J Incl Phenom Macrocycl Chem (2013) 76:87–98
DOI 10.1007/s10847-012-0176-y

ORIGINAL ARTICLE

Novel pyrrolo-quinazolino-quinoline analogues of the natural alkaloids and their inclusion molecular complexes in the native cyclodextrins: experimental versus theoretical study

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Received: 18 October 2011 / Accepted: 3 May 2012 / Published online: 22 June 2012

Abstract A series ten novel analogs based on a novel template pyrrolo-quinazolino-quinolines, containing Luotonin-A (Luot-A) and 14-aza-camptothecin (14-aza-CPT) molecular core as well as their inclusion complexes in the native α- (α-CD), β- (β-CD) and γ- (β-CD) cyclodextrins were obtained. The physical properties of the alkaloids and corresponding molecular complexes with cyclodextrins are elucidated experimentally by the electronic absorption and CD-spectroscopy, electrospray ionization and matrix-assisted laser desorption/ionization mass spectrometry and nuclear magnetic resonance method. The experimental data are supported by the theoretical quantum chemical calculations of the molecular and electronic structures as well as physical properties in condense phase.

Keywords Pyrrolo-quinazolino-quinoline analogues · Neutral alkaloids · Inclusion complexes · Cyclodextrins · Quantum chemical calculations · Optical properties · Mass spectrometry

Introduction

Luotonins as heteroaromatic pyrroloquinazolinoquinoline plant alkaloids, used for the treatment of rheumatism, inflammation, abscesses and other maladies, possess a unique skeleton comprising of pharmacologically important quinoline and quinazoline framework [1–18]. Amongst these derivatives, Luot-A, showed remarkable cytotoxic activity against leukemia, reminiscenting of camptothecin (CPT) an inhibitor of topoisomerase I [1–18]. The molecular skeleton of CPTs, and in particular the 14-Aza-CPT is attractive template for drugs-design of novel biologically active derivatives [1–18]. Hydroxy lactone E-ring (Scheme 1), considered to be an absolute requirement for high topoisomerase I (Top-I) mediated cytotoxicity. Many other E-ring modifications such as CPT-lactol, CPT-lactam, a ring opened hydroxyl amide, α-halo lactone, α-azidolactone, α-aminolactone and α-exo-methylenelactone were either inactive or showed significantly decreased activity in cell assays, comparing to the parent CPT [1–18]. The pyridone D-ring also has been considered to be an absolute requirement for the biological activity [1–18]. Surprisingly Luot-A, which can be regarded as a DE-ring modified analogue of CPT, is cytotoxic and very recently, it has been demonstrated that it's stabilized a Top-I-DNA covalent binary complex. These findings have resulted in increasing interest for novel analogues and hence considerable attention has been focused on their synthesis [1–18]. To overcome the typical stability and/or solubility problems, several approaches have been presented [19–27], including the inclusion molecular complexes in cyclodextrins, which have been extensively used to improve solubility and stability of a variety of poorly soluble and labile drugs [28–30]. Therefore, in this paper, are studied the physical and physical optical properties of ten novel structurally modified pyrrolo-quinazolino-quinolines and their molecular inclusion complexes with native α-, β- and γ-cyclodextrins as part of our ongoing research in the field of the natural products (NPs) and the functional oriented synthesis and drug-design [31–40]. Nevertheless, the large number of the papers focusing on design, synthesis and in vivo analysis of pyrrolo-quinazolino-quinolines

Electronic supplementary material The online version of this article (doi:10.1007/s10847-012-0176-y) contains supplementary material, which is available to authorized users.

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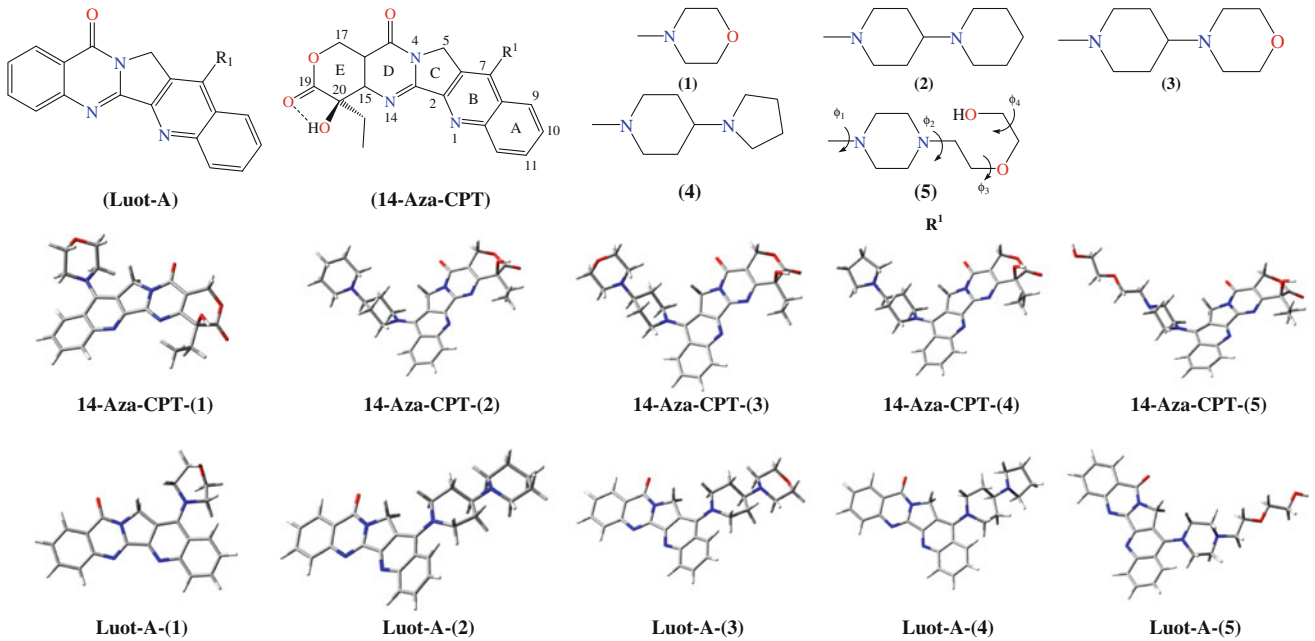
[1–18, 41–52], the comprehensive studies on their physical properties is rare [24, 25, 35–37].

Results and discussion

Molecular structure of the alkaloids and their molecular complexes with cyclodextrins

