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Novel camphane-based anti-tuberculosis agents with nanomolar activity

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ABSTRACT

A series of new amidoalcohols and amidodiols were designed on the base of the camphor scaffold and evaluated for their in vitro activity against *Mycobacterium tuberculosis* H37Rv and MDR strain 43. Some of the new compounds show 25 times higher activity than the classical anti-TB drug ethambutol. Small structural changes in the side chain shift the activity from micromolar to nanomolar inhibitory concentrations. Quantitative structure–activity relationship (QSAR) model is derived to guide the further lead optimization. Two hydrogen bond donors and up to three rings in the molecules are optimal for nanomolar activity. The camphane-based amides present novel promising scaffolds for antimycobacterial agents.

1. Introduction

Tuberculosis (TB) is a global health problem causing substantial morbidity, mortality, negative socioeconomic impact, and human suffering. One-third of the world's population is latently infected with *Mycobacterium tuberculosis* and approximately 9 million cases of active disease occur each year [1]. The recent widespread emergence of multidrug resistant (MDR) strains of *M. tuberculosis* to clinically available drugs puts further impetus to the urgent need for the discovery of new and effective anti-TB agents. Much progress has been done in drug development over the past decade. Currently, there are at least nine compounds in clinical development: two in phase III, four in phase II, and three in phase I trials [2]. Among these, four are existing drugs redeveloped for a TB indication and five are new chemical entities. More than 30 new anti-TB drugs are in preclinical development [3,4].

Monoterpenoids have long been widely used as chiral, enantiopure starting materials in natural product synthesis. Among the numerous monoterpenoids, the camphor derivatives are of particular importance because of their widespread occurrence in plants [5]. In spite of the fact that the chemistry of camphor is as old as the chemistry itself, this natural product and its derivatives still remain attractive as inexpensive source of enantiopure building blocks for organic synthesis [6]. Molecules possessing bornyl fragment exhibit a variety of pharmacological activities, including antibacterial, antifungal, anti-inflammatory, and anesthetic. Although a number of biologically active camphor derivatives are isolated from plants, almost no efforts are dedicated to the development of synthetic analogues [7–12].

Wilkinson and coworkers first reported the synthesis and activity of ethambutol (EMB) (Fig. 1**I**) [13]. EMB was a useful addition to tuberculosis chemotherapy, despite a relatively modest MIC of 10 μM, in part because of very low toxicity and relatively few side-effects. Based on structure–activity relationship (SAR) studies it appeared that the distance between the two nitrogens, the presence of β-aminoalcohols, and the small side chains were critical for determining activity. The configuration of the molecule is decisively important for the activity, since EMB (with *S*,*S*-configuration) is approx. 200–500 fold more potent than its (*R*,*R*)-enantiomer. Removal or significant alteration of the basicity of either amino group resulted in a loss of potency, with the exception that the corresponding amides retained activity in some analogues (Fig. 1**II**) [14].

Reports concerning the structural optimization of EMB have remained rather scarce for many years [15]. Lately, the occurrence of a 'better ethambutol' has been systematically investigated

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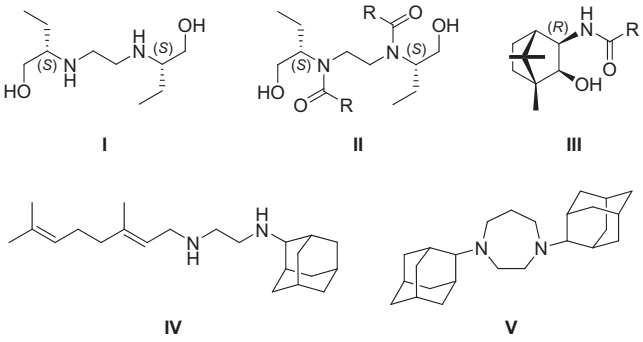


Fig. 1. Ethambutol (I); amide modified ethambutol (II), camphane-based *-amidoalcohol (III), SQ 109 (IV) and SQ 775 (V).

through virtual screening [16] or a combinatorial approach [17]. Several 1,2-diamines, such as SQ 109 (Fig. 1**IV**), displaying up to 14- to 35-fold improved in vitro antimycobacterial potencies and promising pharmacokinetic properties have thus been reported [18]. New cyclic diamine scaffolds with in vivo activity against *M. tuberculosis* have similarly been identified, like for example the homopiperazine SQ 775 (Fig. 1**V**) [19].

Inspired by the β-aminoalcohol fragment in the molecule of EMB and the retention of the activity on its amide analogues, we dedicated our efforts towards the development of camphane based β-amidoalcohol structures (Fig. 1**III**). In the present study we describe the synthesis and antimycobacterial activity of a novel class anti-TB compounds, containing camphane moiety. Quantitative structure–activity relationship (QSAR) model is derived to guide the further lead optimization.

2. Results and discussion

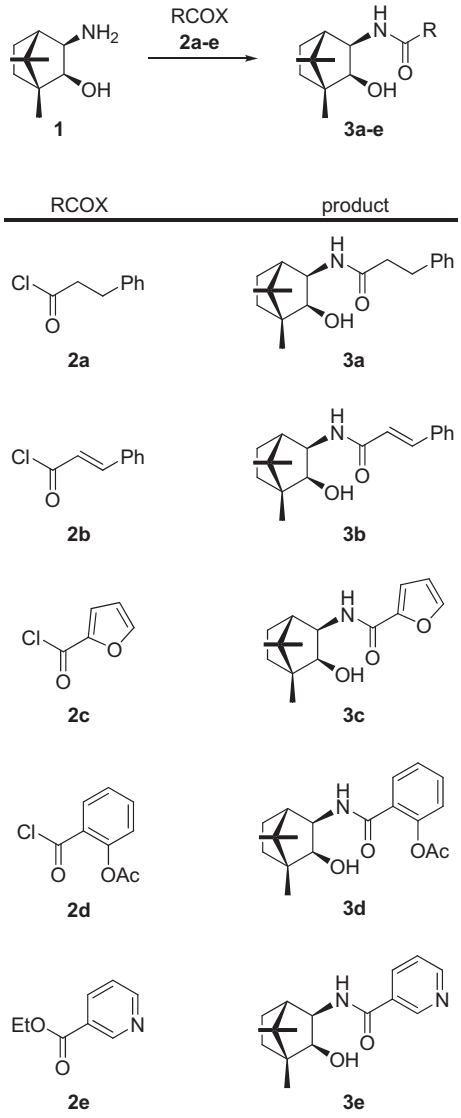
2.1. Chemistry

Since peptides and peptide-related structures have a wide variety of physiological and pharmacological actions, in the present study the concept of peptidomimetics was aimed by the design of amidoalcohols containing important pharmacophore groups. Readily available (+)-camphor-derived 3-*exo*-aminoisoborneol was selected as the key starting compound [20]. Our aim was to obtain the target structures using simple and effective synthetic procedures.

