Design and Synthesis of Novel Polycycles Based on the 3*H*-Pyrrolo/6,7-Dihydropyrido[1,2-*a*]indole Scaffold as Templates for Pharmaceutical Development

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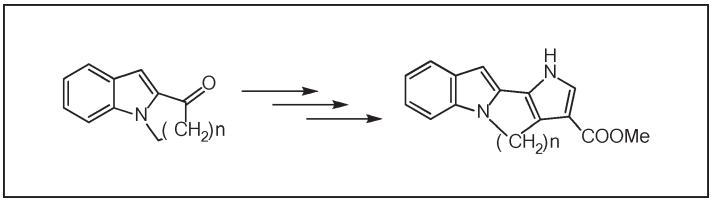

In the course of our work aimed at developing novel heterocycles of pharmaceutical interest, we designed and synthesized several polycyclic templates as potential substrates to be used in drug design. We obtained a set of condensed ring systems as versatile structural platforms to generate potential DNA-interactive agents and/or reversible inhibitors of enzymes such as topoisomerases, poly(ADP-ribose) polymerase-1 (PARP-1), telomerase, and, in particular, cyclin dependent kinases. Herein, we report the design, synthesis, structural investigation, and preliminary DNA-binding affinity of these heteroaromatic systems.

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INTRODUCTION

DNA, due to its fundamental role in the cell cycle, is the pharmacological target of several chemotherapeutic drugs currently in the clinic. In particular, intercalating agents and topoisomerase inhibitors are well-known and extensively studied anticancer drugs [1]. Many of the clinically approved antitumor agents, as well as several compounds under clinical investigation, belong to both classes of therapeutics [2–4].

From a structural point of view, such compounds bear planar aromatic/heteroaromatic polycycle with appropriate side chains in their chemical scaffolds [5,6]. As far as the mechanism of action is concerned, the intercalating agents act through insertion between the bases of the DNA, which results in a change of its conformation and, in turn, this determines a cascade of biochemical events with a subsequent block of the cellular processes. Examples of this class of compounds are mitoxantrone, bisantrene, doxorubicin, amsacrine, and their derivatives. As for the topoisomerase inhibitors, they act either by direct inhibition of the proteins or by interfering with the breaking and rejoining of the DNA molecule, increasing the half-life of the opened form of DNA. Camptothecin, topotecan, and irinotecan are some of the currently approved topoisomerase inhibitors [2–4,6].

Interestingly, pyrrolo[2,3-*a*]carbazole derivatives **I** and pyrazolo[4,3-*h*]quinazolines **II** (Fig. 1) are found to be promising selective inhibitors towards cyclin dependent kinases (CDKs) [7,8] (particularly against CDK1 and CDK2), a family of serine/threonine kinases that play a pivotal role in the regulation of cell cycle progression, and consequently in several physiological processes [9–12].

Since some of CDKs have been proposed to finely regulate each cell cycle phase transition, and the deregulation of CDKs and their modulators occurs in many human tumor, these enzymes represent attractive targets for therapeutic intervention.

In this context, the design and synthesis of novel heterocyclic templates to generate compounds showing specificity and selectivity towards these key biological targets are essential for the development of novel potential antitumor agents.

Recently, we reported the synthesis of a novel heterocyclic scaffold **3** (Fig. 2) [13], which bears two carbonyl groups, suitable to be functionalized (''two-armed'' approach) giving compound **4** and related derivatives [14]. Both computational studies and viscosimetric analysis seem to support the hypothesis that these compounds could interfere with DNA, and further studies

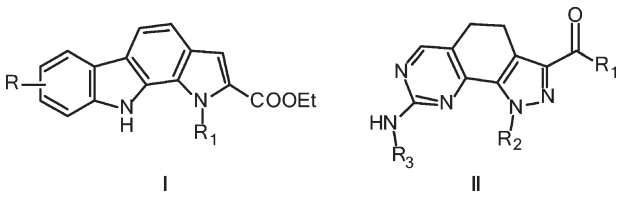


Figure 1. General structure of representative CDKs inhibitors.

are currently in progress to investigate their biological properties [15].

For this reasons, a new series of tetracycles of general structure **1** and **2** have been designed firstly starting from their respective monofunctional intermediates **5** and **6**, which formally derive from the tricycle **3** (Fig. 2). These novel heteroaromatic systems also appeared to be structurally related to the above-mentioned CDKs inhibitors (**I** and **II**). Furthermore, other heterocyclic prototypes, obtained in the course of our work, were also investigated.

RESULTS AND DISCUSSION

The preparation of key intermediates **5** and **6**, as suitable platform to obtain the desired compounds **1** and **2**, has been previously described [16,17]. These compounds showed a similar heterocyclic core, even if the six-membered cycle of **6**, an homologue of **5**, has been planned to modulate the flexibility of the planar framework. Synthetic elaboration of these intermediates was therefore expected to give the designed tetracycles **1** and **2** as final target compounds.

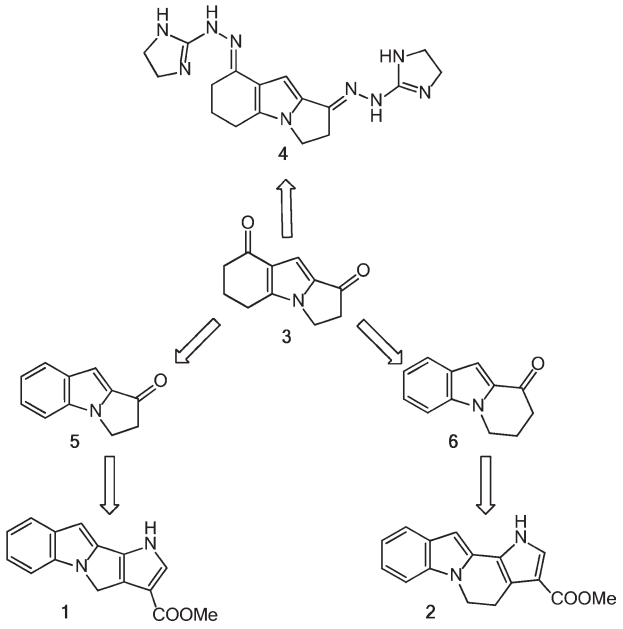
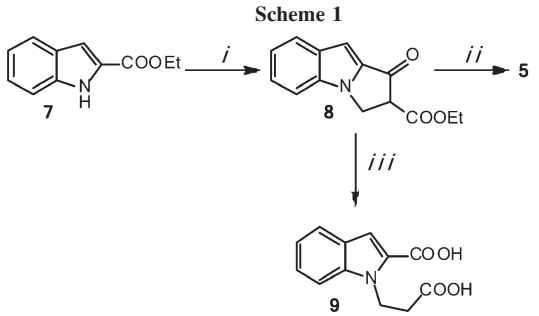


Figure 2. Design of title compounds.

