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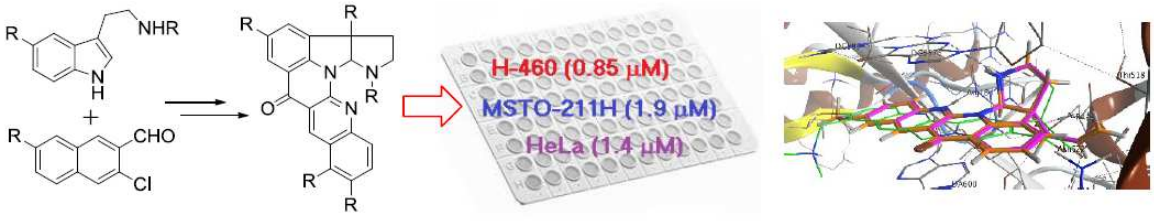

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A new scaffold of Topoisomerase I Inhibitors: Design, Synthesis and Biological Evaluation

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ABSTRACT

The synthesis of a new hexacyclic system was realized starting from tryptamines and exploiting as a key step a sequential Pd-catalyzed *N*-arylation/acylation reaction. Having topoisomerases as biological target and the camphotecins class as benchmark, the new scaffold was decorated with substituents having different polarity and tested as Topoisomerase I inhibitors.

1. Introduction

The topoisomerases are ubiquitous enzymes essential for the vital cellular processes as involved in different steps of DNA replication, transcription, recombination and several reviews describe their characteristics and their activity on DNA in cells[1,2,3,4]. In particular topoisomerase I and topoisomerase II play a key role binding to the DNA double helix inducing temporary single (Topo I) or double strand break (Topo II) allowing relaxation of the DNA for replication. Then the inhibition of these enzymes is lethal and leads to cell death, thus establishing Topo I and II as promising targets for cancer treatment and as a goal for the development of a new class of anticancer drugs.

Recently we realized a new series of bis-heteroarylmaleimides and bis-heterofused imides endowed with antiproliferative effects on human tumor cells (NCI-H460 lung carcinoma) and rat aortic smooth muscle cells (SMC's), due to the ability to stabilize the DNA-intercalator-topoisomerase II ternary complex[5,6].

While many cytotoxic drugs used in the clinical practice act on Topo II, only camptothecins have been developed as inhibitors of Topo I. Camptothecin (CPT) (Fig. 1), a natural alkaloid first isolated from extracts of Camptotheca acuminata, selectively poisons Topo I and exhibits strong antineoplastic activity against colorectal, breast, lung and ovarian cancers[7]. Topotecan (TPT) and Irinotecan (Fig. 1), are the most potent synthetic derivatives of CPT in clinical use. However, several limitations of CPT and its analogs such as solubility, toxicity, resistance and above

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all the instability under physiological conditions have encouraged the development of new CPT analogues and non-CPT topo I inhibitors. The interest in topoisomerase I as a therapeutic target promoted various efforts to identify other chemotypes effective as topoisomerase inhibitors and chemical/modelling efforts to rationally design specific analogs among known inhibitors[8,9,10,11]. During the last years several tetra- and pentacyclic structures containing the indoline fragment has received much attention due to the structural correlation with natural compounds belonging to the alkaloids class endowed with biological activity as cyclooxygenase/5-lipooxygenase inhibitors, characterized by the presence of the pyrrolo[3,2-*de*]acridine subunit[12], indolocarbazoles non-CPT topo I inhibitors[13,14,15,16] and analogues of physostigmine alkaloids[17,18,19,20].

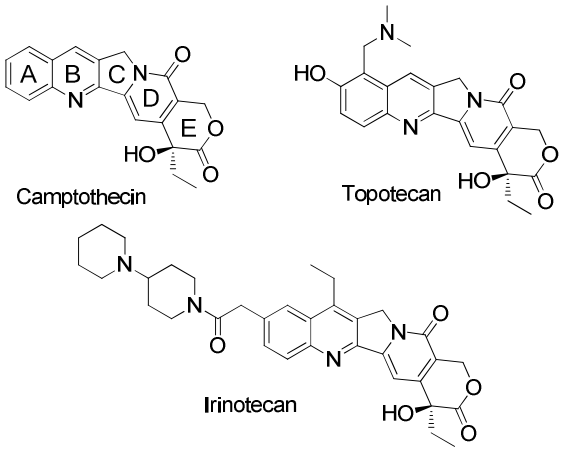
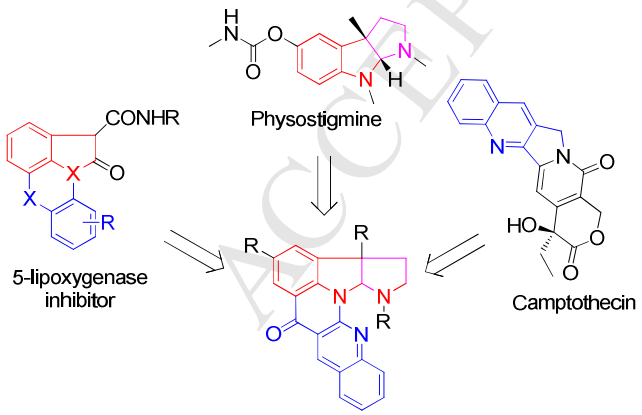


Fig. 1. Topo I inhibitor compounds

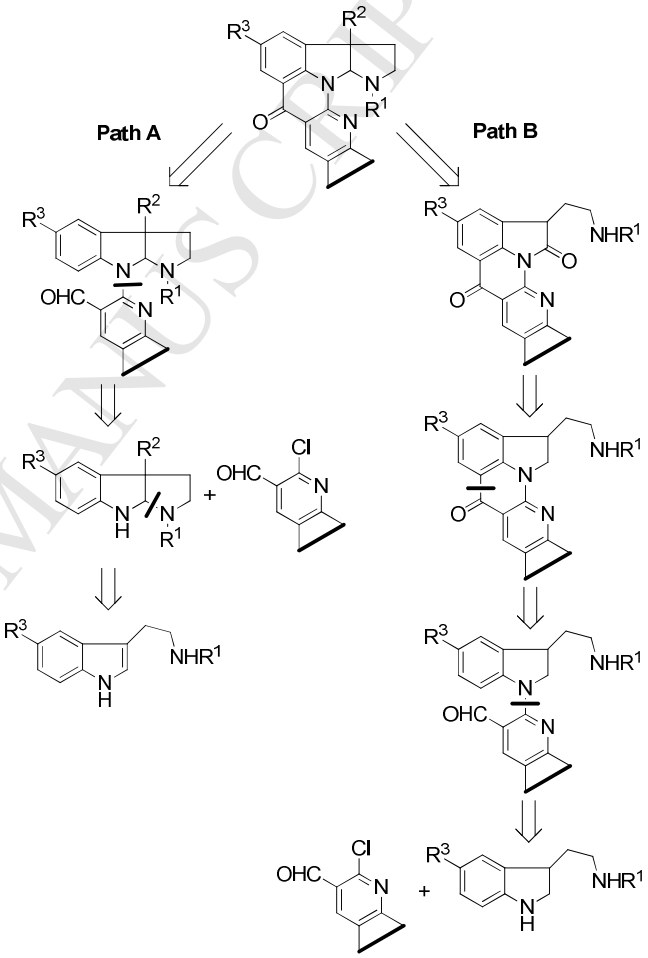
Having topoisomerase I as biological target, our efforts were addressed to realize new heteropolycyclic structure based on scaffold hopping combining some of the structural features of 5-lipooxygenase inhibitors, physostigmine derivatives and CPT derivatives. (Scheme 1)



Scheme 1. Scaffold design

2. Result and discussion

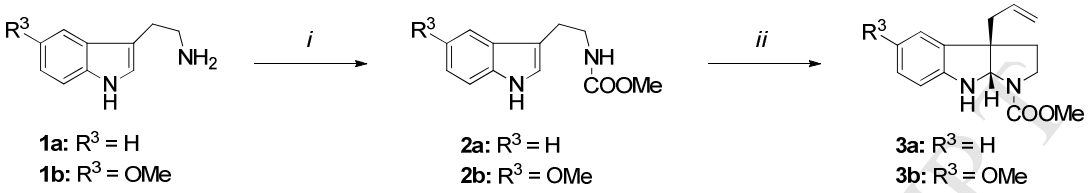
Two possible retrosynthetic pathways were initially identified to obtain the scaffold: path A, starting from triptamine and path B starting from 3-substituted indoline, in both cases followed as a key steps by a sequence of *N*-arylation/aromatic acylation. The minor steps prompted us to follow path A. (Scheme 2, path A).



Scheme 2. Selected strategies for the synthesis of the scaffold

Starting from substituted tryptamines **1**, the first step provided the formation of the tricyclic tetrahydropyrrolo[2,3-*b*]indoles **3** through the intramolecular reaction of the carbamates **2** under the catalysis of Pd-complex/Lewis acid ($Pd(PPh_3)_4/Et_3B$) in THF as solvent at r.t.. The alkylative amination step was performed in the presence of allyl alcohol as electrophile resulting the contemporary insertion of the allyl substituent in position 3a[21]. Both Pd-complex and Et_3B Lewis acid were necessary to obtain allylation. The intramolecular amination was stereoselective giving only the *cis* isomer in the junction of the B and C rings, as stated by ^1H NMR NOESY experiments (see supp. Inf.) (Scheme 3)[22]. The tricyclic systems **3** were in all the cases obtained as enantiomers mixture. Attempts

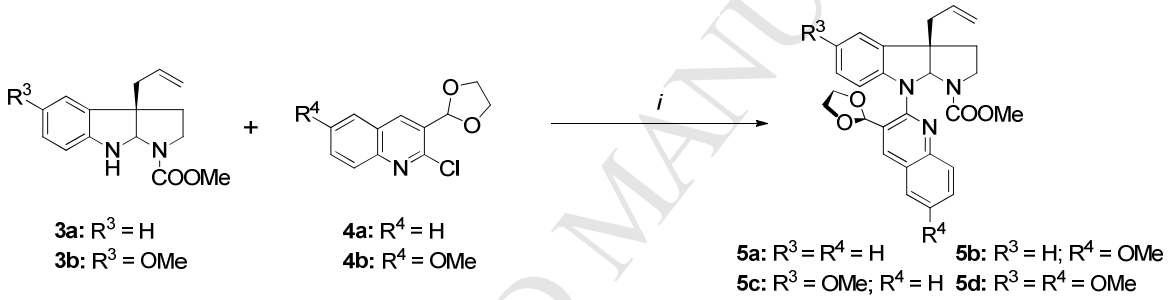
to separate the isomers as diastereoisomers by using chiral carbamate derivatives of the triptamine ((*S*)-(-)-2-methylbutyl carbamate, (1*S*)-(+)-menthyl carbamate, (1*R*)-(-)-myrtenol carbamate) gave unsatisfactory results or failed in the cyclization step.



Scheme 3. Reagents and conditions: (i) $ClCO_2Me$ (1.2 eq), Et_3N (1.2 eq), MeOH, 0 °C, 10 min, rt, 24 h, 90-95 %; (ii) $Pd(PPh_3)_4$ (0.5 mol%), allyl alcohol (1.2 eq), Et_3B (1.2 eq), THF, 0 °C, 30 min, rt, 24 h, 77-91%.

The following key step consisted in the Pd-catalyzed *N*-arylation of the derivatives **3** with 2-chloroquinoline-3-carbaldehydes **4**, affording the products **5**. (Scheme 4) The reaction conditions were optimized as showed in Table 1, the best conditions used the $Pd_2(dba)_3$ as catalyst and triisobutylphosphatrane (IAPU) as ligand, in toluene at 110 °C, in the presence of *t*-BuONa as base. The reaction required the protection of the formyl substituent as acetal. (Table 1, entry 8)

No result was obtained attempting the SNAr reaction.

