Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 1993–1998

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and QSAR Studies of 4-Substituted Phenyl-2,6-dimethyl-3, 5-Bis-*N*-(substituted Phenyl)carbamoyl-1,4-dihydropyridines as Potential Antitubercular Agents†

Bhavik Desai,[a] Dinesh Sureja,[a] Yogesh Naliapara,[a] Anamik Shah[a] and Anil K. Saxena[b,*]

[a]*Department of Chemistry, Saurashtra University, Rajkot-360 005, India*
[b]*Medicinal Chemistry Division, Central Drug Research Institute, Lucknow-226 001, India*

Received 6 December 2000; accepted 3 March 2001

Abstract—Synthesis and QSAR studies of the title compounds have resulted in the identification of structural and physicochemical parameters (MR, σ^o, σ^m, σ^p) contributing to antitubercular activity. Among these, carbamoyl phenyl ring substituted at 3 and 4 position with NO_2 group or 2 position with Cl or OCH_3 group shows >90% inhibition against $H_{37}Rv$ comparable to other substituted phenyls.

Introduction

The acid-fast bacillus Mycobacterium tuberculosis is the causative agent of tuberculosis (TB). The tubercle bacillus is a slow growing organism, which does not elicit a sharp and massive reaction from the host. The tubercle bacillus does not produce any substance, which is toxic to the normal host. It acts as an irritating foreign body and tubercle formation can be produced by virulent, avirulent and nonpathogenic types. The tubercle bacillus is an intracellular parasite, and lives and grows within the host's tissue cells, macrophages and epithelial cells. The recent emergence of outbreaks of multidrug resistant tuberculosis (MDR-TB) pose a serious threat to the treatment of the disease.[1,2] TB will undoubtedly increase in prevalence in most countries due to the human immunodeficiency virus (HIV) epidemic. In response to these alarming statistics and trends, WHO declared TB to be a global public health emergency.[3] Antitubercular drugs available for the treatment were discovered in the period of 1945–1965. No new drugs were synthesized during the last few decades. There are millions of patients suffering from tuberculosis, this tells us about the necessity of searching for and synthesizing new highly active compounds with less side-effects. The search for new anti-tubercular drugs may be done using the Biochemical and Chemical methods.

The preparation and properties of the 3-acetyl pyridine analogue of Nicotinamide Adenine Dinucleotide (NAD) were reported in 1956.[4] The production of the Isonicotinic acid (INA) analogue of NAD disturbs the normal cellular metabolism. The analogue is probably incapable of participating in redox reactions. Goldman[5] has demonstrated that the Isoniazide (INH) analogue of NAD was not reduced by dithionite or in several enzymatic assay systems and did not form a dihydropyridine with cyanide ion. In contrast to this, the 3-acetyl pyridine analogue described by Kaplan et al.[4] is reduced by $S_2O_4^{2-}$, CN^- and yeast alcohol dehydrogenase. The disturbance of metabolism by INH or INA apparently leads to a breakdown of mycolic acid synthesis[6,7] and damage of the cell wall structure; the latter is evidenced by the loss of "acid-fastness".

The pyridine derivatives substituted with alkylated tetrazoles at 3 and 5 position showed better in vitro activity than pyrazinamide against $H_{37}Rv$ *M. Tuberculosis* activity.[8] It was also observed that the compounds designed as lipophilic precursors were more active than the unmodified polar isosteres of pyrazinoic acid and nicotinic acid which may be due to better penetration of the compound into the cell wall of *M. Tuberculosis*. In view of this, it appeared of interest to synthesise some new 1,4-dihydropyridine-3,5-dicarbamoyl derivatives

†CDRI Communication No. 6119

*Corresponding author. Tel.: +91-0522-212411-18, extn. 4268; fax: +91-0522-223405; e-mail: anilsak@hotmail.com

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00141-9

with lipophilic groups. These compounds may act as precursors, and after penetration into the cell wall may lead to the 3,5-carboxylate anions by enzymatic hydrolysis. All these derivatives were screened for their antitubercular activity against *M. Tuberculosis* $H_{37}Rv$ (ATCC 27294; American type culture collection, Rockville, MD). Among them, some derivatives showed >90% inhibition comparable to rifampicin. In order to understand the structure–activity relationship, the QSAR analysis based on physicochemical approach has been performed and the results are reported in this paper.

