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BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Biological Evaluation of Benzo[*d*]isothiazole, Benzothiazole and Thiazole Schiff Bases

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Abstract—Three new series of benzo[*d*]isothiazole, benzothiazole and thiazole Schiff bases were synthesized and tested in vitro with the aim of identifying novel lead compounds active against emergent and re-emergent human and cattle infectious diseases (AIDS, hepatitis B and C, tuberculosis, bovine viral diarrhoea) or against drug-resistant cancers (leukaemia, carcinoma, melanoma, MDR tumors) for which no definitive cure or efficacious vaccine is available at present. In particular, these compounds were evaluated in vitro against representatives of different virus classes, such as a HIV-1 (Retrovirus), a HBV (Hepadnavirus) and the single-stranded RNA^+ viruses Yellow fever virus (YFV) and Bovine viral diarrhoea virus (BVDV), both belonging to Flaviviridae. Title compounds were also tested against representatives of Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Salmonella* spp.), various atypic mycobacterial strains (*Mycobacterium fortuitum* and *Mycobacterium smegmatis*), yeast (*Candida albicans*) and mould (*Aspergillus fumigatus*). None of the compounds showed antiviral or antimicrobial activity. The benzo[*d*]isothiazole compounds showed a marked cytotoxicity ($CC_{50} = 4–9\,\mu M$) against the human $CD4^+$ lymphocytes (MT-4) that were used to support HIV-1 growth. For this reason, the most cytotoxic compounds of this series were evaluated for their antiproliferative activity against a panel of human cell lines derived from haematological and solid tumors. The results highlighted that all the benzo[*d*]isothiazole derivatives inhibited the growth of leukaemia cell lines, whereas only one of the above mentioned compounds (**1e**) showed antiproliferative activity against two solid tumor-derived cell lines.

Introduction

The twentieth century has been characterised both by a drastic reduction in the mortality caused by infectious diseases and by a rise in the control of neoplastic pathologies. Nevertheless, microorganism and viruses, on the one hand, and tumors, on the other, still represent a dreadful menace to men's health and therefore, for a more efficient control, require the steady development of novel and more powerful drugs. To this end, as a continuation of our research program on benzo[*d*]-isothiazole and benzothiazole derivatives of biological interest,[1–7] we synthesized new compounds bearing an azomethine linkage and evaluated in vitro their antiviral, antimicrobial and antiproliferative activities.

This study reports a comparative evaluation of benzo [*d*]isothiazole, benzothiazole and thiazole Schiff bases (azomethines) purposely designed to combine the heterocyclic ring with an arylidene moiety bearing different substituents. The compounds synthesized for this study are shown in Figure 1 where structures **1** and **2** represent the benzo[*d*]isothiazole and the benzothiazole Schiff bases, respectively, which were derived from the isomeric benzo[*d*]isothiazole and benzothiazole heterocycles. Structures **3**, related to the latter compounds, are simple thiazole Schiff bases that can be regarded as simplified versions of structure **2**, which follows an approach that is often applied in rational drug design. Here we report the synthesis of the above compounds and the evaluation of their biological activity.

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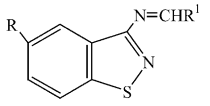
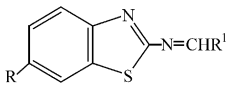


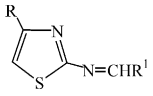
1

1a R= H; R^1=3-ClC_6H_4
1b R= H; R^1=4-$OCH_3C_6H_4$
1c R= H; R^1=2-OH, 3-$OCH_3C_6H_3$
1d R= H; R^1=4-OH, 3-$OCH_3C_6H_3$
1e R= H; R^1=5-NO_2-2-Furanyl
1f R= CH_3; R^1=2-OHC_6H_4
1g R= CH_3; R^1=2-ClC_6H_4
1h R= CH_3; R^1=3-ClC_6H_4
1i R= CH_3; R^1=4-$OCH_3C_6H_4$



