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Original article

Synthesis and *in vitro* antimycobacterial activity of compounds derived from (*R*)- and (*S*)-2-amino-1-butanol – The crucial role of the configuration

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ABSTRACT

The synthesis of 47 structurally diverse compounds incorporating the (*R*)-2-amino-1-butanol motif has been realized. Ten of these compounds were found to exhibit *in vitro* specific activity against *Mycobacterium tuberculosis* H37Rv in a MIC range of 0.65 μM–14.03 μM. Five of the most active compounds **11**, **22**, **23**, **31** and **42** (5.7–11.1 fold more active than ethambutol) can be outlined with very low cytotoxicity towards human embryonal kidney non-tumour cells (SI ranging from 91.2 to 375.4). For the purpose of comparison the (*S*)-enantiomers of these most active compounds have been synthesized and evaluated towards *M. tuberculosis* $H_{37}Rv$ showing no activity even at 20–32 fold higher concentrations.

1. Introduction

Tuberculosis (TB) is one of the most wasteful diseases primarily due to several decades of neglect, HIV infection, immigration and globalization [1]. Approximately one-third of the world's population has been infected with the causative organism *Mycobacterium tuberculosis* (MTB), eight million people become sick with TB and globally it accounts for approximately two million deaths, per year. The synergy of TB/HIV infections [2] and the emergence of multi drug resistance (MDR-TB) and extensively drug resistance tuberculosis (XDR-TB) pose a threatening challenge to chemotherapy of tuberculosis with significant problems and complications [3–5]. One fifth of all deaths of adults in developing countries are due to TB and the problem is particularly re-emerging in many industrialized countries mostly because of the free movement of people in the globalized world. In affected regions, the disease is recognized as serious hindrance to economic and social development.

The leading drugs isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) are currently recommended by the World Health Organization (WHO) for the treatment of TB [6]. The problems with current TB treatment are complex and include: a prolonged standard course regimen of six months, which often result in patient noncompliance; emergency of XDR-TB strains; lack of effective drugs against the latent state. One approach to decrease treatment time is improvement of potency of currently used anti-tuberculosis drugs [7] and discovery of new active compounds [8–10].

The simple diamine EMB (Scheme 1) was synthesized by reacting 1,2-dihaloethane with (*S*)-2-amino-1-butanol [11,12]. An alternative synthetic method was also described [13]. The EMB is primarily a bacteriostatic anti-tuberculosis agent with not fully known mechanism of action. It targets the arabinosyl transferases responsible for arabinogalactan biosynthesis, a key component of the unique mycobacterial cell-wall [14–16]. Despite of modest antimycobacterial activity and due to its synergy with other drugs, and lower toxicity, EMB is used in combination with more potent front-line antimycobacterial agents. Early SAR study indicates that the distance between the two nitrogens, the presence of two hydroxy groups, and the small side chains in the molecule are key pharmacophore elements [17]. 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 [17].

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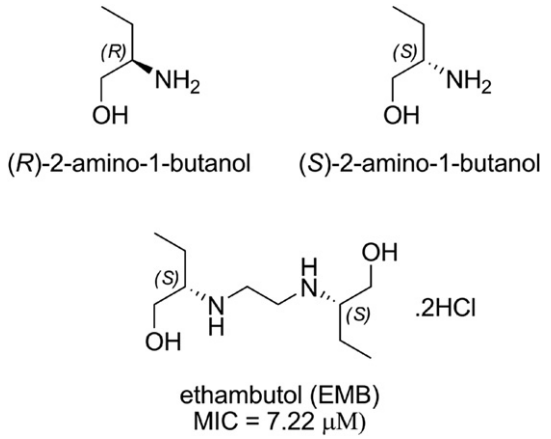


Scheme 1.

In recent years diverse derivatives of (*S*)-2-amino-1-butanol and 1,2-ethylenediamine have been synthesized and evaluated for their antimycobacterial activities and mechanisms of action [18–22]. In general, most of the compounds containing (*S*)-2-amino-1-butanol motif are showing similar but not significantly higher activity than EMB. It is important to note that the utilization of (*R*)-2-amino-1-butanol motif for the synthesis of anti TB drug candidates has been obviously neglected. There are only isolated examples of such derivatives showing results that are not encouraging enough with respect to antimycobacterial activity [18,21,23], but possessing promising antifungal activity [24].

Herein, we report the synthesis of small library of structurally diverse compounds incorporating (*R*)-2-amino-1-butanol motif and the evaluation of their *in vitro* antimycobacterial, antibacterial and antifungal activities. For selected structures the (*S*)-configured analogues have also been synthesized and evaluated.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of compounds *10–17*

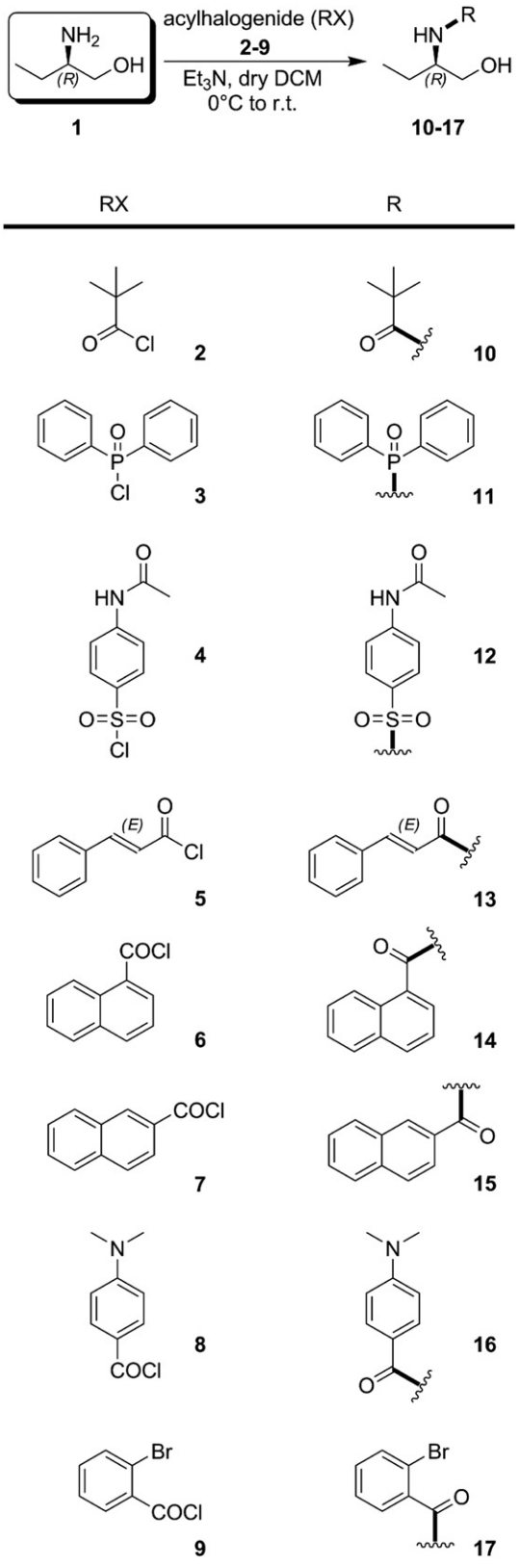
A series of *N*-monoacylated compounds **10–17** was synthesized and good to excellent yields were achieved using standard conditions for acylation of **1** (0 °C and Et_3N in dry DCM) with acid chlorides **2–9** (Scheme 2). All compounds were obtained in excellent purity (>99%) after column chromatography or crystallization. The synthesis of **13–15** and **17** has been published earlier, with no data about the purity whatsoever [25–29]. In the case of **11**, the (*S*)-enantiomer has been obtained through rather complicated procedure in enantiomerically enriched form (75–97 % ee) [25].

