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Scientific Research

Natural and Semi-Synthetic Pseudoguaianolides as Inhibitors of NF-κB

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Abstract

Damsin (1) is a natural pseudoguaianolide sesquiterpene that inhibits NF-κB, a protein complex that controls the transcription of DNA in mammalian cells, and has a potential for standing model for the development of new anti-cancer lead structures. In order to do a preliminary structure-activity study and improve the anti-cancer activity, fourteen derivatives and analogs were prepared and evaluated. These were chosen to represent both structural diversity and structural novelty. The importance of α methylene-γ-lactone moiety for the anti-cancer activity was confirmed, even though other features in the scaffold were shown to be important for the activity. In some cases a new substitution negatively affected the initial activity, however, two analogues, indolo [3,2-c]-4-desoxydamsin (5) and ambrosin (6), were found to be more potent.

Keywords

Damsin, α Methylene-γ-Lactone Sesquiterpenoids, Anti-Cancer Activity

1. Introduction

NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells, is a family of protein complexes that control the transcription of DNA as transcription factors. They are involved in the cellular responses to various stimuli, an important part of the immune system and responsible for cytokine production and cell survival. Poor

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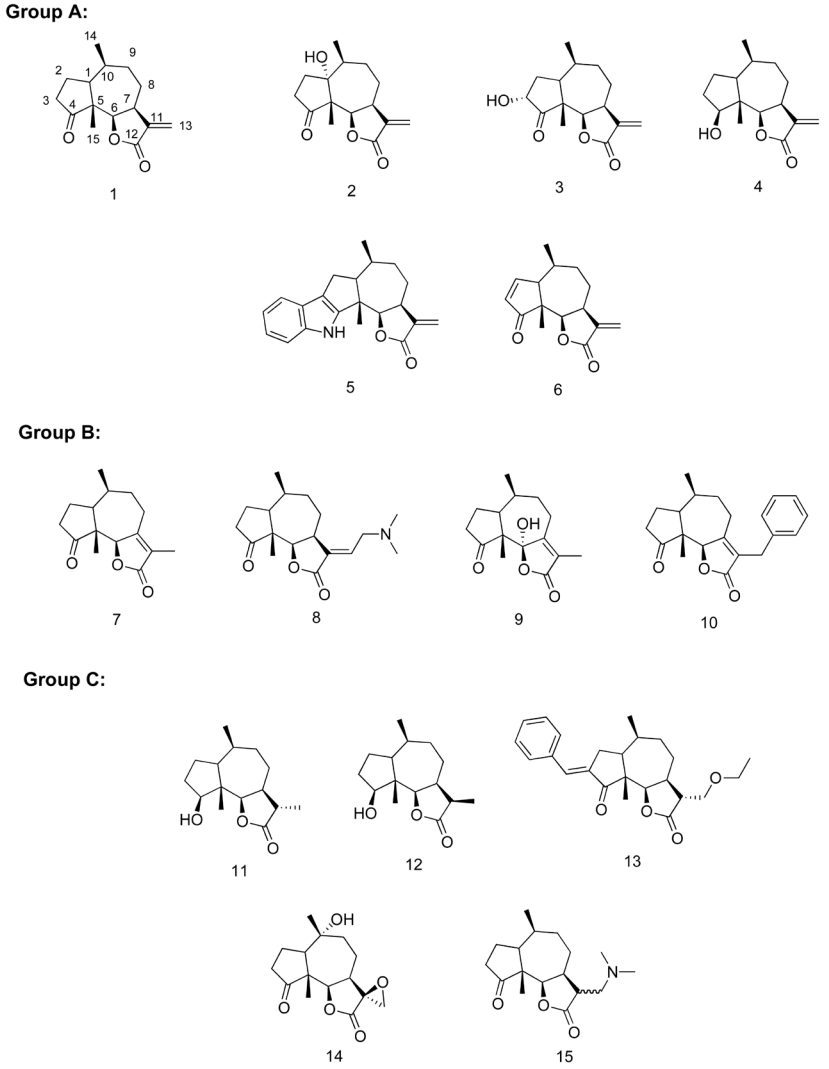
regulation of NF-κB is linked to several serious conditions, including cancer. The NF-κB complexes include the subunits RelA (p65), NF-κB1 (p50; p105), NF-κB2 (p52; p100), c-Rel and RelB. The amino-terminal Rel-homology domain (RHD) is conserved within the family, containing the subdomains for dimerization, nuclear localization and DNA-binding. Therefore, homo-and heterodimers between these proteins can be found *in vivo*, except for RelB, which just forms heterodimers. A dimer is normally associated with the inhibitory protein IκB, and the complex (NF-κB-IκB) remains inactive in the cytoplasm. A broad number of stimuli are responsible for the activation of such complexes, e.g. pathogens, stress signals and pro-inflammatory cytokines, by activating IκB kinases (IKK) that initiate a process that eventually degrades IκB. Free and activated NF-κB can then be translocated into the nucleus and start the transcription of target genes. The most well-known heterodimer, and present in most mammalian cells, is RelA/p50 [1]-[3]. The main biological functions of NF-κB are the activation of immune response and inflammation via the expression of genes encoding for cytokines, cytokine receptors, and cell-adhesion molecules [1].

In recent years, a deeper understanding of the NF-κB pathway and its role in inflammatory processes has been established, creating a molecular link between the chronic inflammatory diseases and cancer [4] [5]. Specifically, this transcription factor has been shown to be crucial in oncogenesis and tumor progression, inhibiting apoptosis by transcribing genes for anti-apoptotic proteins or suppressing genes for apoptotic proteins. In a state of constant activation of NF-κB malignancies may progress even if apoptosis is induced by anti-cancer treatments, leading to chemoresistance [6]. For these reasons, the NF-κB family is considered to be an interesting target for the development of new anti-cancer drugs [7]-[9], although it should be noted that pro-apoptotic and tumor suppression functions of NF-κB have been reported in some cancer cell lines [10]-[15]. Nevertheless, new inhibitors of NF-κB are required in order to study and understand the following steps in the signal transduction: a) the blocking of the binding of NF-κB to DNA; b) the inhibition of the nuclear import system to prevent NF-κB from reaching the nucleus; c) the inhibition of the IκB degradation ensuring that NF-κB remains inactive; d) the inhibition of the IKK kinases that initiate the degradation of IκB [16].

Sesquiterpenes with an *α*-methylene-*γ*-lactone moiety (e.g. damsin (**1**), see **Scheme 1**) constitute a class of natural products with potential as anti-cancer agentsby inhibiting NF-κB [17]-[21]. Structure-activity relationship studies have demonstrated the importance of the rigid skeleton of pseudoguaianes and/or guaianes with an *α*-methylene-*γ*-lactone moiety, as it appears to contribute significantly to both the anti-cancer activity and the NF-κB inhibition [22] [23]. The critical property of these compounds is the ability of the *α*-methylene-*γ*-lactones to react as Michael acceptors with thiol groups of cysteines in proteins, modifying the protein covalently [17] [19] [21] [22] [24]-[26]. The reaction of the thiol group ofcysteine-38 in RelA with a Michael acceptor is considered to be the mechanism by which the *α*-methylene-*γ*-lactone sesquiterpenes prevent the binding of NF-κB to DNA. This cysteine is considered crucial for the interaction between NF-κB and DNA, association with the coactivator RPS3 and antiapoptotic gene expression [27]. The importance of this cysteine residue was also demonstrated by studying mutants of RelA/NF-κB and measuring their ability to bind to DNA in the absence or presence of *α*-methylene-*γ*-lactone sesquiterpenes, showing that this site is affected by this class of natural products [28]-[30].

We have focused our interest on damsin (**1**), a pseudoguaiane sesquiterpene that can be isolated in large amounts from the plant *Ambrosia arborescens* Mill. [31]. This natural product is cytotoxic towards Hep-2 cells (epidermoid carcinoma of larynx) [32] and Eagle's KB cells (nasopharynx carcinoma) [33]. In addition, antiproliferative activity was reported in U937 cells (monocytic leukaemia), Jurkat cells (leukaemia T) and Molt 4 cells (acute lymphoblastic leukaemia) [34]. Recently we reported the antiproliferative activity of damsin (**1**) towards Caco-2 cells (epithelial colorectal adenocarcinoma) and its inhibitory effect on NF-kB and STAT3 (a signal transducer and activator of transcription) [35]. In an effort to prepare new derivatives at least retaining the NF-kB inhibitory capacity, a broad range of chemical transformations were carried out with damsin (**1**). As **1** is chiral, all derivatives obtained from it (see **Scheme 1**) are consequently pure enantiomers with the absolute configuration as shown. The transformations were partly inspired by the reports from the 1960s, when the structure elucidation of sesquiterpene lactones was based on functional group interconversions [36] [37]. In the later years, the derivatisation studies have focused on the improvement of their biological activities, such as cytotoxicity [38] [39], and the development of pro-drugs as anticancer agents [40] and anti-malarial [41]. Such studies usually only utilized specific chemical reactions, but the aim of this study was to use a broad scope of reactions in order to generate novel structures that are not found in plants.

