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SYNTHESIS OF NOVEL LEVAMISOLE DERIVATIVES FOR THEIR ANTICANCER AND ANTIVIRAL ACTIVITY

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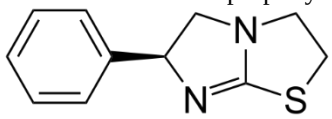
Abstract : All the compounds (CH-69 to CH-84) were evaluated for their cytostatic activity against human HeLa cervix carcinoma cells, human CEM CD4þ T-lymphocytes as well as murine L1210 cells. All assays were performed in 96-well microtiter plates. To each well were added (5-7.5) x 10^4 tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and human cervix carcinoma HeLa cells) at 37 ºC in a humidified CO2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%. The Cytotoxicity and antiviral activity of a new series of 2-arylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)-2H-chromen-2-one against different MDCK cell cultures, HELa cell cultures, Vero cell cultures, CRFK cell cultures is reported. Among the tested compounds, Inhibitory effects of compounds (CH-69 to CH-84) on the proliferation of murine leukemia cells (L1210) and human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa).

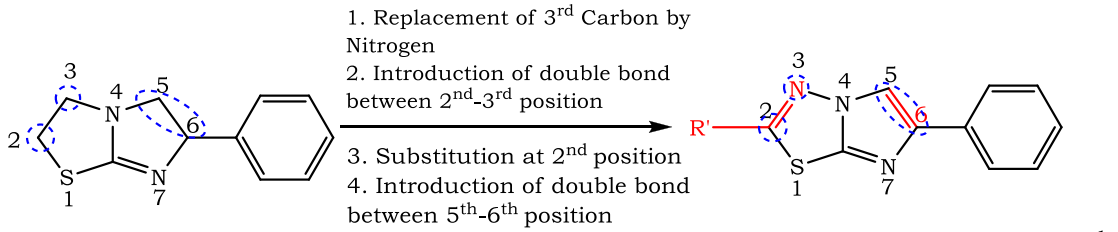
IndexTerms - HeLa, MDCK, CRFK, thymidine kinase-deficient (TK-) HSV-1 Kos strain, herpes simplex virus.

I. INTRODUCTION

Levamisole was introduced by Janssen Pharmaceutica in 1966 as anthelmintic agent to treat worm infestations in both humans and animals[1]. Later it was withdrawn from the market because of the serious side effects like Agranulocytosis[2]. After being pulled out, the molecule has been tested in combination with fluorouracil to treat colon cancer. Evidence from clinical trials supports its addition to fluorouracil therapy to benefit patients with colon cancer[3]. Chemically levamisole is imidazothiazole derivative. Like levamisole, the modified molecule ''Imidazo [2,1-*b*][1,3,4]thiadiazole'' also bears anticancer property.



Levamisole
(6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*][1,3]thiazole)

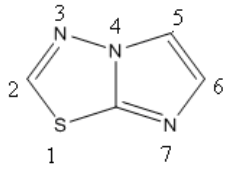


6-phenyl-2,3,5,6-tetrahydroimidazo [2,1-*b*][1,3]thiazole **(Levamisole)**

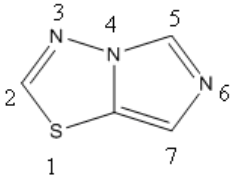
(Fig.-01)

2-substituted-6-phenylimidazo[2,1-*b*] [1,3,4]thiadiazole **(Target Molcule)**

There are two types of bicyclic imidazo[2,1-b]-1,3,4-thiadiazole ring systems are possible. Both the ring systems have nitrogen as a bridgehead atom at 4th position. It is pseudo aromatic in nature containing imidazole as electron rich centre and desired substitution can be done at 2nd, 5th and 6th position by starting with appropriate synthons.



Imidazo[2,1-b]-1,3,4-thiadiazole



Imidazo[5,1-b]-1,3,4-thiadiazole

Kumar *et al*[4,5], Hegde *et al*[6], Karki *et al*[7,8],Terzioglu *et al*[9], Andreani *et al*[10] and other researchers have shown imidazo[2,1-*b*][1,3,4]thiadiazole nucleus as an useful scaffold for the development of novel anticancer agent.

Based on above discussion,2-naphthyl-6-aryl-imidazo[2,1-*b*][1,3,4]-thiadiazole nucleus has been taken as the target molecule for the thesis entitled "Synthesis of Levamisole Derivatives for Anti-cancer Activity".

