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Novel dihydropyrimidines as a potential new class of antitubercular agents

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ABSTRACT

A small library of 30 dihydropyrimidines was synthesized and evaluated for their in vitro antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$. Two compounds, ethyl 4-[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-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 more potent than isoniazid.

Tuberculosis (TB) is a worldwide pandemic caused by different species of mycobacteria. The latest statistics reveals that around 2 million people throughout the world die annually from tuberculosis and there are around 8 million new cases each year, out of which developing countries show major share.[1] Among HIV-infected people with weakened immune system, TB is a leading killer epidemic. Every year about 2 million people living with HIV/AIDS die from TB.[2] Furthermore, in recent times the appearance of multidrug-resistant TB (MDR-TB), a form of TB that does not respond to the standard treatments, is more common. It is a shocking revelation that MDR-TB is present in almost all countries as per the recent survey, made by the World Health Organization (WHO) and its partners. A recent estimation by WHO has revealed that within next 20 years approximately 30 million people will be infected with the bacillus.[3] Keeping in view of the above statistics, WHO declared TB as a global health emergency and aimed at saving 14 million lives between 2006 and 2015.[4]

Despite the efforts of academic institutions and the pharmaceutical companies engaged in the design, synthesis, and development of new antitubercular regimens, the current TB therapeutic arsenal is poor. A number of compounds belonging to different prototypes have shown moderate to excellent antitubercular activities.[5] As on today at least 10 molecules including fluoroquinolones (gatifloxacin and moxifloxacin),[6–9] diarylquinoline (TMC207),[10] nitroimidazoles (OPC67683 and PA824),[11,12] pyrrole (LL3858),[13] 1,2-diamine (SQ109)[14] are in different stages of clinical trial.[15] All the above facts reveal that there is an urgent need for development of new drugs with divergent and unique structure and with a mechanism of action possibly different from that of existing drugs.

In this context, dihydropyrimidines are potential inhibitors of dihydrofolate reductase (DHFR),[16] which is a promising drug target for treatment of mycobacterial infections. DHFR is a key enzyme in the folate cycle[17,18] which supplies one-carbon units, derived from the action of serine hydroxymethyltransferase[19,20] on L-serine, for the biosynthesis of deoxythymidine monophosphate (dTMP). Inhibition of the folate cycle leads to interruption of the supply of thymidine and thus to inhibition of DNA biosynthesis and inhibition of proliferation of cells. Inhibition of proliferation is a useful goal in the therapy of bacterial and protozoal infections.[21] Although DHFR does not represent a new target, there is still enthusiasm for the development of DHFR inhibitors, particularly with regard to mycobacteria.[22–26] A unique feature of DHFR is the selectivity that is possible in the design of inhibitor. This makes it an ideal 'old' target for rational and effective drug design for antimycobacterial agents.

Dihydropyrimidines are 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 and there are very few investigatory reports on dihydropyrimidines as antitubercular agents.[27,28] Recognizing these facts and in continuation of work on pyrimidine derivatives,[29–31] we set upon a programme of making antitubercular agents, using the central dihydropyrimidine as the template and adding versatile substituents on the various positions of dihydropyrimidine ring. The synthetic route for the preparation of dihydropyrimidines derivatives (**4a–e** to **9a–e**) is outlined 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

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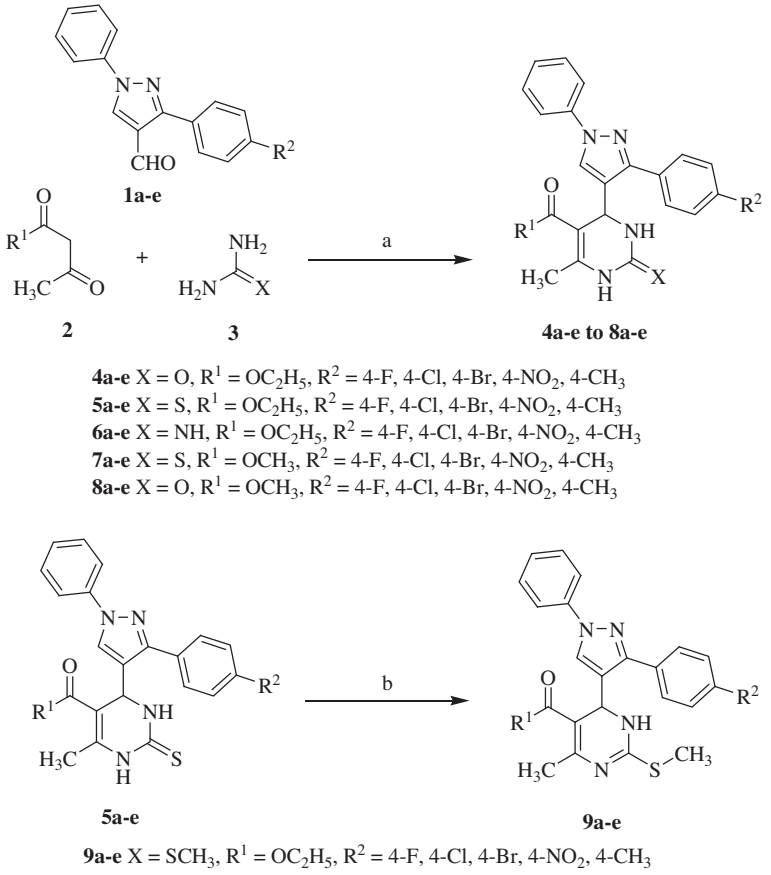