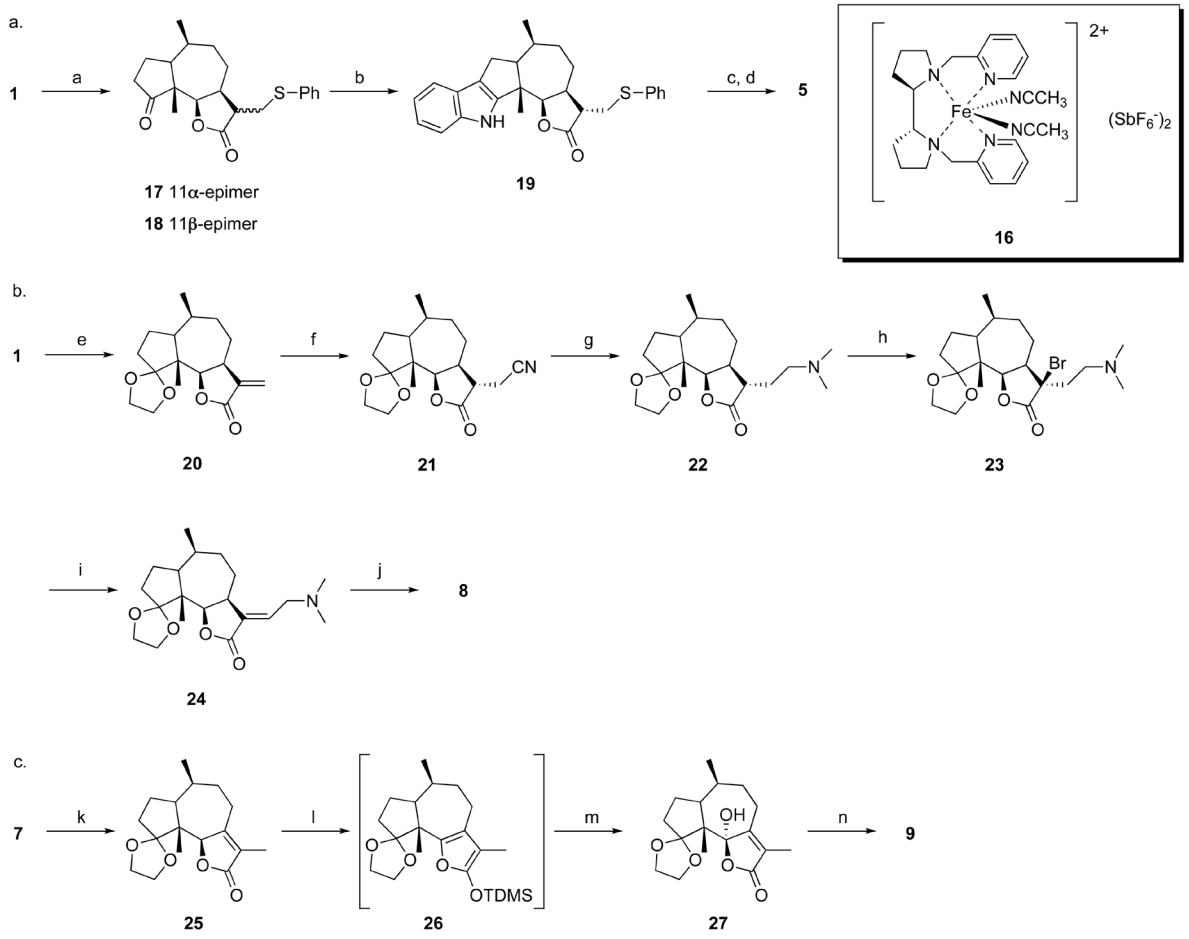


Scheme 1. Sesquiterpenes with an α-methylene-γ-lactone moiety.

2. Results

Damsin (**1**) and coronopilin (**2**) are known pseudoguaiane sesquitepenes that can be isolated from *Ambrosia arborescens* Mill. [34] [36]. As the isolation of **1** in larger amounts was achievable, it was selected as the starting material for all the chemical modifications. Our ambition was not only to modify the Michael acceptor moiety and consequently the reactivity, but also to introduce new groups into the pseudoguaiane skeleton in order to investigate the effect of non-covalent interactions. The compounds prepared can be divided in three categories (**Scheme 1**): Group A, compounds retaining the α-methylene-γ-lactone moiety; Group B, compounds with a modified Michael acceptor in the γ-lactone moiety and Group C, compounds lacking a carbon-carbon double bond in the γ-lactone ring.

Group A. New hydrogen donors/acceptors in **1** were introduced in compounds **3** and **4**. A Rubottom oxidation provided **3** that is hydroxylated in position 3 (**Scheme 1**) [42], while a selective reduction of the keto function through a Luche reduction with $NaBH_4$ in presence of $CeCl_3$ afforded **4** [43]. A Fischer indole synthesis with phenylhydrazine gave **5** that has a planar and rigid structure. To avoid competitive reactions of the hydrazine with the α methylene-γ-lactone moiety, the thiophenoxide-protected adduct (**17**) was used as starting material (**Scheme 2(a)**). The molecule was deprotected by a selective oxidation of the thioether to a sulfoxide with *m*-CPBA at −20˚C, followed by a thermal elimination. Ambrosin (**6**), a natural product, was prepared via a Saegusa-Ito oxidation [42]. **6** is unique in this investigation by containing a second Michael acceptor function.



Scheme 2. a. PhSNa/EtOH; b. H_2NNHPh/AcOH; c. *m*-CPBA /DCM; d. PhMe, reflux; e. $(CH_2OH)_2$, $(CH_3O)_3CH$/ 10% *p*-TsOH, DCM; f. ACH, NaCN/DMSO; g. H_2/Pd-C/Me_2NH, EtOH; h. TMSOTf, TEA /DCM then PTAB, 0˚C; i. TBAF/THF; j. HCl/MeOH; k. $(CH_2OH)_2$, $(CH_3O)_3CH$/10% *p*-TsOH, DCM; l. TBDMSOTf, TEA/DCM; c. m-CPBA/DCM; n. HCl/MeOH.

Group B. The isomerization of **1** to **7** was achieved by treatment with $RhCl_3$ [42], providing a derivative that still is a Michael acceptor but less available for nucleophilic attack and consequently less reactive [40]. **8** was designed to facilitate a Michael addition by providing an intramolecular basic catalysis for an incoming thiol [44]. Several strategies towards **8** were investigated, including metathesis and a Wittig olefination reaction, but the procedure that eventually succeeded (see **Scheme 2(b)**) starts with a Michael addition of a cyanide ion to the acetal **20** to give the nitrile **21**. After the reduction of **21** to the tertiary amine **22** and the oxidation of the lactone ring of **22** via the bromine **23** to the olefine **24**. Finally, **8** was obtained by the hydrolysis of the acetal protection group. Compound **9** was prepared from **7** by a procedure similar to the Rubotton oxidation [45]. Firstly, **7** was protected as the acetal **25**, which wasoxidized to **27** via the 2-hydroxyfuran silylether **26** (not isolated), and finally deprotected by hydrolysis to yield **9** (**Scheme 2(c)**). Compound **10** was prepared from **1** by a Heck coupling reaction, following procedures reported for *α*-methylene-*γ*-lactones [46] [47]. However, the isomerization could not be avoided, yielding the endocyclic *α*, *β*-unsaturated-*γ*-lactone **10**.

Group C. Even though it has been stated that the absence of the *α*-methylene-*γ*-lactone moiety renders this class of terpenes inactive as inhibitors of NF-κB [29], we wanted to include also such compounds in this investigation. The reduction of the *α*-methylene-*γ*-lactone of **1** with $NaBH_4$ gave the two epimers **11** and **12** [42]. The Claisen-Schmidt condensation of **1** with benzaldehyde in ethanol gave **13** [48]. The hydroxylated compound **14** was prepared following an iterative procedure [49] with the hydrogen peroxide and catalyst **16** (**Scheme 2**). Although this catalyst hydroxylated the desired tertiary position, the epoxidation of the double bond was unavoidable. Finally, the reaction of **1** with dimethyl amine in ethanol yielded the amine adduct **15** (as an epimeric

mixture 3:1 11*α*:11*β*) [50], for evaluation of its possible usefulness as a pro-drug.

The configuration of the products and intermediates was determined by comparing the ^{1}H NMR coupling constants and correlations observed in the NOESY spectra, with computational models. The conformational search was carried out using the MMFFs force field and the low energy conformers were optimized using B3LYP/6-31G** basis set. In several cases a second order system was resolved in order to determine the coupling constants, and this was done by approximation using spin simulation. For compound **4**, for example, the coupling constants and atomic distances of the 4*β*-hydroxyl group were experimentally and computationally correlated as follows. While the experimental coupling constants of H-4*α* with H-3*α* and H-3*β* both are 9.0 Hz, the calculated coupling constants were 8.0 Hz for H-3*α* and 8.1 Hz for H-3*β*. In addition, a NOESY correlations was observed from H-4*α* toH-1, corresponding with the calculated distance (2.40 Å), as well as to H-6 (calculated distance 2.26 Å). The chiral centers as well as the double bond configurations were assigned in the corresponding way for all the derivatives. The structural elucidation of **3**, **11** and **12** has previously been reported by us [42].

The coupling constants and atomic distances of each compound with a new chiral center are experimentally and computationally consistent as follows:

Compound **9.** A NOE correlation was observed between OH-6 and H-1, corresponding with the calculated distance of 3.04 Å.

Compound **13**. The configuration of C-11 was assigned considering the experimental coupling constant of H-11*β* with H-7*α* (7.2 Hz), compared with the calculated coupling constant (11.9 Hz). In addition, NOESY correlations were observed between H-11*β* andH_3-15, H-8*α*, H-8*β* and H-9*β* corresponding to the calculated interatomic distances 2.36, 3.01, 3.86 and 2.16 Å, respectively. NOESY correlations between H_2-13 to-$OCH_2C\underline{H_3}$ (weak), H-7, -$OC\underline{H_2}CH_3$ and H-6 correspond to the interatomic distances 4.60, 2.70, 2.40 and 2.54 Å, respectively. A second order system (ABM) was resolved for H-13a and H-13b, to discriminate their coupling constants with H-11*β*. The error is small enough to confirm the proposed stereochemistry, even though the contribution of more than one conformer could explain the divergence. In addition the *E* isomerism of the 3-phenylethenyl, was confirmed trough NOE correlations of H-2' with H-2a and H-2b (2.224 Å and 2.449 Å, respectively).

Compound **15**. The relative amounts of the two diastereomers in the epimeric mixture obtained was made using the integral of H-6 for each epimer. The complete assignation was done by a careful analysis of the 2D spectra. The configuration of C-11 in the two epimers was determined by the NOESY correlation observed between H-11 andH_3-15 in the spectrum of the major epimer (11*β*, 13-dihydro-13-(*N*, *N*-dimethylamino) damsin)),and the correlation between H-11 and H-6 for the minor epimer (11*α*, 13-dihydro-13-(*N*, *N*-dimethylamino) damsin.

Compound **17.** The configuration of C-11 in **17** was suggested by the experimental coupling constant between H-11*β* and H-7 (7.7 Hz) compared with the calculated coupling constant (11.4 Hz). This was confirmed by the NOESY correlations between H-11*β* and H-8*β* (weak), H-9*β* and H_3-15 corresponding to the interatomic distances 3.01, 2.23 and 2.37 Å, respectively.

Compound **18**. The configuration of C-11 in **17** was suggested by the experimental coupling constant between H-11*α* and H-7(6.0 Hz) compared with the calculated coupling constant (6.4 Hz). A second order system (AMNX) was resolved for H-11*α*, H-7, H-13a and H-13b, in order to determine their coupling constants and chemical shifts. In addition, a NOESY correlation was observed between H-11*α* and H-6 corresponding to the interatomic distance of 2.51 Å.

