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Synthesis and topoisomerase I inhibitory activity of a novel diazaindeno[2,1-*b*]phenanthrene analogue of Lamellarin D

Salvatore Cananzi [a], Lucio Merlini [a], Roberto Artali [b], Giovanni Luca Beretta [c], Nadia Zaffaroni [c], Sabrina Dallavalle [a,*]

[a] Department of Molecular and Agrifood Sciences, Università di Milano, Via Celoria 2, 20133 Milano, Italy
[b] Department of Pharmaceutical Sciences 'P. Pratesi', Università di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[c] Department of Experimental Oncology and Molecular Medicine, Molecular Pharmacology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milano, Italy

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ABSTRACT

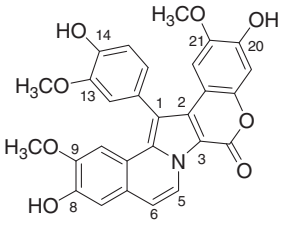
A novel 5-oxa-6a,8-diazaindeno[2,1-*b*]phenanthren-7-one scaffold was designed and synthesized as an active analogue of the cytotoxic marine alkaloid Lamellarin D. The design was based on molecular modeling of the site of interaction of Lamellarin D with DNA–topoisomerase I cleavable complex, whereas the synthesis capitalized on a simple Friedel–Crafts cyclization of indole to a β-carbolinone nucleus. The product exhibited topoisomerase I poisoning activity and submicromolar cytotoxicity on human non-small cell lung cancer H460 cell line.

1. Introduction

Nature continues to be the most prolific source of biologically active compounds. Indeed, more than 60% of the recently marketed drugs have been isolated or derived from natural products.[1]

The marine environment has proven to be a very rich source of potent compounds. The oceans, with their more than two million species of plants and animals, offer a vast reservoir for new compounds: more than 12,000 new chemical entities have so far been discovered, their number being increased every year by several hundreds of new marine compounds, a significant percentage of which typically show antitumor activity.[2]

Lamellarins are a group of marine alkaloids. The first four members of this group, Lamellarins A–D, were originally isolated in 1985 by Faulkner and co-workers[3] from six specimens of the marine prosobranch mollusc *Lamellaria* sp. Since then, more than 70 lamellarins and structurally-related pyrrole alkaloids have been isolated from molluscs, ascidians, and sponge species.[4] Most of them possess a common 6*H*-[1]benzopyrano-[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline ring system, and differ in the number and position of the hydroxy and methoxy groups on the scaffold.



Lamellarin D (1)

Chart 1. Structure of Lamellarin D.

Lamellarins and related pyrrole-derived alkaloids have shown promising biological activities such as antitumor activity, reversal of multidrug resistance (MDR), and HIV-1 integrase inhibition activity.[4] Lamellarin D (Lam-D, **1**, Chart 1) is one of the most cytotoxic compounds in the series.[4a,5]

This compound exhibits potent anticancer activity at nanomolar concentration. Recently, Bailly and co-workers reported that Lam-D is a potent poison of the enzyme topoisomerase I (topI).[6] Interactions between Lam-D and specific aminoacid residues of the topI–DNA complex have been identified from molecular modeling studies.[6] Additionally, the proapoptotic activity of Lam-D has been correlated with its ability to promote DNA cleavage through stabilization of topI–DNA covalent complexes,[7] similarly to what happens with camptothecins.[8]

* Corresponding author. Tel.: +39 2 50316818; fax: +39 2 50316801.
E-mail address: sabrina.dallavalle@unimi.it (S. Dallavalle).

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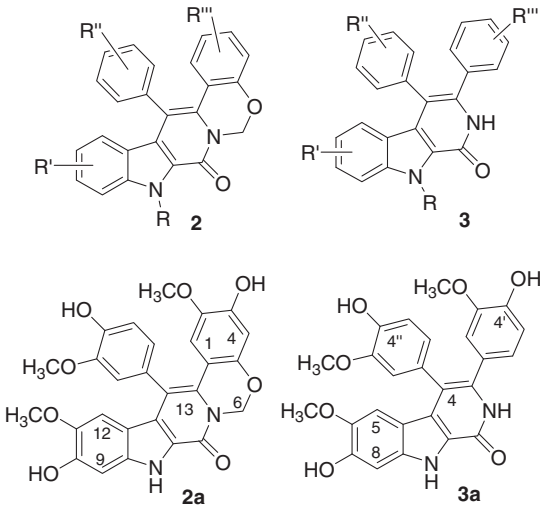


Chart 2. Chemical structure of compounds **2** and **3**.

Several strategies for the synthesis of lamellarins and their analogues have been devised by numerous research groups.[9] The synthesis of Lam-D derivatives with different substitution patterns on the pentacyclic framework would allow to delineate comprehensive structure–activity relationships.[6b,10] Hence, the hydroxy groups at both C-8 and C-20 position and the planar pentacyclic core are crucial for cytotoxic activity and for topI inhibition. Moreover, most of the Lam-D derivatives with an opened lactone ring were found to be considerably less cytotoxic than the parent compound except when a potential lactonization was preserved.[11]

All the analogues of lamellarins so far prepared have maintained completely or in part their pyrrolo[2,1-*a*]isoquinoline skeleton. Nevertheless, the identification of alternative molecular scaffolds endowed with similar properties is highly desirable.

We were intrigued by the possibility of obtaining cytotoxic compounds containing a new scaffold, that could mimic the crucial interactions of lamellarins with the topI–DNA complex. To our knowledge, no attempt of this kind has been reported in the literature.

Therefore, on the basis of Lam-D structure, we designed the replacement of the pyrrolo[2,1-*a*]isoquinoline core with a β-carbolin-1-one moiety and the substitution of the labile lactone ring with a 3,4-dihydro-2*H*-[1,3]oxazine (compounds **2**, Chart 2). Simplified analogues (**3**), that according to the modeling studies, could satisfy these requirements as well, were also synthesized.

2. Results and discussion

2.1. Molecular modeling

Molecular modeling studies were performed on compounds **2a** and **3a** maintaining the crucial hydroxy and methoxy groups of Lam-D. A preliminary simple MM2 overlay study on the isolated molecules indicated a satisfactory superposition of the two 3D structures **2a** and **3a** with that of **1**. Therefore, molecular docking studies were performed to elucidate the mode of interaction between the planned compounds and the DNA–topI complex. We started from the crystal structure[8] of human topI covalently linked to double-stranded DNA and bound to topotecan, a camptothecin in clinical use. In particular, in our initial model, at first topotecan was replaced with compound **2a** and then the structure of the resulting ternary complex was minimized. As shown in Figure 1, the ligands were placed in the DNA–topI complex in almost the same orientation. The comparative analysis of the best poses obtained from the docking experiments shows how the intercalation binding site of both complexes results from conformational changes of the phosphodiester bond between the +1 (upstream) and −1 (downstream) base pairs of the uncleaved strand. This effectively splits the DNA duplex allowing both compounds to intercalate within the site, stabilized mainly by the formation of stacking interactions with both the +1(CG) and −1(AT) base pairs.

The binding mode of compound **2a** revealed that the molecule forms base-stacking interactions with both the −1 and +1 base pairs, with a strong π–π stacking between the pyridone ring and G^{-1}, and that the ternary structure is further stabilized by five hydrogen bonds with specific amino acid residues of the protein. According to the Lam-D binding model,[6a] two hydroxy groups attached to the main aromatic moiety (C-3 and C-10, corresponding to C-20 and C-8, respectively, in Lam-D) are located at hydrogen bond distance from the Asn^{722} and Glu^{356} residues of the enzyme, whereas the carbonyl is interacting with the Arg^{364} residue. This disposition is in perfect agreement with the interaction pattern in the lamellarin series. Interestingly, the phenol ring in the C-13 position of **2a** is pointing out to the protein–DNA interface, showing a so far never reported direct interaction between the methoxy group on this ring and the protein residue Asn^{352}. It is important to note that the presence of this interaction for both compounds **2a** and **3a** could mean that this extra ring (on C-13 in **2a** and C-4 in **3a**, respectively), although not essential for cytotoxicity, can be useful to modulate the binding to the enzyme. Finally, the free indole NH group did not seem to play a role: in fact, the *N*-methyl substituted derivative showed the same interaction pattern and the same free energy of binding found for **2a** (data not shown). The interactions between compound **2a** and the DNA–topI complex are reported in Figure 1.

The same experiment was run also on the compound **3a**. The structure of the complex thus obtained was fully consistent with that of the compound **2a** and Lam-D. Indeed, also compound **3a** is characterized by the presence of base-stacking interactions with both the −1 and +1 base pairs, and the ternary structure is again stabilized by hydrogen bonds with the same residues of the protein, as shown in Figure 1(C). The two hydroxyl groups attached to the main aromatic moiety (C-7 and C-4′, corresponding to C-8 and C-20 in Lam-D) form a hydrogen bond with Asn^{722} and Glu^{356}. Differently from **2a**, in compound **3a** the carbonyl group is closer to Arg^{364} residue. As mentioned above, the phenol ring in the C-4 position shows a hydrogen bond between the methoxy group and the protein residue Asn^{352}. The two compounds are differently oriented in the binding site because of their different torsion angles τ_1 and τ_2, as shown in Figure 2.

In fact, while compound **2a** adopt an almost completely planar conformation of the main skeleton, with angles τ_1 and τ_2 of −2.3° and 82.2°, respectively, **3a** is more flexible and the interaction with the enzyme pocket leads to a partial disruption of the planarity, with angles of −24.1° (τ_1) and 84.0° (τ_2).

The modeling analysis suggests that the two compounds are placed in a similar way at the DNA–topI interface and the obtained molecular models confirm the possibility of the binding of the designed compounds to the enzyme–DNA complex.

The promising binding provided structural support for synthesizing these novel Lam-D analogues. Accordingly, we developed a general synthetic route to compounds with a diazaindeno[2,1-*b*]phenanthrene skeleton.

In order to check the feasibility of the sequence, a route for the synthesis of the structurally simpler 3,4-diphenyl-β-carbolin-1-one **3** was devised, that could be later extended to the preparation of the more complex derivative **2**.

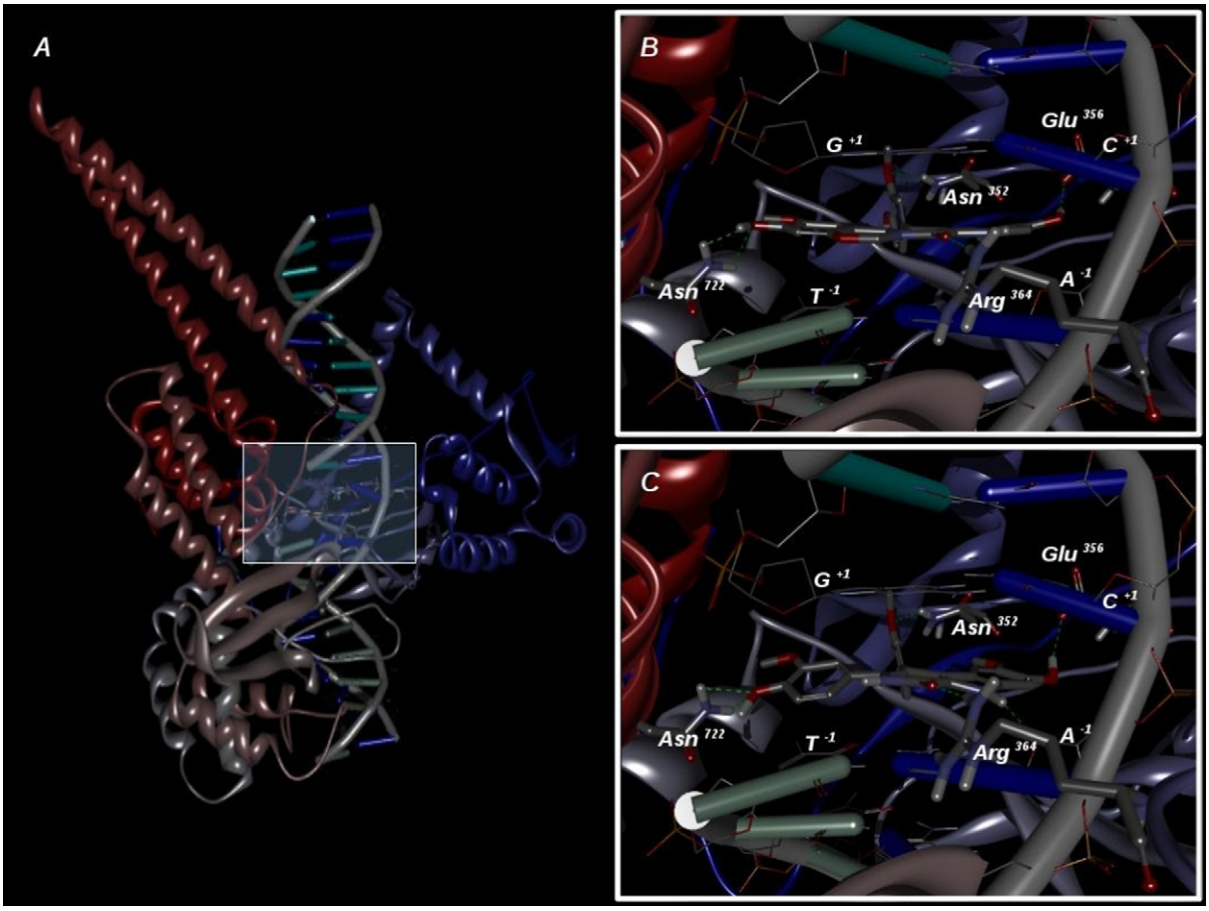


Figure 1. Theoretical model of **2a** and **3a** bound to the covalent topoisomerase I–DNA (PDB code: 1K4T). (A) Solid ribbon (protein) and ladder (DNA) representation of topoisomerase I–DNA. The transparent rectangle correspond to the position of the binding site used for the docking runs. (B) Best pose for compound **2a** (in stick). Topoisomerase I residues forming hydrogen bond interactions with **2a** are in stick, hydrogen bond interactions showed as green dot lines. (C) Best pose for compound **3a** (in stick). Topoisomerase I residues forming hydrogen bond interactions with **3a** are in stick, hydrogen bond interactions shown as green dot lines.

