Med Chem Res (2013) 22:3026–3034
DOI 10.1007/s00044-012-0310-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and analgesic activity of new heterocyclic compounds derived from monoterpenoids

Oksana Mikhalchenko · Irina Il'ina · Alla Pavlova · Ekaterina Morozova · Dina Korchagina · Tat'yana Tolstikova · Evgeny Pokushalov · Konstantin Volcho · Nariman Salakhutdinov

Received: 17 April 2012 / Accepted: 24 October 2012 / Published online: 9 November 2012

Abstract A set of new heterocyclic compounds with different types of framework, including a new type of framework, were synthesized by reactions of verbenol epoxide and (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol with aromatic aldehydes containing three methoxy groups. The analgesic activity of these substances was studied. Three compounds possessed considerable activity in vivo in the acetic acid-induced writhing test. (2*S*,4*R*,4a*R*,8*S*,8a*R*)-4,7-Dimethyl-2-(2,4,5-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2*H*-4,8-epoxychromene exhibited high analgesic activity in both acetic acid-induced writhing and hot plate tests; its selectivity index IS_{50} exceeded 60.

Keywords Verbenol epoxide · Terpenoid · Heterocyclic compounds · Analgesic activity · Acetic acid-induced writhing test · Hot plate test

Introduction

Pain is the most common symptom for which patients seek medical attention (Wolff *et al*., 2011). Despite exponential advances in understanding the biology of pain, new analgesics, and improvements in analgesic delivery methods, the treatment of pain is still inadequate and continues to be a substantial worldwide public health concern especially under treatment of cancer related pain (White *et al*., 2005; Juniper *et al*., 2009). Thus, it is necessary to find highly effective approaches or novel analgesic substances for pain relief.

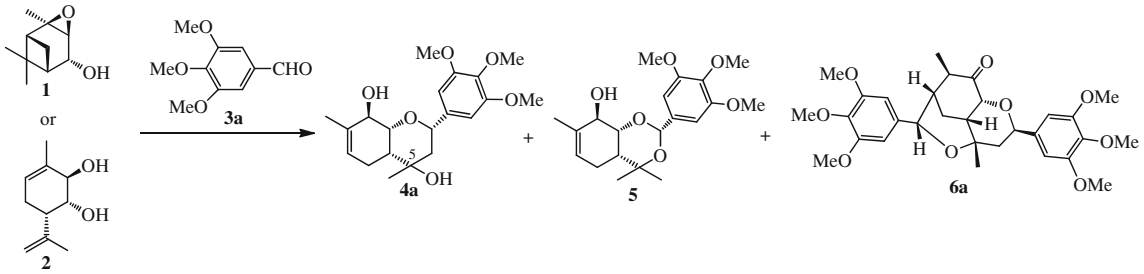
Earlier, we found (Il'ina *et al*., 2011) that the reaction of monoterpenoids (−)-*cis*-verbenol epoxide **1** and (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol **2** with 3,4,5-trimethoxybenzaldehyde **3a** in the presence of montmorillonite clay K10 gave a set of intermolecular products with different types of framework: a mixture of diastereomers (5*R*)- and (5*S*)-**4a** with a chromene framework, compound **5** with a benzodioxine framework, and the product of addition of two aldehyde molecules to epoxide **1** with an (epoxymethano)chromene framework **6a** (Scheme 1). Although rather complex reaction mixtures were formed, the individual products were relatively easy to isolate because of noticeable differences in the molecular mass and chemical nature of these compounds.

Cannabinoids and their analogs with a combination of a monoterpene fragment and the aromatic ring are known to have a considerable analgesic activity (Finn and Chapman, 2004; Costa, 2007; Lambert and Fowler, 2005). On the other hand, it was shown that the introduction of several methoxy groups in the aromatic ring led to increased analgesic activity of compounds with different structural types (Palomba *et al*., 2000; Husain *et al*., 2005; Wagle *et al*., 2008). Therefore, it seemed promising (and is the goal of the present work) to synthesize analogs of compounds **4a**–**6a** with different positions of methoxy groups for further studies of their analgesic activity.

O. Mikhalchenko
Novosibirsk State University, Pirogova 2,
630090 Novosibirsk, Russian Federation

I. Il'ina · A. Pavlova · E. Morozova · D. Korchagina · T. Tolstikova · K. Volcho (✉) · N. Salakhutdinov
Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Lavrentjev Ave., 9, 630090 Novosibirsk, Russian Federation
e-mail: volcho@nioch.nsc.ru

E. Pokushalov
State Research Institute of Circulation Pathology, Rechkunovskaya 15, 630055 Novosibirsk, Russian Federation



Scheme 1 The interaction of compounds **1** and **2** with 3,4,5-trimethoxybenaldehyde **3a**

Chemistry

The interaction of verbenol epoxide **1** with aldehydes **3b–d**, each containing three methoxy groups in different positions of the aromatic ring, in the presence of clay K10 in CH_2Cl_2 for 1.5 h led to the formation of intermolecular products **4b–d** and **6b**, in addition to the products of isomerization **2**, **7,** and **8** (Table 1; Scheme 2).

It is interesting that the structure of the starting aldehyde had a significant effect not only on the composition and yield of intermolecular products but also on the ratio of isomerization products. For example, in the case of 3,4,5-trimethoxybenzaldehyde **3a** and 2,4,5-trimethoxybenzaldehyde **3c**, the reaction mixtures contained considerable amounts of diol **2**, which was absent in reactions with 2,3,4-trimethoxybenzaldehyde **3b** and 2,4,6-trimethoxybenzaldehyde **3d**. It can be supposed that isomerization of verbenol epoxide **1** into monoterpenoids **2**, **7,** and **8** may proceed on various acidic centers of the clay. Aldehydes, depending on the location of methoxy groups at the aromatic ring, in the process of adsorption on clay can block some of the acidic sites, thus affecting the direction of isomerization.

