Synthesis and in Vitro Antibacterial Evaluation of Novel 4-Substituted 1-Menthyl-1,2,3-triazoles

Pooneh Khaligh, Peyman Salehi, Morteza Bararjanian, Atousa Aliahmadi, Hamid Reza Khavasi, and Samad Nejad-Ebrahimia

a Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University; Evin, G.C., Tehran 1983969411, Iran; b Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University; Evin, G.C., Tehran 1983969411, Iran; and c Department of Chemistry, Faculty of Chemistry, Shahid Beheshti University; G.C., Evin, Tehran 1983963113, Iran.

Received June 5, 2016; accepted August 9, 2016

Menthol is a natural compound with three asymmetric carbon atoms which among the optical isomers, (−)-menthol with the 1R, 2S, 5R configuration found widely in nature. In vitro and in vivo researches demonstrated that menthol as a simple monoterpene exhibits significant biological properties such as antitumor, antibacterial, antifungal, antiviral, anti-inflammatory, antipruritic, analgesic and antitussive. It is also used in lots of pharmaceutical remedies such as chest rubs, analgesic balm, nose drop and spray, cough drops and lotion. It has been reported that menthol acts as an enhancer for transdermal delivery of variety of drugs and it is one of the major agonists of transient receptor potential melastatin 8 (TRPM8). There are reports based on synthesis of menthol derivatives and evaluation of their effects such as antitumor, antimicrobial and antifungal activity as well as on penetration of drugs, percutaneous absorption, inhibition of plasminogen activator inhibitor-1 (PAI-1), cooling effect and insecticidal activity.

Previous in vitro antibacterial investigations of menthol have proved strong antibacterial activity of this compound against a range of bacterial strains. Bacterial infections progressively avoid standard treatment as resistance to multiple antibiotics is extending throughout the world, as a result there is an urgent medical need for a sustainable supply of novel, effective, and nontoxic antibacterial drugs without cross-resistance to currently used antibiotics. Among all the medicines currently approved as pharmaceutical remedies such as chest rubs, analgesic balm, nose drop and spray, cough drops and lotion. Previous in vitro antibacterial investigations of menthol have proved strong antibacterial activity of this compound against a range of bacterial strains.

Bacterial infections progressively avoid standard treatment as resistance to multiple antibiotics is extending throughout the world, as a result there is an urgent medical need for a sustainable supply of novel, effective, and nontoxic antibacterial drugs without cross-resistance to currently used antibiotics. Among all the medicines currently approved as antibacterial new chemical entities, a significant percentage of them are either natural products or were derived from a natural product base. As a result, it is not surprising that natural products are hopeful lead structures particularly for antibacterial drugs. Structural and chemical diversity of natural products is more than synthetic compounds, so they have been the major sources of bioactive factors and the main target for discovering and designing new drugs.

Nitrogen-containing heterocycles demonstrate outstanding biological potency. (−)-menthol with 1,4-disubstituted 1,2,3-triazole derivatives of hydroxybenzaldehydes, phenols and bile acids were synthesized via click chemistry. The novel synthesized compounds were evaluated for their in vitro antibacterial activity against Enterococcus faecium, and Staphylococcus aureus as Gram-positive bacteria. Some derivatives illustrated strong inhibitory effect against E. faecium with the minimum inhibitory concentration (MIC) values ranged from 1–3 µM, whereas cefixime as a positive control revealed MIC value of 35 µM. The structures of the synthesized compounds were confirmed by different spectroscopic techniques including 1H-NMR, 13C-NMR, high resolution (HR)-MS, IR and X-ray crystallographic analysis.

Key words menthol; 1,2,3-triazole; click chemistry; antibacterial activity

© 2016 The Pharmaceutical Society of Japan
phenols and bile acids have been synthesized and their antibacterial activities were evaluated and compared with parent compounds.

Results and Discussion

Chemistry In this study, our main strategy was the preparation of menthyl azide (3) from menthol and synthesis of 1,4-disubstituted 1,2,3-triazoles by the regioselective copper(I) (Cu(I))-catalyzed Huisgen 1,3-dipolar cycloaddition reaction with terminal alkynes. Therefore, in the first step (−)-menthol (1) was mesylated in the presence of mesyl chloride followed by reaction with sodium azide which afforded the proper key azide 3 as the building block of all of the target molecules 27,28) (Chart 1). The next step was preparation of the desired alkyne library, therefore three drug-like structures including ortho-salicylaldehyde and para-hydroxybenzaldehyde derivatives (4a–c), phenols (5a–i) and bile acids like deoxycholic acid, cholic acid and ursodeoxycholic acid (6a–c) were selected to increase the diversity of the propargyl building blocks. O-Alkylation of these compounds with propargyl bromide provided the alkyne components 7a–c, 8a–i and 9a–c (Table 1).

