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SYNTHESIS AND CYTOTOXIC ACTIVITY OF α-SANTONIN AMINO-DERIVATIVES

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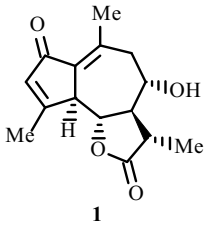
Previously unknown amino-derivatives of the natural sesquiterpene lactone α-santonin were synthesized. The activity of the products against several human tumor-cell lines was studied.

Key words: sesquiterpene lactone, α-santonin, Michael reaction, aminosantonins, cytotoxic activity.

Natural sesquiterpene lactones exhibit various types of biological activity including antitumor activity [1]. A study of structure–activity relationships has found that high cytotoxicity correlates with the presence of an exocyclic double bond in the lactone ring [2]. Furthermore, the presence of an activated double bond allows modification (by a Michael reaction), introduction of new pharmacophores, and production of water-soluble and transportable forms [3].

Compounds containing in a lactone ring not a methylene group but a methyl are found among the large variety of natural sesquiterpene γ-lactones isolated from plants of the family Asteraceae. For example, austricin **(1)** [4] was isolated from *Artemisia leucodes* Schrenk, *A. austriaca* Jacg.; arborescin **(2)** [5], from *A. arborescens* L.; and balchanolide **(3)** [6], from *Achillea millefolium* L. and *A. balchanorum* H. Krasch, etc.



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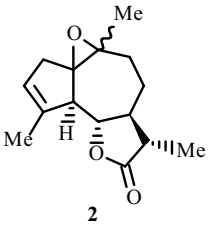

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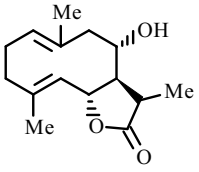

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These and other sesquiterpene lactones with a methyl in the lactone ring are found in plants in large quantities and can be considered promising synthons for modification in order to introduce a new reactive center such as an activated exocyclic double bond that reacts readily with nuclophiles. These transformations could produce new derivatives of natural sesquiterpene lactones containing pharmacophores and their water-soluble or transportable forms.

Our goal was to use α-santonin (**4**), which is isolated from certain *Artemisia* species (Asteracea), as an example to demonstrate the ability to introduce an active methylene into the lactone ring, to use the resulting lactone as a synthon for synthesis of previously unknown amino-derivatives of lactones, and to study their cytotoxic activity.

Chemical and biochemical transformations of α-santonin and its pharmacological properties have been studied already for over 100 years. It has been used previously as an antiparasite preparation [7]. Other types of activity have recently been found for it, for example, antipyretic, anti-inflammatory, and fungicidal [8, 9]. Santonin presents several reactive centers and is a convenient starting material for further chemical modification. Various transformations involving the dienone ring have been reported [10–13]. Methods for opening the lactone ring using various reagents are known [1, 14, 15].