Compound **22**. NOESY correlations were observed between H-11*β* and H-8*β* as well as H_3-15, corresponding with the calculated distance of 2.42 and 2.44 Å, respectively.

NF-κB Inhibition and Structure Activity Relationship

To analyze the NF-κB inhibitory activity of the synthesized compounds we used the stably transfected cell line 5.1, a lymphoid T cell line in which the HIV-1 LTR is activated by TNF-*α* through an NF-κB dependent mechanism [51]. The HIV-1 LTR promoter contains two κB sites that are critically required to respond to TNF*α*, and it is well known that deletion of the two κB sites in the LTR promoter abolishes completely the response to TNF*α*. Thus, 5.1 cells represent an excellent cellular model for the screening of anti-NF-κB compounds. We have previously found that dasmin (**1**) and coronofilin (**2**) possess anti-NF-κB activity, with IC_{50} values of 7.2 and 10.1 μM respectively [35]. As shown in **Table 1**, two compounds from group A (**5** and **6**) have a lower IC_{50}value compared to **1**, suggesting that indolic system slightly favors the ability of the compound to interact

Table 1. Inhibitory activity (IC_{50}) on TNFα-induced NF-κB activation. The results represent the mean of three independent experiments and the SD was less than 10%.

Compound	IC50 (μM)
3	76
4	29
5	6.0
6	0.50
7	>100
8	>100
9	36
10	76
11	>100
12	>100
13	34
14	>100
15	31

with the protein. Compound **6** is the most active, probably due the presence of two *α*, *β*-unsaturated carbonyl moieties that increase the alkylating capacity of the product, and this has been noted previously [18] [23] [52]. On the other hand, compounds **2**, **3** and **4** still possess theoriginal *α*-methylene-*γ*-lactone but are less potent (especially **3** and **4**) compared to **1**. These results strongly indicate that a number of intermolecular interactions between the compound assayed and the protein it affects modulate their effects, and that a more systematic QSAR study to understand such interactions in detail is motivated.

The compounds in group B possess a modified *α*-methylene-*γ*-lactone moiety. Compound **7** is inactive at the maximal concentration tested, which could be explained by the lower reactivityof the endocyclic *α*, *β*-unsaturated-*γ*-lactone moiety and its inability to form stable adducts with thiols [53]. However, compound **8** is also inactive although it was designed to provide support for a thiol attacking C-13. Possibly the steric hindrance posed by the C-13 substituent prevents an attack. Compound **9** is an interesting exception with an intermediate activity, possibly caused by a hydrolysis of the hemi-acetal lactonic ring forming a 1,4-enedione with a higher reactivity. Compound **10** is less potent, but the comparison with **7** and indicates that the aromatic group in position 13 slightly enhance the ability of the compound with the protein.

The compounds of group C as **11** and **12** are inactive as was predictable, confirming the crucial role of the *α*-methylene-*γ*-lactone moiety for the inhibition of NF-κB [23]. The inactivity of compound **14** demonstrate that other electrophilic groups, such as an epoxide, in positions 11, 13 can not replace the *α*-methylene-*γ*-lactone moiety, even though the position is the same. Compounds **13** and **15** are weakly active, this could be explained ifthe Michael addition of etoxide and dimethyl amine to the *α*-methylene-*γ*-lactone moiety is reversible but this was not further investigated. Amines like **15** are classical pro-drugsof sesquiterpene lactones [18] [40] [50], although **15** is considerably less activecompared todamsin (**1**) in this study.

3. Conclusion

In total, fourteen derivatives were prepared, characterized and assayed. Several (**7**, **8**, **11**, **12** and **14**) were inactive at the tested concentrations, confirming the importance of the *α*-methylene-*γ*-lactone moiety for the activity. Nevertheless, compound **10** is slightly active, suggesting that also endocyclic Michael acceptors may be active and that an aromatic group in position 13 can be important. Also, compound **9** was unexpectedly active, probably because the hemi-acetal can be hydrolyzed to a more reactive 1,4-enedione. The compounds **2**, **3** and **4** are

somewhat surprisingly less active compared to **1**, indicating that hydroxyl groups on positions 1, 3 or 4, respectively, have a negative effect on the ability of the sesquiterpene to interact with the protein. For compound **15**, the ability to regenerate the *α*-methylene-*γ*-lactone moiety during the performance of the assay is the probable reason for its modest activity, amine adducts (**15**) have previously been demonstrated to possess such a reversibility [50]. This is not the case for ethers, e.g. **13**, so there may be another reason for its modest activity. Finally, compounds **5** and **6** are more potent compared to **1**. This was expected for **6** as it possesses two Michael acceptors, but unexpected for **5**. Possible explainations are that the indol system imposes a steric strain on the molecule, including the *α*-methylene-*γ*-lactone moiety, which could be relieved if C-11 no longer is part of an unsaturation. Alternatively the indole may be involved in some specific interaction with the molecular environment of the reacting cysteine, favoring the position of the *α*-methylene-*γ*-lactone moiety in the right place. The need for a more systematic investigation is evident.

4. Experimental

a) Chemistry

General

All other chemicals were obtained from commercial suppliers of analytical grade. High Resolution Mass Spectrometry (HMRS) Electrospray Ionization (ESI) spectra were recorded with a Micromass Quadrupole-time of Flight (Q-TOF) Micro spectrometer. Nuclear Magnetic Resonance (NMR) spectra (in $CDCl_3$) were recorded with a Bruker DRX at 400 Mhz (^1H) and at 100 MHz (^{13}C). Chemical shifts are given in ppm relative to the residual $CHCl_3$ in $CDCl_3$ (7.25 ppm ^1H and 77.00 ppm ^{13}C). All flash chromatography was performed with 60 Å 30 - 75 μm silica gel. Thin Layer Chromatography (TLC) analyses were come out on silica Gel 60 F254 (Merck, Darmstadt, Germany) plates.

4*β*-hydroxy-4-deoxydamsin (**4**)**.** To a solution of **1** (100 mg, 0.4027 mmol) and $CeCl_3 \cdot 7H_2O$ (150.0 mg, 0.4027 mmol) in MeOH (2.5 mL), was added $NaBH_4$ (15.2 mg, 0.4027 mmol) in one portion. The reaction mixture was stirred at room temperature for 40 min and then quenched with a 10% HCl solution (1 mL). The mixture was neutralized with $NaHCO_3$ sat. (pH = 7) and the aqueous mixture was extracted with DCM (4 × 10 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (30% EtOAc: Pet. $Et._{40\text{-}60}$) to provide the product **4** (77.2 mg, 76%). $[\alpha]_D^{20}$: −39.9(*c* 1.00, CH_2Cl_2); IR (film) = 3468, 2960, 2920, 2875, 1750, 1659, 1473, 1456, 1386, 1339, 1264, 1149, 1103, 1091, 1044, 1020, 993, 974, 802, 733, 701, 422 cm^{-1}; (400 MHz $CDCl_3$) *δ* 1.67 (1H, *m*, H-1), *δ* 1.52 (1H, *m*, H-2a), *δ* 1.80 (1H, *m*, H-2b), *δ* 1.53 (1H, *m*, H-3a), *δ* 2.05 (1H, *m*, H-3b), *δ* 3.99 (1H, *t*, ^3J 9.0; 9.0 Hz, H-4), *δ* 4.48 (1H, *d*, ^3J 9.5 Hz, H6), *δ* 3.34 (1H, *m*, H-7), *δ* 1.90 (1H, *m*, H-8a), *δ* 2.11 (1H, *m*, H-8b), *δ* 1.56 (1H, *m*, H-9a), *δ* 1.85 (1H, *m*, H-9b), *δ* 2.08 (1H, *m*, H-10), *δ* 5.49 (1H, *d*, ^3J 3.8 Hz, H-13a), *δ* 6.19 (1H, *d*, ^3J 3.5 Hz, H-13b), *δ* 1.01 (3H, *d*, ^3J 7.5 Hz, Me-14), *δ* 0.82 (3H, *s*, Me-15); d_C (100.6 MHz, $CDCl_3$) 47.8 (C-1), 23.9 (C-2), 28.1 (C-3), 82.6 (C-4), 50.8 (C-5), 90.8 (C-6), 43.2 (C-7), 22.8 (C-8), 32.6 (C-9), 33.2 (C-10), 139.8 (C-11), 169.8 (C-12), 119.8 (C-13), 14.0 (C-14), 9.0 (C-15); HRMS-ESI: $[M+Na^+]$, found 273.1467. $C_{15}H_{22}O_3$ requires 273.1467.

11*β*,13-dihydro-13-thiophenoxydamsin (**17**) and **11*α*,13-dihydro-13-thiophenoxydamsin** (**18**). PhSH (300 μL, 2.9409 mmol) was carefully added to metallic sodium (28.0 mg, 1.2080 mmol) and when the reaction was complete, EtOH was added (1 mL) and the solution was stirred at room temperature for 1 h. Then, a solution of **1** (100 mg, 0.4027 mmol) in ethanol (2.5 mL) was added and the reaction was stirred at room temperature for 1 h. The reaction was quenched with 4 drops of AcOH, followed by 2 mL of water. The aqueous phase was extracted with DCM (3 × 10 mL), the combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (from 15% to 25% EtOAc:Pet. $Et._{40\text{-}60}$) to provide the product **17** (74.6 mg, 52%). $[\alpha]_D^{20}$: +55.2(*c* 1.00, CH_2Cl_2); IR (film) = 3056, 2922, 2879, 1761, 1736, 1582, 1478, 1439, 1385, 1358, 1269, 1227, 1175, 1051, 999, 971, 949, 932, 838, 740, 691, 475 cm^{-1}; (400 MHz $CDCl_3$) *δ* 2.01 (1H, *m*, H-1), *δ* 1.84 (1H, *m*, H-2a), *δ* 2.00 (1H, *m*, H-2b), *δ* 2.22 (1H, *m*, H-3a), *δ* 2.46 (1H, *m*, H-3b), *δ* 4.47 (1H, *d*, ^3J 8.5 Hz, H-6), *δ* 2.75 (1H, *m*, H-7), *δ* 1.70 (1H, *m*, H-8*α*), *δ* 1.89 (1H, *m*, H-8*β*), *δ* 1.62 (1H, *m*, H-9a), *δ* 1.70 (1H, *m*, H-9b), *δ* 2.17 (1H, *m*, H-10), *δ* 2.58 (1H, *ddd*, ^3J 8.8; 7.7; 3.9 Hz, H-11), *δ* 3.02 (1H, *dd*, ^3J 13.6; 8.9 Hz, H-13a), *δ* 3.46 (1H, *dd*, ^3J 13.6; 3.9 Hz, H-13b), *δ* 1.05 (3H, *d*, ^3J 7.5 Hz, Me-14), *δ* 1.10 (3H, *s*, Me-15), *δ* 7.37 (2H, *dddd*, ^3J 7.7; 2.2; 1.2; 0.8 Hz, H-2'/6'), *δ* 7.29 (2H, *dddd*, ^3J 7.8; 7.7; 2.2; 0.8 Hz, H-3'/5'), *δ* 7.21 (1H, *dddd*, ^3J 7.8; 7.8; 1.2; 1.2 Hz, H-4'); d_C (100.6 MHz, $CDCl_3$) 45.8 (C-1), 23.7 (C-2),