Library generation of the menthyl 1,4-disubstituted 1,2,3-triazoles was a paramount part of the research. As a result, functionalizations of the azide and alkyne components were extremely important in increasing the diversity of the desired library. Thus compound 3 as a key azide 29,30) and alkyne building blocks 7a–c, 8a–i and 9a–c with different substituents were subjected to synthesis of novel derivatives 10a–c, 11a–i, and 12a–c via 1,3-dipolar cycloaddition in high yields and purity in the presence of Cu(I) sulfate and sodium ascorbate as catalysts in methanol at room temperature (Chart 1, Fig. 1).

The use of ortho-salicylaldehyde and para-hydroxybenzaldehyde derivatives, ended up with the formation of the corresponding 1,2,3-triazoles (10a–c) in 90–98% yields. Other phenolic compounds with different functional groups such as halide, alkyl and acyl (11a–i) were synthesized and sensitive groups survived under the mild reaction condition. Deoxycholic acid, cholic acid and ursodeoxycholic acid were linked to the 4 position of 1-menthyl-1,2,3-triazol moiety with a methylene spacer in excellent yields (12a–c).

The structures of synthesized compounds were confirmed by different spectroscopic techniques including 1H-NMR, 13C-NMR, distortionless enhancement by polarization transfer (DEPT)-135 and HR-MS analyses. In evaluation of 1H-NMR and 13C-NMR spectra of derivatives 10a–c, 11a–i and 12a–c characteristic peaks were clearly evident which indicating certain positions of the molecules. One of these peaks was related to H-1 as a proton in menthyl ring which carbon is attached to the nitrogen of the triazole moiety and it was found that in all derivatives this peak was appeared in the range of δ=4.93–5.07 ppm. Also, evaluation of 1H-NMR

Reagents and conditions: a) MsCl, Et3N, CH2Cl2, r.t., 2h, 2: 90%; b) NaN3, DMF, 40°C, 48h, 3: 70%; c) CuSO4·5H2O (0.2 eq), sodium ascorbate (0.4 eq), MeOH, r.t., 30min, 10a–c, 11a–i and 12a–c: 90–98%.

Chart 1. Methods for Preparation of Azide (3) and Menthyl 1,4-Disubstituted 1,2,3-Triazole Derivatives (10a–c, 11a–i, 12a–c)

Table 1. Structures of Hydroxybenzaldehydes (4a–c), Phenols (5a–i) and Bile Acids (6a–c) and the Synthesis Pathway of Propargyl Ethers (7a–c, 8a–i, 9a–c)
spectra of products demonstrated chemical shift in the range of $\delta = 7.14$–8.34 ppm for H-5 as a proton of the triazole ring, one of the main scaffolds of all derivatives. Additionally, investigation of spectra revealed chemical shifts in the range of $\delta = 5.09$–5.44 and $\delta = 59.3$–66.7 ppm for H-6 and C-6 as protons and carbon of the methylene group attached to the oxygen, respectively. And finally, C-1 as a quaternary aromatic carbon connected to the oxygen in the molecules with the aromatic scaffold, illustrated chemical shift in the range of $\delta = 150.1$–163.3 ppm. In assessments, differences in chemical shifts of some of these certain areas in compound 11d (Fig. 2) compared with others attracted our attention. Differences were related to chemical shifts of protons and carbon in position 6 with $\delta = 3.04$, 3.08 and 37.5 ppm, respectively, and C-1 with $\delta = 186.3$ ppm. The reason for these differences is justified by the type of substituents of this compound. In this analogue, aryl moiety is functionalized by two bulky tert-butyl groups in the ortho positions. These results were probably caused by the influence of bulky substituents on benzene ring which led to a nonplanar mode. The conclusion evidence for the structure of compound 11g was obtained from single-crystal X-ray diffraction. Single crystal of compound 11g was obtained by dissolving it in hot $n$-hexane followed by slow evaporation of the solvent. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center, the deposition number is CCDC 1447884. Its ORTEP view is shown in Fig. 3.

