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α-Amino acid-derived 2-phenylimidazoles with potential antimycobacterial activity

Research Article

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Abstract: α-Amino acid-derived 2-phenylimidazole derivatives were designed, synthesized, and further investigated as potential antimycobacterial agents. The synthesis of target imidazole derivatives involved the transformation of Cbz-protected α-amino acids (Ala, Val, Phe, Leu, iLe, and Pro) into α-diazoketones and α-bromoketones, respectively. Subsequent treatment of α-bromoketones with (4-nitro) benzamidine afforded imidazole derivatives bearing α-amino acid residue appended to the imidazole C4 and (4-nitro)phenyl ring in the position C2. Antimycobacterial activities of both series of compounds against *M. tuberculosis*, *M. avium*, and *M. kansasii* were screened and basic structure-activity relationships were further evaluated.

Keywords: *Imidazole • α-Amino acid • Antimycobacterial agents • SAR*

1. Introduction

Despite the recent progress in treatment of infectious diseases caused by *Mycobacterium* spp., these microorganisms continue to represent a considerable problem in world health control. Over 2 billion people are infected by *Mycobacteria* (mostly *M. tuberculosis*) and each tenth of them suffers from apparent form of these infections (*e.g.* tuberculosis) [1]. Distribution of mycobacterial infections in the world's population steadily increases, mainly due to the raising human mobility as well as the raising resistance of particular strains. Nowadays, the tuberculosis strains become multi drug resistant (MDR-TB), extensively drug resistant (XDR-TB), and very recently also totally drug resistant (TDR-TB). Moreover, some species of *Mycobacteria* such as *M. avium* do not have any universally efficient treatment protocol [2]. The increasing strains resistance, especially the cross-resistance, led to the current extensive explorations and studies on new antituberculotics featuring improved or different mechanism of action [3].

Five-membered heterocycles (azoles) such as pyrroles [4], isoxazoles [5], oxadiazoles [6], and imidazoles [7] constitute one of the structural motives used for the design of therapeutic agents to date. Di(tri) azole derivatives found wide applications as antifungal agents (*e.g.* flukonazole, itraconazole, clotrimazole). Imidazole-derived antibiotic agents belong to a large sub-class of the aforementioned drugs, from which metronidazole and its derivatives are certainly the most widely applied antibiotics against anaerobic bacteria and protozoa (Fig. 1) [8]. 2-(Hetero)arylazole linkage was recently recognized as efficient structural arrangement in pyrrole, oxazole, and oxadiazole derivatives that feature respectable antimycobacterial activities (Fig. 1) [4-6].

Recently, we have screened the antimycobacterial activities of various 2-phenylimidazoline [9] as well as benzimidazole, benzthiazole, and benzoxazole derivatives [10]. This screening revealed very promising activities of compounds **1**-**2** (Fig. 1). In general, these derivatives possess the following important structural features: – i) central azole derivative, ii) 2-(hetero)aryl

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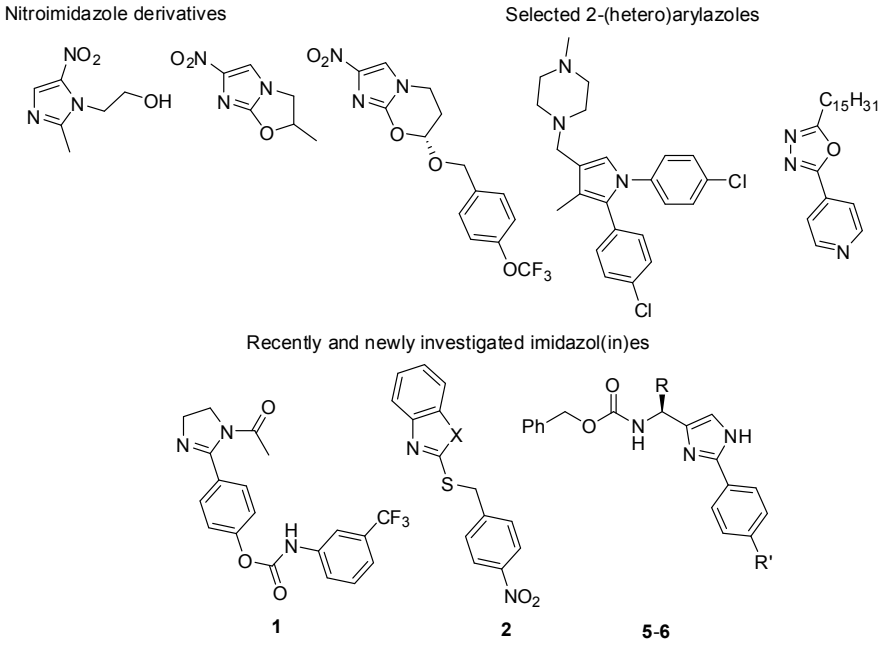


