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Short communication

Synthesis and primary cytotoxicity evaluation of 3-[[(3-phenyl-4(3H)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-1H-2-indolinones

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Abstract

New esters (**2b** and **2c**) and hydrazides (**3b** and **3c**) were synthesized from 6-methyl/fluoro-3-phenyl-4(1H, 3H)-quinazolinone-2-thiones (**1b** and **1c**). Subsequent treatment of 3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetic acid hydrazides (**3a**–**e**) with 1*H*-indole-2,3-diones (**4a**–**e**) furnished the corresponding 3-[[(3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-1*H*-2-indolinones (**5a**–**u**). The structures of new compounds were determined by analytical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR, EIMS) methods. Previously reported 3-[[(3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-5-bromo-1*H*-2-indolinone **5v** and compounds **5b**, **5d** and **5o** chosen as prototypes were evaluated against the full panel of 60 human tumour cell lines at a minimum of five concentrations at tenfold dilutions in the National Cancer Institute in vitro primary cytotoxicity assay. Sulforhodamine B protein assay was used to estimate cell stability or growth. 3-[[(6-Chloro-3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-5-fluoro-1*H*-2-indolinone **5o** showed the most favourable cytotoxicity against a renal cancer cell line UO-31 ($\log_{10}$ GI_{50} value −6.68). Compound **5v** was also tested against human immunodeficiency virus 1 (HIV-1). Compound **5v** was confirmed moderately active against HIV-1.

Keywords: 1*H*-Indole-2,3-diones; 3-Phenyl-4(3*H*)-quinazolinones; Hydrazones; Cytotoxicity

1. Introduction

The non-nucleoside inhibitors of human immunodeficiency virus 1 (HIV-1) reverse transcriptase have also been studied extensively in the laboratory and clinic as antiviral agents for the treatment of immunodeficiency syndrome (AIDS). Williams et al. identified 5-chloro-3-(phenylsulfonyl)-indole-2-carboxamide (**I**) as a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase enzyme in vitro [1]. Modification of the non-nucleoside inhibitor of HIV-1 reverse transcriptase nevirapine by incorporation of a 2-indolyl substitutent (**II**) conferred activity against several mutant forms of the enzyme [2]. Methisazone (**III**) was one of the first antiviral compounds used in clinical practice. This drug plays an important role as a prophylactic agent against several viral diseases [3]. Delaviridine (**IV**) have been approved for use in combination with nucleoside reverse transcriptase inhibitors for the treatment of HIV [4]. Substituted oxoisoindolines (**V**) have been proved to be potent cytotoxic agents in suspended cells derived from solid uterine tumours by Hall et al. [5–7] (Fig. 1). On the other hand, quinazoline derivatives are used in medicine because of their wide-range biological properties. As documented in the literature, many derivatives act as anticancer and anti-HIV agents [8–13]. It was reported that these compounds interact with biological nucleophiles such as L-cysteine and sulfhydryl bearing enzymes in a Michael-type addition reaction and possess cytotoxic and antitumour activity as an alkylating agent [14]. In this study, previously reported 3-[[(3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-5-bromo-1*H*-2-indolinone **5v** [15] was evaluated against the full panel of 60 human tumour cell lines and HIV-1. The cytotoxicity of compound **5v** was comparable to those of thioguanine and 5-fluorouracil used as anticancer agents. Compound **5v** was also confirmed moderately active against HIV-1. In view of these facts, a new

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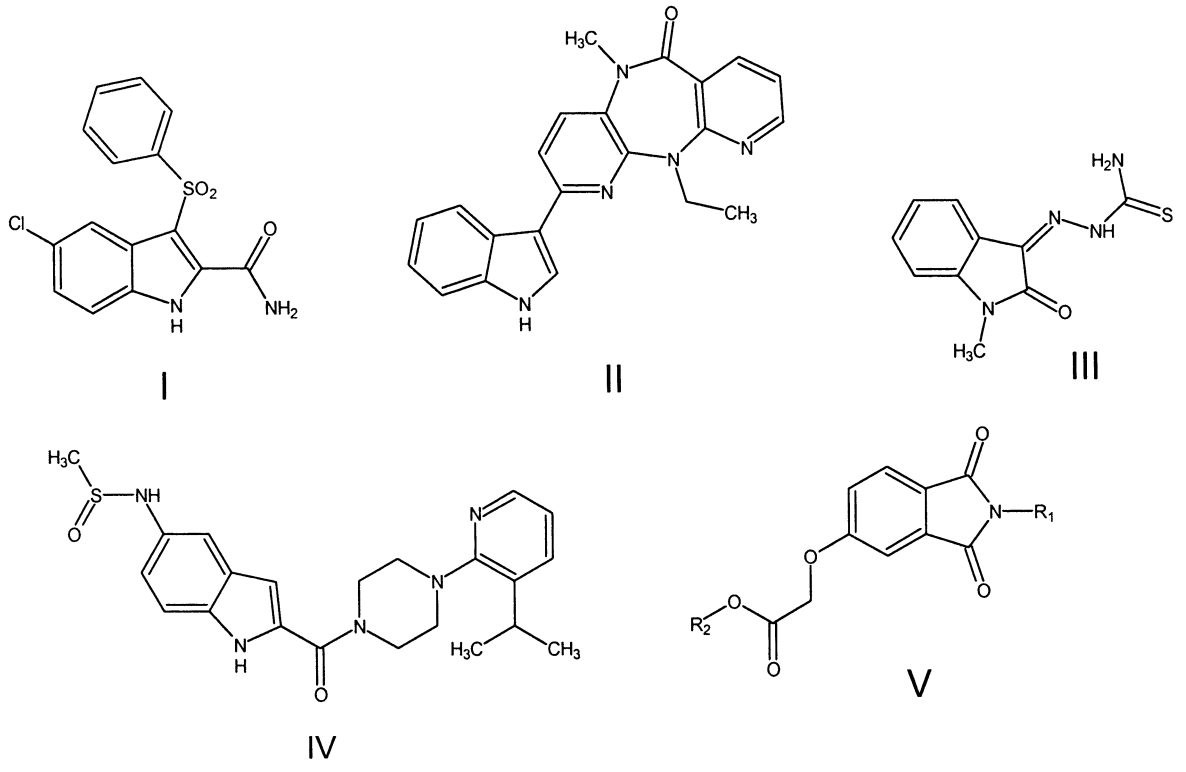
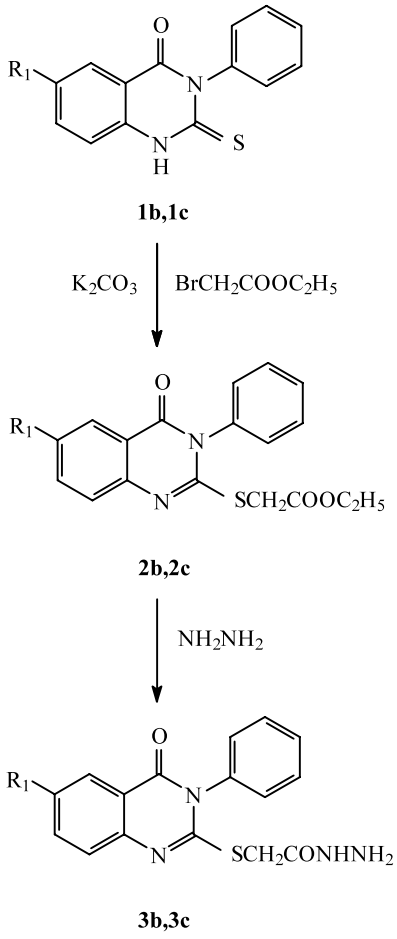


