hz), 12.60 (s, 1H, exchangeable D_2O). MS (IS): 381−383 (M + 1)$^+$. Anal. ($C_{19}H_{13}BrN_2O_2$): C, H, N.

2-Methyl-8-(benzenesulfonyl)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (13). A solution containing compound **11** (550 mg, 1.05 mmol), Bu_4NCl (292 mg, 1.05 mmol), AcONa (172 mg, 2.10 mmol), and PPh_3 (422.1 mg, 2.10 mmol) in dry dioxane (19 mL) was degassed by argon bubbling for 20 min. $Pd(OAc)_2$ (235 mg, 2.10 mmol) was then added in one portion and the reaction mixture immersed into a preheated oil bath at 90 °C for 6 h. After cooling, water (50 mL) and EtOAc (50 mL) were added, and the solution was filtered. The precipitate was washed with EtOAc (2 × 25 mL), and the aqueous layers were separated. The organic layer was evaporated under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/EtOAc 8/2) to afford compound **13** as a yellow solid (344 mg, 83%). Mp: 244 °C dec. ^{1}H NMR (DMSO, 250 MHz): δ 3.08 (s, 3H), 6.85 (m, 2H), 7.15 (m, 2H), 7.42 (m, 2H), 7.63 (t, 1H, J = 7.5 Hz), 7.79 (m, 2H), 8.18 (d, 1H, J = 8.3 Hz), 8.60 (d, 1H, J = 7.8 Hz), 8.71 (m, 1H), 8.94 (m, 1H). MS (IS): 441 (M + 1)$^+$. Anal. ($C_{25}H_{16}N_2O_4S$): C, H, N.

2-Methylbenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (14). Procedure A from 12. A solution containing compound **12** (623 mg, 2.00 mmol) and AcOK (215 mg, 2.2 mmol) in dry DMA (27 mL) was degassed by argon bubbling for 20 min. $Pd(PPh_3)_4$ (119 mg, 0.1 mmol) was then added in one portion and the reaction mixture was immersed into a preheated oil bath at 130 °C for 4 h. After cooling, water (50 mL) and EtOAc (50 mL) were added, and the solution was filtered. The precipitate was washed with EtOAc (2 × 25 mL), and the aqueous layers were separated. The organic layer was evaporated under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/EtOAc 5/5 then MeOH) to afford compound **14** as an orange solid (599 mg, quant.).

Procedure B from 13. The same procedure as described for compound **12** was used. Flash chromatography (petroleum ether/EtOAc 7/3) afforded compound **14** (quant.). Mp: 244 °C dec. ^{1}H NMR (DMSO, 250 MHz): δ 3.11 (s, 3H), 7.34 (t, 1H, J = 8.1 Hz), 7.56 (t, 1H, J = 7.2 Hz), 7.71 (d, 1H, J = 8.1 Hz), 7.81 (m, 2H), 8.65 (m, 1H), 8.87 (d, 1H, J = 8.1), 8.96 (m, 1H), 12.92 (s, 1H, exchangeable D_2O). MS (IS): 301 (M + 1)$^+$. Anal. ($C_{19}H_{12}N_2O_2$): C, H, N.

3-(1-Benzenesulfonyl-1*H*-5-benzyloxyindol-3-yl)-4-(2-bromophenyl)-1-methylpyrrole-2,5-dione (15). The same procedure as described for compound **11**, starting from **10**, was used. The reaction time was 8 h and flash chromatography (petroleum ether/EtOAc 8/2) afforded compound **15** (58%) as a yellow solid. Mp: 139−141 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.08 (s, 3H), 4.48 (s, 2H), 6.19 (d, 1H, J = 3.2 Hz), 6.89 (dd, 1H, J = 7.8, J = 3.2 Hz), 7.32−7.47 (m, 8H), 7.60−7.70 (m, 4H), 7.80 (d, 1H, J = 10.1 Hz), 7.97 (d, 2H, J = 8.3), 8.16 (s, 1H). MS (IS): 627−629 (M + 1)$^+$. Anal. ($C_{32}H_{23}BrN_2O_5S$): C, H, N.

3-(1*H*-5-benzyloxyindol-3-yl)-4-(2-bromophenyl)-1-methylpyrrole-2,5-dione (16). The same procedure as described for compound **12** was used. Flash chromatography afforded compound **16** (86%) as a orange solid. Mp: >250 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.04 (s, 3H), 4.44 (s, 2H), 6.03 (s, 1H), 6.73 (d, 1H, J = 8.8 Hz), 7.35−7.46 (m, 9H), 7.81 (d, 1H, J = 7.5 Hz), 8.11 (s, 1H), 11.94 (s, 1H, exchangeable D_2O). MS (IS): 487−489 (M + 1)$^+$. Anal. ($C_{26}H_{19}BrN_2O_3$): C, H, N.

2-Methyl-5-(benzyloxy)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3-(2*H*,8*H*)-dione (17). The same procedure as described in procedure A for compound **14**, starting from **16**, was used. Flash chromatography afforded compound **17** (quant.) as a orange solid. Mp: 188−190 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.04 (s, 3H), 5.17 (s, 2H), 7.20 (d, 1H, J = 8.1 Hz), 7.35−7.46 (m, 3H), 7.56−7.58 (m, 3H), 7.70−7.73 (m, 2H), 8.40 (s, 1H), 8.49 (d, 1H, J = 8.1 Hz), 8.81 (d, 1H, J = 8.2 Hz), 12.64 (s, 1H, exchangeable D_2O). MS (IS): 407 (M + 1)$^+$. Anal. ($C_{26}H_{18}N_2O_3$): C, H, N.

