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RESEARCH ARTICLE

Stereoselective Synthesis and Antiproliferative Activity of Monoterpene-Fused 2-Imino-1,3-oxazines

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Abstract: ***Background*:** In the recent years the 2-imino-1,3-thiazine and 2-iminothiazolidine ring systems can be found as moieties in biologically relevant compounds, including BACE1 inhibitors, or cannabinoid receptor agonists, while monoterpene-based 2-imino-1,3-thiazines, prepared from chiral 1,3-amino alcohols exhibiting pronounced antiproliferative activity.

***Methods*:** The antiproliferative activities of the prepared compounds were determined *in vitro* against a panel of human adherent cancer cell lines including HeLa, MCF7 and A431 by MTT assay.

***Results*:** Starting from pinane-, apopinane- and carane-based β-amino acid derivatives, 1,3-amino alcohols were prepared *via* two-step syntheses. The reactions of the product 1,3-amino alcohols and aryl isothiocyanates yielded γ-hydroxythioureas, which were transformed to monoterpene-fused 2-imino-1,3-oxazines via base-catalysed ring closure. The antiproliferative activities of these 2-imino-1,3-oxazines were examined and the structure–activity relationships were studied from the aspects of the type and stereochemistry of the monoterpene ring and the substituent effects on the 1,3-oxazine ring system. The *N*-unsubstituted monoterpene-based derivatives exhibited considerable antiproliferative activity against a panel of human adherent cancer cell lines (HeLa, MCF7 and A431).

***Conclusions*:** A mild and efficient method has been developed for the synthesis of 2-imino-1,3-oxazines by the ring closure of thiourea adducts of 1,3-amino alcohols. The resulting 1,3-oxazines exert marked antiproliferative action on a panel of human cancer cell lines.

Keywords: Monoterpene, asymmetric synthesis, enantiopure chiral templates, 1,3-amino alcohol, 1,3-oxazine, antiproliferative.

1. INTRODUCTION

In the past decade, alicyclic 1,3-aminoalcohols have proved to be versatile building blocks and applied as useful starting materials in stereoselective syntheses of compounds of pharmacological interest, serving as chiral ligands and auxiliaries in enantioselective transformations [1-4].

Several natural, chiral terpenes, including (+)-pulegone [5-7], α- and β-pinene [8-11] and fenchone-camphor [12-14], have been found to be excellent sources for the production of various amino alcohols, which have been successfully applied in enantioselective syntheses [1, 4]. The transformation of enantiomerically pure α-pinene to β-amino acid derivatives such as 1,3-aminoalcohols was recently reported [3, 10, 15, 16], and these synthons have proved to be useful chiral auxiliaries in the enantioselective synthesis of secondary alcohols or pharmacons, *e.g.* esomeprasol [17-21].

Besides their value in enantioselective catalysis, 1,3-aminoalcohols are good starting materials for the synthesis of various heterocyclic ring systems, such as 1,3-oxazines, 1,3-thiazines or 1,4-oxazepams [2, 22, 23]. The 2-imino-1,3-thiazine and 2-iminothiazolidine ring systems can be found as moieties in biologically relevant compounds, including BACE1 inhibitors [24] and cannabinoid receptor agonists [25-27].

In recent years, novel pathways have been developed for the synthesis of monoterpene-based chiral β-lactams and β-amino acid derivatives derived from (-)- and (+)-α-pinene, (-)-3-carene, (-)-and (+)-apopinene and peryllic acid [3, 10, 15, 16, 28-31]. These amino acid derivatives have been shown to be excellent building blocks for the syntheses of compounds with MDR antagonist activity [29], while some of the amino carboxamide derivatives displayed marked KDR and Aurora B kinase inhibitor activities [32].

Monoterpene-based 1,3-amino alcohols prepared from the appropriate β-amino acid derivatives are excellent building blocks for the synthesis of 2-imino-1,3-thiazines via CDI-promoted ring closure. The resulting monoterpene-fused 1,3-heterocycles exhibited pronounced antiproliferative activity against a panel of human adherent cancer cell lines [33].

Since the analogues bearing the 2-imino-1,3-oxazine ring system also display noteworthy pharmacological activities [22], *e.g.* as BACE1 inhibitors [34], cannabinoid receptor agonists [35] or antimicrobial agents [36], the aim of the present work was to synthesize new chiral pinane-, apopinane- and carane-fused 2-imino-1,3-oxazines, analogues of pharmacologically active 2-imino-1,3-thiazines, to study their antiproliferative activity on multiple cancer cell lines.

2. MATERIALS AND METHODS

2.1. General Synthetic Procedures

^{1}H-NMR spectra were recorded in $CDCl_3$, CD_3OD or D_2O in a 5-mm tube with a Bruker Avance DRX 400 spectrometer at 400.13 MHz (^{1}H) and 100.61 MHz (^{13}C) [δ=0 (TMS)]. Chemical shifts are expressed in ppm (δ) relative to TMS as internal reference. *J* values are given in Hz. Microanalyses were performed on a Perkin-Elmer 2400 elemental analyser. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. Melting points were determined on a Kofler apparatus and are uncorrected. Chromatographic separations were carried out on Merck Kieselgel 60 (230-400 mesh ASTM).

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Reactions were monitored with Merck Kieselgel 60 F_{254}-precoated tlc plates (0.25 mm thickness). IR spectra were recorded with an FT-IR spectrometer.

The enantiomeric purities of the prepared compounds were based on the enantiomeric purities of their starting materials, determined by means of GC measurements with direct separation of the enantiomers according to literature procedures [15, 16, 28]. During the transformations, ^{1}H-NMR spectra indicated the formation of a single diastereoisomer in each case.

Compounds **3**-**8** and thioureas **15a**, **15c**-**e**, **16a**, **23** and **27**-**29** were prepared according to literature methods [15, 16, 28, 33, 37, 38]; all spectroscopic data and physical properties were similar to those reported previously.

2.2. General Procedure for the Preparation of Aminoalcohols

To a slurry of $LiAlH_4$ (0.93 g, 24.5 mmol) in dry THF (150 ml), 2.00 g (9.5 mmol) of the appropiate amino ester **3**-**5** was added dropwise at 0 °C. After stirring at room temperature for 1.5 h and monitoring the reduction by means of TLC, the mixture was decomposed with a mixture of THF (10 ml) and H_2O (2.0 ml) under ice cooling. The inorganic material was filtered off and washed with THF (3x75 ml). After drying (Na_2SO_4) and evaporation, a pale-yellow oil was obtained. The hydrochloride salt of the resulting aminoalcohol was purified by recrystallization from an Et_2O/EtOH mixture.