Fig. 1. Some antiviral and cytotoxic agents bearing the indole structure.

series of 3-[[(3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-1*H*-2-indolinones (**5a**–**u**) were synthesized for primary cytotoxicity evaluation.

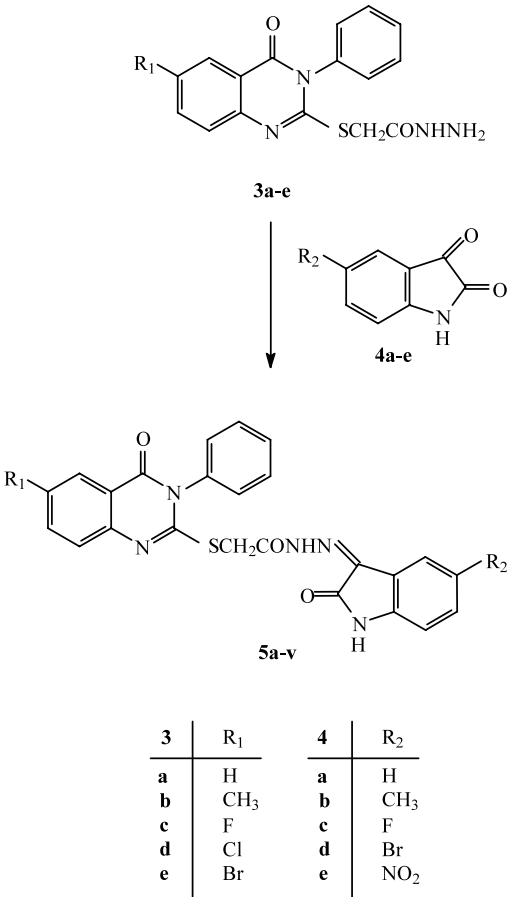
2. Chemistry

6-Methyl/fluoro-3-phenyl-4(1*H*,3*H*)-quinazolinone-2-thiones (**1b** and **1c**) reacted with ethyl bromoacetate in alkaline medium to give the corresponding esters (**2b** and **2c**). The esters (**2b** and **2c**) were treated with hydrazine hydrate to yield 6-methyl/fluoro-3-phenyl-4(3*H*)-quinazolinone-2-ylmercaptoacetic acid hydrazides (**3b** and **3c**) (Fig. 2). Subsequent treatment of 3-phenyl-4(3*H*)-quinazolinone-2-ylmercaptoacetic acid hydrazides (**3a**–**e**) with an appropriate isatin (1*H*-indole-2,3-dione) (**4a**–**e**) furnished the corresponding 3-[[(3-phenyl-4(3*H*)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-1*H*-2-indolinones (**5a**–**v**) (Fig. 3). The structures of new compounds were confirmed by physical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR, EIMS) data (Tables 1–3). IR spectra of **2b** and **2c** showed two bands resulting from the C=O stretching bands of the ester and lactam functions in the 1740, 1692 and 1740, 1693 cm^{-1}, respectively [16]. IR spectra provided evidence for the confirmation of the hydrazide structure (**3b** and **3c**) as the N–H stretching bands (3298 and 3300 cm^{-1}) absorbed, ester C=O stretching bands disappeared and a new C=O stretching bands (1651 and 1651 cm^{-1}) indicative of the hydrazide structure appeared in addition to the lactam stretching bands (1694 and 1695 cm^{-1}). The spectra of **5a**–**u** exhibited the N–H and C=O stretching bands in the 3316–3180 and 1741–1655 cm^{-1} regions, respectively [11,16–18]. In the ^{1}H-NMR spectra of **2b**, **2c**, **3b** and **3c** the SCH_2 protons were shifted to higher fields as singlets (δ 3.81–3.96 ppm), whereas the SCH_2 protons of **5a**, **5h**, **5l**, **5n** and **5r** chosen as prototypes displayed as two separate singlets (δ 4.08–4.36 and 4.44–4.55 ppm). Observation of the NH_2 and NH signals (δ 4.27, 4.26 and 9.31, 9.29 ppm) assigned to the $NHNH_2$ moiety in the spectra of **3b** and **3c** provided support for hydrazide formation. In the spectra of **5a**, **5h**, **5l**, **5n** and **5r**, the indole NH and N–NH protons were observed as singlets or two separate singlets presumably due to the cooperative effects of the C=N and C–N bonds and bulk of the attached 3-phenyl-4(3*H*)-quinazolinone structure which can disrupt free rotation about the cited bonds [19]. The protons of indole and quinazolinone nucleus resonated at the expected regions in the literature [11,20–22]. In the APT ^{13}C-NMR spectra of **5a** and **5n** chosen as prototypes displayed the SCH_2, quinazolinone C_2, indole C_3, indole CO, quinazolinone CO and amid CO peaks (δ 34.65, 156.74, 157.38, 160.55, 162.55, 164.57 and 33.17, 34.55, 156.88, 157.47, 159.63, 162.55, 164.75, 169.50 ppm) which verified the proposed quinazolinoylhydrazonoindolinone structure [23–25] (Table 3). In the EIMS spectra of **2b**, **2c**, **3b**, **3c**, **5a**, **5h**, **5l**, **5n** and **5r**



1-3	R_1
b	CH_3
c	F

Fig. 2. Synthesis of **2b**, **2c**, **3b** and **3c**.



3	R_1	4	R_2
a	H	a	H
b	CH_3	b	CH_3
c	F	c	F
d	Cl	d	Br
e	Br	e	NO_2

Fig. 3. Synthesis of **5a**–**v**.

showed molecular ion (M^+) peaks of different intensity (except **5a**). The major fragmentation pathway was formed by the cleavage of the NH–CO bonds of the mercaptoacetylhydrazono moiety [26]. The fragments peculiar to the quinazolinone and indole moieties were also observed in the spectra of **5** [27–30].