Figure 1. Imidazole- and 2-(hetero)arylazole-derived antimycobacterial agents [4-6], our recently investigated derivatives **1**-**2** [9-10] and general structure of newly synthesized *N*-Cbz α-amino acid-derived 2-phenylimidazoles **5**-**6**.

linkage, and iii) carbamate moiety known as active functional group of many AChE inhibitors [11]. Inspired by the success of these particular structural features, we have proposed to investigate *N*-Cbz α-amino acid-derived 2-phenylimidazoles **5** (Fig. 1). These derivatives, which were originally intended as optically active nitrogen ligands for asymmetric catalysis [12], combine all of the aforementioned structural features into one molecule. According to our previous studies on the terminal nitro group effect on the antimycobacterial activity [10], the series of compounds **5a**-**g** was further completed with nitro analogs **6a**-**g**.

2. Experimental procedure

2.1. General

Reagent and solvents were reagent-grade or HPLC-grade and were purchased from Penta, Aldrich and Acros and used as received. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl radical. Thin-layer chromatography (TLC) was conducted on aluminium sheets coated with silica gel 60 F_{254} obtained from Merck, with visualisation by UV lamp (254 or 365 nm). ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, with a Bruker AVANCE 400 instrument at 25°C. Chemical shifts are reported in ppm relative to the signal of Me_4Si. The residual solvent signal in the ^{1}H and ^{13}C NMR spectra was used as an internal reference (d_6-DMSO – 2.55 and 39.51 ppm). Apparent resonance multiplicities are described as s (singlet), br s (broad singlet), d (doublet) and m (multiplet). Some *N*-Cbz protected 2-phenylimidazole derivatives showed in d_6-DMSO strongly hindered rotation in the carbamate moiety and slow imidazole tautomerism which may result in a set of two signals, broad signals or no observable signals [12b]. Mass spectra were measured on a GC/MS configuration comprised of an Agilent Technologies – 6890N gas chromatograph equipped with a 5973 Network MS detector (EI 70 eV, mass range 33−550 Da). IR spectra were recorded on a Perkin Elmer FT-IR Spectrum BX spectrometer. Optical rotations were measured on a Perkin Elmer 341 polarimeter using the sodium D line (589 nm), specific rotations [α] are given in units of deg cm^2 g^{-1} and concentration *c* is 1 g per 100 cm^3 MeOH. Elemental analyses were performed on a Thermo Flash 2000 CHNS experimental organic analyzer.

N-Cbz protected α-amino acids [13], α-diazoketones **3a**-**g**, α-bromoketones **4a**-**g**, and imidazole derivatives **5a**-**g** were synthesized according to the literature procedure and their analytical data were in accordance with those previously published [12a].

2.2. Synthesis

Preparation of 4-nitrobenzamidine hydrochloride (modification of [14])

Sodium methoxide (0.165 g; 3.1 mmol) was added to a solution of 4-nitrobenzonitrile (4.8 g, 32.4 mmol) in dry methanol (15 mL) and the reaction was refluxed

for 12 h (monitored by TLC and GC/MS). Ammonium chloride (2.85 g, 53.3 mmol) was added and the reaction mixture was allowed to cool to 25°C. The resulting yellow suspension was filtered off and the filtration cake was washed with dry methanol and diethylether to afford the title compound as a yellow solid. Yield 3.85 g (59%). M. p. 288-291°C (Lit. [14] m.p. 285-287°C). R_f = 0.3 (SiO_2; EtOAc; free base); MS (EI), free base: *m/z* = 166 ([M^+], 80), 150 (100), 120 (20), 104 (35), 92 (25), 76 (33); ^{1}H-NMR (400 MHz; d_6-DMSO): δ = 8.15 (d, J^3(H,H) = 9.0 Hz, 1H, Ar*H*), 8.23 (d, J^3(H,H) = 9.0 Hz, 1H, Ar*H*), 8.43 (d, J^3(H,H) = 9.0 Hz, 1H, Ar*H*), 8.45 (d, J^3(H,H) = 9.0 Hz, 1H, Ar*H*), 8.54 ppm (br s, 4H, C(N*H*2)N*H*2); ^{13}C-NMR (100 MHz; d_6-DMSO): δ = 123.8 (*C*H), 124.4 (*C*H), 130.0 (*C*H), 134.1 (*C*), 134.2 (*C*H), 150.3 (*C*), 164.5 (*C*) ppm.

