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In search of cytotoxic abietyl amides

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Abstract: Based on prior research findings with pentacyclic triterpenoids, it was hypothesized that (un)-substituted benzylamides would exhibit enhanced cytotoxic activity compared to parent abietic acid. Conversely, none of these compounds was cytotoxic, but (homo)-piperazinyl amides demonstrated significant cytotoxic activity across multiple cell lines, even at concentrations as low single-digit micromolar levels. Additional staining experiments revealed that the most potent compound (with an EC_{50} value of 2.8 µM for HT29 colon carcinoma cells) primarily induced apoptosis rather than necrosis.

Keywords: Abietic acid; Cytotoxicity; Amides

1. Introduction

Natural products have served as an excellent source for discovering bioactive compounds. These products are excellent starting points for developing new anticancer drugs [1-5]. Cancer treatment is still challenging for scientists, physicians, and patients worldwide. Furthermore, cancer is a complex disease characterized by uncontrolled cell growth and the formation of malignant tumors. Hence, treating cancer requires a multidisciplinary approach and involves various therapeutic strategies. Thereby, challenges in cancer therapy include the problem of heterogeneity of cancers, the resistance to medicines, and the side effects of treatment, especially in chemotherapy and radiation therapy but also in targeted therapies [6,7].

Ethnobotanical literature [8-10] from almost all around the world shows trees of the species "pinus" used in folk medicine; as a consequence, diterpenoids of the pimarane [11,12] and abietane [13] scaffold have attracted many scientists for many decades. Of particular interest is abietic acid (**AB**, Figure 1), a diterpenoid resin acid usually obtained from pines, for example, *Pinus insularis*, *Pinus strobus*, or *Pinus sylvstris* [14,15].

AB is considered a "nonhazardous natural substance" in tall oil but might also acts as a contact allergen [16,17]. Parent **AB** has been shown to hold many biological activities, such as anti-inflammatory, anti-mycotic, and antiviral activity [18-22]. But **AB** also inhibits the multidrug resistance-associated protein 2 (MRP-2) [21,23] and has an antiproliferative effect on NSCLC cell lines [21]. Furthermore, **AB** induces apoptosis in MCF7 cells with a cell cycle arrest in G_0-G_1 and G_2/M phases and the $SubG_0$-G_1 Subpopulation [21]. However, this cytotoxic effect is not high enough for therapy but parallels the biological activities of many terpene carboxylic acids. For example, triterpenoid carboxylic acids such as oleanolic acid, ursolic acid, and glycyrrhetinic acid are of weak or almost no cytotoxicity. Still, their cytotoxic activity can significantly be improved by derivatization. Previously, we could show on maslinic acid, betulinic acid, and platanic acid that benzylamides are especially more cytotoxic than the respective parent compounds [24-28].

Consequently, checking whether this concept could be applied to AB was reasonable. We included the synthesis of (homo)-piperazinyl amides [29] into this study since triterpenoid (homo)-piperazinyl amides were previously shown to exhibit good cytotoxicity.

2. Results and Discussion

Compared to dehydroabietylamine [30,] which is readily available from AB, the investigation of derivatives of parent AB for cytotoxic activity has hardly attracted any attention.

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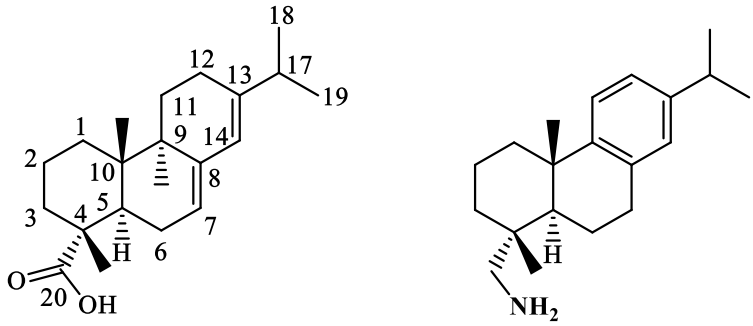
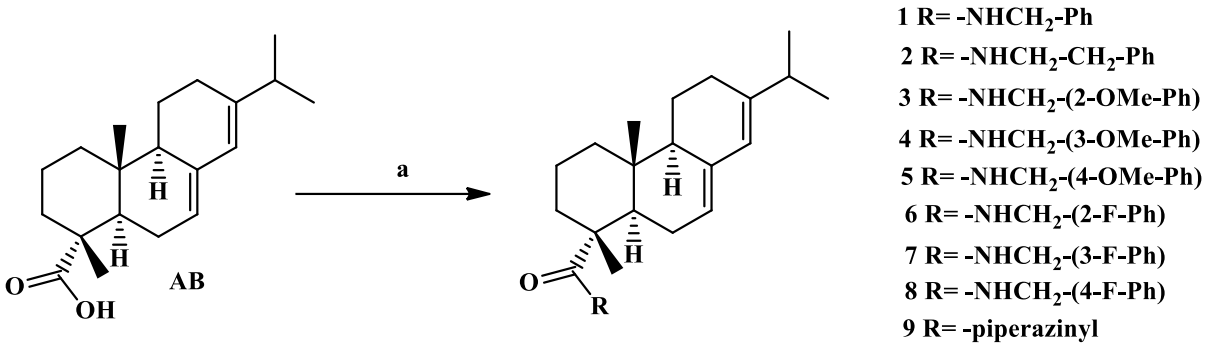


