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Isobornylamine and bornylamine derived amides – synthesis, antimycobacterial activity and cytotoxicity

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A series of ten novel amides was designed and synthesized on the base of the camphane scaffold by coupling of isobornylamine and bornylamine with different carboxylic acids. The compounds were screened for their *in vitro* activity against *M. tuberculosis* H37Rv and cytotoxic activity against the human embryonal kidney cell line HEK-293T. Six of the structures revealed profound anti-tuberculosis activity (MICs up to 0.16 μM) in combination with moderate to low cytotoxicity. The compound derived from bornylamine and furan-2-carboxylic acid can be considered as a promising lead for the development of anti-mycobacterial agents.

Keywords: amides, camphane, cytotoxicity, *M. tuberculosis* H37Rv.

INTRODUCTION

Natural products have served as templates for the development of many anti-mycobacterial agents [1]. Their structural complexity appeared to be crucial for the observed activity due to interaction with specific protein targets [2]. Since tuberculosis (TB) still remains a growing problem in the context of treatment of multidrug-resistant TB (MDR-TB) [3], the development of drugs with novel mechanism of action is of urgent need [4]. Now there is a reemerging interest in natural products as templates for the discovery of new antitubercular leads [5, 6].

Recently we have reported a series of novel (+)-camphor derived compounds as potent inhibitors of *Mycobacterium tuberculosis* (MTB) [7-11]. Some of our camphanes displayed properties which made them promising anti-TB lead compounds: high activity against MTB strain H37Rv, activity against a drug-resistant strain and generally low cytotoxic activity against the human embryonal kidney cell line 293T. We were inspired mostly by the analogy of the camphane scaffold as compact lipophilic moiety to the adamantyl fragment in SQ 109 (Fig. 1), a capable second line anti-TB drug [12-14]. It is very likely that its highly lipophilic adamantane structure helps the penetration of the bacterial wall and is thus decisive for the activity [15].

Initially our investigations started with the synthesis of β-amidoalcohols and amidodiols on the base of 3-*exo*-aminoisoborneol (Fig. 1, **I**) [7]. All compounds were active against MTB strain H37Rv and three of them showed activity against MDR-TB. A quantitative structure - activity relationship (QSAR) study revealed several structural requirements for anti-TB activity. Based on them we expanded the study by the design of isobornylamine (Fig. 1, **III**) and bornylamine (Fig. 1, **IV**) derived amidoalcohols by combining the camphane skeleton with α-hydroxy acids [8, 9].

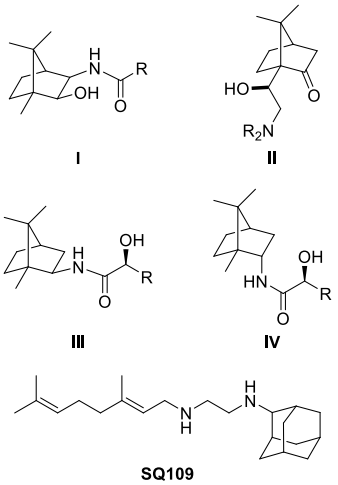

Fig. 1. Structures of camphane based compounds with anti-mycobacterial activity and SQ109.

Four of the new compounds showed activities in the nanomolar range, significantly higher than the activities of the initial set. The QSAR study based on all derivatives provided new directions for

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optimization [10]. Further, a series of aminoalcohols (Fig. 1, **II**) were synthesized by reaction of aminolysis of camphor derived oxiranes with chosen amines. Three compounds demonstrated excellent activities against MDR-TB combined with low to moderate cytotoxic activity [11].

Herein, we present the synthesis, antibacterial activity and cytotoxicity of camphane based amides lacking hydroxyl function in their structures. This work is a continuation of our SAR study of the anti-tuberculosis potency of camphor derived compounds.

EXPERIMENTAL

General

Reagents were of 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 reactions, TLC and column chromatography were used after distillation (and were dried when needed). Melting points of the compounds were determined using the "Electrothermal" MEL-TEMP apparatus (uncorrected). Optical rotation ($[\alpha]_D^{20}$) was measured on a Perkin–Elmer 241 polarimeter. The NMR spectra were recorded on a Bruker Avance II+ 600 spectrometer (600.13 for ^{1}H MHz and 150.92 MHz for ^{13}C NMR) with TMS 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. Elemental analyses were performed at the Microanalytical Service Laboratory of the Faculty of Pharmacy, Medical University of Sofia, using Vario EL*3* CHNS(O). Dimethyl sulfoxide (DMSO) for testing of bioactivities was commercial (spectroscopic grade) and was used without distillation.

