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Short communication

# Synthesis and antimycobacterial activity of 2-substituted halogenobenzimidazoles

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**Abstract**

A series of substituted 2-polyfluoroalkyl and 2-nitrobenzylsulphanyl benzimidazoles was synthesized. The compounds were evaluated for their activity against four *Mycobacterium* strains; the activities were expressed as the minimum inhibitory concentration (MIC). The substances tested showed appreciable antimycobacterial activity, particularly 5,6-dichloro-2-nonafluorobutylbenzimidazole (**2h**), and 5-halogeno- (**5a–c**) and 4,6-dihalogeno- (**5d** and **5g**) 2-(3,5-dinitrobenzylsulphanyl)benzimidazoles, whose MIC values for *Mycobacterium kansasii* and *Mycobacterium avium* exceeded that of isoniazide that was used as a reference compound. Relationships between structure and biological activity of the tested benzimidazole derivatives are discussed.


*Keywords:* Antimycobacterial activity; 2-Nitrobenzylsulphanylbenzimidazoles; 2-Polyfluoroalkylbenzimidazoles, *Mycobacterium tuberculosis*

## 1. Introduction

Tuberculosis, which is caused by single infectious agent *Mycobacterium tuberculosis*, is one of the most important killing infectious diseases. WHO estimated that there were 8.8 million new cases of tuberculosis in 2002, of which 3.9 million were smear-positive. The global incidence rate of tuberculosis is growing at approximately 1.1% per year, and the number of new cases—at 2.4% per year. Currently almost one third of the world's population is infected by the bacterium [1]. The problem is being aggravated by increasing resistance against the frontline drugs and synergy of this disease with HIV and mycotic infections in immunocompromised patients [2]. No new drug against tuberculosis has been developed in the last 30 years. Hence there is an urgent need for new antituberculous agents, preferably having a different mode of action than these presently in use, therefore, the search for new antimycobacterials is the subject of numerous recent studies (e.g. Refs. [3–7]).

Below we report the synthesis and antimycobacterial activity of two groups of halogenobenzimidazole derivatives: one group carrying a polyfluoroalkyl substituent at position 2, and the second one—a nitrobenzylsulphanyl moiety at the same position. Our previous studies on a variety of substituted halogenobenzimidazole have shown that 2-substitution combined with halogenation of the benzene ring of the benzimidazole core endows the resulting derivatives with considerable potential for inhibiting growth of diverse microbial and protozoal species [8–11]. We have also investigated the anticancer activity in series of 2-trifluoromethyl- and 2-pentafluoroethyl-modified benzimidazoles; of these, 5,6-dichloro-2-pentafluoroethyl-1*H*-benzimidazole showed marked activity against human breast cancer and prostate cancer cell lines [12]. Recently, we have also found that polyhalogenated benzimidazoles are potent inhibitors of casein kinase CK2, probably the most pleiotropic protein kinase with more than 300 known protein substrates found in numerous eukaryotic organisms [13,14].

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Our previous studies indicated that various nitrobenzylsulphanyl substituents in position 2 markedly enhanced antimycobacterial activity of benzene ring-unsubstituted benzimidazoles [15]; notably, this effect was even more prominent in the 5-methylbenzimidazole series studied [16]. We have also found in an earlier study that 4,6-dichloro-2-(*p*-nitrobenzylsulphanyl)benzimidazole and 4,6-dibromo-2-(*p*-nitrobenzylsulphanyl)benzimidazole were highly effective against Gram-positive bacteria [17]. Having these facts in mind we have undertaken the synthesis of a variety of halogenated benzimidazole derivatives carrying a mononitro- or dinitrobenzylsulphanyl substituent at position 2. The major objective of the present study was the search for novel benzimidazole compounds that would show a promise to become useful antimycobacterial agents.

## 2. Chemistry

Two series of halogenated benzimidazole derivatives that included novel compounds, namely 2-polyfluoroalkyl- (series **2**) and 2-mono- and dinitrobenzylsulphanyl derivatives (series **5**), and a number of previously described tetrabromobenzimidazoles (series **3**) were synthesized as presented in Fig. 1. As starting materials for the synthesis of 2-polyfluoroalkylated benzimidazoles, the appropriate halogeno-*o*-phenylenediamines (**1**) were used. The condensation conditions employed to synthesize these compounds were similar to those originally described by Phillips [18]. However, the reactions with polyfluoroalkyl carboxylic acids needed prolonged reflux times. Moreover, due to the formation of byproducts, chromatographic purification was usually necessary. Except for the previously described derivatives [8,13,17,19], the structures of the newly obtained compounds were confirmed by physicochemical methods and elemental analysis.