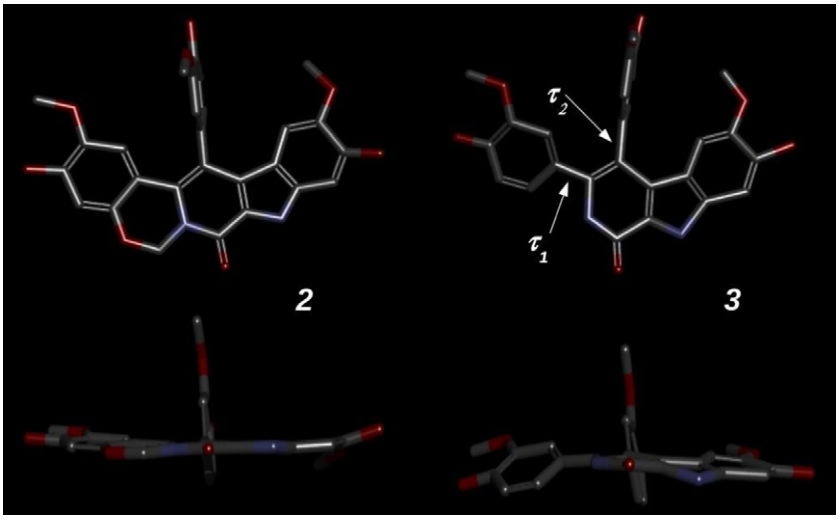
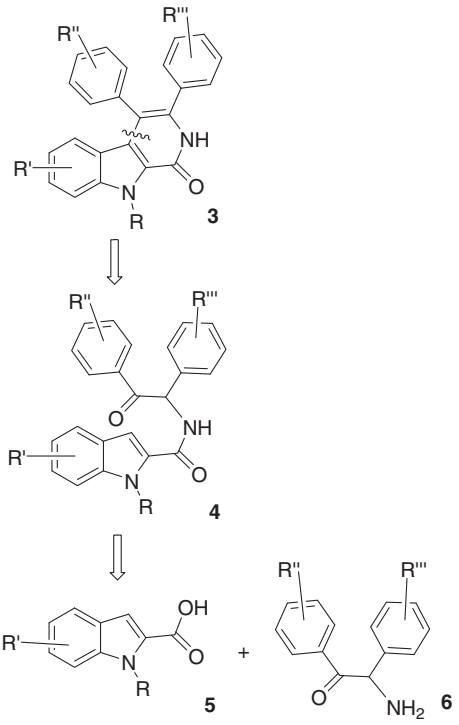


Figure 2. Graphical representation (in stick) of **2a** (left) and **3a** (right). τ_1 and τ_2 refer to the respective torsion angles, as discussed in the text. Atoms are coloured following the CPK notation.

2.2. Chemistry

The synthesis was envisioned to proceed via a key intramolecular Friedel–Crafts cyclization of indole-2-carboxylic acid β-oxoamide (**4**), on its turn obtained by coupling 2-indole carboxylic acid **5** and substituted 2-amino-1,2-diphenyl-ethanone **6** (Scheme 1). This strategy was based on an efficient procedure recently developed by us for the preparation of 3- or 4-substituted β-carbolinones.[12]

The 2-amino-1,2-diphenyl-ethanone fragment **15** was obtained starting from vanillic acid (**7**), following the sequence depicted in



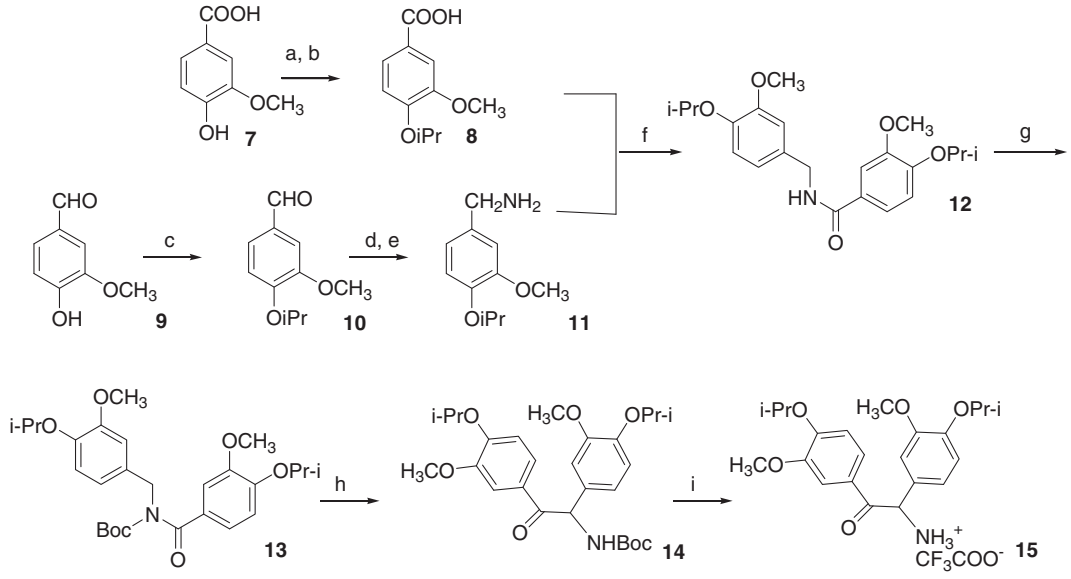
Scheme 1. Retrosynthetic approach to compounds **3**.

Scheme 2. The phenol group was protected as isopropyl ether **8**, that could be selectively cleaved at the end of the synthetic route providing the desired hydroxy–methoxy alternation. Protection of vanillin **9** as isopropylether **10**, followed by conversion into the corresponding oxime and reduction with Raney-Ni alloy, gave the benzylamine derivative **11**, that was coupled with **8** using HOBt (1-hydroxybenzotriazole) and WSC [*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide] in THF. After protection of the amide nitrogen with a BOC group, compound **13** was treated with an excess of LDA to obtain the α-aminoketone **14** via a N–C migration reaction, following a procedure described in the literature on different substrates.[13] The yield was optimized using three equivalents of LDA in the presence of DMPU (3%) at −78 °C. Interestingly, other strong bases, such as NaH, KH, KHMDS and *s*-BuLi with sparteine, did not give any acyl migrated product. Treatment with TFA in DCM at room temperature afforded compound **15** as a trifluoroacetate salt.

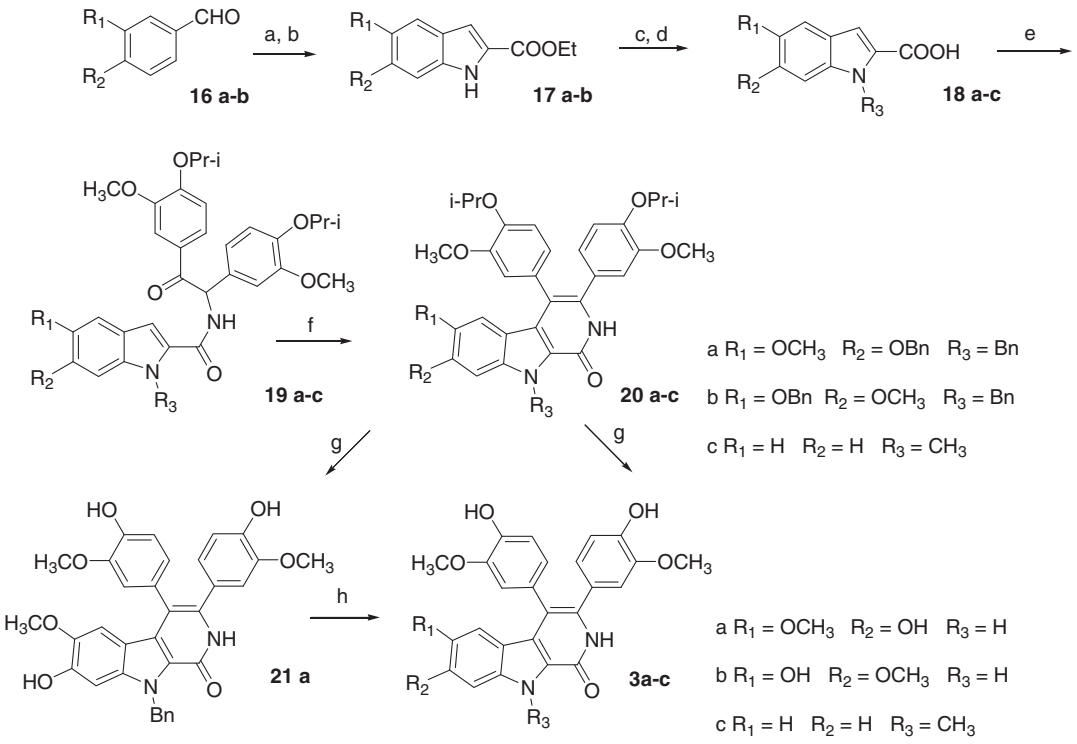
With the protected aminoketone in hand, attention turned to the indole fragment. We planned to synthesize differently substituted rings to verify whether a change in the position of the hydroxy groups could influence the cytotoxic activity. Compounds **17** were obtained by reacting aldehydes **16** with ethyl azidoacetate in the presence of sodium ethoxide at −15 °C in ethanol, followed by cyclization in xylene at reflux. *N*-alkylation and basic hydrolysis of the ester group gave the indolecarboxylic acids **18**, that were coupled with the previously obtained α-aminoketone **15** in the presence of HOBt and WSC in THF (Scheme 3). Subsequently, β-oxoamides **19** were treated with TFA to perform the crucial Friedel–Crafts cyclization step by which the β-carbolin-1-one derivatives **20** were obtained. The cleavage of the protecting groups was carried out with $AlCl_3$ in anisole under heating. The treatment of compound **20a** with $AlCl_3$ in DCM produced the *N*-benzyl protected compound **21a**.

The synthetic strategy reported in Schemes 2 and 3 was applied to the synthesis of compound **2**. To obtain the desired compound it was necessary to introduce an additional OH group on the benzylamine fragment, for the construction of the oxazine ring. Moreover, the new phenolic moiety had to be protected with a differently cleavable protecting group, in order to remove it in a different step with respect to the isopropyl ethers, that were to be maintained throughout the whole process.

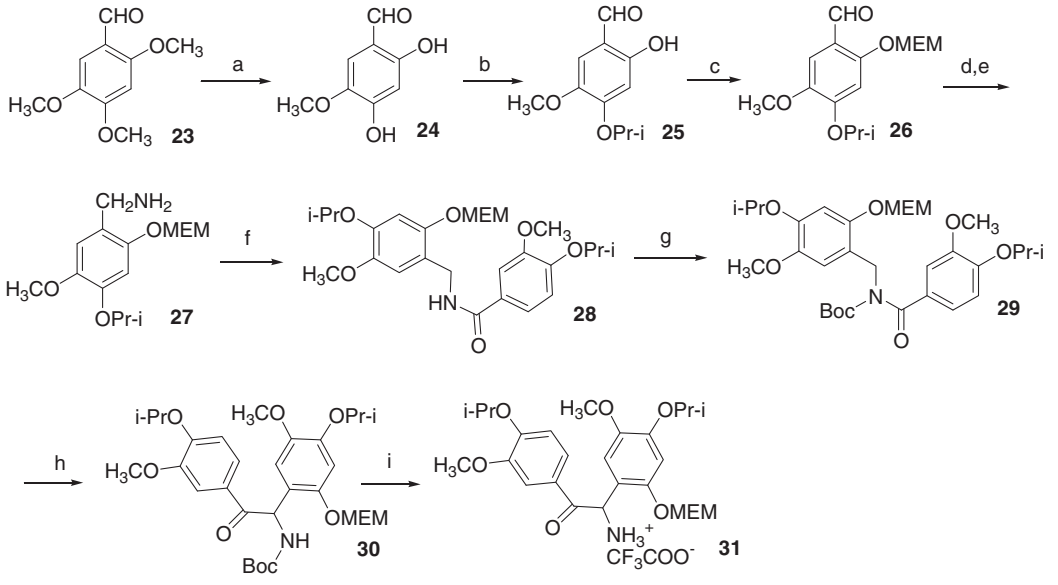
Due to the sensitivity of the free indole ring to acidic reagents, it appeared more appropriate to use indole synthon with a stable protecting group, such as methyl. This choice was supported by the modeling studies that indicated no specific interaction due to the NH indole moiety. The synthesis of compound **27** (Scheme 4) was performed starting from commercially available 2,4,5-trimethoxybenzaldehyde (**23**). Selective *ortho*/*para* methylether cleavage by $AlCl_3$ in DCM provided compound **24** in good yield. The treatment with 2-bromopropane and sodium bicarbonate in DMF allowed a selective protection of the *p*-hydroxy group, due



Scheme 2. Reagents and conditions: (a) iPrBr, K_2CO_3, DMF, reflux, 4 h, 98%; (b) KOH, ethanol, H_2O, reflux, 4 h, 86%; (c) iPrBr, Na_2CO_3, DMF, reflux, 4 h, 91%; (d) $NH_2OH{\cdot}HCl$, Py, ethanol, rt, 90 min, 71%; (e) Ni/Al alloy, NaOH 2 N, ethanol, rt, 90 min, 98%; (f) HOBt, WSC, THF, rt, 24 h, 96%; (g) di-*tert*-butyldicarbonate, DMAP, acetonitrile, rt, 16 h, 95%; (h) LDA, DMPU, THF, −78 °C, 8 h, 55%; (i) CF_3COOH, DCM, rt, 2 h, 98%.



