Isolation of a Library of Aromadendranes from *Landolphia dulcis* and Its Characterization Using the VolSurf Approach

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A library of nine aromadendrane-type sesquiterpenes (**1**−**9**), including eight new natural products (**1**−**5** and **7**−**9**), was isolated from *Landolphia dulcis* var. *barteri* along with a previously described cadinane derivative (**10**) and a new muurolane derivative (**11**). The structures of all compounds were established by means of NMR methods including COSY, NOESY, HSQC, and HMBC experiments, supported by HRMS and optical rotation data. Virtual characterization of the aromadendrane library (**1**−**9**) was performed using chemoinformatics tools. 3D molecular fields were calculated with the GRID program using low-energy structures obtained with the MMFF force field. VolSurf descriptors were calculated from the GRID maps and subsequently analyzed by multivariate statistics. The analysis disclosed the presence of a common motif for possible interactions of the aromadendranes with a putative target receptor. At the same time, a considerable chemical diversity within the library was disclosed, despite a close biosynthetic relationship of its members. The results can be interpreted in terms of evolutionary optimization of structures of secondary metabolites for interaction with macromolecular targets and are of interest in terms of assessment of potential "drug-likeness" of natural products.

One of the most important rationales for isolation and structure elucidation of natural products is their potential pharmaceutical usefulness. With the advent of ultrahigh-throughput screening and large-scale combinatorial synthetic methods that are now the major source of drug leads,[1−4] natural products have experienced a decline of interest in pharmaceutical industries. However, raising costs and a perceived failure of the large-scale technologies to provide actual marketed drugs have led to a rethinking of the approaches used.[5−8] Natural products have an excellent record as a source of new drugs[9−13] and continue to provide new chemical entities to the market.[14−16] With the growing recognition that not the size of a chemical library but rather its molecular diversity, biological functionality, and "drug likeness" are key issues for identification of successful developmental leads, natural products have important roles to play in future drug discovery programs, perhaps preferably as pure compound libraries or scaffolds for combinatorial chemistry.[17−21] In this context, it is of interest to apply to natural products the techniques of chemoinformatics, which are being used to assess and compare chemical diversity of compound libraries for pharmacological screens. In the present work, we use this approach to describe a small library of aromadendrane-type sesquiterpenoids isolated from *Landolphia dulcis* var. *barteri* (Stapf) Pichon. The plant represents a genus of Apocynaceae that is very poorly characterized from the chemical point of view.

L. dulcis, as other *Landolphia* species, is known as a source of latex.[22,23] Apart from that, there are no phytochemical reports on the plant. The present finding of aromadendranes in *L. dulcis* is to our knowledge the first one on the occurrence of this group of terpenoids in Apocynaceae. Aromadendranes are a group of sesquiterpenes named after 10(14)-aromadendrene, a hydrocarbon first isolated from *Eucalyptus* trees.[24,25] Aromadendranes are found especially in species belonging to Asteraceae,[26−30] Fabaceae,[31] and Myrtaceae.[32,33] *ent*-Aromadendranes have been found in marine organisms including soft corals, sponges, and liverworts.[25,34−38]

Results and Discussion

An ethanolic extract of roots of *L. dulcis* var. *barteri* was partitioned between light petroleum, EtOAc, and water. The EtOAc-soluble material was fractionated by vacuum liquid chromatography (VLC) on silica gel, with eluents containing increasing amounts of EtOAc in toluene. Further purification was performed by reversed-phase HPLC to afford nine aromadendrane-type sesquiterpenes, **1**−**9**, a cadinane-type sesquiterpene, **10**, and a muurolane-type sesquiterpene, **11**.

Molecular composition of all compounds was determined by HRFTMS using matrix-assisted laser-desorption ionization (MALDI). All compounds gave abundant MNa^+ pseudomolecular ions. The number of different types of carbon atoms was determined by comparison of ordinary $^{13}C\{^1H\}$ NMR spectra and DEPT135 spectra, which also provided information about the level of oxygenation of the molecules. The structures **1**−**9**, including relative stereochemistry of all substituents, were established on the basis of full analysis of COSY, NOESY, HSQC, and HMBC data. The distinction between the cyclopropane hydrogens H-6 and H-7 was obtained from COSY correlations to either a methine group (H-5) or a methylene group (H-8). ^1H NMR coupling patterns and correlations in the NOESY spectra of **1**−**8** showed that H-6 and H-7, necessarily positioned *cis* to each other ($^3J_{6,7}$ about 9.5 Hz), are opposite to H-4 and H-5. The relative orientation of H-2 (above the ring plane) in **1**−**3** was shown by NOE correlations to H-4 and H-5. The relative configuration of the 9-hydroxy group in **2** was likewise obtained from a NOESY spectrum following

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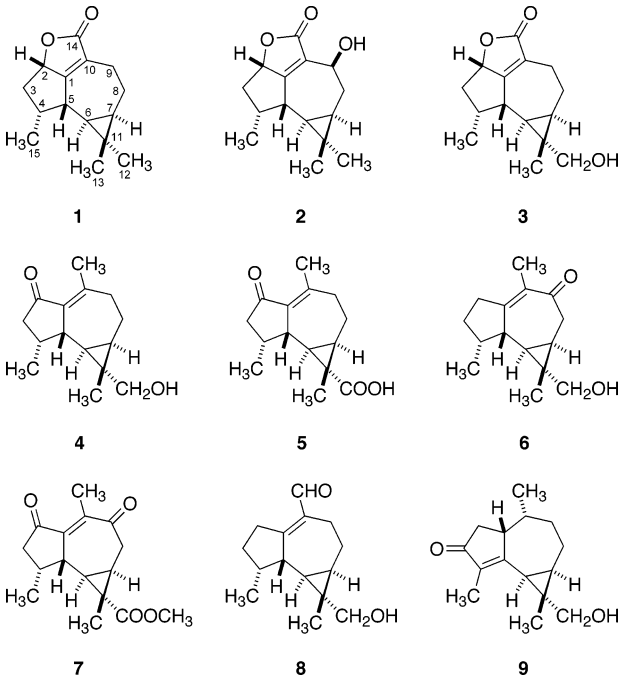


1 **2** **3**

4 **5** **6**

7 **8** **9**

stereospecific assignment of the diastereotopic protons attached to C-8. The geminal methyl groups in **1** and **2** could be assigned on the basis of NOEs from H-12 to H-6 and H-7. In **3**, **4**, **6**, **8**, and **9**, the C-12 methyl group was replaced by a hydroxymethyl group showing a characteristic AB pattern at δ 3.3−3.5 ($^2J_{AB}$ around 11 Hz). The relative configuration of this hydroxymethyl group followed from NOEs to H-6 and H-7. In **5** and **7**, C-12 was further oxidized to a carboxy group and a methoxycarbonyl group, respectively, causing a large downfield shift of H-6 and H-7 due to diamagnetic anisotropy of the carbonyl group. The information summarized above proves the relative configuration of **1**−**9** as shown. ^{13}C and ^{1}H NMR data for the aromadendranes are collected in Tables 1 and 2. Selected HMBC and NOE correlations observed for **1** are shown in Figure 1. Tables with connectivities from 2D NMR spectra (COSY, NOESY, HSQC, and HMBC) of **1**−**3** are included as Supporting Information.

