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Synthesis and biological evolution of hydrazones derived from 4-(trifluoromethyl)benzohydrazide

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ABSTRACT

Reflecting the known biological activity of isoniazid-based hydrazones, seventeen hydrazones of 4-(trifluoromethyl)benzohydrazide as their bioisosters were synthesized from various benzaldehydes and aliphatic ketones. The compounds were screened for their *in vitro* activity against *Mycobacterium tuberculosis*, nontuberculous mycobacteria (*M. avium*, *M. kansasii*), bacterial and fungal strains. The most antimicrobial potent derivatives were also investigated for their cytostatic and cytotoxic properties against three cell lines. Camphor-based molecule, 4-(trifluoromethyl)-*N*′-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene) benzohydrazide, exhibited the highest and selective inhibition of *M. tuberculosis* with the minimum inhibitory concentration (MIC) of 4 μM, while *N*′-(4-chlorobenzylidene)-4-(trifluoromethyl)benzohydrazide was found to be superior against *M. kansasii* (MIC = 16 μM). *N*′-(5-Chloro-2-hydroxybenzylidene)-4-(trifluoromethyl)benzohydrazide showed the lowest MIC values for gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* as well as against two fungal strains of *Candida glabrata* and *Trichophyton mentagrophytes* within the range of ≤0.49–3.9 μM. The convenient substitution of benzylidene moiety at the position 4 or the presence of 5-chloro-2-hydroxybenzylidene scaffold concomitantly with a sufficient lipophilicity are essential for the noticeable antimicrobial activity. This 5-chlorosalicylidene derivative avoided any cytotoxicity on two mammalian cell cultures (HepG2, BMMΦ) up to the concentration of 100 μM, but it affected the growth of MonoMac6 cells.

Development of novel antimicrobial agents represents an up-to-date research topic to achieve a global control of drug-resistant microbial strains. Modification of compounds with a known activity to improve their properties belongs to the powerful trends in medicinal chemistry.

The concept of (bio)isosters could be considered as one of the successful approaches for the rational drug design. It is based on the assumption that single atoms, groups or molecules that exhibit similar volume, shape, and/or physicochemical properties can produce broadly similar pharmacologic effects. However, the outcomes of isosteric replacement cannot be predicted absolutely, since conversion of the action was also observed occasionally. In the molecules of bioisosters, a different substituent or a group exchanges one moiety from the parent lead compound to impart similar biological effects. This change can modify both pharmacokinetics and pharmacodynamics to fine tune of them. Examples of broadly similar isosteric substitution comprise, e.g., replacement of hydrogen by fluorine, ester bond by amide, phenyl ring by thiophene, carboxylic acid by tetrazole etc., that are based on chemical and/or physical similarities.[1,2]

Hydrazones obtained from isoniazid (INH), a first-line antimycobacterial agent, have been reported as potent antimycobacterial,[3–8] antibacterial[5,7,9] and antifungal[5,7,9] agents. An analogue of INH where heterocyclic nitrogen is replaced by a carbon substituted with a strong electron-withdrawing group, 4-(trifluoromethyl)benzohydrazide **1** inhibits the growth of *Mycobacterium tuberculosis*[10] (*Mtb.*). Similarly, 4-(trifluoromethyl)benzohydrazide-derived INH isosters preserved antimycobacterial properties.[11,12] Illustratively, hydrazones of **1** with various aldehydes and ketones have exhibited antimycobacterial,[10,11] antibacterial,[13,14] antifungal,[14] antiparasitic[15] and cytotoxic[16,17] properties.

Abbreviations: BAC, bacitracin; BMMΦ, murine bone marrow culture-derived macrophages; FLU, fluconazole; INH, isoniazid; MRSA, methicillin-resistant *Staphylococcus aureus*; *Mtb.*, *Mycobacterium tuberculosis*; NTM, non-tuberculous (atypical) mycobacteria.

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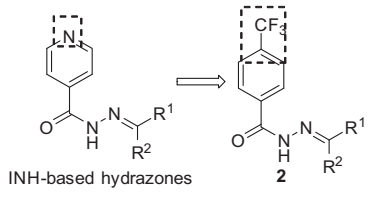


Scheme 1. Bioisosteric design of 4-(trifluoromethyl)benzohydrazide hydrazones **2**.

Based on here summarized facts, we designed and synthesized 4-(trifluoromethyl)benzohydrazide hydrazones **2**, bioisosters of INH hydrazones (Scheme 1), as potential antimicrobial agents.

The synthesis of *N′*-alkyl/cycloalkyl/benzylidene-4-(trifluoromethyl)benzohydrazides **2** is depicted in Scheme 2. It involves the reaction of the hydrazide **1** (1.0 equivalent) with commercially available aldehydes and ketones (1.1 of eq.). If any ketone (acetone, cyclopentanone, cyclohexanone, camphor) was one of the reactants, a catalytic amount of concentrated sulphuric acid was added into the reaction mixture. The refluxing in boiling methanol for 2 h provided targeted compounds **2** in 85–99% yields (aldehydes) and 68–87% for ketones (Scheme 2). The lowest yield was observed when camphor was used as a carbonyl compound.

We chose substituents with different electronic effects as a substitution pattern for benzaldehyde ring: no substituent (R = H), those with electron-donating properties – methyl (+I effect), hydroxy and methoxy groups (−I and +M effects), as well as strong electron-withdrawing groups of NO_2 (−I and −M effects) and CF_3 (−I effect). Halogens (Cl, Br) as weak deactivating substituents with −I and +M electronic effects were involved too. Based on the known antimicrobial activity of 5-chloro-2-hydroxybenzylidene derivatives,[18,19] 5-chlorosalicylaldehyde was used. We also investigated various positional isomers (*ortho*, *meta*, *para*; R = Cl, OH) and the activity of hydrazones obtained from four ketones. In spite of different electronic effects, these derivatives differ also in lipophilic behaviour and steric parameters.