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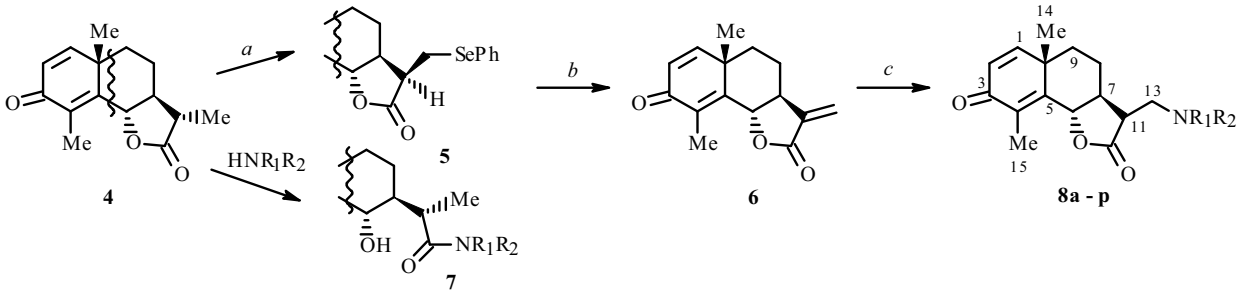
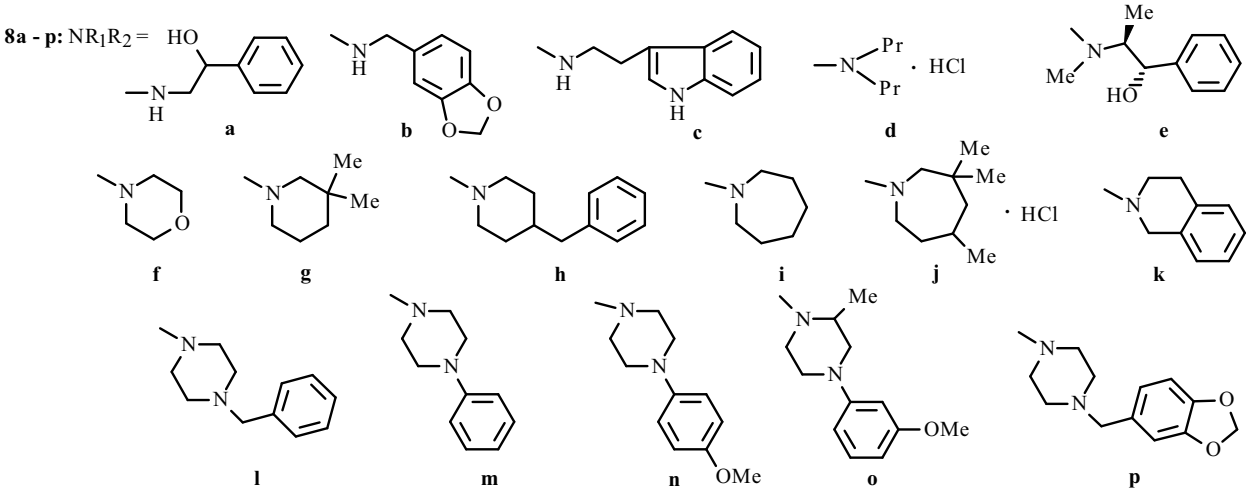
We used a two-step method of selective dehydrogenation of the α-methyl-γ-lactone moiety that included formation of phenylseleno-substituted **5** and subsequent oxidation by H_2O_2 in acetic acid [16] in order to form the exocyclic double bond in santonin. These transformations formed dehydrosantonin **6**. (It is noteworthy that **6** was recently identified in fruit extract of *Laurus nobilis* L. [17].)

Santonin (**4**) is known to react upon heating with primary and secondary amines at the lactone carbonyl with ring opening and amide formation **7** [13–15].



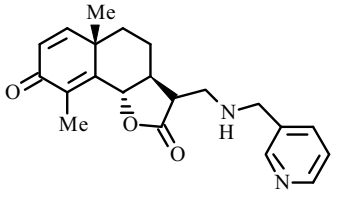
7: NR_1R_2 = NHMe, $NHCH_2Ph$, $NH(CH_2)_2OH$, NMe_2, $N(CH_2)_4$, $N(CH_2)_5$, $N(CH_2CH_2)_2O$



a. Bu^{n}Li, HN^{i}Pr$_2$, THF, –78°C, Ar, Ph_2Se; *b*. H_2O_2, AcOH, THF; *c*. NHR_1R_2, MeOH

We found that dehydrosantonin (**6**), in contrast with **4**, added regioselectively amines at the methylene through a mechanism analogous to the Michael reaction to form aminosantonins **8a**–**p**.

The reaction occurred under mild conditions, i.e., upon storage of equimolar amounts of reagents in a polar solvent (MeOH) at room temperature. The reaction times and yields depended on the nature of the starting amine. We used several primary and secondary aliphatic amines including those containing heterocyclic groups. Sterically hindered 2,6-dimethylmorpholine and weakly nucleophilic aromatic amines (aniline and its derivatives with electron-donating and electron-accepting substituents) were unreactive. However, reaction of **6** with 3-aminomethylpyridine under analogous conditions produced aminosantonin **8q** in comparable amounts with another compound, presumed to be addition product **9**, in which the lactone ring had opened.