**Antibacterial Activity**  Inhibitory effect of menthol on the
The synthesized novel triazole derivatives of menthol (10a–c, 11a–i, 12a–c) were evaluated for their in vitro antibacterial activity using standard techniques by determining the minimum inhibitory concentrations (MICs, μM), defined as the lowest concentration of the compound required to give complete inhibition of visible bacterial growth.22 Evaluations were carried out against Enterococcus faecium (ATCC 35667) and Staphylococcus aureus (ATCC 25923) as Gram-positive bacteria via comparing the results with cefixime as a standard antibiotic. To investigate the role of menthol group on antibacterial activity, standardized compounds 12b, 11d, 10a and 12a–c against strains along with cefixime, menthol and bile acids are listed in Table 2. Obtained data demonstrated stronger effect than cefixime as a standard antibiotic. In the meantime, compounds 12b, 11d, 10a and 12a–c with the lowest MIC values of 1, 1, 2 and 3 μM, respectively, were more potent than the positive control cefixime with MIC of 35 μM. Compounds 12a–c which were derived from triterpenic building blocks (9a–c) demonstrated stronger effect than their parent compounds deoxycholic acid, cholic acid and ursodeoxycholic acid with MIC values of 20, 157 and 10 μM, respectively, in which 12b showed a remarkable change. A similar trend for considerable differences in the antibacterial and antifungal activities of some derivatives of cholic acid and deoxycholic acid has already been reported in the literature.22 Also, the physicochemical and biological properties of bile acids have been related to the balance between hydroxyls at positions 3, 7 and/or 12 and the carboxylic side chain as hydrophilic groups and hydrophobic methyl groups in their structures. This balance and consequently properties can be modified by the distribution of the number, position and stereochemistry of hydroxyl groups and linking proper substituents that increase either the hydrophilicity or the hydrophobicity of these building blocks depending on the nature of the organic group.33

As indicated from the data, for S. aureus, while compounds 10a with MIC of 10 μM along with 11h and i with MIC values of 86 and 90 μM, respectively, exhibited promising activity compared to menthol, others did not show comparable activity than cefixime with MIC of 2 μM. Also, derivatives activity was investigated against two other strain namely Bacillus subtilis and Escherichia coli, but was not observed significant effect. It was especially noteworthy that menthol as the precursor of the target compounds showed moderate inhibitory activity against all tested strains, while the incorporation of 1,2,3-triazole ring, dramatically enhanced the antibacterial activity of all the derivatives against E. faecium and some derivatives against S. aureus.

A survey in the literature revealed the same trend for increasing the antibacterial activity of some lead compounds by linking a triazole ring.20,34–36 This could be due to enhancement of hydrogen bonding and dipole–dipole interaction of the molecules with the biological targets or improvement of their solubility.21,37,38 1,2,3-Triazoles are attractive linker units and molecules with the biological targets or improvement of their interaction of the number, position and stereochemistry of hydroxyl groups and linking proper substituents that increase either the hydrophilicity or the hydrophobicity of these building blocks depending on the nature of the organic group.33

To investigate the role of menthol group on antibacterial activities of the products, we have synthesized compound 13 which is the methyl analogue of compound 10a (Fig. 4). While 10a was one of the strongest antibacterial compounds, the MIC values of 109 and 868 μM were observed for 13 against E. faecium and S. aureus, respectively. These findings clearly show the importance of menthol moiety on the

### Table 2. In Vitro Antibacterial Activity of Compounds 10a–c, 11a–i and 12a–c

| Compounds          | Strains MIC (μM) |
|--------------------|------------------|
|                    | Enterococcus faecium | Staphylococcus aureus |
| 10a                | 2                | 10               |
| 10b                | 22               | 345              |
| 10c                | 94               | 375              |
| 11a                | 21               | 670              |
| 11b                | 42               | 670              |
| 11c                | 23               | 750              |
| 11d                | 1                | 582              |
| 11e                | 24               | 782              |
| 11f                | 22               | 704              |
| 11g                | 26               | 817              |
| 11h                | 22               | 86               |
| 11i                | 11               | 90               |
| 12a                | 3                | 209              |
| 12b                | 1                | 204              |
| 12c                | 7                | 209              |
| 13                 | 109              | 868              |

(–)-Menthol 410 >1638
Deoxycholic acid 20 >652
Cholic acid 157 >626
Ursodeoxycholic acid 10 >652
Cefixime<sup>a</sup> 35 2

<sup>a</sup> Minimum inhibitory concentration. <sup>b</sup> Positive control.
GC analysis was carried out on a Thermoquest Finnigan in -
Molecular Polar Surface Area.

