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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and antimicrobial activity of some novel homodrimane sesquiterpenoids with diazine skeleton

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Abstract Herein we report a feasible study concerning the design, synthesis, and in vitro antimicrobial activity of some novel homodrimane sesquiterpenoids with diazine skeleton. The reaction pathway is efficient and straight, involving the direct N-acylation of diazine with homodrimane sesquiterpenoids bearing acyl chlorides or organic acids functionality. A reliable explication and a feasible reaction mechanism for the obtained compounds are presented. The in vitro antimicrobial activity of the homodrimane sesquiterpenoids with and without diazine skeleton has been evaluated. All the tested compounds have an excellent antibacterial activity against Gram-positive strains *S. aureus* and *B. cereus*. SAR correlations concerning antimicrobial activity are reported.

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Introduction

Infectious diseases caused by bacteria and fungi have substantially increased over the last few years, becoming a major concern in many countries around the world (Grahman, 2001; Greenwood *et al.*, 2007). In particular, the emergences of drug resistance and multidrug resistance have caused life-threatening infectious diseases. Although new generations of antimicrobial drugs have been introduced, bacterial and fungal infections remain a major problem in modern therapy. There is, therefore, a clear need to discover and develop novel structures with antimicrobial properties, which could lead to the development of new useful agents for the management of bacterial and fungal infections.

Natural products have been found to be a relevant source of novel and potent bioactive compounds with a good activity, well tolerated by the organism, with low or no toxicity. One of the promising classes within this area is sesquiterpenoids, which have a wide application in medicine, pharmaceutics, cosmetics, and agriculture (Jansen and de Groot, 2004). Sesquiterpenoids, especially those with a drimane skeleton, are natural, semisynthetic, or synthetic compounds possessing a wide variety of biological activities, including antimalarial (Lhinhatrakool *et al.*, 2011; do Céu de Madureira *et al.*, 2002), antidiabetic (Zhao *et al.*, 2012; Miura *et al.*, 2005), anti-inflammatory (Owoyele *et al.*, 2004; Rüngeler *et al.*, 1998), antimicrobial (Komala *et al.*, 2010), and anticancer (Komala *et al.*, 2010; Allouche *et al.*, 2009) ones.

The majority of pharmaceutical products that mimic natural products with biological activity are heterocycles. Diazines heterocycles (in particular, pyrimidine and

pyrazine) have a wide range of biological properties, including antibacterial and antifungal (Ungureanu *et al.*, 2006; Krátký *et al.*, 2012), antituberculosis (Ballell *et al.*, 2007; Abdel-Aziz and Bdel-Rahman, 2010), anti-inflammatory (Amr *et al.*, 2007; da Silva *et al.*, 2010), anti-HIV (Fujiwara *et al.*, 2008; de Castro *et al.*, 2011), cardiotonic (Kostapanos *et al.*, 2008; Masereel *et al.*, 2003), anticancer (Wagner *et al.*, 2008; Racané *et al.*, 2012), analgesic (Giles *et al.*, 2012; Ferreira and Kaiser, 2012) ones, etc.

As part of our ongoing research in the field of biologically active sesquiterpenes (Kuchkova *et al.*, 2009, 2010, 2011; Vlad *et al.*, 1997) and diazines (Ungureanu *et al.*, 2006; Luca *et al.*, 2010; Balan *et al.*, 2009; Mangalagiu *et al.*, 2001), we decided to synthesize new homodrimane sesquiterpenoids with a diazine skeleton (HSDS) in order to combine their respective biological potentials, our attention being focused on antibacterial and antifungal activities.

Results and discussion

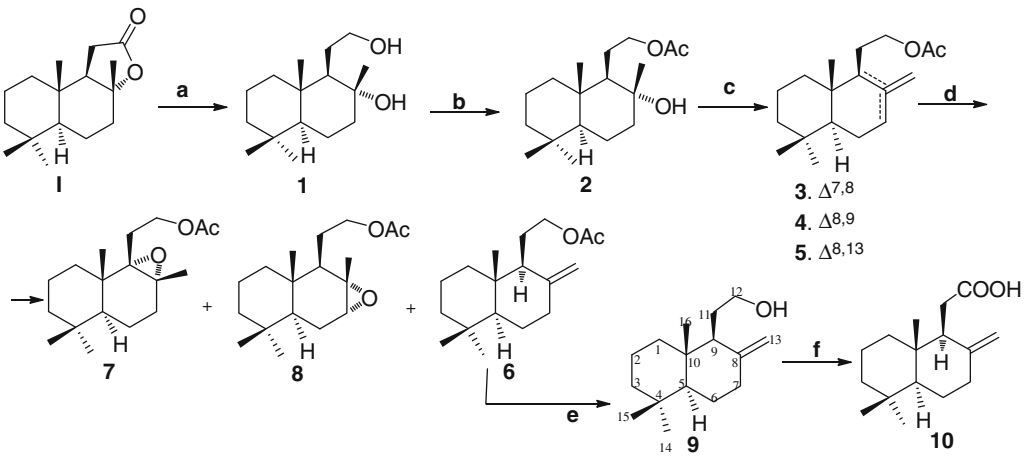
In a preliminary communication (Kuchkova *et al.*, 2013), we designed an efficient route for the obtaining of HSDS. The strategies adopted for the construction of novel homodrimane sesquiterpenoids with diazine skeleton involve several steps and two different methods of synthesis, having $\Delta^{8,13}$-bicyclohomofarnezenic acid **10** as the key intermediary. The starting material for the $\Delta^{8,13}$-bicyclohomofarnezenic acid synthesis (Scheme 1) was commercially available norambreinolide **I**, which was converted into bicyclohomofarnezane-8α,12-diol monoacetate **2** in two steps following the previously described procedure (Vlad *et al.*, 1997; Martres *et al.*, 1993). The dehydration of **2** led to a mixture of unsaturated isomers **3**–**5** in a ratio of 21:14:65 (from ^{1}H-NMR spectral data); further epoxidation with monoperftalic acid (MPFA) of **3**–**5** gave a mixture of compounds **6**–**8**. Acetate **6** was separated from this mixture by flash chromatography. The saponification of **6** led to $\Delta^{8,13}$-bicyclohomofarnezene-12-ol **9**, which was subsequently oxidized with Jones reagent into the key intermediary, $\Delta^{8,13}$-bicyclohomofarnezenic acid **10**.

Once farnezenic acid **10** was obtained, two strategies were used to obtain HSDS derivatives.

The former strategy (Method I, Scheme 2) involved the N-acylation of aminodiazine with acyl chloride. Using this method, the desired HSDS derivatives **13a–c** were obtained by treating $\Delta^{8,13}$-bicyclohomofarnezenic acid chloride **11** [generated in situ from the corresponding acid **10**] with aminopyrazine **12b**, 2-aminopyrimidine **12c**, and 4-aminopyrimidine **12a**.