Scheme 3. Reagents and conditions: (a) ethyl azidoacetate, EtONa, EtOH, −15 °C, 3 h, 73–88%; (b) xylene, reflux, 12 h, 32–76%; (c) for **17a**–**b**: BnBr, NaH, DMF, 0 °C–rt, 2 h, 67–97%; (d) LiOH, H_2O, THF, MeOH, rt, 4 h, 64–76%; (e) HOBt (1-hydroxybenzotriazole), WSC, **15**, TEA, THF, rt, 14 h, 56–58%; (f) TFA, acetonitrile, reflux, 15 min, 58–71%; (g) $AlCl_3$, DCM, 0 °C–rt, 24 h, 30–48%; (h) for **3a**: $AlCl_3$, anisole, 100 °C, 5 h, 20%.



Scheme 4. Reagents and conditions: (a) $AlCl_3$, DCM, N_2, rt, 19 h, 84%; (b) iPrBr, $NaHCO_3$, DMF, 70 °C, 4 h, 34%; (c) MEMCl, NaH, THF, rt, 12 h, 94%; (d) $NH_2OH{\cdot}HCl$, Py, EtOH, 0 °C, 90 min, 89%; (e) Al/Ni alloy, EtOH, rt, 90 min, 97%; (f) **8**, HOBt, WSC, THF, rt, 24 h, 95%; (g) di-*tert*-butyl dicarbonate, DMAP [4-(dimethylamino)pyridine], acetonitrile, rt, 16 h, 67%; (h) LDA (lithium diisopropylamide), DMPU [1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone], THF, −78 °C, 6 h, 29%; (i) CF_3COOH, DCM, 0 °C, 2 h.

to the different reactivity of the two phenolic hydroxyls, one of them H-bonded to the *ortho* carbonyl. Finally, compound **25** was protected with a MEM (2-methoxyethoxymethyl) group by reacting it with MEM chloride in the presence of NaH. The aldehyde **26** was then converted into the corresponding oxime, then reduced using Ni/Al alloy and NaOH in ethanol to give benzylamine **27** in good yield.

Synthesis of the *N*-BOC α-aminoketone was achieved following the same procedure described in Scheme 2. In this case the *N*-BOC removal resulted troublesome, due to the presence of the MEM protecting group, which is cleavable under the same acidic conditions. For this reason, alternative procedures were investigated, such as thermolytic treatment in solid phase under nitrogen, silica gel promoted cleavage, and cerium ammonium nitrate treatment

in acetonitrile. All these efforts failed to produce a selective removal. Finally, a kinetic cleavage of the two protecting groups in acidic conditions was attempted. The reaction was carried out in DCM and TFA under nitrogen at 0 °C (monitoring the course by ^{1}H NMR analysis) and afforded α-aminoketone **31** as a trifluoroacetate salt.

The coupling with indolecarboxylic acid **32** was performed following the same procedure described in Scheme 3 and gave amide **33** (Scheme 5). Attempts to obtain the key β-carbolin-1-one scaffold following the intramolecular Friedel–Crafts cyclization failed, due to the instability of MEM group in acidic conditions: in the presence of TFA it released formaldehyde that immediately attacked the extremely reactive position 3 of the indole moiety. To avoid this side reaction, other conditions were investigated. Interestingly, it was found that treatment of **33** with two equivalents of trimethylsilyl iodide (TMSI) afforded the desired compound **34**. Ring-closure to give the tetracyclic compound **35** was achieved using diiodomethane in DCM under basic conditions. Finally, removal of protecting isopropyl and benzyl ether groups with $AlCl_3$ in DCM at room temperature gave the final compound **2b** in good yield.

To investigate the role of the oxygenated groups on the aromatic rings, the unsubstituted analogue **38** was synthesized following a similar sequence (Scheme 6).

2.3. Biological activity

2.3.1. Cytotoxicity studies

The antiproliferative effect of the new molecules was determined at 1 h exposure on human non-small cell lung cancer H460 cell line (Table 1). Topotecan, a camptothecin derivative in clinical use, and Lam-D were used as reference drugs. The antiproliferative effect of the most active compounds was also evaluated at 72 h exposure.

All the tested derivatives, except for **2b**, exhibited a moderate cytotoxicity, much lower than Lamellarin D. In a general overview, compounds possessing the more flexible skeleton of **3** were less potent in reducing cell growth. It can be seen that the cytotoxic potency varies considerably depending on the presence of free OH substituents on the ring system. Comparing compounds **20a–21a–3a** we can notice that **20a**, with bulky substituents on all the aromatic rings, proved to be almost totally inactive, whereas progressive removal of protecting groups (**21a**, **3a**) corresponded to an increase in cytotoxic activity. Compounds **3a** and **3c** showed the same cytotoxic activity, confirming that the introduction of a methyl group on the indole nitrogen was not deleterious for activity. Collectively, the modest activity shown by this series of compounds may be presumably due to lack of planarity, as already observed for other analogues of Lam-D.[11]

Remarkably, compound **2b**, possessing a planar 3,4-dihydro-2*H*-[1,3]oxazine core, showed a significant increase in cytotoxicity compared to the previously discussed derivatives. This is consistent with the docking experiments. Moreover, comparing **2b** with the unsubstituted analogue **38**, it can be seen that deletion of the OH groups has a dramatic effect on the activity, giving a 30-fold drop in cytotoxicity. This can probably be attributed to the capacity of **2b** to form hydrogen bonds with the active site, as described for Lam-D[6a] and predicted by molecular modeling.

2.3.2. Topoisomerase I-dependent DNA cleavage assay

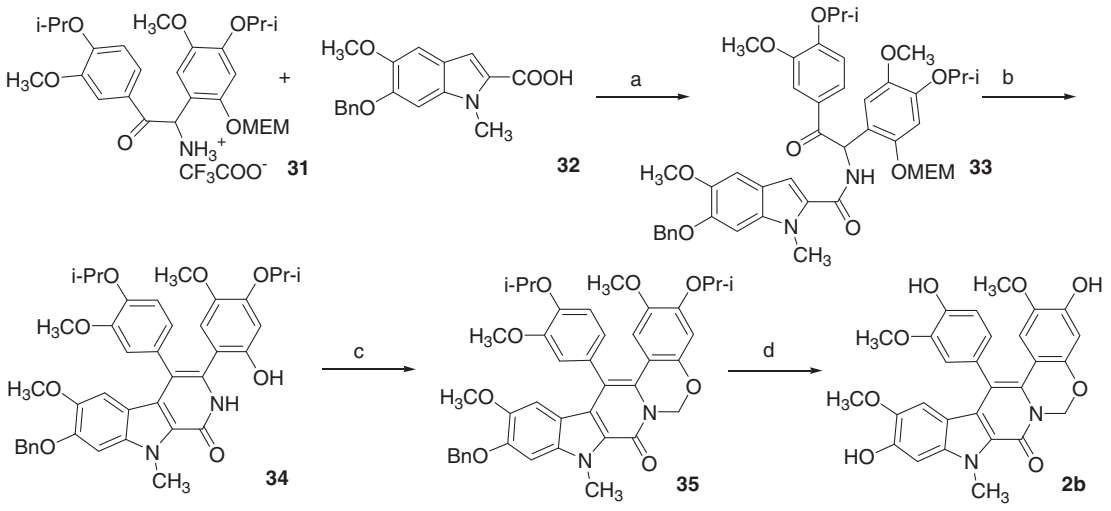
Topoisomerase I-mediated DNA cleavage experiments were performed to investigate the ability of the new compounds to stimulate DNA damage. Lam-D and SN-38 (10-hydroxy-7-ethylcamptothecin) were used as reference compounds.

The results of topoisomerase I-mediated DNA damage are presented in Figure 3. Marked topI poisoning was detected for SN-38, used as a positive control. Similarly, DNA damage was observed in the presence of Lam-D, indicating that this natural product also stabilizes DNA–topI covalent complex. Virtually no cleavage was detected in the presence of **20a**, **21a**, **3a**, this result being consistent with the very low cytotoxicity showed by these compounds. In contrast, compound **2b** was found to stimulate DNA cleavage by topI, although less efficiently than SN-38 and Lam-D. Interestingly, the new molecule showed the same cleavage profile of the controls, therefore validating the conclusion that **2b** can be identified as a new topI poison.

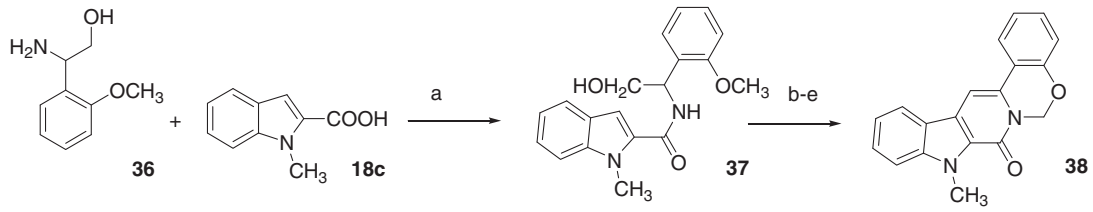
Overall, our results are consistent with previous findings on lamellarin derivatives, such as the critical importance of the planarity of the molecule skeleton.[11] In addition, the presence of OH–hydrogen bond donors positionally equivalent to hydroxy groups at C-8 and C-20 in Lam-D led to a strong gain in cytotoxicity.

3. Conclusions

In conclusion, on the basis of molecular modeling studies, we identified a new molecular scaffold that could mimic the



Scheme 5. Reagents and conditions: (a) HOBt, WSC, TEA, THF, rt, 26 h, 55%; (b) NaI, TMSCl, acetonitrile, −20 °C, 30 min, 31%; (c) CH_2I_2, DCM, reflux, 1 h, 12%; (d) $AlCl_3$, DCM, reflux, 6 h, 83%.



Scheme 6. Reagents and conditions: (a) HOBt, WSC, TEA, THF, rt, 2 h, 97%; (b) IBX (2-iodoxybenzoic acid), DMSO, acetonitrile, rt, 3 h; (c) TFA, acetonitrile, reflux, 15 min, 64%; (d) BBr_3, DCM, rt, overnight, 38%; e) CH_2I_2, DCM, reflux, 1 h, 28%.

Table 1
Antiproliferative activity of Lamellarin D analogues

	IC_{50} (μM)[a]	
	1 h	72 h
Topotecan	1.75 ± 0.22	0.016 ± 0.005
Lam-D	0.066 ± 0.004	0.003 ± 0.0006
20a	>150	–
20c	60.8 ± 2.6	–
21a	118.8 ± 5.6	–
3a	60.8 ± 5.3	–
3b	60.8 ± 4.2	–
3c	60.8 ± 7.1	–
2b	4.02 ± 0.95	0.35 ± 0.030
38	117.5 ± 3	–

[a] Drug concentration required for 50% reduction of cell growth as compared with untreated controls after 1 and 72 h exposure to the drug.

interactions of lamellarins with the topI–DNA complex. We designed the replacement of the pyrrolo[2,1-*a*]isoquinoline core with a β-carbolin-1-one moiety, while maintaining the crucial hydroxy and methoxy groups at the appropriate positions on the aromatic rings. The synthesized compounds were tested to evaluate their antiproliferative effect on human non small cell lung cancer H460 cell line and the topI-mediated DNA cleavage. Collectively, the compounds resulted less active than Lam-D. However, the obtained data provided information about the importance of a planar structure and of hydrogen bond donors at appropriate positions on the aromatic ring. In fact, compound **2b**, possessing a planar 3,4-dihydro-2*H*-[1,3]oxazine core, and phenolic OHs positionally equivalent to hydroxy groups at C-8 and C-20 in Lam-D, showed remarkable cytotoxicity and topI–DNA cleavage ability.