The amidoalcohols **3a**–**d** were obtained by reacting 3-*exo*-aminoisoborneol **1** with **2a**–**d** under standard acylation conditions (0 °C and Et_3N in dry DCM). Compound **3e** was synthesized by simple solvent-free aminolysis of ester **2d** with **1** by heating at 90–100 °C. All compounds were isolated in pure form after flash column chromatography in moderate to good yields (Scheme 1).

With the intention of comparing the carboxamido-function with phosphinic amide function and urea, we synthesized compounds **4** and **5**. The synthesis of diphenylphosphinic amide **4** was performed by reaction of **1** with diphenylphosphinoyl chloride in the presence of NEt_3 in CH_2Cl_2. Mixing **1** with ethyl isocyanate in CH_2Cl_2 as a solvent resulted in the formation of the urea **5** (Scheme 2). Both compounds were obtained in excellent purity after purification on silica.

Another aspect of our work was the synthesis of structures containing additional hydroxyl function. The aim was to investigate the structure–activity relationship of amidodiols compared to amidoalcohols. The key step was the preparation of a series of chiral α-hydroxy acids as starting building blocks. The natural amino acids were used as easily available and cheap chiral auxiliaries. Reaction of L-phenylalanine **6a** with nitrous acid is known to give α-hydroxy acid **7a** with retention of configuration due to the participation of the neighbouring carboxyl group [21]. Applying the reaction to a set of α-amino acids **6b**–**d** afforded the corresponding α-hydroxy analogues **7b**–**d** (Scheme 3).



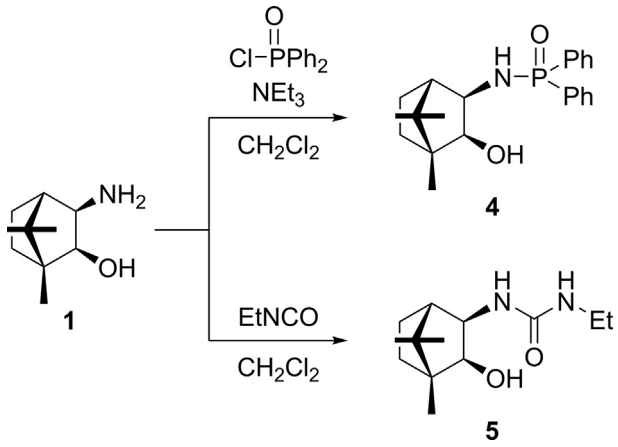
Scheme 1. Synthesis of 3-exo-aminoisoborneol derived amido-alcohols.

The formation of the amide linkage between the α-hydroxy acids and aminoalcohol **1** was accomplished by procedures developed for peptide synthesis. The reaction was optimized for the commercially available mandelic acid **8** in the presence of *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) and 1-Hydroxybenzotriazole hydrate (HOBT) as coupling reagents to yield **9a**. Following the same protocol for α-hydroxy acids **7a**–**d** we synthesized the corresponding amidodiols **9b**–**e** (Scheme 4). The products were obtained in good yields and excellent purity after flash column chromatography.

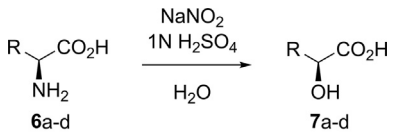
All compounds were identified by elementary analysis, ^1H NMR, ^{13}C NMR and MS data. The spectral analyses were in accordance with the assigned structures.

2.2. Antimycobacterial activity

The synthesized compounds were evaluated for their in vitro activity against *M. tuberculosis* H37Rv and MDR strain 43 using the



Scheme 2. Synthesis of 3-exo-aminoisoborneol derived phosphinic amide and urea.



(a) Phenylalanine, R = $PhCH_2$-; 43%
(b) Valine , R = *i*Pr-; 35%
(c) Isoleucine, R = *s*Bu-; 43%
(d) Methionine, R = $MeS(CH_2)_2$-; 17%

Scheme 3. Synthesis of hydroxy acids.

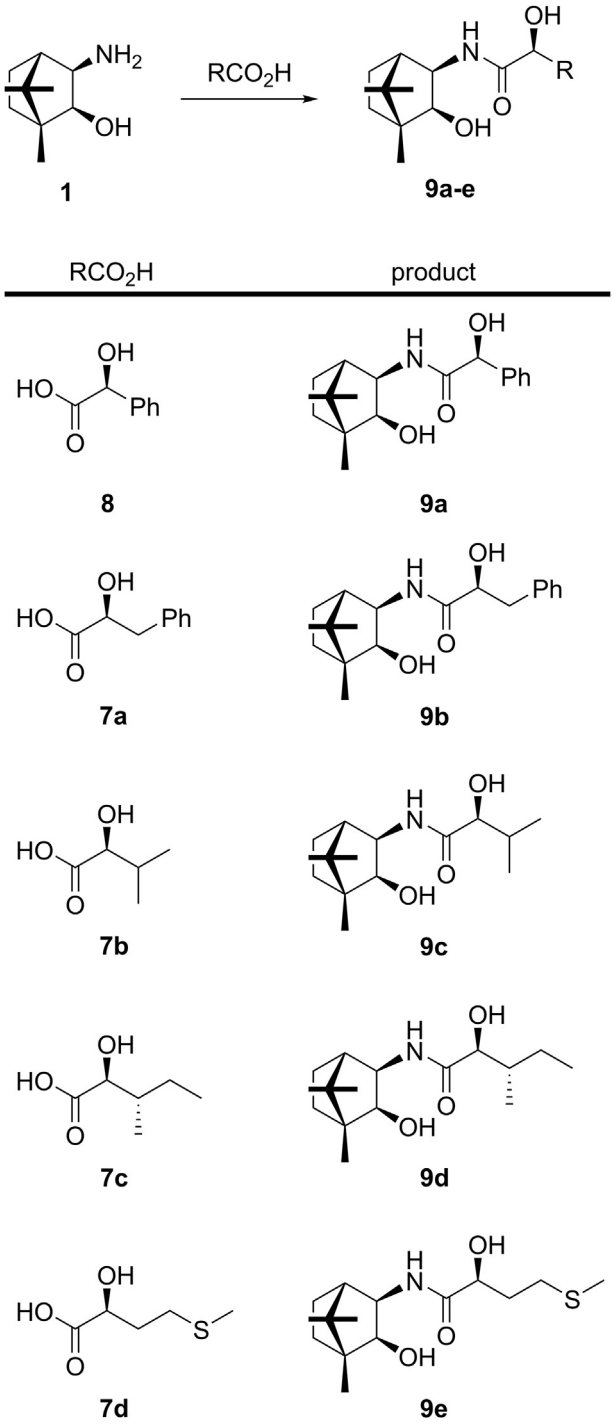
method of Canetti (Table 1). All the compounds synthesized are in agreement with the formal Lipinski's rule of five.