3. Pharmacology

The cytotoxic and/or growth inhibitory effects of previously reported compound **5v** [15] and compounds **5b**, **5d** and **5o** chosen as prototypes were tested in vitro against the full panel of 60 human tumour cell lines derived from nine neoplastic diseases at tenfold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. Primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [31–33]. For the compounds, the 50% growth inhibition (GI_{50}) and total growth inhibition (TGI) were obtained for each cell line. The $\log_{10} GI_{50}$ and $\log_{10} TGI$ were then determined, defined as the mean of the $\log_{10}$'s of the individual GI_{50} and TGI values. Negative values indicated the most sensitive cell lines. Compounds having values -4 and < -4 were declared to be active. As shown in Table 4, $\log_{10} GI_{50}$ values of compounds **5b**, **5d**, **5o** and **5v** were smaller than -4 against all the tested tumour cell lines (except NSCLC NCI-H226). Among the compounds tested, $R_1 = Cl$ and $R_2 = F$ substituted compound **5o** demonstrated the most marked effects in the National Cancer Institute's 60 human tumour cell line in vitro screen on a renal cancer cell line UO-31 ($\log_{10} GI_{50}$ value -6.68). In addition, compound **5o** was highly active on a renal cancer cell line (RFX 393, $\log_{10} GI_{50}$ value -5.78) and a CNS cell line (U251, $\log_{10} GI_{50}$ value -5.77). On the same cancer cell lines, the $\log_{10} GI_{50}$ values of thioguanine and 5-fluorouracil used as anticancer agents were -5.83, -6.12, -5.31 and -5.17, -4.26, -4.37, respectively. When these data were examined, it was observed that compound **5o** was much more active than thioguanine and 5-fluorouracil against a renal cancer cell line (UO-31) and more active than 5-fluorouracil against a renal cancer cell line

Table 1
Physical and IR data of **2b**, **2c**, **3b**, **3c** and **5a–v**

Compound	R_1	R_2	Yield (%)	M.p. (°C)	Formula (M.A.)	Analysis (Calc./Found) C	H	N	IR (KBr, cm^{-1})
2b	CH_3	–	55	145	$C_{19}H_{18}N_2O_3S$	64.39	5.12	7.90	1740, 1692 (CO)
					(354.42)	64.76	5.20	8.06	
2c	F	–	95	128	$C_{18}H_{15}FN_2O_3S\cdot\frac{1}{2}H_2O$	58.84	4.39	7.62	1740, 1693 (CO)
					(367.40)	59.09	4.31	7.74	
3b	CH_3	–	82	210–2	$C_{17}H_{16}N_4O_2S$	59.98	4.74	16.46	3298 (NH)
					(340.41)	60.38	5.14	16.34	1694, 1651 (CO)
3c	F	–	73	192–4	$C_{16}H_{13}FN_4O_2S$	55.80	3.80	16.27	3300 (NH)
					(344.37)	55.66	4.23	16.21	1695, 1651 (CO)
5a	H	CH_3	66	269–70	$C_{25}H_{19}N_5O_3S\cdot 2H_2O$	59.40	4.59	13.85	3290 (NH)
					(505.56)	59.60	4.05	13.34	1699, 1662 (CO)
5b	H	F	80	255–9	$C_{24}H_{16}FN_5O_3S$	60.88	3.41	14.79	3259 (NH)
					(473.49)	60.77	3.46	14.84	1731, 1700, 1660 (CO)
5c	H	NO_2	66	274–6	$C_{24}H_{16}N_6O_5S\cdot 2H_2O$	53.73	3.76	15.66	3199 (NH)
					(536.53)	53.62	3.36	15.47	1703, 1660 (CO)
5d	CH_3	H	77	282–3	$C_{25}H_{19}N_5O_3S\cdot\frac{1}{2}H_2O$	62.75	4.21	14.64	3274 (NH)
					(478.54)	62.40	4.37	14.28	1691, 1665 (CO)
5e	CH_3	CH_3	50	261–3	$C_{26}H_{21}N_5O_3S\cdot 2\frac{1}{2}H_2O$	59.07	4.95	13.24	3255 (NH)
					(528.61)	59.30	4.44	13.14	1692, 1663 (CO)
5f	CH_3	F	84	278–80	$C_{25}H_{18}FN_5O_3S\cdot H_2O$	59.40	3.99	13.85	3283 (NH)
					(505.53)	59.65	4.03	13.85	1732, 1700, 1662 (CO)
5g	CH_3	Br	84	278–9	$C_{25}H_{18}BrN_5O_3S\cdot\frac{1}{2}H_2O$	53.87	3.43	12.56	3217 (NH)
					(557.44)	53.62	3.68	12.25	1699, 1657 (CO)
5h	CH_3	NO_2	70	290	$C_{25}H_{18}N_6O_5S\cdot 3H_2O$	52.81	4.25	14.78	3180 (NH)
					(568.58)	52.49	3.83	14.75	1702, 1655 (CO)
5i	F	H	79	265–8	$C_{24}H_{16}FN_5O_3S$	60.88	3.41	14.79	3273 (NH)
					(473.49)	60.78	3.84	14.71	1725, 1700, 1665 (CO)
5j	F	CH_3	89	270–4	$C_{25}H_{18}FN_5O_3S$	61.59	3.72	14.37	3306 (NH)
					(487.52)	61.47	3.81	14.13	1725, 1698, 1664 (CO)
5k	F	F	97	268–70	$C_{24}H_{15}F_2N_5O_3S\cdot\frac{1}{2}H_2O$	57.59	3.22	13.99	3296 (NH)
					(500.49)	57.78	2.97	13.61	1697, 1664 (CO)
5l	F	Br	95	276–7	$C_{24}H_{15}BrFN_5O_3S$	52.18	2.74	12.68	3248 (NH)
					(552.39)	52.39	2.71	12.58	1697, 1660 (CO)
5m	F	NO_2	81	283–4	$C_{24}H_{15}FN_6O_5S\cdot\frac{1}{2}H_2O$	54.64	3.05	15.93	3190 (NH)
					(527.49)	54.64	2.99	15.76	1702, 1658 (CO)
5n	CI	CH_3	90	274–5	$C_{25}H_{18}ClN_5O_3S$	59.58	3.60	13.90	3313 (NH)
					(503.97)	59.65	3.67	14.15	1698, 1668 (CO)
5o	CI	F	77	275–9	$C_{24}H_{15}ClFN_5O_3S$	56.75	2.98	13.79	3260 (NH)
					(507.93)	56.56	2.98	13.59	1697, 1663 (CO)
5p	CI	NO_2	91	284–5	$C_{24}H_{15}ClN_6O_5S\cdot 1\frac{1}{2}H_2O$	51.29	3.23	14.95	3199 (NH)
					(561.96)	50.91	2.87	14.96	1702, 1660 (CO)
5q	Br	H	62	272–6	$C_{24}H_{16}BrN_5O_3S$	53.94	3.02	13.11	3278 (NH)
					(534.40)	53.91	2.94	13.12	1698, 1665 (CO)
5r	Br	CH_3	63	266–9	$C_{25}H_{18}BrN_5O_3S$	54.75	3.31	12.77	3316 (NH)
					(548.43)	54.72	3.21	13.02	1726, 1699, 1668 (CO)
5s	Br	F	64	270–1	$C_{24}H_{15}BrFN_5O_3S\cdot 1\frac{1}{2}H_2O$	49.74	3.13	12.08	3266 (NH)
					(579.42)	49.43	3.36	11.45	1687 (CO)
5t	Br	Br	70	278–80	$C_{24}H_{15}Br_2N_5O_3S$	47.00	2.46	11.41	3218 (NH)
					(613.30)	46.41	1.68	11.16	1698, 1664 (CO)
5u	Br	NO_2	69	281–2	$C_{24}H_{15}BrN_6O_5S\cdot\frac{1}{2}H_2O$	48.99	2.74	14.28	3194 (NH)
					(588.40)	48.92	2.67	14.63	1741, 1703, 1660 (CO)
5v [a]	H	Br	77	273–4	$C_{24}H_{16}BrN_5O_3S$	53.94	3.02	13.11	3245 (NH)
					(534.40)	54.49	2.93	13.13	1728, 1699 (CO)

