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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) × 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°C in a humidified CO_2 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).

Keywords: HeLa • MDCK • CRFK • Thymidine kinase-deficient (TK-) HSV-1 Kos strain • Herpes simplex virus

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 Later it was withdrawn from the market because of the serious side effects like agranulocytosis [2].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 (Figure 1).

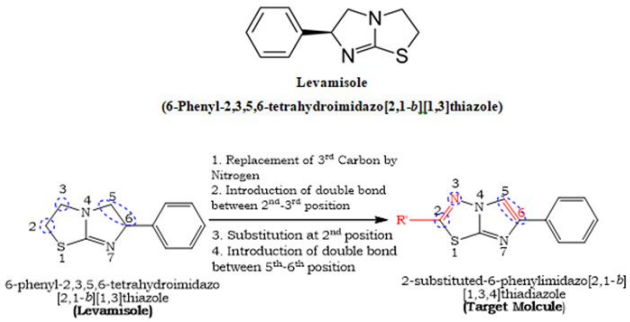

Figure 1. Synthesis of target molecule from levamisole.

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 (Figure 2).

Figure 2. Imidazo [2,1-b]-1,3,4-thiadiazole and Imidazo [5,1-b]-1,3,4-thiadiazole.

Other researchers have shown imidazo[2,1-b][1,3,4]thiadiazole nucleus as an useful scaffold for the development of novel anticancer agent [4-8].

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".

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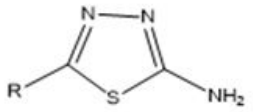
Methods and Materials

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

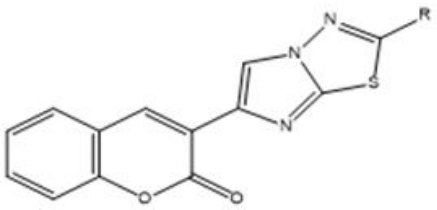
0.034 M of Phosphorous oxychloride was added drop-wise to mixture of 0.01 M of carboxylic acid [E] and thiosemicarbazide [F] with constant stirring. The reaction mixture was refluxed for one hour, cooled and added to 250 ml of ice-cold water and neutralized with 10% potassium hydroxide solution. The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



2-amino-5-substituted-1,3,4-thiadiazole

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

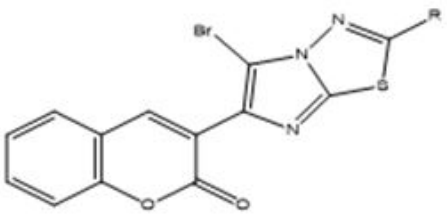
Equimolar quantity of 3-(2-bromoacetyl)-2H-chromen-2-one [D] and 5-substituted-1,3,4-thiadiazole-2-amine [G] in ethanol was refluxed for 10 hours-12 hours. The reaction mixture was poured in ice-cold water and pH of the solution was adjusted to 7.0 with aqueous solution of Na_2CO_3 to get 3-(2-substituted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



3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one

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 0.0050 M of anhydrous sodium acetate and 0.0025 M of an appropriate 3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one, 0.0025 M of bromine was added drop wise at room temperature. The stirring was stirred for 1 hour 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 1.



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

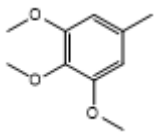
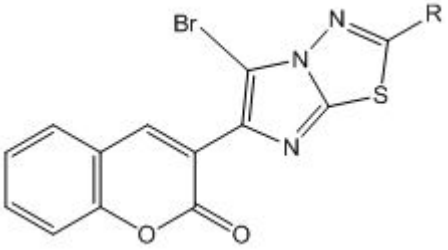
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f Value
CH-69		Yellow, amorphous	62	210-212	$C_{22}H_{17}N_3O_5S$	435.45	0.56
CH-70	2-Methyl thiophene	Brown, crystalline	40	196-98	$C_{18}H_{11}N_3O_2S_2$	365.43	0.54
CH-71	0	Brown, amorphous	68	222-224	$C_{14}H_9N_3O_2S$	283.3	0.52
CH-72	Phenyl	White, amorphous	65	278-80	$C_{19}H_{11}N_3O_2S$	345.37	0.5
CH-73	Thiophene	Brown, crystalline	70	290-292	$C_{17}H_9N_3O_2S_2$	351.4	0.77

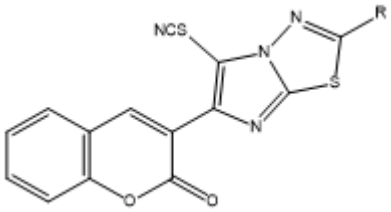
Table 1. Physical parameters of different 5-bromo-3-(2-substituted imidazo [2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one.



