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Indeno[1,2-*c*]isoquinolin-5,11-diones conjugated to amino acids: Synthesis, cytotoxicity, DNA interaction, and topoisomerase II inhibition properties

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ABSTRACT

Three series of indeno[1,2-*c*]isoquinolin-5,11-dione-amino acid conjugates were designed and synthesized. Amino acids were connected to the tetracycle through linkers with lengths of $n = 2$ and 3 atoms using ester (series I), amide (series II), and secondary amine (series III) functions. DNA binding was evaluated by thermal denaturation and fluorescence measurements. Lysine and arginine substituted derivatives with $n = 3$ provided the highest DNA binding. Arginine derivative **32** ($n = 2$, series II) and glycine derivative **34** ($n = 2$, series III) displayed high topoisomerase II inhibition. Incrementing the length of the *N*-6 side chain from two to three methylene units provided a significant increase in DNA affinity but a substantial loss in topoisomerase II inhibition. The most cytotoxic compounds toward HL60 leukemia cells were **19**, **33**, and **34** displaying micromolar IC_{50} values. When tested with the topoisomerase II-mutated HL60/MX2 cell line, little variation of IC_{50} values was found, suggesting that topoisomerase II might not be the main target of these compounds and that additional targets could be involved.

1. Introduction

Topoisomerases are key enzymes required for the survival of all organisms. They regulate the topological state of the DNA in vital cellular processes of replication, transcription, recombination, repair, chromatin assembly, and chromosome segregation. Topoisomerases act by generating transient breaks in the double helix. There are two major classes of topoisomerases, type I and type II, that are distinguished by the number of DNA strands that they cleave, generating either single- or double-stranded breaks, respectively, and the mechanism by which they alter the topological properties of the genetic material.

Eukaryotic type I topoisomerases are monomeric enzymes that require no high-energy co-factor. They have been further classified[1,2] into three different sub-families: types IA, IB, and IC. Type IB topoisomerases are the targets of important anticancer compounds such as camptothecins,[3,4] indolocarbazoles,[5] and indenoisoquinolines.[6]

Eukaryotic type II topoisomerases are homodimers that require divalent metal ions and ATP for complete catalytic activity. Two isoforms are expressed by vertebrate species: topoisomerases IIα and IIβ displaying a high degree of amino acid sequence identity (~70%). Topoisomerase IIα is essential for the survival of actively growing cells and its concentrations are dramatically upregulated during periods of cell growth. It is generally believed to be the isoform α that functions in growth-dependent processes, such as DNA replication and chromosome segregation.[7]

Topoisomerases I and II control tightly DNA topology by cleaving transiently DNA to form a complex known as the 'cleavage complex' changing its topology by a controlled rotation of the cleaved strand around the intact strand, and religating it to give an intact DNA duplex. DNA topoisomerases are targets for several clinically prescribed anticancer agents. Camptothecins, potent topoisomerase I poisons, reversibly trap the topo I in the covalent complex with DNA, inducing an accumulation of DNA damage that triggers cell death. Among topoisomerase I poisons, the only FDA/EMEA-approved compounds are the camptothecin derivatives irinotecan and topotecan used to treat colorectal, ovarian, and lung cancers.

Topoisomerase II poisons represent some of the most important and widely prescribed anticancer drugs. Among them, etoposide,

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doxorubicin, mitoxantrone, and amsacrine are indicated for a variety of malignancies, including leukemias, lymphomas, sarcomas, breast, and lung cancers.

Clinical uses of these molecules are limited by their toxicities and development of multidrug resistance (MDR) phenomenon, essentially due to the induction and action of the membrane ATP-dependent multidrug efflux pump P-glycoprotein (MDR1 and ABCB1) which extrudes cytotoxic molecules, keeping intracellular drug concentration below a cell-killing threshold.[8]

Over the past 20 years, new topoisomerases inhibitors have been designed to overcome limitations cited above. Indenoisoquinolines are novel non-camptothecin topoisomerase I poisons endowed with greater stability compared to camptothecins and potentially lower toxicities. Topoisomerase I cleavage complexes trapped by indenoisoquinolines display better stability, which could increase the duration of action of the drugs. These compounds are also weakly recognized by resistance efflux pumps (MDR-1 and ABCG2). Among the over 400 indenoisoquinolines evaluated, three have been selected as leads for clinical development by the National Cancer Institute,[6] particularly compound **1** (NSC 706744, Fig. 1).

Given the potential of this family, a great number of structural modifications were introduced at both the *N*-6 position and at the indenoisoquinoline skeleton on the aromatic A and D cycles[9–12] to provide structure–activity relationships.[13,14] Modifications at the *N*-6 position such as introduction of aminoalkyl chains provided potent topoisomerase I inhibitors. Non-substituted molecules on the A and D aromatic cycles could also display good topoisomerase I inhibition and antiproliferative activity.[15] A crystallographic study performed with compound **2** (MJ-238, Fig. 1)[16] bound to the topoisomerase I-DNA cleavage complex revealed that the aromatic tetracycle interacts with both DNA and topoisomerase I, whereas the side chain was placed in a hydrophilic binding pocket of the enzyme.[17] Introduction of dimethylaminoethoxy substituent on D-cycle at 8-position and dimethylaminoethyl at *N*-6 position provided a good topoisomerase II inhibitor[18] (compound **3**, Fig. 1).

In recent years, various drug-amino acid conjugates were reported, such as DNA-acting drugs anthraquinones,[19–21] naphthalimides,[22,23] indolocarbazoles[24,25] and camptothecins.[26] In some cases, promising results were obtained with conjugates showing increased water-solubility and cytotoxicity, reduced drug resistance and toxicity. These considerations prompted us to consider a similar approach in the indenoisoquinoline series. In the current work, we report the synthesis (Fig. 2) of a novel series of molecules bearing basic amino acids (lysine, histidine, arginine) on the *N*-6 side-chains of the indenoisoquinolinedione framework. The cytotoxicity, DNA interaction, and topoisomerase I and II inhibition properties of these molecules were evaluated. The goal was to increase the overall basicity of the molecules in order to enhance DNA binding as well as the aqueous solubility. Amino acids were connected to the tetracycle through linkers with lengths of 2 and 3 atoms using ester (series I), amide (series II), and secondary amine (series III) functions. In each of the three series, glycine analogues were synthesized and evaluated as controls.

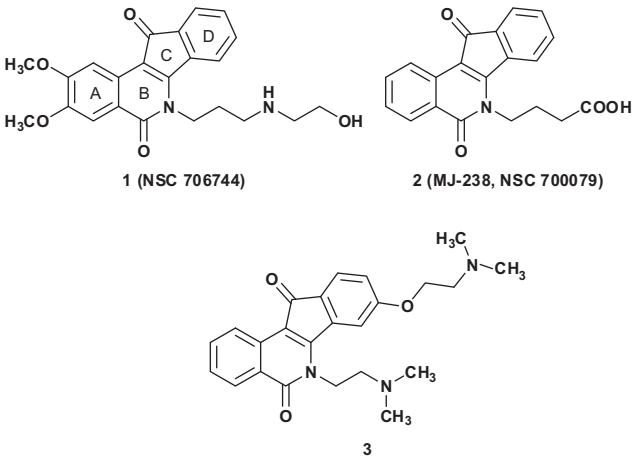


Figure 1. Indenoisoquinolin-5,11-dione inhibitors of topoisomerases I and II.

2. Chemistry

The synthesis of the targeted compounds is depicted in Schemes 1 and 2. The indenopyrandione **38**, obtained by a described procedure[9] slightly modified, was used as a precursor for the synthesis of compounds **4**–**37**. Esters **4**–**17** were prepared by the condensation of lactone **38** with commercially available aminoethanol or aminopropanol followed by the reaction of resulting alcohols **39** and **40** with the corresponding Boc-protected amino acids using EDCI as an activator to provide esters **4a**–**17a**. This operation spared the stereogenic centers and subsequent removal of *tert*-butoxycarbonyl groups by acidolysis (HCl in isopropanol) yielded the desired products as hydrochloride salts.

Condensation of precursor **38** with *N*-Boc-protected *N*,*N*-ethylenediamine **41** or *N*,*N*-propanediamine **42** followed by acidolysis afforded the NH_2 free intermediates **43** and **44**. Amides **18a**–**33a** were obtained, respectively, by reaction with Boc-protected amino acids using either EDCI/HOBt or HBTU/HOBt as coupling agents. Deprotection of the *tert*-butoxycarbonyl groups was performed by acidolysis to provide the desired products **18**–**33** as hydrochloride salts. It is noteworthy that for deprotection of compounds **32a** and **33a**, the usually employed conditions (HCl in isopropanol) failed. The use of $SnCl_4$ was required to secure the formation of arginine-containing derivatives **32** and **33**.

For elaboration of compounds from series III bearing amine functionalities, *N*-Boc-protected glycine and lysine were initially converted into corresponding Weinreb amides **45** and **46** which were subsequently reduced with $LiAlH_4$ to provide the corresponding α-aminoaldehydes. A reductive amination process involving the aminoalkylated derivatives **43** and **44** delivered the *N*-Boc-protected compounds **34a**, **36a**, and **37a**. Subsequent acidolysis with HCl 5 M in isopropanol afforded very satisfactory yields of the desired compounds **34**, **36**, and **37**. Amine **35** was readily assembled through an alternative pathway involving nucleophilic substitution between the tosylate **47** and the *N*-Boc-protected-*N*,*N*-ethylenediamine **41**, followed by standard acidolysis.

Many attempts to introduce *N*-protected arginines in Series I and in Series III were performed. Unfortunately, concerning the Series I, although the esterification reaction was proven to be successful, the harsh conditions used for the deprotection step led to the exclusive formation of the transcription side products. Several strategies to synthesize the expected esters were subsequently envisaged but all attempts to assemble these compounds were invariably doomed to failure.

As for the Series III, taking into account the undesirable racemization observed for D- or L-lysine derivatives, only the syntheses involving the racemic DL-lysine were then considered. Histidine derivatives were also eliminated from the current study due to the failure to synthesize the histidinal: to the best of our knowledge, no description of histidinal synthesis has been found in the literature. And finally, arginine derivatives were dismissed from further studies since even under forcing conditions, the ultimate reductive amination liable to give access to the targeted compounds failed and an inextricable mixture of unidentified products was obtained instead.

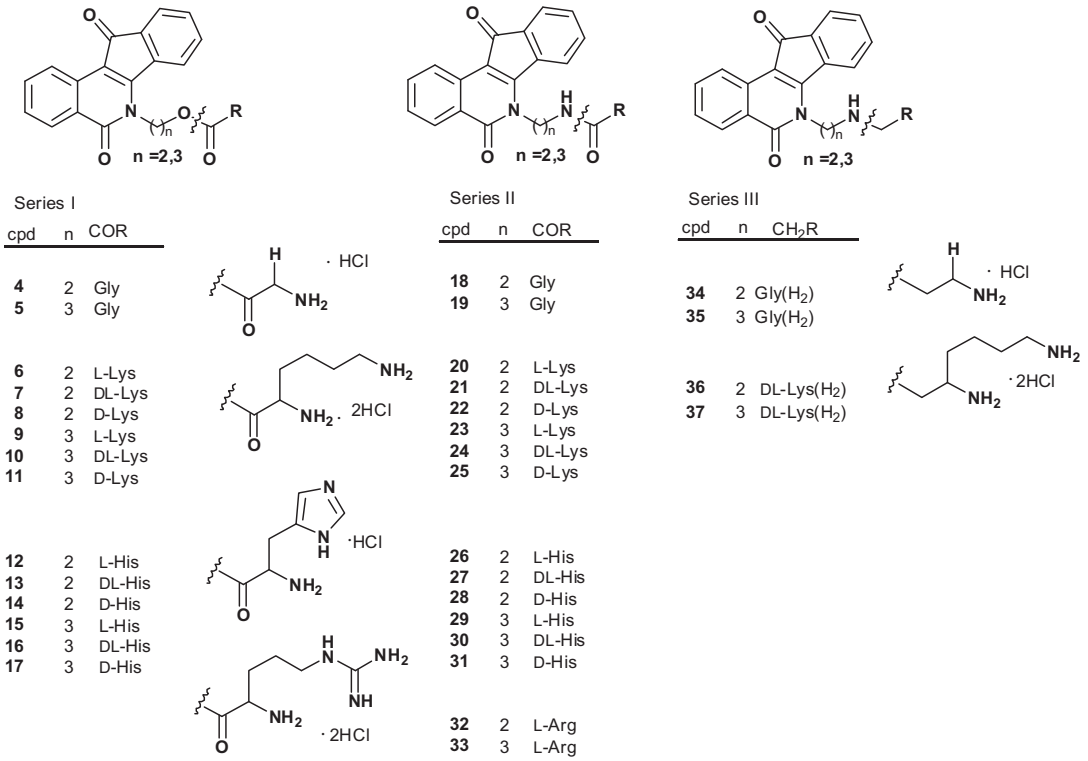
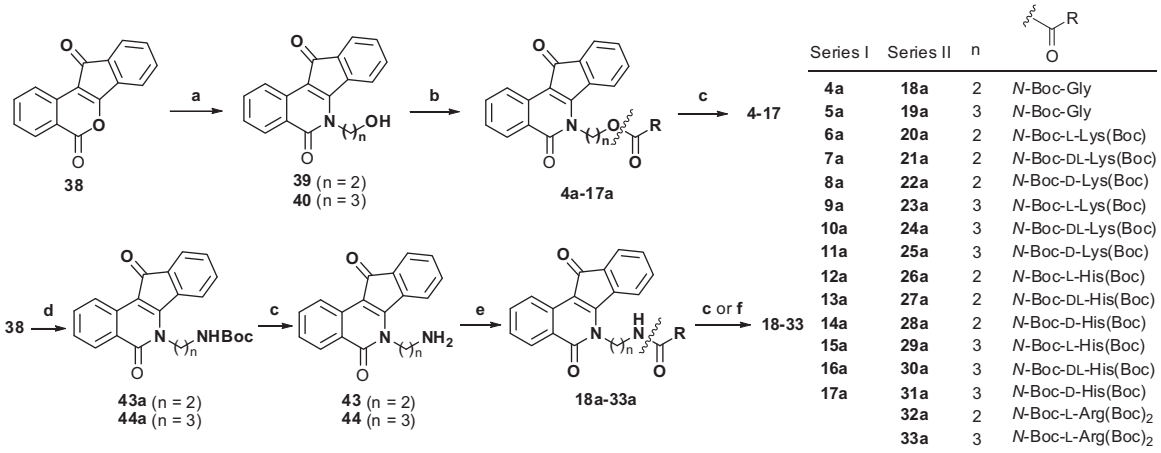


Figure 2. Compounds considered in the present study.