The amidoalcohols **3a**–**e** exhibited excellent activities against *M. tuberculosis* H37Rv with MIC around 0.3 μM (Table 1, Entry 1–5). The camphane-based 3-phenylpropanamidoalcohol **3a** and cinnamidoalcohol **3b** differ only in a double bond, which obviously does not influence the activities: both are with MIC of 0.33 μM. The introduction of heterocyclic fragments in the cases of furan-2-carboxamidoalcohol **3c** and nicotinamidoalcohol **3e** resulted in very similar and again excellent activities. The salicylamidoalcohol **3d** was the most active, with MIC values of 0.30 μM. The camphane-based urea **5** also demonstrated very good activity of 0.41 μM (Table 1, Entry 7). The change of the amido function with diphenylphosphinic amide **4** (Table 1, Entry 6) resulted in a drastic drop of the activity to MIC 13.55 μM. Obviously, the presence of a carboxamido-function is crucial for the activity against *M. tuberculosis* H37Rv.

A logical extension of our studies of amidoalcohol structure – anti-TB activity relationship was the introduction of a second hydroxyl function as an additional hydrogen bond donor. The camphane-based mandelamidodiol **9a** gave the lowest activity against *M. tuberculosis* H37Rv with MIC values of 16.50 μM (Table 1, Entry 8). The phenylalanine derived amidodiol **9b**, which differs only in an additional hydroxyl group with 3-phenylpropanamidoalcohol **3a**, revealed MIC comparable with the one of the reference ethambutol (Table 1, Entry 9). Apparently, the presence of a second hydroxyl function had a negative effect on the activity. Very similar MIC values (7.06 μM) were observed with the isoleucine derived amidodiol **9d**. However, the valine and methionine derived compounds **9c** and **9e** exhibited very high MICs of 0.74 μM and 0.66 μM, respectively (Table 1, Entries 10, 12).

Noteworthy, although that the carbon atom at the nitrogen in all camphane structures possesses (*R*)-configuration, most of the molecules are extremely active. This is opposite to the fact that (*S*,*S*)-EMB is approximately 500 fold more active than (*R*,*R*)-EMB [13].

The most active compounds were tested for antimycobacterial activity towards MDR strain 43 of *M. tuberculosis.* The compound with the highest activity against *M. tuberculosis* H37Rv, salicylamidoalcohol **3d**, and the urea **5** were not active against the MDR strain even at concentrations of 5 mg/ml. Nicotinamidoalcohol **3e** gave low activity with MIC 18.22 μM. Intriguing were the results with 3-phenylpropanamidoalcohol **3a** (MIC 6.63 μM), cinnamamidoalcohol **3b** (MIC 6.68 μM) and furan-2-carboxamidoalcohol **3c**



Scheme 4. Synthesis of 3-exo-aminoisoborneol derived amido-diols.

Table 1
In vitro screening data for antimycobacterial activity.

Entry	Compound	Antimycobacterial activity towards reference strain of *Mycobacterium tuberculosis* H37Rv, MIC (μM)	Antimycobacterial activity towards MDR strain 43 of *Mycobacterium tuberculosis*, MIC (μM)
1	**3a**	0.33	6.63
2	**3b**	0.33	6.68
3	**3c**	0.38	7.59
4	**3d**	0.30	>15.08
5	**3e**	0.36	18.22
6	**4**	13.55	NT
7	**5**	0.41	>20.80
8	**9a**	16.50	NT
9	**9b**	6.30	NT
10	**9c**	0.74	NT
11	**9d**	7.06	NT
12	**9e**	0.66	NT
13	EMB·2HCl	7.22	NT

NT – not tested for low active compounds against H37Rv strain.
EMB·2HCl – ethambutol dihydrochloride (reference compound).

(MIC 7.59 μM). All three compounds revealed activity against the MDR strain comparable with the activity of ethambutol against the used control *M. tuberculosis* H37Rv.

2.3. Quantitative structure – antimycobacterial activity relationships (QSAR)

The antimycobacterial activity of the studied compounds was presented in p-units (*pMIC*). The molecular structures were described by 178 descriptors grouped into five types as described in Experimental section. Variable selection procedure by genetic algorithm (GA) was applied to select the most predictive descriptors followed by a stepwise linear regression. Several models were derived and compared in terms of r^2 and q^2. The best performing one is given below:

$$pMIC = 0.592\ knotp - 0.445\ SHHBd - 0.683\ nrings + 12.992$$

$$n = 12 \quad r^2 = 0.875 \quad SEE = 0.290 \quad F = 18.58 \quad q^2 = 0.675$$

No intercorrelation between the descriptors in the model was observed ($R < 0.7$). The plot calculated *pMIC* versus observed *pMIC* is given in Fig. 2.

By definition, the molecular descriptor *knotp* is the difference between two connectivity indices: χ *cluster 3* and χ *path/cluster 4*. The molecular connectivity indices could be interpreted in terms of intermolecular accessibility [22]. Negative *knotp* values correspond to large molecules with high degree of branching, distal substituents, or adjacent (conjugated) rings (Table 2). The highest negative *knotp* value in the studied dataset belongs to compound **4**. The positive coefficient of *knotp* in the QSAR model means that large branched molecules have low antimycobacterial activity.

The descriptor *SHHBd* represents the sum of atom-type hydrogen E-state indices for hydrogen bond donors. There are two common hydrogen bond donors in the studied structures: the OH and NH groups in the camphene moiety. Compounds **5** and **9a**–**e** have an additional H-bond donor in the side chain. They have the highest values for *SHHBd* (Table 2). The negative coefficient of *SHHBd* in the model points to the disfavored contribution of this additional hydrogen bond donor.

The descriptor *nrings* accounts for the number of rings in the molecular graph. The camphene moiety itself brings two rings (Table 2). Some of the compounds have one additional ring in the side chain and only compound **4** contains four rings. The negative coefficient of *nrings* in the model reflects the negative contribution of many rings in the structures.

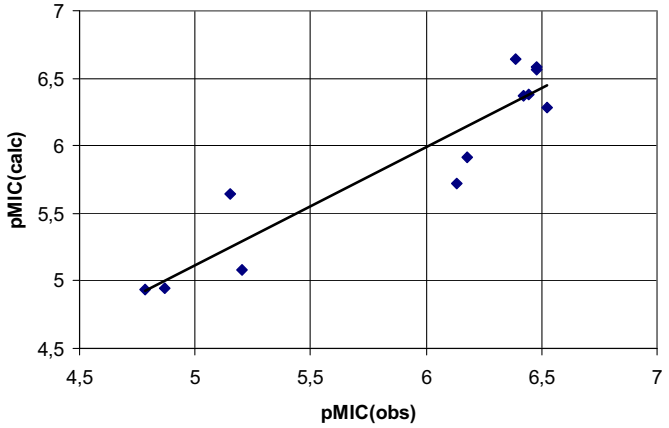


Fig. 2. Calculated *vs.* observed antimycobacterial activity of the studied compounds.

The QSAR study on the camphane-based antimycobacterial compounds provides clear directions for further lead optimization. Two hydrogen bond donors and up to three rings in the molecules are optimal for nanomolar activity.