Molecular weight.

ide (DMSO)-d$_6$

calculation results showed that all compounds except 12a

were obtained in chloroform (CDCl$_3$) and dimethyl sulfoxide

(2) and methyl azide (3) were synthesized ac-

to the known methods reported in the literature.$^{27,28}$

Preparation of Propargylic Compounds 7a–c, 8a–i and 9a–c  -

95%. HR-MS (ESI) +$\alpha$ (c=0.5, MeOH).

5-Bromo-2-((1-(2-isopropyl-5-methylcyclohexyl)-1H-1,2,3-

1593

Preparation of Mesylate 2 and Azide 3  -

Methyl methanesulfonate (2) and methyl azide (3) were synthesized ac-

to the known methods reported in the literature.$^{27,28}$

Preparation of Propargylic Compounds 7a–c, 8a–i and 9a–c -

were synthesized by propargylation of compounds 4a–c, 5a–i and 6a–c,

respectively, according to a known procedure.$^{48}$

General Procedure for Preparation of Methyl 1,4-Disubsti-

tuted 1,2,3-Triazole Derivatives (10a–c, 11a–i, 12a–c)

Synthesis of the target compounds 10a–c, 11a–i and 12a–c

was carried out via alkyne-azole Huisgen cycloaddition re-

action. Accordingly, propargyl ethers 7a–c, 8a–i and 9a–c (1 eq) were treated with compound 3 (200 mg, 1.1 mmol) in

the presence of sodium ascorbate (0.087 mg, 0.44 mmol) and copper sulfate (0.055 mg, 0.22 mmol) in MeOH (5 mL) at room

temperature for 30 min to give exclusively 1,4-disubstituted 1,2,3-triazoles (10a–c, 11a–i, 12a–c). After completion of the reaction confirmed by TLC, aqueous ammonia (10 mL)

was added to remove the excess of copper. In the following

H$_2$O (50 mL) was added to the suspension and extracted with

EtOAc (3×50 mL). The organic layers were washed with H$_2$O

(3×150 mL) and dried over Na$_2$SO$_4$. Solvent was removed

under reduced pressure. Final purification by flash chroma-
tography on silica gel (25% EtOAc–n-hexane) afforded pure products in 90–98% yields.

Preparation of Compound 13  -

One-pot synthesis of compound 13 was performed using a known method with some modifications by the reaction of methyl iodide (Mel) (1.5 eq), Na$_2$CO$_3$ (1.5 eq) and 7a (1.0 eq) in MeOH–H$_2$O (1 : 1) as solvent in the presence of CuSO$_4$·5H$_2$O (0.2 eq) and sodium ascorbate (0.4 eq).$^{49}$

Experimental  -

Table 3. Drug Likeness of the Synthesized Derivatives 10a–c, 11a–i and 12a–c

| Compound   | cLogP$^{(a)}$ | H-Acceptor | H-Donor | tPSA$^{(b)}$ | MW$^{(c)}$ |
|------------|--------------|------------|---------|------------|----------|
| 10a        | 5.77         | 5          | 0       | 54.26      | 420.35   |
| 10b        | 4.54         | 6          | 0       | 63.49      | 371.48   |
| 10c        | 4.87         | 5          | 0       | 54.26      | 341.46   |
| 11a        | 5.90         | 4          | 0       | 37.19      | 382.33   |
| 11b        | 6.54         | 4          | 0       | 37.19      | 382.33   |
| 11c        | 6.15         | 4          | 0       | 37.19      | 341.50   |
| 11d        | 9.30         | 4          | 0       | 37.19      | 439.69   |
| 11e        | 5.65         | 4          | 0       | 37.19      | 327.47   |
| 11f        | 6.33         | 4          | 0       | 37.19      | 363.50   |
| 11g        | 5.15         | 4          | 0       | 37.19      | 313.44   |
| 11h        | 3.47         | 5          | 1       | 80.28      | 370.50   |
| 11i        | 4.89         | 5          | 0       | 54.26      | 355.48   |
| 11j        | 8.64         | 6          | 2       | 94.72      | 611.91   |
| 12a        | 6.55         | 7          | 3       | 114.95     | 627.91   |
| 12b        | 8.64         | 6          | 2       | 94.72      | 611.91   |
| Menthol    | 3.23         | 1          | 1       | 20.23      | 156.27   |
| Deoxoycholic acid | 4.51 | 3     | 3    | 77.76    | 392.58   |
| Cholic acid | 2.43         | 4          | 4       | 97.99      | 408.58   |
| Ursodeoxycholic acid | 4.51 | 3   | 3    | 77.76    | 392.58   |
| Cefxime    | 0.25         | 8          | 4       | 183.98     | 453.44   |