2-Methyl-5-hydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3-(2*H*,8*H*)-dione (18). To a solution containing compound **17** (263 mg, 0.65 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise a solution of BBr_3 (1M in CH_2Cl_2, 0.65 mL, 0.65 mmol). After 1 h at room temperature, the reaction mixture was poured into ice (30 g), and the aqueous layers were extracted with EtOAc (2 × 30 mL). The organic layer was evaporated under reduced pressure to dryness. The crude material was washed with in MeOH (3 × 10 mL) to afford compound **18** as an orange solid. Mp: >250 °C. ^{1}H NMR (DMSO, 250 MHz): δ 3.00 (s, 3H), 7.02 (dd, 1H, J = 9.1 Hz, J = 2.3 Hz), 7.47 (d, 1H, J = 9.1 Hz), 7.64−7.68 (m, 2H), 8.26 (d, 1H, J = 2.3 Hz), 8.44 (dd, 1H, J = 9.1 Hz, J = 4.3 Hz), 8.76 (dd, 1H, J = 9.1 Hz, J = 4.3 Hz), 9.24 (s, 1H), 12.50 (s, 1H, exchangeable D_2O). MS (IS): 317 (M + 1)$^+$. Anal. ($C_{19}H_{12}N_2O_3$): C, H, N.

5*H*-Furo[3,4-*c*]benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(8*H*)-dione (19). To a solution containing compound **14** (500 mg, 1.66 mmol) in EtOH (10 mL) was added at room temperature an aqueous solution of KOH (5 N) under vigorous stirring. The reaction mixture was refluxed during 5 h. After cooling, the reaction mixture was acidified using a hydrochloric solution (5 N) until the pH raised to 5 and then concentrated to 6 mL under reduced pressure. The precipitate was filtered, washed with cold MeOH (3 × 10 mL), and dried under vacuum to afford compound **19** as a red solid (473 mg, 99%). Mp: >250 °C. ^{1}H NMR (DMSO, 250 MHz): δ 7.40 (t, 1H, J = 7.5 Hz), 7.59 (d, 1H, J = 7.5 Hz), 7.75 (d, 1H, J = 7.5 Hz), 7.78 (m, 2H), 8.66 (d, 1H, J = 7.0 Hz), 8.79 (m, 2H), 12.35 (s, 1H, exchangeable D_2O). MS (IS): 288 (M + 1)$^+$. Anal. ($C_{18}H_9NO_3$): C, H, N.

5-Benzyloxy-5*H*-furo[3,4-*c*]benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(8*H*)-dione (20). The same procedure as described for compound **19**, starting from compound **17**, yielded compound **20** as a orange solid (83%). Mp: 243 °C dec. ^{1}H NMR (DMSO, 250 MHz): δ 5.13 (s, 2H), 7.20 (d, 1H, J = 8.2), 7.35−7.46 (m, 6H), 7.76−7.81 (m, 2H), 8.06 (s, 1H), 8.52 (d, 1H, J = 8.2 Hz), 8.63 (d, 1H, J = 8.0 Hz), 12.90 (s, 1H, exchangeable D_2O). MS (IS): 394 (M + 1)$^+$. Anal. ($C_{25}H_{15}NO_4$): C, H, N.

5-Hydroxy-5*H*-furo[3,4-*c*]benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(8*H*)-dione (21). The same procedure as described for compound **18** yielded compound **21** as a red solid (quant.). Mp: >250 °C. ^{1}H NMR (DMSO, 250 MHz): δ 7.07 (dd, 1H, J = 9.1 Hz, J = 2.2 Hz), 7.55 (d, 1H, J = 9.1 Hz), 7.83−7.89 (m, 2H), 8.12 (d, 1H, J = 2.2 Hz), 8.62 (d, 1H, J = 8.4 Hz), 8.73 (d, 1H, J = 9.1 Hz),

9.40 (s, 1H, exchangeable D_2O), 12.92 (s, 1H, exchangeable D_2O). MS (IS): 304 $(M + 1)^+$. Anal. ($C_{18}H_9NO_4$): C, H, N.

Benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (22).[19] To a solution of compound **19** (150 mg, 0.52 mmol) in DMF (2 mL) was added a solution of ammonia (37%, 2 mL). The reaction mixture was refluxed during 8 h. After cooling to room temperature, water (20 mL) was added and pH was adjusted to 7 using hydrochloric acid (1 N). The precipitate was filtered and washed with EtOAc (3 × 10 mL) to afford compound **22** (138 mg, 93%).

5-Hydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (23). The same procedure as described for compound **22** was used. The solution was heated at 120 °C for 24 h. After filtration, the precipitate was dissolved in acetone and purified by flash chromatography (petroleum ether/EtOAc 7/3) to afford compound **23** as a red solid (95 mg, 96%). Mp: >250 °C. ^{1}H NMR (DMSO, 250 MHz): δ 7.03 (d, 1H, J = 9.0 Hz), 7.56 (d, 1H, J = 9.0 Hz), 7.77 (m, 3H), 8.33 (d, 1H, J = 2.0 Hz), 8.44 (d, 1H, J = 9.0 Hz), 9.26 (s, 1H, exchangeable D_2O), 11.07 (s, 1H, exchangeable D_2O), 12.50 (s, 1H, exchangeable D_2O). MS (IS): 303 $(M + 1)^+$. Anal. ($C_{18}H_{10}N_2O_3$): C, H, N.