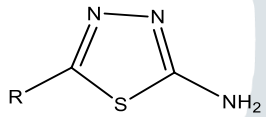
MATERIALS AND METHODS

All chemicals procured for the proposed research work is of high purity. Purity of all chemicals to be confirmed by TLC and solvents to be used after distillation. Proposed research work is comprised of following steps:

1. General method of synthesis of 2-amino-5-substituted-1,3,4-thiadiazole:

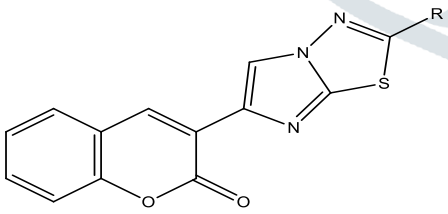
0.034M of Phosphorous oxychloride was added drop-wise to mixture of 0.01M of carboxylic acid **[E]** and thiosemicarbazide **[F]** with constant stirring. The reaction mixture was refluxed for one hour, cooled and added to 250 ml of ice-cold water and neutralized with 10% potassium hydroxide solution. The precipitate of 2-amino-5-substituted-1,3,4-thiadiazole **[G]** was filtered, washed with water and crystallized from DMF-ethanol mixture.

General Structure:



2. General method of synthesis of 3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one:

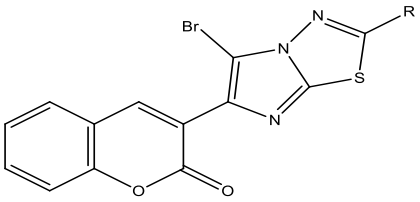
Equimolar quantity of 3-(2-bromoacetyl)-2*H*-chromen-2-one **[D]** and 5-substituted-1,3,4-thiadiazole-2-amine **[G]** in ethanol was refluxed for 10-12 hours. The reaction mixture was poured in ice-cold water and pH of the solution was adjusted to 7.0 with aqueous solution of Na_2CO_3 to get 3-(2-substituted imidazo [2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-one. The compound so obtained was purified from chloroform-ethanol mixture.



3. General method of synthesis of 5-bromo-3-(2-substituted imidazo[2,1-b]- 1,3,4-thiadiazol-6-yl)-2H-chromen-2-one:

To a well stirred mixture of 0.0050M of anhydrous sodium acetate and 0.0025M of an appropriate 3-(2-substituted imidazo[2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-one, 0.0025M of bromine was added drop wise at room temperature. The stirring was stirred for 1hour and later poured into ice cold water. The separated solid was filtered and recrystallized from chloroform-ethanol mixture. Physical constant values are given in **Table No.-06.**

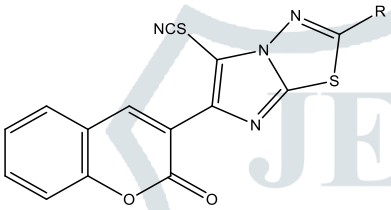
General Structure:



4. <u>General method of synthesis of 5-thiocyanato-3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one:</u>

0.0025M of bromine in glacial acetic acid (10 ml) was added drop wise at 0^0C to a solution of 0.0025M of 3-(2-substituted imidazo[2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-one and 0.004M of potassium thiocyanate in 10 ml of glacial acetic acid,. The reaction mixture was further stirred for 1 hour at 15-18^0C, after which it was poured into ice cold water. Solid separated was filtered and recrystallized from the mixture of chloroform/ethanol. Physical constant values are given in **Table No.-07.**

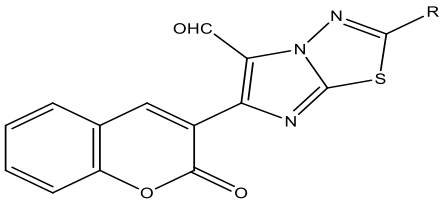
General Structure:



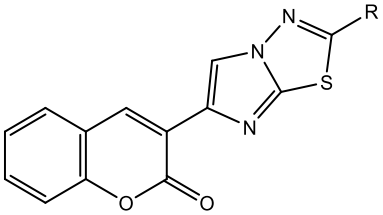
5. <u>General method of synthesis of 5-formyl-3-(2-substituted imidazo[2,1-b]- 1,3,4-thiadiazol-6-yl)- 2H-chromen-2-one:</u>

0.002M of 3-(2-substituted imidazo[2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-one was added to the freshly prepared Villsmeier-Haack Reagent [Prepared by the adding 0.75 ml of $POCl_3$ drop wise to 5 ml of DMF at 0-5 ^{0}C for 30 min] at room temperature. Stirring was continued for 4 hours at 80-90 ^{0}C. The resulting reaction mixture was poured into ice cold water and neutralized to pH-7 with cold aqueous solution of sodium carbonate. The solid so obtained was filtered and recrystallized from ethanol. Physical constant values are given in**.**

General Structure



Physical parameters of different 3-(2-substituted imidazo[2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-ones:



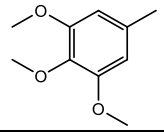
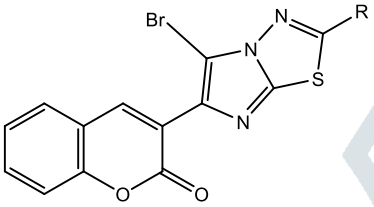
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f Value
CH69		Yellow, Amorphous	62	210-212	$C_{22}H_{17}N_3O_5S$	435.45	0.56
CH70	2-Methyl thiophene	Brown , Crystalline	40	196-98	$C_{18}H_{11}N_3O_2S_2$	365.43	0.54
CH71	$-CH_3$	Brown, Amorphous	68	222-224	$C_{14}H_9N_3O_2S$	283.30	0.52
CH72	Phenyl	White, Amorphous	65	278-80	$C_{19}H_{11}N_3O_2S$	345.37	0.50
CH73	Thiophene	Brown, Crystalline	70	290-292	$C_{17}H_9N_3O_2S_2$	351.40	0.77

Table No.-06: Physical parameters of different 5-bromo-3-(2-substituted imidazo [2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-one.



