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Design, synthesis, and biological evaluation of alcyopterosin A and illudalane derivatives as anticancer agents

Liliana M. Finkielsztein, Ana M. Bruno, Sergio G. Renou and Graciela Y. Moltrasio Iglesias*

Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113, Buenos Aires, Argentina

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Abstract—The synthesis of alcyopterosin A and a series of new derivatives possessing an illudalane skeleton is described. The DNA binding properties of these compounds have been examined and compared to those of reference drugs using a UV spectroscopy technique. The antitumor activity of selected compounds against a panel of 60 human tumor cell lines was tested in the in vitro anticancer screening of the National Cancer Institute. Redox properties were also evaluated. Tested compounds showed significant DNA affinity, derivatives **6** and **15** exhibited remarkable antiproliferative activity and have been identified as new leads in the antitumor strategies.

1. Introduction

Illudalane sesquiterpenoids (Fig. 1) are a class of compounds rare in nature and were isolated from the fungal subdivision of Basidiomycotina[1] and from the fern family of Pteridaceae.[2] Antimicrobial,[3,4] cytotoxic,[5,6] and antispasmodic activities[7] are some of the properties exhibited by members of this class. The alcyopterosins comprise a new set of these compounds whose isolation from sub-Antarctic deep seawater soft coral *Alcyonium paessleri* has been quite recently reported.[8] They represent the first illudalane sesquiterpenoids ever isolated from marine sources.

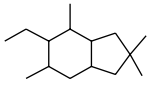
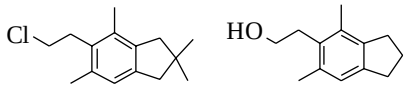


Figure 1. Illudalane skeleton.



1 **7**

Figure 2. Chemical structures of alcyopterosin A (**1**) and alcyopterosin homologue (**7**).

In a preliminary in vitro test, alcyopterosin A (**1**) (Fig. 2) showed cytotoxicity toward HT-29 (human colon carcinoma) cell line.[8] The unusual structure as well as the potential biological activities of these new compounds make them attractive synthetic targets. In contrast to the preparation method already published,[9] we present a versatile route to synthesize alcyopterosin A starting from simple materials and allowing the obtainment of other members of the family of alcyopterosins. In order to establish the appropriate reagents and the optimum reaction conditions for the synthesis, we attempted to prepare an alcyopterosin homologue **7** (Fig. 2).

In the course of the synthetic pathway, a series of compounds possessing the illudalane skeleton was obtained. In this paper, we report the total synthesis of a naturally occurring alcyopterosin A as well as the preparation of new non-natural derivatives as potential antitumor agents. Compounds were evaluated in terms of their non-covalent DNA binding properties. Moreover, we focused on the redox characteristics through the study of cyclic voltammetry. Compounds **6**, **9**, and **15** were

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* Corresponding author. Tel./fax: +54 1149648252; e-mail: gmoltra@ffyb.uba.ar

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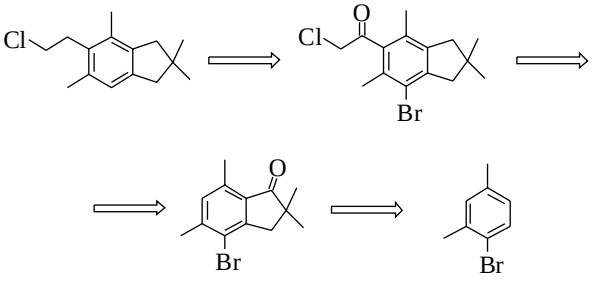
selected and evaluated in vitro against three cancer cell lines: MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS) by NCI (National Cancer Institute). Compounds **6** and **15** were also evaluated in the in vitro anticancer screening against 60 human tumor cell lines. Tested compounds are potential novel templates for anticancer chemotherapy and can serve as new clinical leads.

2. Chemistry

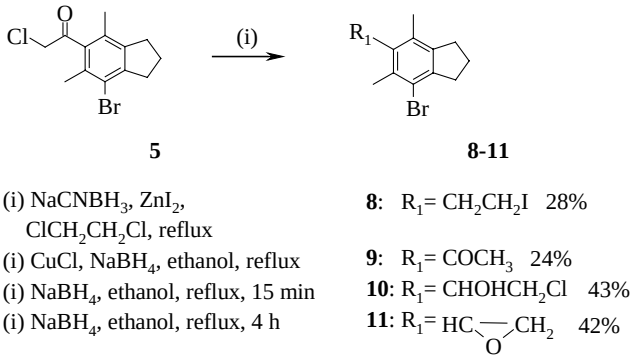
Scheme 1 shows the retrosynthetic strategy for the alcyopterosin A preparation. We started our synthetic approach with the synthesis of β-chloroketone **2**, obtained from acylation of commercially available 4-bromo-*m*-xylene (Scheme 2). The presence of the bromine atom serves as a positional protective group to gain regiochemical control of the products' substitution pattern. Conversion of **2** into indanone **3** was accomplished by cyclization with concentrated sulfuric acid. An efficient deoxygenation of indanone **3** into the corresponding indane **4** became possible by using sodium cyanoborohydride in the presence of zinc iodide.[10] Thereafter, conversion of **4** to α-chloroketone **5** was reached by a classical Friedel–Crafts acylation reaction.