35.9 (C-3), 219.0 (C-4), 54.5 (C-5), 82.1 (C-6), 45.5 (C-7), 25.3 (C-8), 33.5 (C-9), 34.1 (C-10), 46.4 (C-11), 176.7 (C-12), 34.9 (C-13), 16.0 (C-14), 14.1 (C-15), 134.8 (C-1'), 129.8 (C-2'/6'), 129.1 (C-3'/5'), 126.7 (C-4'); HRMS-ESI: [M+H$^+$], found 359.1715. $C_{21}H_{26}O_3S$ requires 359.1715. In addition, the epimer **18** was obtained (67.3 mg, 47%). $[\alpha]_D^{20}$: −8.0(*c* 1.00, CH_2Cl_2); IR (film) = 2979, 2929, 2876, 2858, 1773, 1732, 1579, 1477, 1437, 1389, 1357, 1286, 1164, 1131, 1088, 965, 755, 692, 602, 580, 535, 473 cm^{-1}; (400 MHz $CDCl_3$) *δ* 2.06 (1H, *m*, H-1), *δ* 1.82 (1H, *m*, H-2a), *δ* 2.07 (1H, *m*, H-2b), *δ* 2.12 (1H, *m*, H-3a), *δ* 2.46 (1H, *m*, H-3b), *δ* 4.47 (1H, *d*, ^3J 5.3 Hz, H-6), *δ* 2.59 (1H, *m*, H-7), *δ* 1.51 (1H, *m*, H-8a), *δ* 1.55 (1H, *m*, H-8b), *δ* 1.58 (1H, *m*, H-9a), *δ* 1.85 (1H, *m*, H-9b), *δ* 2.20 (1H, *m*, H-10), *δ* 2.90 (1H, *ddd*, ^3J 10.9; 6.0; 3.5 Hz, H-11), *δ* 2.88 (1H, *dd*, ^3J 13.1; 10.9 Hz, H-13a), *δ* 3.49 (1H, *dd*, ^3J 13.1; 3.5 Hz, H-13b), *δ* 1.07 (3H, *d*, ^3J 7.6 Hz, Me-14), *δ* 1.15 (3H, *s*, Me-15), *δ* 7.36 (2H, *dddd*, ^3J 7.7; 2.0; 1.2; 0.5 Hz, H-2'/6'), *δ* 7.29 (2H, *dddd*, ^3J 7.8; 7.7; 1.9; 0.5 Hz, H-3'/5'), *δ* 7.21 (1H, *dddd*, ^3J 7.8; 7.8; 1.2; 1.2 Hz, H-4'); d_C (100.6 MHz, $CDCl_3$) 45.7 (C-1), 24.1 (C-2), 34.6 (C-3), 221.1 (C-4), 54.7 (C-5), 81.8 (C-6), 45.2 (C-7), 17.8 (C-8), 36.9 (C-9), 34.9 (C-10), 44.9 (C-11), 176.0 (C-12), 29.3 (C-13), 16.6 (C-14), 15.8 (C-15), 134.4 (C-1'), 130.0 (C-2'/6'), 129.2 (C-3'/5'), 126.9 (C-4'); HRMS-ESI: [M+H$^+$], found 359.1708. $C_{21}H_{26}O_3S$ requires 359.1715.

Indolo[3,2-c]-13-thiophenoxy-11b,13-dihydro-4-deoxydamsin (**19**). To a solution of **17** (189.5 mg, 0.5286 mmol) in AcOH (8 mL) was added phenylhydrazine (396 µL, 6.4086 mmol) and the reaction was stirred in a reflux system for 1 day at 120˚C. After that, 3 additional portion of phenylhydrazine (58 µL, 0.5894 mmol) were added, one per day. After a total reaction time of 4 days, the reaction was quenched with sat. Na_2CO_3 solution (100 mL) until pH = 8 and the aqueous mixture was extracted with DCM (5 × 30 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (from 10% to 20% EtOAc:Pet.Et.$_{40\text{-}60}$) to provide the product **19** (147.0 mg, 64%). $[\alpha]_D^{20}$: −87.3(*c* 1.00, CH_2Cl_2); IR (film) = 3396, 3054, 3002, 2921, 2855, 1763, 1582, 1479, 1448, 1440, 1384, 1359, 1305, 1266, 1226, 1176, 1129, 1090, 1025, 995, 740, 691, 474 cm^{-1}. (400 MHz $CDCl_3$) *δ* 2.66 (1H, *m*, H-1), *δ* 2.70 (1H, *dd*, ^3J 13.5; 6.7Hz, H-2a), *δ* 2.91 (1H, *dd*, ^3J 13.4; 10.3Hz, H-2b), *δ* 4.68 (1H, d, ^3J 9.5 Hz, H-6), *δ* 2.94 (1H, m, H-7), *δ* 1.93 (1H, m, H-8a), *δ* 2.06 (1H, *m*, H-8b), *δ* 1.65 (1H, *m*, H-9a), *δ* 1.83 (1H, *m*, H-9b), *δ* 2.31 (1H, *m*, H-10), *δ* 2.78 (1H, *m*, H-11), *δ* 3.19 (1H, *dd*, ^3J 13.5; 7.1 Hz, H-13a), *δ* 3.51 (1H, *dd*, ^3J 13.5; 4.1 Hz, H-13b), *δ* 1.19 (3H, *d*, ^3J 7.5 Hz, Me-14), *δ* 1.24 (3H, *s*, Me-15), *δ* 7.43 (1H, *m*, H-4'), *δ* 7.06 (1H, *m*, H-5'), *δ* 7.10 (1H, *m*, H-6'), *δ* 7.31 (1H, *m*, H-7'), *δ* 8.26 (1H, *bs*, N-H), *δ* 7.39 (2H, *m*, H-2''/6''), *δ* 7.32 (2H, *m*, H-3''/5''), *δ* 7.23 (1H, *m*, H-4''); d_C (100.6 MHz, $CDCl_3$) 46.3 (C-1), 24.8 (C-2), 115.1 (C-3/3'), 148.9 (C-4/2'), 50.7 (C-5), 87.8 (C-6), 44.0 (C-7), 24.8 (C-8), 33.3 (C-9), 34.0 (C-10), 55.9 (C-11), 177.1 (C-12), 35.1 (C-13), 14.9 (C-14), 18.0 (C-15), 124.1 (C-4a'), 118.7 (C-4'), 119.6 (C-5'), 121.0 (C-6'), 111.8 (C-7'), 139.6 (C-7a'), 135.3 (C-1''), 129.8 (C-2''/6''), 129.2 C-3''/5''), 126.8 (C-4''); HRMS-ESI: [M+H$^+$], found 432.2035. $C_{21}H_{23}NO_2$ requires 432.1997.

Indolo[3,2-c]-4-deoxydamsin(**5**). To a solution of **19** (130.1 mg, 0.3014 mmol) in DCM (4 mL) was added *m*-CPBA 77% (67.5 mg, 0.3014 mmol) and the reaction was stirred at −40˚C. After 3 h, the reaction was quenched with sat. $NaHCO_3$ solution (2 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product. Then, it was suspended in PhMe (20 mL) and the suspension was stirred in a reflux system at 110˚C for 3.5 h. The reaction was quenched allowing to reach room temperature and the solvent was evaporated *in vacuo*. The product was purified by silicagel flash chromatography (15% EtOAc:Pet.Et.$_{40\text{-}60}$) to provide the product **5** (64.1 mg, 66%). $[\alpha]_D^{20}$: −183.8(*c* 1.00, CH_2Cl_2); IR (film) = 3395, 3369, 3057, 2996, 2957, 2918, 2858, 1758, 1449, 1383, 1351, 1339, 1306, 1271, 1253, 1228, 1149, 1114, 1084, 1020, 998, 971, 927, 812, 741, 711, 681, 604, 564, 532, 414 cm^{-1}; (400 MHz $CDCl_3$) *δ* 2.69 (1H, *ddd*, ^3J 10.0; 6.8; 3.5Hz, H-1), *δ* 2.73 (1H, *dd*, ^3J 13.2; 6.8Hz, H-2a), *δ* 2.93 (1H, *dd*, ^3J 13.1; 10.0Hz, H-2b), *δ* 4.79 (1H, *d*, ^3J 9.4 Hz, H-6), *δ* 3.46 (1H, *tdt*, ^3J 10.1; 10.1; 6.7; 3.4; 3.4 Hz, H-7), *δ* 2.04 (1H, *m*, H-8a), *δ* 2.16 (1H, *m*, H-8b), *δ* 1.76 (1H, *m*, H-9a), *δ* 1.95 (1H, *m*, H-9b), *δ* 2.35 (1H, *m*, H-10), *δ* 5.59 (1H, *d*, ^3J 3.3 Hz, H-13a), *δ* 6.31 (1H, *d*, ^3J 3.7 Hz, H-13b), *δ* 1.20 (3H, *d*, ^3J 7.5 Hz, Me-14), *δ* 1.16 (3H, *s*, Me-15), *δ* 7.43 (1H, *m*, H-4'), *δ* 7.07 (1H, *m*, H-5'), *δ* 7.11 (1H, *m*, H-6'), *δ* 7.33 (1H, *m*, H-7'); d_C (100.6 MHz, $CDCl_3$) 55.9 (C-1), 28.0 (C-2), 115.0 (C-3/3'), 149.0 (C-4/2'), 50.9 (C-5), 87.3 (C-6), 42.7 (C-7), 24.4 (C-8), 33.2 (C-9), 34.1 (C-10), 139.8 (C-11), 170.4 (C-12), 120.9 (C-13), 14.8 (C-14), 17.3 (C-15),124.0 (C-3a'), 118.7 (C-4'), 119.6 (C-5'), 121.2 (C-6'), 111.8 (C-7'), 139.6 (C-7a'); HRMS-ESI: [M+H$^+$], found 322.1816. $C_{21}H_{23}NO_2$ requires 322.1807.