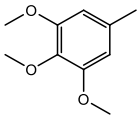
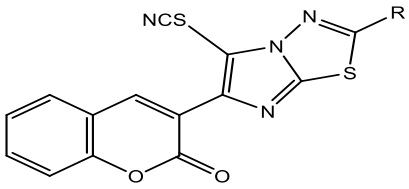
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f Value
CH74		Yellow Amorphous	55	218-220	$C_{22}H_{16}BrN_3O_5S$	514.35	0.63
CH75	$-CH_3$	White, Amorphous	50	199-200	$C_{14}H_8BrN_3O_2S$	362.20	0.55
CH76	Thiophene	Brown, Amorphous	60	248-249	$C_{17}H_8BrN_3O_2S_2$	430.29	0.48

Table-07: Physical parameters of different 5-thiocyanato-3-(2-substituted imidazo[2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-one



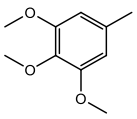
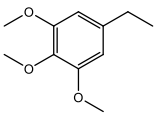
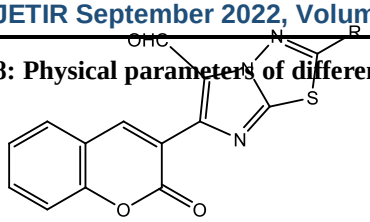
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f
CH77		Yellow, Amorphous	70	242-244	$C_{23}H_{16}N_4O_5S_2$	492.53	0.44
CH78		White, Amorphous	55	180-182	$C_{24}H_{18}N_4O_5S_2$	506.55	0.56

Table -08: Physical parameters of different 5-formyl-3-(2-substituted imidazo [2,1-*b*]-1,3,4-thiadiazol-6-yl)-2*H*-chromen-2-one.



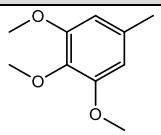
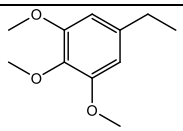
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f Value
CH79		Light, Yellow	35	192-194	$C_{23}H_{17}N_3O_6S$	463.46	0.54
CH80		White, Amorphous	40	178-180	$C_{24}H_{19}N_3O_6S$	477.49	0.66
CH81	Phenyl	Brown, Amorphous	45	250-252	$C_{20}H_{11}N_3O_3S$	373.38	0.72
CH82	Thiophene	Brown, Crystalline	40	278-280	$C_{18}H_9N_3O_3S_2$	379.41	0.58

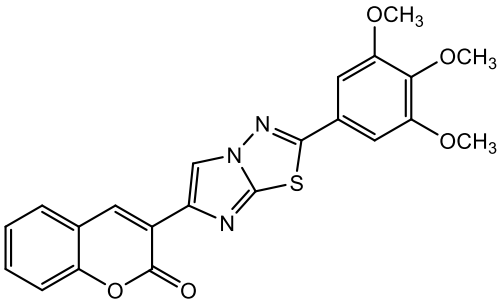
Table No.- 09: IR Spectral data of synthesized derivatives.

Compound Code	Spectral Peaks (cm^{-1})	Molecular Stretch
CH69	3050.01, 2972.73-2734 1724.05, 1590.99 1476.24	C-H (aromatic) C-H (aliphatic) >C=O >C=N >C=C
CH70	3034.44 2968.87-2911.99 1716.34 1605.45 1480.10	C-H (aromatic) -C-H(aliphatic) >C=O (Ketone) >C=N >C=C
CH71	3042.16 2899.45-2844.49 1722.12 1609.31 1462.74	-C-H (aromatic) -C-H (aliphatic) >C=O (Ketone) >C=N >C=C
CH72	3046.98 2972.73 1718.26 1606.41 1432.85	-C-H (aromatic) -C-H (aromatic) >C=O >C=N >C=C
CH73	3058.55 2966.95-2826.17 1713.44 1606.41 1471.42	-C-H (aromatic) -C-H (aliphatic) >C=O (Ketone) >C=N >C=C