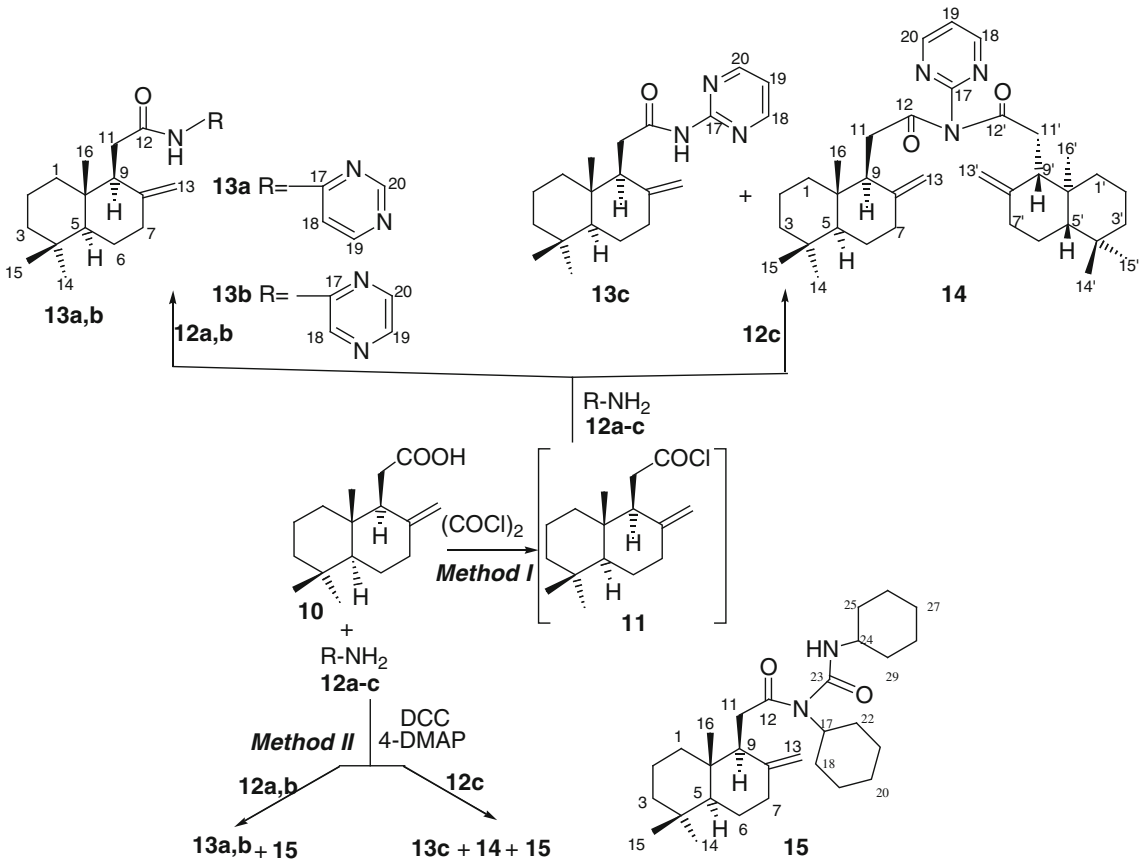
As indicated in Scheme 2, in the case of aminopyrimidines the N-acylation reactions occur in a different way according to the relative position of the amino group on the pyrimidine ring. In the case of 4-aminopyrimidine **12a** the mono-acyl amide **13a** was obtained in good yield in the N-acylation reaction. In the case of 2-aminopyrimidine **12c**, besides the desired mono-N-acylation, which leads to mono-acyl amide **13c**, the bis-N-acylation reaction occurs unexpectedly, leading to the bis-acyl amide **14** as a principal product.

The latter strategy used (Method II, Scheme 2) had several aims: to ascertain if the bis-N-acylation reaction of 2-aminopyrimidine could take place in any conditions, to observe if the method could be applied to the other aminodiazines, and also to increase the reaction yields. For this purpose, we perform the N-acylation of aminodiazines



(a) $LiAlH_4$, Et_2O, Δ , 2h (98%); (b) Ac_2O, Py, 20°C, 2h (100%); (c) $POCl_3$, Py, 0°C, 2h, 24°C, 3h, (96%); (d) MPFA, Et_2O, 0°C, 5h, (65%); (e) KOH, MeOH, 20°C, 2h (98%); (f) Jones reagent, $(CH_3)_2CO$, 20°C, 3h (98%).

Scheme 1 Reaction pathway for the preparation of $\Delta^{8,13}$-bicyclohomofarnezenic acid



Scheme 2 Reaction pathway for the preparation of HSDS

12a–c directly with $\Delta^{8,13}$-bicyclohomofarnezenic acid **10** [in classical conditions: dicyclocarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP)]. Again, besides the desired HSDS derivatives **13a–c**, in the case of 2-aminopyrimidine the bis-acyl amide **14** was obtained. In this synthetic pathway, besides the desired amides, another reaction product was coming up, *N*-$\Delta^{8,13}$-bicyclohomofarnezenoyl-*N,N′*-dicyclohexylurea **15**, as an unwanted by-product.

Having in view the above considerations, it is clear that the N-acylation of aminodiazines with acyl chloride is more efficient in terms of yields and selectivity than the direct N-acylation with organic acids.

As far as, the bis-N-acylation reaction of 2-aminopyrimidine is concerned, a feasible explication could be related to the placement of the amino group between the two nitrogen atoms from the pyrimidine ring. We presume that after the first acylation, the remaining hydrogen from the amide nitrogen atom became very acidic as a result of two factors: the powerful electron withdrawing effect exerted by the two nitrogen atoms and a hydrogen bond with the nonbonding electrons from one nitrogen pyrimidine ring. In the case of 4-aminopyrimidine and 2-aminopyrazine these factors could not take place (there is only a nitrogen atom in the neighboring area and, from sterically point of view hydrogen bonds with nitrogen would not be possible). Consequently, the second electrophilic attack of the acyl group to the amide nitrogen will possibly be rather smooth (Scheme 3).