Therefore, we have reached the proof of concept that the scaffold of lamellarins can be modified to obtain new cytotoxic and topI-active compounds, provided that the crucial peripheral interactions are maintained.

4. Experimental section

4.1. General

All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined

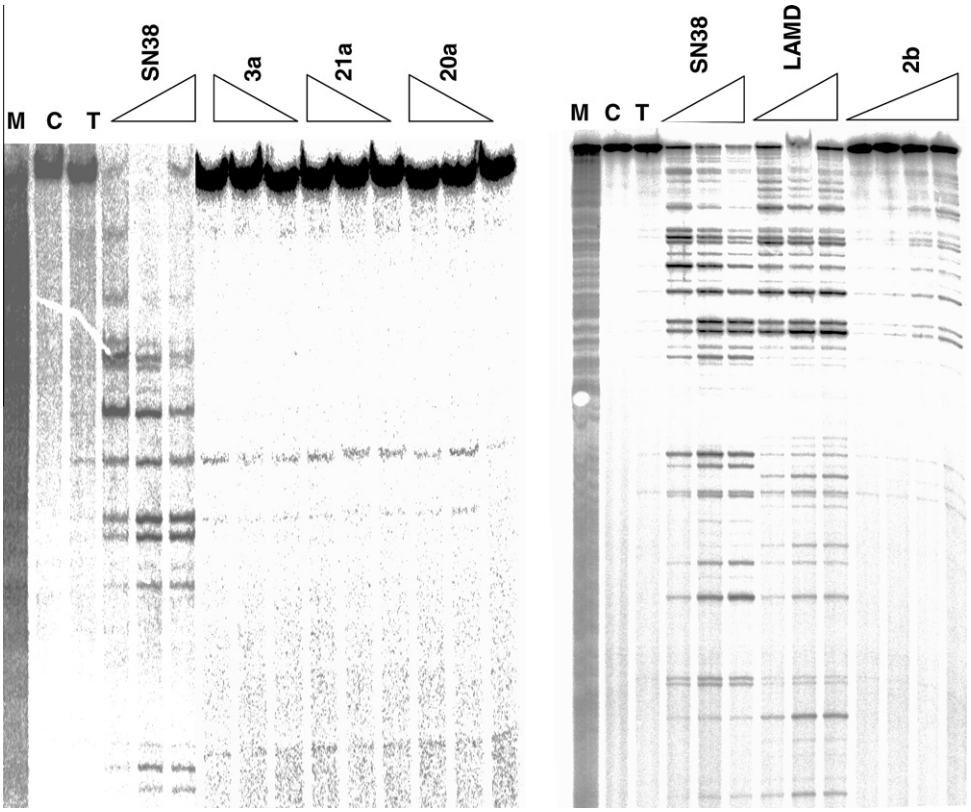


Figure 3. Topoisomerase I-mediated DNA cleavage by SN38, Lamellarin D, and analogues. Samples were reacted with 1, 10, and 50 μM (100 μM for **2b**) drug at 37 °C for 30 min. Reaction was then stopped by adding 0.5% SDS, 0.3 mg/mL of proteinase K, and incubating for 45 min at 42 °C before loading on a denaturing 8% polyacrylamide gel. C, control DNA; T, reaction without drug; M, purine markers.

in open capillaries. NMR spectra were recorded with a Bruker AMX 300 spectrometer. Mass spectra were recorded with a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer APEX II & Xmass software (Bruker Daltonics)-4.7T Magnet (Magnex). The Elemental Analyses were recorded with a CARLO ERBA EA 1108 instrument. Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et_2O) were obtained by distillation from sodium–benzophenone ketyl; dry dichloromethane was obtained by distillation from phosphorus pentoxide. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and all glassware were oven dried and/or flame dried. Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was conducted on TLC plates (silica gel 60 F_{254}, aluminum foil) visualized by UV light and spraying with phosphomolybdic acid and *p*-anisaldehyde.

4.2. 4-Isopropoxy-3-methoxybenzoic acid (8)

Vanillic acid **7** (12 g, 71.4 mmol) was dissolved in DMF (70 mL) under nitrogen atmosphere and K_2CO_3 (24.53 g, 0.177 mol) was added. The suspension was stirred for 15 min at room temperature and subsequently 2-bromopropane (35.11 g, 285.4 mmol, 26 mL) was added dropwise. After refluxing for 4 h, the suspension was filtered and the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate (80 mL) and the solution was washed with $NaHCO_3$ (3 × 20 mL) and brine (3 × 20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure giving 4-isopropoxy-3-methoxybenzoic acid isopropyl ester as a yellow solid (17.65 g, 98%). ^{1}H NMR ($CDCl_3$) δ: 7.62 (1H, dd, J = 1.7 and 8.4 Hz), 7.53 (1H, d, J = 1.7 Hz), 6.87 (1H, d, J = 8.4 Hz), 5.21 (1H, m), 4.62 (1H, m), 3.89 (3H, s), 1.38 (6H, d, J = 6.1 Hz), 1.34 (6H, d, J = 6.1 Hz).

The ester (17.60 g, 69.7 mmol) was dissolved in 90% ethanol (100 mL) and KOH (5.89 g, 104.9 mmol) was added. The solution was refluxed for 4 h. The solvent was evaporated under reduced pressure and the crude product was dissolved in water adding HCl solution until complete precipitation occurred (pH 3). The suspension was extracted with ethyl acetate, the organic layer was dried over Na_2CO_3, filtered and evaporated under reduced pressure to give compound **8** (12.70 g, 86%) as a white solid; mp 120 °C. ^{1}H NMR ($CDCl_3$) δ: 7.73 (1H, dd, J = 2.1 and 8.9 Hz), 7.59 (1H, d, J = 2.1 Hz), 6.91 (1H, d, J = 8.9 Hz), 4.66 (1H, m), 3.91 (3H, s), 1.41 (6H, d, J = 6.10 Hz). Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.88; H, 6.69.

4.3. 4-Isopropoxy-3-methoxybenzaldehyde (10)

Vanillin **9** (3.95 g, 25.9 mmol) was dissolved in DMF (20 mL) under nitrogen atmosphere and K_2CO_3 (4.13 g, 29.9 mmol) was added. The suspension was stirred for 15 min at room temperature and subsequently 2-bromopropane (6.40 g, 52.0 mmol, 4.88 mL) was added dropwise. After refluxing for 4 h, the suspension was filtered and the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate (20 mL) and the solution was washed with brine (3 × 15 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated affording compound **10** as a yellow solid (4.07 g, 81%); mp 114–116 °C. ^{1}H NMR ($CDCl_3$) δ: 9.84 (1H, s), 7.36–7.45 (2H, m), 6.97 (1H, d, J = 8.2 Hz), 4.70 (1H, m), 3.92 (3H, s), 1.43 (6H, d, J = 5.9 Hz). Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 68.09; H, 7.22.

4.4. (4-Isopropoxy-3-methoxyphenyl)methanamine (11)

A solution of **10** (4.0 g, 20.6 mmol) in 95% ethanol (44 mL) was treated with a solution of hydroxylamine hydrochloride (1.82 g, 26.2 mmol) in dry pyridine (8.13 mL) under stirring at room temperature for 90 min, then at 0 °C for 30 min to facilitate 4-isopropoxy-3-methoxybenzaldoxime precipitation. The white solid was filtered, dried (3.20 g, 74%) and used without further purification; mp 130 °C. ^{1}H NMR ($CDCl_3$) δ: 8.09 (1H, s), 7.23 (1H, d, J = 1.9 Hz), 7.03 (1H, dd, J = 1.9 and 8.2 Hz), 6.89 (1H, d, J = 8.2 Hz), 4.60 (1H, m), 3.89 (3H, s,), 1.40 (6H, d, J = 6.1 Hz).

A solution of the oxime (3.15 g, 15.0 mmol) in 95% ethanol (38.5 mL) was treated with an equal volume of 2 M NaOH followed by Al/Ni alloy (4 g) under stirring at room temperature for 90 min. The Al/Ni alloy was removed by filtration and washed with fresh ethanol. Filtrate and washings were combined, acidified with 0.8 M HCl (pH 2) and extracted with DCM (3 × 15 mL). The aqueous phase was treated with solid KOH up to pH 14 and extracted with diethyl ether (3 × 10 mL). The extracts, after drying over anhydrous Na_2SO_4 and removal of the solvent, provided **11** (2.88 g, 98%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 6.90 (1H, dd, J = 2.1 and 8.2 Hz), 6.80 (1H, d, J = 8.2 Hz), 6.75 (1H, d, J = 2.1 Hz), 4.56 (1H, m), 3.89 (3H, s), 3.75 (2H, s), 1.40 (6H, d, J = 6.10 Hz).

Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.62; H, 8.75; N, 7.12.

4.5. *N*-(4-Isopropoxy-3-methoxybenzyl)-4-isopropoxy-3-methoxybenzamide (12)

To a suspension of HOBt (1.89 g, 14.0 mmol) and WSC (2.68 g, 14.0 mmol) in THF (110 mL) were added under nitrogen atmosphere **8** (1.46 g, 6.9 mmol) and **11** (2.70 g, 13.8 mmol). The reaction mixture was stirred at room temperature for 24 h and the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate, the solution washed with $NaHCO_3$, HCl 2 M and brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford **12** as a yellow oil (2.58 g, 96%). ^{1}H NMR ($CDCl_3$) δ: 7.47 (1H, s), 7.28 (1H, s), 6.81–6.93 (4H, m), 6.37 (1H, br s), 4.45–4.70 (4H, m), 3.91 (3H, s), 3.85 (3H, s), 1.40 (6H, d, J = 6.2 Hz), 1.37 (6H, d, J = 6.3 Hz). ^{13}C NMR (DMSO-d_6) δ: 166.2, 150.5, 149.8, 149.6, 146.1, 133.4, 127.2, 120.9, 119.9, 116.5, 114.2, 112.5, 111.7, 71.0, 70.6, 56.0, 42.9, 22.5, 22.4. Anal. Calcd for $C_{22}H_{29}NO_5$: C, 68.20; H, 7.54; N, 3.61. Found: C, 68.24; H, 7.51; N, 3.70.

4.6. (4-Isopropoxy-3-methoxybenzoyl)-(4-isopropoxy-3-methoxybenzyl)-carbamic acid *tert*-butyl ester (13)

Compound **12** (2.20 g, 5.6 mmol) was dissolved in acetonitrile (23 mL) under nitrogen flow and DMAP (0.073 g, 0.6 mmol) and di-*tert*-butyldicarbonate (2.62 g, 12.0 mmol) were added. After stirring the solution at room temperature for 16 h, the solvent was evaporated under reduced pressure and the crude product was dissolved in DCM. The solution was washed with saturated aqueous $NaHCO_3$ and the organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (hexane:ethyl acetate, 6:4) affording **13** (2.60 g, 95%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.60 (1H, dd, J = 1.7 and 8.8 Hz), 7.53 (1H, d, J = 1.7 Hz), 6.77–6.94 (4H, m), 4.63 (1H, m), 4.48 (1H, m), 3.87 (3H, s), 3.83 (3H, s), 3.62 (1H, d, J = 9.9 Hz), 1.45 (9H, s), 1.35 (12H, d, J = 6.5 Hz). Anal. Calcd for $C_{27}H_{37}NO_7$: C, 66.51; H, 7.65; N, 2.87. Found: C, 66.41; H, 7.61; N, 2.80.

4.7. *tert*-Butyl 1,2-bis(4-isopropoxy-3-methoxyphenyl)-2-oxo ethylcarbamate (14)

LDA was prepared by mixing diisopropylamine (1.52 g, 15.0 mmol, 2.10 mL) and DMPU (1.05 mL) with *n*-BuLi (1.59 M in hexane, 9.4 mL, 15.0 mmol) in THF (14 mL) at −78 °C under nitrogen atmosphere. A solution of **13** (2.40 g, 4.9 mmol) in freshly distilled THF (43 mL) was added to the LDA solution at −78 °C. The mixture was stirred for 8 h, then it was quenched with aqueous NH_4Cl, diluted with ethyl acetate, washed with saturated aqueous NH_4Cl and brine, and dried over Na_2SO_4. Solvent evaporation followed by flash chromatography (hexane:ethyl acetate, 7:3) gave product **14** (1.21 g, 55%) as a white solid; mp 120–122 °C. ^{1}H NMR ($CDCl_3$) δ: 7.60 (1H, dd, J = 1.7 and 8.8 Hz), 7.53 (1H, d, J = 1.7 Hz), 6.77–6.94 (4H, m), 4.63 (1H, m), 4.48 (1H, m), 3.87 (3H, s), 3.83 (3H, s), 3.62 (1H, d, J = 9.9 Hz), 1.45 (9H, s), 1.35 (12H, d, J = 6.5 Hz). Anal. Calcd for $C_{27}H_{37}NO_7$: C, 66.51; H, 7.65; N, 2.87. Found: C, 66.47; H, 7.62; N, 2.91.