Obtaining compound **7** from α-chloroketone **5** allowed us to try a variety of reducing agents with different reactivities to be able to perform the transformation in one-pot reaction. As a consequence a series of derivatives (**8**–**11**) was obtained, as summarized in Scheme 3.

Compounds **6**, **10**, and **11** were prepared from **5** at different reaction times using sodium borohydride in refluxing ethanol. After 15 min of reflux, compound **5** was reduced to the corresponding β-chlorohydrin **10**. Upon refluxing over an extended period of time (4 h), epoxide **11** was isolated as the main product. After 16 h of reaction, alcohol **6** was cleanly obtained as a product of epoxide **11** ring opening.



Scheme 1. Retrosynthesis of alcyopterosin A.



Scheme 3. Synthesis of the 1-(7-bromo-4,6-dimethylindan-5-yl)-2-chloroethan-1-one derivatives (**8**–**11**).

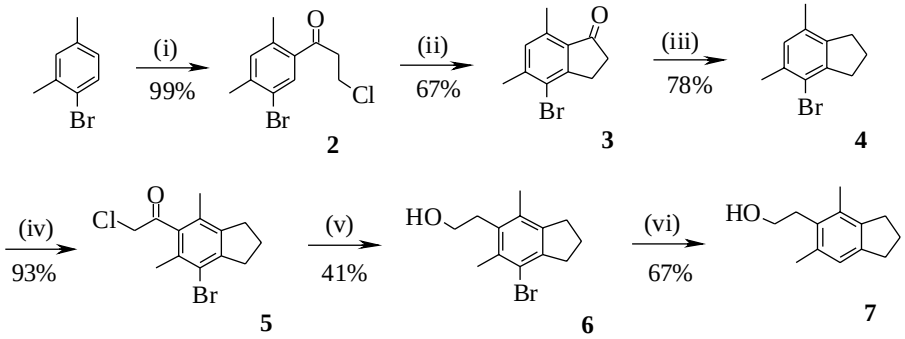
The results obtained encouraged us to carry out the synthesis of **7** from **5** in two steps: first with sodium borohydride to give **6** and finally, with lithium aluminum hydride to afford **7**.

The synthesis of alcyopterosin A was achieved through methylation of indanone **3**. The reaction was successful in a sealed glass tube using both an excess of iodomethane and sodium hydride in toluene. Similarly, compounds **13**–**16** were synthesized under the same conditions as those used for obtaining **4**–**7**. The alcyopterosin A preparation was completed by substituting a hydroxyl group in compound **16** for a chlorine atom (Scheme 4).

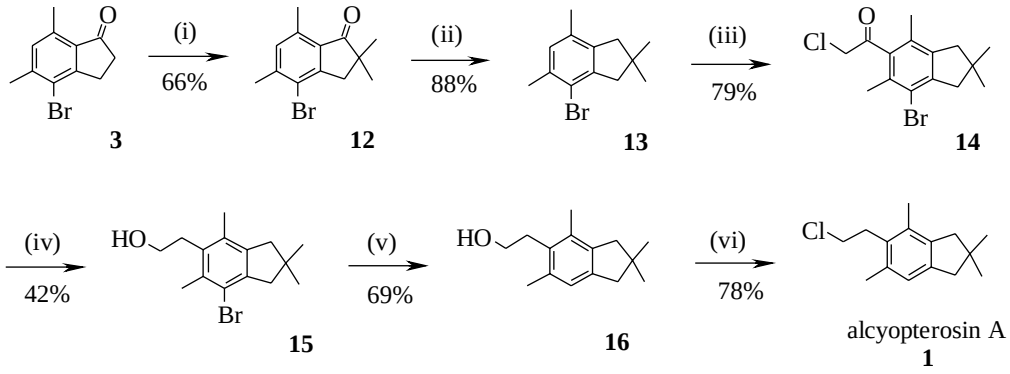
3. Results and discussion

3.1. DNA binding properties

Compounds **1**, **4**–**11**, and **13**–**16** were tested for their ability to bind DNA. The binding capacity of these compounds was evaluated by measuring the hypochromic



Scheme 2. Synthesis of alcyopterosin homologue 2-(4,6-dimethylindan-5-yl)ethan-1-ol (**7**). Reagents and conditions: (i) $ClCOCH_2CH_2Cl$, $AlCl_3$, CS_2; (ii) concentrated H_2SO_4; (iii) $NaCNBH_3$, ZnI_2, CH_2Cl_2; (iv) $ClCOCH_2Cl$, $AlCl_3$, CS_2; (v) $NaBH_4$, ethanol, 16 h; (vi) $LiAlH_4$, THF.



