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Review article

Synthesis and antiviral activity of maleopimaric and quinopimaric acids' derivatives

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ABSTRACT

A series of maleopimaric and quinopimaric acids' derivatives modified in the E-ring, at the carbonyl- and carboxyl-groups were synthesized and evaluated for their activity in vitro against respiratory viruses (influenza; rhinovirus; adenovirus; and SARS), papilloma virus, and hepatitis B and C viruses. The antiviral screening of levopimaric acid diene adducts derivatives was carried out with minimal effect on SARS and influenza type B viruses. Excellent antiviral activity of the ozonolysis product of maleopimaric acid and dihydroquinopimaric methyl-(2-methoxycarbonyl)ethylene amide was found toward papilloma virus (HPV-11 strain) with the selectivity index of SI 30 and 20, respectively. Methyl (2-methoxycarbonyl)ethylene-, 1β-hydroxy-5′-kaprolaktamo- and 4β-hydroxy-4α,14α-epoxy-13(15)-ene-dihydroquinopimaric acid derivatives have also shown activity against replication of HCV nucleic acid and low toxicity.

Contents

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1. Introduction

Tricyclic diterpenoids of abietane series is one of the important groups of the secondary metabolites, which are widespread in nature.[1] Their natural and synthetic derivatives exhibit a broad spectrum of biological activities for example, antimicrobial,[2] antiviral,[3,4] antimalaria,[5] antiulcer,[6] antileishmaniasis,[7] antioxidant,[8,9] and others. Abietane diterpenoids exhibited the antitumor promoting activity[10,11] and they are inhibitors of viruses reproduction[12,13] such as the herpes simplex virus type 1 (HSV-1),[14] cytomegalovirus (CMV),[15] varicella-zoster virus (VZV)[15] and Epstein-Barr virus.[16]

Abietane acids', such as abietic and levopimaric acids', readily available from an oleoresin produced by *Pinus* or commercial disproportionate rosin and easily reacts with dienophiles giving the Diels–Alder adducts in high yields.[17–19] Diterpene derivatives obtained by the diene synthesis and their synthetic derivatives have diverse pharmacological activity, including anti-inflammatory,[20–22] antiulcer,[23] anticancer[24] and antitumor.[25] Despite the variety of biological properties of this compounds family, there are few data on the antiviral activity study of their derivatives. So, for dihydroquinopimaric acid amides[26] and some frame derivatives of quinopimaric acid[27] was set a moderately antiviral activity against influenza A virus. Dihydroquinopimaric acid and its non-trivial product of dimethyldioxirane oxidation proved to be effective inhibitors of papillomavirus (HPV).[28]

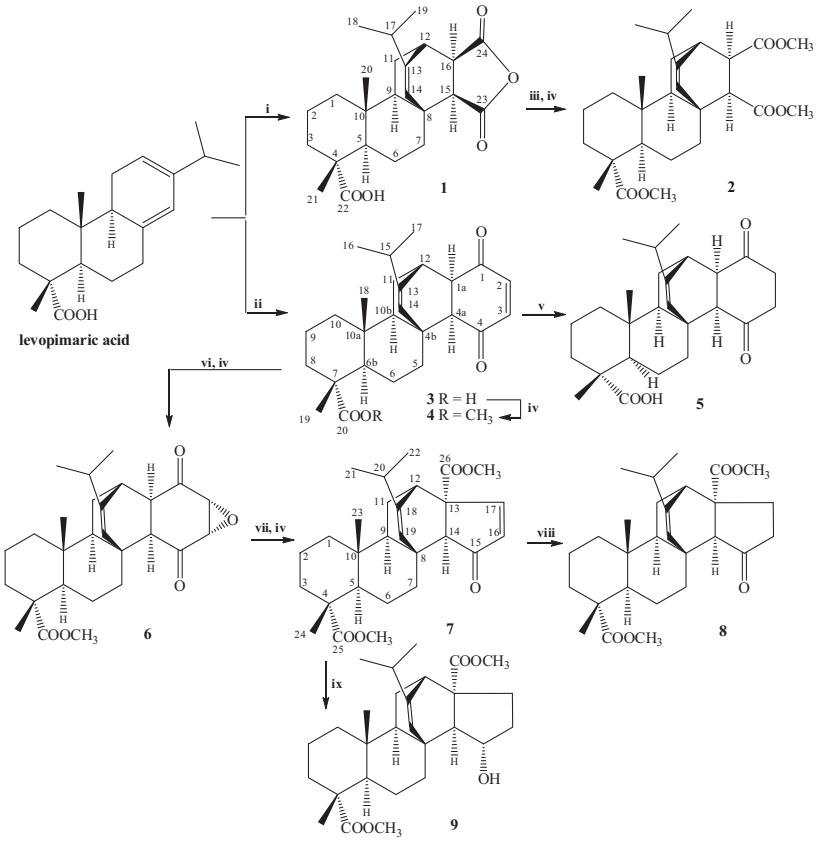
The present work is an extension of our ongoing efforts toward developing promising biologically active agents among the levopimaric acid diene adducts derivatives.[20–24,26,28–30] We have realized the chemical transformations of levopimaric acid diene adducts with maleic anhydride and *p*-benzoquinone, resulting in more than thirty derivatives of maleopimaric and quinopimaric acids' modified in the E-ring, at the carbonyl- and carboxyl-groups were synthesized and their in vitro antiviral activity was evaluated.