Abietic acid (AB) **Dehydroabietylamine**

Figure 1. Structures of abietic acid (**AB**) and dehydroabietylamine

The reaction of **AB** with oxalyl chloride (Scheme 1) followed by the addition of the corresponding amine gave the benzylamides **1** and **3**-**8**; phenethylamine amide compound **2** was obtained. All compounds were fully characterized by their NMR, UV-vis, and IR spectra, optical rotation, and micro-analytical data. Analogous reactions with piperazine yielded amide **9**, from homopiperazine compound **10** was obtained; under these conditions, no dimerization products were observed.



Scheme 1. Reactions and conditions: a) oxalyl chloride, DCM, cat. DMF, 1 h, 21 °C, then amine in DCM, 1 h, 21°C; chromatography: yields: **1** (92%), **2** (93%), **3** (94%), **4** (90%), **5** (92%), **6** (78%), **7** (69%), **8** (74%), **9** (73%), **10** (85%)

AB and amides **1**-**10** were tested for cytotoxic activity in sulforhodamine (SRB) assays employing several human tumor cell lines; non-malignant cell lines (NIH 3T3 and HEK293, respectively) served as a comparison. Doxorubicin (**DX**) was included as a positive standard.

The assays yielded unexpected results (Table 1): Whereas with previously investigated pentacyclic triterpenes, the (substituted) benzylamides proved to be good cytotoxic, the diterpenoid abietyl amides **1**-**8** of this study either showed no cytotoxicity or did cause only negligible cytotoxic effects with all cell lines used. A different picture emerged with the (homo)-piperazinyl amides **9** and **10** where cytotoxic effects were found in the range of single-digit µM concentrations; thereby, piperazinyl amide **9** was found to be slightly more cytotoxic than homopiperazinyl amide **10**. A difference in lipophilicity might be a reason for this increased cytotoxicity of the (homo)-piperazinyl amides. While for **1** a Log P*o/w* (consensus) of 5.57 was calculated (http://www.swissadme.ch), for **9,** a log P*o/w* (consensus) of 4.05 was determined. In contrast to many derivatives of pentacyclic triterpenes that we have investigated this time, not ovarian cancer cell line A2780 proved to be particularly sensitive, but the colon adenocarcinoma cell line HT29.

Some extra staining experiments were performed. The staining system AO/PI (Acridine Orange/Propidium Iodide) causes viable nucleated cells to fluoresce green and non-viable nucleated cells to fluoresce red. Figure 2 shows the results from the AO/PI staining experiments (after an incubation time of 2 days, double EC_{50} concentration of **9** was applied); thus, cells of this cell line have died instead by apoptosis than necrosis upon treatment with compound **9**.

Table 1. Cytotoxicity results from the SRB assays: **AB** and compounds **1**–**10** (EC_{50}-values in µM from SRB-assays) after 72 h of treatment; the values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error; all cell lines malignant (A375, HT29, MCF-7, A2780, HeLa) except (murine fibroblasts NIH 3T3 and human embryonic kidney cells HEK293; positive control: doxorubicin (**DOX**); n.d. not determined.

#	A375	HT29	MCF7	A2780	HeLa	NIH 3T3	HEK293
AB	>30	>30	>30	>30	>30	>30	>30
1	> 30	> 30	27.1±3.1	25.4±3.1	> 30	> 30	24.6±2.0
2	21.0±0.5	26.2±1.7	14.3±0.5	18.0±1.4	> 30	> 30	15.2±4.2
3	25.0±1.3	> 30	18.9±2.0	16.7±1.8	> 30	> 30	20.2±0.9
4	23.7±1.0	25.2±2.5	19.9±2.5	15.5±2.0	> 30	> 30	20.4±0.7
5	> 30	> 30	> 30	> 30	> 30	> 30	> 30
6	> 30	> 30	20.6±2.1	18.6±1.6	> 30	> 30	18.3±2.1
7	27.8±1.6	> 30	21.7±2.9	22.1±1.7	> 30	> 30	22.25±1.7
8	> 30	> 30	>30	> 30	> 30	> 30	> 30
9	3.6±0.3	2.6±0.2	4.0±0.5	4.8±0.6	5.2±0.3	1.7±0.4	2.6±0.8
10	5.6±1.2	2.8±0.8	6.0±1.5	7.3±0.5	5.9±1.6	4.6±1.9	4.5±1.6
DX	n.d.	0.91±0.01	1.1 ± 0.01	0.01±0.01	n.d.	0.4±0.07	n.d.