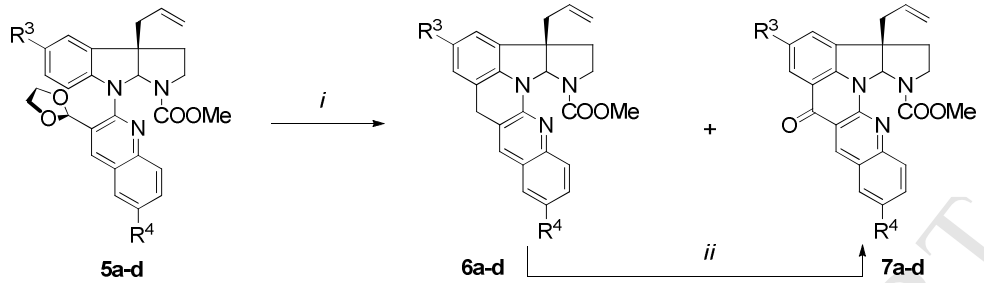


Scheme 4. Reagents and conditions: (i) $Pd_2(dba)_3$ (2 mol%), triisobutylphosphatrane (8 mol%), *t*-BuONa (1.4 eq), toluene, 110 °C, 5h, 87-92%.

Table 1 – Optimization of Pd-catalyzed *N*-arylation reaction

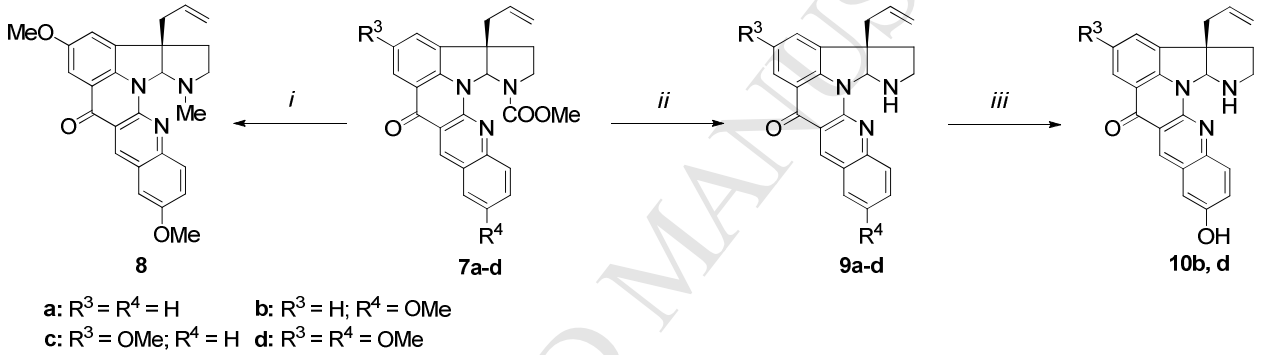
Entry	Catalyst	Ligand	Base	T °C	Time (h)	Yield (%)
1	$Pd(OAc)_2$ (2 mol%)	BINAP (2 mol%)	Cs_2CO_3 (5eq)	110	24	10
2	$Pd(OAc)_2$ (2 mol%)	DPPF (2 mol%)	*t*-BuOK (1.4 eq)	110	24	5
3	$Pd(OAc)_2$ (2 mol%)	triisobutylphosphatrane (4 mol%)	Cs_2CO_3 (5eq)	110	24	25
4	$Pd_2(dba)_3$ (5 mol%)	BINAP (10 mol%)	*t*-BuONa (1.4 eq)	110	24	60
5	$Pd_2(dba)_3$ (5 mol%)	BINAP (10 mol%)	Cs_2CO_3 (2eq)	100	24	70
6	$Pd_2(dba)_3$ (1 mol%)	triisobutylphosphatrane (4 mol%)	*t*-BuONa (1.4 eq)	100	24	75
7	$Pd_2(dba)_3$ (0.5 mol%)	triisobutylphosphatrane (2 mol%)	K_2CO_3 (5 eq)	100	24	75
8	$Pd_2(dba)_3$ (2 mol%)	triisobutylphosphatrane (8 mol%)	*t*-BuONa (1.4 eq)	110	3	85

The subsequent step was the intramolecular Friedel-Craft reaction of the intermediate **5** using the protected formyl group directly[23]. The polyheterocyclic alkylated derivative was obtained as a mixture of two products **6** and **7,** one of which was the oxidized form of the alkylated product **6**. (Scheme 5) The treatment of the mixture with oxidants MnO_2 and mCPBA resulted in the complete transformation of compound **6** in **7**.



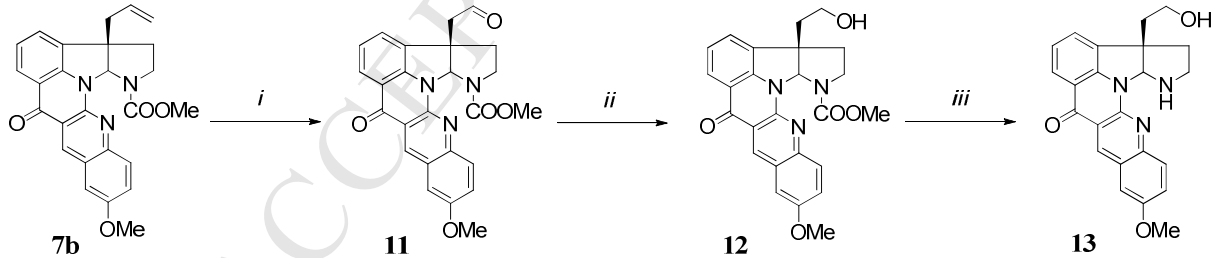
Scheme 5. Reagents and conditions: (i) BF_3-Et_2O (3-5 eq), CH_2Cl_2, 0 °C, 10 min, rt, 48h; (ii) MnO_2 (4 eq), m-CPBA (5 mol%), THF/$H_2$0 (4:1), 60 °C, 12h, 50-80%.

The insertion of different substituents on the polycyclic scaffold **7** has been realized with the aim to evaluate changes in the biological activity, in fact the presence of polar groups may have strong interaction with the enzyme. The functionalization in position 1 may be obtained from **7** by reduction of the carbomethoxy group to give compound **8** or by hydrolysis obtaining derivatives **9a-d**. (Scheme 6)



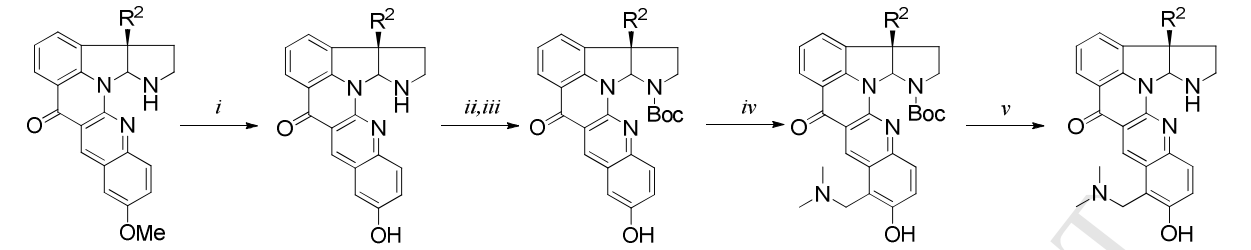
Scheme 6. Reagents and conditions: (i) Red-Al® (10 eq), toluene, 0 °C, 10 min, 110 °C, 5h, 50%; (ii) NaOH (15 eq), EtOH/H_2O (3:1), 80 °C, 5-8h, 65-85%; (iii) BBr_3 (2.5 eq), CH_2Cl_2, 40 °C, 24-48h, 70-80%.

The methoxy substituents may be present in positions 5 and 10, the reaction with BBr_3 of the intermediates **9** resulted in the formation of the phenolic derivatives **10**. The 2-hydroxyethyl substituent selected as polar branch, was formed in position 3a from the allyl susbstituent of the compound **7b**, through the sequence oxidation-reduction affording the compounds **12** and **13**. (Scheme 7)



Scheme 7. Reagents and conditions: (i) OsO_4 (4 mol%), $NaIO_4$ (3 eq), lutidine (2 eq), THF/H_2O (5:1), rt, 24h, 75%; (ii) $NaBH_4$ (1.2 eq), MeOH, rt, 3h, 65%; (iii) NaOH (15 eq), EtOH/H_2O (3:1), 80 °C, 6h, 61%.

The projected decoration of the scaffold involved the insertion of a dimethylaminomethyl group, present also on the TPT, synthetic derivative of CPT. The derivative was obtained through a Mannich reaction effective only on the phenolic derivatives **15a, b** obtained by hydrolysis of the methylethers **9b** and **13** and after protection of the nitrogen atom as Boc derivative. (Scheme 8)



Scheme 8. Reagents and conditions: (i) BBr_3 (2.5 eq), CH_2Cl_2, 40 °C, 12-48h, 50-70%.; (ii) Boc_2O (3 eq), Et_3N (2-4 eq), EtOH, rt, 48h; (iii) Piperidine (3 eq), EtOH, 80 °C, 1-2h, 60-80 %; (iv) CH_2O (4 eq), Me_2NH (3 eq), EtOH, rt, 24-48h, 56-60%; (v) TMSCl (5 eq), MeOH, rt, 6h, 78%.

The Figure 2 and Table 2 showed as convenience the sum of the different substituents on compounds **7-17** evaluated for their antiproliferative activity.

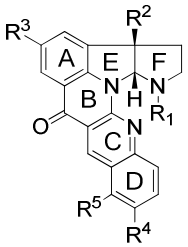


Fig. 2. Different substituents on the hexacyclic scaffold

Table 2 – Different substituents on the scaffold

Compd.	R^1	R^2	R^3	R^4	R^5
7b	CO_2Me	Allyl	H	OMe	H
8	Me	Allyl	OMe	OMe	H
9a	H	Allyl	H	H	H
9b	H	Allyl	H	OMe	H
9c	H	Allyl	OMe	H	H
9d	H	Allyl	OMe	OMe	H
10b	H	Allyl	H	OH	H
10d	H	Allyl	OH	OH	H
13	H	$(CH_2)_2OH$	H	OMe	H
15a	Boc	Allyl	H	OH	H
15b	Boc	$(CH_2)_2OH$	H	OH	H
16a	Boc	Allyl	H	OH	CH_2NMe_2
16b	Boc	$(CH_2)_2OH$	H	OH	CH_2NMe_2
17	H	Allyl	H	OH	CH_2NMe_2

3. Biological evaluation

3.1. Antiproliferative activity. The ability of new derivatives to inhibit cell growth was investigated by an *in vitro* assay on three human tumor cell lines, H460 (large cell lung carcinoma), MSTO-211H (human biphasic mesothelioma) and HeLa (cervix adenocarcinoma). The results, expressed as GI_{50} values, i.e. the concentration (μM) of compound that induces 50% cell death with respect to the control culture, are shown in Table 3. The CPT was used as reference compound.

The results indicate for all test derivatives a detectable antiproliferative activity, with GI_{50} values in the micromolar range. Inside the new class of hexacyclic compounds, the most active is **13**, characterized by the 2-hydroxyethyl substituent in R^2 and a methoxy group in R^4, which shows GI_{50} values in the low micromolar range in all cell lines taken into consideration. For **9b** and **10d**, GI_{50} values lower that 10 μM are obtained in two cell lines (H460 and MSTO-211H), while for all other compounds the cytotoxicity is lower and indeed GI_{50} values ranging from 13.0 to 35.2 μM can be observed.

On the basis of these data, some preliminary structure-activity relationships could be drawn. In particular, the presence of the 2-hydroxyethyl chain in **13** seems to be determinant for the biological activity. Indeed, its replacement with the allyl substituent (**9b**) induces a significant decrease in cytotoxicity, especially in H460 and HeLa cells where an increase of about of one order of magnitude can be observed. This drop in antiproliferative ability is confirmed for all compounds carrying the allyl moiety in R^2 (**8**, **9a-d**, **10b, 10d**, **15a, 16a** and **17**) apart from the different groups inserted in the others position of the hexacyclic scaffold. Nevertheless, the effectiveness of the 2-hydroxyethyl is considerably dampened by the insertion of BOC in R^1 and hydroxyl in R^4 and/or a dimethylaminomethyl side chain in R^5 as suggested by the comparison between **13** and **15b** or **16b**. It is noteworthy that the presence of BOC appears detrimental for the occurrence of the cytotoxic capacity in all compounds (**15a**, **15b, 16a** and **16b**) and indeed they show high GI_{50} values.

Table 3 – Cell growth inhibition in the presence of test derivatives and CPT as reference compound.