[a] **5v** was previously reported [15].

RFX 393 and a CNS cell line U251. Moreover, the cytotoxicity of compound **5o** was comparable to that of thioguanine against a renal cancer cell line RFX 393 and a CNS cell line U251. On the other hand, $R_1 = H$ and $R_2 = F$ substituted compound **5b** exhibited high cytotoxicity on leukemia cell lines (SR, K-562 and MOLT-4). The $\log_{10} GI_{50}$ values of compound **5b**, thioguanine and 5-fluorouracil were −5.82, −5.79, −5.79; −6.71,

−6.39, −6.57 and −5.04, −4.77, −4.86 on the same cancer cell lines. The $\log_{10} GI_{50}$ value of $R_1 = H$ and $R_2 = Br$ substituted compound **5v** was −5.77 on a non-small cell lung cancer cell line A549/ATCC, whereas the $\log_{10} GI_{50}$ values of thioguanine and 5-fluorouracil on this cancer cell line were −5.45 and −5.68. When the results of compounds **5b**, **5o** and **5v** were compared with thioguanine and 5-fluorouracil, it was speculated that the cytotoxicities of compounds **5b**, **5o** and **5v** were comparable to those of anticancer agents. Potency might be related to the electronic nature of the R_1 and R_2 substitutents. $R_2 = F$ or Br substituted derivatives **5b**, **5o** and **5v** were more active than compound **5d** which was R_2 unsubstituted. Among the compounds tested, the most cytotoxic compounds were found to be $R_2 = F$ substituted compounds **5b** and **5o**. $R_1 = Cl$ and $R_2 = F$ substituted compound **5o** showed the most favourable cytotoxicity against a renal cancer cell line (UO31). In conclusion, these preliminary results are promising and some of these compounds may be potential candidates for new anticancer agents.

The National Cancer Institute's AIDS antiviral screen was developed as an effort to discover new compounds capable of inhibiting the HIV virus. HIV assay is a relatively simple method to determine the ability of a drug to protect cells against the cytopathic effects of HIV [34]. T-lymphocyte-derived CEM cells are added to 96-well microtiter plates along with cell-free HIV and the test agent at half-log dilutions over a multi-dose range. Six days after infection, a tetrazolium reagent, 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide (XTT), was added to the wells. In the presence of viable cells, XTT was metabolized to an orange coloured formazan, such that the quantity of viable cells, and thus, the protective ability of the test agent, is proportional to the depth of the colour. Uninfected cells were also treated with drug in order to determine the cytotoxicity of the drug, if any, to the CEM cells. The results of the screening tests were evaluated and placed in one of three categories. Compounds able to provide at least 50% protection to the CEM cells were retested. Compounds that provided at least 50% protection on retest were listed as confirmed moderately active. Compounds that reproducibly provided 100% protection were listed as confirmed active. Compounds not meeting these criteria were listed as confirmed inactive.

Fig. 4 displays a plot of the $\log_{10}$ of compound **5v**'s concentrations (as μg mL^{-1}) against the measured test values expressed as a percentage of the uninfected, untreated control values. The solid line depicts the percentage of surviving HIV-infected cells treated with compound **5v** (at the indicated concentration) relative to uninfected, untreated controls. This line expresses the in vitro anti-HIV activity of compound **5v**. The dashed line depicts the percentage of surviving uninfected cells treated with compound **5v** relative to the same uninfected, untreated controls. This line expresses the in vitro growth inhibitory properties of compound **5v**. The viral cytopathic effect in this particular experiment is indicated by a dotted reference line. This line shows the extent of destruction of cells by the virus in the absence of treatment and is used as a quality control parameter. Survival values of this parameter less than 50% are considered acceptable in the current protocol. The percent of protection has been calculated from the data. Table 5 provides a listing of the numerical data plotted in the graphics section. Approximate values for 50% effective concentration (EC_{50}) against HIV cytopathic effects, 50% inhibitory concentration (IC_{50}) for cell growth, and therapeutic index ($TI = IC_{50}/EC_{50}$) have been calculated for each test. As showing in Fig. 4 and Table 5, compound **5v** that provided at least 50% protection on retest was listed as confirmed moderately active.

4. Experimental

4.1. Chemistry

Melting points were estimated with a Büchi 530 melting point apparatus (Flawil, Switzerland) in open capillaries and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyser (Milano, Italy). IR spectra were recorded on KBr (BDH, Poole, England) discs, using a Perkin–Elmer Model 1600 FT-IR spectrometer (Norwalk, CT). ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker AC 200 (200 and 50.3 MHz) (Rheinstätten, Germany) spectrophotometer using DMSO-d_6 (E. Merck, Darmstadt, Germany). EIMS were determined on a VG Zab Spec (70 eV) mass spectrometer (Manchester, England).

