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Synthesis and evaluation of anti viral, anti tubercular and anticancer activities of some novel thioureas derived from 4-aminobenzohydrazidehydrazones

Mr. Sanjay Kumar Gupta[1], Dr. Parameshwar H[2], Mr. Mohammed Kafeel Urrahman Khan[3]

Associate professor[1,2]. Assistant professor[3]
Department of Pharmaceutics,,
Global College of Pharmacy, Hyderabad. Chilkur (V), Moinabad (M), Telangana- 501504.

ABSTRACT: A class of unique 1-[4-[[2-[(4-substituted phenyl)methylene]hydrazino]carbonyl]p henyl] (By condensing 4-aminobenzoic acid hydrozide with 4-fluorobenzaldehyde or 4-(trifluoromethyl)benzaldeyde, substituted aryl isothiocyanates were added to 4-amino-N'-[(4-substituted phenyl) methylene] benzohydrazide, resulting in -3-substituted thiourea derivatives. We used HeLa, Vero, or HEL cell cultures to test all of the produced compounds in vitro against HIV-1(IIIB) and HIV-2(ROD) strains in MT-4 cells, along with additional chosen viruses including HSV-1, HSV-2, Coxsackie virus B4, Sindbis virus, human cytomegalovirus, and varicella-zoster virus. They also tested it for antimycobacterial activity against Mycobacterium tuberculosis H37Rv.The synthetic compounds were tested for anticancer activity and cytotoxicity using A549 and L929 cell lines.

KEY WORDS: Hydrazones, Thioureas, Antiviral activity, Anticancer activity, Mycobacterium tuberculosisH37Rv

INTRODUCTION

There have been reports of thiacetazone, a tuberculostatic drug, which has a thiosemicarba-zone structure (1). A well-known inhibitor of Mycobacterium tuberculosis is thiocarlide, which is N,NĻ-bis[p-(isoamyloxy) phenyl]-thiourea (2). Thiocarlide structural analogues, N-D-aldopentofuranosyl-NĻ-[p-(isoamyloxy)phenyl] thioureaderivatives, have been shown to be more effective than thiocarlide itself, according to recent reports (3). As seen in Figure 1, methisazone was one of the first antiviral compounds to be utilized in an inclinational fashion. This medicine is effective in preventing the spread of many viral infections. Several 4-aminobenzoic acid-substituted benzalhydrazones have shown antitubercular properties (5).The antioxidant activity of several thiourea derivatives derived from isonicotinoyl hydrazone has been recently reported by Sriram and colleagues.

(6).Some hydrazide-hydrazones and thiou-reas show antitumor (7,8) and antitubercular (9–11) effects.A number of thiou-rea derivatives have also been shown to inhibit the activity of influenza virus nuclease, coxsackie B4 virus, and thymidine kinase positive varicella-zoster virus (TK+ VZV, OKA strain) (12,13).

In continuation of our earlier work on 4-aminobenzoic acid hydrazones (14) and various thiourea derivatives (15,16), we have synthesized a series of novel thioureas incorporating hydrazide-hydrazone and disubstituted thiourea moieties into a single structure. These were derived from 4-amino-N'-[[4-fluoro/4-(trifluoromethyl) phenyl] methylene] benzohydrazide, and their antitubercular, antiviral, and anticancer efficacy were assessed. We used HeLa, Vero, or human embryonic lung (HEL) cells to screen all of the synthesized compounds in vitro against HIV-1 (IIIB) and HIV-2 (ROD) strains in MT-4 cells, as well as other selected viruses like HSV-1, HSV-2, Coxsackie B4 virus, Sindbis virus, cytomegalovirus (CMV), and varicella-zoster virus (VZV).

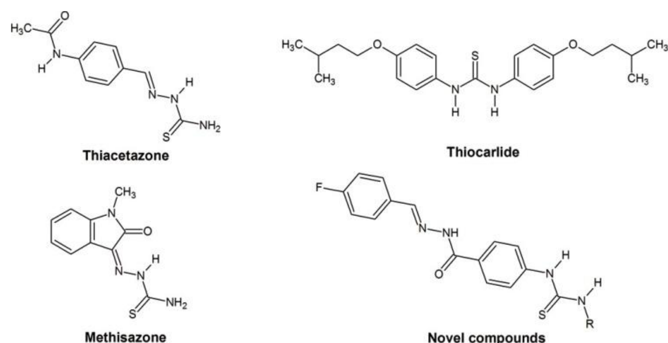


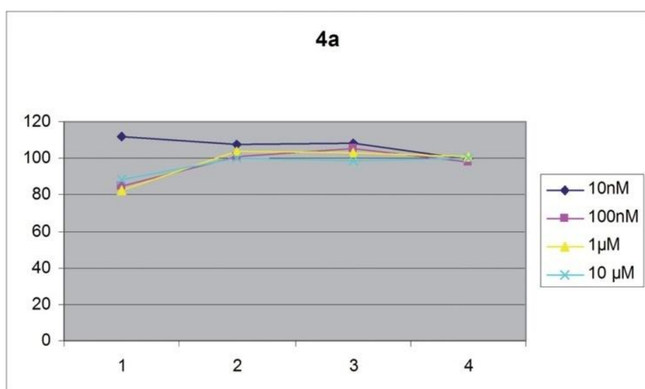
FIGURE1. Somethioureaswithantimycobacterialorantiviralactivity.

