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Synthesis of new heterocyclic dehydroabietylamine derivatives and their biological activity

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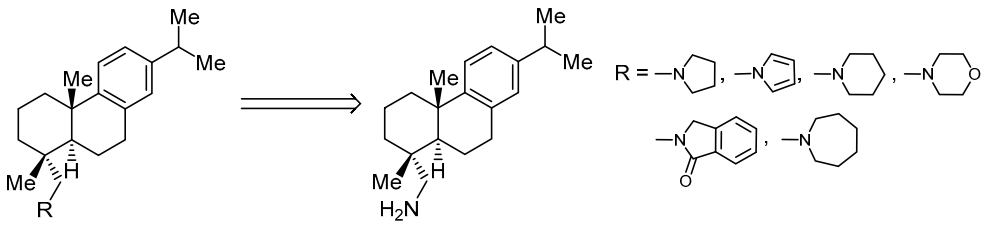


New derivatives of dehydroabietylamine were obtained by transformation of primary amino group to pyrrolidine, piperidine, azepane, morpholine, isoindoline, and pyrrole heterocycles. The obtained compounds were tested for antiviral activity against influenza A/California/07/09 (H1N1)pdm09 strain, as well as the activity against some types of bacteria and fungi.

Keywords: dehydroabietylamine, antibacterial activity, antiviral activity, natural compounds.

One of the most effective current approaches to the synthesis of biologically active compounds is functionalization of already known bioactive molecules with various pharmacophoric groups, such as heterocyclic systems. Of particular interest in this respect are nitrogen-containing heterocycles, which are found in the molecular structures of many classes of biologically active compounds, such as alkaloids.

Our work was devoted to the study of dehydroabietylamine (**1**, DHAAm) – a derivative of dehydroabietic acid (**2**, DHAAc) (Fig. 1), which is found in the resins of coniferous plants of *Pinus, Picea, Abies,* and *Larix* geni. A particularly high content of DHAAc (71%) is found in the resin of *Picea obovata*.[1]

Dehydroabietylamine (**1**) is also known as leelamine and belongs to the list of compounds that are important building blocks in medicinal chemistry.[2] Azomethines (Schiff bases), obtained on the basis of DHAAm (**1**) and benzaldehyde derivatives, have shown bactericidal activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*,[3] as well as antiproliferative activity against lung cancer cell lines A549, NCI-H460 and colon cancer cell line SW620.[4] It was also found that dehydroabietylamine hydrochloride exhibited high cytotoxicity against some cancer cell lines.[5] It was shown that DHAAm (**1**) can effectively and selectively kill melanoma cells by lowering the extent of cell proliferation and inducing apoptosis.[6] The cytotoxic effect and mechanism of apoptosis in the presence of derivative obtained from DHAAm (**1**) and *p*-trifluoromethylbenzaldehyde was studied on carcinoma cell line SMMC-7721.[7] Studies have been published in recent years on the antiprotozoal activity

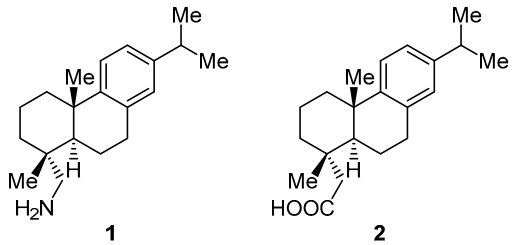


Figure 1. The structures of DHAAm (**1**) and DHAAc (**2**).

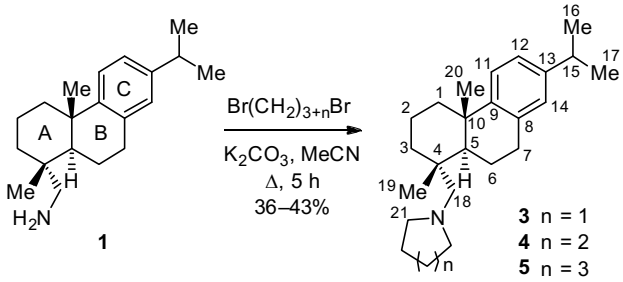
of DHAAm amides[8] against *Leishmania donovani* and *Trypanosoma cruzi* and the activity of amides containing substituents in the ring system.[9]

Previously various amide and imino derivatives with substituents in the aromatic ring were described. Several *N*-alkyl derivatives have also been prepared,[10] A few compounds have been decorated with heterocyclic substituents (phthalimide and polychlorophthalimide).[11,12] The goal of this work was to synthesize similar compounds containing aliphatic and aromatic nitrogen heterocycles.