Compd.	GI_{50}[a] (μM)		
	H-460	MSTO-211H	HeLa
8	› 50	34.2 ± 7.9	18.3 ± 1.2
9a	14.4 ± 0.9	13.0 ± 2.1	16.5 ± 1.0
9b	8.9 ± 1.4	7.8 ± 1.0	14.8 ± 1.5
9c	32.2 ± 4.7	14.4 ± 3.3	30.7 ± 4.8
9d	18.6 ± 0.3	13.5 ± 2.6	16.6 ± 3.8
10b	› 50	18.7 ± 0.6	19.0 ± 0.5
10d	4.8 ± 1.0	7.0 ± 0.9	15.3 ± 0.8
13	0.85 ± 0.09	1.9 ± 0.3	1.4 ± 0.3
15a	32.7 ± 0.8	24.1 ± 1.6	30.5 ± 1.8
15b	26.8 ± 2.0	13.7 ± 0.9	13.0 ± 2.2
16a	17.8 ± 1.7	22.8 ± 2.4	35.2 ± 1.6
16b	16.4 ± 1.4	20.0 ± 1.6	13.0 ± 1.0
17	26.2 ± 1.2	29.1 ± 1.9	29.3 ± 5.6
CPT	0.0020 ± 0.0002	0.0021 ± 0.0001	0.0054 ± 0.0002

[a] Mean values ±SD of at least three independent experiments are reported.

3.2. Interaction with DNA. The interesting antiproliferative effect exerted by the most biologically active compound (**13**) and in particular, the presence of a wide planar heteropolycyclic scaffold, suggested an investigation on the ability to form a molecular complex with DNA through an intercalative mode of binding, in accordance with previous studies[24]. For this purpose flow linear dichroism (LD) experiments were performed with DNA solutions in the absence and in the presence of **13** and the corresponding allyl derivative **9b**. The obtained LD spectra are shown in Figure 3, the UV-vis absorbance spectra of the test compounds (**A**) are also reported as reference.

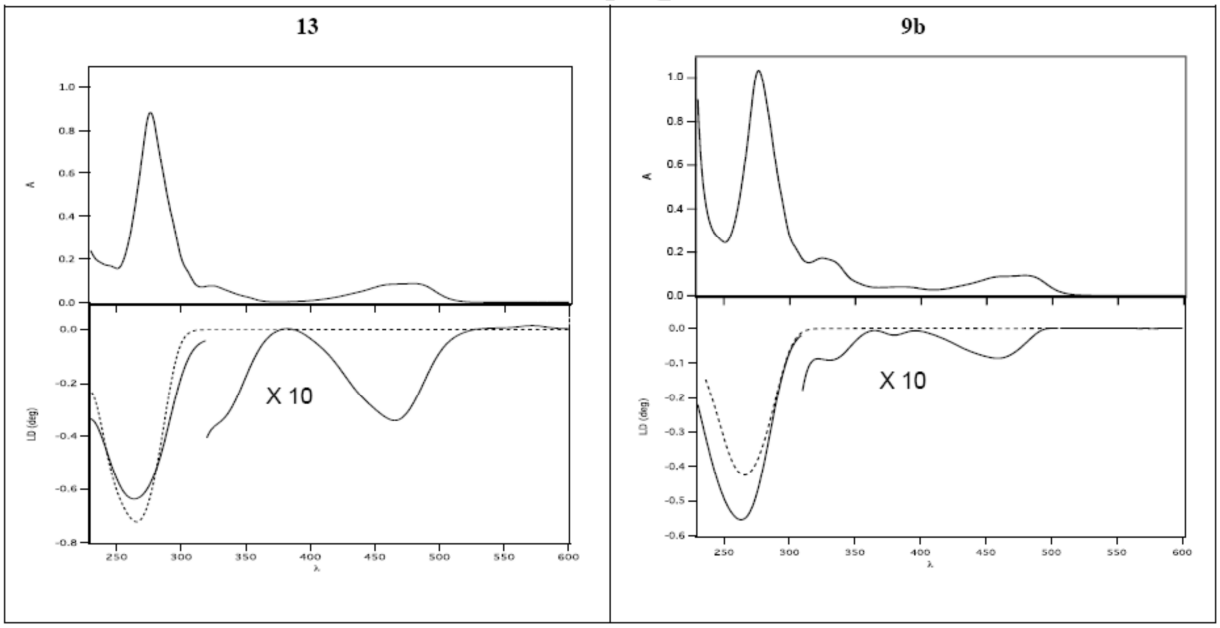


Fig. 3. Absorbance spectra (A) for compounds 13 and 9b at 1.2 x 10^{-5}M. Linear flow dichroism spectra (LD) for compounds 13 and 9b at different [compound]/[DNA] ratios: dotted line=0; continuous line=0.08. [DNA] = 1.9 x 10^{-3}M.

Both LD spectra show an evident negative signal at 260 nm, typical of the macromolecule and due to the strong absorption of DNA base pairs at this wavelength. Moreover, interestingly, in the presence of the considered derivative (continuous lines) a further dichroic signal appears at higher wavelengths (380-520 nm). Because no contribution from the macromolecule exists in this latter spectral region, the occurrence of the signal has to be attributed to the added chromophore, which, otherwise, absorbs at these wavelengths (see absorption spectra, A). Since small molecules, such as **13** and **9b**, cannot become oriented in the flow field, the occurrence of the LD signal has to be attributed to the formation of a

molecular complex with DNA that permit them the orientation. Moreover, the negative sign of the LD signal, as the strong band at 260 nm, indicate a parallel orientation of the planar hexacyclic system of new derivatives with respect to the plane of the purine and pyrimidine base pairs. This means that **13** and **9b** form a complex with DNA via an intercalative mode of binding.

3.3. Effect on topoisomerase. DNA topoisomerase I has been shown to be the target of many anticancer agents, both camptothecin (topotecan and irinotecan) and non-camptothecin (indolocarbazoles and phenanthroline) derivatives[2,3]. From a mechanistic point of view, agents that inhibit topoisomerase I can be grouped into two classes: poisons and suppressors. Both inhibit the catalytic activity of the enzyme (supercoiled DNA relaxation), nevertheless poisons, like CPT, stabilize a covalent intermediate complex, called cleavable complex, thus producing single-stranded DNA breakages. Otherwise, suppressors interfere with other steps of the catalytic cycle without stabilize the cleavable complex, for example through a direct interaction with the enzyme or the formation of a molecular complex with DNA[25,26].

In this connection and based on the above results, we investigate the ability of the most active derivative **13** and of the structurally related **9b**, to affect the catalytic activity of topoisomerase I. Figure 4 shows the effect of the test compounds on the relaxation of supercoiled DNA mediated by topoisomerase I. The enzyme removes supercoils from pBR322 plasmid DNA (lane DNA) giving rise to a population of relaxed DNA topoisomers that migrates differently depending on their linking number (lane topo I). The results shown in Figure 4 indicate that both **13** and **9b** affects the relaxation activity of the enzyme and indeed they induce both a decrease in the number of topoisomers and an increase in the intensity of the band corresponding to the relaxed plus nicked DNA. This behavior, similar to that observed for CPT, a well-known topoisomerase I poison, demonstrate the capacity of test compounds to interfere with the catalytic activity, but does not allow to establish if it is due to a poisoning effect.

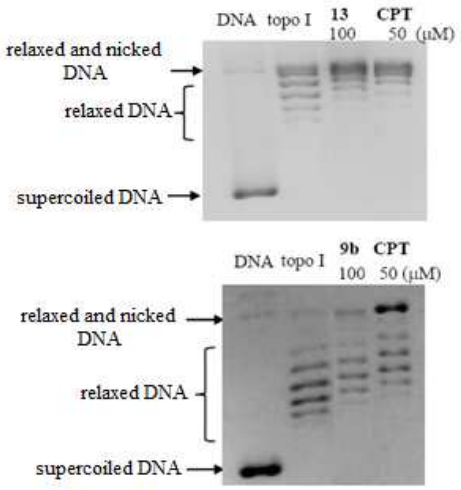


Fig. 4. Effect of compounds 13 and 9b on relaxation of supercoiled pBR322 DNA mediated by topoisomerase I. Supercoiled DNA (lane DNA) was incubated with topoisomerase I in the absence (lane topo I) and in the presence of test compounds at indicated concentration. CPT was used as reference.

Therefore, to discriminate between a specific poisoning action and other possible nonspecific effects, due for example to DNA intercalation, the experiments were performed with agarose gel containing ethidium bromide and the results are showed in Figure 5, in comparison with CPT. Indeed, in these latter experimental conditions the DNA species moving toward the anode become progressively saturated by the intercalative effect of ethidium and this influences significantly their rate of migration. Otherwise, the electrophoretic mobility of the band corresponding to the nicked DNA, resulting from the stabilization of the cleavable complex, is unaffected by the presence of ethidium bromide and thus can be easily detected[27].

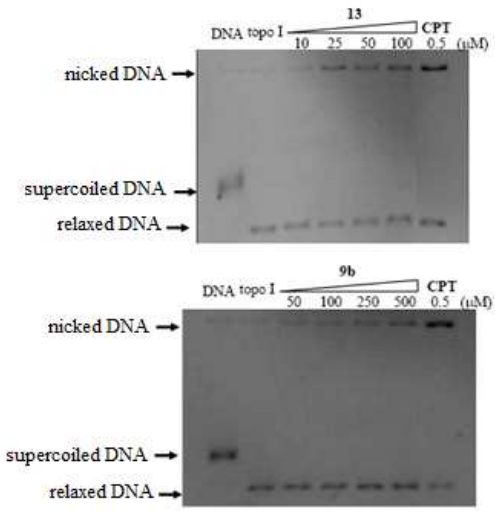


Fig. 5. Effect of compounds 13 and 9b on the stabilization of covalent-DNA-topoisomerase I complex. Supercoiled DNA (lane DNA) was incubated with topoisomerase I in the absence (lane topo I), in the presence of test compounds at indicated concentration and in the presence of ethidium bromide in the gel and buffer. CPT was used as reference

The results obtained by incubating DNA and enzyme in the presence of increasing concentrations (from 10 to 100 μM) of **13** indicate for this compound the ability to induce the formation of the cleavable complex as from 25 μM. For the analogue **9b** a lower poisoning effect emerges from the experiments reported in Figure 5. Indeed, for this latter derivative, a concentration of 500 μM has to be used to detect a significant increase in the band corresponding to the nicked DNA. For the well-known poison CPT, as expected, a notable amount of nicked DNA is formed already at

0.5 µM concentration. It is interestingly to note that these results are in agreement with cytotoxicity data reported in Table 3. Indeed, **13** demonstrates an antiproliferative effect clearly higher with respect to **9b**, with GI_{50} values from 4 to 11 times lower, depending on the cell line taken into consideration. As regard CPT, its cytotoxicity is notably higher with respect to both new derivatives and indeed GI_{50} even in the nanomolar range are obtained (Table 3). Thus, it can be concluded that a correlation can be drawn between the poisoning effect and the antiproliferative ability.

4. Analysis of the binding mode

The binding mode of the most interesting compounds **13** and **9b** was analyzed by docking calculations and compared to that of the reference crystallographic compound topotecan (Fig. 6).