Scheme 1



Reagents and Conditions: *i*) ethyl acrylate, NaH 60% dispersion in mineral oil, anhydrous toluene, N_2, reflux, 5 h; *ii*) 1N HCl, dioxane, reflux, 12 h or CH_3COOH/H_2O, reflux 12 h; *iii*) 2N NaOH, methanol, rt, 5 h then 50 °C, 15 h.

Scheme 1 details the synthesis of compound **5**, which was obtained following the procedure reported by Adams *et al*. [16].

The synthesis of **5** involves a tandem-Michael coupling reaction from the commercially available ethyl indole-2-carboxylate **7** with ethyl acrylate using sodium hydride as a base to give the tricyclic ketoester **8**. The latter was then submitted to hydrolysis/decarboxylation, in one-step reaction, using 1*N* HCl in dioxane or acetic acid in water (both at reflux conditions) to give the desired compound **5** in good yield. Attempts to hydrolyze the ester were not successful giving different products depending on the hydrolysis conditions: in the case of alkaline hydrolysis using 2*N* NaOH the opened dicarboxylic acid **9** was obtained. The possible mechanism of this reaction is depicted in Figure 3 and may involve an attack of the hydroxy group to the ketone carbonyl followed by the opening of the cycle, probably due to the constraint of the five-membered ring. Treatment of **8** with 2*N* NaOH gave the opened dicarboxylic acid **9**.

Scheme 2 shows the synthetic route to obtain the key intermediate **10**, performed following the procedure reported by Bit *et al*. [17].

The ester **7** was reacted with ethyl 4-bromo-butyrate in the presence of sodium hydride to give the opened diester **10**, which was isolated and then cyclized using sodium hydride in toluene as already described for the preparation of **5**. Compound **11** (**11-***keto*) resulted in tautomeric equilibrium with its **11-***enol* derivative,

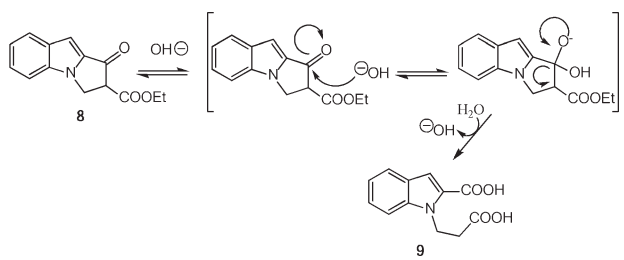
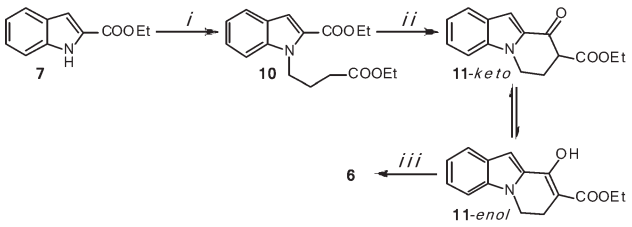


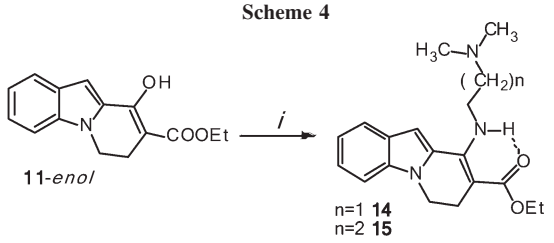
Figure 3. Possible mechanism for the formation of **9**.

Scheme 2



Reagents and Conditions: *i*) Ethyl 4-bromo-butyrate, NaH 60% dispersion in mineral oil, anhydrous DMF, N_2, rt, 18 h; *ii*) NaH 60% dispersion in mineral oil, anhydrous toluene, N_2, reflux, 6 h; *iii*) 1 N HCl, dioxane, reflux, 12 h or 12% aqueous KOH, reflux, 2.5 h; 37% HCl, EtOH, reflux, 1.5 h.

Scheme 4

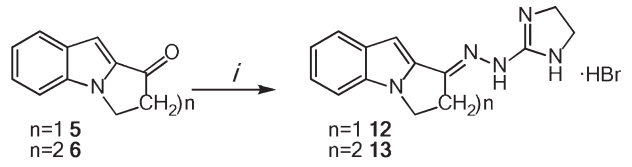


Reagents and Conditions: *i*) *N,N*-dimethylethylenediamine (for **14**) or 3(dimethylamino)-1-propylamine (for **15**), DMAP, anhydrous toluene, N_2, reflux, 22 h (for **14**), 14 h (for **15**).

approximately in 1:3 ratio, for the ketonic and enolic form, respectively. In particular, analysis of ^{1}H NMR spectra, recorded in $CDCl_3$, revealed the presence of two triplets for CH_2 protons, centered at 4.11 and 2.89 ppm resonance, which is coherent with the enolic form. Furthermore, due to exchangeable enolic hydrogen, **11** also showed a broad singlet in a 12.0−12.5 ppm region and besides, a series of small multiplets indicating CH_2 (x2) and C*H* protons in a 2.08−4.36 resonance range were detected, thus confirming the coexistence of the ketonic tautomer. These observations were confirmed by distortionless enhancement by polarization transfer (DEPT) and due to the overlap with other signals, a full characterization of the *keto* tautomer remains however unclear. Hydrolysis using either acid or basic conditions led to the desired ketone **6**. In this case, the acid hydrolysis gave a better yield, 91% using 1*N* HCl or 70% using 37% HCl, compared to the one (≈50%) obtained using 12% aqueous KOH. Unlike **8**, the tricycle **11** resisted to the basic condition leading only compound **6**, and in this case the formation of the dicarboxylic acid was not observed. This result may be due to the higher stability of the six-membered ring of **11** compared to the five-membered ring of **8** as well as to the prevalent more stable enolic form observed for this compound.