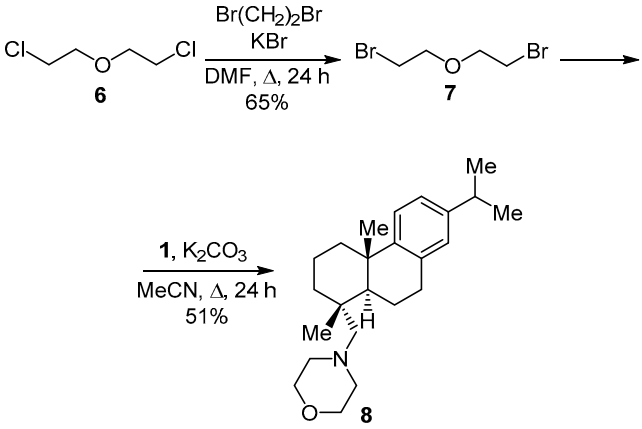
In order to obtain aliphatic five-, six-, and seven-membered nitrogen heterocycles, cyclization reactions of primary amines were performed with aliphatic dihaloalkanes having various carbon chain length, using a method that was proposed earlier.[13] It was shown that the reaction of dehydroabietylamine (**1**) with 1,4-dibromobutane produced compound **3** containing a pyrrolidine moiety, the reaction of DHAAm (**1**) with 1,5-dibromopentane gave compound **4** containing a piperidine moiety, while the reaction of DHAAm (**1**) with 1,6-dibromohexane yielded the azepane derivative **5**. The reactions proceeded during refluxing in acetonitrile with the addition of potassium carbonate (Scheme 1). According to the results of GC-MS analysis, the reaction mixture after refluxing for 5 h did not contain the starting amine **1**, and the conversion was complete. The yields of products after purification by column chromatography were in the range of 36–43%.

We have shown that performing the reaction with microwave activation at 110°C allowed to substantially shorten the reaction time from 5 to 1 h, but increased the formation of intractable products and lowered the yield to 20%. In order to obtain the morpholine moiety, a reaction was attempted with bis(2-chloroethyl) ether (**6**). The conversion degree under these conditions was low (5%), therefore the chlorine atoms in bis(2-chloroethyl) ether were replaced with bromine atoms in order to improve the reactivity. When bis(2-chloroethyl) ether (**6**) was refluxed in dibromoethane with the addition of KBr, bis(2-bromoethyl) ether (**7**) was obtained and was further used in a cyclization reaction with dehydroabietylamine (**1**), forming compound **8** (Scheme 2). The yield after purification by column chromatography was 51%.

Scheme 1



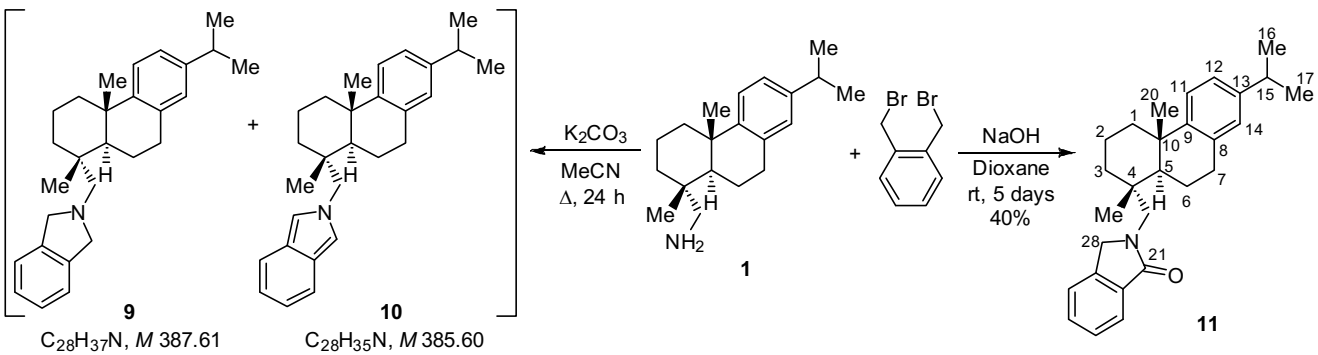
Scheme 2



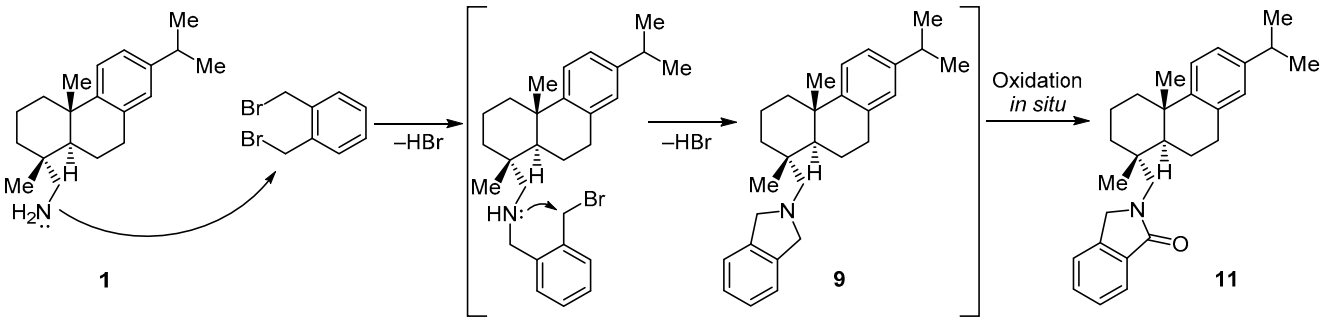
Analogously to previous transformations, it was expected that the reaction of *o*-dibromoxylene with dehydroabietylamine (**1**) under the same conditions (refluxing in acetonitrile) should lead to the formation of compound **9**. However, according to GC-MS data, besides product **9** the reaction mixture also contained a compound that gave a molecular peak with *m*/*z* 385 $[M]^+$. It is possible that performing the reaction under these conditions resulted in the formation of isoindole **10** (Scheme 3). Compounds **9** and **10** could not be isolated from the reaction mixture and their structures were not proved.