Table 1 Yields of products in the reaction of (−)-*cis*-verbenol epoxide **1** with aldehydes **3a–d**

Aldehyde		Yields of products (%)					
		Intramolecular			Intermolecular		
N	Substituents	**2**	**7**	**8**	**4** (5*S*:5*R*)	**5**	**6**
3a[a]	R_2, R_6 = H; R_3, R_4, R_5 = OMe	10	12	7	25 (50:50)	6	5
3b	R_5, R_6 = H; R_2, R_3, R_4 = OMe	–	15	6	15 (60:40)	–	2
3c	R_3, R_6 = H; R_2, R_4, R_5 = OMe	11	17	6	11 (89:11)	–	–
3d	R_3, R_5 = H; R_2, R_4, R_6 = OMe	–	17	13	3 (93:7)	–	–

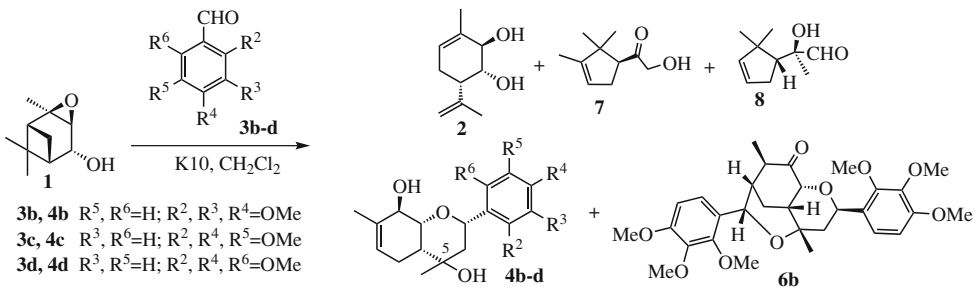
[a] Il'ina *et al.* (2011)

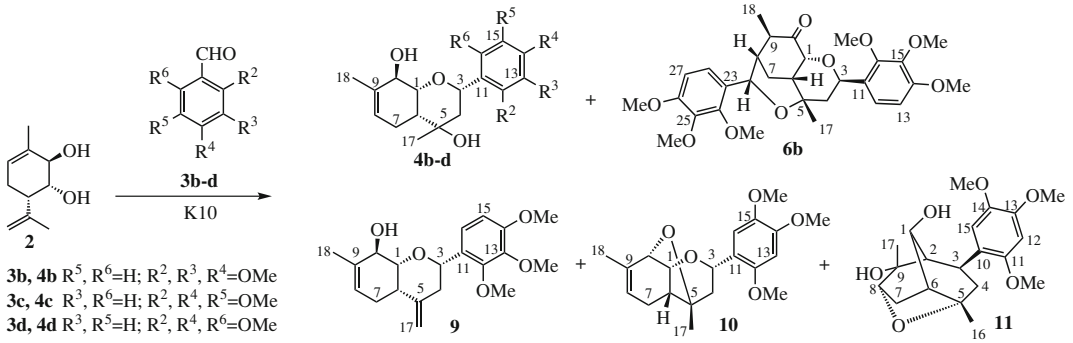
The main intermolecular products were compounds **4** with a chromene framework in all instances, but in the reaction of aldehyde **3d** (containing substituents in both *ortho*-positions) the yield of **4d** was insignificant probably because of steric hindrances. The ratio of (5*S*/5*R*)-diastereomers in **4a–d** depended considerably on the structure of the aldehyde used; the reactions with **3a, b** formed isomers in approximately equal ratios, while the reactions with **3c, d** were much more selective and predominantly led to (5*S*)-isomers.

The reaction of diol **2** with aldehydes **3a–d** in the presence of clay K10 was much more sluggish than similar reactions with epoxide **1**. Although these reactions were performed without a solvent and were thus considerably accelerated (Volcho *et al.*, 1999), the reaction mixture had to be stored for 1–7 days to attain a complete conversion of diol **2**. It should be noted that CH_2Cl_2 was used for evenly deposition of reagents on clay; CH_2Cl_2 then was evaporated.

The yield of compounds **4** with a chromene framework was almost independent of the structure of aldehyde in all reactions, except the reaction involving sterically crowded aldehyde **3d** (Scheme 3; Table 2). As in the case of

Scheme 2 The interaction of verbenol epoxide **1** with aldehydes **3b–d**





Scheme 3 The interaction of compound **2** with aldehydes **3b–d**

Table 2 Yields of products in reactions of diol **2** with aldehydes **3a–d**

Aldehyde		Yields of products (%)					
N	Substituents	**4** (5*S*:5*R*)	**5**	**6**	**9**	**10**	**11**
3a[a]	R_2, R_6 = H; R_3, R_4, R_5 = OMe	39 (60:40)	3	9	–	–	–
3b	R_5, R_6 = H; R_2, R_3, R_4 = OMe	39 (57:43)	–	9	7	–	–
3c	R_3, R_6 = H; R_2, R_4, R_5 = OMe	39 (67:33)	–	–	–	15	2
3d	R_3, R_5 = H; R_2, R_4, R_6 = OMe	14 (75:25)	–	–	–	–	–

[a] Il'ina *et al.* (2011)

verbenol epoxide **1**, the reaction became more diastereoselective on passing from aldehydes **3a, b** to aldehydes **3c, d**, but the effect was less significant. At the same time, the structure of minor intermolecular products influenced by the structure of the aldehyde used. For example, compound **5** with a benzodioxine framework was formed only in the case of aldehyde **3a**, the diene **9** was isolated in the reaction with aldehyde **3b**, and tricyclic compounds **10** and **11** were formed in the reaction with aldehyde **3c**.

Compound **9** is evidently formed from **4b** as a result of dehydration. A possible mechanism of the formation of compound **10** is presented in Scheme 4. It involves intramolecular heterocyclization in compound (5*S*)-**4c**. The formation of **10** from isomer (5*R*)-**4c** seems impossible for steric reasons. The suggested mechanism was confirmed by the presence of **10** in the reaction mixture after storing **4c** under reaction conditions with diastereomer (5*S*)-**4c** spent in predominant amounts (GLC-MS data), just as would be expected in this case. To the best of our knowledge, the framework of epoxychromene **10** is new.

The supposed mechanism of the formation of minor tricyclic compound **11** can involve an attack of double bond of diol **2** at the protonated aldehyde **3c**, the addition of a water molecule to the resulting carbocation **12** and protonation of the allyl hydroxy group, followed by heterocyclization accompanied by the elimination of water leading to intermediate **13** (Scheme 5). The protonation of the benzyl hydroxy group in **13**, subsequent carbocyclization and water elimination-addition can just lead to the formation of **11**.

Thus, we obtained a set of new chiral compounds with different types of framework, including a monoterpene fragment and an aromatic ring with three methoxy groups in different positions by one-pot synthesis from monoterpenoids **1** and **2**.

Biology

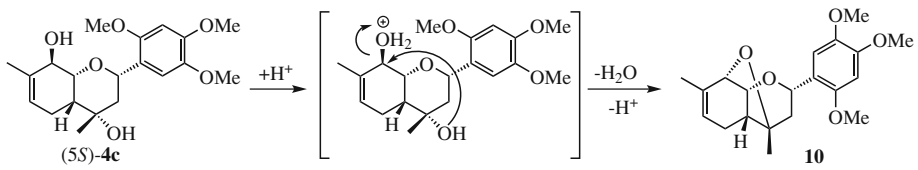
All the synthesized compounds, except **11** obtained with a yield of only 2 %, were tested for analgesic activity. In the case of compounds **4** we used mixtures of diastereomers obtained in the reactions with diol **2** (Table 2) without separation.