Preparation of 2-phenylimidazoles 6a-g – general procedure (modification of [12a])

A solution of α-bromoketone **4a**-**g** (29.0 mmol) in THF (50 mL) was added dropwise into a refluxing solution of 4-nitrobenzamidine hydrochloride (5.85 g; 29.0 mmol) and anhydrous sodium carbonate (12.3 g, 116.0 mmol) in THF (80 mL) and water (20 mL) and the reaction mixture was further refluxed for the indicated time (Table 1, monitored by TLC). The crude reaction mixture was concentrated in vacuo and the residue was portioned between dichloromethane (40 mL) and water (40 mL), the layers were separated, and the water layer was extracted with dichloromethane (2 × 60 mL). Combined organic layers were washed with water (100 mL), brine (100 mL), dried (Na_2SO_4), and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (SiO_2; EtOAc/hexane 1:1).

2-(4-Nitrophenyl)-4-(1-(S)-benzyloxycarbonyl-aminoethyl)-1H-imidazole **(6a)**

The title compound was synthesized from α-bromoketone **4a** (8.7 g, 29.0 mmol) following the general method. Yellowish solid, yield 2.7 g (25%). M. p. 178-179°C. R_f = 0.7 (SiO_2; EtOAc/hexane 2:1); $[\alpha]_D^{20}$ = −31.6 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, d_6-DMSO): δ = 1.46 (d, J^3(H,H) = 7.4 Hz, 3H, CH_3), 4.77-4.80 (m, 1H, CBzNHC*H*), 5.09 ppm (s, 2H, PhCH_2OCO), 7.03+7.21 ppm (2×s, 1H, CH_{im}), 7.36-7.42 (m, 5H, CH_2Ph*H*), 7.62 (br s, 1H, N*H*CBz), 8.19 (d, J^3(H,H) = 8.8 Hz, 2H, Ph*H*NO_2), 8.35 (d, J^3(H,H) = 8.8 Hz, 2H, Ph*H*NO_2), 12.85 ppm (br s, 1H, NH_{im}); ^{13}C NMR (100 MHz, d_6-DMSO): δ = 21.1, 45.1, 65.3, 124.3, 125.4, 127.8, 128.4, 136.6, 137.3, 143.0, 146.4, 155.5 ppm (3 signals missing); FT-IR(HATR): 1047, 1347, 1407, 1445, 1520, 1677, 3300 cm^{-1}; Found: C 62.2, H 5.0, N 15.4. $C_{19}H_{18}N_4O_4$ requires C 62.3, H 4.9, N 15.3.

2-(4-Nitrophenyl)-4-(1-(R)-benzyloxycarbony-laminoethyl)-1H-imidazole **(6b)**

The title compound was synthesized from α-bromoketone **4b** (8.7 g, 29.0 mmol) following the general method. Yellowish solid, yield 3.9 g (37%). M. p. 176-178°C. R_f = 0.7 (SiO_2; EtOAc/hexane 2:1); $[\alpha]_D^{20}$ = +32.0 (*c* 1.0, MeOH); ^{1}H (400 MHz,d_6-DMSO): δ = 1.46 (d, J^3(H,H) = 7.4 Hz, 3H, CH_3), 4.77-4.80 (m, 1H, CBzNHC*H*), 5.09 ppm (s, 2H, PhCH_2OCO), 7.03+7.21 ppm (2×s, 1H, CH_{im}), 7.36-7.42 (m, 5H, CH_2Ph*H*), 7.62 (br s, 1H, N*H*CBz), 8.19 (d, J^3(H,H) = 8.8 Hz, 2H, Ph*H*NO_2), 8.35 (d, J^3(H,H) = 8.8 Hz, 2H, Ph*H*NO_2), 12.85 ppm (br s, 1H, NH_{im}); ^{13}C NMR (100 MHz, d_6-DMSO): δ = 21.1, 45.1, 65.3, 124.3, 125.4, 127.8, 128.4, 136.6, 137.3, 143.0, 146.4, 155.5 ppm (3 signals missing); FT-IR(HATR): *v* = 1047, 1347, 1407, 1445, 1520, 1677, 3300 cm^{-1}; Found: C 64.2, H 5.1, N 15.5. $C_{19}H_{18}N_4O_4$ requires C 64.3, H 4.9, N 15.3.

