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Antimycobacterial activity of chiral aminoalcohols with camphane scaffold

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ABSTRACT

A series of aminoalcohols were synthesized by reaction of aminolysis of camphor derived oxiranes with chosen amines. The compounds were evaluated for their *in vitro* activity against *Mycobacterium tuberculosis* H37Rv. Ten of the new structures show much higher activity than the classical anti-TB drug ethambutol. Some of the most active compounds were tested against MDR strain 43, and four of them demonstrated excellent activities with MICs 0.27–0.72 μM. The cytotoxicity of representative exerting antimycobacterial activity compounds was assessed. Quantitative structure–activity relationship (QSAR) model is derived to estimate the contribution of each structural fragment to the activity. The camphane-based aminoalcohols are promising lead compounds for further development of novel antimycobacterial agents.

1. Introduction

Mycobacterium tuberculosis (MTB) infects latently one-third of the world's population causing approximately 9 million cases of active disease each year [1]. The WHO-recommended anti-TB therapy involves four drugs: isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB), but the emergence of multi drug-resistant bacteria (MDR TB) against which the first-line drugs have become ineffective, requires treatment for up to two years with more toxic, less active and more expensive drugs. The latter usually involve any first-line drugs to which the strain is still susceptible and alternative or second-line drugs [2]. The long current drug regimen, the emergence of drug resistant strains and HIV co-infection necessitate the urgent development of new and effective anti-TB drugs.

The synthesis and activity of EMB (Fig. 1. **I**) was first reported by Wilkinson and coworkers [3]. Despite the relatively modest MIC of 10 μM, EMB is a useful addition to tuberculosis chemotherapy, in part because of its very low toxicity and relatively few side-effects. Based on structure–activity relationship (SAR) studies it appeared that crucial for its activity is the distance between the two nitrogens, the presence of β-aminoalcohol motifs, and the small side chains [4]. Lately, the occurrence of a 'better ethambutol' has been systematically investigated through virtual screening or a combinatorial approach [5,6]. Several 1,2-diamines, such as SQ 109 (Fig. 1. **II**), displaying improved antimycobacterial potencies and promising pharmacokinetic properties have thus been reported [7]. It is very likely that the highly lipophilic adamantane structure was integral for the antitubercular activity.

Camphor and its derivatives are of particular importance among the numerous monoterpenoids. Camphor is a readily available and inexpensive chiral source for the synthesis of a variety of structurally diverse compounds [8,9]. Inspired by the β-aminoalcohol fragment in the molecule of EMB and the analogy of the camphane scaffold as a compact lipophilic moiety to the adamantyl fragment in SQ 109, we dedicated our efforts towards the development of camphor derived structures. Recently, we have accomplished a practical synthesis of new β-amido-alcohols and amido-diols on the base of 3-*exo*-aminoisoborneol (Fig. 1. **III**) and isobornylamine (Fig. 1. **IV**) [10,11]. Some of the compounds show 25 times higher antimycobacterial activity than EMB.

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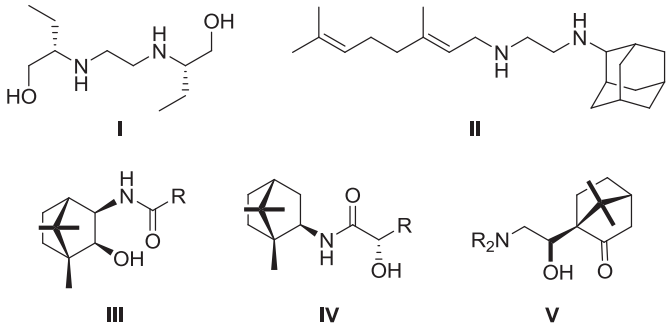


Fig. 1. Structures of ethambutol (**I**), SQ 109 (**II**), 3-*exo*-aminoisoborneol based amido-alcohols (**III**), isobornylamine based amido-alcohols (**IV**), camphor derived amino-alcohols (**V**).

Encouraged by these observations, we expanded the approach to the synthesis of aminoalcohols possessing a camphane skeleton (Fig. 1. **V**). Here, we report the synthesis, antimycobacterial activity and cytotoxicity of 21 compounds and derive a QSAR model to estimate the contribution of each structural fragment to the activity. Thus, we continue the exploration of camphane based structures as a novel class of anti-TB compounds.

2. Results and discussion

2.1. Chemistry

Commercially available 10-camphorsulfonyl chloride **1** was selected as the key (+)-camphor-derived starting compound. Its reaction with diazomethane and triethylamine followed by elimination of sulphur dioxide afforded 7,7-dimethyl-1-vinyl-norbornan-2-on in 77% yield [12]. Subsequent epoxidation using 3-chloroperbenzoic acid led to diastereoisomeric mixtures of oxiranes **2a** and **2b** (Scheme 1) [13].

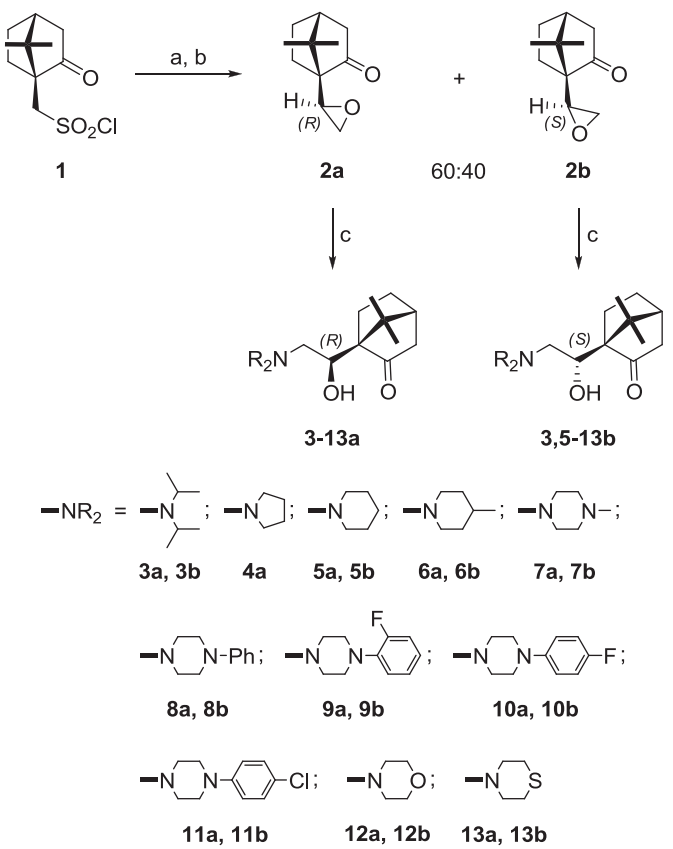
Both oxiranes **2a** and **2b** were isolated as pure diastereoisomers, after chromatographic separation, in 52% and 33% yield respectively [13]. In our previous work we determined the configuration of the newly formed stereogenic centre by advanced NMR experiments and X-ray crystallography [13]. The two products **2a** and **2b** were then converted into aminoalcohols by aminolysis with excess of secondary amines at 50 °C in acetonitrile in the presence of $LiClO_4$ (Scheme 1). The synthesis of compounds **3a,b**–**6a,b** was reported in our previous work considering their use as chiral ligands for asymmetric Zn-mediated catalysis [13]. The initial antimycobacterial activity testing of these compounds directed us towards the creation of a small library of camphane based aminoalcohols and evaluation of their biological properties. The aminolytic cleavage of the oxirane ring with various amines containing groups with pharmacophore properties afforded 14 new compounds **7a,b**–**13a,b**. Consequently, 21 compounds were synthesised and tested for the antimycobacterial activity reported herein.