2-(2-Dimethylaminoethyl)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (24). A solution of compound **19** (100 mg, 0.35 mmol) in 2-dimethylaminoethylamine (0.5 mL) was heated at 120 °C for 12 h. After cooling to room temperature, water (20 mL) was added. The precipitate was filtered and washed with cold MeOH (3 × 10 mL) to afford compound **24** as a yellow solid (100 mg, 80%). Mp: >250 °C. ^{1}H NMR (DMSO, 250 MHz): δ 2.21 (s, 6H), 2.57 (t, 2H, J = 5.6 Hz), 3.79 (t, 2H, J = 5.6 Hz), 7.35 (t, 1H, J = 7.0 Hz), 7.54 (t, 1H, J = 8.0 Hz), 7.72−7.83 (m, 2H), 8.33 (d, 1H, J = 2.0 Hz), 8.66 (d, 1H, J = 5.0 Hz), 8.89 (d, 1H, J = 8.0 Hz), 8.96 (d, 1H, J = 5.0 Hz), 12.95 (s, 1H, exchangeable D_2O). MS (IS): 358 $(M + 1)^+$. Anal. ($C_{22}H_{19}N_3O_2$): C, H, N.

2-(2-Dimethylaminoethyl)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (25). The same procedure as described for compound **24** was used. After treatment, compound **25** was isolated as an orange solid (71%). Mp: >250 °C. ^{1}H NMR (DMSO, 250 MHz): δ 2.25 (s, 6H), 2.52 (m, 2H), 3.74 (m, 2H), 7.04 (d, 1H, J = 8.0 Hz), 7.52 (d, 1H, J = 8.0 Hz), 7.76 (m, 2H), 8.34 (s, 1H), 8.59 (d, 1H, J = 5.0 Hz), 8.92 (d, 1H, J = 5.0 Hz), 9.27 (s, 1H, exchangeable D_2O), 12.62 (s, 1H, exchangeable D_2O). MS (IS): 374 $(M + 1)^+$. Anal. ($C_{22}H_{19}N_3O_3$): C, H, N.

2-[2-Hydroxy-1-(hydroxymethyl)ethyl]benzo[*a*]pyrrolo[3,4-*c*]-carbazole-1,3(2*H*,8*H*)-dione (26). To a solution of compound **19** (150 mg, 0.38 mmol) in DMF (0.5 mL) was added 2-aminopropan-1,3-diol (377 mg, 4.14 mmol). The reaction mixture was refluxed for 12 h. After cooling, water (5 mL) was added and the pH was adjusted to 5 using hydrochloric acid (1 N). The aqueous layer was extracted with EtOAc (3 × 10 mL), and the combined organic layers were evaporated under reduced pressure. The crude material was washed with cold MeOH (2 mL) to afford compound **26** as a orange solid (103 mg, 75%). Mp: >232 °C dec. ^{1}H NMR (DMSO, 250 MHz): δ 3.76 (m, 2H), 3.93 (m, 2H), 4.37 (m, 1H), 4.94 (s, 2H, exchangeable D_2O), 7.39 (t, 1H, J = 7.0 Hz), 7.54 (d, 1H, J = 7.0 Hz), 7.74 (d, 1H, J = 8.2 Hz), 7.85−7.88 (m, 2H), 8.69 (d, 1H, J = 4.7 Hz), 8.92 (d, 1H, J = 7.7 Hz), 9.02 (d, 1H, J = 4.7 Hz), 12.92 (s, 1H, exchangeable D_2O). MS (IS): 361 $(M + 1)^+$. Anal. ($C_{21}H_{16}N_2O_4$): C, H, N.

2-]2-Hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxybenzo[*a*]-pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (27). The same procedure as described for compound **26** was used. After treatment, compound **27** was isolated as a red solid (68%). Mp: 248 °C dec. ^{1}H NMR (DMSO, 250 MHz): δ 3.74 (m, 2H), 3.89 (m, 2H), 4.33 (m, 1H), 4.94 (s, 2H, exchangeable D_2O), 7.08 (dd, 1H, J = 8.9 Hz, J = 3.5 Hz), 7.55 (d, 1H, J = 8.2 Hz), 7.77−7.81 (m, 2H), 8.36 (d, 1H, J = 3.5 Hz), 8.71−8.74 (m, 1H), 8.98−9.01 (m, 1H), 9.29 (s, 1H, exchangeable D_2O), 12.94 (s, 1H, exchangeable D_2O). MS (IS): 377 $(M + 1)^+$. Anal. ($C_{21}H_{16}N_2O_5$): C, H, N.

2-(2-(1*H*-Imidazol-4-yl)ethyl)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (28). In a sealed tube, histamine (28 mg, 1.5 equiv) was added to a solution of compound **19** (50 mg, 0.17 mmol) in DMF (2 mL). The solution was heated to 130 °C for 3 days. After cooling, water (10 mL) was added. The precipitate was filtered and washed with cold MeOH (5 mL) to afford compound **28** as an orange solid (63 mg, 97%). Mp: 158−160 °C. ^{1}H NMR (DMSO, 250 MHz): δ 2.95 (t, 2H, J = 8.0 Hz), 3.88 (t, 2H, J = 8.0 Hz), 6.85 (s, 1H), 7.34 (t, 1H, J = 8.0 Hz), 7.51 (s, 1H), 7.58 (t, 1H, J = 8.0 Hz), 7.74 (d, 1H, J = 8.0 Hz), 7.80−7.83 (m, 2H), 8.63 (d, 1H, J = 5.0 Hz), 8.86 (d, 1H, J = 8.0 Hz), 8.69 (d, 1H, J = 8.0 Hz), 11.85 (s, 1H, exchangeable D_2O), 12.92 (s, 1H, exchangeable D_2O). MS (IS): 381 $(M + 1)^+$. Anal. ($C_{23}H_{16}N_4O_2$): C, H, N.