Performing this reaction under conditions proposed in the literature[14] led to compound **11** as the main product, which contained an isoindoline ring system, and was isolated during this study in 40% yield by using column chromatography (Scheme 3).

Scheme 3



Scheme 4

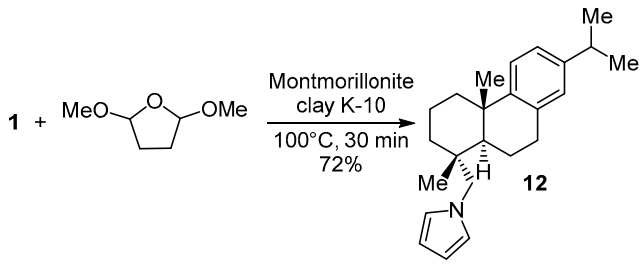


The structure of the obtained isoindolinone **11** was unequivocally confirmed by its physicochemical data. Thus, characteristic signals were observed in NMR spectra for the isoindolinone substituent: ^{1}H NMR signals of the protons bonded to the C(28) carbon atom were observed as AB doublets (4.45 and 4.49 ppm, J_2 = 16.8 Hz) and four signals of the unsymmetrical aromatic system were also observed. ^{13}C NMR spectrum featured the signal of the C(21) atom at 169.9 ppm, corresponding to the amide carbonyl group. The chromato-mass spectrum contained a molecular ion peak with *m*/*z* 401 $[M]^+$, corresponding to the molecular formula of product **11**.

There are no previous reports available about the preparation of isoindolinones in a single step from a primary amine and *o*-dibromoxylene. Various approaches exist for the synthesis of this type of compounds: the simplest and most effective method is selective mono-reduction of readily available phthalimides. The appropriate phthalimides have been catalytically reduced with hydrogen under pressure,[15] by using stoichiometric amounts of tin[16] or zinc[17] in the presence of an acid, or by using polymethylhydrosiloxane with a catalytic amount of fluoride ions.[18] Another approach to the preparation of isoindolinone moiety is the oxidation of isoindolines. Electrochemical oxidation of isoindolines has been performed in pyridine,[19] but this method was not selective and led to the formation of isoindolinones as a mixture with phthalimides. We proposed a mechanism for the reaction of DHAAm (**1**) and *o*-dibromoxylene (Scheme 4), including the initial formation of isoindoline moiety, followed by its oxidation in the reaction mixture. It can be assumed that the isoindolinone compound **11** was formed by oxidation with peroxides derived from dioxane. This possibility was also confirmed by the fact that the amount of product **11** in the reaction mixture increased during prolonged storage, and it was not formed when dioxane was additionally purified from peroxides on alumina.

The pyrrole ring is one of the most significant heterocyclic structural motifs and is present in a large number of biologically active molecules.[20] A simple and convenient method for the synthesis of pyrroles is the Clauson–Kaas reaction. In this reaction, *N*-substituted pyrroles were formed as a result of condensation reactions between primary aliphatic or aromatic amines and 2,5-dimethoxytetrahydrofuran in the presence of acidic catalyst, as a rule, in acetic acid medium.[21] Performing the reaction of DHAAm (**1**) with 2,5-dimethoxytetrahydrofuran in acetic acid led to the formation of the expected product **12**, but was accompanied by significant resinification of the reaction mixture. In order to minimize the resinification and to simplify the isolation of pure product, we relied on a procedure using montmorillonite K-10 clay as a catalyst.[22] When performing this transformation without a solvent in the presence of K-10 clay, while heating to 100°C, the reaction was complete in less than 30 min with the formation of compound **12** (Scheme 5).

Scheme 5



All the obtained compounds were tested for antiviral activity against influenza virus and for activity against some types of bacteria and fungi. Antimicrobial screening was performed by CO-ADD (The Community for Antimicrobial Drug Discovery), funded by the Wellcome Trust (UK) and The University of Queensland (Australia).

The initial screening for antimicrobial activity was performed by testing for the inhibition of cell proliferation, using samples at the same concentration of 32 mg/ml. The tests were repeated twice. In the cases when ≥80% growth inhibition was observed once or twice, the studied compound was considered to be active. The growth inhibition experiments were performed with five different types of bacteria: *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, and two types of fungi: *Candida albicans* and *Cryptococcus neoformans*. The initial screening results are presented in Table 1.