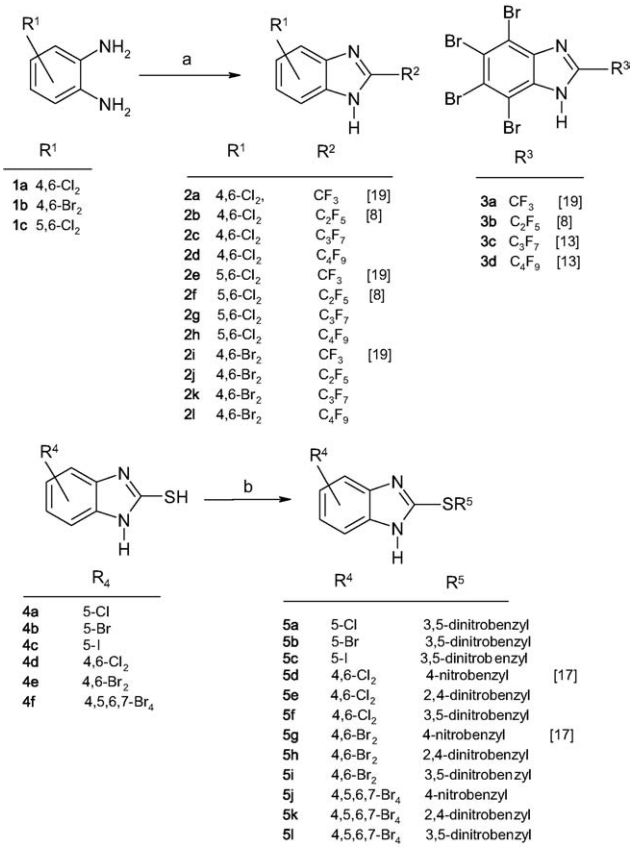


Fig. 1. Synthetic procedures for compounds **2a–l** and **5a–l**: reagents and conditions: (a) $C_nF_{2n+2}COOH$, 3 M HCl, reflux; (b) RCl, $K_2CO_3$, $EtOH/H_2O$.

The other group of halogenobenzimidazoles synthesized and studied was a series of 2-*S*-nitrobenzylated compounds. Using the ethanol/water solution and $K_2CO_3$ as a base, the appropriate benzimidazole-2-thiol were subjected to reaction with 4-nitro-, 2,4-dinitro-, or 3,5-dinitro benzylchloride to give the desired nitrobenzylsulphanyl compounds. The product compounds were characterized by $^1H$ NMR, UV and mass spectra. It is worth mentioning that the singlet signal of the benzylic $CH_2$ group at 4.79–4.95 ppm, which was characteristic of all series **5** benzimidazoles studied, was shifted downfield for 3,5-dinitrobenzylated compounds as compared to that for 2,4-dinitrobenzylated compounds, except in 4,5,6,7-tetrabromobenzimidazoles. Moreover, due to the '*ortho*' effect, the mass spectra of the investigated 2,4-dinitrocompounds showed lower intensities in the molecular peak region then the respective 3,5-dinitroisomers. Mass spectroscopy was particularly useful for identification and characterization of the investigated compounds because of the specific isotopic composition of the halogens.

## 3. Biology

In vitro antimycobacterial activity of the compounds was evaluated against *M. tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *M. kansasii* 6509/96, and *Mycobacterium avium* CNCTC My 330/88. All these strains were obtained from the Czech National Collection of Type Cultures (CNCTC), except *M. kansasii* 6509/96, which was a clinical isolate.

## 4. Results and discussion

The results of the antimycobacterial activity screening of the tested compounds are summarized in Table 2. As a reference compound, INH was used. The minimum inhibitory concentration (MIC) for **5j** and **5l** against *M. avium* could not be determined due to limited solubility of the compound in the test medium. The compounds studied were active against all mycobacterial strains tested; in most cases, MIC ranged between 2 and 125 μmol $l^{-1}$. Almost all tested compounds were more active against *M. kansasii 235/80* and *M. avium* than INH.