(1R,2R,3S,5R)-(2-Amino-6,6-dimethylbicyclo[3.1.1]hept-3-yl)methanol hydrochloride (**9**). It was synthesized from **3** by the general method. The isolated compound was a white solid (1.37 g,70%). Mp 179-183 °C; $[\alpha]_D^{20}$ = -16.4 (*c* 0.5, MeOH); IR = 3123, 2917, 1529, 1457, 1051 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.95 (3H, s), 1.15 (1H, d, *J* = 11.1 Hz), 1.28 (3H, s), 1.44 (1H, dt, *J* = 4.0, 14.1 Hz), 1.97-2.03 (1H, m), 2.09-2.18 (2H, m), 2.27-2.34 (1H, m), 2.59-2.70 (1H, m), 3.73 (2H, ddd, *J* = 5.0, 11.58, 40.2 Hz), 3.98 (1H, d, *J* = 9.6 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 19.9 (Me), 25.2 (CH_2), 25.8 (Me), 29.0 (CH_2), 30.3 (CH), 38.6 (C_q), 40.0 (CH), 45.0 (CH), 52.9 (CH), 65.0 (CH_2). Anal. Calcd. for $C_{10}H_{20}ClNO$ (205.72): C, 58.38; H, 9.80; N, 6.81. Found: C, 58.61; H, 10.11; N, 6.49.

(1S,2S,3R,5S)-(2-Amino-6,6-dimethylbicyclo[3.1.1]hept-3-yl)methanol hydrochloride (**12**). It was synthesized from **5** using the general method. All chemical and physical properties of **12** were similar to those of **9**. The isolated compound was a white solid (1.41 g, 72%). Mp 179-183 °C; $[\alpha]_D^{20}$ = +13.4 (*c* 0.5, MeOH). Anal. Calcd. for $C_{10}H_{20}ClNO$ (205.72): C, 58.38; H, 9.80; N, 6.81. Found: C, 58.59; H, 10.17; N, 6.43.

(1R,2R,3R,5R)-(2-Amino-6,6-dimethylbicyclo[3.1.1]hept-3-yl)methanol hydrochloride (**13**). It was synthesized by the general method from **6**. The isolated compound was a white solid (1.52 g, 78%). Mp 199-202 °C; $[\alpha]_D^{20}$ = -7.9 (*c* 0.52, MeOH); IR = 3298, 2905, 1512, 1040 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (3H, s), 1.32 (3H, s), 1.50-1.59 (1H, m), 1.62 (1H, d, *J* = 10.6 Hz), 2.03-2.20 (4H, m), 2.28-2.36 (1H, m), 3.63 (1H, d, *J* = 8.1 Hz), 3.73 (2H, ddd, *J* = 2.5, 5.5, 11.1 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 19.1 (Me), 23.1 (CH_2), 26.2 (Me), 26.3 (CH_2), 35.1 (CH), 89.7 (CH), 40.0 (C_q), 44.2 (CH), 54.6 (CH), 64.5 (CH_2). Anal. Calcd. for $C_{10}H_{20}ClNO$ (205.72): C, 58.38; H, 9.80; N, 6.81. Found: C, 58.67; H, 10.08; N, 6.51.

(1S,2S,3S,5S)-(2-Amino-6,6-dimethylbicyclo[3.1.1]hept-3-yl)methanol hydrochloride (**14**). Its synthesis from **7** was performed using the general procedure. All chemical and physical properties of **14** were similar to those of **13**. The isolated compound was a white solid (1.52 g, 78%). Mp 199-202 °C; $[\alpha]_D^{20}$ = +8.1 (*c* 0.5, MeOH). Anal. Calcd. for $C_{10}H_{20}ClNO$ (205.72): C, 58.38; H, 9.80; N, 6.81; Found: C, 58.49; H, 9.71; N, 6.93.

(1R,2R,3S,5R)-(2-Benzylamino-6,6-dimethylbicyclo[3.1.1]heptan-3-yl)-methanol hydrochloride (**10**). It was synthesized by the general method from **4**. The isolated compound was a white solid (1.71 g, 61%). Mp 252-253 °C; $[\alpha]_D^{20}$ = -8.5 (*c* 0.5, MeOH); IR = 3177, 2927, 2741, 1597, 1457, 1048 cm^{-1}. ^{1}H-NMR (D_2O) δ (ppm) 0.94 (3H, s), 1.23 (1H, d, *J* = 11.1 Hz), 1.36 (3H, s), 1.44 (1H, dt, *J* = 4.0, 14.1 Hz), 2.02-2.23 (2H, m), 2.37-2.55 (3H, m), 2.64-2.75 (1H, m), 3.74-3.94 (3H, m), 4.23 (1H, d, *J* = 13.1), 4.44 (1H, d, *J* = 13.1 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 19.8 (Me), 25.3 (CH_2), 25.9 (Me), 28.8 (CH_2), 30.7 (CH), 38.5 (C_q), 39.7 (CH), 41.9 (CH), 49.9 (CH_2), 59.8 (CH), 65.6 (CH_2), 129.8 (4xCH_{ar}), 130.1 (CH_{ar}), 131.5 (C_q). Anal. Calcd. for $C_{17}H_{26}ClNO$ (295.85): C, 69.02; H, 8.86; N, 4.73. Found: 68.85; H, 8.67; N, 4.97.

(1R,2R,3S,5R)-(6,6-Dimethyl-2-methylaminobicyclo[3.1.1]-heptan-3-yl)-methanol hydrochloride (**11**). To a slurry of $LiAlH_4$ (2.82 g, 74.32 mmol) in dry THF (150 ml), a THF solution of *N*-Boc amino acid **8** (4.78 g, 16.9 mmol, 10 ml) was added dropwise at room temperature. After stirring at room temperature for 6 h (the reduction was monitored by means of TLC), the mixture was decomposed with a mixture of THF (30 ml) and H_2O (6.0 ml) under ice cooling. The inorganic material was filtered off and washed with THF (3x50 ml). After drying (Na_2SO_4) and evaporation of the solvent, a pale-yellow oil was obtained. The hydrochloride salt of the resulting aminoalcohol was purified by recrystallization from an Et_2O/EtOH mixture. The isolated compound was a white solid (1.44 g, 39%). Mp 192-193 °C; $[\alpha]_D^{20}$ = -15.7 (*c* 0.5, MeOH); IR = 3308, 3123, 2916, 2475, 1595, 1458, 1049 cm^{-1}. ^{1}H-NMR (D_2O) δ (ppm): 0.99 (3H, s), 1.18 (1H, d, *J* = 10.6 Hz), 1.35 (3H, s), 1.48-1.55 (1H, m), 2.03-2.10 (1H, m), 2.15-2.24 (1H, m), 2.33-2.47 (2H, m), 2.74 (3H, s), 2.72-280 (1H, m), 3.73-3.91 (3H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.0 (Me), 25.1 (CH_2), 26.1 (Me), 29.2 (CH_2), 31.1 (CH), 32.1 (CH), 38.4 (C), 39.9 (CH), 41.1 (CH), 62.0 (Me), 65.1 (CH_2). Anal. Calcd. for $C_{11}H_{22}ClNO$ (219.14): C, 60.12; H, 10.09; N, 7.20. Found: C, 60.33; H, 10.27; N, 6.95.