8q

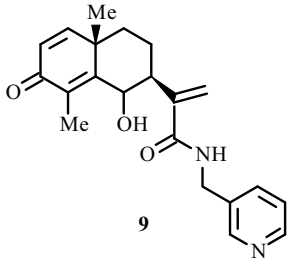


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TABLE 1. PMR Spectral Data (δ, ppm, J/Hz) for **4, 6,** and **8a**–**p** (in $CDCl_3$)

Compound	H-2 (1H, d)	H-1 (1H, d)	H-6 (1H, d)	H-11	H-13 (2H)	H-14 (3H, s)	H-15 (3H, s)	NR_1R_2
4[a]	6.96 (10.0)	6.20 (10.0)	5.04 (11.0)	2.68 m	–	2.10	1.28	–
6[b]	6.70 (9.8)	6.24 (9.8)	4.73 dq (1.6, 11.4)	–	6.22 (1.4) 5.54 (1.4)	2.15	1.28	–
8a	6.59 (10.0)	6.15 (10.0)	4.70 (11.2)	–	see[c]	2.10	1.30	4.65 (1H, m, CHOH), 7.24 (5H, s, $H_{arom.}$)
8b	6.65 (10.0)	6.22 (10.0)	5.04 (9.5)	–	3.21 d (11.6), 3.02 d (11.6)	2.04	1.30	3.6 (1H, br.s, NH), 4.05 (1H, d, CH_2N, J = 12.8), 4.34 (1H, d, CH_2N, J = 12.8), 5.95 (2H, s, OCH_2O), 6.79 (1H, d, H-6′, J = 7.7), 7.02 (1H, d, H-5′, J = 7.7), 7.13 (1H, s, H-2′)
8c[d]	6.65 (10.0)	6.23 (10.0)	4.72 (11.8)	–	see[c]	2.15	1.35	7.05 (1H, s, H-5′), 7.35 (1H, d, H-4′, J = 3.84), 7.60 (1H, d, H-7′, J = 3.84), 7.15 (2H, m, H-5′ and 6′), 8.10 (1H, br.s, NH)
8d[e]	6.67 (10.0)	6.21 (10.0)	4.98 (10.7)	3.24 dd (3.1, 5.6)	3.74 dd (5.6, 12.6), 3.32 dd (3.1,12.6)	2.08	1.30	1.01 (6H, dd, Me, J = 12.6)
8e	6.61 (10.0)	6.18 (10.0)	4.77 (11.8)	–	2.95 m	2.11	1.32	0.76 (3H, d, CHMe, J = 7.7), 2.34 (3H, s, NMe), 4.22 (1H, d, CHOH, J = 10.2), 7.29 (5H, s, $H_{arom.}$)
8f	6.76 (10.0)	6.23 (10.0)	4.77 dd (0.9, 11.4)	–	2.82 m	2.10	1.30	3.65 (4H, m, CH_2OCH_2)
8g	6.65 (9.8)	6.23 (9.8)	4.75 dd (0.9, 11.4)	–	see[c]	2.10	1.29	0.86 (6H, s, 2Me)
8h	6.67 (9.7)	6.25 (9.7)	4.75 (11.3)	–	2.8	2.11	1.30	2.50 (2H, d, CH_2, J = 6.9), 7.10–7.27 (5H, $H_{arom.}$)
8i	6.68 (10.0)	6.24 (10.0)	4.75 dd (0.9, 11.6)	3.06 dd (3.9, 13.0)	see[c]	2.11	1.29	see[f]
8j[e]	6.67 (10.0)	6.24 (10.0)	4.97 (10.4)	–	see[c]	2.08	1.30	0.9–1.0 (9H, d + s, 3Me), 3.00–3.6 (6H, CH_2NCH_2 and H-13)
8k	6.63 (10.0)	6.20 (10.0)	4.79 dd (0.9, 11.3)	–	3.55 d (14.0), 3.68 d (14.0)	2.11	1.29	2.6 (4H, m, CH_2CH_2), 6.99–7.14 (4H, m, $H_{arom.}$)
8l	6.63 (10.0)	6.22 (10.0)	4.74 dd (0.9,11.4)	–	2.84 m	2.10	1.28	3.46 (2H, s, NCH_2), 7.28 (5H, s, $H_{arom.}$)
8m	6.67 (9.8)	6.23 (9.8)	4.79 dd (1.4, 11.4)	2.91 d (8.8)	2.65 d (10.0), 2.55 dd (9.6, 10.0)	2.11	1.30	3.15 (4H, m, $PhNCH_2CH_2$), 6.8–6.95 (3H, $H_{arom.}$), 7.2–7.3 (2H, м, $H_{arom.}$)
8n	6.67 (10.0)	6.23 (10.0)	4.88 dd (0.9, 11.2)	–	2.90 m	2.10	1.32	3.77 (3H, s, OMe), 3.05 (4H, m, NCH_2), 6.87 (4H, dd, $H_{arom.}$, J = 3.9 and 9.3)
8o	6.67 (10.0)	6.22 (10.0)	4.77 dd (1.2, 11.4)	–	see[c]	2.10	1.31	1.00 (3H, d, CHMe, J = 4.4), 3.76 (3H, s, OMe), 3.85 (1H, m, CHMe), 6.35–6.50 (3H, m, $H_{arom.}$), 7.14 (1H, т, H-2′, J = 7.7)
8p	6.67 (10.0)	6.23 (10.0)	4.75 dd (1.2, 11.2)	2.84 d (8.4)		2.09	1.28	3.38 (2H, s, NCH_2), 5.91 (2H, s, OCH_2O), 6.71 (2H, s, H-5′, H-6′), 6.81 (1H, s, H-2′)