CH74	2997.80 2942.84-2826.17 1738.51 1610.27 1478.17	C-H (aromatic) C-H (aliphatic) >C=O >C=N >C=C
CH75	3045.05 2924.52-2961.16 1729.83 1604.48 1471.42	-C-H (aromatic) -C-H (aliphatic) >C=O (Ketone) >C=N >C=C
CH76	3052.76 2942.84-2765.42 1729.83 1597.41 1471.42	-C-H (aromatic) -C-H (aliphatic) >C=O (Ketone) >C=N >C=C
CH77	3028.66 2979.48-2833.48 2167.60 1707.66 1610.27 1466.60	C-H (aromatic) C-H (aliphatic) -CN >C=O >C=N >C=C
CH78	2942.84-2747.10 2158.92 1702.84 1604.48 1465.83	-C-H (aliphatic) -CN >C=O >C=N >C=C
CH79	2942.84-2836.77 1721.16 1677.77 1589.06 1474.31	-C-H(aliphatic) >C=O (Ketone) >C=O (Aldehyide) >C=N >C=C
CH80	3001.20 2906.20-2747.10 1716.34 1654.62 1598.70 1467.56	-C-H (aromatic) -C-H (aliphatic) >C=O (Ketone) >C=O (Aldehyde) >C=N >C=C
CH82	3061.44 2972.73-2869.56 1712.48 1664.27 1610.27 1475.28	-C-H (aromatic) -C-H (aliphatic) >C=O (Ketone) >C=O (Aldehyde) >C=N >C=C

Table No.-10: ^{1}H NMR spectral data of synthesized compounds

Compound Code	Chemical Shift Value (δ) in ppm & Proton Nature
CH69	**8.68(1H, *s*, *ar.*), 8.60(1H, *s*, *ar.*), 7.88-7.85(1H, *m*, *ar.*), 7.67-7.57 (1H, *m*, *ar.*), 7.45(1H, *d*, *J*=8), 7.37(1H, *t J*=16), 7.17(2H, *s*, *ar.*), 3.89 (6H, *s*, 2-OCH_3), 3.75(3H,*s*,-OCH_3).**
CH70	**8.67(1H, *s*, *ar.*), 8.58(1H, *s*, *ar.*),7.88-7.86(1H, *m*, *ar.*), 7.63-7.59(1H, *m*, *ar.*), 7.52-7.50(1H, *d*, *ar.*), 7.47(1H, *d*, *J*=8), 7.38(1H, *t*, *j=16.*), 7.16-7.15(1H, *m*, *ar.*), 4.73(2H, *s*, -CH_2).**
CH71	**8.64(1H, *s*, *ar.*), 8.52(1H, *s*, *ar.*), 7.87-7.85(1H, *m*, *ar.*), 7.62-7.58(1H, *m*, *ar.*), 7.46(1H, *d*, *j=8.*), 7.38(1H, *t*, *j*=16), 2.74(3H, *s*, -CH_3).**
CH72	**8.72(1H, *s*, *ar.*), 8.68(1H, *s*, *ar.*), 7.99(2H, *d*, *j*=8), 7.91(1H, *d*, *j*=8), 7.65-7.59(4H, *m*, *ar.*), 7.49(1H, *d*, *j*=8), 7.40(1, *t*, *j*=16).**
CH73	**10.03(1H, *s*, -CHO), 8.57(1H, *s*, *ar.*), 8.01-7.97(3H, *m*, *ar.*), 7.92-7.89(1H, *m*, *ar.*), 7.73-7.69(2H, *m*, *j*=16), 7.52(1H, d, *j*=8), 7.43(1H, *t*, *j*=16), 7.32-7.30(1H, *m*, *ar.*)**
CH74	**8.39(1H, *s*, *ar.*), 7.87-7.85(1H, *m*, *ar.*), 7.70-7.65(1H, *m*, *ar.*), 7.49(1H, *d*, *J*=8), 7.41(1H, *t*, *J*=16), 7.21(2H, *s*, *ar.*), 3.92(6H, *s*, 2-OCH_3), 3.77(3H, *s*, -OCH_3).**
CH75	**8.33(1H, *s*, *ar.*), 7.85-7.82(1H, *m*, *ar.*), 7.68-7.64(1H, *m*, *ar.*), 7.47(1H, *d*, *j*=8), 7.40(1H, *t*, *j*=16), 2.78(3H, *s*, -CH_3).**
CH76	**8.70(1H, *s*, -*ar.*), 8.63(1H, *s*, *ar.*), 7.97-7.95(1H, *m*, *ar.*), 7.90-7.88(2H, *m*, *ar.*), 7.64-7.60(1H, *m*, *ar.)*, 7.48(1H, *d*, *j*=8), 7.40(1H, *t*, *j*=16), 7.30-7.28(1H, *m*, *ar.*)**
CH77	**8.55(1H, *s*, *ar.*), 7.92-7.90(1H, *m*, *ar.*), 7.73-7.69(1H, *m*, *ar.*), 7.53(1H, *d*, *J*=8), 7.44(1H, *t*, *J*=16), 7.26(2H, *s*, *ar.*), 3.93(6H, *s*, 2-OCH_3), 3.78(3H, *s*, -OCH_3).**
CH78	**8.47(1H, *s*, *ar.*), 7.89(1H, *d*, *j=8.*), 7.69(1H, *t*, *j=16*), 7.51(1H, *d*, *j=8.*), 7.42(1H, *t*, *j=16.*), 6.79(2H, *s*, *ar.*), 4.49(2H, *s*, -CH_2), 3.77(6H, *s*, 2-OCH_3), 3.65(3H, *s*, -OCH_3).**
CH79	**10.07(1H, *s*, -*CHO*), 8.59(1H, *s*, *ar.*), 7.92(1H, *d*, *j=8*), 7.71(1H, *t*, *J*=16), 7.52(1H, *d*, *J*=8), 7.43(1H, *t*, *j=16*), 7.25(2H, *s*, *ar.*), 3.93(6H, *s*, -OCH_3), 3.77(6H, *s*, -OCH_3).**
CH80	**10.05(1H, *s*, -CHO), 8.52(1H, *s*, *ar.*), 7.90(1H, *d*, *j=8*), 7.70(1H, *t*, *j*=16), 7.50(1H, *d*, *j*=8), 7.42(1H, *t*, *j*=16), 6.78(2H, *s*, *ar.*), 4.47(2H, *s*, -CH_2), 3.77(6H, *s*, 2-OCH_3), 3.65(3H, *s*, -OCH_3).**
CH82	**8.37(1H, *s*, -*ar.*), 8.00-7.99(1H, *m*, *ar.*), 7.94-7.93(1H, *m*, *ar.*), 7.87-7.84(1H, *m*, *ar.*), 7.69-7.65(1H, *m*, *ar.*), 7.48 (1H, *d*, *j=8*), 7.43-7.38(1H, *m*, *ar.*), 7.31-7.29(1H, *m*, *ar.*)**



