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Antitubercular activity of quinolizidinyl/pyrrolizidinylalkyliminophenazines

Michele Tonelli [a,*], Federica Novelli [a], Bruno Tasso [a], Anna Sparatore [b], Vito Boido [a], Fabio Sparatore [a], Sara Cannas [c], Paola Molicotti [c], Stefania Zanetti [c,*], Silvia Parapini [d], Roberta Loddo [e]

[a] Dipartimento di Farmacia, Università di Genova, Viale Benedetto XV 3, 16132 Genova, Italy
[b] Dipartimento di Scienze Farmaceutiche, Università di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[c] Dipartimento di Scienze Biomediche, Università di Sassari, Viale San Pietro, 07100 Sassari, Italy
[d] Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Via C. Pascal 36, 20133 Milano, Italy
[e] Dipartimento di Scienze Biomediche, Università di Cagliari, Cittadella Universitaria, 09042 Monserrato (CA), Italy

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ABSTRACT

Novel riminophenazine derivatives, characterized by the presence of the basic and cumbersome quinolizidinylalkyl and pyrrolizidinylethyl moieties, have been synthesized and tested (Rema test) against *Mycobacterium tuberculosis* $H_{37}R_v$ and $H_{37}R_a$, and six clinical isolates of *Mycobacterium avium* and *Mycobacterium tuberculosis*. Most compounds exhibited potent activity against the tested strains, resulting more active than clofazimine, isoniazid and ethambutol.

The best compounds (**4**, **5**, **12** and **13**) exhibited a MIC in the range 0.82–0.86 μM against all strains of *Mycobacterium tuberculosis* and, with the exception of **4** a MIC around 3.3 μM versus *M. avium*. The corresponding values for clofazimine (CFM) were 1.06 and 4.23 μM, respectively. Cytotoxicity was evaluated against three cell lines and compound **4** displayed a selectivity index (SI) versus the human cell line MT-4 comparable with that of CFM (SI = 5.23 vs 6.4). Toxicity against mammalian Vero 76 cell line was quite lower with SI = 79.

1. Introduction

In the recent years the incidence of tuberculosis (TB) is strongly increased worldwide due to the diffusion of multi-drug resistant strains of *Mycobacterium tuberculosis*. In 2012, mostly in the underdeveloped countries, 8.6 million people developed tuberculosis and 1.3 died from the disease.[1] Therefore new effective and safe antitubercular drugs, acting through novel mechanisms, are urgently needed for a combination chemotherapy able to improve efficacy and delay onset of resistance.

Besides searching for new chemotypes, eventually active against new targets of mycobacterial cells, the synthesis of derivatives of proven but not fully exploited antimycobacterial agents, as clofazimine (CFM), is largely pursued.

Indeed, clofazimine is a potent antitubercular drug of the riminophenazine group, firstly described in 1956[2,3] and largely used since 1962 in the treatment of leprosy[4] and, more recently, of the *Mycobacterium avium complex* (MAC) infections in AIDS patients.[5a] The WHO guidelines included CFM within a group of 5 drugs for the treatment of Multidrug Resistant tuberculosis (MDR-TB), especially of cases that are extensively drug-resistant (XDR-TB).[5b] A widespread use of CFM in the treatment of tuberculosis has been hampered by its high lipophilicity, long half-life (70 days) and skin staining side effects. To address the issue, several analogs of CFM with reduced lipophilicity have been prepared by replacing one or both 4-chlorophenyl groups with salt forming pyridyl residues[6] or by replacing the 3-(isopropyl)imino group with several kinds of (alkylaminoalkyl)imino residue or more complex basic moieties as the (2,2,6,6-tetramethylpiperidin-4-yl)imino

Abbreviations: AIDS, acquired immune-deficiency syndrome; CC, column chromatography; $CDCl_3$, deutero chloroform; CFM, clofazimine; DMSO, dimethylsulfoxide; ENL, erythema nodosum leprosum; ESI, electro spray ionization; FC, flash chromatography; G+ bacteria, Gram positive bacteria; HRMS, high resolution mass spectroscopy; INH, isoniazid; MAC, *Mycobacterium avium complex*; *M. avium*, *Mycobacterium avium*; MIC, minimum inhibitory concentration; *M. t.*, *Mycobacterium tuberculosis*; NMR, nuclear magnetic resonance; OADC, oleic acid, albumin, dextrose and catalase; PANTA, polymyxin, amphotericin B, nalidixic acid, trimethoprim and azlocillin; SD, standard deviation; TB, tuberculosis; THF, tetrahydrofuran; TLC, thin layer chromatography.

* Corresponding authors. Tel.: +39 010 3538867; fax: +39 010 3538399 (M.T.); tel.: +39 079 228463; fax: +39 070212345 (S.Z.).

E-mail addresses: michele.tonelli@unige.it (M. Tonelli), zanettis@uniss.it (S. Zanetti).

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group.[7] In some cases the antitubercular activity was very improved and the skin pigmentation potential reduced, however the compounds producing the least discoloration of tissue were not endowed with the lowest lipophilicity and half-life values, suggesting that a rather complex relationship must exist between the physicochemical characteristics of compounds and their pharmacokinetics and tissue staining potentials (Chart 1).