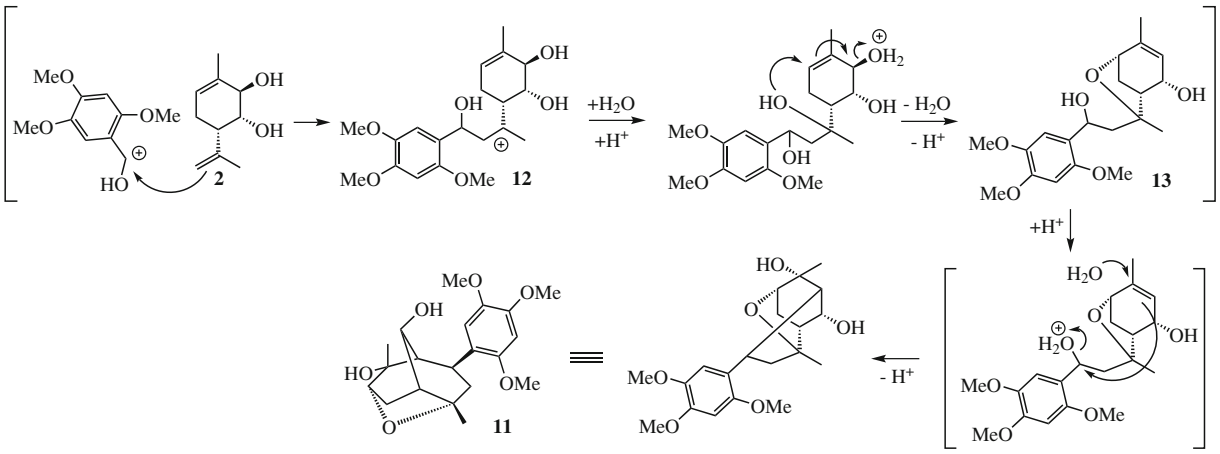
The analgesic activity of the compounds in a dose of 10.0 mg/kg (oral administration) was studied in the standard experimental pain models, namely the acetic acid-induced writhing (0.75 % acetic acid, 0.1 ml for one animal, intraperitoneally) and hot plate (thermal stimulation, T = 54 ± 0.5 °C) tests (Koster *et al.*, 1959; Eddy and Leimbach, 1953). Sodium diclofenac in the same dose was used as a reference drug.

The data of Table 3 show that compounds **4b**, **6a**, and **10** taken in a dose of 10 mg/kg exhibited a considerable analgesic activity in the acetic acid-induced writhing test, significantly reducing the writhing caused by the introduction of acetic acid in animals. The highest activity in this test was observed for compound **4b** with two-thirds of animals showing no writhing at all in this case (in the control group of mice, writhing was observed for all animals).

In the hot plate test, however, only tricyclic compound **10** showed analgesic activity (Table 4). Compound **10** was more effective than the sodium diclofenac reference taken in the same dose in this test.

Scheme 4 The mechanism of formation of compound **10**





Scheme 5 The mechanism of formation of compound **11**

Curiously, analgesic activity was found in compounds of different structural types with methoxy groups in different positions of the aromatic ring, stimulating further search for new analgesics among compounds with combinations of monoterpene and aromatic fragments.

For compound **10**, which exhibited high analgesic activity in both tests, we determined ED_{50} (the "median effective dose" is a dose which produces the 50 % of protection) (22 mg/kg in the acetic acid-induced writhing test and 25 mg/kg in the hot plate test). The acute toxicity of **10** was determined by the Kerber method by a single intragastric administration in mice. LD_{50} (median lethal dose) is the quantity of an agent that will kill 50 % of the test subjects. Compound **10** proved moderately toxic, it's LD_{50} exceeding 1,500 mg/kg. Thus, the selectivity index IS_{50} (LD_{50}/ED_{50}) of **10** exceeded 60 for both tests. For comparison, the IS_{50} of acetylsalicylic acid in the acetic acid-induced writhing test is 10.3 (Syubaev *et al.*, 1986).

Table 3 Analgesic activity of compounds **4a–d**, **5**, **6a, b**, and **10** and sodium diclofenac in the acetic acid-induced writhing test (10 mg/kg dose)

Compound	Mean ± SD	Control	Pain inhibition (%) (percent of animals without symptoms)
4a	8.5 ± 1.9	10.1 ± 0.7	16
4b	2.8 ± 1.6	10.9 ± 0.9	74# (63)
4c	7.5 ± 1.5	10.1 ± 0.7	26
4d	12.1 ± 1.0	13.9 ± 1.0	13
5	12.6 ± 1.3	14.4 ± 0.9	13
6a	6.3 ± 1.6	12.6 ± 1.3	50* (25)
6b	7.8 ± 1.3	10.1 ± 0.7	23
10	3.8 ± 0.9	10.9 ± 0.9	65# (25)
Diclofenac sodium	0.8 ± 0.4	8.4 ± 0.8	90# (87.5)

% of protection = $(t_{exp} - t_{control})/t_{control} \times 100$ %

* $p < 0.01$; # $p < 0.001$ in comparison with control

Conclusion

To summarize, we synthesized a set of new polycyclic compounds with different types of framework, including

Table 4 Analgesic activity of compounds **4a–d**, **5**, **6a, b**, and **10** and sodium diclofenac in the hot plate test (10 mg/kg dose)

Compound	Mean ± SD	Control	Protection (%)
4a	13.1 ± 1.2	12.3 ± 1.7	7
4b	11.8 ± 1.2	10.5 ± 1.8	12
4c	15.4 ± 1.2	12.3 ± 1.7	25
4d	13.3 ± 2.4	11.9 ± 1.3	12
5	14.3 ± 1.0	12.3 ± 0.9	16
6a	14.6 ± 2.0	12.3 ± 0.9	19
6b	13.4 ± 1.9	12.3 ± 1.7	9
10	18.1 ± 1.9	10.5 ± 1.8	72#
Diclofenac sodium	33.4 ± 2.3	20.4 ± 2.2	64*

* $p < 0.01$; # $p < 0.001$ in comparison with control

new ones, by reactions of monoterpenoids verbenol epoxide **1** and diol **2** with aromatic aldehydes each containing three methoxy groups. Studies of the analgesic activity of these compounds showed that three compounds (**4b**, **6a**, and **10**) had a considerable activity in vivo in the acetic acid-induced writhing test. One of these substances (**10**) also showed high analgesic activity in the hot plate test. The selectivity index IS_{50} of **10** exceeded 60 for both tests.

