Development of a highly potent and selective DHFR inhibitor for *Mycobacterium avium* and *Mycobacterium abscessus*

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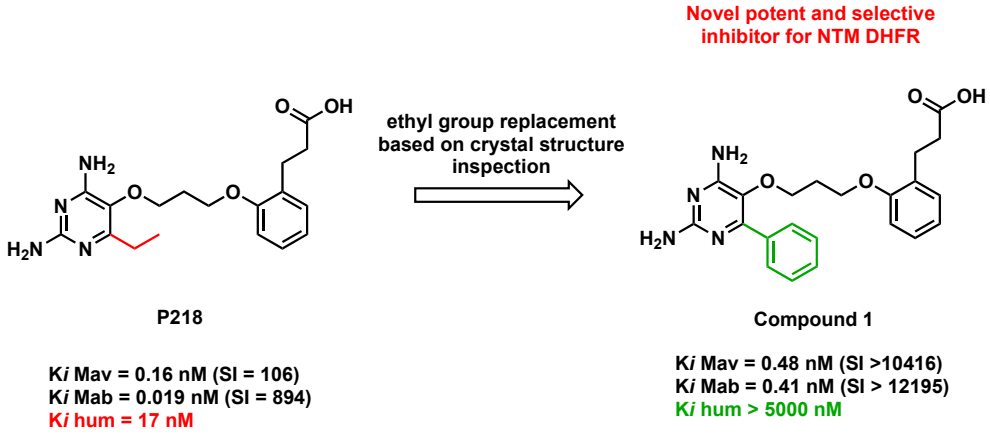
Nontuberculous mycobacteria (NTM) are opportunistic pathogens responsible for pulmonary disease in immunocompromised patients or patients with pre-existing lung conditions such as cystic fibrosis and chronic obstructive pulmonary disease.[1,2] The incidence and prevalence of these diseases are increasing worldwide due to inefficient medicines.[3] We aimed to modify the chemical structure of compound **P218**, a dihydrofolate reductase (DHFR) inhibitor developed for malaria,[4] to access new inhibitors of NTM DHFR. Although **P218** itself is highly potent against DHFR of *M. avium* (K*i* = 0.16 nM) and *M. abscessus* (K*i* = 0.019 nM), the compound is also potent against human DHFR (K*i* = 14 nM). By performing a visual inspection of the crystal structures of *M. abscessus* and the human enzymes complex with **P218**, we envisage that the replacement of the ethyl group with a bulkier substituent would decrease the activity against the human enzyme, furnishing more selective inhibitors. We then developed a synthetic route to allow the desired modifications and we synthesized a series of analogues containing alkyl and aryl groups at the ethyl position. Among the compounds prepared, **Compound 1** demonstrated the best profile. This molecule is highly active against both *M. avium* (K*i*= 0.48 nM) and *M. abscessus* (K*i* = 0.41 nM), and no inhibition was detected against the human enzyme (K*i* > 5000 nM).



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