2.3. General Procedure for the Synthesis of Thioureas 15-17, 21, 23 and 25

These compounds were synthesized by dissolving amino alcohols **9**-**14** (1.62 mmol) and the appropriate isothiocyanates (1.71 mmol) in toluene (100 mL) and stirring the mixtures at room temperature for 6 h. In the case of *N*-benzylamino alcohol **10**, heating at 50 °C for 6 h was applied. The resulting reaction mixtures were then evaporated to dryness, filtered and washed with *n*-hexane. The purities of the products were determined by NMR to be >97%.

(1R,2R,3R,5R)-1-(3-Chlorophenyl)-3-(3-hydroxymethyl-6,6-dimethylbicyclo[3.1.1]hept-2-yl)thiourea (**15b**). It was synthesized by the general method from **9** and 3-chlorophenyl isothiocyanate. The isolated compound was a white solid (0.51 g, 92%). Mp 155-156 °C; $[\alpha]_D^{20}$ = +13.0 (*c* 0.25, MeOH); IR = 3352, 2914, 1536, 1475, 1306, 691 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.00 (3H, s), 1.04 (1H, d, *J* = 10.0 Hz), 1.23 (3H, s), 1.66-1.78 (1H, m), 1.89-2.19 (5H, m), 2.53-2.64 (1H, m), 3.52-2.59 (1H, m), 3.64-3.71 (1H, m), 5.17 (1H, br s), 7.12 (1H, d, *J* = 7.5 Hz), 7.20 (1H, d, *J* = 7.7 Hz), 7.28 (1H, d, *J* = 3.3 Hz), 7.31 (1H, t, *J* = 7.9 Hz), 7.39 (1H, br d, *J* = 7.6 Hz), 8.04 (1H, br s). ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.2 (Me), 26.6 (CH_2), 26.7 (Me), 30.0 (CH_2), 32.4 (CH), 39.4 (C_q), 40.8 (CH), 46.3 (CH), 57.3 (CH), 64.9 (CH_2), 123.2 (CH_{ar}), 125.2 (CH_{ar}), 127.1 (CH_{ar}), 131.2 (CH_{ar}), 135.7 (C_q), 137.8 (C_q), 179.9 (C=S). Anal. Calcd for $C_{17}H_{23}ClN_2OS$ (338.90): C, 60.25; H, 6.84; N, 8.27; S, 9.46%; Found: C, 60.39; H, 8.10; N, 8.32; S, 9.53%.

(1R,2R,3R,5R)-1-Ethyl-3-(3-hydroxymethyl-6,6-dimethylbicyclo [3.1.1]hept-2-yl)thiourea (**15f**). It was synthesized by the general method from **9** and ethyl isothiocyanate. The isolated compound was a white solid (0.37 g, 89%). Mp 158-160 °C; $[\alpha]_D^{20}$ = +6.0 (*c* 0.25, MeOH); IR = 3236, 2916, 1568, 1587, 1518, 1265 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.02 (3H, s), 1.19 (1H, d, *J* = 9.3 Hz), 1.23 (3H, t, *J* = 7.3 Hz), 1.25 (3H, s), 1.75 (1H, ddd, *J* = 2.9, 6.0, 13.3 Hz), 1.93-1.99 (1H, m), 2.04-2.22 (3H, m), 2.55-2.65 (1H, m), 3.24-3.40 (2H, m), 3.63 (1H, dd, *J* = 4.6, 10.8 Hz), 3.74 (1H, dd, *J* = 2.8, 10.9 Hz), 5.05 (1H, br s), 5.89 (1H, br s), 6.83 (1H, d, *J* = 7.9 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.4 (Me), 21.2 (Me), 26.8 (Me),

26.9 (CH_2), 30.2 (CH_2), 32.8 (CH), 38.6 (CH_2), 39.4 (C_q), 40.8 (CH), 46.7 (CH), 56.5 (CH), 65.4 (CH_2), 181.1 (C=S). Anal. Calcd for $C_{13}H_{24}N_2OS$ (256.41): C, 60.89; H, 9.43; N, 10.93; S, 12.51%; Found: C, 60.97; H, 9.32; N, 11.11; S, 12.61%.

(1R,2R,3R,5R)-1-Benzyl-1-(3-chlorophenyl)-3-(3-hydroxymethyl-6,6-dimethylbicyclo[3.1.1]hept-2-yl)thiourea (**16b**). It was synthesized by the general method from **10** and 3-chlorophenyl isothiocyanate. The isolated compound was a white solid (0.59 g, 85%). Mp 143-145 °C; $[\alpha]_D^{20}$ = +12.0 (*c* 0.25, MeOH); IR = 2934, 1687, 1580, 1223, 763, 723 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.10 (3H, s), 1.25 (3H, s), 1.51 (1H, d, *J* = 10.3 Hz), 1.76-1.83 (1H, m), 1.90-2.05 (3H, m), 2.09-2.16 (1H, m), 2.20-2.32 (1H, m), 3.53-3.61 (1H, m), 3.65-3.69 (1H, m), 3.72 (1H, dd, *J* = 2.0, 10.6 Hz), 3.86 (1H, dd, *J* = 3.0, 10.6 Hz), 4.74-4.91 (2H, m), 7.16 (1H, d, *J* = 7.6 Hz), 7.20-7.38 (9H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 19.9 (Me), 25.3 (Me), 27.3 (CH_2), 27.5 (CH_2), 35.6 (CH), 40.0 (C_q), 42.8 (CH), 45.8 (CH), 49.0 (CH_2), 59.6 (CH), 62.1 (CH_2), 123.3 (CH_{ar}), 123.5 (CH_{ar}), 125.3 (CH_{ar}), 125.4 (CH_{ar}), 127.0 (CH_{ar}), 127.6 (CH_{ar}), 131.4 (CH_{ar}), 135.6 (C_q), 135.7 (C_q), 137.9 (C_q), 180.7 (C=S). Anal. Calcd for $C_{24}H_{29}ClN_2OS$ (429.02): C, 67.19; H, 6.81; N, 6.53; S, 7.47%; Found: C, 67.39; H, 6.60; N, 6.65; S, 7.54%.

(*1R,2R,3R,5R)-1-(3-Hydroxymethyl-6,6-dimethylbicyclo[3.1.1]-hept-2-yl)-1-methyl-3-phenylthiourea* (**17a**). It was synthesized by the general method from **11** and phenyl isothiocyanate. The isolated compound was a white solid (0.49 g, 94%). Mp 151-153 °C; $[\alpha]_D^{20}$ = +90.0 (*c* 0.25, MeOH); IR = 3272, 2912, 1514, 1341, 691 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.96 (3H, s), 1.29 (3H, s), 1.52 (1H, d, *J* = 10.2 Hz), 1.72 (1H, dt, *J* = 3.4, 13.9 Hz), 1.91-1.97 (1H, m), 2.03-2.17 (3H, m), 2.36-2.44 (1H, m), 2.79-2.92 (1H, m), 3.14 (3H, s), 3.46-3.58 (1H, m), 3.63-3.73 (1H, m), 5.45 (1H, br s), 7.14 (1H, br s), 7.15-7.37 (5H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.1 (Me), 26.8 (Me), 27.8 (CH_2), 29.7 (CH_2), 34.4 (CH), 39.9 (CH), 40.8 (C_q), 45.7 (CH), 11.2 (Me), 67.3 (CH_2), 126.0 (CH_{ar}), 126.1 (CH_{ar}), 129.1 (CH_{ar}), 140.4 (C_q), 183.2 (C=S). Anal. Calcd for $C_{18}H_{26}N_2OS$ (318.48): C, 67.88; H, 8.23; N, 8.80; S, 10.07%; Found: C, 67.97; H, 8.09; N, 8.92; S, 10.31%.

