Bioorganic Chemistry 90 (2019) 103092

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Novel 12-hydroxydehydroabietylamine derivatives act as potent and selective butyrylcholinesterase inhibitors

Anne Loesche[a], Jana Wiemann[a], Matthias Rohmer[a], Wolfgang Brandt[b], René Csuk[a,*]

[a] Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany
[b] Leibniz Institute of Plant Biochemistry, Bioorganic Chemistry, Weinberg 3, D-06120 Halle (Saale), Germany

A B S T R A C T

The skeleton of the diterpene dehydroabietylamine was modified, and a set of 12-hydroxy-dehydroabietylamine derivatives was obtained. The compounds were screened in colorimetric Ellman's assays to determine their ability to act as inhibitors for the enzymes acetylcholinesterase (AChE, from electric eel) and butyrylcholinesterase (BChE, from equine serum). Additional investigations concerning the enzyme kinetics were performed and showed 12-hydroxy-*N*-(4-nitro-benzoyl) dehydroabietylamine (**13**) and 12-hydroxy-*N*-(isonicotinoyl)dehydroabietylamine (**17**) as selective BChE inhibitors holding good inhibition constants $K_i = 0.72 \pm 0.06\,\mu M$ and $K_i = 0.86 \pm 0.19\,\mu M$, respectively.

1. Introduction

The pathology of Alzheimer's disease (AD) is characterized by an irreversible decline of memory and of recognition caused *inter alia* by a deficit of cholinergic functions. Especially a decreased concentration of the primary neurotransmitter acetylcholine (ACh) has been considered to be a main factor for the progressive cognitive impairment [1–3]. ACh is mainly located in the cholinergic neurons, responsible for various behavioral and physiological functions of the cholinergic neurotransmission. The impulse transmissions at the cholinergic synapse are terminated by the rapid hydrolysis of the ACh by two types of cholinesterases (ChEs): the acetylcholinesterase (AChE) and the butyrylcholinesterase (BChE).[3] The use of ChE inhibitors is among the best established prime therapeutic strategies for alleviating the symptoms of AD. Therefore, the search for new inhibitors continues to play an important role in scientific research [4–8]. Inhibition of these enzymes prevents the degradation of ACh, and its deficit in the cholinergic neurons (that is observed in the brain of AD patients) can be compensated [9]. This is a symptomatic treatment but leads to a significant improvement of the cognitive functions and - as a consequence - enhances the patient's quality of life. In healthy human brains AChE is the predominating enzyme responsible for the hydrolysis of ACh to choline and acetate, whereas BChE was attributed a subordinate, supportive role for a long time [10]. However, in the progression of AD a significant change in the AChE and BChE activities has been observed in certain parts of the brain: the AChE/BChE ratio shifts from 11.0 to 0.2 and BChE dominates the degradation of Ach [10–12]. The effect of drugs targeting AChE on the symptoms of AD is rapidly decreasing with the progression of the disease. Thus, targeting BChE and designing selective inhibitors seems to be a promising option for treating late-stage AD [13–15]. Different studies concerning the use of selective BChE inhibitors pointed out the potential and the advantages of BChE inhibitors as compared to the use of AChE inhibitors in the context of AD [16–18].

Our research is focused on the derivatization of natural products to enhance their biological activity and to increase their selectivity for chosen enzymes. In this context, we investigated the inhibitory potency of several terpene derived substances for cholinesterases. These studies showed the remarkable potential of dehydroabietylamides to act as AChE inhibitors. Several derivatives showed an inhibitory potency comparable or even supreme to standard galantamine hydrobromide, while BChE was significantly less affected [19]. In addition, previous studies pointed out the promising ChE inhibitory potency of ferruginol (holding a hydroxy moiety at C-12 of the C-ring of the aromatic abietane scaffold) [20]. Subsequently, we focused on the investigation of the influence of C-ring modifications of dehydroabietylamine on the inhibitory activity of these derivatives.

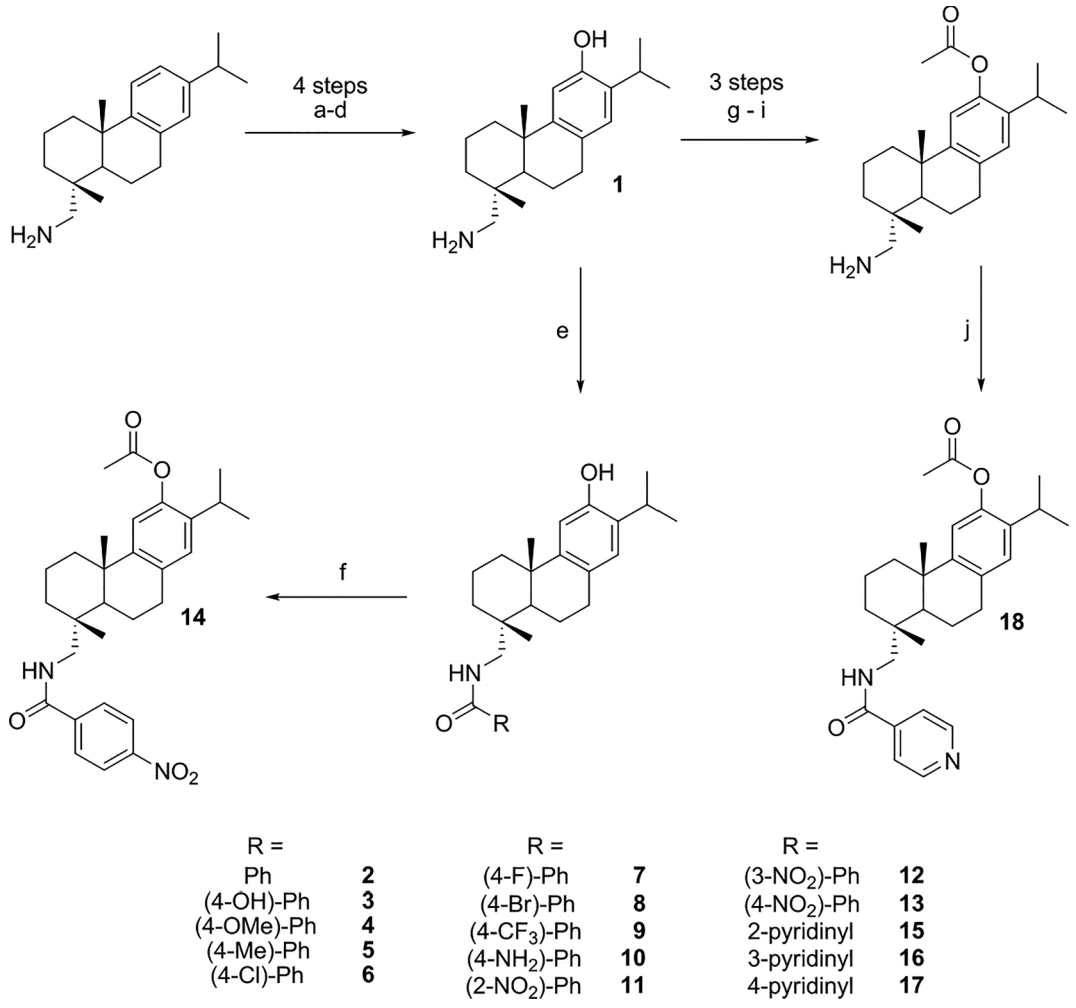
2. Results and discussion

2.1. Chemistry

Starting from the diterpene dehydroabietylamine C-ring modifications were performed according to González *et al.* (Scheme 1), and 12-hydroxydehydroabietylamine (**1**) was obtained after four steps in 51% isolated yield [21]. In the following we focused on modifications of the

* Corresponding author.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

https://doi.org/10.1016/j.bioorg.2019.103092
Received 6 January 2019; Received in revised form 23 June 2019; Accepted 26 June 2019
Available online 02 July 2019



Scheme 1. Synthesis and derivatization of 12-hydroxydehydroabietylamine (**1**): (a) phthalic anhydride, pyridine, reflux, 2.5 h, 72%; (b) AcCl, $AlCl_3$, DCM, 0 °C to rt, 2 h, 92%; (c) *m*CPBA, TFA, DCM, 0 °C to rt, overnight, 86%; (d) $N_2H_4{\cdot}H_2O$, EtOH, 80 °C, 7 h, 89%; (e) aromatic carboxylic acid, EDC, HOBt, DCM, 0 °C to rt, overnight, 23–94%; (f) AcCl, TEA, DCM, 0 °C to rt, 30 min, 61%; (g) Boc_2O, NaOH, THF, 0 °C to rt, 2 h, 74%; (h) AcCl, TEA, DCM, 0 °C to rt, 30 min, 88%; (i) TFA, DCM, 0 °C to rt, 1.5 h, 95%; (j) isonicotinic acid, EDC, HOBt, DCM, 0 °C to rt, overnight, 77%.