The molecular geometries of the studied alkaloids are depicted in Scheme 1. Selected geometry parameters are summarized in Table S1. The molecular geometry of the Luot-A and 14-Aza-CPT skeleton have a coplanar pentacyclic ring geometry, including quinoline moiety, a lactam ring and a β-lactone E-ring with an α-hydroxyl group at the position of carbon 20 with a *S*-configuration in 14-Aza-CPTs. The close ring β-lactone form is not stable and coexists with the E-opening ring (carboxylate form), in equilibrium in aqueous solution, meaning that the stability of the lactone form is pH-dependent. Hydrophobicity, of the CPTs in lactone form is lowered on going to the carboxylate form with the anionic COO^{-}-group, observed at pH range of 9.9–12.0 [1–18]. Similar to our previous study of some protonated CPTs, as well as other reported data [1–18, 35, 36], at low pH values, the lactone form kept. Thus, our spectroscopic and structural study is performed, using the low pH values assuming the stabilization of the lactone form. The most stable conformers are characterized with the intramolecular OH···O bond with lengths within 2.549–2.580 Å in lactone moiety (Scheme 1). The R^1-substituents are turning the plane of the CPT skeleton (Table S1). The shown ϕ_1–ϕ_4 dihedral angles are appeared informative for the elucidation of the molecular complexes with the native cyclodextrins (Schemes 2 and S1). As can be seen the maximal deviation of the relatively extended geometry of the alkaloids is found in the Laur-A-(5) and 14-Aza-CPT-(5) (Table 1) characterizing with the relatively low values of the free Gibbs energies (Table S2). As expected the main factor defining this conformational transformation appears the possibility for intermolecular interactions with the cyclodextrin molecules. It is important to discuss that these bindings in 14-Aza-CPTs are preferred. Moreover, the lactone OH-group takes part in the strong to moderate OH···O interactions with the carbohydrates. Series of non-classical weak interactions are found in the alkaloid-cyclodextrin complexes such as CH···N, with bond lengths within 3.041–3.432 Å in all of the studied thirty complexes (Schemes 2 and S1). In Luot-A alkaloids-β-CD molecular complexes are found moderate (O)H···π interactions with the A-ring of the alkaloids (Scheme 3), explaining the observed atypical bathochromic shifting of the charge transfer (CT) band in the EAs. The large cavity of the γ-CD resulted to the observation of more than one stable conformation of the interacting molecules with close ΔG values (Table S2; Schemes 3 and

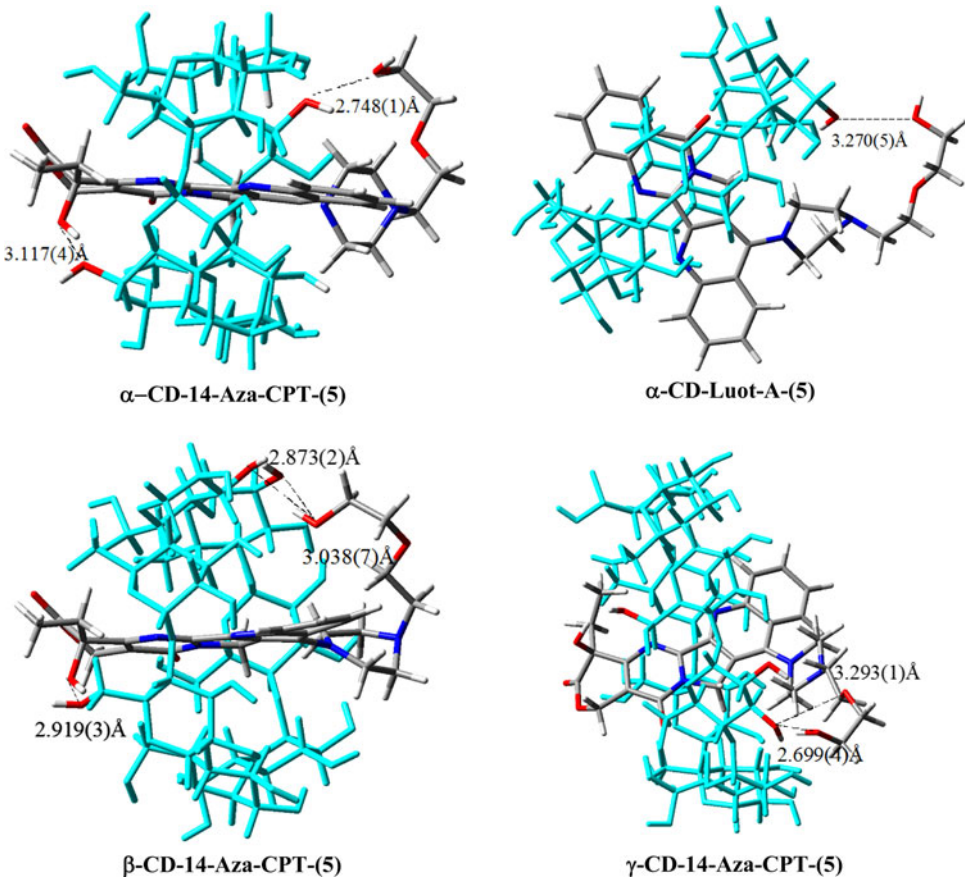


Scheme 1 Chemical diagrams of the novel alkaloids, labeling and the most stable conformers obtained at B3LYP/6-31 ++G(2d, 2p) level of theory; Name definition of the dihedral angles $\phi 1$–ϕ_4, respectively; The labeling of the alkaloid skeleton and the typical intramolecular hydrogen bond in lactone form

S2). The mass spectrometric data provided experimental evidence of the type of the inclusion interactions.

Mass spectrometric study

The mass spectrometry appeared especially informative method for structural elucidation of the complex interacting alkaloids in starting solutions, using the ESI method as well as in solid-state of the obtained polymers of the inclusion complexes by MALDI-MS method (Figs. 1, 2). The observed peaks at high *m/z* values corresponding to native cyclodextrins and the alkaloids are an indication of the stable complexes in gas-phase, *i.e.* 1407.62 ($[C_{53}H_{82}N_4O_{35}]^+$, α-CD-14-Aza-CPT-(1)), 1488.73 ($[C_{59}H_{93}N_5O_{34}]^+$, α-CD-14-Aza-CPT-(2)), 1489.99 ($[C_{58}H_{91}N_5O_{35}]^+$, α-CD-14-Aza-CPT-(3)), 1473.77 ($[C_{58}H_{91}N_5O_{34}]^+$, α-CD-14-Aza-CPT-(4)), 1492.83 ($[C_{57}H_{90}N_5O_{36}]^+$, α-CD-14-Aza-CPT-(5)), 1568.93 ($[C_{65}H_{92}N_4O_{40}]^+$, α-CD-14-Aza-CPT-(1)), 1650.91 ($[C_{71}H_{103}N_5O_{39}]^+$, β-CD-14-Aza-CPT-(2)), 1652.09 ($[C_{70}H_{101}N_5O_{40}]^+$, β-CD-14-Aza-CPT-(3)), 1635.79 ($[C_{70}H_{101}N_5O_{40}]^+$, β-CD-14-Aza-CPT-(4)), 1655.22 ($[C_{69}H_{100}N_5O_{32}]^+$, β-CD-14-Aza-CPT-(5)), 1732.44 ($[C_{71}H_{102}N_4O_{45}]^+$, γ-CD-14-Aza-CPT-(1)), 1812.78 ($[C_{75}H_{113}N_5O_{44}]^+$, γ-CD-14-Aza-CPT-(2)), 1814.33 ($[C_{74}H_{111}N_5O_{45}]^+$, γ-CD-14-Aza-CPT-(3)), 1799.93 ($[C_{74}H_{111}N_5O_{44}]^+$, γ-CD-14-Aza-CPT-(4)), 1819.07 ($[C_{73}H_{110}N_5O_{46}]^+$, γ-CD-14-Aza-CPT-(5)), 1297.84 ($[C_{52}H_{78}N_4O_{32}]^+$, α-CD-Laut-A-(1)), 1423.17 ($[C_{58}H_{89}N_5O_{31}]^+$, α-CD-Laut-A-(2)), 1426.92 ($[C_{57}H_{87}N_5O_{32}]^+$, α-CD-Laut-A-(3)), 1410.01 ($[C_{57}H_{87}N_5O_{31}]^+$, α-CD-Laut-A-(4)), 1430.04 ($[C_{56}H_{86}N_5O_{33}]^+$, α-CD-Laut-A-(5)), 1505.27 ($[C_{64}H_{88}N_4O_{37}]^+$, β-CD-Laut-A-(1)), 1587.09 ($[C_{70}H_{99}N_5O_{36}]^+$, β-CD-Laut-A-(2)), 1589.81 ($[C_{69}H_{97}N_5O_{37}]^+$, β-CD-Laut-A-(3)), 1573.33 ($[C_{69}H_{97}N_5O_{36}]^+$, β-CD-Laut-A-(4)), 1592.81 ($[C_{68}H_{96}N_5O_{38}]^+$, β-CD-Laut-A-(5)), 1668.84 ($[C_{70}H_{98}N_4O_{42}]^+$, γ-CD-Laut-A-(1)), 1749.22 ($[C_{74}H_{109}N_5O_{41}]^+$, γ-CD-Laut-A-(2)), 1731.45 ($[C_{73}H_{107}N_5O_{42}]^+$, γ-CD-Laut-A-(3)), 1735.06 ($[C_{73}H_{107}N_5O_{41}]^+$, γ-CD-Laut-A-(4)), 1753.16 ($[C_{72}H_{106}N_5O_{43}]^+$, γ-CD-Laut-A-(5)), respectively. The methods allow to evaluate the surface interactions of the alkaloids with the cyclodextrins, since the non-covalent bonding [27] of the small molecules leads to the observation of the single peaks for the cyclodextrins and the corresponding low-weight molecular fragments [53–57]. The methods, provided unique experimental structural evidence for the formation of the inclusion complexes, within the frame of the capability the single crystal X-ray diffraction, however does not depending of the crystallization processes, which for the chiral molecules such both for the native cyclodextrins, the studied alkaloids themselves as well as their inclusion