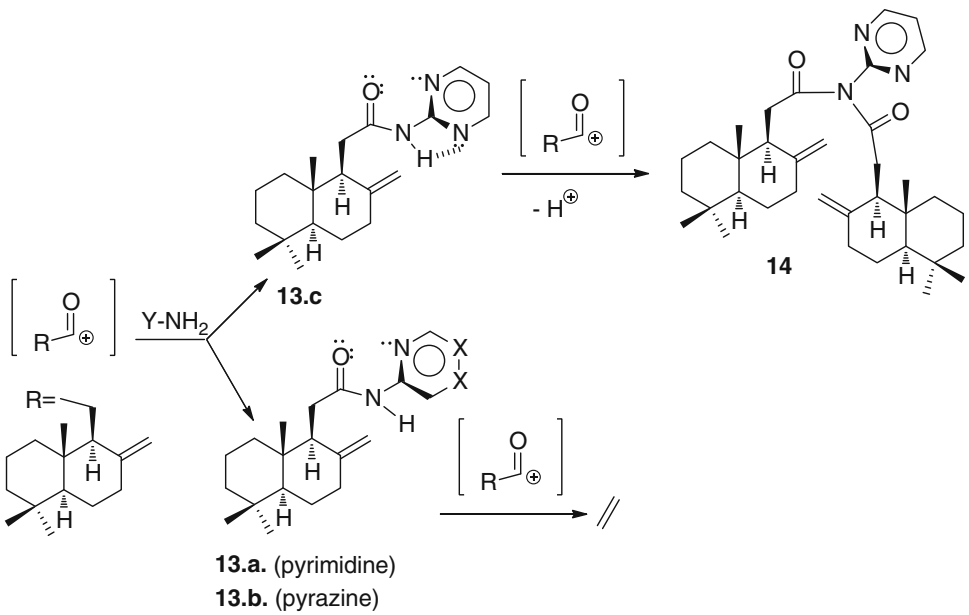
The structures of all new compounds were proved by the elemental and spectral analysis (IR, ^{1}H-NMR, ^{13}C NMR), two-dimensional experiments 2D-COSY, 2D-HETCOR (HMQC), long range 2D-HETCOR (HMBC), MS, and finally, in the case of the bis-acyl amide **14**, by the single crystal X-ray structure determination (Fig. 1).

The compound crystallizes in the orthorhombic system, space group $P2_12_12_1$ with $a = 6.9890(6)$ Å, $b = 14.1283(7)$ Å, $c = 33.8288(17)$ Å, $\alpha = \beta = \gamma = 90.0°$, $V = 3340.3(4)$ Å^3, and $Z = 4$. Full information concerning X-ray structure could be found in the Cambridge Crystallographic Data Centre, the CCDC-893824 structure.

Design and biological activity

Among many other biological activities, natural, semisynthetic, or synthetic sesquiterpenoids were proved to have a

Scheme 3 The proposed reaction mechanism for the obtaining of the bis-acyl amide **14**



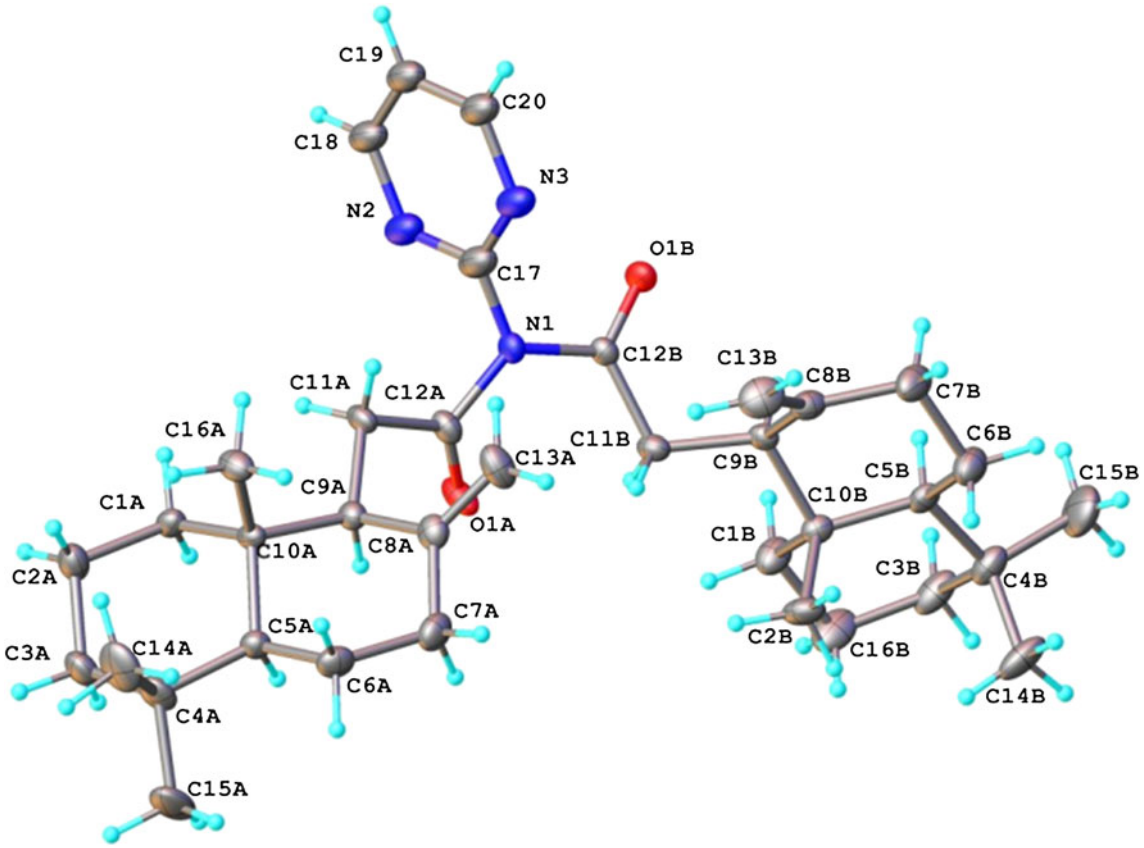


Fig. 1 X-ray molecular structure of **14**. Thermal ellipsoids are drawn at 40 % probability level

good antimicrobial activity (Komala *et al.*, 2010). Recently, we have reported successful results on the identification of new antimicrobial (Ungureanu *et al.*, 2006; Balan *et al.*, 2009; Mangalagiu *et al.*, 2001) compounds which contain a diazine skeleton as pharmacophoric moiety. As a consequence, in an attempt to increase the potential of pharmacophoric models, we decided to combine their respective biological potentials, intending to obtain the compounds with better activity, well

tolerated by the organism, having low or no toxicity, and with minimal or no side effects (Scheme 4).

For this purpose, five of the newly synthesized compounds were preliminary screened for their in vitro antimicrobial activity against six different strains of Gram-positive (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *Bacillus subtilis*) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacteria, respective to three fungal strains (*Candida albicans* ATCC 10231, *Candida glabrata* ATCC MYA 2950, *Candida sake*). The preliminary screening was carried out by agar diffusion assay (Villamizar *et al.*, 2003), using nutrient agar medium (Mueller–Hinton agar for antibacterial tests and Sabouraud agar for antifungal tests). Ampicillin, chloramphenicol, and nystatin were used as reference drugs. Table 1 summarizes the antimicrobial activity of the compounds and reference drugs. The results are expressed as diameters of inhibition zones (mm).