Following the successful isolation of the intermediates **5** and **6**, and to test whether a suitable derivatization of these functionalities could occur, ketones **5** and **6** were coupled with 2-amine-2-imidazoline bromohydrate providing compounds **12** and **13**, in 60% and 27% yields, respectively (Scheme 3).

Scheme 3



Reagents and Conditions: *i*) 2-amine-2-imidazoline bromohydrate, absolute EtOH, reflux, 23 h for 13, and 48 h for14.

Scheme 4 reports the synthesis of new derivatives starting from compound **11**. Treatment of **12** with alkylamines gave, as expected, compounds **16** and **17**, being known the behavior of beta-ketoesters as ethylacetoacetate (see, for example Hantzsch dihydropyridine synthesis). Fourteen and fifteen have been characterized by means of IR and NMR spectroscopy, mass spectrometry (EI) and elemental analysis.

Interestingly, computational studies showed a possible conformation for these enamines involving the formation of a pseudo-forth ring, which may be able to modulate the planarity of the backbone, through a formation of an intramolecular hydrogen bond between the N—H bond and the oxygen of the carbonyl of the ester (Fig. 4).

The DNA-binding properties of **14**, as a representative compound, were studied by viscometric titration, using ethidium bromide (EthBr) as a reference compound, as already performed for compound **4** [15]. Figure 5 shows the effect of increasing concentration of **14** on the relative specific viscosity of DNA solution, in comparison with those of EthBr, thus supporting the potential intercalating activity for these prototypes.

The preparation of the tetracyclic templates **1** and **2** (Scheme 5) was carried out by following a thermal rearrangement of *O*-vinyloximes, adapting a procedure already described by us [18].

The ketones **5** and **6** were converted to their corresponding *E*/*Z* mixture of oximes **16** and **17**, in 80% and 43% yields, respectively. The adducts **18** and **19** were obtained in good yield (43 and 62%, respectively) as a mixture of three of the four geometric isomers *E*/*Z*-*E*/*Z* (approximately in 2:2:1 ratio for *E*-*E*, *Z*-*E*, and E-Z, respectively) by coupling the ketoximes **16** and **17** with methyl propiolate in anhydrous DMSO and in the presence of anhydrous TEA. According to an aza-Cope mechanism ([3,3]sigmatropic shift), the final thermal rearrangement of these *O*-alkenoates, to obtain **1** and **2**, was performed either by heating in normal oil bath or through microwave irradiation.

In the case of the five-membered ring intermediate **18**, the cyclization was not successful and the formation of both the ketoxime **16** and the starting ketone **5** was

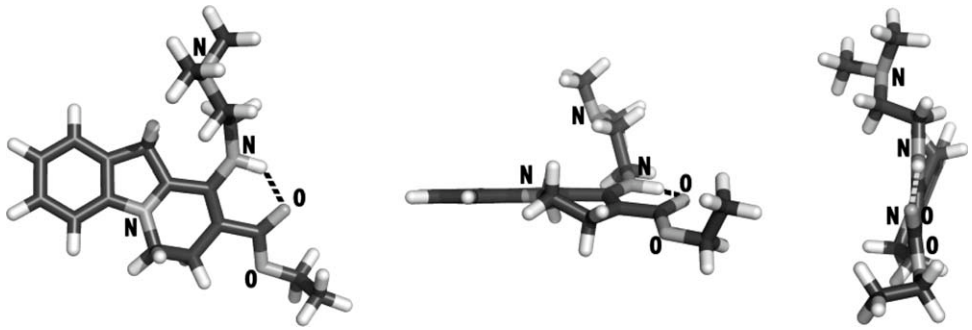


Figure 4. The pseudo-6-membered ring for compound **14** originated by an intramolecular *H*-bond, established between the *O*-carbonyl and the N—H of amide side chain. Conformational analysis was performed by using molecular mechanics methods.

observed, probably due to a competitive retro-Michael reaction addition. In the case of the adduct **19**, the cyclization reaction worked well using both methods, giving 35% and 28% yields, in the case of the thermal and the microwave procedures, respectively. The structure of **2** was unambiguously assigned on the basis of its elemental analysis and EI-mass as well as by the data of ^{1}H NMR experiments.

Once again, the difference in the reactivity may be due to a greater reactivity of the six-membered ring compared to the five-membered ring, as revealed from quantum mechanical studies.

As shown in Figure 6, the HOMO and LUMO coefficients of the orbitals located in the carbon C2-β (Fig. 7) for both adducts, calculated by a quantum mechanical approach, are slight different in terms of absolute values.

In particular, according to the frontier orbital theory, the LUMO coefficients for the C2-β of **18** resulted higher than that of **19**. Therefore, due to the higher electrophilicity of the latter, this carbon might be better subjected to a nucleophilic attack by a lone-pair, which is generated in the transition steps during the evolution of the above-mentioned reaction. Further differences resulted from the comparison of the molecular orbitals, calculated for both enolates (Fig. 6). More experiments should be conducted to support this assumption, eventually considering other possible intermediates that could be originate by this concerted mechanism.

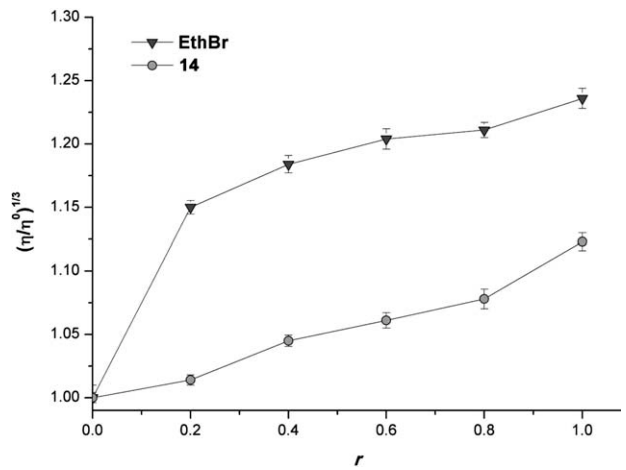


Figure 5. The relative specific viscosity of DNA in the presence (η) and in absence (η^0) of compound **14** and ethidium bromide (EthBr) was calculated from the relation $\eta = (t - t^0)/t^0$, where t is the observed flow time in seconds. The relative viscosity $(\eta/\eta^0)^{1/3}$ is plotted against r, defined as molar ratio of **14** and ethidium bromide to DNA base pair.