5-bromo-3-(2-substituted imidazo [2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one

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

0.0025 M of bromine in glacial acetic acid (10 ml) was added drop wise at 0°C to a solution of 0.0025 M of 3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one and 0.004 M of potassium thiocyanate in 10 ml of glacial acetic acid,. The reaction mixture was further stirred for 1 hour at 15°C-18°C, 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 2.



5-thiocyanato-3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one

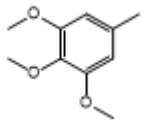
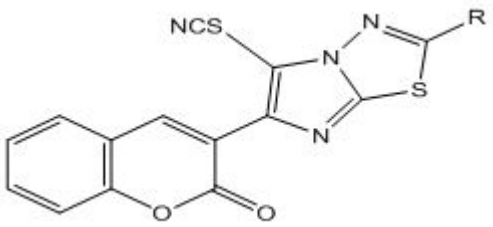
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f
CH-74		Yellow, amorphous	55	218-220	$C_{22}H_{16}BrN_3O_5S$	514.35	0.63
CH-75	$-CH_3$	White, amorphous	50	199-200	$C_{14}H_8BrN_3O_2S$	362.2	0.55
CH-76	Thiophene	Brown, amorphous	60	248-249	$C_{17}H_8BrN_3O_2S_2$	430.29	0.48

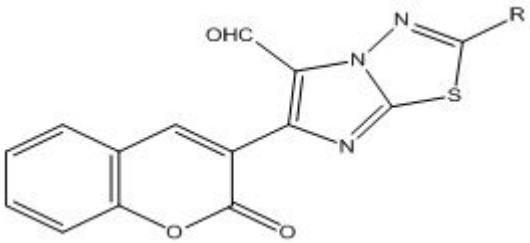
Table 2. Physical parameters of different 5-thiocyanato-3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one.



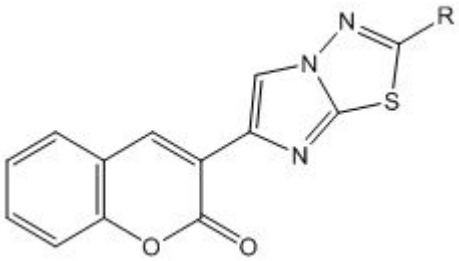
5-thiocyanato-3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one

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

0.002 M of 3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-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°C-5°C for 30 min) at room temperature. Stirring was continued for 4 hours at 80°C-90°C. The resulting reaction mixture was poured into ice cold water and neutralized to pH 7 with cold aqueous solution of sodium carbonate (Figures 3 and 4). The solid so obtained was filtered and recrystallized from ethanol (Tables 3-6).



5-formyl-3-(2-substituted imidazo[2,1-b]- 1,3,4-thiadiazol-6-yl)-2H-chromen-2-one



3-(2-substituted imidazo[2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-ones

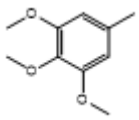
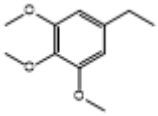
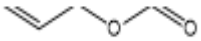
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f
CH-77		Yellow, amorphous	70	242-244	$C_{23}H_{16}N_4O_5S_2$	492.53	0.44
CH-78		White, amorphous	55	180-182	$C_{24}H_{18}N_4O_5S_2$	506.55	0.56

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



5-formyl-3-(2-substituted imidazo [2,1-b]-1,3,4-thiadiazol-6-yl)-2H-chromen-2-one

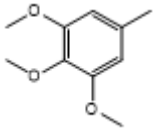
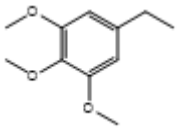
Code	R	Nature	% Yield	M.P (°C)	M.F	M.W	R_f Value
CH-79		Light, yellow	35	192-194	$C_{23}H_{17}N_3O_6S$	463.46	0.54
CH-80		White, amorphous	40	178-180	$C_{24}H_{19}N_3O_6S$	477.49	0.66
CH-81	Phenyl	Brown, amorphous	45	250-252	$C_{20}H_{11}N_3O_3S$	373.38	0.72
CH-82	Thiophene	Brown, crystalline	40	278-280	$C_{18}H_9N_3O_3S_2$	379.41	0.58

Table 4. IR Spectral data of synthesized derivatives.

