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Synthesis and Antitubercular Activity Studies of Some Unsymmetrical 1,4 – Dihydropyridines

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Unsymmetrical 1,4-dihydropyridines having isoxazole and pyridine system been synthesized from 2,6-dimethyl-4[3"-nitrophenyl]-5-carbmethoxy-3-[3"-aryl propene-1"-one]-1,4-dihydropyridines of the type Ia-h. All compounds were tested for antitubercular activity against *M.tuberculosis* ($H_{37}Rv$) strain by using Bactec 460 method. Isoxazole derivatives showed modest activity.

The growing concern worldwide due to resurgence of tuberculosis[1] has drawn attention of many researchers to reinvestigate entire chemotherapeutic agents both from known class of current drugs and also from class of compounds, which were never used for chemotherapeutic intervention. The AIDS related TB is growing at an alarming level and other mycobacterium strain like *M. avium* were found to be highly opportunistic[2]. The MDR-TB[3-6] needs to have newer antitubercular agents with high efficacy and safety profile and therefore one of the approaches to go for new synthetic analogs is by targeted structure modification of known class of compounds.

In our recent findings, we found that 1,4-dihydropyridine (DHP) class of compounds are excellent starting synthon for the development of antitubercular activity provided the most important site of C_3 of DHP is occupied by some heterocyclic ring system preferably containing nitrogen or oxygen atom. Similarly incorporation of unsymmetrical character also addresses to the higher activity in some cases[7]. Looking to this, and in continuation of our earlier study [8-9], hitherto we systematically introduced either a five member isoxazole or a six member pyridine system at C_3 position of DHP. The above mentioned two series of these compounds have been synthesized from 2,6-dimethyl-4-[3"-nitrophenyl]-5-carbmethoxy-3-[3"-aryl propene-1"-one]1,4-dihydropyridines **(1a-h)**.

All compounds were tested against *M.tuberculosis* $H_{37}Rv$ strain. Rifampicin was used as a standard drug.

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Primary screening was conducted at 12.5 µg/ml against *M. tuberculosis* ($H_{37}Rv$)strain in Bactec 12B medium using the Bactec 460 radiometric system [10].

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and uncorreced. Purity of the compounds was checked by precoated TLC plates. IR spectra were recorded using KBr pellets on a Nicolet Megna-IR 550 Series-II and ^{1}H NMR spectra were recorded on a Bruker AC300 MHz FTNMR using TMS as internal standard and chemical shifts have been expressed in δ ppm. EI-MS spectra were recorded at 70 ev on a Jeol D – 300 Mass Spectrometer. Elemental analysis was carried out using a Perkin Elmer–2400 CHN analyser. The compounds **(Ia-h)** were prepared according to the reported method [7]

General method for the preparation of 2,6-dimethyl-4-(3'-nitrophenyl)-5-carbmethoxy-3-[5"-(substituted phenyl) isoxazole-3"-yl]-1,4-dihydropyridines(2):

To a solution of anhydrous sodium acetate (0.73 g, 0.01 mol) dissolved in minimum amount of hot acetic acid,a solution of hydroxylamine hydrochloride (0.79 g, 0.01 mol) in ethanol (10 ml) was added. The reaction mixture was added to a solution of **(1)**(0.01 mol) in ethanol (15 ml). The reaction mixture was heated on a steam bath for 12 h. After completion of reaction, it was cooled and poured into ice-cold water and neutralized with ammonia to get a solid mass. The product so obtained was crystallized from absolute ethanol in order to get a pure product. Eight such compounds **(2a-h)** were synthesized and characterized

(Table 1).

