# Synthesis and Biological Evaluation of Novel Naphthocarbazoles as Potential Anticancer Agents

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We report the efficient synthesis involving palladium-catalyzed reactions and biological evaluation of new naphthocarbazoles designed as potential anticancer agents. The use of 5- and 6-benzyloxyindoles generated three substitution sites which were successively exploited to introduce several hydrophilic side chains. The cytotoxicity of the newly designed compounds was evaluated on three cell lines. Several compounds showed a marked cytotoxicity with $IC_{50}$ values in the sub-micromolar range. This is the case for the 3-hydroxy-naphthopyrrolocarbazoledione **37**, bearing a dimethylaminoethyl side chain, which is extremely cytotoxic to L1210 and DU145 cells ($IC_{50}$: 36 nM, 108 nM) and induces an accumulation of L1210 cells in the G2+M phases of the cell cycle. Some of the most cytotoxic compounds were tested for inhibition of CDK-5, GSK-3 and topoisomerase I, and their interaction with DNA was also evaluated. Interaction with DNA was detected, suggesting that nucleic acids represent a privileged target for these molecules.

## 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 their central role in the regulation of cell cycle progression and specific enzymatic inhibitions.[2,3] Structure activity relationships (SAR) have been reported and can be used as a guide to design more potent compounds. Closely related compounds such as arcyriaflavins have been described as inhibitors of different isoforms of protein kinase C. This is the case for arcyriaflavin-A (Figure 1) which prevents the replication of the human cytomegalovirus via an inhibition of the protein kinase pUL97.[4] In contrast, compounds bearing a pyrroloindolocarbazole with one *N*-glycosidic bond, such as the antibiotic rebeccamycin, generally function as DNA topoisomerase I inhibitors whereas similar compounds with two *N*-glucosidic bonds, such as staurosporine, are mainly nonspecific protein kinase C (PKC) inhibitors.[1] A few rebeccamycin analogues such as NSC655649 (also known as BMS 181176) and NB-506 have entered clinical trials for cancer treatment.[5] The later compound NB-506 has been subsequently replaced with a more potent derivative J-107088 which is also an indolocarbazole with a functionalized maleimide nitrogen.[6,7] Very recently, a promising series of fluorinated analogues targeting topoisomerase I has also been described.[8] One of the key molecular element of these compounds is their hydrophilic side chain (e.g. diethylaminoethyl chain for NSC655649, bis-(hydroxymethyl)methylamino chain for J-107088) which increases the water solubility and enhances the molecular interaction with the target.[9−13] In the same vein, several modifications of the sugar moiety have been proposed to improve the water solubility and modulate the topoisomerase I inhibition property. The SAR relationships in the indolocarbazole series have been extensively studied in the context of topoisomerase I inhibition.[1]

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**.[14] The cytotoxic properties of the *N*-glycosylated derivatives of **I** and **II** have been reported too.[15,16] Recently, macrocyclic polyoxygenated bisindoylmaleimide and 7-azaindolylmaleimide **III** were described as potent and selective inhibitors of the glycogen synthase kinase-3$\beta$ (GSK-3), a multisubstrate kinase directly involved in diverse vital cellular processes and which might represent an interesting therapeutic target.[17,18] GSK-3 inhibitors might be useful for the treatment of diabete, Alzheimer's disease and protection against cell death in general. Cyclin dependent kinases (CDK) have also raised a considerable attention because of their central role in the regulation of cell cycle progression, and these CDKs can be targeted with indolocarbazoles.[19]

To develop selective kinase inhibitors acting in the ATP binding site, modifications of the indolocarbazole moiety appeared as a valid alternative to the restrictive synthesis of glycosylated compounds. For example, some derivatives containing an indeno moiety fused to the carbazole skeleton equipped with a lactam staurosporin-like structure**IV** have been described as potent vascular endothelial growth factor R2 (VEGF−R2) tyrosine kinase inhibitor. VGEF is a key mediator of angiogenesis and evidence has accumulated that tumor VEGF ex-

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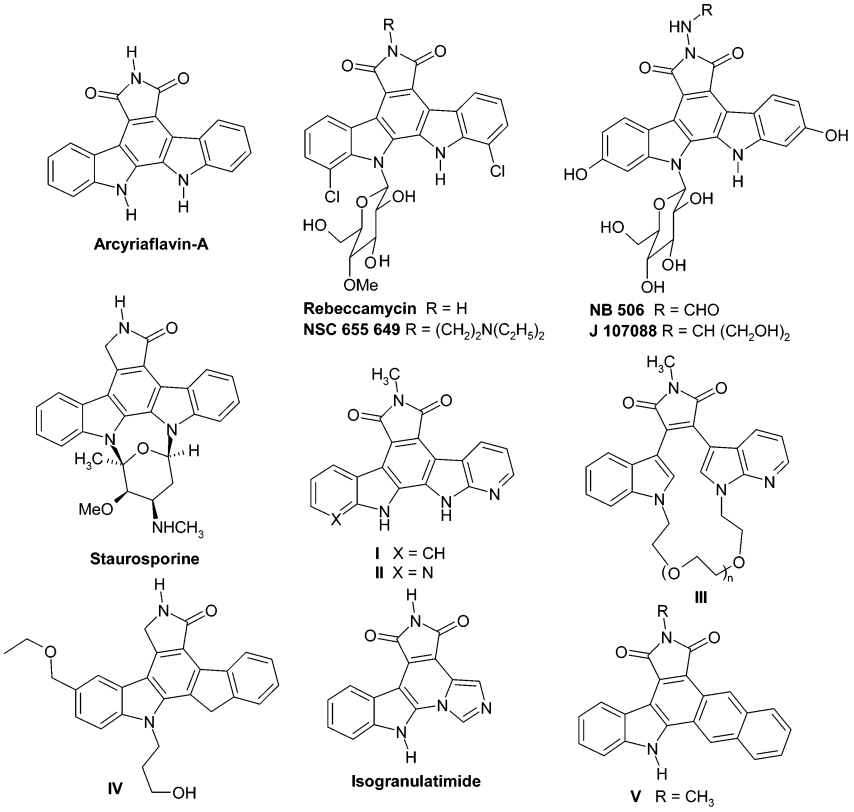



**Figure 1.** Modifications of the indolocarbazole skeleton.

pression was clinically associated with solid malignancies.[20] In the maleimide-containing series of rebeccamycin aglycons, indole versus (hetero)arylcarbazoles replacement represents an interesting strategy. Granulatimide and isogranulatimide (Figure 1) have been described as G2 checkpoints inhibitors.[21−24] For our own part we reported a preliminary synthesis of some naphthalenic compounds **V**.[25] Replacing also one of the two indoles by a phenyl, pyridine, naphthalene, quinoline or isoquinoline led to the identification of novel potent cyclin D1/CDK1 or cyclin D1/CDK4 inhibitors.[26−29] These examples indicated also that a slight chemical modification of the indolocarbazole framework can have a profound impact on the mechanism of action and the biological activity. All these considerations prompted us to describe our extensive efforts to design novel anticancer agents in the naphthalenic series.

## Chemistry

Various synthetic methods[30] have been used to modify the indolocarbazole skeleton and to diversify the representative panel of symmetrical or unsymmetrical indolocarbazoles. Direct formation of the maleimide framework through reaction of indole-3-acetamidate[31] or by an oxidative coupling of indolyl acetate dianion have been reported.[32] Generation of the second indoyl moiety via nitrene insertion, use of 2,2′-bisindole as diene for a (4 + 2) Diels Alder cycloaddition with dimethyl acetylene dicarboxylate or *N*-phenylmaleimide have also been described.[33−37] An alternative and more common synthesis consisted of the selective 3-alkylation of indole with a dibromomaleimide using Grignard reagents to produce bisindoylmaleimides. This procedure often requires an oxidative photochemical benzannulation, carried out by irradiation in a low concentrated solution, which limits the amount of formed product.[38−40] Palladium-catalyzed reactions or Wacker type reactions represent interesting alternatives to the photochemical step. By contrast, cross-coupling reactions were scarcely described.[41−44] Recently such strategy was also used by Cephalon or Lilly groups[20,26−29] using a similar approach. All these studies indicated that Suzuki, Stille and intramolecular Heck reactions were quite efficient but never used in the same sequence to complete the synthesis of indolocarbazoles and related structures.