2-(4-Nitrophenyl)-4-(1-(S)-benzyloxycarbonyl-amino-2-methylpropyl)-1H-imidazole **(6c)**

The title compound was synthesized from α-bromoketone **4c** (9.5 g, 29.0 mmol) following the general method. Yellow-red solid, yield 1.1 g (10%). M. p. 185-187°C. R_f = 0.75 (SiO_2; EtOAc/hexane 2:1); $[\alpha]_D^{20}$ = −58.1 (*c* 1.0, MeOH); ^{1}H (400 MHz, d_6-DMSO): δ = 0.89 (d, J^3(H,H) = 6.6 Hz, 3H, CH_3), 0.92 0.89 (d, J^3(H,H) = 6.6 Hz, 3H, CH_3), 2.08-2.20 (m, 1H, C*H*(CH_3)$_2$), 4.50-4.65 (m, 1H, CBzNHC*H*), 5.05-5.13 (m, 2H, PhCH_2OCO), 7.07+7.25 (2×s, 1H, CH_{im}), 7.35-7.41 (m, 5H, CH_2Ph*H*), 7.54+7.75 (2×d, J^3(H,H) = 9.3 Hz, 1H, N*H*CBz), 8.19 (d, J^3(H,H) = 8.8 Hz, 2H, Ph*H*NO_2), 8.35 (d, J^3(H,H) = 8.8 Hz, 2H, Ph*H*NO_2), 12.76+12.86 (2×br s, 1H, NH_{im}); ^{13}C (100 MHz, d_6-DMSO): δ = 18.4, 19.6, 32.2+32.7, 55.4, 65.2+65.5, 116.3, 124.3, 125.3, 127.7, 127.8, 128.3, 136.6, 137.3, 142.7, 144.5, 146.3, 156.0 ppm; FT-IR(HATR): *v* = 1047, 1297, 1345, 1417, 1445, 1541, 1673, 3300 cm^{-1}; Found: C 63.8, H 5.6, N 14.0. $C_{21}H_{22}N_4O_4$ requires C 63.9, H 5.6, N 14.2.

2-(4-Nitrophenyl)-4-(1-(S)-benzyloxycarbonyl-amino-2-phenylethyl)-1H-imidazole **(6d)**

The title compound was synthesized from α-bromoketone **4d** (10.9 g, 29.0 mmol) following the general method. Red solid, yield 2.1 g (16%). M. p. 163-165°C. R_f = 0.7 (SiO_2; EtOAc/hexane 2:1); $[\alpha]_D^{20}$ = −18.4 (*c* = 1.0, MeOH); ^{1}H (400 MHz, d_6-DMSO): δ = 3.01–3.05 (m, 1H, CHCH_2Ph), 3.20-3.31 (m, 1H, CHCH_2Ph), 4.90-4.93 (m, 1H, CBzNHC*H*), 5.01 (dd, J^2(H,H) = 12.8 and 8.4 Hz, 2H, PhCH_2OCO), 7.08+7.18 (2×s, 1H, CH_{im}), 7.23-7.39 (m, 10H, 2×Ph*H*), 7.73+7.91 (2×d, J^3(H,H) = 8.8 Hz, 1H,

NHCBz), 8.21 (d, J^3(H,H) = 8.8 Hz, 2H, PhHNO$_2$), 8.36 (d, J^3(H,H) = 8.8 Hz, 2H, PhHNO$_2$), 12.89 ppm (br s, 1H, NH$_{im}$); ^{13}C (100 MHz, d_6-DMSO): δ = 40.6, 51.2, 65.0+65.2, 116.1, 124.3, 125.4, 126.0, 127.4, 127.6, 128.1, 128.3, 129.3, 136.5, 137.4, 138.9, 142.9, 145.1, 146.4, 155.6 ppm; FT-IR(HATR): v = 1047, 1347, 1407, 1443, 1518, 1660, 3300 cm^{-1}; Found: C 67.6; H 5.0; N 12.5. $C_{25}H_{22}N_4O_4$ requires C 67.9; H 5.0; N 12.7.