Table 1
Grade of inhibition (I in %) and inhibition constants (K_i and K_i' in μM) for compounds **1–18** and galantamine hydrobromide (**GH**) as a standard, determined by Ellman's assays employing butyrylcholinesterase (BChE, equine serum). Percent inhibition was determined at 10 μM concentration; each experiment was performed at least in duplicate.

Comp.	K_i/K_i' in μM (I in %)	Type of inhibition	SI	Comp.	K_i/K_i' in μM (I in %)	Type of inhibition	SI
GH	3.06 ± 0.11 (57.29 ± 0.25)	competitive	0.2	**10**	(5.16 ± 0.10)[a]		1.0
1	(31.65 ± 0.05)		1.3	**11**	(11.21 ± 0.16)		0.6
2	(31.80 ± 0.41)		1.3	**12**	1.44 ± 0.14/7.00 ± 1.44 (75.46 ± 0.12)	mixed-type	1.9
3	(6.06 ± 0.61)[a]		1.0	**13**	0.72 ± 0.06 (71.01 ± 0.26)	competitive	3.2
4	(44.94 ± 0.46)		1.1	**14**	(7.48 ± 0.36)		0.9
5	(34.66 ± 0.10)		0.9	**15**	(15.84 ± 0.17)		1.0
6	(40.54 ± 0.26)		1.3	**16**	1.02 ± 0.09 (74.53 ± 0.66)	competitive	3.4
7	(41.23 ± 0.42)		1.4	**17**	0.86 ± 0.19 (75.87 ± 0.72)	competitive	3.7
8	(42.11 ± 0.26)		1.4	**18**	(6.90 ± 0.97)		1.1
9	2.61 ± 0.14/11.10 ± 0.39 (59.54 ± 0.11)	mixed-type	0.2				

[a] I at 5 μM (limitation due to reduced solubility); mean ± SE; selectivity index for BChE: $SI = (100–I_{AChE})/(100–I_{BChE})$. The type of inhibition as well as the inhibition constants K_i (competitive part) and K_i' (uncompetitive part) were determined using Dixon, Cornish-Bowden and Lineweaver-Burk plots for those compounds showing the highest rate of inhibition in the preliminary screening.

NH_2 moiety of compound **1**. Thus, amide compounds were synthesized by EDC/HOBt coupling reactions (Scheme 1). The structure of these 12-hydroxydehydroabietylamides was confirmed by observing signals for their carbonyl moieties (C–21, δ = 167.9–164.6 ppm) in the ^{13}C NMR spectra, the signals of the aromatic protons (δ = 9.10–6.50 ppm) and of the NH moiety (δ = 8.53–5.82 ppm) in the ^{1}H NMR spectra.

To prove the importance of the hydroxyl group for the inhibitory efficacy, acetylation of the two most potent inhibitors completed the reaction sequence. Therefore, compound **13** was directly acetylated by the use of acetyl chloride and triethylamine in DCM (Scheme 1), and compound **14** was formed. Regarding an optimization of the synthesis of compound **18**, the acetylation was preceded to the amidation (Scheme 1). The NMR spectra of compound **14** and **18** were characterized by distinct signals assigned to the acetyl moiety. The singlet signal at δ = 2.29 ppm in the ^{1}H NMR spectrum and the signal of the carbonyl moiety (δ = 170.2 ppm) in the ^{13}C NMR spectrum confirmed the structural modification.

2.2. Biology

Compounds **1–18** were screened in colorimetric Ellman's assays to determine their ability to inhibit the enzymes AChE (*Electrophorus electricus*) and BChE (from equine serum) using the anti-AD drug galantamine hydrobromide (**GH**) as a standard. The results are compiled in Table 1.

Compound **1** was a weak inhibitor for AChE and BChE with inhibition rates of 11.51 ± 1.74% and 31.65 ± 0.05%, respectively. The insertion of a benzoyl group did not lead to an increase of activity, and compound **2** showed inhibition rates of 12.41 ± 0.85% (AChE) and 31.80 ± 0.41% (BChE). All tested substances except **9** (see supplementary material) showed no significant inhibition for AChE. The *p*-trifluoromethyl derivative **9** acted as a mixed-type inhibitor for both AChE (93.22 ± 0.09%; K_i = 4.73 ± 0.85 µM, K_i' = 2.19 ± 0.08 µM) and BChE (59.54 ± 0.11%; K_i = 2.61 ± 0.14 µM, K_i' = 11.10 ± 0.39 µM). In part, the substitution of the aromatic ring had a significant impact on BChE inhibition. In general, introducing a *para*-methoxy substituent (**4**) or a halogen moiety (**6–8**) seems to result in moderate inhibition rates (between 40.54 ± 0.26% and 44.94 ± 0.46%). In the case of the nitro derivatives **11–13** the influence of the position of the substituent was tested, too. The investigation showed ortho-substituted **11** as the weakest BChE inhibitor (11.21 ± 0.16%), whereas *meta*- and *para*-substituted compounds held much higher inhibition rates (> 70%). For mixed-type inhibitor **12** low inhibitions constants (K_i = 1.44 ± 0.14 µM and K_i' = 7.00 ± 1.44 µM) were determined. 12-Hydroxy-*N*-(4-nitrobenzoyl)dehydroabietylamine (**13**) acted like **GH** as a competitive inhibitor (Fig. 1) and gave an excellent K_i value as low as 0.72 ± 0.06 µM. Therefore, **13** was the most active compound in the test. The insertion of a pyridinyl moiety gave compounds with similar inhibitory properties. Thus, 12-hydroxy-*N*-(nicotinoyl)dehydroabietylamine **16** and the isonicotinoyl derivative **17** gave splendid K_i values in single-digit micromolar range (K_i = 1.02 ± 0.09 µM and K_i = 0.86 ± 0.19 µM, respectively), showing competitive inhibition of BChE. Compared to anti-AD drug **GH**, these competitive inhibitors showed significantly lower K_i values. Acetylation of the most active compounds (**13** and **17**) reduced the activity significantly, and inhibitory rates for BChE of only 7.48 ± 0.36% (**14**) and 6.90 ± 0.97% (**18**) were found.

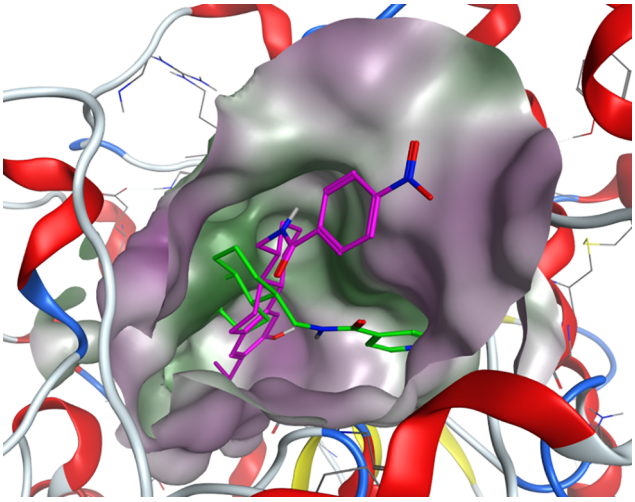


Fig. 2. View from the top to the binding site of human BChE with bound compounds **13** (magenta carbon atoms) and **17** (green carbon atoms) with displayed lipophilic potential (green surface). The more hydrophilic site is colored magenta.

To gain an insight into the interaction of compounds **13** and **17** and the enzyme, some molecular modeling studies were performed.