2

2a R= H; R^1=C_6H_5
2b R= H; R^1=2-ClC_6H_4
2c R= H; R^1=2-$NO_2C_6H_4$
2d R= H; R^1=3-$NO_2C_6H_4$
2e R= F; R^1=3-ClC_6H_4
2f R= F; R^1=4-$NO_2C_6H_4$
2g R= OC_2H_5; R^1=4-OHC_6H_4



3

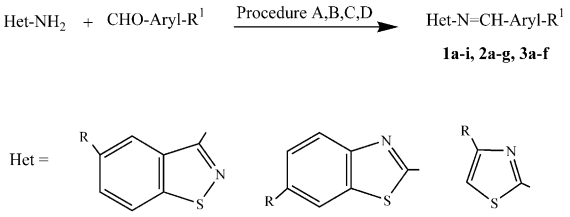
3a R= H; R^1=C_6H_5
3b R= H; R^1=3-ClC_6H_4
3c R= H; R^1=4-$NO_2C_6H_4$
3d R= H; R^1=4-$OCH_3C_6H_4$
3e R= 4-$OCH_3C_6H_4$; R^1= C_6H_5
3f R=4-$OCH_3C_6H_4$; R^1=3-OCH_3,4-OHC_6H_3

Figure 1. Structures of the benzo[*d*]isothiazole, benzothiazole and thiazole Schiff bases.

Chemistry

The general method which is employed to prepare the final compounds **1a–i**, **2a–g** and **3a–f** is outlined in Scheme 1.

The designed Schiff bases were prepared by reacting the appropriate heteroarylamine with the selected aldehyde. Nucleophilic addition of the NH_2 group to the carbonyl function of the aromatic aldehyde is not straightforward[8] and different experimental conditions A, B, C and D were developed to prepare the target compounds. Difficulties connected with the poor reactivity of some starting materials were overcome by the use of suitable catalysts and with azeotropical removal of water by a Dean–Stark trap. Each of the final compound **1a–i**, **2a–g** and **3a–f** was purified by crystallization using absolute ethanol (unless otherwise noted). It emerged that anhydrous crystallization solvents were essential to avoid decomposition by hydrolysis, at varying rates, of the compounds. The various heteroarylamines, key intermediates for the synthesis of the final compounds, were prepared according to the methods described in our previous papers.[7,9] The test compounds already described had constants in agreement with the reported literature values (see Table 1). The structures of the newly described compounds were confirmed by elemental analysis and by IR, MS and ^{1}H NMR spectral data. Physical, chemical and IR relevant spectroscopic data of the compounds are reported in Table 1.



Scheme 1. Reagents: (A) anhydrous benzene 5 h, reflux; (B) absolute ethanol and acetic acid 15 min, reflux; (C) benzene and *p*-toluensulphonic acid 6 h, reflux; (D) absolute ethanol and piperidine 3 h, reflux.

Structural ^{1}H NMR, complete IR data and elemental analysis have only been given in the Experimental for compound **1b.** The complete structural data and the elemental analysis, for all the novel compounds can be obtained from the authors on request.

Results and Discussion

The benzo[*d*]isothiazole, benzothiazole and thiazole Schiff bases were evaluated for cytotoxicity against MT-4 cells, which was carried out in parallel with the evaluation of the anti-HIV-1 activity (Table 2), to determine whether the compounds were endowed with selective antimicrobial/antiviral activity. The compounds were also tested for antiviral, antimicrobial and antiproliferative activities (Tables 3–5).

Contrary to the benzothiazole (**2a–g**) and thiazole Schiff bases (**3a–f**), the benzo[*d*]isothiazoles (**1a–i**) showed significant cytotoxicity against MT-4 cells (Table 2). When tested for antiviral activities, the above compounds resulted inactive against representatives of different virus classes, such as a Retrovirus (HIV-1), a Hepadnavirus (HBV), Yellow fever virus (YFV) and Bovine viral diarrhoea virus (BVDV).