(1S,2S,3R,5S)-1-(3-Hydroxymethyl-6,6-dimethylbicyclo[3.1.1] hept-2-yl)-3-phenylthiourea (**21**). It was synthesized by the general method from **12** and phenyl isothiocyanate. All chemical and physical properties of **21** were similar to those of **15a** reported in the literature [33]. The isolated compound was a white solid (0.47 g, 95%). Mp 151-154 °C; $[\alpha]_D^{20}$= -50.0 (*c* 0.25, MeOH). Anal. Calcd for $C_{17}H_{24}N_2OS$ (304.45): C, 67.07; H, 7.95; N, 9.20; S, 10.53%; Found: C, 67.39; H, 8.13; N, 9.01; S, 10.42%.

(1S,2S,3S,5S)-1-(3-Hydroxymethyl-6,6-dimethylbicyclo[3.1.1] hept-2-yl)-3-phenylthiourea (**25**). It was synthesized by the general method from **14** and phenyl isothiocyanate. All chemical and physical properties of **25** were similar to those of **23** reported in the literature [33]. The isolated compound was a white solid (0.46 g, 94%). Mp 151-155 °C; $[\alpha]_D^{20}$ = +18.0 (*c* 0.25, MeOH). Anal. Calcd for $C_{17}H_{24}N_2OS$ (304.45): C, 67.07; H, 7.95; N, 9.20; S, 10.53%; Found: C, 67.31; H, 7.80; N, 9.30; S, 10.41%.

2.4. General Procedure for the Preparation of 2-Imino-1,3-Oxazines

To the solution of the corresponding thiourea (1.7 mmol of **15-17**, **21**, **23** and **25**) in dry MeOH (20 ml) MeI (0.58 ml, 9.3 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 3 h and then evaporated to dryness. The resulting semisolid material was dissolved in a 2.5 N methanolic solution of KOH (20 ml). The mixture, after stirring for an additional 4 h at room temperature, was evaporated to dryness. The remaining crude product was dissolved in H_2O (30 ml) and extracted with $CHCl_3$ (3x30 ml). The combined organic layer was dried (Na_2SO_4) and evaporated, resulting in white crystalline products, which were purified by recrystallization from an *n*-hexane/EtOAc mixture. As an exception, **18f** was purified as the hydrochloride salt by recrystallisation from an EtOH/Et_2O mixture.

(1R,2R,7S,9R)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}]-undec-4-ylidene)phenylamine (**18a**). It was synthesized by the general method from **15a**. The isolated compound was a white solid (0.36 g, 78%). Mp 164-166 °C; $[\alpha]_D^{20}$ = +32.7 (*c* 0.5, MeOH); IR = 2915, 1667, 1587, 1223, 763 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.91 (3H, s), 1.24 (3H, s), 1.34 (1H, d, *J* = 10.7 Hz), 1.37-1.41 (m, 1H), 1.92-2.17 (4H, m), 2.45-2.65 (1H, m), 3.86-4.12 (3H, m), 6.95-7.25 (5H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.8 (Me), 25.9 (CH_2), 26.9 (Me), 28.6 (CH_2), 28.7 (CH), 39.4 (C_q), 40.9 (CH), 47.2 (CH), 52.2 (CH), 71.5 (CH_2), 122.6 (CH_{ar}), 123.5 (CH_{ar}), 129.1 (CH_{ar}), 131.6 (C_q), 151.6 (C=N). Anal. Calcd. for $C_{17}H_{22}N_2O$ (270.37): C, 75.52; H, 8.20; N, 10.36. Found: C, 75.87; H, 8.25; N, 10.11.

(1S,2S,7R,9S)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}]-undec-4-ylidene)phenylamine (**22**). **22** was synthesized by the general method from **21**. All chemical and physical properties of **22** were similar to those of **21**. The isolated compound was a white solid (0.36 g, 78%). Mp 164-166 °C; $[\alpha]_D^{20}$ = -9 (*c* 0.25, MeOH). Anal. Calcd. for $C_{17}H_{22}N_2O$ (270.37): C, 75.52; H, 8.20; N, 10.36. Found: C, 75.80; H, 8.29; N, 10.09.

(1R,2R,7R,9R)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}]-undec-4-ylidene)phenylamine (**24**). It was synthesized by the general method from **23**. The isolated compound was a white solid (0.29 g, 64%). Mp 118-121 °C; $[\alpha]_D^{20}$ = +77 (*c* 0.25, MeOH); IR = 2904, 1666, 1590, 1205, 695 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.84 (3H, s), 1.31 (3H, s), 1.46 (1H, t, *J* = 12.0 Hz), 1.76 (1H, d, *J* = 10.6 Hz), 1.80-1.87 (1H, m), 1.97-2.31 (4H, m), 3.51 (1H, d, *J* = 9.7 Hz), 4.18 (1H, dd, *J* = 12.4, 9.2 Hz), 4.36 (1H, dd, *J* = 8.7, 5.1 Hz), 6.86-7.31 (5H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.1 (Me), 24.0 (CH_2), 24.6 (CH_2), 28.1 (Me), 33.4 (CH), 41.8 (CH), 42.2 (C_q), 46.6 (CH), 55.0 (CH), 73.4 (CH_2), 119.4 (CH_{ar}), 122.2 (CH_{ar}), 129.4 (CH_{ar}), 136.5 (C_q), 150.7 (C=N). Anal. Calcd. for $C_{17}H_{22}N_2O$ (270.37): C, 75.52; H, 8.20; N, 10.36; Found: C, 75.68; H, 8.41; N, 10.56.

(1S,2S,7S,9S)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}] undec-4-ylidene)phenylamine (**26**). It was synthesized by the general method from **25**. All chemical and physical properties of **26** were similar to those of **24**. The isolated compound was a white solid (0.30 g, 65%). Mp 118-121 °C; $[\alpha]_D^{20}$ = -76 (c 0.25, MeOH); IR = 2904, 1666, 1590, 1205, 695 cm^{-1}. Anal. Calcd. for $C_{17}H_{22}N_2O$ (270.37): C, 75.52; H, 8.20; N, 10.36; Found: C, 75.60; H, 8.35; N, 10.49.