The most favored docking arrangement of **13** (bright magenta carbon atoms) and **17** (green carbon atoms) are shown in Fig. 2. Compound **13** is docked deep into the hydrophobic binding site with the nitro group located at the water accessible surface, whereas compound **17** is docked almost perpendicular to **13**. The docking pose of **17** results from the preferred interaction with hydrophobic residues like W79, F326, L283, and particularly with W228 supported by two hydrogen bonds formed with the hydroxyl group of the ligand and the back-bone carbonyl group of G75 and hydroxyl side chain of Y435 (Fig. 3). The different orientation of compound **13** is caused by the nitro substituent. This substituent prevents the interaction with W228 at the

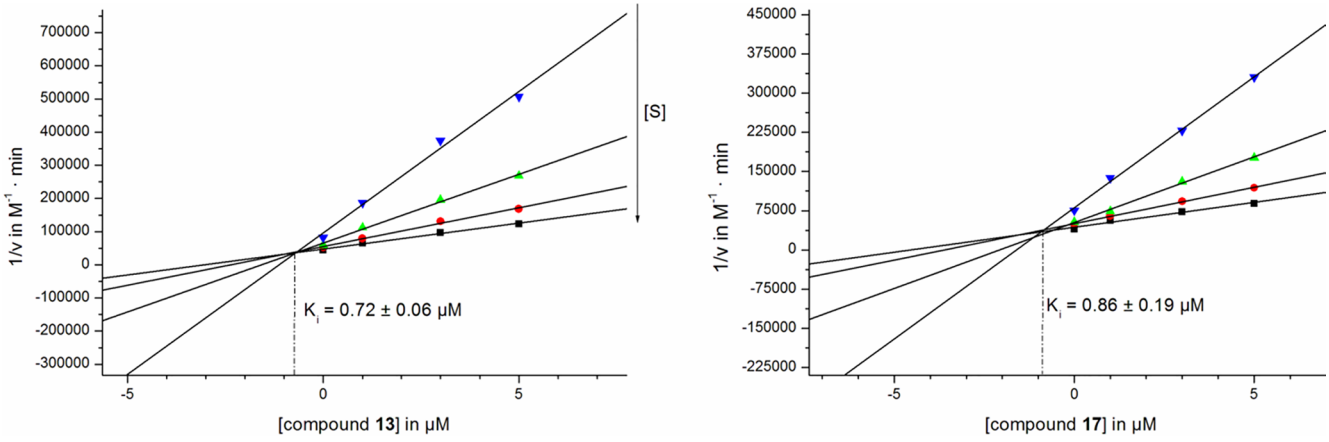


Fig. 1. Dixon plots for BChE inhibitors **13** (left) and **17** (right).

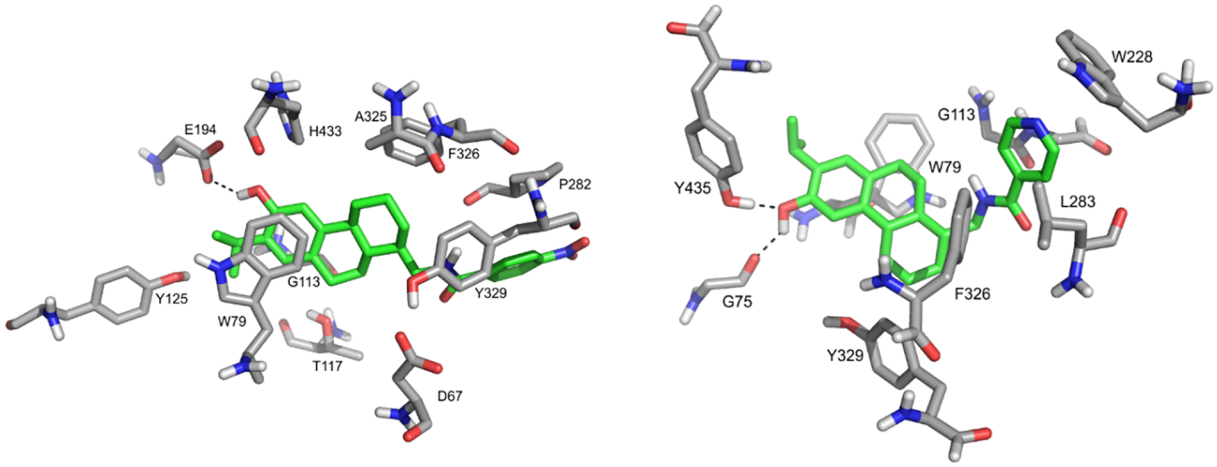


Fig. 3. Details of the most favored docking arrangement of **13** (left); preferentially hydrophobic interactions with W79, F326 and P282 and additionally hydrogen bonds formed with E194 stabilized the docking pose. Details of the most favored docking arrangement of **17** (right); preferentially hydrophobic interactions with W79, F326, L283 and W228 and additionally hydrogen bonds formed with G75 and Y435 stabilized the docking pose.

hydrophobic binding site, whereas a hydrophobic interaction with P282 and the formation of a strong hydrogen bond with E194 supports this docking arrangement (Fig. 3).

3. Conclusion

In summary, 12-hydroxydehydroabietylamine derivatives were easily accessed by synthesis, their structures were determined by the combination of modern spectroscopic techniques, and subsequent screening of the compounds in Ellman's assays showed some of them as selective inhibitors for BChE. Additional enzyme kinetic studies were performed. The most active derivatives were *p*-nitro substituted compound **13** and isonicotinoyl analog **17**, showing K_i values of 0.72 ± 0.06 μM and 0.86 ± 0.19 μM, respectively. These compounds proved to be competitive inhibitors for butyrylcholinesterase.

4. Experimental part

4.1. General

Detailed procedures for the screening and modeling as well as a description of the equipment is given in the Supplementary material.

4.2. General procedure of the amide coupling reaction

The carboxylic acid (1.2 eq.) was suspended in DCM (dry, 10 mL/100 mg carboxylic acid) and cooled to 0 °C. HOBt (1.4 eq.) and EDC (1.4 eq.) were added. After 30 min of stirring at 0 °C, the amine (1 eq.) was added. After completion of the reaction (as indicated by TLC) and usual aqueous work-up the residue was subjected to column chromatography [19].

4.3. Syntheses

4.3.1. 12-Hydroxydehydroabietylamine (1)

Compound **1** was synthesized as previously described [22]; R_F = 0.38 (silica gel, chloroform/methanol, 9:1); mp = 193–198 °C; $[\alpha]_D$ = +50.9° (*c* = 0.36, $CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 302.2 ($[M + H]^+$, 100).

4.3.2. 12-Hydroxy-N-benzoyldehydroabietylamine (2)

Compound **2** was prepared according to the general procedure from benzoic acid (0.05 g, 0.40 mmol) and compound **1** (0.10 g, 0.33 mmol) followed by column chromatography (silica gel, *n*-hexane/ethyl acetate, 7:3). Compound **2** [22] (0.07 g, 53%) was obtained as a colorless solid; R_F = 0.32 (silica gel, *n*-hexane/ethyl acetate, 7:3); mp = 194–196 °C; $[\alpha]_D$ = +6.6° (*c* = 0.34, $CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 406.3 ($[M+H]^+$, 100), 428.3 ($[M+Na]^+$, 52), 460.3 ($[M+Na+MeOH]^+$, 8).

4.3.3. 12-Hydroxy-N-(4-hydroxybenzoyl)dehydroabietylamine (3)

Compound **3** was prepared according to the general procedure from 4-hydroxybenzoic acid (0.08 g, 0.60 mmol) and compound **1** (0.20 g, 0.66 mmol). Column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 1:1:2) gave compound **3** [22] (0.18 g, 65%) as a colorless solid; R_F = 0.47 (silica gel, *n*-hexane/ethyl acetate/chloroform, 1:1:2); mp = 261–263 °C; $[\alpha]_D$ = +30.7° (*c* = 0.34, DMSO); MS (ESI, MeOH): *m/z* (%) = 422.2 ($[M+H]^+$, 20), 444.3 ($[M+Na]^+$, 30), 843.3 ($[2M+H]^+$, 10), 865.2 ($[2M+Na]^+$, 100).

4.3.4. 12-Hydroxy-N-(4-methoxybenzoyl)dehydroabietylamine (4)

Compound **4** was prepared according to the general procedure from 4-methoxybenzoic acid (0.12 g, 0.79 mmol) and compound **1** (0.20 g, 0.66 mmol). Column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 1:1:2) gave compound **4** [22] (0.22 g, 77%) as a colorless solid; R_F = 0.56 (silica gel, *n*-hexane/ethyl acetate, 6:4); mp = 227–229 °C; $[\alpha]_D$ = +5.3° (*c* = 0.36, $CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 436.3 ($[M+H]^+$, 21), 458.3 ($[M+Na]^+$, 20), 871.2 ($[2M+H]^+$, 25), 893.3 ($[2M+Na]^+$, 100).

4.3.5. 12-Hydroxy-N-(4-methylbenzoyl)dehydroabietylamine (5)

Compound **5** was prepared according to the general procedure from 4-methylbenzoic acid (0.08 g, 0.60 mmol) and compound **1** (0.15 g, 0.50 mmol). Column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10) gave compound **5** [22] (0.10 g, 48%) as a colorless solid; R_F = 0.36 (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10); mp = 243–245 °C; $[\alpha]_D$ = +11.3° (*c* = 0.30, $CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 420.3 ($[M+H]^+$, 18), 442.3 ($[M+Na]^+$, 12), 839.3 ($[2M+H]^+$, 48), 861.3 ($[2M+Na]^+$, 100).

4.3.6. 12-Hydroxy-N-(4-chlorobenzoyl)dehydroabietylamine (6)

Compound **6** was prepared according to the general procedure from 4-chlorobenzoic acid (0.09 g, 0.60 mmol) and compound **1** (0.15 g, 0.50 mmol) followed by column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10) to yield compound **6** [22] (0.13 g, 60%) as a colorless solid; R_F = 0.45 (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10); mp = 246–248 °C; $[\alpha]_D$ = +9.8° (*c* = 0.33, DMSO), MS (ESI, MeOH): *m/z* (%) = 440.2 ($[M+H]^+$, 56), 462.3 ($[M+Na]^+$, 35), 901.2 ($[2M+Na]^+$, 100).