The aminoalcohols were obtained in very good to excellent yields as summarized in Table 1. All compounds were characterized by NMR spectroscopy, MS data, $[\alpha]_D^{20}$ and elemental analysis.

2.2. Antimycobacterial activity

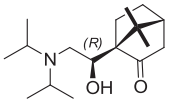
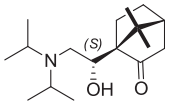
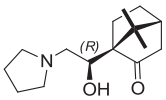
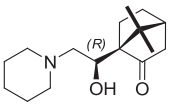
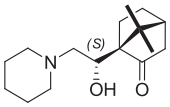
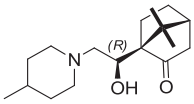
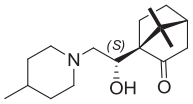
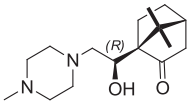
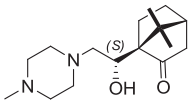
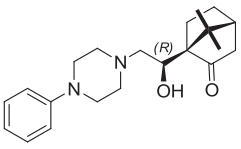
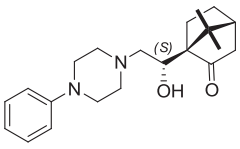
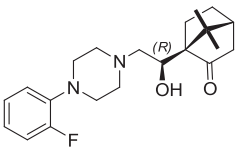
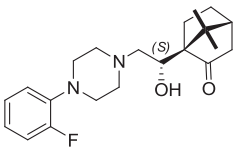
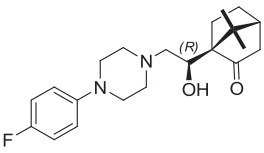
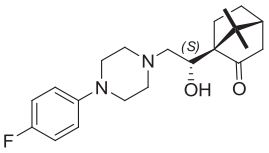
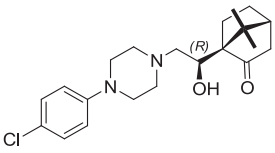
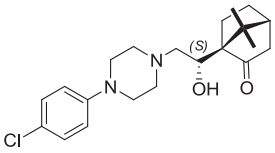
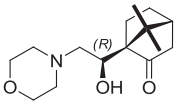
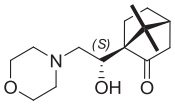
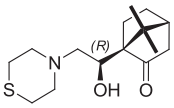
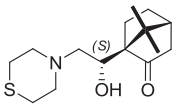
All synthesized camphane-based aminoalcohols were evaluated for their *in vitro* activity against *M. tuberculosis* H37Rv using the method of Canetti (Table 2). Ten of these compounds have shown excellent activity, between 10 and 27 times higher than that of EMB, used as reference (Table 2). The most active compounds possess diisopropyl, piperidine, methylpiperidine, morpholine and fluoro or chloro substituted N-phenylpiperazine moieties. As evident from the calculated log *P* values, most of the compounds are lipophilic and thus have low water solubility (Table 2). However, the compounds possess amino groups, which allow their transformation into corresponding water soluble ammonium salts. The combination of these two features conditions good cell permeability and facilitates preparation of pharmaceutical forms for various applications.

The diisopropylamine and piperidine derived aminoalcohols with *R*-configuration (**3a** and **5a**) exhibited high activity against *M. tuberculosis* H37Rv with MICs of 0.37 and 0.38 μM. The isomers with *S*-configuration **3b** and **5b** gave low activity. Interestingly, both isomers derived from 4-methyl-piperidine **6a,b** demonstrated excellent activity with MIC values of 0.36 and 0.72 μM, respectively. On the other hand, introducing of N-Me-piperazine **7a,b** and N-phenyl-piperazine **8a,b** fragments in the structure of the aminoalcohols resulted in activity lower than that of EMB. Various substituted piperazines (N-*o*-F-phenyl **9a,b**, N-*p*-F-phenyl **10a,b**, N-*p*-Cl-phenyl **11a,b**) were also used as pharmacophores in combination with camphane moiety. The *o*-F-phenyl- and *p*-F-phenyl-piperazine derivatives **9a**,**b**, and **10a**,**b** exhibited extremely high activity for both isomers: MICs of 0.28 μM. In the case of *p*-Cl-phenyl-piperazine, the more active isomer was the one with *S*-configuration **11b**, MIC 0.27 μM. The *R*-isomer **11a** displayed potency comparable to EMB. The morpholine derived aminoalcohol with *R*-configuration **12a** showed excellent MIC of 0.37 μM, while the *S*-isomer **12b** had low activity. The thiomorpholine derivatives **13a,b** were not active.



Scheme 1. Synthesis of aminoalcohols **3a** and **3b**: (a) Et_3N, CH_2N_2, 95 °C, 77%; (b) MCPBA, CH_2Cl_2, 53% **2a** and 32% **2b;** (c) HNR_2, $LiClO_4$, up to 99%.

Table 1
Structures of the synthesized aminoalcohols.

Compound	Starting oxirane **2a**	Yield	Compound	Starting oxirane **2b**	Yield
3a		82	**3b**		71
4a		93	–	–	–
5a		83	**5b**		94
6a		98	**6b**		86
7a		85	**7b**		79
8a		99	**8b**		99
9a		80	**9b**		91
10a		99	**10b**		98
11a		73	**11b**		76
12a		91	**12b**		84
13a		72	**13b**		68

It is worth noting that the stereogenic center at C10 does not influence the activity. Hence, the proper choice of amine containing pharmacophores appears to be crucial.

Some of the most active compounds were tested for anti-mycobacterial activity towards MDR strain 43 of *M. tuberculosis*. Remarkable is that 4-methyl-piperidine **6b,** p-F-phenylpiperazine **10a,b,** and p-Cl-phenylpiperazine **11b** derivatives gave excellent activity with MICs 0.27–0.72 μM. Piperidine **5a** and morpholine **12a** demonstrated low activity: MICs of 18.91 and 18.70 μM, respectively.

Table 2
Data for antimycobacterial activity, cytotoxicity and lipophilicity of synthesized compounds.