2-(4-Nitrophenyl)-4-(1-(S)-benzyloxycarbonyl-amino-2-methylbutyl)-1H-imidazole **(6f)**

The title compound was synthesized from α-bromoketone **4f** (9.9 g, 29.0 mmol) following the general method. Orange solid, yield 0.8 g (7%). M. p. 98 – 101 °C. R_f = 0.8 (SiO$_2$; EtOAc/hexane 2:1); $[\alpha]_D^{20}$ = −26.6 (c 1.0, MeOH); ^{1}H (400 MHz,d_6-DMSO): δ = 0.83 (d, J^3(H,H) = 6.7 Hz, 3H, CHCH$_3$), 0.90 (t, J^3(H,H) = 7.3 Hz, 3H, CH2CH$_3$), 1.14-1.18 (m, 1H, CH$_2$CH$_3$), 1.50-1.56 (m, 1H, CH$_2$CH$_3$), 1.89-1.94 (m, 1H, CHCH$_3$), 4.55-4.66 (m, 1H, CBzNHCH), 5.03-5.08 (m, 2H, PhCH$_2$OCO), 7.05+7.25 (2×s, 1H, CH$_{im}$), 7.35-7.41 (m, 5H, PhH), 7.54+7.75 (2×d, J^3(H,H) = 8.8 Hz, 1H, NHCBz), 8.18 (d, J^3 = 8.8 Hz, 2H, PhHNO$_2$), 8.35 (d, J^3 = 8.8 Hz, 2H, PhHNO$_2$), 12.77+12.86 ppm (2×brs, 1H, NH$_{im}$); ^{13}C (100 MHz, d_6-DMSO): δ = 11.3, 15.8, 24.9, 38.7, 54.1, 65.3, 116.1, 124.3, 125.3, 127.7, 127.8, 128.4, 136.6, 137.4, 142.7, 144.3, 146.3, 156.9 ppm; FT-IR(HATR): v = 841, 934, 1113, 1335, 1410, 1445, 1530, 1685, 3300 cm^{-1}; Found: C 64.5; H 5.9; N 13.5. $C_{22}H_{24}N_4O_4$ requires C 64.7; H 5.9; N 13.7.

2-(4-Nitrophenyl)-4-(1-benzyloxycarbonyl) pyrrolidine-2-(S)-yl-1H-imidazole **(6g)**

The title compound was synthesized from α-bromoketone **4g** (9.5 g, 29.0 mmol) following the general method. Yellow solid, yield 3.1 g (28%). M. p. 101-102°C. R_f = 0.7 (SiO$_2$; EtOAc/hexane 2:1); $[\alpha]_D^{20}$ = −54.4 (c 1.0, MeOH); ^{1}H (400 MHz, d_6-DMSO): δ = 1.93-1.96 (m, 1H, CH$_2$), 2.03-2.07 (m, 2H, CH$_2$), 2.18-2.3 (m, 1H, CH$_2$), 3.40-3.50 (m, 1H, NCH$_2$), 3.59-3.63 (m, 1H, NCH$_2$), 4.96-5.01 (m, 1H, CBzNHCH), 5.07-5.15 (m, 2H, PhCH$_2$OCO), 6.98-7.42 (m, 6H, PhH+CH$_{im}$), 8.19 (d, J^3(H,H) = 8.8 Hz, 2H, PhHNO$_2$), 8.35 (d, J^3(H,H) = 8.8 Hz, 2H, PhHNO$_2$), 12.76+12.86 ppm (2×br s, 1H, NH$_{im}$); ^{13}C (100 MHz, d_6-DMSO): δ = 22.6+23.5, 31.8+32.9, 46.1+46.6, 55.2+55.6, 65.4+65.7, 115.9+116.1, 124.3, 125.3, 126.9+ 127.4, 127.3+127.7, 128.1+128.4, 136.6, 137.3, 142.9, 145.3+145.9, 146.3, 153.9+154.0 ppm; FT-IR(HATR): v = 1047, 1347, 1407, 1445, 1520, 1677, 3300 cm^{-1}; Found: C 64.2; H 5.1; N 14.7. $C_{21}H_{20}N_4O_4$ requires C 64.3; H 5.1; N 15.0.