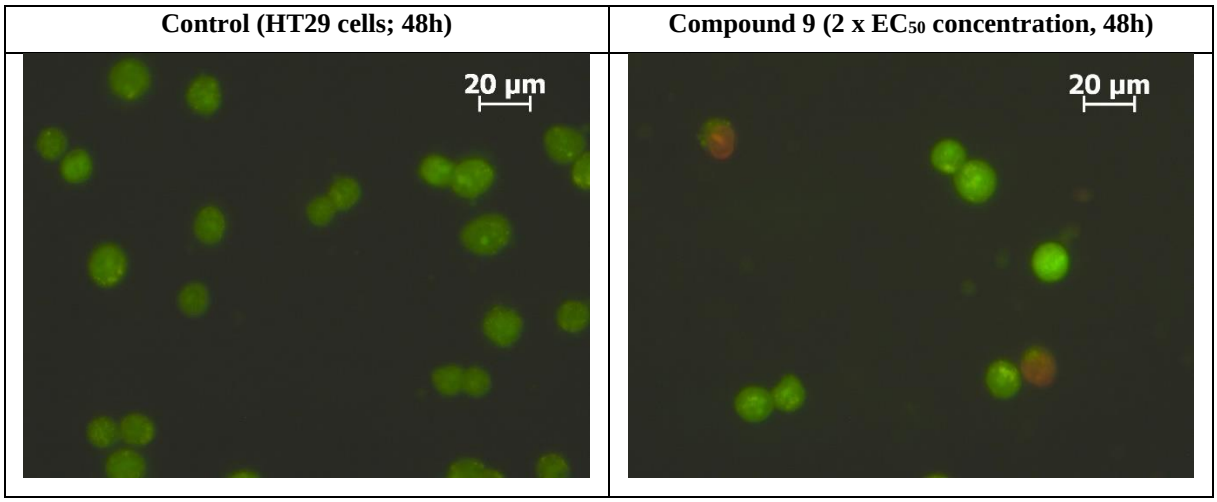


Figure 2. AO/PI staining of HT29 colon carcinoma cells (double EC_{50} concentration of compound 9 applied after 48 h of incubation)

3. Conclusion

The study focuses on abietic acid, a diterpenoid that has previously shown various biological effects, including some antiproliferative effects on cancer cell lines. As outlined in previous studies, we expected that (un)-substituted benzylamides should exert an increased cytotoxic activity. However, none of these compounds was cytotoxic within the cut-off of the assay. (Homo)-piperazinyl amides, however, held good cytotoxicity for most of the cell lines, even in a single-digit mM concentration. Extra staining experiments showed one of the most active compounds, i.e., abieta-7,13-diene-*N*-(piperazin-1-yl)-18-amide (**9**, EC_{50} = 2.8 µM for HT29 colon carcinoma cells) to act instead by apoptosis than by necrosis. However, the selectivity of these compounds to distinguish between malignant and non-malignant cells was low. A further modification is called to access analogs of high selectivity while retaining good cytotoxicity.

4. Experimental

Equipment: According to the literature, 26 abietic acid was obtained from local vendors and purified by repeated re-crystallization [15]. Samples, spectra, and raw biological screening data can be obtained from the authors upon request.

General procedure for the synthesis of the abietyl amides (GP)

To a solution of **AB** (1 eq.) in dry DCM (20 mL), oxalyl chloride (4 eq.) and DMF (cat.) were added, and the mixture was stirred at 20°C for 1 h. The volatiles were removed under reduced pressure, and the residue re-dissolved in dry THF (3 x 20 mL) and again evaporated. The residue was dissolved in dry DCM (20 mL), and the corresponding amine (3 eq.) was added. The mixture was stirred at 20°C for 1 h. Usual aqueous work-up (dil. aq. HCl (5%), extraction with DCM, brine, drying with Na_2SO_4) followed by column chromatography (silica gel) gave compounds **1**–**10** each as a colorless solid.

Benzyl Abieta-7,13-diene-*N*-(benzyl)-18-amide (1)