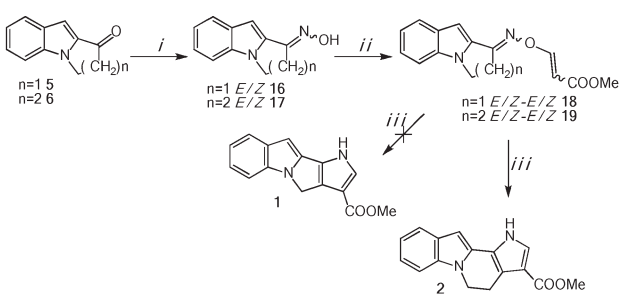
CONCLUSIONS

In this work, a series of novel heterocycles have been designed and synthesized, to be used as versatile platform in drug design process. These results prompted us to propose that this type of chromophores are suitable for extensive modifications and will be undertaken in future studies. Antiproliferative activity and other biological experiments for some related congeners are currently under investigation and will be reported elsewhere.

EXPERIMENTAL

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Merck or Carlo Erba. All reactions involving

Scheme 5



Reagents and Conditions: *i*) $NH_2OH{\cdot}HCl$, sodium acetate, $EtOH/H_2O$, reflux, 6.5 h; *ii*) methyl propiolate, anhydrous TEA, anhydrous DMSO, N_2, 55 °C, 24 h; *iii*) 120 °C, 12-24 h; or 180 °C, 3 h; or 525 W, 8+8+4 min; or 750 W, 5+5+5 min.

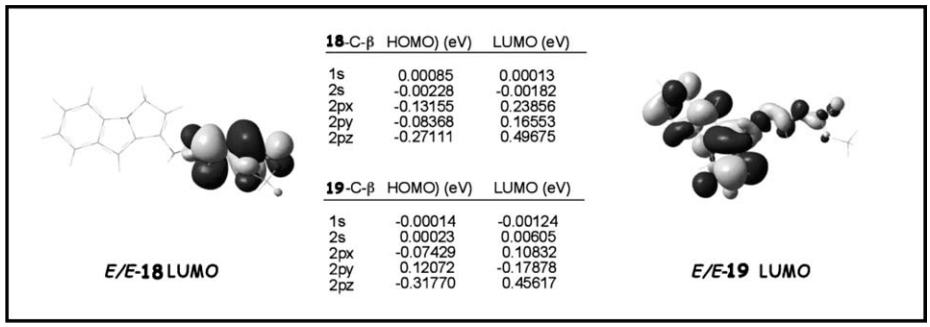


Figure 6. HOMO and LUMO Orbitals coefficients of carbon *b* (for the adducts **18** and **19** as enamines) are performed with Gaussian 98 using the method B3LYP/6.311G.

air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Melting points (mp) were determined using an Electrothermal melting point or a Köfler apparatus and are uncorrected. Infrared (IR) spectra were recorded as thin films or nujol mulls on NaCl plates with a Perkin-Elmer 781 IR spectrophotometer and are expressed in n (cm^{-1}). Nuclear magnetic resonance (^{1}H-NMR, DEPT) spectra were determined in $CDCl_3$, DMSO-d_6 or $CDCl_3$/DMSO-d_6 (in 3/1 ratio) and were recorded at 200 MHz on a Varian XL-200. Chemical shifts (δ scale) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) used as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double doublet. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Electron ionization mass spectra (70 eV) were recorded on a Hewlett-Packard 5989 Mass Engine Spectrometer. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230–400 mesh ASTM) as the stationary phase. Elemental analyses were performed on a Perkin-Elmer 2400 spectrometer at Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari (Italy), and were within ±0.4% of the theoretical values.

Ethyl 1-oxo-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indole-2-carboxylate (8). To a stirred solution of **7** (4.00 g, 21.14 mmol) in anhydrous toluene (200 mL), NaH (60% dispersion in mineral oil) (1.03 g, 25.79 mmol) and ethyl acrylate (2.06 mL, 19.03 mmol) were added under a nitrogen atmosphere, and the reaction mixture was refluxed for 1 h. Then ethyl acrylate (1.6 mL, 14.80 mmol) was added, followed by a further addition after 1 h of NaH (60% dispersion in mineral oil) (0.60 g, 14.80 mmol) and ethyl acrylate (0.46 mL, 4.23 mmol). The reaction mixture was stirred under reflux for further 3 h. After this period the reaction mixture was quenched with ethanol and water, and acidified with 1*N* HCl. The aqueous phase was extracted with ethyl acetate and the organic phase was washed with brine, dried over $NaSO_4$ and concentrated. The residue was purified by column flash chromatography eluting with petroleum ether/ethyl acetate (80/20) to give a gray solid, 3.86 g (75%), mp 97−98°C (lit [19], 100°C); ^{1}H NMR ($CDCl_3$): δ 7.77 (d, 1H, Ar—H), 7.55−7.30 (m, 2H, Ar—H), 7.29−7.15 (m, 1H, Ar—H), 7.08 (s, 1H, Ar—H), 4.96−4.75 (m, 1H, J = 4.2 and 11 Hz, NCH*H*CH), 4.70−4.55 (m, 1H, *J* = 8.4 and 11 Hz, NCH*H*CH), 4.42−4.15 (m, 3H, *J* = 4.2 and 8.4 Hz, NCH_2CH+$COOCH_2CH_3$), 1.47−1.17 (m, 3H, OCH_2CH_3). ms: *m*/*e* 243 (M^+). Mol Formula: $C_{14}H_{13}NO_3$.