Resonances are given only for characteristic protons. [a]Resonances of the C-7 proton appear as a multiplet at 1.92 ppm; the C-11 Me group, at 1.26 ppm (3H, d, J = 6.9); [b]resonances of the C-7 protons, at 2.68 (1H, qt, J_1 = 3.3, J_2 = H-4); [c]not determined because it overlaps with resonances of the amine protons; [d]in $CDCl_3$:CD_3OD mixture; [e]for hydrochlorides NH^+ resonances, at 12.5 ppm; [f]resonances of aliphatic protons appear at 1.5–3.1 ppm; others, from 1.5 to 2.6 ppm.

The presence of a mixture of products **8q** and **9** was established based on PMR spectroscopy (Table 1). The spectrum of amino-derivative **8q** contained resonances for the tricyclic system at 1.93 ppm (3H, s, Me-15), 1.27 (3H, s, Me-14), 2.88 (2H, d, H-13), 4.83 (1H, d, H-6), 6.70 (1H, d, H-1), and 6.24 (1H, d, H-2) in addition to the methylene resonance of the aminomethylpyridyl substituent at 3.99 ppm as a 2H singlet. Compound **9** was characterized by resonances for the bicyclic system at 1.38 (3H, s, Me-14), 2.33 (3H, s, Me-15), 2.6 (1H, m, H-7), 4.68 (1H, d, H-6), 6.32 (1H, d, H-2), and 6.84 (1H, d, H-1) in addition to resonances indicating that the lactone ring had opened (doublets for methylene protons at 5.52 and 5.76 ppm) and the aminopyridyl substituent was present (CH_2Ph doublets at 4.75 and 4.55). Pure **8q** and **9** could not be isolated. Conditions for stereoselective formation of one of the products could not be found.

TABLE 2. Cytotoxic Activity of Santonin Amino-Derivatives Against Human Melanoma (MS) and Ovary Adenocarcinoma (CaOv) Tumor-Cell Cultures

Compound **8**	MS GI_{50}*	CaOv GI_{50}*	Compound **8**	MS GI_{50}*	CaOv GI_{50}*
a	$>1\cdot10^{-5}$	$>1\cdot10^{-5}$	**i**	$>1\cdot10^{-5}$	$>1\cdot10^{-5}$
b	$>1\cdot10^{-5}$	$3\cdot10^{-6}$	**j**	$3\cdot10^{-6}$	$>1\cdot10^{-5}$
c	$>1\cdot10^{-5}$	$>1\cdot10^{-6}$	**k**	$5\cdot10^{-6}$	$9\cdot10^{-6}$
d	$4\cdot10^{-6}$	$2\cdot10^{-6}$	**l**	$3\cdot10^{-6}$	$1\cdot10^{-6}$
e	$>1\cdot10^{-5}$	$8\cdot10^{-6}$	**m**	$>1\cdot10^{-5}$	$3\cdot10^{-6}$
f	$3\cdot10^{-7}$	$4\cdot10^{-7}$	**n**	$3\cdot10^{-6}$	$3\cdot10^{-6}$
g	$3\cdot10^{-6}$	$2\cdot10^{-6}$	**o**	$>1\cdot10^{-5}$	$>1\cdot10^{-5}$
h	$>1\cdot10^{-5}$	$>1\cdot10^{-5}$	**p**	$2\cdot10^{-6}$	$4\cdot10^{-6}$

*Concentration of studied preparation (μM) causing 50% growth inhibition of cells in culture.

Dehydrosantonin (**6**) reacted faster than all with secondary amines, i.e., substituted piperazines. The corresponding adducts **8l**–**p** began to precipitate from the reaction mixture already several minutes after mixing the reagents. They were easily purified by recrystallization from benzene:hexane mixtures. The yields were lowest for 2-hydroxyamines. Compounds **8a** and **8e** could be isolated from the reaction mixture using RP HPLC. As a rule aminosantonins **8a**–**p** were easily crystallized upon evaporation of the reaction mixture. Only **8d** and **8j** were thick oils. Therefore, they were characterized as the hydrochlorides.