The aromadendranes **1**−**5** and **7**−**9** are novel compounds, whereas compound **6** was previously isolated by Collins et al. as a product of microbial hydroxylation of squamulosone [1(10)-aromadendren-9-one].[39,40] The ^{1}H and ^{13}C NMR spectra as well as specific rotation reported for the latter compound[39] are practically identical with those obtained for **6** in the present work. However, the microbial metabolite was formulated as a C-11 epimer of **6**, i.e., with the hydroxy group at C-13 rather than at C-12.[39,40] Since this assignment of the site of hydroxylation was apparently based on chemical shift considerations and did not involve NOE experiments,[39] we believe that the compound isolated by Collins et al.[39,40] has in fact the structure **6**. The absolute configuration of **6** follows therefore from the known[41−43] absolute configuration of squamulosone. Further support for the absolute configuration of the whole aromadendrane library as shown by the formulas **1**−**9** is provided by the unusually large negative specific rotation of **9** at the sodium D-line ([α]$^{25}_D$ −415°). Thus, **9** is the 12-hydroxy derivative of cyclocolorenone,[44,45] both enantiomers of which are known.[46−51] While the cyclocolorenone isolated from liverworts exhibits a large positive rotation,[46−49] the enantiomer isolated from higher plants has a large negative rotation at the sodium D-line,[50,51] similarly as observed for **9**. The absolute configuration of levorotatory cyclocolorenone was confirmed by synthesis.[52] Since aromadendranes are generally biosynthesized from one enantiomer of the same precursor, either (+)-bicyclogermacrene or (−)-bicyclogermacrene, the former being a common precursor in higher plants,[25] the absolute configuration of all aromadendranes **1**−**9** is as shown. Compounds **1**−**3**, containing a lactone ring, represent a novel variation of the basic aromadendrane scaffold and a novel tetracyclic ring system.

Table 1. ^{13}C NMR Spectral Data for Aromadendranes **1**−**9** (100 MHz, $CDCl_3$)[a]

carbon	1	2	3	4	5	6	7[b]	8	9
C-1	176.2	177.8	175.7	134.1	133.0	165.3	145.4	170.7	42.7
C-2	82.8	83.2	85.7	207.4	206.7	34.0	207.7	29.9	40.2
C-3	37.3	37.1	37.4	47.5	47.4	32.3	47.5	32.8	208.2
C-4	37.2	37.1	37.4	29.7	29.4	37.4	29.2	35.8	140.9
C-5	35.0	35.6	34.0	38.5	38.4	44.2	42.4	42.3	174.6
C-6	28.7	27.8	25.3	26.1	31.7	27.8	32.2	24.7	24.8
C-7	26.9	23.5	23.6	22.0	27.6	19.7	23.5	21.6	28.1
C-8	21.4	30.0	21.0	20.8	20.3	41.3	40.4	20.3	20.7
C-9	25.3	65.6	25.0	38.9	37.8	200.3	200.6	26.6	32.3
C-10	125.5	127.4	125.7	151.5	151.6	130.6	143.0	132.5	31.6
C-11	18.1	21.3	25.0	25.8	26.2	31.4	31.2	25.7	31.5
C-12	28.2	28.3	72.7	73.0	181.5	72.2	174.5	73.0	72.4
C-13	15.6	15.6	11.4	11.4	9.3	11.8	10.1	11.3	12.1
C-14	175.9	175.6	175.5	21.4	21.4	15.0	13.3	193.1	17.4
C-15	15.0	15.0	15.1	16.7	16.5	15.5	16.4	15.2	8.2

[a] Assignments based on 2D heteronuclear correlations. [b] Methyl ester resonance at δ 52.4.

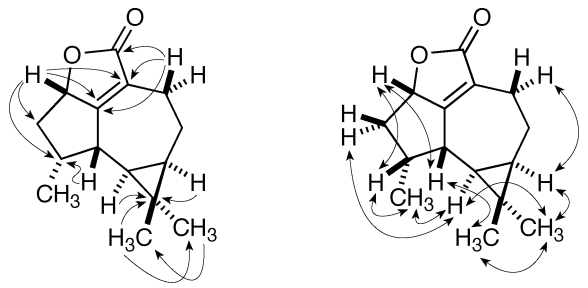
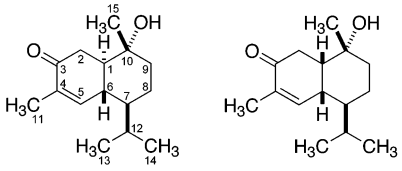


Figure 1. Left: Selected HMBC connectivities observed at 400 MHz for **1** with 70 ms delay for evolution of long-range C,H couplings. Arrows point from H to C. Right: Selected NOE correlations observed at 400 MHz for **1** using 700 ms mixing time.



10 **11**

Compounds **10** and **11** were assigned the molecular formula $C_{15}H_{24}O_2$ by MALDI HRFTMS. Their NMR spectra differed from those of **1**−**9** by the presence of characteristic resonances of an isopropyl group. Detailed analysis of 2D NMR data led to their identification as derivatives of bicyclo[4.4.0]decane. Compound **10** has previously been isolated from *Taiwania cryptomerioides*.[53,54] Compound **11** gave ^{1}H and ^{13}C NMR spectra practically identical with those reported in the literature for (+)-10α-hydroxy-4(5)-muurolen-3-one of fungal origin.[55] However, **11** is levorotatory and must thus be the enantiomer of the fungal metabolite previously described.[55]

The compounds **1**−**9** isolated in this work represent a small library of closely related secondary metabolites differing in the oxidation level. All aromadendranes except for **9** contain a double bond between C-1 and C-10. The

Table 2. ^{1}H NMR Spectral Data for Aromadendranes **1**−**9** (400 or 800 MHz, $CDCl_3$)[a]