*General procedure for preparation of compounds **4a-d** and **5a-d***

To a solution of isobornylamine or bornylamine (1 equiv) and NEt_3 (1.1 equiv) in CH_2Cl_2 the corresponding acyl chloride (1.1 equiv) was added dropwise at 0 °C. The mixture was stirred for 15 min at 0 °C, and left 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.

*3-Phenyl-N-((1R,2R,4R)-1,7,7-trimethylbicyclo [2.2.1]heptan-2-yl)propanamide **4a***. Yield: 72%; white crystals; m.p. 75-78 °C. $[\alpha]^{20}_D$ = -33.3 (c 1.269, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.29-7.27 (m, 2H, arom.), 7.23-7.18 (m, 3H, arom.), 5.24 (d, $J_{H,H}$ = 7.6 Hz, 1H, NH), 3.85 (dq, $J_{H,H}$ = 9.0, 5.1 Hz, 1H, 2-H*endo*), 2.95 (dt, $J_{H,H}$ = 7.5, 2.2 Hz, 2H, C*H*$_2$CO), 2.48 (dt, $J_{H,H}$ = 7.5, 2.2 Hz, 2H, C*H*$_2$Ph), 1.80 (dd, $J_{H,H}$ = 13.3, 9.1, 1H, 3-H*endo*), 1.69-1.64 (m, 2H, 4-H, 5-H*exo*), 1.54-1.49 (m, 1H, 3-H*exo*), 1.42-1.38 (m, 1H, 6-H*exo*), 1.27-1.23 (m, 1H, 6-H*endo*), 1.14-1.10 (m, 1H, 5-H*endo*), 0.78 (s, 3H, 8-H), 0.73 (s, 3H, 9-H), 0.69 (s, 3H, 10-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 171.28 (CO), 140.66 (1 arom. C), 128.53 (2 arom. CH), 128.32 (2 arom. CH), 126.25 (1 arom. CH), 56.56 (2-C), 48.19 (1-C), 46.97 (7-C), 44.75 (4-C), 39.05 (*C*H$_2$Ph), 38.59 (3-C), 35.86 (6-C), 31.77 (*C*H$_2$CO), 26.91 (5-C), 20.16 (9-C), 20.08 (8-C), 11.56 (10-C) ppm. $C_{19}H_{27}NO$ (285.42): calcd. C 79.95, H 9.53, N 4.91, found C 79.76, H 9.21, N 4.83.

*N-((1R,2R,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl)cinnamamide **4b***. Yield: 74%; white crystals; m.p. 65-69°C. $[\alpha]^{20}_D$ = -66.6 (c 0.988, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.61 (d, 1H, $J_{H,H}$ = 15.5 Hz, 1H, C*H*CO), 7.51-7.50 (m, 2H, arom.), 7.36-7.35 (m, 3H, arom.), 6.38 (d, 1H, $J_{H,H}$ = 15.5 Hz, 1H, C*H*Ph), 5.58 (d, $J_{H,H}$ = 8.6 Hz, 1H, NH), 4.06 (dq, $J_{H,H}$ = 9.0, 5.1 Hz, 1H, 2-H*endo*), 1.92 (dd, $J_{H,H}$ = 13.3, 9.0 Hz, 1H, 3-H*endo*), 1.77 (t, $J_{H,H}$ = 4.3 Hz, 1H, 4-H), 1.75-1.70 (m, 1H, 5-H*exo*), 1.99-1.57 (m, 2H, 3-H*exo*, 6-H*exo*), 1.37-1.32 (m, 1H, 6-H*endo*), 1.22-1.17 (m, 1H, 5-H*endo*), 0.97 (s, 3H, 8-H), 0.89 (s, 3H, 10-H), 0.86 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 165.12 (CO), 140.80 (1 arom. C), 134.87 (*C*HCO), 129.53 (1 arom. CH), 128.73 (2 arom. CH), 127.72 (2 arom. CH), 121.02 (*C*HPh), 56.88 (2-C), 48.75 (1-C), 47.14 (7-C), 44.85 (4-C), 39.17 (3-C), 35.91 (6-C), 26.97 (5-C), 20.37 (9-C), 20.25 (8-C), 11.76 (10-C) ppm. $C_{19}H_{25}NO$ (283.41): calcd. C 80.52, H 8.89, N 4.94, found C 80.90, H 9.24, N 4.87.