Surprisingly, the derivatives bearing 5-nitrofurane (**1e**), chlorophenyl (**1a**, **1g**, **1h**, **2b**, **2e**, **3b**) and nitrophenyl (**2c**, **2d**, **2f**, **3c**) substituents, which were expected to be endowed with antimicrobial activity, were inactive against bacteria and fungi (data not shown). As far as the anti-mycobacterial activity was concerned, compounds **1a–i** inhibited Mycobacterium *smegmatis* and/or *fortuitum* at concentrations close to those cytotoxic, thus resulting poorly selective in their anti-mycobacterial activity (Table 3).

Due to the fact that the benzo[*d*]isothiazole Schiff bases (**1a–i**) inhibited the growth of MT-4 cells, they were also evaluated against a panel of human cell lines derived from haematological and solid tumors (Tables 4 and 5). Generally, these compounds showed antiproliferative activity in the micromolar range against both T-lymphoblastic and B-lymphoblastic leukaemia cells and they did not inhibit the proliferation of solid tumor-derived cell lines. It is noteworthy that only compound **1e**, which bears a 5-nitrofurane at position R^1, was active against skin melanoma and breast adenocarcinoma cells. This result suggests that the benzo[*d*]-isothiazole arylidene derivatives might be interesting enough for further investigations on potential antitumor effects.

Table 1. Physico-chemical data of the benzo[*d*]isothiazole, benzothiazole and thiazole Schiff bases **1a–i**, **2a–g**, **3a–f**

Compd	Molecular formula[a] (Mol. Mass)	Procedure	Mp (°C)	Yield (%)	IR ν (cm^{-1})		
					N=C	Azomethine C–H	Substituents
1a	$C_{14}H_9ClN_2S$ (272.75)	A	122–124	70	1619.91	2919.70, 2838.70, 1388.50	—
1b	$C_{15}H_{12}N_2OS$ (268.33)	A	87–88	78	1599.66	2903.31, 2826.17, 1380.78	2964.05–2877.27 (CH_3)
1c	$C_{15}H_{12}N_2O_2S$ (284.33)	B	145–147[b]	75	1597.73	2930.31, 2826.17, 1382.71	3522.34–3251.40 (OH) 2998.77–2826.17 (CH_3)
1d	$C_{15}H_{12}N_2O_2S$ (284.33)	C	141–143[b]	68	1598.70	2929.34, 2838.70, 1394.28	3369.03-2981.41 (OH) 2958.27-2836.77 (CH_3)
1e	$C_{12}H_7N_3O_3S$ (273.27)	B	171–173[b]	69	1596.77	2924.52, 2838.70, 1382.71	1517.70, 1346.07 (NO_2)
1f	$C_{15}H_{12}N_2OS$ (268.33)	A	118–120	40	1601.59	2929.34, 2838.70, 1377.89	3535.84–3316.00 (OH) 2967.91–2822.67 (CH_3)
1g	$C_{15}H_{11}ClN_2S$ (286.78)	A	125–127	52	1599.66	2915.84, 2826.17, 1380.78	2967.91–2812.67 (CH_3)
1h	$C_{15}H_{11}ClN_2S$ (286.78)	A	114–116	43	1618.95	2919.70, 2838.70, 1379.82	2967.91–2812.67 (CH_3)
1i	$C_{16}H_{14}N_2OS$ (282.36)	A	89–91	40	1599.66	2903.31, 2826.17, 1375.00	3000.59–2823.13 (CH_3)
2a	$C_{14}H_{10}N_2S$ (238.31)[10]	C	100–101	96	—	—	—
2b	$C_{14}H_9ClN_2S$ (272.75)[10]	C	176–177	57	—	—	—
2c	$C_{14}H_9N_3O_2S$ (283.31)[10]	C	164–166	67	—	—	—
2d	$C_{14}H_9N_3O_2S$ (283.31)[10]	C	188–189	69	—	—	—
2e	$C_{14}H_8ClFN_2S$ (290.74)	C	106–108	75	1603.52	2970.8, 2908.12, 1380.81.	—
2f	$C_{14}H_8FN_3O_2S$ (301.30)	C	157–159	80	1605.45	2968.87, 2897.54, 1390.15	1527.35, 1347.03 (NO_2)
2g	$C_{16}H_{14}N_2O_2S$ (298.36)[5]	C	228–230	55	—	—	—
3a	$C_{10}H_8N_2S$ (188.25)[8]	D	113–115	92	—	—	—
3b	$C_{10}H_7ClN_2S$ (222.69)[11]	D	138–140	98	—	—	—
3c	$C_{10}H_7N_3O_2S$ (233.25)[8]	D	183–184	60	—	—	—
3d	$C_{11}H_{10}N_2OS$ (218.28)[8]	D	102–103	74	—	—	—
3e	$C_{17}H_{14}N_2OS$ (294.37)	D	123–125	41	1602.56	2932.23, 2834.85, 1385.02	2978.34–2815.41 (CH_3)
3f	$C_{18}H_{16}N_2O_3S$ (340.40)[12]	D	140–142	80	—	—	—

