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Design, Synthesis and Biological Activity of Substituted Dihydropyrimidine-2-(1*H*)-thiones

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Abstract: A series of some dihydropyrimidine-2(1*H*)-thione derivatives have been synthesized and characterized on the basis of IR and NMR spectral data. Antitubercular and antibacterial activities were performed by microbroth dilution and cup-plate method respectively. The compounds were also screened for antioxidant activity by DPPH method. All the synthesized compounds have been subjected for physical parameter evaluation. Though the compounds showed moderate antioxidant activity, few compounds have shown good antitubercular activity and better antibacterial activity compared to the standard drug.

Keywords : Dihydropyrimidine-2(1*H*)-thione, Antitubercular, Antibacterial, *invitro* Antioxidant.

Introduction

Recently, the interest in the synthesis of 3,4-Dihydropyrimidin-2(1*H*)-ones (DHPM) and their thio derivatives have received considerable attention due to their attractive pharmacological profiles. They are mostly used as calcium channel blockers[1], antihypertensive agents[2], alpha-antagonists[2] and neuropeptide antagonists. Alkaloids containing the dihydropyrimidine structure have been isolated from various marine sources which have shown some interesting biological properties[3]. Most important among these alkaloids was betzelladine, which was found to be potent HIV-gp-120-CD_4 inhibitors[4-5]. Synthetic strategies for the dihydropyrimidine nucleus involves one-pot to multi-step approaches.

The classical Biginelli synthesis a one-pot condensation using β-dicarbonyl compounds with aldehydes and urea or thiourea in ethanol solution containing catalytic amounts of acid. In recent years, several methods for the synthesis of DHPM's have been developed to improve and modify this reaction by means of microwave irradiation[6], ultra sound irradiation[7] and ionic liquids[8].

The World Health Organization estimates that about 30 million people will be infected by *M. tuberculosis* within the next 20 years. The incidence of TB infection has steadily risen in the last decade. The reemergence of TB infection has been further complicated by an increase in the prevalence of drug-resistant TB cases. Current control efforts are severely hampered due to M. tuberculosis being a leading opportunistic infection in patients with acquired immuno deficiency syndrome and the spreading of multidrug-resistant strains (MDR-MTB). Since no effective vaccine is available, the major strategy to combat the spreading of TB is chemotherapy and the ever-increasing drug resistance, toxicity, side effects of currently used antituberculosis drugs and the absence

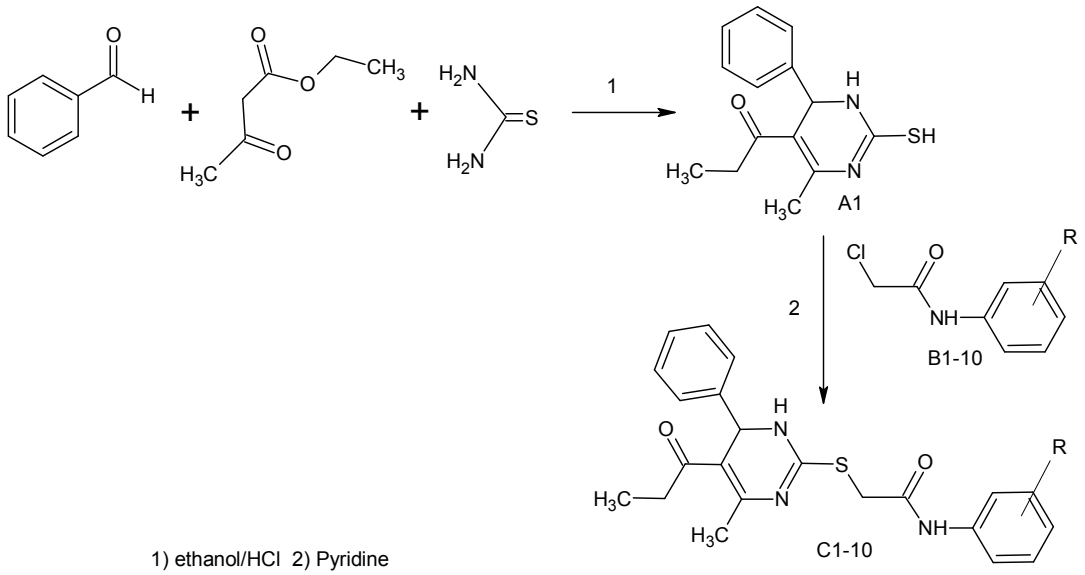
of their bactericidal activity highlight the need for new, safer, and more effective antimycobacterial compounds.