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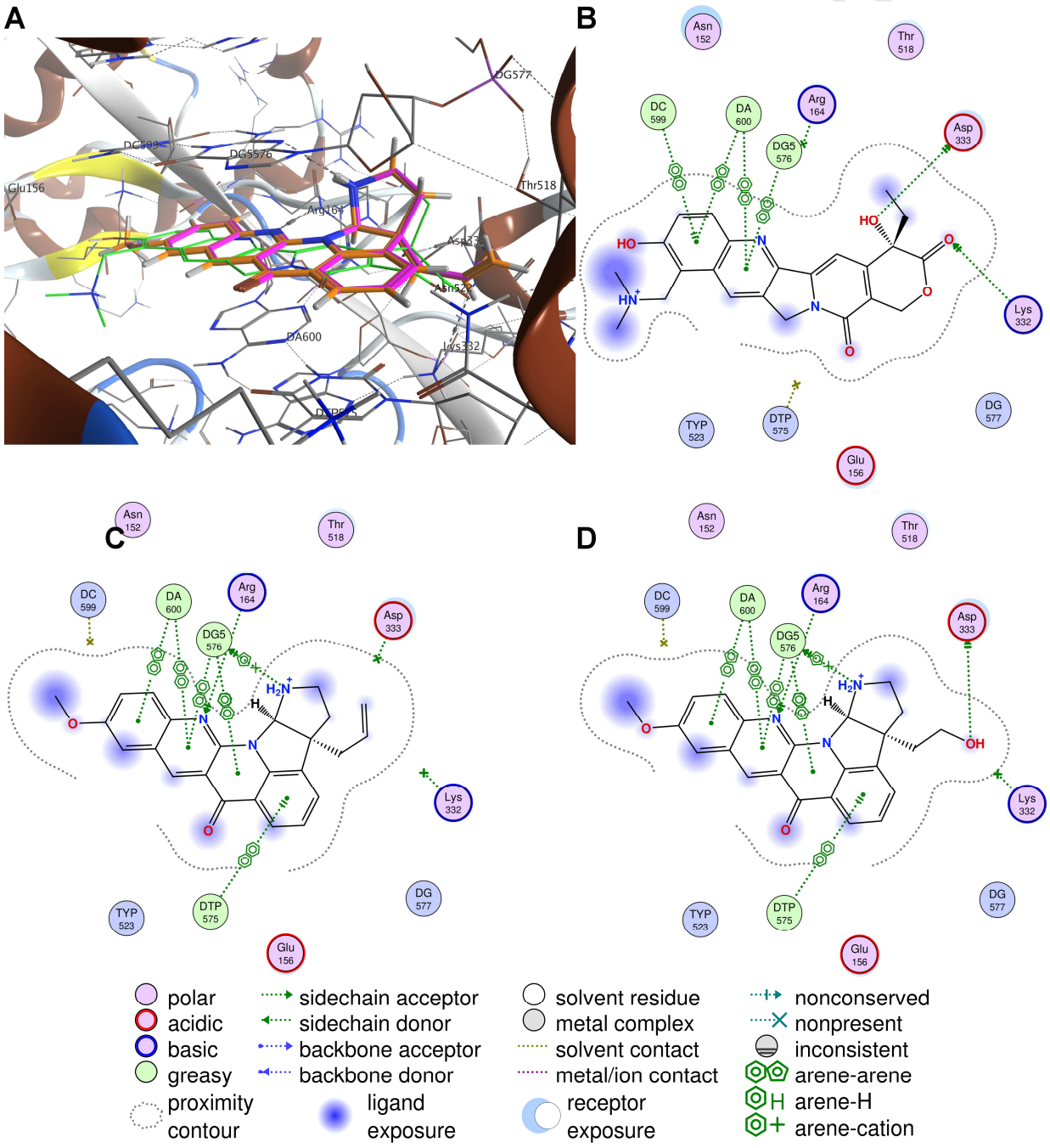

Fig. 6. Panel A: Predicted binding mode for compounds 13 (carbon atoms coloured in magenta) and 9b (carbon atoms coloured in orange). The crystallographic structure of topotecan (PDB code: 1K4T; carbon atoms coloured in green) is also reported as a reference. Panels B, C and D: diagram reporting all ligand interactions for topotecan and compounds 9b and 13, respectively.

The docked conformation of reference compound topotecan is in excellent accord with the crystallographic structure (see Fig. S54, Supporting Information), providing support to the following discussion. Moreover, the binding energies computed for topotecan, **9b** and **13** are -129.4, 128.2 and -128.4 kcal/mol, respectively, thus the docking software correctly ranks the three compounds.

The binding mode predicted for **9b** and **13** (Fig.6, panels A, C and D) shows that the tetracyclic planar moiety is in both cases well packed in-between the two DNA bases pairs made by deoxycytidine 599 and deoxyguanosine 576 (corresponding to the 5' terminus of the cleaved DNA strand) and by deoxyadenosine 600 and thymidine 575, which is covalently bound to Tyr523 through its 3' phosphate group. The benzonaphthyridinone group of **9b** and **13** (rings A-C, Fig. 2) are partially overlaid with the pyrroloquinoline group of topotecan (rings A-C, Fig. 1 and Fig. 6, panels A and B), with the scaffold of the former compounds being slightly shifted toward the α-helix formed by residues Thr518-Tyr523. This shift allows compounds **9b** and **13** to form a moderately strong H-bond involving the nitrogen atom of ring B and the guanidine group of Arg164 (N···H distance = 2.47 and 2.42 Å; N···H-N angle = 169.0 and 170.8 deg. for compounds **9b** and **13**, respectively)[28].

The charged pyrrole group (ring F, Fig. 2) of both **13** and **9b** points toward the hydrophilic pocked made by Asp333 and Arg164 (Fig. 6, panels C and D). Moreover, is involved in a H-bond with nitrogen 3 of deoxyguanosine 576 (N···H distance = 2.15 and 2.22Å; N ···H-N angle = 140.1 and 142.2 deg. for compounds **9b** and **13**, respectively).

The difference in antiproliferative activity (Table 3) and in topoisomerase I poisoning (Figs. 4 and 5) observed for compounds **9b** and **13** are reasonably due to the substituent at C-3a. Indeed, the allyl chain of **9b** is not apparently involved in any interaction with either topoisomerase or DNA. Conversely, a rather strong H-bond is observed between the hydroxyethyl substituent and the acidic group of Asp333 (H···O=C distance = 1.65 Å; O-H···O=C angle = 175.5 deg.).

5. Conclusion

We describes the preparation of a new scaffold starting from triptamines exploiting as a key step the sequential protocol Pd-catalyzed *N*-arylation/intramolecular Friedel-Crafts alkylation.

The antiproliferative activity of the derivatives due to the topoisomerase I inhibition has been evaluated. Although the potency of the new derivatives is two orders of magnitude lower than that of CPT, all compounds resulted active on at least two of the three evaluated cell lines. A computational binding mode analysis has thus been performed on compound **13** to provide insights possibly useful for designing decorations that might improve the activity of the scaffold here described.

6. Experimental section

6.1 Chemistry

6.1.1 General remarks

Melting points were determined on a Büchi B-540 heating apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a VARIAN Gemini 200 M, VARIAN Oxford 300 MHz and XXX 500 MHz instruments. Chemical shifts are given in ppm downfield from $SiMe_4$. ^{13}C NMR spectra are ^{1}H-decoupled and multiplicities were determined by the APT pulse sequence. Mass spectra were determined with a LCQ Advantage Thermo Finningan. Thin-layer chromatographic separations were performed on Merck silica-gel 60-F_{254} precoated. Preparative separations were performed by flash chromatography by using Merck silica gel 0.035-0.070 mm.

6.1.2 Synthesis of methyl (2-(indol-3-yl)ethyl)carbamates **2**.

The products **2a**[29] (yield 85%) and **2b**[30] (yield 91%) were synthesized following the procedure reported in literature[30].

6.1.3 Synthesis of tetrahydropyrrolindoles **3**.

To a solution of $Pd(PPh_3)_4$ (0.005 mmol) in THF (5 ml), cooled at 0 °C, was added the appropriate methyl (2-(indolin-3-yl)ethyl)carbamate **2** (1 mmol), allyl alcohol (1.2 mmol), and Et_3B (1M in THF, 1,2 mmol). The reaction mixture was stirred at rt for 24 h. Then the reaction mixture was poured into sat. $NaHCO_3$, concentrated under vacuum and extracted with AcOEt (10 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

*Methyl 3a-allyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate **(3a)***. Yield: 77%. Eluent: Hexane/AcOEt 5:1. White solid. M.p.: 94-96 °C (Et_2O/Hexane). ^{1}H-NMR (300 MHz; C_6D_6) δ: 6.99 (m, 1H); 6.83 (m, 1H); 6.73 (m, 1H); 6.33 (m, 1H); 5.59 (m, 1H); 5.22 (s, 0.6H, exchange with D_2O); 5.14 (s, 1H); 4.89 (m, 2H); 4.41 (s, 0.4H, exchange with D_2O); 3.70 (t, *J* = 9.2 Hz, 0.3H); 3.49 (s, 1H); 3.45 (s, 2H); 3.32 (t, *J* = 9.2 Hz, 0.7H); 2.92 (m, 1H); 2.16 (d, *J* = 6.9 Hz, 2H); 1.68 (m, 1H); 1.55 (m, 1H) ppm. ^{13}C-NMR (75 MHz; C_6D_6) δ: 154.3 (s); 154.2 (s); 149.8 (s); 149.5 (s); 134.1 (s); 131.6 (s); 128.6 (d); 132.3 (d); 118.9 (d); 118.6 (d); 117.9 (t); 117.8 (t); 109.5 (d); 109.2 (d); 80.6 (d); 79.7 (d); 57.5 (s); 56.2 (t); 51.9 (q); 51.8 (q); 45.9 (t); 45.5 (t); 42.6 (t); 42.2 (t); 34.9 (t) ppm. m/z (ESI$^+$): 259.2 $[M+H]^+$, 258.1 $[M+Na]^+$. IR (ν_{max}/cm^{-1}): = 3352; 2949; 2890; 1686; 1608. Anal. calcd for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.70; H, 7.05; N, 10.87.

*Methyl 3a-allyl-5-methoxy-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate **(3b)***. Yield: 91%. Eluent: Hexane/AcOEt 2:1. White solid. M.p.: 65-67 °C (Et_2O). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 6.58 (m, 3H); 5.73 (m, 1H); 5.09 (m, 3H); 4.87 (bs, 1H, exchange with D_2O); 3.74 (s, 3H); 3.67 (s, 3H); 3.58 (m, 1H); 3.05 (m, 1H); 2.43 (d, *J* = 7.5 Hz, 2H);

2.13 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 155.7 (s); 154.9 (s); 153.9 (s); 153.7 (s); 143.4 (s); 143.1 (s); 133.8 (d); 133.5 (s); 133.4 (s); 118.7 (t); 113.3 (d); 113.2 (d); 110.6 (d); 110.5 (d); 110.2 (d); 110.1 (d); 81.8 (d); 80.5 (d); 58.1 (s); 57.0 (s); 56.2 (q); 52.7 (q); 52.4 (q); 46.0 (t); 45.7 (t); 42.5 (t); 42.2 (t); 35.1 (t) ppm. m/z (ESI$^+$): 289.1 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3350; 2949; 1693. Anal. calcd for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72. Found: C, 66.61; H, 7.03; N, 9.75.

6.1.4 Synthesis of 2-chloroquinoline-3-dioxolanes **4.**

The products **4a**[31] (yield 98%) and **4b**[31] (yield 99%) were synthesized following the procedure reported in literature[31].

6.1.4 General procedure for N-arylation of tetrahydropyrrolindoles **3.**

To a solution of $Pd_2(dba)_3$ (0.02 mmol) and triisobutylphosphatrane (0.08 mmol) in toluene (25 ml) were added **3** (1 mmol), the appropriate 2-chloroquinoline **4** (1 mmol) and *t*-BuONa (1.4 mmol). The reaction mixture was warmed at 110 °C for 5 h. After completion the reaction mixture was filtered through a Celite pad, washed with brine (10 ml) for three time and extracted with AcOEt (15 ml). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified trough silica gel chromatography.

Methyl 8-(3-(1,3-dioxolan-2-yl)quinolin-2-yl)-3a-allyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**5a**). Yield: 87%. Eluent: Hexane/AcOEt 1:1. White solid. M.p.: 127-130 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 8.52 (s, 1H); 8.00 (d, *J* = 8.4 Hz, 1H); 7.86 (d, *J* = 8.1 Hz, 1H); 7.68 (m, 1H); 7.51 (m, 1H); 7.11 (m, 2H); 6.84 (m, 1H); 6.66 (m, 1H); 6.14 (s, 1H); 6.11 (s, 1H); 5.94 (m, 1H); 5.15 (m, 2H); 4.22 (m, 2H); 3.97 (m, 2H); 3.33 (m, 2H); 2.85 (s, 3H); 2.70 (m, 2H); 2.22 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 155.1 (s); 154.5 (s); 149.2 (s); 148.3 (s); 136.8 (d); 134.2 (d); 133.5 (s); 130.2 (d); 129.7 (s); 128.9 (d); 128.6 (d); 128.0 (d); 127.2 (s); 126.5 (d); 123.4 (d); 120.2 (d); 118.9 (t); 110.0 (d); 100.4 (d); 84.8 (d); 65.8 (t); 65.6 (t); 57.6 (s); 51.9 (q); 45.8 (t); 43.4 (t); 36.1 (t) ppm. m/z (ESI$^+$): 480.3 $[M+Na]^+$. IR (ν_{max}/cm^{-1}): = 3423 (br); 2950; 2855; 1706. Anal. calcd for $C_{27}H_{27}N_3O_4$: C, 70.88; H, 5.95; N, 9.18. Found: C, 70.84; H, 5.90; N, 9.22.