Chemistry

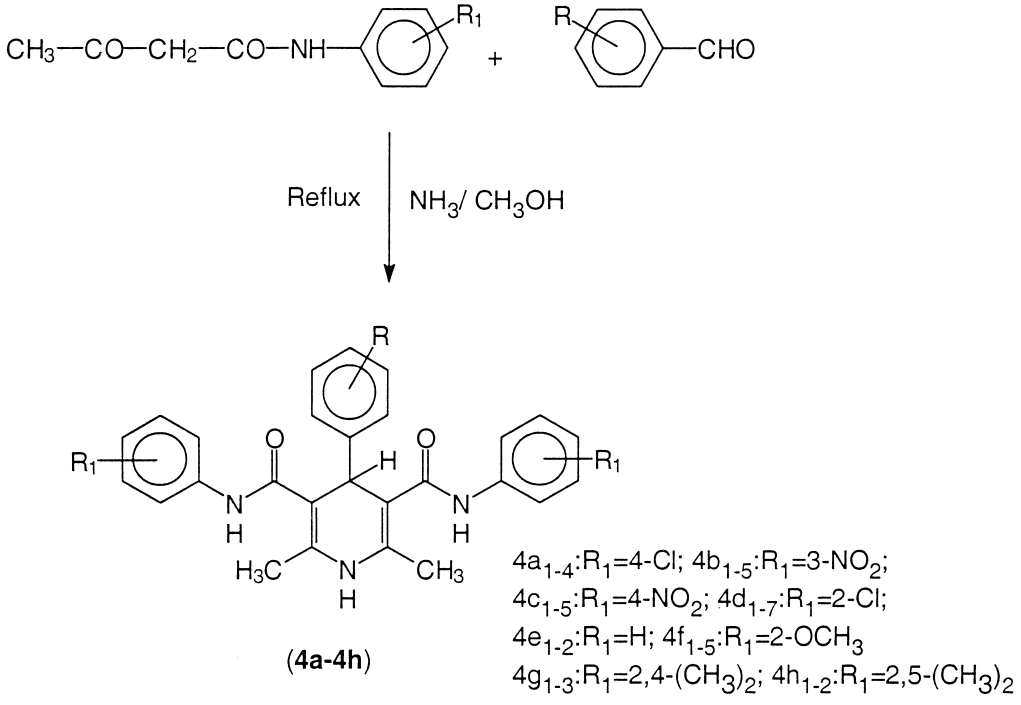
The key intermediate acetoacetanilides were synthesized by simple condensation of ethyl acetoacetate with appropriate aryl amines in toluene according to the reported method.[9] Condensation[10] of acetanilides with different aromatic aldehydes in methanol with excess amount of ammonia (25%) afforded the required title products according to the reaction Scheme 1. Physicochemical parameters and biological activity data is shown in Table 1.

Results and Discussion

In order to establish the quantitative structure–activity relationship (QSAR) the antitubercular activity data at a fixed concentration (12.5 μg/mL) was transformed as log (p/100-p) and was used as a dependent variable. It was first correlated with different topological parameters ($^1\chi$, $^2\chi_v$, information content IC) and physicochemical parameter like hydrophobicity (π), steric (MR) and electronic (σ) parameters. The values of the physicochemical parameters used were taken from the literature,[11] and multiparameter regression analysis was carried out on PC-486 using SYSTAT (version 7.0) software.[12] Preliminary QSAR analysis showed no significant correlation ($r<0.3$) with topological and hydrophobic (π) parameter, however the steric parameter; molar refractivity (MR) for the substituent at the phenyl ring present at 4-position ($r=0.437$) and electronic parameter (σ) for the substituents present at the phenyl ring of carbamoyl group present at 3 and 5 positions showed some correlation ($r=0.3$–0.5) (Table 2). In view of orthogonality among the MR parameter for the substituent R at 4-phenyl group and the electronic Hammett parameters for the ortho (σ^o), meta (σ^m) and para (σ^p) position for the substituent R_1 of the carbamoyl phenyl group, stepwise regression analysis of different combinations of these parameters were studied which led to the derivation of the following equation, with best correlation ($r=0.917$) of high statistical >99.9% significance ($F_{4,28\ \propto\ 0.01}=6.97$, $F_{4,28}=36.983$) also with statistically >99.9% significant value of all the regression coefficients. The calculated activities for the compounds by eq 1 were in good agreement