2.1.2. Synthesis of compounds *21–23*

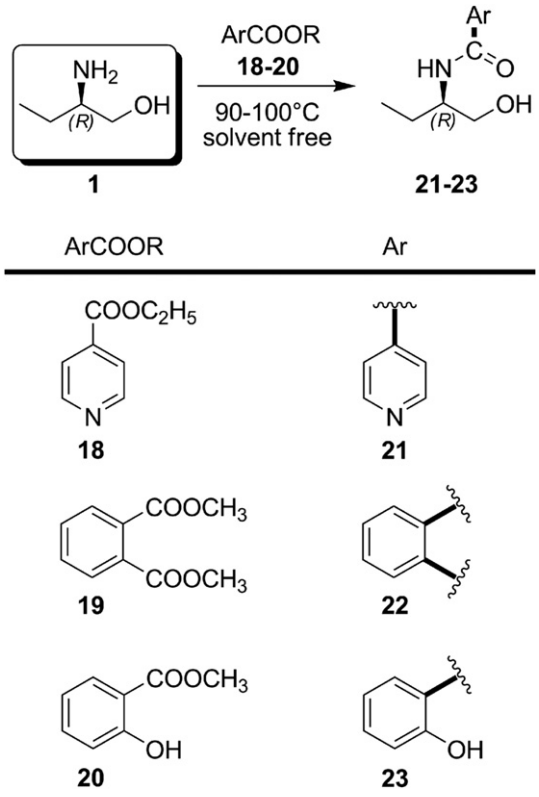
The amide derivatives **21–23** were synthesized using simple solvent-free aminolysis of esters **18–20** with **1** by heating at 90–100 °C (Scheme 3). Compounds **21–23** were isolated in very good yields and purities (by column chromatography or crystallization).

2.1.3. Synthesis of compounds *31–37*

The synthesis of compounds **31–37** (Scheme 4) was performed by applying standard procedures described in literature [30–36] – heating of **1** with **24–30**, and using either solvent-free conditions or different solvents (e.g. Et_2O, THF, EtOH etc.). Compounds **31**, **32** and **35** have been obtained previously [30–32,36], however, the data published were insufficient in respect of purity and of



Scheme 2. Synthesis of compounds **10–17**.



Scheme 3. Synthesis of compounds **21**–**23**.

properties. It is interesting to note that the reaction of **1** with **26** under those particular conditions led to ester **33**. Compound **34** was obtained from **1** and **27** as a result of migration of the double bond. Similar rearrangement reaction has been previously observed [37].

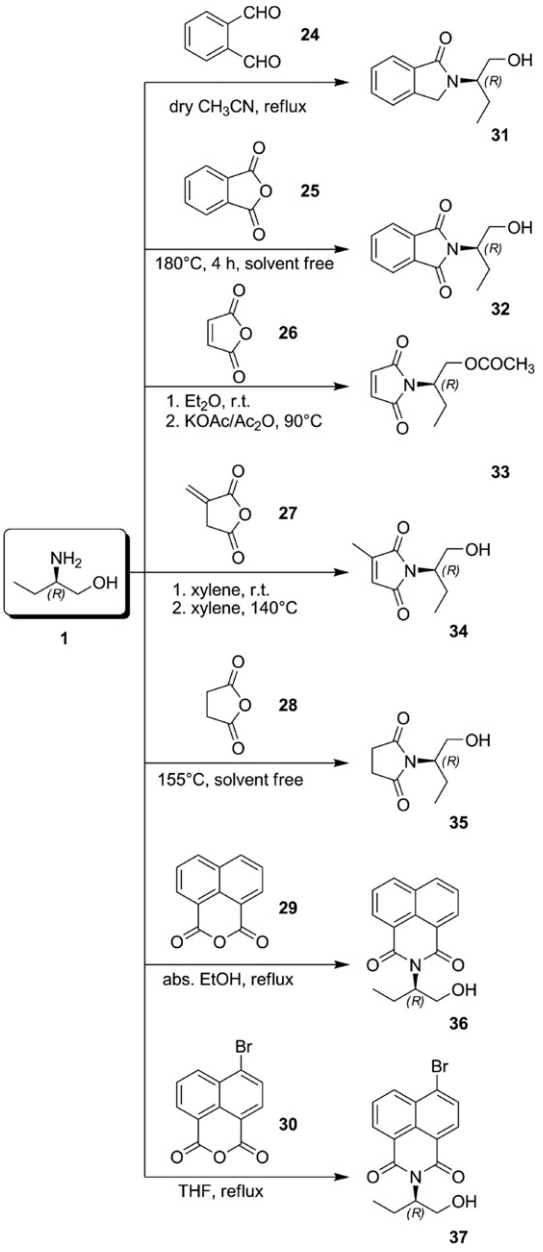
*2.1.4. Synthesis of compounds **41**–**43** and **47**–**49***

It is known that some ureas and thioureas are effective drugs against a range of MDR strains of *M. tuberculosis* [10,38–41]. Therefore it is worthy to evaluate the activity of similar structures incorporating the (*R*)-2-aminobutanol moiety. The synthesis of compounds **41**–**43** was performed by mixing **1** and isothiocyanate **38** and isocyanates **39**, **40** respectively, in THF as a solvent (Scheme 5). Compounds **41**–**43** were obtained in very high yields and excellent purities. The formation of **41** [42–44] and **43** [45] was described elsewhere in connection with different studies, not being related to the present investigations.

The heterocycle **47** was synthesized in two steps. In the first step the salt of **1** was formed and isolated after mixing of **1** and homophthalic acid (**44**) in THF. The salt was then heated without solvent for 5 h to form **47** in good yields. Structures similar to **47** have shown anti-inflammatory and analgesic activity [46].