The first group of tested compounds consisted of 2-polyfluoroalkylated benzimidazoles that were additionally sub-

Table 1
Yields, m.p., $^1$H NMR, UV spectroscopic data and mass spectra of the new compounds prepared

| Compound | Yield (%) | M.p. (°C) | $^1$H NMR $\delta$ (ppm) | UV | MS |
|---|---|---|---|---|---|
| **2c** $C_{10}H_3N_2Cl_2F_7$ (355.05) | 33 | 185–187 | 7.63 (1H, d, $J$ = 2.3 Hz) 7.77 (1H, s), 14.7 (1H, bs) | $H_2O$/MeOH (1:1): 220 (30,400), 263 (9500), 283 (10,500), 292 (10,100); 0.1 N NaOH/MeOH (1:1): 225 (27,000), 290 (10,900) | 357 (10), 355 (60), 354 (11), 353 (93), 334 (11), 320 (28), 235 (100) |
| **2d** $C_{11}H_3N_2Cl_2F_9$ (405.06) | 29 | 177–179 | 7.62 (1H, d, $J$ = 2.3 Hz), 7.77 (1H, s), 14.7 (1H, bs) | $H_2O$/MeOH (1:1): 220 (25,400), 265 (8000), 283 (8800), 292 (8400); 0.1 N NaOH/MeOH (1:1): 225 (23,000), 290 (9100) | 407 (10), 405 (49), 404 (11), 404 (75), 384 (14), 369 (30), 235 (100) |
| **2g** $C_{10}H_3N_2Cl_2F_7$ (355.05) | 37 | 178–179 | 8.07 (2H, s), 14.4 (1H, bs) | $H_2O$/MeOH (1:1): 260 (6100), 291 (7800), 300 (6800); 0.1 N NaOH/MeOH (1:1): 221 (27,900), 294 (9700), 302 (8900) | 355 (60), 354 (11), 353 (93), 334 (12), 319 (28), 235 (100) |
| **2h** $C_{11}H_3N_2Cl_2F_9$ (405.06) | 31 | 181–182 | 8.08 (2H, s), 14.4 (1H, bs) | $H_2O$/MeOH (1:1): 260 (5900), 291 (7500), 300 (6000); 0.1 N NaOH/MeOH (1:1): 294 (6700), 303 (7900) | 405 (49), 404 (110, 403 (75), 384 (14), 369 (30), 235 (100) |
| **2j** $C_9H_3N_2Br_2F_5$ (393.94) | 29 | 199–201 | 7.82 (1H, d, $J$ = 2.4 Hz), 7.92 (1H, s),14.6 (1H, bs) | $H_2O$/MeOH (1:1): 268 (7200), 284 (8100), 292 (7800); 0.1 N NaOH/MeOH (1:1): 223 (25,200), 290 (8600) | 396 (49), 395 (11), 394 (100), 393 (6), 392 (51), 327 (17), 325 (44), 323 (23) |
| **2k** $C_{10}H_3N_2Br_2F_7$ (443.94) | 25 | 179–180 | 7.83 (1H, d, $J$ = 2.4 Hz) 7.92 (1H, s), 14.6 (1H, bs) | $H_2O$/MeOH (1:1): 268 (7500), 284 (9100), 293 (8600); 0.1 N NaOH/MeOH (1:1): 224 (26,600), 291 (8900) | 446 (48), 445 (12), 444 (100), 443 (7), 442 (50), 327 (29), 325 (62), 323 (30) |
| **2l** $C_{11}H_3N_2Br_2F_9$ (493.95) | 21 | 181–183 | 7.82 (1H, d, $J$ = 2.4 Hz) 7.92 (1H, s), 14.6 (1H, bs) | $H_2O$/MeOH (1:1): 220 (30,400), 269 (7800), 283 (10,500), 292 (10,100); 0.1 N NaOH/MeOH (1:1): 225 (27,000), 290 (10,900) | 496 (49), 495 (14), 494 (100), 493 (70, 492 (500, 475 (110, 327 (39), 325 (81), 323 (42) |