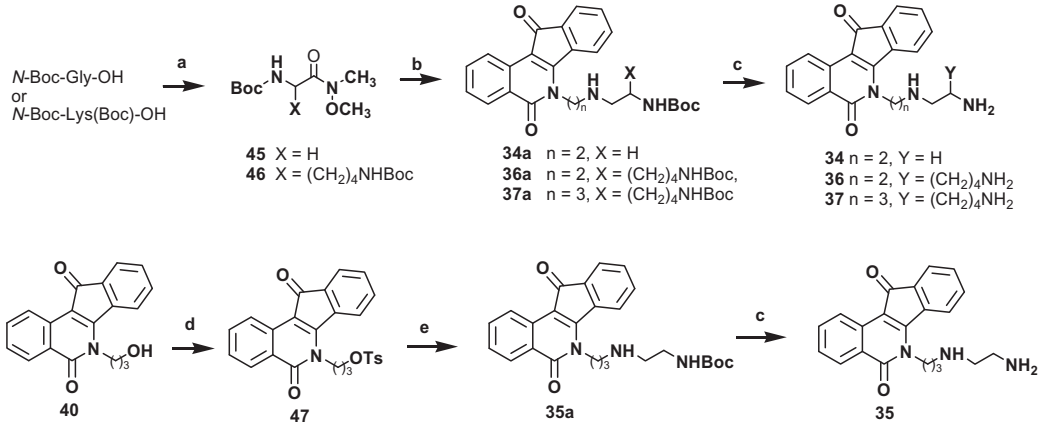
Scheme 1. Synthesis of compounds from series I and II. Reagents and conditions: (a) $NH_2(CH_2)_nOH$, $n = 2$ (**39**) or 3 (**40**), $CHCl_3$, rt; (b) corresponding *N*-Boc protected amino acid, EDCI, DMAP, CH_2Cl_2, rt; (c) HCl, isopropanol, $CHCl_3$, rt; (d) $NH_2(CH_2)_2NHBoc$ (**41**) or $NH_2(CH_2)_3NHBoc$ (**42**), $CHCl_3$, rt; (e) corresponding *N*-Boc protected amino acid, EDCI/HOBt or HBTU/HOBt, DMAP, CH_2Cl_2, rt; (f) $SnCl_4$, AcOEt, rt.

3. Results and discussion

3.1. DNA interactions

3.1.1. DNA thermal denaturation

The ability of the drugs to protect calf thymus DNA (CT DNA, 42% GC bp) against thermal denaturation was used as an indicator of their relative capacity to bind and to stabilize the DNA double helix. Variations of T_m values $\Delta T_m = T_m^{\text{drug}-\text{DNA complex}} - T_m^{\text{DNA alone}}$ are presented in Table 1. They ranged from 2.1 °C (compound **13**) to 26.7 °C (compound **37**). Amides from series II (**18**–**31**) display better ΔT_m values than their ester counterparts (**4**–**17**). Reduction of amide (series II) to amine function (series III) increased significantly duplex stabilization as shown by comparison of series III derivatives (**34**–**37**) with their respective amide counterparts (**18**, **19**, **21**, and **24**). Incrementing the length of the *N*-6 side chain from two to three methylene units enhanced ΔT_m values for all tested compounds from the three series, except for histidine derivatives (**12**-**17** and **26**-**31**). As expected, among all amino acids used in this study, lysine provided the strongest duplex stabilization. In series II and III lysine derivatives were twofold more potent than their reference glycine counterparts (**20**–**22** vs **18**, **23**–**25** vs **19**, **36** vs **34**, and **37** vs **35**) and in series I they display three to four times more potent stabilization (**6**–**8** vs **4**, **9**–**11** vs **5**). In series II, arginine derivatives (**32** and **33**) were equipotent to lysine (**20** and **23**). These values were similar to daunorubicin values



Scheme 2. Synthesis of compounds from series III. Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine, EDCI, DMAP, CH_2Cl_2, rt; (b) (i) $LiAlH_4$, THF, 0 °C, (ii) compound **43** or **44**, $NaHB(OAc)_3$, triethylamine, CH_2Cl_2, rt; (c) HCl, isopropanol, $CHCl_3$, rt; (d) TsCl, triethylamine, CH_2Cl_2, rt; (e) compound **41**, CH_3CN, 80 °C.

reference intercalative compound. Finally, no significant differences were found between L- and D-stereoisomers from series I and II.

The high ΔT_m values obtained with the lysine and arginine derivatives are most likely attributable to additional electrostatic interactions or hydrogen bonding between protonated basic side-chains of the amino acids and the negatively charged DNA backbone.

3.1.2. Fluorescence measurements

DNA binding affinities were quantified by means of fluorescence (Table 1). Since a weak fluorescence was observed upon DNA titration with all compounds, an indirect method was privileged. We used the conventional fluorescence quenching assay based on DNA binding competition between the intercalating drug ethidium bromide and the tested molecules.[27,28] As reported in Table 1, K_{app} values ranged from 0.22×10^6 (compounds **13** and **14**) to 16.97×10^6 (compound **37**). Replacing ester (series I) by amide (series II) connection had no significant effect on histidine and glycine derivatives DNA affinity but provided a net increase for lysine analogues displaying K_{app} values (**20**–**22** vs **6**–**8**, **23**–**25** vs **9**–**11**). Compounds from series III display higher K_{app} values than their amide and ester counterparts as noted for lysine derivatives (**36** vs **21** and **7**, **37** vs **24** and **10**). For glycine derivatives the effects obtained are more pronounced as K_{app} values are 10-fold superior to their amide and ester counterparts (**34** vs **18** and **4**, **35** vs **19** and **5**) which suggest the key role of the secondary amino group as H-donor for the DNA interaction.

When considering the influence of the amino acid in DNA interaction, the best K_{app} values in the three series were obtained for lysine derivatives. The results obtained by the fluorescence assay were consistent. Incrementing the length of the *N*-6 side chain from two to three methylene units leads to a significant increase in DNA affinity for lysine derivatives: 10-fold in series I (**9**–**11** vs **6**–**8**), twofold in series II (**23**–**25** vs **20**–**22**), 1.2-fold in series III (**37** vs **36**).

From both experiments (ΔT_m and K_{app}), DNA binding rank in the order amine > amide > ester which follows the variation of percentages of ionization in the three series (Table 1), suggesting a relationship between the two parameters as protonated forms are more likely to provide DNA binding and stabilization. Compound **37** displayed the highest ΔT_m and K_{app} values, indicating both good affinity for DNA and duplex stabilization.

3.1.3. Mode of binding to DNA

Different binding modes (i.e. intercalation and/or groove binding) can account for the melting temperature evaluation. As ΔT_m and fluorescence experiments suggest an interacting mode, a DNA unwinding assay was used (Fig. 3) to confirm it.

The experiments were based on the relaxation of supercoiled plasmid DNA in the presence or not of topoisomerase I and increasing concentration of the tested compounds. We selected compounds with the highest ΔT_m and K_{app} in each series. We also selected compounds with three carbon lactam substituent chain length ($n = 3$) as they displayed more pronounced effect than their shorter side-chain counterparts ($n = 2$). As shown in Figure 3, we compared DNA relaxation of racemate and enantiomer compounds in series I (Fig. 3A) and II (Fig. 3B). We also compared series II and III with the lysine derivatives (Fig. 3A) and the lysine and arginine derivatives (Fig. 3B). As shown in Figure 3, the DNA (lane DNA) relaxed by topoisomerase I alone (lane Topo) generated a family of DNA topoisomers with a slow electrophoretic mobility. When the drug was added while topoisomerase I was maintained in the reaction mixture, the closed circular duplex DNA was progressively supercoiled, indicating that the drug intercalates into DNA.[29,30]

All tested compounds in series I, II, and III modify the relaxation of the DNA with a passage from negative to positive in the helical superturn with 1 or 2 μM of compound. There are no differences between compounds: any difference between racemate and enantiomers in series I and II, between lysine derivatives in series I and III, between lysine and arginine derivatives in series II.

Only compounds with space length of three methylene units were reported on Figure 3. ($n = 3$) as they displayed more pronounced effect than their shorter side-chain counterparts ($n = 2$).

In series I, compound **10** (Lys) intercalated more efficiently than **16** (His), as judged from the appearance of the supercoiled DNA band, respectively, for 5 and 10 μM (data not shown). In series II, for selected compounds, the ranking was **24** (Lys) > **19** (Gly) > **30** (His), **33** (Arg) (data not shown). Amides displayed better intercalation than ester counterparts as noted for lysine (**24** vs **10**) and histidine (**30** vs **16**) analogues (data not shown). When comparing the nature of the amino acid, the best intercalating profile was obtained for lysine derivative **24** which achieved the same effect at lower drug concentration (compare the 2 μM lanes). They also displayed the highest ΔT_m and K_{app} values in series II. In series III, compound **37** displayed good intercalating profile comparable to the best molecule in the amide series, compound **24**.

At this point, it is worth to mention that none of the compounds was found to function as a topoisomerase I poison. Molecules were tested in a topoisomerase I assay using ethidium bromide containing gels. In contrast to camptothecin, no topoisomerase I-induced strand breaks was observed with any of the compounds.

Table 1

Compd	*n*	Aminoacyl	ΔT_m[a]	K_{app}[b]	% ionization[c]
DN[d]			21.9	nd[e]	
Etop[f]			0	-[g]	
Series I					
4	2	Gly	3	0.68 ± 0.05	62.4
5	3	Gly	5.5	0.66 ± 0.04	66.0
6	2	L-Lys	9.1	0.89 ± 0.07	100
7	2	DL-Lys	9	0.87 ± 0.06	100
8	2	D-Lys	10.4	0.90 ± 0.09	100
9	3	L-Lys	15.2	8.36 ± 0.49	100
10	3	DL-Lys	12.6	8.09 ± 0.39	100
11	3	D-Lys	15.2	8.27 ± 0.52	100
12	2	L-His	3.0	0.58 ± 0.07	61.3
13	2	DL-His	2.1	0.25 ± 0.03	61.3
14	2	D-His	2.2	0.22 ± 0.03	61.3
15	3	L-His	2.3	0.60 ± 0.09	65.1
16	3	DL-His	3.5	0.69 ± 0.07	65.1
17	3	D-His	4.7	0.72 ± 0.06	65.1
Series II					
18	2	Gly	5	0.71 ± 0.05	85.5
19	3	Gly	9.8	0.95 ± 0.08	86.8
20	2	L-Lys	13.2	5.86 ± 0.18	100
21	2	DL-Lys	13.1	6.03 ± 0.29	100
22	2	D-Lys	12.6	5.98 ± 0.22	100
23	3	L-Lys	23.8	10.24 ± 0.32	100
24	3	DL-Lys	20.9	10.59 ± 0.41	100
25	3	D-Lys	18.4	11.09 ± 0.45	100
26	2	L-His	3.4	0.65 ± 0.06	86.7
27	2	DL-His	4.5	0.62 ± 0.03	86.7
28	2	D-His	7.3	0.67 ± 0.05	86.7
29	3	L-His	7.9	0.72 ± 0.08	88
30	3	DL-His	4.9	0.73 ± 0.05	88
31	3	D-His	5.5	0.69 ± 0.08	88
32	2	L-Arg	13.6	7.29 ± 0.44	100
33	3	L-Arg	21.0	10.23 ± 0.55	100
Series III					
34	2	Gly	11.4	6.99 ± 0.38	99.8
35	3	Gly	14.9	12.35 ± 0.58	99.9
36	2	DL-Lys	23.8	13.88 ± 0.59	100
37	3	DL-Lys	26.7	16.97 ± 0.74	100

[a] Variation in melting temperature $\Delta T_m(T_m^{\text{drug DNA complex}} - T_m^{\text{DNA alone}})$. Drug/CT DNA ratio = 0.25; mean values correspond to $n = 3$ with deviation standard ± 10%.
[b] Apparent binding constant measured by fluorescence using [BET]/[DNA] = 1.26, K_{app} in 10^6 M^{-1}.
[c] Calculated percentage of ionized form at pH 7.4.
[d] Daunorubicin (ΔT_m at ratio 1).
[e] Interferences of fluorescence spectra of DNA and BET were observed.
[f] Etoposide.
[g] 28% of displacement of BET for etoposide at 10 μM.

3.2. Topoisomerase II inhibition

A conventional DNA relaxation assay was used to assess the effects of the compounds from the three series on the catalytic activity of human topoisomerase II. In these experiments supercoiled plasmid DNA was treated with topoisomerase II in the presence of two concentrations of the tested drug (20 μM and 50 μM) and the DNA relaxation products were then resolved by gel electrophoresis on agarose gels. The reference drug etoposide produces a marked level of DNA double stranded breaks, corresponding to linear DNA (lin, Fig. 4). We essentially compared results in function of the length of the *N*-6 side chain (two or three) methylene chains in series I (**6**–**8** vs **9**–**11**), II (**32** vs **33**), and III (**34** vs **35**). We also compared results for lysine (**6**–**8** vs **9**–**11**) or arginine (**32** vs **33**) or glycine (**34** vs **35**) derivatives.

As shown in Figure 4A, in series I, lysine ester **6** ($n = 2$) displayed the highest activity. Increasing the spacing arm length totally suppressed the activity (**6** vs **9**, **7** vs **10**). We did not detect a DNA topoisomerase II inhibition with the D-enantiomer **8** compared to the **6** L-enantiomers and the **7** DL-racemic mixture at tested concentrations. Interestingly, for compounds **6** and **7** inhibition at 50 μM was lower than at 20 μM indicating possibly inhibition of topoisomerase II mediated DNA cleavage at 50 μM. They act as topoisomerase II poisons at low concentrations (<20 μM) and as topoisomerase II suppressors at high concentrations (>50 μM). This trend has already been reported for indenoisoquinoline derivatives.[31] Compounds with a three methylene chain of the *N*-6 side chain in series I (**9**, **10**, and **11**) had no effect on the inhibition of topoisomerase II. All other compounds in series I had no effect on the inhibition of topoisomerase II (data not shown).