No	MTB MIC (μM)[a]	MDR MTB MIC (μM)[b,c]	Cytotoxicity IC_{50} (μM)[d]	Log P[e]
3a	0.37	NT	166.3	2.86
3b	10.66	NT	NT	2.86
4a	19.89	NT	NT	1.92
5a	0.38	18.91	143.2	2.49
5b	18.84	NT	NT	2.49
6a	0.72	NT	158.4	2.98
6b	0.36	0.72	169.3	2.98
7a	17.83	NT	NT	0.55
7b	17.83	NT	NT	0.55
8a	14.60	NT	NT	2.96
8b	14.60	NT	NT	2.96
9a	0.28	NT	128.6	3.33
9b	0.28	NT	119.8	3.33
10a	0.28	0.55	82.2	3.05
10b	0.28	0.55	65.1	3.05
11a	7.96	NT	8.6	3.69
11b	0.27	0.27	9.2	3.69
12a	0.37	>18.70	>200	0.83
12b	18.70	NT	NT	0.83
13a	17.64	NT	NT	1.82
13b	17.64	NT	NT	1.82
EMB·2HCl[f]	7.22	NT	NT	0.06[g]

[a] Antimycobacterial activity towards reference strain of *Mycobacterium tuberculosis* H37Rv, MIC (μM).
[b] Antimycobacterial activity towards MDR strain 43 of *Mycobacterium tuberculosis*, MIC (μM).
[c] NT – not tested.
[d] *In vitro* cytotoxicity towards human embryonal kidney cell line 293T, IC_{50} (μM).
[e] Log *P*, octanol–water partitioning coefficient, was calculated using ACDLabs/ChemSketch 12.01.
[f] EMB·2HCl – ethambutol dihydrochloride (reference compound).
[g] Log *P* and water solubility of EMB·2HCl are known in the literature: N.R. Budha, R.E. Lee and B. Meibohm, Curr. Med. Chem. 15 (2008) 809.

2.3. Cytotoxicity

In order to examine the selectivity of the antiproliferative effects, the cytotoxic activity of representative compounds, exerting antimycobacterial activity, was assessed against the human embryonal kidney cell line 293T, after 72 h exposure. Evident from the IC_{50} values summarized in Table 2, the compounds were generally of low-to-moderate cytotoxicity against the human cells; with the exception of **11a** and **11b**. Disappointing is the result for compound **11b**, which demonstrated high activity both against the reference strain and the MDR strain of *M. tuberculosis*. Compounds **10a** and **10b** have moderate cytotoxicity with IC_{50} 82.2 and 65.1 μM respectively. Compounds **3a**, **5a**, **6a**,**b** and **9a**,**b** induced 50% inhibition of cellular viability at concentrations exceeding 100 μM. Compound **12a** did not induce 50% inhibition of cellular viability within the tested range of concentrations (12.5–200 μM), hence its cytotoxicity was exceeding 200 μM.

Structure **6b** exhibits both high antimycobacterial activity against the reference and the MDR strains, and low cytotoxicity, which makes it a promising lead compound for antimycobacterial agents.

2.4. Quantitative structure–antimycobacterial activity relationships (QSAR)

The antimycobacterial activity of the studied compounds towards the reference strain of *M. tuberculosis* H37RV was presented in p-units (*pMIC*). According to these values, the compounds are almost equally distributed into two classes: *class 1* – high active compounds ($pMIC > 5$) and *class 2* – low active compounds ($pMIC < 5$). Eleven (**3a**, **5a**, **6a**, **9a-12a**, **6b**, **9b**, **10b**, **11b**) have $MIC < 10$ μM and enter *class 1*; ten compounds (**4a**, **7a**, **8a**, **13a**, **3b**, **5b**, **7b**, **8b**, **12b**, **13b**) have antimycobacterial activity above 10 μM and enter *class 2*. The chemical structure of the studied compounds was divided into two parts: a camphane scaffold which is common for all derivatives and an N-containing substituent. The N-containing substituents were described by binary coded descriptors. Twelve descriptors were used: *NME* (N-dimethyl), *PYR* (pyrrolidine), *PPD* (piperidine), *MET-PPD* (methyl-piperidine), *PPZ* (piperazine), *PHE-PPZ* (phenyl-piperazine), *oF-PHE-PPZ* (*o*-flouro-phenyl-piperazine), *pF-PHE-PPZ* (*p*-flouro-phenyl-piperazine), *pCL-PHE-PPZ* (*p*-chloro-phenyl-piperazine), *MOR* (morpholine), *THI-MOR* (thiomorpholine) and *(R)-OH* (hydroxyl group at carbon with (*R*)-configuration). If a particular substituent presents in the structure, the corresponding element in the binary string takes 1; otherwise, it takes 0. The coded structures are shown in Table 3.

Discriminant analysis by partial least squares (DA-PLS) was applied to the dataset as implemented in SIMCA 13.0 [14]. The first principal component (*PC*) accounts for 79% of the variance in the set; the second *PC* adds only 1% and it was omitted. The score and loadings plots are given in Fig. 2. The sets of highly and low active compounds are well distinguished on the score plot (Fig. 2a). The loadings plot (Fig. 2b) points the descriptors contributing positively to the *class 1* activity. They are *MET-PPD*, *oF-PHE-PPZ*, *pF-PHE-PPZ*, *pCL-PHE-PPZ* and *(R)-OH*.

The contributions of substituents to *class 1* activity are given below:

$$\mathrm{DA}(class\ 1) = 1.024 - 0.015\ NME - 0.220\ PYR - 0.015\ PPD + 0.290\ MET - PPD - 0.319\ PPZ - 0.319\ PHE - PPZ + 0.290$$
$$oF - PHE - PPZ + 0.290\ pF - PHE - PPZ + 0.290\ pCL - PHE - PPZ - 0.015\ MOR - 0.319\ THI - MOR + 0.222$$
$$(R) - OH\ n = 21 \quad r^2 = 0.790 \quad q^2 = 0.331 \text{ at threshold} = 0.5 \quad \text{sensitivity} = 1.000 \quad \text{specificity} = 1.000 \quad \text{accuracy} = 1.000$$

According to this model, the substituents methyl-piperidine (*MET-PPD*), *o*-fluoro-phenyl-piperazine (*oF-PHE-PPZ*), *p*-fluoro-phenyl-piperazine (*pF-PHE-PPZ*), *o*-chloro-phenyl-piperazine (*oCL-PHE-PPZ*) and hydroxyl group at carbon with (*R*)-configuration ((*R*)-*OH*) increase the antimycobacterial activity of the studied compounds. The remaining substituents decrease the activity. At threshold 0.5, the model classifies the studied compounds with 100% *accuracy* – compounds with predicted values above 0.5 were classified as *class 1*, those with values below 0.5 – as *class 2*.

3. Conclusion

In summary, we are presenting a small library of compounds synthesized by aminolytic cleavage of camphor derived oxiranes with a set of amines. The compounds were screened for their antimycobacterial activity against *M. tuberculosis* H37Rv. Some of the new compounds show 25 times higher activity than the classical anti-TB drug ethambutol. The activity shifts from micromolar to nanomolar inhibitory concentrations depending on the nitrogen containing fragments. Quantitative structure–activity relationship (QSAR) model was derived to estimate the contribution of each

Table 3
Antimycobacterial activity (*pMIC*) and binary coded structures of the studied compounds.