2,3-Dihydro-1H-pyrrolo[1,2-a]indol-1-one (5a). To a solution of **8** (1.50 g, 6.17 mmol) in dioxane (110 mL), 1*N* HCl (30.83 mL, 30.83 mmol) was added and the reaction mixture was stirred at reflux for 12 h. After the solvent removal, the residue was triturated with water to give a pale brown solid, 1.04 g (99%); b) A solution of **8** (1.00 g, 4.11 mmol) in glacial acetic acid (59 mL) and water (3 mL) was stirred at reflux for 12 h. The solvent was removed and the residue dissolved in $CHCl_3$. The organic phase was washed with 5% $NaHCO_3$ aqueous solution and brine, then concentrated. The residue was triturated with petroleum ether and methanol and purified

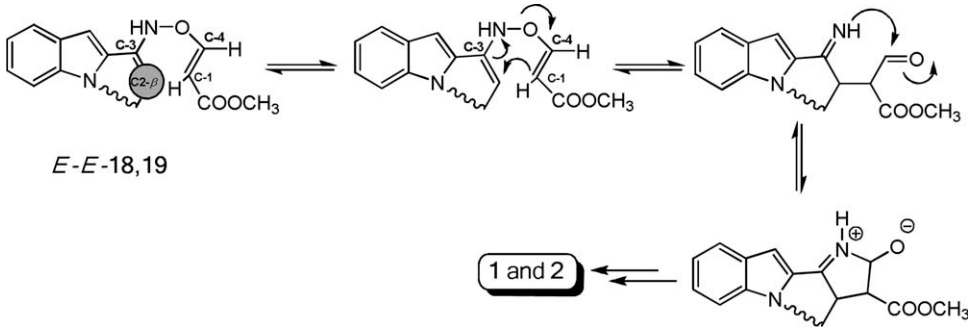


Figure 7. Hypothetical Aza-Cope-like mechanism for the formation of **1** and **2**.

by column flash chromatography eluting with petroleum ether/ethyl acetate (85/15) to give a pale yellow solid, 0.69 g (98%), mp 142−144°C (lit [19], 145−146°C); ^{1}H NMR ($CDCl_3$): δ 7.77 (d, 1H, Ar—H), 7.50−7.30 (m, 2H, Ar—H), 7.24−7.10 (m, 1H, Ar—H), 7.02 (s, 1H, Ar—H), 4.45 (t, 2H, J = 6.4 Hz, N$CH_2$$CH_2$), 3.23 (t, 2H, J = 6.4 Hz, NCH$_2$$CH_2$). ms: *m*/*e* 171 ($M^+$). Mol Formula: $C_{11}H_9NO$.

1-(2-Carboxyethyl)-1H-indole-2-carboxylic acid (9). To a solution of **8** (0.18 g, 0.74 mmol) in methanol (6 mL), 2*N* NaOH (1.5 mL, 3.0 mmol) was added and the mixture was stirred at room temperature for 5 h then at 60°C for 15 h. After this period, the reaction mixture was allowed to cool down, diluted with water, and acidified with 1*N* HCl. The precipitate formed was filtered to give a white solid, 0.14 g (81%), mp 215−217°C ([20], 220°C); ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 7.65 (d, 1H, Ar—H), 7.50 (d, 1H, Ar—H), 7.40−7.11 (m, 2H, Ar—H), 7.12 (t, 1H, Ar—H), 4.86 (t, 2H, J = 7.4 Hz, NCH_2), 2.81 (t, 2H, J = 7.4 Hz, CH_2COOH). ms: *m*/*e* 233 (M^+). Mol Formula: $C_{12}H_{11}NO_4$.

Ethyl 1-(4-ethoxy-4-oxobutyl)-1H-indole-2-carboxylate (10). A solution of **7** (5.00 g, 26.43 mmol) in anhydrous DMF (80 mL) was added, under a nitrogen atmosphere, to a solution of NaH (60% dispersion in mineral oil) (1.10 g, 27.50 mmol) in anhydrous DMF (10 mL). Then ethyl 4-bromo-butyrate (4.50 mL, 31.44 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 18 h. After this period, the reaction mixture was quenched with water (30 mL) and 2*N* HCl (10 mL) and extracted with ethyl acetate. The organic phase was washed with water (5 times), dried over $NaSO_4$ then concentrated to give a yellow oil (lit [17], oil), 6.97 g (87%); ^{1}H NMR ($CDCl_3$): δ 7.67 (d, 1H, Ar—H), 7.46 (d, 1H, Ar—H), 7.40−7.28 (m, 2H, Ar—H), 7.20−7.08 (m, 1H, Ar—H), 4.64 (t, 2H, N$CH_2$$CH_2$$CH_2$), 4.37 (q, 2H, COO$CH_2$$CH_3$), 4.12 (q, 2H, COO$CH_2$$CH_3$), 2.34 (t, 2H, NCH$_2$$CH_2$$CH_2$), 2.28−2.08 (m, 2H, NCH$_2$$CH_2$$CH_2$), 1.41 (t, 3H, COOCH$_2$$CH_3$), 1.32−1.15 (m, 3H, COOCH$_2$$CH_3$). ms: *m*/*e* 303 ($M^+$). Mol Formula: $C_{17}H_{21}NO_4$.

Ethyl 9-hydroxy-6,7-dihydropyrido[1,2-a]indole-8-carboxylate (11). To a solution of **10** (5.00 g, 16.48 mmol) in anhydrous toluene (200 mL), NaH (60% dispersion in mineral oil; 1.32 g, 32.96 mmol) was added under a nitrogen atmosphere and the reaction mixture was stirred at reflux for 6 h. After this period, the reaction mixture was quenched with ethanol and water, acidified with 1*N* HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried over $NaSO_4$ and concentrated. The residue was triturated with petroleum ether to give a beige solid, 2.97 g (70%), mp 100−102°C (lit [21], 101−103°C); ^{1}H NMR ($CDCl_3$): δ 12.17 (bs, 1H, enol), 7.80−7.60 (m, 2H, Ar—H), 7.44−7.08 (m, 2H, Ar—H), 7.03 (s, 1H, Ar—H), 4.36−4.11 (m, 2H, $COOCH_2$), 4.08 (t, 2H, J = 6.6 Hz, NCH_2), 2.89 (t, 2H, J = 6.6 Hz, CH_2), 1.37 (t, 3H, CH_3). ms: *m*/*e* 257 (M^+). Mol Formula: $C_{15}H_{15}NO_3$.