(1R,2R,7S,9R)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}]-undec-4-ylidene)-(3-chlorophenyl)amine (**18b**). It was synthesized by a general method from **15b**. The isolated compound was a white solid (0.26 g, 51%). Mp 157-160 °C; $[\alpha]_D^{20}$ = +4 (*c* 0.25, MeOH); IR = 2904, 1670, 1584, 1219, 781 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.89 (3H, s), 1.18 (3H, s), 1.32 (1H, d, *J* = 10.7 Hz), 1.33-1.45 (m, 1H), 1.72-1.81 (1H, m), 1.90-1.99 (1H, m), 2.01-2.18 (2H, m), 2.49-2.65 (1H, m), 3.74-4.12 (3H, m), 6.87 (1H, d, *J* = 7.3 Hz), 6.93 (1H, d, *J* = 7.3 Hz), 7.03 (1H, s), 7.14 (1H, t, *J* = 7.5 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.4 (Me), 25.6 (Me), 26.6 (CH_2), 28.3 (CH), 28.4 (CH_2), 39.1 (C_q), 40.6 (CH), 46.8 (CH), 51.8 (CH), 71.4 (CH_2), 122.0 (CH_{ar}), 122.3 (CH_{ar}), 123.9 (CH_{ar}), 127.8 (C_q), 129.7 (CH_{ar}), 141.4 (C_q), 153.7 (C=N). Anal. Calcd. for $C_{17}H_{21}ClN_2O$ (304.81): C, 66.99; H, 6.94; N, 9.19; Found: C, 67.27; H, 6.78; N, 9.35.

(1R,2R,7S,9R)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}] undec-4-ylidene)-(4-methylphenyl)amine (**18c**). It was synthesized by the general method from **15c**. The isolated compound was a white solid (0.32 g, 67%). Mp 188-189 °C; $[\alpha]_D^{20}$ = +8 (*c* 0.25, MeOH); IR = 2902, 1684, 1509, 1223 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.90 (3H, s), 1.22 (3H, s), 1.34 (1H, d, *J* = 10.7 Hz), 1.35-1.48 (1H, m), 1.85-2.19 (4H, m), 2.28 (3H, s), 2.49-2.73 (1H, m), 3.68-4.10 (3H, m), 6.80-7.10 (4H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.5 (Me), 20.2 (Me), 25.7 (CH_2), 26.7 (Me), 28.4 (CH_2), 28.5 (CH), 39.1 (C_q), 40.7 (CH), 46.9 (CH), 51.9 (CH), 71.3 (CH_2),

123.0 (2xCH_{ar}), 129.5 (2xCH_{ar}), 131.5 (C_q), 143.4 (C_q), 153.6 (C=N). Anal. Calcd. for $C_{18}H_{24}N_2O$ (284.40): C, 76.02; H, 8.51; N, 9.85; Found: C, 75.81; H, 8.27; N, 9.56.

(1R,2R,7S,9R)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}] undec-4-ylidene)-(4-fluorophenyl)amine (**18d**). It was synthesized by the general method from **15d**. The isolated compound was a white solid (0.28 g, 56%). Mp 224-225 °C; $[\alpha]_D^{20}$ = +17 (*c* 0.25, MeOH); IR = 2930, 1666, 1505, 1208, 849 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.92 (3H, s), 1.25 (3H, s), 1.35 (1H, d, *J* = 10.7 Hz), 1.37-1.47 (m, 1H), 1.87-2.24 (4H, m), 2.53-2.71 (1H, m), 3.80-4.18 (3H, m), 6.87-7.07 (4H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.5 (Me), 25.7 (CH_2), 26.7 (Me), 28.3 (CH_2), 28.5 (CH), 39.2 (C_q), 40.7 (CH), 47.0 (CH), 52.0 (CH), 71.4 (CH_2), 115.4 (d, *J* =22.5 Hz, 2xCH_{ar}), 124.3 (2xCH_{ar}), 142.2 (C_q), 153.6 (C_q), 157.3 (C=N). Anal. Calcd. for $C_{17}H_{21}FN_2O$ (288.36): C, 70.81; H, 7.34; N, 9.71; Found: C, 71.13; H, 7.02; N, 9.56.

(1R,2R,7S,9R)-(10,10-Dimethyl-5-oxa-3-azatricyclo[7.1.1.0^{2,7}] undec-4-ylidene)-(3-methoxyphenyl)amine(**18e**). It was synthesized by the general method from **15e**. The isolated compound was a white solid (0.23 g, 44%). Mp 139-140 °C; $[\alpha]_D^{20}$ = -7 (*c* 0.25, MeOH); IR = 3202, 2904, 1665, 1596, 1263, 1091, 773 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.91 (3H, s), 1.23 (3H, s), 1.34 (1H, d, *J* = 10.7 Hz), 1.34-1.47 (m, 1H), 1.76-2.20 (4H, m), 2.51-2.74 (1H, m), 3.77 (3H, s), 3.81-4.20 (3H, m), 6.45-6.73 (3H, m), 7.09-7.22 (1H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.5 (Me), 25.7 (CH_2), 26.7 (Me), 28.4 (CH_2), 28.5 (Me), 39.1 (C_q), 40.7 (CH), 47.0 (CH), 51.9 (CH), 55.5 (CH), 71.3 (CH_2), 108.0 (CH_{ar}), 109.0 (CH_{ar}), 115.8 (CH_{ar}), 129.4 (CH_{ar}), 135.2 (C_q), 141.4 (C_q), 154.2 (C=N). Anal. Calcd. for $C_{18}H_{24}N_2O_2$ (300.40): C, 71.97; H, 8.05; N, 9.33; Found: C, 71.81; H, 8.39; N, 9.31.

(1R,2R,7S,9R)-Ethyl-(10,10-dimethyl-5-oxa-3-azatricyclo[7.1. 1.0^{2,7}]undec-4-ylidene)amine hydrochloride (**18f**). It was synthesized by the general method from **15f**. The prepared crude product was purified as the hydrochloride salt with recrystallization from an Et_2O/EtOH mixture. The isolated compound was a white solid (0.24 g, 54%). Mp 180-183 °C; $[\alpha]_D^{20}$ = +6 (*c* 0.25, MeOH); IR = 2911, 1690, 1589 cm^{-1}. ^{1}H-NMR (two rotamers, $CDCl_3$) δ (ppm): 1.00 (3H, br s), 1.24 (3H, t, *J* = 7.1 Hz), 1.34 (3H, br s), 2.03-2.36 (5H, m), 2.83-2.99 (1H, m), 3.25-3.45 (2H, m), 3.99-4.60 (3H, m). ^{13}C-NMR (two rotamers, $CDCl_3$) δ (ppm): 13.4 (Me), 14.6 (Me), 19.7 (Me), 24.6 (CH_2), 26.1 (CH), 26.3 (CH), 27.5 (CH_2), 27.9 (CH_2), 36.5 (CH_2), 39.1 (C_q), 40.2 (CH), 45.6 (CH), 51.7 (CH), 52.2 (CH), 72.2 (CH_2), 72.7 (CH_2), 154.1 (C=N). Anal. Calcd. for $C_{13}H_{23}ClN_2O$ (258.79): C, 60.33; H, 8.96; N, 10.82; Found: C, 60.57; H, 8.71; N, 10.97.