*N-((1R,2R,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl)furan-2-carboxamide **4c***. Yield: 97%; oil. $[\alpha]^{20}_D$ = -64.7 (c 1.168, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.43-7.42 (m, 1H, furane H), 7.08 (d, $J_{H,H}$ = 3.4 Hz, 1H, furane H), 6.49 (dd, $J_{H,H}$ = 3.4, 1.5 Hz, 1H, furane H), 6.34 (d, $J_{H,H}$ = 7.1

Hz, 1H, NH), 4.06 (dq, $J_{H,H}$ = 9.1, 5.0 Hz, 1H, 2-H*endo*), 1.93 (dd, $J_{H,H}$ = 13.3, 9.1 Hz, 1H, 3-H*endo*), 1.79 (t, $J_{H,H}$ = 4.3 Hz, 1H, 4-H), 1.77-1.66 (m, 2H, 3-H*exo*, 5-H*exo*), 1.63-1.59 (m, 1H, 6-H*exo*), 1.37-1.33 (m, 1H, 6-H*endo*), 1.22-1.18 (m, 1H, 5-H*endo*), 1.00 (s, 3H, 8-H), 0.89 (s, 3H, 10-H), 0.87 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 157.63 (CO), 148.22 (furane C), 143.56 (furane CH), 113.83 (furane CH), 112.11 (furane CH), 56.23 (2-C), 48.75 (1-C), 47.11 (7-C), 44.89 (4-C), 39.13 (3-C), 35.85 (6-C), 26.99 (5-C), 20.26 (8-C), 20.22 (9-C), 11.79 (10-C) ppm. $C_{15}H_{21}NO_2$ (247.33): calcd. C 72.84, H 8.56, N 5.66, found C 73.01, H 8.47, N 5.71.

2-((1R,2R,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-ylcarbamoyl)phenyl acetate ***4d***. Yield: 86%; white crystals; m.p. 76-80°C. $[\alpha]^{20}_D$ = -50.5 (c 1.109, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.67 (dd, $J_{H,H}$ = 7.2, 1.7 Hz, 1H, arom.), 7.45 (dt, $J_{H,H}$ = 7.5, 1.7 Hz, 1H, arom.), 7.30 (dt, $J_{H,H}$ = 7.6, 1.1 Hz, 1H, arom.), 7.08 (dd, $J_{H,H}$ = 8.2, 1.0 Hz, 1H, arom.), 6.12 (d, $J_{H,H}$ = 7.8 Hz, 1H, NH), 4.09 (dq, $J_{H,H}$ = 8.9, 5.2 Hz, 1H, 2-H*endo*), 2.33 (s, 3H, COC*H*$_3$), 1.95 (dd, $J_{H,H}$ = 13.3, 9.1 Hz, 1H, 3-H*endo*), 1.78 (t, $J_{H,H}$ = 4.3 Hz, 1H, 4-H), 1.76-1.71 (m, 1H, 5-H*exo*), 1.65-1.58 (m, 2H, 3-H*exo*, 6-H*exo*), 1.38-1.34 (m, 1H, 6-H*endo*), 1.22-1.18 (m, 1H, 5-H*endo*), 0.93 (s, 3H, 8-H), 0.91 (s, 3H, 10-H), 0.85 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 169.39 (CO), 165.17 (CO), 147.69 (1 arom. C), 131.41 (1 arom. CH), 129.40 (1 arom. CH), 126.30 (1 arom. CH), 123.16 (1 arom. CH), 57.09 (2-C), 48.72 (1-C), 47.12 (7-C), 44.84 (4-C), 39.03 (3-C), 35.91 (6-C), 26.96 (5-C), 21.16 (CO*C*H$_3$), 20.35 (8-C), 20.21 (9-C), 11.77 (10-C) ppm. $C_{19}H_{25}NO_3$ (315.41): calcd. C 72.35, H 7.99, N 4.44, found C 72.51, H 8.26, N 4.53.