In series II, no inhibition was detected for lysine and histidine derivatives (data not shown). In contrast, as shown in Figure 4B, arginine derivative **32** ($n = 2$) displayed high inhibition, comparable to etoposide. Increasing the spacing arm length suppressed the activity when compared to its counterpart **33** ($n = 3$). In series III, glycine derivative **34** ($n = 2$, Fig. 4B) displayed high inhibition comparable to **32**. Increasing the spacing arm length decreased the inhibition when compared to derivative **35** ($n = 2$). Replacing glycine by lysine totally suppressed the activity (**34**–**35** vs **36**–**37**) (data not shown).

3.3. In vitro antiproliferative activity

The antiproliferative activities of these compounds were tested (Table 2) using two human leukemia cell lines, HL60 and HL60/MX2, respectively sensitive and resistant to the antitumor drug mitoxantrone.

To gain an insight into the involvement of topoisomerase II inhibition in the cytotoxicity of the molecules, their antiproliferative activity was assessed using the HL60/MX2 cell line resistant to mitoxantrone, which displays altered catalytic activity and reduced levels of topoisomerase II[32] (the values for mitoxantrone and etoposide are given as references). All compounds were tested except for D-lysine derivatives. When evaluated on the HL60 cell line, all compounds displayed IC_{50} values ranging from 1.2 μM ± 0.145 (**19** and **34**) to 21.3 μM ± 0.4 (**10**).

Compounds from series I displayed moderate to weak cytotoxicities (IC_{50} values between 4.95 ± 0.05 μM and 21.3 ± 0.4 μM). Glycine derivative which exert a topoisomerase II inhibition (**4**, $n = 2$) displayed the best activity. For intercalative compounds (**9** and **10**), the IC_{50} values were higher. Lysine derivatives which exert an inhibition of topoisomerase II or a DNA intercalation were less active. We hypothesize that low value obtained could be attributable at least partially to the instability of the ester function.

In contrast, in series II, compounds displayed better cytotoxicities, IC_{50} values ranging from 1.2 ± 0.145 μM to 5.8 ± 0.15 μM. Differences in IC_{50} values between racemates and enantiomers were not significative at micromolar range. Lysine amides **20**–**24** displayed IC_{50} values fourfold lower than for their ester counterparts **6**–**10**. One of the highest cytotoxicity was obtained for arginine derivative **33**. This compound also displayed the strongest DNA interaction (ΔT_m and K_{app}) in this series and this could account, at least partially for the good cytotoxicity obtained. In contrast, lysine derivative **23** which displayed similar DNA interaction parameters to compound **33** was four times less cytotoxic, indicating that the aminoacyl plays a role in the cytotoxicity and other parameters could be involved such as membrane uptake or additional cellular targets. DNA interaction was found to correlate neither with topoisomerase poisoning nor with the antiproliferative activity. The highest cytotoxicities were obtained for compounds **19**, **33** and **34**. For compound **33** strong DNA interaction could account for the high cytotoxicity obtained. Compound **34** displayed lower

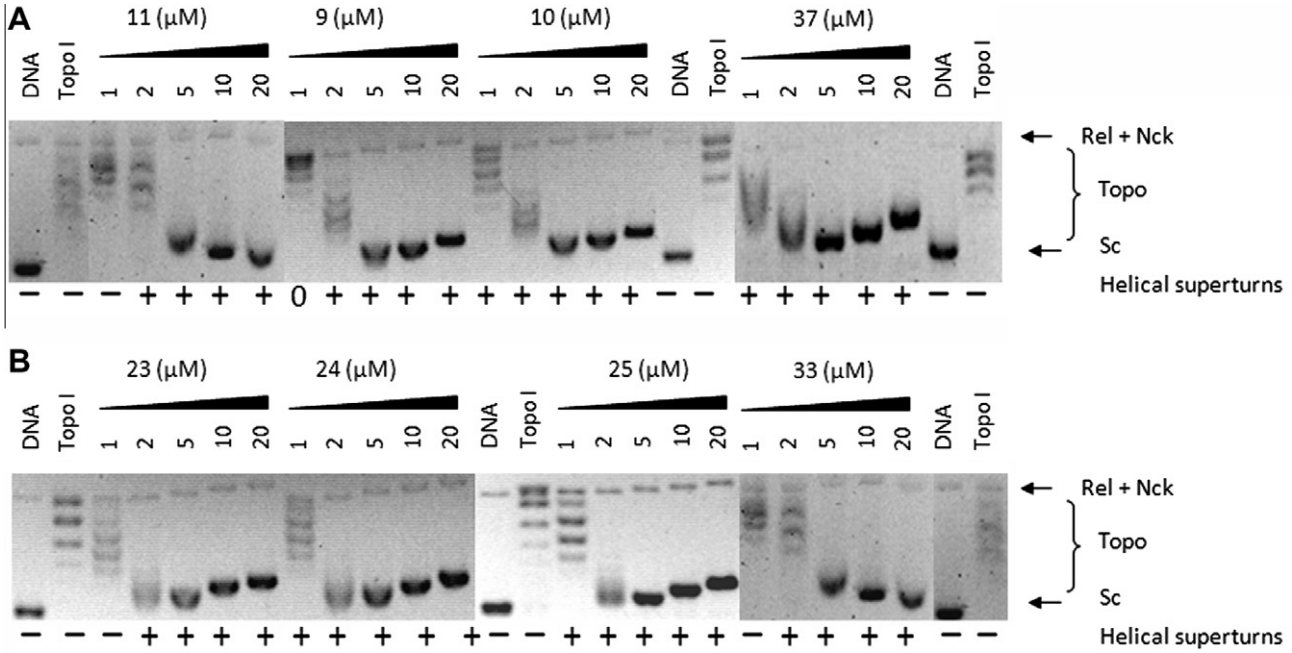


Figure 3. (A) Effects of lysine derivatives from series I (**9**, **10**, and **11**), and series III (**37**) with $n = 3$ on the relaxation of plasmid DNA by human topoisomerase I. (B) Effect of lysine (**23**, **24**, **25**) and arginine (**33**) compounds from series II. Native supercoiled pUC19 (130 ng, lane DNA) was incubated with 4 units of topoisomerase I in the absence (lane Topo) or presence of tested compound at the indicated concentration (from 1 to 20 μM). DNA samples were separated by electrophoresis on a 1% agarose gel which was stained with ethidium bromide after DNA migration. Gels were photographed under UV light. Nck: nicked; Sc: supercoiled; Rel: relaxed; Topo: topoisomer products.

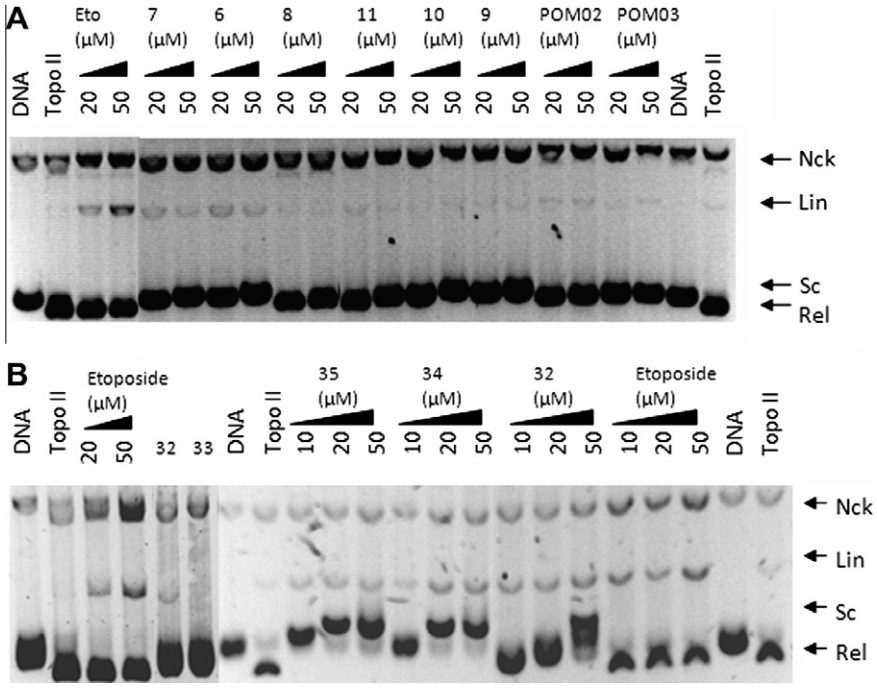


Figure 4. (A) Effect of lysine derivatives from series I (**6**–**11**) compared with reference compounds (POM02 and POM03). (B) Effect of arginine derivatives from series II (**32**, **33**) and glycine derivatives from series III (**34**, **35**) on the relaxation of plasmid DNA by human topoisomerase II. Native supercoiled pUC19 (130 ng, lane DNA) was incubated with 4 units topoisomerase II in the absence (lane Topo) or presence of tested compound at the indicated concentration (A: 20 and 50 μM, B: from 10 to 50 μM and 20 μM for **32**, **33**). Etoposide was used at the same concentrations. DNA samples were separated by electrophoresis on a 1% agarose gel containing 1 μg/mL ethidium bromide. Gels were photographed under UV light. Nck: nicked; Sc: supercoiled; Lin: linear; Rel: relaxed.

DNA interaction but it is a good topoisomerase II inhibitor. Distribution coefficient for compound **19** ($c \log D = 0.1$) was higher than for compounds **34** ($c \log D = -1.0$) and **33** ($c \log D = -2.6$) indicating better membrane uptake which could at least partially account for the good cytotoxicity obtained despite weaker DNA interaction and lack of topoisomerase II inhibition.

Table 2

Compd	*n*	Aminoacyl	*c* log *D*[b]	IC_{50} (μM)[a]		RRI[c]
				HL60	HL60/MX2	
MX[d]				**0.063 ± 0.020**	**1.51 ± 0.08**	24
Etop[e]				**0.486 ± 0.190**	48.5 ± 1.5	100
Series I						
4	2	Gly	1.6	4.95 ± 0.05	27.5 ± 0.85	5.56
5	3	Gly	1.6	nd[f]	nd	nd
6	2	L-Lys	1.2	12.3 ± 0.2	32 ± 3	2.60
7	2	DL-Lys	1.2	14 ± 0.5	28 ± 0.01	2.00
8	2	D-Lys	1.2	nd	nd	nd
9	3	L-Lys	1.1	14.55 ± 0.5	24.5 ± 1.05	1.68
10	3	DL-Lys	1.1	21.3 ± 0.4	22.5 ± 0.5	1.06
11	3	D-Lys	1.1	nd	nd	nd
12	2	L-His	1.2	7.8 ± 0.2	23 ± 2	2.95
13	2	DL-His	1.2	9.2 ± 0.4	21.0 ± 1	2.28
14	2	D-His	1.2	9.2 ± 0.75	16.5 ± 0.95	1.79
15	3	L-His	1.4	15.7 ± 0.2	22.6 ± 1.05	1.44
16	3	DL-His	1.4	8.9 ± 0.85	16.0 ± 0.95	1.80
17	3	D-His	1.4	10.5 ± 0.85	15.25 ± 1.05	1.45
Series II						
18	2	Gly	0.0	2.5 ± 0.3	3.0 ± 0.4	1.20
19	3	Gly	0.1	1.2 ± 0.145	1.6 ± 0.25	1.33
20	2	L-Lys	2.7	1.85 ± 0.05	2.55 ± 0.15	1.38
21	2	DL-Lys	2.7	3 ± 0.1	3.8 ± 0.2	1.27
22	2	D-Lys	2.7	nd	nd	nd
23	3	L-Lys	2.6	3.2 ± 0.2	4.95 ± 0.15	1.55
24	3	DL-Lys	2.6	5.25 ± 0.35	7.25 ± 0.55	1.38
25	3	D-Lys	2.6	nd	nd	nd
26	2	L-His	0.05	3.6 ± 0.4	4.0 ± 0.5	1.11
27	2	DL-His	0.05	5.8 ± 0.15	14.7 ± 0.7	2.53
28	2	L-His	0.05	4.6 ± 0.05	17.5 ± 0.75	3.80
29	3	L-His	0.07	3.0 ± 0.15	2.8 ± 0.05	0.93
30	3	DL-His	0.07	2.45 ± 0.35	4.65 ± 0.55	1.90
31	3	D-His	0.07	2.8 ± 0.05	4.9 ± 0.15	1.75
32	2	L-Arg	2.7	2.0 ± 0.15	3.1 ± 0.3	1.55
33	3	L-Arg	2.6	1.3 ± 0.15	2.3 ± 0.25	1.77
Series						
34	2	Gly	1.0	1.2 ± 0.1	2 ± 0.01	1.67
35	3	Gly	1.0	2.95 ± 0.15	4.85 ± 0.25	1.64
36	2	DL-Lys	2.5	7.6 ± 0.4	5.5 ± 0.35	0.72
37	3	DL-Lys	2.4	2.4 ± 0.1	2.8 ± 0.3	1.17

[a] The cytotoxicity IC_{50} values are the concentrations corresponding to 50% growth inhibition.
[b] Calculated log *D*.[33]
[c] Relative resistance index: $IC_{50}^{(MX\text{-}resistant)}/IC_{50}^{(MX\text{-}sensitive)}$.
[d] Mitoxantrone.
[e] Etoposide.
[f] Not determined.

The best in vitro topoisomerase II poisons **32** and **34** comparable to etoposide displayed weak resistance index on MX2 which could be explained by their binding to the enzyme at a different site from mitoxantrone or specific kinetics.

4. Conclusion

In this study, amino-acid moieties especially with protonable side-chains were introduced on indenoisoquinolin-5,11-dione skeleton. Variations were introduced in the length of the spacing arm, connecting function, amino-acid structure, and chirality to provide structure–activity relationships.

Lysine and arginine derivatives displayed high DNA interaction consistent with the establishment of additional electrostatic and/or hydrogen interactions. The compounds displayed good intercalant profiles at concentrations above 5 μM, and two of them (**32** and **34**) are good topoisomerase II poisons.