The quinazolinone derivative **48** was prepared in acceptable yield, in boiling toluene by mixing **1**, anthranilic acid (**45**) and triethyl orthoformate, in presence of catalytic amounts of p-toluenesulfonic acid (PTSA). In the present case, the published solvent-free procedure for synthesis of quinazolinones [47] was not successful. Some 4(3*H*)-quinazolinones have shown antitubercular [48] and antibacterial [49] activity.

Compound **49** was interesting to synthesize due to the expected lipophilicity and hydrolytic stability caused by the steric hindrance of the camphene skeleton. The reaction was carried out by condensation of (+)-camphor (**46**) and **1** in the presence of catalytic amount of anhydrous $ZnCl_2$, according to published procedure [50].



Scheme 4. Synthesis of compounds **31**–**37**.

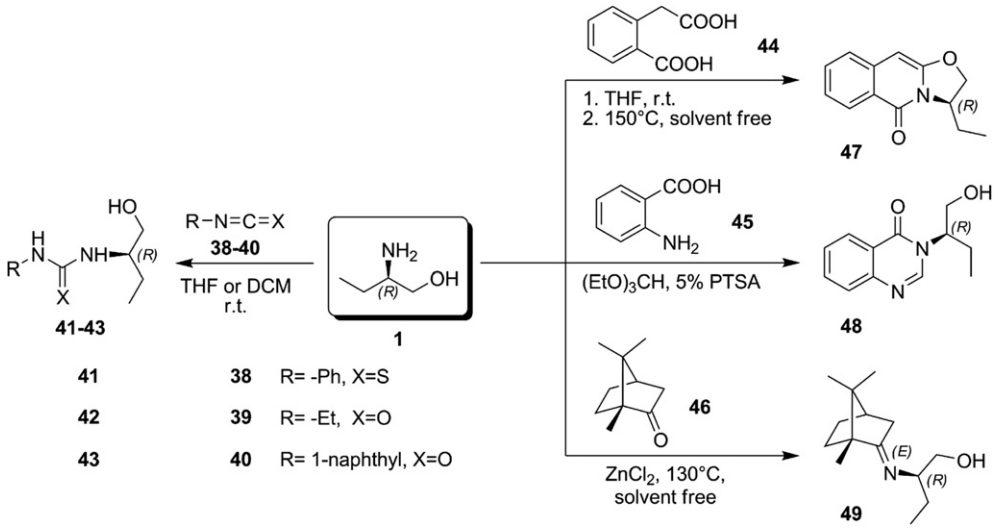
Imine **49** was obtained in excellent purity by column chromatography and crystallization.

*2.1.5. Synthesis of compounds **52**–**54**, **59**–**62** and **65**–**67***

Three series of N-substituted aminoalcohols have been synthesized by using (*R*)-2-amino-1-butanol (**1**) as central chiral unit, in reaction with aryl- and α-aryl-alkyl halogenides (Scheme 6).

Compounds **59**–**62** were prepared in good yields, under standard conditions, by using excess of K_2CO_3 in refluxing acetonitrile. Due to steric reasons, in the case of products **52** and **54** only mono-substitution of the amino group was achieved. The reactions, however, were smooth in both cases (Et_3N/DCM for **52** and K_2CO_3/18-crown-6 for **54**). Attempts for N-acylation of **52** provided only the O-acylated product **53**.

The synthesis of compounds **59** [51–56], **60** [57,58] and **52** has been previously described. The latter two derivatives have been used for preparation of antitumour agents [59–66].



Scheme 5. Synthesis of compounds **41**–**43** and **47**–**49**.

Aminoalcohol **1** reacted under solvent-free conditions with the heteroaryl bromides **37** and **63**, producing compounds **65**–**66**. It is interesting to note that the bromine atom in **66** was not replaced with second (*R*)-2-amino-1-butanol unit. Compound **67** was easily synthesized from **1** and **64** in refluxing EtOH.

*2.1.6. Synthesis of compounds **81**–**93***

A set of benzylic type substituted aminoalcohols **81**–**93** was synthesized (Scheme 7) by applying very efficient reductive amination of aldehydes **68**–**80** with aminoalcohol **1**. Instead of using the commonly applied (and more expensive) reducing agent $NaBH(OAc)_3$ [68–70], we used the cheaper $NaCNBH_3$ [67] and achieved good results.

Some of the isolated products have been previously mentioned elsewhere – the *S*-enantiomer of **81** has been described in patents as catalyst [71,72]; **83** has been a part of study, concerning prevention of drug-induced cytotoxicity [73]; aminoalcohol **84** and its *S*-enantiomer were used as ligands for stereoselective reduction of ketones [74]; the racemate of **86** has been mentioned as intermediate for preparation of ferrocenylmethylaminoalkoxy silanes [75]; the *S*-enantiomers of **87** and **88** have been previously prepared by reductive amination of aldehydes **74** and **75** respectively, by using resin supported cyano borohydride [76]; the formation of **90** through reductive amination has been noted in a study regarding the cytotoxicity of pyrene-containing aminoalcohols [77].

Compounds **81**–**93** have been isolated in excellent purities.

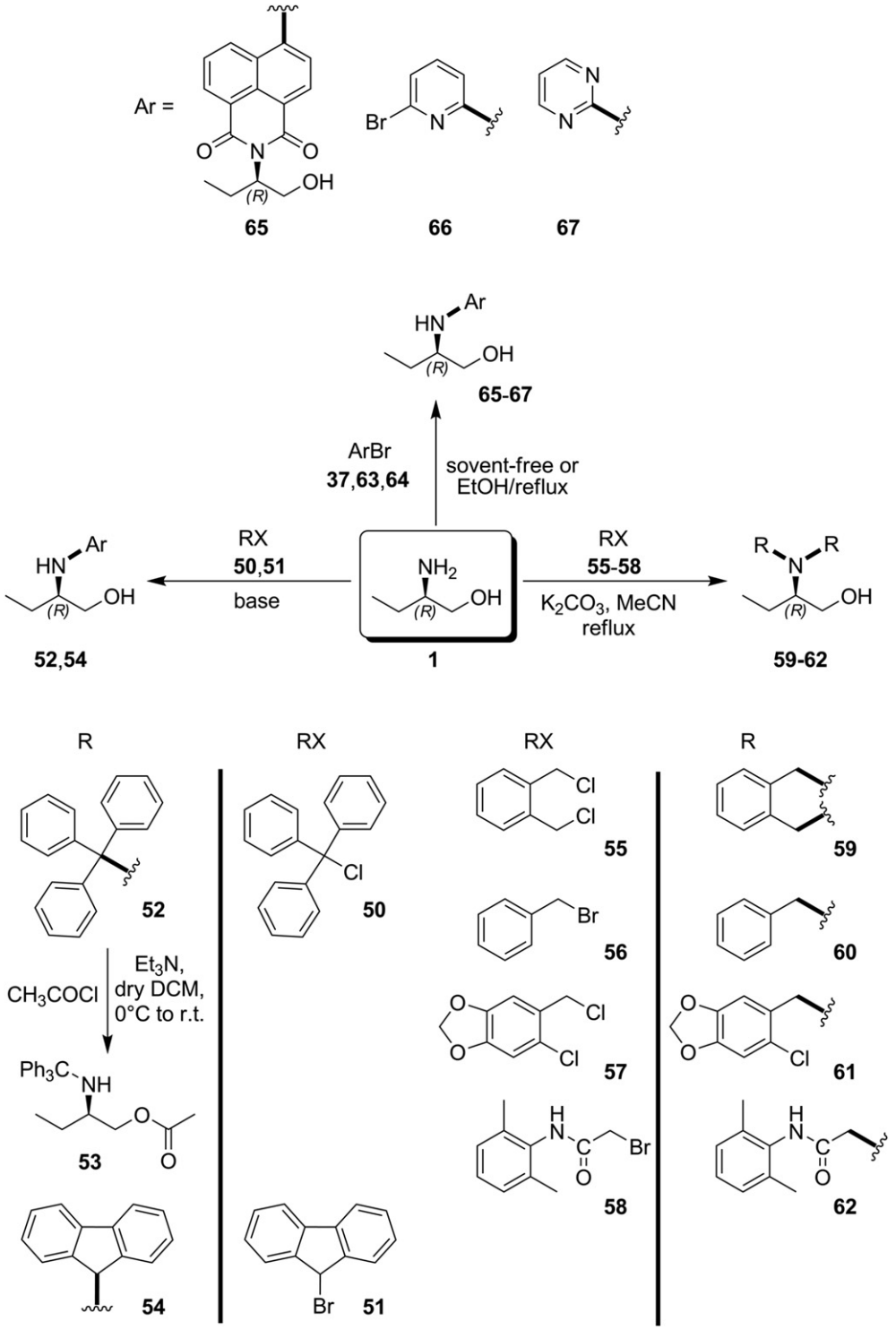
*2.1.7. Synthesis of compounds **95**–**100***