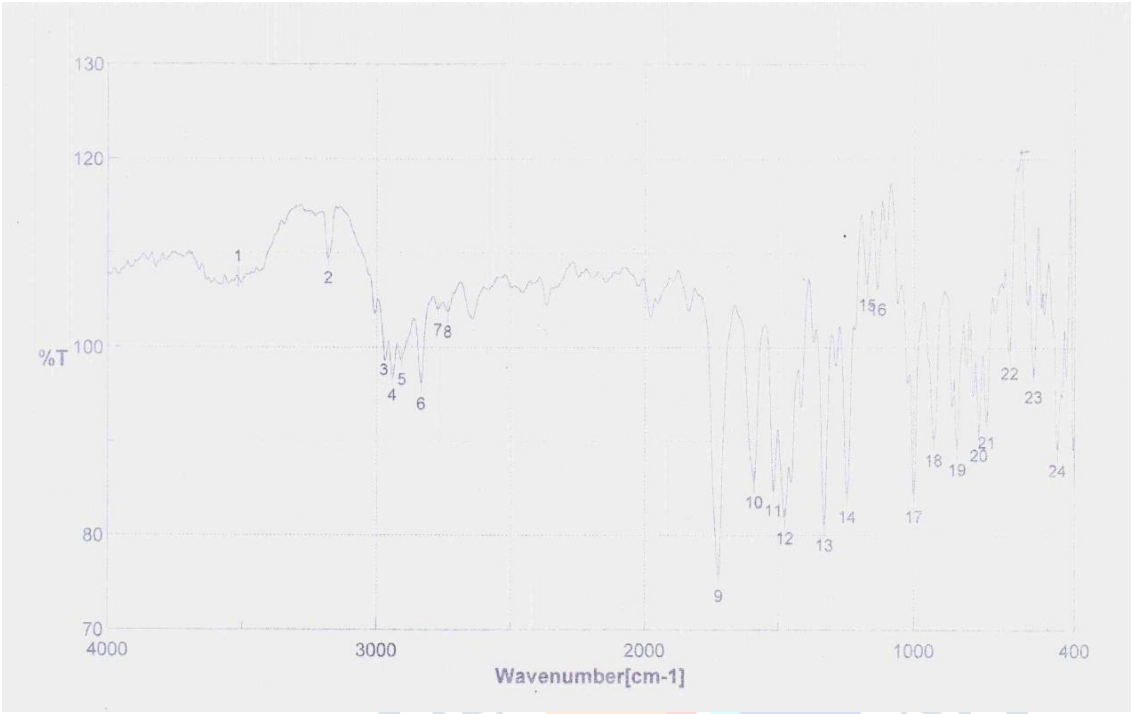
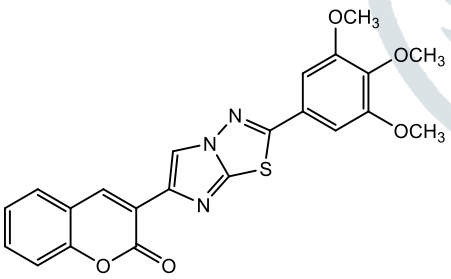


Figure No.-03: IR spectra of 3-(2-(3,4,5-trimethoxyphenyl)imidazo [2,1-*b*] [1,3,4] thiadiazol 6-yl)-2*H*-chromen-2-one.**[CH69]**