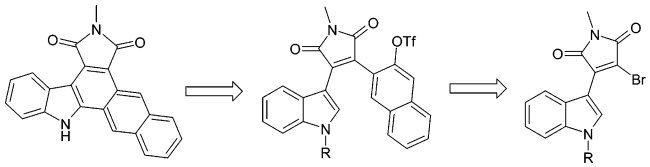
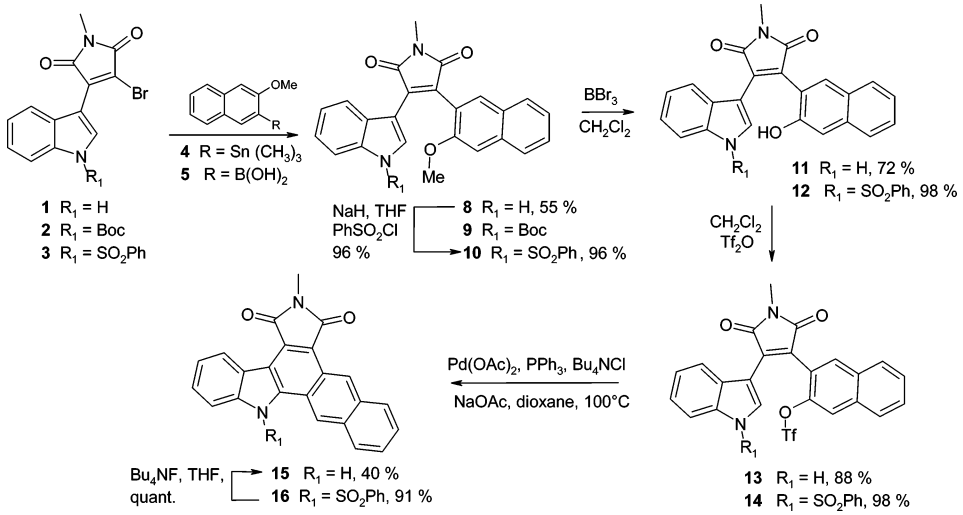



**Figure 2.** Retrosynthetic route.

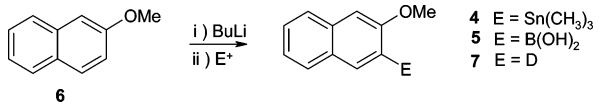
To avoid the inconstant irradiation step, which is always a limiting synthetic factor, we designed an original route to naphthocarbazoles using a multistep catalyzed palladium synthesis. The ideal straightforward retrosynthetic scheme consisted of the introduction of the naphthyl parts by Stille or Suzuki procedures followed by an intramolecular Heck reaction (Figure 2).

Our first efforts[25] consisted of the synthesis of compound **1,** classically obtained from indole and 2,3-dibromo-*N*-methylmaleimide in the presence of LiHMDS as a base according to a reported procedure.[41] Compound **1** was either *N*-Boc or *N*-benzenesulfonyl protected. These protective groups can be easily removed either in acidic or basic conditions. *N*-Boc protected compound **2** was prepared using $Boc_2O$, DMAP, and triethylamine as a base whereas the benzenesulfon-

**Scheme 1**




**Scheme 2**




yl protective group was introduced on compound **1** using NaH, to yield **3** in 85% yield.

Direct introduction of a 2-methoxynaphthyl group on compound **2** was performed using the very efficient Suzuki or Stille coupling reaction with palladium derivatives as catalysts (Scheme 1).

First, the (3-methoxynaphthalen-2-yl)-trimethylstannate **4** or (3-methoxy-naphthalen-2-yl)-boronic acid **5** was prepared by selective lithiation of the 2-methoxynaphthalene **6** at −10 °C with *n*-BuLi (1.85 equiv).[45] To attribute the regioselectivity of proton abstraction, quenching the reaction after 1 h with $D_2O$ showed a complete lithiation with the exclusive formation of the compound **7**. Several assays were then performed with other electrophiles. Addition of trimethylstannyl chloride was performed at −78 °C to afford the compound **4** in 95% yield. Similar conditions using an excess of trimethylborate as electrophile did not afford the desired boronic acid **5**; however, **5** could be obtained in 78% yield by allowing the reaction mixture to reach room temperature. (Scheme 2).

Having obtained the partners for the Stille or Suzuki reactions, we performed several assays to optimize these cross-coupling reactions between indolic derivatives **1**−**3** and naphthalene organometallic agents **4** and **5**. Organostannane **4** and compounds **1**−**3** using $Pd(PPh_3)_4$ as catalyst and CuI in dioxane or DMF gave only limited yield of coupled products **8**−**10**. After some experiments, compound **8** is obtained in 56% yield from **1** using the catalytic system $Pd_2(dba)_3$, $AsPh_3$ as a ligand and CuI as an additive. In the case of the *N*-Boc derivative **2**, compound **8** was only obtained with 12% yield using $Pd(PPh_3)_4$ as a catalyst and CuI in dioxane or DMF with no trace of compound **9**, while in the presence of LiCl (3 equiv) in place of CuI, the yield of **8** from **2** increased to 54%. Finally **10** was obtained from **3** in 64% yield using the same catalytic system. This Stille coupling reaction was more efficient in the presence of a *N*-indolic protected compound. A benzenesulfonyl group insensitive to temperature seems to be the best-adapted protection of indolic moiety compared to the conventional Boc group which was lost by heating.

A similar study was realized using the boronic acid **5** via a Suzuki procedure. Standard conditions involved $Pd(OAc)_2$ (10%) as a catalyst in the presence of $K_2CO_3$ as a base in a mixture of dioxane and water. These cross-coupling reactions appeared more efficient than Stille reactions with a shorter reaction time (2 h). From compound **1** this approach gave access to compound **8** in a moderate 55% yield but obtained after 2 h at 100 °C (or 5 h at 90 °C). A similar yield was obtained using *N*-Boc protected compound **2** and, as described before, only the deprotected compound **8** was obtained. The best result was obtained with the benzenesulfonyl compound **3** which gave compound **10** in 80% yield. In addition, the deprotected compound **8** could be easily converted into compound **10** with a 96% yield using NaH as a base in the presence of benzenesulfonyl chloride.

The *O*-demethylation was equally performed on compounds **8** and **10** using $BBr_3$ to afford **11** and **12** in 72 and 98% yields, respectively (Scheme 1). The hydroxy compounds **11** and **12** were converted into triflates **13** and **14** in 88 and 98% yields by reaction with triflic anhydride in the presence of triethylamine (no reaction was observed on the indolic nitrogen atom with compound **11**). The central six-membered ring was then obtained by an intramolecular Heck reaction (Table 1). This reaction was carried out at 100 °C using $Pd(OAc)_2$ as a catalyst, $PPh_3$ as a ligand, NaOAc (2 equiv) as a base and $Bu_4NCl$ (1 equiv) as a phase transfer agent. The *N*-unprotected indolic compound **13** in DMA or dioxane afforded the desired compound **15** in only a modest 40% yield. Compound **14** gave access to a mixture of **15** and **16** which ratio was under the influence of the conditions used; surprisingly, the benzenesulfonyl group was partially removed under these conditions. Compounds **15** and **16** were obtained in 51% and 10% yields, respectively (entry 2), using the conditions described for **13**.

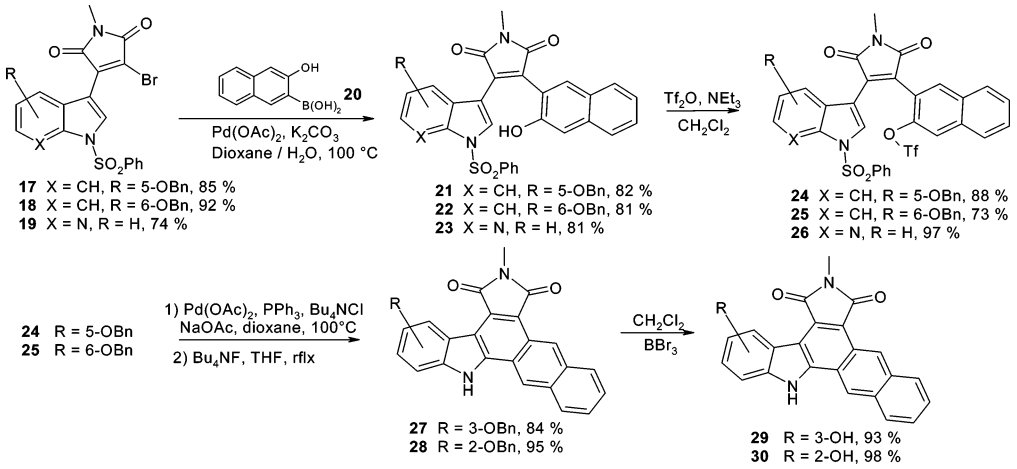
Increasing the amount of catalyst to 30% (entry 3) enhanced the reaction turnover but the deprotected product **15** was formed and the reaction stopped again. After 3 days no trace of the protected compound **16** could be detected. To complete the reaction, we deliberately

**Table 1.** Heck Cyclization Procedure on Compounds **13** and **14**

| entry | reactant | catalyst | conditions | solvent, temp | time, h | product (yield) |
|---|---|---|---|---|---|---|
| 1 | **13** | $Pd(OAc)_2$ 10% | $PPh_3$ 0.2 equiv, $Bu_4NCl$ 1 equiv, NaOAc 2 equiv | DMA, 100°C | 12 | **15** (40) |
| 2 | **14** | $Pd(OAc)_2$ 10% | $PPh_3$ 0.2 equiv, $Bu_4NCl$ 1 equiv, NaOAc 2 equiv | dioxane, 100°C | 16 | **16** (10)<br>**15** (51) |
| 3 | **14** | $Pd(OAc)_2$ 30% | $PPh_3$ 0.2 equiv, $Bu_4NCl$ 1 equiv, NaOAc 2 equiv | dioxane, 100°C | 72 | **15** (63) |
| 4 | **14** | $Pd(OAc)_2$ 1 equiv | $PPh_3$ 0.2 equiv, $Bu_4NCl$ 1 equiv, NaOAc 2 equiv | dioxane, 100°C | 3 | **16** (70),<br>**15** (12);<br>**15** (91)[a] |

[a] Same assay realized with an additional $Bu_4NF$ treatment.