4.8. 2-Amino-1,2-bis(4-isopropoxy-3-methoxyphenyl)ethanone trifluoroacetate (15)

Compound **14** (1.21 g, 2.3 mmol) was dissolved in dry DCM (4 mL) under nitrogen flow and TFA (2 mL) was added at 0 °C. The solution was stirred at rt for 2 h. The solvent was evaporated under vacuo and the crude product was used for the next step without further purification.

4.9. Ethyl 6-(benzyloxy)-5-methoxy-1*H*-indole-2-carboxylate (17a)

Na (1.13 g, 49.2 mmol) was dissolved in anhydrous EtOH (45 mL), treated with 4-benzyloxy-3-methoxybenzaldehyde (3 g, 12.4 mmol), and the mixture was cooled to −15 °C. Ethyl azidoacetate (ethanol solution 25%, 49.2 mmol, 25 mL) was added dropwise to the reaction mixture. The mixture was stirred for 3 h at −15 °C, then warmed to room temperature and treated with saturated aqueous NH_4Cl. The beige solid that formed was collected by filtration and washed with water to afford (*Z*)-ethyl 2-azido-3-(4-(benzyloxy)-3-methoxyphenyl)acrylate (2.95 g, 73%); mp 102–103 °C. ^{1}H NMR ($CDCl_3$) δ: 7.53 (1H, d, J = 1.9 Hz), 7.26–7.50 (6H, m), 6.85–6.93 (2H, m), 5.22 (2H, s), 4.38 (2H, q, J = 7.2 Hz), 3.95 (3H, s), 1.41 (3H, t, J = 7.2 Hz).

The above compound (2.90 g, 8.1 mmol) was dissolved in xylenes (20 mL) and the solution was warmed at 130 °C for 12 h. The solution was cooled to room temperature and hexane was added (5 mL). The beige solid was collected by filtration. The filtrated was concentrated in vacuo and purified by flash chromatography (hexane:ethyl acetate, 8:2). The precipitate was combined with the product isolated by chromatography to afford **17a** (0.847 g, 32%); mp 218–220 °C. ^{1}H NMR ($CDCl_3$) δ: 8.63 (1H, br s), 7.23–7.50 (5H, m), 7.01–7.12 (2H, m), 6.83 (1H, s), 5.20 (2H, s), 4.35 (2H, q, J = 7.1 Hz), 3.92 (3H, s), 1.38 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 161.5, 148.9, 146.6, 136.7, 131.6, 128.4, 127.7, 126.9, 126.0, 120.8, 108.4, 103.0, 96.3, 71.0, 60.5, 56.2, 14.2. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 70.22; H, 5.79; N, 4.35.

4.10. 1-Benzyl-6-(benzyloxy)-5-methoxy-1*H*-indole-2-carboxylic acid (18a)

Indole **17a** (0.840 g, 2.6 mmol) was dissolved in dry DMF (8 mL) under nitrogen. The solution was cooled at 0 °C and NaH (mineral oil susp. 60%, 0.146 g, 3.7 mmol) was added slowly. The reaction mixture was warmed to room temperature and stirred for 45 min, then benzyl bromide (3.7 mmol, 0.43 mL) was added and the mixture was reacted for 2 h. When the reaction was completed the solvent was removed under vacuo. The crude product was dissolved in ethyl acetate and washed with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford ethyl 1-benzyl-6-(benzyloxy)-5-methoxy-1*H*-indole-2-carboxylate as a white solid (0.730 g, 67%); mp 190–191 °C. ^{1}H NMR ($CDCl_3$) δ: 7.11–7.47 (9H, m), 7.07 (1H, s), 6.91–7.00 (2H, m), 6.74 (1H, s), 5.69 (2H, s), 5.11 (2H, s), 4.29 (2H, q, J = 7.1 Hz), 3.91 (3H, s), 1.33 (3H, t, J = 7.1 Hz).

The ester (0.725 g, 1.7 mmol) was dissolved in 3:2:1 THF:MeOH:-H_2O (6 mL), treated with $LiOH{\cdot}H_2O$ (0.292 g, 6.9 mmol), and the mixture was stirred for 4 h at 25 °C. 1 N aqueous HCl was added and the solution was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford **18a** (0.500 g, 76%); mp 200 °C. ^{1}H NMR ($CDCl_3$) δ: 7.13–7.43 (9H, m), 7.07 (1H, s), 6.91–6.99 (2H, m), 6.72 (1H, s), 5.68 (2H, s), 5.12 (2H, s), 3.91 (3H, s). ^{13}C NMR ($CDCl_3$) δ: 165.9, 149.6, 147.1, 138.0, 136.7, 135.5, 129.2, 128.6, 128.2, 127.8, 127.5, 126.8, 126.1, 124.8, 119.5, 95.7, 70.8, 55.5, 53.9. Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.40; H, 5.46; N, 3.62. Found: C, 74.29; H, 5.41; N, 3.69.

4.11. *N*-(1,2-Bis(4-isopropoxy-3-methoxyphenyl)-2-oxoethyl)-1-benzyl-6-(benzyloxy)-5-methoxy-1*H*-indole-2-carboxamide (19a)

To a suspension of HOBt (0.739 g, 5.4 mmol) and WSC (1.04 g, 5.4 mol) in THF (44 mL) under nitrogen atmosphere **18a** (2.09 g, 5.4 mmol) was added. The reaction mixture was stirred at room temperature for 12 h until the activated ester was formed, then compound **15** dissolved in THF (10 mL) and TEA (5.4 mmol, 0.75 mL) were added. After stirring the reaction mixture at room temperature for 14 h, the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate, the solution washed with aqueous $NaHCO_3$, HCl 2 M and brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford **19a** as a yellow solid (2.24 g, 55%); mp 140–141 °C. ^{1}H NMR ($CDCl_3$) δ: 7.50–7.68 (3H, m), 7.21–7.43 (5H, m), 7.09–7.18 (3H, m), 7.06 (1H, s), 6.88–7.00 (5H, m), 6.76–6.87 (2H, m), 6.73 (1H, s), 6.59 (1H, d, J = 7.8 Hz), 5.72 (1H, AB, J = 16.1 Hz), 5.59 (1H, AB, J = 16.1 Hz), 5.10 (2H, s), 4.62 (1H, m), 4.49 (1H, m), 3.91 (3H, s), 3.86 (3H, s), 3.79 (3H, s), 1.38 (6H, d, J = 6.1 Hz), 1.32 (6H, d, J = 6.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 194.8, 162.0, 151.9, 150.2, 149.6, 148.1, 147.0, 146.5, 139.3, 137.5, 133.7, 129.7, 129.6, 128.8, 128.6, 128.4, 127.3, 119.3, 113.5, 107.3, 103.5, 96.3, 70.1, 58.0, 56.9, 56.3, 54.7, 22.5, 22.4. Anal. Calcd for $C_{46}H_{48}N_2O_8$: C, 73.00; H, 6.39; N, 3.70. Found: C, 73.08; H, 6.31; N, 3.77.

4.12. 9-Benzyl-7-(benzyloxy)-3,4-bis(4-isopropoxy-3-methoxy phenyl)-6-methoxy-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (20a)

To a solution of **19a** (1.10 g, 1.4 mmol) in acetonitrile (8 mL) was added TFA (0.16 mL) and the reaction mixture was refluxed for 15 min. The precipitate formed was filtered and washed with acetonitrile to afford **20a** as a white solid (0.764 g, 71%); mp 238 °C. ^{1}H NMR (DMSO-d_6) δ: 7.05–7.51 (12H, m), 6.63–7.03 (7H, m), 6.04 (2H, m), 5.16 (2H, s), 4.36–4.60 (2H, m), 3.60 (3H, s), 3.57 (3H, s), 3.37 (3H, s), 1.25 (6H, d, J = 5.5 Hz), 1.21 (6H, d, J = 5.6 Hz). ^{13}C NMR (DMSO-d_6) δ: 156.1, 150.5, 146.7, 146.3, 139.2, 137.3, 134.7, 130.3, 129.3, 128.7, 128.3, 128.0, 127.7, 127.4, 126.0, 124.5, 114.7, 114.2, 79.5, 70.6, 70.1, 56.7, 54.2, 22.6, 22.3. Anal. Calcd for $C_{46}H_{46}N_2O_7$: C, 74.78; H, 6.28; N, 3.79. Found: C, 74.81; H, 6.25; N, 3.74. HRMS (ESI positive) Calcd for $C_{46}H_{46}N_2O_7Na$: 761.31972. Found: 761.31644 $[M+Na]^+$.

4.13. 9-Benzyl-7-hydroxy-3,4-bis(4-hydroxy-3-methoxyphenyl) -6-methoxy-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (21a)

Anhydrous $AlCl_3$ (0.360 g, 2.7 mmol) was suspended in dry DCM (5 mL) under nitrogen and compound **20a** (0.700 g, 0.9 mmol) was added. After stirring the reaction mixture at room temperature for 24 h, saturated aqueous NH_4Cl was added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The obtained solid was crystallized from ethanol to give product **21a** (0.056 g, 11%); mp 297 °C. ^{1}H NMR (DMSO-d_6) δ: 11.25 (1H, s), 9.30 (1H, s), 9.07 (1H, s), 9.02 (1H, s), 7.15–7.35 (5H, m), 6.56–6.93 (7H, m), 6.13 (2H, s), 6.02 (2H, m), 3.64 (3H, s), 3.60 (3H, s), 3.39 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 156.1, 148.4, 148.0, 147.0, 146.6, 146.2, 144.2, 139.4, 136.5, 134.8, 129.5, 128.4, 128.1, 127.0, 126.5, 126.1, 124.4, 115.3, 114.3, 114.1, 57.0, 55.3, 53.9. Anal. Calcd for $C_{33}H_{28}N_2O_7$: C, 70.20; H, 5.00; N, 4.96. Found: C, 70.14; H, 5.09; N, 4.99. HRMS (ESI positive) Calcd for $C_{33}H_{28}N_2O_7$Na: 587.17887. Found: 587.17698 [M+Na]$^+$.

4.14. 7-Hydroxy-3,4-bis(4-hydroxy-3-methoxyphenyl)-6-metho xy-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (3a)

Anhydrous $AlCl_3$ (0.188 g, 1.4 mmol) was suspended in anisole (10 mL) under nitrogen and compound **21a** (0.05 g, 0.1 mmol) was added. After refluxing the reaction mixture at 100 °C for 5 h, MeOH was added and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (DCM–MeOH, 9:1) affording product **22a** as a white solid (9.5 mg, 20%); mp 312–315 °C. ^{1}H NMR (DMSO-d_6) δ: 11.55 (1H, s), 11.13 (1H, s), 9.25 (1H, s), 9.05 (1H, s), 8.99 (1H, s), 6.58–6.91 (7H, m), 6.22 (1H, s), 3.62 (3H, s), 3.59 (3H, s), 3.42 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 156.0, 147.8, 146.9, 143.2, 143.9, 141.2, 139.5, 139.2, 134.8, 128.7, 126.7, 125.1, 123.1, 122.9, 122.6, 115.4, 114.9, 114.6, 113.9, 112.6, 109.9, 109.3, 55.6, 55.3, 55.2 34.1. Anal. Calcd for $C_{26}H_{22}N_2O_7$: C, 65.82; H, 4.67; N, 5.90. Found: C, 65.86; H, 4.62; N, 5.98. HRMS (ESI positive) Calcd for $C_{26}H_{22}N_2O_7$Na: 497.13192. Found: 497.13346 [M+Na]$^+$.

4.15. Ethyl 5-(benzyloxy)-6-methoxy-1*H*-indole-2-carboxylate (17b)

Na (0.377 g, 16.4 mmol) was dissolved in anhydrous EtOH (15 mL), treated with 3-benzyloxy-4-methoxybenzaldehyde (1.00 g, 4.1 mol), and the mixture was cooled to −15 °C. Ethyl azidoacetate (ethanol solution 25%, 16.0 mol, 1.044 mL) was added dropwise to the reaction mixture. The mixture was stirred for 3 h at −15 °C, then warmed to room temperature and treated with saturated aqueous NH_4Cl. The beige solid that formed was collected by filtration and washed with water to afford (*Z*)-ethyl-2-azido-3-(3-(benzyloxy)-4-methoxyphenyl)acrylate (1.20 g, 88%); mp 94–95 °C. ^{1}H NMR ($CDCl_3$) δ: 7.25–7.50 (7H, m), 6.85–6.95 (2H, m), 5.21 (2H, s), 4.38 (2H, q, *J* = 7.1 Hz), 3.95 (3H, s), 1.39 (3H, t, *J* = 7.1 Hz).

The azido derivative (1.15 g, 3.1 mol) was dissolved in xylenes (5 mL) and the solution was warmed at 130 °C for 12 h. The solution was cooled to room temperature and hexane was added (5 mL). The beige solid was collected by filtration. The filtrated was concentrated in vacuo and purified by flash chromatography (hexane:ethyl acetate, 8:2). The precipitate was combined with the product isolated by chromatography to afford **17b** (0.800 g, 76%); mp 177–178 °C. ^{1}H NMR ($CDCl_3$) δ: 8.69 (1H, s), 7.30–7.58 (6H, m), 7.12 (1H, m), 6.88 (1H, s), 5.25 (2H, s), 4.40 (2H, s), 4.40 (2H, q, *J* = 7.02 Hz), 3.95 (3H, s), 1.40 (3H, t, *J* = 7.07 Hz). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 70.21; H, 5.85; N, 4.27.