Hence in the present research work it was thought worth to synthesise some novel dihydropyrimidine derivatives and screen for their antitubercular, antibacterial and *invitro* antioxidant activities.

Experimental

Melting point of the compounds synthesized was determined by using Thiel's melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellet method in the range of 4000-500 cm^{-1} on a Fourier Transform IR Spectrophotometer, Shimadzu 8700 and frequencies were recorded in wave numbers. ^1H NMR-(400 MHz) spectra was recorded on AMX-400 MHz spectrometer using $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) down field from internal reference TMS. Purity of the compounds were checked by thin layer chromatography using silica gel-G coated aluminium plates as stationary phase, *n*-hexane : ethyl acetate as mobile phase. The scheme for the synthesis is shown in **Fig 1**.

Fig 1 :Scheme of synthesis



Comp	R	Comp	R
C1	H	C6	4-Cl
C2	2-CH_3	C7	3-Br
C3	3-CH_3	C8	4-Br
C4	4-CH_3	C9	3-NO_2
C5	3-Cl	C10	4-NO_2

General procedure for the synthesis of ethyl-2-mercapto-4-methyl-6-phenyl-1,6-dihydropyrimidine-5-carboxylate (A1-10).

Benzaldehyde, ethyl acetoacetate and thiourea were taken in a round bottom flask and refluxed in ethanol (30 mL) containing concentrated HCl (0.5mL) for 5-6 hr. The reaction mixture was cooled and poured into ice-cold water. The precipitate obtained was filtered, washed, dried and recrystallized with ethanol.

General procedure for the Synthesis of N-substituted aryl chloro acetamide (B1-10).

Various substituted aniline was taken in a round bottom flask to which acetone was added and mixed thoroughly; chloroacetyl chloride was added drop wise with continuous shaking. After complete addition, the reaction mixture was refluxed for 6-8 hr. The reaction was monitored by TLC, after the completion of reaction the contents were cooled and poured into ice-cold water with stirring; sodium bicarbonate was added to neutralize the HCl liberated during the reaction. The product obtained was filtered, thoroughly washed with water, dried and recrystallised with ethanol

General procedure for the Synthesis of ethyl 2-[(substituted 2-anilino-2-oxoethyl)thio]-4-methyl-6-phenyl-1,6-dihydropyrimidine-5-carboxylate (C1-10)

Ethyl 2-mercapto-4-methyl-6-phenyl-1,6-dihydro pyrimidine-5-carboxylate was taken in a round bottom flask and dissolved in dry pyridine, various N-substituted arylchloro acetamide was added and refluxed for 25-27 hr. The reaction was monitored by TLC. After the completion of reaction, the contents were cooled and poured into ice-cold water with stirring and kept aside for 2 hr, the solid obtained was filtered at pump, thoroughly washed with water, dried and recrystallised with ethanol.

C1: IR (cm^{-1}) (KBr): 3326 (Ar-NH str), 3174 (NH str), 3109 ArC-H(str), 2977 AliC-H str), 1672 (C=O str), 1577 (C=C, str),

^{1}H NMR ($CDCl_3$): 8.61-8.63 (Pyrim N-H 1H, s), 7.64–7.80 (Pyrim C-H 1H, s), 7.07–7.30(Ar-H 10H, m), 5.40 (CONH 1H, s), 4.04– 4.14 (EthylCH_2 2H, m) 2.43 (Ar-$CH_3$3H,s),1.59 ($SCH_2$2H, s),1.06-1.27 (Ethyl CH_3 3H,t)

C2: IR (cm^{-1}) (KBr): 3326 (Ar-NH str), 3174 (NH str) 3105 (ArC-Hstr), 2979 (AliC-H str), 1670 (C=O str), 1573 (C=C str),

C3: IR (cm^{-1}) (KBr): 3326 (Ar-NH str), 3174 (NH str) 3107 (ArC-Hstr),2981 (AliC-H str), 1672 (C=O str), 1575 (C=C str),

C4: IR (cm^{-1}) (KBr): 3323 (Ar-NH str), 3174 (NH str) 3105 (ArC-Hstr), 2961(AliC-H str),1668(C=O str),1575 (C=C str),

