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Solanocapsine derivatives as potential inhibitors of acetylcholinesterase: Synthesis, molecular docking and biological studies

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ABSTRACT

The investigation of natural products in medicinal chemistry is essential today. In this context, acetylcholinesterase (AChE) inhibitors comprise one type of the compounds most actively studied in the search for an effective treatment of symptoms of Alzheimer's disease. This work describes the isolation of a natural compound, solanocapsine, the preparation of its chemical derivatives, the evaluation of AChE inhibitory activity, and the structure–activity analysis of relevant cases. The influence of structural variations on the inhibitory potency was carefully investigated by modifying different reactive parts of the parent molecule. A theoretical study was also carried out into the binding mode of representative compounds to the enzyme through molecular modeling. The biological properties of the series were investigated. Through this study valuable information was obtained of steroidal alkaloid-type compounds as a starting point for the synthesis of AChE inhibitors.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that affects more than 36 million people worldwide. This disease is characterized by progressive dysfunction of memory and higher cognitive functions [1]. Currently there is a very small number of approved drugs for treating the disease, four of them (tacrine, donepezil, rivastigmine and galantamine) are acetylcholinesterase inhibitors (AChEIs). However, they are only effective for a few months and for half of the patients with milder forms of AD offering symptomatic relief but do not alter the course or the outcome of the disease [2]. Hence, research in this field focuses on drugs for delaying the disease progression or providing prophylaxis. Even though the understanding of the complete pathogenic mechanism of AD still remains unknown, some targets and theories have been proposed. Through the study of crystallographic structure of AChE, it was revealed that this enzyme has a catalytic active site (CAS) at the bottom of a deep, narrow catalytic gorge and a peripheral anionic site (PAS) at the entrance of this gorge [3]. The AChEIs activity produce an increase in the concentration of acetylcholine that finally improves cholinergic transmission. However, achieving strong inhibitors that interact with CAS of AChE does not represent a significant enhancement, unless concomitant interaction with PAS. In this point it is important to emphasize that there are numerous reports about the identification and mechanistic elucidation of the pro-aggregating action of AChE on β-amyloid peptide [4] (a neurotoxic cascade characteristic of AD is associated with the formation of plaques or aggregates of β-amyloid). AChEIs able to interact with PAS have been found to inhibit this pro-aggregating activity. According to this hypothesis, simultaneous interaction with both the CAS and PAS of AChE has been suggested to be important in designing powerful and selective AChEIs. Because of this, in recent reports new AChEIs including two components separated by a spacer group with a suitable length were synthesized, achieving interaction throughout all the active site [5–7]. These compounds are commonly referred to as dual binding site inhibitors.

In the investigation for new AChEIs we should possibly consider research on naturally occurring compounds: nature is a rich source of biological and chemical diversity [8]. The unique and complex structures of natural products cannot be obtained easily by chemical synthesis [9]. Since most AChEIs are known to contain nitrogen, more research is needed to further explore the actions of these alkaloids in the search of a promising treatment for AD [10,11]. Besides, while a large number of very potent inhibitors have been developed in the past decades, there are very few

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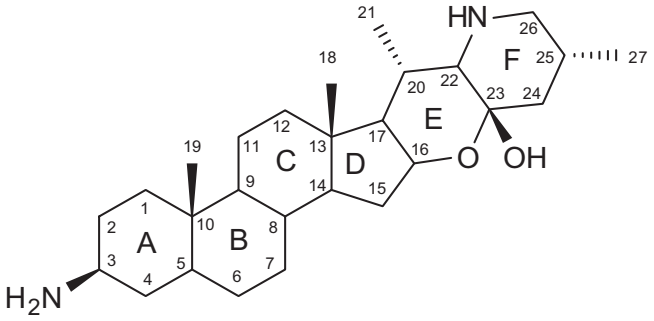

Fig. 1. Structure of solanocapsine: numeration of skeleton positions and ring nomenclature.

reports of steroid-related compounds among them [12,13]. Within this context, we have focused our attention on solanocapsine, a major steroidal alkaloid isolated from *Solanum pseudocapsicum* L. (Fig. 1) which has shown an interesting inhibition of the enzyme AChE with an IC_{50} of 3.22 μM, determined by the Ellman's method [14], according to measurements performed in our laboratory. It has also been recently reported that related steroidal alkaloid-type compounds show AChEI activities [15]. For solanocapsine, molecular docking preliminary studies performed by our research group, located solanocapsine filling most of the enzyme's cavity, interacting with residues of both parts of the active site. In this case, solanocapsine itself may function as a dual inhibitor. However, modifications in its reactive functional groups (primary and secondary amines and hemiketal) or introduction of a new substituent, could greatly improve the AChE inhibitory activity.

Based on the above hypothesis in the first part, inherent solanocapsine dual inhibitor capacity was evaluated. In a second part, with the aim to maximize interactions with the enzyme, synthesis of hybrid molecules was performed. Hybridization and dimerization strategies of scaffold molecule have proven to be successful strategies in the discovery of novel AChEIs [16]. These inhibitors improved pharmacological profiles due their interactions with both the CAS and PAS of enzyme or by other strategies as simultaneously interact with AChE and another biological target related with AD. Thus, a series of new solanocapsine derivatives was designed and synthesized, and their inhibitory activities were tested against AChE. Finally, for all derivatives, structure–activity relationships (SARs) were also discussed. Indeed, molecular docking simulations of solanocapsine and its most active derivative with *Torpedo californica* acetylcholinesterase (*Tc*AChE) were carried-out.

2. Results and discussion

2.1. Chemistry

2.1.1. Natural Products

The aerial parts of *S. pseudocapsicum* L. (444 g) were air-dried and extracted with ethanol (3 × 500 mL). Fresh fruits of this species (1800 g) were extracted exhaustively with methanol (3 L). After concentration and different acid-base extractions of both extracts, a purified alkaloidal fraction was obtained. This fraction consisted solely by solanocapsine (0.94 g), a steroidal alkaloid previously described in this species (Fig. 1) [17,18].

2.1.2. Derivatization of solanocapsine

As discussed above and since a considerable amount of solanocapsine was isolated from *S. pseudocapsicum*, the steroidal alkaloid was proposed as the target compound to be derivatized. Although all reactive groups of the molecule (amines, hemiketal and OH-23) were subjected to modification reactions, the intrinsic reactivity of the compound and the amount of starting material mass available, limited the variability of obtained products. Thus, seven types of structural modifications were investigated in order to improve the anti-AChE activity of solanocapsine, the main modifications being the formation of imine, amide and *N*-substituted derivatives. Preparation of a series of chemical analogues was accomplished using the general methods outlined in Scheme 1. As suggested by our preliminary studies of molecular docking, the primary amino group is a key element in the interactions with the enzyme. Thus, a set of imine derivatives was prepared from aldehydes with the aim of assessing, effectively, if a primary amine group is involved in the receptor interactions. Another important point to assess is whether the change in hybridization of primary amine or if interactions change according to the electronic nature of the substituent on the aromatic ring, which may render the aryl moiety electron rich ("donor") or electron-poor ("acceptor") (compounds **1**–**6**, Scheme 1, pathway A) are important.

In order to determine whether the electron pair of the nitrogen atom is involved in the interaction with the receptor, a series of amide derivatives were prepared. Compounds **7**–**14** were obtained by acylation with several carboxylic acids using phosphorus oxychloride as a coupling reagent [19] and acyl chlorides [20] (Scheme 1, pathway B). Some chemical modifications were introduced into both nitrogen atoms of the molecule as in compounds **7** and **8**.

On the other hand, substitution reactions by different alkyl halides were performed on the amine groups in order to introduce different stereoelectronic groups, lipophilic characteristics and to evaluate the steric hindrance effects on the inhibitory activity (compounds **15**–**18**, Scheme 1, pathway C).

The presence of a double bond or a more flexible skeletal confers differential rigidity to the structure of a substance, as well as potential van der Waals interactions with the receptor. Besides, in order to advance a complex steroidal alkaloid to a preclinical development phase, hemiketal or lactol functionality often needs to be reduced to the corresponding cyclic ether affording additional chemical and metabolic stability required for in vivo assays [21]. Thus, compounds **19** and **20** were synthesized [22] (Scheme 1, pathway D).

The set of derivatives shown in Scheme 1, pathways E, F and G were prepared in order to introduce the maximum of structural diversity into the final hybrid compounds. As we mentioned previously, this approach can also be used to optimize certain biological properties like affinity and selectivity [23]. Due to the high potential of natural products to exhibit pronounced biological activities, two known bioactive alkaloids reported as able to interact with the enzyme, such as ecgonine [24] and quinidine [25] were selected as a component in hybrid molecules (compounds **21** and **22**). Next, compounds **23** and **24** were synthesized to evaluate whether they were able to maximize their interactions as dual binding site AChEIs. For that purpose, solanocapsine was connected to tacrine or a related unit to improve its interactions with the active site, either at the mid-gorge or at the peripheral site with an appropriate linker [26]. Previous to the synthesis of compound **24**, molecular docking studies were carried out to assess the optimal length for the linker (see Section 2.3).

Finally, due to the fact that rhodamine-based dyes have applications as molecular probes in the study of complex biological systems, we proposed the synthesis of a fluorophore-solanocapsine derivative [27], obtaining a double *N*-functionalized analogue (compound **25**, Scheme 1, pathway G).

The structures of all these new derivatives were confirmed on the basis of their spectroscopic data, as provided in the experimental section.

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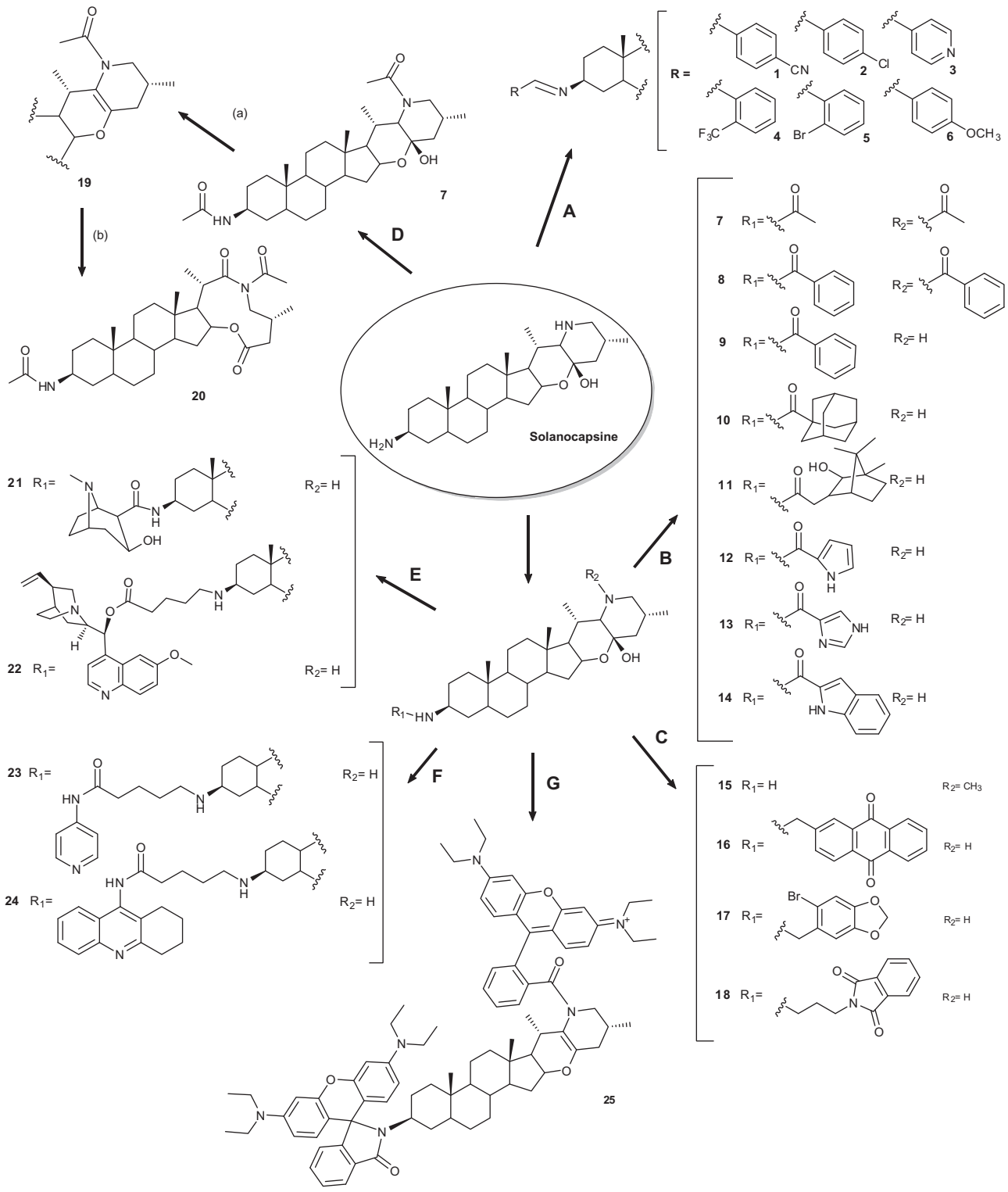

Scheme 1. Synthesis of solanocapsine derivatives. Reagents and conditions: (A) **1**–**6**: RCOH (1 eq.), Na_2SO_4, CH_2Cl_2, RT. (B) **7**: CH_3COCl (2 eq.), Et_3N (5 eq.), CH_2Cl_2, RT; **8**–**9**: BzCOCl (1.5 eq.), $NaHCO_3$, H_2O–CH_2Cl_2, (1:1), RT; **10**–**14**: RCOOH (0.9 eq.), Et_3N (2 eq.), DMAP (0.25 eq.), $POCl_3$ (0.9 eq.), CH_2Cl_2, RT. (C) **15**–**18**: RX (1–2 eq.), base (K_2CO_3 or Et_3N, 1–2 eq.), DMF, RT to 80 °C. (D) **19** (a) CH_3COOH, reflux; **20**: (b) CH_3COOH (50 eq.), CrO_3 (5 eq.), RT. (E) **21**: RCOOH (1.2 eq.), DCC (1 eq, 0 °C), DMAP (0.2 eq.), CH_2Cl_2, RT. **22**: $ROCO(CH_2)_2Br$ (1 eq.), *t*-BuOK (1 eq.), DMF, RT. (F) **23**: $RNHCO(CH_2)_4Br$ (1 eq.), *t*-BuOK (1 eq.), DMF, 60 °C. **24**: $RNHCO(CH_2)_4Br$ (1 eq.), KI (1 eq.), *t*-BuOK (1 eq.), DMF, RT. (G) **25**: (i) rhodamine base (2 eq.), $POCl_3$ (1.3 eq.), $CHCl_3$, reflux.

2.2. Biological activity and structure–activity relationship

2.2.1. Acetylcholinesterase inhibitory activity

To determine the therapeutic potential of the new series of solanocapsine analogues, the inhibitory activities of synthetic derivatives (**1**–**25**) were evaluated against AChE using the method of Ellman et al. [14]. For comparison purposes, tacrine was used as a reference inhibitor. Table 1 summarizes IC_{50} values for the inhibition of AChE.

Viewed as a whole, these results allowed us to classify the compounds into four groups: the first group, comprising solanocapsine and derivatives **1**–**6**, **15**, **16**, **23** that induced the significant inhibi-

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Table 1
In vitro AChE inhibition activity of solanocapsine and their derivatives.[a]

Compound	IC_{50} (μM)	Log IC_{50} ± SD
Solanocapsine	**3.22**	0.51 ± 0.05
1	7.15	0.85 ± 0.05
2	5.42	0.74 ± 0.04
3	6.00	0.78 ± 0.04
4	5.15	0.71 ± 0.05
5	3.43	0.54 ± 0.05
6	8.10	0.91 ± 0.04
7	>100	–
8	>100	–
9	>100	–
10	58.90	1.77 ± 0.09
11	>100	–
12	>100	–
13	16.18	1.21 ± 0.05
14	33.89	1.53 ± 0.16
15	1.56	0.19 ± 0.06
16	7.70	–
17	17.50	1.24 ± 0.14
18	20.03	1.30 ± 0.10
19	>100	–
20	>100	–
21	>100	–
22	14.04	1.15 ± 0.04
23	7.47	0.87 ± 0.05
24	0.090	−1.04 ± 0.02
25	29.24	1.47 ± 0.11
Tacrine	0.029	−1.53 ± 0.06

[a] Results are expressed as IC_{50} values (μM). Log IC_{50} ± SD are also given. Each value is the mean of three replications.

tion of AChE with IC_{50} values below 9 μM; the second group of compounds, including **10**, **13**, **14**, **17**, **18**, **22** and **25**, that exhibited moderate activity (IC_{50} = 14.04–58.9 μM); the third group, compounds **7**–**9**, **11**, **12** and **19**–**21**, which failed to show inhibition (IC_{50} > 100 μM); and finally, the fourth group (compound **24**) with an inhibitory activity in the nanomolar range.

The influence of the substitution pattern on the activity of the solanocapsine derivatives tested in this work was examined. In general terms, we observed that introduction of a lipophilic group into the primary amine through benzyl imine derivatives leads to a slight decrease in inhibitory potency. On the other hand, an effect of the aromatic ring substituent (halogen or donor and acceptor group) on the activities is not clearly observed.

Substitution of the primary and secondary amines by amidation led to compounds (**7**–**8**) showing a depletion of activity, indicating that at least one amine must be free and able to be protonated at physiological pH for inhibition.