3-phenyl-N-((1R,2S,4R)-1,7,7-trimethylbicyclo [2.2.1]heptan-2-yl)propanamide ***5a***. Yield: 97%; white crystals; m.p. 92-96°C. $[\alpha]^{20}_D$ = 0 (c 1.094, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.30-7.26 (m, 2H, arom.), 7.22-7.19 (m, 3H, arom.), 5.29 (d, $J_{H,H}$ = 8.2 Hz, 1H, NH), 4.21-4.17 (m, 1H, 2-H*exo*), 2.97 (dt, $J_{H,H}$ = 7.6, 1.4 Hz, 2H, C*H*$_2$CO), 2.51 (t, $J_{H,H}$ = 7.6 Hz, 2H, C*H*$_2$Ph), 2.32-2.26 (m, 1H, 3-H*exo*), 1.73-1.67 (m, 1H, 5-H*exo*), 1.61 (t, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.28-1.25 (m, 1H, 6-H*exo*), 1.23-1.18 (m, 1H, 6-H*endo*), 1.06-1.02 (m, 1H, 5-H*endo*), 0.92 (s, 3H, 8-H), 0.84 (s, 3H, 9-H), 0.72 (s, 3H, 10-H), 0.62 (dd, $J_{H,H}$ = 13.4, 4.6 Hz, 1H, 3-H*endo*) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 171.99 (CO), 140.81 (1 arom. C), 128.54 (2 arom. CH), 128.37 (2 arom. CH), 128.23 (1 arom. CH), 53.58 (2-C), 49.20 (1-C), 48.04 (7-C), 44.76 (4-C), 38.76 (*CH*$_2$Ph), 37.64 (3-C), 31.87 (C*H*$_2$CO), 28.24 (5-C), 27.80 (6-C), 19.76 (9-C), 18.59 (8-C), 13.53 (10-C) ppm. $C_{19}H_{27}NO$ (285.42): calcd. C 79.95, H 9.53, N 4.91, found C 80.16, H 9.76, N 5.04.

N-((1R,2S,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl)cinnamamide ***5b***. Yield: 99%; white crystals; m.p. 82-85°C. $[\alpha]^{20}_D$ = 0 (c 1.155, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.63 (d, 1H, $J_{H,H}$ = 15.5 Hz, 1H, C*H*CO), 7.52-7.50 (m, 2H, arom.), 7.38-7.34 (m, 3H, arom.), 6.46 (d, 1H, $J_{H,H}$ = 15.5 Hz, 1H, C*H*Ph), 5.73 (d, $J_{H,H}$ = 8.7 Hz, 1H, NH), 4.43-4.39 (m, 1H, 2-H*exo*), 2.44-2.38 (m, 1H, 3-H*exo*), 1.83-1.77 (m, 1H, 5-H*exo*), 1.69 (t, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.58-1.53 (m, 1H, 6-H*endo*), 1.46-1.40 (m, 1H, 6-H*exo*), 1.24-1.19 (m, 1H, 5-H*endo*), 0.98 (s, 3H, 8-H), 0.89 (s, 3H, 9-H), 0.87 (s, 3H, 10-H), 0.88-0.84 (m, 1H, 3-H*endo*) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 165.88 (CO), 140.75 (1 arom. C), 134.92 (*CH*CO), 129.50 (1 arom. CH), 128.74 (2 arom. CH), 127.73 (2 arom. CH), 121.04 (*CH*Ph), 53.91 (2-C), 49.69 (1-C), 48.21 (7-C), 44.90 (4-C), 37.83 (3-C), 28.39 (5-C), 28.04 (6-C), 19.82 (9-C), 18.66 (8-C), 13.73 (10-C) ppm. $C_{19}H_{25}NO$ (283.41): calcd. C 80.52, H 8.89, N 4.94, found C 80.74, H 9.12, N 4.88.