(a) Logarithm of compound partition coefficient between n-octanol and water. b) Molecular Polar Surface Area. c) Molecular weight.

antibacterial activity of the synthesized compounds.

The drug-likeness properties of synthesized compounds evaluated by physicochemical properties calculation based on Lipinski’s rule of five using ChemBio3D package ver-

version 14.0.0.117 (Perkin Elmer, Inc. (United States)). The calculation results showed that all compounds except 12a–c meet the Lipinski rules of the five, suggesting that these compounds theoretically would not have problems with oral bioavailability$^{39}$ (Table 3).

![Structure of Compound 13](image-url)
White solid mp 139–141°C. Yield 98%. IR (KBr) cm⁻¹: 3124, 3072, 2950, 2653, 1699, 1602, 1454, 1251. ¹H-NMR (CDCl₃) δ: 7.65 (s, 1H, H₆₋₋₋), 7.64 (s, 1H, H₃₋₋), 7.04 (m, 2H, H₇₋₋), 3.08 (s, 2H, CH₂–O), 5.06 (s, 2H, CH₂–N), 1.78–1.87 (m, 2H), 0.96 (s, 3H, CH₃). ¹³C-NMR (CDCl₃) δ: 158.5, 143.1, 135.4, 127.4, 126.4, 125.9, 125.8, 125.2, 124.1, 121.9, 120.9, 1057, 62.8, 59.3, 46.7, 40.9, 34.7, 29.7, 26.2, 24.9, 22.3, 21.1, 20.5. NMR purity: >95%. HR-MS (ESI) m/z: Calcd for C$_{23}$H$_{26}$Cl$_2$N$_3$O: 382.1453; Found: 382.1454 [M⁺]$.^1$ δ$^0_{D}$ +0.10 (ε=0.5, MeOH).

White solid mp 112–114°C. Yield 96%. IR (KBr) cm⁻¹: 3080, 2951, 1582, 1480, 1262, 799. ¹H-NMR (CDCl₃) δ: 7.74 (s, 1H, H₆₋₋₋), 7.30 (d, 1H, H₃₋₋), 7.20 (d, 1H, H₆₋₋₋), 7.13 (s, 1H, H₃₋₋), J=2.2 Hz), 6.93 (dd, 1H, H₆₋₋₋), 8.4 (s, 2H, H₃₋₋), 5.05 (bs, 1H, CH–N), 1.67–2.03 (m, 5H), 1.36–1.54 (m, 2H), 1.03–1.13 (m, 2H), 0.89 (s, 3H, CH₃, J=5.9Hz), 0.84 (d, 3H, CH₃, J=6.5Hz), 0.78 (d, 3H, CH₃, J=6.5Hz). ¹³C-NMR (CDCl₃) δ: 154.3, 142.1, 133.1, 130.8, 130.6, 122.0, 121.7, 115.2, 63.8, 59.3, 46.6, 40.8, 34.7, 29.7, 26.2, 25.0, 22.2, 21.1, 20.5. NMR purity: >95%. HR-MS (ESI) m/z: Calcd for C$_{23}$H$_{24}$Cl$_2$N$_3$O: 382.1453; Found: 382.1454 [M⁺]$.^1$ δ$^0_{D}$ +0.08 (ε=0.5, MeOH).