$$\text{Log c} = 0.733(\pm0.117)\sigma^{p}_{R1} + 1.153\ (\pm0.131)\sigma^{m}_{R1} - 0.974\ (\pm0.204)\sigma^{o}_{R1} + 0.078(\pm0.014)\ \text{MR} - 1.070(\pm0.199)$$
$$n = 33, \quad r = 0.917, \quad s = 0.376, \quad F = 36.983 \quad (1)$$

with the observed activity (Table 2) (Fig. 1). The Robertson of this equation was also checked by leave one out cross validated r^2_{cv} value (0.776) and the predicted activity data for these compounds was also in good agreement with the observed activity (Table 2, Fig. 2).



Scheme 1.

Table 1. Physicochemical and antitubercular activity data of the compounds **4a**–**4h**

Compd	R	M.P. (°C)	Yield (%)	Molecular formula	%Inhibition (p) at 2.5 μg/ml	Log c = log[p/(100−p)]
4a$_1$	3-NO_2	190	59	$C_{27}H_{22}Cl_2N_4O_4$	29	−0.3888
4a$_2$	2-OH,4-OCH_3	225	65	$C_{28}H_{25}Cl_2N_3O_4$	81	0.6297
4a$_3$	4-OH	218	62	$C_{27}H_{23}Cl_2N_3O_3$	79	0.5754
4a$_4$	3,4,5-$(OCH_3)_3$	172	57	$C_{30}H_{29}Cl_2N_3O_5$	94	1.1949
4b$_1$	4-SCH_3	226	49	$C_{28}H_{25}N_5O_6S$	98	1.6901
4b$_2$	2-Cl	220	63	$C_{27}H_{22}ClN_5O_6$	97	1.5096
4b$_3$	2,4-$(Cl)_2$	156	65	$C_{27}H_{21}Cl_2N_5O_6$	98	1.6901
4b$_4$	H	153	80	$C_{27}H_{23}N_5O_6$	94	1.1949
4b$_5$	2-OH	204	68	$C_{27}H_{23}N_5O_7$	92	1.0606
4c$_1$	2,4-$(Cl)_2$	130	65	$C_{27}H_{21}Cl_2N_5O_6$	96	1.3802
4c$_2$	2-Cl	215	62	$C_{27}H_{22}ClN_5O_6$	95	1.2787
4c$_3$	4-$N(CH_3)_2$	240	68	$C_{29}H_{28}N_6O_6$	97	1.5096
4c$_4$	4-SCH_3	210	72	$C_{28}H_{25}N_5O_6S$	97	1.5096
4c$_5$	4-Cl	145	68	$C_{27}H_{22}ClN_5O_6$	62	0.2126
4d$_1$	4-OCH_3	199	71	$C_{28}H_{25}Cl_2N_3O_3$	30	−0.3679
4d$_2$	4-SCH_3	196	50	$C_{28}H_{25}Cl_2N_3O_2S$	78	0.5496
4d$_3$	2-Cl	221	62	$C_{27}H_{22}Cl_3N_3O_2$	18	−0.6585
4d$_4$	4-Cl	223	65	$C_{27}H_{22}Cl_3N_3O_2$	17	−0.6886
4d$_5$	2,4-$(Cl)_2$	215	65	$C_{27}H_{21}Cl_4N_3O_2$	11	−0.9079
4d$_6$	2-SH	212	53	$C_{27}H_{23}Cl_2N_3O_2S$	10	−0.9542
4d$_7$	2-OH	205	58	$C_{27}H_{23}Cl_2N_3O_3$	6	−1.1949
4e$_1$	3-NO_2	217	65	$C_{27}H_{24}N_4O_4$	75	0.4771
4e$_2$	4-SCH_3	216	63	$C_{28}H_{27}N_3O_2S$	69	0.3474
4f$_1$	4-$N(CH_3)_2$	232	45	$C_{31}H_{34}N_4O_4$	93	1.1233
4f$_2$	4-SCH_3	222	62	$C_{30}H_{31}N_3O_4S$	93	1.1233
4f$_3$	2,4-$(Cl)_2$	205	58	$C_{29}H_{27}Cl_2N_3O_4$	91	1.0047
4f$_4$	3-NO_2	220	58	$C_{29}H_{28}N_4O_6$	64	0.2498
4f$_5$	2-Cl	170	61	$C_{29}H_{28}ClN_3O_4$	37	−0.2311
4g$_1$	4-SCH_3	222	68	$C_{32}H_{35}N_3O_2S$	59	0.1580
4g$_2$	H	225	53	$C_{31}H_{33}N_3O_2$	12	−0.8653
4g$_3$	3-NO_2	230	68	$C_{31}H_{32}N_4O_4$	53	0.0521
4h$_1$	2-OH	200	39	$C_{31}H_{33}N_3O_3$	27	−0.4319
4h$_2$	4-OCH_3	265	72	$C_{32}H_{35}N_3O_3$	41	−0.1580