In vitro antitubercular activity of novel compounds against*Mycobacterium tuberculosis* H37Rv was evaluated at TAACF.Anticancer potential of the synthesized compounds was deter-mined using the A 549 andL 929 cell lines.

RESULTSANDDISCUSSION

Chemistry

4-Aminobenzoic acid hydrazide**1** was prepared by thereac-tion of ethyl 4-aminobenzoate with hydrazine-hydrate. 4-Ami-no-*N*'-[[4-fluoro/4-(trifluoromethyl)phenyl]methylene]-ben- zohydrazide**2**-**3**(cf.ExperimentalSection)weresynthesizedby condensation of **1** with 4-fluoro (17) /4-(trifluoromethyl)benzaldehyde.1-[4-[[2-[(4-Substitutedphenyl)methylene]hy-drazino]-carbonyl]phenyl]-3-substitutedthioureas**4a-g**and**5a-f**weresynthesizedbythereactionof4-amino-*N*'-[(4- fluoro/4-(trifluoromethyl)phenyl]methylene]benzohydrazide



in a mixture of dry acetonitrile and substituted phenyl isothiocyanates produces yields ranging from 42% to 68% per scheme. It was found that certain dry solvents or mixes might be used to carry out the thiou-rea reaction (18-21). The current investigation demonstrated that dry acetonitrile was beneficial. Tables 1 and 2 provide the physical and spectral data of thioureas 4a-g and 5a-f, respectively.

They were chosen as starting compounds to design several new thioureas, including 4-amino-N'-[(4-fluorophenyl)methylene]benzohydrazide, which was originally synthesized in the present study, and 4-amino-N'-[[4-(trifluoromethyl)phenyl]methylene]benzohydrazide, which was reported to have kinhibitory potency against Mycobacterium tuberculosis H37Rv at 12.5 µg/ml(14). Single signals corresponding to azomethine proton resonances at 8.38-8.51 ppm were seen in the 1H-NMR spectra of 4a-g and 5a-f (22). The predicted thiourea structures are supported by observations in 1H-NMR spectra, such as resonances at 8.44-10.03 and 9.84-10.24 ppm corresponding to thioureaR-NH-CS- and -CS-NH-Ar functions (15), respectively. It is worth noting that there are no resonances related to NH2 function. The predicted values were likewise recorded for the other chemical changes.

With a bias of less than 8 mmu between the experimental and estimated m/z values of either fragment ions or

molecules, high resolution mass spectra (HRMS) verified the molecular weights and empirical formula of compounds 4a-g and 5a-f (Table 2). While compound 4c showed molecular ion peaks when utilizing electron impact (EI) as its ionization mechanism, the other compounds did not. The rapid atomic bombardment (FAB) method, which yields precise MH+ peaks rather than M+ in a 3-nitrobenzylalcohol matrix, was used to examine these compounds. The predicted structure was further confirmed by the fragmentation pattern of the sample molecule 4c, as shown in Scheme 2. The first breakage occurred during thiou-rea moiety cleavage, resulting in the isothiocyanate fragment at m/z 299.0529 due to observed benzyl loss. Additionally, hydrazide-hydrazone characteristic fragmentations were noted. The base peak at m/z 120.0444 was caused by the main fragmentation product, which was identified as a 4-aminophenyl carbo-nyl cation.

Antiviralactivity

Compounds**4a-g**and**5a-f**weretestedforantiviralactivityand cytotoxicity in various viral test systems (Tables 3-5), ac-cording to previously published procedures (23-27). The fol-lowing viruses and host cells were used for the evaluation :