N-((1R,2S,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl)furan-2-carboxamide ***5c***. Yield: 93%; white crystals; m.p. 85-88°C. $[\alpha]^{20}_D$ = +7.7 (c 0.942, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.45 (d, $J_{H,H}$ = 0.9 Hz, 1H, furane H), 7.09 (d, $J_{H,H}$ = 3.1 Hz, 1H, furane H), 6.50 (dd, $J_{H,H}$ = 3.4, 1.7 Hz, 1H, furane H), 6.38 (d, $J_{H,H}$ = 8.1 Hz, 1H, NH), 4.43-4.38 (m, 1H, 2-H*exo*), 2.45-2.39 (m, 1H, 3-H*exo*), 1.85-1.79 (m, 1H, 5-H*exo*), 1.71 (t, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.62-1.57 (m, 1H, 6-H*endo*), 1.47-1.42 (m, 1H, 6-H*exo*), 1.28-1.24 (m, 1H, 5-H*endo*), 0.99 (s, 3H, 8-H), 0.91 (dd, $J_{H,H}$ = 13.6, 4.6 Hz, 1H, 3-H*endo*), 0.90 (s, 3H, 9-H), 0.87 (s, 3H, 10-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 158.37 (CO), 148.26 (furane C), 143.52 (furane CH), 113.87 (furane CH), 112.14 (furane CH), 53.37 (2-C), 49.67 (1-C), 48.19 (7-C), 44.90 (4-C), 37.71 (3-C), 28.38 (5-C), 28.02 (6-C), 19.82 (9-C), 18.66 (8-C), 13.68 (10-C) ppm. $C_{15}H_{21}NO_2$ (247.33): calcd. C 72.84, H 8.56, N 5.66, found C 72.98, H 8.69, N 5.69.

2-((1R,2S,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-ylcarbamoyl)phenyl acetate ***5d***. Yield: 91%; white crystals; m.p. 115-117°C. $[\alpha]^{20}_D$ = +3.3 (c 0.967, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 7.74 (dd, $J_{H,H}$ = 7.7, 1.7 Hz, 1H, arom.), 7.46 (dt, $J_{H,H}$ = 7.6, 1.7 Hz, 1H, arom.), 7.31 (dt, $J_{H,H}$ = 7.6, 1.1 Hz, 1H, arom.), 7.11 (dd, $J_{H,H}$ = 8.1, 1.0 Hz, 1H, arom.), 6.28 (d, $J_{H,H}$ = 8.4 Hz, 1H, NH), 4.44-4.40 (m, 1H, 2-H*exo*), 2.48-2.42 (m, 1H, 3-H*exo*), 2.35 (s, 3H, COC*H*$_3$), 1.84-1.78 (m, 1H, 5-H*exo*), 1.71 (t, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.54-1.49 (m, 1H, 6-H*endo*),

1.47-1.42 (m, 1H, 6-H*exo*), 1.20-1.16 (m, 1H, 5-H*endo*), 0.99 (s, 3H, 8-H), 0.90 (s, 3H, 9-H), 0.89 (s, 3H, 10-H), 0.84 (dd, $J_{H,H}$ = 13.4, 4.6 Hz, 1H, 3-H*endo*) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 169.21 (CO), 165.75 (CO), 147.56 (1 arom. C), 131.42 (1 arom. CH), 129.72 (1 arom. CH), 126.32 (1 arom. CH), 123.07 (1 arom. CH), 54.20 (2-C), 49.51 (1-C), 48.20 (7-C), 44.84 (4-C), 37.83 (3-C), 28.44 (5-C), 28.14 (6-C), 21.19 (CO*CH*$_3$), 19.79 (9-C), 18.66 (8-C), 13.72 (10-C) ppm. $C_{19}H_{25}NO_3$ (315.41): calcd. C 72.35, H 7.99, N 4.44, found C 72.48, H 8.16, N 4.49.

*General procedure for preparation of compounds **4e** and **5e***

1-Hydroxybenzotriazole (HOBt) (1.1 equiv) and nicotinic acid (1.1 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 isobornylamine or bornylamine (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 2M 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.