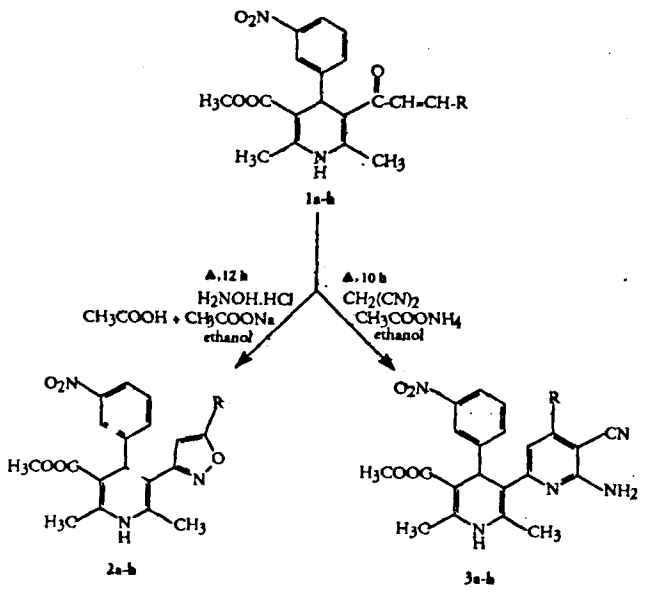
Following the above procedure, 2,6-dimethyl-4-(3'-nitrophenyl)-5-carbmethoxy-3-[5"-4"-methoxy phenyl) isoxazole-3"-yl]-1,4-dihydropyridine **(2a)** was obtained as a crystalline product. IR (KBr): 3346(b), 1527, 1346(s), 1704(s) cm^{-1}; PMR (300 MHz, DMSO-d_6 + $CDCl_3$): 2.28 (s, 6H, 2xCH_3), 3.58 (s, 3H, OCH_3), 3.66 (s,3H, $COOCH_3$), 5.39 (s, 1H, C_4-H), 6.86-7.36 (m, 8H, Ar-H) δ ppm; MS(m/z):461(M^+,5%)327(15%),287(10%), 224(100%), 166(23%), 102(18%); (Found: C 65.10; H 5.02; N 9.05; $C_{25}H_{23}N_3O_6$, required: C 65.07;H 4.99;N 9.11)

General method for the preparation of 2,6-dimethyl-4-(3'-nitrophenyl)-5-carbmethoxy 3-[2"-amino-4"-(4""-substituted phenyl)-3"-cyanopyridine-6"-yl]-1,4-dihdropyridines(3):

A mixture of **(1)** (0.01 mo;) , malononitrile (0.66 g, 0.01 mol) and ammonium acetate (6.16 g,0.08 mol) in 25 ml of ethanol was refluxed for 10 h. The reaction mass was cooled and concentrated. The solid seperated was filtered and dried. The product so obtained was crystallized from absolute ethonal so as to obtain a pure crystalline product. Eight such compounds **(3a-h)** were synthesized and characterized (Table-1).

Following the above procedure 2,6-dimethyl-4-3'-nitro- phenyl)5-carbmethoxy-3-[2"-amino-4"-(4""-methoxyphenyl)-3"-cyanopyridine-6"-yl]-1-4-dihydropyridine **(3a)** was obtained as white crystals IR (KBr): 3351(b), 3250(b),2200(s), 1528(s),1346 (s),1704(s) cm^{-1}, PMR (300MHz,DMSO-d_6+$CDCl_3$): 2.35 (s,6H,2xCH_3), 3.63 (s,3H,OCH_3), 3.68 (s3H,$COOCH_3$) 5.07 (s,1H,c4-H), 6.79-8.16 (m,9H, Ar-H) δ ppm; MS (m/z): 51(M^+,5%) 373 (6%), 332 (100%), 243(4%), 133(6%); Found: C;H;N 13.61; $C_{28}H_{25}N_5O_5$, required C 65.75; H 4.89; N 13.69)

REACTION SCHEME



Antitubercular activity:

Antitubercular activity was determined by using the modified Bactec 460 system[10] Stock solution as test compounds were prepared in dimethylsulfoxide (DMSO) at 1 mg/ml and sterilized by passage through 0.22 μm PTFE filter (Millex – FG, Millipore Bedford, MA). Fifty microliters was added to 4 ml radiometric $7H_{12}$ broth (Bactec 12B; Becton Dickinson Diagnostic Instrument System, Sparks, MD) to achieve a final concentration of 12.5 μg/ml. Controls received 50 μl DMSO. Rifampicin (Sigma Chemicals Co., St. Louis, MO) was included as a positive drug control. Rifampicin was solublized and diluted in DMSO and added to BACTEC-12 broth to achieve a range of concentration for determination of Minimum Inhibitory Concentration (MIC, lowest concentration inhibiting 99% of the inoculum).

M. tuberculosis ($H_{37}Rv$), (ATCC 27294; American type culture collection, Rockville MD)was cultured at 37° on a rotary shaker in Middlebrook $7H_9$ broth (Difco Laboratories, Detroite, MI) supplemented with 0.2 v/v glycerol and 0.05 v/v Tween 80 until the culture turbidity achieved an optical density of 0.45-0.55 at 550 nm. Bacteria were then pelleted by centrifugation, washed twice and resuspended in one fifth the original volume in Dulbecco's phosphate buffered saline (PBS, Irvine Scientific Santa, Nalgene, Rochester, NY) and aliquots were frozen at – 80°. Culture was thawed and an appropriate dilution performed such that a Bactec-12B vial inoculated with a 0.1 ml would reach a growth index (GI) of 999 in 5 d. One tenth of the diluted inoculum was used to inoculate 4 ml fresh Bactec 12B broth containing the test compounds. An additional control vial was included which received a further 1:100 diluted inoculum (as well as 50 μl DMSO) for use in calculating the MIC of rifampicin, respectively by established procedures.