As shown in Table 1, all the tested compounds have a good antibacterial activity against Gram-positive strains *S. aureus* and *B. cereus*. If we compare the activity of homodrimane sesquiterpenoids with diazine skeleton (**13a–c**) with that of homodrimane sesquiterpenoids without diazine skeleton (**15**), we may notice a spectacular increase of antibacterial activity of HSDS compounds, the influence of a diazine skeleton being certain. While HSDS compounds possess a good activity against five bacterial strains (*S. aureus*, *S. lutea*, *B. cereus*, *B. subtilis*, *E. coli*), the homodrimane sesquiterpenoids without a diazine skeleton (**15**), have an activity against only two Gram-positive bacteria (*S. aureus* and *B. cereus*). In the HSDS (**13a–c**, **14**) series, we could notice that pyrimidine derivatives (**13a**, **13c**) have a better activity than pyrazine derivative (**13b**) and that the mono-acyl amides (**13a–c**) have also a better activity compared with the bis-acyl amide (**14**).

As shown in Table 1, no compounds have an antifungal activity.

Conclusion

In conclusion, we reported herein an efficient and general method for the preparation of new homodrimane sesquiterpenoids with diazine skeleton. The reaction pathway involves the direct N-acylation of diazines with homodrimane sesquiterpenoids bearing acyl chlorides or organic acids functionality. A reliable explication and a feasible reaction mechanism for the obtained compounds are presented. The structure of compounds was proven unambiguously by elemental and spectral analyses, in the case of the bis-acyl amide **14**, by a single crystal X-ray structure determination. The in vitro antimicrobial activities of the homodrimane sesquiterpenoids with and without diazine skeleton have been evaluated. The tested compounds have an excellent antibacterial activity against Gram-positive strains *S. aureus* and *B. cereus*. SAR correlation reveals that homodrimane sesquiterpenoids with diazine skeleton

Scheme 4 Design in the class of HSDS derivatives

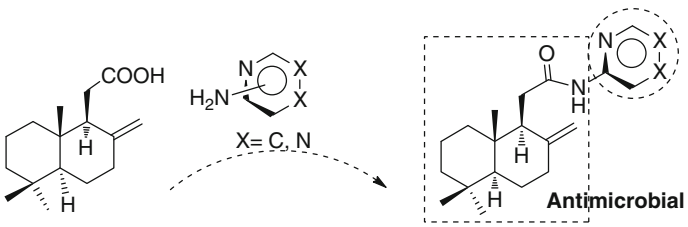


Table 1 Antimicrobial activity of the tested compounds **7–9**

	Diameter of inhibition zone (mm)								
	S. aureus ATCC 25923	*S. lutea* ATCC 9341	*B. cereus* ATCC 14579	*B. subtilis*	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231	*C. glabrata* ATCC MYA 2950	*C. sake*
13a	15	18	10	7	12	0	0	0	0
13b	15	0	10	0	0	0	0	0	0
13c	14	19	12	7	10	0	0	0	0
14	10	0	0	0	0	10	0	0	0
15	19	0	11	0	0	0	0	0	0
Ampicillin (25 μg/disk)	25	30	0	25	20	0	–	–	–
Chloramphenicol (30 μg/disk)	25	30	28	31	29	19	–	–	–
Nystatin (100 mg/disk)	–	–	–	–	–	–	29	26	31

possess a better antibacterial activity compared with homodrimane sesquiterpenoids without diazine skeleton. Also, in the HSDS series, the pyrimidine derivatives have a better activity than the pyrazine ones and, the mono-acyl amide has a better activity than the bis-acyl amide. No tested compounds have an antifungal activity.

Experimental

Melting points were determined on a Boetius heating stage and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. The ^{1}H, ^{13}C, and ^{15}N NMR spectra were recorded in $CDCl_3$ on Avance III Bruker 400 spectrometer (400 and 100 MHz). Chemical shifts are given on the δ scale in ppm relative to $CHCl_3$ resonances as an internal standard (δ 7.24 and 77.00 ppm for H and C, respectively) and TMS for ^{15}N spectra. Resonances in ^{13}C NMR spectra were assigned using DEPT, ^{1}H–^{1}H COSY-45, ^{1}H–^{13}C HMQC, HMBC, NOESY programs and in ^{15}N NMR spectra, a ^{1}H–^{15}N HMBC program. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Optical rotations were determined on a Perkin-Elmer 241 polarimeter with a 1 dm microcell, using $CHCl_3$ as solvent. The X-ray analyses were recorded with an Agilent Xcalibur Eos diffractometer. CCDC-893824 contains supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK: fax: (internat.) +44-1223/336-033; Email: deposit@ccdc.cam.ac.uk]. The course of reactions was monitored by TLC on Silufol plates with the detection by I_2 vapor. Column chromatography used L 100/400 μm silica gel and petroleum ether (bp 30–60 °C). Ether extracts were dried over anhydrous $MgSO_4$.

Preparation of $\Delta^{8,13}$-bicyclohomofarnezen-12-ol (**9**)

A solution of MPFA (35 mL, 3.57 g, 19.6 mmol) in ether was added dropwise at 0 °C to a solution of the mixture of unsaturated isomers **3**–**5** (4.77 g, 17.1 mmol) [obtained from monoacetate of diol (**2**) as described previously (Vlad *et al.*, 1997)] in 30 mL of ether, and the reaction mixture was stirred at the same temperature for 5 h. The obtained precipitate was filtered off and washed successively with ether. The filtrate was washed with 10 % NaOH solution and water, dried, and evaporated under the reduced pressure. The crude product (4.86 g) was chromatographed over silica gel (146 g), and the elution with 2 % ethyl acetate in petroleum ether afforded 3.21 g [65 % from monoacetate (**2**)] of 12-acetoxy-$\Delta^{8,13}$-bicyclohomofarnezen-12-ol (**6**). A solution of KOH (3.37 g, 60 mmol) in 85 mL of methanol was added to acetate (**6**) (3.16 g, 11.3 mmol), and the reaction mixture was stirred at room temperature for 2 h. Methanol was evaporated under the reduced pressure to approximately 10, 100 mL of water and 100 mL of ether were added to the reaction mixture, shacked vigorously, and the organic layer separated. The aqueous phase was extracted again with ether. The combined ether extracts were washed with water, dried, and evaporated to dryness to give 2.66 g (98 %) of $\Delta^{8,13}$-bicyclohomofarnezen-12-ol **9**.