**Scheme 3**




used a stoichiometric amount of palladium acetate. The reaction rate was considerably increased, the starting material **14** was fully converted in only 3 h and the final products **15**, **16** were isolated in an overall yield of 82%. Purification of the protected compound **16** was not so easy, and a small amount was contaminated with **15** during the purification. The full deprotection of the crude mixture, using $Bu_4NF$ in refluxing THF,[46] simplified the purification procedure and afforded the desired compound **15** in a 91% yield for the two steps.

To diversify the naphthocarbazole skeleton, we decided to introduce on the indolic moiety hydroxy groups and to replace the indole moiety by a 7-azaindole unit to modulate the biological activity by creating potential supplementary hydrogen bonds with the biological target(s). Preparation of the 3- indolic or 3-azaindolic bromomaleimide compounds was realized using described procedures. *N*-protected compounds **17** and **18** were synthesized from the corresponding unprotected indolic nitrogen derivatives using benzenesulfonyl chloride in the presence of NaH in 85 and 92% yields, respectively. The 7-azaindolic derivative **19** was obtained using similar conditions in a 74% yield (Scheme 3).[14,41]

Generation of the hydroxy group from the methyl ether on the naphthalene moiety was hampered by the presence of the benzyloxy group on the indolic unit. For this reason, we used a naphthalene boronic acid with a 2-hydroxy substituent instead of a 2-methyl ether substituent. Transformation of compound **5** into (3-hydroxy-naphthalen-2-yl)-boronic acid **20** was realized in a 85% yield using $BBr_3$ in dichloromethane; Suzuki cross-coupling reactions of **17**−**19** with **20** afforded compounds **21**−**23** in 82, 81 and 81% yields, respectively (Scheme 3). These compounds were then reacted with triflic anhydride to give compounds **24**−**26** in 88, 73 and 97% yields, respectively. Then the intramolecular Heck reaction was directly followed by the benzenesulfonyl deprotection using $Bu_4NF$ in boiling THF to afford compounds **27** and **28** in 84 and 95% yields for the two steps. Unfortunately, the cyclization reaction was inefficient using the azaindolic compound **26**. The last step to obtain the fully deprotected derivatives was performed using $BBr_3$ and led to compounds **29** and **30** in 93 and 98% yields, respectively.

The naphthocarbazoles **15**, **28**−**30** could be easily functionalized and several compounds were then synthesized (Scheme 4) with the goal of reinforcing the hydrophilic properties of the compounds and hopefully the biological activity. On one hand, different side chains were introduced on the *N*-free indolic nitrogen atom of compound **15**. On the other hand, substitutions were performed on the maleimide group of **15**, **29** and **30** maintaining in all cases the free indolic NH and/or OH groups. The structures of compounds **31**−**47** were reported in Table 2.

In the presence of NaH as a base, the *N*-indolic atom of **15** was alkylated in THF (entries 1−3). Using 4-(2-chloroethyl)-morpholine or (2-chloroethyl)-dimethylamine, compounds **31** and **32** were synthesized in 49 and 35% yields, respectively. Similarly, reaction of compound **15** with allyl bromide led to **33** in a 51% yield

**Scheme 4**

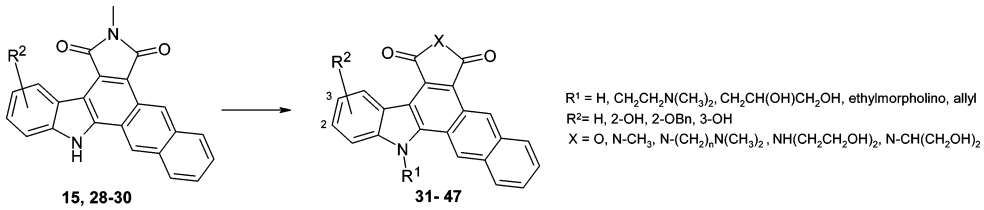
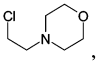
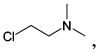
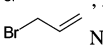
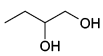
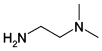
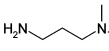
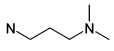
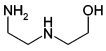
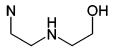
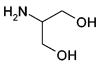
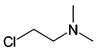
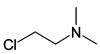
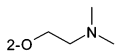



**Table 2.** Substitution of the Naphthocarbazoles **15**, **28**−**30**

| Entry | Reactant | Conditions | Product (% ) | $R^2$ | $R^1$ | X |
|---|---|---|---|---|---|---|
| 1 | **15** |  , NaH, THF, rflx. | **31** (49) | H |  | $NCH_3$ |
| 2 | **15** |  , NaH, THF, rflx. | **32** (35 ) | H |  | $NCH_3$ |
| 3 | **15** |  NaH, THF, r.t. | **33**(51) | H |  | $NCH_3$ |
| 4 | **33** | $KMnO_4$, acetone/water, r.t | **34** (71) | H |  | $NCH_3$ |
| 5 | **15** | KOH, ethanol, water, rflx. | **35** (83)[a] | H | H | O |
| 6 | **15** | | **36** (83)[a] | H | H |  |
| | **35** |  , 80 °C | **36** (88)[a] | | | |
| 7 | **29** | idem, rflx. | **37** (87)[a] | 3-OH | H | idem |
| 8 | **30** | idem | **38** (55)[a] | 2-OH | H | idem |
| 9 | **29** |  , rflx | **39** (87)[a] | 3-OH | H |  |
| 10 | **30** | idem | **40** (80)[a] | 2-OH | H | idem |
| 11 | **29** |  ,120 °C | **41** (quant.) | 3-OH | H |  |
| 12 | **30** | idem | **42** (27)[a] | 2-OH | H | idem |
| 13 | **29** |  , 120 °C | **43** (65)[a] | 3-OH | H |  |
| 14 | **30** | idem | **44** (31)[a] | 2-OH | H | idem |
| 15 | **28** |  , NaH, THF, rflx. | **45** (85) | 2-OBn |  | $NCH_3$ |
| 17 | **30** | TBDMSCl, imidazole | **46** (quant.) | 2-OTBDMS | H | $NCH_3$ |
| 18 | **46** |  , NaH, THF, rflx. | **47** (69) |  | H | $NCH_3$ |
| 19 | **29** | idem | **47** (41) | idem | H | $NCH_3$ |

[a] Quantitative on TLC; derivatives soluble in water.

which was converted to compound **34** in a 71% yield by oxidation using aqueous $KMnO_4$. The creation of this 2,3-propanediol side chain may be considered as a sugar mimic.

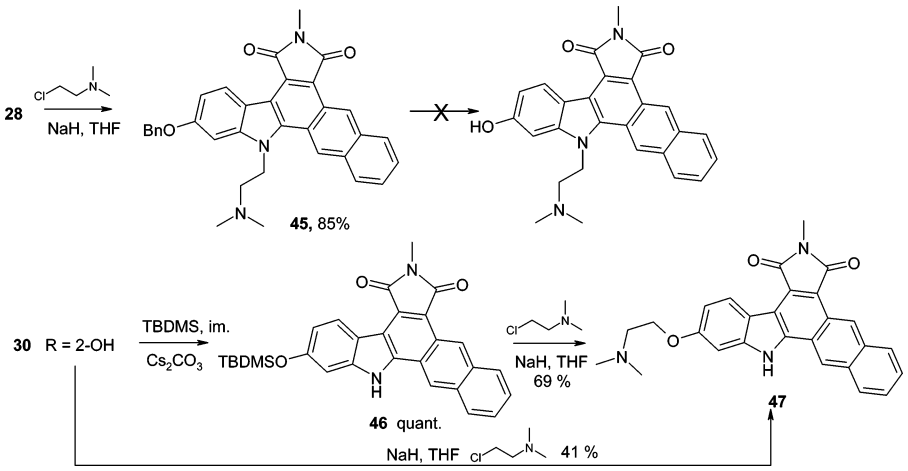
The maleimide function was then modified (entries 5, 6). First, anhydride **35** was obtained by reaction of compound **15** with KOH in ethanol in a 83% yield. Further reaction of **35** with (2-aminoethyl)-dimethylamine generated compound **36** in a 83% yield. This compound could be directly obtained from compound **15** by boiling in (2-aminoethyl)-dimethylamine in a 88% yield.

The selective substitution of compounds **29** and **30** on the maleimide moiety while maintaining the combination of unprotected NH and 2- or 3-OH groups simultaneously was performed with several primary amines. Such structures maintain the presence of the well-known hydroquinonimine system which is considered as an important element for the bioactivity of such anticancer agents. These substitutions were realized with four different chains in order to determine the best added chain. All substitutions were performed by exchange of the *N*-methyl group in boiling aliphatic amine to obtain compounds **37**−**44** in the range 27−100% yield (entries 7−14).

The last goal concerned the preparation of (i) *O*-alkylated compounds in the presence of the unprotected indolic nitrogen atom and (ii) *N*-alkylated compounds in the presence of the hydroxy group in order to determine the influence of the substitution of each atom (O, N) by a similar dimethylaminoethyl side chain. First we realized the direct alkylation of benzyl compound **28** as described for compound **15** to afford compound **45** in a 85 % yield. Unfortunately, the benzyl deprotection was unsuccessful despite numerous efforts (acidic deprotection, hydrogenolysis in the presence of Pd(C) 10%, Pd-$(OH)_2$...). Therefore, we realized a selective *O*-silylation of the deprotected compounds **29** and **30**. Starting from compound **30** the *O*-TBDMS compound **46** was quantitatively obtained, using TBDMSCl, imidazole and cesium carbonate as a base (Scheme 5); under identical conditions, compound **29** gave a degradation of the mixture.

