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Synthesis and Biological Evaluation of Some Diverse Dyhdropyrimidine Dervatives

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ABSTRACT

A novel series of dihydropyrimidine derivatives were synthesized and evaluated for their in vitro antitubercular activity against Mycobacterium tuberculosis $H_{37}Rv$. Two compounds, ethyl 4-[3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 4a and ethyl 4-[3-(4-nitrophenyl)-1-phenyl-1H-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.

Keywords : Dihydropyrimidines, Antitubercular Activity, Antimycobacterial Activity.

I. INTRODUCTION

Tuberculosis (TB) remains a deadly disease and continues to claim approximately 2 million lives annually.[1] In affected regions, the disease is recognized as serious impediment to economic and social development.[2] The prevalence of TB has been increasing, and presently nearly two billion individuals worldwide have been exposed to thetubercle bacillus. Many of these latent infected cases are expected to reactivate sometime later in life. Because of this characteristic of Mycobacterium tuberculosis, about one-third (approx. 200 million) of the world population is estimated to be latently infected with the pathogen. This rise in TB incidents can be attributed to the development of resistance by M. tuberculosis to commonly used anti-tuberculosis drugs, raising incidences of disease in immuno-compromised patients, and longer durations of therapy that are required as a results of resistance development.

A perfect vaccine against TB would be most effective in controlling the disease but it has been elusive so far. BCG, the current vaccine against tuberculosis, prevents the development of severe and fatal TB in young children and has no significant side effects. However, protective efficacy of BCG against pulmonary tuberculosis in adults has been controversial, and no other effective vaccine is available in reducing the greater number of TB cases in adults. There are now a number of potential vaccine candidates being developed, but marketable and more useful vaccine may still be decades away.[3] Thus, research efforts are required to develop new drugs for the ever-pervasive rise of the drug resistance. The pandemic of Acquired Immunodeficiency Syndrome (AIDS) and the evidence of its association with tuberculosis is now of serious concern. Since the containment of tuberculosis infection in an individual depends on intact cellular immunity, Human Immunodeficiency Virus (HIV), due to its ability to destroy the immune system, has now emerged as the most significant risk factor for progression of dormant tuberculosis infection to clinical disease. As a result, tuberculosis epidemic has not only begun to worsen, but also poses an unprecedented medical, social and economic threat to the world.[4]

With the introduction of rifampicin, "short course" regimens using isoniazid and rifampicin, together with streptomycin, ethambutol or pyrazinamide for six months became possible. The emergence of drug resistance and spread of MDR strains, helped to a large extent by coinfection with the HIV, have made this armamentarium of drugs insufficient, calling for the development of new drugs. The past decade has seen dramatic advances in our understanding of the metabolic and intracellular lifestyle of M. tuberculosis, culminating in the recent publication of its complete genomic DNA sequence.[5] The emphasis of mycobacterial research has now shifted from gene hunting to interpretation of the biology of the whole organism in an effort to define the activities, which are likely to be critical for its survival and thus, amenable to the development of new drugs.[6] At present, the best long-term strategy appears to discover and develop an entirely new class of agents possibly acting on completely novel targets through mechanism of actions different from those of existing drugs.[3] They should have better tolerability (lower toxicity) than existing drugs and have improved pharmacokinetics properties, in order to make intermittent chemotherapy feasible. Furthermore, with improved understanding of the disease, it will be possible to target persisting bacteria with new chemotherapeutic agents, and hence reduce the overall duration of therapy to less than existing six months.

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.[7], [8], [9] 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

2.1 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-1H-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.[10] 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 60–74%. Designed series of molecules (Table 1) were characterized by ^{1}H NMR, ^{13}C NMR, and Mass spectrometry techniques before evaluating for antimycobacterial activity.