Methyl 8-(3-(1,3-dioxolan-2-yl)-6-methoxyquinolin-2-yl)-3a-allyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**5b**). Yield: 92%. Eluent: CH_2Cl_2/AcOEt 5:1. White solid. M.p.: 152-155 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 8.41 (s, 1H); 7.89 (d, *J* = 9.5 Hz, 1H); 7.33 (m, 1H); 7.14 (m, 1H); 7.05 (m, 2H); 6.81 (m, 1H); 6.55 (m, 1H); 6.16 (s, 1H); 6.07 (s, 1H); 5.93 (m, 1H); 5.17 (m, 2H); 4.17 (m, 2H); 4.03 (m, 2H); 3.92 (s, 3H); 3.31 (m, 2H); 2.90 (s, 3H); 2.69 (m, 2H); 2.18 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 158.0 (s); 155.2 (s); 152.6 (s); 149.6 (s); 144.4 (s); 135.4 (d); 135.3 (d); 133.4 (s); 130.4 (d); 130.0 (s); 128.6 (d); 128.2 (s); 123.3 (d); 122.9 (d); 120.0 (d); 118.8 (t); 109.7 (d); 105.5 (d); 100.5 (d); 85.0 (d); 65.8 (t); 65.6 (t); 57.6 (s); 55.8 (q): 51.9 (q); 45.8 (t); 43.5 (t); 36.3 (t) ppm. m/z (ESI$^+$): 488.5 $[M+H]^+$; 510.3 $[M+Na]^+$. IR (ν_{max}/cm^{-1}): = 3402 (br); 2951 (br); 1705. Anal. calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62. Found: C, 68.93; H, 5.97; N, 8.66.

Methyl 8-(3-(1,3-dioxolan-2-yl)quinolin-2-yl)-3a-allyl-5-methoxy-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**5c**). Yield: 92%. Eluent: CH_2Cl_2/ AcOEt 5:1. White solid. M.p.: 154-159 °C (Et_2O/ Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 8.49 (s, 1H); 7.97 (d, *J* = 8.0 Hz, 1H); 7.84 (d, *J* = 8.0 Hz, 1H); 7.66 (m, 1H); 7.48 (m, 1H); 6.69 (m, 3H); 6.20 (s, 1H); 6.13 (s, 1H); 5.95 (m, 1H); 5.16 (m, 2H); 4.24 (m, 2H); 4.07 (m, 2H); 3.75 (s, 3H); 3.31 (m, 2H,); 2.93 (s, 3H); 2.70 (m, 2H); 2.18 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 155.2 (s); 154.5 (s); 148.2 (s); 143.3 (s); 136.8 (d); 135.2 (s); 134.9 (s); 134.2 (d); 130.2 (d); 129.5 (s); 128.8 (t); 128.0 (d); 127.0 (s); 126.3 (d); 118.9 (s); 113.2 (d); 110.9 (d); 110.4 (d): 100.5 (d); 85.3 (d); 65.8 (t); 65.6 (t); 57.8 (s); 56.2 (q); 51.9 (q); 45.9 (t); 43.1 (t); 35.8 (t) ppm. m/z (ESI$^+$): 488.4 $[M+H]^+$; 510.3 $[M+Na]^+$. IR (ν_{max}/cm^{-1}): = 3416 (br); 2950 (br); 1705. Anal. calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62. Found: C, 68.94; H, 5.98; N, 8.66.

Methyl 8-(3-(1,3-dioxolan-2-yl)-6-methoxyquinolin-2-yl)-3a-allyl-5-methoxy-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**5d**). Yield: 88%. Eluent: CH_2Cl_2/AcOEt 4:1. White solid. M.p.: 142-145 °C (Et_2O/Hexane). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 8.36 (s, 1H); 7.87 (d, *J* = 9.3 Hz, 1H); 7.32 (d, *J* = 9.3 Hz, 1H); 7.12 (d, *J* = 2.6 Hz, 1H); 6.74 (d, *J* = 1.8 Hz, 1H); 6.60 (m, 2H); 6.10 (s, 1H); 6.01 (s, 1H); 5.95 (m, 1H); 5.18 (m, 2H); 4.20 (m, 2H); 4.04 (m, 2H); 3.91 (s, 3H); 3.74 (s, 3H); 3.30 (m, 2H); 2.95 (s, 3H); 2.70 (m, 2H); 2.12 (m, 2H) ppm. ^{13}C-NMR (75 MHz; $CDCl_3$) δ: 158.0 (s); 155.4 (s); 154.6 (s); 144.5 (s); 144.0 (s); 135.6 (d); 135.3 (s); 134.4 (d); 130.5 (d); 130.0 (s); 128.2 (s); 123.0 (d); 119.0 (t); 118.8 (s); 113.4 (d); 110.8 (d); 110.5 (d); 105.7 (d); 100.7 (d); 85.6 (d); 66.0 (t); 65.8 (t); 57.9 (s); 56.4 (q); 56.0 (q); 52.1 (q); 46.0 (t); 43.4 (t); 36.1 (t) ppm. m/z (ESI$^+$): 518.2 $[M+H]^+$; 540.2 $[M+Na]^+$. IR (ν_{max}/cm^{-1}): = 3393 (br); 2951 (br); 1705. Anal. calcd for $C_{29}H_{31}N_3O_6$: C, 67.30; H, 6.04; N, 8.12. Found: C, 67.26; H, 6.00; N, 8.17.

6.1.5 General procedure for the intramolecular Friedel-Crafts and oxidation reactions.

a) To a solution of compound **5** (1 mmol) in CH_2Cl_2 (10 ml), cooled at 0 °C, was added BF_3-Et_2O (3–5 mmol). The reaction mixture was stirred at rt for 48 h. After completion the mixture was poured into a sat. $NaHCO_3$ (25 ml) soln. at 0 °C, and stirred for 30 min. Then was extracted with CH_2Cl_2 (15 ml) for three time and the organic layer was dried under vacuum. **b)** The crude was dissolved in THF (40 ml) and water (10 ml), and MnO_2 (3 mmol) and m-CPBA (0.05 mmol) were added. The reaction mixture was warmed at 60 °C for 12h. After the complete oxidation of the intermediate, the reaction mixture was filtered through a Celite pad and concentrated under vacuum. Then was extracted with AcOEt (15 ml) and washed with water (10 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified trough silica gel chromatography.

Methyl 3a-allyl-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**7a**). Yield: 50%. Eluent: Hexane/AcOEt 7:1. Yellow solid. M.p.: 133-135 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.28 (d, 1H); 8.12 (d, *J* = 8.1 Hz, 1H); 7.96 (m, 2H); 7.72 (td, *J* = 6.8, 1.4 Hz, 1H); 7.55 (dd, *J* = 7.1, 0.9 Hz, 1H), 7.44 (td, *J* = 7.1, 0.9 Hz, 1H); 7.25 (m, 1H); 7.02 (s, 1H); 5.68 (m, 1H); 5.27 (m, 2H); 4.12 (m, 1H); 3.97 (s, 3H); 2.84 (m, 3H); 2.12 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.7 (s); 155.4 (s); 150.0 (s); 147.9 (s); 145.8 (s); 139.5 (d); 135.5 (s); 132.8 (d); 132.7 (d); 129.9 (d); 128.6 (d); 128.0 (d); 125.0 (d); 124.9 (d); 124.7 (s); 122.7 (d): 120.2 (t); 119.2 (s); 117.7 (s); 81.9 (d); 58.2 (s); 53.2 (q); 46.1 (t); 43.1 (t); 39.7 (t) ppm. m/z (ESI$^+$): 434.2 [M+Na]$^+$. IR (ν_{max}/cm^{-1}): = 3436 (br); 1698; 1655. Anal. calcd for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21. Found: C, 73.03; H, 5.09; N, 10.18.

Methyl 3a-allyl-10-methoxy-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**7b**). Yield: 62%. Eluent: CH_2Cl_2/AcOEt 6:1. Yellow solid. M.p.: 157-160 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.22 (s, 1H); 8.15 (dd, *J* = 8.1 ; 1.1 Hz, 1H); 7.90 (d, *J* = 9.5 Hz, 1H); 7.54 (m, 2H); 7.24 (m, 2H); 7.01 (s, 1H); 5.70 (m, 1H); 5.09 (m, 2H); 4.14 (m, 1H); 3.97 (s, 3H); 3.96 (s, 3H); 2.94 (m, 3H); 2.18 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.7 (s); 156.7 (s); 155.4 (s); 146.5 (s); 146.4 (s); 145.8 (s); 137.6 (d); 135.2 (s); 132.8 (d); 129.5 (d); 128.3 (d); 126.4 (d); 125.4 (s); 125.0 (d); 122.4 (d); 120.1 (t); 119.2 (s); 117.6 (s); 106.1 (d); 81.9 (d); 58.2 (s); 55.8 (q); 53.1 (q); 46.1 (t); 43.1 (t); 39.7 (t) ppm. m/z (ESI$^-$): 440.3 [M]$^-$. IR (ν_{max}/cm^{-1}): = 3467 (br); 170; 1647. Anal. calcd for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52. Found: C, 70.75; H, 5.24; N, 9.49.

Methyl 3a-allyl-5-methoxy-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**7c**). Yield: 70%. Eluent: CH_2Cl_2/AcOEt 6:1. Orange solid. M.p.: 165-167 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.31 (s, 1H); 7.97 (m, 2H); 7.77 (m, 1H); 7.50 (d, *J* = 2.5 Hz, 1H); 7.44 (m, 1H); 7.23 (d, *J* = 2.5 Hz, 1H); 7.02 (s, 1H); 5.71 (m, 1H); 5.18 (m, 2H); 4.12 (m, 1H); 3.97 (s, 3H); 3.91 (s, 3H); 2.84 (m, 3H); 2.16 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.3 (s);156.5 (s); 155.3 (s); 150.0 (s); 147.4 (s); 140.9 (s); 139.5 (d); 137.1 (s); 132.6 (d, 2C); 132.6 (d); 129.9 (d); 128.0 (d) 124.7 (d); 124.6 (s); 120.3 (t); 120.2 (d); 118.7 (s); 117.5 (s); 103.8 (d); 82.1 (d); 58.1 (s); 56.3 (q); 53.2 (q); 46.0 (t); 43.0 (t); 39.6 (t) ppm. m/z (ESI$^+$): 464.3 [M+Na]$^+$. IR (ν_{max}/cm^{-1}): = 3467 (br); 1701; 1647. Anal. calcd for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52. Found: C, 70.75; H, 5.30; N, 9.48.

Methyl 3a-allyl-5,10-dimethoxy-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**7d**). Yield: 80%. Eluent: Hexane/AcOEt 2:1. Orange solid. M.p.: 157-160 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.22 (s, 1H); 7.87 (d, *J* = 9.2 Hz, 1H); 7.48 (m, 2H); 7.24 (m, 2H); 7.00 (m, 1H); 5.71 (m, 1H); 5.16 (m, 2H); 4.14 (m, 1H); 3.97 (s, 3H); 3.95 (s, 3H); 3.92 (s, 3H); 2.83 (m, 3H); 2.36 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.3 (s); 156.6 (s); 156.3 (s); 155.4 (s); 146.5 (s); 146.1 (s); 140.9 (s); 137.5 (d); 137.2 (s); 132.7 (d); 129.5 (d); 126.5 (d); 125.3 (s); 120.3 (t); 120.1 (d); 118.7 (s); 117.3 (s); 106.0 (d); 103.6 (d); 82.1 (d); 58.1 (s); 56.3 (q); 56.8 (q); 53.2 (q); 46.0 (t); 43.0 (t); 39.6 (t) ppm. m/z (ESI$^+$): 472.3 [M+H]$^+$; 494.3 [M+Na]$^+$. IR (ν_{max}/cm^{-1}): = 3437 (br); 2959 (br); 1713; 1645. Anal. calcd for $C_{27}H_{25}N_3O_5$: C, 68.78; H, 5.34; N, 8.91. Found: C, 68.82; H, 5.35; N, 8.88.