In relation to mono-amide analogues, the activity depends on the nature of the acyl substituent. (a) Inspired in the liphophilicity of memantine, usually administrated in combined therapy with AChEIs, we introduced an adamantyl residue into the primary amine position. This bulky and non-polar group gave a derivative (**10**) less potent than the lead compound solanocapsine [28]. (b) The introduction of oxygenated and/or nitrogenated aliphatic bicyclic rings resulted in an activity loss (**11** and **21**). (c) A series of aromatic nitrogenated ring moieties (**12**–**14**) was chosen on the basis of its pharmacological, structural, and electronic properties [29]. The activity for these compounds showed dependency with their p*K*a values. These results could be explained since non-basic compounds failed to show inhibition. A table with the estimated protonation states of the compounds at physiological pH is included in the Supplementary material, Table S1.

On the other hand, amine-substituted derivatives showed different inhibitory activity with IC_{50} values ranging from 20 μM to nanomolar order. Probably they are better inhibitors than their amide counterparts, because these compounds retain their ability to be protonated at both amino groups. Regarding the solanocapsine reactivity in substitution reactions we observed that, under an excess of nucleophile and only in the case of small molecules, substitution was carried out at the secondary nitrogen. The resulting methylated compound (**15**) exhibits an increased inhibitory activity (IC_{50} = 1.56 μM) compared to solanocapsine. With respect to the remaining amine-derivatives, we observed that **16**, **17** and derivatives with elongation and posterior substitution with different moieties (**18**, **22**) showed promising anti-AChE activity; yet, they did not show a clear structure–activity pattern.

Deletion of the rigid moiety (**20**) or introduction of a double bond (**19**) with the purpose of changing the conformation of E-F rings induces a deleterious effect on the activity. This finding could suggest the importance of the spatial disposition of these rings and their substituent for global interactions. This is an important feature to consider for future derivatizations.

Among all the derivatives, compound **24** showed the most potent inhibitory activity against AChE with an IC_{50} value of 90 nM (36-times increased activity compared to that of solanocapsine). This compound was inspired in dual binding site inhibitors with a flexible linker. In addition, its molecular simplification (**23**) induces a decrease in activity, revealing that all parts of tetrahydroacridine moiety are necessary for inhibition.

Finally, with an IC_{50} value of 29 μM, the rhodamine dye derivative (**25**) showed an interesting result due to the possibility of synthesizing a mono-amide derivative with different dyes to be used as fluorescent probes to evaluate the interaction with the enzyme.

Briefly, many modifications were found to be detrimental to biological activity. Maybe most importantly to note is the incorporation of a molecule known as enzyme activity inhibitor with an optimum spacer, capable of improve or reinforce interactions with the residues of the enzyme cavity.

2.2.2. Kinetic characterization of AChE inhibition

Since compound **24** was the most effective AChEI of the series, it was selected beside solanocapsine for the kinetic study of enzyme inhibition. Enzyme activity was evaluated at different fixed substrates concentrations and by increasing inhibitor concentrations. The data were used to elucidate the enzyme inhibition mechanism. The results are illustrated in the form of Lineweaver–Burk plot (Fig. 2). The double-reciprocal plots showed an increasing slope (decreased V_{max}) and an increasing intercept (higher K_m) on the y-axis at a higher concentration of **24** and solanocapsine, indicating a mixed-type inhibition in both cases. Thus, the enzyme kinetic study suggests that these inhibitors binds to both the CAS and PAS sites of AChE [7]. The inhibition constants K_i were equal to 3.6 ± 0.2 μM for solanocapsine and 84 ± 5nM for **24**.

2.3. Molecular docking

To gain insight into the molecular determinants that modulate the inhibitory activity and the mechanism of inhibition, a molecular modeling study was performed to explore the binding interactions of solanocapsine derivatives to the *Tc*AChE enzyme. Before starting the docking simulation, the p*K*as of the compounds were evaluated; the results are included in Table S1. Therefore, under physiological condition the predominant form of the amino groups was expected to be that of the protonated one.

A control search was conducted with the complexes of *Tc*AChE with donepezil, and with bis-tacrine derivatives; they were compared to the experimental ones (PDB: 1EVE, 2CMF and 1UT6) [30,31]. In these simulations, good docking geometries of the donepezil molecule and the tacrine moiety of the different derivatives were achieved. The geometries obtained were close to those of the experimental ones with low RMSD values, thus validating the

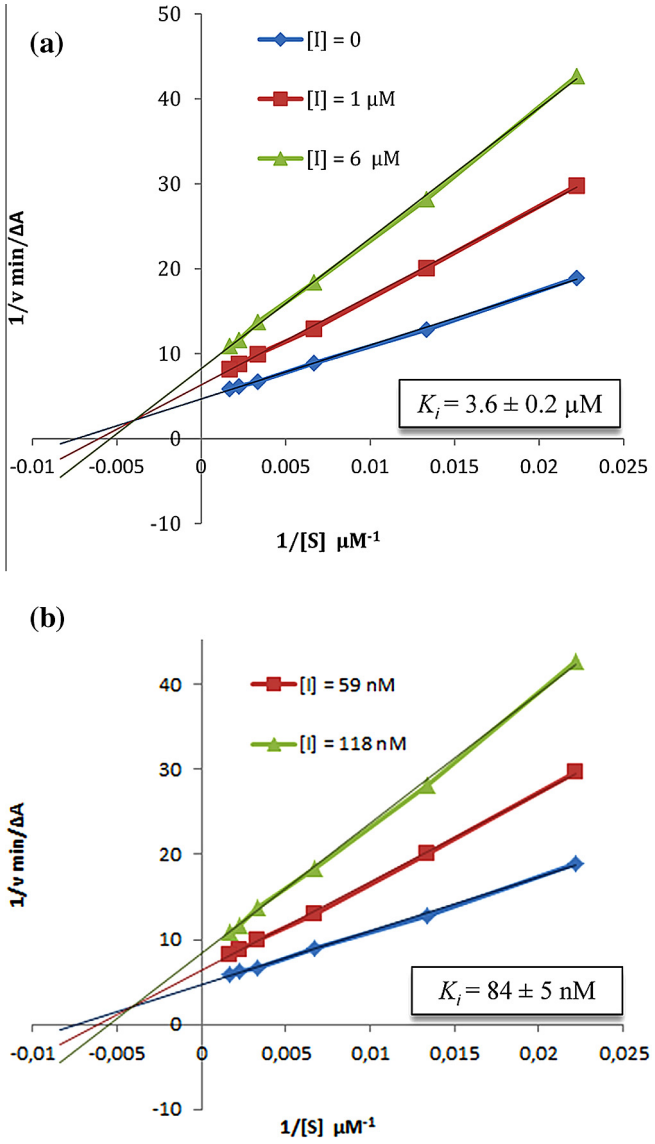

Fig. 2. Lineweaver–Burk plots of the inhibition of AChE by solanocapsine (a) compound **24** (b) with acetylthiocholine (S) as substrate.

protocol used. These geometries are shown in Fig. S1a-c in the Supplementary material.

For evaluating the geometries of the complexes of solanocapsine derivatives, two geometries of the receptor were employed. It was found that, according to the position of the side chain of Phe330, two geometries of the receptor could be formed, one with the gate "open" to the active site (1EVE), suitable for the donepezil but not for the tacrine derivatives, and another with the gate "closed" (2CMF), with a narrower entrance suitable for positioning the tacrine moiety with the linkers. These differences are represented in Fig. S2. A list with the docking energies, for the most stable complexes with each compound, the most appropriate receptor and a detail of the binding mode is included in the Supplementary material, Table S2. In almost all the cases more stable geometries were obtained with receptor 1EVE, with part of the inhibitors reaching the bottom of the active site, depicted for solanocapsine in Fig. 3(a). However, for the more active compound (**24**), the most stable geometries were found with the other receptor allowing a better fit for the tacrine part onto the active site, Fig. 3(b). Then, a good correlation (R^2 = 0.85) between the experimental activities and the docking energies (with the *Chemmgauss4* scoring function, Fig. S4) was obtained and only two of the compounds were outside the trend line (**4** and **22**). This tendency could be important in the prediction of the activity of new solanocapsine derivatives. However, a bigger set of compounds with a wider range of experimental activities should be needed to extend the methodology to compounds from other families.

As seen in Fig. 3, the conformation of the solanocapsine nucleus showed a good fit with the shape of the gorge. With the aim of identifying the residues that stabilize the complex formed, a decomposition of the binding energy per residue was performed. Fig. 4 shows the results of this decomposition. The docking studies revealed that at the active site the protonated primary nitrogen of solanocapsine formed a hydrogen bond with the carboxylate group of Glu199 with a distance of 1.77 Å and cation–π plus van der Waals interactions with Trp84. In addition, the most significant stabilizing interactions in the PAS have non-polar character. It can be seen that rings B and C interact with Phe330 and Phe331 residues, these interactions being added to those established between rings D and E with Tyr334. Finally, a hydrogen bond between the secondary amino group of solanocapsine and the carbonyl of the backbone of Tyr334 (1.94 Å) was also found. The simultaneous interactions of solanocapsine with the peripheral and catalytic sites of AChE could explain the high inhibitory potency of this compound, for more details see Figs. S3 and S5 in Supporting information.

As was noted above, inspired on tacrine hybrid molecules, and previous to the synthesis of **24**, we decided to assess the optimal linker length between tacrine and solanocapsine. For this purpose, binding energies of the complexes with molecules **24**, **26** and **27**, with linkers of four, two and five methylene units respectively, were compared (Fig. S7 in the Supplementary material). The calculated binding energies of the complexes with these compounds are −60.2, −46.3 and −50.6 kcal/mol for **24**, **26** and **27**. The comparative geometries are shown in the Supporting Information (Fig. S7). According to the results, modifications in the length of the linker chain affected the binding energy and the geometries. With a short linker (**26**) worst binding energy was obtained (−46.3 kcal/mol). This could be ascribed to the lost of important interactions between solanocapsine and the residues of PAS. On the other hand, increasing the chain length (**27**) does not improve binding energy. Indeed, the nucleus of solanocapsine rotated, losing important interactions with PAS residues. This analysis helps to conclude that compound **24**, with intermediate length, has a better linker than **26** and **27**. Therefore, it was decided to synthesize compound **24**.

In derivative **24**, tacrine moiety is firmly bound to the active site of the protein, even without being protonated, as seen in the X-ray structures of complexes with tacrine [32] and other tacrine inhibitors [33]. As expected, this group is stacked against the aromatic rings of Trp84 and Phe330 through van der Waals interactions, Fig. S5. In the profile of decomposition of the binding energy per residue, it could also be seen that there are not interactions with Glu199. In addition, carboxylate is close to an apolar group; as a result it has a destabilizing contribution to the binding energy, Fig. 4. Meanwhile, the linker of this derivative interacts with Phe330, Phe331 and Tyr334 by means of hydrophobic interactions. Rings A and B of solanocapsine and the methyl group between them were in front of the aromatic side chain of Trp279. The former primary amino group of solanocapsine formed a weak hydrogen bond with the carboxylate of Asp72 (2.42 Å). Finally there are stabilizing interactions with Phe284, a hydrogen bond between the hydroxyl group of solanocapsine and the carbonyl of the backbone and cation–π interaction between the other amino group and the aromatic side chain. Despite the unfavorable interaction with Glu199, this compound shows important interactions with the other residues of the active site and PAS and is the most active pre-

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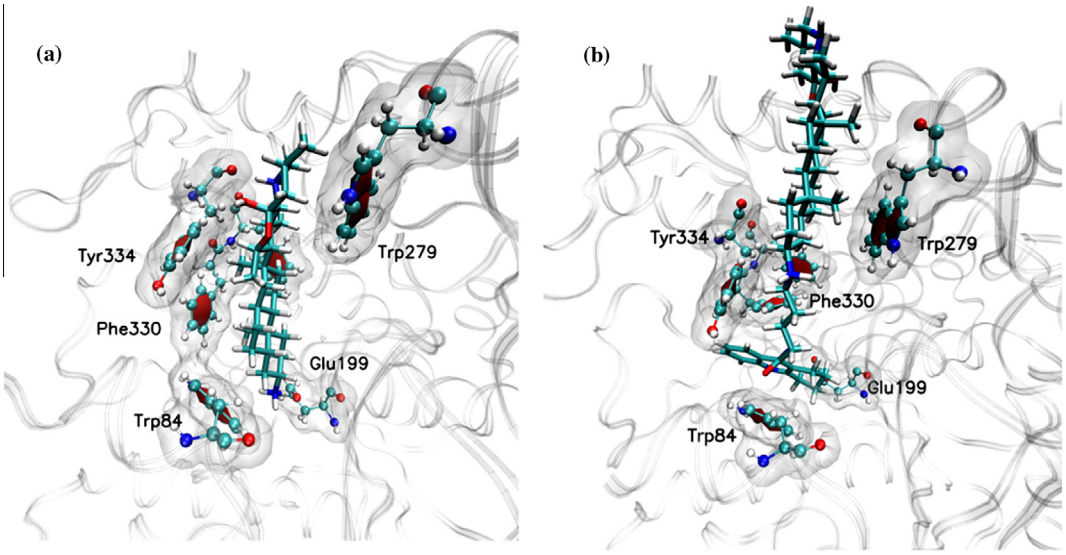

Fig. 3. Calculated position of solanocapsine (a) and compound **24** (b) in the binding pocket of the *Tc*AChE and most relevant residue interactions.

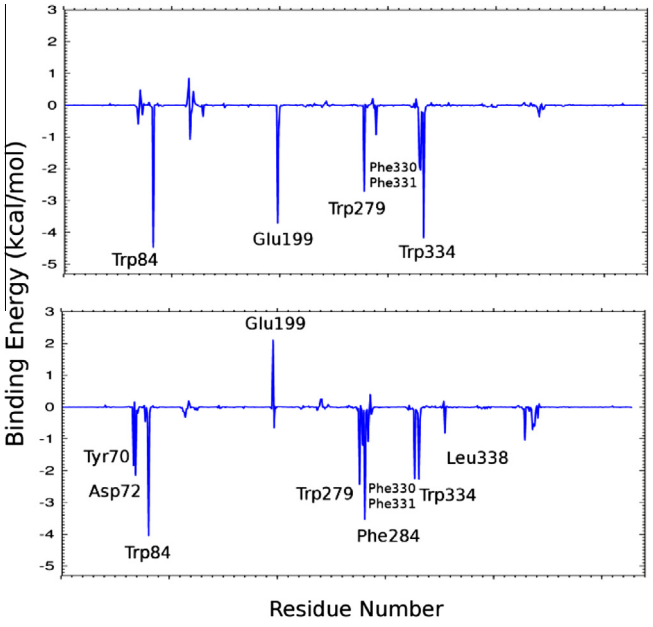

Fig. 4. Per-residue contributions to the binding energy calculated with MM-GBSA from the docking simulation of solanocapsine (up) and **24** (down).

pared derivative. These interactions are shown in Fig. 3b (for more details see Fig. S5 in the Supplementary material).

3. Conclusions

Solanocapsine, a steroidal alkaloid was isolated from fruits and aerial parts of *S. pseudocapsicum*. The synthesis and biological evaluation of a series of solanocapsine analogues led to the design of potent AChEIs. In addition, molecular docking simulations gave a more clarified explanation of the interactions with AChE, in agreement with the mixed-type inhibition mechanism of action.

At present, we consider to synthesize new solanocapsine derivatives according to the docking observations, in addition to perform a complete molecular modeling study, able to predict the AChE binding mode and inhibition value of different steroidal derivatives. The combination of the primary amine substitution with, for instance, the appropriate linker, and the subsequent connection to a compound that can interact efficiently with the active site should allow us to obtain more potent inhibitors. This is the first study of hybrids that combine a steroidal alkaloid with tacrine, hence we consider it to be the base for further studies, especially in relation to the search for new derivatives with enhanced dual binding site inhibitory potencies.

On the basis of these results, the preparation of new solanocapsine derivatives and the rational design of their molecular simplification are in progress. This will provide us with valuable information on these steroidal alkaloids-type compounds pharmacophore.

4. Materials and methods

Optical rotations were measured on JASCO P-1010 polarimeter. IR spectra were obtained in a Nicolet 5-SXC spectrophotometer (each compound was dissolved in a minimum amount of solvent and a drop of solution was added to the AgCl IR plates).

NMR experiments were performed on Bruker AVANCE II 400 MHz instrument. Multiplicity determinations (HSQC-DEPT) and 2D spectra (COSY, HSQC and HMBC) were obtained using standard Bruker software. Chemical shifts are expressed in ppm (δ) units using tetramethylsilane as the standard. HRESIQTOFMS were measured on Micro TOFQ II Bruker Daltonics (MA, USA) mass spectrometer. Chromatographic separations were performed by column chromatography on silica gel 60 (0.063–0.200 mm), and preparative TLC on silica gel 60 F_{254} (0.2 mm thick) plates. Presence of alkaloids was revealed by Dragendorff's reagent.

4.1. Plant material

A voucher specimen of *S. pseudocapsicum* was identified by Professor Gloria Barboza and was deposited at the herbarium of Museo Botánico Córdoba (CORD), Universidad Nacional de Córdoba. *S. pseudocapsicum* was collected in Valle Hermoso, Punilla, Córdoba, Argentina, in December 2012 (code: Barboza et al. 3665 bis).

4.2. Extraction and Isolation

Vegetal parts were processed separately. The air-dried powdered aerial parts of *S. pseudocapsicum* (444 g) were exhaustively

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extracted with EtOH (3 × 500 mL) and the solvent was evaporated at reduced pressure.

On the other side, fresh unripe fruits (1800 g) were minced and extracted on a soxhlet apparatus with MeOH (3 L). The solvent was then evaporated at reduced pressure. The total residue was diluted with (500 mL, 10%) aqueous HCl solution. Diatomaceous earth was added and the homogenate was placed at 2 °C for 12 h. Afterwards, the aqueous phase was vacuum filtrated. The resulting fraction was partitioned in CH_2Cl_2 (3 × 200 mL). The pH of the aqueous acidic fraction was adjusted to 9 with NH_4OH and extracted with CH_2Cl_2 (6 × 150 mL). Organic extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. Finally, after AcOEt:Hexane (1:3) dissolution and evaporation, 0.94 g of solanocapsine as a light yellow amorphous powder was obtained. Analytical and spectral data of this alkaloid were in agreement with those reported in the literature [18].