The *p*-quinoneimine system was too sensitive in this basic medium. The protected compound **46** treated with (2-chloroethyl)-dimethylamine (Scheme 5) afforded the

**Scheme 5**




*O*-alkylated compound **47** in a 69% yield with no *N*-alkylation as a result of a prior desylilation reaction under basic conditions. The same compound **47** was directly synthesized by the alkylation of compound **30**, in a 41% yield.

## Results and Discussion

**In Vitro Antiproliferative Activities.** The antiproliferative activities of the compounds were tested against three tumor cell lines: murine L1210 leukemia, human prostate DU145 and human colon HT29 carcinomes. The cytotoxicity of the compounds was measured using a conventional microculture tetrazolium assay. The $IC_{50}$ values are collected in Table 3. In the L1210 inhibition assay, the *N*-methyl maleimide substituted compounds **15**, **29** and **30** showed micromolar activities. The presence of hydroxyl groups sensibly decreased the cytotoxicity. The comparison of the $IC_{50}$ values obtained with the unsubstituted derivative **15** and the *N*-indolic alkylated compounds **31**−**34** indicates that the introduction of a hydrophilic substituent induces a slight decrease of activity for compounds **31**, **32** and **34** whereas the incorporation of an allyl group decreased more significantly the activity, by 10 times as seen for compound **33**.

Therefore, a hydrophilic substitution might be preferable to a hydrophobic group. Moreover, the presence at this position of the 2,3-dihydroxypropyl side chain seems to be slightly better than no chain or the addition of basic centers. Whereas these modifications on the indolic nitrogen center were unsuccessful, replacing the *N*-methyl group of **15** by an *N*-2-dimethylaminoethyl side chain on **36** increases 5 to 10-fold the cytotoxicity to 210 nM.

The cytotoxicities of the *N*-methyl compounds **29** and **30** are comparable to that of **15** with micromolar $IC_{50}$. The introduction of the 2-dimethylamino ethyl side chain on the maleimide moiety enhances markedly the cytotoxic potential to reach out nanomolar activities as it is the case for compounds **37** and **38** ($IC_{50}$: 36 and 110 nM, respectively). This observation tends to confirm the idea that the addition of a basic side chain on the maleimide generally improves the efficacy of the compound, whatever the series (indolocarbazole or naphthocarbazole).

Substitutions on the northern part of the molecules were performed to further modulate the activity of the hydroxylated compounds. The homologation of the dimethylamino side chain (addition of one supplementary methylene unit) decreased sensibly the activity of compounds **39** and **40**. An extension of the chain longer than four carbon atoms may disfavor the cytotoxic activity. The introduction the mitoxantrone-type basic side chain gave compounds **41** and **42** with reduced activity, thus corroborating the observation that the extension of the basic chain is detrimental to cytotoxicity.

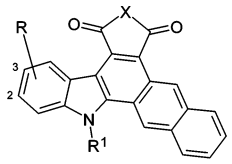
The activity is partially restored with compound **44** bearing the ramified bis-hydroxylated side chain but not with the related molecule **43**. Similarly, the capping of the hydroxy group by a TBDMS protective group or the dimethylamino side chain (compounds **46** and **47**, respectively) decreased the cytotoxicity to the micromolar range. The position of the free hydroxyl groups is apparently crucial. It is however difficult to provide a rational basis for the influence of the position of $R_2$ substituent (compare **37/38** vs **43/44**).

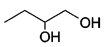
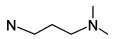
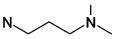
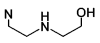
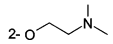
It is worth mentioning here that similar naphthocarbazoles have been recently described by the Lilly's group.[26,27] In their report, the complete suppression of the side chain attached to the maleimide skeleton led to poorly active compounds (micromolar $IC_{50}$), in agreement with our own observations.

The results obtained with the two human cell lines DU145 and HT29 are also very informative. Here again, compounds **36**, **37** and **38** show potent activities. In general, for the different compounds the sensitivity of the cells follows the order L1210 > DU145 > HT29. This is not really surprising as leukemia cells are almost always much more chemosensitive than colon cancer cells. If one focus on the data obtained with the prostate DU145 cell line, it appears that the presence of the indolyl group or the *N*-hydrophilic substitution increased the cytotoxicity. The position of the indolic hydroxyl group combined with the presence of the glycerol-like side chain is more favorable in position-2 than in position-3 (compare activities for compounds **43** and **44**). Interestingly, compound **44** is particularly efficient against the prostate cells ($IC_{50}$: 6.8 nM) and may thus represent an interesting lead molecule.

In conclusion of the cytotoxicity measurements, it appears that the combination of *N*-dimethylamino maleimide with an hydroxy group in position 2 or 3 generated four potent drug candidates **37**−**39** and **44**,

**Table 3.** In Vitro Antiproliferative Activities [$IC_{50}$ ($\mu$M)] against Three Cell Lines: a Murine Leukemia (L1210), a Human Prostate Cancer (DU145), a Colon Carcinoma (HT29)[a]



| Compounds | $R^2$ | $R^1$ | X | L 1210 | DU 145 | HT 29 |
|---|---|---|---|---|---|---|
| **rebeccamycin** | | | | 0.14 | ND | 0.3 |
| **15** | H | H | $NCH_3$ | 2.9 | 1.1 | ND |
| **31** | H |  | $NCH_3$ | 2.4 | ND | ND |
| **32** | H |  | $NCH_3$ | 2.4 | 4.7 | ND |
| **33** | H |  | $NCH_3$ | 34.0 | 59.7 | 65.7 |
| **34** | H |  | $NCH_3$ | 1.26 | 2.31 | 3.69 |
| **36** | H | H |  | 0.21 | 0.290 | ND |
| **29** | 3-OH | H | $NCH_3$ | 3.9 | 6.8 | ND |
| **30** | 2-OH | H | $NCH_3$ | 5.2 | 4.7 | ND |
| **37** | 3-OH | H |  | 0.036 | 0.108 | ND |
| **38** | 2-OH | H |  | 0.11 | 0.25 | ND |
| **39** | 3-OH | H |  | 0.13 | 0.29 | 0.84 |
| **40** | 2-OH | H |  | 0.34 | 0.76 | 1.35 |
| **41** | 3-OH | H |  | 0.29 | 0.63 | 3.76 |
| **42** | 2-OH | H |  | 0.51 | 1.11 | 5.5 |
| **43** | 3-OH | H |  | 1.19 | 2.13 | 2.88 |
| **44** | 2-OH | H |  | 0.2 | 0.0068 | 1.34 |
| **46** | 2-OTBDMS | H | $NCH_3$ | 10.1 | 5.6 | ND |
| **47** |  | H | $NCH_3$ | 1.5 | 4.5 | ND |

[a] $IC_{50}$ values are presented as means of duplicate experiments. ND: Not determined.

which are all significantly more active than the reference compound rebeccamycin.[16] In addition, the introduction on the maleimide moiety of an alkylamino pharmacophore group, frequently found in antitumor agents (e.g. mitoxantrone), led to very cytotoxic compounds **36**−**42**. These new derivatives are the first naphthocarbazoles with nanomolar cytotoxic activities (depending on the cell type). They are also the first isostere analogues of the rebeccamycin aglycone and represent potential candidates for further in vivo evaluations, currently ongoing.

**Cell Cycle Effect and Mechanism of Action.** Three compounds with a dimethylaminoethyl side chain on the maleimide moiety, **32**, **37** and **38**, were used to investigate the cycle cell effects on L1210 leukemia cells. In all three cases, a significant accumulation of the cells in the G2+M phases of the cell cycle was observed, reaching 56, 51 and 61% for compounds **32**, **37** and **38**, respectively. The effect is comparable to that observed with rebeccamycin (69% G2 + M) which exhibited lower cytotoxicity ($IC_{50}$ L1210: 0.14 $\mu$M) than compounds **37** and **38**.[16] The molecular target(s) of these compounds are not known. By analogy with previous studies, we postulated that the compounds may interfere with topoisomerases and certain kinases. Indeed, it was reported recently by the Lilly group that similar naphthocarbazoles can inhibit cyclin dependent kinases with nanomolar $IC_{50}$ values.[27] This prompted us to investigate the effect of our new compounds on CDK-5, but no significant activity was observed ($IC_{50}$ > 10 $\mu$M).[47] Similarly, we tested the compounds for inhibition of the glycogen synthase kinase-3$\beta$ (GSK-3) which is a multisubstrate kinase considered as a promising target for drugs active against diabetes, Alzheimer's disease and possibly cancer, but here again, no active compounds was found ($IC_{50}$ > 10 $\mu$M). Topoisomerases represent another target category for this molecules, as it is the cases for many indolocarbazoles derived from rebeccamycin and NB-506, both inhibiting topoisomerase I,[1] or derived from the synthetic molecule NSC655649 (BMS

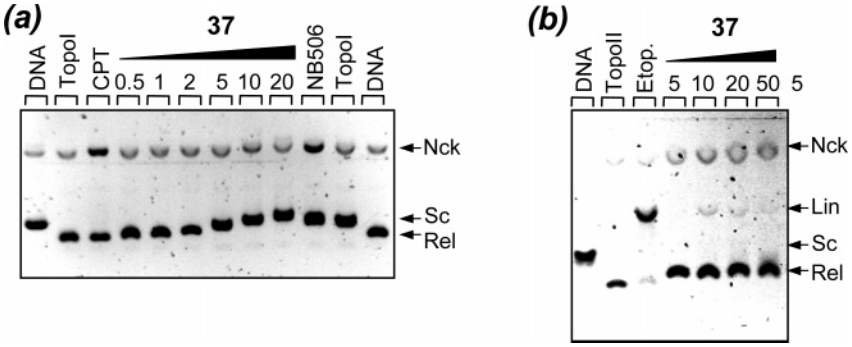



**Figure 3.** Effect of increasing concentrations of the compound **37** on the relaxation of plasmid DNA by human topoisomerases I (a) and II (b). Native supercoiled pKMp27 DNA (0.5 $\mu$g) (lane DNA) was incubated with 4 units topoisomerase in the absence (lane Topo) or presence of the drug at the indicated concentration ($\mu$M). The camptothecin (CPT) and etoposide (Etop.) were used as positive controls (25 $\mu$M each). Reactions were stopped with sodium dodecyl sulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on agarose gels containing ethidium bromide (1 $\mu$g/mL). The gels were photographed under UV light. Nck, nicked; Lin, linear; Rel, relaxed; Sc, supercoiled DNA.