7,8-Dihydropyrido[1,2-*a*]indol-9(6*H*)-one (6a). (a) To a solution of **11** (1.14 g, 4.43 mmol) in dioxane (70 mL), 1*N* HCl (22.0 mL, 22.15 mmol) was added and the reaction mixture was stirred at reflux for 12 h. After this period, the solvent was removed and the residue triturated with water to give a pale brown solid, 0.75 g (91%); (b) A solution of **11** (0.25 g, 0.97 mmol) in 12% KOH aqueous solution (10 mL) was stirred at reflux for 2.5 h. Then, the reaction mixture was allowed to cool down, diluted with water, and acidified with 1*N* HCl. The precipitate obtained was filtered to give a pale brown solid, 0.11 g, (59%); (c) To a solution of **11** (0.60 g, 2.23 mmol) in ethanol (12 mL), 37% HCl (12 mL) was added and the reaction mixture was stirred at reflux for 1.5 h. After this period, the reaction mixture was allowed to cool down, diluted with water, and the precipitate formed was filtered and purified by column flash chromatography gradient elution of petroleum ether/ethyl acetate (80/20 then 70/30) to give a brown solid, 0.30 g (72%), mp 140−142°C for products from (a), (b), and (c) (lit. [17], 138−140°C); ^{1}H NMR ($CDCl_3$): δ 7.74 (d, 1H, Ar—H), 7.47−7.35 (m, 2H, Ar—H), 7.33 (s, 1H, Ar—H), 7.23−7.13 (m, 1H, Ar—H), 4.27 (t, 2H, NCH_2), 2.76 (t, 2H, CH_2), 2.42 (m, 2H, $CH_2$$CH_2$$CH_2$). ms: *m*/*e* 185 ($M^+$). Mol Formula: $C_{12}H_{11}NO$.

(E/Z)-1-(2-(4,5-dihydro-1*H*-imidazol-2-yl)hydrazono)2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indole bromohydrate (12). To a solution of **5** (0.25 g, 1.46 mmol) in absolute ethanol (13 mL), 2-amine-2-imidazoline bromohydrate (0.53 g, 2.92 mmol) was added and the reaction mixture was stirred at reflux for 23 h. After this period, the precipitate formed was filtered to give a beige solid, 0.29 g (60%), mp 263−265°C; ^{1}H NMR ($CDCl_3$ DMSO-d_6): δ 11.34 (bs, 1H, NH), 8.05 (bs, 1H, NH), 7.63 (d, 1H, Ar—H), 7.40 (d, 1H, Ar—H), 7.33−7.00 (m, 2H, Ar—H), 6.71 (s, 1H, Ar—H), 4.42 (t, 2H, N$CH_2$$CH_2$), 3.81 (s, 4H, 2 × NCH_2 imidazolic), 3.47 (t, 2H, NCH$_2$$CH_2$). ms: *m*/*e* 253 ($M^+$). *Anal*. Calcd. for $C_{14}H_{16}BrN_5$: C, 50.31; H, 4.83; N, 20.95. Found C, 50.15; H, 4.93; N, 21.07.

(E/Z)-9-(2-(4,5-dihydro-1*H*-imidazol-2-yl)hydrazono)-6,7,8,9-tetrahydropyrido[1,2-*a*]indole bromohydrate (13). To a solution of **6** (0.20 g, 1.08 mmol) in absolute ethanol (10 mL), 2-amine-2-imidazoline bromohydrate (0.39 g, 2.16 mmol) was added and the reaction mixture was stirred at reflux for 48 h. After this period, the precipitate formed was filtered to give a dark yellow solid, 0.10 g (27%), mp 295−297°C dec; ^{1}H NMR ($CDCl_3$ DMSO-d_6): δ 11.46 (bs, 1H, NH), 8.35 (bs, 1H, NH), 7.60 (d, 1H, Ar—H), 7.47 (d, 1H, Ar—H), 7.30−7.16 (m, 1H, Ar—H), 7.15−7.00 (d, 2H, Ar—H), 4.19 (t, 2H, NCH_2), 3.39 (s, 4H, 2 x CH_2 imidazolic), 2.79 (t, 2H, CH_2), 2.18 (m, 2H, $CH_2$$CH_2$$CH_2$). ms: *m*/*e* 267 ($M^+$). *Anal*. Calcd. for $C_{15}H_{18}BrN_5$: C, 51.73; H, 5.21; N, 20.11. Found C, 51.50; H, 5.03; N, 20.32.

Ethyl 9-(2-(dimethylamino)ethylamino)-6,7-dihydropyrido[1,2-*a*]indole-8-carboxylate (14). A solution of **11** (0.20 g, 0.78 mmol), *N*,*N*-dimethylethylenediamine (0.17 mL, 1.55 mmol) and DMAP (0.03 g, 0.23 mmol) in anhydrous toluene (4 mL) was stirred at reflux for 22 h under nitrogen atmosphere. After this period, the solvent was removed and the residue purified by column flash chromatography eluting with $CHCl_3$/MeOH (95/5) to give a yellow solid, 0.03 g (12%), mp 100−103°C; ^{1}H NMR ($CDCl_3$): δ 9.06 (bs, 1H, NH), 7.64 (d, 1H, Ar—H), 7.38−7.23 (m, 2H, Ar—H), 7.16−7.05 (m, 1H, Ar—H), 6.93 (s, 1H, Ar—H), 4.21 (q, 2H, COO$CH_2$$CH_3$), 4.09 (t, 2H, N$CH_2$$CH_2$), 3.76 (q, 2H, NH$CH_2$$CH_2$), 2.81 (t, 2H, NCH$_2$$CH_2$), 2.59 (t, 2H, $CH_2$$CH_2N(CH_3)_2$), 2.31 (s, 6H, N$(CH_3)_2$), 1.32 (t, 3H, COOCH$_2$$CH_3$). ms: *m*/*e* 327 ($M^+$). Anal. Calcd. for $C_{19}H_{25}N_3O_2$: C, 69.70; H, 7.70; N, 12.83. Found C, 69.99; H, 7.58; N, 12.57.