The evaluation of antimycobacterial activity of the compounds derived from (*R*)-2-amino-1-butanol, provided very promising results (see Table 2). Therefore, studying the *S*-enantiomers of the most active compounds was necessary to compare the activity data. Thus, the synthesis of compounds **95**–**100** (Scheme 8) was achieved by applying the same procedures already described in this article for the *R*-analogues. For the experimental details see Supplementary data.

2.1.8. NMR experiments

The synthesized compounds were studied in detail by NMR spectroscopy by means of $^1H/^{13}C$ spectra, two dimensional routine experiments and additional specific techniques. The structural information obtained is very useful for characterization of similar compounds in future investigations. The routine experiments used are 1D proton and carbon, DEPT, COSY, HSQC, HMBC and NOESY. Some of the synthesized compounds required specific NMR experiments for their assignment. High resolution versions of HSQC and Primitive exclusive HSQC (PE HSQC) experiments were recorded in order to differentiate and assign the highly crowded areas of signals mostly in the aromatic region. The PE HSQC experiment resembles a non-decoupled HSQC experiment [78]. The acquired non-decoupled signals have higher resolved shape, allow multiplicity differentiation of the signals in the aromatic areas (singlet, doublet, triplet) and thus provide additional information. Once the proton and carbon pairs are assigned from the HSQC/PE HSQC experiment a long range correlation is needed (HMBC experiment). The crowded areas of the signals for the aromatic protons and carbons require also a high resolution experiment to be performed. Despite the HSQC experiments, if the HMBC is performed in smaller spectral width, the resulting experiment will contain folded signals and these spurious signals will prevent spectral assignment. These folded signals will be present merely in the indirect dimension (^{13}C) and one way of identifying these spurious signals is to record several HMBC experiments with different spectral widths, therefore these signals that change position will be false and will be differentiated. Another way to overcome the appearance of the folded signals is to selectively irradiate merely the spectral width of interest while leaving the other areas unaffected. Such an experiment where in one dimension selective pulses are applied is called semi-selective. We have performed semi-selective versions of HMBC with different selective pulses depending on the spectral width. Different selective Gaussian pulses were calibrated to cover spectral widths of 10–40 ppm. The high resolution semi-selective HMBC experiments were used to assign the quaternary carbon atoms and also to connect the proton/carbon pairs within one aromatic ring. In some cases TOCSY experiments with different mixing times were applied to provide identification of separate spin systems.

Compounds **11**, **54**, **86**, **89** and **90** couldn't be assigned with the use of the routine NMR experiments and the above specific experiments were applied. Phosphinic amide **11** contains two diastereotopic phenyl rings which showed different chemical shifts. The observed NOE proximities of the methyl group and the methylene proton 3-H_b to the *ortho* protons of one of the phenyl rings assigned its signals as 10-H (see Supplementary data). The $^1H/^{13}C$-signals of the aromatic rings of compound **54** showed small chemical shift variation. Their electron environment is different depending on whether their neighbour is a methyl group or