hydrogen	**1**[b]	**2**[b]	**3**[c]	**4**[b]	**5**[b]
H-2	4.83 (m)	4.93 (dd, $J_{2,3\beta} = 6.7$, $J_{2,3\alpha} = 11.1$)	4.85 (m)		
H-3	α: 1.21 (dt, $J_{3\alpha,3\beta} =$ 11.1, $J_{3\alpha,2} = J_{3\alpha,4} = 12.0$)	α: 1.22 (dt, $J_{3\alpha,3\beta} =$ 12.4, $J_{3\alpha,2} = J_{3\alpha,4} = 11.1$)	α: 1.23 (m)	α: 2.36 (dd, $J_{3\alpha,3\beta} =$ 16.0, $J_{3\alpha,4} = 7.5$)	α: 2.11 (dd, $J_{3\alpha,3\beta} =$ 15.8, $J_{3\alpha,4} = 7.5$)
	β: 2.24 (m)	β: 2.30 (m)	β: 2.26 (m)	β: 2.08 (dd, $J_{3\alpha,3\beta} =$ 16.0, $J_{3\beta,4} = 8.0$)	β: 2.38 (dd, $J_{3\alpha,3\beta} =$ 15.8, $J_{3\beta,4} = 7.4$)
H-4	2.57 (m)	2.64 (m)	2.64 (m)	2.31 (m)	2.32 (m)
H-5	2.77 (m)	2.71 (m)	2.82 (m)	2.99 (m)	2.99 (m)
H-6	0.82 (dd, $J_{6,5} = 8.2$, $J_{6,7} = 9.4$)	0.72 (dd, $J_{6,5} = 7.5$, $J_{6,7} = 9.3$)	1.00 (dd, $J_{6,5} = 8.2$, $J_{6,7} = 9.8$)	1.05 (dd, $J_{6,5} = 10.9$, $J_{6,7} = 9.7$)	1.92 (dd, $J_{6,5} = 10.6$, $J_{6,7} = 9.6$)
H-7	0.60 (ddd, $J_{7,6} = 9.4$, $J_{7,8\beta} = 12.3$, $J_{7,8\alpha} = 4.3$)	0.55 (ddd, $J_{7,6} = 9.3$, $J_{7,8\beta} = 12.4$, $J_{7,8\alpha} = 4.2$)	0.77 (ddd, $J_{7,6} = 9.8$, $J_{7,8\beta} = 12.4$, $J_{7,8\alpha} = 4.3$)	0.79 (ddd, $J_{7,6} = 9.7$, $J_{7,8\beta} = 10.9$, $J_{7,8\alpha} = 5.8$)	1.70 (m)
H-8	α: 2.03 (ddt, $J_{8\alpha,8\beta} =$ 14.1, $J_{8\alpha,7} = J_{8\alpha,9\beta} =$ 4.3, $J_{8\alpha,9\alpha} = 2.9$)	α: 2.28 (m)	α: 2.05 (ddt, $J_{8\alpha,8\beta} =$ 14.3, $J_{8\alpha,7} = J_{8\alpha,9\beta} =$ 4.3, $J_{8\alpha,9\alpha} = 2.9$)	α and β: 1.72 (m, 2H)	α: 1.82 (m)
	β: 1.46 (ddt, $J_{8\alpha,8\beta} =$ 14.1, $J_{8\beta,7} = J_{8\beta,9\alpha} =$ 12.3, $J_{8\beta,9\beta} = 3.3$)	β: 1.42 (ddd, $J_{8\alpha,8\beta} =$ 13.4, $J_{8\beta,7} = 12.4$, $J_{8\beta,9} = 11.5$)	β: 1.52 (dddd, $J_{8\alpha,8\beta} =$ 14.3, $J_{8\beta,9\alpha} = 12.8$, $J_{8\beta,7} = 12.4$, $J_{8\beta,9\beta} = 2.9$)		β:1.76 (m)
H-9	α: 2.37(m)	4.52 (dm, $J_{9,8\beta} = 11.5$)	α: 2.40 (ddq, $J_{9\alpha,8\beta} =$ 12.8, $J_{9\alpha,9\beta} = 15.3$, $J_{9\alpha,8\alpha} = J_{9\alpha,5} = J_{9\alpha,2} = 2.7$)	α and β: 2.45 (m, 2H)	α and β: 2.49 (m, 2H)
	β: 2.44 (m)		β: 2.49 (m)		
H-12	1.05 (s)	1.09 (s)	3.38 and 3.36 (d, $J_{AB} =$ 10.9)	3.31 and 3.39 (d, $J_{AB} = 10.9$)	
H-13	1.10 (s)	1.11 (s)	1.22 (s)	1.23 (s)	1.38 (s)
H-14				2.13 (d, $J_{14,5} = 1.9$)	2.15 (d, $J_{14,5} = 1.9$)
H-15	1.11 (d, $J_{15,4} = 7.0$)	1.12 (d, $J_{15,4} = 7.0$)	1.13 (d, $J_{15,4} = 7.1$)	1.02 (d, $J_{15,4} = 6.9$)	1.02 (d, $J_{15,4} = 4.1$)

hydrogen	**6**[b]	**7**[b,d]	**8**[b]	**9**[c,e]
H-2	α: 2.68 (m)		α: 3.13 (m)	α: 2.09 (ddd, $J_{2\alpha,2\beta} =$ 18.5, $J_{2\alpha,1} = 2.3$, $J_{2\alpha,6} = 0.8$)
	β: 2.36 (m)		β: 2.64 (m)	β: 2.51 (dd, $J_{2\alpha,2\beta} =$ 18.5, $J_{2\alpha,1} = 6.6$)
H-3	α: 1.44 (dddd, $J_{3\alpha,3\beta} =$ 12.3, $J_{3\alpha,2\beta} \approx J_{3\alpha,4} \approx 10.6$, $J_{3\alpha,2\alpha} = 7.1$)	α: 2.30 (dd, $J_{3\alpha,3\beta} = 16.6$, $J_{3\alpha,4} = 9.3$)	α: 1.47 (dddd, $J_{3\alpha,2\beta} \approx$ $J_{3\alpha,4} \approx 9.6$, $J_{3\alpha,3\beta} = 12.2$, $J_{3\alpha,2\alpha} = 7.2$)	
	β: 1.87 (m)	β: 2.57 (dd, $J_{3\alpha,3\beta} = 16.6$, $J_{3\beta,4} = 7.1$)	β: 1.89 (m)	
H-4	2.25 (m)	2.45 (m)	2.16 (m)	
H-5	2.68 (m)	3.00 (m)	2.88 (m)	
H-6	1.01 (dd, $J_{6,5} = 11.0$, $J_{6,7} = 9.7$)	2.02 (dd, $J_{6,5} \approx J_{6,7} \approx 10.1$)	0.89 (dd, $J_{6,5} = 10.7$, $J_{6,7} = 9.7$)	1.65 (d, $J_{6,7} = 8.3$)
H-7	0.88 (ddd, $J_{7,6} = 9.7$, $J_{7,8\beta} = 11.7$, $J_{7,8\alpha} = 5.2$)	1.69 (ddd, $J_{7,6} = 10.1$, $J_{7,8\beta} = 11.5$, $J_{7,8\alpha} = 5.6$)	0.77 (ddd, $J_{6,7} = 9.7$, $J_{7,8\beta} = 10.0$, $J_{7,8\alpha} = 5.4$)	1.39 (ddd, $J_{7,6} = 8.3$, $J_{7,8\beta} = 10.1$, $J_{7,8\alpha} = 7.2$)
H-8	α: 2.82 (dd, $J_{8\alpha,8\beta} = 14.7$, $J_{8\alpha,7} = 5.2$)	α: 2.98 (dd, $J_{8\alpha,8\beta} = 15.1$, $J_{8\alpha,7} = 5.6$)	α: 1.87 (m)	α: 1.97 (m)
	β: 2.41 (dd, $J_{8\alpha,8\beta} = 14.7$, $J_{8\beta,7} = 11.7$)	β: 2.44 (dd, $J_{8\alpha,8\beta} = 15.1$, $J_{8\beta,7} = 11.5$)	β: 1.66 (dddd, $J_{8\beta,9\alpha} = 10.0$, $J_{8\beta,8\alpha} = 14.6$, $J_{8\beta,9\beta} = 6.5$, $J_{8\beta,7} = 10.0$)	β: 1.65 (m)
H-9			α and β: 2.45 (m, 2H)	α: 2.11 (m)
				β: 1.48 (ddt, 17.8, 16.7, 3.5)
H-12	3.36 and 3.40 (d, $J_{AB} = 11.0$)		3.38 and 3.31 (d, $J_{AB} = 10.9$)	3.52 (d, $J_{AB} = 10.9$, $J_{12,OH} = 5.7$)[f]
H-13	1.30 (s)	1.49 (s)	1.22 (s)	1.11 (s)
H-14	1.78 (ddd, $J_{14,2\beta} \approx$ $J_{14,5} \approx 1.6$, $J_{14,2\alpha} = 2.6$)	2.24 (d, $J_{14,5} = 2.3$)	9.97 (s)	0.82 (d, $J_{14,10} = 7.1$)
H-15	1.06 (d, $J_{15,4} = 6.9$)	1.10 (d, $J_{15,4} = 6.8$)	1.03 (d, $J_{15,4} = 7.0$)	1.74 (d, $J_{15,1} = 2.0$)