| **5a** $C_{14}H_9N_4ClO_4S$ (364.77) | 55 | 162–164 | 4.79 (2H, s), 7.16 (1H, dd, $J_1$ = 2.1 Hz, $J_2$ = 8.4 Hz) 7.45–7.60 (2H, m), 8.68 (1H, t, $J$ = 2.2 Hz) 8.83 (2H, 2d, $J$ = 2.2 Hz), 12.8 (1H, bs) | $H_2O$/MeOH (1:1): 230 (27,700), 245 (22,400), 298 (15,000); 0.1 N NaOH/MeOH (1:1): 231 (32,500), 300 (12,900) | 366 (38), 365 (18) 364 (100), 349 (15), 348 (8), 347 (38) |
| **5b** $C_{14}H_9N_4BrO_4S$ (409.21) | 57 | 167–169 | 4.79 (2H, s), 7.22 (1H, dd, $J_1$ = 1.8 Hz, $J_2$ = 8.6 Hz), 7.50–7.70 (2H, m), 8.68 (1H, t, $J$ = 2.2 Hz), 8.90 (2H, d, $J$ = 2.2 Hz) 12.8 (1H, bs) | $H_2O$/MeOH (1:1): 233 (28,100), 239 (24,600), 298 (15,500); 0.1 N NaOH/MeOH (1:1): 232 (32,400), 300 (14,100) | 412 (7), 411 (18), 410 (100), 409 (19), 408 (98), 393 (23) |
| **5c** $C_{14}H_9N_4IO_4S$ (466.22) | 62 | 191–193 | 4.79 (2H, s), 7.28 (1H, dd, $J_1$ = 1.7 Hz, $J_2$ = 8.8 Hz) 7.65–7.85 (2H, m), 8.65 (1H, t, $J$ = 2.3 Hz) 8.90 (2H, 2d, $J$ = 2.2 Hz) 12.8 (1H, bs) | $H_2O$/MeOH (1:1): 230 (39,200), 301 (15,500); 0.1 N NaOH/MeOH (1:1): 233 (37,100), 303 (13,000) | 468 (7), 467 (17), 466 (100), 439 (19), 409 (11) |
| **5e** $C_{14}H_8N_4Cl_2O_4S$ (399.22) | 44 | 151–153 | 4.95 (2H, s), 7.32 (1H, d, $J$ = 1.4 Hz), 7.47 (1H, d, $J$ = 1.0 Hz), 8.12 (1H, d, $J$ = 8.5 Hz), 8.44 (1H, dd, $J_1$ = 2.5 Hz, $J_2$ = 8.4 Hz), 8.73 (1H, d, $J$ = 2.5 Hz), 13.1 (1H, bs) | $H_2O$/MeOH (1:1): 226 (35,100), 291 (14,500), 300 (14,400); 0.1 N NaOH/MeOH (1:1): 229 (29,400), 307 (14,100) | 400 (13), 398 (19), 353 (8), 322 (13), 220 (67), 219 (24), 218 (100), 217 (22) |
| **5f** $C_{14}H_8N_4Cl_2O_4S$ (399.22) | 56 | 184–186 | 4.80 (2H, s), 7.32 (1H, d, $J$ = 1.9 Hz), 7.48 (1H, s), 8.66 (1H, t, $J$ = 2.2 Hz), 8.91 (2H, d, $J$ = 2.2 Hz), 13.1 (1H, bs) | $H_2O$/MeOH (1:1): 223 (33,600), 290 (12,800), 299 (12,700); 0.1 N NaOH/MeOH (1:1): 233 (32,000), 307 (12,800) | 402 (15), 401 (14), 400 (71), 399 (20), 398 (100), 385 (12), 383 (36), 381 (51) |
| **5h** $C_{14}H_8N_4Br_2O_4S$ (488.11) | 26 | 180–182 | 4.94 (2H, s), 7.52 (1H, d, $J$ = 1.6 Hz), 7.62 (1H, d, $J$ = 1.0 Hz.), 8.12 (1H, d, $J$ = 8.4 Hz), 8.40 (1H, dd, $J_1$ = 2.5 Hz, $J_2$ = 8.4 Hz), 8.73 (1H, d, $J$ = 2.5 Hz), 13.1 (1H, bs) | $H_2O$/MeOH (1:1): 223 (39,000), 294 (14,500), 301 (14,400); 0.1 N NaOH/MeOH (1:1): 229 (31,700), 308 (14,300) | 491 (3), 490 (10), 488 (19), 487 (4), 486 (10), 442 (9), 310 (52), 309 (19), 308 (100), 307 (22), 306 (51) |