ID	*pMIC*	*Class*	*NME*[a]	*PYR*[b]	*PPD*[c]	*MET-PPD*[d]	*PPZ*[e]	*PHE-PPZ*[f]	*oF-PHE-PPZ*[g]	*pF-PHE-PPZ*[h]	*pCl-PHE-PPZ*[i]	*MOR*[j]	*THI-MOR*[k]	*(R)–OH*[l]
3a	6.449	1	1	0	0	0	0	0	0	0	0	0	0	1
4a	4.701	2	0	1	0	0	0	0	0	0	0	0	0	1
5a	6.420	1	0	0	1	0	0	0	0	0	0	0	0	1
6a	6.143	1	0	0	0	1	0	0	0	0	0	0	0	1
7a	4.749	2	0	0	0	0	1	0	0	0	0	0	0	1
8a	4.836	2	0	0	0	0	0	1	0	0	0	0	0	1
9a	6.553	1	0	0	0	0	0	0	1	0	0	0	0	1
10a	6.553	1	0	0	0	0	0	0	0	1	0	0	0	1
11a	5.089	1	0	0	0	0	0	0	0	0	1	0	0	1
12a	6.432	1	0	0	0	0	0	0	0	0	0	1	0	1
13a	4.754	2	0	0	0	0	0	0	0	0	0	0	1	1
3b	4.972	2	1	0	0	0	0	0	0	0	0	0	0	0
5b	4.725	2	0	0	1	0	0	0	0	0	0	0	0	0
6b	6.444	1	0	0	0	1	0	0	0	0	0	0	0	0
7b	4.749	2	0	0	0	0	1	0	0	0	0	0	0	0
8b	4.836	2	0	0	0	0	0	1	0	0	0	0	0	0
9b	6.553	1	0	0	0	0	0	0	1	0	0	0	0	0
10b	6.553	1	0	0	0	0	0	0	0	1	0	0	0	0
11b	6.569	1	0	0	0	0	0	0	0	0	1	0	0	0
12b	4.728	2	0	0	0	0	0	0	0	0	0	1	0	0
13b	4.754	2	0	0	0	0	0	0	0	0	0	0	1	0

[a] N-dimethyl.
[b] Pyrrolidine.
[c] Piperidine.
[d] Methyl-piperidine.
[e] Piperazine.
[f] Phenyl-piperazine.
[g] *o*-Flouro-phenyl-piperazine.
[h] *p*-Flouro-phenyl-piperazine.
[i] *o*-Chloro-phenyl-piperazine.
[j] Morpholine.
[k] Thiomorpholine.
[l] Hydroxyl group at carbon with (*R*)-configuration.

structural fragment to the activity. Four of the compounds demonstrate excellent potency against MDR strain 43. Noteworthy, one of them proved to exert low, and two of them moderate cytotoxic activity against a human embryonal kidney cell line 293T. On this basis, the camphane-based aminoalcohols appear to be promising lead compounds for development of antimycobacterial agents.

4. Experimental

4.1. Chemistry

Reagents were of commercial grade and used without further purification. Thin layer chromatography (TLC) was performed on aluminium 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 (Merck). 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 "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 for ^{1}H MHz, 150 MHz for ^{13}C NMR) and Bruker Avance DRX 250 spectrometer (250 for ^{1}H MHz; 62 for ^{13}C MHz) 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. Mass spectra were recorded on Hewlet Packard MS 5973 (30 eV) spectrometer. Elemental analyses were performed in Faculty of Chemistry and Pharmacy, University of Sofia, using Vario EL*III* CHNS(O). Dimethyl sulfoxide (DMSO) for testing of bioactivities was commercial (spectroscopic grade) and was used without distillation.

4.1.1. General procedure for the preparation of aminoalcohols ***3a,b–13a,b***

To a stirred solution of oxirane **2a** or **2b** (0.55 mmol) in 5 ml CH_3CN was added $LiClO_4$ (0.55 mmol) at room temperature and the mixture was stirred until the salt was completely dissolved. Secondary amine (1.7 mmol, 3 eq) was added to the solution and the mixture was stirred at 50 °C until the starting oxirane was consumed (TLC). After aqueous quench, the mixture was acidified with 2N HCl (pH 3–4) and extracted twice with ether to remove traces of starting oxirane. The aqueous layer was basified with 10% aqueous Na_2CO_3 and extracted with CH_2Cl_2 (4 × 15 ml). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (eluent: ether or hexane/ether = 2:3).

4.1.1.1. (1R,4R)-1-((R)-1-hydroxy-2-(4-methylpiperazin-1-yl)ethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***7a***. Yield: 85%; colourless crystals; mp 110–112 °C; $[\alpha]_D^{20} = -12.24$ (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.02 (s, 3H, 8-H), 1.15 (s, 3H, 9-H), 1.25–1.40 (m, 2H, 5-H$_{endo}$, 6-H$_{endo}$), 1.78 (d, 1H, 3-H$_{endo}$, J = 18.3 Hz), 1.95–2.21 (m, 3H, 4-H, 6-H$_{exo}$, 5-H$_{exo}$), 2.29 (dd, 1H, 11-H$_{sin}$, J = 12.1, 10.9 Hz), 2.27 (s, 3H, 16-H), 2.31–2.51 (m, 7H, 3-H$_{exo}$, 12-H, 13-H, 14-H, 15-H), 2.70–2.78 (m, 2H, 13-H, 14-H), 2.92 (dd, 1H, 11-H$_{anti}$, J = 12.2, 3.1 Hz), 3.94 (dd, 1H, 10-H, J = 10.9, 3.1 Hz); ^{13}C NMR (62 MHz, $CDCl_3$): δ = 20.52 (C-8), 21.92 (C-9), 21.94 (C-6), 26.84 (C-5), 43.69 (C-3), 44.18 (C-4), 46.04 (C-16), 48.32 (C-7), 55.33 (C-13, C-12, C-14,

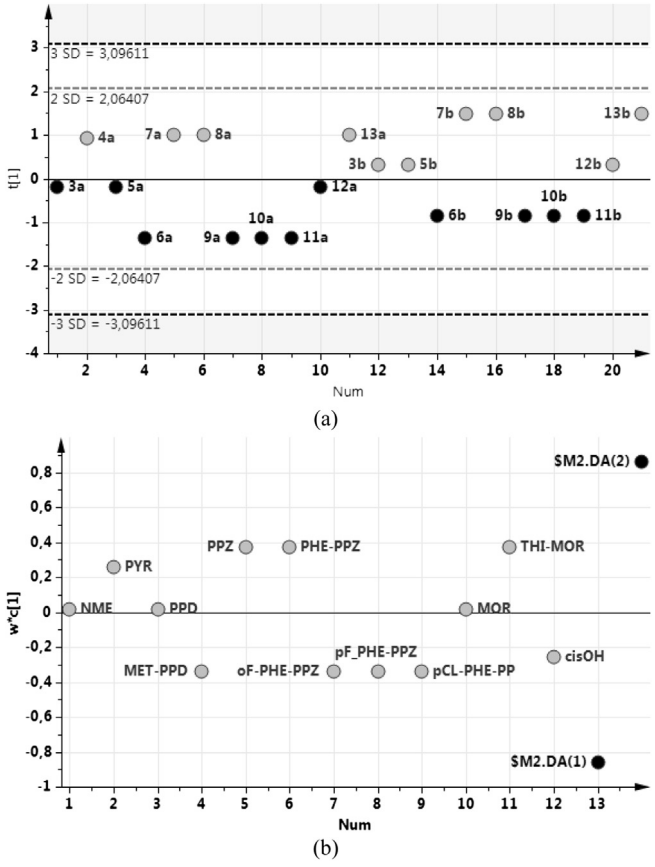


Fig. 2. The score plot (a). The first *PC* accounts for 79% of the variance in the dataset. The highly active compounds with *MIC* < 10 μM (*class 1*) are given as black circles. The low active compounds with *MIC* > 10 μM (*class 2*) are given as grey circles. The loading plot (b). X and Y descriptors are given as grey and black circles, respectively.