[a] Assignments based on 2D homo- and heteronuclear correlations; hydrogens below and above the ring plane are designated as α and β, respectively; coupling constants given in Hz as apparent splittings; s, singlet; d, doublet; t, triplet; m, multiplet. [b] 400 MHz. [c] 800 MHz. [d] Methyl ester resonance at δ 3.70 (s). [e] H-1 at δ 2.98 (broad m), H-10 at δ 2.03 (broad m). [f] Hydroxy group at δ 1.43 (t, $J_{OH,12} = 5.7$).

compounds thus contain a common polycyclic scaffold, but differ in the number and spatial orientation of oxygen functionalities. The functional groups present are capable of accepting (carbonyl and hydroxy groups, lactone/ester and carboxylic oxygen) or donating (hydroxy and carboxylic acid groups) hydrogen bonds. In addition, hydrophobic interactions involving the carbon skeleton and the methyl groups are possible. The aromadendranes isolated in this work, as the secondary metabolites in general,[56−58] should be assumed to have arisen during the evolution in order to play some biological roles, which must involve interactions with some target macromolecules. These target macromolecules may be present within the plant itself, if the compounds **1**−**9** play a specific biological role there. Alternatively, they may be present within some other organisms interacting with the plant, if the structures **1**−**9** have been optimized during co-evolution, e.g., as defense compounds or as attractants.[56,57] With the assumption of an active biological role of **1**−**9**, we have addressed the following questions: Is there a common motif for possible interactions between these compounds and a potential receptor identifiable for the library as the whole, or do the

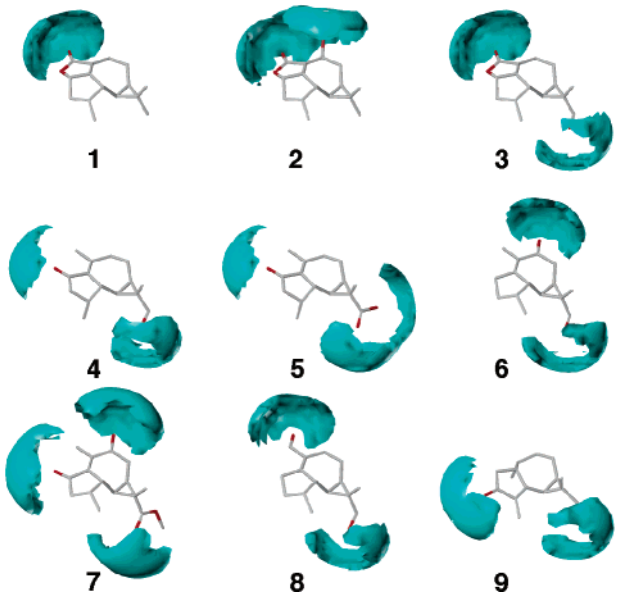


Figure 2. GRID surfaces for **1**−**9**. Interactions with water probe at −4.0 kcal/mol are shown.

structural modifications present within this group represent largely random molecular variations within the parent hydrocarbon skeleton? If a common motif of potential interaction with a receptor is identifiable, what may be a possible advantage of synthesizing a library of related compounds rather than of a single representative?

To shed light on these problems, molecular models of **1**−**9** were constructed and energy-minimized using the MMFF force field.[59] Following Monte Carlo conformational searches,[60] global energy minima were identified and used for calculation of molecular interaction energies with a water probe or a hydrophobic DRY probe using GRID,[61,62] a widely used program for mapping molecular surfaces. Subsequently, VolSurf descriptors[63−65] were calculated from the GRID molecular interaction maps. Finally, multivariate analysis of the VolSurf descriptors was performed.

The GRID maps of the nine compounds (Figure 2) revealed spatial locations for their potential interactions with polar groups or nonpolar regions of a putative receptor. On the basis of comparison of the GRID interaction fields it is obvious that all compounds may be able to fit into the same target site containing three hydrogen bond donors, but only compound **7** will be able to interact with all three sites at the same time. Compounds **3**−**6**, **8**, and **9** may interact with two of these sites, whereas compounds **1** and **2** can interact with only one polar site. Moreover, all compounds can interact with a receptor site via lipophilic interactions with a nonpolar region spanning from C-13 to C-15. This "pharmacophore model" is depicted in Figure 3.

Using the VolSurf program,[63−65] the GRID interaction fields of **1**−**9** were compressed into 56 numerical descriptors representing various molecular properties. The descriptors characterize molecular size and shape, hydrophilic and hydrophobic moments, hydrophilic and hydrophobic interaction energy (integy) moments, capacity factors, and mixed descriptors.[63,65] The VolSurf parameters have been shown to be excellent to describe physicochemical and pharmacokinetic properties.[63−65] A detailed analysis of the VolSurf descriptors was performed using Principal Component Analysis (PCA).[66] A five-dimensional PC model with $R^2 = 0.84$ was constructed. A 3D score plot for the first three principal components is shown in Figure 4. It can be

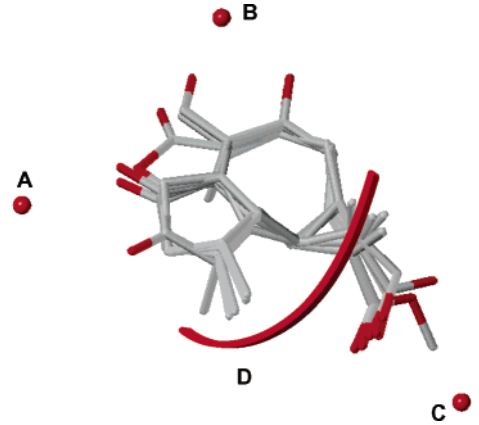


Figure 3. Pharmacophore model for interactions of **1**−**9** with a putative receptor based on 3D molecular fields calculated with GRID. Points A and B represent hydrogen bond donors, point C represents a hydrogen bond donor or acceptor, and line D represents a region of lipophilic interactions. The superposition of the nine compounds is based on the 11 carbon atoms forming the basic tricyclic aromadendrane skeleton.