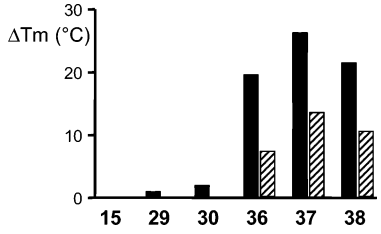



**Figure 4.** Variation in melting temperature ($\Delta T_m = T_m^{complex} - T_m^{DNA}$) for the different drugs bound to calf thymus DNA (grey bars) or poly(dAT)$_2$ (black bars) at a drug−DNA-(nucleotide) ratio 0.5. $T_m$ measurements were performed in BPE buffer pH 7.1 (6 mM $Na_2HPO_4$, 2 mM $NaH_2PO_4$, 1 mM EDTA), at 260 nm with a heating rate of 1 °C/min.

181176) described as a catalytic inhibitor of topoisomerase II (without inducing enzyme-mediated DNA double strand breaks).[5]

This additional screening was not more successful as no potent inhibitor of either topoisomerase I or topoisomerase II was found when using a plasmid relaxation assay (Figure 3). Only a very minor effect on topoisomerase II was noted with compound **37**, but it is considerably weaker than that observed with the reference drug etoposide (Figure 3b). Unlike the reference molecule camptothecin, this compound **37** does not promote DNA cleavage by topoisomerase I, but it induces a dose-dependent reduction of the electrophoretic mobility of DNA in agarose gels (Figure 3a).

Interaction with DNA was evaluated by melting temperature measurements with both calf thymus DNA (CT DNA) and the synthetic polymer poly(dAT)$_2$. The compounds equipped with a dimethylaminoethyl side chain strongly stabilize duplex DNA whereas the neutral compounds (including **44**) showed no significant effect. Under our experimental conditions, poly(dAT)$_2$ and CT DNA melt at 44 °C and 67 °C, and these melting temperatures remain practically unchanged in the presence of the *N*-methyl compounds such as **15**, **29** and **30**. In contrast, the equivalent molecules **36**, **37** and **38** bearing a $(CH_2)_2N(CH_3)_2$ strongly protect DNA against heat denaturation (Figure 4).

The presence of a hydroxyl group on the naphthocarbazole chromophore reinforces the interaction with DNA, and a slightly superior effect was observed with the 3-OH derivative **37** compared to the equivalent 2-OH analogue **38**, both with poly(dAT)$_2$ and CT DNA. The interesting point is that the enhanced interaction with DNA coincides with a superior cytotoxicity.

With L1210 cells, compounds **15**, **29** and **30** show $IC_{50}$ values in the 3−5 $\mu$M range whereas their analogues bearing the cationic side chain exhibit much lower $IC_{50}$, in the sub-micromolar range. The replacement of the *N*-methyl group of **29** with a *N*-dimethylaminoethyl chain strengthens the binding to DNA ($\Delta T_m$ increases from 1 to 13 °C with CT DNA) and increases the cytotoxicity 100 fold ($IC_{50}$ of 3.9 $\mu$M and 36 nM for **29** and **37**, respectively). These observations further illustrate the key role of such a cationic side chain which promotes hydrophilicity and target interaction. Although we have yet no direct evidence that the enhanced cytotoxicity effectively derived from the increased binding to DNA, this hypothesis is however plausible because similar effects have been previously observed with other indolocarbazoles.[1] DNA can be considered as a target for the naphthocarbazoles, at least the cationic molecules. However, the lack of interaction of the *N*-methyl compounds which are also cytotoxic suggests that targets other than nucleic acids must be involved in their mechanism of action, as it is generally the case with these type of molecules.

In conclusion, we have synthesized a large series of naphthocarbazole derivatives. Some of these molecules exhibit potent cytotoxic properties toward cancer cells. DNA binding likely contributes to the antiproliferative activity of the most cytotoxic compounds which warrant further in vitro and in vivo evaluations. The efficient synthetic routes reported here provides novel opportunities to design DNA-targeted antitumor agents.

## Experimental Section

**Chemistry.** $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker 250 instrument using $CDCl_3$ or DMSO-$d_6$. The chemical shifts are reported in ppm ($\delta$ scale), and all *J* values are in Hz. The following abbreviations are used: singlet (s), doublet (d), doubled doublet (dd), triplet (t), multiplet (m), quaternary carbon (Cq). Melting point are uncorrected. IR absorptions were recorded on a Perkin-Elmer PARAGON 1000 PC and values were reported in $cm^{-1}$. MS spectra (ion spray) were recorded on a Perkin-Elmer Sciex PI 300. Monitoring of the reactions was performed using silica gel TLC plates (silica Merck 60 F254). Spots were visualized by UV light at 254 and 356 nm. Column chromatography were performed using silica gel 60 (0.063−0.200 mm, Merck).

**3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-bromo-1-methylpyrrole-2,5-dione (3).** A solution of 3-bromo-4-(1*H*-indol-3-

yl)-1-methyl-pyrrole-2,5-dione[41] (1.1 g, 3.62 mmol) in dry THF (35 mL) was added under argon at 0 °C to a suspension of NaH (217 mg, 5.42 mmol, 60% in oil) in dry THF (10 mL). After 40 min, benzenesulfonyl chloride (1.60 g, 9.04 mmol) was added dropwise. The solution was stirred at room temperature for 12 h, and a saturated solution of $NH_4Cl$ (50 mL) was added. After extraction with EtOAc (2 × 100 mL), the organic layers were washed with a saturated solution of NaCl (20 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Flash chromatography (petroleum ether/EtOAc 5/5) afforded compound **3** as an orange solid in 89% yield (1.443 g). Anal. ($C_{19}H_{13}BrN_2O_4S$): C, H, N.

**(3-Methoxy-naphthalen-2-yl)-trimethyl-stannane (4).** A solution of 2-methoxynaphthalene (**6**) (500 mg, 3.16 mmol) in dry THF (20 mL) was cooled to −10 °C. A solution of n-BuLi (3.65 mL, 584 mmol, 1.6 M in hexanes) was slowly added and the temperature allowed to reach 0 °C. After 2 h, a solution of trimethylstannyl chloride (943 mg, 4.74 mmol) in dry THF (10 mL) was added at −78 °C. The reaction mixture was stirred for 5 h at −78 °C, and water was added (20 mL) at room temperature. The organic layers were extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with water (5 × 20 mL), dried over $MgSO_4$, filtered and removed under reduced pressure to afford compound **4** as a white solid (963 mg, 95%). Anal. ($C_{14}H_{18}OSn$): C, H.

**(3-Methoxy-naphthalen-2-yl)-boronic Acid (5).** Same procedure as described for compound **4** starting from 2-methoxynaphthalene **6** (2 g, 12.65 mmol) and using trimethylborane instead of trimethylstannyl chloride. Trimethylborane (4.03 g, 39.21 mmol) was added at −10 °C, and the reaction was stirred at room temperature for 1 h. Hydrolysis was performed by addition of a hydrochloric solution (0.2 N, 50 mL). The solution was concentrated to 20 mL and extracted with $CHCl_3$ (5 × 20 mL), and the solvent was removed under reduced pressure. The residue was washed with petroleum ether (10 mL) and dried over $MgSO_4$ to afford compound **5** as a white solid (1.99 g, 78%).

**Standard Suzuki Procedure.** A solution containing the desired halide compound (**1**−**3**, **17**−**19**, 1 equiv), the appropriate boronic acid (**5**, **20**, 1.5 equiv), and $K_2CO_3$ (1.8 equiv) in dioxane/water (80/20, 0.8 M) was saturated by argon during 20 min. Palladium acetate (10%) was added, and the mixture was immediately poured in a preheated oil bath at 100 °C. After 2 h the reaction mixture was cooled to room temperature then filtered. The precipitate was washed with EtOAc. The combined organic layers were washed with water, and the solvents after drying over magnesium sulfate were removed under reduced pressure.