White solid mp 110–112°C. Yield 91%. IR (KBr) cm⁻¹: 3100, 2947, 2861, 1603, 1459, 1225. ¹H-NMR (CDCl₃) δ: 7.69 (s, 1H, H₆₋₋₋), 6.55 (s, 1H, H₃₋₋), 5.20 (s, 2H, CH₂–O), 5.05 (bs, 1H, CH–N), 2.30 (s, 6H, 2CH₃), 1.72–2.03 (m, 5H), 1.33–1.54 (m, 2H), 1.01–1.18 (m, 2H), 0.89 (d, 3H, CH₃, J=6.1Hz), 0.86 (d, 3H, CH₃, J=6.6Hz), 0.79 (d, 3H, CH₃, J=6.6Hz). ¹³C-NMR (CDCl₃) δ: 158.4, 143.3, 139.2, 123.7, 123.0, 112.7, 62.3, 59.2, 46.6, 40.8, 34.7, 29.7, 26.2, 25.0, 22.2, 21.0, 23.6, 20.6. NMR purity: >95%. HR-MS (ESI) m/z: Calcd for C$_{24}$H$_{24}$Cl$_2$N$_3$O: 384.2467; Found: 384.2468 [M⁺]$.^1$ δ$^0_{D}$ +0.16 (ε=0.5, MeOH).

White solid mp 79–81°C. Yield 94%. IR (KBr) cm⁻¹: 3136, 2954, 2871, 1644, 1457, 1247. ¹H-NMR (CDCl₃) δ: 7.16 (s, 1H, H₆₋₋₋), 6.65 (s, 1H, H₃₋₋), J=2.9Hz), 6.53 (d, 1H, H₆₋₋₋).

\[ C_{23}H_{26}Cl_2N_3O \]
(m, 2H), 0.83 (d, 3H, CH₃, J=6.2Hz), 0.78 (d, 3H, CH₃, J=6.4Hz). ¹³C-NMR (DMSO-d₆): δ: 173.0, 157.0, 141.9, 130.6, 129.3, 126.3, 114.9, 61.5, 58.5, 46.0, 41.9, 34.9, 29.2, 24.8, 22.6, 21.3, 20.7. NMR purity: >95%. HR-MS (ESI) m/z: Calcd for C₃₁H₄₃N₅O₇: 601.2690; Found: 601.2687 [M]+. [[α]D]²⁰ +0.44 (c=0.5, MeOH).

(1-(2-Isopropyl-5-methylcyclohexyl)-1H,1,2-triazol-4-yl)-methyl 4-(3,7-Dihydroy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (12a)

White solid. mp 97–99°C. Yield 96%. IR (KBr) cm⁻¹: 3465, 2933, 2868, 1731, 1455, 1251. ¹¹H-NMR (CDCl₃) δ: 6.76 (s, 1H, H₁₃); 7.67 (s, 1H, H₁₃); 5.19 (s, 2H, CH₂-O), 5.02 (s, 1H, CH-N), 3.94 (br s, 1H, CH-CH₂), 3.50–3.65 (s, 1H, CH-CH₂), 2.49, 2.49, 2.49, 2.49. ¹³C-NMR (CDCl₃) δ: 174.2, 141.8, 124.8, 71.4, 71.3, 59.4, 57.5, 48.2, 47.2, 46.7, 46.5, 42.1, 40.7, 36.4, 36.0, 35.2, 35.1, 34.6, 34.1, 33.6, 31.1, 30.8, 30.5, 29.1, 28.7, 27.5, 27.1, 26.3, 26.1, 24.9, 23.7, 23.2, 22.2, 21.1, 20.5, 17.3, 12.7. NMR purity: >95%. HR-MS (ESI) m/z: Calcd for C₃₇H₆₂N₃O₇: 628.4689; Found: 628.4698 1H). [[α]D]²⁰ +0.28 (c=0.5, MeOH).

(1-(2-Isopropyl-5-methylcyclohexyl)-1H,1,2-triazol-4-yl)-methyl 4-(3,7-Dihydroy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (12b)