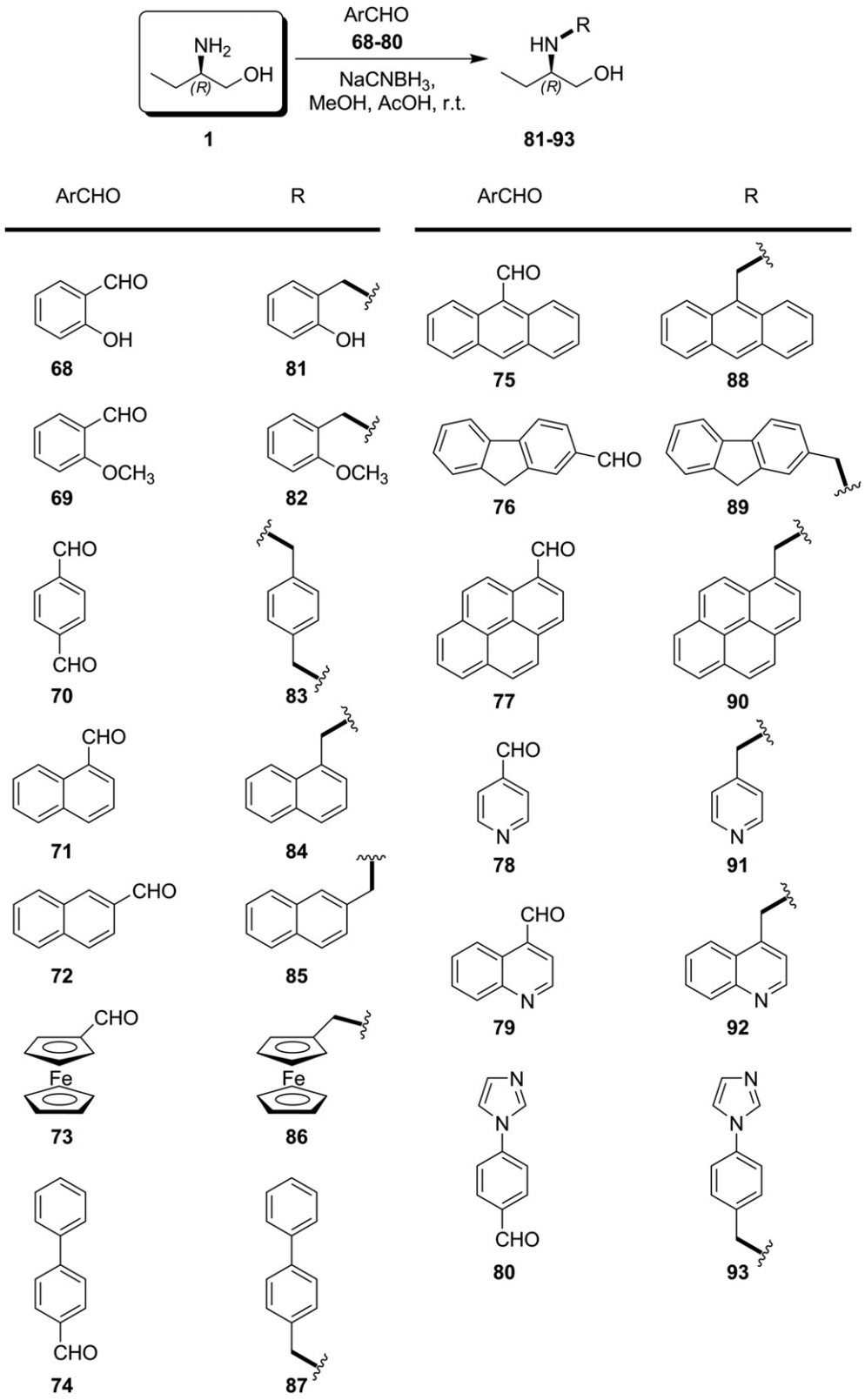


Scheme 6. Synthesis of compounds **52**–**54**, **59**–**62** and **65**–**67**.

a hydroxy group indicating the existence of rotamers. The proton and carbon signals for compounds **89** and **90** were assigned following the above procedure: assignment of proton and carbon pairs, then with the long range correlation fragments were built and quaternary carbons were assigned. Numbering the pairs of atoms with similar chemical shifts for **11** and **54** is tentative (see Supplementary data). Compound **86** contains ferrocenyl moiety and the high resolution experiments in this case were performed for this area to resolve chemical shifts. NMR spectra of compounds **95**–**100** were identical with the spectra of their (*R*)-enantiomers **11**, **13**, **22**, **23**, **31** and **42**, respectively.

2.2. Biology

To the best of our knowledge, there are no data published concerning antimycobacterial, antibacterial and antifungal activity of the compounds synthesized in this study.



Scheme 7. Synthesis of compounds **81**–**93**.

2.2.1. In vitro antimycobacterial activity

The synthesized compounds were evaluated for their *in vitro* activity against *M. tuberculosis* $H_{37}Rv$ (Table 1) using the method of Canetti (see 4.2). Five of the compounds, namely **11**, **22**, **23**, **31** and **42** have shown remarkable high activity, which is between 5 and 11 times better than EMB used as reference. For the purpose of comparison, the most active EMB analogue, the 1,2-diamine SQ 109, is 35 times more potent than EMB [19]. It is interesting to

Table 1
In vitro screening data for antimycobacterial activity and cytotoxicity of synthesized compounds.

Entry	Compound	Antimycobacterial activity towards reference strain of *Mycobacterium tuberculosis* H37Rv, MIC (μM)	*In vitro* cytotoxicity towards human embryonal kidney cell line 293T, IC50 (μM)[a]	Selectivity index, SI[a,b]	Log *P*[c]	Solubility in deionized water at 20 °C (mg/ml)[a]
1	**10**	28.86	NT	NT	0.48±0.31	NT
2	**11**	0.69	155	224.6	1.82±0.57	<1
3	**12**	>17.46	NT	NT	0.79±0.37	NT
4	**13**	9.12	211	23.1	1.70±0.35	<1
5	**14**	>20.55	NT	NT	2.48±0.36	NT
6	**15**	>20.55	NT	NT	2.48±0.36	NT
7	**16**	8.46	173	20.5	1.67±0.39	1.5
8	**17**	>18.37	NT	NT	1.60±0.46	NT
9	**21**	>25.74	NT	NT	−0.01±0.37	NT
10	**22**	0.65	244	375.4	−0.27±0.52	46
11	**23**	0.96	257	267.7	1.59±0.45	2
12	**31**	0.97	89	91.8	0.49±0.36	1
13	**32**	>22.81	NT	NT	1.81±0.28	NT
14	**33**	23.67	NT	NT	0.07±0.33	NT
15	**34**	27.29	NT	NT	0.66±0.33	NT
16	**35**	>29.21	NT	NT	−0.70±0.39	NT
17	**36**	>18.57	NT	NT	1.44±0.62	NT
18	**37**	>14.36	NT	NT	2.21±0.65	NT
19	**41**	>22.29	NT	NT	1.14±0.33	NT
20	**42**	1.25	114	91.2	−0.23±0.35	215
21	**43**	>19.36	NT	NT	2.74±0.40	NT
22	**47**	23.23	NT	NT	2.28±0.75	NT
23	**48**	22.91	NT	NT	0.94±0.29	NT
24	**49**	>22.39	NT	NT	4.62±0.47	NT
25	**52**	>15.09	NT	NT	6.22±0.53	NT
26	**53**	13.39	114	8.5	6.90±0.40	<1
27	**54**	19.74	NT	NT	3.36±0.56	NT
28	**59**	10.46	122	11.7	2.20±0.38	<1
29	**60**	>18.56	NT	NT	4.61±0.42	NT
30	**61**	>11.73	NT	NT	5.79±0.55	NT
31	**62**	>12.15	NT	NT	4.16±0.38	NT
32	**65**	14.03	47	3.4	0.92±0.98	<1
33	**66**	20.40	NT	NT	2.41±0.62	NT
34	**67**	>29.90	NT	NT	0.47±0.57	NT
35	**81**	25.61	NT	NT	1.26±0.35	NT
36	**82**	>23.89	NT	NT	1.91±0.36	NT
37	**83**	17.83	NT	NT	1.78±0.48	NT
38	**84**	21.80	NT	NT	3.23±0.34	NT
39	**85**	>21.80	NT	NT	3.23±0.34	NT
40	**86**	17.41	NT	NT	–[d]	NT
41	**87**	>19.58	NT	NT	3.76±0.39	NT
42	**88**	17.90	NT	NT	4.46±0.34	NT
43	**89**	18.72	NT	NT	3.94±0.40	NT
44	**90**	16.48	NT	NT	4.95±0.34	NT
45	**91**	27.74	NT	NT	0.51±0.35	NT
46	**92**	21.71	NT	NT	1.86±0.35	NT
47	**93**	20.38	NT	NT	1.76±0.62	NT
48	**95**	>17.28	NT	NT	1.82±0.57	NT
49	**96**	>22.80	NT	NT	1.70±0.35	NT
50	**97**	>16.21	NT	NT	−0.27±0.52	NT
51	**98**	>23.90	NT	NT	1.59±0.45	NT
52	**99**	>31.21	NT	NT	−0.23±0.35	NT
53	**100**	>24.36	NT	NT	0.49±0.36	NT
54	EMB·2HCl[e]	7.22	NT	NT	0.06[f]	100[f]