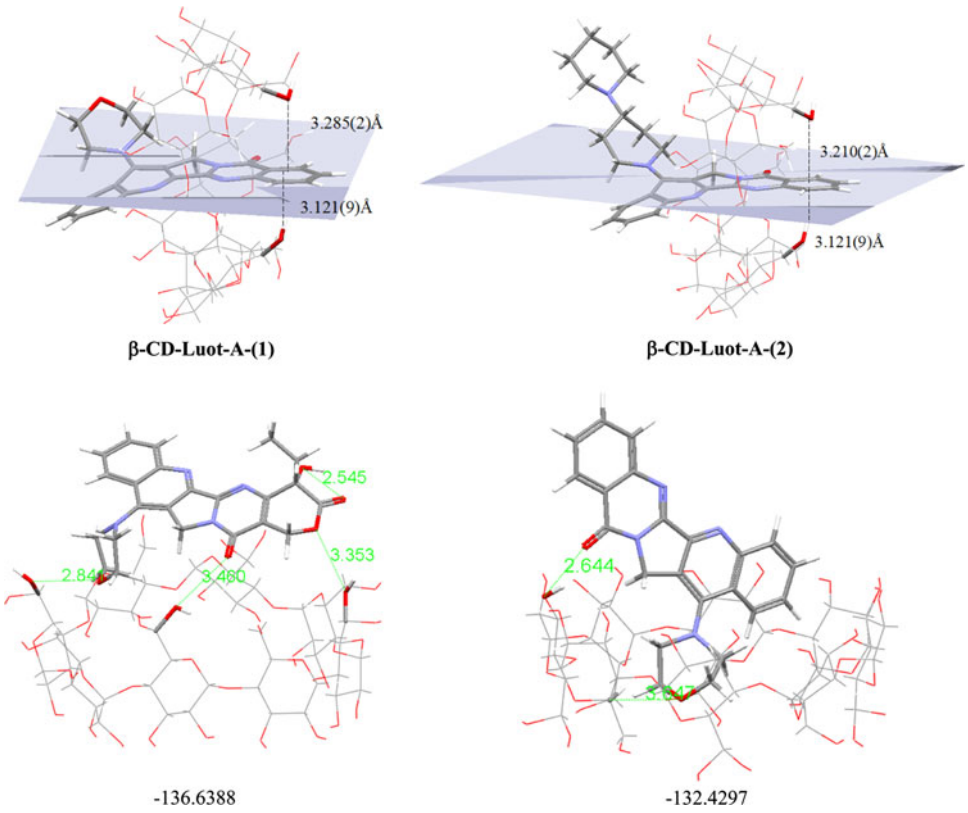


Scheme 2 Most stable conformers of the xCD-14-Aza-(5) (x = α, β or γ; i = 1–5) molecular complexes, obtained at B3LYP/6-31 ++G(2d, 2p) level of theory; Selected intermolecular hydrogen bonds [Å]

Table 1 Intermolecular hydrogen bonding schemes in xCD-14-Aza-(i) and xCD-Luot-A-(i) (x = α, β or γ; i = 1–5) molecular complexes; Bond lengths are given in [Å] as distance between the heavy atoms; In blue are shown the non classical hydrogen bonding (short contacts) in terms of the crystallographic shower contacts with distances longer than 3.45 Å as well as interactions of type CH···O(=C), respectively

	α-CD-14-Aza-CPT-(1)	α-CD-14-Aza-CPT-(2)	α-CD-14-Aza-CPT-(3)	α-CD-14-Aza-CPT-(4)	α-CD-14-Aza-CPT-(5)
$_{(lactone)}$OH···O	2.762(8)	2.901(7)	2.902(1)	2.867(7)	3.117(4)
OH···O(H)	–	–	–	–	2.748(1)
OH···O(=C)	–	–	–	–	–
CH···O(=C)	3.010(3)	3.031(5)	3.055(8)	3.002(0)	3.036(6)
	α-CD-Luot-A-(1)	α-CD-Luot-A-(2)	α-CD-Luot-A-(3)	α-CD-Luot-A-(4)	α-CD-Luot-A-(5)
$_{(lactone)}$OH···O	–	–	–	–	–
OH···O(H)	–	–	–	–	3.270(5)
OH···O(=C)	–	–	–	–	–
CH···O(=C)	3.057(6)	3.072(5)	3.074(4)	3.075(0)	3.012(2), 3.055(7)
	β-CD-14-Aza-CPT-(1)	β-CD-14-Aza-CPT-(2)	β-CD-14-Aza-CPT-(3)	β-CD-14-Aza-CPT-(4)	β-CD-14-Aza-CPT-(5)
$_{(lactone)}$OH···O	3.686(2)	3.645(1)	2.939(4)	2.922(7)	2.919(3)
OH···O(H)	3.535(2)	–	–	–	3.038(7), 2.873(2)
OH···O(=C)	2.673(3)	2.651(3)	2.711(4)	2.716(4)	–
CH···O(=C)	–	–	–	–	3.561(7)
	β-CD-Luot-A-(1)	β-CD-Luot-A-(2)	β-CD-Luot-A-(3)	β-CD-Luot-A-(4)	β-CD-Luot-A-(5)
$_{(lactone)}$OH···O	–	–	–	–	–
OH···O(H)	–	–	–	–	2.758(2)
OH···O(=C)	–	–	–	–	3.681(7)
CH···O(=C)	3.447(1)	3.433(3)	3.372(6)	3.244(2)	3.111(7), 3.319(2)
	γ-CD-14-Aza-CPT-(1)	γ-CD-14-Aza-CPT-(2)	γ-CD-14-Aza-CPT-(3)	γ-CD-14-Aza-CPT-(4)	γ-CD-14-Aza-CPT-(5)
$_{(lactone)}$OH···O	2.690(0)	–	2.655(5)	2.557(6)	3.612(4)
OH···O(H)	–	–	–	–	3.293(1), 2.699(3)
OH···O(=C)	2.669(4)	2.679(3)	2.693(2)	2.682(3)	–
CH···O(=C)	–	–	–	–	3.010(9), 3.001(9)
	γ-CD-Luot-A-(1)	γ-CD-Luot-A-(2)	γ-CD-Luot-A-(3)	γ-CD-Luot-A-(4)	γ-CD-Luot-A-(5)
$_{(lactone)}$OH···O	–	–	–	–	–
OH···O(H)	–	–	–	2.871(4)	2.909(2), 2.708(4)
OH···O(=C)	2.692(7)	2.677(3)	2.655(4)	2.688(2)	2.725(4)
CH···O(=C)	–	–	–	–	3.550(2), 3.013(3)