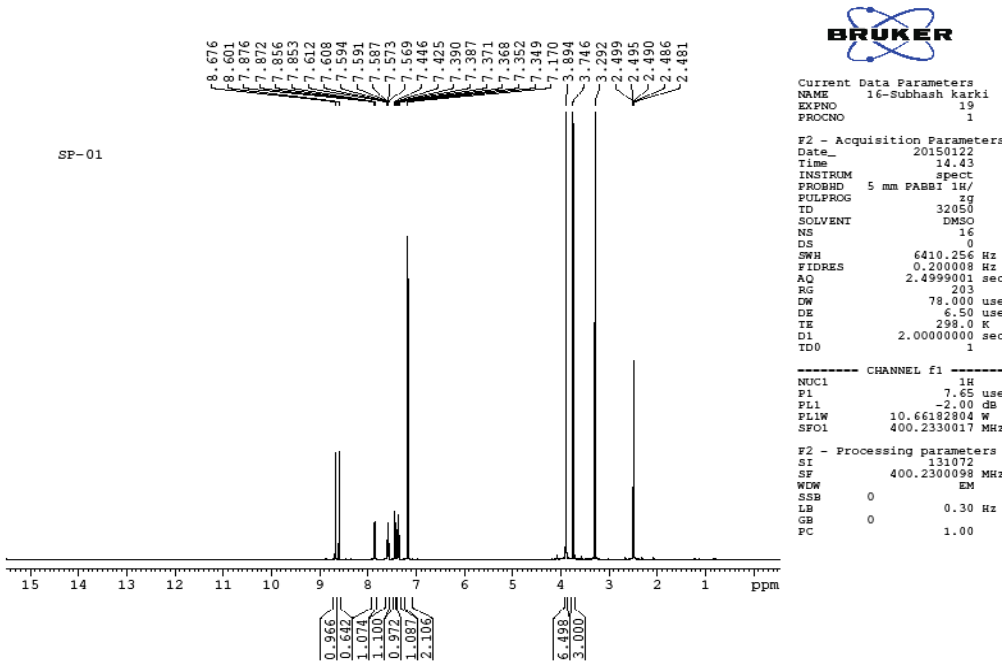


Figure No.-04: ^{1}HNMR Spectra of 3-(2-(3,4,5-trimethoxyphenyl) imidazo [2,1-*b*] [1,3,4] thiadiazol 6-yl)-2*H*-chromen-2-one. **[CH69]**

Results and Discussion

2-aralkyl-6-aryl-imidazo-[2,1-*b*][1,3,4]-thiadiazoles

Series of 2,6-disubstituted-imidazothiadiazoles were prepared. The FTIR spectra find peaks in the range of 3125-3008 and 2969-2764 cm^{-1} for aromatic and aliphatic -CH respectively. The imine (-C=N) and -C=C (Ar.) stretching observed between 1621-1563 and 1545-1463 cm^{-1} respectively. Presence of -C=O stretching at 1702 and 1716 cm^{-1} respectively. The ^{1}H-NMR spectra showed peaks between 8.92-8.49, 8.25-6.93, and 4.95-4.35 δ ppm for imidazole -CH, aromatic -CH, and -CH_2 protons respectively. The 2*H*-chromen-2-one proton of CH-8 and CH-15 appeared at 8.65 and 8.55 δ ppm respectively. The -OCH_3 protons appeared between 3.75-3.74 δ ppm for CH-2, 8 and CH-13. The -CH_3 protons appeared at 2.29 δ ppm in CH-14. ^{13}C-NMR spectra of CH-2 and CH-8 had shown peaks between 165-110 and 37-36 δ ppm for aromatic and -CH_2 carbons respectively. The methyl carbons (-O-CH_3) of CH-2 and 8 appeared at 55 δ ppm. The mass spectra of CH-2 and CH-8 had shown molecular ion peaks in positive mode at m/z 340.02 and 390.08 respectively. The FTIR, ^{1}HNMR, ^{13}C-NMR and HRMS data were summarized in table 4.13, 4.17, 4.21 and 4.22 respectively.

Anticancer activity in human and murine tumor cell lines.[1]

All the compounds (CH-69 to CH-84) were evaluated for their cytostatic activity against human HeLa cervix carcinoma cells, human CEM CD4þ T-lymphocytes as well as murine L1210 cells. All assays were performed in 96-well microtiter plates. To each well were added (5-7.5) x 10^4 tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and human cervix carcinoma HeLa cells) at 37 ºC in a humidified CO2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

Antiviral Activity Assays.