The structures of all products were proved using spectral methods and elemental analysis. Mass spectra of the compounds **8** contained a rather strong peak for the molecular ion. IR spectra showed vibrations for the conjugated C=C–C=O system at 1630 and 1660 cm^{-1} in addition to those for the lactone carbonyl (1775). PMR spectra of the amino-derivatives **8** did not exhibit resonances for the exocyclic $=CH_2$ as doublets at 6.18 and 5.54 ppm. Resonances of the amine protons and C-13 protons appeared at 2.8–3.0. Resonances of the other protons confirmed the assumed structures. Amination of dehydrosantonin **6** was stereospecific, i.e., one stereoisomer was formed. However, special investigations are needed in order to establish accurately the configuration of the new chiral center (C-12).

The synthesized amino-derivatives **8** of santonin were tested against several tumor cell lines. The Microculture Tetrazolium Assay (MTA) was used to study *in vitro* the cytotoxic activity of the sesquiterpene lactones and their synthetic derivatives against human tumor-cell cultures. This colorimetric method for assaying cell growth and viability has been described by Mosmann [18]. We used a modification of the method corresponding to that included in an *in vitro* screening program of antitumor compounds at the National Cancer Institute (USA). The method was based on the ability of living cells to convert soluble yellow bromide 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium (MTT) into insoluble bluish-purple intracellular crystals of MTT-formazan (MTT-f). Non-viable dead cells lacked this capability. Formazan crystals were dissolved in DMSO after exposure of cells to the studied compounds. Optical density was measured at 540 nm on a Titertec Multiscan MCC/344 scanning spectrophotometer. The experiment was carried out by placing cell suspensions in 96-well planchets. The studied compounds were added to tumor cell cultures during the exponential growth phase and incubated for 48 h. The exposure times of the preparations were sufficient to reveal metabolic effects of the studied compounds. The viable part of cells was determined (in percent) from the ratio of partial absorption in experimental samples and a control (tumor cells in growth medium without the studied compounds). The tests were performed on two human tumor-cell cultures, i.e., human melanoma (MS line) and human ovarian adenocarcinoma (CaOv line). Table 2 lists the results for sesquiterpene lactones and their synthetic derivatives on human tumor-cell cultures.

Most of the studied compounds exhibited moderate cytotoxic activity. Compounds **8d**, **8f**–**h**, **8n**, and **8p** were the most active against both human tumor-cell lines. Thus, the level of cytotoxic activity of the most active compounds prepared by us provided a basis for further discovery of cytotoxic compounds in this series.

The results should be viewed as the first stage in a study of aminosantonin derivatives **8** that discovers compounds promising for further testing.

EXPERIMENTAL

IR spectra in KBr disks were recorded on a Bruker ZFS-113 instrument. Mass spectra were recorded in a Finnigan LXQ GC–MS in positive-ion mode using a Kromasil C18 column (2 × 50 mm, 3 μm) and gradient elution with eluent A [trifluoroacetic acid (TFA), 0.1%, pH 2.0] and B (CH_3CN) at flow rate 0.3 mL/min. PMR spectra were taken on a Bruker DPX 200 instrument at operating frequency 200 MHz.

The course of reactions and purity of products were monitored using TLC on Silufol UV-254 plates with elution by benzene:EtOAc (3:2) and GC (Chrom 5 chromatograph, 3.6 m × 3 mm column packed with Inerton Super 0.125–0.160 mm with 5% XE-60, flame-ionization detector, detector and vaporizer temperature 250°C, thermostat from 75 to 225°C). Pure components were isolated by preparative HPLC (Turbo LC 200 chromatograph, Perkin–Elmer, UV detection at 254 nm; analytical Kromasil C18 column, 4 × 100 mm, 5 μm; preparative Kromasil C18 column, 10 × 250 mm, 5 μm) using gradient elution with eluent A (TFA, 0.1% in distilled water, pH 2.0) and B (CH_3CN) at flow rate 1 mL/min for the analytical and 5 mL/min for the preparative column.

We used α-santonin (**4**) and diphenyldiselenide (Aldrich), freshly distilled solvents, and pure grade reagents.