complexes appeared still problematic and represented challenge for the field of molecular crystal growth. Furthermore, for the clinical therapeutic application of the obtained systems and related other ones, developed in the field of the molecular drugs-design for the purpose of the medicinal chemistry, pharmacy and biochemistry, the search of the accurate structural information under physiological conditions, i.e. studies mainly in solution at the physiological pH ranges is crucial for the detail understanding of the biological processes in vivo. For the ESI–MS spectra of the isolated cyclodextrins is typical the observation of the NH_4^+-adducts (Fig. 1). The molecular ion peaks at $[M+H]^+$ was taken for MS/MS studies. The fragmentation scheme, exhibiting the daughter ion peaks is shown in Figs. 1 and 2, respectively. For 14-Aza-CPTs are typical the observation of the peak at *m/z* $[M^++H-44]^+$ which were due to the loss of carbon dioxide moieties from the lactone fragment in the molecules. The mass spectra show as well the peaks for the partial C–N fragmentation of R^1-substituents (Fig. 1). In contrast, spectra of Laut-A-(i), where $i = 1–5$ both of the isolated alkaloids as well as in the molecular complexes, are characterized with the peaks at *m/z* at about 285 and 272, indicating the common fragmentation mode of R^1-substituent. These fragmentation characteristics allow further mass spectrometric study of mixtures of similar alkaloids, both naturally occurred as well as semi- and synthetic derivatives. Their unique character-



Scheme 3 The (O)H···π interactions in the molecular complexes β-CD-Luot-A-(1) and β-CD-Luot-A-(2) at B3LYP/6-31 ++G(2d, 2p) level of theory; The distances are shown in [Å]; Conformers of the β-CD-Luot-A-(1) molecular complexes, labeled as (**a**), close to the local minimum of the free energy show in [kcal/mol], obtained at B3LYP/6-31 ++G(2d, 2p) level of theory; Selected hydrogen bonds [Å]

isation by MALDI-MS method is possible, since the main ions are observed out of the low *m/z* values [53–57], where are found the peaks of the MALDI matrixes (Fig. 2).

Electronic absorption and CD spectroscopic data

Similar to CPT [35–37], the EAs of 14-Aza-CPTs are characterized with the two bands within 320–360 and 365–370 nm assigned to the $\pi \rightarrow \pi^*$ transitions. The presence of the third N-atom in corresponding 14-Aza-derivatives leads only to bathochromic shifting of the CT bands ca. 3–5 nm. Detail analysis of the obtained spectroscopic patterns; reveal the two additional broad maxima at about 330 and 370 nm (Fig. S1). The second band could be associated to the $n \rightarrow \pi^*$ transitions of the non- and conjugated C = O groups. The CD-spectra in methanol:acetonitrile (Fig. S1), show positive Cotton effect of (+3.1)–(+6.3), respectively. The type of R^1-substitient affected weakly on the CD spectra of 14-Aza-CPTs. The Laut-A derivatives are characterized with the CT band within 446–500 nm, a result of the large conjugate molecular skeleton system. In the molecular complexes with β-CD are obtained series of sub-bands within 506–510 nm, which could explain with the theoretically obtained (O)H···π interactions with the A-ring. The complex interaction with the cyclodextrins result to the kept of the configuration of the C-20 chiral centre.

Experimental

Physical methods

HPLC–MS/MS measurements were performed, using TSQ 7000 instrument (Thermo Electron Corporation). Two mobile phase compositions were used: (A) 0.1 % v/v aqueous HCOOH and (B) 0.1 % v/v HCOOH in CH_3CN. Electrospray ionization (ESI) mass spectrometry. A triple quadrupole mass spectrometer (TSQ 7000 Thermo Electron, Dreieich, Germany) equipped with an ESI 2 source was used and operated at the following conditions: capillary temperature 180 °C; sheath gas 60 ψ, corona 4.5 μA and spray voltage 4.5 kV. Sample was dissolved in acetonitrile (1 mg ml^{-1}) and was injected in the ion source by an autosampler (Surveyor) with a flow of pure acetonitrile (0.2 ml min^{-1}). The Excalibur 1.4 software is used. A standard LTQ Orbitrap XL instrument is used for the MALDI-MS measurements, using the UV laser source at 337 nm. An overall mass range of *m/z* 100–2,000 is scanned simultaneously in the Orbitrap analyzer. The ImageQuest 1.0.1 program package is used. The laser energy values are within 11.0–14.5 μJ. The numbers of averaged laser shots lies within 10–110, the MALDI flow rate values are within 26.8–21.5; the acquisition time is within 30.5–118.7 min, the corresponding elapsed scan time range lies within 20.1–3.00 s, respectively. NMR spectroscopy