4.3.7. 12-Hydroxy-N-(4-fluorobenzoyl)dehydroabietylamine (7)

Compound **7** was prepared according to the general procedure from 4-fluorobenzoic acid (0.06 g, 0.43 mmol) and compound **1** (0.10 g, 0.33 mmol) followed by column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10) to yield **7** (0.09 g, 64%) as a colorless solid; R_F = 0.46 (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10); mp = 243–245 °C; $[\alpha]_D$ = +30.9° (*c* = 0.34, DMSO), IR (ATR): *ν* = 3165*br*, 2957 *m*, 2921 *m*, 2846*w*, 1640 *s*, 1550 *s*, 1502*vs*, 1446 *m*, 1421 *s*, 1288 *s*, 1233*vs*, 1197 *m*, 1159 *s*, 853 *s*, 763 *s*, 749 *m*, 733 *m*, 600 *s*, 573 *m* cm^{-1}; UV–vis (DMSO): λ_{max} (log ε) = 264 nm (3.51); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.71 (s, 1H, OH), 8.21 (dd, *J* = 6.3, 6.3 Hz, 1H, NH), 7.91–7.83 (*m*, 2H, 23-H), 7.29–7.20 (*m*, 2H, 24-H), 6.66 (s, 1H, 14-H), 6.61 (s, 1H, 11-H), 3.40 (dd, *J* = 13.4, 7.0 Hz, 1H, 18-H_a) 3.06 (*hept*, *J* = 6.8 Hz, 1H, 15-H), 2.98 (dd, *J* = 13.5, 5.8 Hz, 1H, 18-H_b), 2.73–2.57 (*m*, 2H, 7-H_a + 7-H_b), 2.12–2.05 (*m*, 1H, 1-H_a), 1.98–1.90 (*m*, 1H, 6-H_a), 1.76–1.63 (*m*, 1H, 2-H_a), 1.62–1.51 (*m*, 2H, 6-H_b + 2-H_b), 1.46 (ddd, *J* = 13.3, 13.3, 3.8 Hz, 1H, 3-H_a), 1.38–1.31 (*m*, 2H, 5-H + 3H_b), 1.26–1.16 (*m*, 1H, 1-H_b), 1.12 (s, 3H, 20-H), 1.10 (d, *J* = 6.9 Hz, 3H, 16-H), 1.08 (d, *J* = 6.9 Hz, 3H, 17-H), 0.90 (s, 3H, 19-H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 165.7 (C-21), 163.7 (d, *J* = 248.0 Hz, C-25), 152.0 (C-13), 147.3 (C-9), 131.3 (C-22), 131.3 (C-12), 129.9 (d, *J* = 8.9 Hz, C-24), 125.9 (C-14), 124.7 (C-8), 115.0 (d, *J* = 21.6 Hz, C-23), 110.2 (C-11), 49.4 (C-18), 44.7 (C-5), 38.1 (C-1), 37.8 (C-4), 36.9 (C-10), 35.8 (C-3), 29.1 (C-7), 26.1 (C-15), 25.3 (C-20), 22.6 (C-17), 22.5 (C-16), 18.9 (C-19), 18.9 (C-6), 18.3 (C-2) ppm; ^{19}F NMR (470 MHz, DMSO-d_6): δ = −109.9 (*m*, F) ppm; MS (ESI, MeOH): *m/z* (%) = 424.2 ($[M+H]^+$, 66), 446.2 ($[M+Na]^+$, 16), 847.2 ($[2M+H]^+$, 52), 869.1 ($[2M+Na]^+$, 100), 901.2 ($[2M+Na+MeOH]^+$, 20); analysis calculated for $C_{27}H_{34}FNO_2$ (423.56): C 76.56, H 8.09, N 3.31; found: C 76.31, H 8.10, N 3.07.

4.3.8. 12-Hydroxy-N-(4-bromobenzoyl)dehydroabietylamine (8)

Compound **8** was prepared according to the general procedure from 4-bromobenzoic acid (0.16 g, 0.80 mmol) and compound **1** (0.20 g, 0.66 mmol) followed by column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10) to yield compound **8** (0.21 g, 65%) as a colorless solid; R_F = 0.53 (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10); mp = 242–245 °C; $[\alpha]_D$ = +16.4° (*c* = 0.33, DMSO), IR (ATR): *ν* = 3208*br*, 2949 *m*, 2925 *m*, 2865 *m*, 1629*vs*, 1590 *s*, 1548*vs*, 1482 *s*, 1417 *s*, 1306 *s*, 1236 *s*, 1180 *s*, 1074 *s*, 1011*vs*, 844 *s*, 755 *m*, 711 *m*, 562*vs* cm^{-1}; UV–vis ($CHCl_3$): λ_{max} (log ε) = 264 nm (3.99); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.71 (s, 1H, OH), 8.28 (dd, *J* = 6.3, 6.3 Hz, 1H, NH), 7.78–7.72 (*m*, 2H, 23-H), 7.67–7.60 (*m*, 2H, 24-H), 6.67 (s, 1H, 14-H), 6.61 (s, 1H, 11-H), 3.40 (dd, *J* = 13.4, 7.0 Hz, 1H,18-H_a) 3.07 (*hept*, *J* = 6.9 Hz, 1H, 15-H), 2.98 (dd, *J* = 13.4, 5.8 Hz, 1H, 18-H_b), 2.72–2.59 (*m*, 2H, 7-H_a + 7-H_b), 2.13–2.06 (*m*, 1H, 1-H_a), 1.98–1.90 (*m*, 1H, 6-H_a), 1.75–1.63 (*m*, 1H, 2-H_a), 1.63–1.52 (*m*, 2H, 6-H_b + 2-H_b), 1.45 (ddd, *J* = 13.3, 13.3, 3.7 Hz, 1H, 3-H_a), 1.38–1.31 (*m*, 2H, 5-H + 3H_b), 1.21 (ddd, *J* = 12.8, 12.8, 3.3 Hz, 1H, 1-H_b), 1.12 (s, 3H, 20-H), 1.10 (d, *J* = 6.9 Hz, 3H, 16-H), 1.08 (d, *J* = 6.9 Hz, 3H, 17-H), 0.90 (s, 3H, 19-H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 165.9 (C-21), 152.0 (C-13), 147.3 (C-9), 134.0 (C-22), 131.3 (C-12), 131.1 (C-24), 129.4 (C-23), 125.9 (C-14), 124.7 (C-8), 124.6 (C-25), 110.2 (C-11), 49.5 (C-18), 44.7 (C-5), 38.1 (C-1), 37.8 (C-4), 36.9 (C-10), 35.8 (C-3), 29.1 (C-7), 26.1 (C-15), 25.3 (C-20), 22.6 (C-17), 22.5 (C-16), 18.9 (C-19), 18.9 (C-6), 18.3 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 484.2 ($[M+H]^+$, 53), 508.1 ($[M+Na]^+$, 35), 969.1 ($[2M+H]^+$, 46), 990.9 ($[2M+Na]^+$, 100); analysis calculated for $C_{27}H_{34}BrNO_2$ (484.47): C 66.94, H 7.07, N 2.89; found: C 66.871, H 7.31, N 2.55.