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain Kos, thymidine kinase-deficient (TK-) HSV-1 Kos strain resistant to ACV (ACVr), herpes simplex virus type 2 (HSV-2) strains Lyons and G, varicella zoster virus (VZV) strain Oka, TKVZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis, a clinical isolate of adenovirus type 2 (Ad2), human herpes virus 6 subtype A (HHV-6A) strain GS, vaccinia virus Lederle strain, respiratory syncitial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3, reovirus-1, Sindbis, Punta Toro, yellow fever virus (YFV), human immunodeficiency virus type 1 strain IIIB, human immunodeficiency virus type 2 strain ROD, and hepatitis C virus (HCV). The antiviral, other than anti-HIV and anti-HCV, assays were based on
inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa), or human T-lymphoblasts (HSB-2), according to previously established procedures.[2] Confluent cell cultures in microtiter 96-well plates were inoculated with 100 CCID50 of virus (1 CCID50 being the virus dose to infect 50% of the cell cultures) or with 20 plaque-forming units (PFUs). After a 1-2 h adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying
concentrations of the test compounds. Viral cytopathicity or plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with
the test compounds. Antiviral activity was expressed as the EC50, or the concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

Cytotoxicity Assays.

Cytotoxicity measurements were based on the inhibition of cell growth. HEL cells were seeded at a rate of 5 _ 103 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubationat 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC50, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. CC50 values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Alternatively, cytotoxicity for cell morphology was expressed as the minimum cytotoxic concentration (MCC), or the compound concentration that caused a microscopically detectable alteration of cell morphology.

Inhibitory effects of compounds (CH-69 to CH-84) on the proliferation of murine leukemia cells (L1210) and human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa).

Compound	IC_{50}* (µM)		
	L1210	CEM	HeLa
CH-69	> 250	> 250	> 250
CH-70	**211 ± 14**	**138 ± 35**	> 250
CH-71	≥ 250	**196 ± 4**	> 250
CH-72	> 250	> 250	> 250
CH-73	> 250	> 250	> 250
CH-74	> 250	> 250	> 250
CH-75	> 250	> 250	> 250
CH-76	> 250	> 250	> 250
CH-77	≥ 250	**165 ± 6**	> 250
CH-78	NT	NT	NT
CH-79	> 250	> 250	> 250
CH-80	NT	NT	NT
CH-81	NT	NT	NT
CH-82	> 250	> 250	> 250
CH-83	**23 ± 1**	**3.5 ± 0.8**	**9.5 ± 0.4**
CH-84	**1.6 ± 0.4**	**0.77 ± 0.06**	**0.38 ± 0.03**

*50% inhibitory concentration.

Cytotoxicity and antiviral activity in: MDCK cell cultures (µM)

Compound	Cytotoxicity		Antiviral EC_{50}[c]					
			Influenza A/H1N1 A/Ned/378/05		Influenza A/H3N2 A/HK/7/87		Influenza B B/Ned/537/05	
	CC_{50}[a]	Minimum cytotoxic concentration[b]	visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
CH-69	52.8	100	>100	>100	>100	>100	>100	>100
CH-70	>100	≥100	>100	>100	>100	>100	>100	>100
CH-71	>100	>100	>100	>100	>100	>100	>100	>100
CH-72	>100	≥100	>100	>100	>100	>100	>100	>100
CH-73	>100	≥100	>100	>100	>100	>100	>100	>100
CH-74	>100	≥20	>100	>100	>100	>100	>100	>100
CH-75	51.4	≥20	>100	>100	>100	>100	>100	>100
CH-76	>100	>100	>100	>100	>100	>100	>100	>100
CH-77	>100	20	>100	>100	>100	>100	>100	>100
CH-78	NT	NT	NT	NT	NT	NT	NT	NT
CH-79	>100	>100	**50**	**32.8**	**100**	**>100**	**20**	**11.7**
CH-80	NT	NT	NT	NT	NT	NT	NT	NT
CH-81	NT	NT	NT	NT	NT	NT	NT	NT
CH-82	>100	≥100	>100	>100	>100	>100	>100	>100
CH-83	>100	≥20	>100	>100	>100	>100	>100	>100
CH-84	2.0	≥0.8	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.3	0.05	4	11.6	0.09	0.07
Ribavirin	>100	>100	20	4.0	20	9.1	6.8	3.1
Amantadine	>200	>200	20	9.2	4	2.8	>200	>200
Rimantadine	>200	>200	40	15.9	0.8	0.1	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

MDCK cells: Madin Darby canine kidney cells

Data indicating antiviral activity are shown in red font, and marked in yellow if the SI (ratio of MCC to EC50) is five or higher.

Note that the SI can not be accurately calculated for compounds showing no cytotoxicity at the highest concentration tested (100µM).