Experimental

Chemistry

All the chemicals reagents were of commercial grade. As catalyst, we used K10 clay (*Fluka*). The clay was calcinated at 110 °C for 3 h immediately before use. CH_2Cl_2 was passed through calcined Al_2O_3. (−)-*cis*-Verbenol epoxide (**1**) ($[\alpha]_{580}^{20} = -60$ ($c = 0.41, CHCl_3$)) and (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol ($[\alpha]_D^{31} = -49.1$ ($c = 2.6$, $CHCl_3$)) were synthesized according to (Il'ina *et al*., 2007) from (−)-verbenone (Aldrich), the content of the main substance was not less than 98.0 %. All product yields (except compound **11**) are given for pure compounds isolated by column chromatography. Column chromatography (CC): silica gel (SiO_2; 60–200μ; Macherey–Nagel); hexane/EtOAc 100/0 → 0/100, acetone. GC/MS (purity control and products analysis): Hewlett–Packard 5890/II gas chromatograph with a quadrupole mass spectrometer (HP MSD 5971) as a detector, HP-5MS quartz column, 30 m × 0.25 mm, He as carrier gas. Optical rotation: polAAr 3005 spectrometer, $CHCl_3$ soln. HR-MS: DFS-Thermo-Scientific spectrometer in a full scan mode (15–500 *m/z*, 70 eV electron-impact ionization, direct sample introduction). ^1H and ^{13}C NMR: Bruker DRX-500 apparatus at 500.13 MHz (^1H) and 125.76 MHz (^{13}C) in $CDCl_3$; chemical shifts δ in ppm relative to residual $CHCl_3$ (δ(H) 7.24, δ(C) 76.90 ppm), *J* in Hz; structure determinations by analyzing the ^1H NMR spectra, ^1H–^1H double resonance spectra, *J*-modulated ^{13}C NMR spectra (JMOD), ^{13}C NMR spectra with proton off-resonance saturation, and ^{13}C–^1H 2D heteronuclear correlation with one-bond and long-range spin–spin coupling constants (C–H COSY, ^1J(C,H) = 135 Hz, COLOC, $^{2,3}J$(C, H) = 10 Hz).

Reaction of (−)-*cis*-verbenol epoxide 1 with aldehydes **3b–d** on clay K10: general procedure

An appropriate aldehyde was added to a suspension of clay K10 in CH_2Cl_2 (10 ml). A solution of epoxide **1** in CH_2Cl_2 (20 ml) was added dropwise with stirring and the reaction mixture was stirred for 1.5 h at r.t. Then, ethyl acetate (20 ml) was added. The catalyst was filtered off, the solvent distilled off, and the residue was separated on a SiO_2 column.

*With 2,3,4-trimethoxybenzaldehyde **3b***

The reaction of epoxide **1** (0.800 g) and aldehyde **3b** (0.930 g) in the presence of clay K10 (3.5 g) gave compounds **7** (0.120 g, 15 %), **8** (0.048 g, 6 %), **4b** (5*S*:5*R* = 60:40) (0.260 g, 15 %), and **6b** (0.052 g, 2 %). The spectral data of **7** and **8** coincided with those reported in the literature (Il'ina *et al*., 2007; Ardashov *et al*., 2007).

The NMR spectra of isomers (5*S*)- and (5*R*)-**4b** were recorded for the mixture of isomers.

Here and below, the ratio of diastereomers (5*S*:5*R*) for products of type **4** was determined from the NMR spectrum by the ratio of the peak areas of H_a-3. Methyl group at the carbon atom C(5) (Scheme 2) is axial in (5*S*)-**4**, as indicated by the presence of long range $^4J(C^{17}H_3, H_a\text{-}4)$ of 0.8 Hz, but equatorial in (5*R*)-**4**. In the latter case, as would be expected, the axial OH group causes a paramagnetic shift $\Delta\delta = 0.34$ of the H_a-3 signal due to the 1,3-diaxial interaction.

*(2S,4S,4aR,8R,8aR)-4,7-Dimethyl-2-(2,3,4-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-chromene-4,8-diol ((5S)-**4b**)* ^1H NMR ($CDCl_3$): 1.52 (s, 3H, H-17); 1.62 (ddd, *J*(4e,4a) = 13.4 Hz, *J*(4e,3a) = 2.7 Hz, *J*(4e,6) = 1.1 Hz, 1H, H_e-4); 1.79 (m, all $J \leq 2.5$ Hz, 3H, H-18); 1.77–1.84 (m, 1H, H_a-6); 1.94 (dd, *J*(4a,4e) = 13.4 Hz, *J*(4a,3a) = 12.0 Hz, 1H, H_a-4); 2.16–2.21 (m, 2H, H-7); 3.80 (s, $OC^{21}H_3$); 3.824 (s, $OC^{20}H_3$); 3.86 (s, $OC^{19}H_3$); 3.81 (dd, *J*(1e,6) = 2.4 Hz, *J*(1e,10e) = 2.0 Hz, 1H, H_e-1); 3.87 (br.s, 1H, H_e-10); 4.67 (dd, *J*(3a,4a) = 12.0 Hz, *J*(3a,4e) = 2.7 Hz, 1H, H_a-3); 5.62–5.63 (m, 1H, H-8); 6.63 (d, J(15,16) = 8.5 Hz, 1H, H-15); 7.00 (d, J(16,15) = 8.5 Hz, 1H, H-16).

^{13}C NMR ($CDCl_3$): 77.74 (d, C-1); 72.41 (d, C-3); 42.02 (t, C-4); 71.13 (s, C-5); 38.38 (d, C-6); 22.73 (t, C-7); 124.63 (d, C-8); 131.39 (s, C-9); 70.55 (d, C-10); 127.92 (s, C-11); 150.80 (s, C-12); 141.93 (s, C-13); 153.06 (s, C-14); 107.53 (d, C-15); 121.14 (d, C-16) 26.91 (q, C-17); 20.67 (q, C-18); 61.23 (q, C-19); 60.59 (q, C-20); 55.89 (q, C-21).

HR-MS: 364.1881 (M^+, $C_{20}H_{28}O_6^+$; calc. 364.1880).

*(2S,4R,4aR,8R,8aR)-4,7-Dimethyl-2-(2,3,4-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-chromene-4,8-diol ((5R)-**4b**)* ^1H NMR ($CDCl_3$): 1.21 (s, 3H, H-17); 1.57 (ddd, *J*(4e,4a) = 14.2 Hz, *J*(4e,3a) = 2.7 Hz, *J*(4e,6) = 1.3 Hz, 1H, H_e-4); 1.70 (br.t, *J*(6a,7) = 9 Hz, 1H, H_a-6); 1.79 (m, all $J \leq 2.5$ Hz, 3H, H-18); 1,84 (dd, *J*(4a,4e) = 14.2 Hz; *J*(4a,3a) = 11.8 Hz, 1H, H_a-4); 1.99–2.04 (m, 2H, H-7); 3.79 (s, $OC^{21}H_3$); 3.825 (s, $OC^{20}H_3$); 3.85 (s, $OC^{19}H_3$); 3.89 (br.s, 1H, H_e-10); 4.25 (dd, *J*(1e,6a) = 2.5 Hz,

J(1e,10e) = 2.0 Hz, 1H, H_e-1); 4.98 (dd, J(3a,4a) = 11.8 Hz, J(3a,4e) = 2.7 Hz, 1H, H_a-3); 5.56–5.59 (m, 1H, H-8); 6.61 (d, J(15,16) = 8.5 Hz, 1H, H-15); 6.99 (d, J(16,15) = 8.5 Hz, 1H, H-16).