4,4-(ethylenedioxy)-4-deoxydamsin (**20**). To a stirred solution of **1** (400 mg, 1.6108 mmol), p-TsOH (4.6 mg, 0.2416 mmol), ethylene glycol (898 µL, 16.1082 mmol) in dry DCM (2 mL), was added slowly ethyl orthofor-

mate (603 µL, 3.6243 mmol). The reaction was stirred at room temperature for 24 h. After that, it was quenched with a sat. $NaHCO_3$ solution (5 mL), then 10 mL of brine were added and the aqueous layer was extracted with DCM (3 × 15 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (20% EtOAc:Pet. $Et._{40\text{-}60}$) to provide the product **20** (455.4 mg, 97%). $[\alpha]_D^{20}$: −83.5 (*c* 1.00, CH_2Cl_2); IR (film) = 3323, 2968, 2945, 2903, 2878, 1751, 1659, 1469, 1385, 1302, 1271, 1176, 1135, 1053, 1021, 1003, 978, 953, 912, 804, 734, 701, 459 cm^{-1}; (400 MHz $CDCl_3$) δ 2.09 (1H, *m*, H-1), δ 1.58 (1H, *m*, H-2a), δ 1.76 (1H, *m*, H-2b), δ 1.73 (1H, *m*, H-3a), δ 1.90 (1H, *m*, H-3b), δ 4.95 (1H, *d*, ^3J 9.5 Hz, H-6), δ 3.32 (1H, *m*, H-7), δ 1.87 (1H, *m*, H-8a), δ 2.02 (1H, *m*, H-8b), δ 1.58 (1H, *m*, H-9a), δ 1.86 (1H, *m*, H-9b), δ 2.07 (1H, *m*, H-10), δ 5.42 (1H, *d*, ^3J 3.3 Hz, H-13a), δ 6.13 (1H, *d*, ^3J 3.4 Hz, H-13b), δ 1.00 (3H, *d*, ^3J 7.3 Hz, Me-14), δ 0.93 (3H, *s*, Me-15), δ 3.85 (2H, *m*, -OC$\underline{H_2}$$CH_2$O-), δ 4.05 (2H, *m*, -$OCH_2C\underline{H_2}$O-); d_C (100.6 MHz, $CDCl_3$) 46.9 (C-1), 22.9 (C-2), 33.8 (C-3), 119.9 (C-4), 53.0 (C-5), 82.5 (C-6), 43.0 (C-7), 23.4 (C-8), 32.7 (C-9), 33.6 (C-10), 141.0 (C-11), 170.5 (C-12), 118.8 (C-13), 14.4 (C-14), 12.9 (C-15), 64.8 (-OC$\underline{H_2}$$CH_2$O-), 64.7 (-$OCH_2C\underline{H_2}$O-); HRMS-ESI: [M + Na^+], found 315.1575. $C_{17}H_{24}O_4$ requires 315.1572.

13-cyano-4,4-(ethylenedioxy)-11b,13-dihydro-4-deoxydamsin (**21**). To a solution of **20** (60.8 mg, 0.2081 mmol) and cynohydrin (76.0 µL, 0.8324 mmol) in DMSO (2 mL) was added NaCN (5.0 mg, 0.1020 mmol), and reaction was stirred in a reflux system at 30˚C. After 3.5 h, the reaction was quenched with a sat. NH_4Cl solution (10 mL), extracted with EtOAc (3 × 10 mL), then the combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (20% EtOAc:Pet.$Et._{40\text{-}60}$) to provide the product **21** (58.1 mg, 87%). $[\alpha]_D^{20}$: −136.1(*c* 1.00, CH_2Cl_2); IR (film) = 2965, 2904, 1763, 1474, 1456, 1419, 1388, 1363, 1332, 1285, 1259, 1185, 1066, 1054, 998, 957, 940, 802, 734, 701, 673, 651, 617 cm^{-1};(400 MHz $CDCl_3$) δ 2.10 (1H, *m*, H-1), δ 1.59 (1H, *m*, H-2a), δ 1.77 (1H, *m*, H-2b), δ 1.77 (1H, *m*, H-3β), δ 1.92 (1H, *m*, H-3α), δ 4.89 (1H, *d*, ^3J 9.5 Hz, H-6), δ 2.78 (1H, *m*, H-7), δ 1.83 (1H, *m*, H-8a), δ 2.04 (1H, *m*, H-8b), δ 1.54 (1H, *m*, H-9a), δ 1.80 (1H, *m*, H-9b), δ 2.05 (1H, *m*, H-10), δ 2.64 (1H, *m*, H-11), δ 2.62 (1H, *m*, H-13a), δ 2.81 (1H, *m*, H-13b), δ 1.01 (3H, *d*, ^3J 7.3 Hz, Me-14), δ 1.06 (3H, *s*, Me-15), δ 3.85 (2H, *m*, -OC$\underline{H_2}$$CH_2$O-), δ 4.01 (2H, *m*, -$OCH_2C\underline{H_2}$O-); d_C (100.6 MHz, $CDCl_3$) 47.4 (C-1), 22.8 (C-2), 33.9 (C-3), 119.8 (C-4), 52.6 (C-5), 83.5 (C-6), 44.2 (C-7), 23.2 (C-8), 32.3 (C-9), 33.2 (C-10), 43.1 (C-11), 175.2 (C-12), 18.1 (C-13), 14.3 (C-14), 13.4 (C-15), 64.7 (-OC$\underline{H_2}$$CH_2$O-), 64.6 (-$OCH_2C\underline{H_2}$O-), 116.9 (-$\underline{C}$≡N); HRMS-ESI: [M+$Na^+$], found 342.1694. $C_{18}H_{25}NO_4$ requires 342.1681.

4,4-(ethylenedioxy)-11b,13-dihydro-13-(N,N-dimethylaminomethyl)-4-deoxydamsin (**22**). A solution of **21** (645 mg, 2.0194 mmol), Pd-C 10% (193.0 mg) and Me_2NH (5.9 mL, 33.0400 mmol, sol. ~5.6 M/EtOH) in EtOH (50 mL), was stirred in an autoclave under H_2 (400 psi) at room temperature for 6 days. Additional Pd-C (100 mg) was added and the reaction was completed after one more day. The reaction mixture was filtered trough celite, then the pad was washed with EtOH (2 × 10 mL) and the combined alcoholic extracts were concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography [2% (10% NH_4OH in MeOH)/DCM] to give the provide **22** (674 mg, 95%). $[\alpha]_D^{20}$: −98.9(*c* 1.00, CH_2Cl_2); IR (film) = 2968, 2941, 2901, 2864, 2817, 2766, 1758, 1463, 1385, 1360, 1299, 1225, 1175, 1123, 1073, 1043, 1007, 994, 951, 911, 733, 702, 453 cm^{-1}; (400 MHz $CDCl_3$) δ 2.05 (1H, *m*, H-1), δ 1.55 (1H, *m*, H-2a), δ 1.74 (1H, *m*, H-2b), δ 1.72 (1H, *m*, H-3a), δ 1.91 (1H, *m*, H-3b), δ 4.78 (1H, *d*, ^3J 9.6 Hz, H-6), δ 2.56 (1H, *m*, H-7), δ 1.78 (1H, *m*, H-8a), δ 1.83 (1H, *m*, H-8b), δ 1.48 (1H, *m*, H-9a), δ 1.75 (1H, *m*, H-9b), δ 2.03 (1H, *m*, H-10), δ 2.40 (1H, *m*, H-11), δ 1.70 (1H, *m*, H-13a), δ 1.84 (1H, *m*, H-13b), δ 1.02 (3H, *d*, ^3J 7.3 Hz, Me-14), δ 1.07 (3H, *s*, Me-15), δ 2.45 (2H, *m*, -C$\underline{H_2}$$N(CH_3)_2$), δ 2.23 (6H, *s*, -$CH_2N(C\underline{H_3})_2$), δ 3.84 (2H, *m*, -OC$\underline{H_2}$$CH_2$O-), δ 4.02 (2H, *m*, -$OCH_2C\underline{H_2}$O-); d_C (100.6 MHz, $CDCl_3$) 47.3 (C-1), 22.9 (C-2), 33.9 (C-3), 120.1 (C-4), 52.8 (C-5), 82.7 (C-6), 45.0 (C-7), 23.8 (C-8), 33.0 (C-9), 33.5 (C-10), 44.3 (C-11), 179.0 (C-12), 28.3 (C-13), 14.5 (C-14), 13.7 (C-15), 56.5 (-C$\underline{H_2}$$N(CH_3)_2$), 45.3 (-$CH_2N(C\underline{H_3})_2$), 64.8 (-OC$\underline{H_2}$$CH_2$O-), 64.7 (-$OCH_2C\underline{H_2}$O-); HRMS-ESI: [M+$H^+$], found 352.2494. $C_{20}H_{33}NO_4$ requires 352.2488.