3. Conclusion

In summary, we have synthesized a small number of new amidoalcohols and amidodiols on the base of 3-*exo*-aminoisoborneol. These were screened for antimycobacterial activities against two MTB strains (H37Rv and MDR strain 43). The camphane-based amides present novel scaffolds for antimycobacterial agents. Some of the new compounds show 25 times higher activity than the classical anti-TB drug ethambutol. Small structural changes in the side chain shift the activity from micromolar to nanomolar inhibitory concentrations. The structure–activity relationship study gave clear directions for further lead optimization.

4. Experimental

4.1. Chemistry

Reagents were commercial grade and used without further purification. Thin layer chromatography (TLC) was performed on aluminum sheets pre-coated with Merck Kieselgel 60 F_{254} 0.25 mm (Merck). Flash column chromatography was carried out using Silica Gel 60 230–400 mesh (Fluka). Commercially available solvents for

Table 2
Antimycobacterial activity (*pMIC*) and the most predictive molecular descriptors.

Entry	Compound	*pMIC*	*knotp*	*SHHBd*	*nrings*
1	**3a**	6.481	−4.109	4.327	3
2	**3b**	6.481	−4.109	4.382	3
3	**3c**	6.420	−4.385	4.432	3
4	**3d**	6.523	−4.475	4.523	3
5	**3e**	6.444	−4.385	4.416	3
6	**4**	4.868	−5.560	4.553	4
7	**5**	6.387	−3.880	6.026	2
8	**9a**	4.783	−4.810	7.105	3
9	**9b**	5.201	−4.606	7.049	3
10	**9c**	6.131	−4.784	6.899	2
11	**9d**	5.151	−4.907	6.915	2
12	**9e**	6.180	−4.434	6.939	2

reactions, TLC and column chromatography were used after distillation (and were dried when needed). Melting points of the compounds were determined using "Electrothermal" MEL-TEMP apparatus (uncorrected). Optical rotation ($[\alpha]_D^{20}$) were measured on Perkin–Elmer 241 polarimeter. The NMR spectra were recorded on a Bruker Avance II+ 600 spectrometer (600.13 for ^{1}H MHz, 150.92 MHz for ^{13}C NMR and 242.92 MHz for ^{31}P NMR) with TMS and 85% H_3PO_4 for ^{31}P as internal standards for chemical shifts (δ, ppm). ^{1}H and ^{13}C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), integration, identification. The assignment of the ^{1}H and ^{13}C NMR spectra was made on the basis of DEPT, COSY, HSQC, HMBC and NOESY experiments. Mass spectra (MS) were recorded on a Thermo Scientific DFS (High Resolution Double Focusing Magnetic Sector) mass spectrometer (Bremen, Germany) by negative-ion electrospray ionization (-ESI). Elemental analyses were performed by Microanalytical Service Laboratory of Faculty of Pharmacy, Medical University of Sofia, using Vario EL3 CHNS(O). Dimethyl sulfoxide (DMSO) for testing of bioactivities was commercial (spectroscopic grade) and was used without distillation.

4.1.1. General procedure for preparation of compounds **3a–d**

To a solution of (2*S*)-(−)-3-*exo*-aminoisoborneol (1 equiv) and NEt_3 (1.1 equiv) in CH_2Cl_2 was added dropwise at 0 °C the corresponding acyl chloride (1.1 equiv). The mixture was stirred for 15 min at 0 °C, and overnight at r.t. The reaction was quenched with sat. aq. NH_4Cl and extracted with CH_2Cl_2. The combined organic extracts were washed with sat. aq. $NaHCO_3$ followed by aq. Citric acid, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel.

4.1.1.1. *N-((1S,2R,3S,4R)-3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-3-phenylpropanamide* **3a**.

Yield: 64%; white crystals; m.p. 70–72 °C. $[\alpha]_{20}D_{=+26.9\ (c\ 0.540,\ CHCl3)}$. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.29–7.32 (m, 2H, arom.), 7.21–7.25 (m, 3H, arom.), 6.05 (d, 1H, $J_{H,H}$ = 3.9 Hz, NH), 3.73–3.78 (m, 2H, 2-H, 3-H), 2.94–3.02 (m, 2H, −COCH_2−), 2.53 (*t*, $J_{H,H}$ = 7.7 Hz, 2H, CH_2Ph), 2.12 (brs, 1H, −OH), 1.73 (d, $J_{H,H}$ = 4.4 Hz, 1H, 4-H), 1.68–1.71 (m, 1H, 5-H_{exo}), 1.47–1.52 (m, 1H, 6-H_{exo}), 1.15–1.19 (m, 1H, 5-H_{endo}), 1.03–1.07 (m, 1H, 6-H_{endo}), 0.95 (s, 3H, 8-H), 0.92 (s, 3H, 10-H), 0.79 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 172.07 (CO), 140.85 (1 arom. C), 128.54 (2 arom. CH), 128.43 (2 arom. CH), 126.24 (1 arom. CH), 79.71 (2-C), 57.67 (3-C), 50.28 (4-C), 49.09 (1-C), 46.79 (7-C), 38.61 (−CO−CH_2−), 33.24 (−CH_2-Ph), 31.88 (6-C), 26.16 (1C, 5-C), 21.50 (8-C), 20.85 (9-C), 11.31 (10-C) ppm. ESI-MS: 324 (100, $[M + Na]^+$). $C_{19}H_{27}NO_2$ (301.42): calcd. C 75.71, H 9.03, N 4.65, found C 75.86, H 9.21, N 4.68.

4.1.1.2. *N-((1S,2R,3S,4R)-3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)cinnamamide* **3b**.

Yield: 63%; white crystals; m.p. 136–138 °C. $[\alpha]_{20}D_{=+31.4\ (c\ 0.140,\ CHCl3)}$. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.59 (d, 1H, $J_{H,H}$ = 15.6 Hz, 1H, −CO−C*H*=), 7.47–7.49 (m, 2H, arom.), 7.33–7.35 (m, 3H, arom.), 6.42 (d, $J_{H,H}$ = 15.6 Hz, 1H, =C*H*-Ph), 6.39 (d, $J_{H,H}$ = 4.2 Hz, 1H, NH), 3.91–3.93 (m, 1H, 3-H), 3.87 (d, $J_{H,H}$ = 7.7 Hz, 1H, 2-H), 2.57 (brs, 1H, −OH), 1.89 (d, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.71–1.77 (m, 1H, 5-H_{exo}), 1.50–1.55 (m, 1H, 6H_{exo}), 1.18–1.23 (m, 1H, 5H_{endo}), 1.11 (s, 3H, 8-H), 1.06–1.09 (m, 1H, 6H_{endo}), 0.96 (s, 3H, 10-H), 0.82 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 165.78 (CO), 140.68 (−CO−*C*H=), 134.87 (1 arom. C), 129.61 (1 arom. CH), 128.80 (2 arom. CH), 127.82 (2 arom. CH), 120.96 (=*C*H-Ph), 79.90 (2-C), 58.01 (3-C), 50.43 (4-C), 49.20 (1-C), 46.93 (7-C), 33.30 (6-C), 26.26 (5-C), 21.57 (8-C), 21.11 (9-C), 11.37 (10-C) ppm. ESI-MS: 322 (100, $[M + Na]^+$). $C_{19}H_{25}NO_2$ (299.41): calcd. C 76.22, H 8.42; N 4.68, found C 76.36, H 8.53, N 4.73.