4.3. Preparation of solanocapsine derivatives

4.3.1. (22R, 23S, 25R)-3β-N-(4′-benzonitrile)methyl-imino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**1**)

4-Formylbenzonitrile (8.5 mg, 0.065 mmol) and Na_2SO_4 were added to a solution of solanocapsine (28.0 mg, 0.065 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 20 h. After filtration and removal of the solvent, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH:Et_3N (9:0.9:0.1) to obtain 5 mg of compound **1** (14%) as a white amorphous solid; $[\alpha]_D^{25}$: +3.3 (c 0.09, CH_2Cl_2), IR (film) ν_{max}: 3367.1, 2948.6, 2923.6, 2852.2, 2227.4, 1731.8, 1606.4, 1455.9, 1376.9, 1112.7, 1018.2, 835.0, 736.7, 673.0 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.32 s (1H, H-1′), 7.82 brd (1H, J = 8.3 Hz, H-3′, 7′), 7.67 brd (1H, J = 8.3 Hz, H-4′, 6′), 4.46 ddd (1H, J = 16.6, 9.6, 6.6 Hz, H-16), 3.26 m (1H, H-3), 3.05 dd (1H, J = 11.6, 4.2 Hz, H_2-26a), 2.17 brt (1H, J = 11.6 Hz, H_2-26b), 2.00 d (1H, J = 10.0 Hz, H-22), 1.89 m (1H, H-25), 1.83 m (1H, H_2-12a), 1.82 m (1H, H_2-24a), 1.79 m (1H, H-20), 1.77 m (1H, H_2-1a), 1.65 m (1H, H_2-2a), 1.64 m (1H, H_2-4a), 1.60 m (2H, H_2-7), 1.56 m (2H, H_2-15), 1.53 m (1H, H_2-11a), 1.38 m (1H, H-5), 1.36 m (1H, H-8), 1.31 m (1H, H_2-4b), 1.30 m (1H, H_2-11b), 1.26 m (2H, H_2-6), 1.24 m (1H, H-9), 1.24 m (1H, H_2-12b), 1.20 m (1H, H_2-24b), 1.08 m (1H, H-14), 1.06 m (1H, H_2-1b), 0.96 d (3H, J = 6.4 Hz, H_3-21), 0.89 s (3H, H_3-19), 0.88 m (1H, H_2-2b), 0.86 d (3H, J = 6.4 Hz, H_3-27), 0.76 s (3H, H_3-18), 0.75 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 156.6 (CH, C-1′), 140.3 (C, C-2′), 132.1 (CH, C-4′, 6′), 128.4 (CH, C-3′, 7′), 118.6 (C, C-8′), 113.6 (C, C-5′), 95.7 (C, C-23), 74.2 (CH, C-16), 70.3 (CH, C-3), 68.7 (CH, C-22), 60.2 (CH, C-14), 54.9 (CH, C-5), 54.9 (CH_2, C-26), 54.8 (CH, C-17), 41.7 (C, C-13), 46.0 (CH_2, C-24), 45.0 (CH, C-9), 38.9 (CH_2, C-12), 36.5 (CH_2, C-1), 35.7 (C, C-10), 34.8 (CH, C-8), 36.4 (CH_2, C-4), 32.9 (CH, C-20), 31.7 (CH_2, C-2), 29.5 (CH_2, C-7), 30.0 (CH, C-25), 28.7 (CH_2, C-6), 28.3 (CH_2, C-15), 20.4 (CH_2, C-11), 14.9 (CH_3, C-21), 18.4 (CH_3, C-27), 13.5 (CH_3, C-18), 12.3 (CH_3, C-19). HRESIMS m/z[M-H_2O] 526.3814 (calcd. for $C_{35}H_{48}N_3O$, 526.3797).

4.3.2. (22R, 23S, 25R)-3β-N-(4′-chlorobenzyliden)amino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**2**)

4-Chlorobenzaldehyde (10.0 mg, 0.07 mmol) and Na_2SO_4 were added to a solution of solanocapsine (30.0 mg, 0.07 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 18 h. After filtration and removal of the solvent, the residue was purified by preparative-TLC using Et_2O:MeOH (9.9:0.1) to obtain 4.2 mg of compound **2** (12%) as a white amorphous solid; $[\alpha]_D^{25}$: +5.7 (c 0.06, CH_2Cl_2), IR (film) ν_{max}: 3355.5, 2925.5, 2850.3, 1644.9, 1376.9, 1452.1, 1085.7, 734.8, 661.5 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.26 s (1H, H-1′), 7.66 d (1H, J = 8.5 Hz, H-3′, 7′), 7.36 d (1H, J = 8.5 Hz, H-4′, 6′), 4.47 m (1H, H-16), 3.21 m (1H, H-3), 3.05 m (1H, H_2-26a), 2.17 brt (1H, J = 11.6 Hz, H_2-26b), 2.02 dd (1H, J = 10.4, 2.9 Hz, H-22), 1.95 m (1H, H-25), 1.83 m (1H, H_2-12a), 1.83 m (1H, H_2-24a), 1.81 m (1H, H-20), 1.77 m (1H, H_2-1a), 1.77 m (1H, H-7a), 1.65 m (1H, H_2-2a), 1.65 m (1H, H_2-4a), 1.58 m (1H, H-7b), 1.58 m (2H, H_2-15), 1.51 m (1H, H_2-11a), 1.39 m (1H, H-5), 1.35 m (1H, H-8), 1.32 m (1H, H_2-4b), 1.32 m (1H, H_2-11b), 1.28 m (2H, H_2-6), 1.24 m (1H, H_2-12b), 1.21 m (1H, H_2-24b), 1.12 m (1H, H-9), 1.07 m (1H, H_2-1b), 1.07 m (1H, H-14), 0.96 d (3H, J = 6.3 Hz, H_3-21), 0.89 m (1H, H_2-2b), 0.85 d (3H, J = 6.5 Hz, H_3-27), 0.84 s (3H, H_3-19), 0.76 s (3H, H_3-18), 0.73 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 157.2 (CH, C-1′), 136.3 (C, C-5′), 135.0 (C, C-2′), 129.0 (CH, C-3′, 7′), 128.6 (CH, C-4′, 6′), 95.8 (C, C-23), 74.2 (CH, C-16), 70.3 (CH, C-3), 68.6 (CH, C-22), 60.5 (CH, C-14), 54.8 (CH, C-5), 54.7 (CH_2, C-26), 54.6 (CH, C-17), 46.0 (CH_2, C-24), 45.5 (CH, C-9), 41.5 (C, C-13), 38.9 (CH_2, C-12), 36.9 (CH_2, C-1), 36.4 (CH_2, C-4), 35.2 (C, C-10), 34.8 (CH, C-8), 32.8 (CH, C-20), 31.6 (CH_2, C-2), 29.8 (CH_2, C-7), 29.8 (CH, C-25), 28.3 (CH_2, C-6), 28.2 (CH_2, C-15), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 14.8 (CH_3, C-21), 13.4 (CH_3, C-18), 12.3 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 553.3574 (calcd for $C_{34}H_{50}ClN_2O_2$, 553.3555).

4.3.3. (22R, 23S, 25R)-3β-N-(4′-pyridinbenzyliden)amino-22,26-imino-16β, 23-epoxy-5α-cholestan-23β-ol (**3**)

4-Pyridinecarboxaldehyde (8.0 μL, 0.079 mmol) and Na_2SO_4 were added to a solution of solanocapsine (34.0 mg, 0.079 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 20 h. After filtration and removal of the solvent, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (9.8:0.2) to obtain 16.2 mg of compound **3** (42%) as a white amorphous solid; $[\alpha]_D^{25}$: +12.4 (c 0.43, MeOH), IR (film) ν_{max}: 3357.5, 2925.5, 2850.3, 1727.9, 1643.1, 1600.6, 1558.2, 1450.2, 1413.6, 1378.9, 1276.7, 1112.7, 998.9, 815.7, 734.8, 534.2 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.66 dd (1H, J = 4.4, 1.5 Hz, H-4′, 6′), 8.28 s (1H, H-1′), 7.58 dd (1H, J = 4.4, 1.5 Hz, H-3′, 7′), 4.46 m (1H, H-16), 3.28 m (1H, H-3), 3.04 brdd (1H, J = 11.6, 4.5 Hz, H_2-26a), 2.17 t (1H, J = 11.6 Hz, H_2-26b), 2.01 m (1H, J = 10.4, 2.9 Hz, H-22), 1.94 m (1H, H-25), 1.83 m (1H, H_2-12a), 1.83 m (1H, H_2-24a), 1.79 m (1H, H-20), 1.78 m (1H, H_2-4a), 1.67 m (1H, H_2-1a), 1.66 m (1H, H_2-2a), 1.59 m (2H, H_2-7), 1.58 m (2H, H_2-15), 1.52 m (1H, H_2-11a), 1.39 m (1H, H-5), 1.37 m (1H, H-8), 1.36 m (1H, H_2-11b), 1.32 m (1H, H_2-1b), 1.28 m (2H, H_2-6), 1.25 m (1H, H-9), 1.25 m (1H, H_2-12b), 1.22 m (1H, H_2-24b), 1.08 m (1H, H-14), 1.07 m (1H, H_2-4b), 0.96 d (3H, J = 6.4 Hz, H_3-21), 0.89 m (1H, H_2-2b), 0.89 s (3H, H_3-19), 0.86 d (3H, J = 6.4 Hz, H_3-27), 0.77 s (3H, H_3-18), 0.75 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 156.5 (CH, C-1′), 150.2 (CH, C-4′, 6′), 143.8 (C, C-2′), 121.9 (CH, C-3′, 7′), 96.1 (C, C-23), 74.4 (CH, C-16), 70.4 (CH, C-3), 68.8 (CH, C-22), 60.3 (CH, C-14), 54.9 (CH, C-17), 54.9 (CH_2, C-26), 54.7 (CH, C-5), 46.0 (CH_2, C-24), 44.9 (CH, C-9), 41.8 (C, C-13), 39.0 (CH_2, C-12), 36.7 (CH_2, C-4), 36.2 (CH_2, C-1), 35.2 (C, C-10), 34.6 (CH, C-8), 32.9 (CH, C-20), 32.0 (CH_2, C-2), 29.9 (CH, C-25), 29.4 (CH_2, C-7), 28.4 (CH_2, C-6), 28.0 (CH_2, C-15), 20.2 (CH_2, C-11), 18.7 (CH_3, C-27), 15.1 (CH_3, C-21), 13.3 (CH_3, C-18), 12.2 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 520.3922 (calcd for $C_{33}H_{50}N_3O_2$, 520.3898).

4.3.4. (22R, 23S, 25R)-3β-N-[4′-(trifluoromethylbenzyliden)amino-22,26-imino-16β, 23-epoxy-5α-cholestan-23β-ol (**4**)

2-Trifluoromethylbenzaldehyde (10.0 μL, 0.071 mmol) and Na_2SO_4 were added to a solution of solanocapsine (30.5 mg, 0.071 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 20 h. After filtration and removal of the solvent, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH:Et_3N (9.6:0.3:0.1) to obtain 11.4 mg of compound **4** (29%) as a light yellow amorphous solid; $[\alpha]_D^{25}$: +12.2 (c 0.21, CH_2Cl_2), IR (film) ν_{max}: 3367.1, 2948.6, 2923.6, 2852.2, 2227.4, 1731.8, 1606.4, 1455.9, 1376.9, 1112.7, 1018.2, 835.0, 736.7, 673.0 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.64 s (1H, H-1′), 8.15 d

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(1H, J = 8.0 Hz, H-7′), 7.65 d (1H, J = 7.6 Hz, H-4′), 7.55 t (1H, J = 7.6 Hz, H-5′), 7.46 t (1H, J = 7.6 Hz, H-6′), 4.45 m (1H, H-16), 3.28 m (1H, H-3), 3.02 brdd (1H, J = 11.7, 2, 4 Hz, H_2-26a), 2.15 t (1H, J = 11.7 Hz, H_2-26b), 1.98 m (1H, H-22), 1.98 m (1H, H-25), 1.82 m (1H, H_2-12a), 1.81 m (1H, H_2-24a), 1.77 m (1H, H-20), 1.76 m (1H, H_2-4a), 1.65 m (1H, H_2-1a), 1.62 m (1H, H_2-2a), 1.60 m (2H, H_2-7), 1.59 m (2H, H_2-15), 1.49 m (1H, H_2-11a), 1.37 m (1H, H-5), 1.35 m (1H, H-8), 1.34 m (1H, H_2-11b), 1.32 m (1H, H_2-1b), 1.27 m (2H, H_2-6), 1.26 m (1H, H-9), 1.22 m (1H, H_2-12b), 1.19 m (1H, H_2-24b), 1.07 m (1H, H-14), 1.06 m (1H, H_2-4b), 0.95 d (3H, J = 6.4 Hz, H_3-21), 0.88 s (3H, H_3-19), 0.87 m (1H, H_2-2b), 0.85 d (3H, J = 6.4 Hz, H_3-27), 0.75 s (3H, H_3-18), 0.72 m (1H, H-17).^{13}C NMR ($CDCl_3$ 100.03 MHz): 155.0 (CH, C-1′), 134.6 (C, C-2′), 131.6 (CH, C-5′), 129.5 (CH, C-6′), 128.4 (CH, C-7′), 122.8 (C, C-3′), 125.2 (CH, C-4′), 95.9 (C, C-23), 74.3 (CH, C-16), 70.5 (CH, C-3), 68.5 (CH, C-22), 60.3 (CH, C-14), 54.8 (CH, C-5), 54.8 (CH, C-17), 55.0 (CH_2, C-26), 41.7 (C, C-13), 46.2 (CH_2, C-24), 45.1 (CH, C-9), 36.8 (CH_2, C-4), 38.6 (CH_2, C-12), 36.5 (CH_2, C-1), 36.2 (CH_2, C-7), 35.5 (C, C-10), 34.9 (CH, C-8), 32.8 (CH, C-20), 31.6 (CH_2, C-2), 29.8 (CH, C-25), 28.5 (CH_2, C-6), 28.3 (CH_2, C-15), 20.4 (CH_2, C-11), 18.6 (CH_3, C-27), 14.9 (CH_3, C-21), 13.6 (CH_3, C-18), 12.2 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 587.3848 (calcd for $C_{35}H_{50}F_3N_2O_2$, 587.3819).

4.3.5. (22R, 23S, 25R)-3β-N-(2′-bromobenzyliden)amino-22,26-imino-16β, 23-epoxy-5α-cholestan-23β-ol (**5**)

2-Bromobenzaldehyde (8.2 μL, 0.070 mmol) and Na_2SO_4 were added to a solution of solanocapsine (30.2 mg, 0.070 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 24 h. After filtration and removal of the solvent, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (9.9:0.1) to obtain 8.5 mg of compound **5** (22%) as a white amorphous solid; $[\alpha]_D^{25}$: +8.1 (c 0.18, CH_2Cl_2), IR (film) ν_{max}: 3328.5, 2925.5, 2850.3, 1731.8, 1633.4, 1560.1, 1457.9, 1376.9, 1274.7, 1114.7, 1018.2, 873.6, 754.0, 478.3 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 8.64 s (1H, H-1′), 7.98 dd (1H, J = 7.9, 1.8 Hz, H-7′), 7.55 dd (1H, J = 7.9, 1.0 Hz, H-4′), 7.32 brt (1H, J = 7.9 Hz, H-6′), 7.24 td (1H, J = 7.9, 1.8 Hz, H-5′), 4.46 m (1H, H-16), 3.30 m (1H, H-3), 3.03 brd (1H, J = 11.6 Hz, H_2-26a), 2.17 td (1H, J = 11.6, 2.6 Hz, H_2-26b), 2.10 dd (1H, J = 10.0, 4.0 Hz, H-22), 0.96 d (3H, J = 6.4 Hz, H_3-21), 0.89 s (3H, H_3-19), 0.86 d (3H, J = 6.4 Hz, H_3-27), 0.76 s (3H, H_3-18). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 157.9 (CH, C-1′), 134.9 (C, C-2′), 132.9 (CH, C-4′), 128.9 (CH, C-6′), 131.5 (CH, C-5′), 127.6 (CH, C-7′), 124.8 (C, C-3′), 96.1 (C, C-23), 74.5 (CH, C-16), 70.6 (CH, C-3), 68.8 (CH, C-22), 60.4 (CH, C-14), 55.0 (CH, C-17), 54.9 (CH_2, C-26), 54.8 (CH, C-5), 46.1 (CH_2, C-24), 45.7 (CH, C-9), 41.8 (C, C-13), 39.2 (CH_2, C-12), 37.4 (CH_2, C-1), 36.9 (CH_2, C-4), 35.7 (C, C-10), 34.9 (CH, C-8), 33.0 (CH, C-20), 32.4 (CH_2, C-7), 31.9 (CH_2, C-2), 30.0 (CH, C-25), 28.6 (CH_2, C-6), 28.3 (CH_2, C-15), 20.4 (CH_2, C-11), 18.7 (CH_3, C-27), 15.1 (CH_3, C-21), 13.6 (CH_3, C-18), 12.4 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 587.3848 (calcd for $C_{35}H_{50}BrN_2O_2$, 587.3819).