4.1.1. Synthesis of 6-methyl/fluoro-3-phenyl-4(3*H*)-quinazolinone-2-ylmercaptoacetic acid ethyl ester (**2b** and **2c**)

To a solution of 0.02 mol 6-methyl/fluoro-3-phenyl-4(1*H*, 3*H*)-quinazolinone-2-thione (**1b**/**1c**) in 30 mL of dimethylformamide, 0.024 mol ethyl bromoacetate and 10 g K_2CO_3 were added. The reaction mixture was refluxed on a water bath for 2 h, poured into ice–water and allowed to stand overnight. The precipitate was filtered and recrystallized from EtOH.

4.1.2. Synthesis of 6-methyl/fluoro-3-phenyl-4(3*H*)-quinazolinone-2-ylmercaptoacetic acid hydrazide (**3b** and **3c**)

To a solution of 0.01 mol **2b** or **2c** in absolute EtOH, 0.02 mol hydrazine hydrate was added. The reaction mixture was refluxed on a water bath for 4 h and allowed to stand overnight. The crystals formed were

Table 2
^{1}H-NMR and EIMS data of **2b**, **2c**, **3b**, **3c**, **5a**, **5h**, **5l**, **5n** and **5r**

Compound	^{1}H-NMR (DMSO-d_6, δ, ppm)	EIMS (70 eV) m/z (%)
2b	1.21 (t, J: 7.1 Hz, 3H, CH_2CH_3), 3.96 (s, 2H, SCH_2), 4.13 (q, J: 7.1 Hz, 2H, CH_2CH_3), 7.39–7.68 (m, 7H, aromatic), 7.87 (s, 1H, quin. C_5–H)	354 (M^+, 100), 309 (52), 281 (93), 267 (67), 248 (51), 235 (70), 176 (21), 146 (28), 133 (45), 104 (48), 77 (61)
2c	1.21 (t, J: 7.0 Hz, 3H, CH_2CH_3), 3.96 (s, 2H, SCH_2), 4.13 (q, J: 7.0 Hz, 2H, CH_2CH_3), 7.46–7.77 (m, 8H, aromatic)	358 (M^+, 100), 312 (45), 285 (78), 271 (59), 252 (38), 239 (68), 150 (26), 137 (18), 108 (33), 77 (53)
3b	2.43 (s, 3H, CH_3), 3.81 (s, 2H, SCH_2), 4.27 (br.s, 2H, $NHNH_2$), 7.43–7.58 (m, 6H, aromatic), 7.66, 7.67 (dd, $J_{7.8}$: 8.3, $J_{7.5}$: 1.5 Hz, 1H, quin. C_7-H), 7.87 (s, 1H, quin. C_5–H), 9.31 (br.s, 1H, $NHNH_2$)	340 (M^+, 34), 310 (52), 309 (100), 268 (63), 267 (91), 235 (32), 176 (21), 146 (47), 133 (35), 104 (36), 77 (44)
3c	3.83 (s, 2H, SCH_2), 4.26 (s, 2H, $NHNH_2$), 7.46–7.76 (m, 8H, aromatic), 9.29 (s, 1H, $NHNH_2$)	344 (M^+, 4), 313 (36), 271 (21), 239 (12), 150 (10), 105 (10), 97 (11), 92 (79), 91 (100), 85 (13), 78 (32), 71 (18), 69 (12), 63 (25), 57 (18)
5a	2.28 (s, 3H, CH_3), 4.19, 4.47 (2br.s, 2H, SCH_2), 6.82 (d, $J_{7,6}$: 7.8 Hz, 1H, indole C_7–H), 7.18 (d, $J_{6,7}$: 7.8 Hz, 1H, indole C_6–H), 7.36–7.71 (m, 8H, indole C_4–H, quin. C_8–H, C_6–H, C_6H_5) 7.76, 7.80 (dd, $J_{7.8}$: 7.8, $J_{7.5}$: 1.1 Hz, 1H, quin. C_7–H), 8.07 (d, $J_{5,6}$: 7.1 Hz, 1H, quin. C_5–H), 11.55 (br.s, 1H, indole NH), 13.40 (br.s, 1H, N–NH)	118 (2), 116 (2), 102 (3), 91 (7), 90 (10), 77 (31), 64 (24), 63 (25), 53 (8), 51 (44), 50 (35), 44 (17), 43 (17), 39 (41), 38 (20), 32 (100)
5h	2.28 (s, 3H, CH_3), 4.15, 4.44 (2s, 2H, SCH_2), 6.82 (d, $J_{7,6}$: 7.8 Hz, 1H, indole C_7–H), 7.18 (d, $J_{6,7}$: 7.8 Hz, 1H, indole C_6–H), 7.35–7.60 (m, 7H, indole C_4–H, quin. C_8–H, C_6H_5) 7.78, 7.81 (dd, $J_{7.8}$: 8.6, $J_{7.5}$: 2.6 Hz, 1H, quin. C_7–H), 8.00 (d, $J_{5,7}$: 2.3 Hz, 1H, quin. C_5–H), 10.72, 11.22 (2s, 1H, indole NH), 12.71, 13.42 (2s, 1H, N–NH)	514 (M^+, 5), 310 (30), 309 (100), 282 (15), 277 (15), 268 (52), 267 (65), 249 (28), 235 (25), 206 (17), 176 (9), 146 (29), 133 (19), 104 (19), 77 (26)
5l	4.34, 4.54 (2br.s, 2H, SCH_2), 6.88 (d, $J_{7,6}$: 7.7 Hz, 1H, indole C_7–H), 7.52–7.76 (m, 10H, aromatic), 11.75 (br.s, 1H, indole NH), 12.61, 13.38 (2br.s, 1H, N–NH)	551 (M^+, 2), 313 (22), 281 (7), 272 (84), 271 (100), 253 (8), 239 (35), 224 (7), 212 (10), 180 (9), 150 (6), 137 (19), 108 (13), 91 (16), 81 (13), 77 (19), 69 (21)
5n	2.42 (s, 3H, CH_3), 4.36, 4.47 (2s, 2H, SCH_2), 7.02 (d, $J_{7,6}$: 8.8 Hz, 1H, indole C_7–H), 7.59 (s, 5H, C_6H_5), 7.47–8.31 (m, 5H, indole C_6–H, C_4–H, quin. C_8–H, C_7–H, C_5–H), 11.85 (br.s, 1H, indole NH), 12.87 (br.s, 1H, N–NH)	503 (M^+, 0.01), 330 [20 (332, 7)], 329 [100 (331 (39)], 288 (16), 287 (25), 255 (12), 166 (16), 153 (18), 146 (10), 175 (10), 132 (12), 77 (17)
5r	2.28 (s, 3H, CH_3), 4.08, 4.55 (2s, 2H, SCH_2), 6.82 (d, $J_{7,6}$: 7.8 Hz, 1H, indole C_7–H), 7.18 (d, $J_{6,7}$: 7.3 Hz, 1H, indole C_6–H), 7.36 (s, 1H, indole C_4–H), 7.54–7.60 (m, 6H, quin. C_8–H, C_6H_5), 7.92 (d, $J_{7.8}$: 8.1 Hz, 1H, quin. C_7–H), 8.13 (d, $J_{5,7}$: 2.1 Hz, 1H, quin. C_5–H), 11.21 (s, 1H, indole NH), 12.71, 13.41 (2br.s, 1H, N–NH)	547 [M^+, 0.2 (549, 0.2)], 374 [51 (376,50)], 373 [100 (375, 99)], 345 [10 (347, 10)], 331 [24 (333, 25)], 313 [11 (315, 11)], 299 [8 (301, 9)], 210 [41 (212, 41)], 168 [13 (170, 13)], 146 (21), 132 (19), 91 (21), 77 (20)