Scheme 4. Synthesis of alcyopterosin A. Reagents and conditions: (i) Mel, NaH, toluene; (ii) $NaCNBH_3$, ZnI_2, CH_2ClCH_2Cl; (iii) $ClCOCH_2Cl$, $AlCl_3$, CS_2; (iv) $NaBH_4$, ethanol, 16 h; (v) $LiAlH_4$, THF; (vi) Cl_2SO, Py, $CHCl_3$.

and bathochromic effects of their absorbance in the UV spectra.[11] The typical experiment was enhanced by means of a slow rotation of DNA–drug mixture stirring, in a 5:1 ratio during 24 h. The procedure was validated by repeating assays with well-known intercalating agents (*m*-AMSA and mitoxantrone) and a compound which binds closely in the minor groove (bis-benzimide, Hoechst No. 33258). The degree of interaction was expressed by the ratio between the final absorbance area after 24 h (a_{24}) and that of the compound at the same concentration (a_0), centered at maximal absorbance. Values of 1 or higher indicate a total lack of affinity and value 0 shows that the whole compound was bound to DNA. The coefficient a_{24}/a_0 values obtained are summarized in Table 1.

The DNA binding assay showed that compounds tested are excellent DNA ligands with affinity higher than those of *m*-AMSA and bis-benzimide, except for **7**, **10**, and **16**. The presence of two carbon funtionalized chain is essential (compounds **4** and **13** presented poor affinity). Substitution at C-2 produces no remarkable modification to the affinity (in cases **6**, **15** and **7**, **16**). For hydroxylic compounds (**6**, **7**, **15**, and **16**) the absence of the bromine atom reduces dramatically the DNA binding degree.

3.2. In vitro antitumor activity

In a preliminary test performed by The National Cancer Institute (NCI), compounds **6**, **9**, and **15** were evaluated in a three cell line one dose prescreen.[12–14] The three lines comprise MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS). These have been in use by DTP (Development Therapeutic Program) for several years to evaluate combinatorial libraries and have proven to be an effective test of agents which exhibited some capability level to inhibit the growth of human tumor cells in culture. Two drugs were used as a standard and evaluated against each cell line, NSC 19893 (5-FU) and NSC 123127 (Adriamycin). Results are expressed as percentage test cell growth compared with untreated control cells (PTC). Compounds, which reduce the growth of any of the three cell lines by 32% or less, are considered in vitro active. Table 2 shows the values of PTC of selected targets. Compound **15** has shown 0% of growth inhibition against three cell lines. Similarly, **6** presented the same percentage of inhibition, except for the SF-268 (CNS) line. Meanwhile, compound **9** has not reduced the growth of any cell line by 32% or less. These results suggest that derivatives **6** and **15** produce an important effect on the growth of cancer cell lines.

Compounds **6** and **15**, which passed the first criterion of activity, were further evaluated at five concentrations in 10-fold dilutions (10^{-4} to 10^{-8} M) against 60 different human tumor cell lines organized in subpanels representing melanoma, leukemia, and cancers of breast, prostate, lung, colon, ovary, kidney, and brain. The experimental procedures have been described in detail.[13,15–17]Three dose–response parameters are calculated for each experimental agent: the compound concentration required to cause 50% of net cell growth

Table 1. DNA binding assay [reference drugs: Mitoxantrone (Mx); *m*-AMSA (m-A); and Bis-benzimide (B-b)]

Compound	a_{24}/a_0[a]	Compound	a_{24}/a_0[a]
4	0.90	**13**	0.87
5	0.12	**14**	0.41
6	0.59	**15**	0.40
7	0.89	**16**	0.71
8	0.16	**1**	0.38
9	0.26	Mx	0.00
10	0.69	m-A	0.54
11	0.47	B-b	0.57

[a] a_{24} = final absorbance (DNA-compound) after 24 h; a_0 = initial absorbance (compound) at the same concentration.

Table 2. Three cell line prescreen

Compound	PTC[d]		
	MCF-7[a]	NCI-H460[b]	SF-268[c]
6	0	0	49
9	100	97	116
15	0	0	0

[a] Breast cell line.
[b] Lung cell line.
[c] CNS cell line.
[d] PTC = percent test cell growth compared with untreated control cells.

(GI_{50}); the compound concentration resulting in total growth inhibition (TGI); and the concentration of the compound leading to 50% net cell death (LC_{50}). The meangraph midpoints (MG-MID) represent average values for each of the mentioned parameters and indicate the average sensitivity of all cell lines to each tested compound. The selective activity of a compound against cancer cell lines from a special organ is characterized by a high deviation of the particular cell line parameter compared to the corresponding MG-MID value.

Table 3 shows that evaluated agents demonstrated a remarkable activity in the in vitro antitumor screening, expressed by MG-MID GI_{50} values of −4.77 (compound **6**) and −4.71 (compound **15**). Moreover, these results are of special interest because there is a substantial difference between the cytostatic and the cytotoxic activity in both compounds (MG-MID GI_{50} and MG-MID LC_{50}). Compound **15** displayed broad spectrum activity, almost all subpanels tested showed sensitivity to this agent. Leukemia, cancers of lung, colon, and breast proved to be the most sensitive subpanels to compound **6**. In agreement with the DNA binding assay, the presence of dimethyl substituent in the 2-position does not modify the antiproliferative activity. Representative data for antitumor activity in most sensitive tumor cell lines are given in Table 4.