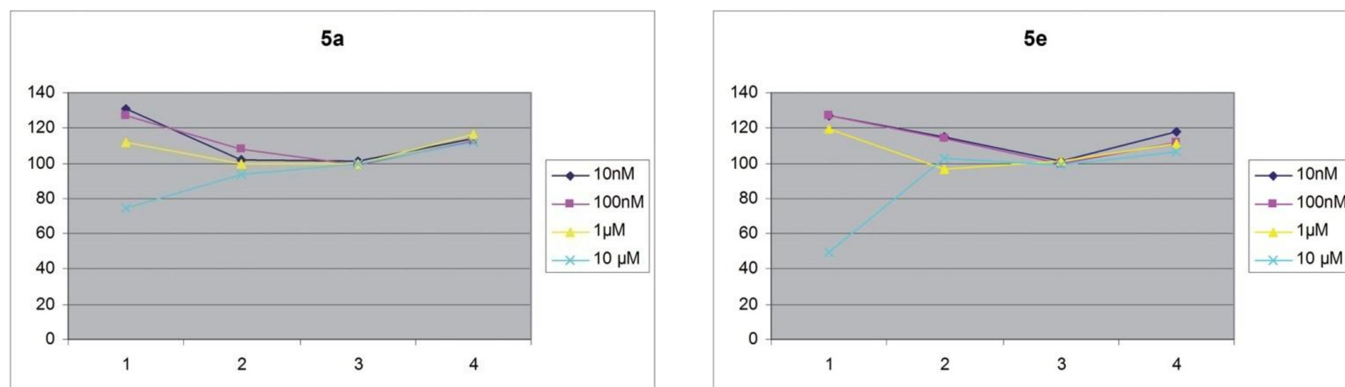
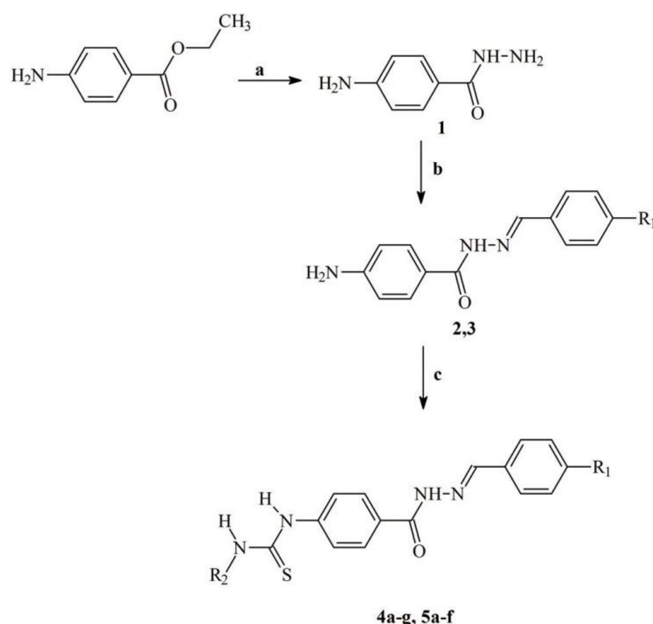


FIGURE2. Cytotoxiceffectsofthecompounds4a,5a,5eatfourdifferentconcentrations(10nM,100nM,1μM,10μM).



SCHEME 1. Synthetic route to compounds 2, 3, 4a-g and 5a-f. Reagents and conditions:(a)H_2N-NH_2 .H_2O/EtOH,reflux;(b)R_1-C_6H_4-CH=O/EtOH,reflux;
(c)R_2-C_6H_4-NCS/dryacetonitrile,reflux.

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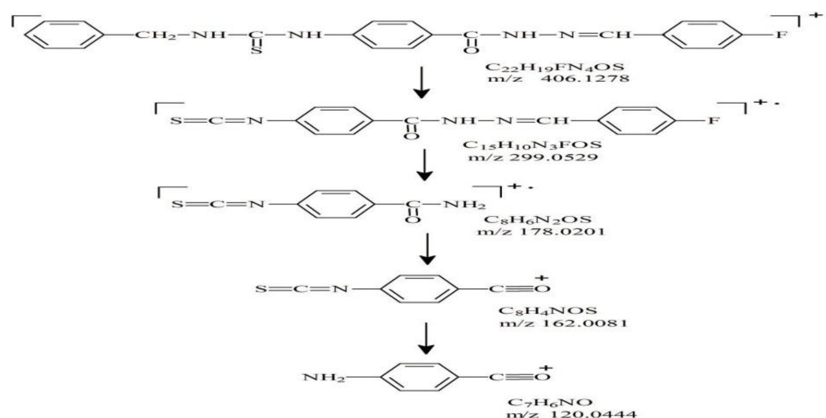
(a) Vero cell kultures : Parainfluenza-3 virus, Reovirus-1, Sind-bis virus, Punto Toro virus and Coxsackie B4 virus.

(b) HeLa cell cultures : Vesicular stomatitis virus (VSV), Cox-sackie B4 virus and respiratory syncytial virus.

TABLE1 .Physicalpropertiesandelementalanalysisdataof4a-gand5a-f.



Compd	R1	R2	Formula	M.W.	Color M.p(°C)	Yield*(%)	ElementalAn alysis (Calculated /Found)			
							C	H	N	S
4a	-F	$-C_6H_5$	$C_{21}H_{17}FN_4OS.½H_2O$	401.456	White	42	62.83	4.52	13.96	7.98
					238		62.57	4.08	14.60	8.30
4b	-F	$-C_6H_4-OCH_3$	$C_{22}H_{19}FN_4O_2S$	422.475	White	55	62.54	4.53	13.26	7.59
					231-4		61.94	4.17	13.83	7.75
4c	-F	$-CH_2C_6H_5$	$C_{22}H_{19}FN_4OS$	406.476	White	58	65.01	4.71	13.78	7.89
					242		64.28	4.52	13.81	8.77
4d	-F	$-C_6H_4-Br$	$C_{21}H_{16}BrFN_4OS$	471.345	Whitish cream	59	53.51	3.42	11.89	6.80
					240		53.09	3.30	11.75	7.72
4e	-F	$-C_6H_4-Cl$	$C_{21}H_{16}ClFN_4OS$	426.894	White	67	59.08	3.78	13.12	7.51
					240		58.88	3.66	13.14	8.24
4f	-F	$-C_6H_4-F$	$C_{21}H_{16}F_2N_4OS.½H_2O$	419.448	Whitish cream	68	60.13	4.08	13.35	7.64
					244		60.26	3.96	14.39	7.33
4g	-F	$-C_6H_4-CH_3$	$C_{22}H_{19}FN_4OS.½H_2O$	415.486	White	45	63.60	4.85	13.48	7.72
					235		63.99	4.50	13.94	8.11
5a	$-CF_3$	$-C_6H_5$	$C_{22}H_{17}F_3N_4OS.½H_2O$	451.464	White	46	58.53	4.02	12.41	7.10
					265-8		58.50	3.65	12.56	7.21
5b	$-CF_3$	$C_6H_4-OCH_3$	$C_{23}H_{19}F_3N_4O_2S.½H_2O$	481.490	White	47	57.37	4,19	11.64	6.66