4.16. 1-Benzyl-5-(benzyloxy)-6-methoxy-1*H*-indole-2-carbox ylic acid (18b)

Indole **17b** (0.740 g, 2.3 mmol) was dissolved in dry DMF (7 mL) under nitrogen. The solution was cooled at 0 °C and NaH (oil mineral susp. 60%, 0.170 g, 4.2 mol) was slowly added. The reaction mixture was warmed to room temperature and stirred for 45 min, then benzyl bromide (4.2 mmol, 0.5 mL) was added and the mixture was stirred for 2 h. The solvent was removed under vacuo, the crude product was dissolved in ethyl acetate and washed with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford ethyl 1-benzyl-5-(benzyloxy)-6-methoxy-1*H*-indole-2-carboxylate (0.900 g, 97%) as a white solid; mp 90–91 °C. ^{1}H NMR ($CDCl_3$) δ: 7.20–7.48 (9H, m), 7.12 (1H, s), 7.02 (2H, m), 6.79 (1H, m), 6.79 (1H, s), 5.72 (2H, s), 5.18 (2H, s), 4.32 (2H, q, *J* = 7.1 Hz), 3.95 (3H, s), 1.36 (3H, t, *J* = 7.1 Hz).

The protected indole (0.890 g, 2.1 mmol) was dissolved in a solution 3:2:1 THF:MeOH:H_2O (5 mL), then treated with LiOH·H_2O (0.359 g, 8.5 mmol). The mixture was stirred for 4 h at 25 °C. 1 N aqueous HCl was added and the solution was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford **18b** (0.600 g, 73%); mp 130 °C. ^{1}H NMR ($CDCl_3$) δ: 7.17–7.48 (9H, m), 7.10 (1H, s), 6.94–7.06 (2H, m), 6.75 (1H, s), 5.71 (2H, s), 5.15 (2H, s), 3.94 (3H, s). ^{13}C NMR ($CDCl_3$) δ: 165.8, 149.7, 147.2, 138.0, 136.5, 135.9, 129.5, 129.0, 128.3, 128.0, 127.9, 127.4, 119.5, 78.2, 57.0, 53.9. Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.40; H, 5.46; N, 3.62. Found: C, 74.45; H, 5.41; N, 3.61.

4.17. *N*-(1,2-Bis(4-isopropoxy-3-methoxyphenyl)-2-oxoethyl)-1-benzyl-5-(benzyloxy)-6-methoxy-1*H*-indole-2-carboxamide (19b)

To a suspension of HOBt (0.335 g, 2.5 mmol) and WSC (0.474 g, 2.5 mmol) in THF (20 mL) under nitrogen atmosphere compound **18b** (0.481 g, 1.2 mmol) was added. The reaction mixture was stirred at room temperature for 12 h until the activated ester was formed. Then compound **15** dissolved in THF (5 mL) and TEA (2.8 mmol, 0.346 mL) were added. After stirring the reaction mixture at room temperature for 14 h, the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate, the solution washed with aqueous $NaHCO_3$, HCl 2 M and brine, dried over Na_2SO_4, and evaporated under reduced pressure to afford **19b** as a yellow solid (0.520 g, 56%); mp 127 °C. ^{1}H NMR ($CDCl_3$) δ: 7.53–7.69 (3H, m), 7.23–7.44 (4H, m), 7.11–7.18 (3H, m), 7.07 (1H, s), 6.92–7.01 (5H, m), 6.77–6.87 (2H, m), 6.74 (1H, s), 6.61 (1H, d, *J* = 7.4 Hz), 5.74 (1H, AB, *J* = 16.2 Hz), 5.61 (1H, AB, *J* = 16.2 Hz), 5.12 (2H, s), 4.65 (1H, m), 4.49 (1H, m), 3.93 (3H, s), 3.88 (3H, s), 3.81 (3H, s), 1.40 (6H, d, *J* = 6.10 Hz), 1.34 (6H, d, *J* = 6.10 Hz). ^{13}C NMR (DMSO-d_6) δ: 194.8, 162.0, 151.9, 150.2, 149.6, 148.1, 147.1, 146.5, 139.3, 137.6, 133.7, 129.8, 129.6, 128.9, 128.8, 128.4, 127.9, 127.4, 123.8, 121.9, 119.4, 115.5, 113.6, 113.5, 112.0, 107.2, 103.7, 96.4, 70.7, 58.3, 56.5, 56.0, 22.5, 22.3. Anal. Calcd for $C_{46}H_{48}N_2O_8$: C, 73.00; H, 6.39; N, 3.70. Found: C, 73.04; H, 6.35; N, 3.71.

4.18. 9-Benzyl-6-(benzyloxy)-3,4-bis(4-isopropoxy-3-methoxy phenyl)-7-methoxy-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (20b)

To a solution of **19b** (0.500 g, 0.66 mmol) in acetonitrile (5 mL) was added TFA (0.9 mmol, 0.07 mL) and the reaction mixture was refluxed for 15 min. The precipitate was filtered and washed with acetonitrile to afford **20b** as a white solid (0.340 g, 70%); mp 230 °C. ^{1}H NMR ($CDCl_3$) δ: 7.11–7.51 (11H, m), 6.65–7.04 (7H, m), 6.43 (1H, s), 5.96 (2H, m), 5.17 (2H, s), 4.56 (1H, m), 4.44 (1H, m), 3.70 (3H, s), 3.57

(3H, s), 3.55 (3H, s), 1.40 (6H, d, J = 5.7 Hz), 1.35 (6H, d, J = 5.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 155.6, 150.0, 148.9, 148.8, 146.5, 145.0, 144.7, 137.7, 136.2, 135.5, 132.9, 129.4, 128.1, 128.0, 127.4, 127.0, 126.8, 126.6, 126.0, 124.0, 120.9, 115.4, 114.7, 114.6, 114.3, 114.0, 112.9, 103.7, 95.0, 71.1, 70.6, 55.5, 55.2, 55.1, 21.6, 21.4. Anal. Calcd for $C_{46}H_{46}N_2O_7$: C, 74.78; H, 6.28; N, 3.79. Found: C, 74.87; H, 6.32; N, 3.70. HRMS (ESI positive) Calcd for $C_{46}H_{46}N_2O_7Na$: 761.31972. Found: 761.32011 $[M+Na]^+$.

4.19. 6-Hydroxy-3,4-bis(4-hydroxy-3-methoxyphenyl)-7-methoxy-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (3b)

Anhydrous $AlCl_3$ (0.094 g, 0.70 mmol) was suspended in dry DCM (4 mL) under nitrogen and compound **20b** (0.100 g, 0.13 mmol) was added. After stirring the reaction mixture at room temperature for 24 h, saturated aqueous NH_4Cl was added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude solid was crystallized from ethanol to give product **3b** (0.030 g, 48%); mp > 350 °C. ^{1}H NMR ($CDCl_3$) δ: 11.53 (1H, s), 11.15 (1H, s), 9.25 (1H, s), 9.08 (1H, s), 9.00 (1H, s), 6.60–6.95 (7H, m), 6.22 (1H, s), 3.65 (3H, s), 3.60 (3H, s), 2.45 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 156.4, 147.4, 147.3, 143.2, 142.4, 140.6, 139.7, 139.2, 134.2, 127.9, 127.7, 125.3, 123.8, 123.5, 122.6, 115.5, 114.8, 114.1, 113.9, 110.7, 109.8, 108.3, 55.6, 55.2, 55.1 34.1. Anal. Calcd for $C_{26}H_{22}N_2O_7$: C, 65.82; H, 4.67; N, 5.90. Found: C, 65.79; H, 4.60; N, 5.82. HRMS (ESI positive) Calcd for $C_{26}H_{22}N_2O_7Na$: 497.13192. Found: 497.13256 $[M+Na]^+$.

4.20. *N*-(1,2-Bis(4-isopropoxy-3-methoxyphenyl)-2-oxoethyl)-1-methyl-1*H*-indole-2-carboxamide (19c)

To a suspension of HOBt (0.988 g, 7.3 mmol) and WSC (1.39 g, 7.3 mmol) in THF (60 mL) under nitrogen atmosphere commercially available **18c** (0.540 g, 3.1 mmol) was added. The reaction mixture was stirred at room temperature for 12 h until the activated ester was formed, then compound **15** dissolved in THF (10 mL) and TEA (7.3 mmol, 1 mL) were added. After stirring the reaction mixture at room temperature for 14 h, the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate, the solution washed with aqueous $NaHCO_3$, HCl 2 M and brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford **19c** as a white solid (0.978 g, 58%); mp 111 °C. ^{1}H NMR (DMSO-d_6) δ: 8.98 (1H, d, J = 7.07 Hz), 7.76 (1H, d, J = 8.2 Hz), 7.40–7.67 (3H, m), 7.23–7.33 (2H, m), 7.19 (1H, s), 6.80–7.15 (4H, m), 6.64 (1H, d, J = 7.07 Hz), 4.70 (1H, m), 4.50 (1H, m), 3.96 (3H, s), 3.79 (3H, s), 3.75 (3H, s), 1.27 (6H, d, J = 6.3 Hz), 1.22 (6H, d, J = 6.3 Hz). ^{13}C NMR (DMSO-d_6) δ: 194.7, 162.0, 151.9, 150.2, 149.6, 147.1, 139.0, 131.9, 129.2, 127.8, 126.1, 124.1, 115.4, 113.5, 113.3, 70.4, 70.1, 62.9, 56.5, 54.4, 22.7, 22.4. Anal. Calcd for $C_{32}H_{36}N_2O_6$: C, 70.57; H, 6.66; N, 5.14. Found: C, 70.65; H, 6.58; N, 5.22.

4.21. 3,4-Bis(4-isopropoxy-3-methoxyphenyl)-9-methyl-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (20c)

To a solution of **19c** (0.970 g, 1.8 mmol) in acetonitrile (10 mL) was added TFA (0.2 mL) and the reaction mixture was refluxed for 15 min. The precipitate formed was filtered and washed with acetonitrile to afford **20c** as a white solid (0.556 g, 58%); mp 287 °C. ^{1}H NMR (DMSO-d_6) δ: 9.10 (1H, br s), 6.50–6.85 (10H, m), 4.62 (1H, m), 4.55 (1H, m), 4.28 (3H, s), 3.60 (3H, s), 3.33 (3H, s), 1.40 (6H, d, J = 6.2 Hz), 1.35 (6H, d, J = 6.2 Hz). ^{1}H NMR (DMSO-d_6) δ: 155.6, 148.9, 148.8, 146.5, 146.0, 144.7, 137.7, 136.2, 135.5, 132.3, 129.4, 128.2, 128.0, 127.4, 127.0, 126.8, 123.1, 120.8, 115.4, 114.7, 114.6, 114.3, 114.0, 112.9, 103.7, 95.0, 71.1, 70.6, 55.5, 55.2, 34.2, 21.5, 21.4. Anal. Calcd for $C_{32}H_{34}N_2O_5$: C, 72.98; H, 6.51; N, 5.32. Found: C, 73.09; H, 6.56; N, 5.42. HRMS (ESI positive) Calcd for $C_{32}H_{34}N_2O_5Na$: 549.23599. Found: 549.23754 $[M+Na]^+$.

4.22. 3,4-Bis(4-hydroxy-3-methoxyphenyl)-9-methyl-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (3c)

Anhydrous $AlCl_3$ (0.197 g, 1.5 mmol) was suspended in dry DCM (5 mL) under nitrogen and compound **20c** (0.300 g, 0.6 mmol) was added. After stirring the reaction mixture at room temperature for 6 h, saturated aqueous NH_4Cl was added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude solid was crystallized from ethanol to give product **3c** (0.08 g, 30%), together with unreacted starting material; mp 315 °C. ^{1}H NMR (DMSO-d_6) δ: 11.40 (1H, br s), 11.32 (1H, br s), 9.05 (1H, br s), 6.53–6.99 (10H, m), 4.28 (3H, s), 3.60 (3H, s), 3.33 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 156.0, 147.4, 147.1, 146.03, 145.6, 142.4, 134.6, 128.0, 127.8, 125.5, 123.8, 123.5, 122.6, 122.2, 119.7, 119.3, 115.5, 115.5, 114.7, 114.3, 113.9, 110.7, 107.3, 55.7, 55.2, 34.0. Anal. Calcd for $C_{26}H_{22}N_2O_5$: C, 70.58; H, 5.01; N, 6.33. Found: C, 70.64; H, 5.09; N, 6.22. HRMS (ESI positive) Calcd for $C_{26}H_{22}N_2O_5Na$: 465.14209. Found: 465.14334 $[M+Na]^+$.