Preparation of $\Delta^{8,13}$-bicyclohomofarnezenic acid (**10**)

1.34 M solution of Jones reagent (5 mL) was added dropwise to a stirred solution of alcohol **9** (1.0 g, 4.23 mmol) in 25 mL acetone at 20 °C. After being stirred for 3 h at room temperature, water was added, and the mixture was extracted with ether. The extract was washed with water, dried, and evaporated to give a colorless low-melting solid (1.04 g, 98 %), of $\Delta^{8,13}$-bicyclohomofarnezenic acid, **10**. Spectral data of alcohol **9** and acid **10** completely correspond to literature data (Villamizar *et al.*, 2003).

Reaction of $\Delta^{8,13}$-bicyclohomofarnezenic acid (**10**) and 2-aminopyrimidine (**12c**)

Method A

A solution of $(COCl)_2$ (0.8 mL, 1.16 g, 9.17 mmol) in 2 mL of dry benzene was added to a solution of acid **10** (200 mg, 0.80 mmol) in 4 mL of dry benzene. Then the reaction mixture was stirred at room temperature for 1 h and was refluxed for 1 h. Benzene and an excess of $(COCl)_2$ were evaporated under the reduced pressure. Dichloromethane (8 mL) and 2-aminopyrimidine **12c** (120 mg, 1.26 mmol) were added to the residue, and the resulting mixture was refluxed with stirring for 15 h. A precipitate was filtered off, washed with dichloromethane, and the filtrate was concentrated to dryness. The residue (279 mg) was dissolved in 3 mL of chloroform and chromatographed on silica gel (8.4 g). The elution with chloroform afforded gradually 120 mg (54 %) of 2-di-$\Delta^{8,13}$-bicyclohomofarnezenoylaminopyrimidine **14**, 80 mg of the two-component mixture, and 10 mg of amide **13c**. This mixture was rechromatographed on silica gel (4 g). The elution with 20 % ether in petroleum ether gave 9 mg (4.5 %) of the unreacted acid **10**. Elution with 60 % ether in petroleum ether afforded 32 mg of amide **13c**. An overall yield of amide **13c** is 42 mg (16 %).

Method B

A solution of DCC (215 mg, 1.04 mmol), 4-DMAP (100 mg, 0.82 mmol), 2-aminopyrimidine **12c** (80 mg, 0.84 mmol), and acid **10** (100 mg, 0.40 mmol) in 4 mL of dichloromethane was stirred at room temperature for 5 h and refluxed with stirring for 15 h. A precipitate was filtered off, washed with dichloromethane, and the filtrate was evaporated under the reduced pressure. The residue (440 mg) was dissolved in 5 mL of chloroform and chromatographed over silica gel (13.2 g). The elution with chloroform gave first 85 mg of the mixture of aminopyrimidine **14** and urea **15**. For the separation of these compounds, the mixture was extracted with petroleum ether. The undissolved precipitate of aminopyrimidine **14** (37 mg, 33 %) was filtered off and washed with petroleum ether. The crystalline residue after the evaporation of the filtrate was recrystallized from acetonitrile to give 40 mg (22 %) of urea **15**. The following elution with chloroform afforded 75 mg of the mixture of amide **13c** and DCC. This mixture was extracted with ether. The undissolved precipitate of DCC (30 mg) was separated, and the ether filtrate was concentrated to dryness. The obtained crystalline residue was recrystallized from acetonitrile to give 30 mg (22 %) of amide **13c**.

*$\Delta^{8,13}$-Bicyclohomofarnezenic acid N-(pyrimidin-2-yl)amide (**13c**)* White crystals; mp: 149–150 °C; $[\alpha]_D^{26}$ −14.8° (c 0.6, $CHCl_3$); IR (KBr): ν/cm^{-1}: 3262, 3189, 3111 (NH amide), 3078, 892 (semicyclic methylene), 1725 (C=O, amide), 1649, 1574, 1435, 1155 (pyrimidine cycle); ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 8.60 (2H, d, J = 8.0 Hz, H-18, H-20), 8.51 (1H, br. s, NH), 6.99 (1H, t, J = 4.9 Hz, H-19), 4.79 (1H, s, Ha-13), 4.54 (1H, s, Hb-13), 2.95 (1H, dd, J = 16.6, 10.1 Hz, Ha-11), 2.79 (1H, dd, J = 16.6, 3.7 Hz, Hb-11), 2.56 (1H, dd, J = 10.1, 3.7 Hz, H-9), 2.41 (1H, ddd, J = 13.0, 4.0, 2.4 Hz, Ha-7), 2.15 (1H, td, J = 13.0, 4.0 Hz, Hb-7), 1.26 (1H, dd, J = 12.6, 2.1 Hz, H-5), 1.80–1.10 (8H, m), 0.90 (3H, s, H-14), 0.83 (3H, s, H-15), 0.76 (3H, s, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 172.64 (s, C-12), 158.32 (d, C-20), 158.32 (d, C-18), 157.70 (s, C-17), 149.27 (s, C-8), 116.14 (d, C-19), 106.51 (t, C-13), 55.21 (d, C-5), 52.27 (d, C-9), 42.12 (t, C-3), 39.24 (s, C-10), 39.09 (t, C-1), 37.67 (t, C-7), 33.57 (q, C-14), 33.56 (t, C-11), 33.53 (s, C-4), 24.13 (t, C-6), 21.77 (q, C-15), 19.35 (t, C-2), 14.75 (q, C-16); ^{15}N NMR (40.5 MHz, $CDCl_3$): δ 263 (N of pyrimidine cycle), 146 (NH amide); EI-MS: 328 (48 %, M^+), 96 (100 %, BP), 329 (7 %), 327 (79 %), 312 (22 %), 232 (57 %), 217 (14 %), 204 (12 %), 191 (82 %), 175 (6 %), 163 (7 %), 150 (11 %), 137 (58 %), 124 (14 %), 122 (16 %), 81 (8 %), 69 (8 %).