C-15), 60.10 (C-11), 62.67 (C-1), 63.90 (C-10), 217.82 (C-2); MS (+ESI) *m/z* (rel. int.): 281 (100, M+1); Anal. Calcd for $C_{16}H_{28}N_2O_2$: C 68.53; H 10.06; N 9.99; Found: C 68.59; H 10.21; N 9.97.

4.1.1.2. (1R,4R)-1-((R)-1-hydroxy-2-(4-phenylpiperazin-1-yl)ethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***8a***. Yield: 99%; colourless crystals; mp 97–100 °C; $[\alpha]_D^{20} = -10.40$ (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 0.97 (s, 3H, 8-H), 1.11 (s, 3H, 9-H), 1.23–1.35 (m, 2H, 5-H$_{endo}$, 6-H$_{endo}$), 1.74 (d, 1H, 3-H$_{endo}$, J = 18.3 Hz), 1.90–2.21 (m, 3H, 4-H, 6-H$_{exo}$, 5-H$_{exo}$), 2.31 (dd, 1H, 11-H$_{sin}$, J = 14.8, 13.7 Hz), 2.25–2.36 (m, 1H, 3-H$_{exo}$), 2.48–2.56 (m, 2H, 12-H, 15-H), 2.78–2.86 (m, 2H, 12-H, 15-H), 2.92 (dd, 1H, 11-H$_{anti}$, J = 12.2, 3.00 Hz), 3.05–3.20 (m, 4H, 13-H, 14-H), 3.94 (dd, 1H, 10-H, J = 10.86, 2.9 Hz), 6.75–6.87 (m, 3H, Ph), 7.16–7.22 (m, 2H, Ph); ^{13}C NMR (62 MHz, $CDCl_3$): δ = 20.53 (C-8), 21.90 (C-6), 21.95 (C-9), 26.88 (C-5), 43.72 (C-3), 44.20 (C-4), 48.38 (C-7), 49.33 (C-13, C-14), 53.06 (C-12, C-15), 60.32 (C-11), 62.68 (C-1), 64.04 (C-10), 116.05, 119.75, 129.11, 151.25 (Ph), 217.89 (C-2); MS (+ESI) *m/z* (rel. int.): 365 (100, M+Na), 343 (86, M+1); Anal. Calcd for $C_{21}H_{30}N_2O_2$: C 73.65; H 8.83; N 8.18; Found: C 73.58; H 8.91; N 8.18.

4.1.1.3. (1R,4R)-1-((R)-2-(4-(2-fluorophenyl)piperazin-1-yl)-1-hydroxyethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***9a***. Yield: 80%; colourless crystals; mp 123–126 °C; $[\alpha]_D^{20} = -11.44$ (c = 1.04, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.04 (s, 3H, 8-H), 1.18 (s, 3H, 9-H), 1.31–1.43 (m, 2H, 6-H$_{endo}$, 5-H$_{endo}$), 1.81 (d, 1H, 3-H$_{endo}$, J = 18.3 Hz), 1.97–2.24 (m, 3H, 4-H, 6-H$_{exo}$, 5-H$_{exo}$), 2.37 (dd, 1H, 11-H$_{sin}$, J = 12.2, 11.0 Hz), 2.33–2.43 (m, 1H, 3-H$_{exo}$), 2.57–2.65 (m, 2H, 12-H, 15-H), 2.87–2.95 (m, 2H, 12-H, 15-H), 3.04 (dd, 1H, 11-H$_{anti}$, J = 12.2, 3.00 Hz), 3.10–3.17 (m, 4H, 13-H, 14-H), 4.01 (dd, 1H, 10-H, J = 10.09, 3.00 Hz), 6.88–7.09 (m, 4H, Ph); ^{13}C NMR (62 MHz, $CDCl_3$): δ = 20.57 (C-8), 21.95 (C-9), 22.01 (C-6), 26.87 (C-5), 43.71 (C-3), 44.20 (C-4), 48.37 (C-7), 50.69 (C-13), 50.74 (C-14), 53.11 (C-15, C-12), 60.28 (C-11), 62.68 (C-1), 64.02 (C-10), 116.14 (d, 1C, C-20, J = 20.7 Hz), 118.92 (d, 1C, C-18, J = 3.00 Hz), 122.45 (d, 1C, C-17, J = 7.9 Hz), 124.43 (d, 1C, C-19, J = 3.6 Hz), 140.04 (d, 1C, C-16, J = 8.5 Hz), 155.72 (d, 1C, C-21, J_{C-F} = 245.7 Hz), 217.89 (C-2); MS (+ESI) *m/z* (rel. int.): 383 (100, M+Na), 361 (91, M+1); Anal. Calcd for $C_{21}H_{29}FN_2O_2$: C 69.97; H 8.11; N 7.77; Found: C 69.82; H 8.24; N 7.78.

4.1.1.4. (1R,4R)-1-((R)-2-(4-(4-fluorophenyl)piperazin-1-yl)-1-hydroxyethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***10a***. Yield: 99%; colourless crystals; mp 104–107 °C; $[\alpha]_D^{20} = -11.48$ (c = 1.02, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 1.04 (s, 3H, 8-H), 1.17 (s, 3H, 9-H), 1.34–1.40 (m, 2H, 6-H$_{endo}$, 5-H$_{endo}$), 1.81 (d, 1H, 3-H$_{endo}$, J = 18.4 Hz), 1.99–2.07 (m, 2H, 4-H, 6-H$_{exo}$), 2.17–2.23 (m, 1H, 5-H$_{exo}$), 2.35–2.40 (m, 2H, 3-H$_{exo}$, 11-H$_{sin}$), 2.57–2.61 (m, 2H, 12-H, 15-H), 2.87–2.91 (m, 2H, 12-H, 15-H), 3.00 (dd, 1H, 11-H$_{anti}$, J = 12.2, 3.00 Hz), 3.08–3.15 (m, 4H, 13-H, 14-H), 4.00 (dd, 1H, 10-H, J = 10.92, 3.00 Hz), 6.86–6.89 (m, 2H, Ph), 6.94–6.98 (m, 2H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 20.55 (C-8), 21.93 (C-6), 21.97 (C-9), 26.88 (C-5), 43.72 (C-3), 44.16 (C-4), 48.39 (C-7), 50.34 (C-13, C-14), 53.03 (C-12, C-15), 60.24 (C-11), 62.70 (C-1), 64.02 (C-10), 115.53 (d, 2C, C-18, C-20, J = 21.7 Hz), 117.80 (d, 2C, C-17, C-21, J = 8.5 Hz), 147.89 (d, 1C, C-16, J = 2.4 Hz), 156.30 (d, 1C, C-19, J_{C-F} = 235.4 Hz), 218.15 (C-2); MS (+ESI) *m/z* (rel. int.): 383 (100, M+Na), 361 (90, M+1); Anal. Calcd for $C_{21}H_{29}FN_2O_2$: C 69.97; H 8.11; N 7.77; Found: C 70.21; H 7.87; N 7.80.