Compound code	Spectral peaks (cm^{-1})	Molecular stretch
CH-69	3050.01, 2972.73-2734	C-H (aromatic)
	1724.05, 1590.99	C-H (aliphatic)
	1476.24	>C=O
		>C=N
		>C=C
CH-70	3034.44	C-H (aromatic)
	2968.87-2911.99	-C-H (aliphatic)
	1716.34	>C=O (Ketone)
	1605.45	>C=N
	1480.1	>C=C

CH-71	3042.16	-C-H (aromatic)
	2899.45-2844.49	-C-H (aliphatic)
	1722.12	>C=O (Ketone)
	1609.31	>C=N
	1462.74	>C=C
CH-72	3046.98	-C-H (aromatic)
	2972.73	-C-H (aromatic)
	1718.26	>C=O
	1606.41	>C=N
	1432.85	>C=C
CH-73	3058.55	-C-H (aromatic)
	2966.95-2826.17	-C-H (aliphatic)
	1713.44	>C=O (Ketone)
	1606.41	>C=N
	1471.42	>C=C
CH-74	2997.8	C-H (aromatic)
	2942.84-2826.17	C-H (aliphatic)
	1738.51	>C=O
	1610.27	>C=N
	1478.17	>C=C
CH-75	3045.05	-C-H (aromatic)
	2924.52-2961.16	-C-H (aliphatic)
	1729.83	>C=O (Ketone)
	1604.48	>C=N
	1471.42	>C=C
CH-76	3052.76	-C-H (aromatic)
	2942.84-2765.42	-C-H (aliphatic)
	1729.83	>C=O (Ketone)
	1597.41	>C=N
	1471.42	>C=C
CH-77	3028.66	C-H (aromatic)
	2979.48-2833.48	C-H (aliphatic)
	2167.6	-CN
	1707.66	>C=O
	1610.27	>C=N
	1466.6	>C=C
CH-78	2942.84-2747.10	-C-H (aliphatic)
	2158.92	-CN
	1702.84	>C=O

	1604.48	>C=N
	1465.83	>C=C
CH-79	2942.84-2836.77	-C-H (aliphatic)
	1721.16	>C=O (Ketone)
	1677.77	>C=O (Aldehyide)
	1589.06	>C=N
	1474.31	>C=C
CH-80	3001.2	-C-H (aromatic)
	2906.20-2747.10	-C-H (aliphatic)
	1716.34	>C=O (Ketone)
	1654.62	>C=O (Aldehyde)
	1598.7	>C=N
	1467.56	>C=C
CH-82	3061.44	-C-H (aromatic)
	2972.73-2869.56	-C-H (aliphatic)
	1712.48	>C=O (Ketone)
	1664.27	>C=O (Aldehyde)
	1610.27	>C=N
	1475.28	>C=C

Table 5. 1H NMR spectral data of synthesized compounds.

Compound code	Chemical shift value (δ) in ppm and proton nature
CH_{-69}	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).
CH_{-70}	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).
CH_{-71}	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).
CH_{-72}	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).
CH_{-73}	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.)
CH_{-74}	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).
CH_{-75}	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).
CH_{-76}	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.)
CH_{-77}	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).
CH_{-78}	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).

CH_{-79}	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, $-OCH_3$).
CH_{-80}	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, $-OCH_3$).
CH_{-82}	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.)

Table 6. Compound code and chemical shift value (δ) in ppm and proton nature.

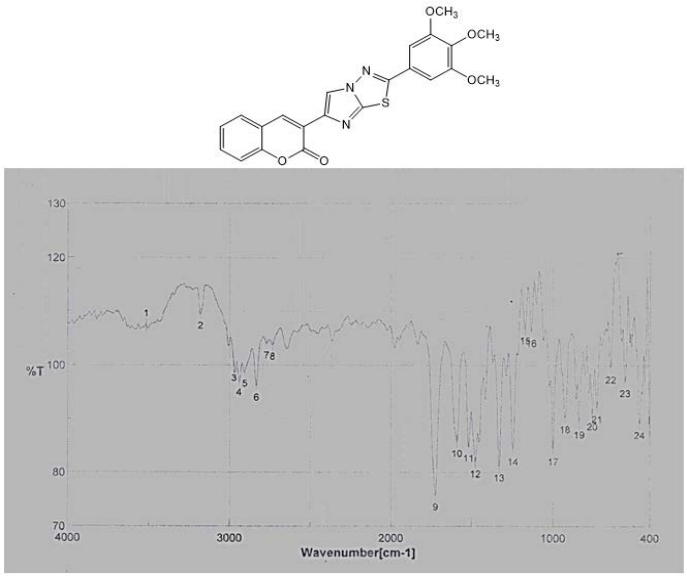


Figure 3. IR spectra of 3-(2-(3,4,5-trimethoxyphenyl)imidazo [2,1-b] [1,3,4] thiadiazol 6-yl)-2H-chromen-2-one.[CH_{69}].