4.1.1.3. *N-((1S,2R,3S,4R)-3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)furan-2-carboxamide* **3c**.

Yield: 54%; white crystals; m.p. 165–169 °C. $[\alpha]_{20}D_{=+56.1\ (c\ 0.490,\ CHCl3)}$. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.41 (dd, $J_{H,H}$ = 1.56, 0.6 Hz, 1H, furane H), 7.08 (brs, 1H, NH), 7.07 (dd, $J_{H,H}$ = 3.4, 0.6 Hz, 1H, furane H), 6.47 (dd, $J_{H,H}$ = 3.4, 1.7 Hz, 1H, furane H), 3.95–3.97 (m, 1H, 3-H), 3.88 (d, $J_{H,H}$ = 7.6 Hz, 1H, 2-H), 2.20 (brs, 1H, −OH), 1.92 (d, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.73–1.78 (m, 1H, 5-H_{exo}), 1.51–1.56 (m, 1H, 6-H_{exo}), 1.21–1.25 (m, 1H, 5-H_{endo}), 1.14 (s, 3H, 8-H), 1.07–1.12 (m, 1H, 6-H_{endo}), 0.97 (s, 3H, 10-H), 0.83 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 158.28 (CO), 148.25 (furane C), 143.88 (furane CH), 113.84 (furane CH), 112.02 (furane CH), 79.90 (2-C), 57.39 (3-C), 50.42 (4-C), 49.24 (1-C), 46.94 (7-C), 33.26 (6-C), 26.20 (5-C), 21.55 (8-C), 21.00 (9-C), 11.35 (10-C) ppm. ESI-MS: 286 (100, $[M + Na]^+$). $C_{15}H_{21}NO_3$ (263.33): calcd. C 68.42, H 8.04; N 5.32, found C 68.54, H 8.13, N 8.14.

4.1.1.4. *2-((1S,2R,3S,4R)-3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-ylcarbamoyl)phenyl acetate* **3d**.

Yield: 74%; white crystals; m.p. 129–131 °C. $[\alpha]_{20}D_{=+35.7\ (c\ 0.470,\ CHCl3)}$. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.82–7.84 (m, 1H, arom.), 7.44–7.47 (m, 1H, arom.), 7.29–7.31 (m, 1H, arom.), 7.14 (d, $J_{H,H}$ = 4.9 Hz, 1H, NH), 7.09–7.10 (m, 1H, arom.), 3.99 (m, 1H, 2-H), 3.87 (d, $J_{H,H}$ = 7.8 Hz, 1H, 3-H), 2.35 (s, 3H, −CO−CH_3), 1.92 (d, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.72–1.78 (m, 1H, 5-H_{exo}), 1.51–1.56 (m, 1H, 6-H_{exo}), 1.22–1.26 (m, 1H, 5-H_{endo}), 1.12 (s, 3H, 8-H), 1.07–1.10 (m, 1H, 6-H_{endo}), 0.96 (s, 3H, 10-H), 0.83 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 169.67 (CO), 165.50 (CO), 148.49 (1 arom. C), 132.05 (1 arom. CH), 130.28 (1 arom. CH), 128.24 (1 arom. C), 126.52 (1 arom. CH), 123.51 (1 arom CH), 80.19 (2-C), 58.38 (3-C), 50.66 (4-C), 49.56 (1-C), 47.22 (7-C), 33.54 (6-C), 26.60 (5-C), 21.85 (−CO−CH_3), 21.63 (8-C), 21.41 (9-C), 11.64 (10-C) ppm. ESI-MS: 354 (100, $[M + Na]^+$). $C_{19}H_{25}NO_4$ (331.41): calcd. C 68.86, H 7.60; N 4.23, found C 68.94, H 7.73, N 4.25.

4.1.2. *N-((1S,2R,3S,4R)-3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)nicotinamide* **3e**

A mixture of (2*S*)-(−)-3-*exo*-aminoisoborneol (0.333 g, 1.97 mmol) and ethyl nicotinate (0.327 g, 2.16 mmol) was heated for 4 days at 90–100 °C. After cooling, the reaction was directly subjected to flash column chromatography (silica gel, CH_2Cl_2/EtOAc = 2:3). Yield: 51%; white crystals; m.p. 173–175 °C. $[\alpha]_{20}D_{=+19.8\ (c\ 0.455,\ CHCl3)}$. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 8.91–8.92 (m, 1H, Py H), 8.67–8.68 (m, 1H, Py H), 8.09–8.11 (m, 1H, Py H), 7.36–7.38 (m, 1H, Py H), 7.19 (d, $J_{H,H}$ = 5.0 Hz, 1H, NH), 3.97 (m, 1H, 2-H), 3.89 (d, $J_{H,H}$ = 7.55, 1H, 3-H), 3.37 (brs, 1H, −OH), 2.00 (d, $J_{H,H}$ = 4.4 Hz, 1H, 4-H), 1.73–1.79 (m, 1H, 5-H_{exo}), 1.52–1.57 (m, 1H, 6-H_{exo}), 1.22–1.26 (m, 1H, 5-H_{endo}), 1.13 (s, 3H, 8-H), 1.06–1.10 (m, 1H, 6-H_{endo}), 0.97 (s, 3H, 10-H), 0.83 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 164.85 (CO), 151.87 (Py CH), 147.59 (Py CH), 135.24 (Py CH), 130.75 (Py C), 123.65 (Py CH), 79.38 (2-C), 57.79 (3-C), 50.20 (4-C), 49.27 (1-C), 46.85 (7-C), 33.18 (6-C), 26.11 (5-C), 21.64 (8-C), 21.10 (9-C), 11.38 (10-C) ppm. ESI-MS: 297 (100, $[M + Na]^+$). $C_{16}H_{22}N_2O_2$ (274.36): calcd. C 70.04, H 8.08; N 10.21, found C 69.94, H 8.22, N 10.25.