4.1.1.5. (1R,4R)-1-((R)-2-(4-(4-chlorophenyl)piperazin-1-yl)-1-hydroxyethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***11a***. Yield: 73%; colourless crystals; mp 140–143 °C; $[\alpha]_D^{20} = -10.84$ (c = 1.02, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 1.04 (s, 3H, 8-H), 1.17 (s, 3H, 9-H), 1.33–1.40 (m, 2H, 5-H$_{endo}$, 6-H$_{endo}$), 1.81 (d, 1H, 3-H$_{endo}$, J = 18.4 Hz), 1.99–2.07 (m, 2H, 4-H, 6-H$_{exo}$), 2.17–2.23 (m, 1H, 5-H$_{exo}$), 2.37–2.41 (m, 1H, 3-H$_{exo}$), 2.39 (dd, 1H, 11-H$_{sin}$ J = 12.5, 10.9 Hz), 2.58–2.62 (m, 2H, 12-H, 15-H), 2.89–2.91 (m, 2H, 12-H, 15-H), 3.01 (dd, 1H, 11-H$_{anti}$, J = 12.2, 2.8 Hz), 3.13–3.20 (m, 4H, 13-H, 14-H), 4.01 (dd, 1H, 10-H, J = 10.9, 2.8 Hz), 6.82–6.85 (m, 2H, Ph), 7.19–7.22 (m, 2H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 20.53 (C-8), 21.90 (C-6), 21.98 (C-9), 26.88 (C-5), 43.72 (C-3), 44.14 (C-4), 48.40 (C-7), 49.27 (C-13, C-14), 52.90 (C-12, C-15), 60.38 (C-11), 62.66 (C-1), 64.05 (C-10), 117.27, 124.53, 128.97, 149.879 (Ph), 218.16 (C-2); MS (+ESI) *m/z* (rel. int.): 399 (100, M+Na), 377 (35, M+1); Anal. Calcd for $C_{21}H_{29}ClN_2O_2$: C 66.92; H 7.76; N 7.43; Found: C 66.99; H 7.79; N 7.44.

4.1.1.6. (1R,4R)-1-((R)-1-hydroxy-2-morpholinoethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***12a***. Yield: 91%; colourless crystals, mp 55–57 °C; $[\alpha]_D^{20} = -16.68$ (c = 1.02, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.03 (s, 3H, 8-H), 1.17 (s, 3H, 9-H), 1.32–1.42 (m, 2H, 5-H$_{endo}$, 6-H$_{endo}$), 1.80 (d, 1H, 3-H$_{endo}$, J = 18.3 Hz), 1.96–2.35 (m, 4H, 3-H$_{exo}$, 4-H, 6-H$_{exo}$, 5-H$_{exo}$), 2.29 (dd, 1H, 11-H$_{sin}$, J = 12.2, 11.00 Hz), 2.39–2.47 (m, 2H, 13-H, 14-H), 2.68–2.76 (m, 2H, 13-H, 14-H), 2.95 (dd, 1H, 11-H$_{anti}$, J = 12.2, 2.9 Hz), 3.64–3.77 (m, 4H, 12-H, 15-H), 3.97 (dd, 1H, 10-H, J = 10.9, 2.9 Hz); ^{13}C NMR (62 MHz, $CDCl_3$): δ = 20.51 (C-8), 21.92 (C-6), 21.94 (C-9), 26.85 (C-5), 43.69 (C-3), 44.16 (C-4), 48.36 (C-7), 53.50 (C-13, C-14), 60.84 (C-11), 62.61 (C-1), 63.80 (C-10), 67.13 (C-12, C-15), 217.90 (C-2); MS (+ESI) *m/z* (rel. int.): 290 (100, M+Na), 268 (61, M+1); Anal. Calcd for $C_{15}H_{25}NO_3$: C 67.38; H 9.42; N 5.24; Found: C 67.33; H 9.54; N 5.26.

4.1.1.7. (1R,4R)-1-((R)-1-hydroxy-2-thiomorpholinoethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***13a***. Yield: 72%; colourless crystals, mp 70–71 °C; $[\alpha]_D^{20} = -12.89$ ($c = 0.43$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) $\delta = 1.01$ (s, 3H, 8-H), 1.15 (s, 3H, 9-H), 1.25–1.43 (m, 2H, 5-H_{endo}, 6-H_{endo}), 1.79 (d, 1H, 3-H_{endo}, $J = 18.3$ Hz), 1.96–2.20 (m, 3H, 4-H, 5-H_{exo}, 6-H_{exo}), 2.20 (dd, 1H, 11-H_{sin}, $J = 12.4$, 10.77 Hz), 2.35 (ddd, 1H, 3-H_{exo}, $J = 18.3$, 4.7, 3.00 Hz), 2.58–2.76 (m, 6H, 12-H, 15-H), 2.91–3.06 (m, 2H, 13-H, 14-H), 3.03 (dd, 1H, 11-H_{anti}, $J = 12.4$, 3.00 Hz), 3.93 (dd, 1H, 10-H, $J = 10.8$, 3.00 Hz); ^{13}C NMR (62 MHz, $CDCl_3$): $\delta = 20.46$ (C-8), 21.80 (C-6), 21.91 (C-9), 26.86 (C-5), 28.15 (C13, C14), 43.68 (C-3), 44.14 (C-4), 48.38 (C-7), 55.11 (C-12, C-15), 61.12 (C-11), 62.64 (C-1), 63.82 (C-10), 217.84 (C-2); MS (+ESI) *m/z* (rel. int.): 285 (100, M+1), 267 (42, M−H_2O); Anal. Calcd for $C_{15}H_{25}NO_2S$: C 63.56; H 8.89; N 4.94; Found: C 63.71; H 8.96; N 4.94.

4.1.1.8. (1R,4R)-1-((S)-1-hydroxy-2-(4-methylpiperazin-1-yl)ethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***7b***. Yield: 79%; colourless crystals; mp 76–79 °C; $[\alpha]_D^{20} = +23.44$ ($c = 1.02$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) $\delta = 0.99$ (s, 3H, 9-H), 1.06 (s, 3H, 8-H), 1.26–1.38 (m, 2H, 5-H_{endo}, 6-H_{endo}, 13-H), 1.72–1.76 (m, 1H, 4-H), 1.74 (d, 1H, 3-H_{endo}, $J = 18.4$ Hz), 1.87–1.92 (m, 2H, 6-H_{exo}, 5-H_{exo}), 2.12 (dd, 1H, 11-H_{anti}, $J = 12.1$, 3.2 Hz), 2.22 (s, 3H, 16-H), 2.38 (ddd, 1H, 3-H_{exo}, $J = 18.2$, 3.6, 4.3 Hz), 2.18–2.67 (m, 8H, 12-H, 13-H, 14-H, 15-H), 3.09 (dd, 1H, 11-H_{sin}, $J = 12.00$, 11.3 Hz), 3.75 (dd, 1H, 10-H, $J = 11.0$, 3.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 20.06$ (C-9), 22.30 (C-8), 25.62 (C-6), 26.78 (C-5), 44.07 (C-3), 44.10 (C-4), 46.06 (C-16), 48.11 (C-7), 55.28 (C-13, C-12, C-14, C-15), 59.91 (C-11), 61.68 (C-1), 65.39 (C-10), 217.48 (C-2); MS (+ESI) *m/z* (rel. int.): 281 (100, M+1), 303 (19, M+Na); Anal. Calcd for $C_{16}H_{28}N_2O_2$: C 68.53; H 10.06; N 9.99; Found: C 68.61; H 10.29; N 9.90.