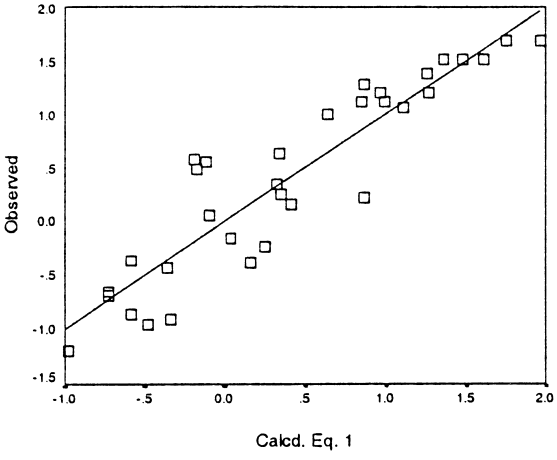


Figure 1. Relationship between observed and calculated activity.

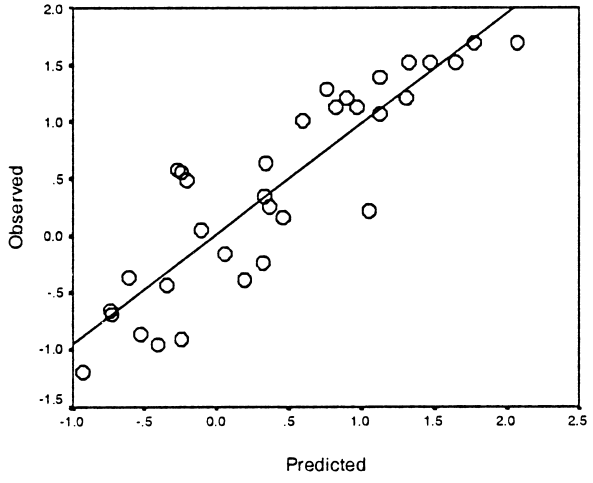


Figure 2. Relationship between observed and predicted activity.

Conclusion

These results indicate that the presence of bulkier substituents in the phenyl ring at 4-position of dihydropyridine positively contributes for the activity possibly due to steric interaction in polar space suggesting that the phenyl ring at 4-position may be involved in binding of these molecules with the target. The electronic influence of the substituents at the carbamoyl phenyl ring present at 3 and 5 position of DHP is important for antitubercular activity, in the order $\sigma^m > \sigma^p > \sigma^o$. The presence of the electron withdrawing groups at *meta* and para positions increases activity while at *ortho* position decrease the activity. This electronic effect may influence the enzymatic hydrolysis of the amide bond present at 3 and 5 position of substituted DHP to corresponding acids inside the *M. Tuberculosis*.