6.1.6 Synthesis of 3a-Allyl-5,10-dimethoxy-1-methyl-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**8**).

To a solution of compound **7d** (1 mmol) in toluene (10 ml), cooled at 0 °C, Red-Al® (65% in toluene, 10 mmol) was added dropwise. After 10 min at rt, the reaction mixture was warmed at 110° C for 5 h. After completion the reaction mixture was poured into H_2O at 0 °C, stirred at rt for 30 min. Then the mixture was filtered through a Celite pad with AcOEt (30 ml) and washed with water (15 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

3a-Allyl-5,10-dimethoxy-1-methyl-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**8**). Yield: 50%. Eluent: CH_2Cl_2/AcOEt 1:1. Orange solid. M.p.: 173-175 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.23 (s, 1H); 7.91 (d, *J* = 9.1 Hz, 1H); 7.50 (m, 2H); 7.24 (m, 1H); 7.18 (d, *J* = 2.4 Hz, 1H); 5.99 (s, 1H); 5.68 (m, 1H); 5.11 (m, 2H); 3.95 (s, 3H); 3.91 (s, 3H); 2.91 (s, 3H); 2.82 (m, 2H); 2.66 (m, 2H); 2.34 (m, 1H); 2.18 (m, 1H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.4 (s); 156.4 (s); 156.2 (s); 147.2 (s); 146.3 (s); 141.2 (s); 139.1 (s); 137.5 (d); 133.3 (d); 129.1 (d); 126.4 (d); 125.2 (s); 119.9 (d); 119.6 (t); 118.7 (s); 117.5 (s); 106.0 (d); 103.1 (d); 89.5 (d); 58.6 (s); 56.3 (q); 55.8 (q); 54.7 (t); 43.5 (t); 40.9 (q); 37.7 (t) ppm. m/z (ESI$^+$): 429.3 [M+Na]$^+$. IR (ν_{max}/cm^{-1}): = 3401 (br); 2785 (br); 1640. Anal. calcd for $C_{26}H_{25}N_3O_3$: C, 73.05; H, 5.89; N, 9.83. Found: C, 73.01; H, 5.92; N, 9.80.

6.1.7 General procedure for hydrolysis of methylcarbamate derivatives **7**.

To a solution of compound **7** (1 mmol) in EtOH (30 ml) was added NaOH (15 mmol) in H_2O (5 ml). The reaction mixture was warmed at 80 °C until the consumption of the starting material (monitored by TLC). After completion the reaction mixture was concentrated under vacuum, extracted with AcOEt (25 ml) and washed with water (15 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

3a-Allyl-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**9a**). Yield: 66%. Eluent: Hexane/AcOEt 1:2. Yellow solid. M.p.: 125-128 °C (Et_2O/Hex). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.36 (s, 1H); 8.08 (dd, *J* = 8.1; 0.8 Hz, 1H); 7.98 (d, *J* = 9.1 Hz, 2H); 7.77 (td, *J* = 7.0; 1.4 Hz, 1H); 7.57 (dd, *J* = 7.0; 0.8 Hz, 1H); 7.44 (td, *J* = 8.1; 0.8 Hz, 1H); 7.21 (m, 1H); 6.20 (s, 1H); 5.80 (m, 1H); 5.17

(m, 2H); 3.42 (bs, 1H, exchange with D_2O); 3.16 (m, 1H); 2.80 (m, 3H); 2.20 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.7 (s); 150.0 (s); 148.1 (s); 146.1 (s); 139.9 (d); 137.0 (s); 133.8 (d); 138.8 (d); 130.8 (d); 128.6 (d); 127.6 (d); 124.6 (d); 124.5 (s); 124.4 (d); 122.6 (d); 119.4 (t); 119.3 (s); 117.2 (s); 84.7 (d); 56.9 (s); 44.9 (t); 43.5 (t); 40.6 (t) ppm. m/z (ESI$^+$): 354.2 $[M+H]^+$; 376.1 $[M+Na]^+$. IR (ν_{max}/cm^{-1}): = 3351 (br), 2930 (br), 1651. Anal. calcd for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89. Found: C, 78.20; H, 5.45; N, 11.85.

3a-Allyl-10-methoxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**9b**). Yield: 85%. Eluent: Hexane/AcOEt 1:1. Yellow solid. M.p.: 94-96 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.16 (s, 1H); 8.07 (dd, *J* = 8.1; 1.1 Hz, 1H); 7.88 (d, *J* = 9.2 Hz, 1H); 7.53 (dd, *J* = 7.0; 1.1 Hz, 1H); 7.43 (dd, *J* = 9.2; 2.6 Hz, 1H); 7.20 (m, 2H); 6.18 (s, 1H); 5.76 (m, 1H); 5.13 (m, 2H); 3.92 (s, 3H); 3.27 (bs, 1H, exchange with D_2O); 3.14 (m, 1H); 2.75 (m, 3H); 2.17 (m, 2H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.6 (s); 156.4 (s); 146.8 (s); 146.4 (s); 146.1 (s); 137.9 (d); 137.0 (s); 133.9 (d); 129.0 (d); 128.4 (d); 126.4 (d); 125.2 (s); 124.3 (d); 122.3 (d); 119.3 (t); 119.0 (s); 117.0 (s); 106.2 (d); 84.7 (d); 56.9 (s); 55.8 (q); 44.9 (t); 43.5 (t); 40.6 (t) ppm. m/z (ESI$^+$): 384.3 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3435 (br), 2932 (br), 1647. Anal. calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.22; H, 5.54; N, 10.93.

3a-Allyl-5-methoxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**9c**). Yield: 68%. Eluent: Hexane/AcOEt 1:2. Orange solid. M.p.: 165-168 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.30 (s, 1H); 7.97 (d, *J* = 8.8 Hz, 2H); 7.76 (td, *J* = 7.7; 0.7 Hz, 1H); 7.42 (m, 2H); 7.23 (d, *J* = 2.3 Hz, 1H); 6.21 (s, 1H); 5.77 (m, 1H); 5.15 (m, 2H); 3.90 (s, 3H); 3.43 (bs, 1H, exchange with D_2O); 3.15 (m, 1H); 2.78 (m, 3H); 2.25 (m, 1H); 2.10 (m, 1H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.2 (s); 156.5 (s); 150.0 (s);147.6 (s); 141.3 (s); 139.8 (d); 139.0 (s); 133.7 (d); 132.7 (d); 130.1 (d); 127.6 (d) 124.4 (d); 124.3 (s); 120.4 (d); 119.5 (t); 118.7 (s); 116.8 (s); 103.1 (d) 84.9 (d); 56.9 (s); 56.3 (q); 44.9 (t); 43.3 (t); 40.5 (t) ppm. m/z (ESI$^+$): 384.3 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3435 (br), 1932 (br), 1645. Anal. calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.21; H, 5.49; N, 10.94.

3a-Allyl-5,10-dimethoxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**9d**). Yield: 65%. Eluent: Hexane/AcOEt 1:2. Orange solid. M.p.: 165-168 °C (Et_2O/Hexane). ^{1}H-NMR (200 MHz; $CDCl_3$) δ: 9.24 (s, 1H); 7.92 (d, *J* = 9.1 Hz, 1H); 7.43 (m, 2H); 7.22 (m, 2H); 6.21 (s, 1H); 5.75 (m, 1H); 5.16 (m, 2H); 3.96 (s, 3H); 3.92 (s, 3H); 3.43 (bs, 1H, exchange with D_2O); 3.16 (m, 1H); 2.78 (m, 3H); 2.24 (m, 1H); 2.10 (m, 1H) ppm. ^{13}C-NMR (50 MHz; $CDCl_3$) δ: 179.0 (s); 156.3 (s, 2C); 146.5 (s); 146.3 (s); 141.3 (s); 139.0 (s); 137.8 (d); 133.7 (d); 129.0 (d); 126.4 (d); 125.1 (s); 120.2 (d); 119.4 (t); 118.7 (s); 116.6 (s); 106.1 (d); 102.9 (d) 84.9 (d); 56.9 (s); 56.2 (q); 55.8 (q); 44.9 (t); 43.3 (t) 40.5 (t) ppm. m/z (ESI$^+$): 414.3 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3435 (br), 2933 (br), 1640. Anal. calcd for $C_{25}H_{23}N_3O_3$: C, 72.62; H, 5.61; N, 10.16. Found: C, 72.65; H, 5.57; N, 10.12.

6.1.8 General procedure for hydrolysis of methylether derivatives **9**.

To a solution of compound **9** (1 mmol) in CH_2Cl_2 (10 ml), cooled at -78 °C, was added dropwise BBr_3 (1M in Hexane, 2.5 mmol). After 1 h at rt the mixture was warmed at 40 °C until the consumption of the starting material (monitored by TLC). Then the mixture, cooled at 0 °C, was poured into a sat. $NaHCO_3$ (25 ml) and was extracted with CH_2Cl_2 (15 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The product was purified by crystallization (AcOEt/Hexane).

3a-Allyl-10-hydroxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**10b**). Yield: 70%. Red solid. M.p.: 296-299 °C (AcOEt/Hexane). ^{1}H-NMR (200 MHz; DMSO) δ: 10.04 (s, 1H, exchange with D_2O); 9.12 (s, 1H); 7.88 (m, 2H); 7.70 (d, *J* = 6.2 Hz, 1H); 7.45 (m, 2H); 7.22 (m, 1H); 6.08 (s, 1H); 5.70 (m, 1H); 5.12 (d, *J* = 17.1 Hz, 1H); 5.00 (dd, *J* = 10.1; 2.2 Hz, 1H); 4.20 (bs, 1H, exchange with D_2O); 2.95 (m, 1H); 2.65 (m, 3H); 2.10 (m, 2H) ppm. ^{13}C-NMR (50 MHz; DMSO) δ: 179.0 (s); 154.6 (s); 146.4 (s); 146.1 (s); 145.3 (s); 137.7 (s); 137.4 (d); 135.0 (d); 129.4 (d); 129.0 (d); 126.9 (d); 125.8 (s); 123.8 (d); 122.7 (d); 119.4 (s); 119.2 (t); 116.5 (s); 110.2 (d); 84.5 (d); 57.4 (s); 57.4 (t); 45.3 (t); 43.1 (t) ppm. m/z (ESI$^+$): 370.3 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3419 (br); 2961 (br) ; 1643. Anal. calcd for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.37. Found: C, 74.74; H, 5.22; N, 11.40.

3a-Allyl-5,10-dihydroxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**10d**). Yield: 80%. Red solid. M.p.: 342-345 °C (AcOEt/Hexane). ^{1}H-NMR (200 MHz; DMSO) δ: 10.02 (bs, 1H, exchange with D_2O); 9.70 (bs, 1H, exchange with D_2O); 9.10 (s, 1H); 7.85 (d, *J* = 9.2 Hz, 1H); 7,51-7.23 (m, 4H); 6.16 (s, 1H); 6.57 (bs, 1H, exchange with D_2O); 5.66 (m, 1H); 5.08 (m, 2H); 3.14 (m, 1H); 2.73 (m, 3H); 2.17 (m, 2H) ppm. ^{13}C-NMR (50 MHz; DMSO) δ: 178.4 (s); 154.4 (s); 154.3 (s); 145.7 (s); 145.2 (s); 139.7 (s); 138.4 (s); 137.3 (d); 134.6 (d); 128.9 (d); 127.0 (d); 125.7 (s); 120.4 (d); 119.7 (s); 118.5 (t); 117.0 (s); 110.2 (d); 106.4 (d); 83.3 (d); 57.7 (s); 57.7 (t); 45.5 (t); 42.7 (t) ppm. m/z (ESI$^+$): 386.3 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3323 (br); 1623. Anal. calcd for $C_{23}H_{19}N_3O_3$: C, 71.67; H, 4.97; N, 10.90. Found: C, 71.65; H, 5.01; N, 10.92.