*(continued on next page)*

Table 1
(continued)

| Compound | Yield (%) | M.p. (°C) | $^1$H NMR $\delta$ (ppm) | UV | MS |
|---|---|---|---|---|---|
| **5i** $C_{14}H_8N_4Br_2O_4S$ (488.11) | 42 | 194–197 | 4.80 (2H, s), 7.52 (1H, d, $J$ = 1.7 Hz), 7.62 (1H, s), 8.65 (1H, t, $J$ = 2.3 Hz), 8.93 (2H, d, $J$ = 2.2 Hz), 13.1 (1H, bs) | $H_2O$/MeOH (1:1): 225 (40,200), 301 (14,500); 0.1 N NaOH/MeOH (1:1): 229 (38,800), 307 (12,600) | 491 (10), 490 (55), 489 (19), 488 (100), 487 (11), 486 (51), 473 (15), 471 (25), 469 (12) |
| **5j** $C_{14}H_7N_3Br_4O_2S$ (600.91) | 56 | 254–255 | 4.71 (2H, s), 7.80–7.90 (2H, m, AA'BB'), 8.10–8.20 (2H, m, AA'BB'), 13.5 (1H, bs) | $H_2O$/MeOH (1:1): 240 (36,800), 271 (21,400), 313 (16,300); 0.1 N NaOH/MeOH (1:1): 242 (33,900) | 606 (3), 605 (19), 604 (12), 603 (68), 602 (19), 601 (100), 600 (14), 599 (66) |
| **5k** $C_{14}H_6N_4Br_4O_4S$ (645.90) | 21 | 211–213 | 4.75 (2H, s), 8.23 (1H, d, $J$ = 8.3 Hz), 8.43 (1H, dd, $J_1$ = 2.4 Hz, $J_2$ = 8.4 Hz), 8.72 (1H, d, $J$ = 2.5 Hz), 13.5 (1H, bs) | $H_2O$/MeOH (1:1): 233 (42,800), 312 (15,000); 0.1 N NaOH/MeOH (1:1): 236 (41,300), 314 (10,800) | 647 (12), 645 (17), 643 (11), 469 (19), 467 (69), 465 (100), 463 (68) |
| **5l** $C_{14}H_6N_4Br_4O_4S$ (645.90) | 34 | 282–284 | 4.70 (2H, s), 8.68 (1H, t, $J$ = 2.2 Hz), 8.92 (2H, d, $J$ = 2.2 Hz),13.4 (1H, bs) | $H_2O$/MeOH (1:1): 240 (52,300), 312 (12,700); 0.1 N NaOH/MeOH (1:1): 241 (46,800), 314 (10,800) | 649 (19), 648 (12), 647 (68), 646 (19), 645 (100), 644 (13), 643 (65), 641 (16), 598 (15) |

stituted in the benzene part of the heterocyclus (**2a–l** and **3a–d**). In most cases, the biological activity correlated positively with the length of the polyfluoroalkylated substituent in position 2 of the benzimidazole nucleus; this may be related to increased lipophylicity of the side chain. The data in Table 2 show that the dichlorinated compounds (**2a–h**) were slightly more effective against mycobacteria than their dibrominated analogs (**2i–l**), except that **2a** = **2i**. This relationship was more significant for *M. tuberculosis* than for the *M. kansasii* strains tested, whereas there was no difference between the activities of the two analog series toward *M. avium*. Of all 2-polyfluoroalkylated benzimidazoles stud-

Table 2
In vitro antimycobacterial activity of compounds **2**, **3** and **5** expressed as MIC (μmol l$^{-1}$)