White solid. mp 85–87°C. Yield 95%. IR (KBr) cm⁻¹: 3395, 2940, 2870, 1734, 1457, 1241. ¹¹H-NMR (CDCl₃) δ: 6.78 (s, 1H, H₁₃); 5.19 (s, 2H, CH₂-O), 5.02 (s, 1H, CH-N), 3.92 (br s, 1H, CH-CH₂), 3.78 (s, 1H, CH-CH₂), 3.35–3.55 (m, 4H, CH-CH₂, 3OH), 2.17–2.45 (m, 4H), 1.17–2.00 (m, 25 H), 0.98–1.14 (m, 4H), 0.93 (d, 3H, CH₃, J=5.2Hz) 0.86 (s, 3H, CH₃), 0.85 (d, 3H, CH₃, J=5.2Hz), 0.82 (d, 3H, CH₃, J=6.6Hz), 0.76 (d, 3H, CH₃, J=6.6Hz), 0.63 (s, 3H, CH₃). ¹³C-NMR (CDCl₃) δ: 174.3, 141.8, 124.8, 73.1, 71.9, 59.3, 57.6, 46.9, 46.7, 46.4, 41.6, 41.5, 40.7, 39.5, 35.3, 34.8, 34.7, 34.6, 31.2, 30.8, 30.3, 29.1, 28.2, 27.5, 26.9, 26.3, 24.9, 23.2, 22.5, 22.3, 21.1, 20.6, 17.3, 12.5. NMR purity: >95%. HR-MS (ESI) m/z: Calcd for C₁₁H₁₁BrN₃O₂: 296.0035; Found: 296.0065 1H). [[α]D]²⁰ +0.44 (c=0.5, MeOH).

Full results have been published in Comprehensive Spectroscopy 2021.
Conflict of Interest  The authors declare no conflict of interest.

Supplementary Materials  The online version of this article contains supplementary materials.