6.1.9 Synthesis of 10-methoxy-7-oxo-3a-(2-oxoethyl)-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**11**).

To a solution of compound **7b** (1 mmol) in THF (15 ml) and H_2O (3 ml) was added $NaIO_4$ (3 mmol), lutidine (2 mmol) and OsO_4 (4% in H_2O, 0.05 mmol). The reaction mixture was stirred at rt. After 24 h the reaction mixture was concentrated under vacuum and was extracted with AcOEt

(20 ml). The organic layer was washed with H_2O (10 ml) for three time, dried Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

Methyl 10-methoxy-7-oxo-3a-(2-oxoethyl)-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**11**). Yield: 75%. Eluent: CH_2Cl_2/AcOEt 2:1. Orange solid. M.p.: 241-243 °C (CH_2Cl_2/Hexane). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 9.77 (s, 1H); 9.16 (s, 1H); 8.12 (dd, *J* = 8.0, 1.1 Hz, 1H); 7.86 (d, *J* = 9.3 Hz, 1H); 7.60 (dd, *J* = 7.1, 1.1 Hz, 1H); 7.46 (dd, *J* = 9.3, 2.8 Hz, 1H); 7.22 (m, 2H); 7.12 (s, 1H); 4.16 (m, 1H); 3.96 (s, 3H); 3.94 (s, 3H); 3.27 (ddd, *J* = 22.5, 18.1, 0.8 Hz, 2H); 2.95 (dt, *J* = 12.1, 5.5 Hz, 1H); 2.38 (m, 1H); 2.22 (m, 1H) ppm. ^{13}C-NMR (75 MHz; $CDCl_3$) δ: 198.8 (d); 179.5 (s); 156.7 (s); 155.4 (s); 146.3 (s); 145.6 (2C, s); 137.7 (d); 134.2 (s); 129.4 (d); 128.7 (d); 126.5 (d); 125.5 (s); 125.4 (d); 122.6 (d); 119.1 (s); 117.6 (s); 106.1 (d); 82.5 (d); 55.9 (q); 54.9 (s); 53.3 (q); 52.2 (t); 45.7 (t); 40.2 (t) ppm. m/z (ESI$^+$): 444.1 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3437 (br); 2945; 1718; 1701. Anal. calcd for $C_{25}H_{21}N_3O_5$: C, 67.71; H, 4.77; N, 9.48. Found: C, 67.75; H, 4.73; N, 9.45.

6.1.10 Synthesis of methyl 3a-(2-hydroxyethyl)-10-methoxy-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**12**).

To a solution of compound **11** (1 mmol) in MeOH (10 ml), cooled at 0 °C, was added $NaBH_4$ (1.2 mmol) and the reaction mixture was stirred at rt. After 3 h the reaction mixture was poured into H_2O (25 ml) and concentrated under vacuum. Then the mixture was extracted with AcOEt (15 ml) and washed with brine (10 ml) for three time. The organic layer was dried Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

Methyl 3a-(2-hydroxyethyl)-10-methoxy-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**12**). Yield: 65%. Yellow solid. Eluent: EtOAc. M.p.: 121-123 °C (CH_2Cl_2/Hexane). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 8.95 (s, 1H); 7.91 (d, *J* = 8.1 Hz, 1H); 7.73 (d, *J* = 9.3 Hz, 1H); 7.48 (d, *J* = 7.1 Hz, 1H); 7.35 (dd, *J* = 9.3; 2.6 Hz, 1H); 7.21 (s, 1H); 7.17 (m, 1H); 7.10 (d, *J* = 2.6 Hz, 1H); 4.05 (m, 1H); 3.94 (s, 3H); 3.91 (s, 3H); 3.72 (m, 2H); 2.83 (m, 1H); 2.58 (br s, 1H, exchange with D_2O); 2.34 (m, 2H); 2.17 (m, 2H) ppm. ^{13}C-NMR (75 MHz; $CDCl_3$) δ: 179.4 (s); 156.5 (s); 155.5 (s); 146.2 (s, 2C); 145.8 (s); 137.4 (d); 134.6 (s); 129.1 (d); 128.3 (d); 126.4 (d); 125.2 (s); 124.9 (d); 122.2 (d); 118.8 (s); 117.3 (s); 106.1 (d); 82.2 (d); 59.7 (t); 57.0 (s); 55.8 (q); 53.1 (q); 45.5 (t); 41.0 (t); 40.7 (t) ppm. m/z (ESI$^+$): 446.1 $[M+H]^+$. IR (ν_{max}/cm^{-1}): = 3451; 2956; 1673; 1650; 1626; 1606; 1598. Anal. calcd for $C_{25}H_{23}N_3O_5$: C, 67.41; H, 5.20; N, 9.43. Found: C, 67.45; H, 5.24; N, 9.40.

6.1.11 Synthesis of 3a-(2-hydroxyethyl)-10-methoxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**13**).

To a solution of compound **12** (1 mmol) in EtOH (30 ml) was added NaOH (15 mmol) in H_2O (5 ml). The reaction mixture was warmed at 80 °C. After 6 h the reaction mixture was concentrated under vacuum, extracted with AcOEt (25 ml) and washed with H_2O (15 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

3a-(2-Hydroxyethyl)-10-methoxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**13**). Yield: 61%. Eluent: AcOEt/MeOH 20:1. Yellow solid. M.p.: 211-213 °C (CH_2Cl_2/Hexane). ^{1}H-NMR (300 MHz; DMSO) δ: 9.21 (s, 1H); 7.90 (m, 2H); 7.69 (d, *J* = 7.1 Hz, 1H); 7.64 (d, *J* = 2.8 Hz, 1H); 7.54 (dd, *J* = 9.2; 2.8 Hz, 1H); 7.23 (m, 1H); 6.30 (s, 1H); 4.15 (t, *J* = 4.8 Hz, 1H, exchange with D_2O); 3.90 (s, 3H); 3.69 8 (br s, 1H, exchange with D_2O); 3.38 (m, 2H); 2.95 (m, 1H); 2.44 (m, 1H); 2.27-1.97 (m, 4H) ppm. ^{13}C-NMR (75 MHz; DMSO) δ: 178.9 (s); 156.3 (s); 146.9 (s); 146.3 (s): 146.2 (s); 137.9 (d); 137.8 (s); 129.4 (d); 129.1 (d); 126.8 (d); 125.4 (s); 123.5 (d); 122.8 (d); 119.3 (s); 116.6 (s); 107.6 (d); 85.3 (d); 58.6 (s); 56.4 (t); 56.3 (q); 44.8 (t); 41.7 (t); 41.4 (t) ppm. m/z (ESI$^+$): 388.1 $[M+H]^+$; 410.0 $[M+Na]^+$. IR (ν_{max}/cm^{-1}): = 3434 (br); 2915; 2876; 1651. Anal. calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.33; H, 5.49; N, 10.82.

6.1.12 Synthesis of 10-hydroxy-3a-(2-hydroxyethyl)-7-oxo-1,2,3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-1-ium bromide (**14**).

To a solution of compound **13** (1 mmol) in CH_2Cl_2 (10 ml), cooled at -78 °C, was added dropwise BBr_3 (1M in Hexane, 2.5 mmol). After 1 h at rt the mixture was warmed at 40 °C for 12 h. Then the reaction mixture was poured over ice and the organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum. The crude product was used directly for the subsequent reaction without purification.

10-Hydroxy-3a-(2-hydroxyethyl)-7-oxo-1,2,3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-1-ium bromide (**14**). Yield: 50%. Red solid. 255-256 °C (CH_2Cl_2/AcOEt). ^{1}H-NMR (300 MHz; DMSO) δ: 10.47 (br s, 1H, exchange with D_2O); 9.57 (br s, 1H, exchange with D_2O); 9.16 (s, 1H); 8.00-7.83 (m, 3H); 7.60 (m, 1H); 7.48 (d, *J* = 2.7 Hz, 1H); 7.36 (m, 1H); 6.68 (s, 1H); 4.70 (br s, 2H, exchange with D_2O); 3.46 (m, 2H); 3.11 (m, 1H); 2.88 (m, 1H); 2.47 (m, 2H); 2.24 (m, 2H) ppm. ^{13}C-NMR (75 MHz; DMSO) δ: 170.1 (s); 155.1 (s); 145.7 (s); 144.7 (s); 137.8 (d); 134.6 (s); 130.0 (d); 129.0 (d); 127.3 (d); 126.5 (s); 126.2 (s); 124.8 (d); 123.8 (d); 119.2 (s); 117.7 (s); 110.6 (d); 80.2 (d); 57.9 (t); 57.7 (s); 57.6 (t); 45.0 (t); 37.9 (t) ppm. m/z (ESI$^+$): 374.3; (ESI$^-$): 372.3. IR (ν_{max}/cm^{-1}): = 3435; 2929; 2709; 1670; 1641; 1640.

6.1.13 General procedure for synthesis of t-butylcarbamates **15.**

To a solution of compound **10b** or **14** (1 mmol) in EtOH (10 ml) was added TEA (3 mmol) and $(Boc)_2O$ (3 mmol). The reaction mixture was

stirred at rt for 48 h. After consumption of starting material (monitored by TLC) the mixture was concentrated under vacuum and extracted with AcOEt (15 ml). The organic layer was washed with H_2O (10 ml) for three time, dried over Na_2SO_4 and concentrated under vacuum. The crude, consisting of a mixture of mono- and di-protected product, was diluted in EtOH (10 ml) and piperidine (3 mmol) was added, in order to remove the Boc on the OH in position 10. The reaction mixture was stirred at 80 °C until the consumption of the di-protected intermediate (monitored by TLC). Then the mixture was poured in H_2O (25 ml) and was extracted with CH_2Cl_2 (15 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

tert-Butyl 3a-allyl-10-hydroxy-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**15a**). Yield: 60%. Eluent: Hexane/AcOEt 1:1. Orange solid. M.p.: 147-149 °C (CH_2Cl_2/Hexane). ^{1}H-NMR (300 MHz; DMSO) δ: 10.15 (s, 1H, exchange with D_2O); 9.06 (s, 1H); 7.91 (d. *J* = 8.1 Hz, 1H); 7.76 (m, 2H); 7.50 (dd, *J* = 9.1; 2.6 Hz, 1H); 7.39 (d, *J* = 2.6 Hz, 1H); 7.25 (m, 1H); 6.88 (s, 1H); 5.67 (m, 1H); 5.18 (d, *J* = 17.1 Hz, 1H); 5.07 (d, *J* = 9.9 Hz, 1H); 3.89 (m, 1H); 2.72 (m, 3H); 2.09 (m, 2H); 1.53 (s, 9H) ppm. ^{13}C-NMR (75 MHz; DMSO) δ: 179.1 (s); 157.7 (s); 153.7 (s); 146.1 (s); 145.9 (s); 145.2 (s); 137.1 (d); 135.5 (s); 134.3 (d); 129.3 (d); 128.9 (d); 126.9 (d); 125.9 (t); 124.2 (d); 122.9 (d); 120.1 (s); 119.2 (s); 117.0 (s); 110.2 (d); 82.0 (d); 80.2 (s); 57.8 (s); 46.1 (t); 42.4 (t); 40.0 (t); 28.9 (q, 3C) ppm. m/z (ESI$^+$): 470.0 [M+H]$^+$; 492.0 [M+Na]$^+$. IR (ν_{max}/cm^{-1}): = 3403; 2976; 2930; 1700; 1623; 1606; 1587. Anal. Calcd for $C_{28}H_{27}N_3O_4$: C, 71.62; H, 5.80; N, 8.95. Found: C, 71.58; H, 5.78; N, 8.99.

tert-Butyl 10-hydroxy-3a-(2-hydroxyethyl)-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**15b**). Yield: 80%. Eluent: Hexane/AcOEt: 1:4. M.p.: 207-209 °C dec. (CH_2Cl_2/Hexane). ^{1}H-NMR (300 MHz; C_3D_6O) δ: 9.06 (s, 1H); 8.96 (s, 1H, exchange with D_2O); 8.00 (dd, *J* = 8.1; 1.1 Hz, 1H); 7.98 (d, *J* = 9.2 Hz, 1H); 7.72 (dd, *J* = 7.1; 1.1 Hz, 1H); 7.56 (dd, *J* = 9.2; 2.7 Hz, 1H); 7.44 (d, *J* = 2.7 Hz, 1H); 7.32 (s, 1H); 7.27 (dd, *J* = 8.1; 7.1 Hz, 1H); 4.06 (m, 1H); 3.71-3.53 (m, 3H, 2H after exchange with D_2O); 2.80 (m, 1H); 2.42-2.14 (m, 4H); 1.62 (s, 9H). ^{13}C-NMR (75 MHz; C_3D_6O) δ: 178.8 (s); 154.3 (s); 154.2 (s); 146.4 (s); 146.3 (s); 145.6 (s); 136.4 (d); 135.8 (s); 129.3 (d); 128.7 (d); 125.8 (d); 125.7 (s); 123.9 (d); 122.1 (d); 119.3 (s); 117.2 (s); 109.6 (d); 82.7 (d); 79.6 (s); 58.7 (t); 58.6 (s); 45.2 (t); 41.4 (t); 40.1 (t); 28.1 (q, 3C). m/z (ESI$^+$): 474.2 [M+H]$^+$. IR (ν_{max}/cm^{-1}): 3414; 2959; 2927; 1702; 1638; 1622; 1608; 1586. Anal. Calcd for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87. Found: C, 68.44; H, 5.72; N, 8.91.