4.23. 2,4-Dihydroxy-5-methoxy-benzaldehyde (24)

To a stirred suspension of $AlCl_3$ (68 g, 509.9 mmol) in dry DCM (450 mL), a solution of 2,4,5-trimethoxybenzaldehyde **23** (25 g, 127.4 mmol) in dry DCM (125 mL) was added dropwise. After stirring for 4 h at room temperature, another portion of $AlCl_3$ (68 g, 509.9 mmol) was added. The suspension was further stirred for 19 h and the reaction mixture was poured into 1 kg of ice to which 45 mL of concentrated hydrochloric acid were added. The organic layer was separated and the aqueous phase was extracted twice with DCM (200 mL). The combined organic layers were filtered over silica gel, dried over Na_2SO_4, evaporated and the residue crystallized from ethyl acetate to give compound **24** (17.95 g, 84%); mp 150 °C. ^{1}H NMR ($CDCl_3$) δ: 11.95 (1H, s), 9.71 (1H, s), 7.29 (1H, s), 6.90 (1H, s), 6.55 (1H, s), 3.92 (3H, s). Anal. Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.17; H, 4.85.

4.24. 2-Hydroxy-4-isopropoxy-5-methoxy-benzaldehyde (25)

2,4-Dihydroxy-5-methoxybenzaldehyde **24** (35.18 g, 209.2 mmol) was dissolved in dry DMF (345 mL). $NaHCO_3$ (26.29 g, 313.0 mmol) and 2-bromopropane (38.5 g, 313.0 mol) were added under nitrogen flow. The reaction mixture was heated at 70 °C and stirred. After 4 h, the suspension was filtered and the organic layer was evaporated. Ethyl acetate was added to the crude product and the suspension was filtered. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (hexane:ethyl acetate, 1:1) affording a yellow oil (15 g, 34%). ^{1}H NMR ($CDCl_3$) δ: 11.36 (1H, s), 9.66 (1H, s), 6.89 (1H, s), 6.44 (1H, s), 4.63 (1H, s), 3.85 (3H, s), 1.42 (6H, d, J = 5.9 Hz). ^{13}C NMR ($CDCl_3$) δ: 194.0, 159.4, 156.2, 143.7, 114.2, 112.7, 101.6, 72.8, 56.8, 22.2, 21.9. Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.77; H, 6.78.

4.25. 4-Isopropoxy-5-methoxy-2-(2-methoxy-ethoxymethoxy)-benzaldehyde (26)

To a suspension of K_2CO_3 (29.16 g, 211.0 mmol) in dry DMF (150 mL) was added compound **25** (14.80 g, 70.4 mmol). After stirring for 15 min, MEMCl (26.63 g, 213.8 mmol) was added slowly and the reaction mixture was stirred for 12 h. The solvent was

removed under reduced pressure and the crude product was dissolved in ethyl acetate, washed with brine, dried over Na_2SO_4, filtered and evaporated in vacuo, affording a yellow oil (19.70 g, 94%). ^{1}H NMR ($CDCl_3$) δ: 10.29 (1H, s), 7.29 (1H, s), 6.82 (1H, s), 5.31 (2H, s), 4.65 (1H, m), 3.80–3.90 (5H, m), 3.53–3.60 (2H, m), 3.36 (3H, s), 1.40 (6H, d, J = 6.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 188.2, 157.2, 155.1, 146.1, 118.8, 110.0, 102.8, 95.1, 72.9, 70.8, 59.1, 57.2, 57.1, 22.1, 22.0. Anal. Calcd for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 60.48; H, 7.46.

4.26. 4-Isopropoxy-5-methoxy-2-(2-methoxy-ethoxymethoxy)-benzylamine (27)

A solution of **26** (17.70 g, 59.3 mmol) in 95% ethanol (400 mL) was treated with a solution of hydroxylamine hydrochloride (5.73 g, 82.4 mmol) in dry pyridine (40 mL) under stirring at room temperature for 90 min and at 0 °C for 30 min to facilitate the oxime precipitation. The white solid 4-isopropoxy-5-methoxy-2-(2-methoxy-ethoxymethoxy)-benzaldehyde oxime was filtered, dried, and used (18.48 g, 89%) without further purification. ^{1}H NMR ($CDCl_3$) δ: 8.94 (1H, br s), 8.40 (1H, s), 7.19 (1H, s), 6.80 (1H, s), 5.20 (2H, s), 4.55 (1H, m), 3.76–3.85 (5H, m), 3.50–3.58 (2H, m), 3.35 (3H, s), 1.35 (6H, d, J = 6.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 150.4, 149.2, 147.0, 118.0, 114.0, 105.2, 94.6, 71.7, 71.2, 61.0, 59.7, 58.9, 57.3, 53.5, 22.1.

A solution of the oxime (18.40 g, 58.7 mmol) in 95% ethanol (154 mL) was treated with an equal volume of 2 M NaOH followed by Al/Ni alloy (15.97 g) under stirring at room temperature for 90 min. The Al/Ni alloy was removed by filtration and washed with fresh ethanol. Filtrate and washings were combined, acidified with 0.8 M HCl (pH 2) and extracted with DCM (3 × 50 mL). The aqueous phase was treated with solid KOH up to pH 14 and extracted with diethyl ether (3 × 30 mL). The extracts after drying over anhydrous Na_2SO_4 and removal of the solvent afforded **27** (17 g, 97%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 6.80 (1H, s), 6.77 (1H, s), 5.20 (2H, s), 4.46 (1H, m), 3.71–3.83 (7H, m), 3.50–3.58 (2H, m), 3.38 (3H, s), 1.31 (6H, d, J = 6.1 Hz). Anal. Calcd for $C_{15}H_{25}NO_5$: C, 60.18; H, 8.42; N, 4.68. Found: C, 60.09; H, 8.51; N, 4.63.

4.27. 4-Isopropoxy-*N*-[4-isopropoxy-5-methoxy-2-(2-methoxy-ethoxymethoxy)-benzyl]-3-methoxy-benzamide (28)

To a suspension of HOBt (6.16 g, 45.6 mmol) and WSC (8.79 g, 45.6 mmol) in THF (250 mL) under nitrogen atmosphere, **8** (7.9 g, 37.6 mmol) and **27** (16.90 g, 56.4 mmol) were added. The reaction mixture was stirred at room temperature for 24 h and the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate, the solution washed with $NaHCO_3$, HCl 2 M and brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford **28** as a yellow oil (17.70 g, 95%). ^{1}H NMR ($CDCl_3$) δ: 7.42 (1H, s), 7.21 (1H, dd, J = 1.7 and 8.4 Hz), 6.75–6.93 (3H, m), 6.65 (1H, t, J = 5.7 Hz), 5.24 (2H, s), 4.40–4.65 (4H, m), 3.88 (3H, s), 3.79–3.85 (2H, m), 3.80 (3H, s), 3.50–3.56 (2H, m), 3.30 (3H, s), 1.36 (6H, d, J = 6.5 Hz), 1.34 (6H, d, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 166.6, 149.8, 149.4, 147.2, 145.5, 127.2, 119.7, 119.0, 114.1, 113.6, 111.2, 105.3, 94.8, 71.6, 71.4, 71.1, 67.9, 58.8, 56.4, 55.9, 39.2, 21.8, 21.7. Anal. Calcd for $C_{26}H_{37}NO_8$: C, 63.53; H, 7.59; N, 2.85. Found: C, 63.57; H, 7.54; N, 2.78.

4.28. (4-Isopropoxy-3-methoxy-benzoyl)-[4-isopropoxy-5-methoxy-2-(2-methoxy-ethoxymethoxy)-benzyl]-carbamic acid *tert*-butyl ester (29)

Compound **28** (17.65 g, 35.9 mmol) was dissolved in acetonitrile (150 mL) under nitrogen flow and DMAP (2.99 g, 24.5 mmol) and di-*tert*-butyldicarbonate (15.71 g, 72.0 mmol) were added. After stirring the solution at rt for 16 h, the solvent was evaporated under reduced pressure and the crude product was dissolved in DCM. The solution was washed with saturated aqueous $NaHCO_3$ and the organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product was purified by flash chromatography (hexane:ethyl acetate, 6:4) affording **29** (13.99 g, 67%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.05–7.17 (2H, m), 6.88 (1H, s), 6.78–6.85 (2H, m), 5.10 (2H, s), 4.89 (2H, s), 4.57 (1H, m), 4.47 (1H, m), 3.81 (3H, s), 3.76 (3H, s), 3.70–3.74 (2H, m), 3.46–3.52 (2H, m), 3.33 (3H, s), 1.35 (6H, d, J = 6.1 Hz), 1.31 (6H, d, J = 6.1 Hz), 1.15 (9H, s). ^{13}C NMR ($CDCl_3$) δ: 172.7, 153.6, 150.2, 149.5, 149.1, 146.9, 145.1, 129.6, 121.5, 119.1, 113.2, 111.7, 104.7, 94.4, 82.2, 71.4, 71.1, 67.2, 58.7, 56.5, 55.8, 44.0, 27.3, 22.5, 21.8, 21.7. Anal. Calcd for $C_{31}H_{45}NO_{10}$: C, 62.93; H, 7.67; N, 2.37. Found: C, 62.88; H, 7.60; N, 2.46.

4.29. 1-[4-Isopropoxy-5-methoxy-2-(2-methoxy-etxy-methoxy)-phenyl]-2-(4-isopropoxy-3-methoxy-phenyl)-2-oxo-ethyl]-carbamic acid *tert*-butyl ester (30)

LDA was prepared by mixing diisopropylamine (7.29 g, 72.0 mmol, 10.1 mL) and DMPU (10.9 mL) with *n*-BuLi (2.7 M in heptane, 26.67 mL, 72.0 mmol) in THF (70 mL) at −78 °C under nitrogen atmosphere. A solution of the amide **29** (13.90 g, 23.5 mmol) in freshly distilled THF (209 mL) was added to the LDA solution at −78 °C and the mixture was stirred for 6 h. The mixture was quenched with aqueous NH_4Cl, diluted with ethyl acetate, washed with saturated aqueous NH_4Cl and brine, and dried over Na_2SO_4. Concentration in vacuo followed by flash chromatography (hexane:ethyl acetate, 7:3) gave product **30** (4.11 g, 29%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.69 (1H, d, J = 8.2 Hz), 7.57 (1H, s), 6.68–6.85 (3H, m), 6.49 (1H, d, J = 7.8 Hz), 5.85 (1H, d, J = 7.8 Hz), 5.27 (2H, m), 4.62 (1H, m), 4.49 (1H, m), 3.74–3.95 (2H, m), 3.86 (3H, s), 3.77 (3H, s), 3.55–3.65 (2H, m), 3.38 (3H, s), 1.44 (9H, s), 1.28–1.40 (12H, m). Anal. Calcd for $C_{31}H_{45}NO_{10}$: C, 62.93; H, 7.67; N, 2.37. Found: C, 63.03; H, 7.54; N, 2.43.

4.30. 1-[4-Isopropoxy-5-methoxy-2-(2-methoxy-ethoxymethoxy)-phenyl]-2-(4-isopropoxy-3-ethoxy-phenyl)-2-oxo-ethyl-ammonium trifluoro-acetate (31)

Compound **30** (1.50 g, 2.53 mmol) was dissolved in dry DCM (30 mL) under nitrogen flow and TFA (3 mL) was added at 0 °C. The solution was stirred at rt for 2 h. Solvent was evaporated under vacuo and the crude product (1.24 g) was used for the next step without further purification.

4.31. 6-Benzyloxy-5-methoxy-1-methyl-1*H*-indole-2-carboxylic acid [1-[4-isopropoxy-5-methoxy-2-(2-methoxyethoxymethoxy)-phenyl]-2-(4-isopropoxy-3-methoxyphenyl)-2-oxo-ethyl]-amide (33)

To a suspension of HOBt (0.459 g, 3.4 mmol) and WSC (0.650 g, 3.4 mmol) in THF (30 mL) was added under nitrogen atmosphere **32** (0.519 g, 1.67 mmol).[14] The reaction mixture was stirred at room temperature for 12 h until the activated ester was formed. Then compound **31** (1.21 g, 1.85 mmol) dissolved in THF (10 mL) and TEA (3.4 mmol, 0.47 mL) were added. After stirring the reaction mixture at room temperature for 14 h, the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate, the solution washed with aqueous $NaHCO_3$, HCl 2 M and brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford **33** as a yellow solid (0.720 g, 55%); mp 134 °C. ^{1}H NMR (DMSO-d_6) δ: 8.79 (1H, d, J = 7.5 Hz), 7.67 (1H, d, J = 8.6 Hz), 7.46–7.58 (3H, m), 7.32–7.46, 7.03–7.25 (4H, m), 6.83–6.98 (3H, m), 5.30 (2H, m), 5.30 (2H, m), 5.15 (2H, s), 4.69

(1H, s), 4.52 (1H, s), 3.90 (3H, s), 3.78 (6H, s), 3.67 (3H, s), 3.17 (3H, s), 1.20–1.29 (12H, m). Anal. Calcd for $C_{44}H_{52}N_2O_{11}$: C, 67.33; H, 6.68; N, 3.57; Found: C, 67.24; H, 6.53; N, 3.66.