**3-(1*H*-Indol-3-yl)-4-(3-methoxy-naphthalen-2-yl)-1-methyl-pyrrole-2,5-dione (8).** Suzuki procedure from compound **1** and boronic acid **5**: compound **8** was purified by flash chromatography (petroleum ether/EtOAc 6:4) to afford an orange solid (55%). Anal. ($C_{24}H_{18}N_2O_3$): C, H, N.

**3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(3-methoxy-naphthalen-2-yl)-1-methyl-pyrrole-2,5-dione (10).** Suzuki procedure from compound **3** and boronic acid **5**: compound **10** was purified by flash chromatography (petroleum ether/EtOAc 8:2) to afford an orange solid (80%). Anal. ($C_{30}H_{22}N_2O_5S$): C, H, N.

**3-(3-Hydroxy-naphthalen-2-yl)-4-(1*H*-indol-3-yl)-1-methyl-pyrrole-2,5-dione (11).** A solution containing compound **8** (200 mg, 0.261 mmol) in distilled dichloromethane (5 mL) was cooled under argon to 0 °C. A solution of $BBr_3$ (1 M in dichloromethane, 0.5 mL, 0.5 mmol) was slowly added to the mixture. After 1 h, the reaction mixture was poured into ice and the aqueous layer was extracted with $CH_2Cl_2$ (2 × 20 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and filtered, and the solvents were removed under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/EtOAc 5/5) to afford compound **11** as an orange solid (207 mg, 72%). Anal. ($C_{23}H_{16}N_2O_3$): C, H, N.

**3-(1-Benzenesulfonyl-1*H*-indol-3-yl)-4-(3-hydroxy-naphthalen-2-yl)-1-methyl-pyrrole-2,5-dione (12).** Same procedure as described for compound **11** starting from compound **10** (200 mg, 0.393 mmol) and $BBr_3$ (2.5 M in dichloromethane, 1.00 mL, 2.5 mmol). The crude residue was purified by flash chromatography. Anal. ($C_{29}H_{20}N_2O_5S$): C, H, N.

**Trifluoromethanesulfonic Acid 3-[4-(1*H*-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-naphthalen-2-yl Ester (13).** A solution containing compound **11** (203 mg, 0.551 mmol) in dichloromethane (6 mL) and $NEt_3$ (0.231 mL, 1.67 mmol) was cooled to 0 °C. Triflic anhydride (0.31 mL, 1.84 mmol) was added, and the temperature was allowed to reach room temperature. After 5 h water (20 mL) was added, the aqueous layer was extracted with $CH_2Cl_2$ (2 × 20 mL), the combined organic layers were dried over $MgSO_4$ and filtered and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/ EtOAc 4/6, $NEt_3$ 10%) to afford compound **13** as a yellow solid (243 mg, 88%). Anal. ($C_{24}H_{15}F_3N_2O_5S$): C, H, N.

**Trifluoro-methanesulfonic Acid 3-[4-(1-Benzenesulfonyl-1*H*-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-naphthalen-2-yl Ester (14).** Same procedure as described for compound **13** starting from compound **12** (750 mg, 1.47 mmol), $NEt_3$ (0.725 mL, 5.29 mmol), and $Tf_2O$ (1 mL, 5.29 mmol), time reaction: 5 h. Compound **14** was obtained directly after extraction without further purification as a yellow solid (1.004 g, 98 Anal. ($C_{30}H_{19}F_3N_2O_7S_2$): C, H, N.

**Heck Cyclization Procedure.** A solution containing the desired triflate compound (**13**−**14**, **24**−**25**; 1 equiv), $Bu_4NCl$ (1 equiv), NaOAc (2 equiv), and $PPh_3$ (0.2 equiv) in dry dioxane (0.1 N) was saturated by argon during 20 min at room temperature. Palladium acetate was added, and the mixture was immediately immersed in a preheated oil bath at 100 °C. After the required time, the reaction mixture was cooled to room temperature and filtered and the precipitate was washed with $CH_2Cl_2$. The combined organic layers were washed with water and dried over $Na_2SO_4$, and the solvents were removed under reduced pressure

**6-Methylnaphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,-14*H*)-dione (15).** (i) Heck cyclization procedure from **13** using $Pd(OAc)_2$ 10%, time reaction: 12 h, flash chromatography (petroleum ether/EtOAc 2/6) afforded compound **15** as a red solid (40%). (ii) A solution containing compound 16 (400 mg, 0.82 mmol) and Bu4NF (1.63 mL, 1.63 mmol, 1 M in THF) was refluxed in dry THF (20 mL) for 2 h. After cooling, water was added and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over $MgSO_4$. The crude product was purified by flash chromatography (petroleum ether/EtOAc 2/6) to afford compound **15** (287 mg, quant.). Anal. ($C_{23}H_{14}N_2O_2$): C, H, N

**6-Methyl-14-benzenesulfonyl-naphtho[2,3-*a*]pyrrolo-[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (16).** Heck cyclization procedure from **14** using $Pd(OAc)_2$ 1.0 equiv, 3 h, flash chromatography (petroleum ether/EtOAc 7/3) afforded compound **16** as a red solid (91%). Anal. ($C_{29}H_{18}N_2O_4S$): C, H, N

**3-(1-Benzenesulfonyl-5-benzyloxy-1*H*-indol-3-yl)-4-bromo-1-methyl-pyrrole-2,5-dione (17).** Same procedure as described for compound **3**: 3-(5-Benzyloxy-1*H*-indol-3-yl)-4-bromo-1-methyl-pyrrole-2,5-dione[41] (1 g, 2.26 mmol), NaH (234 mg, 7.5 mmol, 60% in oil), benzenesulfonyl chloride (1.34 g, 2.44 mmol), time reaction 12 h, flash chromatography (petroleum ether/EtOAc 3:7). Compound **17** was obtained as an orange solid in 85% yield (1.143 g). Anal. ($C_{26}H_{19}BrN_2O_5S$): C, H, N.

**3-(1-Benzenesulfonyl-6-benzyloxy-1*H*-indol-3-yl)-4-bromo-1-methyl-pyrrole-2,5-dione (18)**. Same procedure as described for compound **3**: 3-(6-Benzyloxy-1*H*-indol-3-yl)-4-bromo-1-methyl-pyrrole-2,5-dione[41] (1 g, 2.26 mmol), NaH (234 mg, 7.5 mmol, 60% in oil), benzenesulfonyl chloride (1.34 g, 2.44 mmol), 4 h, flash chromatography (petroleum ether/EtOAc 3:7). Compound **18** was obtained as an orange solid in 92% yield (2.467 g). Anal. ($C_{26}H_{19}BrN_2O_5S$): C, H, N.

**(3-Hydroxy-naphthalen-2-yl)-boronic Acid (20).** A stirred solution of compound **5** (1.500 g, 7.42 mmol) in distilled dichloromethane (30 mL) was cooled at 0 °C. A solution of $BBr_3$ (2.11 mL, 22.27 mmol) was slowly added. After 2 h at room

temperature, the reaction mixture was poured into cold water (10 mL) and extracted with EtOAc (4 × 20 mL). The combined organic layers were evaporated under reduced pressure to afford compound **20** as a white solid (1.185 g, 85%).

**3-(1-Benzenesulfonyl-5-benzyloxy-1*H*-indol-3-yl)-4-(3-hydroxy-naphthalen-2-yl)-1-methyl-pyrrole-2,5-dione (21).** Suzuki procedure starting from compound **17** and boronic acid **20**: time reaction 2 h, flash chromatography (petroleum ether/EtOAc 7/3). Compound **21** was obtained as an orange solid in 82% yield. Anal. ($C_{36}H_{26}N_2O_6S$): C, H, N.

**3-(1-Benzenesulfonyl-6-benzyloxy-1*H*-indol-3-yl)-4-(3-hydroxy-naphthalen-2-yl)-1-methyl-pyrrole-2,5-dione (22).** Suzuki procedure starting from compound **18** and boronic acid **20**: time reaction 2 h, flash chromatography (petroleum ether/EtOAc 7/3). Compound **22** was obtained as an orange solid in 81% yield. Anal. ($C_{36}H_{26}N_2O_6S$): C, H, N.

**3-(1-Benzenesulfonyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)-4-(3-hydroxy-naphthalen-2-yl)-1-methyl-pyrrole-2,5-dione (23).** Suzuki procedure from 7-azaindole derivatives **19**[14] and boronic acid **20**: time reaction 2 h, flash chromatography (petroleum ether/EtOAc 7/3). Compound **23** was obtained as an yellow solid in 81% yield. $^1$H NMR ($CDCl_3$): 3.22 (s, 3H), 6.61 (m, 2H), 7.22−7.57 (m, 10H), 8.24 (m, 3H), 7.49 (s, 1H); $^{13}$C NMR ($CDCl_3$): 25.0 (CH3), 107.2 (Cq), 112.3 (CH), 117.9 (Cq), 118.5 (CH), 123.3 (CH), 125.1 (CH), 126.9 (Cq), 127.1 (CH), 127.3 (2CH), 128.1 (2CH), 129.1 (Cq), 129.5 (CH + Cq), 130.2 (Cq), 131.3 (CH), 131.9 (Cq), 133.4 (CH), 134.4 (Cq), 136.6 (Cq), 141.8 (CH), 144.3 (CH), 150.3 (CH + Cq), 168.9 (CO), 171.7 (CO); MS (IS) 510 $(M + 1)^+$. Anal. ($C_{28}H_{19}N_3O_5S$): C, H, N.