[a]Known products are given with references, the others are new.
[b]Crystallized from benzene.

Table 2. Cytotoxicity and anti-HIV-1 activity of the benzo[*d*]isothiazole, benzothiazole and thiazole Schiff bases **1a–i**, **2a–g**, **3a–f**

Compd	CC_{50}[a] (MT-4)	EC_{50}[b] (HIV-1)
1a	9	>9
1b	7	>7
1c	6	>6
1d	6	>6
1e	4	>4
1f	7	>7
1g	8	>8
1h	7	>7
1i	5	>5
2a	>100	>100
2b	>100	>100
2c	25	>25
2d	60	>60
2e	>100	>100
2f	≥100	>100
2g	≥100	>100
3a	>100	>100
3b	>100	>100
3c	63	>63
3d	>100	>100
3e	≥100	>100
3f	≥100	>100
AZT[c]	150	0.01

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b]Compound concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogeneticy, as determined by the MTT method.
[c]Azidothymidine (AZT) was used as reference drug.

Table 3. Antimycobacterial activity of the benzo[*d*]isothiazole, benzothiazole and thiazole Schiff bases **1a–i**, **2a–g**, **3a–f**

Compd	CC_{50}[a] MT-4	MIC_{50}[b]/MIC_{90}[c]	
		M. smegmatis	*M. fortuitum*
1a	9	>100	70≯100
1b	7	>100	57≯100
1c	6	78≯100	50≯100
1d	6	>100	55≯100
1e	4	42≯100	11\49
1f	7	>100	52≯100
1g	8	>100	>100
1h	7	>100	65≯100
1i	5	83≯100	55≯100
2a	>100	>100	>100
2b	>100	73≯100	>100
2c	25	>100	>100
2d	60	>100	>100
2e	>100	>100	>100
2f	≥100	>100	>100
2g	≥100	>100	>100
3a	>100	>100	>100
3b	>100	>100	>100
3c	63	>100	>100
3d	>100	>100	>100
3e	≥100	>100	>100
3f	≥100	>100	>100
Ciprofloxacin[d]	60	0.6\2.3	0.5\8.5
Isoniazid[d]	>100	1.8\8.5	>100

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b]Minimum inhibitory concentration (μM) required to reduce the number of viable mycobacteria by 50%, as determined by the MTT method.
[c]Minimum inhibitory concentration (μM) required to reduce the number of viable mycobacteria by 90%, as determined by the MTT method.
[d]Ciprofloxacin and Isoniazid were used as reference drugs.

Table 4. Antiproliferative activity against leukaemia-/lymphoma-derived cell lines of the benzo[*d*]isothiazole Schiff bases **1a–i**

Compd	IC_{50}[a] (μM)			
	MT4[b]	CCRF-CEM[c]	WIL-2NS[d]	CCRF-SB[e]
1a	6	2	10	4
1c	5	2	10	5
1d	5	2.5	10	4
1e	2	2	6.5	5
1f	5	2	6	3
1g	3	1.5	5	3
1h	2	1.5	7	4
1i	4	1.5	6.5	4
2A-6-MP[f]	0.1	0.3	0.5	0.3

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication.
[b]$CD4^+$ human T-lymphoblastoid cells.
[c]$CD4^+$ human acute T-lymphoblastic leukaemia.
[d]Human splenic B-lymphoblastoid cells.
[e]Human acute B-lymphoblastic leukaemia.
[f]2-Amino-6-mercaptopurine (2A-6-MP) was used as reference drug.