					250		57.43	3.63	12.28	6.03
5c	$-CF_3$	$-C_6H_4-Br$	$C_{22}H_{16}BrF_3N_4OS$	521.353	White	59	50.68	3.09	10.75	6.15
					245-8		50.72	3.13	10.86	6.63
5d	$-CF_3$	$-C_6H_4-Cl$	$C_{22}H_{16}ClF_3N_4OS$	476.902	Whitish cream	58	55.41	3.38	11.75	6.72
					247		54.96	3.36	11.71	7.27
5e	$-CF_3$	C_6H_4-F	$C_{22}H_{16}F_4N_4OS.½H_2O$	469.455	White	57	56.28	3.65	11.93	6.83
					260		55.94	3.43	12.66	6.38
5f	$-CF_3$	$-C_6H_4-CH_3$	$C_{23}H_{19}F_3N_4OS.2H_2O$	419.514	White	54	56.09	4.71	11.38	6.51
					245		56.39	3.78	12.06	6.67

SCHEME2.HR-EImassspectralfragmentationof4c.

TABLE2.IR,^{1}H-NMRandHRmassspectraldataof4a-gand5a-f.

Compd	IRν(cm^{-1}) NH,C=O, C=S	^{1}H-NMR(DMSO-d_6,ppm)	HR-MS(m/z) Calculated	Found	
4a	3321,1655,1238	7.12-7.90(m,13H,Ar-H);8.44(s,1H,CH=N);9.96-10.07(d,1H,NH-CS-); 10,22(b,1H,NH-CS-);11.78(d,1H,CO-NH).	393.1180	(FAB)	393.1202(MH$^+$)
4b	3317,3236,1650,1238	3.74(t,3H,O-CH$_3$);6.90-7.90(m,12H,Ar-H);8.44(s,1H,CH=N);9.81,9.84	423.1286	(FAB)	423.1328(MH$^+$)
4c	3283,1651,1234	4.75(d,2H,N-CH$_2$);7.24-7.87(m,13H,Ar-H);8.38-8.44(d,2H,CH=Nand	406.1264	(EI)	406.1278(M$^+$)
4d	3294,3232,1654,1242	7.25-7.87(m,12H,Ar-H);8.43(s,1H,CH=N);10.01-10.07(d,2H,NH-CS- NH); 11.77 (s, 1H, CO-NH).	471.0285	(FAB)	471.0301(MH$^+$) 473.0308(MH$^+$+2)
4e	3320,3236,1659,1245	7.24-7.87(m,12H,Ar-H);8.44(s,1H,CH=N);10.02-10.07(d,2H,NH-CS- NH); 11.76 (s, 1H, CO-NH).	427.0790	(FAB)	427.0827(MH$^+$) 429.0705(MH$^+$+2)
4f	3217,1643,1238	7.14-7.90(m,12H,Ar-H);8.44(s,1H,CH=N);9.91,10.05(2s,1H,NH-CS-); 10.21(s,1H,NH-CS-);11.76-11.78(d,1H,CO-NH).	411.1086	(FAB)	411.1108(MH$^+$)
4g	3321,3236,1655,1238	2.27(s,3H,C$_6$H$_4$CH$_3$);7.13-7.90(m,12H,Ar-H);8.44(s,1H,CH=N);9.87-	407.1336	(FAB)	407.1324(MH$^+$)
5a	3321,3236,1655,1238	7.12-7.94(m,13H,Ar-H);8.51(s,1H,CH=N);9.97-10.02(d,1H,NH-CS); 10.24(b1H,NH-CS-);11.95(s,1H,CO-NH).	443.1148	(FAB)	443.1132(MH$^+$)
5b	3301,3240,1651,1249	3.83(t,3H,O-CH$_3$);6.90-7.92(m,12H,Ar-H);8.51(s,1H,CH=N);9.79-9.86	473.1254	(FAB)	473.1258(MH$^+$)
5c	3309,3232,1655,1257	7.42-7.94(m,12H,Ar-H);8.51(s,1H,CH=N);10.03(s,1H,NH-CS);10.10(s, 1H,CS-NH);11.95(s,1H,CO-NH).	521.0253	(FAB)	521.0250(MH$^+$) 523.0242(MH$^+$+2)
5d	3309,1655,1172	7.38-7.94(m,12H,Ar-H);8.51(s,1H,CH=N);10.03-10.09(d,2H,NH-CS- NH); 11.96 (s, 1H, CO-NH).	477.0758	(FAB)	477.0743(MH$^+$) 479.0733(MH$^+$+2)
5e	3317,3202,1651,1172	7.15-7.92(m,12H,Ar-H);8.51(s,1H,CH=N);9.92-10.02(2s,1H,NH-CS); 10.24(s,1H,CS-NH);11.97(s,1H,CO-NH).	461.1054	(FAB)	461.1049(MH$^+$)
5f	3317,3236,1651,1165	2.32(s,3H,C$_6$H$_4$CH$_3$);7.13-7.92(m,12H,Ar-H);8.51(s,1H,CH=N);9.91-	457.1304	(FAB)	457.1335(MH$^+$)

10.24(d,b,2H,NH-CS-NH);11.95(s,1H,CO-NH).