Following GP from **AB** (500 mg, 0.52 mmol) and benzylamine (0.1 mL, 0.92 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 20%)] **1** (190 mg, 92%) was obtained as a colorless solid; R_f = 0.68 (SiO_2, toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p. = 87-90°C; $[\alpha]_D^{20}$ = -14.5° (*c* 0.164, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (3.98);
IR (ATR): ν = 3355*w*, 2927*m*, 1631*s*, 1521*s*, 1454*m*, 1384*m*, 1265*m*, 1028*w*, 885*w*, 822*w*, 697*s*, 602*w* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 – 7.19 (*m*, 5H, 24-H, 25-H, 26-H, 27-H, 28-H), 5.75 (*s*, 1H, 14-H), 5.36 – 5.31 (*m*, 1H, 7-H), 4.58 – 4.29 (*m*, 2H, 21-H), 2.22 (*hept*, *J* = 6.8 Hz, 1H, 17-H), 2.17 – 1.67 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.65 – 1.47 (*m*, 3H, 2-H, 3-H_b), 1.27 (*s*, 3H, 15-H), 1.25 – 1.14 (*m*, 2H, 6-H_b, 1-H_b), 1.01 (*m*, 6H, 18-H, 19-H), 0.83 (*s*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.2 (C-20), 145.2 (C-13), 138.7 (C-23), 135.6 (C-8), 128.7 (C-24, C-28), 127.8 (C-25, C-27), 127.4 (C-26), 122.4 (C-14), 120.4 (C-7), 50.9 (C-5), 46.4 (C-4), 45.7 (C-9), 43.9 (C-21), 38.3 (C-1), 37.7 (C-3), 34.9 (C-17), 34.7 (C-10), 27.4 (C-12), 25.4 (C-11), 23.9 (C-15), 22.5 (C-6), 21.4 (C-18), 20.8 (C-19), 18.3 (C-2), 14.1 (C-16) ppm;
MS (ESI, MeOH/$CHCl_3$, 4:1): *m*/*z* (%) = 392 (100%, $[M+H]^+$), 430 (52%, $[M+K]^+$);
analysis calcd for $C_{27}H_{37}NO$ (391.59): C 82.81, H 9.52, N 3.58; found: C 82.60, H 9.73, N 3.41.

Abieta-7,13-diene-*N*-(phenethyl)-18-amide (2)

Following GPA from **AB** (500 mg, 0.52 mmol) and phenethylamine (0.1 mL, 0.79 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 20%)] **2** (200 mg, 92%) was obtained as a colorless solid; R_f = 0.64 (SiO_2, toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p. = 78-81°C; $[\alpha]_D^{20}$ = -33.6° (*c* 0.055, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (4.24); IR (ATR): ν = 3356*w*, 2927*m*, 1628*s*, 1523*s*, 1497*m*, 1455*m*, 1384*m*, 1262*w*, 1196*w*, 1030*w*, 884*w*, 822*w*, 748*m*, 698*s*, 498*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 – 7.14 (*m*, 5H, 24-H, 25-H, 26-H, 27-H, 28-H), 5.75 (*s*, 1H, 14-H), 5.31 (*s*, 1H, 7-H), 3.61 – 3.43 (*m*, 2H, 21-H), 2.89 – 2.75 (*m*, 2H, 22-H), 2.22 (*hept*, *J* = 6.8 Hz, 1H, 17-H), 2.12 – 1.62 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.61 – 1.43 (*m*, 3H, 2-H, 3-H_b), 1.27 – 1.17 (*m*, 2H, 6-H_b, 1-H_b), 1.16 (*s*, 3H, 15-H), 1.01 (*m*, 6H, 18-H, 19-H), 0.80 (*s*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.4 (C-20), 145.3 (C-13), 139.2 (C-23), 135.7 (C-8), 129.0 (C-24, C-28), 128.8 (C-25, C-27), 126.6 (C-26), 122.6 (C-14), 120.6 (C-7), 51.1 (C-5), 46.4 (C-4), 45.8 (C-9), 41.0 (C-21), 38.4 (C-1), 37.7 (C-3), 35.8 (C-22), 35.0 (C-17), 34.7 (C-10), 27.6 (C-12), 25.4 (C-11), 24.1 (C-15), 22.6 (C-6), 21.6 (C-18), 21.0 (C-19), 18.4 (C-2), 14.3 (C-16) ppm;
MS (ESI, MeOH/$CHCl_3$, 4:1): *m*/*z* (%) = 444 (100%, $[M+K]^+$), 406 (96%, $[M+H]^+$);
analysis calcd for $C_{28}H_{39}NO$ (405.62): C 82.91, H 9.69, N 3.45; found: C 82.75, H 9.83, N 3.20.

Abieta-7,13-diene-*N*-(2-methoxybenzyl)-18-amide (3)