All of the compounds **2** were characterized by spectroscopic data involving ^{1}H, ^{13}C and IR spectra (see Supplementary information), and the purity was checked additionally by TLC and elemental analysis. In the ^{1}H NMR spectra, hydrazone (CONHN=) protons appeared as singlets at 12.31–11.83 ppm for benzylidene scaffold-based compounds **2a**–**m** concomitantly with azomethine singlets (N=CH) observed at 8.88–8.36 ppm. Ketone-derived hydrazones **2n**–**q** share CONH proton signals shifted to upfield within the range of 10.84–10.41 ppm. The ^{13}C NMR spectra contain C=O and C=N peaks at 162.43–161.83 ppm and 149.14–144.65 ppm, respectively. In the spectra of ketone-based hydrazones **2n**–**q**, the C=N singlet is shifted to 174.92–161.53 ppm.

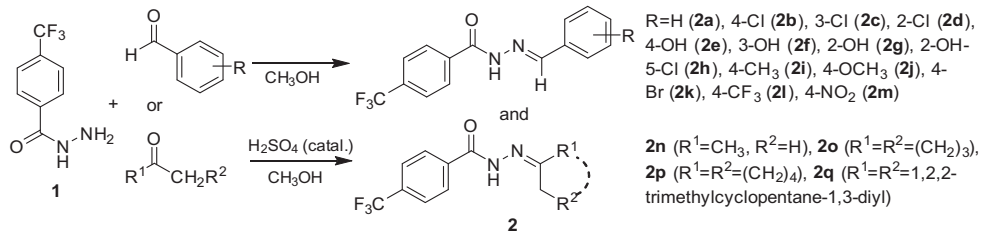
The hydrazide **1** and all of the hydrazones **2a**–**q** were screened *in vitro* for their antimicrobial properties (see Supplementary information). The panel of pathogens involved *Mycobacterium tuberculosis* 331/88 (*i.e.*, H_{37}Rv), *Mycobacterium avium* 330/88 (resistant to INH, rifamycines, ethambutol and ofloxacin), *Mycobacterium kansasii* 235/80 and 6509/96 (a clinical isolate; Table 1); gram-positive bacteria: *Staphylococcus aureus* CCM 4516/08, methicillin-resistant *Staphylococcus aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08, *Enterococcus faecalis* J 14365/08; *Escherichia coli* CCM 4517, *Klebsiella pneumoniae* D 11750/08, extended spectrum beta-lactamase (ESBL)-positive *Klebsiella pneumoniae* J 14368/08, and *Pseudomonas aeruginosa* CCM 1961 (gram-negative strains),[20] and fungal species of *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, *Candida glabrata* 20/I, *Trichosporon asahii* 1188, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272, and *Trichophyton mentagrophytes* 445 (Table 2).[21] This panel of twenty microbial species covers a wide range of important human pathogens including those with an acquired resistance. It is a useful tool for an initial identification of potential antimicrobial activity of novel compounds. Bacitracin (BAC), fluconazole (FLU) and isoniazid were employed as the comparative drugs for antibacterial, antifungal and antimycobacterial activity, respectively.

The results from antimycobacterial evaluation expressed as minimum inhibitory concentrations (MICs) are overviewed in Table 1; only active molecules are involved.

Eleven hydrazones (**2b**–**c**, **2e**–**h**, **2m**–**q**) exhibited an antimycobacterial activity, remaining derivatives (**2a**, **2d**, **2i**–**l**) were inactive. Hydrazide **1** itself showed only a mild activity against *Mtb.* (250/500 μM). *M. avium* inhibited only by phenolic derivatives was the most resistant strain (MICs ≥ 62.5 μM). The lowest MIC values for *Mtb.* were produced by terpenic hydrazone obtained from camphor **2q** (4 μM concomitantly with an evident selectivity for this strain) followed by 4-chlorobenzylidene derivative **2a**. This molecule was also found to be the most active against both strains of *M. kansasii* (16 μM). *N′*-(4-Nitrobenzylidene)-4-(trifluoromethyl) benzohydrazide **2m** inhibited significantly only the growth of the collection strain of *M. kansasii* 235/80 with MIC values starting from 32 μM.

In sum, for the antimycobacterial activity it is essential the presence of one chlorine atom at the position of 4 (**2b**) since its structural isomers are virtually (**2c**) or totally inactive (**2d**). The phenolic group represents another favourable substituent preferably at the position 4 again (**2e**), but in this case isomeric hydroxybenzaldehydes provided derivatives (**2f**–**g**) with a similar biological response. The halogenation of salicylaldehyde improves antimycobacterial properties up to four times (**2g** vs. **2h**). Generally, an increased lipophilicity is translated into an enhanced antimycobacterial action (**2q**, **2b** vs. **2e** and **2m**, **2g** vs. **2h**). On the other hand, the most lipophilic benzaldehyde-based hydrazones (i.e., 4-Br **2k** and 4-CF_3 **2l** with C log*P* > 5) did not display any growth inhibition. Although essential, the lipophilicity is not the only factor determining this property and similarly, the occupation of the 4-position does not ensure bioactivity automatically.

The chemical modification of the parent hydrazide **1** by carbonyl compounds led mainly to more potent antimycobacterial agents. Four derivatives (**2e**–**h**) exceeded the *in vitro* activity of INH for *M. avium* and *M. kansasii* 235/80, additional four



Scheme 2. Synthesis of *N′*-substituted 4-(trifluoromethyl)benzohydrazides **2**.