As noted in our previous study,[18] compounds bearing a spacing linker with three methylene units ($n = 3$) were more favorable to DNA interaction than the ones with a spacing linker with two methylene units ($n = 2$). Strong affinity to DNA is detrimental to topoisomerase II inhibition as the best topoisomerase II poison (**32**) displayed only moderate interaction to DNA and the best DNA ligand (**37**) displayed no topoisomerase II inhibition. No significant differences were observed for enantioselectivity of these compounds in all of the experiments.

This present study revealed two new topoisomerase II poison, arginine derivative **32** and glycine derivative **34** displaying potency comparable to the reference topoisomerase II poison etoposide and cytotoxicity in the micromolar range. Further studies about the role of guanidine group in topoisomerase II inhibition are in progress.

No obvious relationship was found between cytotoxicity, DNA interaction and topoisomerase II inhibition. This could be the result of several contributions such as differences in cellular uptake and/or involvement of targets and mechanisms different from topoisomerase II-mediated DNA cleavage. Uptake studies as well as stability of the cleavage complex will be conducted on our compounds in attempt to rationalize the results obtained.

5. Experimental

5.1. General

All starting materials were purchased from Acros Organics, Alfa Aesar, Sigma–Aldrich and Bachem. Common organic solvents such as petroleum spirit, ethyl acetate, dichloromethane, chloroform, acetone, and toluene were purchased from VWR. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were pre-dried with anhydrous Na_2SO_4, then distilled over sodium benzophenone ketyl under Ar and stored over sodium wire before use. When required, an inert atmosphere was obtained by using dry Ar. ^1H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz on a BRUKER AM300WB spectrometer. Chemical shifts (ppm) were reported with tetramethylsilane ($SiMe_4$) as internal reference. Analytical thin layer chromatography (TLC) was carried out on aluminum plates pre-coated with Merck Kieselgel 60 GF_{254} and products visualized by UV light. For flash column chromatography, Merck silica gel 60 (40–63 μm) was used. Melting points were determined by using an electrothermal REICHERT THERMOPAN and are uncorrected. Optical rotation values were given by Perkin Elmer Model 343 Polarimeter emitting lights with a Na/Hal lamp at a wavelength of 589 nm, at 20.0 °C. High-resolution mass spectra (HRMS) were obtained on an Orbitrap Exactive Thermo Scientific mass spectrometer by positive mode electrospray ionization.

5.2. Benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (38)

To a suspension of 2-carboxybenzaldehyde (3.00 g, 20.0 mmol, 1.0 equiv) and phthalide (2.68 g, 20.0 mmol, 1.0 equiv) in EtOAc (10 mL) a solution of sodium methoxide (25 mL of 3.2 M methanolic solution) was added at 65 °C. The resulting mixture was maintained at 65 °C for 18 h under stirring. The mixture was subsequently diluted at 0 °C with distilled water (50 mL), washed with Et_2O (3 × 50 mL) and acidified with HCl 12 M until pH 1. The resulting yellow precipitate was filtered, dried in vacuo and was diluted with acetic anhydride (60 mL). The mixture was stirred at 100 °C for 3 h and cooled to room temperature. The resulting solid was filtrated and washed with Et_2O to give the desired product as bright orange solid. Yield: 2.75 g, 55%. mp: 257–259 °C. ^1H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.40 (d, *J* = 7.9 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H),

7.83 (dd, J = 8.9 Hz, J = 1.2 Hz, 1H), 7.62 (d, J = 6.9 Hz, 1H), 7.57–7.49 (m, 4H).

5.3. General procedure for the synthesis of 6-hydroxyalkyl-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 39 and 40

2-Aminoethanol or 3-aminopropan-1-ol (40.0 mmol, 10.0 equiv) were added to a solution of **38** (1.00 g, 4.0 mmol, 1.0 equiv) in $CHCl_3$ (12 mL). After stirring at room temperature for 48 h, the reaction mixture was diluted in $CHCl_3$ (100 mL) and subsequently washed with distilled water (2 × 50 mL), HCl 0.1 M (1 × 25 mL), and brine (1 × 50 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to afford dark-orange needles. The crude product was used in the next step without further purification.

5.3.1. 6-(2-Hydroxyethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (39)

Compound **39** was prepared from 2-aminoethanol (2.4 mL). Yield: 1.04 g, 89%. mp: 210–212 °C. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.74 (d, J = 8.0 Hz, 1H), 8.36 (d, J = 8.2 Hz, 1H), 7.77 (dd, J = 6.0 Hz, J = 1,3 Hz, 1H), 7.68–7.60 (m, 2H), 7.53–7.40 (m, 3H), 4.78 (t, J = 5.9 Hz, 2H), 4.20 (q, J = 5.8 Hz, 2H), 2.45 (t, J = 5.3 Hz, 1H).

5.3.2. 6-(3-Hydroxypropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (40)

Compound **40** was prepared from 3-aminopropan-1-ol (3.0 mL). Yield: 1.16 g, 95%. mp: 191–192 °C. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.47 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.79–7.65 (m, 2H), 7.54–7.42 (m, 4H), 4.75 (t, J = 6.6 Hz, 2H), 3.72 (t, J = 5,0 Hz, 2H), 3.26 (t, J = 6.3 Hz, 1H), 2.19–2.15 (m, 2H).

5.4. General procedure for the synthesis of 6-(*N*-Boc-aminoacyl-oxyalkyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 4a–17a

To a suspension of **39** or **40** (1.1 mmol, 1.0 equiv) in CH_2Cl_2 (8 mL) DMAP (1.1 mmol, 1.0 equiv) and the *N*-Boc-protected amino acid (1.2 mmol, 1.1 equiv) were added. The resulting mixture was cooled to 0 °C and EDCI (1.3 mmol, 1.2 equiv) was subsequently added. The mixture was stirred at 0 °C for 2 h; then at room temperature for 18 h. The solvent was evaporated under reduced pressure and the residue was taken up in EtOAc-distilled water mixture (2:1, 60 mL). The organic layer was separated, washed with saturated $NaHCO_3$ (2 × 15 mL) and distilled water (2 × 15 mL), dried on Na_2SO_4, filtered and concentrated in vacuo. Purification by flash column chromatography on silica gel (CH_2Cl_2/EtOAc, 8:2, as eluent) provided the desired products.

5.4.1. 6-(*N*-Boc-glycyl-oxyethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (4a)

Compound **4a** was obtained as a red amorphous solid from **39** (320 mg) and N^{α}-Boc-glycine (212 mg). Yield: 301 mg, 61%. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.75 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 7.6 Hz, 1H), 7.76 (dd, J = 7.7 Hz, J = 1.2 Hz, 1H), 7.69–7.66 (m, 2H), 7.53–7.40 (m, 3H), 4.93 (s, 1H), 4.85 (t, J = 6.0 Hz, 2H), 4.65 (t, J = 6.0 Hz, 2H), 3.81 (d, J = 5.7 Hz, 2H), 1.42 (s, 9H).

5.4.2. 6-(*N*-Boc-glycyl-oxypropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (5a)

Compound **5a** was obtained as red amorphous solid from **40** (336 mg) and N^{α}-Boc-glycine (212 mg). Yield: 295 mg, 58%. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.73 (d, J = 8.2 Hz, 1H), 8.35 (d, J = 8.0 Hz, 1H), 7.75 (dd, J = 7.5 Hz, J = 1.2 Hz, 1H), 7.67–7.61 (m, 2H), 7.52–7.35 (m, 3H), 5.01 (s, 1H), 4.66 (t, J = 7.6 Hz, 2H), 4.41 (t, J = 6.0 Hz, 2H), 4.00 (d, J = 5.6 Hz, 2H), 2.36–2.19 (m, 2H), 1.43 (s, 9H).

5.4.3. 6-(N^{α},N^{ε}-Di-Boc-lysyl-oxyethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 6a–8a

Compounds **6a**–**8a** were obtained from compound **39** (320 mg) and appropriate N^{α},N^{ε}-di-Boc-lysine (419 mg) as bright red amorphous solids. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.74 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 7.5 Hz, 1H), 7.79–7.72 (m, 2H), 7.66 (dd, J = 7.0 Hz, J = 1.3 Hz, 1H), 7.53–7.41 (m, 3H), 5.01 (d, J = 7.6 Hz, 1H), 4.92–4.85 (m, 2H), 4.64–4.58 (m, 3H), 4.14 (q, J = 7.1 Hz, 1H), 3.00 (t, J = 6.0 Hz, 2H), 1.65–1.58 (m, 2H), 1.35–1.21 (m, 4H), 1.51 (s, 9H), 1.41 (s, 9H).

5.4.3.1. 6-(N^{α},N^{ε}-Di-Boc-L-lysyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (6a). Compound **6a** was obtained from N^{α},N^{ε}-di-Boc-L-lysine. Yield: 436 mg, 64%.

5.4.3.2. 6-(N^{α},N^{ε}-Di-Boc-DL-lysyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (7a). Compound **7a** was obtained from N^{α},N^{ε}-di-Boc-DL-lysine. Yield: 266 mg, 39%.

5.4.3.3. 6-(N^{α},N^{ε}-Di-Boc-D-lysyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (8a). Compound **8a** was obtained from N^{α},N^{ε}-di-Boc-D-lysine. Yield: 559 mg, 82%.

5.4.4. 6-(N^{α},N^{ε}-Di-Boc-lysyl-oxypropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 9a–11a

Compounds **9a**–**11a** were obtained from compound **40** (336 mg) and appropriate N^{α},N^{ε}-di-Boc-lysine (419 mg) as bright red amorphous solids. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.73 (d, J = 7.9 Hz, 1H), 8.35 (d, J = 7.4 Hz, 1H), 7.76 (td, J = 7.5 Hz, J = 1.3 Hz, 1H), 7.68–7.62 (m, 2H), 7.56–7.41 (m, 3H), 5.19 (d, J = 7.4 Hz, 1H), 4.76–4.58 (m, 3H), 4.47–4.32 (m, 3H), 3.17–3.11 (m, 2H), 2.36–2.27 (m, 2H), 1.91–1.73 (m, 2H), 1.56–1.50 (m, 2H), 1.48–1.43 (m, 20H).

5.4.4.1. 6-(N^{α},N^{ε}-Di-Boc-L-lysyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (9a). Compound **9a** was obtained from N^{α},N^{ε}-di-Boc-L-lysine. Yield: 502 mg, 72%.

5.4.4.2. 6-(N^{α},N^{ε}-Di-Boc-DL-lysyl-oxypropyl)-6H-indeno[1,2-c]-isoquinoline-5,11-dione (10a). Compound **10a** was provided from N^{α},N^{ε}-di-Boc-DL-lysine. Yield: 411 mg, 59%.

5.4.4.3. 6-(N^{α},N^{ε}-Di-Boc-D-lysyl-oxypropyl)-6H-indeno[1,2-c]-isoquinolin-5,11-dione (11a). Compound **11a** was obtained from N^{α},N^{ε}-di-Boc-D-lysine. Yield: 181 mg, 26%.

5.4.5. 6-(N^{α},N^{π}-Di-Boc-histidyl-oxyethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 12a–14a

Compounds **12a**–**14a** were obtained from compound **39** (320 mg) and appropriate N^{α},N^{π}-di-Boc-histidine (430 mg) as bright red amorphous solids. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.70 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 7.4 Hz, 1H), 7.80 (d, J = 6.7 Hz, 1H), 7.79 (s, 1H), 7.74 (td, J = 7.7 Hz, J = 1.3 Hz, 1H), 7.64 (dd, J = 6.5 Hz, J = 1.2 Hz, 1H), 7.50–7.38 (m, 3H), 7.04 (s, 1H), 5.59 (d, J = 8.0 Hz, 1H), 4.85 (t, J = 4.2 Hz, 2H), 4.77–4.45 (m, 3H), 2.99–2.90 (m, 2H), 1.60 (s, 9H), 1.42 (s, 9H).

5.4.5.1. 6-(N^{α},N^{π}-Di-Boc-L-histidyl-oxyethyl)-6H-indeno[1,2-c]-isoquinolin-5,11-dione (12a). Compound **12a** was obtained from N^{α},N^{π}-di-Boc-L-histidine. Yield: 567 mg, 82%.

5.4.5.2. 6-(N^{α},N^{π}-Di-Boc-DL-histidyl-oxyethyl)-6H-indeno[1,2-c]-isoquinolin-5,11-dione (13a). Compound **13a** was obtained from N^{α},N^{π}-di-Boc-DL-histidine. Yield: 436 mg, 63%.

5.4.5.3. 6-(N^{α},N^{π}-Di-Boc-D-histidyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (14a). Compound **14a** was obtained from N^{α},N^{π}-di-Boc-D-histidine. Yield: 553 mg, 80%.

5.4.6. 6-(N^{α},N^{π}-Di-Boc-histidyl-oxypropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 15a–17a

Compounds **15a–17a** were obtained from compound **40** (336 mg) and appropriate N^{α},N^{π}-di-Boc-histidine (430 mg) as bright red amorphous solids. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.71 (d, J = 7.9 Hz, 1H), 8.33 (d, J = 7.4 Hz, 1H), 7.93 (s, 1H), 7.73 (td, J = 7.1 Hz, J = 1.3 Hz, 1H), 7.67–7.61 (m, 2H), 7.55–7.38 (m, 3H), 7.19 (s, 1H), 5.87 (d, J = 8.0 Hz, 1H), 4.68–4.58 (m, 3H), 4.45–4.30 (m, 2H), 3.18–3.05 (m, 2H), 2.33–2.24 (m, 2H), 1.56 (s, 9H), 1.42 (s, 9H).

5.4.6.1. 6-(N^{α},N^{π}-Di-Boc-L-histidyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (15a). Compound **15a** was obtained from N^{α},N^{π}-di-Boc-L-histidine. Yield: 502 mg, 71%.

5.4.6.2. 6-(N^{α},N^{π}-Di-Boc-DL-histidyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (16a). Compound **16a** was obtained from N^{α},N^{π}-di-Boc-DL-histidine. Yield: 198 mg, 28%.