2. Results and discussion

2.1. Chemistry

For the synthesis of maleopimaric acid **1**[31] and quinopimaric acid **3**[32] pine resin *Pinus silvestris* containing about 25% levopimaric acid was used. Dihydroquinopimaric acid **5**,[33] trimethyl fumaropimarate **2**,[34] and methyl 2,3-epoxyquinopimarate **6**,[33] as well as dimethyl cyclopentenonepimarate **7**[33] and dimethyl cyclopentanonepimarate **8**,[33] were obtained by procedures described before (Scheme 1).

The reaction of the diester **7** with sodium borohydride in refluxing methanol showed recovery not only of the carbonyl group, but



Scheme 1. Reagents and conditions: (i) maleic anhydride, 200 °C (ii) 1,4-benzoquinone, $CHCl_3$–CH_3CN (1:4), 7 days, rt (iii) 15% KOH/MeOH, reflux, 2 h. (iv) CH_2N_2/Et_2O, EtOH, 0 °C (v) Zn/AcOH, 100 °C (vi) 35% H_2O_2, 6 M NaOH/MeOH, Et_2O, 0 °C (vii) 10% NaOH, EtOH, rt (viii) H_2, 20% Ni/Raney, MeOH (ix) $NaBH_4$, MeOH, reflux.

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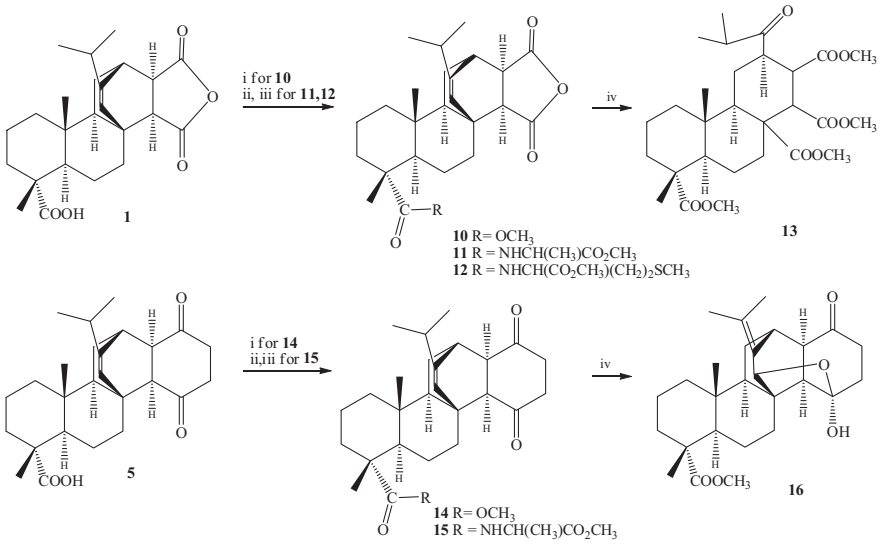
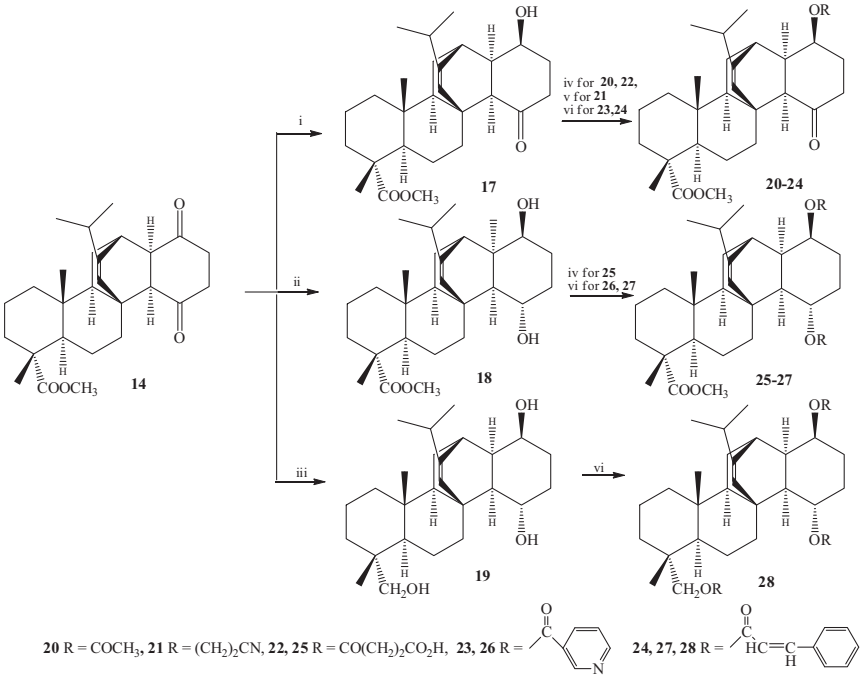


Scheme 2. Reagents and conditions: (i) CH_2N_2/Et_2O, 0 °C (ii) $(COCl)_2$, $CHCl_3$, rt (iii) $NH_2CH(CH_3)CO_2CH_3$ or $NH_2CH(CO_2CH_3)(CH_2)_2SCH_3$, Et_3N, $CHCl_3$, reflux (iv) O_3, CH_2Cl_2, 0 °C.