Table 5. Antiproliferative activity against solid tumour-derived cell lines of the benzo[*d*]isothiazole Schiff bases **1a–i**

Compd	IC_{50}[a] (μM)			
	SK-MEL-28[b]	MCF7[c]	SKMES-1[d]	DU145[e]
1a	>20	>20	>20	>20
1c	>20	>20	>20	>20
1d	>20	>20	>20	>20
1e	6	10	>20	>20
1f	>20	>20	>20	>20
1g	>20	>20	>20	>20
1h	>20	>20	>20	>20
1i	>20	>20	>20	>20
2A-6-MP[f]	5	4	2	2

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication.
[b]Human skin melanoma.
[c]Human breast adenocarcinoma.
[d]Human lung squamous carcinoma.
[e]Human prostate carcinoma.
[f]2-Amino-6-mercaptopurine (2A-6-MP) was used as reference drug.

Experimental

Chemistry

Melting points (°C) were determined with a Buchi 512 apparatus and are uncorrected. New compounds were analysed in the analytical laboratory of Dipartimento Farmaceutico, Università di Parma, on a ThermoQuest (Italia) FlashEA 1112 Elemental Analyzer, for C H N and S. The found values for C H N S were always ±0.4% of the theoretical ones.

IR spectra were recorded, as KBr pellets, on a Jasco FT-IR 300E spectrophotometer (Jasco Ltd., Tokyo, Japan). Spectral IR data were consistent with the assigned structure in all cases and the reported wavenumbers are given in cm^{-1}. ^1H NMR spectra, in DMSO-d_6 solutions, were recorded on a Bruker AC 300 instrument at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard. The progress of the reactions was monitored by thin-layer chromatography with F_{254} silica-gel precoated sheets (Merck) using methylene chloride/ethanol 99.5/0.5 as eluent; UV light was used for detection. Solvents, unless otherwise specified, were of analytical reagent grade or of the highest quality commercially available. Synthetic starting material, reagents and solvents were purchased from Aldrich Chemical Co. and were rigorously dried.

General procedure A for synthesis of benzo[*d*]isothiazol-3-yl-(arylidene)-amines 1a,b,f–h

A solution of benzo[*d*]isothiazol-3-ylamine or 5-methyl-benzo[*d*]isothiazol-3-ylamine (5 mmol) and the appropriately substituted benzaldehyde (6 mmol) in anhydrous benzene (25 mL) was refluxed for 6 h. The mixture kept overnight at room temperature gave a precipitate which was removed by filtration and purified by recrystallization.

Experimental data of benzo[*d*]isothiazol-3-yl-(4-methoxy-benzylidene)-amine 1b. Anal. calcd for $C_{15}H_{12}N_2OS$ (268.33): C, 67.14; H, 4.51; N, 10.44; S, 11.95. Found: C, 67.47; H, 4.53; N, 10.16; S, 11.65. IR (KBr) ν cm^{-1}: 3097.12–2974.05 (C–H aromatic), 2964.05–2877.27 (CH_3), 2903.31, 2826.17, 1380.78 (C–H azomethine), 1606.41 (N=C), 1573.63, 1511.92, 1259.29. ^1H NMR (300 MHz, DMSO-d_6): 9.18 (s, 1H, CH), 8.19–8.16 (m, 2H, H-4,7), 8.11–8.08 (m, 2H, H-2′, 6′), 7.69–7.63 (m, 1H, H-5), 7.57–7.52 (m, 1H, H-6), 7.15–7.12 (m, 2H, H-3′, 5′), 3.88 (s, 3H, CH_3).

General procedure B for synthesis of benzo[*d*]isothiazol-3-yl-(arylidene)-amines 1c,e. A well stirred solution of benzo[*d*]isothiazol-3-ylamine (5 mmol), the appropriate arylaldehyde (6 mmol) and acetic acid (0.5 mL), in ethanol (18 mL), was heated for 15 min, until no starting amine was detected by TLC. After cooling at room temperature, the precipitate was collected, filtered and washed with diethylether to give the crude product which was purified by recrystallisation.