(3*S*,3*aS*,5*aS*,9*bS*)-3,5*a*,9-trimethyl-3*a*,4,5,9*b*-tetrahydro-3*H*-benzo[*g*]benzofuran-2,8-dione (3-Oxo-6β*H*-eudesm-1,4,11-trien-6,12-olide) (6) was prepared by a modified literature method [16]. A solution of lithium diisopropylamide in anhydrous THF (30 mL) that was prepared from diisopropylamine (8 mL, 56 mmol) and butyllithium (40 mL, 1.47 M, 56 mmol) in hexane was stirred, cooled to –78°C under Ar, treated dropwise with α-santonin (**4**, 10 g, 40 mmol) in THF (120 mL), stirred for 1 h, treated with diphenyldiselenide (15 g, 48 mmol) in THF (40 mL) with added hexamethylphosphoramide (8.4 mL), and stirred for 3 h at –(60–70)°C. Samples were taken every 0.5 h to monitor the course of the reaction by PMR. The temperature was raised to 0°C. The solution was treated with HCl (60 mL, 10%) and benzene (300 mL) and worked up at room temperature. The organic layer was separated, washed with water and saturated Na_2CO_3 solution, and evaporated. The solid was passed over a column of SiO_2 with elution by hexane:EtOAc (with EtOAc gradient) to afford **5** (6.5 g) that was used in the oxidation reaction without further purification. A solution of **5** (6.5 g, 16 mmol) in THF (200 mL) was stirred, cooled to 0°C, treated with H_2O_2 (11 mL, 30%) and acetic acid (2.2 mL), stored for 1 h, treated with saturated Na_2CO_3 solution (40 mL), and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated. The solid was purified over a column of SiO_2 with elution by hexane:EtOAc (with EtOAc gradient) to afford **6** (5.12 g, 23% calculated for starting santonin), mp 144–145°C, $[\alpha]_D^{20}$ –211° (*c* 0.2, EtOH), lit. [16] mp 145–147°C, $[\alpha]_D^{20}$ –10.4° (*c* 1.12, MeOH).

General Amination Method. A solution of **6** (224 mg, 1 mmol) in MeOH (1 mL) was stirred, treated in portions with a solution of the appropriate amine (1.1 mmol) in MeOH, and left at room temperature until the reaction was complete (TLC monitoring). Table 2 lists the PMR spectra of **8a-p**. The following derivatives were prepared by this method.

(3*aS*,5*aS*,9*bS*)-3-[(2-Hydroxy-2-phenylethylamino)methyl]-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8a) was prepared from α-hydroxyphenethylamine (2-amino-1-phenylethanol) (150 mg). After 3 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from MeOH, yield 140 mg (37%), mp 142–143°C, $[\alpha]_D^{20}$ –80°C (*c* 0.2, EtOH), $C_{23}H_{27}NO_4$. Mass spectrum (*m*/*z*): 381 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 3366br.s (NH), 2938m, 1775vs (C=O), 1663vs and 1632m (C=C–C=O), 1613m, 1450m, 1149m, 1045m.

(3*aS*,5*aS*,9*bS*)-3-{[(Benzo[1,3]dioxol-5-ylmethyl)amino]methyl}-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8b) was prepared from piperonylamine (330 mg). After 1 d the solid was filtered off and recrystallized from benzene:hexane, yield 330 mg (84%), mp 210–212°C, $[\alpha]_D^{20}$ –70° (*c* 0.2, EtOH), $C_{23}H_{25}NO_5$. Mass spectrum (*m*/*z*): 395 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 3880br.s (NH), 2926m, 2359m, 1775s (C=O), 1663s and 1636m (C=C–C=O), 1250m, 1211m, 1038m.

(3*aS*,5*aS*,9*bS*)-3-{[2-(Indol-3-yl)ethylamino]methyl}-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8c) was prepared from tryptamine (176 mg). After 3 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from aqueous MeOH, yield 395 mg (80%), mp 114–116°C, $[\alpha]_D^{20}$ –56° (*c* 0.2, EtOH), $C_{25}H_{28}N_2O_3$. Mass spectrum (*m*/*z*): 494 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 3476br.s (NH), 2936m, 1774s (C=O), 1663s and 1632m (C=C–C=O), 1613m, 1453m, 1149m, 1048m.