(1R,2R,7S,9R)-(3-Benzyl-10,10-dimethyl-5-oxa-3-azatricyclo[7. 1.1.0^{2,7}]undec-4-ylidene)phenylamine (**19a**). **19a** was synthesized by the general method from **16a**. The isolated compound was a white solid (0.22 g, 36%). Mp 105-108 °C; $[\alpha]_D^{20}$ = -35 (*c* 0.25, MeOH); IR = 2940, 2918, 2860, 1630, 1579, 1264, 1098, 993, 693. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.84 (3H, s), 1.25 (3H, s), 1.32 (1H, d, *J* = 11.1 Hz), 1.72-1.78 (1H, m), 1.91-1.96 (1H, m), 2.12-2.23 (2H, m), 2.32-2.37 (1H, m), 2.63-2.73 (1H, m), 3.69-3.73 (1H, m), 3.83 (1H, dd, *J* = 3.0, 10.6 Hz), 4.08 (1H, d, *J* = 15.1 Hz), 4.15 (1H, dd, *J* = 4.5, 10.6 Hz), 5.13 (1H, d, *J* = 15.1 Hz), 6.90-6.98 (3H, m), 7.19-7.38 (7H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (Me), 27.0 (CH_2), 27.6 (Me), 30.0 (CH), 32.0 (CH_2), 39.8 (C_q), 40.8 (CH), 44.7 (CH), 50.3 (CH_2), 57.1 (CH), 70.1 (CH_2), 122.3 (CH_{ar}), 124.1 (CH_{ar}), 127.7 (CH_{ar}), 128.6 (CH_{ar}), 129.0 (CH_{ar}), 139.1 (C_q), 149.0 (C_q), 154.3 (C=N). Anal. Calcd. for $C_{24}H_{28}N_2O$ (360.22): C, 79.96; H, 7.83; N, 7.77. Found: C, 79.83; H, 8.01; N, 7.52.

(1R,2R,7S,9R)-(3-Benzyl-10,10-dimethyl-5-oxa-3-azatricyclo[7. 1.1.0^{2,7}]undec-4-ylidene)-3-chlorophenylamine (**19b**). It was synthesized by the general method from **16b**. The isolated compound was a white solid (0.26 g, 39%). Mp 95-97 °C; $[\alpha]_D^{20}$ = -64 (0.25, MeOH); IR = 2988, 2925, 2364, 1635, 1585, 1239, 773. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.85 (3H, s), 1.22-1.29 (4H, m), 1.76 (1H, ddd, *J* = 2.5, 5.0, 13.6 Hz), 1.92-1.98 (1H, m), 2.13-2.23 (2H, m), 2.34 (1H, q, *J* = 5.5, 8.6 Hz), 2.65-2.75 (1H, m,), 3.72 (1H, dd, *J* = 2.0, 10.6 Hz), 3.86 (1H, dd, *J* = 3.0, 10.6 Hz), 4.08 (1H, d, *J* = 15.1 Hz), 4.15 (1H, dd, *J* = 4.5, 10.6 Hz), 5.10 (1H, d, *J* = 15.1 Hz), 6.80 (1H, d, *J* = 8.1 Hz), 6.87-6.96 (2H, m), 7.12 (1H, t, *J* = 8.1 Hz), 7.23-7.37 (5H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (Me), 27.0 (CH_2), 27.6 (Me), 29.8 (CH), 32.0 (CH_2), 39.8 (C_q), 40.8 (CH), 44.7 (CH), 50.4 (CH_2), 57.2 (CH), 70.2 (CH_2), 122.2 (CH_{ar}), 122.5 (CH_{ar}), 124.2 (CH_{ar}), 127.8 (CH_{ar}), 128.5 (CH_{ar}), 129.1 (CH_{ar}), 129.9 (CH_{ar}), 134.4 (CCl), 138.8 (C_q), 150.4 (C_q), 154.7 (C=N). Anal. Calcd. for $C_{24}H_{27}ClN_2O$ (394.18): C, 72.99; H, 6.89; N, 7.09; Found: C, 73.21; H, 6.55; N, 7.17.

(1R,2R,7S,9R)-(3-Methyl-10,10-dimethyl-5-oxa-3-azatricyclo [7.1.1.0^{2,7}]undec-4-ylidene)phenylamine (**20a**). **20** was synthesized by the general method from **17a**. The isolated compound was a white solid (0.26 g, 54%). Mp 89-91 °C; $[\alpha]_D^{20}$ = -168 (*c* 0.25, MeOH); IR = 3062, 2948, 2870, 1924, 1636, 1584, 1056, 750, 693. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.96 (3H, s), 1.21 (1H, d, *J* = 10.6 Hz), 1.28 (3H, s), 1.65-1.73 (1H, m), 1.93-1.97 (1H, m), 2.11-2.21 (2H, m), 2.35 (1H, q, *J* = 5.5, 9.1 Hz), 2.67-2.76 (1H, m), 2.88 (3H, s), 3.70 (1H, ddd, *J* = 1.5, 3.5, 10.6 Hz), 3.81 (1H, dd, *J* = 4.0, 10.6 Hz), 4.08 (1H, dd, *J* = 4.5, 10.6 Hz), 6.90-6.96 (3H, m), 7.19-7.25 (2H, m). ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.1 (Me), 26.6 (CH_2), 27.7 (Me), 29.8 (CH), 31.7 (CH_2), 36.0 (CH), 39.6 (C_q), 40.9 (CH), 44.7 (CH), 60.1 (Me), 70.0 (CH_2), 122.2 (CH_{ar}), 124.1 (CH_{ar}), 129.0 (CH_{ar}), 149.2 (C_q), 154.7 (C=N). Anal. Calcd. for $C_{18}H_{24}N_2O$ (284.19): C, 76.02; H, 8.51; N, 9.85. Found: C, 75.67; H, 8.40; N, 10.03.

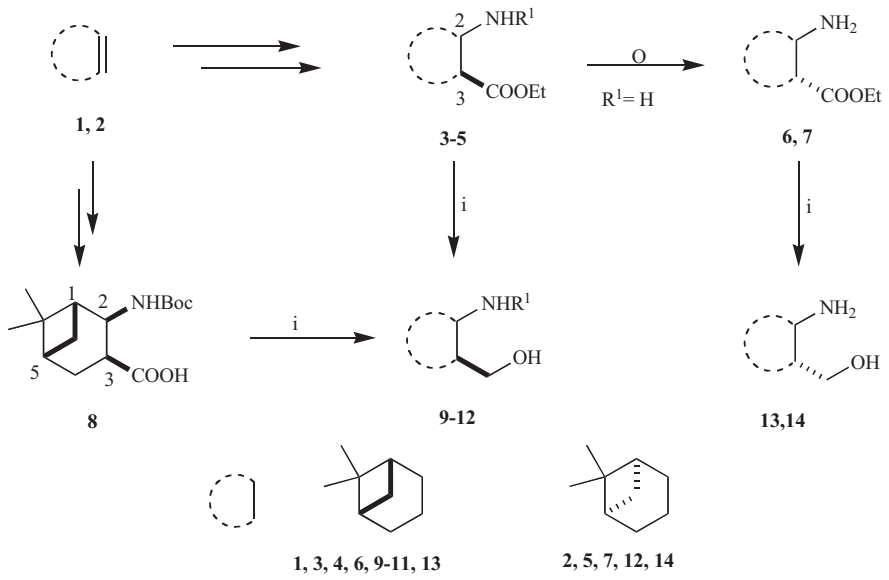
2.5. Determination of Antiproliferative Activities