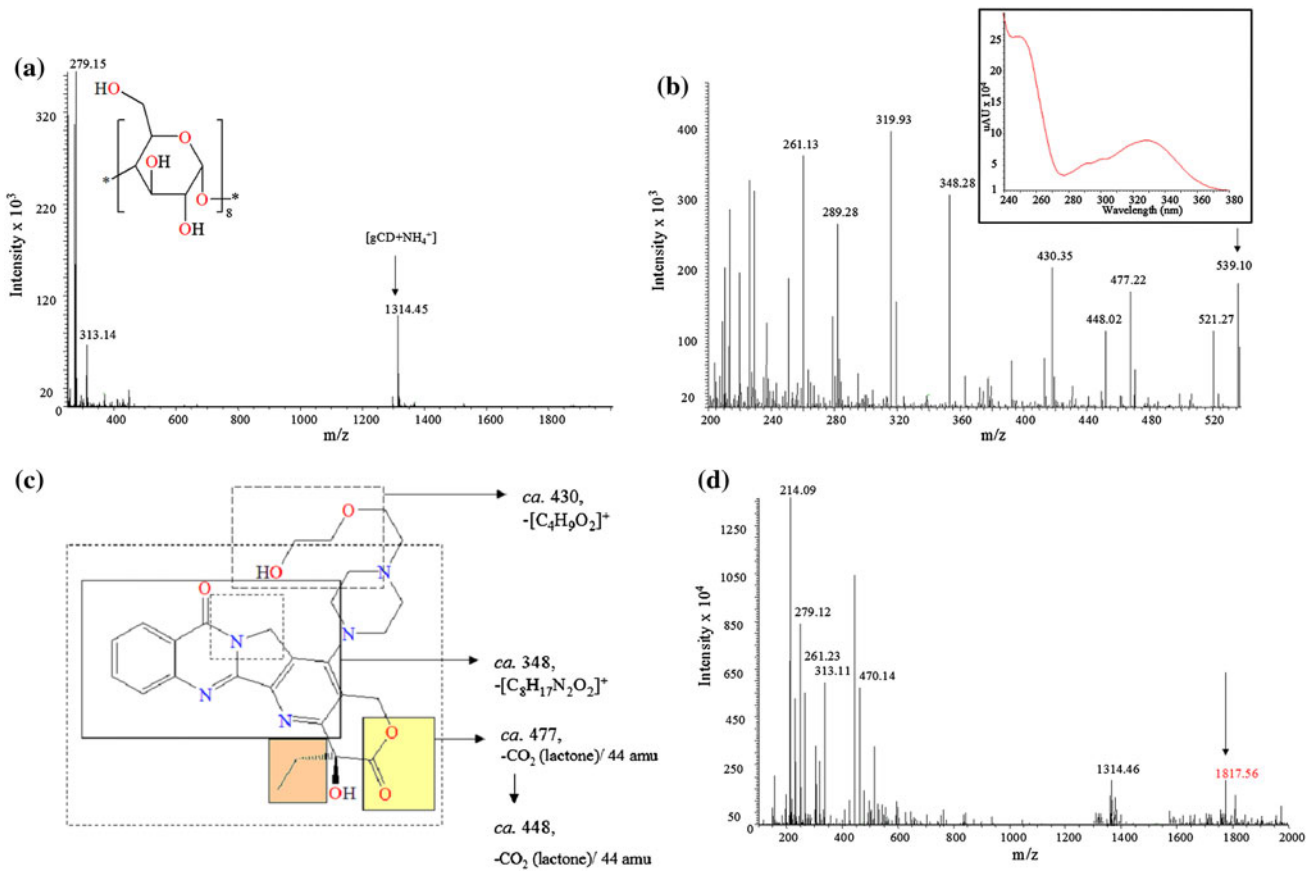


Fig. 1 ESI-MS data and chemical diagrams: γ-CD (**a**); 14-Aza-CPT-(5) and the corresponding electronic transition (**b**) and γ-CD-14-Aza-CPT-(5) (**d**); Fragmentation scheme of 14-Aza-CPT-(5) (**c**)

was performed with a Varian (Palo Alto, CA, USA) Inova 600, a 600 MHz instrument, with anhydrous DMSO-D_6 used as solvent. The NMR spectra were recorded in both one-dimensional (1-D) and two dimensional (2-D) modes. The 1-D mode involved recording ^{1}H and ^{13}C APT spectra. 2-D NMR techniques included correlation spectroscopy (gCOSY), heteronuclear multibond correlation (gHMBC), and heteronuclear single quantum coherence (gHSQC). gCOSY was performed to investigate the coupling of two protons. gHMBC reflects long-range CH correlation over two or three bonds, whereas gHSQC establishes direct CH correlation. Chromatographic purification was performed with a Gynkotek (Germering, Germany) HPLC equipped with a preparative Kromasil 100 C18 column (250 × 20 mm, 7 μm; Eka Chemicals, Bohus, Sweden) and a UV detector set at 250 nm. The mobile phase was acetonitrile:water (90:10, v/v) at a flow rate of 4 ml/min. The analytical HPLC was performed on a Phenomenex (Torrance, CA, USA) RP-18 column (150 × 2 mm, 3 μm) under the same chromatographic conditions as above, except for a flow rate of 0.2 ml/min. The UV–VIS–NIR spectra within 200 and 800 nm, using solvent acetonitrile (Uvasol, Merck product) at a concentration of 2.5×10^{-5} M in 0.921 cm quartz cells were recorded on Tecan Safire Absorbance/Fluorescence XFluor 4 V 4.40 spectrophotometer. The CD spectra were measured on JASCO J-715 polarimeter with 0.5 nm resolution.

Sample preparation for the MALDI-MS measurements

As standard solvent for sample solutions, a mixture of methanol/water (1:1, v/v) was used (UVASOL, Merck). The MALDI measurements in the matrixes in the samples, according the corresponding techniques are compared with the analysis, using the standard dried droplet method [53–57] in 2,5-dihydroxybenzoic acid (DHB). The dried droplet techniques were applied, using the 1 mol/l concentration of the of matrix solution plus and analyte solutions (10^{-4}–10^{-3} M), both dissolved in methanol/water (1:1, v/v) solvent mixtures. The obtained solutions were mixed on the MALDI target and dried by a gentle flow of air. The same analyte solutions were used for ESI–MS measurements, using however the acetonitrile:methanol solvent mixtures 1:1. The polymeric complexes of the cyclodextrins with the alkaloids were analyzed directly, using the

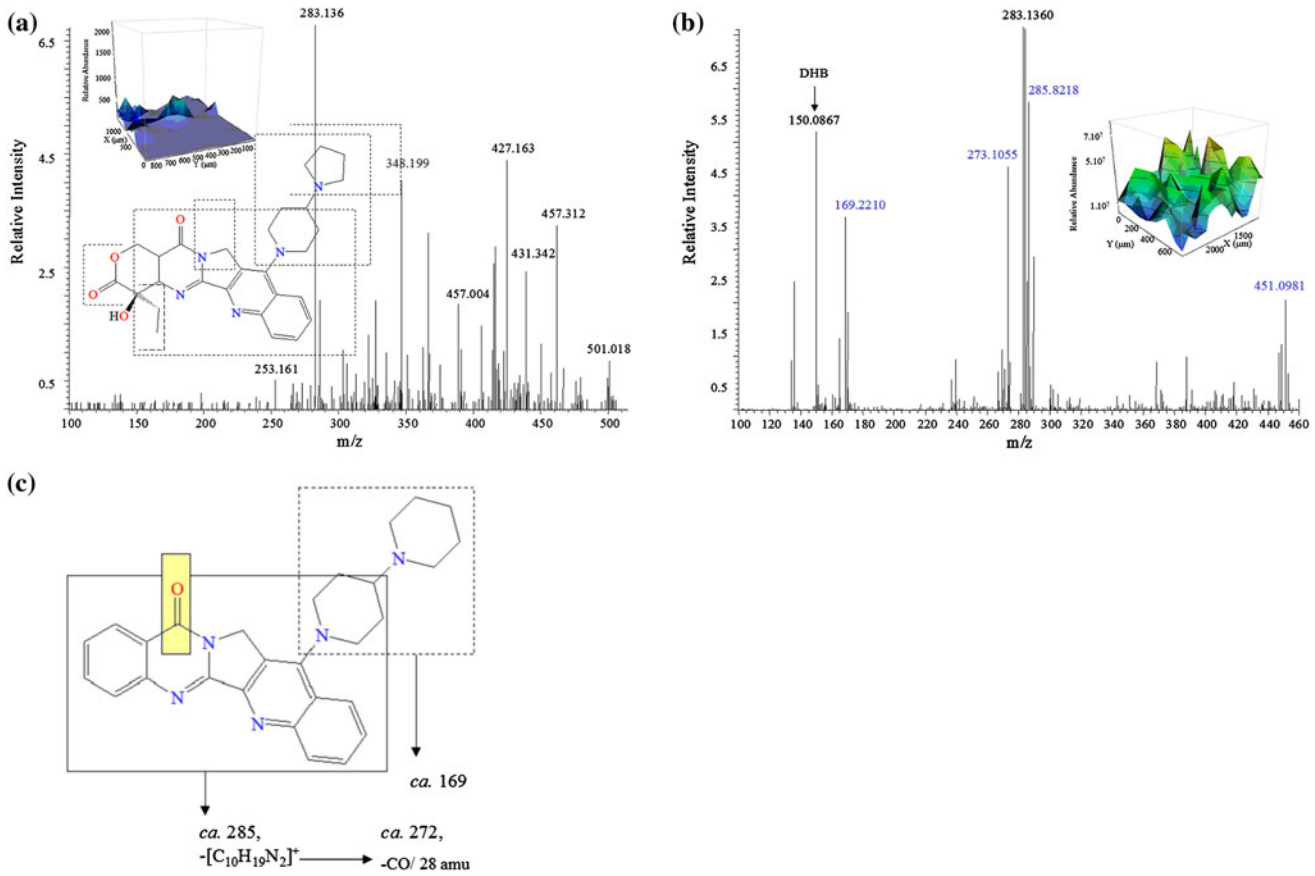


Fig. 2 3D image by logarithm plotting of Δx, Δy versus the signal amplitude (grid) of selected ion range of 14-Aza-CPT-(3) (**a**) and Laut-A-(2) (**b**); Chemical diagram with the fragmentation pathway and MALDI-MS data (**a**, **c**)

spray method for adding the matrix DHB and inner standard.