**Trifluoromethanesulfonic Acid 3-[4-(1-Benzenesulfonyl-5-benzyloxy-1*H*-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-naphthalen-2-yl Ester (24).** Same procedure as for compound **13**: Compound **21** (1.00 g, 1.62 mmol), $NEt_3$ (0.687 mL, 4.86 mmol), triflic anhydride (0.912 mL, 5.42 mmol), 3 h. A flash chromatography (petroleum ether/EtOAc 7/3 + $NEt_3$ 1%) afforded compound **24** as an orange solid (1.066 g, 88%). $^1$H NMR ($CDCl_3$,): 3.27 (s, 3H), 5.89 (s, 2H), 6.76−6.81 (m, 3H), 7.22−7.27 (m, 4H), 7.49−7.65 (m, 6H), 7.82−7.97 (m, 5H), 8.23 (s, 1H), 8.44 (s, 1H); $^{13}$C NMR ($CDCl_3$,): 24.6 (CH3), 69.1 (Cq), 103.4 (CH), 111.0 (Cq), 114.4 (CH), 115.6 (CH), 120.1 (Cq), 120.8 (CH), 122.3 (Cq), 127.0 (Cq + 3CH), 127.5 (Cq), 127.7 (2CH), 128.1 (2CH + Cq), 128.4 (2CH), 128.8 (CH), 129.2 (CH), 129.4 (2CH), 131.5 (CH), 131.8 (CH), 132.8 (CH), 133.2 (Cq), 133.4 (Cq), 134.1 (CH), 135.8 (Cq), 137.5 (Cq), 144.9 (Cq), 155.3 (Cq), 169.6 (CO), 169.9 (CO); MS (IS) 747 $(M + 1)^+$. Anal. ($C_{37}H_{25}F_3N_2O_8S_2$): C, H, N.

**Trifluoromethanesulfonic Acid 3-[4-(1-Benzenesulfonyl-6-benzyloxy-1*H*-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-naphthalen-2-yl Ester (25).** Same procedure as for compound **13**: Compound **22** (1.00 g, 1.62 mmol), $NEt_3$ (0.687 mL, 4.86 mmol), triflic anhydride (0.912 mL, 5.42 mmol), time reaction 3 h. A flash chromatography (petroleum ether/EtOAc 7/3 + $NEt_3$ 1%) afforded compound **25** as an orange solid (887 mg, 73%). Anal. ($C_{37}H_{25}F_3N_2O_8S_2$): C, H, N.

**Trifluoromethanesulfonic Acid 3-[4-(1-Benzenesulfonyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]-naphthalen-2-yl Ester (26).** Same procedure as described for compound **13**: Compound **23** (0.888 g, 1.75 mmol), $NEt_3$ (0.730 mL, 5.24 mmol), and triflic anhydride (0.881 mL, 5.24 mmol), 2 h. A flash chromatography (petroleum ether/EtOAc 6/4 + $NEt_3$ 1%) afforded compound **26** as a yellow solid (1.118 g, 97%). Anal. ($C_{29}H_{18}F_3N_3O7S2$): C, H, N.

**3-(Benzyloxy)-6-methylnaphtho[2,3-*a*]pyrrolo[3,4-*c*]-carbazole-5,7(6*H*,14*H*)-dione (27)**. Heck cyclization procedure from **24** using $Pd(OAc)_2$ (1 equiv), time reaction 4 h, followed by the $Bu_4NF$ deprotection, time reaction 1 h. Flash chromatography (petroleum ether/EtOAc 7/3) afforded compound **27** as a red solid (84%). Anal. ($C_{30}H_{20}N_2O_3$): C, H, N.

**2-(Benzyloxy)-6-methylnaphtho[2,3-*a*]pyrrolo[3,4-*c*]-carbazole-5,7(6*H*,14*H*)-dione (28).** Heck cyclization procedure from **25** using $Pd(OAc)_2$ (1 equiv), time reaction 6 h, followed by the $Bu_4NF$ deprotection, time reaction 1 h. Crude compound **28** was washed with EtOAc to afford **28** as a red solid without further purification (95%). Anal. ($C_{30}H_{20}N_2O_3$): C, H, N.

**3-(Hydroxy)-6-methylnaphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (29).** Same procedure as described for compound **11**: compound **27** (500 mg, 1.09 mmol), $BBr_3$ (1 M in dichloromethane, (2.34 mL, 2.34 mmol), time reaction 6 h. A flash chromatography (petroleum ether/EtOAc 8/2 then 5/5) afforded compound **29** as a red solid (371 mg, 93%). Anal. ($C_{23}H_{14}N_2O_3$): C, H, N.

**2-(Hydroxy)-6-methylnaphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (30).** Same procedure as described for compound **11**: compound **28** (200 mg, 0.439 mmol), $BBr_3$ (1 M in dichloromethane, 6.0 mL, 6 mmol), time reaction 2 h. A flash chromatography (petroleum ether/EtOAc 6/4 then EtOAc) afforded compound **30** as a red solid (157 mg, 98%). Anal. ($C_{23}H_{14}N_2O_3$): C, H, N.

**6-Methyl-14-[2-(4-Morpholinyl)ethyl]-naphtho[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (31)**. A solution of compound **15** (77 mg, 0.219 mmol) in freshly distillated DMF (2 mL) was stirred under argon at 0 °C and NaH (17 mg, 0.70 mmol, 60% in oil) was added. After 30 min, a suspension of *N*-(2-chloroethyl)-morpholine hydrochloride (158 mg, 1.0 mmol), NaH (41 mg, 1.71 mmol, 60% in oil) in dry DMF (5 mL) was added. The reaction mixture was warmed to 100 °C for 6 h. After cooling, cold water (30 mL) was added and the aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic layers were evaporated under reduced pressure, and the crude residue was purified by flash chromatography (EtOAc) to afford compound **31** as a red solid (50 mg, 49%). Anal. ($C_{29}H_{25}N_3O_3$): C, H, N.

**6-Methyl-14-[2-dimethylamino)-ethyl]-naphtho[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (32).** Same procedure as described for compound 31: compound **15** (100 mg, 0.285 mmol), NaH (17 mg, 0.7 mmol), and (2-chloroethyl)-dimethylamine hydrochloride (122 mg, 0.847 mmol) with NaH (41 mg, 1.61 mmol), 90 °C, time reaction, 12 h. After evaporation, the crude residue was dissolved in EtOAc (10 mL) and filtered off. The filtrate was concentrated under reduced pressure, and the organic material was purified by flash chromatography (EtOAc/MeOH 9/1) to afford compound **32** as a red solid (45 mg, 35%). Anal. ($C_{27}H_{23}N_3O_2$): C, H, N

**6-Methyl-14-allyl-naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (33).** A suspension containing compound **15** (100 mg, 0.285 mmol) and NaH (18 mg, 0.75 mmol) in freshly distilled DMF (2 mL) was stirred for 30 min at 0 °C. Allyl bromide (0.150 mL, 1.7 mmol) was added, and the reaction mixture was stirred at room temperature for 9 h. After evaporation, the crude residue was precipitated in MeOH (10 mL) and filtered off to afford compound **33** as an orange solid (57 mg, 51%). Anal. ($C_{26}H_{18}N_2O_2$): C, H, N

**6-Methyl-14-(2,3-dihydroxypropyl)-naphtho[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (34).** A solution containing $KMnO_4$ (34.5 mg, 0.218 mmol) dissolved in a mixture acetone/water (3/1, 20 mL) was slowly added to a solution of compound **33** (40 mg, 0.102 mmol) in acetone (4 mL) at 0 °C. The resulting mixture was stirred at room temperature for 6 h and filtered off, and the precipitate was washed with $CHCl_3$ (30 mL). The filtrate was extracted with $CHCl_3$ (20 mL), and the combined organic layers were removed under reduced pressure. The crude material was purified by flash chromatography (petroleum ether/EtOAc 1/9) to afford compound **34** as a red solid (31 mg, 71%). Anal. ($C_{26}H_{20}N_2O_4$): C, H, N.

**5H-Furo[3,4-*c*]naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(14H)-dione (35).** To a solution of compound **15** (100 mg, 0.285 mmol) in ethanol (40 mL) was added a solution of aqueous KOH (5 N, 10 mL) at room temperature. The reaction mixture was refluxed for 5 h. After coolingbeing cooled to room temperature, the solution was concentrated then neutralized by addition of a 10% HCl aqueous solution. After filtration, the precipitate was washed with water (10 mL) then with

EtOAc (2 × 10 mL), dried under vacuum to afford compound **35** as a red solid (80 mg, 83%). Anal. ($C_{22}H_{11}NO_3$): C, H, N.

**6-[2-(Dimethylamino)ethyl]-naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*,14*H*)-dione (36).** (i) A solution of compound **15** (50 mg, 0.148 mmol) was heated at 80 °C in the presence of a large excess of *N*1,*N*1-dimethyl-ethane-1,2-diamine (1 mL). After 8 h, water (50 mL) and EtOAc (50 mL) were added and the aqueous layers were extracted with EtOAc (2 × 30 mL). The solvents were removed under reduced pressure, and the crude residue was purified by flash chromatography (acetone then THF) to afford compound **36** as a red solid (50 mg, 83%). (ii) Same procedure starting from compound **35** (50 mg, 0.142 mmol) affording compound **36** (51 mg, 88%). Anal. ($C_{26}H_{21}N_3O_2$): C, H, N.