2.3. Biological activity

The antimycobacterial activity screening was carried out in the Laboratory for Mycobacterium diagnostics of the Institute of Public Health in Ostrava. The antimycobacterial activities of the compounds **5**-**6** were evaluated *in vitro* against *Mycobacterium tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80, *M. kansasii* 6509/96 and *M. avium* CNCTC My 330/88. Micromethod for the determination of the minimum inhibition concentration (MIC) was used. All tested strains were obtained from the Czech National Collection of Type Cultures (CNCTC), except of *M. kansasii* 6509/96, which was clinically isolated. The activities of the compounds were determined in the Šula semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethylsulfoxide solutions. The following concentrations were used: 1000, 500, 250, 125, 62, 32, 16, 8, 4 and 2 μmol L^{-1} (binary dilution steps). The MIC values were determined upon incubation at 37°C for 14 and 21 days and in the case of *M. kansasii* 7, 14, and 21 days, respectively. The MIC values were determined as the lowest concentration of a substance in binary dilution steps performing the inhibition of the growth of *Mycobacteria*. Isonidazid (INH) was used as a standard.

3. Results and discussion

3.1. Synthesis of target compounds

The synthesis of target compounds **5** and **6** is outlined in Scheme 1. The synthesis started from commercially available nonpolar α-amino acids such as (*S*)-Ala (**a**), (*R*)-Ala (**b**), (*S*)-Val (**c**), (*S*)-Phe (**d**), (*S*)-Leu (**e**), (*S*)-iLe (**f**), and (*S*)-Pro (**g**) and their *N*-protection using benzylchloroformate to afford *N*-Cbz protected α-amino acids in the yields of 93-97% [13]. Activation of the carboxylic acids via formation of mixed anhydride and its subsequent treatment with ethereal diazomethane solution afforded smoothly α-diazoketones **3a**-**g** in the yields of 90-98%. These α-diazoketones easily release nitrogen if treated with hydrobromic acid (48%), yielding α-bromoketones **4a**-**g**. Compounds **4a**-**g** proved to be very useful 1,2-dielectrophiles and may react with a variety of nucleophiles, e.g. the reaction with 1,3-dinucleophiles such as amidines led to imidazole derivatives [15]. Thus, the condensation of either commercially available benzamidine or 4-nitrobenzamidine hydrochlorides in the system of THF/H$_2$O/Na$_2$CO$_3$ afforded target 2-phenylimidazole derivatives **5** and **6**. Starting 4-nitrobenzamidine was prepared from 4-nitrobenzonitrile and its sodium methoxide-catalyzed

conversion to imidoester and subsequent treatment with ammonium chloride in 59% overall yield [14]. Whereas the reaction of α-bromoketones **4a-g** with benzamidine proceeded smoothly within 12 h affording the desired 2-phenylimidazole derivatives **5a-g** in good yields (42-89%), the reaction with electron poor 4-nitrobenzamidine required significantly prolonged reaction times up to 72 h. However, the attained reaction yields were modest (7-37%, Table 1). The compound **6e** (derived from (*S*)-Leu) was detected in the crude reaction mixture, however, its attempted isolation was always unsuccessful.

Table 1. Isolated yields, optical rotations, and melting points of 2-phenylimidazoles **5** and **6**.

Comp.	Yields [%][a]	$[\alpha]_D^{20}$ (c 1, MeOH)	M.p. [°C]
5a/6a	89/25	-25.4/-31.6	152-153/178-179
5b/6b	85/37	+24.9/+32.0	158-159/176-178
5c/6c	76/10	-45.2/-58.1	138-139/185-187
5d/6d	65/16	-13.5[b]/-18.4	171-172/163-165
5e/6e	88/0	-48.7/---	146-147/---
5f/6f	42/7	-50.2/-26.6	128-129/98-101
5g/6g	70/28	-34.2/-54.4	52-54/101-102

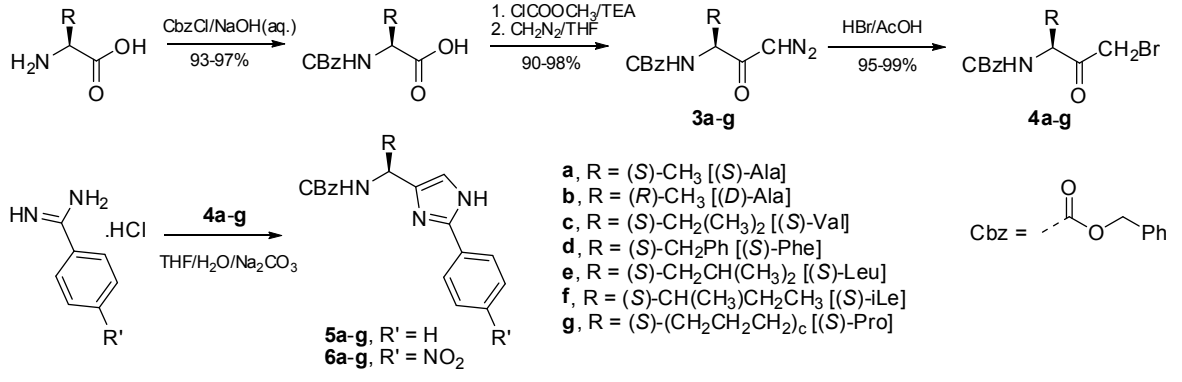
[a]*Isolated yields after 12/72 h.* [b]*Measured in $CHCl_3$ (c = 1).*