2-(2-(1*H*-Imidazol-4-yl)ethyl)-5-hydroxybenzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (29). The same procedure as described for compound **28** was used. After treatment, compound **29** was obtained as a red solid (91%). Mp: 183−185 °C. ^{1}H NMR (acetone-d_6, 250 MHz): δ 2.45 (t, 2H, J = 7.0 Hz), 3.40 (t, 2H, J = 6.8 Hz), 6.39 (s, 1H), 6.58 (dd, 1H, J = 9.0 Hz, J = 2 Hz), 7.07 (s, 1H), 7.10 (d, 1H, J = 9 Hz), 7.30−7.33 (m, 2H), 7.45 (s, 1H), 7.88 (d, 1H, J = 2.0 Hz), 8.15 (dd, 1H, J = 8.0 Hz, J = 4.0 Hz), 8.83 (s, 1H, exchangeable D_2O), 11.53 (s, 1H, exchangeable D_2O), 12.21 (s, 1H, exchangeable D_2O). MS (IS): 397 $(M + 1)^+$. Anal. ($C_{23}H_{16}N_4O_3$): C, H, N.

DNA Binding Measurements. Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. Titrations of the drug with DNA, covering a large range of drug/DNA-phosphate ratios (D/P), were performed by adding aliquots of a concentrated drug solution to a constant DNA solution (20 μM). T_m measurements were performed in pH 7.1 BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette (10 mm path length) was increased over the range 20−100 °C with a heating rate of 1 °C/min. The "melting" temperature T_m was taken as the midpoint of the hyperchromic transition.

DNase I Footprinting. The complete procedure has been recently detailed.[35]

Cell Culture and Survival Assay. Human CEM and CEMC2 leukemia cells were obtained from the American Tissue Culture Collection. Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 4.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, penicillin (100 IU/mL), and streptomycin (100 μg/mL). The cytotoxicity of the tested compounds was assessed using a cell proliferation assay developed by Promega (CellTiter 96 aqueous one-solution cell proliferation assay). Briefly, 2×10^4 exponentially growing cells were seeded in 96-well microculture plates with graded drug concentrations in a volume of 100 μL. After 72 h incubation at 37 °C, 20 μL of the tetrazolium dye was added to each well, and the samples were incubated for a further 2 h at 37 °C. Plates were analyzed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

Cell Cycle Analysis. For flow cytometric analysis of DNA content, 0.7×10^6 cells in exponential growth were treated with graded concentrations of the tested drug for 24 h and then washed with 1 mL of PBS. After centrifugation, the cell pellet was resuspended in 1 mL of cold ethanol for 24 h at −20 °C. The ethanol was removed, and the pellet was washed with 1 mL of PBS and then incubated for 30 min in a solution containing 50 μg/mL PI and 100 μg/mL RNase. Samples were analyzed on a Becton Dickinson FACScan flow cytometer using CellQuest software, which was also used to determine the percent of cells in the different phases of the cell cycle. PI was excited at 488 nm and the fluorescence analyzed at 620 nm on channel Fl-2.

Protein Kinase Assays. Kinase activities were assayed in buffer A or C, at 30 °C, at a final ATP concentration of 15 μM. Blank values were subtracted and activities calculated as picomoles of phosphate incorporated for a 10-min incubation. The activities are usually expressed in percent of the maximal activity, i.e., in the absence of inhibitors. Controls were performed with appropriate dilutions of dimethyl sulfoxide.

GSK-3α/β was purified from porcine brain by affinity chromatography on immobilized axin.[36] It was assayed, following a 1/100 dilution in 1 mg of BSA/mL of 10 mM DTT, with 5 μL of 40 μM GS-1 peptide as a substrate, in buffer A [10 mM $MgCl_2$, 1 mM

EGTA, 1 mM DTT, 25 mM Tris-HCl (pH 7.5), 50 μg heparin/mL], in the presence of 15 μM [γ-^{33}P]ATP (3000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After 30 min incubation at 30 °C, 25-μL aliquots of supernatant were spotted onto 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper, and 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL of phosphoric acid/L of water. The wet filters were counted in the presence of 1 mL of ACS (Amersham) scintillation fluid.

CDK1/cyclin B was extracted from M phase starfish oocytes and purified by affinity chromatography on p9^{CKShs1}-sepharose beads as previously described.[37] The kinase activity was assayed in buffer C [60 mM β-glycerophosphate, 15 mM *p*-nitrophenyl phosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM phenyl phosphate], with 1 mg of histone H1/mL, in the presence of 15 μM [γ-^{33}P]ATP (3000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After 10 min incubation at 30 °C, 25-μL aliquots of supernatant were spotted onto P81 phosphocellulose papers and treated as described above.

CDK5/p25 was reconstituted by mixing equal amounts of recombinant mammalian CDK5 and p25 expressed in *Escherichia coli* as GST (glutathione-*S*-transferase) fusion proteins and purified by affinity chromatography on glutathione-agarose (p25 is a truncated version of p35, the 35 kDa CDK5 activator). Its activity was assayed in buffer C as described for CDK1/cyclin B.