4.3.6. (22R, 23S, 25R)-3β-N-(4′-methoxybenzyliden)amino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**6**)

p-Anisaldehyde (9.0 μL, 0.070 mmol) and Na_2SO_4 were added to a solution of solanocapsine (30.2 mg, 0.070 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 36 h. After filtration and removal of the solvent, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (9.5:0.5) to obtain 7.8 mg of compound **6** (19%) as a light yellow amorphous solid; $[\alpha]_D^{25}$: −51.4 (c 0.09, MeOH), IR (film) ν_{max}: 3363.3, 2925.5, 2850.3, 1729.8, 1658.5, 1606.4, 1511.9, 1452.1, 1378.9, 1247.7, 1112.7, 1079.9, 1008.6, 831.2, 734.8, 470.6 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 8.23 s (1H, H-1′), 7.65 d (1H, J = 8.9 Hz, H-3′, 7′), 6.90 d (1H, J = 8.9 Hz, H-4′, 6′), 4.46 ddd (1H, J = 16.7, 9.9, 6.7 Hz, H-16), 3.82 s (3H, H_3-8′), 3.16 m (1H, H-3), 3.03 dd (1H, J = 11.6, 4.3 Hz, H_2-26a), 2.16 t (1H, J = 11.6 Hz, H_2-26b), 1.99 m (1H, H-22), 1.94 m (1H, H-25), 1.81 m (1H, H_2-12a), 1.81 m (1H, H_2-24b), 1.78 m (1H, H-20), 1.67 m (1H, H-7a), 1.66 m (1H, H_2-1a), 1.63 m (1H, H_2-2a), 1.57 m (2H, H_2-15), 1.49 m (1H, H_2-11a), 1.42 m (1H, H_2-4a), 1.37 m (1H, H-5), 1.32 m (1H, H-8), 1.32 m (1H, H_2-11b), 1.25 m (2H, H_2-6), 1.23 m (1H, H-7b), 1.22 m (1H, H_2-12b), 1.20 m (1H, H_2-24a), 1.11 m (1H, H_2-4b), 1.11 m (1H, H-9), 1.07 m (1H, H-14), 0.96 m (1H, H_2-1b), 0.95 d (3H, J = 6.4 Hz, H_3-21), 0.87 m (1H, H_2-2b), 0.86 d (3H, J = 6.5 Hz, H_3-27), 0.78 s (3H, H_3-19), 0.74 s (3H, H_3-18), 0.69 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 161.3 (C, C-5′), 157.9 (CH, C-1′), 130.6 (C, C-2′), 129.4 (CH, C-3′, 7′), 113.8 (CH, C-4′, 6′), 95.6 (C, C-23), 74.6 (CH, C-16), 70.4 (CH, C-3), 68.7 (CH, C-22), 60.5 (CH, C-14), 55.1 (C, C-8′), 54.9 (CH_2, C-26), 54.6 (CH, C-5), 54.6 (CH, C-17), 46.1 (CH_2, C-24), 45.5 (CH, C-9), 41.6 (C, C-13), 39.1 (CH_2, C-4), 39.1 (CH_2, C-12), 37.3 (CH_2, C-1), 35.4 (C, C-10), 34.9 (CH, C-8), 32.8 (CH, C-20), 32.1 (CH_2, C-7), 31.6 (CH_2, C-2), 29.9 (CH, C-25), 28.6 (CH_2, C-6), 28.3 (CH_2, C-15), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 14.9 (CH_3, C-21), 13.5 (CH_3, C-18), 12.3 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 549.4076 (calcd for $C_{35}H_{53}N_2O_3$, 549.4051).

4.3.7. (22R, 23S, 25R)-N,N′-diacetyi-3β-amino-22,26-imino-16β, 23-epoxy-5α-cholestan-23β-ol (**7**)

Acetyl chloride (9.0 μL, 0.120 mmol) and 42 μL of triethylamine (0.300 mmol) were added to a solution of solanocapsine (24.6 mg, 0.057 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 4 h. Once the solvent was removed, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (9.5:0.5) to obtain 17.8 mg of compound **7** (60%) as a light pink amorphous solid; $[\alpha]_D^{25}$: −3.0 (c 0.40, MeOH), IR (film) ν_{max}: 3305.4, 3081.7, 2929.3, 2850.3, 1666.2, 1644.9, 1637.3, 1629.6, 1558.2, 1446.4, 1373.0, 1268.9, 1180.2, 1083.8, 1024.0, 950.7, 734.8, 605.5 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz):5.38 brs (1H, NH), 4.35 m (1H, H-16), 3.76 m (1H, H_2-26a), 3.75 m (1H, H-3), 3.40 d (1H, J = 12.0 Hz, H_2-24a), 3.26 m (1H, H_2-24b), 3.25 m (1H, H-20), 2.69 m (1H, H_2-26b), 2.56 d (1H, J = 12.0 Hz, H-22), 2.12 s (3H, H_3-2″), 2.04 m (1H, H-25), 1.94 s (3H, H_3-2′), 1.81 brd (1H, J = 8.0 Hz, H_2-4a), 1.68 m (1H, H_2-1a), 1.61 m (1H, H-7a), 1.58 m (1H, H_2-2a), 1.58 m (2H, H_2-15), 1.51 m (1H, H_2-11a), 1.41 m (1H, H-5), 1.33 m (1H, H-8), 1.30 m (1H, H_2-11b), 1.26 m (1H, H_2-4b), 1.25 m (2H, H_2-6), 1.21 m (1H, H-9), 1.19 m (1H, H-14), 1.09 m (1H, H_2-2b), 1.03 m (1H, H_2-1b), 0.93 d (3H, J = 6.4 Hz, H_3-27), 0.92 m (1H, H-17), 0.91 d (3H, J = 6.4 Hz, H_3-21), 0.87 m (1H, H-7b), 0.79 s (3H, H_3-19), 0.75 s (3H, H_3-18). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 174.7 (C, CO-1″), 169.2 (C, CO-1′), 98.1 (C, C-23), 74.8 (CH, C-16), 73.0 (CH, C-22), 61.7 (CH, C-17), 60.2 (CH, C-14), 58.5 (CH_2, C-26), 54.7 (CH, C-5), 48.9 (CH, C-3), 46.6 (CH_2, C-24), 45.4 (CH, C-9), 42.1 (C, C-13), 37.0 (CH_2, C-1), 38.8 (CH_2, C-4), 35.6 (CH_2, C-2), 34.6 (CH, C-8), 35.5 (C, C-10), 31.7 (CH_2, C-7), 31.3 (CH, C-25), 32.0 (CH, C-20), 28.6 (CH_2, C-15), 28.5 (CH_2, C-6), 22.9 (CH_3, C-2″), 23.2 (CH_3, C-2′), 20.1 (CH_2, C-11), 17.7 (CH_3, C-27), 15.8 (CH_3, C-21), 13.3 (CH_3, C-18), 12.1 (CH_3, C-19). HRESIMS m/z[M+Na]$^+$ 537.3687 (calcd for $C_{31}H_{50}N_2O_4Na$, 537.3663).

4.3.8. (22R, 23S, 25R)-N,N′-dibenzoyl-3β-amino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**8**) and (22R, 23S, 25R)-N-benzoyl-3β-amino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**9**)

To a solution of solanocapsine (29.0 mg, 0.068 mmol) in CH_2Cl_2 (1 mL), 2 mL of saturated 2 M aqueous $NaHCO_3$ solution was added. This mixture was vigorously stirred for a few minutes. Then 12.0 μL of benzoyl chloride (0.102 mmol) in CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred at room temperature for 2 h. The organic phase was separated and extracted with aqueous Na_2CO_3 solution (1 M, 3 × 15 mL). The organic extracts were dried with anhydrous Na_2SO_4 and filtered. Once the solvent was removed, the residue was purified by preparative-TLC using

CH_2Cl_2:MeOH (9.7:0.3) to obtain 9.8 mg of compound **8** (23%) and 5.9 mg of **9** (16%) as a white amorphous solid; **(22*R*, 23*S*, 25*R*)-*N*, *N*′-dibenzoyl-3β-amino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (8)** $[\alpha]_D^{25}$: +5.8 (c 0.28, MeOH), IR (film) ν_{max}: 3330.5, 3056.6, 3027.7, 2925.5, 2854.1, 1731.8, 1633.5, 1535.1, 1430.9, 1268.9, 1209.2, 1172.5, 1174.4, 1120.4, 1159.0, 1078.0, 1020.2, 873.6, 798.4, 698.1, 445.5 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.09 d (1H, *J* = 7.6 Hz, H-5′), 7.74 brd (2H, *J* = 7.0 Hz, H-3′, 7′), 7.57 brd (2H, *J* = 7.0 Hz, H-3″, 7″), 7.50 m (1H, H-6″), 7.48 m (1H, H-4′), 7.46 m (1H, H-5″), 7.43 m (1H, H-4″), 7.41 m (1H, H-6′), 5.94 d (1H, *J* = 8.1 Hz, NH), 4.48 c (1H, *J* = 8.4 Hz, H-16), 3.97 m (1H, H-3), 3.77 dd (1H, *J* = 13.0 Hz, H_2-26a), 3.25 m (1H, H-20), 2.78 m (1H, *J* = 13.0 Hz, H_2-26b), 2.75 d (1H, *J* = 10.2 Hz, H-22), 2.05 m (1H, H_2-24a), 1.97 m (1H, H-25), 1.83 m (1H, H_2-4a), 1.72 m (1H, H_2-1a), 1.70 m (1H, H-7a), 1.64 m (1H, H_2-2a), 1.64 m (2H, H_2-15), 1.50 m (1H, H_2-11a), 1.43 m (1H, H-5), 1.36 m (1H, H-8), 1.35 m (1H, H_2-11b), 1.32 m (1H, H_2-24b), 1.28 m (1H, H_2-4b), 1.27 m (2H, H_2-6), 1.25 m (1H, H-9), 1.24 m (1H, H-7b), 1.09 m (1H, H_2-1b), 1.05 m (1H, H-14), 0.97 d (3H, *J* = 6.2 Hz, H_3-21), 0.91 m (1H, H_2-2b), 0.82 s (3H, H_3-19), 0.81 s (3H, H_3-18), 0.75 m (1H, H-17), 0.75 d (3H, *J* = 6.6 Hz, H_3-27). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 175.5 (C, CO-1″), 166.7 (C, CO-1′), 135.1 (C, C-2′), 134.9 (C, C-2″), 131.4 (CH, C-4′), 131.2 (CH, C-5″), 130.0 (CH, C-5′), 128.8 (CH, C-3″), 128.8 (CH, C-7″), 128.6 (CH, C-4″), 126.7 (CH, C-3′), 128.5 (CH, C-6′), 126.7 (CH, C-7′), 128.5 (CH, C-6″), 97.9 (C, C-23), 74.6 (CH, C-16), 72.0 (CH, C-22), 61.4 (CH, C-14), 59.9 (CH2, C-26), 55.0 (CH, C-17), 54.5 (CH, C-5), 49.3 (CH, C-3), 46.5 (CH2, C-24), 45.5 (CH, C-9), 42.0 (C, C-13), 38.9 (CH2, C-4), 38.9 (CH2, C-12), 37.2 (CH2, C-1), 35.4 (C, C-10), 35.2 (CH2, C-7), 34.7 (CH, C-8), 31.6 (CH2, C-2), 31.5 (CH, C-25), 31.0 (CH, C-20), 28.6 (CH2, C-15), 28.3 (CH2, C-6), 20.1 (CH2, C-11), 17.6 (CH3, C-27), 15.5 (CH3, C-21), 13.6 (CH3, C-18), 11.9 (CH3, C-19). HRESIMS *m*/*z*[M+Na]$^+$ 661.3994 (calcd for $C_{41}H_{54}N_2O_4Na$, 661.3976). **(22*R*, 23*S*, 25*R*)-*N*-benzoyl-3β-amino-22, 26-imino-16β,23-epoxy-5α-cholestan-23β-ol (9)**: white amorphous solid; $[\alpha]_D^{25}$: +57.8 (c 0.23, CH_2Cl_2), IR (film) ν_{max}: 3332.4, 3058.6, 2927.4, 2852.2, 1729.8, 1633.4, 1538.9, 1448.3, 1380.8, 1274.7, 1157.1, 1081.9, 1016.3, 873.6, 833.1, 802.2, 715.5, 547.7 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.07 brd (1H, *J* = 8.8 Hz, H-6′), 7.75 brd (1H, *J* = 7.2 Hz, H-4′), 7.50 m (1H, H-5′), 7.44 m (1H, H-3′), 7.42 m (1H, H-7′), 5.93 d (1H, *J* = 8.0 Hz, NH), 4.47 c (1H, *J* = 8.4 Hz, H-16), 3.97 m (1H, H-3), 3.26 brd (1H, *J* = 13.0 Hz, H_2-26a), 2.30 t (1H, *J* = 13.0 Hz, H_2-26b), 2.24 d (1H, *J* = 10.2 Hz, H-22), 2.12 m (1H, H-25), 2.03 m (1H, H-20), 1.94 m (1H, H_2-24a), 1.93 m (1H, H_2-4a), 1.81 m (1H, H_2-12a), 1.73 m (1H, H_2-1a), 1.71 m (1H, H-7a), 1.63 m (1H, H_2-2a), 1.57 m (2H, H_2-15), 1.51 m (1H, H_2-11a), 1.39 m (1H, H-5), 1.32 m (1H, H-8), 1.31 m (1H, H_2-24b), 1.30 m (2H, H_2-6), 1.29 m (1H, H_2-11b), 1.27 m (1H, H_2-12b), 1.25 m (1H, H-9), 1.24 m (1H, H_2-4b), 1.24 m (1H, H-7b), 1.11 m (1H, H_2-1b), 1.08 m (1H, H-14), 1.05 d (3H, *J* = 6.2 Hz, H_3-21), 0.91 m (1H, H_2-2b), 0.91 d (3H, *J* = 6.4 Hz, H_3-27), 0.82 s (3H, H_3-19), 0.76 m (1H, H-17), 0.74 s (3H, H_3-18). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 166.7 (C, CO-1′), 135.1 (C, C-2′), 131.3 (CH, C-5′), 129.7 (CH, C-6′), 128.1 (CH, C-7′), 127.0 (CH, C-3′), 126.6 (CH, C-4′), 95.4 (C, C-23), 74.3 (CH, C-16), 67.9 (CH, C-22), 60.3 (CH, C-14), 54.6 (CH, C-5), 54.6 (CH, C-17), 53.4 (CH_2, C-26), 49.3 (CH, C-3), 45.3 (CH_2, C-24), 45.1 (CH, C-9), 41.7 (C, C-13), 38.9 (CH_2, C-12), 37.1 (CH_2, C-1), 35.4 (CH_2, C-7), 35.3 (C, C-10), 34.7 (CH, C-8), 32.5 (CH, C-20), 31.6 (CH_2, C-2), 28.8 (CH_2, C-4), 28.5 (CH_2, C-6), 28.4 (CH, C-25), 28.1 (CH_2, C-15), 20.0 (CH_2, C-11), 18.2 (CH_3, C-27), 14.8 (CH_3, C-21), 13.6 (CH_3, C-18), 11.9 (CH_3, C-19). HRESIMS *m*/*z* [M+H]$^+$ 535.3913 (calcd for $C_{34}H_{51}N_2O_3$, 535.3894).

4.3.9. General procedure for preparation of amides from acids

To a solution of acid (0.060 mmol), solanocapsine (30.0 mg, 0.070 mmol) and triethylamine (19.0 μL, 0.140 mmol) in CH_2Cl_2 (1.5 mL), a solution of 4-dimethylaminopyridine (DMAP, 2.0 mg, 0.018 mmol) in CH_2Cl_2 (0.5 mL) was added. The reaction was stirred for 5 min before adding $POCl_3$ (0.06 mmol) in CH_2Cl_2 (1 mL). After completion of reaction at room temperature (ca. 3 h, monitored through TLC), the organic phase was extracted sequentially with ice-cooled water (10 mL), 10% aqueous HCl (10 mL), saturated aqueous $NaHCO_3$ solution (10 mL), and brine. The final organic extract was dried over anhydrous Na_2SO_4. Removal of solvent under vacuum, and purification of the residue by preparative-TLC using CH_2Cl_2:MeOH (9:1) afforded the pure product.

*4.3.9.1. (22R, 23S, 25R)-3β-N-(adamantane-1′-carboxamide)-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**10**).* Following the general procedure, using 3-noradamantanecarboxylic acid (10 mg) and after purification, 8.4 mg of compound **10** (20%) was obtained as a white amorphous solid; $[\alpha]_D^{25}$: +3.7 (c 0.30, MeOH), IR (film) ν_{max}: 3342.0, 2925.5, 2859.9, 2852.2, 1729.8, 1633.4, 1529.3, 1457.9, 1380.8, 1081.9, 1014.4, 869.7, 734.6, 528.4 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz):4.46 brc (1H, *J* = 8.0 Hz, H-16), 3.76 m (1H, H-3), 3.11 m (1H, H_2-26a), 2.62 t (1H, *J* = 6.7 Hz, H-8′), 2.28 brs (2H, H-5′, 3′), 2.22 t (1H, *J* = 11.8 Hz, H_2-26b), 2.08 m (1H, H-22), 2.02 m (1H, H-25), 1.94 brd (2H, *J* = 10.4 Hz, H-2′), 1.89 m (1H, H-20), 1.84 m (1H, H_2-24a), 1.81 m (1H, H-6a), 1.80 m (2H, H_2-6′, 7′), 1.79 m (1H, H_2-4a), 1.73 dd (2H, *J* = 10.4, 2.4 Hz, H_2-10′), 1.68 m (1H, H_2-1a), 1.63 m (2H, H_2-4′), 1.62 m (1H, H_2-2a), 1.61 m (2H, H_2-6′, 7′), 1.58 m (1H, H_2-9a′), 1.56 m (2H, H_2-15), 1.50 m (1H, H_2-11a), 1.37 m (1H, H-5), 1.32 m (1H, H-8), 1.32 m (1H, H_2-11b), 1.29 m (2H, H_2-12), 1.27 m (1H, H-6b), 1.24 m (1H, H_2-4b), 1.23 m (1H, H_2-24b), 1.19 m (1H, H-9), 1.09 m (1H, H_2-9b′), 1.05 m (1H, H-14), 1.04 m (1H, H_2-1b), 0.99 d (3H, *J* = 6.6 Hz, H_3-21), 0.89 m (1H, H_2-2b), 0.89 d (3H, *J* = 6.3 Hz, H_3-27), 0.79 s (3H, H_3-19), 0.75 s (3H, H_3-18), 0.73 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 176.7 (CO), 95.6 (C, C-23), 74.3 (CH, C-16), 68.3 (CH, C-22), 60.3 (CH, C-14), 54.6 (CH, C-5), 54.5 (CH, C-17), 54.3 (CH_2, C-26), 48.7 (CH, C-3), 47.6 (CH_2, C-2′, 10′), 46.0 (CH_2, C-24), 45.3 (CH, C-9), 43.9 (CH_2, C-6′, 7′), 42.8 (CH, C-8′), 41.7 (C, C-13), 39.1 (CH_2, C-4), 38.8 (CH_2, C-12), 37.6 (CH, C-3′, 5′), 37.1 (CH_2, C-1), 36.8 (C, C-1′), 35.3 (CH_2, C-9′), 35.2 (C, C-10), 34.8 (CH, C-8), 34.7 (CH_2, C-4′), 32.7 (CH, C-20), 31.6 (CH_2, C-2), 29.1 (CH, C-25), 28.5 (CH_2, C-6), 27.9 (CH_2, C-15), 20.3 (CH_2, C-11), 18.5 (CH_3, C-27), 15.0 (CH_3, C-21), 13.4 (CH_3, C-18), 12.2 (CH_3, C-19). HRESIMS *m*/*z* [M+H]$^+$ 593.4682 (calcd for $C_{38}H_{61}N_2O_3$, 593.4677).