Cytotoxicity and antiviral activity in: HEL cell cultures (Concentration µM)

Compound	Minimum cytotoxic concentration[a]	EC_{50}[b] Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Herpes simplex virus-1 TK^- KOS ACV^r	Vaccinia virus	Adeno virus-2	Human Coronavirus (229E)
CH-69	>100	>100	>100	>100	>100	>100	>100
CH-70	>100	>100	>100	>100	>100	>100	>100
CH-71	100	>100	>100	>100	>100	>100	>100

CH-72	>100	>100	>100	>100	>100	>100	>100
CH-73	>100	>100	>100	>100	>100	>100	>100
CH-74	>100	>100	>100	>100	>100	>100	>100
CH-75	>100	>100	>100	>100	>100	>100	>100
CH-76	>100	>100	>100	>100	>100	>100	>100
CH-77	>100	>100	>100	>100	>100	>100	>100
CH-78	NT	NT	NT	NT	NT	NT	NT
CH-79	20	>100	>100	>100	>100	>100	>100
CH-80	NT	NT	NT	NT	NT	NT	NT
CH-81	NT	NT	NT	NT	NT	NT	NT
CH-82	100	>100	>100	>100	>100	>100	>100
CH-83	100	>100	>100	>100	>100	>100	>100
CH-84	20	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.08	112	>250	10	-	-
Cidofovir	>250	5	2	2	14	10	-
Acyclovir	>250	0.08	0.8	>250	>250	-	-
Ganciclovir	>100	0.3	0.094	20	>100	-	-
Zalcitabine	>250	-	-	-	-	5.8	-
Alovudine	>250	-	-	-	-	10	-
UDA	>100	-	-	-	-	-	1.8
Ribavirin	>250	-	-	-	-	-	85

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Cytotoxicity and antiviral activity in: HeLa cell cultures

Compound	**Concentration unit**	**Minimum cytotoxic concentration[a]**	**EC_{50}[b]**		
			Vesicular stomatitis virus	**Coxsackie virus B4**	**Respiratory syncytial virus**
69	µM	>100	>100	>100	>100
70	µM	>100	>100	>100	>100
71	µM	>100	>100	>100	>100
72	µM	>100	>100	>100	>100
73	µM	>100	>100	>100	>100
74	µM	100	>100	>100	>100
75	µM	>100	>100	>100	>100
76	µM	>100	>100	>100	>100
77	µM	100	>100	>100	>100
78	--	NT	NT	NT	NT
79	µM	≥100	>100	>100	>100
80	--	NT	NT	NT	NT
81	--	NT	NT	NT	NT
82	µM	>100	>100	>100	>100
83	µM	100	>100	>100	>100
84	µM	4	>100	>100	>100
DS-10.000	µg/ml	>100	1.4	>100	0.5
Ribavirin	µM	>250	5	146	3.4

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Compound	Concentration unit	Minimum cytotoxic concentration[a]	EC_{50}[b]					
			Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus	Yellow Fever virus
CH-69	µM	≥20	>100	>100	>100	>100	>100	>100
CH-70	µM	>100	>100	>100	>100	>100	>100	>100
CH-71	µM	100	>100	>100	>100	>100	>100	>100
CH-72	µM	>100	>100	>100	>100	>100	>100	>100
CH-73	µM	>100	>100	>100	>100	>100	>100	>100
CH-74	µM	≥20	>100	>100	>100	>100	>100	>100
CH-75	µM	100	>100	>100	>100	>100	>100	>100
CH-76	µM	>100	>100	>100	>100	>100	>100	>100
CH-77	µM	≥20	>100	>100	>100	>100	>100	>100
CH-78	--	NT	NT	NT	NT	NT	NT	NT
CH-79	µM	100	>100	>100	>100	>100	>100	>100
CH-80	--	NT	NT	NT	NT	NT	NT	NT
CH-81	--	NT	NT	NT	NT	NT	NT	NT
CH-82	µM	>100	>100	>100	>100	>100	>100	>100
CH-83	µM	100	>100	>100	>100	>100	>100	>100
CH-84	µM	≥0.8	>100	>100	>100	>100	>100	>100
DS-10.000	µg/ml	>100	>100	>100	>100	58	50	0.4
Ribavirin	µM	≥250	19	111	>250	>250	25	>250
Mycophenolic acid	µM	>100	0.4	0.6	>100	>250	2.3	0.8

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	Concentration unit	CC_{50}[a]	EC_{50}[b]	
			Feline Corona Virus (FIPV)	Feline Herpes Virus
CH-69	µM	>100	>100	>100
CH-70	µM	>100	>100	>100
CH-71	µM	>100	>100	>100
CH-72	µM	>100	>100	>100
CH-73	µM	>100	>100	>100
CH-74	µM	>100	>100	>100
CH-75	µM	>100	>100	>100
CH-76	µM	>100	>100	>100
CH-77	µM	13.0	>100	>100
CH-78	--	NT	NT	NT
CH-79	µM	39.6	>100	>100
CH-80	--	NT	NT	NT
CH-81	--	NT	NT	NT
CH-82	µM	>100	>100	>100
CH-83	µM	>100	>100	>100
CH-84	µM	4.9	>100	>100
HHA	µg/ml	>100	3.3	2.7
UDA	µg/ml	>100	14.4	9.1
Ganciclovir	µM	>100	>100	1.6

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

CRFK cells: Crandell-Rees Feline Kidney cells.

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