The initial screening allowed to identify the antimicrobial activity of two of the studied compounds, heterocyclic dehydroabietylamine derivatives **3** and **5**, containing a pyrrolidine or azepane ring, respectively.

Table 1. The results of primary screening for antimicrobial activity

Compound	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Candida albicans*	*Cryptococcus neoformans*
3	+	–	–	–	–	+	+
4	–	–	–	–	–	–	–
5	+	–	–	–	–	+	+
8	–	–	–	–	–	–	–
11	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–

These compounds showed activity against the bacterial culture of *Staphylococcus aureus,* as well as against the fungi *Candida albicans* and *Cryptococcus neoformans*. The minimum inhibitory concentrations (MIC, mg/ml) were determined for these compounds against the aforementioned bacterial and fungal cultures, as well as the concentration that was lethal for 50% of the cells CC_{50} (mg/ml) was established for the human embryonic kidney cell line HEK293 (Table 2).

Thus, it was shown that some derivatives of DHAAm (**1**) are able to effectively inhibit the growth of pathogenic microorganisms, such as *Staphylococcus aureus* bacteria and certain types of fungi. These observations provide foundation for a broader structure–activity study and motivate efforts to decrease the toxicity of the obtained compounds, because derivatives of DHAAm (**1**) can potentially become a promising new class of antifungal agents.

Practically nothing is known about the antiviral activity of dehydroabietylamine derivatives. Antiviral activity has been studied for close structural analogs of this class of aromatic diterpenoids.[23] Compounds that were hydroxylated in the ring A, analogs of dehydroabietic acid, showed strong inhibitory activity against the HSV-2 herpes virus.[24] Significant antiherpetic properties were also found in the case of dehydroabietinol acetate.[25] The antiviral activity of dehydroabietylamine derivatives against the influenza A virus were studied for the first time in this work. The antiviral properties of the obtained compounds were studied with respect to the influenza A/California/07/09 (H1N1)pdm09 strain.

The greatest activity against influenza A virus was shown by compounds **5** and **12**, containing in their structures azepane and pyrrole rings, respectively. The selectivity index for these compounds was ≥10, which was achieved due to their relatively low toxicity (Table 3).

Table 2. The antimicrobial and cytotoxic activity of compounds **3** and **5**

Compound	MIC, mg/ml (% of maximum inhibition)			CC_{50}, mg/ml
	S. aureus	*C. albicans*	*C. neoformans*	HEK293
3	8 (101) 8 (102)	32 (98) 32 (97)	8 (96) 8 (94)	7.64 (86)
5	32 (83) >32 (62)	32 (100) 32 (99)	8 (104) 16 (98)	25.4 (59)

Table 3. Antiviral activity

Compound	CC_{50},* mmol	IC_{50},** mmol	SI***
3	21.2 ± 2.8	5.9 ± 1.4	4
4	50.7 ± 5.1	12.5 ± 2.3	4
5	>800	83.7 ± 6.0	10
8	40.3 ± 2.7	19.7 ± 1.7	2
11	48.6 ± 2.3	12.5 ± 2.5	4
12	>900	70.7 ± 12.3	13

* CC_{50} – the concentration resulting in 50% cell death.
** IC_{50} – the concentration resulting in 50% viral inhibition.
*** SI – selectivity index, the ratio of CC_{50}/IC_{50}.

Thus, in this work we studied the alkylation reaction of primary amino group in dehydroabietylamine with dibromoalkanes and synthesized new compounds containing a range of heterocyclic substituents: pyrrolidine, piperidine, azepane, and morpholine rings. A possibility was identified for obtaining isoindolinone moiety in a single step from primary amine and *o*-dibromoxylene. A method was found that gave a high yield of a derivative containing a pyrrole moiety. All of the obtained compounds were tested for biological activity. 1-{[(1*R*,4a*S*)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}pyrrolidine and 1-{[(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}azepane were shown to effectively inhibit the growth of *Staphylococcus aureus* bacterial culture, as well as the fungal cultures of *Candida albicans* and *Cryptococcus neoformans*. The studied derivatives were shown to lack a pronounced antiviral activity.

Experimental

^1H and ^{13}C NMR spectra were acquired on a Bruker DRX-500 spectrometer (500 and 125 MHz, respectively) in $CDCl_3$, with residual solvent signals used as internal standards (7.24 ppm for ^1H nuclei, 76.9 ppm for ^{13}C nuclei). High-resolution mass spectra were recorded on a Thermo Scientific DFS instrument in full scan mode over the *m*/*z* range of 0–500, ionization by electron impact at 70 eV, and direct introduction of samples. The reaction products were separated by column chromatography (60-200 μm silica gel, Masherey-Nagel). GC-MS analysis was performed on an Agilent 7890 A gas chromatograph with an Agilent 5975C quadrupole mass spectrometer as

detector, HP-5MS 30000×0.25 mm fused silica column, carrier gas – helium. The identification of compounds was performed by comparing the retention times with a series of authentic samples and comparing the complete experimentally obtained mass spectra with mass spectral database. Melting points were determined on a Kofler bench. The structures of the obtained compounds were established from ^{1}H and ^{13}C NMR spectra, two-dimensional homonuclear ^{1}H–^{1}H COSY and NOESY experiments, as well as two-dimensional heteronuclear ^{1}H–^{13}C HSQC and HMBC experiments. The atom numbering in the compounds is intended for the assignment of NMR signals and does not necessarily match the IUPAC nomenclature.