[a] NT – not tested; cytotoxicity, SI and water solubility were tested/calculated/measured only for selected active compounds.
[b] Selectivity index SI = IC_{50}/MIC.
[c] Log *P*, octanol-water partitioning coefficient, was calculated using ACDLabs/ChemSketch 12.01 (www.acdlabs.com).
[d] Log *P* was not calculated for this compound because of software limitations.
[e] EMB·2HCl – ethambutol dihydrochloride (reference compound).
[f] 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.

note that no analogue structures of **11**, **22**, **31** and **42** were found, that possess antimycobacterial activity. Known antimycobacterial activity of salicylanilide phenolic esters [79,80], shows that the salicylamide fragment is important for the activity of **23**. Compounds **13**, **16**, **53**, **59** and **65** have shown 50–80% activity compared to EMB. The cinnamide group of **13** has been incorporated in diverse structures possessing antimycobacterial activity [81,82]. The remaining (*R*)-2-aminobutanol derivatives that were synthesized have no perceptible activity. All of the compounds mentioned above (except **53**) are in agreement with the formal Lipinski's rule of five (Table 1).

The evaluation of the antimycobacterial activity of the *S*-enantiomers **95**–**100** has shown absence of activity (100% growth) at concentrations ca. 2–3 fold higher than MIC of EMB. These results are in contrast with the high activity observed for the *R*-configured enantiomers (**11**, **13**, **22**, **23**, **31** and **42**, respectively). This is opposite to the fact that (*S*,*S*)-EMB is approximately 500 fold more active than (*R*,*R*)-EMB [17]. Therefore, the direct comparison of the

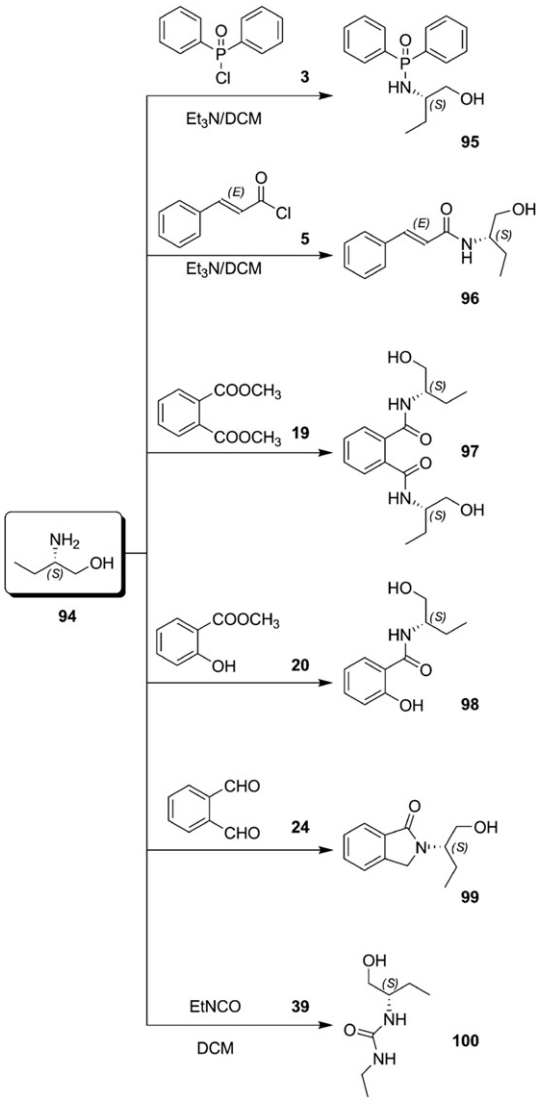
Table 2
Evaluation of *in vitro* antibacterial and antifungal activity of (*R*)-2-amino-1-butanol derivatives (50 mg/ml DMSO) against conditioned pathogenic microorganisms.

Compound	Microorganisms												
	1	2	3	4	5	6	7	8	9	10	11	12	13
10	15	11	NA	11	16	NA	15	NA	NA	NA	NA	NA	NA
11	18	15	16	15	15	17	18	18	15	NA	22	16	NT
12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NT
13	NA	NA	12	12	12	NA	12	15	NA	NA	11	13	NT
14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	NA	NA	NA	NA	NA	NA	NA	NA	NA	NT	NA	NA	NA
21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
23	NA	11	13	12	11	13	11	13	11	NA	13	15	NA
31	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
32	NA	NA	NA	NA	NA	NA	NA	NA	NA	11 (NT)	NA	NA	NA
33	40 (0.06)	35 (0.06)	40 (0.06)	38 (0.06)	40 (0.0125)	39 (0.06)	>40 (0.06)	38 (0.06)	38 (0.3)	29 (1.25)	>40 (0.006)	>40 (0.006)	>40 (0.0125)
34	21	23	20	24	28	21	20	21	22	32	20	21	25
35	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
36	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10
37	NA	NA	NA	NA	NT	NA	NA	NT	NA	NT	NA	NA	NA
41	NA	NA	13	12	14	12	NA	12	12	16	16	15	NT
42	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
43	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
47	NA	15	13	15	18	10	12	13	15	0	20	11	35
48	NA	14	NA	NA	NA	14	NA	NA	NA	NA	NA	NA	NA
49	NA	NA	10	11	NA	NA	NA	NA	NA	NA	NA	NA	NA
52	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
53	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
54	NA	15	22	17	NA	16	12	17	17	12	17	22	20
59	11	20	NT	23	25	NT	25	NT	NT	25	21	16	10
60	NA	11	NA	NA	11	11	11	12	12	NA	10	NA	NA
61	NA	NA	10	NA	NA	NA	NA	10	NT	NA	NA	NA	NA
62	NA	10	NA	12	11	NA	15	NA	12	NA	NA	NA	NA
65	NA	NA	NA	NA	NA	NA	NA	NA	NT	NT	NT	NA	NA
66	13	17	15	13	19	14	14	17	18	17	25	10	17
67	NA	NA	NA	NA	12	NA	NA	NA	NA	NA	NA	NA	NA
81	17	11	12	14	14	13	12	12	11	NA	NA	NA	12
82	10	15	11	11	13	10	10	NT	11	NA	NA	16	NA
83	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
84	17	18	10	18	15	20	16	13	17	28	30	32	22
85	19	19	17	17	14	17	19	17	19	22	10	10	35
86	15	16	16	20	15	15	18	17	17	16	12	14	12
87	18	18	19	18	15	20	20	17	17	28	18	18	40
88	18 (0.3)	19 (1.25)	20 (0.6)	20 (0.6)	19 (0.3)	17 (0.6)	19 (0.03)	18 (0.3)	19 (0.3)	12	31 (0.0125)	32 (0.0125)	16 (0.06)
89	18 (1.25)	17 (0.6)	16 (1.25)	20 (1.25)	16 (1.25)	17 (1.25)	17 (1.25)	18	16	16 (1.25)	21 (0.06)	20 (1.25)	30
90	14	30	13	15	14	14	13	13	14	NA	40	35	14
91	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
92	10	12	11	16	13	13	14	13	10	20	NT	NT	20
93	12	16	NA	18	13	18	16	18	16	NA	NA	NA	12
Streptomycin[a] (reference)	35/30/25	30/27/21	30/25/20	35/30/25	30/28/23	30/24/20	32/26/20	27/22/19	30/25/20	35/26/23	NT	NT	NT
Gentamycin[b] sulphate (reference)	30/27	35/29	36/30	32/28	29/26	39/31	35/27	34/28	38/28	31/25	NT	NT	NT
Fluconazole[c] (reference)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	35	–	–
Itraconazole[d] (reference)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	–	30	–