Following GPA from **AB** (500 mg, 0.52 mmol) and 2-methoxybenzylamine (0.1 mL, 0.77 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 20%)] **3** (210 mg, 97%) was obtained as a colorless solid; R_f = 0.65 (SiO_2, toluene/ethyl acetate/heptane/formic acid/formic acid, 80:26:10:5); m.p. = 84-87°C; $[\alpha]_D^{20}$ = -14.5° (*c* 0.152, MeOH);
UV-Vis (MeOH): λ_{max} (log ε) = 224 nm (4.39);
IR (ATR): ν = 2928*m*, 1636*m*, 1491*s*, 1240*s*, 1029*m*, 479*s* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26 – 7.20 (*m*, 2H, 26-H, 28-H), 6.96 – 6.81 (*m*, 2H, 25-H, 27-H), 5.73 (*s*, 1H, 14-H), 5.33 – 5.21 (*m*, 1H, 7-H), 4.49 – 4.32 (*m*, 2H, 21-H), 3.85 (*s*, 3H, 29-H), 2.21 (*d*, *J* = 12.4 Hz, 1H), 2.12 – 1.65 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.66 – 1.43 (*m*, 3H, 2-H, 3-H_b), 1.23 (*s*, 3H, 15-H), 1.29 – 1.11 (*m*, 2H, 6-H_b, 1-H_b), 1.00 (*m*, 6H, 18-H, 19-H), 0.81 (*s*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.4 (C-20), 158.0 (24), 145.5 (C-13), 135.8 (C-8), 130.4 (C-26), 129.1 (C-28), 126.9 (C-23), 122.8 (C-14), 121.1 (C-7), 121.0 (C-27), 110.7 (C-25), 55.7 (C-29), 51.4 (C-5), 46.7 (C-4), 46.2 (C-9), 40.4 (C-21), 38.7 (C-1), 37.8 (C-3), 35.3 (C-10), 35.0 (C-17), 27.8 (C-12), 25.6 (C-11), 24.4 (C-15), 22.9 (C-6), 21.8 (C-18), 21.3 (C-19), 14.5 (C-16) ppm;
MS (ESI, MeOH/$CHCl_3$, 4:1): *m*/*z* (%) = 422 (100%, $[M+H]^+$), 460 (18%, $[M+K]^+$);
analysis calcd for $C_{28}H_{39}NO_2$ (421.62): C 79.76, H 9.32, N 3.22; found: C 79.53, H 9.48, N 3.07.

Abieta-7,13-diene-*N*-(3-methoxybenzyl)-18-amide (4)

Following GPA from **AB** (500mg, 0.52 mmol) and 3-methoxy-benzylamine (0.1 mL, 0.77 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 25%)] **4** (200 mg, 90%) was obtained as a colorless solid; R_f = 0.69 (SiO_2, toluene/ethyl acetate/

heptane/formic acid, 80:26:10:5), m.p. = 73-75°C; $[\alpha]_D^{20}$ = -7.0° (*c* 0.151, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 224 nm (4.27);
IR (ATR): ν = 3355*w*, 2927*m*, 1632*s*, 1601*m*, 1520*s*, 1489*m*, 1384*w*, 1261*s*, 1155*m*, 1044*m*, 996*w*, 884w, 776*m*, 690*m* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 7.26 – 7.16 (*m*, 1H, 27-H), 6.89 – 6.73 (*m*, 3H, 24-H, 26-H, 28-H), 5.75 (*s*, 1H, 14-H), 5.33 (*s*, 1H, 7-H), 4.49 – 4.32 (*m*, 2H, 21-H), 3.79 (*s*, 3H, 29-H), 2.32 – 2.12 (*m*, 1H, 17-H), 2.11 – 1.66 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.59 (*m*, 3H, 2-H, 3-H_b), 1.28 – 1.27 (*m*, 2H, 6-H_b, 1-H_b), 1.25 – 1.16 (*m*, 3H, 15-H), 1.00 (*m*, 6H, 18-H, 19-H), 0.83 (*s*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.3 (C-20), 160.0 (C-25), 145.3 (C-13), 140.5 (C-23), 135.7 (C-8), 129.8 (C-27), 122.5 (C-14), 120.5 (C-7), 120.1 (C-28), 113.2 (C-26), 113.2 (C-24), 55.3 (C-29), 51.1 (C-5), 46.5 (C-4), 45.9 (C-9), 44.0 (C-21), 38.4 (C-1), 37.8 (C-3), 35.0 (C-17), 34.8 (C-10), 27.5 (C-12), 25.5 (C-11), 24.1 (C-15), 22.6 (C-6), 21.5 (C-18), 21.0 (C-19), 18.5 (2), 14.3 (16) ppm;
MS (ESI, MeOH/$CHCl_3$, 4:1): *m*/*z* (%) = 422 (100%, $[M+H]^+$), 460 (44%, $[M+K]^+$);
analysis calcd for $C_{28}H_{39}NO_2$ (421.62): C 79.76 H 9.32, N 3.22; found: C 79.47, H 9.58, N 3.04.