C5: IR (cm^{-1}) (KBr): 3325 (Ar-NH str), 3174 (NH str) 3107 (ArC-H str), 2979 (AliC-H str), 1670 (C=O str), 1573 (C=C str), 759 (C-Cl str)

C6: IR (cm^{-1}) (KBr): 3328 (Ar-NH str), 3174 (NH str) 3105 (ArC-H str), 2979 (AliC-H str), 1670 (C=O str), 1575 (C=C str), 756 (C-Cl str)

C7: IR (cm^{-1}) (KBr): 3325 (Ar-NH str), 3172 (NH str) 3101 (ArC-H str), 2979 (AliC-H str), 1668 (C=O str), 1577 (C=C str), 651 (C-Br str)

C8: IR (cm^{-1}) (KBr): 3325 (Ar-NH str), 3174 (NH str) 3105 (ArC-H str), 2961 (AliC-H str), 1668 (C=O str), 1573 (C=C str), 648 (C-Br str),

^{1}H NMR ($CDCl_3$): 8.61-8.63 (Pryi N-H 1H, s), 7.4 (Pryi C-H 1H, s),7.21- 7.74 (Ar-H 9H,m) 5.40 (CONH 1H, s), 4.04- 4.14(Ethyl CH_2 2H, m) 2.43 (Ar-CH_3 3H,s), 1.62(S-CH_2 ,2H, s), 1.06-1.20 (Ethyl CH_3, 3H, t)

C9: IR (cm^{-1}) (KBr): 3323 (Ar-NH str), 3171 (NH str), 3113 (ArC-Hstr), 2984 (AliC-H str),1675 (C=O str), 1572 (C=C str), 1469 (-NO_2 str).

C10: IR (cm^{-1}) (KBr): 3325(Ar-NH str), 3174(N str) 3103 (ArC-H str), 2983 (AliC-H str), 1670 (C=O str),1575 (C=C str), 1467 (-NO_2 str)

Table 1: Physical Data of the Synthesized Compounds

Sl No	Comp Code	Mol Formula	Mol Wt	M.P (^oC)	% Yield	R_f	MilogP[a]	TPSA[b]	HBD[c]	HBA[d]	nrotb[e]
Lipinski[f]			≤500				≤5.0		≤10		
1	C1	$C_{22}H_{23}N_3O_3S$	409	182	33.05	0.47	3.96	79.79	2	6	8
2	C2	$C_{23}H_{25}N_3O_3S$	423	184	34.78	0.76	4.36	79.79	2	6	8
3	C3	$C_{23}H_{25}N_3O_3S$	423	101	28.0	0.71	4.38	79.79	2	6	8
4	C4	$C_{23}H_{25}N_3O_3S$	423	181	37.68	0.41	4.40	79.79	2	6	8
5	C5	$C_{22}H_{22}N_3O_3SCl$	443	208	37.0	0.61	4.61	79.79	2	6	8
6	C6	$C_{22}H_{22}N_3O_3SCl$	443	201	42.0	0.69	4.64	79.79	2	6	8
7	C7	$C_{22}H_{22}N_3O_3SBr$	488	190	41.28	0.42	4.74	79.79	2	6	8
8	C8	$C_{22}H_{22}N_3O_3SBr$	488	182	36.69	0.54	4.77	79.79	2	6	8
9	C9	$C_{22}H_{22}N_4O_5S$	454	209	23.58	0.37	3.99	125.62	2	9	9
10	C10	$C_{22}H_{22}N_4O_5S$	454	204	37.73	0.39	3.92	125.62	2	9	9

a= MilogP value, b= topological polar surface area, c=hydrogen bond donor,
d= hydrogen bond acceptor, e= number of rotatable bonds,
f= Lipinski's Rule of 5 for pharmaceuticals [9]

Physical property evaluation

Since some of the designed compounds of the present study showed good antitubercular, antibacterial and antioxidant activity when compared with the standard, a computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, TPSA and simple molecular descriptors used by Lipinski in formulating his "rule of five" calculations by using ACD lab software, ChemDraw Ultra and www.molinspiration.com. **Table 1** represents the calculated ClogP, SMV, TPSA and other Lipinski parameters of the synthesized compounds C1-10**.** Polar surface area and lipophilicity favors a molecule to cross the biological membranes. Very high TPSA value contributes for a low bioavailability of the molecule, in the present work it was interesting to find that the TPSA of molecules with a nitro group substitution and a MilogP in the range of 3.9 have shown better activity when compared to other molecules. The study of molecular properties of any small molecule can be considered as a unique tool in the field of drug design and also proves that there is a relationship between the physical parameter and the biological activity. Each structure was fully geometry optimized using the ChemDraw Ultra version 8.0 force field.