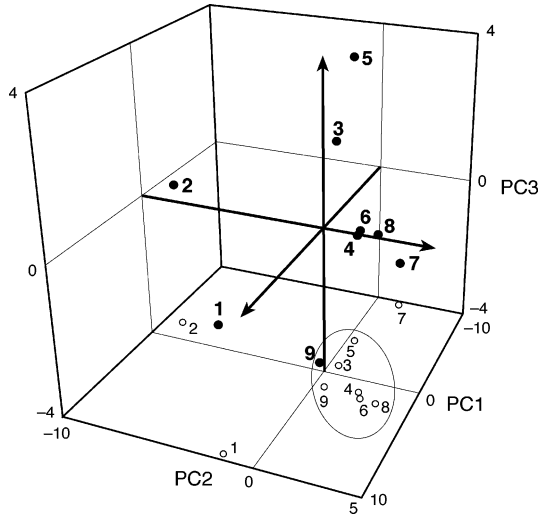


Figure 4. Three-dimensional PCA score plot for VolSurf parameters calculated for **1**−**9**. The first three principal components of a five-dimensional model with $R^2 = 0.84$ are displayed as filled circles. Projections of the data points on the plane formed by PC1 and PC2 are shown as open circles.

seen that **1**, **2**, and **7** are the most different compounds in the series, which is not surprising, as these compounds are characterized by one or three hydrophilic areas, whereas the remaining compounds have two hydrophilic areas. Compounds **4** and **6** are very similar to each other despite the different position of the carbonyl group, and both are similar to **8**, which has an aldehyde group. This indicates similarity in the overall physicochemical properties, which is relevant to phenomena such as passive transport. Compounds **3**, **5**, and **9** are placed closely to each other in the plane formed by PC1 and PC2 despite differences in the functional groups present, but a considerable shift in PC3 is observed (Figure 4). Compounds **3** and **9** appear from a visual inspection of the GRID maps (Figure 2) to be able to interact with different sites of a putative receptor (Figure 3) and are located on the opposite ends of the PC3 axis (Figure 4). Individual plots of scaled VolSurf descriptors (Figure 5) further demonstrate the mutual differences and similarities between the members of the library **1**−**9**. Compounds **1** and **7**, which are located at each end of the PC1 axis (Figure 4) and therefore represent the largest chemical difference within the set, display nearly comple-

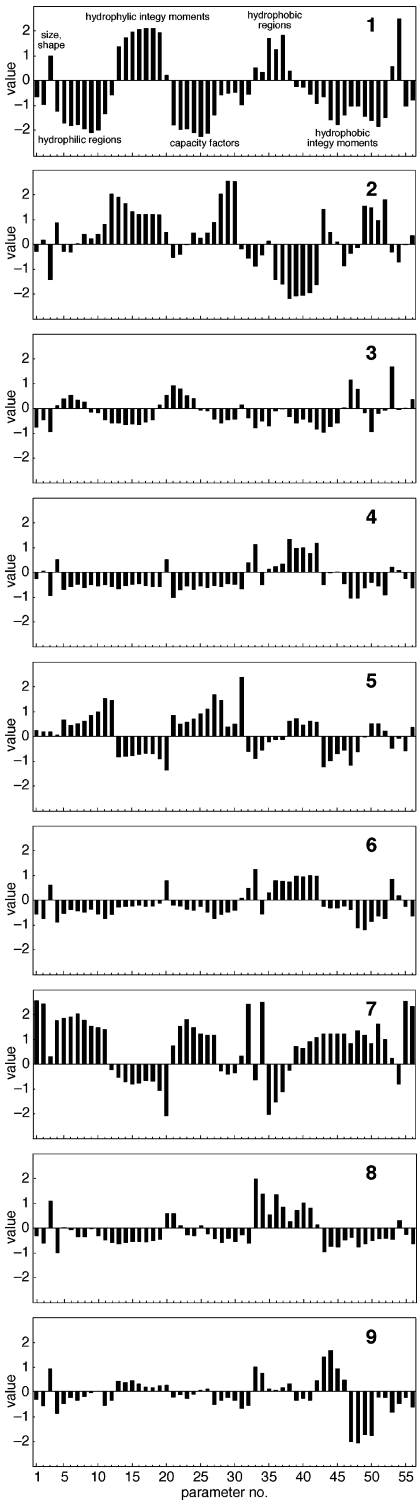


Figure 5. Plots of autoscaled VolSurf descriptors calculated for aromadendranes **1**−**9**. The descriptors[63,65] are as follows: 1, total water accessible volume at 0.20 kcal/mol; 2, total water accessible surface at 0.20 kcal/mol; 3, volume/surface ratio; 4, globularity (total surface area/surface of sphere with the same volume); 5−12, hydrophilic regions at eight levels of integy (interaction energy) with water probe (−0.2, −0.5, −1.0, −2.0, −3.0, −4.0, −5.0, and −6.0 kcal/mol); 13−20, hydrophilic integy moments at the eight energy levels as above; 21−28, capacity factors at eight energy levels as above; 29−31, three lowest energy minima for interaction energy with water probe; 32−34, distances between the three energy minima; 35−42, hydrophobic regions at eight levels of interaction with DRY probe[63,65] (−0.2, −0.4, −0.6, −0.8, −1.0, −1.2, −1.4, and −1.6 kcal/mol); 43−50, hydrophobic integy moments at the eight energy levels as above; 51 and 52, hydrophilic/lipophilic balance parameters; 53, amphiphilic moment; 54, critical packing parameter; 55, average molecular polarizability; 56, molecular weight.

mentary parameter profiles. Contrary to this, the profiles for compounds **4** and **6** are very similar, and accordingly they are placed closely in the PC scores plot (Figure 4).

In conclusion, the analysis shows several interesting properties of the aromadendrane library isolated in this work. The library is homogeneous in the sense that its members are capable of interacting with the same macromolecular target site with interaction points depicted in Figure 3. However, details of the interaction (number and nature of possible contacts with the putative receptor site) vary within the group, suggesting that the library as a whole may be able to trigger various responses at a putative binding site or may be able to interact with a range of related receptor sites, for example representing mutants of the original receptor site. At the same time, a considerable molecular diversity in terms of physicochemical properties is encountered (cf. Figures 4 and 5), despite a small size of the library and the identical basic skeleton. The differences in physicochemical properties will determine transport properties of the individual compounds, which are of interest in relation to the access to the putative macromolecular targets. We believe that these properties of the library **1**−**9** are exactly what would be optimal for an array of compounds designated to play a defensive role via interaction with a macromolecular site: a common interaction motif with the target receptor, various ways of binding within the common motif (in order to mediate various biological responses or to accommodate a possible heterogeneity of the target), and a range of transport properties to optimize access to the target through various biological barriers. This "multilayer design" of a defense system should be assumed to be more effective than defense based on a single chemical entity. At the same time, we believe that the described properties make natural products of special interest as potential drugs, providing a "preprogramming" for interaction with macromolecular sites and at the same time offering a broad range of chemical diversity, functional heterogeneity, and pharmacokinetic properties.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 Fourier transform spectrometer as films on KBr plates. NMR spectra were recorded at 25 °C on a Bruker AMX 400 spectrometer (proton frequency 400.13 MHz) or a Varian Unity Inova spectrometer (proton frequency 799.79 MHz) in chloroform-*d*, using TMS as internal standard. HMBC spectra were optimized for $^{n}J_{C,H}$ = 5, 7, or 17 Hz. High-resolution mass spectra were obtained on an IonSpec Ultima 4.7 T Fourier transform mass spectrometer equipped with a MALDI source based on a 337 nm nitrogen laser. A saturated solution of 2,5-dihydroxybenzoic acid in methanol was used for matrix preparation. All spectra were peak-matched using *m*/*z* 273.03936 ([2M − $2H_2O$ + H]$^+$) as a reference peak. VLC separations were performed on Merck TLC-grade silica gel 60H (90% with particle size less than 45 μm). Column chromatography was performed on Matrex silica gel 60A (particle size 70−200 μm). Preparative HPLC was performed on a Phenomenex Luna 5 C_{18} column (250 × 21.2 mm, 5 μm) eluted with H_2O−MeCN mixtures, with spectrophotometric detection at 220 nm.