| Compound | *M. tuberculosis* My 331/88 | | *M. kansasii* My 235/80 | | | *M. kansasii* 6509/96 | | | *M. avium* My 330/88 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 14 d | 21 d | 7 d | 14 d | 21 d | 7 d | 14 d | 21 d | 14 d | 21 d |
| **2a** | 32 | 32 | 32 | 62.5 | 62.5 | 32 | 62.5 | 62.5 | 62.5 | 62.5 |
| **2b** | 16 | 32 | 32 | 62.5 | 62.5 | 32 | 62.5 | 62.5 | 62.5 | 62.5 |
| **2c** | 8 | 8 | 8 | 8 | 16 | 16 | 32 | 32 | 32 | 32 |
| **2d** | 8 | 8 | 16 | 16 | 16 | 8 | 16 | 16 | 16 | 16 |
| **2**[e] | 8 | 8 | 32 | 32 | 32 | 32 | 62.5 | 62.5 | 32 | 62.5 |
| **2f** | 4 | 8 | 8 | 16 | 16 | 16 | 32 | 32 | 16 | 32 |
| **2g** | 8 | 8 | 8 | 16 | 16 | 16 | 16 | 32 | 32 | 32 |
| **2h** | 4 | 4 | 4 | 4 | 4 | 4 | 8 | 8 | 4 | 8 |
| **2i** | 32 | 32 | 32 | 62.5 | 62.5 | 32 | 62.5 | 62.5 | 62.5 | 62.5 |
| **2j** | 16 | 16 | 16 | 32 | 32 | 16 | 32 | 32 | 32 | 32 |
| **2k** | 16 | 16 | 16 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| **2l** | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| **3a** | 32 | 32 | 62.5 | 62.5 | 62.5 | 32 | 62.5 | 62.5 | 125 | 125 |
| **3b** | 8 | 16 | 32 | 32 | 32 | 32 | 32 | 62.5 | 125 | 125 |
| **3c** | 4 | 8 | 32 | 32 | 32 | 32 | 32 | 32 | 62.5 | 62.5 |
| **3d** | 16 | 16 | 16 | 16 | 16 | 16 | 32 | 32 | 32 | 32 |
| **5a** | 2 | 2 | 4 | 8 | 8 | 4 | 8 | 8 | 32 | 32 |
| **5b** | 4 | 4 | 4 | 8 | 8 | 4 | 8 | 8 | 32 | 62.5 |
| **5c** | 2 | 4 | 2 | 4 | 4 | 2 | 4 | 4 | 32 | 32 |
| **5d** | 2 | 4 | 4 | 4 | 8 | 4 | 4 | 4 | 4 | 4 |
| **5**[e] | 32 | 32 | 16 | 32 | 62.5 | 16 | 32 | 32 | 62.5 | 62.5 |
| **5f** | 4 | 4 | 8 | 16 | 16 | 8 | 16 | 16 | 62.5 | 125 |
| **5g** | 2 | 4 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 16 |
| **5h** | 16 | 32 | 32 | 62.5 | 62.5 | 32 | 62.5 | 62.5 | 500 | 1000 |
| **5i** | 4 | 4 | 4 | 4 | 4 | 8 | 16 | 16 | 62.5 | 62.5 |
| **5j** | 16 | 16 | 16 | 16 | 32 | 16 | 32 | 32 | >250 | >250 |
| **5k** | 16 | 16 | 8 | 16 | 32 | 8 | 16 | 32 | 62.5 | 125 |
| **5l** | 8 | 16 | >250 | >250 | >250 | 4 | 4 | 4 | >250 | >250 |
| INH | 0.5 | 1 | >250 | >250 | >250 | 2 | 4 | 4 | >250 | >250 |

INH, isoniazide used as a reference compound.

ied, the regioisomeric 5,6-dichlorobenzimidazoles (**2e–h**), and especially the 2-nonafluorobutyl compound **2h**, showed the highest activity against all *Mycobacterium* strains tested.

Introduction of four bromine atoms into the 2-substituted benzimidazoles did not enhance the activity of the daughter compounds against *M. kansassii* and *M. avium* (see the data for compounds **3a–d** in Table 2) compared to that of the respective 4,6-dibromo analogs (**2i–l**); however, *M. tuberculosis* was more susceptible to the tetrabromo- than dibromo-derivatives.