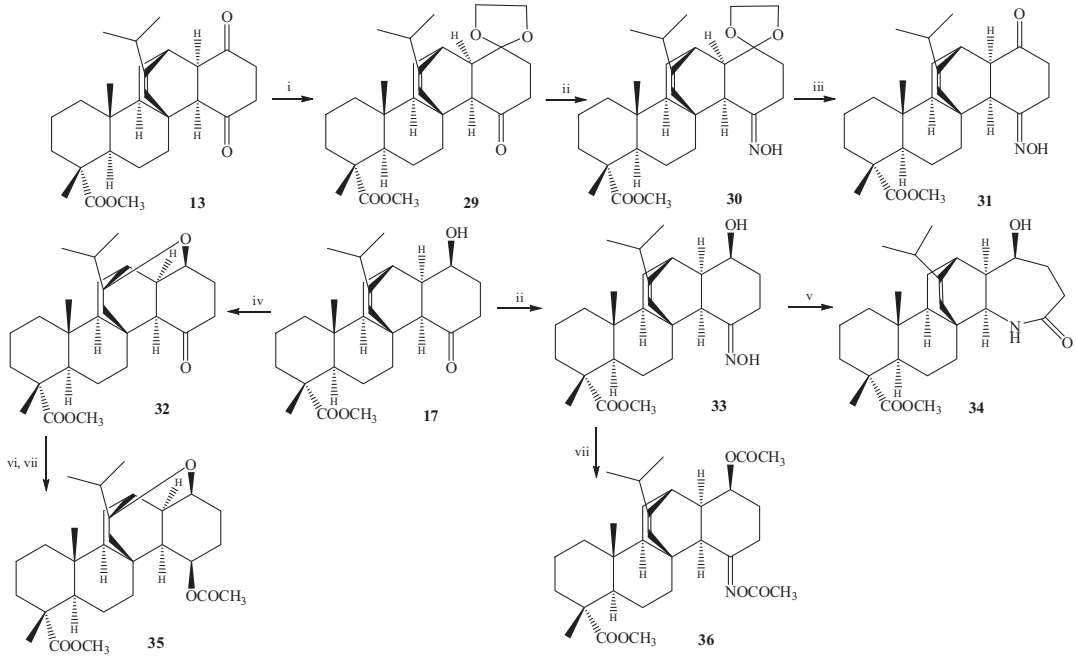
Scheme 3. Reagents and conditions: (i) $NaBH_4$, MeOH, rt (ii) $NaBH_4$, EtOH, reflux (iii) $LiAlH_4$, THF, rt (iv) $(CH_3CO)_2O$ or $(CH_2CO)_2O$, Py, 115 °C (v) $H_2C{=}CHCN$, dioxane, BTEAC, 30% KOH, rt, 2 h (vi) cinnamoyl chloride or nicotinic chloride, Py, 115 °C.

a double bond in the cyclopentane ring too. Compound **9** was obtained in 76% yield after recrystallization from methanol.

Methyl esters of quinopimaric acid **4**,[32] maleopimaric acid **10**[31] and dihydroquinopimaric acid **14**[33] were prepared by methylation of the corresponding acid with an ethereal solution of diazomethane. Functionalization of the carboxylic group of maleopimaric **1**[22] and dihydroquinopimaric **5**[26] acids with the introduction into the molecule of bioactive amino acids fragments was performed by chloride method. By reacting amides with fragments of L-alanine **11**, **15** and L-methionine **12** was obtained (Scheme 2).

Ozonolysis of the methyl esters **10** and **14** at 0 °C in methylene chloride led to the secoacid **13**[22] and hemiacetal **16**,[30] respectively (Scheme 2).

Hydroxy derivatives **17** and **18** were obtained by reducing dihydroquinopimaric acid methyl ester **14** with sodium borohydride,[35] and triol **19**—in the reduction of lithium aluminum hydride[35] (Scheme 3). The alcohol **17** is also characterized as the acetate **20**.[35] Cyanethylation of the 1β-hydroxy derivative **17** with acrylonitrile in dioxane in the presence of alkali and a phase transfer catalyst benzyltriethylammonium chloride (BTEAC) leads to compound **21**, isolated in 40% yield after column chromatography (Scheme 3).



Scheme 4. Reagents and conditions: (i) $HO(CH_2)_2OH$, benzene, 80 °C (ii) $NH_2OH{\cdot}HCl$, Py, 115 °C (iii) HCl, acetone, 53 °C (iv) HCl, MeOH, reflux (v) PCl_5, Et_2O, reflux (vi) $NaBH_4$, MeOH, rt (vii) $(CH_3CO)_2O$, Py, 115 °C.

Table 1
In vitro activities of compounds **1**–**15**, **17**–**36** against the respiratory viruses (concentration in μM)