Synthesis of compounds 3–5 by alkylation of amine 1. A suspension of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), the appropriate dibromoalkane (3.5 mmol), and K_2CO_3 (1.0 g, 7.2 mmol) in MeCN (50 ml) was refluxed for 5 h, then cooled to room temperature and the solids were removed by filtration. The filtrate was evaporated under vacuum, acidified with concd HCl (1 ml), then washed with Et_2O (3×10 ml). The aqueous solution was then diluted with H_2O (1 ml), neutralized with aqueous 10% NaOH solution, and extracted with Et_2O (3×10 ml). The organic extract was dried over anhydrous Na_2SO_4, evaporated under vacuum, and the residue was purified by silica gel column chromatography (hexane–EtOAc, 20:1).

1-{[(1*R*,4a*S*)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}-pyrrolidine (3). Yield 0.511 g (43%), light-brown powder, mp 40–44°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.82 (3H, s, 19-CH_3); 1.19 (3H, s, 20-CH_3); 1.22 (6H, d, *J* = 6.9, 16,17-CH_3); 1.36–1.45 (3H, m, 5-CH, 6-CH_2); 1.55–1.77 (10H, m, 1,2,3,22,23-CH_2); 2.47 (1H, d, *J* = 13.9) and 2.13 (1H, d, *J* = 13.9, 18-CH_2); 2.53–2.63 (4H, m, 21,24-CH_2); 2.74–2.83 (3H, m, 15-CH, 7-CH_2); 6.88 (1H, s, H-14); 6.98 (1H, d, $J_{12,11}$ = 8.2, H-12); 7.18 (1H, d, $J_{11,12}$ = 8.2, H-11). ^{13}C NMR spectrum, δ, ppm: 147.7 (C-9); 145.1 (C-13); 134.8 (C-8); 126.6 (C-14); 124.1 (C-11); 123.5 (C-12); 68.2 (C-18); 57.3 (C-21,24); 44.2 (C-5); 38.3 and 38.2 (C-4 and C-1); 37.2 (C-10); 36.6 (C-3); 33.2 (C-15); 30.2 (C-7); 25.5 (C-20); 24.1 (C-22,23); 23.8 (C-16,17); 19.2 (C-19); 18.8 (C-6); 18.7 (C-2). Found, *m*/*z*: 339.2914 $[M]^+$. $C_{24}H_{37}N$. Calculated, *m*/*z*: 339.2921.

1-{[(1*R*,4a*S*)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}-piperidine (4). Yield 0.446 g (36%), light-brown powder, mp 73–75°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.80 (3H, s, 19-CH_3); 1.18 (3H, s, 20-CH_3); 1.21 (6H, d, *J* = 6.9, 16,17-CH_3); 1.25–1.35 (3H, m, 5-CH, 6-CH_2); 1.40–1.56 (6H, m, 22,23,24-CH_2); 1.56–1.60 (2H, m, 1-CH_2); 1.64–1.74 (2H, m, 7-CH_2); 1.76–1.86 (4H, m, 2,3-CH_2); 2.21–2.27 (2H, m, 18-CH_2); 2.32–2.45 (4H, m, 21,25-CH_2); 2.82 (1H, sept, *J* = 6.9, 15-CH); 6.86 (1H, s, H-14); 6.97 (1H, d, $J_{12,11}$ = 8.2 H-12); 7.17 (1H, d, $J_{11,12}$ = 8.2, H-11). ^{13}C NMR spectrum, δ, ppm: 147.8 (C-9); 145.1 (C-13); 134.9 (C-8); 126.8 (C-14); 124.2 (C-11); 123.6 (C-12); 69.7 (C-18); 57.6 (C-21,25); 44.2 (C-5); 38.9 (C-4); 38.4 (C-1); 37.5 (C-10); 36.2 (C-3); 33.3 (C-15); 30.4 (C-7); 26.4 (C-22,24); 25.8 (C-20); 24.0 (C-23); 23.9 (C-16,17); 19.2 (C-6); 18.8 (C-2). Found, *m*/*z*: 353.3072 $[M]^+$. $C_{25}H_{39}N$. Calculated, *m*/*z*: 353.3082.