4.1.1.9. (1R,4R)-1-((S)-1-hydroxy-2-(4-phenylpiperazin-1-yl)ethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***8b***. Yield: 99%; colourless crystals; mp 103–105 °C; $[\alpha]_D^{20} = +7.48$ ($c = 1.03$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) $\delta = 1.09$ (s, 3H, 9-H), 1.14 (s, 3H, 8-H), 1.35–1.47 (m, 2H, 5-H_{endo}, 6-H_{endo}), 1.83 (d, 1H, 3-H_{endo}, $J = 18.24$ Hz), 1.81–1.87 (m, 1H, 5-H_{exo}), 1.95–2.01 (m, 2H, 4-H, 6-H_{exo}), 2.27 (dd, 1H, 11-H_{anti}, $J = 12.2$, 3.2 Hz), 2.38 (ddd, 1H, 3-H_{exo}, $J = 18.2$, 4.6, 3.3 Hz), 2.59–2.63 (m, 2H, 12-H, 15-H), 2.83–2.86 (m, 2H, 12-H, 15-H), 3.17–3.24 (m, 5H, 11-H_{sin}, 13-H, 14-H), 3.88 (dd, 1H, 10-H, $J = 11.0$, 3.2 Hz), 6.87–6.85 (m, 1H, Ph), 6.93–6.94 (m, 2H, Ph), 7.25–7.28 (m, 2H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 20.11$ (C-9), 22.33 (C-8), 25.67 (C-6), 26.79 (C-5), 44.08 (C-3), 44.15 (C-4), 48.14 (C-7), 49.31 (C-13, C-14), 52.97 (C-12, C-15), 59.11 (C-11), 61.76 (C-1), 65.57 (C-10), 116.09, 119.81, 129.14, 151.24 (Ph), 217.62 (C-2); MS (+ESI) *m/z* (rel. int.): 365 (88, M+Na), 343 (100, M+1); Anal. Calcd for $C_{21}H_{30}N_2O_2$: C 73.65; H 8.83; N 8.18; Found: C 73.64; H 8.98; N 8.12.

4.1.1.10. (1R,4R)-1-((S)-2-(4-(2-fluorophenyl)piperazin-1-yl)-1-hydroxyethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***9b***. Yield: 91%; colourless crystals; mp 102–104 °C; $[\alpha]_D^{20} = +22.53$ ($c = 1.01$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) $\delta = 1.08$ (s, 3H, 9-H), 1.14 (s, 3H, 8-H), 1.35–1.39 (m, 1H, 5-H_{endo}), 1.43–1.48 (m, 1H, 6-H_{endo}), 1.83 (d, 1H, 3-H_{endo}, $J = 18.2$ Hz), 1.82–1.86 (m, 1H, 6-H_{exo}), 1.96–2.00 (m, 2H, 4-H, 5-H_{exo}), 2.28 (dd, 1H, 11-H_{anti}, $J = 12.1$, 3.1 Hz), 2.47 (ddd, 1H, 3-H_{exo}, $J = 18.2$, 4.4, 3.3 Hz), 2.61–2.65 (m, 2H, 12-H, 15-H), 2.85–2.89 (m, 2H, 12-H, 15-H), 3.08–3.15 (m, 4H, 13-H, 14-H), 3.22 (dd, 1H, 11-H_{sin}, $J = 11.76$, 11.52 Hz), 3.88 (dd, 1H, 10-H, $J = 10.98$, 3.1 Hz), 6.92–6.96 (m, 2H, Ph), 7.00–7.07 (m, 2H, Ph); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 20.11$ (C-9), 22.31 (C-9), 25.62 (C-6), 26.80 (C-5), 44.08 (C-3), 44.12 (C-4), 48.14 (C-7), 50.70 (C-13, C-14), 53.02 (C-12, C-15), 59.09 (C-11), 61.74 (C-1), 65.49 (C-10), 116.12 (d, 1C, C-20, $J = 20.7$ Hz), 118.93 (d, 1C, C-18, $J = 2.8$ Hz), 122.46 (d, 1C, C-17, $J = 7.9$ Hz), 124.48 (d, 1C, C-19, $J = 3.4$ Hz), 140.06 (d, 1C, C-16, $J = 8.6$ Hz), 155.74 (d, 1C, C-21, $J_{C-F} = 246.5$ Hz), 217.61 (C-2); MS (+ESI) *m/z* (rel. int.): 383 (100, M+Na), 361 (74, M+1); Anal. Calcd for $C_{21}H_{29}FN_2O_2$: C 69.97; H 8.11, N 7.77; Found: C 70.11; H 8.34; N 7.75.

4.1.1.11. (1R,4R)-1-((S)-2-(4-(4-fluorophenyl)piperazin-1-yl)-1-hydroxyethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***10b***. Yield: 98%; colourless crystals; mp 134–138 °C; $[\alpha]_D^{20} = +24.96$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) $\delta = 1.08$ (s, 3H, 9-H), 1.14 (s, 3H, 8-H), 1.32–1.50 (m, 2H, 6-H_{endo}, 5-H_{endo}), 1.82 (m, 1H, 3-H_{endo}), 1.79–2.01 (m, 3H, 4-H, 6-H_{exo}, 5-H_{exo}), 2.27 (dd, 1H, 11-H_{anti}, $J = 12.2$, 3.2 Hz), 2.46 (ddd, 1H, 3-H_{exo}, $J = 18.3$, 4.3, 3.6 Hz), 2.57–2.65 (m, 2H, 12-H, 15-H), 2.80–2.88 (m, 2H, 12-H, 15-H), 3.07–3.18 (m, 4H, 13-H, 14-H), 3.20 (dd, 1H, 11-H_{sin}, $J = 12.2$, 11.0 Hz), 3.88 (dd, 1H, 10-H, $J = 10.88$, 3.15 Hz), 6.83–7.01 (m, 4H, Ph); ^{13}C NMR (62 MHz, $CDCl_3$): $\delta = 20.10$ (C-9), 22.30 (C-8), 25.68 (C-6), 26.77 (C-5), 44.05 (C-3), 44.17 (C-4), 48.11 (C-7), 50.31 (C-15), 52.98 (C-12), 59.13 (C-11), 61.77 (C-1), 65.66 (C-10), 115.51 (d, 2C, C-18, C-20, $J = 22.1$ Hz), 117.81 (d, 2C, C-17, C-21, $J = 7.6$ Hz), 147.90 (d, 1C, C-16, $J = 2.2$ Hz), 157.20 (d, 1C, C-19, $J_{C-F} = 238.8$ Hz), 217.55 (C-2); MS (+ESI) *m/z* (rel. int.): 383 (100, M+Na), 361 (74, M+1); Anal. Calcd for $C_{21}H_{29}FN_2O_2$: C 69.97; H 8.11; N 7.77; Found: C 69.93; H 8.04; N 7.78.