4.1.3. *N-((1S,2R,3S,4R)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-P,P-diphenylphosphinic amide* **4**

To a solution of (2*S*)-(−)-3-*exo*-aminoisoborneol (0.54 g, 3.19 mmol) and NEt_3 (0.49 ml, 3.51 mmol) in CH_2Cl_2 (20 ml) was added dropwise at 0 °C diphenylphosphinic chloride (0.67 ml, 3.51 mmol). The mixture was stirred for 30 min at 0 °C, and overnight at r.t. The reaction was quenched with sat. aq. NH_4Cl and extracted with CH_2Cl_2. The combined organic extracts were washed with sat. aq. $NaHCO_3$ followed by aq. Citric acid, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified

by flash column chromatography (silica gel, CH_2Cl_2/EtOAc/MeOH = 3:2:0.005). Yield: 41%; white crystals; m.p. 204–207 °C. $[\alpha]_{20}D$ = +67.0 (c 0.470, $CHCl_3$). ^1H NMR ($CDCl_3$, 600 MHz) δ = 7.96–7.92 (m, 2H, arom.), 7.83–7.79 (m, 2H, arom.), 7.54–7.52 (m, 1H, arom.), 7.49–7.46 (m, 3H, arom.), 7.42–7.40 (m, 2H, arom.), 3.70 (d, $J_{H,H}$ = 7.1 Hz, 1H, 2-H), 3.54 (d, $J_{H,H}$ = 7.8, Hz, 1H, NH), 3.17–3.12 (m, 1H, 3-H), 1.74 (d, $J_{H,H}$ = 4.4 Hz, 1H, 4-H), 1.67–1.61 (m, 1H, 5-H_{exo}), 1.44–1.39 (m, 1H, 6-H_{exo}), 1.19 (s, 3H, 8-H), 0.99–0.96 (m, 1H, 6-H_{endo}), 0.95 (s, 3H, 10-H), 0.82–0.87 (m, 1H, 5-H_{endo}), 0.79 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 132.69 (d, $J_{^{31}P,^{13}C}$ = 9.4 Hz, 2arom. CH), 132.56 (d, $J_{^{31}P,^{13}C}$ = 126.0 Hz, 1arom. C), 131.47 (d, $J_{^{31}P,^{13}C}$ = 2.9 Hz, 2arom. CH), 131.33 (d, $J_{^{31}P,^{13}C}$ = 9.7 Hz, 2arom. CH), 130.94 (d, $J_{^{31}P,^{13}C}$ = 132.5 Hz, 1arom. C), 128.67 (d, $J_{^{31}P,^{13}C}$ = 12.5 Hz, 2arom. CH), 128.58 (d, $J_{^{31}P,^{13}C}$ = 12.3 Hz, 2arom. CH), 80.18 (2-C), 60.16 (3-C), 52.10 (d, $J_{^{31}P,^{13}C}$ = 8.2 Hz, 4-C), 49.05 (1-C), 47.20 (7-C), 33.30 (6-C), 26.88 (5-C), 21.67 (9-C), 21.48 (8-C), 11.59 (10-C) ppm. ESI-MS: 392 (100, $[M + Na]^+$). $C_{22}H_{28}NO_2P$ (369.44): calcd. C 71.52, H 7.64; N 3.79, found C 71.64, H 7.72, N 3.81.

4.1.4. 1-Ethyl-3-((1S,2R,3S,4R)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)urea **5**

To a solution of (2*S*)-(−)-3-*exo*-aminoisoborneol (0.500 g, 2.95 mmol) in CH_2Cl_2 (15 ml) was added dropwise at 0 °C ethyl isocyanate (0.26 ml, 3.25 mmol). The mixture was stirred for 30 min at 0 °C, and 2 h at r.t. The reaction was quenched with sat. aq. NH_4Cl and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/EtOAc = 1:1). Yield: 76%; white crystals; m.p. 141–144 °C. $[\alpha]_{20}D$ = +39.8 (c 0.465, $CHCl_3$). ^1H NMR ($CDCl_3$, 600 MHz) δ = 5.22 (brs, 1H, NH), 4.80 (brs, 1H, NH), 3.75 (d, $J_{H,H}$ = 7.5 Hz, 1H, 3-H), 3.64 (d, $J_{H,H}$ = 7.4 Hz, 1H, 2-H), 3.18 (q, $J_{H,H}$ = 7.2 Hz, 2H, $-CH_2-CH_3$), 2.19 (brs, 1H, −OH), 1.72 (d, $J_{H,H}$ = 3.1 Hz, 1H, 4-H), 1.67–1.71 (m, 1H, 5-H_{exo}), 1.46–1.51 (m, 1H, 6-H_{exo}), 1.14 (*t*, $J_{H,H}$ = 7.2 Hz, 3H, CH_2-CH_3), 1.11–1.16 (m, 1H, 5-H_{endo}), 1.08 (s, 3H, 8-H), 1.03–1.06 (m, 1H, 6-H_{endo}), 0.93 (s, 3H, 10-H), 0.80 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 158.67 (CO), 79.82 (2-C), 58.34 (3-C), 50.71 (4-C), 48.92 (1-C), 46.77 (7-C), 35.26 ($-CH_2-CH_3$), 33.29 (6-C), 26.20 (5-C), 21.54 (8-C), 20.93 (9-C), 15.29 ($-CH_2-CH_3$), 11.33 (10-C) ppm. ESI-MS: 263 (100, $[M + Na]^+$). $C_{13}H_{24}N_2O_2$ (240.34): calcd. C 64.97, H 10.07, N 11.66, found C 65.11, H 10.12, N 11.70.

4.1.5. General procedure for the preparation of the amides (**9a**–**e**)

1-Hydroxybenzotriazole (HOBt) (1.1 equiv) and the respective acid (1 equiv) were suspended in dichloromethane, and the mixture was stirred for 5 min. Then, *N*-[3-(dimethylamino)propyl]-*N* -ethylcarbodiimide (EDC) (1.1 equiv) was added, followed by (2*S*)-(−)-3-*exo*-aminoisoborneol (1 equiv). Stirring was continued at room temperature until the starting material was completely consumed (TLC). The mixture was quenched with water, extracted with CH_2Cl_2, washed with 2 M HCl, sat. aq. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel.

4.1.5.1. (S)-2-Hydroxy-N-((1S,2R,3S,4R)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-2-phenylacetamide **9a**.

Yield: 59%; white crystals; m.p. 179–181 °C. $[\alpha]_{20}D$ = +28.6 (c 0.466, CH_3OH). ^1H NMR (CD_3OD, 600 MHz) δ = 7.44–7.43 (m, 2H, arom.), 7.34–7.32 (m, 2H, arom.), 7.30–7.27 (m, 1H, arom.), 5.00 (s, 1H, PhC*H*OH), 3.72 (d, $J_{H,H}$ = 7.9 Hz, 1H, 2-H), 3.64 (d, $J_{H,H}$ = 7.9 Hz, 1H, 3-H), 1.85 (d, $J_{H,H}$ = 4.4 Hz, 1H, 4-H), 1.75–1.69 (m, 1H, 5-H_{exo}), 1.55–1.50 (m, 1H, 6-H_{exo}), 1.17–1.13 (m, 1H, 5-H_{endo}), 1.07 (s, 3H, 8-H), 1.07–1.03 (m, 1H, 6-H_{endo}), 0.94 (s, 3H, 10-H), 0.83 (s, 3H, 9-H) ppm. ^{13}C NMR (CD_3OD, 150.9 MHz) δ = 174.38 (CO), 141.88 (1 arom. C), 129.33 (2 arom. CH), 129.04 (1 arom. CH), 128.13 (2 arom. CH), 79.72 (2-C), 75.51 (Ph*C*HOH), 58.11 (3-C), 51.60 (4-C), 50.06 (1-C), 47.53 (7-C), 34.22 (6-C), 26.90 (5-C), 22.15 (9-C), 21.55 (8-C), 11.83 (10-C) ppm. ESI-MS: 326 (100, $[M + Na]^+$). $C_{18}H_{25}NO_3$ (303.40): calcd. C 71.26, H 8.31, N 4.62, found C 71.31, H 8.42, N 4.70.