4.3.9. 12-Hydroxy-N-(4-trifluoromethylbenzoyl)dehydroabietylamine (9)

Compound **9** was prepared according to general procedure from 4-(trifluoromethyl)benzoic acid (0.15 g, 0.79 mmol) and compound **1** (0.20 g, 0.66 mmol) followed by column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10) to yield compound **9** (0.26 g, 83%) as a colorless solid; R_F = 0.62 (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10); mp = 279–282 °C; $[\alpha]_D$ = +27.6° (*c* = 0.37, DMSO), IR (ATR): *ν* = 3325*br*, 2945*w*, 2929*w*, 2871*w*, 2855*w*, 1653 *m*, 1539 *m*, 1510 *m*, 1419 *m*, 1325*vs*, 1289 *m*, 1237 *m*, 1169 *s*, 1134*vs*, 1068 *s*, 1018 *m*, 853 *s*, 767 *m* cm^{-1}; UV–vis (DMSO): λ_{max} (log ε) = 270 nm (3.50); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.72 (s, 1H, OH), 8.43 (dd, *J* = 6.2, 6.2 Hz, 1H, NH), 7.99 (d, *J* = 8.1 Hz, 2H, 23-H), 7.80 (d, *J* = 8.3 Hz, 2H, 24-H), 6.67(s, 1H, 14-H), 6.61 (s, 1H, 11-H), 3.42 (dd, *J* = 13.4, 6.9 Hz, 1H,18-H_a) 3.13–2.98 (*m*, 2H, 15-H + 18-H_b), 2.76–2.58 (*m*, 2H, 7-H_a + 7-H_b), 2.14–2.05 (*m*, 1H, 1-H_a), 1.99–1.90 (*m*, 1H, 6-H_a), 1.79–1.64 (*m*, 1H, 2-H_a), 1.63–1.53 (*m*, 2H, 6-H_b + 2-H_b), 1.51–1.41 (*m*, 1H, 3-H_a), 1.40–1.31 (*m*, 2H, 5-H + 3H_b), 1.30–1.16 (*m*, 1H, 1-H_b), 1.13 (s, 3H, 20-H), 1.10 (d, *J* = 7.0 Hz, 3H, 16-H), 1.08 (d, *J* = 7.0 Hz, 3H, 17-H), 0.92 (s, 3H, 19-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.8 (C-21), 152.0 (C-13), 147.3 (C-9), 138.7 (C-22), 131.4 (C-12), 130.9 (q, *J* = 31.8 Hz, C-25), 128.2 (C-23), 125.9 (C-14), 125.2 (q, *J* = 3.7 Hz, C-24), 124.7 (C-8), 123.9 (q, *J* = 272.4 Hz, C-26), 110.2 (C-11), 49.5 (C-18), 44.7 (C-5), 38.1 (C-1), 37.9 (C-4), 37.0 (C-10), 35.8 (C-3), 29.1 (C-7), 26.1 (C-15), 25.3 (C-20), 22.6 (C-17), 22.5 (C-16), 18.9 (C-19), 18.9 (C-6), 18.3 (C-2) ppm; ^{19}F NMR (376 MHz, DMSO-d_6): δ = -61.3 (s, F) ppm; MS (ESI, MeOH): *m/z* (%) = 474.2 ($[M+H]^+$, 62), 496.1 ($[M+Na]^+$, 17), 947.1 ($[2M+H]^+$, 36), 969.1 ($[2M+Na]^+$, 100); analysis calculated for $C_{28}H_{34}F_3NO_2$ (473.57): C 71.01, H 7.24, N 2.96; found: C 70.84, H 7.39, N 2.73.

4.3.10. 12-Hydroxy-N-(4-aminobenzoyl)dehydroabietylamine (10)

Compound **10** was prepared according to the general procedure from 4-aminobenzoic acid (0.09 g, 0.66 mmol) and compound **1** (0.16 g, 0.53 mmol) followed by column chromatography (silica gel, ethyl acetate/chloroform, 1:2) to yield compound **10** (0.07 g, 23%) as a colorless solid; R_F = 0.44 (silica gel, ethyl acetate/chloroform, 1:2); mp = 280–284 °C; $[\alpha]_D$ = +32.2° (*c* = 0.32, DMSO), IR (ATR): *ν* = 3478*w*, 3354 *m*, 2933*w*, 2865*w*, 1625 *m*, 1604 *s*, 1597 *s*, 1551 *s*, 1509 *s*, 1418 *m*, 1301*vs*, 1239 *m*, 1187 *s*, 840 *m*, 768 *m* cm^{-1}; UV–vis (DMSO): λ_{max} (log ε) = 304 nm (4.41); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.70 (s, 1H, OH), 7.69 (dd, *J* = 6.3, 6.3 Hz, 1H, NH), 7.54 (d, *J* = 8.6 Hz, 2H, 23-H), 6.66 (s, 1H, 14-H), 6.60 (s, 1H, 11-H), 6.50 (d, *J* = 8.6 Hz, 2H, 24-H), 5.53 (s, 2H, NH_2), 3.36 (dd, *J* = 13.4, 7.1 Hz, 1H,18-H_a) 3.06 (*hept*, *J* = 6.8 Hz, 1H, 15-H), 2.92 (dd, *J* = 13.4, 5.7 Hz, 1H, 18-H_b), 2.72–2.55 (*m*, 2H, 7-H_a + 7-H_b), 2.14–2.04 (*m*, 1H, 1-H_a), 2.00–1.89 (*m*, 1H, 6-H_a), 1.76–1.62 (*m*, 1H, 2-H_a), 1.62–1.50 (*m*, 2H, 6-H_b + 2-H_b), 1.44 (ddd, *J* = 13.2, 13.2, 3.5 Hz, 1H, 3-H_a), 1.36–1.27 (*m*, 2H, 5-H + 3H_b), 1.24–1.15 (*m*, 1H, 1-H_b), 1.11 (s, 3H, 20-H), 1.10 (d, *J* = 7.2 Hz, 3H, 16-H), 1.09 (d, *J* = 6.9 Hz, 3H, 17-H), 0.87 (s, 3H, 19-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 166.6 (C-21), 152.0 (C-13), 151.4 (C-25), 147.4 (C-9), 131.3 (C-12), 125.8 (C-14), 124.8 (C-8), 128.8 (C-23), 121.5 (C-22), 112.4 (C-24), 110.2 (C-11), 49.1 (C-18), 44.6 (C-5), 38.2 (C-1), 37.8 (C-4), 36.9 (C-10), 35.8 (C-3), 29.2 (C-7), 26.1 (C-15), 25.3 (C-20), 22.6 (C-17), 22.5 (C-16), 18.9 (C-19), 18.8 (C-6), 18.3 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 421.3 ($[M+H]^+$, 100), 443.3 ($[M+Na]^+$, 4), 841.3 ($[2M+H]^+$, 68), 863.2 ($[2M+Na]^+$, 50); analysis calculated for $C_{27}H_{36}N_2O_2$ (420.59): C 77.10, H 8.63, N 6.66; found: C 76.80, H 8.79, N 6.41.

4.3.11. 12-Hydroxy-N-(2-nitrobenzoyl)dehydroabietylamine (11)

Compound **11** was prepared according to the general procedure from 2-nitrobenzoic acid (0.13 g, 0.78 mmol) and compound **1** (0.20 g, 0.66 mmol) followed by column chromatography (silica gel, ethyl acetate/chloroform, 5:95) to yield compound **11** (0.19 g, 64%) as a colorless solid; R_F = 0.47 (silica gel, ethyl acetate/chloroform, 0,5:9,5); mp = 124–126 °C; $[\alpha]_D$ = +40.3° (*c* = 0.33, DMSO); IR (ATR): *ν* = 3291*br*, 2954*w*, 2927*w*, 2867*w*, 1645 *m*, 1530*vs*, 1417 *m*, 1347 *s*, 1309 *m*, 1160 *m*, 1080 *m*, 857 *m*, 751 *s*, 697 *s* cm^{-1}; UV–vis (DMSO): λ_{max} (log ε) = 281 nm (3.68); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02 (dd, *J* = 8.1, 0.9 Hz, 1H, 24-H), 7.64 (ddd, *J* = 7.5, 7.5, 1.2 Hz, 1H, 26-H),

7.55 (ddd, J = 7.8, 7.8, 1.5 Hz, 1H, 25-H), 7.47 (dd, J = 7.5, 1.4 Hz, 1H, 27-H), 6.83 (s, 1H, 14-H), 6.63 (s, 1H, 11-H), 5.82 (dd, J = 6.0, 6.0 Hz, 1H, NH), 4.80 (s, 1H, OH), 3.48 (dd, J = 13.7, 7.1 Hz, 1H, 18-H_a), 3.29 (dd, J = 13.6, 5.6 Hz, 1H, 18-H_b), 3.10 (*hept*, J = 6.9 Hz, 1H, 15-H), 2.93–2.71 (*m*, 2H, 7-H_a + 7-H_b), 2.24–2.15 (*m*, 1H, 1-H_a), 1.95–1.85 (*m*, 1H, 6-H_a), 1.84–1.62 (*m*, 3H, 2-H_a + 2-H_b + 6-H_b), 1.57–1.49 (*m*, 1H, 3-H_a), 1.47–1.30 (*m*, 3H, 1-H_b + 3-H_b + 5-H), 1.25–1.19 (*m*, 6H, 16-H + 17-H), 1.22 (s, 3H, 20-H), 1.02 (s, 3H, 19-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.9 (C-21), 151.0 (C-13), 148.2 (C-9), 146.6 (C-23), 133.8 (C-26), 133.3 (C-22), 131.9 (C-12), 130.6 (C-25), 128.8 (C-27), 126.9 (C-8), 126.8 (C-14), 124.7 (C-24), 111.0 (C-11), 51.0 (C-18), 46.1 (C-5), 38.5 (C-1), 37.7 (C-4), 37.6 (C-10), 36.3 (C-3), 29.5 (C-7), 26.9 (C-15), 25.3 (C-20), 22.8 (C-16), 22.7 (C-17), 19.3 (C-6), 18.8 (C-19), 18.7 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 451.2 ($[M+H]^+$, 100), 473.3 ($[M+Na]^+$, 32); analysis calculated for $C_{27}H_{34}N_2O_4$ (450.57): C 71.97, H 7.61, N 6.22; found: C 71.70, H 7.43, N 5.99.