3.2. Antimycobacterial activities

The antimycobacterial activities of both series of compounds **5a-g** and **6a-g** were screened *in vitro* against *M. tuberculosis*, *M. avium*, and two strains of *M. kansasii* and their minimum inhibition concentrations (MIC) were compared with the MIC values of the conventional antimycobacterial agent - isoniazid (INH).

Table 2. The MIC values of target compounds **5** and **6**.

Comp.	Inhibiting activity (μmol L^{-1})			
	M. tuberculosis My 331/88 14/21 days	M. avium My 330/88 14/21 days	M. kansasii My 235/80 7/14/21 days	M. kansasii My 6509/96 7/14/21 days
5a	125/125	125/250	125/250/250	125/125/125
5b	125/125	250/250	125/250/250	62.5/125/125
5c	16/62.5	>250/>500	62.5/62.5/62.5	32/62.5/62.5
5d	500/>250	>250/>500	>250/>500/>500	>250/>250/>250
5e	125/>250	>250/>500	>250/>500/>500	>125/>125/>250
5f	32/125	>250/>500	32/62.5/62.5	32/32/32
5g	62.5/62.5	>250/>500	32/62.5/62.5	62.5/62.5/62.5
6a	>125/>125	>125/>125	>125/>125/>125	>125/>125/>125
6b	>125/>125	>125/>125	>125/>125/>125	>125/>125/>125
6c	16/16	>125/>125	32/62.5/62.5	16/32/32
6d	16/16	>62.5/>62.5	16/32/>62.5	8/16/16
6f	16/32	>125/>125	32/62.5/62.5	16/16/32
6g	32/32	>250/>250	62.5/125/250	32/62.5/62.5
INH	0.5/1	>250/>250	>250/>250/>250	2/4/8



Scheme 1. Synthetic approach to α-amino acid-derived *N*-Cbz protected 2-phenylimidazoles **5** and **6**.

The results are summarized in Table 2. The MIC values of compounds **5** and **6** against *M. tuberculosis* My 331/88 and *M. kansasii* 6 509/96 (clinical isolate) are generally higher than those observed for INH. However, the antimycobacterial activities against *M. avium* My 330/88 and *M. kansasii* My 235/80 are comparable or, in some cases, slightly higher. In contrast to INH, the compounds were comparably active against both *M. kansasii* strains. The structure-activity relationships indicate that the nitro substituted 2-phenylimidazoles **6a-g** possess higher antimycobacterial activities than unsubstituted analogs **5a-5g**. Whereas the absolute configuration of the α-amino acid side chain did not play any role on the antimycobacterial activity (compare **5a**/**5b** or **6a**/**6b**), the activity is mostly affected by the increasing bulkiness of the substituent R (**6c**-**f**). Thus, compound **6d** (R = benzyl, R´ = NO_2) is the most active compound from the evaluated series of agents.

4. Conclusions

We have designed, synthesized, and further tested *N-Cbz* protected α-amino acid-derived 2-phenylimidazoles as potential antimycobacterial agents. Overall thirteen compounds with different α-amino acid side chains (**a-g)** and 4-nitro substitution at the appended phenyl ring were investigated. These compounds were accessible from the commercially available *N-Cbz* protected α-amino acids in a three step synthesis involving formation of α-diazoketones, α-bromoketones, and their subsequent condensation with amidines. The yield of the final condensation step was strongly affected by the electron behavior of the benzamidine pendant. However, target compounds **5a-g** and **6a-g** can be obtained in a multigram scale in this way.

The anitmycobacterial activities of compounds **5** and **6** were tested *in vitro* against four strains. Whereas the activities against *M. tuberculosis* were lower than those of commercial isoniazid, the activities against *M. avium* and *M. kansasii* CNTCT exceeded the activity of INH. The main structural features affecting the antimycobacterial activities of tested compounds **5** and **6** are i) the presence of nitro group and ii) the bulkiness of the α-amino acid residue. 2-Phenylimidazole derivatives bearing nitro group and larger α-amino acid residue such as *iPr, Bn or iBu* (**6c-f)** showed to be the most effective compounds from the studied series.