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Supporting Information Available: ^{13}C NMR, IR, and MS data and results of elemental analysis for compounds **3**−**6**, **11**−**21**, **23**−**29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Synthesis and Biological Evaluation of Novel Phenylcarbazoles as Potential Anticancer Agents

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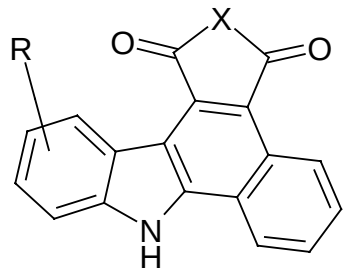
Table of contents :



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Elemental analysis Table.

Compound #	Anal.	Calculated, C, H, N	Found, C, H, N
3	$C_{26}H_{20}N_2O_5S$	66.09, 4.27, 5.93.	65.72, 4.41, 6.04.
4	$C_{25}H_{18}N_2O_5S$	65.49, 3.96, 6.11.	65.78, 3.80, 6.24.
5	$C_{26}H_{17}F_3N_2O_7S_2$	52.88, 2.90, 4.74.	53.21, 3.04, 4.93.
6	$C_{19}H_{14}N_2O_3$	71.69, 4.43, 8.80.	71.40, 4.56, 8.65.
11	$C_{25}H_{17}BrN_2O_4S$	57.59, 3.29, 5.37.	57.30, 3.43, 5.49.
12	$C_{19}H_{13}BrN_2O_2$	59.86, 3.44, 7.35.	60.30, 3.27, 7.52.
13	$C_{25}H_{16}N_2O_4S$	68.17, 3.66, 6.37.	68.44, 3.51, 6.47.
14	$C_{19}H_{12}N_2O_2$	75.99, 4.03, 9.33.	75.65, 4.21, 9.52.
15	$C_{32}H_{23}BrN_2O_5S$	61.25, 3.69, 4.46.	61.54, 3.82, 4.59.
16	$C_{26}H_{19}BrN_2O_3$	64.08, 3.93, 5.75.	63.73, 4.08, 5.87.
17	$C_{26}H_{18}N_2O_3$	76.83, 4.46, 6.89.	77.12, 4.63, 6.75.
18	$C_{19}H_{12}N_2O_3$	72.15, 3.82, 8.86.	72.46, 3.69, 8.99.
19	$C_{18}H_9NO_3$:	75.26, 3.16, 4.88.	74.86, 3.30, 4.71.
20	$C_{25}H_{15}NO_4$	76.33, 3.84, 3.56.	76.01, 3.97, 3.68.
21	$C_{18}H_9NO_4$	71.29, 2.99, 4.62.	71.63, 2.84, 4.79.
23	$C_{18}H_{10}N_2O_3$	71.52, 3.33, 9.27.	71.28, 3.50, 9.13.
24	$C_{22}H_{19}N_3O_2$	73.93, 5.36, 11.76	74.27, 5.18, 11.83.
25	$C_{22}H_{19}N_3O_3$	70.76, 5.13, 11.25.	71.03, 5.02, 11.09.
26	$C_{21}H_{16}N_2O_4$	69.99, 4.48, 7.77.	70.33, 4.35, 7.86.
27	$C_{21}H_{16}N_2O_5$	67.02, 4.28, 7.44.	66.79, 4.45, 7.50.
28	$C_{23}H_{16}N_4O_2$	72.62, 4.24, 14.73.	72.96, 4.10, 14.87.
29	$C_{23}H_{16}N_4O_3$	69.69, 4.07, 14.13.	69.31, 4.23, 14.04.

Routine spectroscopic data

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-methoxy-phenyl)-1-methyl-pyrrole-2,5-dione (3). Rf (petroleum ether/EtOAc 7/3) 0.86 ; IR (KBr, cm^{-1}) ν 3402, 2918, 1752, 1684, 1594, 1386, 1168, 734; ^{13}C NMR (DMSO, 62.5 MHz) : δ 26.6 (CH_3), 56.2 (CH_3), 113.8 (CH), 115.3 (CH), 115.5 (Cq), 120.7 (Cq), 122.5 (CH), 123.2 (CH), 125.6 (CH), 127.6 (CH), 129.3 (2CH), 129.8 (Cq), 130.1 (CH), 132.4 (2CH), 132.8 (Cq), 133.5 (CH), 133.8 (CH), 134.9 (Cq), 136.2 (Cq), 137.4 (Cq), 138.8 (CH), 159.6 (Cq), 172.4 (CO), 172.7 (CO).

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-hydroxy-phenyl)-1-methyl-pyrrole-2,5-dione (4). Rf (petroleum ether /EtOAc 3/7) 0.55; IR (KBr, cm^{-1}) ν 3426, 2946, 1696, 1430, 1174, 752 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 27.2 (CH_3), 115.5 (CH), 116.0 (Cq), 118.8 (Cq), 119.8 (CH), 121.8 (CH), 124.6 (CH), 126.5 (CH), 128.2 (CH), 129.8 (2CH), 130.9 (Cq), 131.3 (CH), 133.1 (2CH + Cq), 134.0 (Cq), 134.1 (CH), 136.9 (Cq), 137.4 (Cq), 138.1 (Cq), 139.5 (CH), 158.8 (Cq), 173.3 (CO), 173.4 (CO).