4.3.12. 12-Hydroxy-N-(3-nitrobenzoyl)dehydroabietylamine (12)

Compound **12** was prepared according to the general procedure from 3-nitrobenzoic acid (0.13 g, 0.78 mmol) and compound **1** (0.20 g, 0.66 mmol) followed by column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10) to yield compound **12** (0.22 g, 74%) as a colorless solid; R_F = 0.33 (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10); mp = 114–120 °C; $[\alpha]_D$ = +19.3° (*c* = 0.37, DMSO); IR (ATR): ν = 3307*br*, 2955*w*, 2927*w*, 2867*w*, 1645 *m*, 1616 *m*, 1528*vs*, 1417 *m*, 1349*vs*, 1160 *m*, 754 *s*, 717 *s* cm^{-1}; UV–vis ($CHCl_3$): λ_{max} (log ε) = 270 nm (3.89); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.55 (dd, J = 1.8, 1.8 Hz, 1H, 23-H), 8.34 (ddd, J = 8.2, 2.1, 1.0 Hz, 1H, 25-H), 8.12–8.07 (*m*, 1H, 27-H), 7.62 (dd, J = 8.0, 8.0 Hz, 1H, 26-H), 6.83 (s, 1H, 14-H), 6.63 (s, 1H, 11-H), 6.25 (dd, J = 6.1, 6.1 Hz, 1H, NH), 4.82 (s, 1H, OH), 3.47 (dd, J = 13.7, 6.5 Hz, 1H, 18-H_a), 3.37 (dd, J = 13.7, 6.5 Hz, 1H, 18-H_b), 3.10 (*hept*, J = 6.9 Hz, 1H, 15-H), 2.93–2.83 (*m*, 1H, 7-H_a), 2.81–2.66 (*m*, 1H, 7-H_b), 2.24–2.14 (*m*, 1H, 1-H_a), 2.00–1.92 (*m*, 1H, 6-H_a), 1.85–1.65 (*m*, 3H, 2-H_a + 2-H_b + 6-H_b), 1.57–1.51 (*m*, 1H, 3-H_a), 1.44 (dd, J = 12.3, 1.9 Hz, 1H, 5-H), 1.41–1.29 (*m*, 2H, 1-H_b + 3-H_b), 1.25–1.18 (*m*, 6H, 16-H + 17-H), 1.22 (s, 3H, 20-H), 1.02 (s, 3H, 19-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.5 (C-21), 151.1 (C-13), 148.4 (C-24), 148.0 (C-9), 136.5 (C-22), 133.1 (C-27), 131.9 (C-12), 130.0 (C-26), 127.0 (C-8), 126.9 (C-14), 126.2 (C-25), 121.9 (C-23), 111.0 (C-11), 50.9 (C-18), 46.0 (C-5), 38.5 (C-1), 37.9 (C-4), 37.7 (C-10), 36.6 (C-3), 29.7 (C-7), 27.0 (C-15), 25.4 (C-20), 22.8 (C-16), 22.7 (C-17), 19.4 (C-6), 18.9 (C-19), 18.7 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 451.1 ($[M+H]^+$, 58), 473.2 ($[M+Na]^+$, 20), 901.3 ($[2M+H]^+$, 23), 923.1 ($[2M+Na]^+$, 100); analysis calculated for $C_{27}H_{34}N_2O_4$ (450.57): C 71.97, H 7.61, N 6.22; found: C 71.68, H 7.94, N 6.00.

4.3.13. 12-Hydroxy-N-(4-nitrobenzoyl)dehydroabietylamine (13)

Compound **13** was prepared according to the general procedure from 4-nitrobenzoic acid (0.13 g, 0.78 mmol) and compound **1** (0.20 g, 0.66 mmol) followed by column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 7:3:10) to yield compound **13** (0.28 g, 94%) as a colorless solid; R_F = 0.44 (silica gel, *n*-hexane/ethyl acetate/chloroform, 7:3:10); mp = 185–187 °C; $[\alpha]_D$ = +22.81° (*c* = 0.35, DMSO); IR (KBr) = 3396*br*, 2942 *s*, 2928 *s*, 2866 *m*, 2848 *m* 1638*vs*, 1598*vs*, 1488 *m*, 1464 *m*, 1440 *m*, 1390 *m*, 1344 *s*, 1298 *s*, 1232 *m*, 1178 *m*, 1108 *m*, 1014*w*, 868 *m*, 858 *m*, 752 *s*, 722 *m* cm^{-1}; UV–vis (DMSO): λ_{max} (log ε) = 303 nm (4.11); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.72 (s, 1H, OH), 8.53 (dd, J = 6.3, 6.3 Hz, 1H, NH), 8.30–8.25 (*m*, 2H, 24–H), 8.05–8.00 (*m*, 2H, 23–H), 6.67 (s, 1H, 14–H), 6.61 (s, 1H, 11–H), 3.42 (dd, J = 13.3, 6.9 Hz, 1H, 18–H_a), 3.11–2.99 (*m*, 2H, 15–H + 18–H_b), 2.75–2.59 (m, 2H, 7–H_a + 7–H_b), 2.13–2.06 (*m*, 1H, 1–H_a), 1.98–1.90 (*m*, 1H, 6–H_a), 1.77–1.52 (*m*, 3H, 2–H_a + 2–H_b + 6–H_b), 1.47 (ddd, J = 13.2, 13.2, 3.7 Hz, 1H, 3–H_a), 1.41–1.33 (*m*, 2H, 5–H + 3–H_b), 1.23 (ddd, J = 13.1, 13.1, 3.4 Hz, 1H, 1–H_b), 1.13 (s, 3H, 20–H), 1.10 (d, J = 6.9 Hz, 3H, 16–H), 1.08 (d, J = 6.9 Hz, 3H, 17–H), 0.92 (s, 3H, 19–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.3 (C–21), 152.1 (C–13), 148.8 (C–25), 147.3 (C-9), 140.6 (C–22), 131.3 (C–12), 128.8 (C–24), 125.9 (C–14), 124.7 (C-8), 123.4 (C–23), 110.2 (C–11), 49.6 (C–18), 44.7 (C-5), 38.1 (C-1), 37.9 (C-4), 37.0 (C–10), 35.8 (C-3), 29.1 (C-7), 26.1 (C–15), 25.3 (C–20), 22.6 (C–16), 22.5 (C–17), 18.9 (C-6), 18.9 (C–19), 18.3 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 451.1 ($[M+H]^+$, 85), 473.1 ($[M+Na]^+$, 12), 901.1 ($[2M+H]^+$, 25), 923.0 ($[2M+Na]^+$, 100); analysis calculated for $C_{27}H_{34}N_2O_4$ (450.57): C 71.97, H 7.61, N 6.22; found: C 71.64, H 7.93, N 6.01.

4.3.14. 12-Acetoxy-N-(4-nitrobenzoyl)dehydroabietylamine (14)