Compound	Virus Flu A (H1N1), strain California/07/2009 cell line MDCK			Virus Flu A (H3N2), strain Brisbane/10/2007 cell line MDCK			Virus Flu A (H5N1), strain Vietnam/1203/2004H cell line MDCK			Virus Flu B, strain Florida/4/2006 cell line MDCK		
	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI
1	31	48	1.5	37	44	1.2	32	35	1.1	27	36	1.3
2	3.8	9.4	2.5	>7.6	7.6	0	–	–	–	–	–	–
3	32	33	1	>27	27	0	>30	30	0	>20	20	0
4	>5.5	5.5	0	3.2	3.9	1.2	>2.9	2.9	0	>2.9	2.9	0
5	–	–	–	32	36	1.1	–	–	–	8.6	34	4
6	29	34	1.2	>23	23	0	3.2	3.3	1	3.2	3.3	1
7	3.1	3.8	1.2	3.2	3.9	1.2	>13	13	0	>25	25	0
8	31	34	1.1	32	33	1	32	32	1	>31	31	0
9	32	35	1,1	>24	24	0	3.2	3.2	1	>3.1	3.1	0
10	31	35	1.1	32	33	1	21	32	1.5	>8.7	8.7	0
11	32	34	1.1	32	33	1	32	33	1	32	33	1
12	31	34	1.1	>30	30	0	32	32	1	32	32	1
13	32	33	1	32	33	1	3.2	3.3	1	3.2	3.5	1.1
14	>100	>100	0	>100	>100	0	>100	>100	0	32	>100	>3.1
15	–	–	–	–	–	–	31	33	1.1	22	33	1.5
17	>100	>100	0	>100	>100	0	>100	>100	0	>100	>100	0
18	48	93	1.9	>64	64	0	>100	>100	0	>100	>100	0
19	>11	11	0	32	33	1	>29	29	0	>31	31	0
20	>100	>100	0	>100	>100	0	>100	>100	0	>100	>100	0
21	>100	>100	0	34	68	2	>31	31	0	>52	52	0
22	>100	>100	0	>100	>100	0	88	>100	>1.1	>100	>100	0
23	32	35	1.1	32	33	1	3.2	3.2	1	3.2	3.2	1
24	31	35	1.1	>17	17	0	>9	9	0	10	24	2.4
25	–	–	–	–	–	–	3.2	3.2	1	3.1	3.4	1.1
26	29	32	1,1	>4.9	4.9	0	>8.5	8.5	0	>11	11	0
27	>17	17	0	>4	4	0	>8.2	8.2	0	>9.3	9.3	0
28	31	35	1.1	31	33	1,1	>100	>100	0	>46	46	0
29	27	34	1.3	>26	26	0	>24	24	0	>27	27	0
30	>2.9	2.9	0	3.2	3.3	1	3.2	3.3	1	3.2	3.2	1
31	31	31	1	>13	13	0	>1.7	1,7	0	>2.4	2.4	0
32	>100	>100	0	>100	>100	0	>100	>100	0	>100	>100	0
33	3.2	3.5	1.1	>1.6	1.6	0	>1.2	1.2	0	>1.2	1.2	0
34	32	33	1	>28	28	0	>28	28	0	24	35	1.5
35	3.2	3.5	1.1	>5	5	0	>12	12	0	>10	10	0
36	>4.8	4.8	0	3.1	3.3	1.1	>2.5	2.5	0	>2.8	2.8	0

EC_{50}, virus inhibitory concentration, 50% endpoint; CC_{50}, cell inhibitory concentration 50% endpoint; SI: CC_{50}/EC_{50}.

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Table 2
In vitro activities of compounds **13**, **15**, **25**, **30**, **34** against other viruses (concentration in μM)

Compound	Virus rhinovirus type 2 strain HGP cell line MDCK			Virus adeno strain 65089/ Chicago cell line A-549			Virus SARS strain Urbani cell line vero 76			Virus HPV strain HPV-11		
	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI
13	32	32	1	32	32	1	>30	30	0	>50	<50	30[a]
15	>25	25	0	>47	47	0	30	32	1.1	<25	<25	20[a]
25	31	33	1.1	>10	10	0	3.2	3.6	1.1	>50	<50	N/A[b]
30	3.2	3.2	1	>9.8	9.8	0	>2.4	2.4	0	>50	<50	N/A[b]
34	>28	28	0	>29	29	0	6.3	32	5.1	<50	<50	N/A[b]

EC_{50}, virus inhibitory concentration, 50% endpoint; CC_{50}, cell inhibitory concentration 50% endpoint.
SI: CC_{50}/EC_{50}.
[a] Excellent anti-viral activity at single dose tested; some cellular cytotoxicity.
[b] Cellular cytotoxicity at dilution point tested—retest needed.

Table 3
In vitro primary anti-HBV activity of compounds **5**, **13**, **15**, **16**, **25**, **30**, **34** (concentration in μM)

Compound	Virus HBV, VIR assay		
	CC_{50}	EC_{50}	EC_{90}
5	62	>10	>10
13	189	>10	>10
15	86	>10	>10
16	59	>10	>10
25	21	>10	>10
30	20	>10	>10
34	58	>10	>10
Control drug 3TC, EC_{50} 2399			

EC_{50}, virus inhibitory concentration, 50% endpoint; EC_{90}, virus inhibitory concentration, 90% endpoint.
CC_{50}, cell inhibitory concentration 50% endpoint; VIR data are based on extracellular virion HBV DNA.

Functionalization of the hydroxyl groups of the compounds **17**–**19** was also carried out by introducing into the diterpenoids structure of succinic anhydride (hemisuccinates **22**, **25**),[35] and nicotinic acid chloride (nicotinates **23**, **26**)[35] and cinnamoyl chloride (cinnamates **24**,[35] **27**, **28**) (Scheme 3). Acylation of compounds **18** and **19** was carried by the acid chloride, the compounds **27** и **28** were obtained after purification by column chromatography in a 61% and 45% yield respectively.