Culture was incubated in 37° and the GI determined daily until control cultures achieved a GI of 999. Assays were usually completed in 5-8 d. Percent inhibition was defined as 1-(GI of test sample/GI of control)x100. Minimum inhibitory concentration of the compound effecting a reduction in daily change in GI, which was less that

TABLE 1: PHYSICAL, ANALYTICAL AND ANTITUBERCULAR ACTIVITY DATA OF COMPOUNDS 2a-h AND 3a-h.

Compd.	R.	M.P. *	Yield %	Mol. Formula (mol. wt.)	% of nitrogen$		Antitubercular Activity % Inhibition
					Calcd.	Found	
2a	4 – methoxy phenyl	117	36	$C_{25}H_{23}N_3O_6$ (461)	9.11	9.00	39
2b	2– methoxy phenyl	92	32	$C_{25}H_{23}N_3O_6$ (461)	9.11	9.18	49
2c	2 – nitro phenyl	102	56	$C_{24}H_{26}N_4O_7$ (482)	11.76	11.70	3
2d	3,4-dichloro phenyl	110	34	$C_{24}H_{19}N_3O_5Cl_2$ (500)	8.40	8.25	46
2e	3–nitro phenyl	132	52	$C_{24}H_{20}N_4O_7$ (476)	11.76	11.65	7
2f	Phenyl	115	57	$C_{24}H_{21}N_3O_5$ (431)	9.74	9.68	63
2g	Furyl	112	38	$C_{22}H_{19}N_3O_6$ (421)	9.97	9.85	0
2h	4-N,N-dimethylamino phenyl	100	60	$C_{26}H_{26}N_4O_5$ (474)	11.81	11.72	42
3a	4 – methoxy phenyl	157	57	$C_{28}H_{25}N_5O_5$ (511)	13.70	13.72	0
3b	2 - methoxy phenyl	168	58	$C_{28}H_{25}N_5O_5$ (511)	13.70	13.79	0
3c	2-nitro phenyl	146	60	$C_{27}H_{22}N_6O_6$ (526)	16.00	16.12	0
3d	3-4-dichloro phenyl	157	61	$C_{27}H_{21}N_5O_4Cl_2$ (550)	12.73	12.76	3
3e	3 – nitro phenyl	151	62	$C_{27}H_{22}N_6O_6$ (526)	15.96	16.05	0
3f	Phenyl	162	39	$C_{27}H_{23}N_5O_4$ (481)	14.55	14.50	0
3g	Furyl	174	47	$C_{25}H_{21}N_5O_5$ (471)	14.86	14.80	0
3h	4-N,N-Dimethylamino Phenyl	137	37	$C_{29}H_{28}N_6O_4$ (524)	16.03	16.14	
	Rifampicin (standard drug)	-	-	-	-	-	>98

*Recrystallized from absolute alcohol. $CHN analysis indicated that the calculated and observed- values were within the acceptable limits.

observed with a 1:100 diluted control culture on day the latter reached a GI of a least 30.

RESULTS AND DISCUSSION

The compounds **(Ia-h)** possessing α,β- unsaturated ketones at C_3 position of DHP, which are believed to the most important factor leading to cyclisation affording isoxazoles with the help of hydroxylamine hydrochloride. It was found that during the reaction, firstly the oxime was formed by the condensation of ketone to hydroxylamine and then oxime readily loses the water molecules followed by cyclization to give corresponding isoxazole **(2a-h)**. Acetic acid and sodium acetate were used as buffer. Further it was also observed that α,β unsaturated ketones **(Ia-h)** condensed with malononitrile in presence of ammonium acetate gave corresponding cyanopyridine analogs of DHP **(3a-H)**.

The comparative study of newly synthesized two series revealed that while isoxozolyl system present at the 3rd position of 1,4-dihydropyridine exhibit moderate actvity, pyridine ring system at the same position exhibited very feeble activity against *M. tuberculosis* strain. The presence of six member aromatic ring instead of five-member ring at 3rd position of 1,4-dihydropyridine does not contribute to the activity. Further, the substitution pattern on aromatic ring attached to the isoxazole system also gave the clue that within the same series, the presence of electron withdrawing group is not favoured. It was also observed that the presence of substituents in parent isoxazole skeleton should not be considered as an essential part, because in case of compound **2f**, the phenyl ring has no substituents and yet possesses highest activity in the same series.

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