Theoretical calculations [58–65]

Quantum chemical calculations are performed with GAUSSIAN 09 and Dalton 2.0 program packages. The geometries of the studied species were preoptimized employing B3LYP method, CAM-B3LYP, and M06-2× functionals. Molecular geometries of the studied species were fully optimized by the force gradient method using Bernys' algorithm. For every structure the stationary points found on the molecule potential energy hypersurfaces were characterized using standard analytical harmonic vibrational analysis. The absence of the imaginary frequencies, as well as of negative eigenvalues of the second-derivative matrix, confirmed that the stationary points correspond to minima of the potential energy hypersurfaces. The calculation of vibrational frequencies and infrared intensities were checked to establish which kind of performed calculations agree best with the experimental data. The calculations of the molecular vibrations are utilized by the 6-31 + G(d,p) and large "correlation consistent" basis sets aug-cc-pVDZ and aug-cc-pVTZ (augmented correlation-consistent polarized valence double and triple zeta levels). The obtained vibrational characteristics, by the preliminary optimization of the molecular geometry are correlated to those, obtained by the crystallographic inputs according the method described in. The UV–VIS spectra are calculated, using TDDFT method as above levels and PCM (respectively IPCM) approach. To describe the species in aqueous solution we used both an explicit super molecule and micro hydration approach, in which several water molecules are coordinated to the solute at the optimized geometry of the super molecule, and a polarizable continuum approach. The geometries of all the super molecules in the present study were obtained by a similar approach, but utilizing the polarized SBK basis set at the MP2 level. The geometries have been verified to be local energy minima by frequency analysis, but are not necessarily the global minima. These species are in fact only simple approximations to the real hydrated species. We have not systematically studied the effect of varying the number of water molecules in the super molecule, although we have employed a very large super molecule.

Generally better results are obtained for solutes in aqueous environments if the solute is immersed in a

polarizable continuum. For the most recent application of such an approach to spectral properties. It is also possible to use a "mixed" approach, employing both microhydration and a polarizable continuum. However, many of the studies done so far with this approach have limited the number of water molecules, usually employing only one or two coordinated to the chromophoric group of the molecule. We have tried to approach the problem in a fairly simple yet systematic manner for the present species, using several waters of hydration in the microhydrated species and then immersing this species in a polarizable continuum. The COSMO or CPCM version of the polarizable continuum model was utilized. The COSMO solvation approach has been applied both to bare anionic solutes and to the microhydrated species. For the alkaloide-cyclodextrin complexes were employed the ONIOM method. Molecular mechanics calculations were performed, using consequently DREIDING and UFF force fields. The nonbonded interactions are evaluated for every possible pair of atoms, including the solvent interactions by ONIOM method applying the solvent mixed approach. The interactions between pairs of atoms, separated by three bonds are scaled down. First, are calculate the interactions between all pairs, without taking the scaling into account, than the data set is subtract out the contributions that should have been scaled.

Statistical and mathematical (Chemometrics) methods [66–75]

The experimental and theoretical spectroscopic patterns were processed by R4Cal Open Office STATISTICs for Windows 7 program package. Baseline corrections and curve-fitting procedures were applied. The statistical significance of each regression coefficient was checked by the use of *t* test. The model fit was determined by *F* test as shown in [66–75].

Synthesis

The synthetic pathways are according the literature procedures for synthesis of the Luot-A and 14-Aza-CPT derivatives [24, 41–52]. For the corresponding derivatives of Luot-A is used the method in [41, 48–51].

*Ethyl-4-hydroxy-11-morpholin-4-yl-1,4a,12,13a-tetrahydro-4H-2-oxa-5,6,12a-triaza-dibenzo [b,h] fluorene-3,13-dione (14-Aza-CPT-(**1**))*

Found, C, 63.19; H, 5.12; Calcd. [$C_{23}H_{22}N_4O_5$], C, 63.59; H, 5.10 %; ^{1}H NMR: (600 MHz, $CDCl_3$) δ, 1.00 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.42 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.56 (m, 6H), 1.6 (broar, s, 1H, OH), 2.57 (m, 4H, N–$(CH_2)_2$), 3.13 (q, 2H, $J = 1.4$ Hz, $(CH_2CH_3)_{py}$), 3.62 (s, 2H, CH_2CH_3), 3.62 (broar, s, 2H, N–CH_2), 3.80 (broad, s, 2H, N–CH_2), 5.20 (dd, 2H, $J = 2.3$ and 4.6 Hz, CH2), 5.40 (d, 1H, $J = 4.6$ Hz, CH), 5.67 (d, 1H, $J = 4.6$ Hz, CH), 7.6 (dd, 1H, $J = 2.3$ and 0.7 Hz, ph-H), 7.85 (d, 1H, $J = 0.7$ Hz, ph-H), 8.20 (d, 1H, $J = 2.3$ Hz, ph-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 4.5 (C26), 10.3 (C19), 20.2 (C24), 24.3 (C25), 30.2 (C18), 32.1 (C23), 50.2 (C5), 66.9 (C17), 75.55 (C20), 96.9, 121.1, 121.6, 125.9, 127.5, 128.5, 131.5, 136.7, 145.0, 144.0, 146.2, 148.0, 153.1, 157.2, 169.1, 176.8; MS (ESI) $[M+H]^+$ 436.02.

*1-[1,4′]Bipiperidinyl-1′-yl-4-ethyl-4-hydroxy-1,4a,12,13a-tetrahydro-4H-2-oxa-5,6,12a-triaza-dibenzo[b,h]fluorene-3,13-dione (14-Aza-CPT-(**2**))*

Found, C, 67.13; H, 6.16; Calcd. [$C_{29}H_{33}N_5O_4$], C, 67.55; H, 6.45 %; ^{1}H NMR: (600 MHz, $CDCl_3$) δ, 1.01 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.41 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.61 (m, 6H), 1.7 (broar, s, 1H, OH), 2.56 (m, 4H, N–$(CH_2)_2$), 3.12 (q, 2H, $J = 1.4$ Hz, $(CH_2CH_3)_{py}$), 3.61 (s, 2H, CH_2CH_3), 3.60 (broar, s, 2H, N–CH_2), 3.82 (broad, s, 2H, N–CH_2), 5.22 (dd, 2H, $J = 2.1$ and 4.5 Hz, CH2), 5.41 (d, 1H, $J = 4.6$ Hz, CH), 5.66 (d, 1H, $J = 4.6$ Hz, CH), 7.6 (dd, 1H, $J = 2.3$ and 0.7 Hz, ph-H), 7.85 (d, 1H, $J = 0.7$ Hz, ph-H), 8.20 (d, 1H, $J = 2.3$ Hz, ph-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 4.5 (C26), 10.3 (C19), 20.2 (C24), 24.3 (C25), 30.0 (C18), 32.0 (C23), 50.2 (C5), 66.9 (C17), 75.6 (C20), 96.9, 121.1, 121.6, 125.9, 127.5, 128.5, 131.5, 136.7, 145.0, 144.0, 146.2, 148.0, 153.1, 157.2, 169.0, 176.8; MS (ESI) $[M+H]^+$ 517.11.