5.4.6.3. 6-(N^{α},N^{π}-Di-Boc-D-histidyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (17a). Compound **17a** was obtained from N^{α},N^{π}-di-Boc-D-histidine. Yield: 318 mg, 45%.

5.5. General procedure for the syntheses of compounds 4–17

To a solution of **4a–17a** (0.2 mmol) in $CHCl_3$ (5 mL), HCl 5 M in 2-propanol (1 mL) was slowly added at 0 °C. The resulting mixture was stirred at room temperature for 18 h. The precipitated product was filtered, washed with $CHCl_3$ and Et_2O and dried in vacuo to afford the desired products.

5.5.1. 6-(Glycyl-oxyethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochloride (4)

Compound **4** was obtained from **4a** (90 mg) as a red amorphous solid. Yield: 48 mg, 62%. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 8.61–8.58 (m, 1H), 8.37 (br s, 2H), 8.24–8.22 (m, 1H), 8.13 (br s, 1H), 7.91–7.83 (m, 2H), 7.61–7.52 (m, 4H), 4.81 (d, J = 5.3 Hz, 2H), 4.63 (br s, 2H), 3.59 (br s, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 190.25, 168.00, 162.96, 156.56, 136.80, 134.56, 134.46, 134.15, 132.10, 131.68, 128.38, 127.54, 124.13, 123.02, 122.95 (broad, 2C), 107.67, 63.08, 43.24, 40.30. HRMS calcd for $C_{20}H_{17}N_2O_4$ $[M+H]^+$ 349.1183, found 349.1180.

5.5.2. 6-(Glycyl-oxypropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochloride (5)

Compound **5** was obtained from compound **5a** (92 mg) as a red amorphous solid. Yield: 41 mg, 52%. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 8.54 (d, J = 8.2 Hz, 1H), 8.45 (br s, 2H), 8.19 (d, J = 7.9 Hz, 1H), 7.83–7.79 (m, 2H), 7.63–7.47 (m, 4H), 4.59 (t, J = 6.9 Hz, 2H), 4.35 (t, J = 6.2 Hz, 2H), 3.81 (d, J = 4.7 Hz, 2H), 2.19 (m, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.89, 167.63, 162.53, 156.12, 136.31, 134.31, 134.06, 133.98, 131.76, 131.29, 128.04, 127.13, 123.70, 122.74, 122.67, 122.55, 107.08, 63.29, 41.21, 39.73, 27.96. HRMS calcd for $C_{21}H_{19}N_2O_4$ $[M+H]^+$ 363.1339, found 363.1337.

5.5.3. 6-(Lysyl-oxyethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochlorides 6–8

Compounds **6–8** were obtained from compounds **6a–8a** (124 mg) as red amorphous solids. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 8.57 (br d, J = 8.0 Hz, 4H), 8.22 (d, J = 8.0 Hz, 1H), 8.00 (br s, 3H), 7.95 (d, J = 7.5 Hz, 1H), 7.83 (t, J = 7.0 Hz, 1H), 7.62–7.50 (m, 4H), 4.85 (br s, 2H), 4.70–4.54 (m, 2H), 3.77 (br s, 1H), 2.62 (br s, 2H), 1.05–1.59 (m, 6H). ^{13}C NMR (DMSO-d_6, δ ppm): 190.35, 169.77, 162.96, 156.70, 136.88, 134.58, 134.53, 134.30, 132.13, 131.81, 128.47, 127.72, 124.47 (br, 2C), 123.06, 123.01, 107.65, 63.56, 52.23, 43.14, 38.48, 29.33, 26.60, 21.71.

5.5.3.1. 6-(L-Lysyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (6). Yield: 51 mg, 52%. $[\alpha]_D^{20}$: +10.1 (c 0.2, MeOH). HRMS calcd for $C_{24}H_{26}N_3O_4$ $[M+H]^+$ 420.1918, found 420.1916.

5.5.3.2. 6-(DL-Lysyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (7). Yield: 70 mg, 71%. HRMS calcd for $C_{24}H_{26}N_3O_4$ $[M+H]^+$ 420.1918, found 420.1920.

5.5.3.3. 6-(D-Lysyl-oxyethyl)-6H-indeno[1,2-c]isoquinoline-5,11-dione dihydrochloride (8). Yield: 69 mg, 70%. $[\alpha]_D^{20}$: −6.7 (c 0.2, MeOH). HRMS calcd for $C_{24}H_{26}N_3O_4$ $[M+H]^+$ 420.1918, found 420.1917.

5.5.4. 6-(Lysyl-oxypropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochlorides 9–11

Compounds **9–11** were obtained from compounds **9a–11a** (127 mg) as red amorphous solids. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 8.69 (br s, 3H), 8.52 (d, J = 8.0 Hz, 1H), 8.19–8.13 (m, 4H), 7.82–7.77 (m, 2H), 7.62–7.47 (m, 4H), 4.59 (br s, 2H), 4.38–4.32 (m, 2H), 4.00 (br s, 1H), 2.76 (br s, 2H), 2.19 (t, J = 6.1 Hz, 2H), 1.88 (d, J = 6.7 Hz, 2H), 1.60–1.50 (m, 4H). ^{13}C NMR (DMSO-d_6, δ ppm): 190.32, 169.90, 162.98, 156.04, 136.22, 134.24, 134.00 (br, 2C), 131.68, 131.32, 128.05, 127.11, 123.69, 122.68, 122.63, 122.50, 107.04, 63.48, 51.64, 41.19, 38.10, 29.23, 27.96, 26.25, 21.21.

5.5.4.1. 6-(L-Lysyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (9). Yield: 63 mg, 79%. $[\alpha]_D^{20}$: +11.1 (c 0.2, MeOH). HRMS calcd for $C_{25}H_{28}N_3O_4$ $[M+H]^+$ 433.2074, found 433.2075.

5.5.4.2. 6-(DL-Lysyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (10). Yield: 64 mg, 80%. HRMS calcd for $C_{25}H_{28}N_3O_4$ $[M+H]^+$ 433.2074, found 433.2070.

5.5.4.3. 6-(D-Lysyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (11). Yield: 63 mg, 79%. $[\alpha]_D^{20}$: −8.1 (c 0.2, MeOH). HRMS calcd for $C_{25}H_{28}N_3O_4$ $[M+H]^+$ 433.2074, found 433.2067.

5.5.5. 6-(Histidyl-oxyethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochlorides 12–14

Compounds **12–14** were obtained from **12a–14a** (126 mg) as dark red amorphous solids. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 9.06 (s, 1H), 8.73 (br s, 2H), 8.59 (d, J = 7.9 Hz, 1H), 8.22 (d, J = 7.4 Hz, 1H), 7.92 (d, J = 7.5 Hz, 1H), 7.83 (td, J = 7.7 Hz, J = 1.3 Hz, 1H), 7.60–7.47 (m, 5H), 7.43 (s, 1H), 4.83 (t, J = 5.4 Hz, 2H), 4.72–4.57 (m, 2H), 4.34 (t, J = 6.9 Hz, 1H), 3.25–3.07 (m, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 190.48, 168.53, 163.17, 156.81, 136.96, 134.75, 134.69, 134.51, 134.37, 132.28, 131.77, 128.58, 127.73, 126.89, 124.33, 123.20, 123.16, 123.09, 118.56, 107.76, 63.81, 51.39, 43.04, 24.56.

5.5.5.1. 6-(L-Histidyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (12). Yield: 68 mg, 73%. $[\alpha]_D^{20}$: −7.0 (c 0.2, MeOH). HRMS calcd for $C_{24}H_{21}N_4O_4$ $[M+H]^+$ 429.1557, found 429.1558.

5.5.5.2. 6-(DL-Histidyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (13). Yield: 81 mg, 87%. HRMS calcd for $C_{24}H_{21}N_4O_4$ $[M+H]^+$ 429.1557, found 429.1556.

5.5.5.3. 6-(D-Histidyl-oxyethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (14). Yield: 66 mg, 71%. $[\alpha]_D^{20}$: +7.6 (*c* 0.2, MeOH). HRMS calcd for $C_{24}H_{21}N_4O_4$ $[M+H]^+$ 429.1557, found 429.1551.

5.5.6. 6-(Histidyl-oxypropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochlorides 15–17

Compounds **15**–**17** were obtained from **15a**–**17a** (129 mg) as red amorphous solids. ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 9.09 (s, 1H), 8.92 (br s, 2H), 8.50 (d, *J* = 7.9 Hz, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 7.76 (m, 2H), 7.57–7.48 (m, 4H), 4.60–4.48 (m, 3H), 4.34 (t, *J* = 6.2 Hz, 2H), 3.50–3.36 (m, 2H), 2.19–2.10 (br t, *J* = 6.1 Hz, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.71, 168.12, 162.38, 155.85, 136.06, 134.13, 134.06, 133.98, 133.88, 131.59, 131.20, 127.95, 126.99, 126.59, 123.62, 122.59, 122.53, 122.42, 118.16, 107.00, 64.28, 51.56, 41.58, 28.37, 25.59.

5.5.6.1. 6-(L-Histidyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (15). Yield: 57 mg, 60%. $[\alpha]_D^{20}$: +4.5 (*c* 0.2, MeOH). HRMS calcd for $C_{25}H_{23}N_4O_4$ $[M+H]^+$ 443.1714, found 443.1713.

5.5.6.2. 6-(DL-Histidyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (16). Yield: 58 mg, 61%. HRMS calcd for $C_{25}H_{23}N_4O_4$ $[M+H]^+$ 443.1714, found 443.1712.

5.5.6.3. 6-(D-Histidyl-oxypropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (17). Yield: 0.10 g, 70%. $[\alpha]_D^{20}$: −4.0 (*c* 0.2, MeOH). HRMS calcd for $C_{25}H_{23}N_4O_4$ $[M+H]^+$ 443.1714, found 443.1701.

5.6. 2-(Boc-amino)-ethylamine (41)

A solution of di-*tert*-butyl dicarbonate (7.27 g, 33.3 mmol, 1.0 equiv) in THF (30 mL) was added dropwise at 0 °C, over 30 min to a stirred solution of ethylenediamine (6.7 mL, 100 mmol, 3.0 equiv) dissolved in THF at room temperature for 18 h. The solvent was removed under reduced pressure, and the residue taken up in EtOAc-brine mixture (1:1, 100 mL). The organic phase was washed with brine (2 × 15 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to afford a colorless oil. The crude desired product was used in the next step without further purification. Yield: 4.62 g, 87%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 5.01 (br s, 1H), 3.20–3.12 (m, 2H), 2.78 (t, *J* = 6.0 Hz, 2H), 1.43 (s, 9H), 1.31 (s, 2H).

5.7. 3-(Boc-amino)-propylamine (42)

A solution of di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol, 1.0 equiv) in $CHCl_3$ (20 mL) was added dropwise over 3 h to a stirred solution of 1,3-diaminepropane (4.1 mL, 50.0 mmol, 5.0 equiv) dissolved in $CHCl_3$ (200 mL). The reaction mixture was stirred at room temperature for 18 h and filtered. The filtrate was subsequently concentrated in vacuo and the resulting oil was dissolved in EtOAc (100 mL), washed with brine (3 × 30 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to afford a colorless oil. The crude product was used in the next step without further purification. Yield: 1.39 g, 80%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 4.96 (br s, 1H), 3.29–3.18 (m, 2H), 2.78–2.74 (t, *J* = 6.6 Hz, 2H), 1.65–1.56 (m, 2H), 1.44 (s, 9H).

5.8. General procedure for the synthesis of 6-(Boc-aminoalkyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 43a, 44a

To a suspension of **38** (5.6 mmol, 1.0 equiv, 1.39 g) in $CHCl_3$ (28 mL), appropriate Boc-aminoalkylamine (8.4 mmol, 1.5 equiv) was added. After stirring at room temperature for 72 h, the reaction mixture was diluted with $CHCl_3$ (125 mL), washed with distilled water (3 × 50 mL) and brine (1 × 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the desired product as an orange-red solid. The crude product was used in the next step without further purification.

5.8.1. 6-(2-Boc-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (43a)

Compound **43a** was prepared from compound **41** (1.35 g). mp: 243–245 °C. Yield: 2.07 g, 95%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.75 (d, *J* = 8.0 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 7.4 Hz, 1H), 7.76 (td, *J* = 7.0 Hz, *J* = 1.3 Hz, 1H), 7.67–7.63 (m, 1H), 7.54–7.35 (m, 3H), 5.05 (br s, 1H), 4.69 (t, *J* = 6.5 Hz, 2H), 3.67 (t, *J* = 6.5 Hz, 2H), 1.41 (s, 9H).

5.8.2. 6-(3-Boc-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (44a)

Compound **44a** was prepared from compound **42**(1.46 g). mp: 269–270 °C. Yield: 2.06 g, 91%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.69 (d, *J* = 7.9 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.74 (t, *J* = 7.3 Hz, 1H), 7.63 (d, *J* = 6.7 Hz, 1H), 7.57–7.38 (m, 4H), 5.39 (s, 1H), 4.63 (t, *J* = 6.9 Hz, 2H), 3.28 (t, *J* = 6.1 Hz, 2H), 2.10 (m, 2H), 1.46 (s, 9H).

5.9. General procedure for the synthesis of 6-aminoalkyl-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochlorides 43 and 44

To a solution of compound **43a** or **44a** (2.5 mmol) in $CHCl_3$ (125 mL) a solution of HCl 5 M in 2-propanol (10 mL) was added dropwise. The resulting mixture was stirred at room temperature for 18 h. The precipitated product was filtered, washed with $CHCl_3$ and Et_2O and dried in vacuo to afford the desired product as an orange-red solid. The crude product was used in the next step without further purification.

5.9.1. 6-(2-Aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochloride (43)

Compound **43** was prepared from compound **43a** (0.98 g). Yield: 0.62 g, 76%. ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 8.53 (d, *J* = 7.9 Hz, 1H), 8.44 (br s, 3H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 7.3 Hz, 1H), 7.81 (td, *J* = 7.0 Hz, *J* = 1.2 Hz, 1H), 7.57–7.47 (m, 4H), 4.75 (t, *J* = 6.7 Hz, 2H), 3.24 (t, *J* = 6.0 Hz, 2H).