Abieta-7,13-diene-*N*-(4-methoxybenzyl)-18-amide (5)
Following GPA from **AB** (500mg, 0.52 mmol) and 4-methoxy-benzylamine (0.1 mL, 0.77 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 20%)] **5** (205 mg, 92%) was obtained as a colorless solid; R_f = 0.64 (SiO_2, toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p. = 85-88°C; $[\alpha]_D^{20}$ = -1.3° (*c* 0.480, MeOH);
UV-Vis (MeOH): λ_{max} (log ε) = 227 nm (4.67);
IR (ATR): ν = 3559*w*, 2928*m*, 1631*m*, 1511*s*, 1462*w*, 1245*s*, 1174*m*, 1035*m*, 829*m*, 755*m* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 7.21 – 7.13 (*m*, 2H, 24-H, 28-H), 6.92 – 6.79 (*m*, 2H, 25-H, 27-H), 5.75 (*s*, 1H, 14-H), 5.37 – 5.30 (*m*, 1H, 7-H), 4.47 – 4.24 (*m*, 2H, 21-H), 3.80 (*s*, 3H, 29-H), 2.26 – 2.15 (*m*, 1H, 17-H), 2.15 – 1.68 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.66 – 1.47 (*m*, 3H, 2-H, 3-H_b), 1.25 (*s*, 3H, 15-H), 1.23 – 1.15 (*m*, 2H, 6-H_b, 1-H_b), 1.00 (*m*, 6H, 18-H, 19-H), 0.84 – 0.81 (*m*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.2 (C-20), 159.1 (C-26), 145.4 (C-13), 135.7 (C-8), 130.9 (C-23), 129.3 (C-24, C-28), 122.6 (C-14), 120.6 (C- C-7), 114.3 (C-25, C-27), 55.4 (C-29), 51.1 (C-5), 46.5 (C-4), 45.9 (C-9), 43.6 (C-21), 38.4 (C-1), 37.8 (C-3), 35.0 (C-17), 34.8 (C-10), 27.6 (C-12), 25.5 (C-11), 24.1 (C-15), 22.6 (C-6), 21.5 (C-18), 21.0 (C-19), 18.5 (C-2), 14.3 (C-16) ppm;
MS (ESI, MeOH/$CHCl_3$, 4:1): *m*/*z* (%) = 422 (100%, $[M+H]^+$), 460 (80%, $[M+K]^+$);
analysis calcd for $C_{28}H_{39}NO_2$ (421.62): C 79.76, H 9.32, N 3.22; found: C 79.50, H 9.56, N 3.07.

Abieta-7,13-diene-*N*-(2-fluorobenzyl)-18-amide (6)
Following GPA from **AB** (500mg, 0.52 mmol) and 2-fluoro-benzylamine (0.1 mL, 0.87 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 20%)] **6** (170 mg, 78%) was obtained as a colorless solid; R_f = 0.73 (SiO_2, toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p. = 78-81°C; $[\alpha]_D^{20}$ = -21.5° (*c* 0.135, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (4.14);
IR (ATR): ν = 3348*w*, 2927*m*, 1633*s*, 1521*s*, 1488*s*, 1457*m*, 1384*w*, 1361*w*, 1227*m*, 1001*w*, 833*w*, 753*s*, 515*w* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 7.31 (*dd*, *J* = 7.5 Hz, 1H, 25-H), 7.28 – 7.21 (*m*, 1H, 27-H), 7.10 (*dd*, *J* = 7.5 Hz, 1H, 26-H), 7.06 – 7.01 (*m*, 1H, 24-H), 6.14 – 6.01 (*m*, 1H, NH), 5.74 (*s*, 1H, 14-H), 5.36 – 5.30 (*m*, 1H, 7-H), 4.55 – 4.36 (*m*, 2H, 21-H), 2.21 (*hept*, *J* = 6.7 Hz, 1H, 17-H), 2.13 – 1.65 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.63 – 1.47 (*m*, 3H, 2-H, 3-H_b), 1.26 (*s*, 3H, 15-H), 1.24 – 1.12 (*m*, 2H, 6-H_b, 1-H_b), 1.07 – 0.93 (*m*, 6H, 18-H, 19-H), 0.82 (*s*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.3 (C-20), 161.05 (*d*, *J* = 245.8 Hz, C-23), 145.2 (C-13), 135.5 (C-8), 130.38 (*d*, *J* = 4.3 Hz, C-25), 129.16 (*d*, *J* = 8.2 Hz, C-27), 125.55 (*d*, *J* = 14.7 Hz, C-22), 124.28 (*d*, *J* = 3.6 Hz, C-26), 122.4 (C-14), 120.5 (C-7), 115.32 (*d*, *J* = 21.3 Hz, C-24), 50.9 (C-5), 46.4 (C-4), 45.7 (C-9), 38.3 (C-1), 37.94 (*d*, *J* = 3.7 Hz, C-21), 37.6 (C-3), 34.9 (C-17), 34.6 (C-10), 27.4 (C-12), 25.2 (C-11), 22.5 (C-6), 21.4 (C-18), 20.8 (C-19), 18.3 (C-2), 17.0 (C-15), 14.1 (C-16) ppm;
MS (ESI, MeOH/$CHCl_3$, 4:1): *m*/*z* (%) = 410 (100%, $[M+H]^+$), 448 (64%, $[M+K]^+$);
analysis calcd for $C_{27}H_{36}FNO$ (409.58): C 79.18, H 8.86, N 3.42; found: C 78.85, H 9.03, N 3.11.