11b-bromo-4,4-(ethylenedioxy)-13-hydro-13-(N,N-dimethylaminomethyl)-4-deoxydamsin (**23**). To a solution of **22** (56.9 mg, 0.1620 mmol) and dry Et_3N (135.1 µL, 0.9720mmol) in dry DCM (1.8 mL), was added TMSOTf (93.9 µL, 0.4860 mmol) and the mixture was stirred at 0˚C for 2 h. Then, TMPAP (182.7 mg, 0.4860 mmol) was added as solution in dry DCM (2.5 mL) and the reaction was stirred at 0˚C for exactly 1.75 h. The reaction was quenched with a 10% $Na_2S_2O_3$ sol. (10 mL) and sat. $NaHCO_3$ solution (10 mL), extracted with DCM (4 × 10 mL), whereafter the combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography [from 1% to 3% (10% NH_4OH

in MeOH)/DCM] to give the product **23** (38.9 mg, 67%). $[\alpha]_D^{20}$: −58.6(*c* 1.00, CH_2Cl_2); IR (film) = 3440, 2961, 2901, 2824, 2770, 2623, 2465, 1750, 1673, 1474, 1387, 1345, 1264, 1197, 1106, 1049, 1013, 985, 954, 923, 800, 731, 699, 566, 411 cm^{-1}; (400 MHz $CDCl_3$) δ 2.12 (1H, *td*, ^3J 9.5; 9.5; 3.5Hz, H-1), δ 1.65 (1H, *m*, H-2a), δ 1.68 (1H, *m*, H-2b), δ 1.72 (1H, *m*, H-3a), δ 1.87 (1H, *m*, H-3b), δ 5.29 (1H, *d*, ^3J 5.3 Hz, H-6), δ 2.67 (1H, *m*, H-7), δ 1.58 (1H, *m*, H-8a), δ 1.58 (1H, *m*, H-8b), δ 1.54 (1H, *m*, H-9a), δ 1.81 (1H, *m*, H-9b), δ 2.01 (1H, *m*, H-10), δ 1.99 (1H, *ddd*, ^3J 14.8; 10.6; 4.0 Hz, H-13a), δ 2.38 (1H, *ddd*, ^3J 15.0; 11.5; 4.7 Hz, H-13b), δ 1.00 (3H, *d*, ^3J 7.5 Hz, Me-14), δ 1.15 (3H, *s*, Me-15), δ 2.52 (1H, *td*, ^3J 11.8; 11.8; 3.9 Hz, -C$\underline{H_a}H_bN(CH_3)_2$), δ 2.82 (1H, *ddd*, ^3J 11.9; 10.8; 4.7 Hz, -C$H_a\underline{H_b}N(CH_3)_2$), δ 2.31 (6H, *s*, -C$H_aH_bN(C\underline{H_3})_2$), δ 3.93 (2H, *m*, -OC$\underline{H_2}CH_2$O-), δ 3.98 (2H, *m*, -OC$H_2C\underline{H_2}$O-); d_C (100.6 MHz, $CDCl_3$) 44.1 (C-1), 23.3 (C-2), 31.1 (C-3), 119.8 (C-4), 51.1 (C-5), 79.8 (C-6), 56.1 (C-7), 19.9 (C-8), 36.8 (C-9), 34.4 (C-10), 64.7 (C-11), 173.6 (C-12), 32.1 (C-13), 16.1 (C-14), 16.4 (C-5), 55.6 (-$\underline{C}H_2N(CH_3)_2$), 45.3 (-$CH_2N(\underline{C}H_3)_2$), 65.3 (-O$\underline{C}H_2CH_2$O-), 64.7 (-OC$H_2\underline{C}H_2$O-); HRMS-ESI: $[M+H^+]$, found 430.1561. $C_{20}H_{32}BrNO_4$ requires 430.1593.

(E)-4,4-(ethylenedioxy)-13-(N,N-dimethylaminomethyl)-4-deoxydamsin (**24**). To a solution of **23** (157.2 mg, 0.3653 mmol) in dry THF (7 mL) was added TBAF (730.6 µL, 0.7306 mmol), and the reaction was stirred at room temperature. After 11.5 h, the reaction was quenched adding a sat. $NaHCO_3$ solution (20 mL) and the mixture was extracted with DCM (3 × 20 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography [2% (10% NH_4OH in MeOH)/DCM] to provide the product **24** (95.1 mg, 75%). $[\alpha]_D^{20}$: -80.9(*c* 1.00, CH_2Cl_2); IR (film) = 2966, 2943, 2875, 2819, 2769, 1749, 1674, 1455, 1385, 1312, 1263, 1234, 1206, 1175, 1153, 1125, 1040, 1006, 985, 953, 911, 838, 728, 643, 466 cm^{-1}; (400 MHz $CDCl_3$) δ 2.10 (1H, *td*, ^3J 9.6; 9.6; 4.0Hz, H-1), δ 1.62 (1H, *m*, H-2a), δ 1.69 (1H, *m*, H-2b), δ 1.71 (1H, *m*, H-3a), δ 1.90 (1H, *m*, H-3b), δ 4.92 (1H, *d*, ^3J 8.2 Hz, H-6), δ 3.24 (1H, *m*, H-7), δ 1.66 (1H, *m*, H-8a), δ 1.97 (1H, *m*, H-8b), δ 1.59 (1H, *m*, H-9a), δ 1.78 (1H, *m*, H-9b), δ 2.02 (1H, *m*, H-10), δ 6.72 (1H, *ddd*, ^3J 7.8; 5.5; 2.6 Hz, H-13), δ 1.02 (3H, *d*, ^3J 7.4 Hz, Me-14), δ 1.11 (3H, *s*, Me-15), δ 3.13 (1H, *dd*, ^3J 14.4; 7.7 Hz, -C$\underline{H_a}H_bN(CH_3)_2$), δ 3.27 (1H, *m*, -C$H_a\underline{H_b}N(CH_3)_2$), δ 2.31 (6H, *s*, -C$H_aH_bN(C\underline{H_3})_2$), δ 3.88 (2H, *m*, -OC$\underline{H_2}CH_2$O-), δ 3.97 (2H, *m*, -OC$H_2C\underline{H_2}$O-); d_C (100.6 MHz, $CDCl_3$) 45.2 (C-1), 23.2 (C-2), 32.5 (C-3), 119.9 (C-4), 52.5 (C-5), 80.1 (C-6), 44.6 (C-7), 24.9 (C-8), 35.7 (C-9), 34.2 (C-10), 134.7 (C-11), 170.7 (C-12), 135.1 (C-13), 15.4 (C-14), 14.9 (C-15), 56.9 (-$\underline{C}H_2N(CH_3)_2$), 45.1 (-$CH_2N(\underline{C}H_3)_2$), 64.7 (-O$\underline{C}H_2CH_2$O-), 65.0 (-OC$H_2\underline{C}H_2$O-); HRMS-ESI: $[M+H^+]$, found 350.2313. $C_{20}H_{31}NO_4$ requires 350.2331.

(E)-13-(N,N-dimethylaminomethyl)damsin (**8**). To a solution of **24** (19.7 mg, 0.0564 mmol) in MeOH (0.4 mL) was added $HCl_{(Conc)}$ (200 µL, 2.3303 mmol) and the reaction stirred at room temperature. After 6 h, the reaction was quenched with sat. $NaHCO_3$ solution (15 mL) and the aqueous solution was extracted with DCM (5 × 10 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography [2% (10% NH_4OH in MeOH)/DCM] to provide the product **8** (14.5 mg, 84%). $[\alpha]_D^{20}$: +3.0(*c* 1.00, CH_2Cl_2); IR (film) = 2940, 2862, 2821, 2772, 2723, 1738, 1677, 1454, 1407, 1386, 1372, 1333, 1315, 1263, 1209, 1153, 1132, 1096, 1050, 1012, 987, 838, 798, 536 cm^{-1}; (400 MHz $CDCl_3$) δ 2.05 (1H, *m*, H-1), δ 1.84 (1H, *m*, H-2a), δ 2.06 (1H, *m*, H-2b), δ 2.20 (1H, *m*, H-3a), δ 2.46 (1H, *m*, H-3b), δ 4.45 (1H, *d*, ^3J 7.3 Hz, H-6), δ 3.15 (1H, *m*, H-7), δ 1.47 (1H, *dd*, ^3J 15.0; 6.8 Hz,H-8a), δ 1.94 (1H, *m*, H-8b), δ 1.72 (1H, *m*, H-9a), δ 1.85 (1H, *m*, H-9b), δ 2.21 (1H, *m*, H-10), δ 6.74 (1H, *ddd*, ^3J 7.7; 5.5; 2.0 Hz, H-13a), δ 1.09 (3H, *d*, ^3J 7.6 Hz, Me-14), δ 1.19 (3H, *s*, Me-15), δ 3.05 (1H, *dd*, ^3J 15.1; 7.8 Hz, -C$\underline{H_a}H_bN(CH_3)_2$), δ 3.19 (1H, *dd*, ^3J 15.0; 5.6 Hz, -C$H_a\underline{H_b}N(CH_3)_2$), δ 2.28 (6H, *s*, -C$H_aH_bN(C\underline{H_3})_2$); d_C (100.6 MHz, $CDCl_3$) 46.0 (C-1), 24.3 (C-2), 34.7 (C-3), 220.4 (C-4), 54.8 (C-5), 80.7 (C-6), 45.1 (C-7), 25.9 (C-8), 36.6 (C-9), 35.1 (C-10), 134.2 (C-11), 170.1 (C-12), 136.4 (C-13), 16.2 (C-14), 15.1 (C-15), 57.3 (-$\underline{C}H_2N(CH_3)_2$), 45.3 (–$CH_2N(\underline{C}H_3)_2$); HRMS-ESI: $[M+H^+]$, found 306.2070. $C_{18}H_{27}NO_3$ requires 306.2069.

4,4-(ethylenedioxy)-4-deoxyisodamsin (**25**). To a stirred solution of **7** (100 mg, 0.4027 mmol), p-TsOH (2.3 mg, 0.1208 mmol), ethylene glycol (450 µL, 8.0540 mmol) in dry DCM (2 mL) was added slowly ethyl orthoformate (301 µL, 1.8121 mmol), and the reaction was stirred at room temperature for 24 h. The reaction was quenched with a sat. $NaHCO_3$ solution (20 mL) and the aqueous mixture was extracted with DCM (5x20 mL). The combined organic layers were washed with brine (15 mL), dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (from 10% to 20% EtOAc: $Pet.Et._{40\text{-}60}$) to provide the product **25** (113.0 mg, 96%). $[\alpha]_D^{20}$: −33.6(*c* 1.00, CH_2Cl_2); IR (film) = 3055, 2967, 2945, 2917, 2879, 1743, 1663, 1471, 1458, 1422, 1384 1322, 1303, 1263, 1180, 1147, 1100, 1077, 1050, 1017, 998, 952, 916, 880, 800, 731, 701, 648, 533 cm^{-1}; (400 MHz $CDCl_3$) δ 2.25 (1H, *m*, H-1), δ 1.58 (1H, *m*, H-2a),

δ 1.72 (1H, *m*, H-2b), δ 1.80 (1H, *m*, H-3a), δ 1.92 (1H, *m*, H-3b), δ 5.07 (1H, *s*, H-6), δ 2.60 (1H, *m*, H-8a), δ 2.64 (1H, *m*, H-8b), δ 1.73 (1H, *m*, H-9a), δ 1.80 (1H, *m*, H-9b), δ 2.09 (1H, *m*, H-10), δ 1.77 (3H, *d*, ^3J 1.5 Hz, Me-13), δ 0.94 (3H, *d*, ^3J 7.5 Hz, Me-14), δ 0.70 (3H, *s*, Me-15), δ 3.86 (2H, *m*, -O$\underline{CH_2}CH_2$O-), δ 4.09 (2H, *m*, -OCH$_2\underline{CH_2}$O-); d_C (100.6 MHz, $CDCl_3$) 48.1 (C-1), 23.1 (C-2), 34.5 (C-3), 119.1 (C-4), 51.7 (C-5), 85.3 (C-6), 163.8 (C-7), 22.2 (C-8), 31.2 (C-9), 33.2 (C-10), 123.5 (C-11), 174.9 (C-12), 8.4 (C-13), 13.7 (C-14), 11.1 (C-15), 66.0 (-O$\underline{CH_2}$CH$_2$O-), 64.8 (-OCH$_2\underline{CH_2}$O-); HRMS-ESI: [M+H$^+$], found 293.1750. $C_{17}H_{24}O_4$ requires 293.1753.