5.9.2. 6-(3-Aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochloride (44)

Compound **44** was prepared from compound **44a** (1.00 g). Yield: 0.74 g, 87%. ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 8.53 (d, *J* = 8.0 Hz, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.08 (br s, 3H), 7.83–7.78 (m, 2H), 7.60–7.47 (m, 4H), 4.54 (t, *J* = 6.9 Hz, 2H), 3.01–2.86 (m, 2H), 2.13 (m, 2H).

5.10. General procedure for the synthesis of 6-(*N*-Boc-amino-acyl-aminoalkyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 18a–33a

5.10.1. Procedure A

EDCI (0.86 mmol, 2.0 equiv), HOBt (0.86 mmol. 2.0 equiv) and triethylamine (1.1 mmol, 2.5 equiv) were added successively to a solution of the appropriate *N*-Boc-protected amino acid (0.86 mmol, 2.0 equiv) in dry THF (5 mL) at room temperature. The mixture was stirred for 15 min, then a solution **43** or **44** (0.43 mmol, 1.0 equiv) and TEA (0.64 mmol, 1.5 equiv) in dry THF (6 mL) were added. The reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated and the residue was diluted with $CHCl_3$ (50 mL). The organic layer was washed with HCl 0.1 M (2 × 15 mL), distilled water (2 × 15 mL), $NaHCO_3$ 0.5 M (2 × 15 mL), brine (2 × 15 mL), dried over Na_2SO_4 and concentrated

in vacuo. Purification by flash column chromatography on silica gel (CH_2Cl_2/EtOAc, 8:2, as eluent) furnished the desired products.

5.10.2. Procedure B

Appropriate *N*-Boc-protected amino acid (0.64 mmol, 1.5 equiv), HBTU (0.86 mmol, 2.0 equiv), HOBt (0.86 mmol 2.0 equiv), and *N*,*N*-diisopropylethylamine (2.15 mmol, 5.0 equiv) were added successively to a solution of compound **43** or **44** (0.43 mmol, 1.0 equiv) in CH_2Cl_2 (3 mL). The resulting mixture was stirred at room temperature for 4 h, diluted with CH_2Cl_2 (50 mL) and washed with aq $NaHCO_3$ 1.0 M solution (2 × 15 mL). The organic layer was separated and dried over Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography on silica gel (CH_2Cl_2/EtOAc, 8:2, as eluent) yielded the desired products.

5.10.3. 6-(*N*-Boc-glycyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (18a)

Compound **18a** was obtained from **43** (141 mg) and N^{α}-Boc-glycine (151 mg) according to procedure A as a red amorphous solid. Yield: 129 mg, 67%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.74 (d, *J* = 7.9 Hz, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.77 (td, *J* = 7.7 Hz, *J* = 1.3 Hz, 1H), 7.67–7.63 (m, 1H), 7.57–7.40 (m, 3H), 6.91 (t, *J* = 6.4 Hz, 1H), 4.71 (t, *J* = 6.7 Hz, 2H), 3.83–3.78 (m, 4H), 1.47 (s, 9H).

5.10.4. 6-(*N*-Boc-glycyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (19a)

Compound **19a** was obtained from **44** (147 mg) and N^{α}-Boc-glycine (151 mg) according to procedure A as a bright red amorphous solid. Yield: 161 mg, 81%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz); 8.61 (d, *J* = 8.0 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.68 (t, *J* = 7.3 Hz, 1H), 7.55 (d, *J* = 7.0 Hz, 1H), 7.47–7.32 (m, 5H), 5.43 (t, *J* = 5.7 Hz, 1H), 4.54 (t, *J* = 6.3 Hz, 2H), 3.89 (d, *J* = 5.7 Hz, 2H), 3.39 (q, *J* = 6.1 Hz, 2H), 2.09 (m, 2H), 1.44 (s, 9H).

5.10.5. 6-(N^{α},N^{ε}-Di-Boc-lysyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 20a–22a

Compounds **20a**–**22a** were obtained from **43** (141 mg) and appropriate N^{α},N^{ε}-di-Boc-lysine (298 mg) according to procedure A as red amorphous solids. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.72 (d, *J* = 8.1 Hz, 1H), 8.33 (d, *J* = 7.4 Hz, 1H), 7.99 (d, *J* = 7.5 Hz, 1H), 7.75 (td, *J* = 7.7 Hz, *J* = 1.3 Hz, 1H), 7.64 (d, *J* = 7.2 Hz, 1H), 7.53–7.24 (m, 3H), 6.97 (t, *J* = 6.3 Hz, 1H), 5.12 (br s, 1H), 4.69 (td, *J* = 6.1 Hz, *J* = 2.2 Hz, 2H), 4.61 (br s, 1H), 4.07 (br s, 1H), 3.78 (m, 2H), 3.05 (q, *J* = 6.1 Hz, 2H), 1.86–1.80 (m, 1H), 1.61–1.57 (m, 1H), 1.45 (br s, 18H), 1.44–1.27 (m, 4H).

5.10.5.1. 6-(N^{α},N^{ε}-Di-Boc-L-lysyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (20a).

Compound **20a** was obtained from N^{α},N^{ε}-di-Boc-L-lysine. Yield: 210 mg, 79%.

5.10.5.2. 6-(N^{α},N^{ε}-Di-Boc-DL-lysyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (21a).

Compound **21a** was obtained from N^{α},N^{ε}-di-Boc-DL-lysine. Yield: 138 mg, 52%.

5.10.5.3. 6-(N^{α},N^{ε}-Di-Boc-D-lysyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (22a).

Compound **22a** was obtained from N^{α},N^{ε}-di-Boc-D-lysine. Yield: 176 mg, 66%.

5.10.6. 6-(N^{α},N^{ε}-Di-Boc-lysyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 23a–25a

Compounds **23a**–**25a** were obtained from compound **44** (147 mg) and appropriate N^{α},N^{ε}-di-Boc-lysine (298 mg) according to procedure A as bright red amorphous solids. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.73 (d, *J* = 8.0 Hz, 1H), 8.35 (d, *J* = 7.4 Hz, 1H), 7.76 (td, *J* = 7.8 Hz, *J* = 1.2 Hz, 1H), 7.65 (dd, *J* = 6.7 Hz, *J* = 1.3 Hz, 1H), 7.55–7.40 (m, 4H), 7.21 (s, 1H), 5.25 (s, 1H), 4.72–4.53 (m, 3H), 4.17–4.10 (m, 1H), 3.49–3.21 (m, 2H), 3.14 (t, *J* = 6.3 Hz, 2H), 2.17–2.10 (m, 2H), 1.76–1.65 (m, 2H), 1.57–1.50 (m, 2H), 1.47–1.44 (m, 20H).

5.10.6.1. 6-(N^{α},N^{ε}-Di-Boc-L-lysyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (23a).

Compound **23a** was obtained from N^{α},N^{ε}-di-Boc-L-lysine. Yield: 117 mg, 43%.

5.10.6.2. 6-(N^{α},N^{ε}-Di-Boc-DL-lysyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (24a).

Compound **24a** was obtained from N^{α},N^{ε}-di-Boc-DL-lysine.Yield: 162 mg, 60%.

5.10.6.3. 6-(N^{α},N^{ε}-Di-Boc-D-lysyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (25a).

Compound **25a** was obtained from N^{α},N^{ε}-di-Boc-D-lysine. Yield: 202 mg, 75%.

5.10.7. 6-(N^{α},N^{π}-Di-Boc-histidyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 26a–28a

Compounds **26a**–**28a** were obtained from compound **43** (141 mg) and appropriate N^{α},N^{π}-di-Boc-histidine according to either procedure A or B as red amorphous solids. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.73 (d, *J* = 8.0 Hz, 1H), 8.33 (d, *J* = 7.4 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.83 (s, 1H), 7.76 (td, *J* = 7.6 Hz, *J* = 1.2 Hz, 1H), 7.63 (dd, *J* = 7.0 Hz, *J* = 1.0 Hz, 1H), 7.57–7.39 (m, 4H), 7.15 (s, 1H), 6.16 (m, 1H), 4.70–4.50 (m, 2H), 4.46–4.38 (m, 1H), 3.72 (td, *J* = 6.9 Hz, *J* = 6.3 Hz, 2H), 3.13–2.86 (m, 2H), 1.58 (s, 9H), 1.47 (s, 9H).

5.10.7.1. 6-(N^{α},N^{π}-Di-Boc-L-histidyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (26a).

Compound **26a** was obtained from N^{α},N^{π}-di-Boc-L-histidine (306 mg) according to procedure A. Yield: 224 mg, 83%.

5.10.7.2. 6-(N^{α},N^{π}-Di-Boc-DL-histidyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (27a).

Compound **27a** was obtained from N^{α},N^{π}-di-Boc-DL-histidine (229 mg) according to procedure B. Yield: 177 mg, 64%.

5.10.7.3. 6-(N^{α},N^{π}-Di-Boc-D-histidyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (28a).

Compound **28a** was obtained from N^{α},N^{π}-di-Boc-D-histidine (229 mg) according to procedure B. Yield: 237 mg, 86%.

5.10.8. 6-(N^{α},N^{π}-Di-Boc-histidyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 29a–31a

Compounds **29a**–**31a** were obtained from **44** (147 mg) and appropriate N^{α},N^{π}-di-Boc-histidine according either procedure A or B. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 8.72 (d, *J* = 8.2 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.05 (s, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.65 (d, *J* = 6.9 Hz, 2H), 7.53–7.39 (m, 4H), 7.21 (s, 1H), 6.22 (br s, 1H), 4.68–4.44 (m, 2H), 3.49–3.47 (m, 2H), 3.19–2.99 (m, 2H), 2.10–2.01 (m, 2H), 1.57 (s, 9H), 1.48 (s, 9H).

5.10.8.1. 6-(N^{α},N^{π}-Di-Boc-L-histidyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (29a).

Compound **29a** was obtained from N^{α},N^{π}-di-Boc-L-histidine (306 mg) according to procedure A. Yield: 190 mg, 69%.

5.10.8.2. 6-(N^{α},N^{π}-Di-Boc-DL-histidyl-aminopropyl)-6H-indeno-[1,2-c]isoquinolin-5,11-dione (30a).

Compound **30a** was obtained from N^{α},N^{π}-di-Boc-DL-histidine (306 mg) according to procedure A. Yield: 185 mg, 67%.

5.10.8.3. 6-(N^{α},N^{π}-Di-Boc-D-histidyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione (31a). Compound **31a** was obtained from N^{α},N^{π}-di-Boc-D-histidine (229 mg) according to procedure B. Yield: 240 mg, 87%.

5.10.9. 6-(N^{α},N^{ω},$N^{\omega'}$-Tri-Boc-L-arginyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (32a)

Compound **32a** was prepared from **43** (141 mg) and commercially available N^{α},N^{ω},$N^{\omega'}$-tri-Boc-L-arginine (408 mg, 1.1 mmol) according to procedure A. The crude product was purified by flash column chromatography on silica gel ($CHCl_3$/acetone, 8:2, as eluent). Yield: 222 mg, 69%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 9.14 (br s, 2H), 8.67 (d, *J* = 8.0 Hz, 1H), 8.29 (*J* = 8.0 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.70 (td, *J* = 7.7 Hz, *J* = 1.3 Hz, 1H), 7.59–7.34 (m, 5H), 6.05 (br s, 1H), 4.73–4.58 (m, 2H), 4.21 (br s, 1H), 3.90–3.52 (m, 6H), 3.20 (q, *J* = 7.5 Hz, 2H), 1.43–1.41 (m, 27H).

5.10.10. 6-(N^{α},N^{ω},$N^{\omega'}$-Tri-Boc-L-arginyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (33a)

Compound **33a** was prepared from **44** (0.15 g) and commercially available N^{α},N^{ω},$N^{\omega'}$-tri-Boc-L-arginine (0.41 g, 1.1 mmol) according to procedure A. The crude product was purified by flash column chromatography on silica gel ($CHCl_3$/acetone, 8:2, as eluent). Yield: 291 mg, 89%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 9.31 (br s, 2H), 8.65 (d, *J* = 7.9 Hz, 1H), 8.28 (d, *J* = 7.5 Hz, 1H), 7.70 (td, *J* = 7.7 Hz, *J* = 1.3 Hz, 1H), 7.58 (d, *J* = 6.9 Hz, 1H), 7.51–7.41 (m, 4H), 7.36 (t, *J* = 7.2 Hz, 1H), 5.93 (d, *J* = 8.3 Hz, 1H), 4.54 (t, *J* = 7.0 Hz, 2H), 4.36–4.28 (m, 1H), 3.98–3.73 (m, 2H), 3.51–3.31 (m, 2H), 2.13–2.01 (m, 2H), 1.85–1.81 (m, 2H), 1.75–1.61 (m, 2H), 1.51 (s, 9H), 1.46 (s, 9H), 1.45 (s, 9H).

5.11. General procedure for the synthesis of compounds 18–33

To a solution of **18a**–**33a** (0.2 mmol) in $CHCl_3$ (5 mL), a solution of HCl 5 M in 2-propanol (1 mL) was slowly added at 0 °C. The resulting mixture was stirred at room temperature for 18 h. The precipitated product was filtered, washed with $CHCl_3$ and Et_2O and dried in vacuo to provide the desired product.

5.11.1. 6-(Glycyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochloride (18)

Compound **18** was obtained as an orange amorphous solid from **18a** (89 mg). Yield: 29 mg, 38%. ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 8.93 (t, *J* = 5.7 Hz, 1H), 8.57 (d, *J* = 8.0 Hz, 1H), 8.21 (d, *J* = 7.9 Hz, 1H), 8.16 (br s, 3H), 8.09 (d, *J* = 7.0 Hz, 1H), 7.83 (t, *J* = 7.7 Hz, 1H), 7.60–7.49 (m, 4H), 4.56 (t, *J* = 6.6 Hz, 2H), 3.60–3.54 (m, 2H), 3.46 (br s, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 190.43, 167.23, 163.04, 156.82, 136.99, 134.75, 134.54, 134.29, 132.29, 131.81, 128.51, 127.58, 124.17, 123.27, 122.01, 107.52, 43.86, 40.14, 37.73. HRMS calcd for $C_{20}H_{18}N_3O_3$ $[M+H]^+$ 348.1343, found 348.1342.