^{13}C NMR ($CDCl_3$): 75.26 (d, C-1); 71.14 (d, C-3); 40.97 (t, C-4); 70.79 (s, C-5); 37.87 (d, C-6); 24.56 (t, C-7); 123.95 (d, C-8); 131.90 (s, C-9); 70.52 (d, C-10); 128.56 (s, C-11); 150.95 (s, C-12); 142.04 (s, C-13); 152.93 (s, C-14); 107.53 (d, C-15); 121.36 (d, C-16); 28.30 (q, C-17); 20.77 (q, C-18); 61.29 (q, C-19); 60.59 (q, C-20); 55.89 (q, C-21).

HR-MS: 364.1881 (M^+, $C_{20}H_{28}O_6^+$; calc. 364.1880).

(2R,4S,4aR,6S,7R,8aR,9S)-4,7-Dimethyl-2,9-bis(2,3,4-trimethoxyphenyl)hexahydro-2H-4,6-(epoxymethano) chromen-8(8aH)-one ***(6b)*** ^{1}H NMR ($CDCl_3$): 1.07 (d, J(18,9) = 7.5 Hz, 3H, H-18); 1.40 (s, 3H, H-17); 1.67 (dd, J(4a,4e) = 13.8 Hz, J(4a,3e) = 12.0 Hz, 1H, H_a-4); 1.92 (m, all $J \leq$ 3.1 Hz, 1H, H_e-8); 1.93 (dd, J(4e,4a) = 13.8 Hz, J(4e,3a) = 2.6 Hz, 1H, H_e-4); 2.00 (ddd, J(7a,7e) = 14.2 Hz, J(7a,6e) = 3.3 Hz, J(7a,8) = 3.0 Hz, 1H, H_a-7); 2.31 (dddd, J(6e,1a) = 5.9 Hz, J(6e,7a) = 3.3 Hz, J(6e,7e) = 3.1 Hz, J(6e,8e) = 0.5 Hz, 1H, H_e-6); 2.43 (dddd, J(7e,7a) = 14.2 Hz, J(7e,6e) = 3.1 Hz, J(7e,8) = 3.1 Hz, J(7e,9e) = 1.8 Hz, 1H, H_e-7); 2.46 (qdd, J(9e,18) = 7.5 Hz, J(9e,8e) = 2.2 Hz, J(9e,7e) = 1.8 Hz, 1H, H_e-9); 3.809 s and 3.822 s ($OC^{21}H_3$, $OC^{31}H_3$); 3.816 (s, $OC^{30}H_3$); 3.85 (s, $OC^{20}H_3$); 3.87 (s, $OC^{29}H_3$); 3.96 (s, $OC^{19}H_3$); 4.38 (d, J(1a, 6e) = 5.9 Hz, 1H, H_a-1); 5.24 (d, J(22,8e) = 2.1 Hz, H-22); 5.38 (dd, J(3a,4a) = 12.0 Hz, J(3a,4e) = 2.6 Hz, 1H, H_a-3); 6.65 (d, J(15,16) = 8.6 Hz, H- 15); 6.70 (d, J(27,28) = 8.6 Hz, 1H, H-27); 7.04 (d, J(28,27) = 8.6 Hz, 1H, H-28); 7.14 (d, J(16,15) = 8.6 Hz, 1H, H-16).

^{13}C NMR ($CDCl_3$): 76.49 (d, C-1); 64.80 (d, C-3); 45.79 (t, C-4); 72.92 (s, C-5); 41.22 (d, C-6); 22.38(t, C-7); 40.36 (d, C-8); 43.75 (d, C-9); 210.11 (s, C-10); 128.37 (s, C-11); 151.09 (s, C-12); 141.97 (s, C-13), 152.79 (s, C-14); 107.50 (d, C-15); 121.25 (d, C-16); 21.89 (q, C-17); 17.54 (q, C-18); 61.17 (q, C-19); 60.58, 60.61 and 60.53 (3q, C-20, C-29, C-30); 55.80 and 55.88 (2 q, C-21, C-31); 71.04 (d, C-22); 125.73 (s, C-23); 149.56 (s, C-24); 141.42 (s, C-25); 152.71 (s, C-26); 107.19 (d, C-27); 122.11 (d, C-28).

$[\alpha]_D^{21} = -46{,}7$ (c = 1.99); HR-MS: 542.2513 (M^+, $C_{30}H_{38}O_9^+$; calc. 542.2510).

With 2,4,5-trimethoxybenzaldehyde ***3c***

The reaction of epoxide **1** (0.700 g) and aldehyde **3c** (0.800 g) in the presence of clay K10 (3.0 g) gave compounds **7** (0.100 g, 17 %), **8** (0.033 g, 6 %), **2** (0.075 g, 11 %), **4c** (5*S*:5*R* = 89:11) (0.160 g, 11 %).

The NMR spectra of isomers (5*S*)- and (5*R*)-**4c** were recorded for the mixture of isomers.

*(2S,4S,4aR,8R,8aR)-4,7-Dimethyl-2-(2,4,5-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-chromene-4,8-diol ((5S)-****4c****)* ^{1}H NMR ($CDCl_3$): 1.52 (s, 3H, H-17); 1.68 (ddd, J(4e,4a) = 13.2 Hz, J(4e,3a) = 2.7 Hz, J(4e,6) = 1.1 Hz, 1H, H_e-4); 1.77–1.85 (m, 2H, H- 6, H_a-4); 1.81 (m, all $J \leq$ 2.5 Hz, 3H, H-18); 2.15–2.20 (m, 2H, H-7); 3.78 s, 3.79 s, 3.84 s, (3 OCH_3); 3.81 (dd, J(1e,6) = 2.4 Hz, J(1e,10e) = 2.0 Hz, 1H, H_e-1); 3.90 (br.s, 1H, H_e-10); 4.76 (dd, J(3a,4a) = 11.4 Hz, J(3a,4e) = 2.7 Hz, 1H, H_a-3); 5.63–5.67 (m, 1H, H-8); 6.46 (s, 1H, H-13); 6.88 (s, 1H, H-16).

^{13}C NMR ($CDCl_3$): 77.72 (d, C-1); 71.58 (d, C-3); 41.87 (t, C-4); 71.16 (s, C-5); 38.50 (d, C-6); 22.71 (t, C-7); 124.58 (d, C-8); 131.43 (s, C-9); 70.61 (d, C-10); 122.09 (s, C-11); 150.24 (s, C-12); 97.67 (d, C-13); 149.03 (s, C-14); 143.31 (s, C-15); 111.10 (d, C-16); 27.00 (q, C-17); 20.67 (q, C-18); 56.07, 56.49 and 56.77 (3q, C-19, C-20, C-21).