*4.3.9.2. (22R, 23S, 25R)-3β-N-[2′-((1′R,2′S,3′R,4′S)-3′-hydroxy-4,7,7-trimethylbicyclo [2.2.1]heptan-2′-yl)acetamide]-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**11**).* Following the general procedure, using isoborneol acetic acid (13 mg) and after purification, 6.9 mg of compound **11** (16%) was obtained as a white amorphous solid; $[\alpha]_D^{25}$: +11.8 (c 0.28, MeOH), IR (film) ν_{max}: 3284.2, 3083.6, 2927.4, 2869.6, 1633.4, 1554.3, 1446.4, 1388.5, 1295.9, 1238.1, 1079.9, 1014.4, 858.2, 734.8, 526.5 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 5.88 d (1H, *J* = 8.3 Hz, NH), 4.45 brc (1H, *J* = 8.4 Hz, H-16), 3.74 m (1H, H-3), 3.20 d (1H, *J* = 3.2 Hz, H-4′), 3.09 brd (1H, *J* = 11.6, 4.0 Hz, H_2-26a), 2.36 m (1H, H-3′), 2.26 m (2H, H-1′), 2.19 m (1H, H_2-26b), 2.06 d (1H, *J* = 10.0 Hz, H-22), 1.99 m (1H, H-25), 1.86 m (1H, H-20), 1.83 m (1H, H_2-24a), 1.83 m (1H, H-7a′), 1.82 m (1H, H_2-4a), 1.69 m (1H, H_2-1a), 1.63 m (1H, H_2-2a), 1.59 m (1H, H-2′), 1.57 m (2H, H_2-15), 1.57 m (1H, H-6a′), 1.53 m (1H, H-7a), 1.52 m (1H, H_2-11a), 1.36 m (1H, H-5), 1.32 m (1H, H-8), 1.30 m (1H, H-7b′), 1.26 m (1H, H_2-11b), 1.25 m (1H, H_2-4b), 1.24 m (2H, H_2-6), 1.20 m (1H, H-9), 1.20 m (1H, H_2-24b), 1.12 m (1H, H-6b′), 1.11 s (3H, H_3-10′), 1.05 m (1H, H_2-1b), 1.05 m (1H, H-14), 0.99 d (3H, *J* = 6.9 Hz, H_3-21), 0.94 m (1H, H-7b), 0.90 m (1H, H_2-2b), 0.89 s (3H, H_3-9′), 0.86 d (3H, *J* = 6.6 Hz, H_3-27), 0.85 s (3H, H_3-11′), 0.78 s (3H, H_3-19), 0.75 s (3H, H_3-18), 0.71 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 172.7 (CO), 95.5 (C, C-23), 85.7 (CH, C-4′), 74.0 (CH, C-16), 68.2 (CH, C-22), 60.4 (CH, C-14),

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54.6 (CH, C-5), 54.6 (CH, C-17), 54.3 (CH_2, C-26), 49.3 (CH, C-2′), 49.3 (C, C-5′), 48.9 (CH, C-3), 47.5 (C, C-8′), 46.0 (CH_2, C-24), 45.4 (CH, C-9), 45.2 (CH, C-3′), 41.8 (C, C-13), 39.0 (CH_2, C-4), 38.5 (CH_2, C-12), 38.4 (CH_2, C-1′), 37.2 (CH_2, C-1), 35.5 (C, C-10), 35.2 (CH_2, C-6′), 34.8 (CH, C-8), 34.5 (CH_2, C-7), 32.5 (CH, C-20), 31.7 (CH_2, C-2), 29.1 (CH, C-25), 28.8 (CH_2, C-7′), 28.3 (CH_2, C-6), 28.0 (CH_2, C-15), 20.2 (CH_2, C-11), 19.5 (CH_3, C-10′), 18.4 (CH_3, C-27), 15.0 (CH_3, C-21), 13.6 (CH_3, C-18), 12.3 (CH_3, C-19), 11.4 (CH_3, C-9′), 11.0 (CH_3, C-11′). HRESIMS m/z[M+H]$^+$ 625.4968 (calcd for $C_{39}H_{65}N_2O_4$, 625.4939).

*4.3.9.3. (22R, 23S, 25R)-3β-N-(1′H-pyrrole-2′-carboxamide)-22,26-imino-16β, 23-epoxy-5α-cholestan-23β-ol (**12**).* Following the general procedure, using pyrrole-2-carboxylic acid (7 mg) and after purification, 3.3 mg of compound **12** (9%) was obtained as a white amorphous solid; $[\alpha]_D^{25}$: +37.7 (c 0.06, MeOH), IR (film) ν_{max}: 3293.8, 2927.4, 2852.2, 1733.7, 1623.8, 1560.1, 1527.4, 1446.4, 1413.6, 1376.9, 1338.4, 1295.9, 1199.5, 1112.7, 1095.4, 1081.9, 1008.6, 873.6, 792.6, 738.6 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 6.86 brs (1H, H-5′), 6.45 brs (1H, H-3′), 6.18 dd (1H, J = 6.0, 2.8 Hz, H-4′), 4.43 brc (1H, J = 8.9 Hz, H-16), 3.86 m (1H, H-3), 3.04 brd (1H, J = 12.0 Hz, H_2-26a), 2.16 brt (1H, J = 12.0 Hz, H_2-26b), 2.01 m (1H, H-22), 1.95 m (1H, H-25), 1.85 m (1H, H-6), 1.81 m (1H, H-20), 1.80 m (1H, H_2-24a), 1.78 m (1H, H_2-4a), 1.68 m (1H, H_2-1a), 1.64 m (2H, H_2-2), 1.62 m (2H, H_2-7), 1.54 m (2H, H_2-15), 1.47 m (2H, H_2-11), 1.35 m (1H, H-5), 1.33 m (1H, H-6), 1.30 m (1H, H-8), 1.22 m (1H, H_2-4b), 1.22 m (1H, H-9), 1.18 m (1H, H_2-24b), 1.05 m (1H, H_2-1b), 1.05 m (1H, H-14), 0.95 d (3H, J = 6.6 Hz, H_3-21), 0.83 d (3H, J = 6.9 Hz, H_3-27), 0.78 s (3H, H_3-19), 0.72 s (3H, H_3-18), 0.71 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 160.2 (CO), 130.3 (C, C-2′), 120.9 (CH, C-5′), 109.7 (CH, C-4′), 108.0 (CH, C-3′), 95.7 (C, C-23), 74.3 (CH, C-16), 68.6 (CH, C-22), 60.3 (CH, C-14), 54.6 (CH, C-5), 54.5 (CH_2, C-26), 54.3 (CH, C-17), 48.6 (CH, C-3), 45.9 (CH_2, C-24), 45.1 (CH, C-9), 41.6 (CH_2, C-12), 39.1 (C, C-13), 39.0 (CH_2, C-4), 37.1 (CH_2, C-1), 35.4 (CH_2, C-7), 35.2 (C, C-10), 34.8 (CH, C-8), 32.7 (CH, C-20), 31.5 (CH_2, C-2), 29.4 (CH, C-25), 28.8 (CH_2, C-6), 28.1 (CH_2, C-15), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 14.9 (CH_3, C-21), 13.4 (CH_3, C-18), 11.9 (CH_3, C-19). HRESIMS m/z [M+H]$^+$ 524.3872 (calcd for $C_{32}H_{50}N_3O_3$, 524.3847).

*4.3.9.4. (22R, 23S, 25R)-3β-N-(1′H-imidazole-4′-carboxamide)-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**13**).* Following the general procedure, using 4-imidazolecarboxylic acid (7 mg) and after purification, 3.5 mg of compound **13** (10%) was obtained as a white amorphous solid; $[\alpha]_D^{25}$: +25.9 (c 0.10, MeOH), IR (film) ν_{max}: 3311.2, 2925.5, 2850.3, 1735.6, 1648.8, 1538.9, 1452.1, 1376.9, 1083.8, 1027.9, 759.8, 536.1 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 8.22 dd (1H, J = 5.0, 1.4 Hz, H-2′), 6.52 dd (1H, J = 5.0, 1.4 Hz, H-5′), 4.46 brc (1H, J = 7.9 Hz, H-16), 3.75 m (1H, H-3), 3.04 m (1H, H_2-26a), 2.15 m (1H, H_2-26b), 2.01 m (1H, H-22), 1.96 m (1H, H-25), 1.82 m (1H, H_2-4a), 1.82 m (1H, H_2-24a), 1.68 m (1H, H_2-1a), 1.64 m (1H, H_2-2a), 1.58 m (2H, H_2-7), 1.57 m (2H, H_2-15), 1.49 m (1H, H_2-11a), 1.38 m (1H, H-5), 1.34 m (1H, H-8), 1.31 m (1H, H_2-11b), 1.26 m (2H, H_2-6), 1.24 m (1H, H_2-4b), 1.22 m (1H, H_2-24b), 1.11 m (1H, H-9), 1.07 m (1H, H-14), 1.01 m (1H, H_2-1b), 0.96 d (3H, J = 6.5 Hz, H_3-21), 0.90 m (1H, H_2-2b), 0.88 m (1H, H-20), 0.86 d (3H, J = 6.4 Hz, H_3-27), 0.78 s (3H, H_3-19), 0.74 s (3H, H_3-18), 0.70 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 154.9 (CO), 148.4 (CH, C-2′), 122.0 (C, C-4′), 106.4 (CH, C-5′), 98.2 (C, C-23), 74.2 (CH, C-16), 68.5 (CH, C-22), 60.3 (CH, C-14), 54.7 (CH_2, C-26), 54.6 (CH, C-5), 54.6 (CH, C-17), 48.2 (CH, C-3), 45.4 (CH, C-9), 41.7 (C, C-13), 38.8 (CH_2, C-4), 46.0 (CH_2, C-24), 36.9 (CH_2, C-1), 34.9 (CH, C-8), 35.2 (CH_2, C-7), 29.8 (CH, C-25), 31.5 (CH_2, C-2), 35.1 (C, C-10), 29.6 (CH_2, C-6), 29.6 (CH, C-20), 28.2 (CH_2, C-15), 18.4 (CH_3, C-27), 20.1 (CH_2, C-11), 13.3 (CH_3, C-18), 15.0 (CH_3, C-21), 12.0 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 525.3833 (calcd for $C_{31}H_{49}N_4O_3$, 525.3799).

*4.3.9.5. (22R, 23S, 25R)-3β-N-(1′H-Indole-2′-carboxamide)-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**14**).* Following the general procedure, using 2-indolecarboxylic acid (10 mg) and after purification, 6.0 mg of compound **14** (15%) was obtained as a white amorphous solid; $[\alpha]_D^{25}$: +51.8 (c 0.23, MeOH), IR (film) ν_{max}: 3288.0, 2925.5, 2854.1, 1644.9, 1558.2, 1455.9, 1376.9, 1313.3, 997.0 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 9.30 s (1H, NH-1′), 7.63 d (1H, J = 8.0 Hz, H-4′), 7.43 dd (1H, J = 8.0, 0.7 Hz, H-7′), 7.28 ddd (1H, J = 8.0, 7.0, 1.0 Hz, H-6′), 7.13 ddd (1H, J = 8.0, 7.0, 1.0 Hz, H-5′), 6.78 brd (1H, J = 1.3 Hz, H-3′), 6.00 d (1H, J = 6.0 Hz, NH), 4.47 m (1H, H-16), 3.97 m (1H, H-3), 3.05 m (1H, H_2-26a), 2.18 m (1H, H_2-26b), 2.02 brd (1H, J = 11.0 Hz, H-22), 1.97 m (1H, H-7a), 1.96 m (1H, H-25), 1.86 m (1H, H_2-4a), 1.82 m (1H, H-20), 1.81 m (1H, H_2-24a), 1.75 m (1H, H_2-1a), 1.64 m (1H, H_2-2a), 1.58 m (2H, H_2-15), 1.50 m (1H, H_2-11a), 1.40 m (1H, H-7b), 1.38 m (1H, H-5), 1.35 m (1H, H-8), 1.32 m (1H, H_2-11b), 1.32 m (2H, H_2-12), 1.28 m (2H, H_2-6), 1.27 m (1H, H-9), 1.26 m (1H, H_2-4b), 1.21 m (1H, H_2-24b), 1.09 m (1H, H_2-1b), 1.09 m (1H, H-14), 0.96 d (3H, J = 6.4 Hz, H_3-21), 0.90 m (1H, H_2-2b), 0.87 d (3H, J = 6.4 Hz, H_3-27), 0.84 s (3H, H_3-19), 0.76 s (3H, H_3-18), 0.75 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 160.9 (CO), 136.3 (C, C-2′), 131.0 (C, C-8′), 127.2 (C, C-9′), 124.4 (CH, C-6′), 121.7 (CH, C-4′), 120.6 (CH, C-5′), 111.8 (CH, C-7′), 100.9 (CH, C-3′), 96.1 (C, C-23), 74.3 (CH, C-16), 68.5 (CH, C-22), 60.4 (CH, C-14), 54.7 (CH, C-5), 54.6 (CH, C-17), 54.6 (CH_2, C-26), 49.1 (CH, C-3), 45.8 (CH_2, C-24), 45.5 (CH, C-9), 41.6 (C, C-13), 39.0 (CH_2, C-4), 39.0 (CH_2, C-12), 36.9 (CH_2, C-1), 34.9 (CH, C-8), 35.5 (C, C-10), 32.6 (CH, C-20), 31.7 (CH_2, C-2), 29.7 (CH, C-25), 28.7 (CH_2, C-7), 28.5 (CH_2, C-6), 28.0 (CH_2, C-15), 20.3 (CH_2, C-11), 18.7 (CH_3, C-27), 15.1 (CH_3, C-21), 13.6 (CH_3, C-18), 12.3 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 574.4024 (calcd for $C_{36}H_{52}N_3O_3$, 574.4003).

*4.3.10. (22R, 23S, 25R)-3β-amino-N′-methyl-22,26-imino-16β, 23-epoxy-5α-cholestan-23β-ol (**15**)*

To a solution of solanocapsine (30.0 mg, 0.070 mmol) in DMF (2 mL), methyl iodide (13 μL, 0.140 mmol) and 20 mg of K_2CO_3 (0.140 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. Diethyl ether (10 mL) and aqueous Na_2CO_3 solution (1 M, 10 mL) were added, the mixture was afterwards stirred. The organic phase was separated, and the aqueous phase was extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and filtered. Once the solvent was removed, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (8:2) to obtain 9 mg of compound **15** (29%) as a light yellow amorphous solid; $[\alpha]_D^{25}$: +40.5 (c 0.39, MeOH), IR (film) ν_{max}: 3374.8, 2927.4, 2852.2, 1662.3, 1454.0, 1378.9, 1197.6, 1079.9, 997.0, 962.3, 667.3 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 4.44 m (1H, H-16), 2.87 m (1H, H_2-26a), 2.71 m (1H, H-3), 2.44 s (3H, N-Me), 2.24 m (1H, H_2-26b), 2.23 brd (1H, J = 11.0 Hz, H-22), 2.22 m (1H, H-25), 2.03 m (1H, H-20), 1.85 m (1H, H_2-4a), 1.70 m (1H, H_2-24a), 1.69 m (1H, H_2-1a), 1.69 m (2H, H-2), 1.55 m (2H, H_2-15), 1.48 m (1H, H_2-11a), 1.46 m (1H, H_2-12a), 1.38 m (1H, H-5), 1.33 m (1H, H-8), 1.31 m (1H, H_2-11b), 1.29 m (2H, H_2-7), 1.28 m (1H, H_2-24b), 1.25 m (1H, H_2-4b), 1.25 m (2H, H_2-6), 1.16 m (1H, H_2-12b), 1.11 m (1H, H-9), 1.09 m (1H, H-14), 0.97 m (1H, H_2-1b), 0.97 d (3H, J = 6.4 Hz, H_3-21), 0.83 d (3H, J = 6.4 Hz, H_3-27), 0.79 s (3H, H_3-19), 0.77 s (3H, H_3-18), 0.70 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 97.9 (C, C-23), 74.1 (CH, C-16), 69.8 (CH, C-22), 62.5 (CH_2, C-26), 61.9 (CH, C-14), 54.8 (CH, C-5), 54.6 (CH, C-17), 50.9 (CH, C-3), 47.4 (CH_2, C-24), 45.5 (CH, C-9), 42.1 (C, C-13), 39.2 (CH_2, C-4), 38.4 (CH_2, C-12), 37.2 (CH_2, C-1), 35.9 (CH_3, N-Me), 35.4 (C, C-10), 34.7 (CH, C-8), 31.9 (CH_2, C-7), 31.5 (CH_2, C-2), 31.1 (CH, C-20), 29.6 (CH_2, C-6), 28.2 (CH_2,

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C-15), 23.6 (CH, C-25), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 15.1 (CH_3, C-21), 13.5 (CH_3, C-18), 12.1 (CH_3, C-19). HRESIMS *m/z*[M+H]$^+$ 445.3808 (calcd for $C_{28}H_{49}N_2O_2$, 445.3789).