Another group of novel benzimidazole derivatives studied consisted of 2-nitrobenzylsulfanylbenzimidazoles carrying halogens in the benzene part of the molecule. Our previous study on substituted benzylsulfanylbenzimidazoles indicated that of various modified benzyl substituents tested, the respective nitro compounds were the most powerful antimycobacterials. It is worth to note that 4,6-dichloro- and 4,6-dibromo-2-(4-nitrobenzylsulfanyl)benzimidazoles (**5d** and **5g**), which were very active against *M. avium*, were previously shown to be highly active against Gram-positive bacteria [17]. In the present study, 3,5-dinitrobenzylsulfanylbenzimidazoles halogenated at position 5 with either chlorine, bromine, or iodine (**5a–c**) showed comparable, high activity against *M. tuberculosis* and the two *M. kansasii* strains used, but only a moderate activity against *M. avium*. Introducing an additional chlorine or bromine atom onto benzene part of the benzimidazole core (see compounds **5d–i**) did not improve the antimycobacterial activity. The tetrabrominated compounds (**5j–l**) were even less active then their dibrominated analogs.

Another structure–activity relationship evidenced by the present as well as few related studies [15,16] is the difference between the activities of 2,4-dinitro (**5e** and **5h**) and 3,5-dinitro (**5f** and **5i**) isomers. The 3,5-dinitro compounds were several times more effective against *M. tuberculosis* and *M. kansasii* than the respective isomeric 2,4-dinitro derivatives.

## 5. Experimental

### 5.1. Chemistry

All chemicals and solvents were purchased from Sigma–Aldrich. Melting points (m.p. uncorrected) were measured in open capillary tubes on a Gallenkamp-5 m.p. apparatus. Ultraviolet absorption spectra were recorded in a Kontron Uvikon 940 spectrophotometer. $^1$H NMR spectra (in ppm) were measured with a Varian Gemini 200 MHz (or a Varian UNITY plus 500 MHz) spectrometer at 298 K in $D_6$(DMSO) using tetramethylsilane as internal standard. Flash chromatography was performed with Merck silica gel 60 (200–400 mesh). Analytical TLC was carried out on precoated silica gel $F_{254}$ (Merck) plates (0.25 mm thickness). Analyses of the new compounds, indicated by the symbols of the elements, were within ±0.4% of the respective theoretical values.

The following compounds as shown in Fig. 1 had been described in the literature: **2a**, **2b**, **2e**, **2f**, **2i**, **3a–d**, **5d**, **5g**.

#### 5.1.1. General procedure for the preparation of 2-polyfluoroalkyl halogenobenzimidazoles (*2*)

The mixture containing halogenated *o*-phenylenediamine (**1**) (5 mmol), 3 M HCl (10 ml) and corresponding polyfluoroalkylcarboxylic acid (40 mmol) was heated under reflux for 72 h. The resulting deep dark solution was evaporated to dryness, next twice evaporated with ethanol and toluene. The residue was adsorbed on silica gel and placed on the top of silica gel column (3 × 16 cm). The chromatography was performed with $CHCl_3$ (200 ml) then with $CHCl_3$/MeOH (95:5, v/v). The product-containing fractions were evaporated to dryness, and the residue was crystallized from EtOH/water to give the corresponding product. The yields, m.p. and spectral data of new compounds are listed in Table 1.

#### 5.1.2. General procedure for the preparation of 2-(4-nitro-, 2,4-dinitro- and 3,5-dinitrobenzyl)sulfanyl halogenobenzimidazoles (*5*)

To the stirred solution of halogenated benzimidazole-2-thiol (**4**) (2 mmol) in the mixture of water (20 ml) and EtOH (15 ml) containing $K_2CO_3$ (0.69 g, 5 mmol) the corresponding nitrobenzylated chloride (2.5 mmol) was added portionwise within 4 h at room temperature. The stirring was continued overnight. Then the mixture was brought to pH 6 with acetic acid, the precipitate formed was filtered off, adsorbed on silica gel and deposited on the top of silica gel column (3 × 12 cm). The chromatography was performed with $CH_2Cl_2$. The product-containing fractions were evaporated to dryness, and the residue was crystallized from EtOH/water to give the product. The yields, m.p. and spectral data of the new compounds are listed in Table 1.

### 5.2. Antimycobacterial activity

The antimycobacterial activities were determined in Šula semisynthetic medium (SEVAC, Prague, Czech Republic). The compounds tested were added to the medium as dimethylsulfoxide solutions. The concentrations used were as follows: 500, 250, 125, 62, 32, 16, 8, 4, and 2 μmol $l^{-1}$. MIC values were determined after incubation at 37 °C for 7, 14, and 21 d. MIC was the lowest substance concentration showing an inhibitory effect on the *Mycobacterium* growth. The present results were obtained from three independent measurements.

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