5.11.2. 6-(Glycyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochloride (19)

Compound **19** was obtained as an orange amorphous solid from **19a** (92 mg). Yield: 43 mg (54%). ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 8.70 (br s, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 8.15 (br s, 3H), 7.83–7.74 (m, 2H), 7.63–7.47 (m, 4H), 4.51 (t, *J* = 7.0 Hz, 2H), 3.57 (br s, 2H), 3.37–3.31 (m, 2H), 1.97 (br t, *J* = 7.0 Hz, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.80, 165.95, 162.36, 155.98, 136.22, 134.27, 134.00 (br, 2C), 131.68, 131.24, 127.99, 127.07, 123.65, 122.68, 122.61, 122.51, 106.98, 42.12, 40.05, 36.31, 29.03. HRMS calcd for $C_{21}H_{20}N_3O_3$ $[M+H]^+$ 362.1499, found 362.1498.

5.11.3. 6-(Lysyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochlorides 20–22

Compounds **20**–**22** were obtained from **20a**–**22a** (124 mg) as orange amorphous solids. ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 9.14 (t, *J* = 5.4 Hz, 1H), 8.56 (dd, *J* = 8.0 Hz, *J* = 3.6 Hz, 1H), 8.30 (br s, 3H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.19–8.08 (m, 4H), 7.83 (t, *J* = 7.3 Hz, 1H), 7.57–7.49 (m, 4H), 4.56 (br s, 2H), 3.70 (br s, 2H), 3.51–3.45 (m, 1H), 2.70 (br s, 2H), 1.62–1.52 (m, 4H), 1.31 (br d, *J* = 7.0 Hz, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 190.32, 169.78, 162.92, 156.68, 136.82, 134.63, 134.42, 134.34, 132.15, 131.76, 128.44, 127.55, 124.38, 123.14, 122.99, 122.93, 107.44, 52.35, 43.74, 38.58, 37.69, 30.41, 26.67, 21.68.

5.11.3.1. 6-(L-Lysyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (20). Compound **20** was obtained from **20a**. Yield: 87 mg, 89%. $[\alpha]_D^{20}$: +26.6 (*c* 0.2, MeOH). HRMS calcd for $C_{24}H_{27}N_4O_3$ $[M+H]^+$ 419.2078, found 419.2073.

5.11.3.2. 6-(DL-Lysyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (21). Compound **21** was afforded from **21a**. Yield: 54 mg, 55%. HRMS calcd for $C_{24}H_{27}N_4O_3$ $[M+H]^+$ 419.2078, found 419.2075.

5.11.3.3. 6-(D-Lysyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (22). Compound **22** was afforded from **22a**. Yield: 89 mg, 91%. $[\alpha]_D^{20}$: −23.3 (*c* 0.2, MeOH). HRMS calcd for $C_{24}H_{27}N_4O_3$ $[M+H]^+$ 419.2078, found 419.2074.

5.11.4. 6-(Lysyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochlorides 23–25

Compounds **23**–**25** were obtained from **23a**–**25a** (127 mg) as red amorphous solids. ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 8.94 (br s, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 8.33 (br s, 3H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.04 (br s, 3H), 7.85–7.78 (m, 2H), 7.63–7.49 (m, 4H), 4.51 (m, 2H), 3.79 (br s, 1H), 3.44–3.25 (m, 2H), 2.76 (br s, 2H), 1.95 (br t, *J* = 6.1 Hz, 2H), 1.77 (m, 2H), 1.59 (m, 2H), 1.45–1.38 (m, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.68, 168.55, 162.27, 155.85, 136.10, 134.18, 134.00, 133.87, 131.57, 131.22, 127.97, 127.01, 123.63, 122.59, 122.53, 122.43, 106.93, 51.88, 42.24, 38.12, 36.33, 30.01, 28.89, 26.25, 21.16.

5.11.4.1. 6-(L-Lysyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochloride (23). Yield: 83 mg, 82%. $[\alpha]_D^{20}$: +19.2 (*c* 0.2, MeOH). HRMS calcd for $C_{25}H_{29}N_4O_3$ $[M+H]^+$ 433.2234, found 433.2232.

5.11.4.2. 6-(DL-Lysyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochlorides (24). Yield: 48 mg, 47%. HRMS calcd for $C_{25}H_{29}N_4O_3$ $[M+H]^+$ 433.2234, found 433.2231.

5.11.4.3. 6-(D-Lysyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione dihydrochlorides 25. Yield: 70 mg, 69%. $[\alpha]_D^{20}$: −15.5 (*c* 0.2, MeOH). HRMS calcd for $C_{25}H_{29}N_4O_3$ $[M+H]^+$ 433.2234, found 433.2237.

5.11.5. 6-(Histidyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochlorides 26–28

Compounds **26**–**28** were obtained as red amorphous solids from **26a**–**28a** (126 mg). ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 9.29 (br s, 1H), 8.99 (br s, 1H), 8.57 (br d, *J* = 7.9 Hz, 3H), 8.20 (d, *J* = 7.9 Hz, 1H), 8.10 (d, *J* = 7.0 Hz, 1H), 7.83 (t, *J* = 7.7 Hz, 1H), 7.60–7.47 (m, 5H), 4.55 (t, *J* = 6.5 Hz), 4.21–4.15 (m, 1H), 3.68–3.44 (m, 2H), 3.25–3.07 (m, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.80, 167.90, 162.40, 156.08, 136.24, 134.11, 133.91 (br, 2C), 131.65, 131.23, 127.90, 127.00, 126.80, 123.78, 122.65, 122.44 (br, 3C), 117.74, 106.98, 51.27, 43.08, 37.30, 25.95.

5.11.5.1. 6-(L-Histidyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (26). Yield: 47 mg, 51%. $[\alpha]_D^{20}$: +13.1 (*c* 0.2, MeOH). HRMS calcd for $C_{24}H_{23}N_5O_3$ $[M+H]^+$ 428.1717, found 428.1714.

5.11.5.2. 6-(DL-Histidyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (27). Yield: 42 mg, 45%. HRMS calcd for $C_{24}H_{23}N_5O_3$ $[M+H]^+$ 428.1717, found 428.1720.

5.11.5.3. 6-(D-Histidyl-aminoethyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (28). Yield: 65 mg, 70%. $[\alpha]_D^{20}$: −12.1 (*c* 0.2, MeOH). HRMS calcd for $C_{24}H_{23}N_5O_3$ $[M+H]^+$ 428.1717, found 428.1719.

5.11.6. 6-(Histidyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochlorides 29–31

Compounds **29**–**31** were obtained as red amorphous solids from **29a**–**31a** (128 mg). ^{1}H NMR (DMSO-d_6, δ ppm, *J* Hz): 9.04 (s, 1H), 8.97 (t, *J* = 5.4 Hz, 1H), 8.57 (d, *J* = 7.9 Hz, 1H), 8.54 (br s, 2H), 8.22 (d, *J* = 7.6 Hz, 1H), 7.86–7.77 (m, 2H), 7.65–7.49 (m, 5H), 4.65–4.44 (m, 2H), 4.29–4.23 (br s, 1H), 3.17–3.74 (m, 4H), 1.96 (m, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.59, 167.26, 162.19, 155.68, 135.98, 134.08, 134.00, 133.76, 131.52, 131.12, 127.87, 126.96, 126.89, 123.55, 122.52, 122.44, 122.41, 122.39, 117.72, 106.90, 51.36, 42.14, 36.56, 28.69, 26.30.

5.11.6.1. 6-(L-Histidyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (29). Yield: 74 mg, 77%. $[\alpha]_D^{20}$: +27.3 (*c* 0.2, MeOH). HRMS calcd for $C_{25}H_{24}N_5O_3$ $[M+H]^+$ 442.1874, found 442.1869.

5.11.6.2. 6-(DL-Histidyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (30). Yield: 76 mg, 80%. HRMS calcd for $C_{25}H_{24}N_5O_3$ $[M+H]^+$ 442.1874, found 442.1866.

5.11.6.3. 6-(D-Histidyl-aminopropyl)-6H-indeno[1,2-c]isoquinolin-5,11-dione hydrochloride (31). Yield: 67 mg, 70%. $[\alpha]_D^{20}$: −25.6 (*c* 0.2, MeOH). HRMS calcd for $C_{25}H_{24}N_5O_3$ $[M+H]^+$ 442.1874, found 442.1865.

5.12. General procedure for synthesis of compounds 32, 33

To a stirred solution of **32a** or **33a** (0.3 mmol) in EtOAc (4 mL) under Ar atmosphere, $SnCl_4$ (0.2 mL, 1.3 mmol) was added. The resulting mixture was stirred at room temperature for 4 h under Ar atmosphere. The solvent and the excess of $SnCl_4$ were evaporated in vacuo. The residue was subsequently dissolved in MeOH (10 mL) and Et_2O was then added until an orange precipitate was formed. The precipitate was filtered, washed with Et_2O and dried in vacuo to afford the desired product as orange amorphous solids.

5.12.1. 6-(L-Arginyl-aminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochloride (32)

Compound **32** was obtained from **32a** (224 mg). Yield: 62 mg, 40%. ^{1}H NMR (CD_3OD, δ ppm, *J* Hz): 8.46 (d, *J* = 7.9 Hz, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.90 (d, *J* = 7.6 Hz, 1H), 7.64 (td, *J* = 7.6 Hz, *J* = 1.2 Hz, 1H), 7.52–7.36 (m, 5H), 4.71–4.57 (m, 2H), 3.94–3.82 (m, 2H), 3.67–3.58 (m, 1H), 3.20 (t, *J* = 6.7 Hz, 2H), 1.92–1.65 (m, 4H). ^{13}C NMR (CD_3OD, δ ppm): 190.36, 169.02, 163.63, 157.18, 155.68, 136.57, 134.48, 133.68, 133.62, 132.08, 130.87, 127.73, 126.91, 123.41, 122.98, 122.85, 122.49, 108.19, 52.68, 43.34, 40.48, 38.13, 28.09, 23.98. $[\alpha]_D^{20}$: +18.6 (*c* 0.4, MeOH). HRMS calcd for $C_{24}H_{27}N_6O_3$ $[M+H]^+$ 447.2139, found 447.2137.

5.12.2. 6-(L-Arginyl-aminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochloride (33)

Compound **33** was obtained from **33a** (228 mg). Yield: 24 mg, 15%. ^{1}H NMR (CD_3OD, δ ppm, *J* Hz): 8.53 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 7.72–7.65 (m, 2H), 7.57–7.39 (m, 5H), 4.56 (br s, 2H), 4.01 (t, *J* = 6.3 Hz, 1H), 3.66–3.40 (m, 2H), 3.36–3.31 (m, 2H), 2.15–1.97 (m, 4H), 1.85–1.77 (m, 2H). ^{13}C NMR (CD_3OD, δ ppm): 189.88, 168.58, 163.53, 155.56, 136.53, 134.29, 133.68, 133.62, 132.09, 130.92, 127.76, 126.91, 123.05, 122.96, 122.87, 122.59, 108.78, 52.79, 42.16, 40.50, 36.59, 33.50, 28.99, 28.38, 24.14. $[\alpha]_D^{20}$: +18.1 (*c* 0.4, MeOH). HRMS calcd for $C_{25}H_{29}N_6O_3$ $[M+H]^+$ 461.2296, found 461.2294.

5.13. General procedure for the synthesis of Weinreb amides of *N*-Boc-protected α-amino acids 45 and 46

N,*O*-Dimethylhydroxylamine-HCl (371 mg, 3.8 mmol, 1.3 equiv), and DMAP (35 mg, 1 mol %) were added to a solution of the appropriate *N*-Boc-protected amino acid (2.9 mmol, 1.0 equiv) in CH_2Cl_2 (14.5 mL). The resulting mixture was cooled to 0 °C. Triethylamine (0.7 mL, 4.9 mmol, 1.7 equiv) was added slowly over 5 min then EDCI (657 mg, 3.4 mmol, 1.2 equiv) was introduced. The solution was stirred for 1 h at 0 °C and for 18 h at room temperature. The reaction mixture was subsequently diluted with 50 mL of CH_2Cl_2 (50 mL) and washed with HCl 1 M (2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), and brine (2 × 20 mL). The organic layer was dried over Na_2SO_4, concentrated, and dried in vacuo to provide the crude desired product which was used in the next step without further purification.

5.13.1. N^{α}-Boc-glycyl-*N*-methoxy-*N*-methylamine (45)

Compound **45** was obtained from N^{α}-Boc-glycine (508 mg) as a yellow amorphous solid. Yield: 563 mg, 89%. ^{1}H NMR ($CDCl_3$, δ ppm, *J* Hz): 5.29 (br s, 1H), 4.10 (d, *J* = 4.5 Hz, 2H), 3.73 (s, 3H), 3.22 (s, 3H), 1.47 (s, 9H).

5.13.2. N^{α},N^{ε}-Di-Boc-(DL)-lysyl-*N*-methoxy-*N*-methylamine (46)

Compound **46** was obtained from N^{α},N^{ε}-di-Boc-DL-lysine (1.00 g) as a yellow oil. Yield: 1.03 g, 91%. NMR ($CDCl_3$, δ ppm, *J* Hz): 5.24 (d, *J* = 8.5 Hz, 1H), 4.66 (br s, 2H), 3.78 (s, 3H), 3.22 (s, 3H), 3.15–3.06 (m, 2H), 1.58–1.50 (m, 6H), 1.45 (s, 18H).