To get nitrogen-containing derivatives and dihydroquinopimaric acid derivatives modifications of the carbonyl function at position C-4 we used the reaction of ketones **17**[35] and **29**[32] with hydroxylamine hydrochloride (Scheme 4). Individual *E*-oximes **30** and **33** were obtained by recrystallization of the mixture of *E*/*Z* isomers from MeOH.[22,36] Under acidic conditions, the removal ethyleneketal protection of the compound **30** to form the *E*-oxime **31** in 80% yield after purification by column chromatography occurs. Hemiketal **32** is readily formed from compound **17** by refluxing in acidic conditions,[37] its reaction with sodium borohydride and subsequent acylation with acetic anhydride resulting in a yield of 79% to the compound **35**. Caprolactam **34** was synthesized by reacting the *E*-oxime **33** with PCl_5 in ether.[22] Acetate **36** was synthesized by reacting compound **33** with acetic anhydride.[22]

Thus, we have performed chemical modifications of the quinopimaric acid comprising of double bond reduction at C-2 and C-3 and double bond epoxidation with the subsequent contraction of cycle E to pentacyclic. The reaction of dimethyl cyclopentenonepimarate 7 and sodium borohydride proceeds with the reduction of the keto group and double bond in the pentacyclic cycle. The functionalization of carboxyl-groups of levopimaric acid diene adducts (maleopimaric and dihyroquinopimaric acids) by chloride method led to amides with fragments of the biogenic amino acids. By acylation of the dihydroquinonopimaric acid hydroxyderivatives mono-, di- and tri-acylates were prepared. The synthesis of nitrogen-containing diterpenoids was carried out.

2.2. Evaluation of antiviral activity

The synthesized compounds **1**–**36** were evaluated in vitro for their inhibitory activity against respiratory viruses (influenza type A strains (H1N1), (H3N2), (H5N1); influenza type B; rhinovirus; adenovirus; and SARS), papilloma virus (HPV), and hepatitis B virus (HBV) and C (HCV) according to the agreement with National Institute of Allergy and Infectious Diseases (NIAID), using standard protocols, published on the website NIAID-AACF.[38]

The results of evaluation of the antiviral activity of compounds **1**–**15**, **17**–**36** against influenza viruses types A and B are shown in Table 1. The results of the antiviral activity of compounds **13**, **15**, **25**, **30**, **34** against rhinovirus type 2, adenovirus, SARS and papillomavirus HPV-11 are presented in Table 2. Compounds **1**–**15**, **17**–**36**

Table 4
In vitro primary anti-HCV activity of compounds **5**, **13**, **15**, **16**, **25**, **30**, **34** (concentration in μM)

Compound	HCV dose–response assay, cell line Huh7 ET		
	Antiviral activity HCV RNA % control	Toxicity β-actin RNA % control	Selectivity index (SI) toxicity/antiviral activity
5	0	88	<1[a]
13	0.6	88.1	<1[b]
15	60.8	54	>1[a]
16	68.4	151.2	>1[b]
25	96.6	6.4	<1[c]
30	97.9	0.2	<1[c]
34	97.3	102.2	>1[d]
Positive control Interferon-α (2 IU/mL)	96.7	99.1	>1

[a] Not active against HCV replicon.
[b] Weakly active against replicon.
[c] Cytotoxic.
[d] Active against replicon.

showed a minimal inhibitory effect against four strains of influenza virus with selectivity index from 0 to 4. Compound **5** shown only minor activity against influenza type B strain with a 50% effective concentration (EC_{50}) 8.6 μM and the selectivity index (SI) 4.0 and the compound **34**—against strain SARS (EC_{50} = 6.3 μM, SI = 5.1).

At the same time, the compounds **13** and **15** showed excellent activity against papillomavirus (HPV-11 strain). For the compound **13** the selectivity index SI was 30, for the compound **15** SI = 20 (Table 2).

The results of biological screening for compounds **5**, **13**, **15**, **16**, **25**, **30**, **34** against hepatitis B and C are shown in Tables 3 and 4, respectively. It is established that the test compounds at 10 μM concentration have no effect on DNA replication of hepatitis B virus (HBV), but the compound **13** has low toxicity ($CC_{50} \geqslant 100$ μM). Compounds **25**, **30** showed activity on the replication of the nucleic acid HCV, but were proved toxic in the investigated concentration. Percent at inhibition of nucleic acid replication of hepatitis C virus (HCV) for these compounds in a concentration of 20 μM was 96.6% and 97.9%, and the cytotoxicity (percentage of live cells)—6.4% and 0.2%, the selectivity index was SI < 1. Compounds **15**, **16** и **34** showed a low toxicity and a pronounced inhibitory effect on the replication of the nucleic acid HCV (60.8, 68.4, 97.3% respectively, SI > 1), and can be recommended for further study of the activity of different compounds concentrations.

3. Conclusions

Thus, a number of maleopimaric and quinopimaric derivatives modified in carboxyl and carbonyl groups and ring E are synthesized and evaluated as potential antiviral agents. We observed the following structure–activity relationship: the modifications of levopimaric acid diene adducts does not give rise to activity against viral respiratory infections. Only dihydroquinopimaric acid and 5′-caprolactam—a product of Beckmann rearrangement of 1β-dihydroquinopimaric acid monooxime—have minimal activity against influenza virus type B and SARS respectively. At the same time, the functionalization of the carboxyl group at C-20 dihydroquinopimaric acid with introduction in diterpenoid structure of L-alanine fragment leads to high activity against papilloma virus and hepatitis C virus. The functionalization of the maleopimaric acid derivatives carboxyl group does not have an antiviral effect. The products of maleopimaric and dihydroquinopimaric acids oxidation reactions and the mentioned 1β-hydroxy-5′-caprolactam has also show activity against papilloma and hepatitis C viruses. The received results encourage us to continue research on the synthesis of new derivatives levopimaric acid to obtain new biologically active compounds.