TABLE3. CytotoxicityandantiviralactivityofcompoundsinHel,HeLaandVerocellcultures

Compound	HELcell cultures						HELAcell cultures				Verocellcultures					
	Min.	Min.inhibitoryconc.^{b}(µg/ml)					Min.	Min.inhibitoryconc.^{b}(µg/ml)			Min.	Min.inhibitoryconc.^{b}(µg/ml)				
	cytotoxic conc.^{a}(µg/ml)	Herpessimplex virus-1 (KOS)	Herpessimplex virus-2 (G)	Vaccinia virus	Vesicularstomatitisvirus	Herpessimplex virus-1 TKKOSACVr	cytotoxic conc. a (µg/ml)	Vesicularstomatitisvirus	CoxsackieB4 virus	Respiratorysyncytialvirus	cytotoxic conc. a (µg/ml)	Parainfluenza-3virus	Reovirus-1	Sindbis virus	CoxsackieB4 virus	PuntaToro virus
4a	8	>1.6	>1.6	>1.6	>1.6	>1.6	40	>8	>8	>8	40	>8	>8	>8	>8	>8
4b	8	>1.6	>1.6	>1.6	>1.6	>1.6	40	>8	>8	>8	40	>8	>8	>8	>8	>8
4c	8	>1.6	>1.6	>1.6	>1.6	>1.6	8	>1.6	>1.6	>1.6	8	>1.6	>1.6	>1.6	>1.6	>1.6
4d	8	>1.6	>1.6	>1.6	>1.6	>1.6	200	>40	>40	>40	40	>8	>8	>8	>8	>8
4e	8	>1.6	>1.6	>1.6	>1.6	>1.6	40	>8	>8	>8	40	>8	>8	>8	>8	>8
4f	8	>1.6	>1.6	>1.6	>1.6	>1.6	40	>8	>8	>8	40	>8	>8	>8	>8	>8
4g	8	>1.6	>1.6	>1.6	>1.6	>1.6	40	>8	>8	>8	40	>8	>8	>8	>8	>8
5a	8	>1.6	>1.6	>1.6	>1.6	>1.6	40	>8	>8	>8	40	>8	>8	>8	>8	>8
5b	8	>1.6	>1.6	>1.6	>1.6	>1.6	200	>40	>40	>40	40	>8	>8	>8	>8	>8
5c	8	>1.6	>1.6	>1.6	>1.6	>1.6	200	>40	>40	>40	40	>8	>8	>8	>8	>8
5d	8	>1.6	>1.6	>1.6	>1.6	>1.6	40	>8	>8	>8	40	>8	>8	>8	>8	>8
5e	8	>1.6	>1.6	>1.6	>1.6	>1.6	200	>40	>40	>40	40	>8	>8	>8	>8	>8

Requiredtoreducevirus-inducedcytopathogenicityby50%.

5f	8	>1.6	>1.6	>1.6	>1.6	>1.6	200	>40	>40	>40	40	>8	>8	>8	>8	>8
Brivudin(μM)	>250	0.08	10	2	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
Ribavirin(μM)	>250	250	250	150	150	>250	>250	30	150	50	>250	150	150	>250	>250	250
Acyclovir(μM)	>250	0.4	0.4	>250	>250	50										
Ganciclovir(μM)	>100	0.032	0.0064	100	>100	2.4										
(S)-DHPA(μM)							>250	150	150	>250	>250	50	250	>250	>250	>250

TABLE4. Cytoxicityandantiviralactivityofcompounds4a-gand5a-fagainstcytomegalovirus(CMV)andvaricella-zostervirus(VZV)inhumanembryoniclung (HEL)cells.

Compd.	Antiviralactivity EC_{50}(μg/ml)[a]				Cytotoxicity(μg/ml)		
	CMV(cytomegalovirus)		VZV(varicella-zostervirus)		Cell morphology MCC[b]		Cellgrowth CC_{50}[c]
	AD-169strain	Davisstrain	TK^+(OKA strain)	TK^-(07/1strain)	CMVassay	VZVassay	
4a	>4	>4	>4	>4	20	20	10.5
4b	>20	>20	>20	>4	100	≥20	>100
4c	>4	>4	>4	>4	20	20	12.6
4d	>20	>4	>4	>20	≥20	≥20	>100
4e	>20	>20	>20	>20	100	≥20	>100
4f	>20	>100	>20	>20	≥100	100	>100
4g	>20	>20	>20	>20	100	100	>100
5a	>20	>4	>4	>20	≥20	≥20	>100
5b	>20	>20	>0.8	>4	100	≥0.8	>100
5c	>4	>4	>4	>4	20	≥4	62.2
5d	>4	>20	>4	>4	≥20	20	>100
5e	>20	>20	>4	>4	100	20	>100
5f	>20	>4	>4	>4	≥20	≥4	>100
Ganciclovir	1.4	1.7	-	-	400	-	80
Cidofovir	0.24	0.37	-	-	400	-	57
Acyclovir	-	-	1.0	15	-	>50	190
Brivudin	-	-	0.0095	12.6	-	>50	244