3.3. Electrochemical properties

In an attempt to know if these illudalane derivatives are capable of generating highly reactive radicals, which can damage DNA and be implicated in the probable antineoplastic mechanism, we investigated the electrochemical behavior of the target compounds by cyclic voltammetry.[18] This methodology allows the distinction between reversible and irreversible processes and moreover, when none of them occurs. For compound **9** the result was in accordance with an irreversible process. For the selected agents (compounds **6** and **15**), the cyclovoltammograms exhibited no variations with respect to the support electrolyte. As a consequence this set of compounds is not capable of forming oxy radicals and then, the effect observed is not due to this mechanism.

Table 3. Average values (MG-MID) for in vitro antitumor activity on 60 human cell lines

Compound	MG-MID[a]		
	$Log_{10}GI_{50}$[b]	$Log_{10}TGI$[c]	$Log_{10}LC_{50}$[d]
6	−4.77	−4.40	−4.12
15	−4.71	−4.41	−4.14

[a] MG-MID = meangraph midpoint = arithmetical mean value for all tested cell lines.
[b] $Log_{10}GI_{50}$ = log of molar concentration that inhibits 50% net cell growth.
[c] $Log_{10}TGI$ = log of molar concentration that produces a total growth inhibition.
[d] $Log_{10}LC_{50}$ = log of molar concentration that leads to 50% net cell death.

Table 4. Antitumor activity of compounds **6** and **15** in most sensitive human tumor cell lines

Compound **6**		Compound **15**	
Subpanel/cell line	$Log_{10}GI_{50}$[a]	Subpanel/cell line	$Log_{10}GI_{50}$[a]
Leukemia		*Leukemia*	
CCRF-CEM	−4.95	SR	−4.88
HL-60 (TB)	−4.81	MOLT-4	−4.75
MOLT-4	−4.86	*NSLC*[b]	
RMPI-8226	−5.02	HOP-92	−4.81
NSLC[b]		NCI-H460	−4.82
EKVX	−4.98	NCI-H522	−4.83
Colon cancer		*CNS cancer*	
HCT-15	−4.86	SF-268	−4.78
KM12	−4.86	SF-295	−4.77
CNS Cancer		SNB-75	−4.76
SF-295	−4.82	*Melanoma*	
SF-539	−4.83	SK-MEL-5	−4.83
Melanoma		UACC-62	−4.77
SK-MEL-5	−4.82	SK-MEL-2	−4.77
UACC-62	−4.92	M14	−4.77
Renal cancer		*Renal cancer*	
CAKI-1	−4.92	786-0	−4.78
SN12C	−4.86	CAKI-1	−4.82
Breast cancer		UO-31	−4.85
MDA-MB-231	−4.96	*Ovarian cancer*	
MDA-MB-435	−4.82	IGROV1	−4.77

[a] $Log_{10}GI_{50}$ = log of molar concentration that inhibits 50% net cell growth.
[b] NSLC = non-small cell lung cancer.

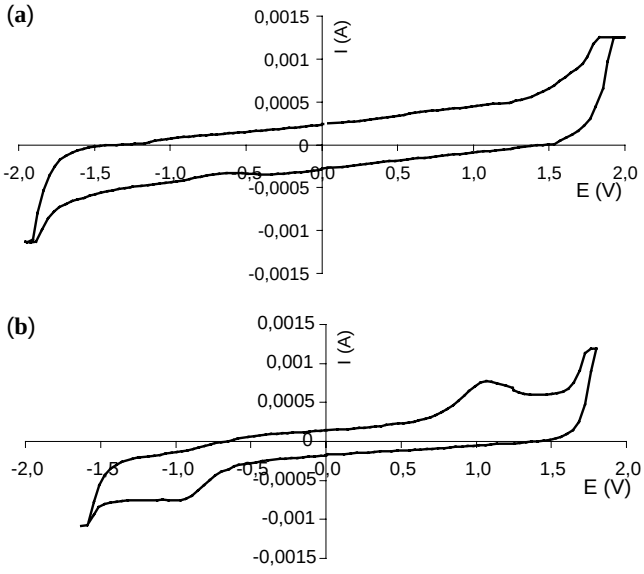


Figure 3. Cyclovoltammograms of compounds **6** (a) and **9** (b).

Figure 3 shows the cyclic voltammetry patterns of compounds **6** and **9**.