1-{[(1*R*,4a*S*)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}-azepane (5). Yield 0.515 g (40%), yellow oil. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.78 (3H, s, CH_3-19); 1.18 (3H, s, 20-CH_3); 1.20 (6H, d, *J* = 6.9, 16,17-CH_3); 1.28–1.43 (3H, m, 5-CH, 6-CH_2); 1.45–1.60 (8H, m, 22,23,24,25-CH_2); 1.58–1.63 (2H, m, 1-CH_2); 1.63–1.72 (2H, m, 7-CH_2); 1.76–1.86 (4H, m, 2,3-CH_2); 2.47 and 2.11 (2H, d, *J* = 14.5, 18-CH_2); 2.64–2.71 (4H, m, 21,26-CH_2); 2.82 (1H, sept, *J* = 6.9, 15-CH); 6.86 (1H, s, H-14); 6.96 (1H, d, $J_{12,11}$ = 8.2, H-12); 7.16 (1H, d, $J_{11,12}$ = 8.2, H-11). ^{13}C NMR spectrum, δ, ppm: 146.3 (C-9); 145.5 (C-13); 134.1 (C-8); 126.6 (C-14); 123.9 (C-11); 123.8 (C-12); 58.9 (C-18); 51.6 (C-21,26); 46.6 (C-5); 37.8 (C-22,25); 37.6 (C-4); 36.2 (C-1); 35.8 (C-10); 35.6 (C-3); 33.2 (C-15); 29.6 (C-23,24); 29.3 (C-7); 25.1 (C-20); 23.8 (C-17,16); 19.1 (C-6); 17.8 (C-2); 17.6 (C-19). Found, *m*/*z*: 367.3234 $[M]^+$. $C_{26}H_{41}N$. Calculated, *m*/*z*: 367.3239.

Bis(2-bromoethyl) ether (7). A suspension of bis-(2-chloroethyl) ether (**6**) (5.0 g, 0.035 mol) and KBr (8.3 g, 0.07 mol) in 2:1 mixture of DMF–1,2-dibromoethane (150 ml) was refluxed for 24 h. Saturated NaCl solution (50 ml) was then added to the reaction mixture, followed by extraction with Et_2O. The solution was dried over anhydrous Na_2SO_4 and evaporated under vacuum, the residue was purified by vacuum distillation at 4–5 Torr. Yield 5.25 g (65%), dark-brown oil. NMR spectra matched the literature.[26]

4-{[(1*R*,4a*S*)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}-morpholine (8). A suspension of dehydroabietylamine (**1**) (0.42 g, 1.5 mmol), bis(2-bromoethyl) ether (**7**) (0.35 g, 1.5 mmol), and K_2CO_3 (0.2 g, 1.4 mmol) in MeCN (30 ml) was refluxed for 24 h; the solids were removed by filtration and the filtrate was evaporated under vacuum. The residue was acidified with concd HCl (1 ml) and washed with Et_2O (3×10 ml). The aqueous solution was then diluted with H_2O (1 ml), neutralized with aqueous 10% NaOH solution, and extracted with Et_2O (3×10 ml). The organic extract was dried over anhydrous Na_2SO_4 and evaporated under vacuum, the residue was purified by silica gel column chromatography (hexane–EtOAc, 20:1). Yield 0.267 g (51%), orange powder. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.83 (3H, s, 19-CH_3); 1.20 (3H, s, 20-CH_3); 1.22 (6H, d, *J* = 6.9, 16,17-CH_3); 1.36–1.54 (3H, m, 5-CH, 6-CH_2); 1.55–1.77 (4H, m, 2,3-CH_2); 1.31 (1H, d, ^2J = 12.8) and 2.26 (1H, d, ^2J = 12.8, 1-CH_2); 1.93 (1H, d, *J* = 14.3) and 2.32 (1H, d, *J* = 14.3, 18-CH_2); 2.49 (4H, t, *J* = 4.4, 21,24-CH_2); 2.71–2.90 (3H, m, 15-CH, 7-CH_2); 3.62 (4H, t, *J* = 4.4, 22,23-CH_2); 6.88 (1H, s, H-14); 6.99 (1H, d, $J_{12,11}$ = 8.2, H-12); 7.18 (1H, d, $J_{11,12}$ = 8.2, H-11). ^{13}C NMR spectrum, δ, ppm: 146.1 (C-9); 145.5 (C-13); 133.2 (C-8); 126.3 (C-14); 123.8 (C-11); 123.6 (C-12); 72.8 (C-22,23); 62.6 (C-18); 55.8 (C-21,24); 47.0 (C-5); 37.8 (C-4); 37.4 (C-1); 37.3 (C-10); 36.4 (C-3); 33.0 (C-15); 29.3 (C-7); 24.9 (C-20); 23.5 (C-17,16); 19.1 (C-6); 17.8 (C-2,19). Found, *m*/*z*: 355.2859 $[M]^+$. $C_{24}H_{37}NO$. Calculated, *m*/*z*: 355.2870.