4.32. 7-Benzyloxy-3-(2-hydroxy-4-isopropoxy-5-methoxy-phenyl)-4-(4-isopropoxy-3-methoxy-phenyl)-6-methoxy-9-methyl-2,9-dihydro-β-carbolin-1-one (34)

TMSCl (0.67 mmol, 0.08 mL) and NaI (0.100 g, 0.67 mmol) were added to a solution of **33** (0.450 g, 0.67 mmol) in acetonitrile (40 mL) at −20 °C under nitrogen flow. After stirring at this temperature for 15 min, an additional equivalent of both NaI and TMSCl was added and the mixture stirred at −20 °C until no starting material remained (TLC analysis). The yellow-orange mixture was quenched with methanol and the solvent removed in vacuo. The residue was extracted with ethyl acetate and the organic layer washed with saturated sodium thiosulfate and brine. The crude product was purified by flash chromatography (hexane:ethyl acetate, 7:3) affording **34** (0.140 g, 31%) as a white solid; mp 220 °C. ^{1}H NMR (DMSO-d_6) δ: 11.00 (1H, s), 9.09 (1H, s), 7.19–7.56 (7H, m), 6.67–7.00 (3H,m), 6.54 (1H, s), 6.41 (1H, s), 6.27 (1H, s), 5.19 (2H, s), 4.50 (1H, s), 4.38 (1H, s), 4.24 (3H, s), 3.58 (3H, s), 3.43 (3H, s), 3.39 (3H, s), 1.22 (12H, m). Anal. Calcd for $C_{40}H_{42}N_2O_8$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.74; H, 6.29; N, 4.20. HRMS (ESI positive) Calcd for $C_{40}H_{43}N_2O_8$: 679.30139. Found: 679.30333 $[M+H]^+$. Calcd for $C_{40}H_{42}N_2O_8Na$: 701.28334. Found: 701.28543 $[M+Na]^+$.

4.33. 10-Benzyloxy-3-isopropoxy-13-(4-isopropoxy-3-methoxy-phenyl)-2,11-dimethoxy-8-methyl-8*H*-5-oxa-6a,8-diazaindeno[2,1-*b*]phenanthren-7-one (35)

A suspension of **34** (0.132 g, 0.2 mmol) and K_2CO_3 (0.072 g, 52.1 mmol) in DMF (6 mL) was stirred for 30 min at room temperature, then treated with diiodomethane (21.96 g, 82.0 mmol). After being heated at 100 °C for 1 h, the solvent was evaporated under reduced pressure and the crude product was dissolved in dichloromethane, washed with brine, dried over Na_2SO_4 and purified by flash chromatography (petroleum ether–ethyl acetate, 1:1) to afford **35** as a white solid (15 mg, 12%). ^{1}H NMR (DMSO-d_6) δ: 7.17–7.58 (5H, m), 6.74–7.17 (4H, m), 6.46–6.72 (2H, m), 5.82–6.14 (3H, m), 5.23 (2H, s), 4.42–4.72 (2H, m), 4.24 (3H, s), 3.78 (3H, s), 3.52 (3H, s), 3.26 (3H, s), 1.07–1.48 (12H, m). Anal. Calcd for $C_{41}H_{42}N_2O_8$: C, 71.29; H, 6.13; N, 4.06. Found: C, 71.38; H, 6.04; N, 4.18. HRMS (ESI positive) Calcd for $C_{41}H_{42}N_2O_8Na$: 713.28334. Found: 713.28421 $[M+Na]^+$.

4.34. 3,10-Dihydroxy-13-(4-hydroxy-3-methoxy-phenyl)-2,11-dimethoxy-8-methyl-8*H*-5-oxa-6a,8-diazaindeno[2,1-*b*]phenanthren-7-one (2b)

Anhydrous $AlCl_3$ (15.5 mg, 0.116 mmol) was suspended in dry DCM (5 mL) under nitrogen and compound **35** (10 mg, 0.014 mmol) was added. After refluxing the reaction mixture for 6 h, saturated aqueous NH_4Cl was added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by preparative TLC to give **2b** (6 mg, 83%); mp 290 °C. ^{1}H NMR (DMSO-d_6) δ: 7.03 (1H, d, J = 8.1 Hz), 6.94 (1H, d, J = 1.9 Hz), 6.88 (1H, s), 6.76 (1H, dd, J = 8.1, 1.9 Hz), 6.63 (1H, s), 6.45 (1H, s), 5.87 (1H, AB, J = 9.6 Hz), 5.80 (1H, s), 5.75 (1H, AB, J = 9.6 Hz), 4.11 (3H, s), 3.69 (3H, s), 3.16 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 153.3, 149.9, 149.5, 149.3, 148.9, 147.2, 144.4, 143.5, 137.6, 128.7, 126.9, 125.9, 123.3, 117.0, 113.5, 112.6, 104.2, 103.9, 71.8, 56.5, 55.6, 55.1. Anal. Calcd for $C_{28}H_{24}N_2O_8$: C, 65.11; H, 4.68; N, 5.42. Found: C, 65.19; H, 4.64; N, 5.49. HRMS (ESI positive) Calcd for $C_{28}H_{24}N_2O_8Na$: 539.14249. Found: 539.14201 $[M+Na]^+$.

4.35. 1-Methyl-1*H*-indole-2-carboxylic acid [2-hydroxy-1-(2-methoxyphenyl)ethyl]amide (37)

To a suspension of HOBt (3.24 g, 23.9 mmol) and WSC (4.60 g, 23.9 mmol) in THF (40 mL) under nitrogen atmosphere, **36** (1.90 g, 11.4 mmol) and **18c** (1.29 g, 7.4 mmol) were added. The reaction mixture was stirred at room temperature for 24 h and the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate, the solution washed with $NaHCO_3$, HCl 2 M and brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford **37** as a white solid (2.33 g, 97%); mp 93–95 °C. ^{1}H NMR ($CDCl_3$) δ: 7.64 (1H, d, J = 8.3 Hz), 7.21–7.43 (5H, m), 7.15 (1H, m), 6.29–7.03 (2H, m), 6.90 (1H, s), 5.51 (1H, m), 4.05 (3H, s), 3.93–4.03 (2H, m), 3.93 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 162.1, 156.9, 138.9, 132.9, 129.4, 128.6, 127.6, 126.2, 124.0, 122.0, 120.8, 120.7, 111.3, 111.0, 105.1, 63.8, 56.0, 50.9. Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.30; H, 6.27; N, 8.60.

4.36. 8-Methyl-8*H*-5-oxa-6a,8-diazaindeno[2,1-*b*]phenanthren-7-one (38)

The above alcohol (0.907 g, 2.8 mmol) was added to a solution of IBX (1.55 g, 5.6 mmol) in dry DMSO (10 mL) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 h, treated with ethyl acetate, washed with $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure to afford *N*-(formyl(2-methoxyphenyl)ethyl)-1-methyl-1*H*-indole-2-carboxamide as a yellow oil (0.900 g), used for the next step without further purification.

To a solution of the above aldehyde (0.900 g, 2.8 mmol) in acetonitrile (10 mL) was added TFA (1 mL) and the reaction mixture was refluxed for 15 min. The precipitate formed was filtered and washed with acetonitrile to afford 3-(2-methoxyphenyl)-9-methyl-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one as a white solid (0.540 g, 64.3%); mp 250 °C. ^{1}H NMR (DMSO) δ: 7.88–8.12 (6H, m), 7.63–7.86 (4H, m), 3.90 (3H, s), 3.31 (3H, s), 2.52 (3H, s).

To an ice-cooled suspension of the above compound (0.540 g, 1.8 mmol) in dry dichloromethane (13 mL) 1 M BBr_3 in dichloromethane (1.33 g, 5.3 mmol, 5.3 mL) was added dropwise under nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature, stirred overnight, quenched with aqueous HCl and extracted with dichloromethane. The extract was washed with brine, dried over Na_2SO_4, concentrated under reduced pressure and purified by flash chromatography (dichloromethane:methanol, 9.5:0.5) to afford 3-(2-hydroxyphenyl)-9-methyl-2*H*-pyrido[3,4-*b*]indol-1(9*H*)-one (0.200 g, 38%) as a yellow solid; mp 270 °C. ^{1}H NMR (DMSO-d_6) δ: 7.88–8.12 (6H, m), 7.63–7.86 (4H, m), 3.31 (3H, s), 2.52 (3H, s).

A suspension of the above compound (0.04 g, 0.14 mmol) and K_2CO_3 (0.05 g, 0.34 mmol) in DMF (4 mL) was stirred for 30 min at room temperature, then treated with iodomethane (0.150 g, 0.56 mmol). After being refluxed for 1 h, the solvent was evaporated and the crude product was dissolved in dichloromethane, washed with brine, dried over Na_2SO_4 and purified by flash chromatography (dichloromethane:methanol, 9.5:0.5) to afford **38** as a white solid (12 mg, 28%); mp 280 °C. ^{1}H NMR (DMSO-d_6) δ: 8.02–8.19 (2H, m), 7.81 (1H, s), 7.65 (1H, d, J = 8.4 Hz), 7.52 (1H, m), 7.38 (1H, m), 7.18–7.32 (2H, m), 7.13 (1H, d, J = 8.1 Hz), 5.92 (2H, s), 4.23 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 153.5, 152.7, 140.8, 130.2, 129.9, 127.1, 125.5, 124.1, 124.0, 123.7, 121.6, 121.2, 120.3, 119.7, 117.2, 110.8, 96.4, 70.8, 31.2. Anal. Calcd for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27. Found: C, 75.42; H, 4.71; N, 9.32. HRMS (ESI positive) Calcd

for $C_{19}H_{14}N_2O_2Na$: 325.09474. Found: 325.09499 $[M+Na]^+$. Calcd for $C_{38}H_{28}N_4O_4Na$: 627.20027. Found: 627.20145 $[2M+Na]^+$.

4.37. Molecular modeling

The three-dimensional structure of the two examined ligands were built and energy minimized within Ghemical.[14] All calculations were performed on a 3.0 GHz Quad-Xeon 64-bit workstation running under MacOSX Tiger. The 2.10 Å resolution crystal structure of human topoisomerase I covalently linked to double-stranded DNA and bound to topotecan[8] (Protein Data Bank entry 1k4t) was used as the reference structure to model the topoisomerase I–DNA complex with **2a** and **3**. After the removal of topotecan and water molecules, hydrogens and partial charges (using the dictionary of partial charges for nucleic acid and protein atoms of the AMBER force-field) were added to the complex. Partial charges were also assigned to the ligands atoms. The two derivatives were docked at the putative binding site using Autodock Vina[15] with a radius of 7 Å centered on the topotecan molecule, with a grid resolution of <0.36 Å. The resulting orientations were clustered, considering a root-mean-square deviation (rmsd) tolerance of 2.0 Å, into families, and the lowest docking-energy conformations were then equilibrated for 1.2 ns by unrestrained MD. The simulations were performed at constant temperature and pressure (NPT ensemble) in a periodic cubic box of pre-equilibrated TIP3P water molecules. The water bond distances and angle were forced using the SETTLE algorithm[16] while the bond lengths within the protein were constrained with the LINCS algorithm.[17] The coupling time was set to 1.0 ps, and the isothermal compressibility was set to 4.6×10^{-5} bar^{-1}. The protein, ligand, and solvent were independently coupled at a temperature of 298 K, coupling time 0.1 ps, and the pressure was held at 1 bar, coupling time 0.2 ps, using a Berendsen thermostat to maintain temperature and pressure unvarying. The time step used was 1.0 fs. Snapshots of the receptor–substrate system were saved every 0.2 ps. Hydrogen bonds and contacts were automatically identified using a contact module of CCP4[18,19] while the other interactions were identified visually.

4.38. Cytotoxic activity

The human non-small cell lung cancer carcinoma cell line NCI-H460 (ATCC, HTB-177) was used. Cells were cultured in RPMI-1640 containing 10% foetal calf serum. Cytotoxicity was assessed by a growth inhibition assay after 1 and 72 h drug exposure. Cells in the logarithmic phase of growth were harvested and seeded in duplicates into six-well plates. Twenty-four hours after seeding, cells were exposed to the drug for 1 and 72 h. Cells were counted 72 h after drug exposure for 1 h of treatment and at the end of drug exposure in the case of 72 h of treatment. Cells were counted with a Coulter counter. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth compared to the untreated control.

4.39. Topoisomerase I-dependent DNA cleavage assay

A gel purified 751-bp BamHI–EcoRV fragment of SV40 DNA was used for the cleavage assay. DNA fragment was uniquely 3′-end labeled. Cleavage reactions (10,000 cpm/sample) were performed in 20 mL of 10 mM Tris–HCl (pH 7.6), 150 mM KCl, 5 mM $MgCl_2$, 0.1 mM dithiotreitol, and the human recombinant enzyme (full length topoisomerase I)[20] for 30 min at 37 °C. Reactions were stopped by 0.5% SDS and 0.3 mg/mL of proteinase K for 45 min at 42 °C. After precipitation DNA was resuspended in denaturing buffer (80% formamide, 10 mM NaOH, 0.01 M EDTA, and 1 mg/mL dyes) before loading on a denaturing 8% polyacrylamide gel in TBE buffer.

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