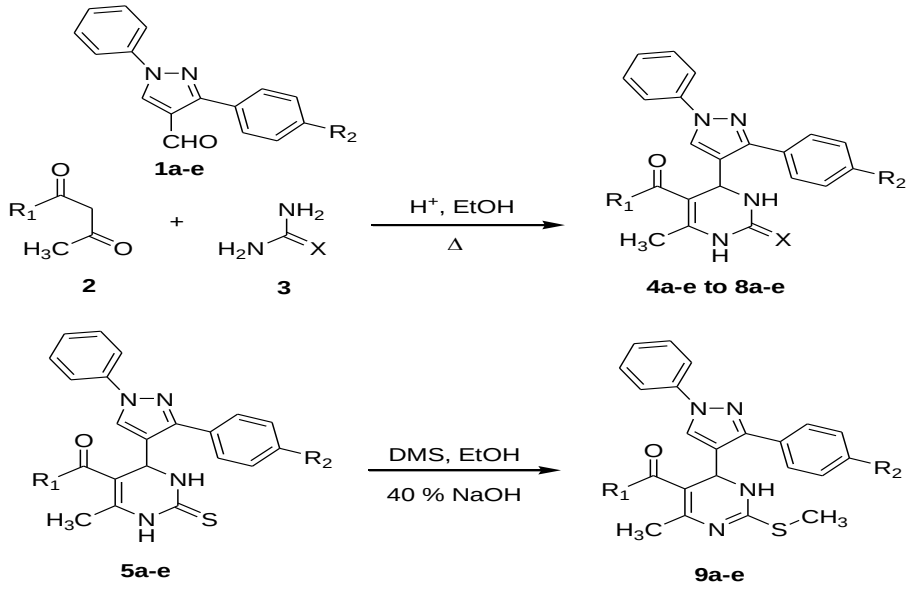

Figure 1

Table 1. In vitro antitubercular screening data of Dihydropyrimidines **4a-e** to **9a-e**

Sr. No.	R_1	R_2	X	% Inhibition	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	OCH_3	4-F	S	73	n.d.	n.d.	n.d.
7b	OCH_3	4-Cl	S	68	n.d.	n.d.	n.d.
7c	OCH_3	4-Br	S	43	n.d.	n.d.	n.d.
7d	OCH_3	4-NO_2	S	91	3.13	9.6	3.0
7e	OCH_3	4-CH_3	S	46	n.d.	n.d.	n.d.
8a	OCH_3	4-F	O	62	n.d.	n.d.	n.d.
8b	OCH_3	4-Cl	O	75	n.d.	n.d.	n.d.
8c	OCH_3	4-Br	O	90	6.25	>10	>1.6
8d	OCH_3	4-NO_2	O	39	n.d.	n.d.	n.d.
8e	OCH_3	4-CH_3	O	98	1.56	>10	>6.4
9a	OC_2H_5	4-F	SCH_3	08	n.d.	n.d.	n.d.
9b	OC_2H_5	4-Cl	SCH_3	13	n.d.	n.d.	n.d.
9c	OC_2H_5	4-Br	SCH_3	13	n.d.	n.d.	n.d.
9d	OC_2H_5	4-NO_2	SCH_3	37	n.d.	n.d.	n.d.
9e	OC_2H_5	4-CH_3	SCH_3	97	1.56	7.4	4.7

2.2 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 [11] (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**, **7d**, **8c**, **8e** 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**, **8e**, **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, 7d** and **9e** were somewhat more toxic than the **4a, 4d, 5d, 6b, 8c, 8e**. 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,[12] 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.

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

III. CONCLUSIONS

In the present paper, we report the synthesis, spectral studies and antimycobacterial activity of various dihydropyrimidine derivatives. The high bioactivity of these compounds makes them suitable hits for additional *in vitro* and *in vivo* evaluations, in order to develop new class of antimycobacterial drugs or prodrugs with potential use in the tuberculosis treatment. Further studies in this area are in progress in our laboratory.

IV. EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. ^{1}H NMR was determined in CDCl3 solution on a Bruker DPX 300 MHz spectrometer. ^{13}C-NMR (75 and 125MHz) spectra were registered on a Bruker AC 200, DPX 300 and ARX 500, at 25 ºC, in CDCl3. Elemental analysis of the newly synthesized compounds was carried out on Carlo Erba 1108 analyzer and are found within the range of theoretical value.

4.1 Synthesis of 3-aryl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (1)

Synthesis of 3-aryl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**1**) was achieved by reported method.[10]

4.2 General procedure for the synthesis of Ethyl 4-(3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a-e)

A mixture of 3-aryl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (0.01 mol), ethyl acetoacetate (0.01 mol) and urea (0.01 mol) in ethanol (30 ml) was heated under reflux for 6-8 h. The reaction mixture was kept at room temperature for 2 h. The yellow crystalline product so obtained was isolated and recrystallized from ethanol.

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