References
1) Galeotti N., Mannelli L. D. C., Mazzanti G., Bartolini A., Ghelardini C., Neurosci. Lett., 322, 145–148 (2002).
2) Kamatou G. P., Vermaak I., Viljoen A. M., Lawrence B. M., Phytochemistry, 96, 15–25 (2013).
3) Klijn, J., M., Sereenpasun A., Chanchao C., Asian Pac. J. Cancer Prev., 15, 1551–1556 (2014).
4) Alvi M., Ahad A., Sultana Y., Ali A., Drug Discov. Today, 21, 2016–2017 (2008).
5) Zhou C.-H., Wang Y., Curr. Med. Chem., 56, 92–103 (2008).
6) Obata Y., Sato H., Li C. J., Takayama K., Higashiyama K., Nagai T., Drug Discov. Today, 4, 17–31 (2013).
7) Dewang P. M., Nikumbh V. P., Tare V. S., Mahulikar P. P., J. Sci. Pest Manag. Sci., 280, 290–295 (2008).
8) Samarasekera R., Weerasinghe I. S., Hemalal K., Molecules, 63, 290–295 (2008).
9) Ye B., Bauer S., Buckman B. O., Ghannam A., Griezman L. L., Weiss D. G., Tlegenov R. T., Zyk N. V., Zefirov N. S., Tetrahedron Lett., 21, 4260–4264 (2008).
10) von Nussbaum F., Brands M., Hinzen B., Weigand S., Häbich D., Angew. Chem. Int. Ed., 45, 5072–5129 (2006).
11) Klein A. H., Carstens M. I., McCluskey T. S., Blanchar G., Simons C. T., Slack J. P., Burrell R., Carstens E., Adv. Synth. Catal., 353, 4–17 (2012).
12) Zefirova O. N., Nuriev E. V., Nuriev V. N., Kuznetsov S. A., Weiss D. G., Tlegenov R. T., Zyk N. V., Zefirov N. S., Moscow Univ. Chem. Bull., 62, 261–263 (2007).
13) Sokovic M., Glamočlija J., Marin P. D., Brkić D., van Griensven L. J. L. D., Molecules, 15, 7352–7354 (2010).
14) Kazemi M., Rostami H., Shahiei S., J. Plant Sci., 7, 55–66 (2012).
15) Kotan R., Kordali S., Cakir A., Z. Naturforsch. C, 62, 507–513 (2007).
16) von Nussbaum F., Brands M., Hinzen B., Weigand S., Häbich D., Angew. Chem. Int. Ed., 45, 5072–5129 (2006).
17) Brown D. G., Lister T., May-Dracka T. L., Bioorg. Med. Chem. Lett., 24, 413–418 (2014).
18) Lahlou M., Pharmacol. Pharm., 4, 17–31 (2013).
19) Al-Omair M. A., Sayed A. R., Yousef M. M., Molecules, 20, 2591–2610 (2015).
20) Wang X.-L., Wang K., Zhou C.-H., Eur. J. Med. Chem., 45, 4631–4639 (2010).
21) Zhou C.-H., Wang Y., Curr. Med. Chem., 19, 2591–2610 (2012).
22) Vatmurgar N. S., Hazra B. G., Por V. S., Shirazi F., Chavan P. S., Deshpande M. V., Bioorg. Med. Chem. Lett., 18, 2043–2047 (2008).
23) Totozenzara J., Burke A. J., Tetrahedron Lett., 56, 2853–2859 (2015).
24) Alonso Valdés F., Morigli Y., Radiyov G., Yus Astiz M., Heterocycles, 84, 1033–1044 (2012).
25) Schulze B., Schubert U. S., Chem. Soc. Rev., 43, 2527–2527 (2014).
26) Ma N., Wang Y., Zhao B.-X., Ye W.-C., Jiang S. Drug., Drug. Des. Ther., 9, 1585–1599 (2015).
27) Shi X.-X., Shen C.-L., Yau J.-Z., Nie L.-D., Quan N., Tetrahedron Asymmetry, 21, 277–284 (2010).
28) Welschhoff N., Waldvogel S. R., Synthesis, 2010, 3596–3601 (2010).
29) Mohan A., Ramkumar V., Sankaranaram S., J. Organomet. Chem., 799, 115–121 (2015).
30) Nagender P., Mall Reddy G., Naresh Kumar R., Chandrashekar Reddy A., Reddy Velatourou L., Pamanji R., Venkateswara Rao J., Narasiah L., Lett. Drug Des. Discov., 10, 865–871 (2013).
31) Zhang J., Wu J., Shen L., Jin G., Cao S., Adv. Synth. Catal., 353, 580–584 (2011).
32) Jorgensen J. H., Turnidge J. D., “Manual of clinical microbiology,” Vol. 1, Chap. 73, ed. by Murray P. R., Baron E. J., Jorgensen J. H., Pfaffer M. A., Louise Lander M., American Society for Microbiology, Washington DC, 2007, pp. 1152–1172.
33) Monti M. J., Marin J., Antelo A., Vaquez-Tato J., World J. Gastroenterol., 15, 804–816 (2009).
34) North L., Desjardins C., Houshyar A., Safavi-Sohi S., Rahmasi J., Med. Chem. Res., 23, 4531–4541 (2014).
35) Aly M. R. E., Saad H. A., Mohamed M. A. M., Bioorg. Med. Chem. Lett., 25, 2824–2830 (2015).
36) Petrova K., Totev T. M., Correia-da-Silva P., Barros M. T., Calheira R. C., Cirec A., Sokovic M., Ferreira I. C., Carbohydr. Res., 417, 66–71 (2015).
37) Collin M. P., Hobbie S. N., Böttger E. C., Vasella A., Helv. Chim. Acta, 91, 1838–1848 (2008).
38) Wei J. J., Jin L., Wan K., Zhou C. H., Bull. Korean Chem. Soc., 32, 229–238 (2011).
39) Liapis C. A., Lombardo F., Dominy B. W., Adv. Drug Deliv. Rev., 64, 4–17 (2012).
40) Khoshkhohlg M. J., Balalai S., Bijnazdeh H. R., Grosse J. H., Arkivoc, 9, 114–121 (2009).
41) Vedachalam S., Wong Q. L., Maji B., Zeng J., Ma J., Liu X. W., Adv. Synth. Catal., 353, 219–225 (2011).
42) Pal M., Parasuraman K., Yeleswarapu K. R., Org. Lett., 5, 349–352 (2003).
43) Lingam V. S. P. R., Vinodkumar R., Makkanti K., Thomas A., Gopal B., Tetrahedron Lett., 49, 4260–4264 (2008).
44) Wang Y., Ji K., Lan S., Zhang L., Angew. Chem. Int. Ed., 51, 1915–1918 (2012).
45) Miyamoto Y., Kalsiak J., Korthals K., Lauterwae T., Cheung D. Y., Lozano R., Cobo E. R., Upercof P., Upercof J. A., Berg D. E., Gillin F. D., Fokin V. Y., Sharpless K. B., Eckmann L., Proc. Natl. Acad. Sci. U.S.A., 110, 17564–17569 (2013).
46) Zhao L., Mao L., Hong G., Yang X., Liu T., Bioorg. Med. Chem. Lett., 25, 2540–2544 (2015).
47) Ikonen S., Maciêkova-Cahová H., Pohl R., Šanda M., Hocek M., Bioorg. Med. Chem. Lett., 21, 7532–7546 (2010).
48) Ikonen S., Maciêkova-Cahová H., Pohl R., Šanda M., Hocek M., Bioorg. Med. Chem. Lett., 21, 4824–4827 (2008).