4.1.1.12. (1R,4R)-1-((S)-2-(4-(4-chlorophenyl)piperazin-1-yl)-1-hydroxyethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***11b***. Yield: 76%; colourless crystals; mp 138–144 °C; $[\alpha]_D^{20} = +21.99$ ($c = 1.30$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) $\delta = 1.08$ (s, 3H, 9-H), 1.14 (s, 3H, 8-H), 1.32–1.50 (m, 2H, 5-H_{endo}, 6-H_{endo}), 1.76–2.01 (m, 3H, 4-H, 6-H_{exo}, 5-H_{exo}), 1.82 (d, 1H, 3-H_{endo}, $J = 18.3$ Hz), 2.27 (dd, 1H, 11-H_{anti}, $J = 12.2$, 3.1 Hz), 2.46 (ddd, 1H, 3-H_{exo}, $J = 18.3$, 4.3, 3.7 Hz), 2.56–2.64 (m, 2H, 12-H, 15-H), 2.79–2.87 (m, 2H, 12-H, 15-H), 3.10–3.22 (m, 5H, 13-H, 14-H, 11-H_{sin}), 3.88 (dd, 1H, 10-H, $J = 10.9$, 3.1 Hz), 6.80–6.86 (m, 2H, Ph), 7.17–7.23 (m, 2H, Ph); ^{13}C NMR (62 MHz, $CDCl_3$): $\delta = 20.10$ (C-9), 22.30 (C-8), 25.67 (C-6), 26.76 (C-5), 44.04 (C-3), 44.17 (C-4), 48.10 (C-7), 49.29 (C-13, C-14), 52.83 (C-12, C-15), 59.17 (C-11), 61.78 (C-1), 65.69 (C-10), 117.23, 124.58, 128.94, 149.84 (Ph), 217.58 (C-2); MS (+ESI) *m/z* (rel. int.): 399 (100, M+Na), 377 (67, M+1); Anal. Calcd for $C_{21}H_{29}ClN_2O_2$: C 66.92; H 7.76; N 7.43; Found: C 67.11; H 7.72; N 7.44.

4.1.1.13. (1R,4R)-1-((S)-1-hydroxy-2-morpholinoethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***12b***. Yield: 84%; colourless crystals; mp 55–57 °C; $[\alpha]_D^{20} = +30.68$ ($c = 1.02$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) $\delta = 1.07$ (s, 3H, 9-H), 1.29 (s, 3H, 8-H), 1.34–1.45 (m, 2H, 5-H_{endo}, 6-H_{endo}), 1.82 (d, 1H, 3-H_{endo}, $J = 18.4$ Hz), 1.80–1.84 (m, 1H, 6-H_{exo}), 1.94–2.00 (m, 2H, 4-H, 5-H_{exo}), 2.22 (dd, 1H, 11-H_{anti}, $J = 12.2$, 3.1 Hz), 2.43–2.47 (m, 3H, 3-H_{exo}, 13-H, 14-H), 2.67–2.69 (m, 2H, 13-H, 14-H), 3.13 (dd, 1H, 11-H_{sin}, $J = 12.1$, 11.1 Hz), 3.69–3.75 (m, 4H, 12-H, 15-H), 3.85 (dd, 1H, 10-H, $J = 10.9$, 3.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 20.09$ (C-9), 22.30 (C-8), 25.63 (C-6), 26.77 (C-5), 44.05 (C-3), 44.13 (C-4), 48.08 (C-7), 53.47 (C-13, C-14), 59.67 (C-11), 61.71 (C-1), 65.36 (C-10), 67.12 (C12, C15), 217.80 (C-2); MS (+ESI) *m/z* (rel. int.): 290 (100, M+Na), 268 (27, M+1); Anal. Calcd for $C_{15}H_{25}NO_3$: C 67.38; H 9.42; N 5.24; Found: C 67.44; H 9.71; N 5.17.

4.1.1.14. (1R,4R)-1-((S)-1-hydroxy-2-thiomorpholinoethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-one ***13b***. Yield: 68%; colourless crystals; mp 60–63 °C; $[\alpha]_D^{20} = +23.60$ ($c = 0.99$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) $\delta = 1.06$ (s, 3H, 9-H), 1.13 (s, 3H, 8-H), 1.34–1.44 (m, 2H, 5-H_{endo}, 6-H_{endo}), 1.78–1.83 (m, 1H, 6-H_{exo}), 1.82 (d, 1H, 3-H_{endo}, $J = 18.4$ Hz), 1.95–2.00 (m, 2H, 4-H, 5-H_{exo}), 2.29 (dd, 1H, 11-H_{anti}, $J = 12.4$, 3.2 Hz), 2.44 (ddd, 1H, 3-H_{exo}, $J = 18.4$, 4.6, 3.1 Hz), 2.64–2.74 (m, 6H, 12-H, 13-H, 14-H, 15-H), 2.92–2.95 (m, 2H, 13-H, 14-H), 3.09 (dd, 1H, 11-H_{sin}, $J = 12.3$, 11.00 Hz), 3.81 (dd, 1H, 10-H, $J = 10.9$, 3.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 20.04$ (C-9), 22.31 (C-8), 25.62 (C-6), 26.77 (C-5), 28.16 (C-12, C-15), 44.07 (C-3), 44.08 (C-4), 48.30 (C-7), 54.99 (C-13, C-14), 59.78 (C-11), 61.73 (C-1),

65.30 (C-10), 217.86 (C-2); MS (+ESI) *m/z* (rel. int.): 285 (100, M+1), 267 (29, M−H_2O); Anal. Calcd for $C_{15}H_{25}NO_2S$: C 63.56; H 8.89; N 4.94; Found: C 63.62; H 9.08; N 4.90.

4.2. Antimycobacterial activity

The antimycobacterial activity was determined through the proportional method of Canetti towards reference strain *M. tuberculosis* H37Rv and multi-drug resistant strain 43 (resistant to Rifampicin and Isoniazid), recovered from Bulgarian adult HIV-negative pulmonary TB patient, who was a permanent resident of the country. This method, recommended by the WHO, is the most commonly used one worldwide for investigation of sensitivity/resistance of tuberculosis strains towards chemotherapeutics [15–19]. 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 five 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. 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 foetal calf serum and 2 mM L-glutamine, at 37 °C 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 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 for each concentration a set of at least 8 separate wells was used. 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 °C 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).

4.4. Discriminant analysis by partial least squares (DA-PLS)

Discriminant analysis (*DA*) is a statistical analysis to predict a categorical dependent variable by one or more continuous or binary independent variables. Partial least squares (*PLS*) is a projection method [22] that can handle matrices with more variables than observations and with noisy and highly collinear data. PLS forms new variables, called principal components (*PC*), as linear combinations of the initial variables and then uses them to predict the dependent variable. The *DA-PLS* used in the present study was implemented in SIMCA 13.0 [14].

The predictive ability of the derived model was evaluated by leave-one-out cross-validated coefficient q^2. At threshold 0.5, *sensitivity* (correctly predicted *class 1* compounds/all *class 1* compounds), *specificity* (correctly predicted *class 2* compounds/all *class 2* compounds) and *accuracy* (correctly predicted *class 1* and *class 2* compounds/all compounds) were calculated.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.007.

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