Biological Activity:

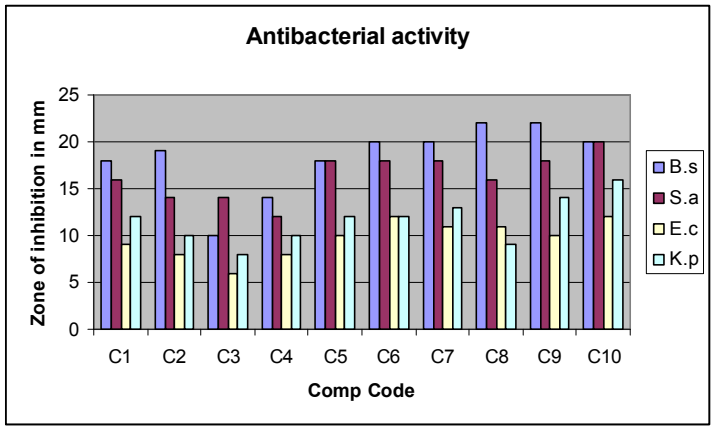
Antibacterial Activity

All the synthesized compounds were tested for their antibacterial activity against both gram positive and gram negative organisms viz., *Bacillus subtilis* (NCIM 2697)*, Staphylococcus auereus* (NCIM 2079)*, Escherichia coli* (NCIM 2065) *and Klebsella pneumonia* (NCIM 5082). The activity was performed by following the procedure of cup plate agar diffusion method[10]. A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms. 0.1 mL of inoculums (of 10^4 to 10^6 CFU / mL population prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. Accurately measured (0.1 mL) solution of each sample and standard were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 ºC for 24 h. The presence of definite zones of inhibition around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of DMSO, which was used as a solvent for sample. The results are shown in **Table II** and **Fig 2.**

Table II: Antibacterial and Antioxidant activity of the compounds C1-10.

Sl.No	Comp. Code	Antioxidant Activity	Zone of Inhibition in mm			
		IC_{50} in µg/ml	*B.subtilis* (NCIM 2697)	*S.aureus* (NCIM 2079)	*E.coli* (NCIM2065)	*K.pneumonia* (NCIM 5082)
1	C1	25	18	16	09	12
2	C2	35	19	14	08	10
3	C3	40	10	12	06	08
4	C4	55	14	12	08	10
5	C5	45	18	16	10	12
6	C6	50	20	18	12	12
7	C7	15	20	18	11	13
8	C8	20	22	16	11	09
9	C9	20	22	17	10	14
10	C10	15	20	20	12	16
	Ampicillin	--	28	26	22	20
	Ascorbic acid	10	--	--	--	--

Fig 2: Antibacterial Activity of the compounds C1-10



Antioxidant Activity

Free radical scavenging activity of the test compounds were determined by the 1,1- diphenyl picryl hydrazyl (DPPH) assay method[11]. Drug stock solution (1 mg/ml) was diluted to final concentrations of 2, 4, 6, 8 and 10 mg mL–1 in methanol. DPPH methanol solution (1 mL, 0.3 mmol) was added to 2.5 mL of drug solutions of different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity. Methanol was used as the solvent and ascorbic acid as the standard. The standard drug used was ascorbic acid at a concentration of 10µg/ml. The results in IC_{50} is shown in **Table II**.

Antitubercular Activity:

All the synthesized compounds were tested for their *invitro* antitubercular activity against *mycobacterium tuberculosis* by agar dilution method[12] with the use of Middlebrook 7H-9 broth and standard strain of *M. tuberculosis* $H_{37}Rv$. The basal medium was prepared according to manufacture's instructions (Hi-Media) and sterilized by autoclaving. 4.5 ml of broth was poured into each one of the sterile bottles. To this, 0.5ml of ADC supplement is added. This supplement contains catalase, dextrose and bovine serum albumin fraction. Then a stock solution of the compound was prepared (10mg / ml). From this appropriate amount of solution is transferred to media bottles to achieve final concentrations of 25, 50, 100ug / ml. Finally 10ul suspension of *M.tuberculosis* strain (100000 organisms/ml, adjusted by Mc Farland's turbidity standard) was transferred to each of the tube and incubated at 37ºC. Along with this one growth control without compound and drug controls were also maintained. The bottles were inspected for growth twice a week for a period of three weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. The results are produced in **Table III.**

Table III: Antitubercular Activity of the compounds C1-10.