*N-((1R,2R,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl)nicotinamide **4e***. Yield: 90%; white crystals; m.p. 133-136°C. $[\alpha]^{20}_D$ = -55.4 (c 1.093, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 8.90 (dd, $J_{H,H}$ = 2.3, 0.8 Hz, 1H, arom.), 8.72 (dd, $J_{H,H}$ = 4.8, 1.8 Hz, 1H, arom.), 8.08 (ddd, $J_{H,H}$ = 7.9, 2.3, 1.8 Hz, 1H, arom.), 7.39 (ddd, $J_{H,H}$ = 7.9, 4.8, 0.8 Hz, 1H, arom.), 6.10 (d, $J_{H,H}$ = 7.4 Hz, 1H, NH), 4.12 (dq, $J_{H,H}$ = 8.9, 5.0 Hz, 1H, 2-H*endo*), 1.95 (dd, $J_{H,H}$ = 13.4, 9.1 Hz, 1H, 3-H*endo*), 1.82 (t, $J_{H,H}$ = 4.3 Hz, 1H, 4-H), 1.79-1.74 (m, 1H, 5-H*exo*), 1.73-1.68 (m, 1H, 3-H*exo*), 1.66-1.61 (m, 1H, 6-H*exo*), 1.39-1.35 (m, 1H, 6-H*endo*), 1.24-1.29 (m, 1H, 5-H*endo*), 1.01 (s, 3H, 8-H), 0.93 (s, 3H, 10-H), 0.88 (s, 3H, 9-H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 164.78 (CO), 152.11 (1 arom. CH), 147.41 (1 arom. CH), 135.02 (1 arom. CH), 130.71 (1 arom. C), 123.56 (1 arom. CH), 57.30 (2-C), 48.84 (1-C), 47.18 (7-C), 44.86 (4-C), 39.10 (3-C), 35.85 (6-C), 26.96 (5-C), 20.32 (8-C), 20.20 (9-C), 11.81 (10-C) ppm. $C_{16}H_{22}N_2O$ (258.36): calcd. C 74.38, H 8.58, N 10.84, found C 74.61, H 8.26, N 10.92.

*N-((1R,2S,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl)nicotinamide **5e***. Yield: 90%; white crystals; m.p. 77-81°C. $[\alpha]^{20}_D$ = -4.8 (c 1.107, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 8.97 (d, $J_{H,H}$ = 2.0 Hz, 1H, arom.), 8.72-8.71 (m, 1H, arom.), 8.12-8.10 (m, 1H, arom.), 7.40-7.38 (m, 1H, arom.), 6.22 (brs, 1H, NH), 4.49-4.45 (m, 1H, 2-H*exo*), 2.49-2.43 (m, 1H, 3-H*exo*), 1.87-1.80 (m, 1H, 5-H*exo*), 1.74 (t, $J_{H,H}$ = 4.5 Hz, 1H, 4-H), 1.60-1.56 (m, 1H, 6-H*endo*), 1.51-1.46 (m, 1H, 6-H*exo*), 1.27-1.23 (m, 1H, 5-H*endo*), 1.01 (s, 3H, 8-H), 0.92 (dd, $J_{H,H}$ = 13.6, 4.6 Hz, 1H, 3-H*endo*), 0.92 (s, 3H, 9-H), 0.90 (s, 3H, 10-H), ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 165.73 (CO), 152.08 (1 arom. CH), 147.64 (1 arom. CH), 135.05 (1 arom. CH), 130.80 (1 arom. C), 123.50 (1 arom. CH), 54.39 (2-C), 49.74 (1-C), 48.27 (7-C), 44.88 (4-C), 37.70 (3-C), 28.38 (5-C), 28.12 (6-C), 19.79 (9-C), 18.63 (8-C), 13.77 (10-C) ppm. $C_{16}H_{22}N_2O$ (258.36): calcd. C 74.38, H 8.58, N 10.84, found C 74.54, H 8.37, N 10.90.

Anti-mycobacterial activity

The anti-mycobacterial activity was determined through the proportional method of Canetti towards reference strain *M. Tuberculosis* H37Rv. This method, recommended by the WHO, is the most commonly used one worldwide for exploration of sensitivity/resistance of tuberculosis strains towards chemotherapeutics [16-19].

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 –2 μg/ml, 0.2 μg/ml, 0.1 μg/ml and 0.05 μg/ml in DMSO. Tubes with Löwenstein-Jensen medium (5 ml) containing the 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 the medium containing compounds and the number of colonies in the control medium were calculated and expressed as percentage of inhibition. The MIC is defined as the minimum concentration of compound required to completely inhibit bacterial growth (0% growth). The MIC values are calculated and given as μM.