Abieta-7,13-diene-*N*-(3-fluorobenzyl)-18-amide (7)
Following GPA from **AB** (500mg, 0.52 mmol) and 3-fluoro-benzylamine (0.1 mL, 0.87 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 20%)] **7** (150 mg, 69%) was obtained as a colorless solid; R_f = 0.68 (SiO_2, toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p. = 78-81°C; $[\alpha]_D^{20}$ = -17.4° (*c* 0.168, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (4.06);
IR (ATR): ν = 3352*w*, 2927*m*, 1633*s*, 1591*m*, 1487*s*, 1449*s*, 1385*w*, 1251*s*, 1135*w*, 1000*w*, 885*w*, 778*m*, 682*m*, 520*m* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 7.31 – 7.27 (*m*, 1H, 27-H), 7.05 – 7.00 (*m*, 1H, 28-H), 6.99 – 6.91 (*m*, 2H, 24-H, 26-H), 6.10 – 5.99 (*m*, 1H, NH), 5.75 (*s*, 1H, 14-H), 5.35 – 5.30 (*m*, 1H, 7-H), 4.54 – 4.31 (*m*, 2H, 21-H), 2.21 (*hept*, *J* = 7.0 Hz, 1H, 17-H), 2.10 – 1.67 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.66 – 1.46 (*m*, 3H, 2-H, 3-H_b), 1.28 (*s*, 3H, 15-H), 1.25 – 1.14 (*m*, 2H, 6-H_b, 1-H_b), 1.04 - 0.97 (*m*, 6H, 18-H, 19-H), 0.87 – 0.81 (*m*, 3H, 16-H) ppm;

^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.3 (C-20), 162.98 (*d*, *J* = 246.5 Hz, C-25), 145.3 (C-13), 141.34 (*d*, *J* = 7.1 Hz, C-23), 135.6 (C-8), 130.18 (*d*, *J* = 8.2 Hz, C-27), 123.18 (*d*, *J* = 2.9 Hz, C-28), 122.4 (C-14), 120.3 (C-7), 114.51 (*d*, *J* = 21.6 Hz, C-24), 114.27 (*d*, *J* = 21.1 Hz, C-26), 51.0 (C-5), 46.4 (C-4), 45.7 (C-9), 43.4, 43.33 (*d*, *J* = 1.9 Hz, C-21), 38.3 (C-1), 37.8 (C-3), 34.9 (C-17), 34.7 (C-10), 27.4 (C-12), 25.4 (C-11), 22.5 (C-6), 21.4 (C-18), 20.8 (C-19), 18.3 (C-2), 17.1 (C-15), 14.1 (C-16) ppm;
MS (ESI, MeOH/$CHCl_3$ 4:1): *m*/*z* (%) = 410 (100%, $[M+H]^+$), 448 (38%, $[M+K]^+$);
analysis calcd for $C_{37}H_{36}FNO$ (409.58): C 79.18, H 8.86, N 3.42; found: C 78.86, H 9.09, N 3.19.

Abieta-7,13-diene-*N*-(4-fluorobenzyl)-18-amide (8)

Following GPA from **AB** (500mg, 0.52 mmol) and 4-fluoro-benzylamine (0.1 mL, 0.87 mmol) and chromatography [silica gel, *n*-hexane/ethyl acetate (5% → 20%)] **8** (160 mg, 74%) was obtained as a colorless solid; R_f = 0.68 (SiO_2, toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p. = 79-81°C; R_f = 0.71 (SiO_2, toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); $[\alpha]_D^{20}$ = -8.9° (*c* 0.166, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (4.23); IR (ATR): ν = 3351*w*, 2927*m*, 1631*m*, 1509*s*, 1384*w*, 1221*m*, 1096*m*, 1000*w*, 885*w*, 823*m*, 572*w*, 488*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 – 7.19 (*m*, 2H, 24-H, 28-H), 7.05 – 6.97 (*m*, 2H, 25-H, 27-H), 6.03 - 5.93 (*m*, 1H, NH), 5.75 (*s*, 1H, 14-H), 5.35 – 5.29 (*m*, 1H, 7-H), 4.45 – 4.31 (*m*, 2H, 21-H), 2.21 (*hept*, *J* = 7.0 Hz, 1H, 17-H), 2.15 – 1.64 (*m*, 9H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b), 1.64 – 1.45 (*m*, 3H, 2-H, 3-H_b), 1.26 (*s*, 3H, 15-H), 1.18 – 1.14 (*m*, 2H, 6-H_b, 1-H_b), 1.08 – 0.96 (*m*, 6H, 18-H, 19-H), 0.83 (*s*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 178.2 (C-20), 162.13 (*d*, *J* = 245.5 Hz, C-26), 145.3 (C-13), 135.6 (C-8), 134.54 (*d*, *J* = 3.7 Hz, C-23), 129.42 (*d*, *J* = 8.0 Hz, C-24, C-28), 122.4 (C-14), 120.3 (C-7), 115.50 (*d*, *J* = 21.4 Hz, C-25, C-27), 51.0 (C-5), 46.4 (C-4), 45.7 (C-9), 43.2 (C-21), 38.3 (C-1), 37.7 (C-3), 34.9 (C-17), 34.7 (C-10), 29.9 (C-12), 27.4 (C-11), 22.5 (C-6), 21.4 (C-18), 20.8 (C-19), 18.3 (C-2), 17.0 (C-15), 14.1 (C-16) ppm;
MS (ESI, MeOH/$CHCl_3$, 4:1): *m*/*z* (%) = 448 (100%, $[M+K]^+$), 410 (94%, $[M+H]^+$);
analysis calcd for $C_{37}H_{36}FNO$ (409.58): C 79.18, H 8.86, N 3.42; found: C 78.84, H 9.02, N 3.21.