4.1.5.2. (S)-2-Hydroxy-N-((1S,2R,3S,4R)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-3-phenylpropanamide **9b**.

Yield: 64%; white crystals; m.p. 147–149 °C. $[\alpha]_{20}D$ = −59.7 (c 0.452, CH_3OH). ^1H NMR (CD_3OD, 600 MHz) δ = 7.26–7.25 (m, 4H, arom.), 7.20–7.17 (m, 1H, arom.), 4.22–4.20 (m, 1H, $PhCH_2CHOH$), 3.68 (d, $J_{H,H}$ = 7.9 Hz, 1H, 2-H), 3.62 (d, $J_{H,H}$ = 7.9 Hz, 1H, 3-H), 3.14 (dd, $J_{H,H}$ = 13.9, 3.3 Hz, 1H, $PhCH_2CHOH$), 2.75 (dd, $J_{H,H}$ = 13.9, 8.8 Hz, 1H, $PhCH_2$), 1.82 (d, $J_{H,H}$ = 4.4 Hz, 1H, 4-H), 1.75–1.69 (m, 1H, 5-H_{exo}), 1.54–1.49 (m, 1H, 6-H_{exo}), 1.20–1.15 (m, 1H, 5-H_{endo}), 1.07–1.02 (m, 1H, 6-H_{endo}), 1.01 (s, 3H, 8-H), 0.91 (s, 3H, 10-H), 0.84 (s, 3H, 9-H) ppm. ^{13}C NMR (CD_3OD, 150.9 MHz) δ = 175.54 (CO), 139.58 (1 arom. C), 130.58 (2 arom. CH), 129.17 (2 arom. CH), 127.35 (1 arom. CH), 79.66 (2-C), 74.23 ($PhCH_2CHOH$), 58.01 (3-C), 51.46 (4-C), 50.02 (1-C), 47.47 (7-C), 41.80 ($PhCH_2$), 34.19 (6-C), 26.89 (5-C), 22.15 (9-C), 21.50 (8-C), 11.80 (10-C) ppm. ESI-MS: 340 (100, $[M + Na]^+$). $C_{17}H_{27}NO_3$ (317.42): calcd. C 71.89, H 8.57, N 4.41, found C 71.95, H 8.01, N 4.50.

4.1.5.3. (S)-2-Hydroxy-N-((1S,2R,3S,4R)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-3-methylbutanamide **9c**.

Yield: 52%; white crystals; m.p. 184–186 °C. $[\alpha]_{20}D$ = −40.5 (c 0.395, CH_3OH). ^1H NMR (CD_3OD, 600 MHz) δ = 3.87 (dd, $J_{H,H}$ = 3.1, 0.5 Hz, 1H, *CH*OH), 3.72 (d, $J_{H,H}$ = 7.9 Hz, 1H, 2-H), 3.66 (d, $J_{H,H}$ = 7.9 Hz, 1H, 3-H), 2.11 (dq, $J_{H,H}$ = 10.1, 6.9 Hz, 1H, $(CH_3)_2CH$), 1.82 (d, $J_{H,H}$ = 4.4 Hz, 1H, 4-H), 1.75–1.69 (m, 1H, 5-H_{exo}), 1.55–1.50 (m, 1H, 6-H_{exo}), 1.20–1.15 (m, 1H, 5-H_{endo}), 1.05 (s, 3H, 8-H), 1.08–1.04 (m, 1H, 6-H_{endo}), 1.01 (d, $J_{H,H}$ = 7.0 Hz, 3H, $(CH_3)_2CH$), 0.93 (s, 3H, 10-H), 0.82 (d, $J_{H,H}$ = 6.8 Hz, 3H, $(CH_3)_2CH$), 0.81 (s, 3H, 9-H) ppm. ^{13}C NMR (CD_3OD, 150.9 MHz) δ = 175.89 (CO), 79.75 (2-C), 77.03 (*CH*OH), 57.94 (3-C), 51.55 (4-C), 50.06 (1-C), 47.49 (7-C), 34.24 (6-C), 32.67 ($(CH_3)_2CH$), 26.91 (5-C), 22.15 (9-C), 21.54 (8-C), 19.75 ($(CH_3)_2CH$), 15.94 ($(CH_3)_2CH$), 11.83 (10-C) ppm. ESI-MS: 292 (100, $[M + Na]^+$). $C_{15}H_{27}NO_3$ (269.38): calcd. C 66.88, H 10.10, N 5.20, found C 66.95, H 10.09, N 5.26.

4.1.5.4. (2S,3S)-2-Hydroxy-N-((1S,2R,3S,4R)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-3-methylpentanamide **9d**.

Yield: 67%; white crystals; m.p. 186–188 °C. $[\alpha]_{20}D$ = −34.9 (c 0.410, CH_3OH). ^1H NMR (CD_3OD, 600 MHz) δ = 3.91 (dd, $J_{H,H}$ = 3.3, 0.4 Hz, 1H, *CH*OH), 3.72 (d, $J_{H,H}$ = 7.9 Hz, 1H, 2-H), 3.65 (d, $J_{H,H}$ = 7.9 Hz, 1H, 3-H), 1.87–1.83 (m, 1H, CH_3CH), 1.82 (d, $J_{H,H}$ = 4.3 Hz, 1H, 4-H), 1.74–1.69 (m, 1H, 5-H_{exo}), 1.55–1.50 (m, 1H, 6-H_{exo}), 1.42–1.35 (m, 1H, CH_3CH_2), 1.22–1.14 (m, 2H, 5-H_{endo}; CH_3CH_2), 1.05 (s, 3H, 8-H), 1.08–1.04 (m, 1H, 6-H_{endo}), 0.98 (d, $J_{H,H}$ = 7.0 Hz, 3H, CH_3CH), 0.93 (s, 3H, 10-H), 0.87 (*t*, $J_{H,H}$ = 7.5 Hz, 3H, CH_3CH_2), 0.82 (s, 3H, 9-H) ppm. ^{13}C NMR (CD_3OD, 150.9 MHz) δ = 175.85 (CO), 79.74 (2-C), 77.09 (*CH*OH), 57.95 (3-C), 51.55 (4-C), 50.06 (1-C), 47.48 (7-C), 39.73 (CH_3CH), 34.24 (6-C), 26.90 (5-C), 24.19 (CH_3CH_2), 21.16 (9-C), 21.55 (8-C), 16.29 (, CH_3CH), 12.37 (CH_3CH_2), 11.83 (10-C) ppm. ESI-MS: 306 (100, $[M + Na]^+$). $C_{16}H_{29}NO_3$ (283.41): calcd. C 67.81, H 10.31, N 4.94, found C 67.95, H 10.39, N 4.98.