[a]Effectiveconcentrationrequiredtoreducevirus-inducedcytopathiceffectby50%.Virusinputwas20(VZV)or100(CMV)plaqueformingunits(PFU).
[b]Minimumcytotoxic concentrationthat causesa microscopicallydetectable alterationof cellmorphology.
[c]Cytotoxicconcentrationrequiredtoreducecellgrowthby 50%.

HEL cell culture : Herpes simplex virus type 1 (HSV-1) (KOS strain), Herpes simplex virus type 2 (HSV-2) (G strain), Vaccinia virus , Vesicular stomatitis virus , HSV-1 thymidine kinase deficient virus (TK^-KOS ACV^r).

(c) HELcellculture:Cytomegalovirus(CMV)(strainsAD-169 and Davis), Varicella-zoster virus (VZV) (TK^+VZV strain OKA strain and 07/1 strain).

(d) MT-4cells:HIV-1(IIIB)andHIV-2(ROD)strains.

Brivudin, (S)-DHPA, ribavirin, acyclovir, cidofovir and ganci-clovir were used as the reference compounds. In the tests withviruses decribed in (a), (b), (c), (d) and (e) antiviral activity andcytotoxicityweredeterminedwiththecompounds**4a-g** and**5a-f**. None of synthesized compounds had selective activity atsubtoxic concentrations against the viruses tested.

Antitubercularactivity
Compounds **4a-g** and **5a-f** were also tested for in vitro antitu-bercular activity against *M. tuberculosis* H37Rv (ATCC 27294)using the BACTEC 12B medium and a broth microdilution as-say, the Microplate Alamar Blue Assay (MABA) (28,29). Ri-fampicin was used as the standard in the antitubercular as-says. None of the tested compounds were considered for fur-ther antitubercular evaluation as they exhibited less than 90% inhibition in the primary screen (MIC>6.25 μg/mL).

Anticanceractivity

Both cytotoxicity and anticancer assay results showed that,none of the tested concentrations of the compounds gave IC_{50}values. Therefore, it was concluded that there were no signifi-cantdifferencesfoundbetweencytotoxicandanticanceref- caused 10-20% cytotoxic effect at the highest concentration on4thday of the incubation period (30) (Figure 2)

EXPERIMENTAL

Chemistry

Allchemicalcompoundswerepurchasedfromfluka.Melting pointsweretakenonBuchi-530apparatus.Mercksilicagel60 F254 plates were used for analytical TLC and visualized with UV.The IR spectra were obtained with a Shimadzu FTIR– 8400.^{1}HNMRspectrainDMSO-$d_6$wereobtainedonaBruker Avance-DPX 400 instrument. HR-Mass spectra using EI and FAB ionization techniques, were performed using a Jeol JMS- 700 instrument.

Synthesisof4-Aminobenzoicacidhydrazide1(17)

Ethyl 4-aminobenzoate(0.01 mol) was added to hydrazine-hydrate (99%, 3 mL) . The reaction mixture was heated for 1 hand this reaction mixture was refluxed in the presence of etha-nol. The compound thus obtained was allowed to stand over-night.Theprecipitatedsolidwaswashedwithwater,driedand cleaned twice using hot methanol.

4-Amino-N'-[(4-fluoro/4-(trifluoromethyl)phenyl)methylene] benzohydrazide 2 (17), 3

Generalprocedure

A solution of 0.01 mol of **1** and equimolar amount of appropri-ate aldehyde in 60 mL of ethanol was heated under reflux for 1h (15 min for compound **3**). The precipitate obtained was fil-tered off, washed with water and cleaned twice with boilingEtOH.
zone);

TABLE 5. Cytoxicityandantiviralactivityofcompounds4a-gand5a-f against HIV-I (IIIB) and HIV-II (ROD).

Compounds	HIV-I(III_B)		HIV-II(ROD)	
	EC_{50} (μg/ml)[a]	CC_{50} (μg/ml)[b]	EC_{50} (μg/ml)[a]	CC_{50} (μg/ml)[b]
4a	>53.85	53.85	>53.85	53.85
4b	>125	>125	>125	>125
4c	>20.3	94.00	>16.8	94.00
4d	>125.00	>125.00	>125.00	>125.00
4e	>125.00	>125.00	>125.00	>125.00
4f	>59.10	59.10	>59.10	59.10
4g	>125.00	>125.00	>125.00	>125.00
5a	>70.9	>125	>79.2	>125
5b	>125.00	>125.00	75.25	>125.00
5c	>58.10	>58.10	>58.10	>58.10
5d	>68.10	68.10	>68.10	68.10
5e	>75.70	75.70	>75.70	75.70
5f	>118.00	>125.00	>125.00	>125.00

[a]Effectiveconcentrationrequiredtoprotect50%ofthecellsagainst destruction by the virus.
[b]Cytotoxicconcentrationrequiredtodestroy50%oftheuninfectedhost cells.