(3*aS*,5*aS*,9*bS*)-5*a*,9-Dimethyl-3-dipropylaminomethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione hydrochloride (8d) was prepared from dipropylamine (111 mg). After 3 d the reaction mixture was evaporated in a

rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was treated with methanolic HCl to convert it to the hydrochloride of **8d**, yield 267 mg (70%), mp 167–170°C, $[\alpha]_D^{20}$ –218° (*c* 0.2, EtOH), $C_{21}H_{32}NO_3Cl$. Mass spectrum (*m*/*z*): 345 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 3675w (NH salt), 2972m, 2937m, 2358m, 2215m, 1779s (C=O), 1663s and 1632s (C=C–C=O), 1454m, 1041m.

(3*aS*,5*aS*,9*bS*)-3{[(*S*)-2-Hydroxy-1-methyl-2-phenylethyl)methylamino]methyl}-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8e) was prepared from L-ephedrine ((*S*)-2-methylamino-1-phenylpropan-1-ol) (182 mg, 1.1 mmol). After 7 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from benzene:hexane, yield 225 mg (55%), mp 174–175°C, $[\alpha]_D^{20}$ –7° (*c* 0.2, EtOH), $C_{25}H_{31}NO_4$. Mass spectrum (*m*/*z*): 409 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 3476br.s (OH), 2936m, 1774s (C=O), 1663s and 1632m (C=C–C=O), 1613m, 1453m, 1149m,1048m.

(3*aS*,5*aS*,9*bS*)-5*a*,9-Dimethyl-3-(morpholin-1-ylmethyl)-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8f) was prepared from morpholine (96 mg). After 1 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from benzene, yield 260 mg (78%), mp 186–187°C, $[\alpha]_D^{20}$ –61.5° (*c* 0.2, EtOH), $C_{19}H_{25}NO_4$. Mass spectrum (*m*/*z*): 331 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2937m, 1779s (C=O), 1662s and 1633m (C=C–C=O), 1613m, 1294m, 1147m, 1131m, 1112s, 1050m, 1006m.

(3*aS*,5*aS*,9*bS*)-5*a*,9-Dimethyl-3-(3,3-dimethylpiperidin-1-ylmethyl)-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8g) was prepared from 3,3-dimethylpiperidine (124 mg). After 1 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from benzene:hexane, yield 250 mg (70%), mp 138–141°C, $[\alpha]_D^{20}$ –151° (*c* 0.2, EtOH), $C_{22}H_{31}NO_3$. Mass spectrum (*m*/*z*): 351 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2937m, 2246m, 1775s (C=O), 1659m and 1632s (C=C–C=O), 1613m, 1212m, 1150m, 1045m.

(3*aS*,5*aS*,9*bS*)-3-(4-Benzylpiperidin-1-ylmethyl)-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8h) was prepared from 4-benzylpiperidine (193 mg) in MeOH (1 mL). After 3 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from aqueous MeOH, yield 240 mg (57%), mp 145–146°C, $[\alpha]_D^{20}$ –46° (*c* 0.2, EtOH), $C_{27}H_{33}NO_3$. Mass spectrum (*m*/*z*): 381 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2938m, 1779s (C=O), 1663vs and 1632s (C=C–C=O), 1613m, 1451m, 1150m, 1134m, 1049m.

(3*aS*,5*aS*,9*bS*)-3-(Azepan-1-ylmethyl)-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione hydrochloride (8i) was prepared from hexamethylenimine (110 mg). After 1 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from benzene:hexane, yield 260 mg (76%), mp 127–128°C, $[\alpha]_D^{20}$ –12° (*c* 0.2, EtOH), $C_{21}H_{29}NO_3$. Mass spectrum (*m*/*z*): 343 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2930m, 1775s (C=O), 1660vs and 1632s (C=C–C=O), 1613m, 1145m, 1045m.

(3*aS*,5*aS*,9*bS*)-5*a*,9-Dimethyl-3-(3,3,5-trimethylazepan-1-ylmethyl)-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8j) was prepared from 3,3,5-trimethylazepan (155 mg) in MeOH (1 mL). After 3 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was treated with methanolic HCl to convert it to the hydrochloride of **8j**, yield 300 mg (72%), mp 150–152°C, $[\alpha]_D^{20}$ –81.5° (*c* 0.2, EtOH), $C_{24}H_{36}NO_3Cl$. Mass spectrum (*m*/*z*): 385 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2961s, 2934m, 1779vs (C=O), 1663vs and 1632s (C=C–C=O), 1613m, 1458m, 1234m, 1153m, 1130m, 1041m.