Compound **13** (0.09 g, 0.20 mmol) was dissolved in dry DCM (10 mL), acetyl chloride (0.02 mL, 0.28 mmol) and triethylamine (0.01 mL, 0.07 mmol) were added. After 15 min of stirring followed by usual aqueous work-up the residue and column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 7:3:10), **14** (0.06 g, 61%) was obtained as a colorless solid; R_F = 0.68 (silica gel, *n*-hexane/ethyl acetate/chloroform, 6:4:10); mp = 114–117 °C; $[\alpha]_D$ = +21.11° (*c* = 0.34, $CHCl_3$); IR (KBr): ν = 3422*vs*, 2962 *m*, 2930 *m*, 2868 *m*, 1756 *m*, 1654 *s*, 1618 *m*, 1602 *m*, 1526*vs*, 1496 *m*, 1458 *m*, 1368 *m*, 1346 *s*, 1290 *m*, 1210 *s*, 1176 *m*, 1108*w*, 1016 *m*, 720 *m* cm^{-1}; UV–vis ($CHCl_3$): λ_{max} (log ε) = 283 nm (4.02); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.27–8.22 (*m*, 2H, 23–H), 7.90–7.86 (*m*, 2H, 24–H), 6.93 (s, 1H, 14–H), 6.81 (s, 1H, 11-H), 6.25 (dd, J = 6.2, 6.2 Hz, 1H, NH), 3.49 (dd, J = 13.7, 6.6 Hz, 1H, 18–H_a), 3.27 (dd, J = 13.8, 6.3 Hz, 1H, 18–H_b), 2.97–2.84 (*m*, 2H, 7–H_a + 15–H), 2.84–2.73 (*m*, 1H, 7–H_b), 2.29 (s, 3H, 27–H), 2.23–2.16 (*m*, 1H, 1–H_a), 2.01–1.93 (*m*, 1H, 6–H_a), 1.85–1.72 (*m*, 2H, 2–H_a + 6–H_b), 1.71–1.63 (*m*, 1H, 2–H_b), 1.53–1.47 (*m*, 1H, 3–H_a), 1.45 (dd, J = 12.4, 2.1 Hz, 1H, 5–H), 1.42–1.30 (*m*, 2H, 3–H_b + 1–H_b), 1.22 (s, 3H, 20–H), 1.18 (d, J = 6.9 Hz, 1H, 16–H), 1.14 (d, J = 6.9 Hz, 1H, 17–H), 1.01 (s, 3H, 19–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2 (C–26), 165.8 (C–21), 149.7 (C–25), 148.2 (C–13), 146.3 (C-9), 140.5 (C–22), 137.1 (C–12), 132.9 (C-8), 128.2 (C–24), 127.2 (C–14), 124.0 (C–23), 118.0 (C–11), 50.6 (C–18), 45.3 (C-5), 38.3 (C-1), 37.9 (C-4), 37.7 (C–10), 36.5 (C-3), 29.9 (C-7), 27.3 (C–15), 25.4 (C–20), 23.1 (C–16), 23.1 (C–17), 21.1 (C–27), 19.2 (C-6), 19.0 (C–19), 18.6 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 493.2 ($[M+H]^+$, 45), 510.1 ($[M+NH_4]^+$, 15), 515.2 ($[M+Na]^+$, 55), 985.1 ($[2M+H]^+$, 85), 1007.0 ($[2M+Na]^+$, 100), 1023.1 ($[2M+K]^+$, 40); analysis calculated for $C_{29}H_{36}N_2O_5$ (492.26): C 70.71, H 7.37, N 5.69; found:; C 70.51, H 7.32, N 5.53.

4.3.15. 12-Hydroxy-N-(picolinoyl)dehydroabietylamine (15)

Compound **15** was prepared according to the general procedure from picolinic acid (0.10 g, 0.81 mmol) and compound **1** (0.20 g, 0.66 mmol). Column chromatography (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10) afforded compound **15** (0.19 g, 72%) as a colorless solid; R_F = 0.42 (silica gel, *n*-hexane/ethyl acetate/chloroform, 8:2:10); mp = 106–109 °C; $[\alpha]_D$ = +27.9° (*c* = 0.31, DMSO); IR (ATR): ν = 3372*br*, 2956 *m*, 2926 *m*, 2867*w*, 1664 *s*, 1535*vs*, 1464 *m*, 1418 *s*, 1237 *s*, 1185 *m*, 1162 *m*, 1089 *m*, 998 *m*, 818 *m*, 748 *s*, 663 *s*, 621 *s* cm^{-1}; UV–vis (DMSO): λ_{max} (log ε) = 267 nm (3.71); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53 (ddd, J = 4.8, 1.6, 0.9 Hz, 1H, 23-H), 8.25–8.16 (*m*, 2H, 26-H + NH), 7.82 (ddd, J = 7.7, 7.7, 1.7 Hz, 1H, 25-H), 7.40 (ddd, J = 7.6, 4.8, 1.2 Hz, 1H, 24-H), 6.82 (s, 1H, 14-H), 6.65 (s, 1H, 11-H), 5.15 (s, 1H, OH), 3.46 (dd, J = 13.6, 6.9 Hz, 1H, 18-H_a), 3.31 (dd, J = 13.6, 6.7 Hz, 1H, 18-H_b), 3.12 (*hept*, J = 6.9 Hz, 1H, 15-H), 2.90–2.70 (*m*, 2H, 7-H_a + 7-H_b), 2.20–2.11 (*m*, 1H, 1-H_a), 2.02–1.93 (*m*, 1H, 6-H_a), 1.83–1.61 (*m*, 3H, 6-H_b + 2-H_a + 2-H_b), 1.57–1.46 (*m*, 2H, 3-H_a + 5-H), 1.45–1.31 (*m*, 2H, 1-H_b + 3-H_b), 1.26–1.19 (*m*, 6H, 16-H + 17-H), 1.21 (s, 3H, 20-H), 1.00 (s, 3H, 19-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.6 (C-21), 150.0 (C-22), 151.1 (C-13), 148.2 (C-9), 148.2 (C-23), 137.5 (C-25), 131.8 (C-12), 127.1 (C-8), 126.8 (C-14), 126.2 (C-24), 122.5 (C-26),111.1 (C-11),

50.2 (C-18), 45.7 (C-5), 38.4 (C-1), 37.9 (C-4), 37.7 (C-10), 36.4 (C-3), 29.8 (C-7), 27.0 (C-15), 25.5 (C-20), 22.8 (C-16), 22.7 (C-17), 19.4 (C-6), 19.0 (C-19), 18.8 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 407.3 ($[M+H]^+$, 38), 429.3 ($[M+Na]^+$, 15), 813.2 ($[2M+H]^+$, 20), 835.2 ($[2M+Na]^+$, 100); analysis calculated for $C_{26}H_{34}N_2O_2$ (406.56): C 76.81, H 8.43, N 6.89; found: C 76.69, H 8.63, N 6.71.

4.3.16. 12-Hydroxy-N-(nicotinoyl)dehydroabietylamine (16)

Compound **16** was prepared according to the general procedure from nicotinic acid (0.10 g, 0.81 mmol) and compound **1** (0.20 g, 0.66 mmol). Column chromatography (silica gel, ethyl acetate/chloroform, 9:1) afforded compound **16** (0.19 g, 71%) as a colorless solid; R_F = 0.46 (silica gel, ethyl acetate/chloroform, 9:1); mp = 109–111 °C; $[\alpha]_D$ = +16.4° (*c* = 0.32, DMSO); IR (ATR): *ν* = 3284*br*, 2956 *m*, 2926 *m*, 2867*w*, 1643 *s*, 1593 *m*, 1543 *m*, 1509 *m*, 1417 *s*, 1306 *s*, 1238 *m*, 1193 *m*, 1160 *m*, 1028 *m*, 998 *m*, 824 *m*, 745 *s*, 705*vs* cm^{-1}; UV–vis (DMSO): λ_{max} (log *ε*) = 364 nm (3.78); ^1H NMR (500 MHz, $CDCl_3$): *δ* = 9.10–9.07 (*m*, 1H, 24-H), 8.69 (dd, *J* = 4.9, 1.6 Hz, 1H, 23-H), 8.21 (ddd, *J* = 8.0, 1.9, 1.9 Hz, 1H, 26-H), 7.46 (dd, *J* = 7.9, 4.9 Hz, 1H, 25-H), 6.82 (s, 1H, 14-H), 6.62 (s, 1H, 11-H), 6.53–6.47 (*m*, 1H, NH), 3.45 (dd, *J* = 13.6, 6.4 Hz, 1H, 18-H_a), 3.37 (dd, *J* = 13.7, 6.5 Hz, 1H, 18-H_b), 3.10 (*hept*, *J* = 6.9 Hz, 1H, 15-H), 2.91–2.83 (*m*, 1H, 7-H_a), 2.82–2.71 (*m*, 1H, 7-H_b), 2.21–2.13 (*m*, 1H, 1-H_a), 2.00–1.92 (*m*, 1H, 6-H_a), 1.83–1.72 (*m*, 2H, 6-H_b + 2-H_a), 1.71–1.63 (*m*, 1H, 2-H_b), 1.57–1.49 (*m*, 1H, 3-H_a), 1.49–1.42 (*m*, 1H, 5-H), 1.43–1.29 (*m*, 2H, 1-H_b + 3-H_b), 1.24–1.19 (*m*, 6H, 16-H + 17-H), 1.22 (s, 3H, 20-H), 1.01 (s, 3H, 19-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ* = 165.7 (C-21), 151.8 (C-23), 151.1 (C-13), 148.1 (C-9), 147.4 (C-24), 135.9 (C-26), 131.9 (C-12), 130.9 (C-22), 127.0 (C-8), 126.9 (C-14), 123.9 (C-25), 111.0 (C-11), 50.6 (C-18), 46.0 (C-5), 38.5 (C-1), 37.9 (C-4), 37.7 (C-10), 36.6 (C-3), 29.8 (C-7), 27.0 (C-15), 25.4 (C-20), 22.8 (C-16), 22.7 (C-17), 19.4 (C-6), 19.0 (C-19), 18.8 (C-2) ppm; MS (ESI, MeOH): *m/z* (%) = 407.3 ($[M+H]^+$, 100), 813.2 ($[2M+H]^+$, 28), 835.2 ($[2M+Na]^+$, 18); analysis calculated for $C_{26}H_{34}N_2O_2$ (406.56): C 76.81, H 8.43, N 6.89; found: C 76.67, H 8.65, N 6.71.