Sl.No	Compound	5µg/ml	10µg/ml	25µg/ml
1	C1	R	S	S
2	C2	R	R	S
3	C3	S	S	S
4	C4	S	S	S
5	C5	R	R	R
6	C6	S	S	S
7	C7	R	R	R
8	C8	R	S	S
9	C9	R	S	S
10	C10	R	S	S
	Streptomycin	S	S	S
	Pyrazinamide	S	S	S

R= Resistant, S= Susceptible

Results and Discussion

All the synthesized compounds were subjected to antitubercular, antibacterial and *invitro* antioxidant activities. The compounds with a nitro group substitution have shown good antitubercular activity and was found to possess a broad spectrum of activity as it was active on both Gram positive and Gram negative organism and moreover this compound was also found to suppress free radicals. Hence this molecule could be taken for further exploitation. The compounds with methyl, chloro and bromo substitution have shown moderate activity against both Gram positive and Gram negative organisms and also on *Mycobacterium tuberculi*.

References

1. Kappe C.O. Biologically active dihydropyrimidones of the Biginelli typer-a literature survey Review article. Eur J Med Chem. 2000, 35, 1043.
2. Atwal K.S, Swanson B.N, Umger S.E, Floyd D.M, Moreland S, Hedberg A, Reilly B.C. Dihydropyrimidine calcium channel blockers, 3,3-carbomoyl-4-aryl-1,2,3,4-tetrahydro-6-methyl-5-pyrimidine carboxylic acid esters as orally effective antihypertensive agents. J Med Chem. 1991, 34, 806.
3. Hu E.H, Sidler D.R, Dolling U.H, Unprecedented Catalytic Three Component One-Pot Condensation Reaction: An Efficient Synthesis of 5-Alkoxycarbonyl- 4-aryl-3,4-dihydropyrimidin-2(1H)-ones. J Org Chem. 1998, 63, 3454.
4. Patil A.D, Kumar N.V, Kokko W.C, Bean M.F, Freyer A.J, Debrosse C et al. Novel Alkaloids from the Sponge Batzella sp.: Inhibitors of HIV gp120-Human CD4 Binding. J Org Chem. 1995, 60, 1182.
5. Snider B.B, Chen J.J, Patil A.D, Freyer A. Synthesis of the tricyclic portions of Batzelladines A,B and D.Revision of the stereochemistry of Batzelladines A and D. Tetrahedron Letters. 1996, 37, 6977.
6. Banik B.K, Reddy A.T, Datta A, Mukopadhyay C. Microwave induced Bismuth Nitrate catalysed synthesis of dihydropyrimidones via Beginelli condensation under solventless conditions. Tetrahedron Letters, 2007, 48, 7392.
7. Li J.T, Han J.F, Yang J.H, Li T.S. An efficient synthesis of 3,4-dihydropyrimidin-2-ones catalysed by NH_2SO_3H under Ultrasound irradiation. Ultrason Sonochem, 2003, 10, 119.
8. Peng J.J, Deng Y.Q. Ionic liquids catalysed Biginelli reaction under solvent free conditions. Tetrahedron Letters. 2001, 42, 5917.
9. Lipinski C.A, Lombardo F, Dominy B.W, Feeney P.J, *Adv. Drug Deliv. Rev.,* 1997, 23, 3-25.
10. Barry A.L, The Antimicrobial Susceptibility Test, Principle and Practices, 1999, 4th Edition, ELBS: London, 180.
11. Mensor L.L, Menzes F.S, Leitao G.G, Reis A.S, Dosantos T.C, Coube C.S *Phytother. Res*., 2001, 15, 127–130.
12. G.K.Rao, R.B.Kotnal, P.N.S.Pai. Synthesis and evaluation of N-((sub phenyl) methylidene)-2(3-methyl-2-oxo quinoxaline-1-(2H)yl)acetohydrazide for possible antibacterial and antifungal activities. *Int.J.Biolog.Chem*., 2009, 3(2), 71-74.

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