Diameter of zone of inhibition are given in mm. Values in brackets are the lowest concentrations (in mg/ml) provoking zone of inhibition >11 mm. Values in brackets are given only for compounds preserving their activity at concentrations 1.25 mg/ml or less.
NA – not active according first zone inhibition test (at concentration 50 mg/ml); further tests not performed.
NT – zone inhibition test not performed.
[a]Zone inhibition for Streptomycin was measured at three concentrations – 25, 5 and 1 mg/ml.
[b]Zone inhibition for Gentamycin sulphate was measured at concentrations 20 and 4 mg/ml.
[c]Zone inhibition for Fluconazole was measured at concentration 25 mg/ml.
[d]Zone inhibition for Itraconazole was measured at concentration 12.5 mg/ml, but is not completely soluble in DMSO at this concentration.
List of microorganisms: 1 *Bacillus subtilis*, 2 *Bacillus idosus*, 3 *Bacillus megaterium*, 4 *Bacillus mycoides*, 5 *Bacillus cereus*, 6 *Acinetobacter johnstonii*, 7 *Staphylococcus aureus*, 8 *Sarcina lutea*, 9 *Micrococcus luteus*, 10 *Escherichia coli*, 11 *Candida tropicalis*, 12 *Saccharomyces cerevisiae*, 13 *Penicillium chrysogenum*.

structures presented in this paper with EMB should be handled with care. Since **95**–**100** were not interesting as antimycobacterial agents, their cytotoxicity and other antibacterial activity was not investigated.

There is no correlation between antimycobacterial activity and activity against other microorganisms, for the compounds in this study (see below and compare Tables 1 and 2). This indicates the specific activity of all potent derivatives of (*R*)-2-aminobutanol to



Scheme 8. Synthesis of compounds **95**–**100**.

the *M. tuberculosis*, as it is established for the majority of first-line antimycobacterial agents (including EMB) in clinical use. Furthermore, since all compounds are partial structure analogues of EMB, it can be assumed that they share similar mode of antimycobacterial action [14–16], although this needs clarification [83]. Therefore, investigating the mechanism of action and the role of chirality of these compounds would aid further discovery of more potent analogues.

2.2.2. Evaluation of in vitro antibacterial and antifungal activity

For the synthesized derivatives of (*R*)-2-amino-1-butanol a qualitative evaluation of *in vitro* antibacterial and antifungal activities against conditioned pathogenic microorganisms was performed (Table 2), using agar diffusion test (see 4.3).

Antibacterial activities were examined against the Gram (+) strains: *Bacillus subtilis* ATCC 6633, *Bacillus idosus* B 241, *Bacillus megaterium* NRRL 1353895, *Bacillus mycoides* DSMZ 274, *Bacillus cereus* ATCC 11778, *Acinetobacter johnsonii* ATCC 17909, *Staphylococcus aureus* NRRL B 313, *Sarcina lutea* ATCC 9341, *Micrococcus luteus* ATCC 9631, and the Gram (−) strain *Escherichia coli* ATCC 8739. Antibiotics streptomycin and gentamicine sulphate were used as reference compounds.

Antifungal activities were examined against the yeast strains *Candida tropicalis* ATCC 20336 and *Saccharomyces cerevisiae* ATCC 9763, and the fungal strain *Penicillium chrysogenum* CECT 2802. Fluconazole and itraconazole were used as antifungal reference compounds.

The most of the compounds tested were not active even at concentrations 50 mg/ml and consequently are not appropriate for antimicrobial agents (Table 2). Some of the compounds (**11**, **33**–**34**, **47**, **54**, **59**, **66**, **84**–**85**, **87**–**89**) demonstrated wide spectrum of low-to-moderate activity at lower concentrations − 25, 10, 5, 2, 1.25 or 1 mg/ml. The data in Table 2 (values in brackets) show that almost all of the compounds are not active at concentrations 2 mg/ml or less. Only compounds **33**, **88** and **89** were active against all microorganisms (except *E. coli*) in significantly lower concentrations. The most potent compound **33** exhibited emphasized activity against the fungi *C. tropicalis*, *S. cerevisiae* and *P. chrysogenum* even at concentrations 6, 6 and 12.5 μg/ml, respectively. The intact maleimido ring of **33** is probably necessary for the observed activity. On the contrary, the substituted maleimide **34** and the dihydro analogue **35** showed significantly lower activity and absence of activity, respectively. Similar trend has been observed in recent report concerning the antifungal activity of analogous *N*-alkyl-aryl substituted maleimide derivatives [84].

2.2.3. In vitro cytotoxicity

In order to examine the selectivity of the antiproliferative effects, the cytotoxic activity of representative compounds, exerting antimycobacterial activity, was assessed against a human embryonal kidney non-tumour cell line 293T, after 72 h continuous exposure. Evident from the IC_{50} values summarized in Table 1, the compounds were generally of low-to-moderate cytotoxicity against the human cells; with few exceptions the compounds induced 50% inhibition of cellular proliferation and viability at concentrations greatly exceeding 100 μM. It is noteworthy that the structural peculiarities affording the highest antimycobacterial activity within the series e.g. phosphinic amide (**11**), phthalic diamide (**22**), salicylamide (**23**), isoindolinone (**31**) and urea (**42**) were also generally associated with very low antiproliferative/cytotoxic effects against human cells with selectivity indices ranging from 91.2 to 375.4. The values of SI for compounds **13**, **16**, **53**, **59** and **65** are significantly lower, near the acceptable limit values.