Table 3
^{13}C-NMR data of **5a** and **5n**

Compound	^{13}C-NMR (DMSO-d_6, δ, ppm)
5a	20.41 (CH_3), 34.65 (SCH_2), 110.87 (indole C_7), 119.48 (quin. C_{4a}), 119.68 (indole C_{7a}), 121.08 (phenyl C_4), 126.09 (indol C_4), 126.51 (quin. C_8), 129.39 (phenyl C_2, C_6), 129.52 (phenyl C_3, C_5), 130.01 (quin. C_5), 131.68 (quin. C_6), 132.08 (quin. C_7), 134.21 (phenyl C_1), 134.86 (indole C_6), 135.81 (indole C_5), 140.16 (indole C_{3a}), 146.93 (quin. C_{8a}), 156.74 (quin. C_2), 157.38 (indole C_3), 160.55 (indole CO), 162.55 (quin. CO), 164.57 (amid CO)
5n	20.44 (CH_3), 33.17, 34.55 (SCH_2), 110.88 (indole C_7), 119.62 (quin. C_{4a}), 120.81 (indole C_{7a}), 121.11 (phenyl C_4), 125.43 (indole C_4), 128.24 (quin. C_8), 129.30 (phenyl C_2, C_6), 129.61 (phenyl C_3, C_5), 130.19 (quin. C_5), 131.70 (quin. C_6), 132.11 (quin. C_7), 134.89 (phenyl C_1), 135.40 (indole C_6), 137.76 (indole C_5), 140.19 (indole C_{3a}), 145.67 (quin. C_{8a}), 156.88 (quin. C_2), 157.47 (indole C_3), 159.63 (indole CO), 162.55 (quin. CO), 164.75, 169.50 (amid CO)

filtered, washed with water and after drying recrystallized from EtOH.

4.1.3. Synthesis of 3-[[(3-phenyl-4(3H)-quinazolinone-2-yl)mercaptoacetyl]hydrazono]-1H-2-indolinones (**5a–v**)

To a solution of **3a–e** (0.005 mol) in EtOH (20 mL) was added a solution of an appropriate isatin (**4a–e)** (0.005 mol) in EtOH (25 mL). After addition of a drop concentrated H_2SO_4, the mixture was refluxed on a water bath for 3 h. The product formed after cooling was filtered, washed with EtOH or recrystallized from EtOH.

4.2. In vitro evaluation of anticancer activity

The human tumour cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40 000 cells well^{-1} depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line were fixed in situ with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg mL^{-1} gentamicin. Additional four, tenfold or half-log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (T_z), control growth, (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$$[(T_i - T_z)/(C - T_z)] \times 100 \text{ for concentrations for which } T_i \geq T_z$$

$$[(T_i - T_z)/T_z] \times 100 \text{ for concentrations for which } T_i < T_z.$$

Three dose response parameters were calculated for each experimental agent. GI_{50} was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in TGI was calculated from $T_i = T_z$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that

Table 4
In vitro tumour cell growth inhibition of **5b**, **5d**, **5o** and **5v**