HR-MS: 364.1878 (M^+, $C_{20}H_{28}O_6^+$; calc. 364.1880).

*(2S,4R,4aR,8R,8aR)-4,7-Dimethyl-2-(2,4,5-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-chromene-4,8-diol ((5R)-****4c****)* ^{1}H NMR ($CDCl_3$): 1.20 (s, 3H, H-17); 1.62 (ddd, J(4e,4a) = 14.2 Hz, J(4e,3a) = 3.2 Hz, J(4e,6) = 1.3 Hz, 1H, H_e-4); 1.65–1.69 (m, 1H, H- 6); 1.69 (dd, J(4a,4e) = 14.2 Hz; J(4a,3a) = 11.4 Hz, 1H, H_a-4); 1.99–2.04 (m, 2H, H-7); 3.77 s, 3.79 s, 3.83 s (3 OMe); 3.91 (br.s, 1H, H_e-10); 4.24 (dd, J(1e,6) = 2.4 Hz, J(1e,10e) = 2.1 Hz, 1H, H_e-1); 5.08 (dd, J(3a,4a) = 11.4 Hz, J(3a,4e) = 3.2 Hz, 1H, H_a-3); 5.57–5.61 (m, 1H, H-8); 6.47 (s, 1H, H-13); 6.89 (s, 1H, H-16).

^{13}C NMR ($CDCl_3$): 75.28 (d, C-1); 70.09 (d, C-3); 40.91 (t, C-4); 70.86 (s, C-5); 38.04 (d, C-6); 24.60 (t, C-7); 123.91 (d, C-8); 131.94 (s, C-9); 70.58 (d, C-10); 122.78 (s, C-11); 150.33 (s, C-12); 97.79 (d, C-13); 148.89 (s, C-14); 143.29 (s, C-15); 111.32 (d, C-16); 28.32 (q, C-17); 20.77 (q, C-18); 56.12, 56.51 and 56.77 (3 q, C-19, C-20, C-21).

HR-MS: 364.1878 (M^+, $C_{20}H_{28}O_6^+$; calc. 364.1880).

With 2,4,6-trimethoxybenzaldehyde ***3d***

The reaction of epoxide **1** (0.800 g) and aldehyde **3d** (0.800 g) in the presence of clay K10 (3.0 g) gave compounds **7** (0.140 g, 17 %), **8** (0.103 g, 13 %), **4d** (5*S*:5*R* = 93:7) (0.057 g, 3 %), and **6b** (0.052 g, 2 %).

The NMR spectra of isomers (5*S*)- and (5*R*)-**4d** were recorded for the mixture of isomers.

*(2S,4S,4aR,8R,8aR)-4,7-Dimethyl-2-(2,4,6-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-chromene-4,8-diol ((5S)-****4d****)* ^{1}H NMR ($CDCl_3$): 1.31 (ddd, J(4e,4a) = 13.1 Hz, J(4e,3a) = 3.1 Hz, J(4e,6a) = 1.1 Hz, 1H, H_e-4); 1.49 (s, 3H, H-17); 1.73 (dddd, J(6a,7a) = 11.0 Hz, J(6a,7e) = 5.8 Hz, J(6a,1e) = 2.4 Hz, J(6a,4e) = 1.1 Hz, 1H, H_a-6); 1.77

(br.s, 3H, H-18); 2.13 (dddq, J(7e,7a) = 17.5 Hz, J(7e,6a) = 5.8 Hz, J(7e,8) = 5.2 Hz, J(7e,18) = 1.5 Hz, 1H, H_e-7); 2.40 (ddm, J(7a,7e) = 17.5 Hz, J(7a,6a) = 11.0 Hz, 1H, H_a-7); 2.66 (dd, J(4a,4e) = 13.1 Hz, J(4a,3a) = 12.2 Hz, 1H, H_a-4); 3.73 (s, 6H, 3H-19, 3H-21); 3.73 (br.s, 1H, H_e-1); 3.75 (s, 3H, $OC^{20}H_3$); 3.88 (br.s, 1H, H_e-10); 5.01 (dd, J(3a,4a) = 12.2 Hz, J(3a,4e) = 3.1 Hz, 1H, H_a-3); 5.63–5.67 (m, 1H, H-8), 6,09 (s, 2H, H-13, H-15).

^{13}C NMR ($CDCl_3$): 77.64 (d, C-1); 69.39 (d, C-3); 38.47 (t, C-4); 71.47 (s, C-5); 38.98 (d, C-6); 22.70 (t, C-7); 124.97 (d, C-8); 131.30 (s, C-9); 70.74 (d, C-10); 110.44 (s, C-11); 159.55 (s, C-12); 91.83 (d, C-13); 160.70 (s, C-14); 91.83 (d, C-15); 159.55 (s, C-16); 26.82 (q, C-17); 20.58 (q, C-18); 56.04 (q, C-19); 55.16 (q, C-20); 56.04 (q, C-21).

HR-MS: 364.1877 (M^+, $C_{20}H_{28}O_6^+$; calc. 364.1880).

*(2S,4R,4aR,8R,8aR)-4,7-Dimethyl-2-(2,4,6-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-chromene-4,8-diol ((5R)-**4d**)* ^{1}H NMR ($CDCl_3$): 1.21 (s, 3H, H-17); 1.25 (ddd, J(4e,4a) = 14.0 Hz, J(4e,3a) = 3.2 Hz, J(4e,6) = 1.3 Hz, 1H, H_e-4); 1.59–1.64 (m, 1H, H-6); 1.77 (br.s, 3H, H-18); 1.97 (dddq, J(7e,7a) = 17.5 Hz, J(7e,6) = 5.9 Hz, J(7e,8) = 5.2 Hz, J(7e,18) = 1.3 Hz, 1H, H_e-7); 2.20–2.29 (m, 1H, H_a-7); 2.56 (dd, J(4a,4e) = 14.0 Hz, J(4a,3a) = 12.1 Hz, 1H, H_a-4); 3.72 (s, 6H, $OC^{19}H_3$, $OC^{21}H_3$); 3.74 (s, 3H, $OC^{20}H_3$); 3.89 (br.s, 1H, H_e-10); 4.17 (dd, J(1e,6) = 2.4 Hz, J(1e,10e) = 2.0 Hz, 1H, H_e-1); 5.33 (dd, J(3a,4a) = 12.1 Hz, J(3a,4e) = 3.2 Hz, 1H, H_a-3); 5.56–5.60 (m, 1H, H-8); 6.09 (2H, H-13, H-15).

^{13}C NMR ($CDCl_3$): 75.16 (d, C-1); 67.69 (d, C-3); 37.38 (t, C-4); 71.13 (s, C-5); 38.45 (d, C-6); 24.50 (t, C-7); 124.41 (d, C-8); 131.73 (s, C-9); 70.70 (d, C-10); 111.05 (s, C-11); 159.71 (s, C-12); 92.05 (d, C-13); 160.60 (s, C-14); 92.05 (d, C-15); 159.71 (s, C-16); 28.61 (q, C-17); 20.69 (q, C-18); 56.14 (q, C-19); 55.16 (q, C-20); 56.14 (q, C-21).