5.14. General procedure for the synthesis of 6-(2-Boc-aminoalkylamino)alkyl-6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones 34a, 36a, 37a

Appropriate Weinreb amide **45** or **46** (2.4 mmol, 1.0 equiv) was dissolved in dry THF (20 mL) and the solution was cooled to 0 °C. $LiAlH_4$ (110 mg, 2.9 mmol, 1.2 equiv) was added in small portions and the resulting mixture was stirred for 40 min at 0 °C. The reaction mixture was quenched at 0 °C by a slow addition of a 0 °C precooled $KHSO_4$ 0.4 M solution (10 mL). The resulting mixture was stirred for 12 min at room temperature. Distilled water (20 mL) was added to the mixture and the aqueous layer was extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with HCl 2 M (3 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL), and brine (2 × 25 mL), dried over Na_2SO_4, concentrated on a rotary evaporator, and dried under vacuum (30 min). The resulting clear oil was immediately dissolved in CH_2Cl_2 (15 mL) then **43** or **44** (2.4 mmol, 1.0 equiv) and triethylamine (0.34 mL, 2.4 mmol, 1.0 equiv) were carefully added to the reaction mixture and the solution was stirred for 5 min. $NaHB(OAc)_3$ (615 mg, 2.9 mmol, 1.2 equiv) was subsequently added to the reaction mixture and the solution was stirred for 90 min. The resulting solution was diluted with a mixture of saturated $NaHCO_3$ (17 mL) and saturated K_2CO_3 (25 mL) then stirred for 10 min. The resulting mixture was extracted with CH_2Cl_2 (4 × 15 mL). The combined organic layers were dried over Na_2SO_4, concentrated and dried in vacuo. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5, as eluent) to give the desired product.

5.14.1. 6-(2-(2-Boc-aminoethylamino)ethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (34a)

Compound **34a** was obtained from **45** (524 mg) and **43** (784 mg) as a dark red amorphous solid. Yield: 385 mg, 37%. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.73 (d, J = 8.0 Hz, 1H), 8.37 (d, J = 7.4 Hz, 1H), 7.76 (td, J = 7.7 Hz, J = 1.3 Hz, 1H), 7.64 (dd, J = 7.2 Hz, J = 7.0 Hz, 2H), 7.52–7.40 (m, 3H), 5.00 (br s, 1H), 4.67 (t, J = 7.0 Hz, 2H), 3.25 (td, J = 6.0 Hz, J = 5.6 Hz, 2H), 3.15 (t, J = 7.02 Hz, 2H), 2.86 (t, J = 5.8 Hz, 2H), 1.46 (s, 9H).

5.14.2. 6-(2-(2,6-Di-Boc-diaminohexylamino)ethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (36a)

Compound **36a** was obtained from **46** (934 mg) and **43** (784 mg) as a dark red amorphous solid. Yield: 421 mg, 29%. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.72 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 8.2 Hz, 1H), 7.75 (t, J = 7.6 Hz, 2H), 7.64 (t, J = 6.2 Hz, 2H), 7.51–7.39 (m, 3H), 4.66 (br t, J = 6.8 Hz, 5H), 3.66 (br s, 1H), 3.17–3.10 (m, 5H), 2.74 (d, J = 5.6 Hz, 2H), 1.56–1.45 (m, 26H).

5.14.3. 6-(3-(2,6-Di-Boc-diaminohexylamino)propyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (37a)

Compound **37a** was obtained from **46** (934 mg) and **44** (818 mg) as a dark red amorphous solid. Yield: 0.33 g, 52%. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.73 (d, J = 8.0 Hz, 1H), 8.36 (d, J = 8.2 Hz, 1H), 7.74 (t, J = 6.7 Hz, 2H), 7.66 (d, J = 7.0 Hz, 1H), 7.54–7.36 (m, 3H), 4.77–4.66 (m, 4H), 3.74–3.67 (m, 1H), 3.14 (br d, J = 6.0 Hz, 3H), 2.83 (t, J = 6.2 Hz, 2H), 2.69 (br d, J = 5.3 Hz, 2H), 2.13–1.98 (m, 2H), 1.55–1.41 (m, 26H).

5.14.4. 3-(5,11-Diketo-6*H*-indeno[1,2-*c*]isoquinolin-6-yl)propyl 4-methylbenzenesulfonate (47)

TsCl (159 mg, 0.8 mmol, 1.1 equiv) was added to a solution of **40** (212 mg, 0.7 mmol, 1.0 equiv) in CH_2Cl_2 (8 mL). Triethylamine (0.3 mL, 2.1 mmol, 3.0 equiv) was added and the mixture was stirred at room temperature for 18 h. The resulting mixture was diluted with CH_2Cl_2 (50 mL) and washed with distilled water (2 × 20 mL) and then brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. Purification by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 99:1, as eluent) afforded the desired products as a dark red solid. Yield: 180 mg, 56%. mp: 180–182 °C. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 8.64 (d, J = 7.9 Hz, 1H), 8.27 (d, J = 8.0 Hz, 1H), 7.91 (t, J = 7.6 Hz, 1H), 7.83 (d, J = 8.2 Hz, 3H), 7.66–7.51 (m, 4H), 7.50 (d, J = 8.2 Hz, 2H), 4.60 (t, J = 7.2 Hz, 2H), 4.35 (t, J = 5.9 Hz, 2H), 2.47 (s, 3H), 2.23 (m, 2H).

5.14.5. 6-(3-(2-Boc-aminoethylamino)propyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (35a)

Compound **41** (370 mg, 2.3 mmol, 10 equiv) was added to a solution of compound **47** (106 mg, 0.2 mmol) in acetonitrile (10 mL). The resulting mixture was heated at 80 °C for 18 h. The solvent was evaporated in vacuo. The residue was taken up in CH_2Cl_2 (50 mL), washed with brine (2 × 25 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/EtOAc, 7:3, as eluent) to yield the desired product as a red amorphous solid. Yield: 83 mg, 80%. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz): 8.73 (d, J = 8.2 Hz, 1H), 8.36 (d, J = 8.0 Hz, 1H), 7.76 (td, J = 7.7 Hz, J = 1.3 Hz, 1H), 7.67 (td, J = 7.6 Hz, J = 1.0 Hz, 2H), 7.52–7.39 (m, 3H), 5.25 (br s, 1H), 4.67 (t, J = 7.2 Hz, 2H), 3.35 (q, J = 5.2 Hz, 4H), 2.88–2.84 (m, 4H), 2.18 (m, 2H), 1.48 (s, 9H).

5.15. General procedures for the syntheses of 6-(aminoalkylamino)alkyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione hydrochlorides 34–37

To a solution of **34a**–**37a** (0.4 mmol) in $CHCl_3$ (10 mL) a solution of HCl 5 M in 2-propanol (2 mL) was added. The resulting mixture was stirred at room temperature for 18 h. The precipitated product was filtered, washed with $CHCl_3$ and Et_2O then dried in vacuo to provide the desired products.

5.15.1. 6-(2-(2-Aminoethylamino)ethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochloride (34)

Compound **34** was obtained from **34a** (173 mg) as an orange amorphous solid. Yield: 111 mg, 68%. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 10.01 (br s, 2H), 8.54 (br s, 3H), 8.43 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 7.2 Hz, 1H), 7.74 (t, J = 7.7 Hz, 1H), 7.52–7.38 (m, 4H), 4.76 (t, J = 6.2 Hz, 2H), 3.46 (br s, 2H), 3.33 (br s, 2H), 3.24 (br s, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.86, 162.79, 155.90, 135.90, 134.05, 133.95, 133.88, 131.63, 131.25, 127.99, 127.07, 123.88, 122.78, 122.57, 122.42, 107.30, 45.11, 44.39, 40.37, 35.02. HRMS calcd for $C_{20}H_{20}N_3O_2$ $[M+H]^+$ 334.1550, found 334.1543.

5.15.2. 6-(3-(2-Aminoethylamino)propyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione dihydrochloride (35)

Compound **35** was afforded from **35a** (179 mg) as an orange amorphous solid. Yield: 155 mg, 92%. ^{1}H NMR (DMSO-d_6, δ ppm): 9.43 (br s, 2H), 8.59 (d, J = 7.9 Hz, 1H), 8.30 (br s, 3H), 8.23 (d, J = 7.5 Hz, 1H), 7.87–7.82 (m, 2H), 7.63–7.50 (m, 4H), 4.59 (t, J = 6.7 Hz, 2H), 3.18 (br s, 6H), 2.22 (m, 2H). HRMS calcd for $C_{21}H_{22}N_3O_2$ $[M+H]^+$ 348.1706, found 348.1703.

5.15.3. 6-(2-(2,6-Diaminohexylamino)ethyl)-6*H*-indeno[1,2-*c*]-isoquinolin-5,11-dione trihydrochloride (36)

Compound **36** was afforded from **36a** (242 mg) as a red amorphous solid. Yield: 117 mg, 57%. ^{1}H NMR (DMSO-d_6, δ ppm, J Hz): 10.14 (br s, 2H), 8.76 (br s, 3H), 8.39 (d, J = 8.0 Hz, 1H), 8.17 (br s, 3H), 8.10 (d, J = 7.9 Hz, 2H), 7.72 (t, J = 7.6 Hz, 1H), 7.51–7.42 (m, 4H), 4.78 (br s, 2H), 3.60 (br s, 2H), 3.45–3.33 (m, 3H), 2.77 (br s, 2H), 1.73–1.48 (m, 6H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.85, 162.71, 155.87, 135.82, 133.98 (br, 2C), 133.83, 131.60, 131.24, 127.96, 127.07, 123.90, 122.76, 122.55, 122.39, 107.31, 48.70, 47.74, 45.32, 40.24, 38.15, 29.59, 26.15, 21.16. HRMS calcd for $C_{24}H_{29}N_4O_2$ $[M+H]^+$ 405.2285, found 405.2283.

5.15.4. 6-(3-(2,6-Diaminohexylamino)propyl)-6*H*-indeno[1,2-*c*]-isoquinolin-5,11-dione trihydrochloride (37)

Compound **37** was afforded from **37a** (248 mg) as a red amorphous solid. Yield: 116 mg, 55%. ^{1}H NMR (DMSO-d_6, δ ppm): 9.80 (br s, 2H), 8.75 (br s, 3H), 8.29 (d, J = 8.0 Hz, 1H), 8.17 (br s, 3H), 8.02 (d, J = 7.9 Hz, 1H), 7.70–7.60 (m, 2H), 7.49 (t, J = 6.5 Hz, 1H), 7.43–7.33 (m, 3H), 4.40 (br s, 2H), 3.57 (br s, 1H), 3.39–2.98 (m, 4H), 2.75 (br s, 2H), 2.21 (br s, 2H), 1.87–1.31 (m, 6H). ^{13}C NMR (DMSO-d_6, δ ppm): 189.57, 162.36, 155.56, 135.76, 134.03, 133.93, 133.79, 131.46, 131.12, 127.82, 126.91, 123.68, 122.47, 122.40, 122.34, 107.01, 48.29, 47.80, 44.89, 41.56, 38.13, 29.46, 26.10, 25.56, 21.16. HRMS calcd for $C_{25}H_{31}N_4O_2$ $[M+H]^+$ 419.2441, found 419.2438.

5.16. DNA and drugs solutions

Calf thymus DNA (CT DNA, Pharmacia) was deproteinized with sodium dodecyl sulfate (SDS, protein content <0.2%) and extensively dialyzed against the required experimental buffer. An extinction coefficient of 6600 M^{-1} cm^{-1} was used to measure the nucleotide-concentration of DNA solutions.[34] All synthesized compounds, as well as camptothecin and etoposide (Sigma), were dissolved as 10 mM solutions in DMSO. Further dilutions were made in the appropriate aqueous buffer.

5.17. Melting temperature studies

Melting curves were obtained using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. Typically, 20 μM of the various drugs were prepared in 1 mL of BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 7.1) in the presence or absence of 20 μM of CT DNA and transferred into a quartz cuvette of 10 mm path length. The spectra were recorded from 230 nm to 500 nm and are referenced against a cuvette containing the same DNA concentration in the same buffer. For the absorption titration, CT DNA was added gradually from 1 to 20 μM with a spectrum recorded after each addition. To perform the melting temperature measurement, CT DNA (20 μM) was incubated alone (control T_m) or with increasing concentrations of the tested compound in 1 mL of BPE buffer, thus resulting in a drug/base pair ratio of 0.05, 0.1, 0.25, 0.5. The sample was transferred into a quartz cell and the absorbance at 260 nm was measured every min over the range of 20–100 °C with an increment of 1 °C per min. The T_m values were obtained from first-derived plots.

5.18. Fluorescence measurements

Since indeno[1,2-*c*]isoquinolin-5,11-dione derivatives show weak fluorescence variation with DNA titration, the binding studies were carried out through a competitive displacement fluorometry assay using DNA-bound ethidium bromide.[27,28] Excitation was set at 546 nm and the fluorescence emission was monitored over the range 560–700 nm. Experiments were performed with an ethidium bromide/DNA molar ratio of 12.6:10 and a drug concentration range of 0.01–100 μM in a BPE buffer, pH 7.1. C_{50} values for ethidium bromide displacement were calculated using a fitting function incorporated into Prism 3.0 and the apparent binding constant was calculated as follows: $K_{app} = (1.26/C_{50})K_{ethidium}$, with $K_{ethidium} = 10^7\ M^{-1}$.

5.19. Topoisomerase inhibition

The experimental procedure has been previously detailed.[35] Supercoiled pUC19 plasmid DNA (130 ng) was incubated with 4 units of human topoisomerase I or II (TopoGen) at 37 °C for 45 min in 20 μL of relaxation buffer (50 mM tris(hydroxymethyl)aminomethane, pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA, and 1 mM ATP) in the presence of graded concentrations (from 1.0 to 50 μM) of the tested compound. Reactions were terminated by adding of SDS to 0.25% and proteinase K to 250 μg/mL and incubating at 50 °C for a further 30 min. 3 μL of the electrophoresis dye mixture was then added to DNA samples which were then separated by electrophoresis in a 1% agarose gel containing ethidium bromide (1 μg/mL; topoisomerase DNA cleavage gel) or not (inhibition of the relaxation of DNA) at room temperature for 2 h at 120 V. Gels run without ethidium bromide were then stained using a bath containing ethidium bromide. Both gels were finally washed and photographed under UV light.

5.20. Cell cultures and antiproliferative assay

Human HL60 and HL60/MX2 leukemia cells were obtained from the American Tissue Culture Collection. Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, penicillin (100 IU/mL), and streptomycin (100 μg/mL). The cytotoxicity of the tested compounds was assessed using a cell proliferation assay developed by Promega (CellTiter 96 AQueous one solution cell proliferation assay). Briefly, 2×10^4 exponentially growing cells were seeded in 96-well microculture plates with various drug concentrations in a volume of 100 μL. After 72 h incubation at 37 °C, 20 μL of the tetrazolium dye was added to each well and the samples were incubated for a further 2 h at 37 °C. Plates were analyzed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

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