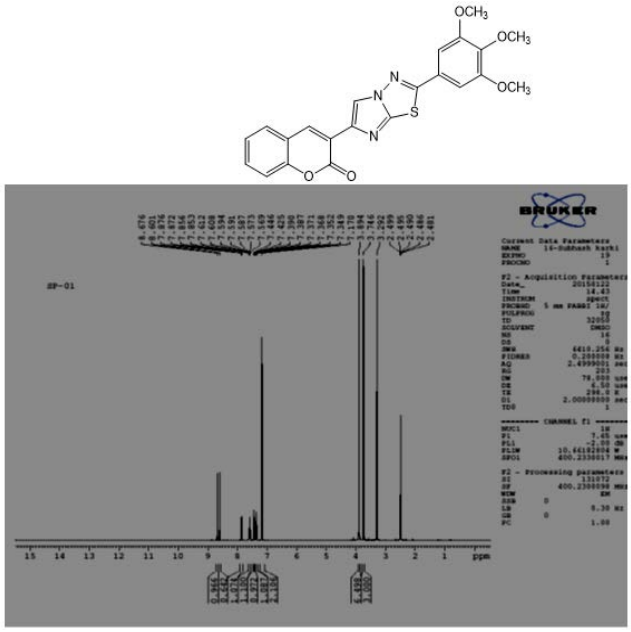


Figure 4. ^{1}HNMR spectra of 3-(2-(3,4,5-trimethoxyphenyl) imidazo [2,1-b] [1,3,4] thiadiazol 6-yl)-2H-chromen-2-one. [CH_{69}].

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 cm^{-1}-2764 cm^{-1} for aromatic and aliphatic -CH respectively. The imine (-C=N) and -C=C (Ar.) stretching observed between 1621 cm^{-1}-1563 cm^{-1} and 1545 cm^{-1}-1463 cm^{-1} respectively. Presence of -C=O stretching at 1702 cm^{-1} and 1716 cm^{-1} respectively. The 1H-NMR spectra showed peaks between 8.92-8.49, 8.25-6.93, and 4.95 -4.35 δδ ppm for imidazole -CH, aromatic -CH, and $-CH_2$ protons respectively. The 2H-chromen-2-one proton of CH-8 and CH-15 appeared at 8.65 δand 8.55 δppm respectively. The $-OCH_3$ protons appeared between 3.75 δ-3.74 δppm for CH-2, 8 and CH-13. The $-CH_3$ protons appeared at 2.29 δppm in CH-14. 13C-NMR spectra of CH-2 and CH-8 had shown peaks between 165-110 and 37 δ-36 δppm for aromatic and $-CH_2$ carbons respectively. The methyl carbons (-O-CH_3) of CH-2 and 8 appeared at 55 δppm. 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.

Anticancer activity in human and murine tumor cell lines

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) × 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°C in a humidified CO_2 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. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 Plaque-Forming Units (PFUs). After a 1 h-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 EC_{50}, 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 cells-10^3 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 CC_{50}, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} 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) (Tables 7 and 8).

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.

Table 7. Inhibitory effects of compounds.

Cytotoxicity			Antiviral EC_{50}[C]		
Compound	CC_{50}[a]	Minimum cytotoxic concentration[b]	Influenza A/H_1N_1 A/Ned/378/05	Influenza A/H_3N_2 A/HK/7/87	Influenza B B/Ned/537/05

			Visual score	CPE	MTS	Visual score	CPE	MTS	Visual score	CPE	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	≥ 0.8	>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	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

Note: [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 EC_{50}) is five or higher (Tables 9-12).

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

Table 8. Cytotoxicity and antiviral activity in: MDCK cell cultures (µM).

EC_{50}[b]							
Compound	**Minimum cytotoxic concentration[a]**	**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

Note: [a]Required to cause a microscopically detectable alteration of normal cell morphology; [b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 9. Cytotoxicity and antiviral activity in: HEL cell cultures (Concentration μM).

EC50[b]							
Compound	**Concentration unit**	**Minimum concentration[a]**	**cytotoxic**	**Vesicular virus**	**stomatitis**	**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

Note: [a]Required to cause a microscopically detectable alteration of normal cell morphology; [b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 10. Cytotoxicity and antiviral activity in: HeLa cell cultures.

			EC50[b]					
Compound	**Concentration unit**	**Minimum cytotoxic concentration[a]**	**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

Note: [a]Required to cause a microscopically detectable alteration of normal cell morphology; [b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 11. Cytotoxicity and antiviral activity in: Vero cell cultures.

			EC50[b]	
Compound	**Concentration unit**	**CC_{50}[a]**	**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	>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

Note: [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.

Table 12. Cytotoxicity and antiviral activity in: CRFK cell cultures.

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