HR-MS: 364.1877 (M^+, $C_{20}H_{28}O_6^+$; calc. 364.1880).

Reaction of diol 2 with aldehydes **3b–d** on clay K10: general procedure

An appropriate aldehyde was added to a suspension of clay K10 in CH_2Cl_2 (10 ml). A solution of diol **2** in CH_2Cl_2 (20 ml) was added dropwise with stirring. The solvent was distilled off. The mixture was stored at r.t. for the required period of time. Then, ethyl acetate (20 ml) was added. The catalyst was filtered off, the solvent distilled off, and the residue was separated on a SiO_2 column.

*With 2,3,4-trimethoxybenzaldehyde **3b***

The reaction of diol **2** (0.800 g) and aldehyde **3b** (0.930 g) for 24 h in the presence of clay K10 (3.5 g) gave compounds **4b** (5*S*:5*R* = 57:43) (0.747 g, 43 %), and **9** (0.108 g, 7 %).

*(2S,4aS,8R,8aR)-7-Methyl-4-methylene-2-(2,3,4-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-chromen-8-ol (**9**)* ^{1}H NMR ($CDCl_3$): 1.80 (br.s, 3H, H-18); 1.95 (dddq, J(7e,7a) = 17.7 Hz, J(7e,6a) = 6.4 Hz, J(7e,8) = 5.1 Hz, J(7e,18) = 1.5 Hz, 1H, H_e-7); 2.26 (dd, J(4e,4a) = 14.1 Hz, J(4e,3a) = 2.9 Hz, 1H, H_e-4); 2.37 (dddqd, J(7a,7e) = 17.7 Hz, J(7a,6a) = 10.8 Hz, J(7a,8) =2.5 Hz, J(7a,18) = 2.5 Hz, J(7a,10e) = 1.5 Hz, 1H, H_a-7); 2.50–2.57 (m, 2H, H_a-6, H_a-4); 3,73 (dd, J(1e,6a) = 2.4 Hz; J(1e,10e) = 2.0 Hz, 1H, H_e-1); 3.81 (s, 3H, $OC^{21}H_3$); 3.84 (s, 3H, $OC^{20}H_3$); 3.86 (s, 3H, $OC^{19}H_3$); 3.88 (br.s, 1H, H_e-10); 4.61 (dd, J(3a,4a) = 11.6 Hz, J(3a,4e) = 2.9 Hz, 1H, H_a-3); 4.78 (dd, J(17,17′) = 2.5 Hz, J(17,6a) = 2.0 Hz, 1H, H-17); 4.88 (dd, J(17′,17) = 2.5 Hz, J(17′,4a) = 2.0 Hz, 1H, H-17′); 5.60-5.64 (m, 1H, H-8); 6.64 (d, J(15,16) = 8.5 Hz, 1H, H-15); 7.05 (d, J(16,15) = 8.5 Hz, 1H, H-16).

^{13}C NMR ($CDCl_3$): 80.55 (d, C-1); 75.64 (d, C-3); 37.59 (t, C-4); 147.15 (s, C-5); 36.81 (d, C-6); 26.28 (t, C-7); 124.43 (d, C-8); 131.52 (s, C-9); 70.36 (d, C-10); 128.27 (s, C-11); 150.91 (s, C-12); 141.98 (s, C-13); 153.05 (s, C-14); 107.51 (d, C-15); 121.12 (d, C-16); 109.45 (t, C-17); 20.85 (q, C-18); 61.24 (q, C-19); 60.59 (q, C-20); 55.90 (q, C-21).

$[\alpha]_D^{21} = -32.25$ ($c = 0.8$); HR-MS: 346.1769 (M^+, $C_{26}H_{30}O_5^+$; calc. 346.1774).

*With 2,4,5-trimethoxybenzaldehyde **3c***

The reaction of diol **2** (0.700 g) and aldehyde **3c** (0.800 g) for 1 week in the presence of clay K10 (3.0 g) gave mixture **4c** and **11** (0.216 g **4c** and 0.029 g **11**, calc. yield of **11** is 2 %), **4c** (5*S*:5*R* = 67:33) (0.371 g, 24 %), overall yield of **4c** is 39 %, **10** (0.217 g, 15 %).

*(2S,4R,4aR,8S,8aR)-4,7-Dimethyl-2-(2,4,5-trimethoxyphenyl)-3,4,4a,5,8,8a-hexahydro-2H-4,8-epoxychromene (**10**)* ^{1}H NMR ($CDCl_3$): 1.36 (s, 3H, H-17); 1.52 (dd, J(4a,4e) = 13.1 Hz, J(4a,3a) = 10.6 Hz, 1H, H_a-4); 1.75 (m, all $J \leq 2.5$ Hz, 3H, H-18); 1.91 (dd, J(4e,4a) = 13.1 Hz; J(4e,3a) = 4.2 Hz, 1H, H_e-4); 2.08 (br.d, J(6,7) = 5.6 Hz; 1H, H-6); 2.37 (dddq, ^2J = 18.6 Hz, J(7,6) = 5.6 Hz, J(7,8) = 3.0 Hz, J(7,18) = 2.2 Hz, 1H, H-7); 2.53 (dm, ^2J = 18.6 Hz, 1H, H'-7); 3.77 (s, 3H, $OC^{19}H_3$); 3.84 (s, 3H, $OC^{21}H_3$); 3.85 (s, 3H, $OC^{20}H_3$); 4.25 (br.s, 1H, H-10); 4.44 (d, J(1,6) = 1.2 Hz, 1H, H-1); 5.13–5.17 (m, 1H, H-8); 5.42 (dd, J(3a,4a) = 10.6 Hz; J(3a,4e) = 4.2 Hz, 1H, H_a-3); 6.47 (s, 1H, H-13); 7.01 (s, 1H, H-16).

^{13}C NMR ($CDCl_3$): 81.07 (d, C-1); 68.21 (d, C-3); 45.69 (t, C-4); 83.33 (s, C-5); 45.66 (d, C-6); 28.20 (t, C-7); 120.66 (d, C-8); 139.86 (s, C-9); 80.21 (d, C-10); 122.15 (s,

C-11); 150.31 (s, C-12); 97.44 (d, C-13); 148.54 (s, C-14); 143.20 (s, C-15); 110.67 (d, C-16); 21.48 (q, C-17); 20.91 (q, C-18); 56.27 (q, C-19); 56.07 (q, C-20); 56.51 (q, C-21). $[\alpha]_D^{23} = -4.3$ ($c = 0.6$). HR-MS: 346.1774 (M^+, $C_{20}H_{26}O_5^+$; calc. 346.1774).