4. Experimental

4.1. Materials and methods

The ^1H and ^{13}C NMR spectra (δ, ppm; J, Hz) were recorded on a Bruker AM-300 (Germany) spectrometer (300.13 and 75.5 MHz, respectively) in a $CDCl_3$ solution using tetramethylsilane as the internal standard. Melting points were determined on a Boetius apparatus. Optical rotations were measured on a Perkin Elmer MC polarimeter (Switzerland) in a 1 dm tube. TLC analysis was carried out on Sorbfil plates (Sorbpolimer, Russia) using chloroform–ethyl acetate (40:3) as the solvent system, detection with a 10% solution of sulfuric acid (2–3 min at 100–120 °C).

For the synthesis of maleopimaric acid **1**[31] and quinopimaric acid **3**[32] pine resin *Pinus silvestris* containing about 25% levopimaric acid was used. Compounds **2**,[34] **4**,[32] **5–8**,[33] **10**,[31] **11**,[22] **12**,[26] **13**,[22] **14**,[33] **15**,[22] **16**,[30] **17–20**,[35] **22–26**,[35] **29**,[32] **30**,[36] **32**,[37] **33**,[22] **34**,[37] **36**[22] were obtained according to the methods described previously.

4.2. Chemistry

4.2.1. Dimethyl 15-hydroxy-18-isopropyl-4,10-dimethyltetradecahydro-8,12-ethenocyclopenta[*a*]phenanthrene-4,13(1*H*)-dicarboxylate (dimethyl 15-hydroxy-cyclopentanonepimarate) 9

A solution of 1 mmol (0.45 g) of the compound **7** in 10 ml MeOH was added portionwise 5 mmol (0.2 g) $NaBH_4$. The reaction mass was refluxed for 2 h, poured into 20 ml of 5% HCl and the precipitate was filtered off, washed with water, dried and recrystallized from methanol. Yield 0.35 g (76%), mp 204–205 °C, $[\alpha]_D^{20}$ +31 (*c* 0.04, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 0.53 (s, 3H, CH_3), 0.92 and 0.93 (both d, 6H, *J* = 6.8 Hz, $2CH_3$), 1.00–1.10 (m, 3H), 1.15 (s, 3H, CH_3), 1.30–1.59 (m, 6H), 1.78–1.90 (m, 6H), 2.11 (sept, 1H, *J* = 6.8 Hz, *J* = 1.2 Hz, H-20), 2.30–2.51 (m, 3H), 2.55 (br s, 1H, H-12), 2.61 (br s, 1H, H-14), 3.42 (br s, 1H, OH), 3.60 and 3.65 (both s, 6H, $2CH_3$), 4.20 (br s, 1H, H-15), 5.69 (br s, 1H, H-19). ^{13}C NMR (75.5 MHz, $CDCl_3$): 15.3, 16.6, 16.9, 20.2, 20.8, 21.7, 26.3, 33.4, 33.6, 34.2 (C-17), 34.3 (C-16), 36.6, 37.1, 37.9, 39.8, 40.6, 47.0, 49.3, 51.7, 52.0, 53.4, 60.0, 60.1, 74.9 (C-15), 127.2 (C-19), 147.7 (C-18), 177.48 (C-26), 179.2 (C-25). Anal. calcd for: $C_{28}H_{42}O_5$ C, 73.3; H, 9.2. Found: C, 73.1; H, 9.7.

4.2.2. Methyl 1-(2-cyanoethoxy)-13-isopropyl-7,10a-dimethyl-4-oxohexadecahydro-1*H*-4b,12-ethenochrysene-7-carboxylate (methyl 1-cyanoethyl-dihydroquinopimarate) 21

A mixture of 1 mmol (0.43 g) of the compound **17**, 21.5 mmol (1.4 ml) of acrylonitrile, 0.5 mmol (0.11 g) of BTEAC, and 0.5 ml of 30% KOH in 15 mL of dioxane was stirred for 2 h at room temperature. The mixture was poured into a mixture of ice with HCl, the precipitate was filtered off, washed with water until neutral washings, air dried, and extracted with methylene chloride (3 × 80 mL) with heating, the solution was filtered, and the filtrate was evaporated. The residue was purified by Al_2O_3 column chromatography with methylene chloride as the eluent. Yield 0.19 g (40%), mp 180–182 °C, $[\alpha]_D^{20}$ +75 (*c* 0.01, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 0.55 (s, 3H, CH_3), 0.81–0.95 (m, 2H), 1.07 and 1.12 (both d, 6H, *J* = 6.9 Hz, $2CH_3$), 1.15 (s, 3H, CH_3), 1.35–1.90 (m, 15H), 2.20–2.39 (m, 6H), 2.78–3.09 (m, 2H), 3.51 (m, 2H, CH_2), 3.69 (s, 1H, H-21), 3.80 (m, 2H, CH_2), 5.51 (br s, 1H, H-14). ^{13}C NMR (75.5 MHz, $CDCl_3$): 15.9, 16.8, 17.0, 19.3 (CH_2), 19.6, 21.4, 21.8, 23.9, 29.9, 32.9, 34.4, 35.2, 36.3, 36.6, 37.9, 38.1, 40.5, 44.9, 47.2, 49.4, 51.9, 55.3, 62.2 (CH_2), 63.1, 76.2 (C-1), 117.8 (CN), 123.5 (C-14), 148.4 (C-13), 179.3 (C-20), 212.9 (C-4). Anal. calcd for: $C_{30}H_{43}NO_4$ C, 74.8; H, 9.0; N, 2.9. Found: C, 75.1; H, 9.5; N, 2.8.