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-trifluoromethylsulfonate-phenyl)-1-methyl-pyrrole-2,5-dione (5). Rf (petroleum ether /EtOAc 7/3) 0.35 ; IR (KBr, cm^{-1}) ν 3156, 2962, 1772, 1708, 1448, 1212, 750, 595 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 24.6 (CH_3), 62.5 (Cq), 111.0 (CH), 113.5 (CH), 120.5 (Cq), 121.0 (CH), 122.3 (Cq), 123.5 (CH), 123.8 (Cq), 125.6 (Cq), 125.8 (Cq), 126.9 (Cq), 127.0 (2CH), 129.4 (CH), 129.6 (CH), 130.3 (2CH), 132.5 (CH), 132.9 (CH), 134.1 (CH), 135.4 (Cq), 136.5 (CH), 146.9 (Cq), 169.4 (CO), 169.5 (CO).

3-(2-Hydroxy-phenyl)-4-(1*H*-indol-3-yl)-1-methyl-pyrrole-2,5-dione (6). Rf (petroleum ether /EtOAc 3/7) 0.28 ; IR (KBr, cm^{-1}) ν 3556, 3105, 2952, 1724, 1703, 1452, 1386, 1176, 994 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 24.1 (CH_3), 112.5 (CH), 116.5 (Cq), 121.5 (CH), 123.5 (CH), 124.7 (CH), 126.1

(Cq), 127.6 (Cq), 125.5 (CH), 127.2 (2CH), 128.3 (CH), 128.8 (CH), 130.5 (Cq), 136.9 (Cq), 141.5 (CH), 164.8 (Cq), 172.6 (CO), 172.7 (CO).

3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(2-bromo-phenyl)-1-methyl-pyrrole-2,5-dione (11). Rf (petroleum ether /EtOAc 8/2) 0.68 ; IR (ATR, cm^{-1}) ν 3119, 2927, 2846, 1696, 1441, 1374, 1137, 738 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 26.0 (CH_3), 113.7 (CH), 115.2 (Cq), 123.0 (CH), 124.8 (Cq), 125.7 (CH), 127.5 (CH), 128.8 (2CH), 129.3 (CH), 129.7 (Cq), 131.3 (CH), 131.9 (2CH), 132.7 (Cq), 133.4 (CH), 133.9 (CH), 134.3 (Cq), 134.7 (Cq), 135.9 (Cq), 137.0 (CH), 137.2 (Cq), 138.2 (Cq), 171.3 (CO), 171.5 (CO).

3-(2-Bromo-phenyl)-4-(1*H*-indol-3-yl)-1-methyl-pyrrole-2,5-dione (12). Rf (petroleum ether /EtOAc 8/2) 0.18 ; IR (ATR, cm^{-1}) ν 3343, 2956, 2919, 2850, 1684, 1627, 1413, 1374, 1093, 746; ^{13}C NMR (DMSO, 62.5 MHz) : δ 24.7 (CH_3), 105.8 (Cq), 112.9 (CH), 120.8 (Cq), 120.9 (CH), 122.9 (CH), 124.6 (Cq), 125.3 (Cq), 128.2 (CH), 131.4 (CH), 132.2 (Cq), 132.4 (CH), 132.9 (Cq), 133.3 (CH), 133.4 (CH), 135.4 (Cq), 137.2 (Cq), 170.8 (CO), 171.5 (CO).

2-(Methyl)-8-(benzenesulfonyl)-benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (13). Rf (petroleum ether /EtOAc 8/2) 0.38 ; IR (ATR, cm^{-1}) ν 3121, 2932, 2856, 1703, 1461, 1401, 1183, 752 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 24.6 (CH_3), 119.5 (CH), 123.5 (Cq), 124.9 (CH), 125.1 (CH), 125.9 (CH), 126.8 (Cq), 127.1 (Cq), 127.2 (CH), 127.3 (2CH), 127.9 (Cq), 128.0 (Cq), 128.4 (CH), 128.8 (CH), 129.3 (Cq), 129.6 (2CH), 129.8 (CH), 134.0 (CH), 135.3 (Cq), 141.5 (Cq), 142.1 (Cq), 168.5 (CO), 169.6 (CO).

2-(Methyl)-benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (14). Rf (petroleum ether /EtOAc 7/3) 0.27 ; IR (ATR, cm^{-1}) ν 3323, 2915, 2854, 1680, 1423, 1378, 1072, 734 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 24.9 (CH_3), 113.2 (CH), 119.0 (Cq), 122.1 (CH), 122.6 (Cq), 124.1 (CH), 125.3 (CH), 126.1

(CH), 127.2 (CH), 127.9 (Cq), 129.0 (CH), 129.5 (CH), 131.4 (Cq), 132.5 (Cq), 133.7 (Cq), 141.3 (Cq), 141.7 (Cq), 170.5 (CO), 171.4 (CO).

-(1-Benzenesulfonyl-1*H*-5-benzyloxyindol-3-yl)-4-(2-bromo-phenyl)-1-methyl-pyrrole-2,5-dione (15). Rf (petroleum ether /EtOAc 7/3) 0.77 ; IR (ATR, cm^{-1}) ν 3143, 2919, 1704, 1439, 1378, 1150, 1089, 754 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 25.8 (CH_3), 70.7 (CH_2), 105.6 (CH), 113.3 (2Cq), 115.7 (CH), 116.8 (CH+Cq), 124.6 (Cq), 128.2 (2CH), 128.9 (CH), 129.2 (Cq), 129.3 (Cq), 129.9 (2CH), 130.1 (Cq), 131.4 (CH+Cq), 132.6 (CH), 132.9 (Cq), 133.4 (Cq), 133.6 (Cq), 134.4 (CH), 135.6 (Cq), 136.5 (CH), 137.7 (CH), 137.8 (CH+Cq), 156.5 (Cq), 170.9 (CO), 171.7 (CO).