*4-Ethyl-4-hydroxy-11-(4-morpholin-4-yl-piperidin-1-yl)-1,4a,12,13a-tetrahydro-4H-2-oxa-5,6,12a-triaza-dibenzo[b,h]fluorene-3,13-dione (14-Aza-CPT-(**3**))*

Found, C, 64.90; H, 6.10; Calcd. [$C_{28}H_{31}N_5O_5$], C, 64.98; H, 6.04 %; ^{1}H NMR: (600 MHz, $CDCl_3$) δ, 1.00 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.41 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.56 (m, 6H), 1.6 (broar, s, 1H, OH), 2.55 (m, 4H, N–$(CH_2)_2$), 3.13 (q, 2H, $J = 1.4$ Hz, $(CH_2CH_3)_{py}$), 3.62 (s, 2H, CH_2CH_3), 3.62 (broar, s, 2H, N–CH_2), 3.80 (broad, s, 2H, N–CH_2), 5.20 (dd, 2H, $J = 2.2$ and 4.6 Hz, CH2), 5.40 (d, 1H, $J = 4.6$ Hz, CH), 5.67 (d, 1H, $J = 4.6$ Hz, CH), 7.6 (dd, 1H, $J = 2.3$ and 0.7 Hz, ph-H), 7.85 (d, 1H, $J = 0.7$ Hz, ph-H), 8.20 (d, 1H, $J = 2.3$ Hz, ph-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 4.5 (C26), 10.3 (C19), 20.2 (C24), 24.3 (C25), 30.2 (C18), 32.2 (C23), 50.2 (C5), 66.9 (C17), 75.5 (C20), 96.9, 121.6, 121.6, 125.9, 127.5, 128.5, 131.5, 136.7, 145.0, 144.1, 146.2, 148.0, 153.1, 157.1, 169.1, 176.8; MS (ESI) $[M+H]^+$ 518.06.

*4-Ethyl-4-hydroxy-11-(4-pyrrolidin-1-yl-piperidin-1-yl)-1,4a,12,13a-tetrahydro-4H-2-oxa-5,6,12a-triaza-dibenzo[b,h]fluorene-3,13-dione (14-Aza-CPT-(**4**))*

Found, C, 67.52; H, 6.25; Calcd. [$C_{28}H_{31}N_5O_4$], C, 67.05; H, 6.23 %; MS (ESI) $[M+H]^+$ 503.24; ^{1}H NMR: (600 MHz, $CDCl_3$) δ, 1.00 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.39 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.54 (m, 6H), 1.2 (broar, s, 1H, OH), 2.02 (m, 4H, N–$(CH_2)_2$), 3.00 (q, 2H, $J = 1.2$ Hz, $(CH_2CH_3)_{py}$), 3.65 (s, 2H, CH_2CH_3), 3.66 (broar, s, 2H, N–CH_2), 3.81 (broad, s, 2H, N–CH_2), 5.10 (dd, 2H, $J = 2.2$ and 4.6 Hz, CH2), 5.41 (d, 1H, $J = 4.5$ Hz, CH), 5.79 (d, 1H, $J = 4.6$ Hz, CH), 7.55 (dd, 1H, $J = 2.3$ and 0.7 Hz, ph-H), 7.85 (d, 1H, $J = 0.8$ Hz, ph-H), 8.22 (d, 1H, $J = 2.3$ Hz, ph-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 4.2 (C26), 10.3 (C19), 20.1 (C24), 24.2 (C25), 30.5 (C18), 32.0 (C23), 50.2 (C5), 66.9 (C17), 75.5 (C20), 96.9, 121.1, 121.6, 125.9, 127.5, 128.5, 131.5, 136.6, 145.0, 144.0, 146.2, 148.0, 153.1, 157.2, 169.1, 176.8.

*4-Ethyl-4-hydroxy-11-{4-[2-(2-hydroxy-ethoxy)-ethyl]-piperazin-1-yl}-1,4a,12,13a-tetrahydro-4H-2-oxa-5,6,12a-triaza-dibenzo [b,h] fluorene-3,13-dione (14-Aza-CPT-(**5**))*

Found, C, 62.27; H, 5.49; Calcd. [$C_{27}H_{30}N_5O_6$], C, 62.30; H, 5.81 %; ^{1}H NMR: (600 MHz, $CDCl_3$) δ, 1.01 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.41 (t, 3H, $J = 1.4$ Hz, CH_2CH_3), 1.56 (m, 6H), 1.5 (broar, s, 1H, OH), 2.55 (m, 4H, N–$(CH_2)_2$), 3.11 (q, 2H, $J = 1.2$ Hz, $(CH_2CH_3)_{py}$), 3.61 (s, 2H, CH_2CH_3), 3.62 (broar, s, 2H, N–CH_2), 3.80 (broad, s, 2H, N–CH_2), 5.20 (dd, 2H, $J = 2.2$ and 4.6 Hz, CH2), 5.41 (d, 1H, $J = 4.6$ Hz, CH), 5.66 (d, 1H, $J = 4.6$ Hz, CH), 7.6 (dd, 1H, $J = 2.3$ and 0.7 Hz, ph-H), 7.85 (d, 1H, $J = 0.8$ Hz, ph-H), 8.22 (d, 1H, $J = 2.3$ Hz, ph-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 4.2 (C26), 10.3 (C19), 20.2 (C24), 24.3 (C25), 30.2 (C18), 32.1 (C23), 50.2 (C5), 66.9 (C17), 75.5 (C20), 96.9, 121.1, 121.6, 125.9, 127.5, 128.5, 131.5, 136.7, 145.0, 144.0, 146.2, 148.0, 153.1, 157.2, 169.1, 176.8; MS (ESI) $[M+H]^+$ 521.02.

*13-Morpholin-4-yl-12H-5,6,11a-triaza-dibenzo[b,h]fluoren-11-one (Luot-A-(**1**))*

Found, C, 71.13; H, 5.06; Calcd. [$C_{22}H_{18}N_4O_2$], C, 71.34; H, 4.90 %; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.41 (d, 1H, $J = 8.4$ Hz, H1), 8.34 (d, 1H, $J = 8.2$ Hz, H4), 8.10 (d, 1H, $J = 8.3$ Hz, H7), 8.10 (d, 1H, $J = 8.3$ Hz, H10), 7.81 (td, 1H, $J = 8.3$, 0.8 Hz, H2), 7.79 (td, 1H, $J = 8.2$, 1.1 Hz, H3), 7.65 (td, 1H, $J = 8.0$, 0.8 Hz, H8), 7.54 (t, 1H, $J = 8.0$ Hz, H9), 5.32 (s, 2H), 2.85 (d, 4H), 3.32 (d, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.5, 153.1, 150.0, 149.2, 140.5, 134.5, 131.3, 130.3, 128.6, 128.7, 128.2, 127.4, 126.4, 123.5, 121.2, 47.0, 15.1, 15.5, 17.2, and 17.1; MS (ESI) $[M+H]^+$ 371.21.

*13-[1,4′]Bipiperidinyl-1′-yl-12H-5,6,11a-triaza-dibenzo[b,h]fluoren-11-one (Luot-A-(**2**))*

Found, C, 74.92; H, 6.80; Calcd. [$C_{28}H_{29}N_5O$], C, 74.48; H, 6.47 %; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.40 (d, 1H, $J = 8.4$ Hz, H1), 8.33 (d, 1H, $J = 8.2$ Hz, H4), 8.11 (d, 1H, $J = 8.3$ Hz, H7), 8.08 (d, 1H, $J = 8.3$ Hz, H10), 7.82 (td, 1H, $J = 8.3$, 0.8 Hz, H2), 7.80 (td, 1H, $J = 8.2$, 1.1 Hz, H3), 7.67 (td, 1H, $J = 8.0$, 0.8 Hz, H8), 7.54 (t, 1H, $J = 8.0$ Hz, H9), 5.33 (s, 2H), 2.85 (d, 4H), 3.31 (d, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.4, 153.1, 150.0, 149.2, 140.5, 134.5, 131.3, 130.3, 128.6, 128.7, 128.2, 127.4, 126.4, 123.5, 121.1, 46.9, 27.8, 15.1, 15.4, 17.2, 17.0, and 13.6; MS (ESI) $[M+H]^+$ 452.18.