Plant Material. Roots of *Landolphia dulcis* var. *barteri* (Stapf) Pichon (Apocynaceae) were collected in a secondary forest near Otwetre Village at Mamfe-Adawso Road, Southern Ghana, in October 1999. Voucher specimens were deposited in Herbarium GC (Ghana Herbarium, Department of Botany, University of Ghana, Legon, Ghana) and in Herbarium C

(Botanical Museum, University of Copenhagen, Copenhagen) under accession numbers GC47666 and DFHJJ40, respectively.

Extraction and Isolation. Air-dried and powdered roots (503 g) were macerated with 4 × 1.25 L of EtOH. The combined extract was evaporated, and the residue (22.7 g) was partitioned between light petroleum, EtOAc, and H_2O. The EtOAc fraction (9.21 g) was further fractionated on a VLC column (9 × 15 cm i.d.) to afford nine fractions: fraction 1 (3 mg) was eluted with 1 L of light petroleum, fraction 2 (5 mg) was eluted with 0.5 L of toluene, fraction 3 (65 mg) was eluted with 1 L of toluene and 0.5 L of toluene−EtOAc (9:1), fraction 4 (0.94 g) was eluted with 0.5 L of toluene−EtOAc (4:1), fraction 5 (1.65 g) was eluted with 1 L of toluene−EtOAc (7:3), fraction 6 (0.66 g) was eluted with 0.5 L of toluene−EtOAc (3:2), fraction 7 (1.00 g) was eluted with 1 L of toluene−EtOAc (1:1), fraction 8 (1.26 g) was eluted with 2 L of EtOAc, and fraction 9 (3.03 g) was eluted with 1 L of MeOH.

VLC fraction 4 was resolved by preparative reversed-phase HPLC using MeCN−H_2O (7:3) to give 12.6 mg of **1** and, after repeated purification using MeCN−H_2O (3:2), 2.0 mg of **7**. VLC fraction 5 was chromatographed on a silica gel column (40 × 3.5 cm i.d.) eluted with light petroleum−EtOAc (3:1) to give three LC fractions, subjected to preparative reversed-phase HPLC with MeCN−H_2O (2:3). LC fraction 2 gave 4.8 mg of **4** and 5.0 mg of **8**. LC fraction 3 gave 7.1 mg of **2**. VLC fraction 6 was subjected to preparative reversed-phase HPLC with MeCN−H_2O (2:3) and gave 5.6 mg of **6**, 11.1 mg of **2**, 2.4 mg of **11**, and 5.3 mg of **10**. VLC fraction 7 was subjected to preparative reversed-phase HPLC with MeCN−H_2O (1:1) and gave 4.3 mg of **9**, 1.2 mg of **3**, and 9.4 mg of **5**. VLC fraction 8 contained only tarry material.

(2*S*,4*R*,5*S*,6*R*,7*R*)-2-Hydroxy-1(10)-aromadendren-14-oic acid 2,14-lactone (1): colorless gum, yield 12.6 mg (0.0025%); $[\alpha]^{25}_D$ +239° (*c* 0.44, $CHCl_3$); IR (film) ν_{max} 2956, 2925, 2868, 1752, 1682, 1035 cm^{-1}; MALDI HRFTMS *m/z* 255.1355 (MNa^+), $C_{15}H_{20}O_2Na^+$ requires 255.1356.

(2*S*,4*R*,5*S*,6*R*,7*R*,9*S*)-2,9-Dihydroxy-1(10)-aromadendren-14-oic acid 2,14-lactone (2): colorless gum, yield 18.2 mg (0.0036%); $[\alpha]^{25}_D$ +240° (*c* 0.34, $CHCl_3$); IR (film) ν_{max} 3444, 2956, 2928, 2869, 1749, 1682, 1049 cm^{-1}; MALDI HRFTMS *m/z* 271.1326 (MNa^+), $C_{15}H_{20}O_3Na^+$ requires 271.1305.

(2*S*,4*R*,5*S*,6*R*,7*R*,11*S*)-2,12-Dihydroxy-1(10)-aromadendren-14-oic acid 2,14-lactone (3): colorless gum, yield 1.2 mg (0.0002%); $[\alpha]^{25}_D$ +338° (*c* 0.05, $CHCl_3$); MALDI HRFTMS *m/z* 271.1307 (MNa^+), $C_{15}H_{20}O_3Na^+$ requires 271.1305.

(4*R*,5*S*,6*R*,7*R*,11*S*)-12-Hydroxy-1(10)-aromadendren-2-one (4): colorless gum, yield 4.8 mg (0.0010%); $[\alpha]^{25}_D$ +6.5° (*c* 0.24, $CHCl_3$); MALDI HRFTMS *m/z* 257.1513 (MNa^+), $C_{15}H_{22}O_2Na^+$ requires 257.1512.

(4*R*,5*S*,6*R*,7*R*,11*S*)-2-Oxo-1(10)-aromadendren-12-oic acid (5): colorless gum, yield 9.4 mg (0.0019%); $[\alpha]^{25}_D$ −1° (*c* 0.42, $CHCl_3$); IR (film) ν_{max} 2966, 2935, 2878, 1703, 1176, 1616, 1179 cm^{-1}; MALDI HRFTMS *m/z* 271.1309 (MNa^+), $C_{15}H_{20}O_3Na^+$ requires 271.1305.

(4*R*,5*S*,6*R*,7*R*,11*S*)-12-Hydroxy-1(10)-aromadendren-9-one (6): colorless gum, yield 5.6 mg (0.0011%); $[\alpha]^{25}_D$ −144° (*c* 0.23, $CHCl_3$), lit.[39] $[\alpha]^{25}_D$ −118° (*c* 4.4, $CHCl_3$); IR (film) ν_{max} 3430, 2954, 2935, 2871, 1712, 1644 cm^{-1}; MALDI HRFTMS *m/z* 257.1522 (MNa^+), $C_{15}H_{22}O_2Na^+$ requires 257.1512.

Methyl (4*R*,5*S*,6*R*,7*R*,11*S*)-2,9-dioxo-1(10)-aromadendren-12-oate (7): colorless gum, yield 2.0 mg (0.0004%); $[\alpha]^{25}_D$ −69° (*c* 0.17, $CHCl_3$); IR (film) ν_{max} 2956, 2935, 2878, 1714, 1668 cm^{-1}; MALDI HRFTMS *m/z* 299.1262 (MNa^+), $C_{16}H_{20}O_4Na^+$ requires 299.1254.

(4*R*,5*S*,6*R*,7*R*,11*S*)-12-Hydroxy-1(10)-aromadendren-14-al (8): colorless gum, yield 5.0 mg (0.0010%); $[\alpha]^{25}_D$ +67.5° (*c* 0.27, $CHCl_3$); MALDI HRFTMS *m/z* 257.1517 (MNa^+), $C_{15}H_{22}O_2Na^+$ requires 257.1512.

