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Synthesis of Several Dihydropyrimidines as Antimycobacterial Agents

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ABSTRACT

A small series of 30 dihydropyrimidine compounds were synthesized and assessed for their in vitro antimycobacterial potential against Mycobacterium tuberculosis $H_{37}Rv$. The lowest MIC value, 0.02 µg/mL, was found for compounds 4a and 4d. Both the compounds showed comparatively low cytotoxicity, and were potent against M. tuberculosis strains resilient to known drugs.

Keywords : Dihydropyrimidines, Antimycobacterial Activity, MDR-TB.

I. INTRODUCTION

Fueled by the growing incidence of HIV infection, the tuberculosis (TB) epidemic is becoming a greater global public health emergency. With one-third of the world's population harboring the tuberculous bacillus, 2 million deaths due to TB occurring each year, 3 million people becoming infected with both HIV and *Mycobacterium tuberculosis,* and the growing incidence of both multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), the need for more effective chemotherapy for the treatment of TB has never been greater [1]. Unfortunately, the last truly novel drug that was approved for the treatment of TB was discovered >40 years ago; this partially explains the inadequacy of the present armamentarium of antituberculous drugs.

The currently recommended treatment regimens for active pulmonary TB are both lengthy and cumbersome. The treatment duration is a minimum of 6 months, with 4 drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) typically given daily for the first 2 months and with 2 drugs (isoniazid and rifampin) administered for 4 additional months [2]. In part because of this lengthy and complex treatment regimen, the World Health Organization (WHO), in 1993, introduced a global strategy for TB control known as "directly observed therapy, short-course" (DOTS) [3]. One of the crucial components of this strategy is the direct observation by trained personnel of patients taking their medications, to ensure compliance and to help prevent the emergence of drug resistance. Although the direct observation and monitoring of patient adherence to the regimen is important to treatment success, it also increases the cost of treatment and makes TB therapy more burdensome.

One additional difficulty associated with the presently available treatment regimens is the potential for drug-drug interactions, primarily those between rifampin and many of the antiretroviral drugs used for the treatment of AIDS. Rifampin induces some of the cytochrome P-450 enzymes that metabolize certain of the protease inhibitors and nonnucleoside reverse-transcriptase inhibitors commonly used to treat HIV/AIDS. Therefore, it is difficult to co-administer effective treatment for TB and AIDS.

The treatment of MDR-TB is characterized by relatively less effective, poorly tolerated, and expensive drugs that may need to be administered for years. Equally inadequate is the treatment available for latent TB infection. It has been estimated that 2 billion individuals are infected with *M. tuberculosis,* so there is an enormous human reservoir of the infecting organism. The currently recommended treatment for latent TB infection is isoniazid given for 6–9 months. Because of the long duration of this therapy and the potential toxicities of isoniazid, there is a major compliance problem associated with the treatment regimen. Although new drugs are needed to shorten the duration of treatment of latent TB infection, the safety profile for these drugs must be excellent, because most patients with latent infection are destined never to experience activation of their TB.

Therefore, the need for newer, more effective drugs that can achieve multiple goals in improving TB control is pressing. Recognizing these serious facts, we initiated a program to synthesize and screen diverse heterocyclic entities like pyrimidines, phenothiazines and pyrazolo[3,4-*d*]pyrimidines as potential anti-tubercular agents [4-6]. Inspired by results of these various heterocyclic entities, we set upon a programme of making anti-tubercular agents, using the central dihydropyrimidine as the template and adding substituents as we deemed necessary to impart activity, on the various positions of dihydropyrimidine ring. Dihydropyrimidine is not represented in the current clinical antitubercular regimens, suggesting that this class of compounds may target new biochemical mechanisms, potentially allowing treatment of MDR-TB.

II. RESULTS AND DISCUSSION

Chemistry

The synthetic route for the preparation of dihydropyrimidines derivatives (**4a-e** to **9a–e**) is summarized in Scheme 1. Various 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes (**1a-e**) bearing a range of electron withdrawing and electron releasing substituents, viz., 4-F; 4-Cl; 4-Br; 4-NO_2; 4-CH_3 were prepared according to the previously reported procedure [7]. The aldehydes thus obtained, were used along with 1,3-diketones and urea derivatives as adducts for the multi-component Biginelli reaction. All the dihydropyrimidines derivatives (**4a-e** to **9a-e**) were synthesized by the three-component coupling reaction involving substituted aldehydes, ethyl/methyl acetoacetate and urea derivatives. The yields of the products were obtained in the range of 45–67%. Designed series of molecules (Table 1) were characterized by ^{1}H NMR, ^{13}C NMR, and Mass spectrometry techniques before evaluating for antimycobacterial activity.