4. Conclusion

A method for the synthesis of alcyopterosin A was developed. In contrast to other more complicated methods, the compound was prepared in a simple manner and with readily available reagents. Furthermore, we

synthesized a new class of derivatives possessing the illudalane skeleton. These compounds exhibited relevant DNA binding properties with an affinity higher than that of standard drugs. This study revealed that dimethyl substituent in the 2-position produces no change in the DNA binding and the presence of the bromine atom leads to an increase of DNA binding ability. Compounds **6** and **15** showed significant growth inhibition activity against several human cancer lines. The study of cyclic voltammetry demonstrated that these derivatives are not capable of forming oxy radicals. Therefore, their antitumor activity is not due to the production of reactive oxygen species. Finally, **6** and **15** constitute new potential anticancer leads. The mechanism of antitumor activity is the subject of further investigations.

5. Experimental

Melting points (uncorrected) were determined with a Thomas Hoover apparatus. Preparative thin layer chromatography (pTLC) was performed on a 20×20 cm glass coated with silica gel 60 F_{254} (0.50 mm). ^1H and ^{13}C NMR spectra were recorded with a Bruker 400 MHz spectrometer with $CDCl_3$ as solvent, employing TMS as internal standard. Chemical shifts are reported as δ values (parts per million). The following NMR abbreviations are used: br (broad), s (singlet), d (doublet), t (triplet), m (multiplet), and ex (exchangeable with D_2O). UV spectra were measured with a Jasco V-570 Spectrophotometer. The cyclic voltammetry experiments were carried out with an EQMAT-S1 potentiostat controlled by EQSOFT software. Elemental analysis was carried out in our laboratories with a Colleman Analyser.

5.1. General procedure for the preparation of 2, 5, and 14

$AlCl_3$ (35.0 mmol) was added to a magnetically stirred solution of aryl bromide (12.4 mmol) and the correspondent acyl chloride (16.8 mmol) in CS_2 (12 mL) at 0 °C over a period of 30 min. The reaction mixture was heated under reflux for a further 30 min. The resulting dark-brown solution was cooled at room temperature and carefully poured onto ice and was extracted with Cl_2CH_2 (25 mL), washed with water ($2\times$ 10 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo to yield the crude product.

5.1.1. 1-(5-Bromo-2,4-dimethylphenyl)-3-chloropropan-1-one (2)[19]. Yield: 99%; white solid, mp: 39–41 °C (ethanol). ^1H NMR: δ 2.42 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.37 (t, $J = 6.8$ Hz, 2H, CH_2), 3.90 (t, $J = 6.8$ Hz, 2H, CH_2), 7.16 (s, 1H, ar), 7.84 (s, 1H, ar). ^{13}C NMR: δ 21.6, 23.5, 39.5, 44.1, 122.1, 133.3, 135.2, 136.5, 138.7, 142.8, 198.9. Anal. Calcd for $C_{11}H_{12}BrClO$: C, 47.94; H, 4.39. Found: C, 48.01; H, 4.38.

5.1.2. 1-(7-Bromo-4,6-dimethylindan-5-yl)-2-chloroethan-1-one (5). Yield: 93%; white solid, mp: 103–105 °C, purified by pTLC using hexane–ethyl acetate (8:2) as eluent. ^1H NMR: δ 2.10 (s, 3H, CH_3), 2.13 (m, $J = 7.6$ Hz, 2H, CH_2), 2.27 (s, 3H, CH_3), 2.96 (t, $J = 7.6$ Hz, 2H, CH_2), 3.01 (t, $J = 7.6$ Hz, 2H, CH_2), 4.39 (s, 2H, CH_2). ^{13}C NMR: δ 16.9, 20.5, 24.1, 33.5, 36.5, 50.6, 121.4, 128.8, 131.4, 138.7, 143.5, 147.0, 200.9. Anal. Calcd for $C_{13}H_{14}BrClO$ C, 51.77; H, 4.68. Found: C, 51.73; H, 4.66.

5.1.3. 1-(7-Bromo-2,2,4,6-tetramethylindan-5-yl)-2-chloroethan-1-one (14). Yield: 79%; colorless oil, purified by pTLC using hexane–ethyl acetate (8:2) as eluent. ^1H NMR: δ 1.19 (s, 6H, $2\times CH_3$), 2.06 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.76 (s, 2H, CH_2), 2.82 (s, 2H, CH_2), 4.40 (s, 2H, CH_2). ^{13}C NMR: δ 16.5, 20.2, 29.6, 39.1, 47.9, 50.4, 50.7, 121.3, 128.6, 131.1, 138.4, 142.7, 146.0. Anal. Calcd for $C_{15}H_{18}BrClO$: C, 54.65; H, 5.50. Found: C, 54.67; H, 5.50.