4.3.17. 12-Hydroxy-N-(isonicotinoyl)dehydroabietylamine (17)

Compound **17** was prepared according to general procedure from isonicotinic acid (0.07 g, 0.57 mmol) and compound **1** (0.15 g, 0.50 mmol). Column chromatography (silica gel, ethyl acetate/chloroform, 1:1) afforded **17** [22] (0.16 g, 78%) as a colorless solid; R_F = 0.27 (silica gel, ethyl acetate/chloroform, 1:1); mp = 120–125 °C; $[\alpha]_D$ = +8.8° (*c* = 0.38, $CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 407.4 ($[M+H]^+$, 100), 813.2 ($[2M+H]^+$, 26), 835.2 ($[2M+Na]^+$, 6).

4.3.18. 12-Acetoxy-N-(isonicotinoyl)dehydroabietylamine (18)

Compound **18** was prepared according to literaturefrom 12-Acetoxydehydroabietylamine (0.10 g, 0.29 mmol) and isonicotinic acid (0.05 g, 0.4 mmol). Column chromatography (silica gel, *n*-hexane/ethyl acetate 3:7) gave compound **18** [22] (0.10 g, 77%) as a colorless solid; R_F = 0.3 (silica gel, *n*-hexane/ethyl acetate, 3:7); mp = 85–89 °C; $[\alpha]_D$ = +29.6° (*c* = 0.34, $CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 449.3 ($[M+H]^+$, 100), 471.3 ($[M+Na]^+$, 5), 897.1 ($[2M+H]^+$, 30), 918.9 ($[2M+Na]^+$, 10).

4.4. Cholinesterase assay

A mixture of the DTNB solution (125 μL), enzyme solution (25 μL) and compounds solutions (25 μL, 3 different concentrations and once blank water) was prepared and incubated at 30 °C for 20 min. The substrate (25 μL, 4 different concentrations) was added to start the enzymatic reaction. The absorbance data (λ = 415 nm) was recorded under a controlled temperature of 30 °C for 30 min at 1 min intervals. The substrate concentrations in the test were as follows: [ATChI] = 0.9375 mM, 0.625 mM, 0.325 mM, 0.1875 mM. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges. The used substrate concentration was 0.625 mM. The absorbance data was recorded under a controlled temperature of 30 °C for 10 min.

Acknowledgments

We would like to thank Dr. R. Kluge for the measurement of the ESI-MS spectra, Dr. D. Ströhl and his team for the NMR spectra and B.Sc. V. Simon for measuring optical rotations, IR and UV-vis spectra. Financial support by the "WissenschaftsCampus Halle WCH" (W13004216 to R.C.) is gratefully recognized.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103092.

References

[1] D.A. Drachman, J. Leavitt, Human memory and cholinergic system – relationship to aging, Arch. Neurol.-Chicago 30 (1974) 113–121.
[2] G.T. Grossberg, Cholinesterase inhibitors for the treatment of Alzheimer's disease: Getting on and staying on, Curr. Ther. Res. Clin. E 64 (2003) 216–235.
[3] M.B. Colovic, D.Z. Krstic, T.D. Lazarevic-Pasti, A.M. Bondzic, V.M. Vasic, Acetylcholinesterase inhibitors: pharmacology and toxicology, Curr. Neuropharmacol. 11 (2013) 315–335.
[4] C. Bayrak, P. Taslimi, H.S. Karaman, I. Gulcin, A. Menzek, The first synthesis, carbonic anhydrase inhibition and anticholinergic activities of some bromophenol derivatives with S including natural products, Bioorg. Chem. 85 (2019) 128–139.
[5] S. Burmaoglu, A.O. Yilmaz, M.F. Polat, R. Kaya, İ. Gulcin, O. Algul, Synthesis and biological evaluation of novel tris-chalcones as potent carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase and α-glycosidase inhibitors, Bioorg. Chem. 85 (2019) 191–197.
[6] N. Öztaşkın, R. Kaya, A. Maraş, E. Şahin, İ. Gülcin, S. Göksu, Synthesis and characterization of novel bromophenols: Determination of their anticholinergic, antidiabetic and antioxidant activities, Bioorg. Chem. 87 (2019) 91–102.
[7] U. Atmaca, R. Kaya, H.S. Karaman, M. Çelik, İ. Gülçin, Synthesis of oxazolidinone from enantiomerically enriched allylic alcohols and determination of their molecular docking and biologic activities, Bioorg. Chem. 88 (2019) 102980.
[8] M. Boztas, P. Taslimi, M.A. Yavari, I. Gulcin, E. Sahin, A. Menzek, Synthesis and biological evaluation of bromophenol derivatives with cyclopropyl moiety: Ring opening of cyclopropane with monoester, Bioorg. Chem. 89 (2019) 103017.
[9] Y. Sun, M.S. Lai, C.J. Lu, R.C. Chen, How long can patients with mild or moderate Alzheimer's dementia maintain both the cognition and the therapy of cholinesterase inhibitors: a national population-based study, Eur. J. Neurol. 15 (2008) 278–283.
[10] E. Giacobini, Cholinergic function and Alzheimer's disease, Int. J. Geriatr. Psych. 18 (2003) S1–S5.
[11] E.K. Perry, R.H. Perry, G. Blessed, B.E. Tomlinson, Changes in brain cholinesterases in senile dementia of alzheimer type, Neuropath. Appl. Neuro. 4 (1978) 273–277.
[12] A. Ciro, J. Park, G. Burkhard, N. Yan, C. Geula, Biochemical differentiation of cholinesterases from normal and Alzheimer's disease cortex, Curr. Alzheimer Res. 9 (2012) 138–143.
[13] N.H. Greig, T. Utsuki, D.K. Ingram, Y. Wang, G. Pepeu, C. Scali, Q.S. Yu, J. Mamczarz, H.W. Holloway, T. Giordano, D.M. Chen, K. Furukawa, K. Sambamurti, A. Brossi, D.K. Lahiri, Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer beta-amyloid peptide in rodent, P. Natl. Acad. Sci. USA 102 (2005) 17213–17218.
[14] U. Kosak, B. Brus, D. Knez, R. Sink, S. Zakelj, J. Trontelj, A. Pislar, J. Slenc, M. Gobec, M. Zivin, L. Tratnjek, M. Perse, K. Salat, A. Podkowa, B. Filipek, F. Nachon, X. Brazzolotto, A. Wieckowska, B. Malawska, J. Stojan, I.M. Rascan, J. Kos, N. Coquelle, J.P. Colletier, S. Gobec, Development of an in-vivo active reversible butyrylcholinesterase inhibitor, Sci. Rep. 6 (2016) 39495.
[15] J. Hartmann, C. Kiewert, E.G. Duysen, O. Lockridge, N.H. Greig, J. Klein, Excessive hippocampal acetylcholine levels in acetylcholinesterase-deficient mice are moderated by butyrylcholinesterase activity, J. Neurochem. 100 (2007) 1421–1429.
[16] B.M. McGleenon, K.B. Dynan, A.P. Passmore, Acetylcholinesterase inhibitors in Alzheimer's disease, Br. J. Clin. Pharmacol. 48 (1999) 471–480.
[17] E. Giacobini, Cholinesterase inhibitors: new roles and therapeutic alternatives, Pharmacol. Res. 50 (2004) 433–440.
[18] N.H. Greig, T. Utsuki, Q.S. Yu, X.X. Zhu, H.W. Holloway, T. Perry, B. Lee, D.K. Ingram, D.K. Lahiri, A new therapeutic target in Alzheimer's disease treatment: Attention to butyrylcholinesterase, Curr. Med. Res. Opin. 17 (2001) 159–165.
[19] J. Wiemann, A. Loesche, R. Csuk, Novel dehydroabietylamine derivatives as potent inhibitors of acetylcholinesterase, Bioorg. Chem. 74 (2017) 145–157.
[20] G. Topcu, U. Kolak, M. Ozturk, M. Boga, S. Damla Hatipoglu, F. Bahadori, B. Culhaoglu, T. Dirmenci, Investigation of anticholinesterase activity of a series of salvia extracts and the constituents of Salvia staminea, Nat Prod. J. 3 (2013) 3–9.
[21] M.A. Gonzalez, D. Perez-Guaita, Short syntheses of (+)-ferruginol from (+)-dehydroabietylamine, Tetrahedron 68 (2012) 9612–9615.
[22] J. Wiemann, L. Fischer, M. Rohmer, R. Csuk, Syntheses of C-ring modified dehydroabietylamides and their cytotoxic activity, Eur. J. Med. Chem. 156 (2018) 861–870.