Table 2. Topological and physicochemical parameter of the compounds **4a–4h**

Comp.	MR_R	σ^{o}_{R1}	σ^{m}_{R1}	σ^{p}_{R1}	Activity calcd eq 1	Activity predicted
$4a_1$	11.48	0.0	0.0	0.46	0.1626	0.1923
$4a_2$	13.81	0.0	0.0	0.46	0.3444	0.3387
$4a_3$	6.97	0.0	0.0	0.46	−0.1891	−0.2695
$4a_4$	25.67	0.0	0.0	0.46	1.2694	1.3076
$4b_1$	17.94	0.0	1.42	0.0	1.9666	2.0741
$4b_2$	10.15	0.0	1.42	0.0	1.3595	1.3251
$4b_3$	15.15	0.0	1.42	0.0	1.7490	1.7724
$4b_4$	5.15	0.0	1.42	0.0	0.9690	0.8945
$4b_5$	6.97	0.0	1.42	0.0	1.1109	1.1281
$4c_1$	15.15	0.0	0.0	1.56	1.2552	1.1249
$4c_2$	10.15	0.0	0.0	1.56	0.8652	0.7565
$4c_3$	19.67	0.0	0.0	1.56	1.6077	1.6446
$4c_4$	17.94	0.0	0.0	1.56	1.4728	1.4730
$4c_5$	10.15	0.0	0.0	1.56	0.8652	1.0507
$4d_1$	11.99	0.46	0.0	0.0	−0.5828	−0.6083
$4d_2$	17.94	0.46	0.0	0.0	−0.1187	−0.2471
$4d_3$	10.15	0.46	0.0	0.0	−0.7263	−0.7323
$4d_4$	10.15	0.46	0.0	0.0	−0.7263	−0.7277
$4d_5$	15.15	0.46	0.0	0.0	−0.3363	−0.2398
$4d_6$	13.34	0.46	0.0	0.0	−0.4775	−0.4032
$4d_7$	6.97	0.46	0.0	0.0	−0.9744	−0.9278
$4e_1$	11.48	0.0	0.0	0.0	−0.1746	−0.2027
$4e_2$	17.94	0.0	0.0	0.0	0.3293	0.3352
$4f_1$	19.67	−0.54	0.0	0.0	0.9902	0.9730
$4f_2$	17.94	−0.54	0.0	0.0	0.8553	0.8202
$4f_3$	15.15	−0.54	0.0	0.0	0.6376	0.5969
$4f_4$	11.48	−0.54	0.0	0.0	0.3514	0.3695
$4f_5$	10.15	−0.54	0.0	0.0	0.2477	0.3209
$4g_1$	17.94	−0.34	0.0	−0.34	0.4113	0.4570
$4g_2$	5.15	−0.34	0.0	−0.34	−0.5864	−0.52512
$4g_3$	11.48	−0.34	0.0	−0.34	−0.0926	−0.1032
$4h_1$	6.97	−0.34	−0.14	0.0	−0.3566	−0.3420
$4h_2$	11.99	−0.34	−0.14	0.0	0.0413	0.0562

Pearson correlation matrix

	MR	σ^{o}	σ^{m}	σ^{p}	Logc
MR	1.000				
σ^{o}	−0.071	1.000			
σ^{m}	−0.159	0.062	1.000		
σ^{p}	0.184	0.105	−0.181	1.000	
Logc	0.437	−0.301	0.503	0.407	1.000

Experimental

Melting points were determined on an electro thermal capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC spectrometer at 300 MHz in $CDCl_3$ + DMSO-d_6 with TMS as internal standard and Hitachi 1200 NMR Spectrometer at 60 MHz using TFA as solvent. IR spectra (ν_{max} in cm^{-1}) were recorded on Shimadzu FT-IR 8101 (KBr pellets). All compounds were checked for their homogeneity by TLC using silica as stationary phase. All compounds were analyzed for C, H and N contents on a Carlo Erba Strum. DP200 analyser and observed values were within ±0.4% of the calculated values. Mass spectra were run on a Jeol JMS D300 instrument using direct inlet system.

General procedure for the preparation of the title compounds

A mixture of acetoacetanilide (0.02 mol) and aldehyde (0.01 mol) was dissolved in methanol. 5–7 mL of ammonia (25%) solution was added and it was refluxed for 5–6 h. A further addition of 2–3 mL of ammonia was carried out at every 3–4 h interval and reflux was further continued for 24 h and kept overnight. The crystalline product was separated out. It was then filtered, washed with chilled methanol 2–3 times and dried. It was recrystallized from suitable solvent. All products were found to be light sensitive and darken on exposure to light. Select data for one compound of each type is given below.

4′-Nitrophenyl-2,6-dimethyl-3,5-bis-*N*-[3′-chlorophenyl]-carbamoyl-1,4-dihydro-pyridine ($4a_1$). MS:*m*/*z* 537(M^+). ^{1}H NMR (60 MHz, TFA) δ: 2.312 (s, 6H, 2×CH_3), 5.631 (s, 1H, CH), 8.425 (brs, 2H, 2×CONH), 5.968 (brs, 1H, NH), 7.401–7.821 (m, 12H, ArH). IR(KBr, c m^{-1}): 3210 (DHP NH), 3367 (amide NH), 1690 (amido C=O).