*4.3.11. (22R, 23S, 25R)-3β-N-[2′-(methyl)anthracene-9′,10′-dione] amino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**16**)*

To a solution of solanocapsine (35.0 mg, 0.080 mmol) in DMF (2 mL), 2-(chloromethyl)-antraquinone (21 mg, 0.080 mmol) and 9 mg of K_2CO_3 (0.080 mmol) were added. The reaction mixture was stirred at 60 °C for 24 h. Additional 0.080 mmol of triethylamine (11 μL) was then added, and the reaction mixture was left for 1 h. Diethyl ether (10 mL) and water (10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and filtered. Once the solvent was removed, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (9.8:0.2) to obtain 17.6 mg of compound **16** (33%) as a yellow amorphous solid; $[\alpha]_D^{25}$: +3.5 (c 0.97, MeOH), IR (film) ν_{max}: 3386.4, 2929.3, 2854.1, 1729.8, 1675.8, 1592.9, 1455.9, 1324.9, 1290.1, 1078.0, 931.5, 736.7, 711.6 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 8.25 m (2H, H-5′, 9′), 8.21 m (1H, H-6′), 8.17 m (1H, H-2′), 7.74 m (2H, H-7′, 8′), 7.73 m (1H, H-4′), 4.38 brc (1H, *J* = 8.0 Hz, H-16), 3.94 brs (2H, H_2-1′), 2.98 brd (1H, *J* = 8.0 Hz, H_2-26a), 2.46 m (1H, H-3), 2.11 m (1H, H_2-26b), 1.95 m (1H, H-22), 1.89 m (1H, H-25), 1.76 m (1H, H_2-24a), 1.73 m (1H, H_2-12a), 1.73 m (1H, H-20), 1.72 m (1H, H_2-1a), 1.63 m (1H, H_2-4a), 1.55 m (1H, H_2-2a), 1.50 m (2H, H_2-15), 1.49 m (2H, H_2-7), 1.41 m (1H, H_2-11a), 1.30 m (1H, H-5), 1.27 m (1H, H-8), 1.25 m (1H, H_2-11b), 1.20 m (2H, H_2-6), 1.16 m (1H, H_2-12b), 1.13 m (1H, H_2-24b), 1.03 m (1H, H-9), 1.01 m (1H, H_2-1b), 1.00 m (1H, H-14), 0.89 m (1H, H_2-4b), 0.89 m (3H, H_3-21), 0.80 m (1H, H_2-2b), 0.79 m (3H, H_3-27), 0.73 s (3H, H_3-19), 0.67 s (3H, H_3-18), 0.62 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 183.5 (CO-10′), 183.1 (CO-11′), 147.9 (C, C-3′), 141.8 (C, C-15′), 133.9 (CH, C-4′), 134.1 (CH, C-7′), 134.0 (CH, C-8′), 132.7 (C, C-12′), 130.7 (C, C-13′), 128.8 (C, C-14′), 127.4 (CH, C-5′), 127.3 (CH, C-6′), 127.2 (CH, C-9′), 126.3 (CH, C-2′), 96.4 (C, C-23), 74.5 (CH, C-16), 68.6 (CH, C-22), 60.6 (CH, C-14), 56.9 (CH, C-3), 54.8 (CH, C-5), 54.7 (CH_2, C-26), 54.6 (CH, C-17), 50.4 (CH_2, C-1′), 45.8 (CH_2, C-24), 45.2 (CH, C-9), 41.5 (C, C-13), 38.9 (CH_2, C-12), 37.1 (CH_2, C-4), 36.7 (CH_2, C-1), 36.5 (C, C-10), 35.3 (CH_2, C-7), 34.7 (CH, C-8), 32.9 (CH, C-20), 31.8 (CH_2, C-2), 29.7 (CH, C-25), 28.5 (CH_2, C-6), 28.1 (CH_2, C-15), 20.2 (CH_2, C-11), 18.4 (CH_3, C-27), 14.9 (CH_3, C-21), 13.4 (CH_3, C-18), 12.1 (CH_3, C-19). HRESIMS *m/z*[M+H]$^+$ 651.4183 (calcd for $C_{42}H_{55}N_2O_4$, 651.4156).

*4.3.12. (22R, 23S, 25R)-3β-N-[(6′-bromobenzo[d] [1′,3′]dioxol-5′-yl) methan]amine-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**17**)*

To a solution of solanocapsine (30.0 mg, 0.070 mmol) in CH_3CN: $CHCl_3$ (1:1, 3 mL), bromomethyl-dioxolane (21 mg, 0.070 mmol) and 0.070 mmol of triethylamine (8 μL) were added. The reaction mixture was stirred at room temperature for 24 h. CH_2Cl_2 (10 mL) and water (10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and filtered. After removing the solvent, the residue was purified by preparative-TLC using CH_2Cl_2: MeOH (9:1) to obtain 9.9 mg of compound **17** (22%) as a light yellow amorphous solid; $[\alpha]_D^{25}$: +11.9 (c 0.43, MeOH), IR (film) ν_{max}: 3351.7, 2925.5, 2850.3, 1492.6, 1477.2, 1376.9, 1234.2, 1112.7, 1037.5, 933.4, 863.9, 831.2, 736.7 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz):6.99 s (1H, H-7′), 6.94 s (1H, H-4′), 5.94 s (1H, H_2-8′), 4.46 m (1H, H-16), 3.81 s (2H, H_2-1′), 3.06 dd (1H, *J* = 11.5, 4.4 Hz, H_2-26a), 2.51 m (1H, H-3), 2.18 t (1H, *J* = 11.5 Hz, H_2-26b), 2.02 m (1H, H-22), 1.97 m (1H, H-25), 1.83 m (1H, H_2-24a), 1.82 m (1H, H-20), 1.81 m (1H, H_2-4a), 1.80 m (1H, H-7a), 1.69 m (1H, H_2-1a), 1.64 m (1H, H_2-2a), 1.57 m (2H, H_2-15), 1.55 m (1H, H_2-12a), 1.51 m (1H, H_2-11a), 1.36 m (1H, H-5), 1.34 m (1H, H-7b), 1.34 m (1H, H-8), 1.34 m (1H, H_2-11b), 1.27 m (2H, H_2-6), 1.24 m (1H, H_2-4b), 1.21 m (1H, H_2-12b), 1.21 m (1H, H_2-24b), 1.11 m (1H, H-9), 1.07 m (1H, H-14), 0.97 m (1H, H_2-1b), 0.97 d (3H, *J* = 6.4 Hz, H_3-21), 0.89 m (1H, H_2-2b), 0.87 d (3H, *J* = 6.5 Hz, H_3-27), 0.79 s (3H, H_3-19), 0.75 s (3H, H_3-18), 0.70 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 146.9 (C, C-6′), 147.5 (C, C-5′), 132.1 (C, C-3′), 114.2 (C, C-2′), 112.7 (CH, C-7′), 110.3 (CH, C-4′), 101.7 (CH_2, C-8′), 95.1 (C, C-23), 74.3 (CH, C-16), 68.7 (CH, C-22), 60.5 (CH, C-14), 56.4 (CH, C-3), 54.9 (CH, C-17), 54.8 (CH, C-5), 54.7 (CH_2, C-26), 50.6 (CH_2, C-1′), 46.0 (CH_2, C-24), 45.4 (CH, C-9), 41.9 (C, C-13), 39.2 (CH_2, C-4), 37.2 (CH_2, C-1), 36.1 (C, C-10), 35.6 (CH_2, C-12), 34.8 (CH, C-8), 32.9 (CH, C-20), 31.8 (CH_2, C-2), 29.7 (CH, C-25), 28.8 (CH_2, C-7), 28.6 (CH_2, C-6), 28.2 (CH_2, C-15), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 15.0 (CH_3, C-21), 13.5 (CH_3, C-18), 12.2 (CH_3, C-19). HRESIMS *m/z*[M+H]$^+$ 643.3137 (calcd for $C_{35}H_{52}BrN_2O_4$, 643.3105).

*4.3.13. (22R, 23S, 25R)-3β-N-(phthalimidopropyl)amino-22, 26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**18**)*

To a solution of solanocapsine (33.0 mg, 0.076 mmol) in DMF (3 mL), *N*-bromopropylphthalimide (21 mg, 0.078 mmol) and 11 mg of K_2CO_3 (0.076 mmol) were added. The reaction mixture was stirred at 80 °C for 24 h. AcOEt (10 mL) and aqueous Na_2CO_3 solution (1 M, 10 mL) were added. The organic phase was separated, and the aqueous phase was extracted with AcOEt (3 × 10 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and filtered. Once the solvent was removed, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (8:2) to obtain 9.9 mg of compound **18** (23%) as a light yellow amorphous solid; $[\alpha]_D^5$: +12.3 (c 0.55, MeOH), IR (film) ν_{max}: 3386.4, 2927.4, 2852.2, 1712.5, 1650.8, 1558.2, 1540.8, 1450.2, 1394.3, 1081.9, 721.3, 669.2 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 7.83 m (2H, H-7′, H-10′), 7.71 m (2H, H-8′, H-9′), 4.47 brc (1H, *J* = 8.7 Hz, H-16), 3.75 m (2H, H_2-3′), 3.16 m (1H, H-3), 3.06 brdd (1H, *J* = 12.0, 4.0 Hz, H_2-26a), 2.73 brt (2H, *J* = 8.0 Hz, H_2-1′), 2.18 m (1H, H_2-26b), 2.02 m (1H, H-22), 1.95 m (1H, H-25), 1.93 m (1H, H_2-2a′), 1.83 m (1H, H_2-24a), 1.82 m (1H, H_2-4a), 1.81 m (1H, H-20), 1.78 m (1H, H_2-2b′), 1.69 m (1H, H_2-1a), 1.63 m (1H, H_2-2a), 1.58 m (2H, H_2-15), 1.49 m (1H, H_2-11a), 1.36 m (1H, H-5), 1.32 m (1H, H-8), 1.29 m (1H, H_2-11b), 1.27 m (2H, H_2-6), 1.24 m (1H, H_2-4b), 1.22 m (1H, H_2-24b), 1.10 m (1H, H-9), 1.09 m (1H, H-14), 0.96 d (3H, *J* = 6.6 Hz, H_3-21), 0.93 m (1H, H_2-1b), 0.88 m (1H, H_2-2b), 0.87 d (3H, *J* = 6.4 Hz, H_3-27), 0.78 s (3H, H_3-19), 0.76 s (3H, H_3-18), 0.69 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 168.5 (CO, C-4′, 5′), 133.8 (CH, C-8′, 9′), 132.4 (C, C-6′, 11′), 123.1 (CH, C-7′, 10′), 96.1 (C, C-23), 74.5 (CH, C-16), 68.9 (CH, C-22), 60.3 (CH, C-14), 54.9 (CH_2, C-26), 54.6 (CH, C-17), 54.4 (CH, C-5), 46.5 (CH, C-3), 46.1 (CH_2, C-24), 45.3 (CH, C-9), 43.4 (CH_2, C-1′), 42.0 (C, C-13), 39.0 (CH_2, C-4), 37.2 (CH_2, C-1), 35.7 (CH_2, C-3′), 34.6 (CH, C-8), 32.9 (CH, C-20), 31.7 (CH_2, C-2), 36.2 (C, C-10), 29.9 (CH, C-25), 28.4 (CH_2, C-6), 28.2 (CH_2, C-15), 28.2 (CH_2, C-2′), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 14.8 (CH_3, C-21), 13.4 (CH_3, C-18), 12.1 (CH_3, C-19). HRESIMS *m/z*[M+H]$^+$ 618.4297 (calcd for $C_{38}H_{56}N_3O_4$, 618.4265).

*4.3.14. (25R)-N,N′-diacetyi-3β-amino-22,26-imino-16β,23-epoxy-5α-22,23-cholestene (**19**)*

A mixture of solanocapsine (82 mg, 0.190 mmol) and acetic anhydride (0.25 mL, 2.600 mmol) in pyridine (4 mL) was stirred at room temperature for 48 h. After this time, the mixture was heated in a bath at 50 °C and diluted with $CHCl_3$ (10 mL). The resulting solution was washed sequentially with aqueous (10%) acetic acid, Na_2CO_3 (1 M) and water. The combined organic phases were dried with anhydrous Na_2SO_4 and filtered. After removing the solvent, the orange residue was purified by column chromatogra-

phy using CH_2Cl_2: MeOH (10:0 to 8:2) as elution solvent to obtain 13.6 mg of compound **7** (14%) and 10.2 mg of compound **19** (11%) as a light pink amorphous solid; $[\alpha]_D^{25}$: +20.8 (c 0.31, MeOH), IR (film) ν_{max}: 3305.4, 3077.8, 2927.4, 2852.2, 1648.8, 1635.3, 1542.8, 1444.4, 1405.9, 1373.1, 1249.7, 1191.8, 1085.7, 956.5, 732.8, 609.4, 457.1 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): mixture of interconverting rotational isomers with respect to the N-formyl bond: 6.46 brs (1H, NH), 4.54 dd (1H, *J* = 12.0, 4.0 Hz, H_2-26a, minor isomer), 4.04 td (1H, *J* = 8.0, 4.0 Hz, H-16, major isomer), 3.96 td (1H, *J* = 8.0, 4.0 Hz, H-16, minor isomer), 3.71 m (1H, H-3), 3.64 dd (1H, *J* = 12.0, 4.0 Hz, H_2-26a, major isomer), 3.15 m (1H, H-20, major isomer), 2.68 m (1H, H-20, minor isomer), 2.64 m (1H, H_2-26b, major isomer), 2.23 m (1H, H_2-2a), 2.23 m (1H, H_2-24a), 2.10 s (3H, H_3-2′), 2.07 m (1H, H_2-26b, minor isomer), 2.06 s (3H, H_3-2″, major isomer), 2.02 s (3H, H_3-2″, minor isomer), 1.90 m (1H, H-25), 1.77 m (1H, H_2-12a), 1.77 m (1H, H_2-24b), 1.76 m (1H, H_2-2b), 1.73 m (1H, H-15a, minor isomer), 1.64 m (1H, H_2-1a), 1.63 m (1H, H-15a, major isomer), 1.58 m (1H, H_2-4a), 1.58 m (1H, H-7a), 1.44 m (1H, H_2-11a), 1.35 m (1H, H-5), 1.30 m (1H, H-8), 1.26 m (1H, H_2-11b), 1.22 m (2H, H_2-6), 1.22 m (1H, H_2-12b), 1.13 m (1H, H_2-4b), 1.13 m (1H, H-9), 1.07 m (1H, H-15b, minor isomer), 1.00 m (1H, H-15b, major isomer), 0.99 m (1H, H-14), 0.97 m (1H, H_2-1b), 0.96 d (3H, *J* = 6.8 Hz, H_3-27, major isomer), 0.90 d (3H, *J* = 6.5 Hz, H_3-27, minor isomer), 0.89 d (3H, *J* = 6.5 Hz, H_3-21, minor isomer), 0.85 m (1H, H-7b), 0.76 d (3H, *J* = 6.8 Hz, H_3-21, major isomer), 0.73 s (3H, H_3-19), 0.68 s (3H, H_3-18), 0.67 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz):171.2 (C, CO-1″, major isomer), 170.3 (C, CO-1″), 169.5 (C, CO-1′), 150.0 (C, C-23, minor isomer), 146.0 (C, C-23, major isomer), 120.0 (C, C-22, minor isomer), 117.0 (C, C-22, major isomer), 80.5 (CH, C-16, minor isomer), 80.3 (CH, C-16, major isomer), 58.6 (CH, C-14), 54.8 (CH, C-5), 54.6 (CH, C-17), 54.2 (CH2, C-26, major isomer), 50.5 (CH2, C-26, minor isomer), 49.2 (CH, C-3), 45.5 (CH, C-9), 38.6 (CH_2, C-12), 37.0 (CH_2, C-1), 34.8 (CH_2, C-4), 34.8 (CH, C-8), 42.0 (C, C-13), 33.8 (CH_2, C-24), 35.5 (C, C-10), 34.1 (CH, C-20, minor isomer), 34.0 (CH_2, C-2), 31.9 (CH, C-20, major isomer), 31.5 (CH_2, C-7), 29.9 (CH, C-25, major isomer), 29.3 (CH, C-25, minor isomer), 28.8 (CH_2, C-15, major isomer), 28.2 (CH_2, C-6), 28.0 (CH2, C-15, minor isomer), 22.7 (CH_3, C-2″), 22.1 (CH_3, C-2′), 20.4 (CH_2, C-11), 19.3 (CH3, C-27, major isomer), 19.0 (CH3, C-27, minor isomer), 16.2 (CH3, C-21, major isomer), 16.0 (CH3, C-21, minor isomer), 13.3 (CH_3, C-18), 12.4 (CH_3, C-19). HRESIMS *m*/*z*[M+H]$^+$ 497.3757 (calcd for $C_{31}H_{49}N_2O_3$, 497.3738).