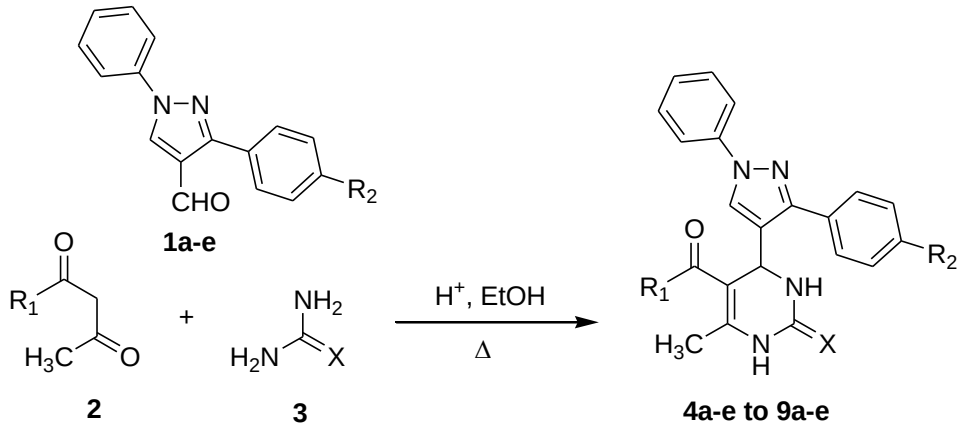

Table 1. Antimycobacterial Activity against M. tuberculosis and Cytotoxic Activity of Dihydropyrimidines **4a-e** to **10a-e**

Sr. No.	R_1	R_2	X	% Inhibi-tion	MIC µg/mL	IC_{50} VERO cells	SI (SI $=IC_{50}$/MIC)
4a	OC_2H_5	4-F	O	100	0.02	> 10	>500
4b	OC_2H_5	4-Cl	O	49	n.d.	n.d.	n.d.
4c	OC_2H_5	4-Br	O	33	n.d.	n.d.	n.d.
4d	OC_2H_5	4-NO_2	O	100	0.02	> 10	>500
4e	OC_2H_5	4-CH_3	O	68	n.d.	n.d.	n.d.
5a	OC_2H_5	4-F	S	30	n.d.	n.d.	n.d.
5b	OC_2H_5	4-Cl	S	75	n.d.	n.d.	n.d.
5c	OC_2H_5	4-Br	S	87	n.d.	n.d.	n.d.
5d	OC_2H_5	4-NO_2	S	92	3.13	> 10	>3.2
5e	OC_2H_5	4-CH_3	S	96	1.56	8.9	5.7
6a	OC_2H_5	4-F	NH	18	n.d.	n.d.	n.d.
6b	OC_2H_5	4-Cl	NH	94	3.13	> 10	>3.2
6c	OC_2H_5	4-Br	NH	51	n.d.	n.d.	n.d.
6d	OC_2H_5	4-NO_2	NH	58	n.d.	n.d.	n.d.
6e	OC_2H_5	4-CH_3	NH	78	n.d.	n.d.	n.d.
7a	OC_2H_5	4-F	SCH_3	08	n.d.	n.d.	n.d.
7b	OC_2H_5	4-Cl	SCH_3	13	n.d.	n.d.	n.d.
7c	OC_2H_5	4-Br	SCH_3	13	n.d.	n.d.	n.d.
7d	OC_2H_5	4-NO_2	SCH_3	37	n.d.	n.d.	n.d.
7e	OC_2H_5	4-CH_3	SCH_3	97	1.56	7.4	4.7
8a	OCH_3	4-F	S	73	n.d.	n.d.	n.d.
8b	OCH_3	4-Cl	S	68	n.d.	n.d.	n.d.
8c	OCH_3	4-Br	S	43	n.d.	n.d.	n.d.
8d	OCH_3	4-NO_2	S	91	3.13	9.6	3.0
8e	OCH_3	4-CH_3	S	46	n.d.	n.d.	n.d.
9a	OCH_3	4-F	O	62	n.d.	n.d.	n.d.
9b	OCH_3	4-Cl	O	75	n.d.	n.d.	n.d.
9c	OCH_3	4-Br	O	90	6.25	>10	>1.6
9d	OCH_3	4-NO_2	O	39	n.d.	n.d.	n.d.
9e	OCH_3	4-CH_3	O	98	1.56	>10	>6.4