(1*R*,6*R*,7*R*,10*R*,11*S*)-12-Hydroxy-4(5)-aromadendren-3-one (9): colorless gum, yield 4.3 mg (0.0009%); $[\alpha]^{25}_D$ −415° (*c* 0.19, $CHCl_3$); IR (film) ν_{max} 3422, 2956, 2923, 2873, 1681, 1617 cm^{-1}; MALDI HRFTMS *m/z* 257.1514 (MNa^+), $C_{15}H_{20}O_3Na^+$ requires 257.1512.

(1*R*,6*R*,7*S*,10*R*)-10-Hydroxy-4(5)-cadinen-3-one (10): colorless gum, yield 5.3 mg (0.0011%); $[\alpha]^{25}_D$ −81° (*c* 0.18, $CHCl_3$), lit.[53] $[\alpha]^{22}_D$ −123.2° (*c* 1.16, $CHCl_3$); IR (film) ν_{max} 2958, 2940, 2869, 1675, 1387, 1119 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.80 (1H, m, H-5), 2.77 (1H, dd, J = 16.0, 3.1 Hz, H-2α), 2.12 (1H, dd, J = 16.0, 14.1 Hz, H-2β), 2.23 (1H, dsp, J = 6.9, 2.6 Hz, H-12), 2.01 (1H, m, H-6), 1.86 (1H, m, H-9β), 1.84 (1H, m, H-1), 1.79 (3H, dd, J = 2.4, 1.3 Hz, H-11), 1.69 (1H, dt, J = 9.7, 3.7 Hz, H-8β), 1.48 (1H, m, H-9α), 1.23 (1H, m, H-8α), 1.21 (1H, m, H-7), 1.16 (3H, s, H-15), 0.99 (3H, d, J = 6.9 Hz, H-14), 0.84 (3H, d, J = 6.9 Hz, H-13); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.1 (C-3), 146.0 (C-5), 135.5 (C-4), 71.2 (C-10), 51.2 (C-1), 45.1 (C-7), 41.6 (C-9), 40.9 (C-6), 38.3 (C-2), 26.2 (C-12), 21.6 (C-8), 21.5 (C-14), 21.0 (C-15), 16.0 (C-11), 15.2 (C-13); MALDI HRFTMS *m/z* 259.1674 (MNa^+), $C_{15}H_{24}O_2Na^+$ requires 257.1669.

(1*S*,6*R*,7*S*,10*R*)-10-Hydroxy-4(5)-muurolen-3-one (11): colorless gum, yield 2.4 mg (0.0005%); $[\alpha]^{25}_D$ −61.5° (*c* 0.13, $CHCl_3$), lit.[55] $[\alpha]^{24}_D$ +96.3° (*c* 0.18, $CHCl_3$) for the enantiomer; IR (film) ν_{max} 2957, 2935, 2869, 1668, 1368, 1118 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.84 (1H, dq, J = 6.1, 1.3 Hz, H-5), 2.68 (1H, dd, J = 17.4, 4.4 Hz, H-2β), 2.48 (1H, dd, J = 17.4, 13.8 Hz, H-2α), 2.35 (1H, m, H-6), 2.20 (1H, ddd, J = 13.8, 4.4 Hz, H-1), 1.89 (1H, dsp, J = 6.9, 3.5 Hz, H-12), 1.79 (3H, dd, J = 1.3 Hz, H-11), 1.62 (1H, m, H-8α), 1.59 (2H, m, H-9), 1.56 (1H, m, H-7), 1.32 (3H, s, H-15), 1.22 (1H, m, H-8β), 0.92 (3H, d, J = 6.9 Hz, H-14), 0.89 (3H, d, J = 6.9 Hz, H-13); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.8 (C-3), 149.0 (C-5), 135.4 (C-4), 71.4 (C-10), 45.4 (C-1), 43.3 (C-7), 37.8 (C-6), 35.3 (C-2), 34.5 (C-9), 27.7 (C-15), 27.4 (C-12), 21.6 (C-8), 21.4 (C-14), 16.0 (C-11), 15.9 (C-13); MALDI HRFTMS *m/z* 259.1687 (MNa^+), $C_{15}H_{24}O_2Na^+$ requires 257.1669.

Calculations. All compounds were constructed with the SYBYL version 6.9 molecular modeling system (Tripos Inc., St. Louis, MO) and initially energy-minimized using the MMFF force field. A Monte Carlo conformational search was performed on each compound and the global energy-minimum conformations used for the subsequent calculations. Atomic coordinates are included as Supporting Information. The calculation of GRID interactions fields, the VolSurf descriptors, and the multivariate statistical analysis were done with the VolSurf version 3.07 program (Molecular Discovery Ltd., London). PCA scores are included as Supporting Information.

Acknowledgment. The 800 MHz ^1H NMR spectra were obtained using the Varian Unity Inova spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules. We are grateful to personnel of Tripos Inc. and Molecular Discovery Ltd. for support in connection with our use of SYBYL, GRID, and VolSurf.

Supporting Information Available: Tables with connectivities observed in 2D NMR spectra (COSY, NOESY, HSQC, and HMBC) for **1**−**3**. Atomic coordinates of **1**−**9** used for GRID calculations and a table of principal components calculated from the VolSurf data. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Oldenburg, K. R. *Ann. Rep. Med. Chem.* **1998**, *33*, 301−311.
(2) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2001**, *5*, 273−284.
(3) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2003**, *7*, 308−325.
(4) Geysen, H. M.; Schoenen, F.; Wagner, D.; Wagner, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 222−230.
(5) Proudfoot, J. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1647−1650.
(6) Ulrich, R.; Friend, S. H. *Nat. Rev. Drug Discovery* **2002**, *1*, 84−88.
(7) Walters, W. P.; Namchuk, M. *Nat. Rev. Drug Discovery* **2003**, *2*, 259−266.
(8) Bleicher, K. H.; Böhm, H.-J.; Müller, K.; Alanine, A. I. *Nat. Rev. Drug Discovery* **2003**, *2*, 369−378.
(9) Wrigley, S. K.; Chicarelli-Robinson, M. I. *Ann. Rep. Med. Chem.* **1997**, *32*, 285−294.
(10) Cragg, G. M.; Newman, D. J.; Snader, K. M. *J. Nat. Prod.* **1997**, *60*, 52−60.
(11) Shu, Y.-Z. *J. Nat. Prod.* **1998**, *61*, 1053−1071.
(12) Newman, D. J.; Cragg, G. M.; Snader, K. M. *Nat. Prod. Rep.* **2000**, *17*, 215−234.