The antiproliferative activities of the prepared compounds were determined *in vitro* against a panel of human adherent cancer cell lines including HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (squamous carcinoma). All cell lines were purchased from the European Collection of Cell Cultures (ECCAC, Salisbury, UK). The cells were maintained in minimal essential medium (Lonza Ltd, Basel, Switzerland) supplemented with 10% foetal bovine serum, 1% non-essential amino acids and an antibiotic-antimycotic mixture. Near-confluent cancer cells were seeded onto a 96-well microplate (5000 cells/well) and, after overnight standing, new medium (200 µL) containing the tested compound at 10 and 30 µM was added. After incubation for 72 h at 37 ºC in humidified air containing 5% CO_2, the viability of the cells was determined by the addition of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) solution. During a 4-h contact period, the MTT was converted by intact mitochondrial reductase and the precipitated formazan crystals were dissolved in 100 µL DMSO. Finally, the reduced MTT was assayed at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls [40]. When compounds elicited substantial growth inhibition at 30 µM (at least 40%), the assays were repeated with a set of dilutions, sigmoidal dose-response curves were fitted to the determined results and the IC_{50} values were calculated by means of GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA). All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Stock solutions of the tested substances (10 mM) were prepared in DMSO. The highest DMSO content of the medium (0.3%) did not have any substantial effect on cell proliferation. Cisplatin (Ebewe Pharma GmbH, Unterach, Austria) was used as reference agent.

3. RESULTS AND DISCUSSION

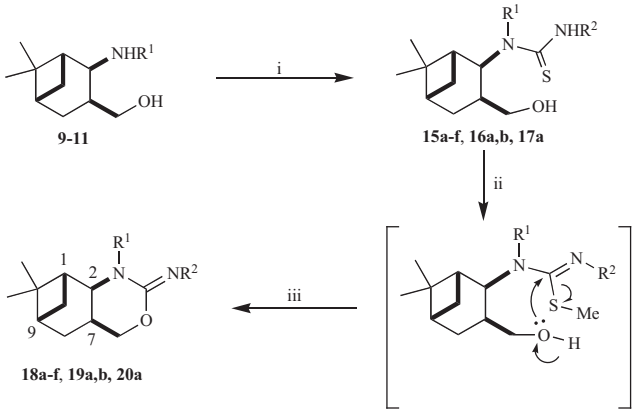
3.1. Synthesis of Alicyclic and Monoterpene-Based 1,3-Aminoalcohols

The synthetic routes applied for the preparation of 1,3-aminoalcohols **9-14** are presented in Scheme **1**. The corresponding



3, 4, 9-11: 2*R*,3*S*; **5, 12**: 2*S*,3*R*; **6, 13**: 2*R*,3*R*; **7, 14**: 2*S*,3*S*;
3, 5, 9, 12: R^1 = H; **4, 10:** R^1 = CH_2Ph; **11**: R^1 = Me

Scheme 1. Synthesis of starting material 1,3-amino alcohols **9-14**: (i) 3 equiv. $LiAlH_4$, THF, rt, 1.5-6 h, yield: 39-78%.



9, **15a-f**, **18a-f**: R^1 = H; **10**, **16a,b**, **19a,b**: R^1 = CH_2Ph;
11, **17a**, **20a** : R^1 = Me
a: R^2 = Ph; **b**: R^2 = 3-ClC_6H_4; **c**: R^2 = 4-MeC_6H_4;
d: R^2 = 4-FC_6H_4; **e**: R^2 = 3-$MeOC_6H_4$; **f**: R^2 = Et

Scheme 2. Synthesis of 2-imino-1,3-oxazines **18-20**: (i) 1.05 equiv. R^2NCS, toluene, rt, yield: 85-95%; (ii) MeI/MeOH, rt, 3 h; (iii) 2.5 N KOH/MeOH, rt, 4 h, yield: 36-67%.

β-lactams were prepared by the stereoselective cycloaddition of chlorosulfonyl isocyanate to (1*S*,5*S*)- and (1*R*,5*R*)-apopinene (**1**, **2**), followed by ring opening using literature methods, which resulted in the formation of *cis*-fused β-amino esters **3** and **5**. Under alkaline conditions, the *cis*-amino esters underwent fast and complete isomerization at the carboxylic function, furnishing *trans*-amino esters **6** and **7** in excellent yields [28]. Reduction of **3** and **5-7** with $LiAlH_4$ led to primary aminoalcohols **9** and **12-14** (Scheme **1**). *N*-Benzyl derivative **10** and *N*-methyl derivative **11**, respectively, were prepared by $LiAlH_4$ reduction of *N*-benzylamino ester **4** and *N*-Boc amino acid **8** [29, 37].

3.2. Synthesis of 2-Imino-1,3-Oxazine Derivatives

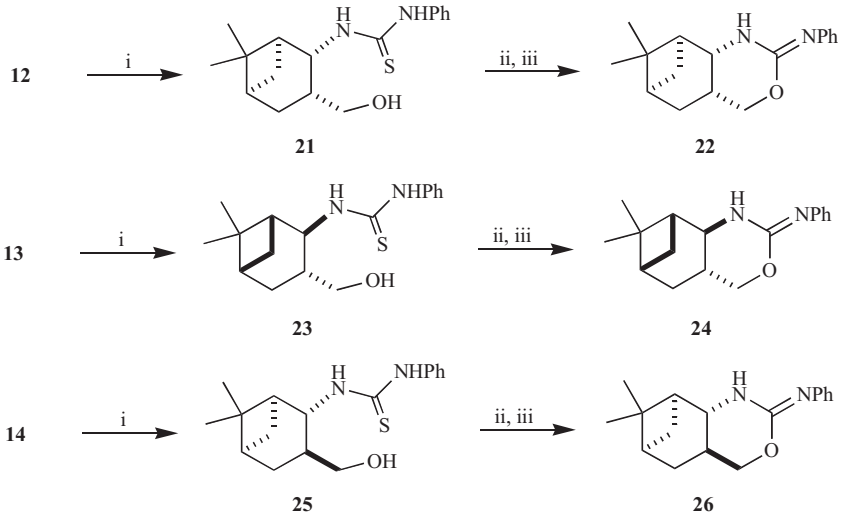
The intermediate thiourea adducts **15-17** were prepared in good to excellent yields [33] by the reaction of the appropriate aryl isothiocyanates or ethyl isothiocyanate and 1,3-aminoalcohols **9-11**. Methyl iodide treatment of thioureas **15-17** gave thioether intermediates. These were then transformed, without isolation, to 2-imino-1,3-oxazine derivatives **18-20** by KOH-promoted methyl mercaptan elimination under mild conditions [38, 39] (Scheme **2**).

To study the influence of the chiral ring system on pharmacological activity, all enantiomers and diastereoisomers of 2-phenylimino-1,3-oxazines (**18a**, **22**, **24** and **26**), were prepared successfully according to the general pathway (Scheme **3**).