III. ANTIMYCOBACTERIAL ACTIVITY

All compounds were initially screened for their antimycobacterial activity at 6.25 µg/mL against MTB $H_{37}Rv$ strain by the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) in BACTEC 12B medium using the Microplate Alamar Blue Assay [8] (Table 1). Compounds exhibiting ≥90% inhibition in the initial screen were retested at and below 6.25 µg/mL using 2-fold dilution to determine the actual MIC.

In the preliminary screening, nine compounds (**4a**, **4d**, **5d**, **5e**, **6b**, **7e**, **8d**, **9c** and **9e**) inhibited MTB with 90–100%. In the secondary level, two compounds (**4a** and **4d**) inhibited MTB with MIC of <1 µg/mL and three compounds (**5e**, **7e**, **9e**) with MIC of <2 µg/mL. When compared to isoniazid (MIC: 0.36 µg/mL), two compounds, ethyl 4-[3-(4-fluorophenyl)-1-phenyl-

1*H*-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetra hydropyrimidine-5-carboxylate **4a** and ethyl 4-[3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **4d** were found to be the most active compounds in vitro with MIC of 0.02 μg/mL against MTB and were 18 times more potent than isoniazid. Substituents with different electronic properties at 4th position of C-3 phenyl ring of pyrazolyl substitution exhibited high inhibitory activity against MTB, indicating that the electronic properties of the substituents have only minor influence on the antimycobacterial activity. The preliminary antimycobacterial evaluation results show that compounds with fluoro and nitro substituents at 4th position of C-3 phenyl ring of pyrazolyl substitution and an oxo substitution at C-2 position of dihydropyrimidine nucleus have shown most promising activity along with C-5 carbethoxy group. Extensive structure-activity relation could be derived in future with various other modifications.

Having identified good number of active antimycobacterial dihydropyrimidines, the next step was to examine the toxicity of the drug candidates. Compounds exhibiting reasonably low MICs (from 0.02 to 6.25 μg/mL) were tested for cytotoxicity (IC_{50}) in VERO cells, and a selectivity index (SI), defined as IC_{50}: MIC, was calculated. The IC_{50} and SI values are shown in Table 1. The compounds **5e, 7e** and **8d** were somewhat more toxic than the **4a, 4d, 5d, 6b, 9c, 9e**. Generally, compounds with an MIC ≤6.25 μg/mL and an SI ≥10 are interesting compounds, and an MIC ≤1 μg/mL in a novel compound class is considered an excellent lead [10], which makes the ethyl 4-[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate **4a** and **e**thyl 4-[3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **4d** very promising antimycobacterial compounds.

It has been estimated that up to 50 million people are infected with drug-resistant forms of TB. The development of drug resistance in the population has increased concern that TB may once again become an incurable disease. Of particular concern is the development of multidrug-resistant forms of the disease (MDR-TB), defined as forms resistant to two or more of the front line anti-TB agents [9]. These forms of the disease are more often fatal and are difficult and expensive to treat [10]. The most active compounds **4a** and **4d** were tested against *M. tuberculosis* strains resistant to isoniazid and rifampin (Table 2). These results demonstrated absolutely no cross resistance with isoniazid and rifampin as both the compounds inhibited the resistant strains with MIC of 0.02 μg/mL. The mechanism by which the dihydropyrimidines described herein exhibit their antimycobacterial activity is not known. However, the lack of cross-resistance with both the known antimycobacterial drugs suggests that these compounds do not share entirely the same antibacterial mode of action as isoniazid and rifampin.

Further in vitro studies of compounds **4a** and **4d** as well as synthesis of analogues of this lead compounds are currently in progress.

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