4.2.3. Methyl 13-isopropyl-7,10a-dimethyl-1-{[(2*E*)-3-phenylprop-2-enoyl]oxy}-4-{[(2*Z*)-3-phenylprop-2-enoyl]oxy}hexadecahydro-1*H*-4b,12-ethenochrysene-7-carboxylate (methyl 1,4–dicynnamoyl-dihydroquinopimarate) 27

A solution of 1 mmol (0.43 g) of compound **18** in 15 ml of anhydrous pyridine was added 4 mmol (0.67 g) of cinnamoyl chloride and refluxed for 6 h. The mixture was poured into 20 ml of a 5% solution of HCl, and the precipitate was filtered, washed and air dried. The residue was purified by Al_2O_3 column chromatography with methylene chloride as the eluent. Yield 0.42 g (61%), mp 105–107 °C, $[\alpha]_D^{20}$ +25 (*c* 0.01, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 0.63 (s, 3H, CH_3), 0.89–1.08 (m, 2H), 1.09 and 1.13 (both d, 6H, *J* = 6.9 Hz, $2CH_3$), 1.15 (s, 3H, CH_3), 1.19–1.79 (m, 12H), 1.91–2.50 (m, 6H), 2.78 (dt, 1H, J_1 = 4.4 Hz, J_2 = 3.7 Hz, J_3 = 13.4 Hz, H-1a), 3.20 (br s, 1H, H-12), 3.67 (s, 3H, H-21), 5.10 (m, 2H, H-1, H-4), 5.61 (br s, 1H, H-14), 6.41 and 6.83 (both d, 2H, *J* = 15.9 Hz, 2CH), 7.26–7.81 (m, 12H, H-Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): 15.7, 16.8, 17.0, 19.6, 20.0, 21.3, 30.2, 31.9, 32.9, 35.2, 35.5, 35.9, 36.6, 37.8, 38.2, 40.2, 45.1, 47.1, 49.4, 51.8, 54.6, 61.8, 74.3 (C-4), 77.6 (C-1), 117.9 (CH), 118.7 (CH), 124.3 (C-14), 127.6 (C-Ar), 128.0 (C-Ar),

128.1 (C-Ar), 128.3 (C-Ar), 128.6 (C-Ar), 128.9 (C-Ar), 129.3 (C-Ar), 129.9 (C-Ar), 130.3 (C-Ar), 131.0 (C-Ar), 142.1 (C-Ar), 142.9 (C-Ar), 145.2 (CH), 145.6 (CH), 147.8 (C-13), 170.0 (OC═O), 171.9 (OC═O), 179.2 (C-20). Anal. calcd for: $C_{45}H_{54}O_6$ C, 78.2; H, 7.9. Found: C, 77.8; H, 7.6.

4.2.4. 13-Isopropyl-7,10a-dimethyl-7-({[(2*E*)-3-phenylprop-2-enoyl]oxy}methyl)hexa-decahydro-1*H*-4b,12-ethenochrysene-1,4-diyl (2*E*,2′*Z*)bis(3-phenylacrylate) (1,4,20-tricynnamoyl-dihydroquinopimarate) 28

A solution of 1 mmol (0.40 g) of compound **19** in 15 ml of anhydrous pyridine was added 6 mmol (1.00 g) of cinnamoyl chloride and refluxed for 12 h. The mixture was poured into 20 ml of a 5% solution of HCl, and the precipitate was filtered, washed and air dried. The residue was purified by Al_2O_3 column chromatography with methylene chloride as the eluent. Yield 0.36 g (45%), mp 125–127 °C, $[\alpha]_D^{20}$ +15 (*c* 0.01, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 0.63 (s, 3H, CH_3), 0.89–1.08 (m, 2H), 1.09 and 1.13 (both d, 6H, *J* = 6.9 Hz, $2CH_3$), 1.15 (s, 3H, CH_3), 1.19–1.79 (m, 12H), 1.91–2.50 (m, 7H), 3.20 (br s, 1H, H-12), 5.03 (m, 2H, CH_2), 5.10 (m, 2H, H-1, H-4), 5.61 (br s, 1H, H-14), 6.41 and 6.53 and 6.82 (all d, 3H, *J* = 15.9 Hz, 3CH), 7.26–7.81 (m, 18H, H-Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): 15.0, 16.8, 17.0, 19.3, 20.0, 22.9, 30.7, 31.9, 32.5, 35.2, 35.8, 36.1, 37.8, 38.1, 40.7, 45.1, 47.1, 49.3, 51.8, 54.2, 61.8, 73.1 (C-20), 74.3 (C-4), 77.6 (C-1), 117.6 (CH), 117.9 (CH), 118.7 (CH), 124.3 (C-14), 127.5 (C-Ar), 127.8 (C-Ar), 127.9 (C-Ar), 128.0 (C-Ar), 128.1 (C-Ar), 128.3 (C-Ar), 128.4 (C-Ar), 128.6 (C-Ar), 128.9 (C-Ar), 129.2 (C-Ar), 129.5 (C-Ar), 129.8 (C-Ar), 130.0 (C-Ar), 130.5 (C-Ar), 131.0 (C-Ar), 142.1 (C-Ar), 142.5 (C-Ar), 142.9 (C-Ar), 145.0 (CH), 145.2 (CH), 145.6 (CH), 147.8 (C-13), 170.0 (OC═O), 170.2 (OC═O), 171.9 (OC═O). Anal. calcd for: $C_{53}H_{60}O_6$ C, 80.3; H, 7.6. Found: C, 79.8; H, 7.6.