On the other hand, it is interesting to note that clofazimine and related riminophenazines, besides the antimycobacterial activity, display also activity against several G+ bacteria[8] and protozoa (plasmodia, chloroquine sensitive and resistant, and leishmania).[9] Clofazimine and its analogs act also as potent resistance circumventing anticancer agents and inhibit the growth of several human cancer lines.[10] Finally the anti-inflammatory activity of CFM is well known and plays an important role in suppressing ENL (erythema nodosum leprosum) and has been shown to be of some value in the treatment of rheumatoid arthritis, discoid lupus erythematosum and other inflammatory diseases.[11]

Based on these data we deemed interesting to investigate the antitubercular activity of novel riminophenazine derivatives, characterized by the replacement of the isopropyl on the 3-imino group with quinolizidinylalkyl residues which we have already observed to confer antimycobacterial activity to a number of different aromatic and heteroaromatic moieties, to which are linked. Examples of the most active compounds are collected in Chart 2.[12–18]

Compounds **A** and **D** are particularly interesting; the former for its high potency against *Mycobacterium tuberculosis*[14] associated with a large spectrum of activity against G+ bacteria[13,14] the latter, on the contrary, for its selective activity against only *Mycobacterium tuberculosis* and for maintaining the activity also in vivo (125 mg/kg, p. os, in infected mice).[16]

Recently, even the simple lupinyl palmitate has been shown to inhibit the growth of *Mycobacterium tuberculosis* for 98%, at the concentration of 6.25 μg/mL.[19]

Thus, thirteen novel riminophenazine derivatives (**1**–**13**; Chart 3) have been synthesized, which bear on position 3 a ω-(quinolizidin-1α/1β-yl)alkylimino group.

The variable length of the linker (from one to three methylenes) and the two possible spatial connections to the position 1 of the quinolizidine ring (stereochemistry corresponding to that of l-lupinine and d-*epi*-lupinine, respectively) allow the bulky basic moiety and the quasi-planar phenazine to assume largely variable reciprocal dispositions, that could influence the biological activity. To further explore a such influence, we have also tested two riminophenazine derivatives (**14** and **15**, Chart 3), where the quinolizidine bicycle is replaced by the smaller pyrrolizidine one. Moreover, in these compounds the two methylenes spacer is attached to the bridgehead achiral carbon **7a**, thus also avoiding any stereochemical issue. The prepared compounds, but two (**6** and **11**), were tested in vitro against *Mycobacterium tuberculosis* $H_{37}R_v$ and $H_{37}R_a$, *Mycobacterium avium* and five clinical isolates (29/10; 778/10; 917/10; 439/10; 756/10). The results are collected in Table 1, together with results of reference drugs (clofazimine, isoniazid, rifampin, streptomycin and ethambutol).

2. Results and discussion

2.1. Chemistry

Compounds of Chart 3 were synthesized by reacting, in dioxane solution, the 2-[(4-*R*-phenyl)amino]-10-(4-*R*-phenyl)-2,10-dihydro-3-iminophenazine hydrochlorides (**16**–**18**) with the suitable

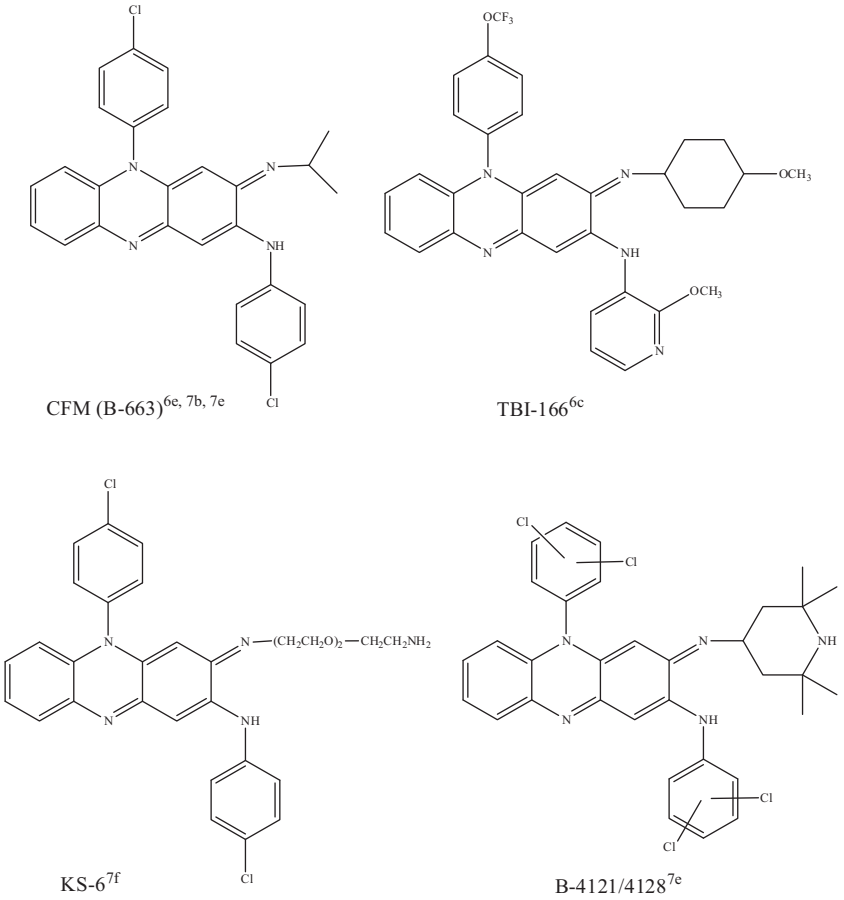

Chart 1. Structures of clofazimine (CFM) and some analogues in development as anti-TB agents.