Abieta-7,13-diene-*N*-(piperazin-1-yl)-18-amide (9)

Following GPA from **AB** (1.0 g, 1.05 mmol) and piperazine (100 mg, 3.15 mmol) and chromatography [silica gel, $CHCl_3$/MeOH (0% → 5%)] **9** (285 mg, 73%) was obtained as a colorless solid; R_f = 0.16 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. = 71-74°C;
$[\alpha]_D^{20}$ = -40.6° (*c* 0.141, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (4.17);
IR (ATR): ν = 2926*m*, 1616*s*, 1406*m*, 1383*m*, 1257*m*, 1226*s*, 1142*w*, 1021*m*, 885*w*, 751*s* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.76 (*s*, 1H, 14-H), 5.36 (*s*, 1H, 7-H), 3.90 – 3.58 (*m*, 4H, 21-H, 24-H), 2.97 – 2.84 (*m*, 4H, 22-H, 23-H), 2.43 – 1.38 (*m*, 13H, 17-H, 12-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b, 2-H, 3-H_b), 1.32 (*s*, 3H, 15-H), 1.30 – 1.17 (*m*, 2H, 6-H_b, 1-H_b), 1.01 (*m*, 6H, 18-H, 19-H), 0.85 (s, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 177.2 (C-20), 145.4 (C-13), 135.7 (C-8), 122.9 (C-14), 121.4 (C-7), 51.8 (C-5), 46.9 (C-22, C-23), 46.6 (C-21, C-24), 46.0 (C-4), 45.1 (C-9), 38.2 (C-1), 36.1 (C-3), 35.3 (C-17), 35.2 (C-10), 27.8 (C-12), 26.3 (C-11), 24.3 (C-15), 23.0 (C-6), 21.8 (C-18, C-19), 18.8 (C-2), 14.5 (C-16) ppm; MS (ESI, MeOH/$CHCl_3$ 4:1): *m*/*z* (%) = 371 (100%, $[M+H]^+$); analysis calcd for $C_{24}H_{36}N_2O$ (368.56): C 78.21, H 9.85, N 7.60; found: C 77.95, H 10.04, N 7.44.

Abieta-7,13-diene-N-(Homopiperazin-1-yl)-18-amide (10)

Following GPA from **AB** (1.0 g, 1.05 mmol) and homopiperazine (120 mg, 3.15 mmol) and chromatography [silica gel, $CHCl_3$/MeOH (0% → 5%)] **10** (385 mg, 85%) was obtained as a colorless solid; R_f = 0.12 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. = 80-82°C; $[\alpha]_D^{20}$ = -35.6° (*c* 0.075, $CHCl_3$);
UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (4.15);
IR (ATR): ν = 2927*m*, 1611*s*, 1456*m*, 1404*m*, 1382*m*, 1286*w*, 1216*w*, 1145*w*, 885*w*, 750*s*, 663*w* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.75 (*s*, 1H, 14-H), 5.37 (*s*, 1H, 7-H), 3.80 – 3.58 (*m*, 4H, 21-H, 25-H), 2.98 (*m*, 4H, 23-H, 24-H), 2.40 – 1.39 (*m*, 15H, 17-H, 12-H, 22-H, 9-H, 5-H, 11-H_a, 1-H_a, 3-H_a, 6-H_a, 11-H_b, 2-H, 3-H_b), 1.32 (*s*, 3H, 15-H), 1.32 – 1.18 (*m*, 2H, 6-H_b, 1-H_b), 1.00 (*m*, 6H, 18-H, 19-H), 0.86 (*s*, 3H, 16-H) ppm;
^{13}C NMR (101 MHz, $CDCl_3$): δ = 177.8 (C-20), 145.3 (C-13), 135.8 (C-8), 122.9 (C-14), 121.4 (C-7), 51.9 (C-5), 51.0 (C-21), 48.7 (C-24), 48.3 (C-25), 47.4 (C-4), 47.0 (C-23), 45.0 (C-9), 38.4 (C-1), 36.1 (C-3), 35.3 (C-17), 35.2 (C-10), 29.7 (C-22), 27.8 (C-12), 26.4 (C-11), 24.4 (C-15), 23.0 (C-6), 21.8 (C-18), 21.2 (C-19), 18.9 (C-2), 14.6 (16) ppm;
MS (ESI, MeOH/$CHCl_3$ 4:1): *m*/*z* (%) = 385 (100%, $[M+H]^+$);
analysis cald for $C_{25}H_{40}N_2O$ (384.60): C 78.07, H 10.48, N 7.28; found: C 77.83, H 10.61, N 7.00.

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