To collect more information on structure-activity relationships, two further monoterpene-based 2-phenylimino-1,3-oxazines (**27** and **28**) and a cyclohexane analogue (**29**) were prepared according to the literature (**27**: [15], **28**: [16], **29**: [38], Fig. **1**).

3.3. Antiproliferative Activities

The prepared 2-imino-1,3-oxazines were subjected to *in vitro* pharmacological studies in order to characterize their antiproliferative actions on a panel of human adherent cancer cell lines. The results of the viability assays are presented in Table **1**. The substituents of the 2-imino function have a crucial impact on the activity



Scheme 3. Synthesis of enantio- and diastereomeric oxazines: (i) 1.05 equiv. R^2NCS, toluene, rt, yield: 95%; (ii) MeI/MeOH, rt, 3 h, (iii) 2.5 N KOH/MeOH, rt, 4 h, yield: 64-78%.

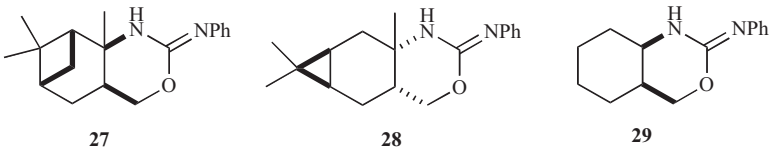


Fig. (1). Analogue monoterpene- and cyclohexane-fused 2-phenylimino-1,3-oxazines **27-29**.

Table 1. Antiproliferative effects of 2-imino-1,3-oxazines 18-29 on human cancer cell lines.

		Growth Inhibition, % ± SEM[a] [Calculated IC_{50} Value (μM)][b]		
		HeLa	**A431**	**MCF7**
18a	10 μM	45.15 ± 1.48	48.18 ± 2.76	45.83 ± 2.07
	30 μM	70.10 + 0.95 [12.26]	81.34 ± 0.66 [10.47]	74.35 ± 1.17 [11.35]
18b	10 μM	67.37 ± 2.42	82.97 ± 0.53	51.83 ± 2.96
	30 μM	96.45 ± 0.29 [8.75]	95.57 ± 0.45 [5.48]	93.76 ± 1.33 [9.73]
18c	10 μM	52.36 ± 1.69	58.30 ± 1.38	40.68 ± 2.77
	30 μM	79.23 ± 1.50 [9.38]	95.14 ± 0.73 [8.88]	95.40 ± 1.80 [10.89]
18d	10 μM	32.75 ± 1.48	22.20 ± 1.17	21.29 ± 2.56
	30 μM	55.94 ± 2.44	54.92 ± 1.95	50.46 ± 2.58
18e	10 μM	46.72 ± 0.97	33.98 ± 2.13	20.63 ± 2.86
	30 μM	79.92 ± 1.69 [11.26]	92.61 ± 0.16	66.65 ± 1.84
18f	10 μM	-	-	-
	30 μM	26.02 ± 2.87	-	-
19a	10 μM	20.70 ± 1.59	-	-
	30 μM	43.91 ± 2.13	37.67 ± 2.93	-
19b	10 μM	-	30.34 ± 1.75	-
	30 μM	-	35.96 ± 2.26	-

Table 1. contd…

		Growth Inhibition, % ± SEM[a] [Calculated IC_{50} Value (μM)][b]		
		HeLa	**A431**	**MCF7**
20a	10 μM	-	-	-
	30 μM	39.34 ± 2.85	35.23 ± 2.31	-
22	10 μM	55.81 ± 2.05	56.77 ± 1.20	64.96 ± 2.39
	30 μM	78.40 ± 1.54 [7.44]	87.16 ± 0.53 [8.09]	78.85 ± 2.24 [5.03]
24	10 μM	43.30 ± 1.29	38.08 ± 1.41	56.23 ± 1.78
	30 μM	69.59 ± 0.69 [16.04]	87.11 ± 0.46	75.43 ± 2.79 [7.79]
26	10 μM	50.60 ± 2.33	32.73 ± 2.23	54.55 ± 2.31
	30 μM	68.90 ± 2.11 [10.73]	85.24 ± 1.12	79.23 ± 1.27 [8.49]
27	10 μM	45.10 ± 0.89	32.87 ± 2.99	42.58 ± 2.92
	30 μM	69.74 ± 1.89 [13.42]	92.65 ± 0.68	94.09 ± 0.98 [11.15]
28	10 μM	-	-	39.10 ± 2.34
	30 μM	81.27 ± 1.50	90.30 ± 1.14	85.95 ± 0.83
29	10 μM	-	-	-
	30 μM	34.31 ± 2.94	-	-
Cisplatin	10 μM	42.61 ± 2.33	88.54 ± 0.50	53.03 ± 2.29
	30 μM	99.93 ± 0.26 [12.43]	90.18 ± 1.78 [2.84]	86.90 ± 1.24 [9.63]

[a] Substances eliciting less than 20% inhibition of cell proliferation were regarded as ineffective and the results are not presented.
[b] The concentration at which 50% inhibition of cell proliferation is exhibited.

of the molecules: aromatic substituents are favored (**18a-e**), and substitution of the aryl group has a limited and inconsequential impact on the efficacy, although the *m*-chlorophenyl (**18b**) group seems to be the most efficient. Without an aromatic function, the activity is negligible (**18f**). *N*-Benzyl (**19a,b**) or *N*-methyl (**20a**) substitution of the oxazine ring at position 3 resulted in a pronounced decrease in activity. Since no substantial differences were observed between the effects of **18a** and **22-26**, the configuration of C-3 (*cis* or *trans* ring fusion for both enantiomers, Schemes **2** and **3**) also appears to be irrelevant. Compounds with analogous monoterpene ring systems (**27** and **28**) have similar antiproliferative actions. Replacing the monoterpene ring system with cyclohexane (**29**) led to ineffective congeners, demonstrating that the bicyclic monoterpene ring as a pharmacophore part of the present molecules is essential for the design and synthesis of novel antiproliferative agents.

CONCLUSION

In conclusion, we have developed a mild and efficient method for the synthesis of 2-imino-1,3-oxazines by the ring closure of thiourea adducts of 1,3-aminoalcohols in the presence of MeI followed by KOH treatment. The resulting 1,3-oxazines exert marked antiproliferative action on a panel of human cancer cell lines. The *in vitro* pharmacological studies have clearly shown that the lipophilic monoterpene ring system and the 2-arylimino function are essential, while *N*-substitution on the 1,3-oxazine ring decreases the activity. The stereochemistry of the 1,3-oxazines has no influence on the antiproliferative effect.

LIST OF ABBREVIATIONS

BACE1	=	beta-secretase 1
MDR	=	multiple drug resistance
KDR	=	kinase insert domain receptor
THF	=	tetrahydrofurane
TLC	=	thin layer chromatography
CDI	=	1,1'-carbonyldiimidzole
MCF7	=	breast adenocarcinoma
A431	=	squamous carcinoma
HeLa	=	cervix adenocarcinoma
MTT	=	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide

CONFLICT OF INTEREST

The author declares that this article content has no conflict of interest.

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