*13-(4-Morpholin-4-yl-piperidin-1-yl)-12H-5,6,11a-triaza-dibenzo[b,h]fluoren-11-one (Luot-A-(**3**))*

Found, C, 71.33; H, 6.10; Calcd. [$C_{27}H_{27}N_5O_2$], C, 71.50; H, 6.00 %; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.42 (d, 1H, $J = 8.4$ Hz, H1), 8.35 (d, 1H, $J = 8.3$ Hz, H4), 8.11 (d, 1H, $J = 8.3$ Hz, H7), 8.08 (d, 1H, $J = 8.3$ Hz, H10), 7.82 (td, 1H, $J = 8.2$, 0.9 Hz, H2), 7.80 (td, 1H, $J = 8.2$, 1.1 Hz, H3), 7.67 (td, 1H, $J = 8.0$, 1.5 Hz, H8), 7.55 (t, 1H, $J = 8.0$ Hz, H9), 5.55 (m, 4H), 2.90 (m, 4H), 3.33 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.5, 153.1, 150.1, 149.1, 140.4, 134.5, 131.2, 130.3, 128.6, 128.7, 128.2, 127.4, 126.4, 123.5, 121.2, 47.0, 27.5, 24.5, 24.4, 22.9, 22.5, 15.1, 15.5, 17.2, and 17.1; MS (ESI) $[M+H]^+$ 454.37.

*13-(4-Pyrrolidin-1-yl-piperidin-1-yl)-12H-5,6,11a-triaza-dibenzo[b,h]fluoren-11-one (Luot-A-(**4**))*

Found, C, 74.40; H, 6.50; Calcd. [$C_{27}H_{27}N_5$], C, 74.12; H, 6.22 %; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.40 (d, 1H, $J = 8.3$ Hz, H1), 8.35 (d, 1H, $J = 8.1$ Hz, H4), 8.09 (d, 1H, $J = 8.3$ Hz, H7), 8.11 (d, 1H, $J = 8.4$ Hz, H10), 7.88 (td, 1H, $J = 8.3$, 0.8 Hz, H2), 7.80 (td, 1H, $J = 8.2$, 1.1 Hz, H3), 7.62 (td, 1H, $J = 8.0$, 0.9 Hz, H8), 7.55 (t, 1H, $J = 8.0$ Hz, H9), 5.33 (s, 2H), 2.86 (d, 4H), 3.33 (d, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.5, 153.3, 150.1, 149.1, 140.3, 134.2, 131.1, 130.2, 128.5, 128.5, 128.1, 127.4, 126.4, 123.5, 121.2, 47.0, 29.3, 15.1, 15.5, 17.2, 17.1, 13.8, 13.6, 13.2, and 13.4; MS (ESI) $[M+H]^+$ 437.91.

*13-{4-[2-(2-Hydroxy-ethoxy)-ethyl]-piperazin-1-yl}-12H-5,6,11a-triaza-dibenzo[b,h]fluoren-11-one (Luot-A-(**5**))*

Found, C, 68.94; H, 5.91; Calcd. [$C_{26}H_{26}N_5O$], C, 68.41; H, 5.74 %, ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.40 (d, 1H,

$J = 8.3$ Hz, H1), 8.35 (d, 1H, $J = 8.1$ Hz, H4), 8.11 (d, 1H, $J = 8.2$ Hz, H7), 8.10 (d, 1H, $J = 8.3$ Hz, H10), 7.81 (td, 1H, $J = 8.3$, 0.8 Hz, H2), 7.79 (td, 1H, $J = 8.2$, 1.1 Hz, H3), 7.65 (td, 1H, $J = 8.0$, 0.8 Hz, H8), 7.54 (t, 1H, $J = 8.0$ Hz, H9), 5.32 (s, 2H), 4.21 (m, 4H), 3.89 (m, 4H), and 3.68 (m, 8H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.4, 153.1, 150.1, 149.2, 140.5, 134.5, 131.3, 130.3, 128.6, 128.7, 128.2, 127.4, 126.1, 123.5, 121.1, 47.0, 15.1, 15.5, 17.2, 17.9, and 17.1; MS (ESI) $[M+H]^+$ 456.34.

Conclusions

The objects of the presented study are ten novel alkaloids of the pyrrolo-quinazolino-quinoline type, containing Luotonin-A and 14-aza-CPT as molecular skeleton templates as well as their inclusion complexes in the native α-, β- and γ-cyclodextrins. The design and synthesis of the obtained alkaloids include the tuning of the molecular architecture by the N-containing aliphatic cyclic piperazine, pyrrolidine and morpholie derivatives at the B-ring. The physical properties of the isolated compounds and corresponding molecular complexes are elucidated experimentally by the electronic absorption and CD-spectroscopy, electrospray ionization and matrix-assisted laser desorption/ionization mass spectrometry and nuclear magnetic resonance method in condense phase as well as separative methods and techniques. The experimental data are supported by the theoretical quantum chemical calculations of the molecular and electronic structures as well as physical properties in condense phase. The results presented in this study highlighted the interactions between novel derivatives and cyclodextrins elucidated experimentally by the mass spectrometry allow us to determine the 1:1 stoichiometry ratio of the molecular complexes. Moreover even in the cases of the alkaloids-γ-cyclodextrin complexes, where the large cavity of the hydrocarbon allow to obtain theoretically as well as experimentally more than one inclusion complex, including the surface interactions, the MALDI mass spectrometric data in solid-state unambiguously define that the alkaloids are embedded in the cyclodextrin cavity, thus stabilizing the whole molecular system by the moderate to weak non-covalent interactions of OH···O type (2.78–3.38 Å). In 14-Aza-CPT derivatives, the lactone OH-group takes part in the intermolecular OH···O interactions with the cyclodextrins. Lautonin-A derivatives are found non-classical hydrogen bonding (O)H···π with the π-system of the A-ring of the molecules in corresponding complexes with β-cyclodextrins, thus explaining the anomalous bathochromic shifting of the CT band in the alkaloids up to 510 nm, respectively.

Supporting information

Structural parameters of the synthesised CPTs 14-Aza-(i) and Luot-A-(i) ($i = 1$–5), and the molecular complexes xCD-14-Aza-(i) and xCD-Luot-A-(i) (x = α, β or γ; i = 1–5) (Table S1); The free Gibbs energies (ΔG) [kcal/mol] for the studied molecular complexes (Table S2); Electronic absorption spectra (Figure S1); Most stable conformers of the xCD-14-Aza-(i) and xCD-Luot-A-(i) (x = a, b or g; $i = 1$–5) molecular complexes, obtained at B3LYP/6-31 ++G(2d, 2p) level of theory(Scheme S1).

Acknowledgments The authors thank the Deutscher Akademischer Austausch Dienst (DAAD), the Deutsche Forschungsgemeinschaft (DFG), the central instrumental laboratories for structural analysis at University of Dortmund (Germany) and the analytical and computational laboratories at the Institute of Environmental Research (INFU) at same University.

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