*(2S,3aR,4R,5R,6S,7aS,8R)-7a,8-Dimethyl-6-(2,4,5-trimethoxyphenyl)octahydro-2,5-methanobenzofuran-4,8-diol (**11**): the NMR spectra of **11** were recorded for the mixture with **4c*** ^{1}H NMR ($CDCl_3$): 1.30 (s, 3H, H-16); 1.58 (s, 3H, H-17); 1.90–1.97 (m, 2H, H-2, H-7); 1.96 (dd, J(4e,4a) = 13.1 Hz, J(4e,3a) = 8.2 Hz, 1H, H_e-4); 2.02–2.06 (m, 1H, H-6); 2.14 (d, ^2J = 11.4 Hz, 1H, H'-7); 2.47 (dd, J(4a,4e) = 13.1 Hz, J(4a,3a) = 10.4 Hz, 1H, H_a-4); 3.65 (dd, J(3a,4a) = 10.4 Hz, J(3a,4e) = 8.2 Hz, 1H, H_a-3); 3.77–3.79 (m, 1H, H-8); 3.78 (s, 3H, $OC^{18}H_3$); 3.80 (s, 3H, $OC^{20}H_3$); 3.85 (s, 3H, $OC^{19}H_3$); 4.35 (d, J(1,2) = 4.4 Hz, 1H, H-1); 6.47 (s, 1H, H-12); 7.20 (s, 1H, H-15).

^{13}C NMR ($CDCl_3$): 72.50 (d, C-1); 54.00 (d, C-2); 30.55 (d, C-3); 35.17 (t, C-4); 80.80 (s, C-5); 46.43 (d, C-6); 32.17 (t, C-7); 83.21 (d, C-8); 75.28 (s, C-9); 126.11 (s, C-10); 151.42 (s, C-11); 97.45 (d, C-12); 147.49 (s, C-13); 142.34 (s, C-14); 113.43 (d, C-15); 27.95 (q, C-16); 26.33 (q, C-17); 55.68 (q, C-18); 56.12 (q, C-19); 56.71 (q, C-20).

*With 2,4,6-trimethoxybenzaldehyde **3d***

The reaction of diol **2** (0.700 g) and aldehyde **3d** (0.700 g) for 1 week in the presence of clay K10 (3.0 g) gave compounds **4d** (5*S*:5*R* = 75:25) (0.214 g, 14 %).

Pharmacology

Animals

All studies were carried out on non-breeding albino mice (male) weighting 20–25 g, eight animals in each group (SPF-vivarium of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences). Mice were maintained at 22–25 °C on a 12 h light–dark cycle with food and water available ad libitum. All work with animals was performed in strict accordance with the legislation of the Russian Federation, the regulations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, and the requirements and recommendations of the Guide for the Care and Use of Laboratory Animals.

Analgesic tests

Agents were dissolved in saline containing 0.5 % Tween 80 just before use and were administered per os, 1 h before testing. Analgesic activity of test agents was assessed using acetic acid-induced writhing test and hot plate test.

In the acetic acid-induced writhing test, the pain reaction was determined by the number of abdominal convulsions, recorded from the fifth to eighth minute following the acetic acid injection (0.75 %, 0.1 mL/mouse) (Koster *et al*., 1959). The percentage of pain reaction inhibition was calculated according to the following equation: % inhibition = 100 × (A−B)/A, where A is the mean number of writhes in the control group, and B is the mean number of writhes in the test group.

In the hot plate test, animals were placed individually on a metallic plate warmed to 54 ± 0.5 °C and the time until either licking of the hind paw or jumping occurred was recorded by a stopwatch (Eddy and Leimbach, 1953).

Statistical data processing was carried out by a Statistica 6.0 program.

Acknowledgments Authors are grateful to the Presidium of Russian Academy of Sciences (Program No. 28) and Russian Foundation for Basic Research (Grant No. 10-03-00230a) for the financial support.

References

Ardashov OV, Il'ina IV, Korchagina DV, Volcho KP, Salakhutdinov NF (2007) Unusual α-hydroxyaldehyde with a cyclopentane framework from verbenol epoxide. Mendeleev Commun 17:303–305

Costa B (2007) On the pharmacological properties of Δ^9-tetrahydrocannabinol (THC). Chem Biodivers 4:1664–1677

Eddy NB, Leimbach D (1953) Studies of anastetics. J Pharmacol Exp Ther 107:385–393

Finn DP, Chapman V (2004) Cannabinoids as analgesic agents: evidence from in vivo studies. Curr Neuropharmacol 2:75–89

Husain MSY, Khan SM, Hasan MMA (2005) 2-Arylidene-4-(4-phenoxy-phenyl)but-3-en-4-olides: synthesis, reactions and biological activity. Eur J Med Chem 40:1394–1404

Il'ina IV, Volcho KP, Korchagina DV, Barkhash VA, Salakhutdinov NF (2007) Reactions of allyl alcohols of the pinane series and of their epoxides in the presence of montmorillonite clay. Helv Chim Acta 90:353–368

Il'ina IV, Volcho KP, Mikhalchenko OS, Korchagina DV, Salakhutdinov NF (2011) Reactions of verbenol epoxide with aromatic aldehydes containing hydroxy or methoxy groups in the presence of montmorillonite clay. Helv Chim Acta 94:502–513

Juniper M, Le TK, Mladsi D (2009) The epidemiology, economic burden, and pharmacological treatment of chronic low back pain in France, Germany, Italy, Spain and the UK: a literature-based review. Expert Opin Pharmacother 10:2581–2592

Koster R, Anderson M, De Beer EJ (1959) Acetic acid for analgesic screening. Fed Proc 18:412–415

Lambert DM, Fowler CJ (2005) The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. J Med Chem 48:5059–5087

Palomba M, Pau A, Boatto G, Asproni B, Auzzas L, Cerri R, Arenare L, Filippelli W, Falcone G, Motola G (2000) Anti-inflammatory and analgesic amides: new developments. Arch Pharm Med Chem 333:17–26

Syubaev RD, Mashkovskii MD, Shvarts GY, Pokryshkin VI (1986) Comparative pharmacological activity of modern nonsteroidal antiinflammatory preparations. Pharm Chem J 20:17–22

Volcho KP, Salakhutdinov NF, Barkhash VA (1999) A new way to accelerate reactions catalyzed by clays. Russ J Org Chem 35:1554–1555

Wagle S, Adhikari AV, Kumari NS (2008) Synthesis of some new 2-(3-methyl-7-substituted-2-oxoquinoxalinyl)-5-(aryl)-1,2,4-oxadiazoles as potential non-steroidal anti-inflammatory and analgesic agents. Indian J Chem 47B:439–448

White FA, Bhangoo SK, Miller RJ (2005) Chemokines: integrators of pain and inflammation. Nat Rev Drug Discov 4:834–844

Wolff R, Clar C, Lerch C (2011) Epidemiology of chronic non-malignant pain in Germany. Schmerz 25:26–44