Cytotoxicity

The human embryonal kidney cell line 293T cells were obtained from the German Collection of Microorganisms and Cell Cultures. Cells were kept in controlled environment e RPMI-1640 medium, supplemented with 10% heat-inactivated fetal calf serum and 2mM L-glutamine, at 37 ºC in a 'Heraeus' incubator with 5% CO_2 humidified atmosphere.

The cytotoxicity of the newly synthesized compounds was assessed using the MTT [3-(4,5-

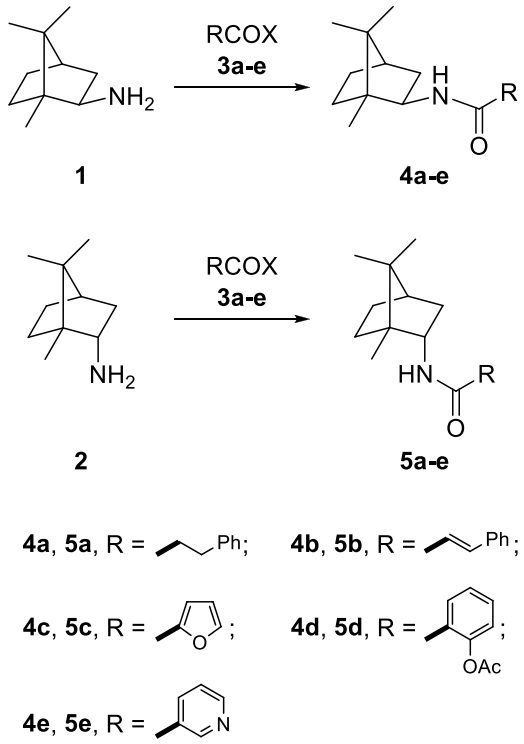
dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-dye reduction assay as described by Mossman with some modifications [20, 21]. In brief, exponentially growing cells were seeded in 96-well microplates (100 µl/well) at a density of 3.5 105 cell/ml and allowed to grow for 24 h prior to the exposure to the studied compounds. Stock solutions of the tested compounds were freshly prepared in DMSO and thereafter were subset to serial dilutions with growth medium in order to obtain the desired final concentrations. At the final dilutions the solvent concentration never exceeded 0.5%. Cells were exposed to the tested agents for 72 h, whereby a set of at least 8 separate wells was used for each concentration. After the exposure period MTT solution (10 mg/ml in phosphate-buffered saline) aliquots (100 µl/well) were added to each well. The plates were further incubated for 4 h at 37 ºC and the MTT-formazan crystals formed were dissolved through addition of 110 ml of 5% HCOOH in 2-propanol. The MTT-formazan absorption of the samples was measured by a multimode microplate reader DTX 880 (Beckman Coulter) at 580 nm. Cell survival fractions were calculated as percentage of the untreated control. The experimental data were fitted to sigmoidal concentration-response curves and the corresponding IC_{50} values (concentrations causing 50% reduction of cellular survival vs. the untreated control) via non-linear regression (GraphPad Prizm software for PC).

RESULTS AND DISCUSSION

Chemistry

We focused on the synthesis of a library of compounds analogues to the one already reported for 3-*exo*-aminoisoborneol [7]. The pharmacophore groups combined with the latter were attached to camphane skeletons lacking hydroxyl functionality (Scheme 1). Isobornylamine **1** and bornylamine **2**, readily available from natural (+)-camphor, were selected as key starting compounds [22, 23].

The target amides **4a-d** and **5a-d** were obtained by condensation of **1** and **2** with acid chlorides **3a-d** using standard conditions for acylation (0 °C and Et_3N in dry CH_2Cl_2). The synthesis of the amides **4e** and **5e** was accomplished by coupling reactions of **1** and **2** with nicotinic acid **3e** in the presence of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBT) as activating agents. All products were obtained in excellent yields and purity after flash column chromatography. The compounds were identified by elemental analysis, ^{1}H NMR and ^{13}C NMR. The spectral analyses were in accordance with the assigned structures.



Scheme 1. Synthesis of isobornylamine and bornylamine based amides.