2-{[(1*R*,4a*S*,10a*R*)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}-isoindolin-1-one (11). A solution of dehydroabietylamine (**1**) (0.16 g, 0.58 mmol), *o*-dibromoxylene (0.15 g, 0.58 mmol), and NaOH (0.06 g, 1.5 mmol) in dioxane (50 ml) was stirred at room temperature for 5 days. Water (5 ml) was then added, the mixture was extracted with $CHCl_3$ (3×10 ml) and the solvent was evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAc, 20:1). Yield 0.09 g (40%), white powder, mp 168–170°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.04 (3H, s, 19-CH_3); 1.19 (6H, d, J = 6.9, 16,17-CH_3); 1.22 (3H, s, 20-CH_3); 1.35 (1H, ddd, ^2J = $J_{1ax,2ax}$ = 13.0, $J_{1ax,2eq}$ = 3.8, 1-CH_{ax}); 1.49–1.61 (2H, m, 3-CH_2); 1.53 (1H, dd, $J_{5,6ax}$ = 12.6, $J_{5,6eq}$ = 2.2, 5-CH); 1.65 (1H, ddd, ^2J = 13.5, $J_{2eq,1ax}$ = 3.8, $J_{2eq,1eq}$ = 3.0, 2-CH_{eq}); 1.75 (1H, ddddd, ^2J = $J_{2ax,3ax}$ = 13.5, $J_{2ax,1ax}$ = 13.0, $J_{2ax,3eq}$ = $J_{2ax,1eq}$ = 3.5, 2-CH_{ax}); 1.83 (1H, dddd, ^2J = 13.4, $J_{6ax,5ax}$ = 12.6, $J_{6ax,7ax}$ = 12.0, $J_{6ax,7eq}$ = 6.9, 6-CH_{ax}); 2.12 (1H, dddd, ^2J = 13.4, $J_{6eq,7ax}$ = 7.4, $J_{6eq,5ax}$ = 2.2, $J_{6eq,7eq}$ = 1.6, 6-CH_{eq}); 2.26 (1H, ddd, ^2J = 13.0, $J_{1eq,2ax}$ = 3.5, $J_{1eq,2eq}$ = 3.0, 1-CH_{eq}); 2.79 (1H, sept, J = 6.9, 15-CH); 2.90 (1H, ddd, ^2J = 17.3, $J_{7ax,6ax}$ = 12.0, $J_{7ax,6eq}$ = 7.4, 7-CH_{ax}); 2.90 (1H, ddd, ^2J = 17.3, $J_{7eq,6ax}$ = 6.9, $J_{7eq,6eq}$ = 1.6, 7-CH_{eq}); 3.44 (1H, d, ^2J = 14.1) and 3.49 (1H, d, ^2J = 14.1, 18-CH_2); 4.45 (1H, d, ^2J = 16.8) and 4.49 (1H, d, ^2J = 16.8, 28-CH_2); 6.88 (1H, d, $J_{14,12}$ = 1.8, H-14); 6.95 (1H, dd, $J_{12,11}$ = 8.2, $J_{12,14}$ = 1.8, H-12); 7.13 (1H, d, $J_{11,12}$ = 8.2, H-11); 7.37 (1H, ddd, $J_{26,25}$ = 7.6, $J_{26,24}$ = 0.8, $J_{26,23}$ = 0.8, H-26); 7.42 (1H, ddd, $J_{24,23}$ = 7.7, $J_{24,25}$ = 7.4, $J_{24,26}$ = 0.8, H-24); 7.49 (1H, ddd, $J_{25,26}$ = 7.6, $J_{25,24}$ = 7.4, $J_{25,23}$ = 1.2, H-25); 7.81 (1H, ddd, $J_{23,24}$ = 7.7, $J_{23,25}$ = 1.2, $J_{23,26}$ = 0.8, H-23). ^{13}C NMR spectrum, δ, ppm: 169.9 (C-21); 147.1 (C-9); 145.5 (C-13); 141.4 (C-22); 134.5 (C-8); 132.4 (C-27); 131.2 (C-25); 127.8 (C-24); 126.8 (C-14); 123.9 (C-11); 123.7 (C-12); 123.6 (C-23); 122.3 (C-26); 55.0 (C-18); 54.0 (C-28); 45.4 (C-5); 39.5 (C-4); 38.2 (C-1); 37.5 (C-10); 37.4 (C-3); 33.3 (C-15); 30.0 (C-7); 25.5 (C-20); 23.8 (C-16,17); 19.2 (C-19); 19.1 (C-6); 18.6 (C-2). Found, *m*/*z*: 401.2712 $[M]^+$. $C_{28}H_{35}NO$. Calculated, *m*/*z*: 401.2713.