(d,2H,o-NH_2,J=8.6Hz),7.69(d,2H,m-NH_2,J=8.6Hz),7.80(d, 2H,o-CH,J=8.3Hz),7.91(d,2H,m-NH,J=8.3Hz),8.46(d,1H,-CH=N),11.65(s,1H,-CONHN=CH-);HR-MS(EI,70eV):m/z (calculated/found)for$C_{15}H_{12}F_3N_3O$307.0932[M^+],307.0903.

1-[4-[[2-[(4-substitutedphenyl)methylene]hydrazino]carb onyl]phenyl]-3-substitutedthioureas4a-g,5a-f

Generalprocedure

Adryacetonitrilesolutionof4-amino-*N*'-[(4-fluoro/4-trifluorophenyl)methylene]-benzohydrazide and equimolar substitutedphenylisothiocyanatesindryacetonitrilewasheatedunderre-fluxfor9-

15h.Thecompletionofreactionwascheckedby TLC(petroleumether:acetone,50:50,v/v).Theprecipitateobtained wasfilteredoffandrecrystallizedtwicewithdryacetonitrile.

Biologicalactivity

Antiviralactivity

Compounds**4a-g**and**5a-f**were tested for antiviral activity andcytotoxicityinvariousviraltestsystems,accordingtopre- viously published procedures (23-27).The synthesized com- pounds were tested against HIV-1 (IIIB) and HIV-2 (ROD), vesicular stomatitis virus, Coxsackie B4 virus, respiratory syn- cytial virus, parainfluenza-3 virus, reovirus, Sindbisvirus, Punto Toro virus, herpes simplex virus type 1 and 2 and vac- cinia virus-induced cytopathogenicity at subtoxic concentra- tions in MT-4 cells , HeLa, Vero or Hel cell culture. Brivudin, (S)-DHPA,ribavirin,acyclovir,cidofovirandganciclovirwere used as the reference compounds.

Antitubercularactivity

Antituberculareevaluationwascarriedoutinthe Tuberculosis AntimicrobialAcquisitionandCoordinatingFacility(TAACF). Primaryscreenwasconductedat6.25µg/mlagainstM.tuber- culosis H37Rv in BACTEC 12B medium using both BACTEC 460radiometricsystemandMicroplateAlamarBlueAssay (MABA) (28, 29) . Compounds effecting< 90 % inhibition in theprimaryscreen(MIC>6.25g/ml)werenotfurtherevalu- ated. Compounds demonstrating at least 90 % inhibition in theprimary screen were considered for re-testing at lower concen- tration (MIC) in a broth microdilution MABA.

Anticanceractivity

The synthesized compounds were tested for anticancer activi- tyandcytotoxicity.TheCellTiter96AqueousONESolution(Promega, Madison, WI) was used to evaluate cellular viabilityutilizingreductionof3-(4,5-dimethylthiazol-2-yl)-5-(3-car-boxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium(MTS).

Cellcultureandviabilityassay

To assess cytotoxicity and anticancer effects, the A 549 and L 929 cell lines were used. The cells were cultured in a 75 mm flask with 5% CO2 and passed every three days. We used the MTS test to check the cell viability. The cells were cultured in a 75 mm flask with 5% CO2 and cultured every three days according to protocol. We used the MTS test to check the cell viability. A 96-well tissue culture plate was used, with 5,000 cells per well. The cells were cultured for 4 days (30) after the media was changed to include different concentrations of chemicals (10 nM, 100 nM, 1 µM, and 10 µM).

The MTS assay was carried out in accordance with the manufacturer-provided protocol.Briefly, 20 microliters of MTS solution was added to each well, and the cells were incubated at 37ºC for 1 to 3 hours.Next, we measured the wells' absorbance at 490 nm. The data was expressed as a percentage of the values obtained from chemical-free cell cultures grown under identical circumstances.The L929 cells were exposed to the same dose of chemicals used to detect the carcinogen effect in order to conduct the time-course study of their cytotoxicity.The viability of the cells was assessed using the MTS assay one to four days after treatment began.

A final dosage of 0.1% DMSO was used to dissolve all test substances. In these tests, the sol-vent failed to exhibit any activity at the screening level. Doxorubicin and taxol were chosen as reference medicines for the purpose of comparing the test compounds' anticancer activity and cytotoxicity testing.