Ethyl 9-(3-(dimethylamino)propylamino)-6,7-dihydropyrido[1,2-*a*]indole-8-carboxylate (15). A solution of **11** (0.20 g, 0.78 mmol), N^1,N^1-dimethylpropane-1,3-diamine (0.20 mL, 1.55 mmol) and DMAP (0.03 g, 0.23 mmol) in anhydrous toluene (4 mL) was stirred at reflux for 14 h under nitrogen

atmosphere. After this period, the solvent was removed and the residue purified by column flash chromatography eluting with $CHCl_3$/MeOH (95/5) to give a yellow solid, 0.06 g (11%), mp 114−117°C; ^{1}H NMR ($CDCl_3$): δ 8.98 (bs, 1H, NH), 7.65 (d, 1H, Ar—H), 7.40−7.20 (m, 2H, Ar—H), 7.15−7.02 (m, 1H, Ar—H), 6.94 (s, 1H, Ar—H), 4.20 (q, 2H, $COOCH_2$), 4.12 (t, 2H, NCH_2CH_2), 3.75−6.62 (m, 4H, $NHCH_2$), 2.80 (t, 2H, NCH_2CH_2), 2.27 (s, 6H, $N(CH_3)_2$), 1.94−1.80 (m, 2H, $CH_2CH_2CH_2$), 1.32 (t, 3H, CH_2CH_3). ms: *m*/*e* 341 (M^+). Anal. Calcd. for $C_{20}H_{27}N_3O_2$: C, 70.35; H, 7.97; N, 12.31. Found C, 70.63; H, 7.68; N, 12.44.

(*E*/*Z*) 2,3-dihydro-1H-pyrrolo[1,2-*a*]indol-1-one oxime (16). A solution of **5** (1.21 g, 7.07 mmol), hydroxylamine chlorohydrate (1.47 g, 21.20 mmol) and sodium acetate (3.47 g, 42.41 mmol) in a 2/1 mixture of ethanol/water (30 mL) was stirred at reflux for 6.5 h. After this period the reaction mixture was allowed to cool down and the precipitate formed was filtered to give a pale brown solid, 1.05 g (80%), mp > 300°C (lit [22], 220°C dec.); ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 11.04 (bs, 1H, NOH), 7.57 (d, 1H, Ar—H), 7.32 (d, 1H, Ar—H), 7.23−6.95 (m, 2H, Ar—H), 6.56 (s, 1H, Ar—H), 4.28 (t, 2H, NCH_2CH_2), 3.40 (t, 2H, NCH_2CH_2). ms: *m*/*e* 186 (M^+). Mol Formula: $C_{11}H_{10}N_2O$.

(*E*/*Z*) 7,8-dihydropyrido[1,2-*a*]indol-9(6*H*)-one oxime (17). A solution of **6** (0.75 g, 4.05 mmol), hydroxylamine chlorohydrate (0.84 g, 12.15 mmol) and sodium acetate (2.03 g, 24.70 mmol) in a 2/1 mixture of ethanol/water (18 mL) was stirred at reflux for 4.5 h. After this period the reaction mixture was allowed to cool down and the precipitate formed was filtered to give a pale brown solid, 0.35 g (43%), mp 138−140°C; ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 11.01 (bs, 1H, NOH), 7.72 (d, 1H, Ar—H), 7.64 (d, 1H, Ar—H), 7.38−7.08 (m, 2H, Ar—H), 6.97 (s, 1H, Ar—H), 4.26−4.05 (m, 2H, $NCH_2CH_2CH_2$), 2.96 (t, 2H, $CH_2CH_2CH_2C{=}NOH$), 2.33−2.10 (m, 2H, $CH_2CH_2CH_2$). ms: *m*/*e* 200 (M^+). *Anal.* Calcd. for $C_{12}H_{12}N_2O$: C, 71.97; H, 6.04; N, 14.00. Found C, 71.70; H, 6.13; N, 13.85.

(*E*/*Z*-*E*/*Z*) methyl 2,3-dihydro-1H-pyrrolo[1,2-*a*]indol-1-ylideneaminooxy)acrylate (18). To a solution of **16** (0.79 g, 4.24 mmol) in anhydrous DMSO (15 mL) and anhydrous triethylamine (0.5 mL), a solution of methyl propiolate (1.89 mL, 21.21 mmol) in anhydrous DMSO (15 mL) was added dropwise under nitrogen atmosphere and the reaction mixture was stirred at 50−60°C for 24 h. After this period, the reaction mixture was allowed to cool down and poured into water and ice. The aqueous solution was extracted with dichloromethane and the combined organic phases were washed with water, then dried over $NaSO_4$, and concentrated. The residue was purified by column flash chromatography eluting with petroleum ether/ethyl acetate (70/30) to give a yellow-orange solid, 0.49 g (43%), mp 110−112°C; ^{1}H NMR ($CDCl_3$): δ 8.04 (d, 2H, J = 13 Hz, CH), 7.64 (d, 5H, Ar—H), 7.57 (d, 2H, J = 12.4 Hz, CH), 7.45 (d, 1H, J = 7.4 Hz, CH), 7.34−7.15 (m, 15H, Ar—H), 6.82 (s, 5H, Ar—H), 5.71 (d, 1H, J = 7.4 Hz, CH), 5.65 (d, 2H, J = 13 Hz, CH), 4.97 (d, 2H, J = 12.4 Hz, CH), 4.32 (t, 10H, NCH_2), 3.98 (s, 3H, $COOCH_3$), 3.76 (s, 12H, $COOCH_3$), 3.56 (t, 10H, CH_2). ms: *m*/*e* 270 (M^+). Anal. Calcd. for $C_{15}H_{14}N_2O_3$: C, 66.64; H, 5.22; N, 10.37. Found C, 66.42; H, 5.37; N, 10.33.