3-(1*H*-5-benzyloxyindol-3-yl)-4-(2-bromo-phenyl)-1-methyl-pyrrole-2,5-dione (16). Rf (petroleum ether /EtOAc 7/3) 0.57 ; IR (ATR, cm^{-1}) ν 3359, 2919, 2846, 1680, 1619, 1419, 1382, 1064, 732 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 24.9 (CH_3), 70.0 (CH_2), 104.1 (Cq), 113.9 (CH), 114.0 (Cq), 124.9 (Cq), 126.1 (Cq), 127.5 (Cq), 127.7 (2CH), 128.2 (2CH), 128.3 (Cq), 128.5 (CH), 129.3 (2CH), 131.6 (Cq), 132.4 (CH), 133.1 (Cq), 133.7 (CH), 134.1 (Cq), 135.4 (CH), 137.7 (CH), 153.9 (Cq), 171.0 (CO), 171.8 (CO).

2-(Methyl)-5-(benzyloxy)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (17). Rf (petroleum ether /EtOAc 7/3) 0.47 ; IR (ATR, cm^{-1}) ν 3347, 3041, 2874, 1676, 1427, 1374, 1296, 1223, 766 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 24.2 (CH_3), 71.2 (CH_2), 108.9 (CH), 112.8 (Cq), 117.7 (CH), 118.2 (Cq), 122.7 (Cq), 123.4 (CH+Cq), 125.6 (CH), 126.7 (Cq), 128.1 (Cq), 128.5 (3CH), 128.7 (CH+Cq), 129.1 (3CH), 125.9 (Cq), 135.9 (Cq), 141.7 (CH), 154.2 (Cq), 169.9 (CO), 170.7 (CO).

2-(Methyl)-5-(hydroxy)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (18). Rf (petroleum ether /EtOAc 5/5) 0.32 ; IR (ATR, cm^{-1}) ν 3347, 1676, 1427, 1328, 1190, 799, 746 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 23.2 (CH_3), 108.4 (CH), 111.5 (Cq), 112.0 (CH), 116.1 (Cq), 111.5 (CH), 121.9 (CH),

122.1 (Cq), 122.4 (Cq), 124.5 (CH), 125.5 (Cq), 127.1 (CH), 127.6 (Cq), 133.8 (CH+Cq), 140.5 (Cq), 151.8 (Cq), 168.9 (CO), 169.8 (CO).

5*H*-Furo[3,4-*c*]benzo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-1,3(8*H*)-dione (19). Rf (petroleum ether /EtOAc 4/6) 0.15 ; IR (ATR, cm^{-1}) ν 3221, 3054, 2960, 1708, 1366, 1170, 1097, 154, 722 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 112.5 (Cq), 113.1 (CH), 118.5 (Cq), 121.7 (Cq), 122.2 (CH), , 123.9 (CH), 124.1 (CH), 124.2 (Cq), 125.6 (Cq), 126.7 (Cq), 127.6 (CH), 128.2 (CH), 127.9 (CH), 129.9 (2Cq+CH), 165.1 (CO), 165.2 (CO).

5-Benzyloxy-5*H*-furo[3,4-*c*]benzo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-1,3(8*H*)-dione (20). Rf (petroleum ether /EtOAc 7/3) 0.27 ; IR (ATR, cm^{-1}) ν 3306, 2935, 2858, 1725, 1313, 1186, 1019, 775; ^{13}C NMR (DMSO, 62.5 MHz) : δ 70.9 (CH_2), 107.7 (CH), 112.8 (Cq), 113.6 (CH), 117.9 (CH), 122.5 (Cq), 123.7 (Cq+CH), 124.5 (Cq), 125.6 (CH), 126.8 (Cq), 128.6 (2CH+Cq), 129.2 (Cq), 129.5 (2CH), 129.6 (CH), 129.9 (Cq), 136.1 (Cq), 138.6 (Cq), 142.9 (Cq), 154.9 (Cq), 165.2 (CO), 165.3 (CO).

5-Hydroxy-5*H*-furo[3,4-*c*]benzo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-1,3(8*H*)-dione (21). Rf (petroleum ether /EtOAc 3/7) 0.24 ; IR (ATR, cm^{-1}) ν 3321, 3229, 1733, 1688, 1460, 1333, 1243, 738 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 110.3 (CH), 114.4 (Cq), 115.5 (CH), 119.5 (Cq), 119.7 (CH), 124.5 (Cq), 125.7 (CH), 126.0 (Cq), 127.6 (CH), 128.4 (CH), 130.4 (Cq), 131.6 (Cq), 132.8 (CH), 136.7 (Cq), 144.4 (Cq), 155.3 (Cq), 167.1 (2CO).

5-Hydroxy-benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (23). Rf (petroleum ether /EtOAc 7/3) 0.47 ; IR (ATR, cm^{-1}) ν 3349, 3327, 1726, 1447, 1337, 1182, 771 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 109.3 (CH), 112.5 (Cq), 113.1 (CH), 117.2 (CH), 118.5 (Cq), 122.9 (Cq), 123.5 (CH), 125.7 (CH), 126.3 (Cq), 126.8 (Cq), 128.4 (CH), 128.8 (CH), 129.6 (Cq), 134.7 (Cq), 141.6 (Cq), 152.8 (Cq), 171.5 (CO), 172.4 (CO).