Panel/cell line	**5b**		**5d**		**5o**		**5v**		**A** [a]		**B** [b]	
	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI
Leukemia												
CCRF-CEM	−5.63	−5.30	−4.74	−4.46	−5.55	−5.16	−5.25	> −4.00	−6.81	−4.59	−4.52	−2.63
HL-60(TB)	−5.74	−5.40	−4.59	−4.21	−5.44	−5.14	−5.49	−4.89	−5.96	−4.75	−4.81	−2.77
K-562	−5.79	−5.47	−4.63	−4.21	−5.06	−4.57	−5.61	−5.19	−6.39	−4.15	−4.77	−2.63
MOLT-4	−5.79	−5.46	−4.75	−4.48	−5.35	−5.12	−5.46	> −4.00	−6.57	−4.81	−4.86	−2.85
RPMI-8226	−5.64	−5.33	−4.75	−4.45	−5.74	−5.38	−5.46	−4.84	−6.40	−4.68	−5.24	−2.73
SR	−5.82	−5.38	−4.71	−4.44	−5.22	−4.65	−5.61	−5.15	−6.71	−4.74	−5.04	−3.14
Non-small cell lung cancer												
A549/ATCC	−5.40	−4.67	−4.82	−4.54	−5.52	−5.09	−5.77	−5.46	−5.45	−3.98	−5.68	−2.74
EKVX	−5.61	−5.25	−4.81	−4.53	−5.42	−4.79	−5.67	−5.10	−5.72	−3.91	−3.27	−2.61
HOP−62	−5.68	−5.17	−4.91	−4.61	−5.69	−5.30	−5.60	−5.18	−6.13	−4.54	−4.92	−2.76
HOP-92	−5.74	−5.38	−4.85	−4.53	−5.60	−5.07	−5.43	−4.93	−6.13	−4.62	−4.30	−2.70
NCI−H226	−5.64	–	−4.79	−4.45	−5.46	−4.90	> −4.00	> −4.00	−5.39	−4.01	−3.56	−2.64
NCI-H23	−5.52	−5.04	−4.79	−4.48	−5.53	−5.12	−5.49	−5.00	−5.97	−4.64	−4.94	−2.64
NCI-H322M	−5.68	−5.32	−4.89	−4.59	−5.43	−4.74	−5.26	−4.32	−5.13	−3.65	−4.49	−2.62
NCI-H460	−5.66	−5.33	−4.81	−4.54	−5.15	−4.70	−5.51	−5.03	−6.17	−4.70	−5.97	−3.38
NCI-H522	–	–	–	–	–	–	−4.97	−4.26	−6.04	−5.20	−4.44	−2.85
Colon cancer												
COLO 205	−5.58	−5.24	−4.87	−4.48	−5.63	−5.32	−5.29	> −4.00	−5.77	−5.05	−5.16	−2.95
HCC-2998	−5.66	−5.32	−4.83	−4.54	−5.76	−5.19	−5.38	> −4.00	−6.00	−4.74	−5.91	−2.81
HCT-116	−5.69	−5.35	−4.82	−4.55	−5.64	−5.34	−5.52	> −4.00	−6.27	−4.79	−5.39	−3.49
HCT-15	−5.70	−5.41	−4.76	−4.39	−5.69	−5.39	−5.56	−5.09	−5.96	−4.29	−5.19	−2.66
HT29	−5.51	−5.16	−4.71	−4.47	−5.67	−5.43	−5.73	−5.43	−5.94	−3.98	−5.22	−2.62
KM12	−5.43	−4.49	−4.86	−4.55	−5.48	−4.86	−5.47	> −4.00	−5.76	−4.50	−4.93	−2.69
SW-620	−5.37	−4.82	−4.63	−4.21	−5.62	−5.22	−5.53	–	−5.81	−3.86	−4.64	−2.60
CNS cancer												
SF-268	−5.65	−5.31	−4.86	−4.58	−5.38	−4.77	−5.13	> −4.00	−5.94	−3.80	−4.28	−2.62
SF-295	−5.73	−5.42	−4.80	−4.53	−5.29	−4.67	−5.53	−5.00	−5.99	−3.80	−4.28	−2.62
SF-539	−5.50	−5.04	−5.24	−4.72	−5.58	−5.22	−5.68	−5.21	−5.99	−4.16	−6.08	−3.93
SNB-19	−5.39	−4.17	−4.76	−4.39	−5.23	−4.48	−5.61	−4.89	−4.10	−3.61	−3.91	−2.61
SNB-75	–	–	–	–	−5.66	−5.29	−5.61	−5.21	−5.84	−4.38	−3.78	−2.68
U251	−5.05	> −4.00	−4.91	−4.61	−5.77	−5.50	−5.72	−5.44	−5.31	−3.62	−4.37	−2.61
Melanoma												
LOX IMVI	−5.37	> −4.00	−4.81	−4.53	−5.75	−5.49	−5.50	−5.00	−6.68	−5.04	−5.23	−3.10
MALME-3M	−5.70	−5.35	−4.69	−4.46	−5.32	−4.75	−5.46	−4.95	−5.87	−4.54	−4.68	−2.64
M14	−5.72	−5.34	−4.79	−4.53	−5.36	−4.64	−5.67	−5.38	−6.23	−4.84	−4.49	−2.61
SK-MEL-2	−5.73	−5.44	−5.77	−4.88	−5.75	−5.27	−5.16	−4.39	−6.03	−4.97	−3.44	−2.65
SK-MEL-28	−5.68	−5.34	−4.81	−4.51	−5.52	−4.97	−5.45	–	−5.05	−3.81	−4.56	−2.62
SK-MEL-5	−5.48	−4.81	−4.80	−4.54	−5.42	−4.85	−5.45	> −4.00	−5.45	−4.78	−4.96	−3.23
UACC-257	−5.61	−5.22	−4.62	−4.25	−5.44	−4.96	−5.25	−4.16	−5.70	−4.01	−4.00	−2.63
UACC-62	−5.65	−5.32	−4.77	−4.51	−5.50	−5.00	−5.44	−4.65	−6.30	−5.34	−4.98	−2.99

Table 4 (*Continued*)

Panel/cell line	**5b**		**5d**		**5o**		**5v**		**A** [a]		**B** [b]	
	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	$\log_{10}$ GI_{50}	$\log_{10}$ TGI
Ovarian cancer												
IGROV1	–	–	–	–	–	–	−5.51	−5.08	−5.32	−3.84	−4.77	−2.94
OVCAR-3	−5.72	−5.41	−4.73	−4.38	−5.67	−5.32	−5.36	−4.70	−6.15	−5.17	−4.53	−2.96
OVCAR-4	−5.74	−5.36	−4.88	−4.59	−5.70	−5.28	−5.26	> −4.00	−5.82	−4.00	−4.20	−2.74
OVCAR-8	−5.69	−5.33	−4.80	−4.53	−5.46	−4.88	−5.52	−5.12	−6.18	−4.32	−3.92	−2.74
SK-OV-3	–	–	–	–	–	–	−5.64	−5.31	−6.16	−4.22	−4.77	−2.62
	−5.74	−5.43	−4.86	−4.57	−5.12	−4.69	−5.29	−4.53	−6.26	−4.78	−3.90	−2.64
Renal cancer												
786-O	−5.63	−5.30	−4.80	−4.53	−5.64	−5.26	−5.63	−5.21	−6.08	−3.97	−4.89	−2.76
A498	−5.73	−5.41	−4.83	−4.55	−5.57	−4.87	−5.29	> −4.00	−5.17	−4.02	−5.20	−3.22
ACHN	−5.72	−5.41	−4.86	−4.57	−5.66	−5.30	−5.38	> −4.00	−5.71	−3.95	−4.99	−2.74
CAKI-1	−5.76	−5.46	−4.73	−4.36	−4.90	−4.56	−5.56	–	−6.27	−4.80	−5.28	−2.90
RXF 393	−5.71	−5.39	−4.85	−4.57	−5.78	−5.37	−5.46	−4.90	−6.12	−5.15	−4.26	−3.08
SN12C	−5.71	−5.41	−4.80	−4.54	−5.66	−5.22	−5.56	−5.10	−5.90	−3.76	−4.61	−2.62
TK-10	−5.72	−5.41	−4.81	−4.53	−5.27	−4.65	−5.68	−5.32	−5.98	−4.10	−3.61	−2.64
UO-31	−5.70	−5.43	−5.69	−4.89	−6.68	−6.30	−5.73	−5.36	−5.83	−4.49	−5.17	−3.34
Prostate cancer												
PC-3	−5.73	−5.42	−4.77	−4.44	−5.52	−4.95	−5.73	−5.36	−5.75	−3.76	−4.40	−2.62
DU-145	−5.72	−5.38	−4.87	−4.58	−5.74	−5.41	−5.69	−5.37	−6.10	−3.87	−5.23	−2.66
Breast cancer												
MCF7	−5.66	−5.37	−4.79	−4.53	−5.70	−5.36	−5.65	−5.11	−6.15	−4.37	−5.77	−2.85
NCI/ADR-RES	−5.66	−5.26	−4.88	−4.58	−5.62	−5.19	−5.52	> −4.00	−6.23	−4.44	−4.29	−2.62
MDA-MB 231/ATCC	−5.70	−5.32	−4.96	−4.64	−5.56	−5.24	−5.67	−5.38	−5.71	−3.78	−3.49	−2.61
HS 578T	−5.38	> −4.00	−4.75	−4.34	−5.54	−5.14	−5.24	> −4.00	−5.13	−3.74	−3.68	−2.63
MDA-MB-435	−5.44	> −4.00	−4.78	−4.47	−5.61	−5.24	−4.67	> −4.00	−6.07	−4.49	−5.00	−2.62
MDA-N	−5.46	−4.85	−4.72	−4.42	−5.49	−4.94	−5.43	> −4.00	−5.99	−4.57	−5.11	−2.61
BT-549	–	–	–	–	–	–	−5.18	> −4.00	−5.44	−3.79	−4.07	−2.66
T-47D	−5.73	−5.40	−4.81	−4.47	−5.71	−5.31	−5.56	–	−5.69	−3.97	−4.33	−2.66
MG-MID	−5.63	−5.16	−4.84	−4.51	−5.54	−5.09	−5.44	−4.71	−5.90	−4.33	−4.66	−2.79