**3-(Hydroxy)-6-[2-(dimethylamino)ethyl]naphtho[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (37).** Same procedure as described for compound **36**: compound **29** (200 mg, 0.546 mmol), *N*1,*N*1-dimethyl-ethane-1,2-diamine (4 mL), time reaction 8 h, rflx. A flash chromatography (acetone) afforded compound **37** as a red solid (201 mg, 87%). Anal. ($C_{26}H_{21}N_3O_3$): C, H, N.

**2-(Hydroxy)-6-[2-(dimethylamino)ethyl]naphtho[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (38).** Same procedure as described for compound **36** : compound **30** (50 mg, 0.136 mmol), *N*1,*N*1-dimethyl-ethane-1,2-diamine (1 mL), 24 h, rflx. A flash chromatography (acetone) afforded compound **38** as a red solid (32 mg, 55%). Anal. ($C_{26}H_{21}N_3O_3$): C, H, N.

**3-(Hydroxy)-6-[3-(dimethylamino)propyl]naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (39).** Same procedure as described for compound **36**: compound **29** (100 mg, 0.273 mmol), *N*1,*N*1-dimethyl-propyl-1,3-diamine (2 mL), 5 h, rflx. A flash chromatography (acetone, $NEt_3$ 5%) afforded compound **39** as a red solid (201 mg, 87%). Anal. ($C_{27}H_{23}N_3O_3$): C, H, N.

**2-(Hydroxy)-6-[3-(dimethylamino)propyl]naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (40).** Same procedure as described for compound **36**: compound **30** (50 mg, 0.136 mmol), *N*1,*N*1-dimethyl-propyl-1,3-diamine (0.5 mL), time reaction 3 h, rflx. A flash chromatography (acetone, $NEt_3$ 5%) afforded compound **40** as a red solid (47 mg, 80%). Anal. ($C_{27}H_{23}N_3O_3$): C, H, N.

**3-(Hydroxy)-6-{2-[(2-hydroxyethyl)amino]ethyl}-naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (41).** Same procedure as described for compound **36**: compound **29** (85 mg, 0.232 mmol), 2-(2-aminoethylamino)-ethanol (2 mL), time reaction 3 h, 120 °C. After evaporation of the solvents, the residue was precipitared with MeOH (20 mL), filtered, washed three time with MeOH (10 mL), and dried under vacuum to afford compound **41** as a red solid (100 mg, quant.). Anal. ($C_{26}H_{21}N_3O_4$): C, H, N.

**2-(Hydroxy)-6-{2-[(2-hydroxyethyl)amino]ethyl}-naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (42).** Same procedure as described for compound 36: compound **30** (50 mg, 0.136 mmol), 2-(2-aminoethylamino)-ethanol (1 mL), time reaction 5 h, 120 °C. After evaporation of the solvents, the residue was washed with water (4 × 20 mL) then with EtOAc (4 × 20 mL), dried under vacuum to afford compound **42** as a red solid (16 mg, 27%). Anal. ($C_{26}H_{21}N_3O_4$): C, H, N.

**3-(Hydroxy)-6-[2-hydroxy-1-(hydroxymethyl)ethyl]-naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (43)**. Same procedure as described for compound **36**: compound **29** (50 mg, 0.136 mmol), 2-amino-1,3-propandiol (0.2 mL), time reaction 3 h, 120 °C. A flash chromatography (EtOAc) afforded compound **43** as a red solid (38 mg, 65%). Anal. ($C_{25}H_{18}N2O_5$): C, H, N.

**2-(Hydroxy)-6-[2-hydroxy-1-(hydroxymethyl)ethyl]-naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (44)**. Same procedure as described for compound **36**: compound **30** (50 mg, 0.136 mmol), 2-amino-1,3-propandiol (0.5 mL), time reaction 3 h, 120 °C. A flash chromatography (petroleum ether/EtOAc 2/8) afforded compound **44** as a red solid (18 mg, 31%). Anal. ($C_{25}H_{18}N_2O_5$): C, H, N.

**2-(Benzyloxy)-6-methyl-14-[2-(dimethylamino)ethyl]-naphtho[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (45).** Same procedure as described for compound **31**: compound **28** (100 mg, 0.219 mmol), NaH (17 mg, 0.7 mmol), (2-chloroethyl)-dimethylamine hydrochloride (122 mg, 0.847 mmol) with NaH (41 mg, 1.61 mmol), time reaction 12 h, 90 °C. After evaporation, the crude residue was dissolved in EtOAc (10 mL) and filtered off. The filtrate was concentrated under reduced pressure, and the organic material was purified by flash chromatography (EtOAc) to afford compound **45** as a red solid (98 mg, 85%). Anal. ($C_{34}H_{29}N_3O_3$): C, H, N.

**2-{[(*tert*-Butyldimethyl)silyl]oxy}-6-methylnaphtho-[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (46).** A solution containing imidazole (330 mg, 4.97 mmol) and TB-DMSCl (620 mg, 4.13 mmol) dissolved in DMF (15 mL) was stirred under argon at 0 °C. After 30 min, cesium carbonate (267 mg, 0.82 mmol) and compound **30** (150 mg, 0.41 mmol) were added. After stirring for 4 h at room temperature, water (20 mL) was added and extractions were performed with EtOAc (3 × 20 mL). The combined organic layers were removed under reduced pressure, and the crude residue was purified by flash chromatography (petroleum ether/EtOAc 7/3, $NEt_3$ 1%) to afford compound **46** as an orange solid (196 mg, quant.). Anal. ($C_{29}H_{28}N_2O_3Si$): C, H, N.

**2-[2-(Dimethylamino)ethyl]-6-methylnaphtho[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*,14*H*)-dione (47).** (i) Same procedure as described for compound **31**: compound **46** (50 mg, 0.10 mmol), NaH (7 mg, 0.29 mmol, 60% in oil), DMF, (2-chloroethyl)-dimethylamine hydrochloride (77 mg, 0.534 mmol), de NaH (35 mg 1.45 mmol, 60% in oil), time reaction 3 h, 100 °C. After treatment, the product was dissolved in EtOAc, and petroleum ether was added until a precipitate appeared. The suspension was filtered, the precipitate was dried under vacuum to afford compound **47** as a red solid (31 mg, 69%). (ii) Idem. starting from compound **30** (130 mg, 0.355 mmol), (2-chloroethyl)-dimethylamine hydrochloride (111 mg, 0.77 mmol), NaH (90 mg, 3.75 mmol, 60% in oil) in one portion, DMF, 100 °C, 3 h. Precipitation in MeOH afforded compound **47** (65 mg, 41%). Anal. ($C_{27}H_{23}N_3O_3$): C, H, N

**Growth Inhibition Assays.** Tumor cells were provided by American Type Culture Collection (Frederick, MD). Three cell lines were used: one murine leukemia (L1210), one human prostate cancer (DU145), and one colon carcinomia (HT29) were cultivated in RPMI 1640 medium (Life cience Technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 units/mL penicillin, 100 $\mu$g/mL streptomycin and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay described.48 Cells were continuously exposed to graded concentrations of the compounds for four doubling times, 15 $\mu$L of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well, and the plates were incubated for 4 h at 37 °C. The medium was then aspirated and the formazan solubilized by 100 $\mu$L of DMSO. Results are expressed as $IC_{50}$, the concentration which was reduced by 50% the optical density of treated cells with respect to untreated controls. $IC_{50}$ were determined by nine serial dilutions performed in duplicate.

**Cell Cycle Analysis.** For the cell cycle analysis, L1210 cells ($2.5 \times 10^5$ cells/mL) were incubated for 21 h with various concentrations of the compounds and fixed by 70% ethanol (v/v), washed, and incubated in PBS containing 100 $\mu$g/mL RNAase and 25 $\mu$g/mL propidium iodide for 30 min at 20 °C for each sample, 104 cells were analyzed on a XL/MCL flow cytometer (Beckman Coulter). The fluorescence of propidium iodide was collected through a 615 nm long-pass filter. These experiments were performed at drug concentrations between $IC_{50}$ and $IC_{90}$ values. Dose-dependent effects were observed in all cases.

**DNA Relaxation Experiments.** Supercoiled pKMp27 DNA (0.5 $\mu$g) was incubated with 4 units human topoisomerase I or II (TopoGen Inc.) at 37 °C for 1 h in relaxation buffer (50 mM Tris pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA) in the presence of varying concentra-

tions of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 mg/mL. DNA samples were then added to the electrophoresis dye mixture (3 mL) and electrophoresed in a 1% agarose gel containing ethidium bromide (1 mg/mL), at room temperature for 2 h at 120 V. Gels were washed and photographed under UV light.[49]

**DNA Binding Measurements.** Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of measurements, 12 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm path length) were heated by circulating water. The $T_m$ measurements were performed in BPE buffer pH 7.1 (6 mM $Na_2HPO_4$, 2 mM $NaH_2PO_4$, 1 mM EDTA). The temperature inside the cuvette was measured with a platinum probe; it 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.

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**Supporting Information Available:** $^1$H NMR, $^{13}$C NMR, IR, and MS data and results of elemental analysis for compounds **3**−**5**, **8**, **10**−**18**, **20**−**47** are available free of charge via the Internet at http://pubs.acs.org.

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