4.3.15. (25R)-N,N′-diacetyi-3β-amino-22,26-imino-16β,23-oxido-5α-cholestan-22,23 dione (**20**)

18 mg of compound **7** (0.035 mmol) was heated to reflux in acetic acid (0.3 mL, 1.750 mmol) for 2 h. After cooling the solution at room temperature, CrO_3 (11 mg, 0.110 mmol) was added. The reaction mixture was stirred additionally for 2 h. The excess of CrO_3 was then eliminated with aqueous Na_2SO_3 solution (1 M), and extracted with Et_2O. The organic phase was washed with aqueous Na_2CO_3 solution (1 M), dried with anhydrous Na_2SO_4 and filtered. Once the solvent was removed, 11.2 mg of compound **20** (59%) was obtained as a white amorphous solid; $[\alpha]_D^{25}$: −18.8 (c 0.50, MeOH), IR (film) ν_{max}: 3502.1, 3305.4, 3075.9, 2929.3, 2854.1, 1725.9, 1704.8, 1650.8, 1542.8, 1444.4, 1369.2, 1326.8, 1286.3, 1157.1, 1035.6, 734.7, 605.5, 501.4 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 6.30 brs (1H, NH), 5.01 td (1H, *J* = 8.0, 4.0 Hz, H-16), 4.48 dd (1H, *J* = 16.0, 12.0 Hz, H_2-26a), 3.69 m (1H, H-3), 3.56 dc (1H, *J* = 12.0, 8.0 Hz, H-20), 2.93 dd (1H, *J* = 16.0, 4.0 Hz, H_2-26b), 2.52 m (1H, H-25), 2.27 s (3H, H_3-2″), 2.22 brd (1H, *J* = 12.0 Hz, H_2-24a), 2.04 m (1H, H_2-24b), 1.99 s (3H, H_3-2′), 1.94 m (1H, H-17), 1.91 brd (1H, *J* = 8.0 Hz, H_2-4a), 1.65 m (1H, H_2-1a), 1.54 m (1H, H_2-2a), 1.52 m (2H, H_2-15), 1.51 m (1H, H-7a), 1.49 m (1H, H_2-11a), 1.35 m (1H, H_2-4b), 1.35 m (1H, H-5), 1.29 m (1H, H_2-11b), 1.26 m (1H, H-8), 1.19 m (2H, H_2-6), 1.15 m (1H, H-9), 1.15 d (3H, *J* = 6.6 Hz, H_3-21), 1.13 m (1H, H_2-2b), 0.99 m (1H, H_2-1b), 0.99 d (3H, *J* = 6.7 Hz, H_3-27), 0.83 m (1H, H-7b), 0.71 s (3H, H_3-19), 0.68 m (1H, H-14), 0.68 s (3H, H_3-18). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 182.0 (CO, C-22), 174.8 (C, CO-1″), 174.2 (CO, C-23), 170.1 (C, CO-1′), 79.7 (CH, C-16), 61.0 (CH, C-17), 53.9 (CH, C-14), 53.8 (CH, C-5), 49.4 (CH, C-3), 49.2 (CH_2, C-26), 45.4 (CH, C-9), 43.2 (CH, C-20), 40.6 (CH_2, C-24), 39.0 (C, C-13), 38.9 (CH_2, C-4), 36.9 (CH_2, C-1), 35.4 (C, C-10), 34.9 (CH_2, C-2), 34.9 (CH, C-8), 32.7 (CH, C-25), 31.6 (CH_2, C-7), 28.4 (CH_2, C-15), 27.9 (CH_2, C-6), 24.4 (CH_3, C-2″), 22.7 (CH_3, C-2′), 20.3 (CH_2, C-11), 19.4 (CH_3, C-27), 15.6 (CH_3, C-21), 12.7 (CH_3, C-18), 12.2 (CH_3, C-19). HRESIMS *m*/*z*[M+H]$^+$ 529.3652 (calcd for $C_{31}H_{48}N_2O_5$, 529.3636).

4.3.16. (22R, 23S, 25R)-3β-N-[(1′R, 2′R, 3′S, 5′S)-3′-hydroxy-8′-methyl-8-azabicyclo[3.2.1] octane-2′-carboxamide]-22,26-imino-16β, 23-epoxy-5α-cholestan-23β-ol (**21**)

To a solution of ecgonine (20.8 mg, 0.112 mmol) and DMAP (3.0 mg, 0.020 mmol) in CH_2Cl_2 (3 mL), solanocapsine (40.0 mg, 0.093 mmol) was added. *N,N′*-dicyclohexylcarbodiimide (DCC, 20 mg, 0.093 mmol) was added to the reaction mixture to 0 °C and stirred for 15 min and then for 5 h at 20 ° C. Then, the precipitate was filtrated and dried with anhydrous Na_2SO_4. Once the solvent was removed, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (8:2) to obtain 8.9 mg of compound **21** (16%) as a light yellow amorphous solid; $[\alpha]_D^{25}$: +9.9 (c 0.29, MeOH), IR (film) ν_{max}: 3286.1, 2929.3, 2854.1, 1621.8, 1558.2, 1450.2, 1371.1, 1348.0, 1259.3, 1245.8, 1081.9, 890.9, 730.9, 669.2 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 4.46 m (1H, H-16), 4.06 dt (1H, *J* = 12.3, 6.8 Hz, H-3′), 3.36 m (1H, H-1′), 3.27 m (1H, H-3), 3.25 m (1H, H-5′), 3.06 m (1H, H_2-26a), 2.65 brs (1H, H-2′), 2.29 s (3H, H_3-8′), 2.19 m (1H, H_2-26b), 2.11 m (1H, H_2-7a′), 2.06 m (1H, H_2-6a′), 2.03 m (1H, H-22), 1.97 m (1H, H-25), 1.93 m (1H, H_2-4a′), 1.83 m (1H, H_2-24a), 1.81 m (1H, H_2-4a), 1.81 m (1H, H-20), 1.80 m (1H, H-7a), 1.80 m (1H, H_2-6b′), 1.73 m (1H, H_2-4b′), 1.72 m (1H, H_2-1a), 1.68 m (1H, H_2-7b′), 1.63 m (1H, H_2-2a), 1.56 m (2H, H_2-15), 1.48 m (1H, H_2-11a), 1.35 m (1H, H-5), 1.34 m (1H, H-8), 1.33 m (1H, H_2-11b), 1.32 m (2H, H_2-12), 1.29 m (1H, H-7b), 1.26 m (2H, H_2-6), 1.22 m (1H, H_2-4b), 1.20 m (1H, H_2-24b), 1.12 m (1H, H-9), 1.06 m (1H, H-14), 0.99 m (1H, H_2-1b), 0.97 d (3H, *J* = 6.2 Hz, H_3-21), 0.88 m (1H, H_2-2b), 0.87 d (3H, *J* = 6.5 Hz, H_3-27), 0.83 s (3H, H_3-19), 0.75 s (3H, H_3-18), 0.71 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 171.9 (CO), 95.9 (C, C-23), 74.1 (CH, C-16), 68.4 (CH, C-22), 60.6 (CH, C-5′), 60.5 (CH, C-14), 63.2 (CH, C-1′), 62.1 (CH, C-3′), 54.7 (CH, C-17), 54.6 (CH_2, C-26), 54.5 (CH, C-), 53.6 (CH, C-3), 52.0 (CH, C-2′), 45.9 (CH_2, C-24), 45.4 (CH, C-9), 41.7 (C, C-13), 40.0 (CH_3, C-8′), 39.5 (CH_2, C-12), 39.2 (CH_2, C-4), 38.7 (CH_2, C-4′), 37.2 (CH_2, C-1), 35.6 (C, C-10), 34.7 (CH, C-8), 32.7 (CH_2, C-7), 32.6 (CH, C-20), 31.6 (CH_2, C-2), 29.6 (CH, C-25), 28.9 (CH_2, C-6), 28.1 (CH_2, C-15), 25.2 (CH_2, C-7′), 24.8 (CH_2, C-6′), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 15.1 (CH_3, C-21), 13.6 (CH_3, C-18), 12.2 (CH_3, C-19). HRESIMS *m*/*z*[M+H]$^+$ 598.4541 (calcd for $C_{36}H_{60}N_3O_4$, 598.4578).

4.3.17. (22R, 23S, 25R)-3β-N-(O-6′-quinidine-5′-pentanoate)amino-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**22**)

To a solution of 300 mg quinidine sulfate (0.760 mmol) in DMF (3 mL), 5-bromovaleryl chloride (150 μL, 1.128 mmol) and DMAP (93 mg, 0.760 mmol) were added. This mixture was stirred at room temperature for 2 h. Afterwards, Et_2O (10 mL) and H_2O (10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and filtered. After removing the solvent, 75 mg of quinidine bromovaleryl ester was obtained (40%). After that 35 mg of this product (0.070 mmol) was redissolved in DMF (2 mL), solanocapsine (30.0 mg, 0.070 mmol) and *t*-BuOK (8.0 mg,

0.070 mmol) were added. The reaction mixture was stirred for 24 h at room temperature. Et_2O (10 mL) and aqueous Na_2CO_3 solution (10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with diethyl ether (3 × 15 mL) and dried with anhydrous Na_2SO_4. Once the solvent was removed, the residue was purified by preparative-TLC using CH_2Cl_2:MeOH (9:1) to obtain 9.1 mg of compound **22** (16%) as a light pink amorphous solid; $[\alpha]_D^{25}$: +156.7 (c 0.31, MeOH), IR (film) ν_{max}: 3316.9, 3070.1, 2931.3, 2869.6, 1729.8, 1666.2, 1621.8, 1509.9, 1454.1, 1241.9, 1228.4, 1106.9, 1027.9, 998.9, 916.0, 831.2, 734.8, 640.3, 464.8 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.70 d (1H, *J* = 4.5 Hz, H-9′), 7.98 d (1H, *J* = 9.3 Hz, H-11), 7.56 d (1H, *J* = 4.4 Hz, H-8′), 7.29 dd (1H, *J* = 9.3, 2.6 Hz, H-12′), 7.20 d (1H, *J* = 2.6 Hz, H-14′), 6.00 dddd (1H, *J* = 17.4, 11.6, 9.5, 7.4 Hz, H-24′), 5.73 d (1H, *J* = 3.7 Hz, H-6′), 5.08 brs (1H, H_2-25′), 5.05 brd (1H, *J* = 5.7 Hz, H_2-25′), 4.46 brc (1H, *J* = 8.2 Hz, H-16), 3.85 s (3H, H_3-16′), 3.42 dd (2H, *J* = 13.6, 7.7 Hz, H_2-20′), 3.13 m (1H, H-17′), 3.11 m (1H, H-3), 3.03 m (1H, H_2-26a), 2.95 dd (2H, *J* = 13.6, 7.7 Hz, H_2-21′), 2.81 m (2H, H_2-23′), 2.27 c (1H, *J* = 8.0 Hz, H-23′), 2.26 m (1H, H_2-1a′), 2.15 m (1H, H_2-26b), 2.09 m (1H, H_2-18a′), 1.99 m (1H, H-22), 1.99 m (1H, H_2-18b′), 1.94 m (1H, H-25), 1.82 m (1H, H_2-24a), 1.81 m (1H, H_2-4a), 1.79 m (1H, H_2-4a′), 1.79 m (1H, H-19′), 1.78 m (1H, H-20), 1.69 m (1H, H_2-1a), 1.61 m (1H, H_2-2a), 1.56 m (1H, H_2-1b′), 1.55 m (2H, H_2-15), 1.55 m (2H, H_2-2′), 1.48 m (1H, H_2-11a), 1.36 m (1H, H-5), 1.33 m (1H, H_2-11b), 1.32 m (1H, H-8), 1.25 m (1H, H_2-4b′), 1.24 m (1H, H_2-4b), 1.24 m (2H, H_2-6), 1.21 m (1H, H_2-3a′), 1.20 m (1H, H_2-24b), 1.18 m (1H, H-9), 1.11 m (1H, H_2-3b′), 1.07 m (1H, H-14), 1.03 m (1H, H_2-1b), 0.94 d (3H, *J* = 6.6 Hz, H_3-21), 0.85 m (1H, H_2-2b), 0.85 d (3H, *J* = 6.4 Hz, H_3-27), 0.78 s (3H, H_3-19), 0.73 s (3H, H_3-18), 0.70 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 179.1 (CO, C-5′), 157.8 (C, C-13′), 146.7 (C, C-7′), 147.2 (CH, C-9′), 143.6 (C, C-10′), 139.9 (CH, C-24′), 131.4 (CH, C-11′), 126.2 (C, C-15′), 121.3 (CH, C-12′), 118.2 (CH, C-8′), 114.5 (CH_2, C-25′), 100.8 (CH, C-14′), 95.6 (C, C-23), 74.5 (CH, C-16), 68.6 (CH, C-22), 71.3 (CH, C-6′), 60.1 (CH, C-14), 59.3 (CH, C-17′), 55.3 (CH_3, C-16′), 54.9 (CH, C-3), 54.7 (CH_2, C-26), 54.4 (CH, C-5), 54.3 (CH, C-17), 49.7 (CH_2, C-22′), 49.4 (CH_2, C-1′), 49.3 (CH_2, C-21′), 49.0 (CH_2, C-20′), 45.9 (CH_2, C-24), 45.5 (CH, C-9), 41.7 (C, C-13), 38.9 (CH_2, C-12), 32.9 (CH, C-20), 39.2 (CH, C-23′), 39.1 (CH_2, C-4), 37.0 (CH_2, C-1), 35.1 (C, C-10), 34.5 (CH, C-8), 31.4 (CH_2, C-2), 29.8 (CH, C-25), 29.2 (CH_2, C-4′), 28.0 (CH_2, C-6), 27.9 (CH_2, C-15), 27.7 (CH, C-19′), 25.7 (CH_2, C-2′), 20.7 (CH_2, C-3′), 20.5 (CH_2, C-18′), 20.1 (CH_2, C-11), 18.5 (CH_3, C-27), 14.7 (CH_3, C-21), 13.5 (CH_3, C-18), 12.0 (CH_3, C-19). HRESIMS *m*/*z* $[M+H]^+$ 837.5928 (calcd for $C_{52}H_{77}N_4O_5$, 837.5888).

4.3.18. (22R, 23S, 25R)-3β-N-(pyridin-4-yl)pentanamide-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**23**)

To a solution of 50 mg *p*-aminepyridine (0.500 mmol) in DMF (3 mL) were added 5-bromovaleryl chloride (200 μL, 1.500 mmol) and Et_3N (145 μL, 1.000 mmol). This reaction mixture was stirred at room temperature for 2 h. Afterwards, Et_2O (10 mL) and aqueous Na_2CO_3 solution (1 M, 10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and filtered. After removing the solvent, 41 mg of bromo-amide was obtained (30%). After that, 18 mg of this product (0.070 mmol) was redissolved in DMF (2 mL), solanocapsine (30.0 mg, 0.070 mmol) and *t*-BuOK (8.0 mg, 0.070 mmol) was added. The reaction mixture was stirred for 24 h at 60 °C. Et_2O (10 mL) and aqueous Na_2CO_3 solution (1 M, 10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with Et_2O (3 × 15 mL) and dried with anhydrous Na_2SO_4. After removing the solvent, the residue was purified by preparative-TLC using a CH_2Cl_2:MeOH:Et_3N (9.7:0.2:0.1) to obtain 17.0 mg of compound **23** (40%) as a white amorphous solid; $[\alpha]_D^{25}$: +12.2 (c 0.87, MeOH), IR (film) ν_{max}: 3394.1, 2927.4, 2850.3, 1658.5, 1608.3, 1558.2, 1454.1, 1378.9, 1114.7, 997.0, 734.8 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 8.20 dd (2H, *J* = 4.8, 1.7 Hz, H-8′, 9′), 6.52 dd (2H, *J* = 4.8, 1.7 Hz, H-7′, 10′), 4.46 ddd (1H, *J* = 16.6, 9.8, 6.9 Hz, H-16), 3.03 brdd (1H, *J* = 11.6, 4.6 Hz, H_2-26a), 2.71 m (1H, H-3), 2.66 m (2H, H_2-1′), 2.16 brt (1H, *J* = 11.6 Hz, H_2-26b), 2.08 m (1H, H_2-4a′), 1.99 d (1H, *J* = 10.6 Hz, H-22), 1.95 m (1H, H-25), 1.81 m (1H, H_2-4a), 1.81 m (1H, H_2-24a), 1.79 m (1H, H-20), 1.72 m (1H, H_2-7a), 1.67 m (1H, H_2-1a), 1.63 m (1H, H_2-2a), 1.57 m (2H, H_2-15), 1.49 m (1H, H_2-11a), 1.46 m (1H, H_2-3a′), 1.38 m (2H, H_2-2′), 1.36 m (1H, H-5), 1.34 m (1H, H_2-11b), 1.32 m (1H, H-8), 1.31 m (1H, H_2-7b), 1.26 m (2H, H_2-6), 1.23 m (1H, H_2-4b), 1.22 m (1H, H_2-24b), 1.16 m (1H, H_2-3b′), 1.12 m (1H, H-9), 1.06 m (1H, H-14), 0.99 m (1H, H_2-4b′), 0.97 m (1H, H_2-1b), 0.95 d (3H, *J* = 6.4 Hz, H_3-21), 0.87 m (1H, H_2-2b), 0.84 d (3H, *J* = 6.6 Hz, H_3-27), 0.79 s (3H, H_3-19), 0.74 s (3H, H_3-18), 0.69 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 177.9 (CO, C-5′), 152.9 (C, C-6′), 149.9 (CH, C-8′, 9′), 109.5 (CH, C-7′, 10′), 95.6 (C, C-23), 74.2 (CH, C-16), 68.7 (CH, C-22), 60.3 (CH, C-14), 54.9 (CH, C-17), 54.8 (CH, C-5), 54.8 (CH_2, C-26), 50.7 (CH, C-3), 46.0 (CH_2, C-24), 45.6 (CH_2, C-1′), 45.5 (CH, C-9), 41.6 (C, C-13), 39.2 (CH_2, C-4′), 39.1 (CH_2, C-4), 38.2 (CH_2, C-12), 38.1 (CH_2, C-3′), 37.3 (CH_2, C-1), 35.5 (C, C-10), 34.6 (CH, C-8), 32.9 (CH, C-20), 31.6 (CH_2, C-2), 31.4 (CH_2, C-7), 29.7 (CH, C-25), 28.4 (CH_2, C-6), 28.3 (CH_2, C-2′), 28.1 (CH_2, C-15), 20.2 (CH_2, C-11), 18.5 (CH_3, C-27), 14.6 (CH_3, C-21), 13.3 (CH_3, C-18), 12.3 (CH_3, C-19). HRESIMS *m*/*z* $[M+H]^+$ 607.4612 (calcd for $C_{37}H_{59}N_4O_3$, 607.4582).