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References

[1] S. T. Cole, G. Riccardi, Curr. Opin. Microbiol. 14, 570 (2011)
[2] D.E. Griffith, T. Aksamit, B.A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, S.M. Holland, R. Horsburgh, G. Huitt, M.F. Iademarco, M. Iseman, K. Olivier, S. Ruoss, C.F. von Reyn, R.J. Wallace Jr., K. Winthrop, Am. J. Resp. Crit. Care, 175, 367 (2007)
[3] (a) L. Ballel, R.A. Field, K. Duncan, R.J. Young, Antimicrob. Agents Chemother. 49, 2153 (2005); (b) R.J. O'Brien, M. Spigelman, Clin. Chest. Med. 26, 327 (2005); (c) M. Krátký, J. Vinšová, Chem. Listy 104, 998 (2010)
[4] (a) M. Biava, G.C. Porretta, G. Poce, A. De Logu, R. Meleddu, E. De Rossi, F. Manetti, M. Botta, Eur. J. Med. Chem. 44, 4734 (2009); (b) D. Deidda, G. Lampis, R. Fioravanti, M. Biava, G.C. Porretta, S. Zanetti, R. Pompei, Antimicrob. Agents Chemother. 42, 3035 (1998)
[5] A. Lilienkampf, M. Pieroni, B. Wan, Y. Wang, S.G. Franzblau, A.P. Kozikowski, J. Med. Chem. 53, 678 (2010)
[6] G. Navarrete-Vázquez, G.M. Molina-Salinas, Z.V. Duarte-Fajardo, J. Vargas-Villarreal, S. Estrada-Soto, F. Gonzáles-Salazar, E. Hernández-Núñez, S. Said-Fernández, Bioorg. Med. Chem. 15, 5502 (2007)
[7] (a) M. Boiani, M. González, Mini-Rev. Med. Chem. 5, 409 (2005); (b) L. De Luca, Curr. Med. Chem. 13, 1 (2006); (c) F. Bellina, S. Cauteruccio, R. Rossi, Tetrahedron 63, 4571 (2007); (d) B. Dolensky, G. Nam, W.-P. Deng, J. Narayanan, J. Fan, K. L. Kirk, J. Fluorine Chem. 125, 501 (2004)
[8] (a) C.K. Stover, P. Warrener, D.R. VanDevanter, D.R. Sherman, T.R. Arain, M.H. Langhorne, S.W. Anderson, J.A. Towell, Y. Yuan, D.N. McMurray, B.N. Kreiswirth, C.E. Barry, W.R. Baker, Nature 405, 962 (2000); (b) M. Matsumoto, H. Hashizume, T. Tomishige, M. Kawasaki, H. Tsubouchi, H. Sasaki, Y. Shimokawa, M. Komatsu, PLoS Med. 3, 2131 (2006)
[9] P. Pařík, J. Jansa, S. Holešová, A. Marek,

V. Klimešová, J. Heterocyclic Chem., accepted (2012)
[10] O. Pytela, V. Klimešová, Chem. Pharm. Bull. 59, 179 (2011)
[11] W.O. Foye, T.L. Lemke, D.A. Williams, Foye's principles of medicinal chemistry, 6th edition (Lippincott Williams&Wilkins, a Wolters Kluwer, Baltimore, 2008)
[12] (a) F. Bureš, J. Kulhánek, Tetrahedron:Asymmetry 16, 1347 (2005); (b) F. Bureš, T. Szotkowski, J. Kulhánek, O. Pytela, M. Ludwig, M. Holčapek, Tetrahedron: Asymmetry 17, 900 (2006); (c) A. Marek, J. Kulhánek, M. Ludwig, F. Bureš, Molecules 12, 1183 (2007); (d) A. Marek, J. Kulhánek, F. Bureš, Synthesis 325 (2009); (e) A. Marek, J. Kulhánek, W. B. Schweizer, F. Bureš, Synthesis 3188 (2010).
[13] F. Bureš, PhD thesis (University of Pardubice, Pardubice, Czech Republic, 2005)
[14] F.C. Schaefer, G.A. Peters, J. Org. Chem. 26, 412 (1961)
[15] A.R. Katritzky, A.F. Pozharskii, Handbook of heterocyclic chemistry, 2nd edition (Elsevier Science Ltd, Oxford, 2000)