4′-Methylthio phenyl-2, 6-dimethyl-3,5-bis-*N*-[3′-nitrophenyl] carbamoyl-1,4-dihydropyridine ($4b_1$). MS: *m*/*z* 511 (M^+). ^{1}H NMR (60 MHz, TFA) δ: 2.493 (s, 6H, 2×CH_3), 2.784 (s,3H,SCH_3), 4.901 (s, 1H, CH), 5.642 (brs, 1H, NH), 8.854 (s, 2H, 2×CONH), 7.260–7.843 (m, 12H, ArH) IR (KBr, cm^{-1}): 3200 (DHP NH), 3373 (amide NH), 1700 (amido C=O).

2′,4′-Dichloro phenyl-2, 6-dimethyl-3,5-bis-*N*-[4′-nitrophenyl] carbamoyl-1,4-dihydropyridine ($4c_1$). MS: *m*/*z* 582 (M^+). ^1H NMR (60 MHz, TFA) δ: 2.452 (s, 6H, 2×CH_3), 5.502 (s,1H,CH), 5.898 (brs, 1H, NH), 8.912 (brs, 2H, 2×CONH), 7.012–8.122 (m, 10H, ArH) IR (KBr, cm^{-1}): 3198 (DHP NH), 3355 (amide NH), 1680 (amido C=O).

4′-Methoxy phenyl-2,6-dimethyl-3,5-bis-*N*-[2′-chloro phenyl] carbamoyl-1,4-dihydropyridine ($4d_1$). MS: *m*/*z* 522 (M^+). ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 2.272 (s, 6H, 2×CH_3), 3.751 (s, 3H, OCH_3), 4.840 (s, 1H, CH), 5.682 (brs, 1H, NH), 8.205 (brs, 2H, 2×CONH), 6.825–7.720 (m, 12H, ArH) IR (KBr, cm^{-1}): 3215 (DHP NH), 3352 (amide NH), 1680 (amido C=O).

3′-Nitrophenyl-2,6-dimethyl-3,5-bis-*N*-[phenyl] carbamoyl-1,4-dihydropyridine ($4e_1$). MS: *m*/*z* 468 (M^+). ^1H NMR (60 MHz, TFA) δ: 2.311 (s, 6H, 2×CH_3), 5.505 (s,1H,CH), 5.789 (brs, 1H, NH), 8.429 (brs, 2H, 2×CONH), 7.228–7.653 (m, 14H, ArH) IR (KBr, cm^{-1}): 3210 (DHP NH), 3327 (amide NH), 1701 (amido C=O).

4′-*N*,*N*-dimethylaminophenyl-2,6-dimethyl-3,5-bis-*N*-[2′-methoxyphenyl]carbamoyl-1,4-dihydropyridine ($4f_1$). MS: *m*/*z* 526 (M^+). ^1H NMR (60 MHz, TFA) δ: 2.308 (s, 6H, 2×CH_3), 2.689 (s, 3H, SCH_3), 3.618 (s, 3H, OCH_3), 5.625 (s, 1H, CH), 6.024 (brs, 1H, NH), 8.385 (brs, 2H, 2×CONH), 7.296–7.853 (m, 12H, ArH) IR (KBr, cm^{-1}): 3233 (DHP NH), 3370 (amide NH), 1702 (amido C=O).

2′-Methylthio phenyl-2,6-dimethyl-3,5-bis-*N*-[2′,4′-dimethyl phenyl] carbamoyl-1,4-dihydropyridine,($4g_1$). MS: *m*/*z* 525 (M^+). ^1H NMR (60 MHz,TFA) δ: 2.231 (s, 3H, $ArCH_3$), 2.334 (s, 3H, $ArCH_3$), 2.451 (s, 3H, CH_3), 5.542 (s, 1H, CH), 2.823 (s,3H,SCH_3), 5.979 (brs, 1H, NH), 7.239–7.985 (m, 10H, ArH) IR (KBr, cm^{-1}): 3200 (DHP NH), 3352 (amide NH), 1690 (amido C=O).