*2-Bis-$\Delta^{8,13}$-bicyclohomofarnezenoylaminopyrimidine (**14**)* White crystals; mp 205–206 °C; $[\alpha]_D^{26}$ 27.78° (c 0.33, $CHCl_3$); IR (KBr): ν/cm^{-1}: 3088, 897 (semicyclic methylene), 1704 (C=O, amide), 1644, 1567, 1460, 1410, 1162 (pyrimidine cycle); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.87 (2H, d, J = 4.9 Hz, H-18, H-20), 7.34 (1H, t, J = 4.9 Hz, H-19), 4.80 (2H, s, Ha-13, Ha-13′), 4.52 (2H, s, Hb-13, Hb-13′), 2.72 (2H, dd, J = 16.9, 2.6 Hz, Ha-11, Ha-11′), 2.56 (2H, dd, 16.9, 10.2 Hz, Hb-11, Hb-11′), 2.49 (2H, dd, J = 10.0, 2.0 Hz, H-9, H-9′), 2.39 (2H, ddd, J = 13.0, 4.0, 2.3 Hz, Ha-7, Ha-7′), 2.10 (2H, td, J = 13.0, 5.0, Hz, Hb-7, Hb-7′), 1.18 (2H, dd, J = 12.5, 2.5 Hz, H-5, H-5′), 1.80–1.03 (16H, m), 0.87 (6H, s, H-14, H-14′), 0.79 (6H, s, H-15, H-15′), 0.59 (6H, s, H-16, H-16′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.22 (s, C-12), 159.95 (s, C-17), 159.34 (d, C-18), 159.34 (d, C-20), 148.92 (s, C-8), 120.20 (d, C-19), 106.41 (t, C-13), 55.12 (d, C-5), 51.90 (d, C-9), 42.05 (t, C-3), 39.03 (t, C-1), 38.90 (s, C-10), 37.54 (t, C-7), 34.38 (t, C-11), 33.56 (q, C-14), 33.49 (s, C-4), 23.97 (t, C-6), 21.73 (q, C-15), 19.25 (t, C-2), 14.63 (q, C-16); ^{15}N NMR (40.5 MHz, $CDCl_3$): δ 293 (N of pyrimidine cycle); EI-MS: 560 (3 %, M^+), 328 (100 %, BP), 545 (2 %), 531 (1 %), 356 (6 %), 342 (2 %), 329 (18 %), 327 (39 %), 312 (13 %), 294 (3 %), 232 (16 %), 217 (2 %), 204 (2 %), 191 (13 %), 137 (14 %), 124 (6 %), 122 (6 %), 96 (14 %), 81 (4 %), 69 (4 %).

*N-$\Delta^{8,13}$-Bicyclohomofarnezenoyl-N,N′-dicyclohexylurea (**15**)* White crystals; mp 166–167 °C; $[\alpha]_D^{26}$ 11.9° (c 1.34, $CHCl_3$); IR (KBr): ν/cm^{-1}: 3266 (NH amide), 3074, 879 (semicyclic methylene), 1698 (C=O amide), 1658 (C=O amide); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.47 (1H, br. s, NH), 4.75 (1H, s, Ha-13), 4.43 (1H, s, Hb-13), 4.02 (1H, m, H-17), 3.72 (1H, m, H-24), 2.49 (3H, m, Ha-11, Hb-11, H-9), 2.38 (1H, ddd, J = 12.9, 3.8, 2.2 Hz, Ha-7), 2.13 (1H, td, J = 12.9, 4.9 Hz, Hb-7), 2.05–1.05 (28H, m), 1.22 (1H, dd, J = 12.7, 2.1 Hz, H-5), 0.90 (3H, s, H-14), 0.82 (3H, s, H-15), 0.71 (3H, s, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.51 (s, C-12), 154.32 (s, C-23), 149.45 (s, C-8), 105.97 (t, C-13), 55.15 (d, C-5), 52.09 (d, C-9), 49.86 (d, C-17), 49.13(d, C-24), 42.04 (t, C-3), 39.09 (t, C-1), 39.02 (s, C-10), 37.68 (t, C-7), 33.56 (q, C-14), 33.50 (s, C-4), 32.80 (t, C-25 and C-29), 31.55 (t, C-11), 31.22 (t, C-22), 30.69 (t, C-18), 26.25 (t, C-19 and C-21), 25.48 (t, C-27), 25.40 (t, C-20), 24.73 (t, C-26 and C-28), 24.05 (t, C-6), 21.73 (q, C-15), 19.29 (t, C-2), 14.81 (q, C-16); EI-MS: 457 (38 %, M^+), 332 (100 %, BP), 458 (9 %), 456 (24 %), 441 (27 %), 375 (4 %), 360 (13 %), 346 (3 %), 331 (25 %), 316 (40 %), 232 (28 %), 225 (17 %), 223 (8 %), 196 (11 %), 191 (14 %), 175 (3 %), 154 (4 %), 141 (12 %), 126 (8 %), 121 (2 %), 98 (16 %), 97 (6 %), 83 (36 %), 69 (8 %).

Reaction of $\Delta^{8,13}$-bicyclohomofarnezenic acid (**10**) and 4-aminopyrimidine (**12a**)

Method A

Following the same procedure described for the reaction of acid (**10**) and 2-aminopyrimidine (**12c**), amide (**13a**) (156 mg, 60 %) was synthesized from acid (**10**) (200 mg) and amine (**12a**) (120 mg).

Method B

Amide (**13a**) (73 mg, 53 %), and urea (**15**) (39 mg, 20 %) were prepared in the reaction of acid (**10**) (105 mg) and 4-aminopyrimidine (**12a**) (86 mg), which was realized following the procedure described for this reaction with 2-aminopyrimidine (**12c**).

*$\Delta^{8,13}$-Bicyclohomofarnezenic acid N-(pyrimidin-4-yl)amide (**13a**)* White crystals; mp: 132–133 °C; $[\alpha]_D^{27}$ −32.7° (c 3.6, $CHCl_3$); IR (KBr): ν/cm^{-1}: 3210, 3147 (NH amide), 3072, 993 (semicyclic methylene), 1708 (C=O amide), 1645, 1572, 1510, 1390, 1160 (pyrimidine cycle); ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 8.85 (1H, s, H-20), 8.61 (1H, d, $J = 4.0$ Hz, H-18), 8.34 (1H, br. s, NH), 8.16 (1H, d, $J = 8.0$, H-19), 4.83 (1H, s, H_a-13), 4.52 (1H, s, H_b-13), 2.66 (1H, dd, $J = 22.5$, 10.0 Hz, H_a-11), 2.48 (1H, dd, $J = 22.5$, 10.3 Hz, H_b-11), 2.48 (1H, dd, $J = 10.3$, 10.0 Hz, H-9), 2.43 (1H, ddd, $J = 13.0$, 4.2, 2.3 Hz, H_a-7), 2.15 (1H, td, $J = 13.0$, 5.2, H_b-7), 1.85–1.1 (8H, m), 0.90 (3H, s, H-14), 0.82 (3H, s, H-15), 0.73 (3H, s, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 172.49 (s, C-12), 158.40 (d, C-18), 158.19 (d, C-20), 157.03 (s, C-17), 148.91 (s, C-8), 110.29 (d, C-19), 106.70 (t, C-13), 55.14 (d, C-5), 52.18 (d, C-9), 41.91 (t, C-3), 39.16 (s, C-10), 39.08 (t, C-1), 37.55 (t, C-7), 33.87 (t, C-11), 33.55 (q, C-14), 33.53 (s, C-4), 23.98 (t, C-6), 21.71 (q, C-15), 19.24 (t, C-2), 14.65 (q, C-16); ^{15}N NMR (40.5 MHz, $CDCl_3$): δ 280 and 260 ($N_{(3)}$ and $N_{(1)}$ of pyrimidine cycle), 142 (NH amide); EI-MS: 328 (100 %, M^+, BP), 329 (23 %), 327 (13 %), 312 (9 %), 232 (7 %), 217 (2 %), 204 (4 %), 191 (16 %), 175 (2 %), 163 (2 %), 150 (3 %), 137 (9 %), 124 (11 %), 122 (3 %), 96 (23 %), 95 (3 %), 81 (2 %), 69 (1 %).