(*E*/*Z*-*E*/*Z*) methyl 3-(7,8-dihydropyrido[1,2-*a*]indol-9(6*H*)-ylideneaminooxy)acrylate (19). To a solution of **17** (0.34 g, 1.70 mmol) in anhydrous DMSO (6 mL) and anhydrous triethylamine (0.25 mL), a solution of methyl propiolate (0.75 mL, 8.49 mmol) in anhydrous DMSO (6 mL) was added dropwise under nitrogen atmosphere. The reaction mixture was stirred at 50−60°C for 18 h and then allowed to cool down and poured into water and ice. The aqueous solution was extracted with dichlomethane and the organic phase was washed with water, dried over $NaSO_4$ and concentrated. The residue was purified by column flash chromatography eluting with petroleum ether/ethyl acetate (80/20) to give a yellow solid, 0.30 g (62%), mp 105−108°C; ^{1}H NMR ($CDCl_3$): δ 8.13 (d, 2H, J = 9.4 Hz, CH), 8.06 (d, 2H, J = 12.4 Hz, CH), 7.69 (d, 5H, Ar—H), 7.59 (d, 1H, J = 7.6 Hz, CH), 7.36−7.08 (m, 20H, Ar—H), 5.78 (d, 2H, J = 9.4 Hz, CH), 5.70 (d, 2H, J = 12.4 Hz, CH), 5.68 (d, 1H, J = 7.6 Hz, CH), 4.16 (t, 10H, NCH_2), 3.75 (s, 15H, $COOCH_3$), 2.99 (t, 10H, CH_2), 2.21 (m, 10H, CH_2). ms: *m*/*e* 284 (M^+). Anal. Calcd. for $C_{16}H_{16}N_2O_3$: C, 67.58; H, 5.68; N, 9.86. Found C, 67.39; H, 5.74; N, 9.77.

Methyl 4,5-dihydro-1*H*-pyrrolo[2′,3′:3,4]pyrido[1,2-*a*]-indol-3-carboxylate (2). (a) The acrylate derivative (**19;** 0.21 g, 0.74 mmol) was placed in an oil bath at 120°C for 20 h. After cooling down, the residue was purified by column flash chromatography gradient elution of ethyl acetate/petroleum ether (70/30 then 85/15) to give a dark yellow solid, 0.07 g (35%); b) **19** (0.07 g, 0.25 mmol) was irradiated with microwave at 525W (8 + 8 + 4 min) or at 750W (5 + 5 + 5 min). After cooling down, the residue was purified by column chromatography gradient elution of ethyl acetate/petroleum ether (70/30 then 85/15) to give a dark yellow solid, 0.02 g (28%), mp 265−268°C; ^{1}H NMR ($CDCl_3$): δ 9.30 (bs, 1H, NH), 7.60 (d, 1H, Ar—H), 7.43−7.04 (m, 3H, Ar—H), 6.83 (s, 1H, Ar—H pyrrole), 6.60 (s, 1H, Ar—H), 4.22 (t, 2H, NCH_2CH_2), 3.91 (s, 3H, OCH_3), 3.06 (t, 2H, NCH_2CH_2). ms: *m*/*e* 266 (M^+). Anal. Calcd. for $C_{16}H_{14}N_2O_2$: C, 72.15; H, 5.30; N, 10.52. Found C, 71.86; H, 5.32; N, 10.50.

Dna Binding Experiments. ***DNA.*** Calf thymus DNA was purchased from Sigma-Aldrich as highly polymerized sodium salt. The DNA was dissolved in Tris buffer solution and the concentration was determined by absorbance measurements at 260 nm with the molar extinction coefficient 6600 cm^{-1}. The purity of the DNA was checked by monitoring the value of A_{260}/A_{280}. The ratio was in excess of 1.80 for all samples used in the experiments so that the contents of residual proteins should be small. This spectral data is consistent with published values [23].

Viscometric titration. Viscosity measurements were carried out using an Ostwald type viscometer immersed in a water bath maintained at 25 ± 0.05°C. Solutions of DNA, the compound **14,** and ethidium bromide (control), were prepared with Tris buffer (5 m*M*, pH 7.2). Ten milliliter of calf thymus DNA solution ($2.47 \times 10^{-4}M$) was placed in the viscometer and titration were conducted by adding aliquots of solutions of **14** and ethidium bromide ($3.41 \times 10^{-4}M$). Following each addition, the solution was carefully mixed with a small flow of air through the dilution bulb of the viscometer for 2−3 min. The flow times were recorded at least in triplicate to an accuracy of ±0.2 s with a stopwatch, and an average time was calculated. The relative specific viscosity of DNA in the presence (η) and in absence (η^0) of the **16** and ethidium bromide was calculated from the relation $\eta = (t - t^0)/t^0$, where t is the observed flow time in seconds. The values of relative viscosity $(\eta/\eta^0)^{1/3}$ were plotted against r value definite as molar ratio of

14 and ethidium bromide to DNA base pair. The data were processed and analyzed by Origin® software (version 7.0 SR0, OriginLab Corporation). The statistical analysis was evaluated by a Student's *t*-test and *P*-values < 0.05 were considered statistically significant.

Molecular Modeling. ***Molecular mechanic method.*** Model compound **14** was constructed with standard bond lengths and angles from the fragment database with MacroModel 6.0 [24] using a Silicon Graphics O2 workstation running on IRIX 6.3. Sybyl 6.2 (2001) [25] was used as graphic platform. The atomic charges were assigned using the Gasteiger-Marsili method [26]. Minimization of structure was performed with the MacroModel/BachMin 6.0 program using the AMBER force field. Extensive conformational search was carried out using the Monte Carlo/Energy minimization [27] for the compound considered in the study (Ei-Emin < 5 Kcal/mol, energy difference between the generated conformation and the current minimum). Final minimization of the structure was performed with Sybyl 6.2 by using Tripos force field.

Ab Initio ***calculations.*** The computations were performed with Gaussian 1998 [28] package (and confirmed with Gaussian 2003) using Hartree-Fock e STO3G Basis Set. The geometries of compounds *E-E*-**18** and *E-E*-**19**, for graphical representation, were constructed wih Sybyl and fully optimized by the DFT (Density Functional Theory) using B3LYP and 6-311G [29,30] basis set [31]. Atomic charges have been obtained at the same level using a Mulliken population analysis, whereas HOMO and LUMO coefficients have been computed at the HF/STO-3G level using the same geometries.

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