(13) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022−1037.
(14) Gaudilliére, B.; Berna, P. *Ann. Rep. Med. Chem.* **2000**, *35*, 331−356.
(15) Gaudilliére, B.; Bernardelli, P.; Berna, P. *Ann. Rep. Med. Chem.* **2001**, *36*, 293−318.
(16) Bernardelli, P.; Gaudilliére, B.; Vergne, F. *Ann. Rep. Med. Chem.* **2002**, *37*, 257−277.
(17) Bindseil, K. U.; Jakupovic, J.; Wolf, D.; Lavayre, J.; Leboul, J.; van der Pyl, D. *Drug Discovery Today* **2001**, *6*, 840−847.
(18) Ganesan, A. *Pure Appl. Chem.* **2001**, *73*, 1033−1039.
(19) Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2878−2890.
(20) Kingston, D. G. I.; Newman, D. J. *Curr. Opin. Drug Discovery Devel.* **2002**, *5*, 304−316.
(21) Frormann, S.; Jas, G. *Business Brief. Future Drug Discovery* **2002**, 84−90.
(22) Nwadinigwe, C. A. *Phytochemistry* **1981**, *20*, 2301−2302.
(23) Adikwu, M. U. *Acta Pharm. (Zagreb)* **1997**, *47*, 31−37.
(24) Smith, H. G. *Proc. R. Soc. N. S. Wales* **1901**, *35*, 124−126.
(25) Gijsen, H. J. M.; Wijnberg, J. B. P. A.; de Groot, A. In *Fortschritte der Chemie Organischer Naturstoffe*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C, Eds.; Springer-Verlag: Wien, 1995; Vol. 64, pp 149−193.
(26) Bohlmann, F.; Singh, P.; Jakupovic, J. *Phytochemistry* **1982**, *21*, 2531−2535.
(27) Martinez, M.; Flores, G.; Romo de Vivar, A.; Reynolds, G.; Rodriguez, E. *J. Nat. Prod.* **1986**, *49*, 1102−1103.
(28) Pizza, C.; De Tommasi, N. *Phytochemistry* **1988**, *27*, 2205−2208.
(29) Jakupovic, J.; Grenz, M.; Bohlmann, F.; Rustaiyan, A.; Koussari, S. *Planta Med.* **1988**, *54*, 254−256.
(30) De Tommasi, N.; Pizza, C.; Conti, C.; Orsi, N.; Stein, M. L. *J. Nat. Prod.* **1990**, *53*, 830−835.
(31) Bowyer, R. C.; Jefferies, P. R. *Chem. Ind.* **1963**, 1245−1246.
(32) Swords, G.; Hunter, G. L. K. *J. Agric. Food Chem.* **1978**, *26*, 734−737.
(33) Jones, T. G. H.; Haenke, W. L. *Proc. R. Soc. Queensland* **1938**, *49*, 95.
(34) Friedel, H. D.; Matusch, R. *Helv. Chim. Acta* **1987**, *70*, 1753−1759.
(35) Warmers, U.; Wihstutz, K.; Bülow, N.; Fricke, C.; König, W. A. *Phytochemistry* **1998**, *49*, 1723−1731.
(36) Melching, S.; Warmers, U.; König, W. A.; Muhle, H. *Phytochemistry* **1999**, *51*, 277−280.
(37) Liu, H.-J.; Wu, C.-L.; Becker, H.; Zapp, J. *Phytochemistry* **2000**, *53*, 845−849.
(38) Wessels, M.; Koenig, G. M.; Wright, A. D. *J. Nat. Prod.* **2001**, *64*, 370−372.
(39) Collins, D. O.; Buchanan, G. O.; Reynolds, W. F.; Reese, P. B. *Phytochemistry* **2001**, *57*, 377−383.
(40) Collins, D. O.; Ruddock, P. L. D.; de Grasse, J. C.; Reynolds, W. F.; Reese, P. B. *Phytochemistry* **2002**, *59*, 479−488.
(41) Batey, I. L.; Hellyer, R. O.; Pinhey, J. T. *Aust. J. Chem.* **1971**, *24*, 2173−2177.
(42) Büchi, G.; Hofheinz, W.; Paukstelis, J. V. *J. Am. Chem. Soc.* **1966**, *88*, 4113−4114.
(43) Büchi, G.; Hofheinz, W.; Paukstelis, J. V. *J. Am. Chem. Soc.* **1969**, *91*, 6473−6478.
(44) Wu, C.-L.; Chen, C.-L. *Phytochemistry* **1992**, *31*, 4213−4217.
(45) Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; van Ofwegen, L.; Kunzmann, A. *J. Nat. Prod.* **1997**, *60*, 716−718.
(46) Asakawa, Y.; Toyota, M.; Takemoto, T.; Kubo, I.; Nakanishi, K. *Phytochemistry* **1980**, *19*, 2147−2154.
(47) Asakawa, Y.; Matsuda, R.; Toyota, M.; Hattori, S.; Ourisson, G. *Phytochemistry* **1981**, *20*, 2187−2194.
(48) Asakawa, Y.; Matsuda, R.; Cheminat, A. *Phytochemistry* **1987**, *26*, 1117−1122.
(49) Tori, M.; Aoki, M.; Nakashima, K.; Asakawa, Y. *Phytochemistry* **1995**, *39*, 99−103.
(50) Corbett, R. E.; Speden, R. N. *J. Chem. Soc.* **1958**, 3710−3715.
(51) Brown, G. D. *Phytochemistry* **1994**, *35*, 975−977.
(52) Büchi, G.; Kauffman, J. M.; Loewenthal, H. J. E. *J. Am. Chem. Soc.* **1966**, *88*, 3403−3408.
(53) Lin, Y. T.; Cheng, Y. S.; Kuo, Y. H. *Tetrahedron Lett.* **1968**, *36*, 3881−3882.
(54) He, K.; Zeng, L.; Shi, G.; Zhao, G.-X.; Kozlowski, J. F.; McLaughlin, J. L. *J. Nat. Prod.* **1997**, *60*, 38−40.
(55) Zapf, S.; Wunder, A.; Anke, T.; Klostermeyer, D.; Steglich, W.; Shan, R.; Sterner, O.; Scheuer, W. *Z. Naturforsch. C* **1996**, *51*, 487−492.
(56) Fraenkel, G. S. *Science* **1959**, *129*, 1466−1470.
(57) Harborne, J. B. In *Comprehensive Natural Products Chemistry*; Mori, K., Ed.; Elsevier: Amsterdam, 1999; Vol. 8, pp 137−196.
(58) Jarvis, B. B. *Recent Adv. Phytochem.* **2000**, *34*, 1−24.
(59) Halgren, T. *J. Comput. Chem.* **1996**, *17*, 490−519.
(60) Goodman, J. M.; Still, W. C. *J. Comput. Chem.* **1991**, *12*, 1110−1117.
(61) Goodford, P. J. *J. Med. Chem.* **1985**, *28*, 849−857.
(62) Bobbyer, D. N. A.; Goodford, P. J.; McWhinnie, P. M. *J. Med. Chem.* **1989**, *32*, 1083−1094.
(63) Cruciani, G.; Crivori, P.; Carrupt, P.-A.; Testa, B. *J. Mol. Struct. (THEOCHEM)* **2000**, *503*, 17−30.
(64) Cruciani, G.; Pastor, M.; Guba, W. *Eur. J. Pharm. Sci.* **2000**, *11* (Suppl. 2), S29−S39.
(65) Cruciani, G.; Clementi, S.; Crivori, P.; Carrupt, P.-A.; Testa, B. In *Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical, and Computational Strategies*; Testa, B., Ed.; Verlag Helvetica Chimica Acta: Zürich, 2000; pp 539−550.
(66) Eriksson, L.; Johansson, E.; Kettaneh-Wold, N.; Wold, S. *Introduction to Multi- and Megavariate Data Analysis using Projection Methods (PCA and PLS)*; Umetrics: Umeå, 1999.

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