(3*aS*,5*aS*,9*bS*)-3-(1,2,3,4-Tetrahydroisoquinolin-2-ylmethyl)-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8k) was prepared from 1,2,3,4-tetrahydroisoquinoline (146 mg). After 4 d the reaction mixture was evaporated in a rotary evaporator. The solid was dissolved in CH_3CN and purified by RP HPLC. The solution was evaporated. The solid was recrystallized from aqueous MeOH, yield 300 mg (80%), mp 165–167°C, $[\alpha]_D^{20}$ +8° (*c* 0.2, EtOH), $C_{24}H_{27}NO_3$. Mass spectrum (*m*/*z*): 377 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2934m, 2246m, 1779s (C=O), 1660vs and 1632s (C=C–C=O), 1613m, 1149m, 1045m.

(3*aS*,5*aS*,9*bS*)-3-(4-Benzylpiperazin-1-ylmethyl)-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8l) was prepared from 4-benzylpiperazine (190 mg). After 10 h a precipitate formed. The precipitate was recrystallized from benzene:hexane, yield 380 mg (90%), mp 169–170°C, $[\alpha]_D^{20}$ –28° (*c* 0.2, EtOH), $C_{25}H_{30}N_2O_3$. Mass spectrum (*m*/*z*): 420 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2939m, 2817m, 1778s (C=O), 1633m and 1661s (C=C–C=O), 1612m, 1292m, 1149m, 1133m, 1049m, 1006m.

(3*aS*,5*aS*,9*bS*)-5*a*,9-Dimethyl-3-(1-phenylpiperazin-1-ylmethyl)-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8m) was prepared from 1-phenylpiperazine (180 mg). After 2 h a precipitate formed. The precipitate was recrystallized from benzene:hexane, yield 390 mg (96%), mp 172–175°C, $[\alpha]_D^{20}$ +27° (*c* 0.2, EtOH), $C_{26}H_{32}N_2O_3$. Mass spectrum (*m*/*z*): 406 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2938m, 2862m, 1775s (C=O), 1663vs and 1632s (C=C–C=O), 1613m, 1458m, 1234m, 1150m, 1049m, 1006m.

(3*aS*,5*aS*,9*bS*)-3-[4-(4-Methoxyphenyl)piperazin-1-ylmethyl]-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8n) was prepared from 4-(4-methoxyphenyl)piperazine (210 mg). After 4 h a precipitate formed. The precipitate was recrystallized from benzene:hexane, yield 420 mg (96%), mp 87–90°C, $[\alpha]_D^{20}$ –1° (*c* 0.2, EtOH), $C_{26}H_{32}N_2O_4$. Mass spectrum (*m*/*z*): 436 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2942m, 2825m, 1775s (C=O), 1663vs and 1633s (C=C–C=O), 1613m, 1509m, 1450m, 1242m, 1149m, 1033m.

(3*aS*,5*aS*,9*bS*)-5*a*,9-Dimethyl-3-{2-methyl-[4-(3-methoxyphenyl)]piperazin-1-ylmethyl}-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8o) was prepared from 2-methyl-4-(3-methoxyphenyl)piperazine (225 mg). After 12 h a precipitate formed. The precipitate was recrystallized from benzene:hexane, yield 360 mg (80%), mp 90–92°C, $[\alpha]_D^{20}$ +2° (*c* 0.2, EtOH), $C_{27}H_{34}N_2O_4$. Mass spectrum (*m*/*z*): 450 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2939m, 1775s (C=O), 1661s and 1631m (C=C–C=O), 1608m, 1293m, 1204m, 1169m, 1046m.

(3*aS*,5*aS*,9*bS*)-3-(4-Benzo[1,3]dioxol-5-ylmethylpiperazin-1-ylmethyl)-5*a*,9-dimethyl-3*a*,5,5*a*,9*b*-tetrahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione (8p) was prepared from 4-benzo[1,3]dioxol-5-ylmethylpiperazine (240 mg, 1.1 mmol). After 4 h a precipitate formed. The precipitate was recrystallized from benzene:hexane, yield 436 mg (94%), mp 86–87°C, $[\alpha]_D^{20}$ –15° (*c* 0.2, EtOH), $C_{27}H_{32}N_2O_5$. Mass spectrum (*m*/*z*): 464 $[M]^+$. IR spectrum (KBr, ν, cm^{-1}): 2939m, 1775s (C=O), 1661s and 1633m (C=C–C=O), 1486m, 1244s, 1149m, 1038s, 1006m.

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