1-{[(1*R*,4a*S*)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]methyl}-1*H*-pyrrole (12). K-10 clay (0.5 g) was added to a solution of dehydroabietylamine (**1**) (0.21 g, 0.74 mmol) and 2,5-dimethoxytetrahydrofuran (0.14 g, 1.11 mmol) in Et_2O (3 ml). The mixture was stirred for 5 min, followed by evaporation of the solvent under vacuum. The dry residue was heated until the color of reaction mixture became light-brown, then Et_2O (10 ml) was added, clay was removed by filtration and washed with Et_2O (3×10 ml) to extract the remaining product. The obtained material was purified by column chromatography ($CHCl_3$) on silica gel that was prepared by washing with aqueous ammonia. Yield 0.178 g (72%), brown powder, mp 100–102°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.97 (3H, s, 19-CH_3); 1.19 (6H, d, J = 6.9, 16,17-CH_3); 1.22 (3H, s, 20-CH_3); 1.36–1.40 (1H, m, 1-CH_{ax}); 1.49–1.61 (3H, m, 3-CH_2, 5-CH); 1.58–1.66 (1H, m, 2-CH_{eq}); 1.68–1.75 (1H, m, 2-CH_{ax}); 1.75–1.86 (1H, m, 6-CH_{ax}); 1.88–1.97 (1H, m, 6-CH_{ax}); 2.19–2.26 (1H, m, 1-CH_{eq}); 2.79 (1H, sept, J = 6.9, 15-CH); 2.80–2.95 (2H, m, 7-CH_2); 3.84 (1H, d, J = 14.3) and 3.59 (1H, d, J = 14.3, 18-CH_2); 6.06 (2H, t, J = 2.1, H-22,23); 6.56 (2H, t, J = 2.1, H-21,24); 6.88 (1H, d, $J_{14,12}$ = 1.8, H-14); 6.95 (1H, dd, $J_{12,11}$ = 8.2, $J_{12,14}$ = 1.8, H-12); 7.13 (1H, d, $J_{11,12}$ = 8.2, H-11). ^{13}C NMR spectrum, δ, ppm: 147.1 (C-9); 145.5 (C-13); 134.3 (C-8); 126.9 (C-14); 124.3 (C-11); 123.7 (C-12); 122.8 (C-22,23); 107.4 (C-21,24); 61.1 (C-18); 44.8 (C-5); 38.5 (C-4); 38.2 (C-1); 37.5 (C-10); 37.4 (C-3); 33.3 (C-15); 30.1 (C-7); 25.5 (C-20); 23.8 (C-16,17); 19.2 (C-19); 19.1 (C-6); 18.6 (C-2). Found, *m*/*z*: 335.2604 $[M]^+$. $C_{24}H_{33}N$. Calculated, *m*/*z*: 335.2608.

Testing of compounds for cytotoxicity. All the synthesized compounds were tested for toxicity against Madin Darby canine kidney (MDCK) cells. A series of triple dilutions was prepared from each compound from 300 to 3 μg/ml, using MEM medium. The cells were incubated for 24 h at 37°C, under 5% CO_2 atmosphere in the presence of dissolved study compounds, after which the extent of cell monolayer destruction was evaluated using microtetrazolium test (MTT).[27] An MTT solution (Calbiochem No. 475989) was prepared (0.5 μg/ml) on the basis of physiological solution. The MTT solution was introduced in previously washed 0.1-ml wells containing the cells. After contacting the cells with MTT for 2 h, the wells were washed and DMSO (0.1 ml) was added, followed by reading the optical density of cells with a PerkinElmer Victor 2 1420 reader at 535 nm wavelength. On the basis of the obtained data, the 50% cytotoxic concentration (the concentration that caused the destruction of 50% of the cell monolayer) was calculated for each of the compounds.

Testing of compounds for antiviral activity. A series of dilutions (300–3 μg/ml) was prepared from the study compounds. After dissolving in the cell medium, the study compounds were added to monolayer MDCK cell culture on a 96-well plate and maintained for 1 h under 5% CO_2 atmosphere in incubator at 37°C. The cell culture was then infected with influenza A/California/07/09 (H1N1)pdm09 strain at the viral loading of 0.01$TCID_{50}$ per cell. The microplates containing the virus with compounds were incubated for 24 h at 37°C under 5% CO_2 atmosphere. The infective activity of the virus was estimated by titration of viral reproduction in MDCK cell culture. Series of tenfold dilutions (10^{-1} – 10^{-7}) were prepared for this purpose from the cultured liquid phase. These dilutions were used to infect the cell monolayer, followed by incubation for 48 h at 37°C under 5% CO_2 atmosphere. The presence of virus in the wells was detected by using hemagglutination reaction with chicken erythrocytes. For this purpose, 100-μl portions of virus-containing medium from the appropriate wells of cell culture plate were transferred to the wells of round-bottomed microplate for immunological reactions, followed by the addition of an equal amount of 1% chicken erythrocyte suspension. The reaction was observed after 30–40 min at room temperature. The greatest dilution of virus that caused complete agglutination of erythrocytes was reported as the virus titer. On the basis of the obtained data, the 50% inhibitory concentration (IC_{50}) was calculated for

each of the compounds, i.e., the concentration that reduced the virus titer by one half compared to the control without added compounds. The selectivity index (SI, ratio CC_{50}/ IC_{50}) was then calculated.

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