2′-hydroxy phenyl-2,6-dimethyl-3,5-bis-*N*-[2′,5′-dimethyl phenyl] carbamoyl-1,4-dihydropyridine ($4h_1$). MS: *m*/*z* 495 (M^+). ^1H NMR (60 MHz, TFA) δ: 2.189 (s,3H,$ArCH_3$), 2.315 (s, 3H, $ArCH_3$), 2.597 (s, 3H, CH_3), 5.506 (s, 1H, CH), 7.110–7.899 (m, 10H, ArH) IR (KBr, cm^{-1}): 3225 (DHP NH), 3358 (amide NH), 1689 (amido C=O).

Biological activity

Antitubercular activity was determined using the modified BACTEC 460 system in which stock solution of test compounds were prepared in dimethylsulfoxide (DMSO) at 1mg/mL and sterilized by passage through 0.22 μm PFTE filters (Millex-FG, Millipore, Bedford, MA) 50 mL was added to 4 mL radiometric $7H_{12}$ broth (BACTEC 12B, Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) to achieve a final concentration of 12.5 μg/mL. Controls received 50 μL DMSO. Rifampicin (Sigma Chemical Co., St. Louis, MO) was included as a positive drug control. Rifampicin was solubilized and diluted in DMSO and added to BACTEC 12 broth to achieve a range of concentrations for determination of minimum inhibitory concentration (MIC, lowest concentration inhibiting 99% of the inoculums; MIC value of Rifampicin is 0.25 g/mL @ 95% Inhibition of $H_{37}Rv$ strain).

M. Tuberculosis $H_{37}Rv$ (ATCC 27294; American type culture collection, Rockville, MD) was cultured at 37 °C on a rotary shaker in middle brook $7H_9$ broth (Difco laboratories, Detroit, 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 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 Ana, (A)]. Large bacterial clumps were removed by passage through an 8 μm filter (Malgene, Rochester, NY) and aliquots were frozen at −80 °C. Culture was thawed and an appropriate dilution performed such that a BACTEC 12 B vial inoculated with 0.1 mL would reach a growth index (GI) of 999 in 5 days. One tenth of the diluted inoculum was used to inoculate. Four millilitres of fresh BACTEC 12 B 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 establishing procedures.

Cultures were incubated at 37 °C and the GI determined daily until control cultures achieved a GI of 999. Assays were usually completed in 5–8 days. Percent inhibition was defined as 1−(GI of test sample/GI of control)×100. Minimum inhibitory concentration of compound effecting a reduction in daily change in GI, which was less than that, observed with a 1:100 diluted control culture on day the latter reached a GI of at least 30.

Acknowledgements

The authors are very thankful to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), funded by the National Institute of Allergy and Infectious Diseases, a division of the National Institute of Health, USA, for providing biological screening results. The authors are thankful to the director, CDRI, Lucknow for providing necessary training and computer facilities. The authors are thankful to Mr. A. S. Kushwaha for excellent technical assistance. One of us (BD) is thankful to the state government for research fellowship.

References and Notes

1. Bloom, B. R.; Murray, C. J. L. *Science* **1992**, *257*, 1055.
2. WHO report on the Tuberculosis Epidemic stop TB at the source. Tuberculosis Programme World Health Organization, Geneva, Switzerland, 1995.

3. Raviglione, M. C.; Snider, D. E., Jr.; Kochi, A. *JAMA* **1995**, *273*, 220.
4. Kaplan, N. O.; Ciotti, M. M. *J. Biol. Chem.* **1956**, *221*, 823.
5. Goldman, D. S. *J. Am. Chem. Soc.* **1954**, *76*, 2841.
6. Winder, F. G.; Collins, P. *Am. Rev. Respir. Dis.* **1969**, *100*, 101.
7. Takayama, K.; Ann, N. Y. *Acad. Sci.* **1974**, *235*, 426.
8. Arnold, M.; Gerald, A. W.; Matt, C. D.; Scott, G. F. *J. Med. Chem.* **1998**, *41*, 2436.
9. Jadhav, G. V. *J. Indian Chem. Soc.* **1930**, *7*, 669.
10. Swamy, S. K.; Reddy, T. M.; Reddy, V. M. *Indian J. Pharm. Sci.* **1998**, *60*, 102.
11. Hansch, C.; Leo, A. Substituent constants for Correlation Analysis in Chemistry and Biology, 1983.
12. SYSTAT, version 7.0, SPSS Inc., 444 North Michigan Avenue, Chicago, IL 60611, USA.