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REFERENCES

1. 1. Bernalar P, Kolonoski P, Young LS, Inder-lied CB, Reynolds R.. Activated against Mycobacterium avium in mice by a thiosemicarbazone (thiacetazone-like) chemical. Inhibitors of Microbes Chemother, 2003, 47: 2685–2687.
2. 2. Bhattacharyya et al., Baulard v. Cooper, Minnikin v. Douglas, Besra GS, and Brennan PJ. By blocking the production of mycolic acid, isoxyl and its novel derivatives exhibit antimycobacterial properties. Chapter 43: Antimicrobial Agents and Chemotherapy, 1991.
3. Authors: Liav, Angala, Brennan, and Jackson.Benzothioureadiene derivatives: a novel class of anti-tuberculosis medicines have been developed. Science in Biomedicine Science Letters, 18(8), 2649–2651 (2008).
4.
5. 4. Sliva K, Schnierle B. Novel anti-poxvirus drugs: from very harmful to very specific. Virol Journal, 4:8, 2007.
6. 5. The authors are Patel, Dave, Langalia, and Thaker. Preparation of 1-(4-aminobenzoyl)-2-benzalhydrazine and 1-[4-(phenylthioureido)benzoyl]-2-substituted benzalhydrazine: studies on antititubercular and antibacterial compounds. Published in 1984 in the Journal of the Indian Chemical Society, volume 61, pages 718–720.
7. 6. Sriram, Yogeeswari, and Madhu wrote the article.Some 1-[(4-sub)phenyl]-3-(4-{1-[(pyridine-4-carbonyl)hydrazono]ethyl]phenyl) thiourea: its synthesis and in vitro antitubercular efficacy.Biology and Medicinal Chemistry Letters, 16(6), 876–878 (2006).
8. 7. Rollas S, Küçükgüzel ŞG. Hydra-zone derivatives and their biological activities.Publishing year: 2007 in the journal Molecules, volume 12, pages 1910–1939.
9. 9. Karakuş S, KüçükgüzelŞG, Küçükgüzelİ, De Clercq E, Pannecouque C, Andrei G, Noeck R, Şahin F, BayrakÖF. N-(4-nitro-2-phenoxyphenyl)-methanesulfonamide-derived new thioureas: synthesis, antiviral and anticancer activities. Published in 2009 in the European Journal of Medical Chemistry, volume 44, pages 3591–3595.
10. Sriram D., Yogeeswari P., Dinakaran M., and Thirumurugan R. Antimycobacterial activity of new 1-(5-cyclobutyl-1,3- oxazol-2-yl)-3-(sub)phenyl/pyridylthiourea compounds endowed with strong activity versus multidrug-resistant Mycobacterium tuberculosis (1999). Published in the Journal of Antimicrobial Chemistry, volume 59, pages 1194–1196, 2007.
11. Ünsalan S, Kaymakçıoğlu BK, Oruç EE, Kandemirli F, Sh- vets N, Rollas S, Anatholy D. and others. Characterization and synthesis
12. discovered new hydrazide-hydrazone compounds and investigated their anti-TB effects based on their structural makeup. The European Journal of Medical Chemistry, volume 41, pages 1253–1261, 2006.
13. Section eleven: Dixit PP, Patil VJ, Nair PS, Jain S, Sinha N, and Arora SK.Genealogy of12-oxo-oxazolidin-5-ylmethyl [3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)]3-substituted derivatives of thiourea as potential anti-TB medications. The European Journal of Medical Chemistry, 41:423-428, 2006.
14. 12. SobhiaME and NairPC.Research on thiourea analogues as influenza virus neuraminidase inhibitors using quantitative structure-activity relationship analysis.Europe Journal of Medical Chemistry, 43: 293–299, 2008.
15. 13. The authors of the document are Küçükgüzelİ, Tatar, KüçükgüzelŞ, Rollas, and DeClercq. 4. A number of new thiourea derivatives have been synthesized using 5-[(4-aminophenoxy)methyl]2-(2,4-triazole-3-thiones) and their potential as antiviral and anti-HIV medicines. The European Journal of Medical Chemistry, 43: 381-392, 2008.
16. 14. Küçükgüzel ŞG, Kiraz M., Rollas S., Küçükgüzel İ. Chemistry and anti-mycobacterial action of some conjugation products derived from 4-aminobenzoic acid hydrazones. Article published in the European Journal of Medical Chemistry in 1999, volume 34, pages 1093–1100.
17. Küçükgüzelİ, G., Rollas, S., and Kiraz, M. (2015).Antimycobacterial potential of some 3-thioxo/alkylthio-

1,2,4-triazoles with a substituted thiourea group. Biology and Medicine Letters,11, 1703–1707, 2001.

18. Kalakuş S. and Rollas S. 16. Novel N-phenyl-N'-[4-(5-alkyl/arylamino-1,3,4-thia-diazole-2-yl)phenyl]thioureas: synthesis and antituberculosis activity.Farmaco, 2002, 57: 577–581.

19. Koümürcü ŞG, Rollas S, Ülgen M, Gorrod JW, and Çevikbaş A. Evaluation of certain arylhydrazones ofp-Aminobenzoic acidhydrazideasantimicrobialagentsandtheirin-vitro hepatic microsomal metabolism. Published in the Journal of Pharmaceutical Science, volume 134, pages 375-379, 1995.

20. ÇevikbaşA, Büyüktimkin S, and Rollas S. 18. Chemical synthesis and antimicrobial effects of N-[4-(3H-1,3,4- oxadiazoline-2-thion-5-yl)phenyl]-N'-substitutedthioureas. Published in the Journal of the American Pharmaceutical Association, Weinheim, 324: 189–190, 1991.

21. AJ Vlietinck, Ram VJ, Vidyottama D, Pieters LAC, 19. The hypoerocyclic gene Science, 26(625), 625-628, 1989.

22. The synthesis and uncoupling activities of hydrophobic thioureas were investigated by Kubota et al. (2021).Chapter 33: 662–666, 1985.