4,4-(ethylenedioxy)-6α-hydroxy-4-deoxyisodamsin (**27**). i) To a stirred solution of **25** (303.8 mg, 1.0398 mmol) and TDMSOTf (465.0 µL, 2.0247 mmol) in dry DCM (10 mL) at 0˚C, was added dry TEA (303.0 µL, 2.1800 mmol) dropwise. The reaction was allowed to reach room temperature and after 3 days, quenched with water (10 mL). Then the mixture was extracted with DCM (3 × 10 mL), dried with Na_2SO_4 and concentarted *in vacuo* to give the crude intermediate. ii) The crude of the previous step was dissolved in dry DCM (9 mL) and *m*-CPBA 77% (233.0 mg, 1.0398 mmol) was added, the reaction was stirred at room temperature for 5 h. The reaction was quenched with a 10% $Na_2S_2O_3$ solution (1 mL), then brine was added (10 mL) and the aqueous mixture was extracted with DCM (4 × 15 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo*. The product was purified by silicagel flash chromatography (from 15% to 20% EtOAc: Pet.Et.$_{40\text{-}60}$) to provide the product **27** (233.3 mg, 73%). $[\alpha]_D^{20}$: -21.1(*c* 1.00, CH_2Cl_2); IR (film) = 3378, 2943, 2915, 2882, 1753, 1674, 1473, 1430, 1385, 1304, 1223, 1182, 1155, 1131, 1116, 1077, 1050, 1001, 973, 941, 903, 762, 646, 535, 453 cm^{-1}; (400 MHz $CDCl_3$) δ 2.73 (1H, *ddd*, ^3J 12.4; 7.4; 4.8Hz, H-1), δ 1.59 (1H, *m*, H-2a), δ 1.71 (1H, *m*, H-2b), δ 1.85 (1H, *m*, H-3a), δ 1.92 (1H, *m*, H-3b), δ 2.60 (1H, *m*, H-8a), δ 2.60 (1H, *m*, H-8b), δ 1.62 (1H, *m*, H-9a), δ 2.28 (1H, *m*, H-9b), δ 2.14 (1H, *m*, H-10), δ 1.77 (3H, *t*, ^3J 1.4; 1.4 Hz, Me-13), δ 0.95 (3H, *d*, ^3J 7.6 Hz, Me-14), δ 0.82 (3H, *s*, Me-15), δ 3.90 (2H, *m*, -O$\underline{CH_2}CH_2$O-), δ 4.15 (2H, *m*, -OCH$_2\underline{CH_2}$O-), δ 6.53 (1H, *s*, -O$\underline{H}$); d_C (100.6 MHz, $CDCl_3$) 46.1 (C-1), 23.1 (C-2), 36.2 (C-3), 121.4 (C-4), 53.4 (C-5), 110.1 (C-6), 160.5 (C-7), 21.9 (C-8), 29.8 (C-9), 33.4 (C-10), 125.3 (C-11), 172.9 (C-12), 8.2 (C-13), 14.1 (C-14), 14.1 (C-15), 64.9 (-O$\underline{CH_2}$CH$_2$O-), 64.4 (-OCH$_2\underline{CH_2}$O-); HRMS-ESI: [M + H$^+$], found 309.1711. $C_{17}H_{24}O_5$ requires 309.1702.

6α-hydroxyisodamsin (**9**). To a solution of **27** (152.1 mg, 0.4932 mmol) in MeOH (5 mL) was added $HCl_{(Conc)}$ (1 mL, 11.6511 mmol) and the reaction was stirred at room temperature. After 4.5 h, the reaction was quenched with sat. $NaHCO_3$ solution (10 mL) and the aqueous mixture was extracted with DCM (4 × 30 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (45% EtOAc:Pet.Et.$_{40\text{-}60}$) to provide the product **9** (94.3 mg, 72%). $[\alpha]_D^{20}$: +43.6(*c* 1.00, CH_2Cl_2); IR (film) = 3292, 2966, 2951, 2931, 2867, 1727, 1677, 1453, 1411, 1376, 1270, 1242, 1214, 1169, 1153, 1087, 1058, 1013, 976, 958, 885, 867, 802, 759, 733, 702, 688, 662, 554, 418 cm^{-1}; (400 MHz $CDCl_3$) δ 3.01 (1H, *dt*, ^3J 12.4; 6.0; 6.0Hz, H-1), δ 1.74 (1H, *m*, H-2a), δ 1.85 (1H, *m*, H-2b), δ 2.32 (1H, *m*, H-3a), δ 2.32 (1H, *m*, H-3b), δ 2.41 (1H, *m*, H-8a), δ 2.62 (1H, *ddd*, ^3J 14.0; 6.6; 1.2 Hz, H-8b), δ 1.12 (1H, *m*, H-9a), δ 2.20 (1H, *m*, H-9b), δ 2.19 (1H, *m*, H-10), δ 1.73 (3H, *s*, Me-13), δ 0.92 (3H, *d*, ^3J 6.8 Hz, Me-14), δ 0.82 (3H, *s*, Me-15), δ 4.71 (1H, *bs*, ^3J Hz, -O$\underline{H}$); d_C (100.6 MHz, $CDCl_3$) 41.4 (C-1), 23.6(C-2), 39.6 (C-3), 218.3 (C-4), 56.1 (C-5), 107.7 (C-6), 161.4 (C-7), 22.3 (C-8), 32.8 (C-9), 33.0 (C-10), 125.0 (C-11), 172.6 (C-12), 7.9 (C-13), 17.6 (C-14), 14.0 (C-15); HRMS-ESI: [M + H$^+$], found 265.1466. $C_{15}H_{20}O_4$ requires 265.1440.

13-phenylisodamsin (10). A solution of **1** (150 mg, 0.6040 mmol), $Pd(OAc)_2$ (5.0 mg, 0.0242 mmol), Tri(p-Tolyl)P (29.0 mg, 0.0966 mmol), PhI (81.0 µL, 0.7248 mmol) and Et_3N (151 µL, 1.0872 mmol) in DMF (1.6 mL), in a sealed tube was stirred at 120˚C for 4.5 days. After that time the reaction was not complete, therefore a second addition of $Pd(OAc)_2$ (5.0 mg, 0.0242 mmol), Tri(p-Tolyl)P (29.0 mg, 0.0966 mmol), PhI (81.0 µL, 0.7248 mmol) and Et_3N (151 µL, 1.0872 mmol) as solution in DMF (1.6 mL) was done. The reaction was stirred for was stirred at 120˚C for 30 h. When the reaction was complete, it was quenched with water (25 mL), and the aqueous mixture was extracted with DCM (5 × 20 mL). The combined organic layers were dried with Na_2SO_4 and contrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (30% EtOAc:Pet. Et.$_{40\text{-}60}$) to provide the product **10** (112.0 mg, 57%). $[\alpha]_D^{20}$: −14.1(*c* 1.00, CH_2Cl_2); IR (film) = 2961, 2923, 2878, 1737, 1664, 1494, 1454, 1301, 1154, 1121, 1068, 1022, 990, 967, 884, 805, 732, 700 cm^{-1}; (400 MHz $CDCl_3$) δ 2.30 (1H, *m*, H-1), δ 1.92 (1H, *m*, H-2a), δ 1.94 (1H, *m*, H-2b), δ 2.32 (1H, *m*, H-3a), δ 2.47 (1H, *ddd*, ^3J 18.3; 7.1; 1.6Hz, H-3b), δ 4.64 (1H, *s*, H-6), δ 2.31 (1H, *m*, H-8a), δ 2.82 (1H, *dd*, ^3J 14.9; 8.0 Hz, H-8b), δ 1.15 (1H, *m*, H-9a), δ 2.03 (1H, *m*, H-9b), δ 2.21 (1H, *m*, H-10), δ 3.58 (1H, *dso*, ^3J 14.8 Hz,

H-13a), δ 3.65 (1H, *dso*, ^3J 14.8 Hz, H-13b), δ 0.94 (3H, *d*, ^3J 7.2 Hz, Me-14), δ 0.79 (3H, *s*, Me-15), δ 7.28 (2H, *m*, H-2'/6'), δ 7.25 (2H, *m*, H-3'/5'), δ 7.21 (1H, *m*, H-4'); d_C (100.6 MHz, $CDCl_3$) 44.1 (C-1), 23.7 (C-2), 37.3 (C-3), 217.4 (C-4), 52.7 (C-5), 84.7 (C-6), 162.5 (C-7), 23.4 (C-8), 32.0 (C-9), 32.9 (C-10), 127.6 (C-11), 174.0 (C-12), 29.3 (C-13), 17.2 (C-14), 11.2 (C-15), 138.2 (C-1'), 128.3 (C-2'/6'), 128.5 (C-3'/5'), 126.4 (C-4'); [M+H$^+$], found 325.1834. $C_{21}H_{24}O_3$ requires 325.1804.