5.2. 4-Bromo-5,7-dimethylindan-1-one (3)

Chloroketone **2** (2.94 g, 10.7 mmol) was added in small portions with swirling to concentrated H_2SO_4 (33 mL). The resulting solution was heated on a silicon bath at 90 °C. After 1 h, the reaction mixture was cautiously poured onto ice and then extracted with ethyl acetate (50 mL). The organic layer was washed with a solution of 10% NaOH ($2\times$ 20 mL), water (20 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by pTLC using hexane–ethyl acetate (8:2) as eluent to yield **3** (1.71 g, 67%) as a white solid, mp: 101–103 °C. ^1H NMR: δ 2.47 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.71 (t, $J = 6.1$ Hz, 2H, CH_2), 3.02 (t, $J = 6.1$ Hz, 2H, CH_2), 7.03 (s, 1H, ar). ^{13}C NMR: δ 18.3, 23.5, 27.8, 37.5, 121.6, 132.7, 134.8, 137.7, 144.8, 156.5, 207.3. Anal. Calcd for $C_{11}H_{11}BrO$: C, 55.26; H, 4.64. Found: C, 55.22; H, 4.66.

5.3. 4-Bromo-2,2,5,7-tetramethylindan-1-one (12)

A solution of indanone **3** (0.40 g, 1.7 mmol) in toluene (5 mL) was added to a glass tube containing NaH (0.22 g, 9.2 mmol). The tube was stoppered and stirred at room temperature for 2 h. Then, methyl iodide (0.72 mL, 11.6 mmol) was added in one portion, the tube was sealed, and the resulting mixture was heated in a silicon bath at 110 °C for 90 h. The reaction mixture was poured onto ice, extracted with dichloromethane (20 mL), washed with a solution of 5% HCl ($2\times$ 10 mL) and water (15 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by pTLC using hexane–ethyl acetate (8:2) as eluent to render **12** (0.29 g, 66%) as a pale yellow solid, mp: 85–87 °C. ^1H NMR: δ 1.25 (s, 6H, $2\times CH_3$), 2.47 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.90 (s, 2H, CH_2), 7.05 (s, 1H, ar). ^{13}C NMR: δ 18.1, 23.4, 25.8, 44.5, 46.2, 121.5, 132.7, 138.3, 144.8, 153.5. Anal. Calcd for $C_{13}H_{15}BrO$: C, 58.44; H, 5.66. Found: C, 58.38; H, 5.66.

5.4. General procedure for the preparation of 4, 8, and 13

ZnI_2 (3 mmol) and $NaCNBH_3$ (15 mmol) were added to a solution of the correspondent α-chloroketone or inda-

none (2 mmol) in ethylene dichloride (10 mL) at room temperature. The reaction mixture was stirred at room temperature or refluxed and, after completion of the reaction (monitored by TLC), the mixture was cooled and poured onto a solution of 6 N HCl (40 mL). Lastly, the mixture was extracted with Cl_2CH_2 (20 mL) and the organic extract was washed with water (15 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo to yield the crude product.

5.4.1. 4-Bromo-5,7-dimethylindane (4)[20]. Yield: 78%; white solid, mp: 32–34 °C, purified by pTLC using hexane–ethyl acetate (9:1) as eluent. ^{1}H NMR: δ 2.11 (m, J = 7.6 Hz, 2H, CH_2), 2.19 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.93 (t, J = 7.6 Hz, 2H, CH_2), 2.98 (t, J = 7.6 Hz, 2H, CH_2), 6.87 (s, 1H, ar). ^{13}C NMR: δ 19.0, 22.8, 24.3, 32.9, 35.8, 119.7, 130.1, 132.7, 135.6, 142.2, 144.8. Anal. Calcd for $C_{11}H_{13}Br$: C, 58.69; H, 5.82. Found: C, 58.75; H, 5.84.

5.4.2. 7-Bromo-5-(2-iodoethyl)-4,6-dimethylindane (8). Yield: 28%; white solid, mp: 80–81 °C, purified by pTLC using hexane–ethyl acetate (9:1) as eluent. ^{1}H NMR: δ 2.05 (m, J = 7.6, 2H, CH_2), 2.17 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.92–2.97 (m, 4H, 2× CH_2), 3.07–3.11 (m, 2H, CH_2), 3.25–3.29 (m, 2H, CH_2). ^{13}C NMR: δ 1.9, 16.6, 19.9, 23.8, 33.9, 36.4, 36.6, 121.6, 131.2, 133.6, 137.9, 142.9, 143.6. Anal. Calcd for $C_{13}H_{16}BrI$: C, 41.19; H, 4.25. Found: C, 41.14; H, 4.25.

5.4.3. 4-Bromo-2,2,5,7-tetramethylindane (13). Yield: 88%; colorless oil, purified by pTLC using hexane–ethyl acetate (9:1) as eluent. ^{1}H NMR: δ 1.20 (s, 6H, 2× CH_3), 2.16 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.75 (s, 2H, CH_2), 2.81 (s, 2H, CH_2), 6.87 (s, 1H, ar). ^{13}C NMR: δ 18.9, 22.8, 29.8, 39.3, 47.8, 50.4, 119.9, 130.1, 133.0, 135.6, 141.4, 144.0. Anal. Calcd for $C_{13}H_{17}Br$: C, 61.67; H, 6.77. Found: C, 61.72; H, 6.79.