6.1.14 General procedure for Mannich reaction.

To a solution of compound **15** (1 mmol) in EtOH (25 ml) was added CH_2O (37% in H_2O, 4 mmol) and Me_2NH (40% in H_2O, 3 mmol). The reaction mixture was stirred at rt for 24-48 h. After consumption of starting material (monitored by TLC) the mixture was concentrated under vacuum and H_2O (25ml) was added. The mixture was extracted with CH_2Cl_2 (15 ml) for three time. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The product was purified trough silica gel chromatography.

tert-Butyl 3a-allyl-9-((dimethylamino)methyl)-10-hydroxy-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**16a**). Yield: 56%. Eluent: AcOEt/MeOH 20:1. Orange solid. M.p.: 125-127 °C dec. (CH_2Cl_2/Hexane). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 9.34 (s, 1H); 8.16 (dd, *J* = 8.1; 1.0 Hz, 1H); 7.97 (d, *J* = 9.1 Hz, 1H); 7.57 (dd, *J* = 7.1; 1.0 Hz, 1H); 7.47 (d, *J* = 9.1 Hz, 1H); 7.26 (dd, *J* = 8.1; 7.1 Hz, 1H); 7.08 (s, 1H); 5.78 (m, 1H); 5.23 (dd, *J* = 8.5; 1.4 Hz, 1H); 5.15 (d, *J* = 10.5 Hz, 1H); 4.28 (s, 2H), 4.10 (m, 1H); 2.88 (m, 2H); 2.73 (m, 2H); 2.50 (s, 6H); 2.18 (m, 2H); 1.64 (s, 9H) ppm. ^{13}C-NMR (75 MHz; $CDCl_3$) δ: 179.8 (s); 155.5 (s); 153.6 (s); 146.0 (s); 145.7 (s); 145.4 (s); 135.4 (s); 132.8 (d); 131.7 (d); 129.1 (d); 128.1 (d); 126.7 (d); 124.6 (d); 123.8 (s); 122.0 (d); 119.9 (t); 118.3 (s); 117.1 (s); 111.9 (s); 81.9 (d); 80.5 (s); 57.5 (t); 57.5 (s); 45.8 (t); 45.6 (q, 2C); 42.9 (t); 39.9 (t); 28.5 (q, 3C) ppm. m/z (ESI$^+$): 527.0 [M+H]$^+$; 550.0 [M+Na]$^+$. IR (ν_{max}/cm^{-1}): 3436; 3061; 2976; 2784; 1704; 1644; 1618; 1606; 1586. Anal. Calcd for $C_{31}H_{34}N_4O_4$: C, 70.70; H, 6.51; N, 10.64. Found: C, 70.68; H, 6.48; N, 10.67.

tert-Butyl 9-((dimethylamino)methyl)-10-hydroxy-3a-(2-hydroxyethyl)-7-oxo-3,3a,7,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridine-1(2H)-carboxylate (**16b**). Yield: 60%. Eluent: AcOEt/MeOH 19:1. Orange solid. M.p.: 157-159 °C dec. (AcOEt/Hexane). ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 9.11 (s, 1H); 7.86 (d, *J* = 7.7 Hz, 1H); 7.63 (d, *J* = 9.1 Hz, 1H); 7.41 (d, *J* = 6.9 Hz, 1H); 7.22 (d, *J* = 9.1 Hz, 1H); 7.16 (s, 1H); 7.10 (m, 1H); 6.50 (br s, 1H, exchange with D_2O); 4.40 (d, *J* = 14.7 Hz, 1H); 4.22 (d, *J* = 14.7 Hz, 1H); 3.90 (m, 1H); 3.75 (br s, 2H); 2.68 (m, 1H); 2.48 (s, 6H); 2.38 (m, 3H, 2H after exchange with D_2O); 2.09 (m, 2H); 1.51 (s, 9H) ppm. ^{13}C-NMR (75 MHz; $CDCl_3$) δ: 179.5 (s); 155.5 (s); 153.9 (s); 145.8 (s); 145.6 (s); 144.9 (s); 134.9 (s); 131.8 (d); 128.6 (d); 128.0 (d); 126.7 (d); 124.5 (d); 123.7 (s); 121.9 (d); 118.0 (s); 116.8 (s); 112.3 (s); 82.0 (d); 80.8 (s); 59.7 (t); 57.9 (s); 56.4 (t); 45.6 (t); 44.9 (q, 2C); 41.4 (t); 40.6 (t); 28.7 (q; 3C) ppm. m/z (ESI$^+$): 531.8 [M+H]$^+$. IR (ν_{max}/cm^{-1}): 3435; 2974; 2954; 2928; 2882; 1698; 1645; 1619; 1606; 1584. Anal. Calcd for $C_{30}H_{34}N_4O_5$: C, 67.91; H, 6.46; N, 10.56. Found: C, 67.88; H, 6.43; N, 10.56.

6.1.15 Synthesis of 3a-allyl-9-((dimethylamino)methyl)-10-hydroxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**17**).

To a solution of compund **16a** (1 mmol) in MeOH (20 ml) was added TMSCl (5 mmol). The reaction mixture was stirred at rt for 6 h. After completion the mixture was poured into a sat. $NaHCO_3$ (25 ml) and was

extracted with CH_2Cl_2 (15 ml) for three time. The organic layer was dried over Na_2SO_4 and was concentrated under vacuum. The product was purified trough silica gel chromatography.

3a-Allyl-9-((dimethylamino)methyl)-10-hydroxy-1,3,3a,14a-tetrahydrobenzo[g]pyrrolo[2',3':2,3]indolo[1,7-ab][1,8]naphthyridin-7(2H)-one (**17**). Yield: 78%. Eluent: AcOEt/MeOH 19:1. Orange solid. M.p.: 285-287 °C dec. (CH_2Cl_2/Hexane). ^{1}H-NMR (300 MHz; DMSO) δ: 9.30 (s, 1H); 7.88 (d, *J* = 7.1 Hz, 1H); 7.79 (d, *J* = 9.1 Hz, 1H); 7.70 (d, *J* = 7.1 Hz, 1H); 7.47 (d, *J* = 9.1 Hz, 1H); 7.20 (m, 1H), 6.07 (s, 1H); 5.70 (ddd, *J* = 17.0; 10.1; 2.0 Hz, 1H); 5.12 (d, *J* = 17.0, 1H); 5.00 (dd, *J* = 10.1; 2.0 Hz. 1H); 4.04 (s, 2H); 2.97 (m, 1H); 2.80 (m, 1H); 2.65 (m, 1H); 2.50 (m, 1H); 2.29 (s, 6H); 2.05 (m, 2H) ppm. ^{13}C-NMR (75 MHz; DMSO) δ: 178.9 (s); 154.1 (s); 146.1 (s); 145.9 (s); 145.6 (s); 173.8 (s); 135.0 (d); 134.0 (d); 129.3 (d); 128.4 (d); 126.6 (d); 124.6 (s); 123.8 (d); 122.7 (d); 119.3 (t); 118.5 (s); 116.5 (s); 115.4 (s); 84.6 (d); 58.5 (s); 57.3 (t); 55.4 (t); 45.3 (t); 45.1 (q, 2c); 43.1 (t) ppm. m/z (ESI$^+$): 427.0 $[M+H]^+$. IR (ν_{max}/cm^{-1}): 3435; 3067; 2919; 2850; 1648; 1618; 1605; 1586. Anal. Calcd for $C_{26}H_{26}N_4O_2$: C, 73.22; H, 6.14; N, 13.14. Found: C, 73.26; H, 6.18; N, 13.11.

6.2 Biological evaluation.

6.2.1 Inhibition Growth Assay.

HeLa (human cervix adenocarcinoma cells) were grown in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.); H-460 (large cell lung carcinoma) and MSTO-211H (human biphasic mesothelioma cells) were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 2.38 g/L Hepes, 0.11 g/L pyruvate sodium and 2.5 g/L glucose. 1.5g/L $NaHCO_3$, 10% heat-inactivated fetal calf serum (Invitrogen), 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B (Sigma Chemical Co.) were added to the media.

The cells were cultured at 37°C in a moist atmosphere of 5% carbon dioxide in air. Cells (3-4 x 10^4) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, various concentrations of the test agents were added to the complete medium and incubated for a further 72 h. A Trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as GI_{50} values, i.e., the concentration of the test agent inducing 50% reduction in cell number compared with control cultures.

6.2.2 Linear flow dichroism.

Linear dichroism (LD) measurements were performed on a Jasco J500A circular dichroism spectropolarimeter, converted for LD and equipped with an IBM PC and a Jasco J interface. Linear dichroism was defined as:

$LD_{(\lambda)} = A_{//(\lambda)} - A_{\perp(\lambda)}$

where $A_{//}$ and $A_{\perp}$ correspond to the absorbances of the sample when polarized light was oriented parallel or perpendicular to the flow direction, respectively. The orientation was produced by a device designed by Wada and Kozawa[6] at a shear gradient of 500-700 rpm, and each spectrum was accumulated twice.

Aqueous solutions of DNA (1.9 x 10^{-3}M) in 10 mM TRIS, 1 mM EDTA (pH 7.0) and 0.01 M NaCl were used (ETN buffer). Spectra were recorded at 25°C at [drug]/[DNA]=0 and 0.08.

6.2.3 Topoisomerase I-mediated DNA relaxation.

Supercoiled pBR322 plasmid DNA (0.25 µg, Fermentas Life Sciences) was incubated with 2U topoisomerase I (human recombinant topoisomerase I, TopoGen) and the test compounds as indicated, for 60 min at 37 °C in 20 µL reaction buffer.

Reactions were stopped by adding 4 µL stop buffer (5% sodium dodecyl sulfate (SDS), 0.125% bromophenol blue, and 25% glycerol), 50 µg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel at room temperature. The gels were stained with ethidium bromide 1 µg/mL in TAE buffer (0.04 M Tris-acetate and 0.001 M EDTA), transilluminated by UV light, and fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

6.2.4 Topoisomerase I-mediated DNA cleavage.

Reaction mixtures (20 µL) containing 35 mM Tris-HCl (pH = 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, 0.01% bovine serum albumin, 20 ng pBR322 plasmid DNA (Fer- mentas Life Sciences), 5 U topoisomerase I (human recombinant topoisomerase I, TopoGen) and test compounds at indicated concentrations were incubated for 60 min at 37 °C. Reactions were stopped by adding 4 µL of stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol), 50 µg/ mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel containing ethidium bromide 0.5 µg/mL (Sigma) at room temperature in TBE buffer (0.09 M Tris-borate and 0.002 M EDTA), transilluminated by UV light, and fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

6.3 Computational analysis.

The structure of the receptor was obtained from the crystal structure of the topoisomeraseI-DNA-topotecan ternary complex (PDB code: 1K4T) and prepared as previously described[32]. Docking calculations were performed with the software PLANTS[33]. The search space was defined by using the crystallographic ligand center of mass coordinates as the binding site center (x = 49.3764, y = 46.9081 and z = 48.2818) and the binding site radius was set to 14 Å, corresponding to the ligand radius of gyration augmented by 6 Å. Maximum accuracy was requested by setting the search_speed parameters to “speed1”. All the other parameters were used as default. Ligands were prepared using MOE 2015[34], and processed by

SPORES in order to assign the correct atom types required for docking calculations with PLANTS[35]. By using this setup, the overlay between the lowest energy docked conformation of topotecan (binding energy = -129.4 kcal/mol, computed with the CHEMPLP scoring function)[36] and its crystallographic structure was excellent, with a RMSD of 1.52 Å, computed with VMD 1.9.1[37].

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- Design and synthesis of a new scaffold of Topo I inhibitors
- The sequential Pd-catalyzed N-arylation/acylation reaction was the key step for the synthesis
- The new compounds were evaluated for their antiproliferative activity against three cancer cell lines such as H-460, HeLa and MSTO-211H
- Compound 13 exhibited the most potent inhibitory activity
- Computational binding mode analysis has been performed on the most active compounds