(E)-11*β*,13-dihydro-13-ethoxy-3-(phenylmethylene)-damsin (**13**).To a solution of **1** (100 mg, 0.4027 mmol) and benzaldehyde (82.0 µL, 0.8052 mmol) in EtOH (1 mL), was added TBAH (265 µL, 0.4027 mmol, solution 40% in water) and the reactionwas stirred at room temperature. After 24 h, the reaction was acidified with a 10% HCl solution (pH = 2), then brine (15 mL) was added and the aqueous mixture was extracted with DCM (5x20 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (from 20% to 30% EtOAc:Pet. Et.$_{40\text{-}60}$) to provide the product **13** (77.2 mg, 50%). $[\alpha]_D^{20}$: +29.1(*c* 1.00, CH_2Cl_2); IR (film) = 2973, 2927, 2865, 1763, 1715, 1624, 1492, 1449, 1359, 1251, 1230, 1182, 1163, 1108, 1027, 982, 772, 734, 693, 517 cm^{-1}; (400 MHz $CDCl_3$) δ 2.06 (1H, *m*, H-1), δ 2.84 (1H, *m*, H-2a), δ 2.95 (1H, *ddd*, ^3J 16.7; 12.3; 3.2Hz, H-2b), δ 4.61 (1H, *d*, ^3J 8.6 Hz, H-6), δ 2.79 (1H, *m*, H-7), δ 1.71 (1H, *m*, H-8a), δ 1.97 (1H, *m*, H-8b), δ 1.64 (1H, *m*, H-9a), δ 1.82 (1H, *m*, H-9b), δ 2.26 (1H, *m*, H-10), δ 2.50 (1H, *ddd*, ^3J 7.2; 5.2; 3.9 Hz, H-11), δ 3.67 (1H, *dd*, ^3J 9.6; 3.8 Hz, H-13a), δ 3.70 (1H, *dd*, ^3J 9.6; 5.2 Hz, H-13b), δ 1.16 (3H, *d*, ^3J 7.1 Hz, Me-14), δ 1.20 (3H, *s*, Me-15), δ 7.41 (1H, *m*, H-1'), δ 7.54 (2H, *m*, H-2''/6''), δ 7.41 (2H, *m*, H-3''/5''), δ 7.35 (1H, *m*, H-4''), δ 3.48 (2H, *m*, -O$\underline{CH_2}$CH$_3$), δ 1.14 (3H, *t*, ^3J 7.0; 7.0 Hz, -OCH$_2\underline{CH_3}$); d_C (100.6 MHz, $CDCl_3$) 43.6 (C-1), 31.2 (C-2), 133.5 (C-3), 208.0 (C-4), 54.6 (C-5), 82.8 (C-6), 43.7 (C-7), 25.6 (C-8), 34.3 (C-9), 34.0 (C-10), 48.8 (C-11), 177.1 (C-12), 68.7 (C-13), 15.8 (C-14), 14.7 (C-15), 133.7 (C-1'), 135.4 (C-1''), 130.5 (C-2''/6''), 128.7 (C-3''/5''), 129.4 (C-4''), 66.8 (-O$\underline{CH_2}$CH$_3$), 14.9 (-OCH$_2\underline{CH_3}$); HRMS-ESI: [M+H$^+$], found 383.2228. $C_{24}H_{30}O_4$ requires 383.2222.

11*β*,13-epoxy-10-hydroxydamsin (**14**). Two stock solutions were prepared: I) 42 mg of **16** and AcOH (35.0 µL) in ACN (1.8 mL). II) 99 µL of H_2O_2 50%wt in ACN (13.5 mL). To a stirred solution of 100 mg of **1** (0.4027 mmol) in 0.6 mL of Stock I [Containing: **16** (14.0 mg, 0.0201 mmol), AcOH (11.5 µL, 0.2013 mmol)], was added 4.5 mL of Stock II [Containing: H_2O_2 50%wt (33.0 µL, 0.4832mmol)] dropwise during 5 minutes. The addition of the same amount of both Stock solutions was repeated two times more, with intervals of 20 min and following the same procedure. After the last addition, the reaction was stirred at room temperature for 2 h. It was quenched with sat. $NaHCO_3$ solution (1 mL) and the organic solvent was evaporated *in vacuo*. The remaining aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was purified by silicagel flash chromatography (EtOAc) to provide the product **14** (44.0 mg, 39%). $[\alpha]_D^{20}$: −2.0(*c* 1.00, CH_2Cl_2); IR (film) = 3498, 2961, 2926, 2856, 1781, 1737, 1460, 1383, 1321, 1260, 1173, 1094, 1069, 1011, 978, 925, 873, 799, 734, 701, 623, 585, 527, 495, 449, 399 cm^{-1}; (400 MHz $CDCl_3$) δ 2.08 (1H, *dd*, ^3J 12.7; 5.4Hz, H-1), δ 1.81 (1H, *m*, H-2a), δ 2.23 (1H, *m*, H-2b), δ 2.24 (1H, *m*, H-3a), δ 2.42 (1H, *m*, H-3b), δ 4.69 (1H, *d*, ^3J 9.3 Hz, H-6), δ 3.05 (1H, *m*, H-7), δ 1.43 (1H, *m*, H-8a), δ 1.83 (1H, *m*, H-8b), δ 1.57 (1H, H-9a), δ 1.86 (1H, H-9b), δ 3.06 (1H, *d*, ^3J 5.5 Hz, H-13a), δ 3.15 (1H, *d*, ^3J 5.7 Hz, H-13b), δ 1.25 (3H, *s*, Me-14), δ 1.01 (3H, *s*, Me-15); d_C (100.6 MHz, $CDCl_3$) 51.8 (C-1), 20.3 (C-2), 36.0 (C-3), 217.3 (C-4), 52.1 (C-5), 81.8 (C-6), 39.1 (C-7), 19.2 (C-8), 39.7 (C-9), 72.8 (C-10), 57.9 (C-11), 173.3 (C-12), 50.8 (C-13), 24.9 (C-14), 12.6 (C-15); HRMS-ESI: [M+H$^+$], found 281.1402. $C_{15}H_{20}O_5$ requires 281.1426.

***epi*-11,13-dihydro-13-(*N*,*N*-dimethylamino)damsin** (**15**). To a solution of **1** (100 mg, 0.4027 mmol) in EtOH (5 mL)was added Me_2NH (720 µL, 4.0270mmol, 5.6 M solution in EtOH) and the reaction was stirred at 0˚C. After 9 h, the reaction was quenched evaporating the solvent *in vacuo* to give the crude product, which was not further purified. The product **15** (114.3 mg, 96%) was obtained as an unseparable mixture of epimers 3:1 (11*β*:11*α*) according ^{1}H-NMR integrals (H-6).$[\alpha]_D^{20}$: +44.2(*c* 1.00, CH_2Cl_2); IR (film) = 2921, 2862, 2821, 2768, 1759, 1736, 1459, 1408, 1382, 1347, 1316, 1268, 1224, 1175, 1154, 1127, 1088, 1051, 1030, 1007, 976, 953, 930, 845, 713, 684, 655, 627, 536 cm^{-1};**11*β*,13-dihydro-13-(*N*,*N*-dimethylamino)damsin** (400 MHz $CDCl_3$) δ 2.01 (1H, *m*, H-1), δ 1.77 (1H, *m*, H-2a), δ 1.97 (1H, *m*, H-2b), δ 2.15 (1H, *m*, H-3a), δ 2.37 (1H, *m*, H-3b), δ 4.41 (1H, *d*, ^3J8.4 Hz, H6), δ 2.68 (1H, *m*, H-7), δ 1.64 (1H, *m*, H-8a), δ 1.87 (1H, *m*, H-8b), δ 1.65 (1H, *m*, H-9a), δ 1.65 (1H, *m*, H-9b), δ 2.12 (1H, *m*, H-10), δ 2.39 (1H, *m*, H-11), δ 2.48 (2H, *m*, H-13a), δ 1.02 (3H, *d*, ^3J7.5 Hz, Me-14), δ 1.07 (3H, *s*, Me-15), δ 2.16 (6H, *s*, H-16a); d_C (100.6 MHz, $CDCl_3$) 45.9, 23.7, 35.8, 219.3, 54.6, 82.1, 44.7, 25.3, 33.9, 34.2, 46.0, 177.8, 59.5, 15.9, 14.1, 45.6; **11*α*,13-dihydro-13-(*N*,*N*-dimethylamino) damsin** (400 MHz $CDCl_3$) δ 2.01 (1H, *m*, H-1), δ 1.77 (1H, *m*, H-2a), δ 1.97 (1H, *m*, H-2b), δ 2.10 (1H, *m*, H-3a), δ 2.39 (1H, *m*, H-3b), δ 4.46 (1H, *d*, ^3J5.4 Hz, H6), δ 2.53 (1H, *m*, H-7), δ 1.44 (1H, *m*, H-8a), δ 1.60 (1H,

m, H-8b), δ 1.57 (1H, *m*, H-9a), δ 1.79 (1H, *m*, H-9b), δ 2.12 (1H, *m*, H-10), δ 2.83 (1H, *m*, H-11), δ 2.48 (2H, *m*, H-13a), δ 1.02 (3H, *d*, ^3J7.5 Hz, Me-14), δ 1.10 (3H, *s*, Me-15), δ 2.17 (6H, *s*, H-16a); d_C (100.6 MHz, $CDCl_3$) 45.7, 23.9, 34.6, 219.3, 54.6, 81.7, 45.5, 17.8, 36.9, 34.8, 43.3, 176.8, 54.5, 16.5, 15.7, 45.3; HRMS-ESI: $[M+H^+]$, found 294.2070. $C_{17}H_{27}NO_3$ requires 294.2069.

b) Biological assays

Cells and luciferase assays. The 5.1 clone line is a Jurkat derived clone stably transfected with a plasmid containing the luciferase gene driven by the HIV-LTR promoter were grown at 37˚C and 5% CO_2 in supplemented RPMI 1640 containing 10% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin (50 U/mL) and streptomycin (50 μg/mL) and supplemented with G418 (200 μg/m). For the anti-NF-κB activity 5.1 cells were stimulated with TNFα (20 ng/mL) in the presence or the absence of the compounds for six h. The cells were washed twice in PBS and lysed in 25 mM Tris-phosphate pH 7.8, 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100 and 7% glycerol during 15 min at RT in a horizontal shaker. After centrifugation, the supernatant was used to measure luciferase activity using an Autolumat LB 9510 (Berthold) following the instructions of the luciferase assay kit (Promega, Madison, WI, USA).

c) Computational

Conformational search calculations for structural elucidation of all the molecules were performed with MacroModel (version 9.9, Schrödinger, LLC, New York, NY, 2011) using the force-field MMFFs, which was generated different conformers for each of molecule. The minimum energy conformer was chosen for full geometry optimization using the ab initio calculations with the basis set B3LYP/6-31G** (Jaguar, version 7.8, Schrödinger, LLC, New York, NY, 2011). The resulting geometries were used for subsequent comparison with the experimental NMR data.

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