5.5. General procedure for the preparation of 6, 10, 11, and 15

$NaBH_4$ (12 mmol) was added over a period of 5 min to a refluxing solution of the correspondent α-chloroketone (1 mmol) in ethanol (18 mL). The reaction mixture was heated under reflux until TLC showed the presence of the desired product. The mixture was cooled at room temperature and the solvent was removed in vacuo. The residue was partitioned between $CHCl_3$ (20 mL) and water (10 mL). The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuo to yield the crude product.

5.5.1. 2-(7-Bromo-4,6-dimethylindan-5-yl)ethan-1-ol (6). Yield: 41%; white solid, mp: 100–101 °C, purified by pTLC using hexane–ethyl acetate (7:3) as eluent. ^{1}H NMR: δ 1.46 (br s, 1H, OH, ex), 2.08 (m, J = 7.6 Hz, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.98 (m, 4H, 2× CH_2), 3.04 (t, J = 7.4 Hz, 2H, CH_2), 3.77 (t, J = 7.4 Hz, 2H, CH_2). ^{13}C NMR: δ 16.8, 20.1, 23.8, 34.0, 34.3, 36.4, 62.3, 121.4, 132.1, 134.3, 134.5, 142.6, 143.1. Anal. Calcd for $C_{13}H_{17}BrO$: C, 58.01; H, 6.37. Found: C, 57.98; H, 6.39.

5.5.2. 1-(7-Bromo-4,6-dimethylindan-5-yl)-2-chloroethan-1-ol (10). Yield: 43%; colorless oil, purified by pTLC using hexane–ethanol (9:1) as eluent. ^{1}H NMR: δ 2.05–2.11 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.65 (br s, 1H, OH, ex), 2.55 (s, 3H, CH_3), 2.96–3.02 (m, 4H, 2× CH_2), 3.65 (dd, J = 3.8 Hz, J = 11.4 Hz, 1H, *H*CH), 3.98 (dd, J = 10.3 Hz, J = 11.4 Hz, 1H, HC*H*), 5.34–5.41 (m, 1H, CH). Anal. Calcd for $C_{13}H_{16}BrClO$: C, 51.43; H, 5.31. Found: C, 51.47; H, 5.33.

5.5.3. 2-(7-Bromo-4,6-dimethylindan-5-yl)oxirane (11). Yield: 42%; white solid, mp: 81–82 °C, purified by pTLC using hexane–ethanol (8:2) as eluent. ^{1}H NMR: δ 2.10 (m, J = 7.5 Hz, 2H, CH_2), 2.30 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 2.74 (dd, J = 3.0 Hz, J = 5.6 Hz, 1H, *H*CH), 2.95–3.01 (m, 4H, 2× CH_2), 3.24 (dd, J = 4.0 Hz, J = 5.6 Hz, 1H, HC*H*), 3.96 (m, 1H). Anal. Calcd for $C_{13}H_{15}BrO$: C, 58.44; H, 5.66. Found: C, 58.39; H, 5.65.

5.5.4. 2-(7-Bromo-2,2,4,6-dimethylindan-5-yl)ethan-1-ol (15). Yield: 42%; white solid, mp: 74–76 °C, purified by pTLC using hexane–ethyl acetate (7:3) as eluent. ^{1}H NMR: δ 1.15 (s, 6H, 2× CH_3), 1.39 (br s, 1H, OH, ex) 2.17 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.76 (s, 2H, CH_2), 2.78 (s, 2H, CH_2), 3.00 (t, J = 7.5 Hz, 2H, CH_2), 3.71 (m, 2H, CH_2). ^{13}C NMR: δ 16.7, 20.1, 29.8, 34.3, 38.5, 48.9, 51.0, 62.3, 121.6, 132.4, 134.2, 134.5, 141.8, 142.3. Anal. Calcd for $C_{15}H_{21}BrO$: C, 60.61; H, 7.12. Found: C, 60.57; H, 7.10.

5.6. General procedure for the preparation of 7 and 16

The correspondent alcohol (0.6 mmol) in dry THF (3 mL) was added to a suspension of $LiAlH_4$ (5 mmol) in dry THF (15 mL) at 0 °C. The reaction mixture was heated under reflux during 24 h. After being cooled at room temperature, the mixture was carefully poured onto ice and a solution of 10% NaOH (5 mL) was added. The aqueous phase was extracted with Cl_2CH_2 (2× 10 mL). The extract was washed with water (10 mL) dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield the crude product.

5.6.1. 2-(4,6-Dimethylindan-5-yl)ethan-1-ol (7). Yield: 67%; white solid, mp: 53–55 °C, purified by pTLC using hexane–ethyl acetate (8:2) as eluent. ^{1}H NMR: δ 1.42 (br s, 1H, OH, ex), 2.07 (m, J = 7.6 Hz, 2H, CH_2), 2.29 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.85 (t, J = 7.6 Hz, 2H, CH_2), 2.90 (t, J = 7.6 Hz, 2H, CH_2), 2.99 (t, J = 7.6 Hz, 2H, CH_2), 3.77 (t, J = 7.6 Hz, 2H, CH_2), 6.95 (s, 1H, ar). ^{13}C NMR: δ 16.6, 20.8, 25.2, 32.5, 33.2, 33.4, 62.4, 124.3, 132.3, 133.1, 135.3, 141.8, 142.3. Anal. Calcd for $C_{13}H_{18}O$: C, 82.06; H, 9.54. Found: C, 82.11; H, 9.52.