3. Conclusion

The synthesis of 47 structurally diverse compounds incorporating the (*R*)-2-amino-1-butanol motif has been realized. They are partial structure analogues (only in respect of the 2-aminobutanol motif) of the clinically used essential antimycobacterial drug ethambutol. After purification and unambiguous structure characterization the antibacterial and antimycobacterial activity was evaluated. Three compounds (**33**, **88** and **89**) showed interesting antifungal activity. Ten of the compounds showed prominent antimycobacterial activity with MICs ranging from less than 1 to ca. 10 μM. Interestingly, the most potent and promising compounds from the large series also proved to exert low level of cytotoxic activity against a human embryonal non-tumour cell line 293T. On these basis, the (*R*)-2-amino-1-butanol derivatives bearing phosphinic amide (**11**), phthalic diamide (**22**), salicylamide (**23**), isoindolinone (**31**) and urea (**42**) moieties (possessing 5.7–11.1 fold higher antimycobacterial activities than EMB) could be regarded as potential lead compounds for elaboration of antimycobacterial agents. Cinnamide **13** could be perspective as lead compound, as well.

The (*S*)-enantiomers of the 6 compounds mentioned above have been synthesized as well. They have shown absence of activity

towards *M. tuberculosis* $H_{37}Rv$ even at 20–32 fold higher concentrations. Therefore, further investigations of the mechanism of action and the role of chirality of similar compounds would contribute to discovery of more potent analogues.

4. Experimental

4.1. Chemistry

For thin layer chromatography (TLC) aluminium sheets precoated with silica gel 60 F_{254} (Merck) were used. Flash column chromatography was carried out using silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM, Merck). Commercially available solvents for reactions, TLC and column chromatography were used after distillation (and were dried when needed) – hexane, diethyl ether (Et_2O), dichloromethane (DCM), methyl tert-butyl ether (MTBE), tetrachloroethylene, tetrahydrofurane (THF), methanol (MeOH), ethanol (EtOH), acetonitrile, xylene. Melting temperatures were determined in capillary tubes on an Electrothermal MEL-TEMP 1102D-230 VAC apparatus without corrections. The NMR spectra were recorded on a Bruker Avance DRX-250 (250.13 for ^1H and 62.90 MHz for ^{13}C) and on a Bruker Avance II+ 600 (600.13 for ^1H and 150.92 MHz and for ^{13}C NMR) spectrometers. In case of $CDCl_3$ TMS was used as internal standard for chemical shifts (δ, ppm) and ^1H spectra were calibrated to the signal of TMS ($\delta = 0.0000$). For other solvents ^1H spectra were calibrated to the residual solvent peaks (DMSO-d_6 $\delta = 2.50$). ^{13}C spectra were calibrated in all cases to the residual solvent peaks ($CDCl_3$ $\delta = 77.00$, DMSO-d_6 $\delta = 39.52$). ^{31}P NMR spectra were recorded with full proton decoupling and using 85% H_3PO_4 as external standard. The calibration of the ^{31}P NMR spectra was performed through changing of the spectrum reference frequency (specific for the used NMR probe). The following additional NMR techniques were used for all compounds: DEPT 135, COSY, HSQC and HMBC. ^1H and ^{13}C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, identification, and coupling constants (in Hz). For numbering of the atoms see Supplementary data. Mass spectra (MS) were recorded on a Thermo Scientific High Resolution Magnetic Sector MS DFS by chemical ionization (CI) or electrospray ionization (ESI), and are reported as fragmentation in m/z with relative intensities (%). Optical rotation $[\alpha]_D^{20}$ measurements were obtained using a Perkin–Elmer 241 polarimeter. Enantiomeric purity of selected compounds was determined by using Agilent 1100 HPLC system with diode-array detector (DAD) and Chiralpak IC chiral column (for conditions see Supplementary data). Elemental analyses were performed by the Microanalytical Laboratory for Elemental Analysis of the Institute of Organic Chemistry, Bulgarian Academy of Sciences. All starting chemicals were commercially available (from Sigma–Aldrich, Merck, Fluka, Acros, Alfa Aesar) or are synthesized according literature procedures. Dimethylsulfoxyde (DMSO) for testing of bioactivities was commercial (with HPLC grade) and was used without distillation. (*R*)-2-Amino-1-butanol (**1**) was available from Merck (98% chemical purity (GC), enantiomeric purity ee>96%, spec. rotation $[\alpha]_D^{20} = -10.5$ to -9.5° (undiluted)). (*S*)-2-Amino-1-butanol (**94**) was available from Alfa Aesar (98% chemical purity (GC), spec. rotation $[\alpha]_D^{20} = +12^\circ$ ($c = 2$ in EtOH)). Intermediate compounds **58** [85] and **80** [86] were not commercially available and were prepared according described procedures.

4.1.1. Synthetic procedures

The individual procedures for synthesis of the described compounds are introduced in detail in the part named "Supplementary data".

4.2. Methodology for evaluation of antimycobacterial activity

The antimycobacterial activity was determined through the proportional method of Canetti towards reference strain *M. tuberculosis* $H_{37}Rv$. This method, recommended by the WHO, is the most commonly used one worldwide for exploration of sensibility/resistance of tuberculosis strains towards chemotherapeutics [87–90]. 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 three concentrations – 5 mg/ml, 2 mg/ml and 0.2 mg/ml (in DMSO). For compounds showing 0% growth at 0.2 mg/ml, additional test at concentrations 0.1 mg/ml was performed. Tubes with Löwenstein–Jensen medium (5 ml) containing tested compounds and such without them (controls) were inoculated with a suspension of *M. tuberculosis* $H_{37}Rv$ (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 give complete inhibition of bacterial growth (0% growth). The MIC values are given as μM.

4.3. Methodology for evaluation of antibacterial and antifungal activity

Antimicrobial and antifungal activities were determined by the agar diffusion test (or zone inhibition test) according to European Pharmacopoeia [91]. Test organisms were suspended in melted nutrient agar and poured into Petri dishes. Holes of 8 mm were cut in the agar and filled with 50 μl solution (in DMSO) of various concentrations of the compound. The diameter of clear zone around the point of application of the compound was measured. Bacterial strains were grown for 24 h at 37 °C in nutrient agar (Serva), yeast strains were grown in yeast peptone dextrose agar (YEPD) and fungi – in potato dextrose agar (PDA). Yeast and fungal strains were incubated for 72 h at 28 °C.

4.4. Methodology for evaluation of 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 – RPMI-1640 medium, supplemented with 10% heat-inactivated fetal 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 [92] with some modifications [93]. In brief, exponentially growing cells were seeded in 96-well microplates (100 μl/well) at a density of 3.5×10^5 cell/ml and allowed to grow for 24 h prior 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 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 μl 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).

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.11.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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