4.1.5.5. (S)-2-Hydroxy-N-((1S,2R,3S,4R)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-4-(methylthio)butanamide **9e**.

Yield: 55%; white crystals; m.p. 157–159 °C. $[\alpha]_{20}D$ = 29.6 (c 0.270, CH_3OH). ^1H NMR ($CDCl_3$, 600 MHz) δ = 7.19 (d, $J_{H,H}$ = 5.3 Hz, 1H, NH), 4.29–4.26 (m, 1H, *CH*OH), 3.83 (dd, $J_{H,H}$ = 7.5, 4.0 Hz, 1H, 2-H), 3.80–3.78 (m, 1H, 3-H), 3.74 (d, $J_{H,H}$ = 5.2 Hz, 1H, CH*OH*), 2.73–2.63 (m, 2H, CH_3SCH_2), 2.47 (d, $J_{H,H}$ = 4.1 Hz, 1H, OH), 2.18–2.13 (m, 1H, $CH_3SCH_2CH_2$), 2.12 (s, 3H, CH_3S), 1.97–1.91 (m, 1H, $CH_3SCH_2CH_2$), 1.82 (d, $J_{H,H}$ = 4.4 Hz, 1H, 4-H), 1.77–1.71 (m, 1H, 5-H_{exo}), 1.54–1.49

(m, 1H, 6-H_{exo}), 1.21–1.16 (m, 1H, 5-H_{endo}), 1.09 (s, 3H, 8-H), 1.10–1.05 (m, 1H, 6-H_{endo}), 0.94 (s, 3H, 10-H), 0.82 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 173.19 (CO), 79.94 (2-C), 71.72 (*C*HOH), 57.54 (3-C), 50.17 (4-C), 49.19 (1-C), 46.93 (7-C), 33.22 (6-C), 32.88 ($CH_3SCH_2CH_2$), 30.25 (CH_3SCH_2), 26.19 (5-C), 21.45 (9-C), 20.96 (8-C), 15.22 (CH_3S), 11.30 (10-C) ppm. ESI-MS: 324 (100, $[M + Na]^+$). $C_{15}H_{27}NO_3S$ (301.44): calcd. C 59.77, H 9.03, N 4.65, found C 59.85, H 9.12, N 4.68.

4.2. Antimycobacterial activity

The antimycobacterial activity was determined through the proportional method of Canetti towards reference strain *M. tuberculosis* H37Rv and multidrug resistant strain 43 (resistant to Rifampin and Isoniazid), recovered from Bulgarian adult HIV-negative pulmonary TB patient, who was permanent resident of the country. This method, recommended by the WHO, is the most commonly used one worldwide for exploration of sensitivity/resistance of tuberculosis strains towards chemotherapeutics [23–27]. It allows precise determination of the proportion of resistant mutants to a certain drug.

A sterile suspension/solution of each tested compound was added to Löwenstein–Jensen egg based medium before its coagulation (30 min at 85 °C). Each compound was tested at four concentrations – 5 mg/ml, 2 mg/ml, 0.2 mg/ml and 0.1 mg/ml (in DMSO). Tubes with Löwenstein–Jensen medium (5 ml) containing tested compounds and those without them (controls) were inoculated with a suspension of *M. tuberculosis* H37Rv (10^5 cells/ml) and incubated for 45 days at 37 °C. The ratio between the number of colonies of *M. tuberculosis* grown in medium containing compounds and the number of colonies in control medium were calculated and expressed as percentage of inhibition. The MIC is defined as the minimum concentration of compound required to inhibit bacterial growth completely (0% growth). The MIC values are calculated and given as μM.

4.3. Molecular descriptors

The chemical structure of the studied compounds was described by 178 molecular descriptors computed using the software package MDL QSAR version 2.2 (MDL Information Systems, Inc., San Leandro, USA). The descriptors were grouped into five types: molecular connectivity χ (chi) indices [28], which represent molecular structure by encoding significant topological features of whole molecule; κ shape indices – a family of graph-based structure descriptors that represent shape [29]; electrotopological state (E-state) indices, which represent the electron density at each atom and the ability of those electrons to participate in intermolecular interactions [29]; molecular properties – weight, log *P*, log $D_{7.4}$, number of rings, number of hydrogen bond donors and acceptors, etc.; and 3D molecular properties such as polarizability, surface area, volume, etc.

4.4. Variable selection

A genetic algorithm (GA) [30], as implemented in the MDL QSAR package, was used as a variable selection procedure in the present study. GA allows one to select a subset of the most significant predictors using two evolutionary operations: random mutation and genetic recombination (crossover). The algorithm was used in the study with default values for the size of initial population (32), choice of parents (tournament selection), types of crossover (uniform crossover) and mutation (one-point mutation), and fitness function (Friedman's lack-of-fit scoring function with 2 parameters) [31]. The selected variables entered a stepwise linear regression, as implemented in the MDL QSAR package. It was used in a forward mode with default value for F-to-enter (4.00) and F-to-remove (3.99).

4.5. Models assessment

Final models were assessed by explained variance (r^2), standard error of estimate (SEE), and cross-validated r^2 (q^2) according to the following equations:

$$r^2 = 1 - \frac{\sum_{i=1}^{n}\left(pMIC_{\mathrm{obs},i} - pMIC_{\mathrm{calc},i}\right)}{\sum_{i=1}^{n}\left(pMIC_{\mathrm{obs},i} - pMIC_{\mathrm{obs,mean}}\right)},$$

$$\mathrm{SEE} = \sqrt{\frac{\sum_{i=1}^{n}\left(pMIC_{\mathrm{obs},i} - pMIC_{\mathrm{calc},i}\right)}{n-d-1}},$$

$$q^2 = 1 - \frac{\sum_{i=1}^{n}\left(pMIC_{\mathrm{obs},i} - pMIC_{\mathrm{pred},i}\right)}{\sum_{i=1}^{n}\left(pMIC_{\mathrm{obs},i} - pMIC_{\mathrm{obs,mean}}\right)},$$

where $pMIC_{obs,i}$ is the observed *pMIC* of the *i*-th compound, $pMIC_{calc,i}$ is the calculated by the model *pMIC*, $pMIC_{pred,i}$ is the predicted by the model *pMIC*, *n* – the number of compounds in the dataset, *d* – the number of molecular descriptors in the model. Fisher statistics (*F*) for models also was calculated. In the leave-one-out cross-validation (LOO-CV) procedure, one compound is excluded from the training subset, the model is derived based on the remaining $n - 1$ compounds and used to predict the $pMIC_{pred,i}$ of the excluded *i*-th compound.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.10.015.

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