4a-e X = O, $R^1 = OC_2H_5$, R^2 = 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3
5a-e X = S, $R^1 = OC_2H_5$, R^2 = 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3
6a-e X = NH, $R^1 = OC_2H_5$, R^2 = 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3
7a-e X = S, $R^1 = OCH_3$, R^2 = 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3
8a-e X = O, $R^1 = OCH_3$, R^2 = 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3

9a-e X = SCH_3, $R^1 = OC_2H_5$, R^2 = 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3

Scheme 1. Synthetic protocol of title compounds **4a**–**e** to **9a**–**e**. Reagents and conditions: (a) concd HCl, EtOH, reflux; (b) DMS, EtOH, 40% NaOH, stirring.

according to the previously reported procedure.[32] All the dihydropyrimidines derivatives (**4a–e** to **9a–e**) were synthesized by the multi-component Biginelli reaction involving substituted aldehydes, ethyl/methyl acetoacetate and urea derivatives. Dihydropyrimidines **5a–e** on S-methylation with dimethylsulfate in the presence of 40% NaOH afforded S-methylated adducts **9a–e**. The compounds **4a–e** to **6a–e**, and **9a–e** have carbethoxy group in the 5-position, while compounds **7a–e** to **8a–e** have carbmethoxy group in the 5-position. The yields of the products were obtained in the range of 60–74%. Designed series of molecules were characterized by ^{1}H NMR, ^{13}C NMR, and Mass spectrometry techniques before evaluating for antimycobacterial activity.

^{1}H NMR spectra of dihydropyrimidines derivatives (**4a–e** to **9a–e**) showed singlets at δ 4.96–5.75 ppm corresponding to methine group and δ 8.69–9.01 ppm corresponding to NH group of pyrimidine ring confirming the formation of dihydropyrimidine nucleus. Further, formation of S-methylated adducts **9a–e** was confirmed by a singlet at δ 3.51–3.61 ppm corresponding to *S*-methyl group. On the other hand, ^{13}C NMR spectra displayed chemical shift at δ 38.7–46.8 ppm for carbon of methine proton. Also, *S*-methyl carbon was observed at δ 12.3–14.3 ppm for compounds 9a–**e**. The elemental analysis results were within ±0.4% of the theoretical values.

All compounds were initially screened for their in vitro antimycobacterial activity at 6.25 μg/mL against MTB H_{37}Rv strain by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) in BACTEC 12B medium using the Microplate Alamar Blue Assay.[33] Compounds exhibiting ⩾90% inhibition in the initial screen were retested at and below 6.25 μg/mL using two-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**, and **9e**) with MIC of <2 μg/mL. Among all the compounds, compounds **4a** and **4d** having 4-F and 4-NO_2 substituents at the 4-phenyl ring of pyrazolyl substitution were the most potent compounds (MIC of 0.02 μg/mL and SI >500) of the series. These compounds are better than the existing drug INH (MIC: 0.03 μg/mL) in the in vitro studies. Therefore, these compounds provide an excellent lead for further development as novel antitubercular molecule. Substituents with different electronic properties at fourth 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. With the sizable number of compounds no definite conclusion could be drawn from this study, yet the detailed SAR, in vivo evaluation and mode of action of the series would be undertaken in due course.

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**, and **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,[34] which makes the ethyl 4-[3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydro

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	8	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
Isoniazid	—	—	—	—	0.03	—	—

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.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.046.

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