Anti-mycobacterial activity

The synthesized compounds were screened for their *in vitro* activity against *M. tuberculosis* H37Rv using the method of Canetti (Table 1) [16-19]. The compounds exhibited excellent activities against the referent strain *M. tuberculosis* H37Rv with MIC between 0.16 µM and 0.81 µM. Only the derivative of bornylamine with cinnamic acid **5b** displayed activity (MIC 7.06 µM) comparable to the one of ethambutol, used as reference. We were interested in the effect of the chirality of the camphane fragment on the structure activity relationship. Except in the case of cinnamic acid derived **4b** and **5b**, no influence of the chirality on the activity was detected. In all other cases we observed activity correlation between the bornylamine and the isobornylamine derivatives.

Cytotoxicity

The excellent anti-mycobacterial activity of the compounds forced us to evaluate their cytotoxic activity, and hence to assess the selectivity of the antiproliferative effects (Table 1). The cytotoxicity was evaluated against the human embryonal kidney cell line 293T after 72 h exposure [20, 21]. With the only exception of **5c** all tested compounds induced 50% inhibition of cellular viability within

the tested range of concentrations (12.5–200 μM) exerting moderate to high cytotoxicity. Unfortunately, the exceptionally active salicylic acid derivatives **4d**, **5d** displayed high cytotoxicity. The same was relevant for cinnamamides **4b**, **5b**. The 3-phenylpropanamides **4a**, **5a**, differing from the latter only in a double bond, revealed moderate cytotoxicity, which combined with the high antituberculous activity gave promising selectivity indices (SI>255). Interestingly, the furanecarboxamides **4c** and **5c** differ drastically in their cytotoxicity depending on the chirality of the camphane scaffold. Diastereoisomer **5c** revealed low cytotoxicity and high activity towards MTB, which made it a promising lead for further investigation. The same conclusion can be made for nicotinamides **4e**, **5e**. They exhibit appreciable anti-mycobacterial activity and low cytotoxicity, which is represented by their selectivity indexes: 329.7 for **4e**, and 181.3 for **5e**.

Table 1. *In vitro* screening data for anti-mycobacterial activity and cytotoxicity.

Compound	Anti-MTB[a] MIC (μM)	Cytotoxicity[b] IC_{50} (μM)	SI[c] IC_{50}/MIC.
4a	0.17	43.5	255.9
5a	0.17	46.1	271.2
4b	0.18	30.6	170.0
5b	7.06	28.9	4.1
4c	0.81	54.2	66.9
5c	0.81	>200	>247
4d	0.16	10.82	67.6
5d	0.16	12.63	78.9
4e	0.38	125.3	329.7
5e	0.77	139.6	181.3
EMB.2HCl[d]	7.22		

[a]Anti-mycobacterial activity towards reference strain of *Mycobacterium tuberculosis* H37Rv; [b]*In vitro* cytotoxicity towards human embryonal kidney cell line 293T; [c]Selectivity index; [d]EMB.2HCl – ethambutol dihydrochloride (reference compound).

CONCLUSIONS

In summary, we synthesized bornylamine and isobornylamine derived amides, by combining the camphane scaffolds with chosen pharmacophore groups. The anti-TB activity of the compounds was screened *in vitro* against the referent strain *M. tuberculosis* H37Rv. All compounds demonstrated appreciable activity. The compounds were additionally evaluated for their cytotoxic activity against a human embryonal kidney cell line 293T. Six of the structures revealed high activity in combination with moderate to low cytotoxicity. The compound derived from bornylamine and furan-2-carboxylic acid can reasonably be used as a template for further structural modifications.

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АМИДИ НА ИЗОБОРНИЛАМИН И БОРНИЛАМИН – СИНТЕЗ, АНТИМИКОБАКТЕРИАЛНА АКТИВНОСТ И ЦИТОТОКСИЧНОСТ

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(Резюме)

Серия от десет нови амида беше синтезирана на базата на камфанов скелет с помощта на свързване на изоборниламин и борниламин с различни карбоксилови киселини. Веществата бяха изследвани за тяхната *in vitro* активност срещу *Mycobacterium tuberculosis* H37Rv и цитотоксична активност спрямо човешка ембрионална бъбречна клетъчна линия HEK-293T. Шест от структурите показаха забележителна анти-туберкулозна активност (MIC до 0.16 μM) в комбинация с умерена до ниска цитотоксичност. Веществото получено при кондензация на борниламин и фуран-2-карбоксилова киселина може да бъде считано за перспективен лекарствен прототип за разработване на антимикобактериални агенти.