General procedure C for synthesis of benzo[*d*]isothiazol-3-yl-(3-methoxy-4-hydroxy-benzylidene)-amine 1d and of benzothiazol-2-yl-(arylidene)-amines 2a–g

A solution of amine (5 mmol), the appropriate arylaldehyde (6 mmol) and *p*-toluenesulphonic acid (a small amount), in anhydrous benzene (30 mL) was refluxed for 6 h while water was removed in a Dean–Stark trap. The solid product obtained after cooling was collected by filtration and purified by recrystallisation.

General procedure D for synthesis of thiazol-2-yl-(arylidene)-amines 3a–f

The properly substituted thiazol-2-ylamine (5 mmol), a slight excess of the aldehyde (6 mmol) and a few drops

of piperidine in ethanolic solution, were refluxed in a water bath for 3 h. After cooling the final products were precipitated, filtered and recrystallized.

Microbiology

Test compounds were dissolved in DMSO at 100 mM and then diluted in culture medium.

Cytotoxicity and antiproliferative assays

Exponentially growing human $CD4^+$ lymphocytes (MT-4), baby hamster kidney (BHK-21), Madin Darby bos kidney (MDBK) and human cell lines derived from hematological and solid tumor were resuspended in growth medium containing serial dilutions of the drugs. Cell viability was determined after 96 h at 37 °C by the 3 - (4,5 - dimethylthiazol - 2 - yl) - 2,5 - diphenyl - tetrazolium bromide (MTT) method[13]

Antiviral assay

Activity of compounds against HIV-1 was based on inhibition of virus-induced cytopathogenicity in acutely infected MT-4 cells at a multiplicity of infection of 0.01.[14] Briefly, 50 μL of culture medium containing 1×10^4 cells were added to each well of flat-bottom microtiter trays containing 50 μL of culture medium with or without test compounds. Then 20 μL of an HIV suspension containing 100 ($HIV\text{-}1_{IIIB}$) $CCID_{50}$ was added. After a 4-day incubation, cell viability was determined by 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method.[13]

Activity against YFV and BVDV was based on inhibition of virus-induced cytopathogenicity in acutely infected BHK-21 and MDBK cells, respectively. Cells were seeded overnight at a rate of 3×10^4–5×10^4/well into 96-well plates and incubated in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were infected with 50 μL of a proper virus dilution to give a m.o.i. = 0.01. Then, serial dilutions of test compounds in Minimum essential medium with Earle's salts supplemented with 2% inactivated foetal calf serum, respectively, were added. After a 3-day incubation at 37 °C, the number of viable cells was determined by the MTT method.

Antibacterial and antimycotic assays

Staphylococcus aureus, *Salmonella* spp and *Aspergillus fumigatus* are clinical isolates, *Candida albicans* 10231 is an ATCC strain. Assays were carried out in Triptosio agar for S. aureus and Salmonella spp, and in Sabouraud dextrose broth for *C. albicans* and *A. fumigatus*, with an inoculum of 10^3 bacteria/mL and 5×10^3 yeast/mL. *A. fumigatus* inocula were obtained from cultures grown at 37 °C for 1 days and then diluting to 0.05 OD_{50}/mL. Minimum inhibitory concentrations (MIC) were determined after incubations at 37 °C for 18 h in the presence of serial dilutions of test compounds.

Antimycobacterial assays

Mycobacterium tuberculosis 27294 and *Mycobacterium smegmatis* 19420 are ATCC strains, *Mycobacterium fortuitum* is a clinical isolate. MICs were assessed in microtiter plates by adding 20 μL aliquots of a culture suspension to 80 μL of Middlebrook 7H9 medium containing serial dilutions of test compounds. At the end of incubation, the number of viable mycobacteria was determined by the MTT method.[13]

Linear regression analysis

Viral and tumor cell growth at each drug concentration was expressed as percentage of untreated controls and the concentrations resulting in 50% (EC_{50}, EC_{50}) growth inhibition was determined by linear regression analysis.

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