4.2.5. Methyl (4*E*)-4-(hydroxyimino)-13-isopropyl-7,10a-dimethyl-1-oxohexadecahydro-1*H*-4b,12-ethenochrysene-7-carboxylate (methyl 4-oxime-dihydroquinopimarate) 31

A solution of 1 mmol (0.47 g) of the compound **30** in 20 ml of acetone was added 1 mL HCl and the reaction mixture was refluxed for 3 h. The mixture was poured into a 20 ml of a 5% solution of $NaHCO_3$, the precipitate was filtered, washed with water and air dried. The residue was purified by Al_2O_3 column chromatography with chloroform as the eluent. Yield 0.38 g (80%), mp 121–123 °C, $[\alpha]_D^{20}$ +45.5° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 0.56 (s, 3H, CH_3), 0.95 and 0.99 (both d, 6H, *J* = 6.9 Hz, $2CH_3$), 1.13 (s, 3H, CH_3), 1.30–1.79 (m, 12H), 1.80–2.78 (m, 9H), 3.05 (br s, 1H, H-12), 3.65 (s, 3H, H-21), 5.51 (br s, 1H, H-14), 6.05 (d, 1H, *J* = 4.2 Hz, NOH). ^{13}C NMR (75.5 MHz, $CDCl_3$): 16.1, 16.5, 16.9, 18.6, 19.6, 20.6, 21.8, 27.3, 32.6, 35.4, 36.6, 37.4, 37.8, 38.5, 39.3, 40.9, 47.1, 49.6, 51.6, 52.1, 54.6, 56.4, 126.0 (C-14), 147.9 (C-13), 158.5 (C-4), 179.1 (C-20), 211.6 (C-1). Anal. calcd for: $C_{27}H_{39}NO_4$ C, 73.4; H, 8.9; N, 3.2. Found: C, 73.0; H, 7.6; N, 2.8.

4.2.6. Methyl 1-(acetyloxy)-5-isopropyl-6b,10-dimethyloctadecahydro-5,12a-methanochryseno[1,12-*bc*]furan-10-carboxylate (methyl 1,13-epoxy-4-acetoxy-dihydroquinopimarate) 35

A solution of 1 mmol (0.45 g) of the compound **7** in 10 ml MeOH was added portionwise 5 mmol (0.2 g) $NaBH_4$. The reaction mass was refluxed for 2 h, poured into 20 ml of 5% HCl and the precipitate was filtered off, washed with water and air dried. The residue was dissolved in 10 ml of pyridine was added 0.3 ml of acetic anhydride and allowed to stand for 15 h. The reaction mixture was poured into 50 mL of 5% HCl solution, cooled to 0 °C, the precipitate was filtered off, washed with water, dried and recrystallized from methanol. Yield 0.37 g (79%), mp 125–128 °C, $[\alpha]_D^{20}$ +25.5° (*c* 0.01, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 0.84 (s, 3H, CH_3), 1.02 and 1.07 (both d, 6H, *J* = 7.1 Hz, $2CH_3$), 1.28 (s, 3H, CH_3), 1.32–1.76 (m, 17H), 2.01 (s, 3H, OCH_3), 2.10–2.24 (m, 6H), 3.05 (br s, 1H, H-12), 3.65 (s, 3H, H-21), 4.05 (br s, 1H, H-1), 4. 50 (br s, 1H, H-4). ^{13}C NMR (75.5 MHz, $CDCl_3$): 15.5, 16.6, 16.9, 17.0, 19.2, 20.7, 21.4 (CH_3), 21.8, 26.7, 33.7, 35.0, 36.0, 36.7, 37.3, 38.5, 38.8, 39.2, 41.0 (C-14), 41.1, 47.1, 50.0, 51.1 (C-21), 51.6, 55.1, 70.9 (C-4), 75.8 (C-1), 86.6 (C-13), 170.4 (OAc), 177.8 (C-20). Anal. calcd for: $C_{29}H_{44}O_5$ C, 73.7; H, 9.4. Found: C, 73.3; H, 8.6.

4.3. Evaluation of antiviral activity

The National Institute of Allergy and Infectious Diseases (NIAID) established the AACF under a contract with Southern Research Institute. The NIAID, through the AACF, provides free and confidential services for suppliers, who are interested in submitting compounds to be evaluated for antiviral activity. Tested compounds were delivered in standard DMSO solutions. The methods applied for the different assays can be found at the URL via the internet at http://niaid-aacf.org.

Primary antiviral assay was performed on a respiratory viruses panel (Flu A (H1N1), Flu A (H3N2), Flu A (H5N1), Flu B, rhinovirus type 2, adenovirus and SARS).[39]

Primary anti-HPV assay was determined using standard AACF screening assay protocols.[40]

Primary Anti-HBV assay and toxicity against confluent 2.2.15 cells were determined by the published procedure of Korba and Gerin.[41]

Primary HCV RNA replicon assay was examined by the effect of drugs added in triplicate at a single high-test concentration of 20 μM on HCV RNA-derived LUC activity and cytotoxicity.[42]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.09.006.

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