Reaction of $\Delta^{8,13}$-bicyclohomofarnezenic acid (**10**) and aminopyrazine (**12b**)

Method A

Amide (**13b**) (10 mg, 15 %) was obtained in the reaction of acid (**10**) (50 mg) and aminopyrazine (**12b**) (30 mg), which was carried out as described above for this reaction with 2-aminopyrimidine (**12c**).

Method B

Amide (**13b**) (68 mg, 52 %) and urea (**15**) (31 mg, 17 %) were prepared in the reaction of acid (**10**) (100 mg) and aminopyrazine (**12b**) (80 mg), which was realized following the procedure described for this reaction of acid (**10**) with 2-aminopyrimidine (**12c**).

*$\Delta^{8,13}$-Bicyclohomofarnezenic acid N-(pyrazinil-3-yl)amide (**13b**)* White crystals; mp: 265–266 °C; $[\alpha]_D^{27}$ −22.5° (c 0.87, $CHCl_3$); IR (KBr): ν/cm^{-1}: 3253, 3094 (NH amide), 3043, 894 (semicyclic methylene), 1719 (C=O, amide), 1697, 1544, 1417 (pyrazine cycle); ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 9.53 (1H, s, H-18), 8.33 (1H, d, $J = 4.0$ Hz, H-20), 8.22 (1H, d, $J = 4.0$ Hz, H-19), 8.06 (1H, br. s, NH), 4.84 (1H, s, H_a-13), 4.56 (1H, s, H_b-13), 2.68 (1H, dd, $J = 21.0$, 8.8 Hz, H_a-11), 2.50 (1H, dd, $J = 21.0$, 10.6 Hz, H_b-11). 2.50 (1H, dd, $J = 10.6$, 8.8 Hz, H-9), 2.43 (1H, ddd, $J = 13.1$, 4.2, 2.3 Hz, H_a-7), 2.16 (1H, td, $J = 13.1$, 5.2 Hz, H_b-7), 1.25 (1H, dd, $J = 12.7$, 2.6 Hz, H-5), 1.80–1.15 (8H, m), 0.90 (3H, s, H-14), 0.83 (3H, s, H-15), 0.74 (3H, s, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 171.49 (s, C-12), 149.01 (s, C-8), 148.12 (s, C-17), 141.87 (d, C-19), 140.06 (d, C-20), 137.10 (d, C-18), 106.71 (t, C-13), 55.14 (d, C-5), 52.31 (d, C-9), 41.93 (t, C-3), 39.19 (s, C-10), 39.06 (t, C-1), 37.59 (t, C-7), 33.56 (q, C-14), 33.56 (s, C-4), 33.47 (t, C-11), 24.00 (t, C-6), 21.71 (q, C-15), 19.25 (t, C-2), 14.65 (q, C-16); ^{15}N NMR (40.5 MHz, $CDCl_3$): δ 338 and 294 ($N_{(4)}$ and $N_{(1)}$ of pyrazine cycle), 138 (NH amide); EI-MS: 328 (100 %, M^+, BP), 329 (12 %), 327 (97 %), 312 (28 %), 294 (4 %), 232 (66 %), 217 (17 %), 204 (17 %), 191 (70 %), 176 (4 %), 163 (5 %), 150 (18 %), 124 (20 %), 122 (22 %), 96 (58 %), 81 (12 %), 69 (9 %).

Biological activity

Agar diffusion assay

Antibacterial and antifungal activities of the compounds were evaluated by agar diffusion assay (Reeves *et al.*, 1978). The microbial suspension (0.5 McFarland turbidity standard) was mixed (1/10 ratio) with agar nutrient (Mueller–Hinton agar for antibacterial tests, respectively Sabouraud agar for antifungal tests). Sterile stainless steel cylinders (5 mm internal diameter; 10 mm height) were applied on the agar surface in Petri plates. Each compound (10 mg/mL in dimethylsulfoxide; 0.1 mL) was added to another cylinder. After 24 h of incubation at 30 °C for bacteria and 48 h of incubation at 28 °C for fungi, the diameter of inhibition zone (mm) was measured (Table 1). *Ampicillin* (25 μg/disk), *Chloramphenicol* (30 μg/disk), and *Nystatin* (100 μg/disk) were purchased from Himedia

(Mumbai, India) and were used as reference drugs for antibacterial and antifungal assays. All the experiments were performed in triplicate; the results were expressed as diameter of inhibition zones (mm).

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References

Abdel-Aziz M, Bdel-Rahman HMA (2010) Synthesis and antimycobacterial evaluation of some pyrazine-2-carboxylic acid hydrazide derivatives. Eur J Med Chem 45:3384–3388

Allouche N, Apel C, Martin MT, Dumontet V, Guéritte F, Litaudon M (2009) Cytotoxic sesquiterpenoids from Winteraceae of Caledonian rainforest. Phytochemistry 70:546–553

Amr AE, Nermien MS, Abdulla MM (2007) Synthesis, reactions, and anti-inflammatory activity of heterocyclic systems fused to a thiophene moiety using citrazinic acid as synthon. Monatsh Chem 138:699–707

Balan AM, Florea O, Moldoveanu C, Zbancioc G, Iurea D, Mangalagiu II (2009) Diazinium salts with dihydroxyacetophenone skeleton: syntheses and antimicrobial activity. Eur J Med Chem 44:2275–2279

Ballell L, Field RA, Chung GAC, Young RJ (2007) New mercaptopyrazolo[3,4-*d*]pyrimidine derivatives as anti-mycobacterial agents. Bioorg Med Chem Lett 17:1736–1740

da Silva YKC, Augusto CV, Barbosa MLD, Melo GMD, de Queiroz AC, Dias TDMF, Bispo W, Barreiro EJ, Lima LM, Alexandre-Moreira MS (2010) Synthesis and pharmacological evaluation of pyrazine N-acylhydrazone derivatives designed as novel analgesic and anti-inflammatory drug candidates. Bioorg Med Chem 18:5007–5015

de Castro S, Familiar O, Andrei G, Snoeck R, Balzarini J, Camarasa MJ, Velázquez S (2011) From beta-amino-gamma-sultone to unusual bicyclic pyridine and pyrazine heterocyclic systems: synthesis and cytostatic and antiviral activities. ChemMedChem 6:686–697

do Céu de Madureira M, Martins AP, Gomes M, Paiva J, Proença da Cunha A, do Rosário V (2002) Antimalarial activity of medicinal plants used in traditional medicine in S. Tomé and Príncipe island. J Ethnopharmacol 81:23–29