2-(2-Dimethylaminoethyl)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (24). Rf (petroleum ether /EtOAc 5/5) 0.14 ; IR (ATR, cm^{-1}) ν 2919, 2838, 1688, 1460, 1374, 1243, 811, 738 ^{13}C NMR (DMSO, 62.5 MHz) : δ 38.05 (CH_2), 47.95 ($2CH_3$), 59.63 (CH_2), 114.6 (CH), 120.1 (Cq), 123.5 (CH), 123.9 (Cq), 125.2 (Cq), 125.5 (CH), 126.7 (CH), 127.5 (CH), 128.2 (CH), 128.6 (Cq), 129.3 (Cq), 130.2 (CH), 130.4 (Cq), 130.9 (CH), 142.6 (Cq), 143.1 (Cq), 171.7 (CO), 172.6 (CO)

2-(2-Dimethylaminoethyl)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (25). Rf (petroleum ether /EtOAc 5/5) 0.17 ; IR (ATR, cm^{-1}) ν 3274, 2939, 2833, 2780, 1696, 1468, 1390, 1256, 995, 771 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 36.15 (CH_2), 46.0 ($2CH_3$), 57.7 (CH_2), 109.3 (CH), 112.7 (Cq), 113.2 (CH), 117.4 (CH), 122.9 (Cq), 123.4 (CH), 123.6 (Cq), 125.7 (CH), 126.7 (Cq), 128.5 (CH), 128.6 (Cq), 129 (CH), 134.9 (Cq), 136.2 (Cq), 141.7 (Cq), 153.0 (Cq), 170.0 (CO), 170.9 (CO).

2-[2-Hydroxy-1-(hydroxymethyl)ethyl]benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (26). Rf (petroleum ether /EtOAc 7/3) 0.57 ; IR (ATR, cm^{-1}) ν 3319, 3217, 3066, 1684, 1455, 1341, 1236, 738 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 57.5 (CH), 59.5 ($2CH_2$), 112.8 (CH), 112.9 (Cq), 118.6 (Cq), 121.8 (CH), 122.2 (Cq), 123.6 (CH), 123.9 (CH), 125.1 (CH+Cq), 125.8 (Cq), 127.0 (Cq), 127.6 (CH), 128.8 (CH), 129.3 (CH), 141.0 (Cq), 141.4 (CH), 170.7 (CO), 171.6 (CO).

2-]2-Hydroxy-1-(hydroxymethyl)ethyl]-5-(hydroxy)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (27). Rf (petroleum ether /EtOAc 7/3) 0.62 ; IR (ATR, cm^{-1}) ν 3355, 3253, 2960, 2923, 2850, 1684, 1455, 1366, 1019, 942, 771 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 55.6 (CH), 58.2 ($2CH_2$), 108.0 (CH), 111.1 (CH), 111.9 (Cq), 115.9 (Cq), 116.0 (CH), 121.5 (Cq), 122.2 (Cq), 122.8(Cq), 124.2 (CH), 125.4 (CH), 126.9 (CH), 127.6 (Cq), 127.7 (CH), 133.6 (Cq), 140.4 (Cq), 151.6 (Cq), 169.3 (CO), 170.2 (CO).

2-(2-(1*H*-Imidazol-4-yl)-ethyl)-5-(hydroxy)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (28). Rf (petroleum ether /EtOAc 5/5) 0.17 ; IR (ATR, cm^{-1}) ν 3551, 3319, 2923, 2850, 1688, 1460,

1362, 1239, 1023, 742 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 29.75 (CH_2), 34.8 (CH_2), 110.8 (CH), 116.5 (Cq), 118.7 (CH), 119.9 (Cq), 120.2 (CH), 121.4 (CH), 121.8 (2Cq), 122.9 (CH), 123.8 (Cq), 124.9 (CH), 125.6 (Cq), 126.6 (Cq), 126.7 (CH), 127.2 (CH), 133.9 (CH), 138.9 (CH), 139.4 (Cq), 161.3 (Cq), 167.9 (CO), 168.8 (CO) ; MS (IS) : 381 $(M+1)^+$; Anal. calcd for $C_{23}H_{16}N_4O_2$: C, 72.62; H, 4.24; N, 14.73. Found: C, 72.96 ; H, 4.10 ; N, 14.88.

2-(2-(1*H*-Imidazol-4-yl)-ethyl)benzo[*a*]pyrrolo[3,4-*c*]carbazole-1,3(2*H*,8*H*)-dione (29). Rf (petroleum ether /EtOAc 5/5) 0.17 ; IR (ATR, cm^{-1}) ν 3355, 2827, 2842, 1684, 1476, 1376, 1190, 795 ; ^{13}C NMR (DMSO, 62.5 MHz) : δ 39.2 (CH_2), 43.5 (CH_2), 106.9 (CH), 110.2 (Cq), 110.7 (CH), 114.8 (Cq), 115.1 (CH), 120.5 (CH), 120.9 (Cq), 121.1 (Cq), 123.2 (CH), 124.3 (Cq), 126.0 (2CH), 126.3 (Cq), 126.5 (CH), 132.4 (Cq), 138.8 (CH), 139.2 (Cq), 139.3 (Cq), 150.5 (Cq), 167.5 (CO), 168.4 (CO) ; MS (IS) : 397 $(M+1)^+$; Anal. calcd for $C_{23}H_{16}N_4O_3$: C, 69.69; H, 4.07; N, 14.13. Found: C, 69.31 ; H, 4.23 ; N, 14.04.