[a] **A**, Thioguanine.
[b] **B**, 5-Fluorouracil.

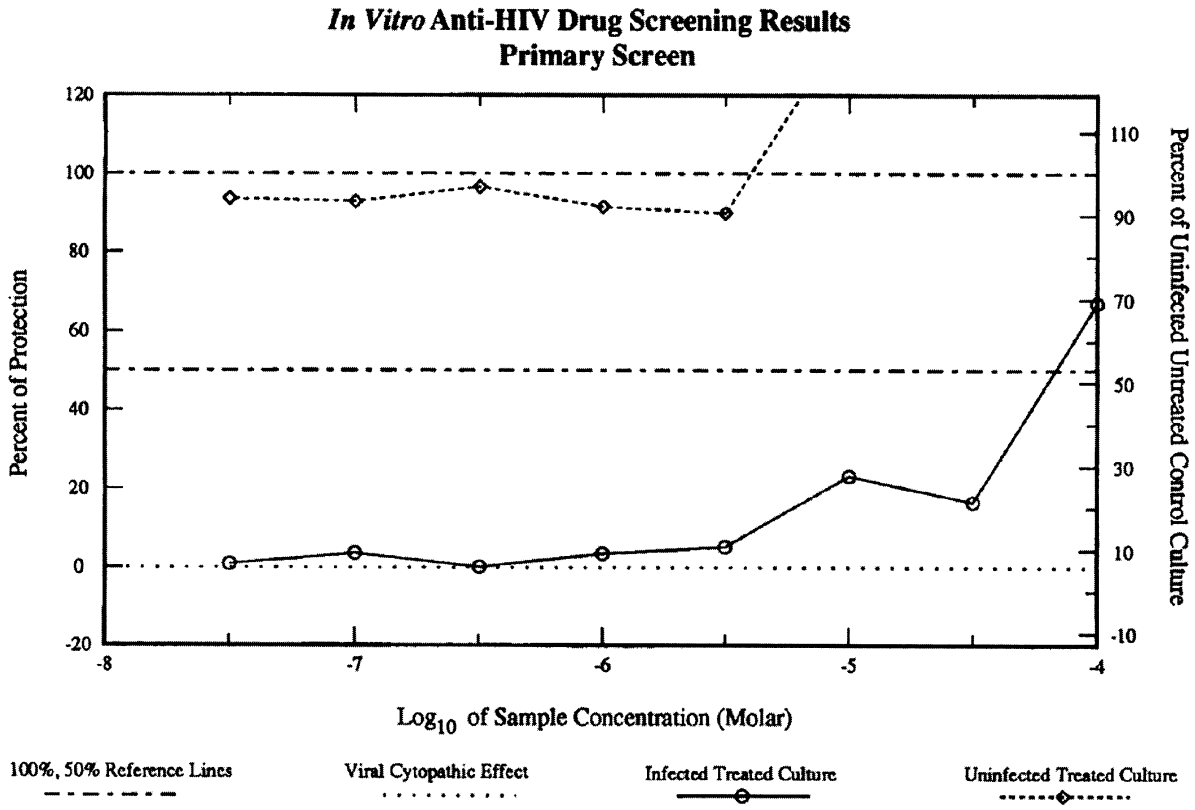


Fig. 4. In vitro anti-HIV drug screening results primary screen.

Table 5
In vitro anti-HIV screening results of **5v**

Dose (Molar)	Percent of protection	Percent of control	
		Infected	Uninfected
3.17×10^{-8}	0.80	6.75	93.94
1.00×10^{-7}	3.51	9.30	93.30
3.17×10^{-7}	0.06	6.06	96.76
1.00×10^{-6}	3.41	9.21	92.02
3.16×10^{-6}	5.16	10.85	90.56
1.00×10^{-5}	23.11	27.72	136.34
3.16×10^{-5}	16.41	21.43	179.57
1.00×10^{-4}	67.16	69.13	173.55
IC_{50} (Molar)	$> 1.00 \times 10^{-4}$		
EC_{50} (Molar)	6.81×10^{-5}		
TI_{50} (IC/EC)	> 1.47		

parameter was expressed as greater or less than the maximum or minimum concentration tested.

4.3. In vitro evaluation of anti-HIV activity

The procedure used in the National Cancer Institute's test for agents active against HIV is designed to detect agents acting at any stage of the virus reproductive cycle. The assay basically involves the killing of T4 lymphocytes by HIV. Small amounts of HIV are added to cells, and two cycles of virus reproduction are necessary to obtain the required cell killing. Agents that interact with virions, cells, or virus gene-products to interfere with viral activities will protect a cell from cytolysis. Compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in this screen. All tests were compared with at least one positive control done at the same time under identical conditions.

Compound **5v** was dissolved in dimethyl sulfoxide, then diluted 1:100 in cell culture medium before preparing serial half-$\log_{10}$ dilutions. T4 lymphocytes (CEM cell line) were added and after a brief interval HIV-1 was added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere for 6 days. The tetrazolium salt, XTT, was added to all wells, and cultures were incubated to allow formazan colour development by viable cells. Individual wells were analysed spectrophotometrically to quantitate formazan production, and in addition were viewed microscopically to detect viable cells and confirm protective activity. Drug-treated virus-infected cells were compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc.) on the same plate. Data were reviewed in

comparison with other tests done at the same time and a determination about activity was made.

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