Ferreira SB, Kaiser CR (2012) Pyrazine derivatives: a patent review (2008 present). Expert Opin Ther Pat 22:1033–1051

Fujiwara N, Nakajima T, Ueda Y, Fujita H, Kawakami H (2008) Novel piperidinylpyrimidine derivatives as inhibitors of HIV-1 LTR activation. Bioorg Med Chem 16:9804–9816

Giles D, Roopa K, Sheeba FR, Gurubasavarajaswamy PM, Divakar G, Vidhya T (2012) Synthesis pharmacological evaluation and docking studies of pyrimidine derivatives. Eur J Med Chem 58:478–484

Grahman PL (2001) An introduction to medicinal chemistry, 2nd edn. Oxford University Press, New York

Greenwood D, Finch R, Davey P, Wilcocs M (2007) Antimicrobial chemotherapy, 5th edn. Oxford University Press, New York

Jansen BJM, de Groot A (2004) Occurrence, biological activity and synthesis of drimane sesquiterpenoids. Nat Prod Rep 21:449–477

Komala I, Ito T, Nagashima F, Yagi Y, Asakawa Y (2010) Cytotoxic, radical scavenging and antimicrobial activities of sesquiterpenoids from the Tahitian liverwort *Mastigophora diclados* (Brid.) Nees (Mastigophoraceae). J Nat Med 64:417–422

Kostapanos MS, Milionis HJ, Elisaf MS (2008) An overview of the extra-lipid effects of rosuvastatin. J Cardiovasc Pharmacol Ther 13:157–174

Krátký M, Vinšová J, Buchta V (2012) In vitro antibacterial and antifungal activity of salicylanilide pyrazine-2-carboxylates. Med Chem 8:732–741

Kuchkova KI, Aricu AN, Vlad PF (2009) Synthesis of 11-aminodrim-7-ene from drimenol. Chem Nat Compd 45:367–370

Kuchkova KI, Aricu AN, Vlad PF, Deleanu C, Nicolescu A (2010) Synthesis of N-containing drimane sesquiterpenoids from 11-dihomodriman-8α-ol-12-one. Chem Nat Compd 46:539–544

Kuchkova KI, Aricu AN, Barba AN, Vlad PF, Lipkovskii J, Simonov YS, Kravtov VK (2011) Synthesis of nitrogen-containing drimane sesquiterpenoids from 11-dihomodrim-8(9)-en-12-one. Chem Nat Compd 47:223–228

Kuchkova KI, Aricu AN, Barba AN, Vlad PF, Shova S, Secara E, Ungur N, Zbancioc G, Mangalagiu II (2013) An efficient and straightforward method to new organic compounds: homodrimane sesquiterpenoids with diazine units. Synlett 24:697–700

Lhinhatrakool T, Prabpai S, Kongsaeree P, Sutthivaiyakit S (2011) Antiplasmodial sesquiterpene alkaloids from the roots of Maytenus mekongensis. J Nat Prod 74:1386–1391

Luca MC, Tura V, Mangalagiu II (2010) Considerations concerning design and mechanism of action of a new class of dual DNA intercalators. Med Hypotheses 75:627–629

Mangalagiu G, Ungureanu M, Grosu G, Mangalagiu II, Petrovanu M (2001) New pyrrolopyrimidine derivatives with antifungal or antibacterial properties in vitro. Ann Pharm Fr 59:139–140

Martres P, Perfetiti P, Zahra JP, Waegell B (1993) Synthesis of norambracetal: a new ambergris derivative. Tetrahedron Lett 34:3127–3128

Masereel B, Pochet L, Laeckmann D (2003) An overview of inhibitors of Na(+)/H(+) exchanger. Eur J Med Chem 38:547–554

Miura T, Nosaka K, Ishii H, Ishida T (2005) Antidiabetic effect of Nitobegiku, the herb *Tithonia diversifolia*, in KK-Ay diabetic mice. Biol Pharm Bull 28:2152–2154

Owoyele VB, Wuraola CO, Soladoye AO, Olaleye SB (2004) Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. J Ethnopharmacol 90:317–321

Racané L, Kraljević Pavelić S, Ratkaj I, Stepanić V, Pavelić K, Tralić-Kulenović V, Karminski-Zamola G (2012) Synthesis and antiproliferative evaluation of some new amidino-substituted bis-benzothiazolyl-pyridines and pyrazine. Eur J Med Chem 55:108–116

Reeves DS, Phillips I, Williams JD, Wise R (1978) Disc methods of sensitivity testing and other semiquantitative methods. In: Brown D, Blowers R (eds) Laboratory methods in antimicrobial chemotherapy, part I. Churchill Livingstone, Edinburgh, pp 8–23

Rüngeler P, Lyss G, Castro V, Mora G, Pahl HL, Merfort I (1998) Study of three sesquiterpene lactones from *Tithonia diversifolia* on their anti-inflammatory activity using the transcription factor NF-kappa B and enzymes of the arachidonic acid pathway as targets. Planta Med 64:588–593

Ungureanu M, Moldoveanu C, Poeata A, Drochioiu G, Petrovanu M, Mangalagiu II (2006) New pyrimidine compounds with in vitro antimicrobial or antifungal activity. Ann Pharm Fr 64:287–288

Villamizar J, Fuentes J, Salazar F, Tropper E, Alonso R (2003) Facile access to opti-cally active labdane-type diterpenes from (+)-manool: synthesis of (+)-coronarin E,(+)-15,16-epoxy-8(17),13(16),14-labdatriene, and (+)-labda-8(17),13(Z)-diene-15,16-diol. J Nat Prod 66:1623–1627

Vlad PF, Ungur ND, Aricu AN, Andreeva IY (1997) Regioselective dehydration of axial and equatorial tertiary alcohols with α-methyl group in the cyclohexane ring by Swern's reagent. Russ Chem Bull 46:800–803

Wagner E, Al-Kadasi K, Zimecki M, Sawka-Dobrowolska W (2008) Synthesis and pharmacological screening of derivatives of isoxazolo[4,5-*d*]pyrimidine. Eur J Med Chem 43:2498–2504

Zhao G, Li X, Chen W, Xi Z, Sun L (2012) Three new sesquiterpenes from *Tithonia diversifolia* and their anti-hyperglycemic activity. Fitoterapia 83:1590–1597