4.3.19. (22R, 23S, 25R)-3β-N-(1,2,3,4-tetrahydroacridin-9-yl) pentanamide-22,26-imino-16β,23-epoxy-5α-cholestan-23β-ol (**24**)

To a solution of 40 mg tetrahydroacridine hydrochloride (0.170 mmol) in DMF (3 mL) were added 5-bromovaleryl chloride (68 μL, 0.510 mmol) and Et_3N (35 μL, 0.340 mmol). This mixture was stirred at room temperature for 2 h. Then, Et_2O (10 mL) and aqueous Na_2CO_3 solution (1 M, 10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and filtered. After removing the solvent, 34 mg of corresponding bromo-amide was obtained (55%). After that 27 mg of this product (0.070 mmol) was redissolved in DMF (2 mL), solanocapsine (30.0 mg, 0.070 mmol) and KI (27.0 mg, 0.210 mmol) were added. The reaction mixture was stirred for 48 h at room temperature. CH_2Cl_2 (10 mL) and aqueous Na_2CO_3 solution (0.1 M, approximately pH = 11, 10 mL) were added, and the mixture was stirred. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL) and dried with anhydrous Na_2SO_4. After removing the solvent, the residue was purified by preparative-TLC using a CH_2Cl_2:MeOH:Et_3N (9.7:0.2:0.1) to obtain 19.0 mg of compound **24** (39%) as a yellow amorphous solid; $[\alpha]_D^{25}$: +15.2 (c 1.07, MeOH), IR (film) ν_{max}: 3478.9, 3357.5, 3247.5, 3056.6, 2927.4, 2854.1, 1648.8, 1575.6, 1565.9, 1500.4, 1444.4, 1376.9, 1303.6, 1280.5, 1265.1, 1170.6, 1112.7, 1079.9, 1004.7, 759.8, 736.7, 701.9 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 7.90 dd (1H, *J* = 8.4, 0.7 Hz, H-17′), 7.71 dd (1H, *J* = 8.4, 0.7 Hz, H-15′), 7.56 ddd (1H, *J* = 8.4, 6.8, 1.2 Hz, H-16′), 7.36 ddd (1H, *J* = 8.4, 6.8, 1.2 Hz, H-14′), 4.47 ddd (1H, *J* = 16.9, 9.7, 7.2 Hz, H-16), 3.04 m (2H, H_2-9′), 3.03 m (1H, H_2-26a), 2.64 m (1H, H-3), 2.61 m (2H, H_2-12′), 2.56 m (2H, H_2-1′), 2.16 brt (1H, *J* = 11.8 Hz, H_2-26b), 2.08 m (1H, H_2-4a′), 2.00 m (1H, H-22), 1.95 m (1H, H-25), 1.95 m (4H, H_2-10′, 11′), 1.82 m (1H, H_2-4a), 1.82 m (1H, H_2-24a), 1.79 m (1H, H-20), 1.68 m (1H, H_2-7a), 1.67 m (1H, H_2-1a), 1.63 m (2H, H_2-2), 1.58 m (2H, H_2-15), 1.49 m (1H, H_2-11a), 1.42 m (1H, H_2-12a), 1.38 m (1H, H-5), 1.34 m (1H, H-8), 1.33 m (1H, H_2-11b), 1.27 m (2H, H_2-2′), 1.26 m (2H, H_2-6), 1.25 m (1H, H_2-12b), 1.23 m (1H, H_2-7b), 1.21 m (1H,

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H_2-4b), 1.20 m (1H, H_2-24b), 1.19 m (2H, H_2-3′), 1.11 m (1H, H-9), 1.07 m (1H, H-14), 1.02 m (1H, H_2-4b′), 0.96 m (1H, H_2-1b), 0.95 d (3H, J = 6.7 Hz, H_3-21), 0.85 d (3H, J = 6.4 Hz, H_3-27), 0.77 s (3H, H_3-19), 0.75 s (3H, H_3-18), 0.70 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 168.1 (CO, C-5′), 158.3 (C, C-8′), 146.5 (C, C-6′), 146.3 (C, C-18′), 128.6 (CH, C-17′), 128.3 (CH, C-16′), 123.9 (CH, C-14′), 119.5 (CH, C-15′), 1 17.1 (C, C-13′), 110.3 (C, C-7′), 96.2 (C, C-23), 74.4 (CH, C-16), 68.9 (CH, C-22), 60.5 (CH, C-14), 54.9 (CH, C-5), 54.9 (CH_2, C-26), 54.8 (CH, C-17), 50.8 (CH, C-3), 46.3 (CH_2, C-1′), 46.0 (CH_2, C-24), 45.6 (CH, C-9), 41.6 (C, C-13), 39.2 (CH_2, C-4′), 39.2 (CH_2, C-12), 39.0 (CH_2, C-4), 38.9 (CH_2, C-3′), 37.4 (CH_2, C-1), 34.9 (CH, C-8), 32.9 (CH, C-20), 35.4 (C, C-10), 33.6 (CH, CH_2-9′), 32.2 (CH_2, C-7), 31.5 (CH_2, C-2), 29.8 (CH, C-25), 28.9 (CH_2, C-2′), 28.5 (CH_2, C-6), 28.1 (CH_2, C-15), 23.5 (CH, CH_2-12′), 22.5 (CH, CH_2-10′, 11′), 20.2 (CH_2, C-11), 18.7 (CH_3, C-27), 15.1 (CH_3, C-21), 13.4 (CH_3, C-18), 12.3 (CH_3, C-19). HRESIMS m/z[M+H]$^+$ 711.5240 (calcd for $C_{45}H_{67}N_4O_3$, 711.5208).

*4.3.20. (25R)-3β-N-[2-(3′,6′-bis(diethylamino)spiro[isoindoline-1,9′-xanthen]-3-one)] lactame-N′-[9″-(2″-(cyclohexylcarbamoyl)phenyl)-6″-(diethylamino)-3″H-xanthen-3″-ylidene)-N-ethylethanaminium] amide-22,26-imino-16β,23-epoxy-5α-22,23-cholestene (**25**)*

Rhodamine B (117 mg, 0.250 mmol) was dissolved and partitioned between aqueous 1 M NaOH and EtOAc. After isolation of the organic layer, the aqueous layer was extracted with two additional portions of EtOAc. The organic layer was then washed with NaOH (10%) and brine. The resulting organic solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 85.5 mg of product as light pink foam (Rhodamine B base, 68%) [34]. After that, 72 mg de Rhodamine B base (0.150 mmol) and solanocapsine (30 mg, 0.070 mmol) were dissolved in $CHCl_3$ (2 mL). The mixture was stirred and $POCl_3$ (9 μL, 0.091 mmol) was added with temperature increasing to about 50° C. The reaction mixture was refluxed for 3 h. After this time, 3 mL of water was added, after which distillation was continued until $CHCl_3$ no longer passed over. Then, 10% NaOH solution (1 mL) was added and the stirred mixture was left to cool [35]. An amorphous solid was obtained and filtered off under suction, and the pH of remaining solution was adjusted to 9 and then extracted with CH_2Cl_2 (3 × 15 mL). Organic phases were grouped and dried with anhydrous Na_2SO_4. Once the solvent was removed, the residue was purified by column chromatography using CH_2Cl_2:MeOH:Et_3N (9.7:0.2:0.1) as elution solvent and then by preparative-TLC using CH_2Cl_2:MeOH (9:1) to obtain 9.1 mg of compound **25** (10%) as a pink amorphous solid; $[\alpha]_D^{25}$: +80.1 (c 0.22, CH_2Cl_2), IR (film) ν_{max}: 3367.1, 2971.8, 2929.3, 1729.8, 1681.6, 1589.1, 1515.8, 1415.5, 1336.4, 1180.2, 1118.5, 1074.2, 1012. 5, 921.8, 821.5, 786.8, 684.6, 545.8 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): 7.81 m (1H, H-3′), 7.67 m (1H, H-3″), 7.59 m (1H, H-5″), 7.37 m (1H, H-6″), 7.36 m (1H, H-6′), 7.33 m (1H, H-4″), 7.27 m (1H, H-5′), 7.06 brd (2H, J = 8.9 Hz, H-10″, 19″), 6.96 m (1H, H-4′), 6.91 brt (2H, J = 8.9 Hz, H-11″, 18″), 6.78 dd (2H, J = 8.9, 2.0 Hz, H-13″, 16″), 6.48 brd (2H, J = 8.9 Hz, H-10′, 19′), 6.36 dd (2H, J = 3.7, 2.7 Hz, H-13′, 16′), 6.24 dt (2H, J = 8.9, 2.7 Hz, H-11′, 18′), 3.93 td (1H, J = 9.7, 4.2 Hz, H-16), 3.68 m (1H, H_2-26a), 3.64 m (CH_2, N$CH_2$$CH_3$″), 3.33 c ($CH_2$, N$CH_2$$CH_3$-′), 2.95 m (1H, H-3), 2.92 m (1H, H-20), 2.65 m (1H, H_2-26b), 2.38 m (1H, H_2-2a), 2.24 m (1H, H_2-4a), 2.06 m (1H, H_2-24a), 1.76 m (1H, H_2-24b), 1.67 m (1H, H_2-12a), 1.59 m (1H, H-25), 1.58 m (2H, H_2-15), 1.49 m (1H, H_2-1a), 1.46 m (1H, H_2-7a), 1.38 m (1H, H_2-11a), 1.31 m (CH_3, NCH$_2$$CH_3$″), 1.26 m (1H, H-5), 1.26 m (2H, H_2-6), 1.25 m (1H, H-8), 1.20 m (1H, H_2-11b), 1.12 t (CH_3, NCH$_2$$CH_3$′), 1.11 m (1H, H_2-12b), 1.02 m (1H, H_2-2b), 0.97 m (1H, H-14), 0.91 d (3H, J = 6.6 Hz, H_3-27), 0.82 s (3H, H_3-19), 0.80 m (1H, H_2-4b), 0.78 m (1H, H-9), 0.73 m (1H, H_2-7b), 0.63 m (1H, H_2-1b), 0.61 s (3H, H_3-18), 0.61 m (3H, H_3-21), 0.52 m (1H, H-17). ^{13}C NMR ($CDCl_3$ 100.03 MHz): 166.8 (CO, C-1″), 167.5 (CO, C-1′), 157.8 (C, C-14″, 15″), 157.7 (C, C-8″), 155.7 (C, C-2′), 155.6 (C, C-2″), 155.6 (C, C-12″, 17″), 153.3 (C, C-14′, 15′), 149.2 (C, C-12′, 17′), 148.7 (C, C-7″), 146.4 (C, C-23), 136.5 (C, C-7′), 132.5 (CH, C-5′), 131.8 (CH, C-6′), 131.2 (CH, C-10″, 19″), 130.1 (CH, C-4″), 129.8 (CH, C-3″), 127.9 (CH, C-5″), 127.6 (CH, C-6″), 129.3 (CH, C-10′, 19′), 123.6 (CH, C-4′), 122.2 (CH, C-3′), 118.8 (C, C-22), 114.8 (C, C-9″, 20″), 113.9 (CH, C-11″, 18″), 107.8 (CH, C-11′, 18′), 105.8 (C, C-9′, 20′), 97.8 (CH, C-13′, 16′), 96.2 (CH, C-13″, 16″), 80.0 (CH, C-16), 65.5 (C, C-8′), 58.1 (CH, C-14), 55.2 (CH_2, C-26), 54.3 (CH, C-5), 54.3 (CH, C-17), 53.4 (CH, C-3), 46.0 (CH_2, N$CH_2$$CH_3$″), 45.9 (CH, C-9), 44.0 (CH_2, N$CH_2$$CH_3$′), 41.8 (C, C-13), 39.0 (CH_2, C-12), 37.5 (CH_2, C-1), 35.0 (C, C-10), 34.6 (CH, C-8), 33.7 (CH_2, C-24), 31.6 (CH_2, C-7), 31.4 (CH, C-20), 31.0 (CH_2, C-4), 29.6 (CH, C-25), 29.4 (CH_2, C-6), 28.8 (CH_2, C-15), 24.2 (CH_2, C-2), 20.4 (CH_2, C-11), 18.8 (CH_3, C-27), 16.0 (CH_3, C-21), 13.2 (CH_3, C-18), 12.4 (CH_3, NCH$_2$$CH_3$′), 12.4 ($CH_3$, NCH$_2$$CH_3$″), 11.9 ($CH_3$, C-19). HRESIMS m/z[M]$^+$ 1261.7870 (calcd for $C_{83}H_{101}N_6O_5^+$, 1261.7828).

4.4. Biological assays

4.4.1. Materials

All starting materials were commercially available research-grade chemicals and used without further purification. UV spectra were recorded on a JASCO V-630BIO spectrophotometer. Acetylcholinesterase from electric eel (type VI-S), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI) and tacrine were purchased from Sigma.

4.4.2. Cholinesterase inhibition assay

Electric eel was used as a source of cholinesterase. AChE inhibiting activity was measured *in vitro* by the spectrophotometric method developed by Ellman with slight modification [14]. The lyophilized enzyme (500U) was prepared in buffer A (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4) to obtain 5 U/mL stock solution. Further enzyme dilution was carried out with buffer B (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4, 0.15 M NaCl, 0.05% Tween 20, pH 7.6) to produce 0.126 U/mL enzyme solution. Samples were dissolved in buffer B with 2.5% of MeOH as cosolvent. Enzyme solution (300 μL) and 300 μL of sample solution were mixed in a test tube and incubated for 60 min at room temperature. The reaction was started by adding 600 μL of the substrate solution (0.5 mM DTNB, 0.6 mM ATCI, 0.1 M Na_2HPO_4, pH 7.5). The absorbance was read at 405 nm for 120 s at 27 °C. Enzyme activity was calculated by comparing reaction rates for the sample to the blank. All the reactions were performed in triplicate. IC_{50} values were determined with GraphPad Prism 5. Tacrine (99%) was used as the reference AChE inhibitor. To prove the stability of compound **24** in the enzymatic conditions, we repeated the inhibition assay of AChE with compound **24** and was carefully extracted with CH_2Cl_2. After having analyzed the organic extract by TLC, no trace of tacrine was detected. We have concluded that under the experimental assay conditions, compound **24** does not decompose or hydrolyze [36].

4.4.3. Kinetic characterization of AChE inhibition

The enzyme reaction was carried out at three fixed inhibitor concentrations for solanocapsine (0.0, 1.0 and 6.0 μM) and for **24** (0.0, 59.0 and 118.0 nM). In each case the initial velocity measurements were obtained at varying substrate (S) concentrations ([ATCI] = 45–600 μM) and the reciprocal of the initial velocity ($1/v$) was plotted as a function of 1/[S]. The data were analyzed with GraphPad Prism 5. The Lineweaver–Burk plot showed a pattern of lines with increasing slopes, characteristic of mixed-type inhibition (K_i = 3.61 ± 0.20 μM for solanocapsine and K_i = 83.86 ± 5.17nM, for 24). The nonlinear regression of these data fitted with mixed-type inhibition with a R^2 = 0.9974 and 0.9926 for **24** and solanocapsine, respectively.

4.5. Molecular modeling studies

The complex between the ligands and *Tc*AChE were obtained by molecular docking employing the coordinates of the complex *Tc*AChE-donepezil (PDB: 1EVE) and *Tc*AChE-bistacrine dimer (PDB: 2CMF) [31] for the receptor, removing ligand and water molecules. Before starting docking simulations, the p*K*a of compounds was evaluated employing *Marvin Sketch v.5.12.4* software package, assuming a pH of 7.4 as a physiological value. The ligand conformer libraries were obtained with *Omega2 v2.5.1.4* software, with the default settings. The receptor was prepared using *Make_Receptor* software in combination with a shape-based site detection algorithm and the position of well-known bounded ligand, with a binding box volume of 43,281 Å^3 (PDB: 1EVE) and 39,813 Å^3 (PDB: 2CMF). The docking runs were performed with *Fred v3.0.1* software [37,38] and ranked using the *Chemmgauss4* scoring functions. The visualization of the docking result was performed with *Vida v 4.2.1*. For each complex obtained a refinement of the binding energy was performed with *Amber 11* [39].

The construction of the ligand units to be used was achieved with the antechamber module, using GAFF force field and AM1-BCC fitted charges. The input files for the simulations were built with the *xleap* package included in *amber tools*. For the refinement, two minimizations of 5000 steps of the complexes were performed employing *Sander* software. The first one kept the protein heavy atoms retrained at their initial positions and the second one kept the whole system free.

After the minimization steps, the binding free energies were calculated with molecular mechanics-Poisson–Boltzmann surface area (MM-PBSA) and molecular mechanics-Generalized Born surface area (MM-GBSA) approximations [40].

Conflict of interest

The authors state no conflict of interest.

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Appendix A. Supplementary data

Supplementary material with a copy of ^{1}H NMR and ^{13}C NMR spectra of compounds **1–24** and graphics with more information are available in the on-line version. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2015.09.001.

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