5.6.2. 2-(2,2,4,6-Tetramethylindan-5-yl)ethan-1-ol (16)[7]. Yield: 69%; white solid, mp: 65–67 °C, purified by pTLC using hexane–ethyl acetate (8:2) as eluent. ^{1}H NMR: δ 1.14 (s, 6H, 2× CH_3), 1.43 (br s, 1H, OH, ex) 2.21 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.65 (s, 2H, CH_2), 2.69 (s, 2H, CH_2), 2.94 (t, J = 7.4 Hz, 2H, CH_2), 3.73 (m, 2H, CH_2), 6.85 (s, 1H, ar). ^{13}C NMR: δ 16.5, 20.8, 29.7, 33.2, 39.6, 47.6, 48.3, 62.4, 124.6, 132.3, 133.3,

135.2, 141.1, 141.6. Anal. Calcd for $C_{15}H_{22}O$: C, 82.52; H, 10.16. Found: C, 82.58; H, 10.18.

5.7. 1-(7-Bromo-4,6-dimethylindan-5-yl)ethan-1-one (9)

$NaBH_4$ (0.49 g, 10.2 mmol) was added, in small portions over a period of 15 min at 0 °C, to a solution of α-chloroketone **5** (0.22 g, 0.6 mmol) and CuCl (0.20 g, 2 mmol) in MeOH (26 mL). After being heated under reflux for 30 min, the resulting black precipitate was removed by filtration, and the filtrate was acidified with 5% aqueous HCl (10 mL) and extracted with Et_2O (2× 10 mL). The organic phase was washed with water (2× 5mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by pTLC using hexane–ethanol (8:2) as eluent to render **9** (0.04 g, 24%) as white solid, mp: 63–66 °C. ^{1}H NMR: δ 2.07–2.15 (m, 2H, CH_2), 2.11 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.95 (t, J = 7.6 Hz, 2H, CH_2), 3.00 (t, J = 7.6 Hz, 2H, CH_2). Anal. Calcd for $C_{13}H_{15}BrO$: C, 58.44; H, 5.66. Found: C, 58.47; H, 5.67.

5.8. Alcyopterosin A (1)

A solution of thionyl chloride (0.1 mL, 1.2 mmol) in $CHCl_3$ (1 mL) was added dropwise to a stirred solution of **16** (0.10 g, 0.5 mmol) and pyridine (0.1 mL, 1.1 mmol) in $CHCl_3$ (1 mL) at 0 °C. It was stirred for 10 min and heated under reflux for 45 min. The reaction mixture was diluted with water (5 mL), extracted with $CHCl_3$ (2× 5mL), and washed with a solution of 10% HCl (2× 5mL) and water (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified by pTLC using hexane as eluent to render **1** (0.09 g, 78%) as a colorless oil. ^{1}H NMR: δ 1.14 (s, 6H, 2× CH_3), 2.20 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.64 (s, 2H, CH_2), 2.68 (s, 2H, CH_2), 3.11 (t, J = 8.3 Hz, 2H, CH_2), 3.52 (t, J = 8.3 Hz, 2H, CH_2), 6.85 (s, 1H, ar). Anal. Calcd for $C_{15}H_{21}Cl$: C, 76.09; H, 8.94. Found: C, 76.15; H, 8.97.

5.9. DNA affinity assay

DNA solution: Calf thymus DNA (12.5 mg) was slowly magnetically stirred in Tris–HCl buffer 10 mM, pH 7.4 (5 mL), for 24 h at 4 °C. 0.6 mL was taken from this solution and diluted to 25 mL with the same buffer.

The test compound solution was prepared at a 10^{-4} M concentration using a minimal volume of ethanol and then diluted adding water to a concentration of 2×10^{-5} M. A 3 mL sample of this solution was mixed with 3 mL of the DNA solution. The mixture was slowly rotated during 24 h and, then, its UV spectra were recorded at 20 °C using a 1 cm cell.

5.10. Cyclic voltammetry

Studies were carried out using a glassy carbon working electrode (A = 3.03 cm^2), a platinum auxiliary electrode, a Ag/AgCl reference electrode, and MeOH of electrochemical purity. The potentials were scanned from −2000 to +2000 mV employing scan rates of 0.2 mV/s. The glassy carbon electrode was polished intensively with aluminum oxide on a smooth polishing cloth and degreased in methanol prior to each electrochemical measurement. All the solutions examined by electrochemical techniques were first deaerated for at least 10 min with N_2, after which a continuous stream of N_2 was passed over the solution during measurements. Lithium perchlorate 0.1 M was used as support electrolyte. Sample concentration was 0.5 mM.

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